MECHANISMS AND PATHWAYS CONTRIBUTING TO THE DIVERSITY OF AGING ACROSS THE TREE OF LIFE

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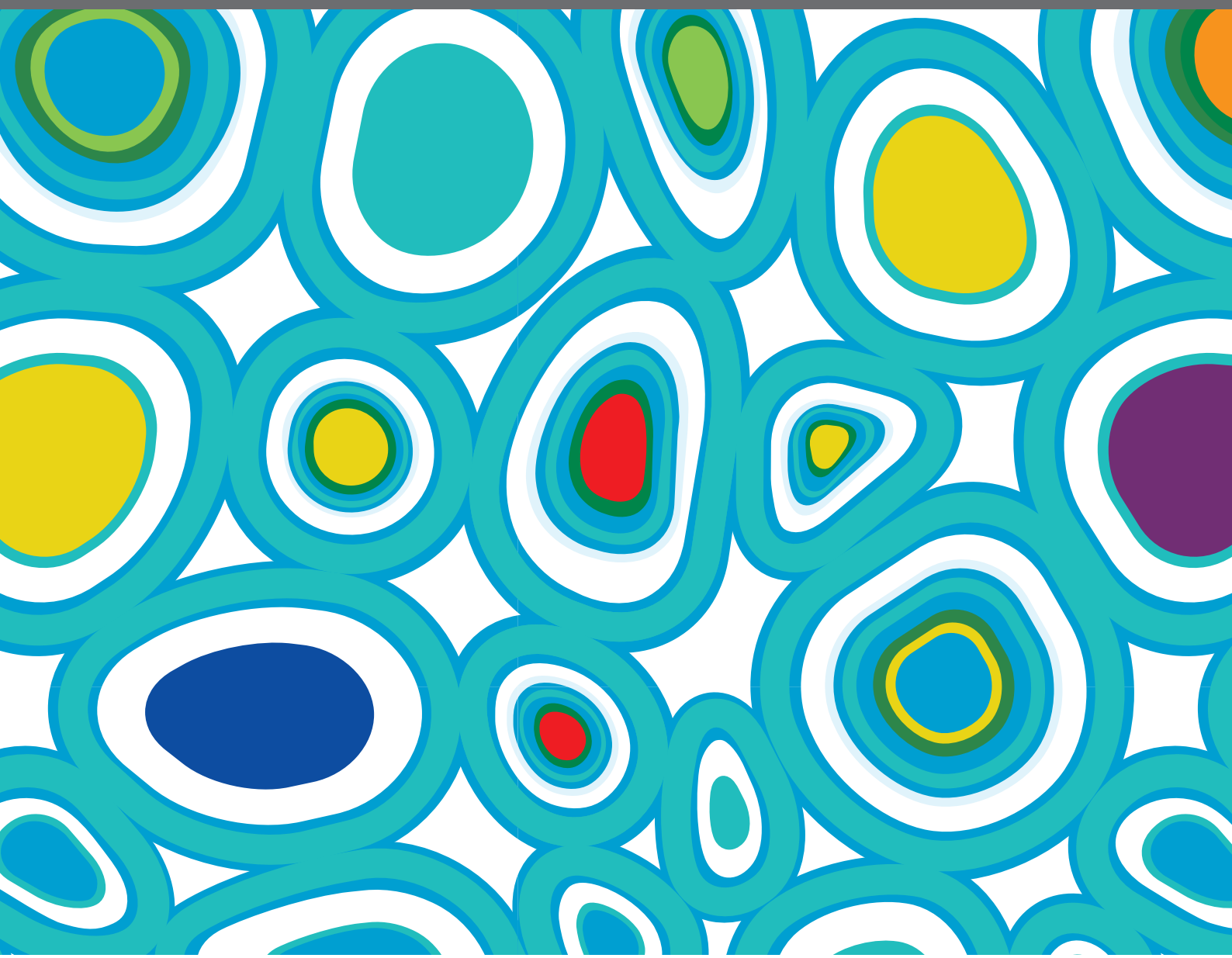

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MECHANISMS AND PATHWAYS CONTRIBUTING TO THE DIVERSITY OF AGING ACROSS THE TREE OF LIFE

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EDITORIAL
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Editorial: Mechanisms and Pathways Contributing to the Diversity of Aging Across the Tree of Life

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Editorial on the Research Topic

Mechanisms and Pathways Contributing to the Diversity of Aging Across the Tree of Life

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INTRODUCTION

The steadily increasing number of elderly individuals in our societies imposes an increased burden on our healthcare system due to an accompanying rise in chronic age-related diseases, including cardiovascular and neurodegenerative diseases (Niccoli and Partridge 2012). It is therefore of utmost importance to identify and understand mechanisms of aging across the tree of life and translate these discoveries into health-promoting interventions in humans (Partridge et al., 2018). Roughly 30 years ago, two important publications shaped our thinking on the evolution of aging and lifespan. Caleb Finch's "Longevity, Senescence, and the Genome" (Finch 1990) provided an authoritative overview of what was known about the diversity of aging patterns across the tree of life, showing that some species appeared not to age. Kenyon and others (Kenyon et al., 1993) showed that simple genetic tweaks could dramatically extend lifespan in *Caenorhabditis elegans*, paving the way for an industry of research into conserved genetic pathways, driven by the idea of universal aging processes. Since then, the complexity and diversity of demographic aging patterns has been fleshed out (Jones et al., 2014; Jones and Vaupel 2017), while at the same time we are increasingly realizing that the mechanisms are also diverse (Cohen 2018). We are starting to move from a model of studying a few canonical model organisms (e.g. fruit flies and nematodes) complemented by a few exceptionally long-lived model organisms (e.g. naked mole rats, ocean quahogs, and hydra) to a need to characterize more systematically how mechanisms vary across species (Austad 2009). Beyond the basic science interest, this is essential from a translational perspective: a failure to understand the diversity of aging mechanisms among and within species, and how they interact, could be dangerous for proposed geroscience interventions. This Research Topic thus assembles cutting-edge work that sheds light on the mechanistic evolution of aging, from surveys of how single mechanisms vary across species to portraits of understudied species to eco-evolutionary perspectives and even novel theories of aging. Our hope is that these studies, presented together, will stimulate a new wave of thinking on how variation in mechanisms across species drives variation in aging, and on the underlying ecological, physiological, and evolutionary forces that may drive the mechanisms.

RESEARCH COMPARING MECHANISMS ACROSS SPECIES

Our Research Topic contains several papers focusing on the conservation of aging mechanisms across species. These contain well-known mechanisms, such as mitochondrial dysfunction, protein toxicity and reactive oxygen species (ROS), but also understudied phenomena, such as reproductive suicide and eusociality. The review by van der Rijt and others (van der Rijt et al., 2020) focusses on mitochondrial dysfunction, one of the nine hallmarks of aging. The authors show that this hallmark is connected to many of the other hallmarks across the tree of life and argue that future studies on aging should thus take these interactions into account. Pras and Nollen (Pras and Nollen 2021) focused on another hallmark of aging, namely loss of proteostasis, and describe the different mechanisms responsible for maintaining a healthy proteome. They also provide examples of long-lived species that seem to have optimized protein homeostasis, showing that this hallmark is conserved across the tree of life. Oxidative damage, caused by ROS, is another mechanism often implicated in aging. The review by Shields and others (Shields et al., 2021) provides an overview of studies linking ROS and lifespan, showing the relationship between the two is complex, with ROS showing beneficial or detrimental effects depending on the species under study. Huang and others (Huang et al., 2021) focused their work on an understudied group of proteins in aging; flavin-containing monooxygenases (FMOs). They previously identified FMOs to be involved in lifespan regulation in *C. elegans* and now show that mammalian homologs affect stress resistance and metabolism in cellular models, which makes them an interesting target for further studies in model organisms and humans. Another understudied factor in aging is sociality and accompanying kin selection, which can modify selection pressures and the magnitude and direction of trade-offs (Bourke 2007). Increased sociality is associated with a longer lifespan (*see* (Arnold and Owens 1998; Thorley 2020) for examples) and eusociality, in particular, presents a fascinating window into the evolution of aging with greatly extended lifespans compared to non-social relatives, but also diversity in aging among castes (Keller and Jemielity 2006; Kramer et al., 2016). Giraldo and others (Giraldo et al., 2021) provide a mini-review examining the relationship between eusociality and brain senescence in eusocial species. They conclude that, though different taxa may show similarly extended lifespans and similar senescence phenotypes, the mechanisms involved are different.

NOVEL APPROACHES TO STUDY AGING MECHANISMS

In addition, several papers in our Research Topic used novel approaches to study interactions between aging pathways and mechanisms. Simons and others (Simons et al., 2021), for example, showed that androgens do not mediate a simple trade-off between survival/maintenance and reproduction: relationships are often quadratic. Ukraintseva and others (Ukraintseva et al., 2021), on the other hand, looked at the interaction between genetic variants in genes involved in different aging-related pathways. They identified several combinations of genetic variants in genes implicated in different pathways that show an epistatic effect on survival to 85 years of age in two independent human studies.

GENERAL INSIGHTS FROM INDIVIDUAL SPECIES

Most articles in our Research Topic set their work in a comparative (cross-species) context, and some also highlight the value of undertaking detailed studies using non-canonical model organisms. For example, Yun (2021) provides a fascinating perspective on the regenerative abilities of salamanders. These exceptionally long-lived animals can famously regrow limbs and even complex organs and are also markedly cancer-resistant. Yun explores the cellular and molecular basis for these traits and probes the tantalizing mechanistic connections between these abilities and their extraordinary longevity and negligible senescence. Meanwhile, Steiner (Steiner 2021) examined cell senescence in bacteria, which for a long time were thought to escape senescence by dividing into two identical "daughter" cells. It is now well known that this division is asymmetric and that bacteria such as *Escherichia coli* do indeed senesce. Steiner reviews progress into bacterial senescence and highlights that aging trajectories are sensitive to environmental conditions and genotypic variation that make a mechanistic understanding elusive. Nevertheless, they optimistically point out that rapid developments in molecular toolkits and microfluidic techniques open the path to exciting opportunities to understand aging more generally.

ECO-EVOLUTIONARY INSIGHTS INTO MECHANISMS

Our Research Topic also includes some papers addressing aging in the wild. Aging in wild animals was for a long time neglected because individuals were thought to be eliminated by predation or starvation before age-related physiological decline could take hold. Work by Nussey and others (Nussey et al., 2008) and Jones and others (Jones et al., 2008) convincingly showed that this was not the case, and that senescence was in fact readily detectable with sufficient data. This opened the door to studies examining the eco-evolutionary aspects of senescence in the wild. In our Research Topic, the study by Pigeon and others (Pigeon et al., 2021) on bighorn sheep is an excellent example of this work. Their study asks whether patterns of senescence in reproduction and survival may be influenced by environmental conditions during early development. This is an important question because the disposable soma theory of aging (Kirkwood 1977) would suggest that variation in resource availability could influence the balance of resource allocation to the competing processes of maintenance, growth and reproduction. Such effects have been detected in other taxa before (*see* (Cooper and Kruuk 2018; Spagopoulou et al., 2020) for examples), but this study shows that although early-life conditions influence the magnitude of survival probability and reproduction in later life, they do not

necessarily influence rates of senescence. Pigeon et al. also highlight the need for clear thinking on how trajectories may be influenced by early conditions. The magnitude, age at onset, and rate of senescence combine to determine the trajectory and may be hard to distinguish. Kumar and others (Kumar et al., 2021) also tackle senescence in non-canonical model species, but from a very different perspective. They use samples from 106 bird species to examine the membrane pacemaker hypothesis (MPH), which supposes that cell membranes trade off metabolic rate with oxidative damage: cells with more unsaturated membrane fatty acids have enhanced metabolism, but are prone to oxidative damage. Thus, short-lived species are expected to have a greater degree of membrane unsaturation than long-lived species. The hypothesis offers a convenient mechanistic explanation for the cross-species apparent trade-off between longevity, fecundity and metabolic rate. Ultimately, however, Kumar et al. find little general support for the MPH and, rather, suggest that long lifespans co-evolve with long-chain fatty acids independently of the degree of unsaturation.

EVOLUTIONARY THEORIES EXPLAINING AGING MECHANISMS

Several papers in the collection have important implications for our understanding of the evolution of aging and lifespan. Two, in fact, present novel evolutionary theories of aging. The adaptive hitchhike model by Omotoso and others (Omotoso et al., 2021) suggests that pro-longevity mutations may arise for reasons unrelated to longevity and then hitchhike around the tree of life. In a complementary perspective, Wensink and Cohen (Wensink and Cohen 2021) propose the Danaid theory of aging: that complex organisms are often unmaintainable, i.e. structured in ways that inadvertently prevent immortality, with clade-specific chance events having large impacts on the distribution of aging, lifespan, and the underlying mechanisms. Gems and others (Gems et al., 2021) contribute additional evidence for the evolutionary theory of semelparity, showing that *C. elegans* may be considered semelparous much like salmon, but that the pathways triggering reproductive death are also active in iteroparous organisms with more limited effects. The papers by Kumar et al. (Kumar et al., 2021) and by Simons et al. (Simons et al., 2021) provide additional evidence against straightforward, linear mediation of trade-offs by two different hypothesized mechanisms. Taken together, and with the species-specific studies mentioned above, these studies start to paint a coherent picture of the evolution of aging as a much more rich and textured landscape than previously thought, one in which no mechanistic or evolutionary theory will simply explain everything, yet in which mechanisms and evolutionary forces are tightly intertwined.

FUTURE PERSPECTIVES

The manuscripts presented in this Research Topic bring us a small step closer to the identification and understanding of mechanisms of aging across the tree of life, but also show that there is still a lot of work to be done before we will have a full understanding of the aging process. We hope that our Research Topic will stimulate new research focusing on more non-canonical model organisms and understudied mechanisms and will lead to the development of new and improved theories of aging that, for example, also deal with currently unexplained phenomena, such as asexual reproduction and vegetative growth. We believe that the recent emergence of state-of-the-art methods, including gene editing and tracking of individual animals in the wild, and the use of more comparative approaches, taking into account eco-evolutionary and environmental contexts, will also tremendously benefit such studies. The subsequent translation of findings from (non-)canonical model organisms to humans will hopefully assist in managing the upcoming Silver Tsunami.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing of the article and approved the submitted version.

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REFERENCES

Arnold, K. E., and Owens, I. P. F. (1998). Cooperative Breeding in Birds: A Comparative Test of the Life History Hypothesis. *Proc. R. Soc. Lond. B* 265 (1398), 739–745. doi:10.1098/rspb.1998.0355

Austad, S. N. (2009). Comparative Biology of Aging. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 64A (2), 199–201. doi:10.1093/gerona/gln060

Bourke, A. F. G. (2007). Kin Selection and the Evolutionary Theory of Aging. *Annu. Rev. Ecol. Evol. Syst.* 38 (1), 103–128. doi:10.1146/annurev.ecolsys.38.091206.095528

Cohen, A. A. (2018). Aging Across the Tree of Life: The Importance of a Comparative Perspective for the Use of Animal Models in Aging. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864 (9 Pt A), 2680–2689. doi:10.1016/j.bbadis.2017.05.028

Cooper, E. B., and Kruuk, L. E. B. (2018). Ageing with a Silver-Spoon: A Meta-Analysis of the Effect of Developmental Environment on Senescence. *Evol. Lett.* 2 (5), 460–471. doi:10.1002/evl3.79

Finch, C. E. (1990). *Longevity, Senescence, and the Genome*. Chicago, IL: University of Chicago Press.

Gems, D., Kern, C. C., Nour, J., and Ezcurra, M. (2021). Reproductive Suicide: Similar Mechanisms of Aging in *C. elegans* and Pacific Salmon. *Front. Cel Dev. Biol.* 9, 688788. doi:10.3389/fcell.2021.688788

Giraldo, Y. M., Muscedere, M. L., and Traniello, J. F. A. (2021). Eusociality and Senescence: Neuroprotection and Physiological Resilience to Aging in Insect and Mammalian Systems. *Front. Cel Dev. Biol.* 9, 673172. doi:10.3389/fcell.2021.673172

Huang, S., Howington, M. B., Dobry, C. J., Evans, C. R., and Leiser, S. F. (2021). Flavin-Containing Monooxygenases Are Conserved Regulators of Stress

Resistance and Metabolism. *Front. Cel Dev. Biol.* 9, 630188. doi:10.3389/fcell.2021.630188

Jones, O. R., Gaillard, J.-M., Tuljapurkar, S., Alho, J. S., Armitage, K. B., Becker, P. H., et al. (2008). Senescence Rates Are Determined by Ranking on the Fast-Slow Life-History Continuum. *Ecol. Lett.* 11 (7), 664–673. doi:10.1111/j.1461-0248.2008.01187.x

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of Ageing Across the Tree of Life. *Nature* 505 (7482), 169–173. doi:10.1038/nature12789

Jones, O. R., and Vaupel, J. W. (2017). Senescence Is Not Inevitable. *Biogerontology* 18 (6), 965–971. doi:10.1007/s10522-017-9727-3

Keller, L., and Jemielity, S. (2006). Social Insects as a Model to Study the Molecular Basis of Ageing. *Exp. Gerontol.* 41 (6), 553–556. doi:10.1016/j.exger.2006.04.002

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A *C. elegans* Mutant That Lives Twice as Long as Wild Type. *Nature* 366 (6454), 461–464. doi:10.1038/366461a0

Kirkwood, T. B. L. (1977). Evolution of Ageing. *Nature* 270 (5635), 301–304. doi:10.1038/270301a0

Kramer, B. H., van Doorn, G. S., Weissing, F. J., and Pen, I. (2016). Lifespan Divergence Between Social Insect Castes: Challenges and Opportunities for Evolutionary Theories of Aging. *Curr. Opin. Insect Sci.* 16, 76–80. doi:10.1016/j.cois.2016.05.012

Kumar, S. A., Albrecht, T., Tomášek, O., and Tomasek, O. (2021). No Evidence for Trade-Offs between Lifespan, Fecundity, and Basal Metabolic Rate Mediated by Liver Fatty Acid Composition in Birds. *Front. Cel Dev. Biol.* 9, 638501. doi:10.3389/fcell.2021.638501

Niccoli, T., and Partridge, L. (2012). Ageing as a Risk Factor for Disease. *Curr. Biol.* 22 (17), R741–R752. doi:10.1016/j.cub.2012.07.024

Nussey, D. H., Coulson, T., Festa-Bianchet, M., and Gaillard, J.-M. (2008). Measuring Senescence in Wild Animal Populations: Towards a Longitudinal Approach. *Funct. Ecol.* 22 (3), 393–406. doi:10.1111/j.1365-2435.2008.01408.x

Omotoso, O., Gladyshev, V. N., and Zhou, X. (2021). Lifespan Extension in Long-Lived Vertebrates Rooted in Ecological Adaptation. *Front. Cel Dev. Biol.* 9, 704966. doi:10.3389/fcell.2021.704966

Partridge, L., Deelen, J., and Slagboom, P. E. (2018). Facing up to the Global Challenges of Ageing. *Nature* 561 (7721), 45–56. doi:10.1038/s41586-018-0457-8

Pigeon, G., Landes, J., Festa-Bianchet, M., and Pelletier, F. (2021). Do Early-Life Conditions Drive Variation in Senescence of Female Bighorn Sheep? *Front. Cel Dev. Biol.* 9, 637692. doi:10.3389/fcell.2021.637692

Pras, A., and Nollen, E. A. A. (2021). Regulation of Age-Related Protein Toxicity. *Front. Cel Dev. Biol.* 9, 637084. doi:10.3389/fcell.2021.637084

Shields, H. J., Traa, A., and Van Raamsdonk, J. M. (2021). Beneficial and Detrimental Effects of Reactive Oxygen Species on Lifespan: A Comprehensive Review of Comparative and Experimental Studies. *Front. Cel Dev. Biol.* 9, 628157. doi:10.3389/fcell.2021.628157

Simons, M. J. P., Sebire, M., Verhulst, S., and Groothuis, T. G. G. (2021). Androgen Elevation Accelerates Reproductive Senescence in Three-Spined Stickleback. *Front. Cel Dev. Biol.* 9, 752352. doi:10.3389/fcell.2021.752352

Spagopoulou, F., Teplitsky, C., Chantepie, S., Lind, M. I., Gustafsson, L., and Maklakov, A. A. (2020). Silver-spoon Upbringing Improves Early-life Fitness but Promotes Reproductive Ageing in a Wild Bird. *Ecol. Lett.* 23 (6), 994–1002. doi:10.1111/ele.13501

Steiner, U. K. (2021). Senescence in Bacteria and its Underlying Mechanisms. *Front. Cel Dev. Biol.* 9, 668915. doi:10.3389/fcell.2021.668915

Thorley, J. (2020). The Case for Extended Lifespan in Cooperatively Breeding Mammals: A Re-appraisal. *PeerJ* 8, e9214. doi:10.7717/peerj.9214

Ukraintseva, S., Duan, M., Arbeev, K., Wu, D., Bagley, O., Yashkin, A. P., et al. (2021). Interactions Between Genes from Aging Pathways May Influence Human Lifespan and Improve Animal to Human Translation. *Front. Cel Dev. Biol.* 9, 692020. doi:10.3389/fcell.2021.692020

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Wensink, M. J., and Cohen, A. A. (2021). The Danaid Theory of Aging. *Front Cel Dev Biol*.107, 2411–2502. doi:10.3389/fcell.2021.671208

Yun, M. H. (2021). Salamander Insights into Ageing and Rejuvenation. *Front. Cel Dev. Biol.* 9, 689062. doi:10.3389/fcell.2021.689062

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REVIEW
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Integrating the Hallmarks of Aging Throughout the Tree of Life: A Focus on Mitochondrial Dysfunction

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Since the identification and definition of the hallmarks of aging, these aspects of molecular and cellular decline have been most often described as isolated or distinct mechanisms. However, there is significant evidence demonstrating interplay between most of these hallmarks and that they have the capacity to influence and regulate one another. These interactions are demonstrable across the tree of life, yet not all aspects are conserved. Here, we describe an integrative view on the hallmarks of aging by using the hallmark "mitochondrial dysfunction" as a focus point, and illustrate its capacity to both influence and be influenced by the other hallmarks of aging. We discuss the effects of mitochondrial pathways involved in aging, such as oxidative phosphorylation, mitochondrial dynamics, mitochondrial protein synthesis, mitophagy, reactive oxygen species and mitochondrial DNA damage in relation to each of the primary, antagonistic and integrative hallmarks. We discuss the similarities and differences in these interactions throughout the tree of life, and speculate how speciation may play a role in the variation in these mechanisms. We propose that the hallmarks are critically intertwined, and that mapping the full extent of these interactions would be of significant benefit to the aging research community.

Keywords: aging, mitochondria, interplay, hallmarks of aging, tree of life

INTRODUCTION

The Nine Hallmarks of Aging

Aging is a multifactorial and complex process, affecting organisms at the molecular, cellular, tissue, and system levels (Gems and Partridge, 2013; Cohen, 2018). This progressive deterioration throughout life leads to age-related diseases such as cardiovascular diseases, cancer, osteoarthritis, diabetes mellitus type II and neurodegenerative diseases such as Alzheimer's Disease (AD) and Parkinson's Diseases (PD) (López-Otín et al., 2013; Franceschi et al., 2018). While lifespan has steadily increased in humans (Oeppen and Vaupel, 2002), many of these diseases remain prevalent and possess no cures. With the aim of uncovering mechanisms of aging and discovering interventions that promote healthier aging, scientists have utilized model organisms from across the phylogenetic tree.

The most common model organisms are short lived, with well annotated genomes and molecular toolkits to intervene genetically in aging, such as *S. cerevisiae*, *C. elegans*, *D. melanogaster,* and *M. musculus* (Mitchell et al., 2015). Outside these traditional model organisms, aging researchers have studied extremely long-lived species, such as Hydra and the naked mole rat (Buffenstein, 2005; Boehm et al., 2012), as well as emerging models for aging research such as the killifish (Harel and Brunet, 2016).

Geroprotective interventions, either environmental or genetic, can extend the lifespan of model organisms (Mitchell et al., 2015). Through such experiments, nine hallmarks of aging have been defined, with indications that these are also conserved in humans (López-Otín et al., 2013). While more hallmarks are likely to be described in the future, as was the case for the hallmarks of cancer (Hanahan and Weinberg, 2011), we provide evidence of the interplay of between these current nine hallmarks of aging. The nine cellular and molecular hallmarks of aging are divided into three groups; (a) the primary hallmarks, (b) antagonistic hallmarks, and (c) integrative hallmarks (López-Otín et al., 2013). The primary hallmarks, which are unequivocally deleterious to the cell, include genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis. The antagonistic hallmarks, which are beneficial at low levels but at high levels become deleterious, are deregulated nutrient sensing, cellular senescence, and mitochondrial dysfunction. Finally, the integrative hallmarks, affecting tissue homeostasis and function, are stem cell exhaustion and altered intercellular communication. These hallmarks of aging have been presented throughout various research disciplines as nine separate hallmarks, and, while possessing crosstalk, are still considered largely independent. In this review, we provide evidence of the interplay between hallmarks, by highlighting one, mitochondrial dysfunction, and how it interacts with all others. In doing so, we aim to provide a broader view of the mitochondria's role in the aging process throughout the tree of life and suggest reflection on the integrative nature of complex biological processes such as aging.

Mitochondrial Dysfunction and Aging

The role of mitochondrial dysfunction in aging is well documented and for comprehensive reviews on mitochondrial function in aging we refer the reader to Sun et al. (2016), Zhang et al. (2018), Bar-Ziv et al. (2020), Rath (2020). Here we summarize the various aspects of mitochondrial biology and aging across the prominent model organisms that are relevant to this review and the hallmark of mitochondrial dysfunction.

Mitochondria generate energy in the cell, via the electron transport chain (ETC) and oxidative phosphorylation (OXPHOS). In general, mitochondrial function is impaired with age, marked by altered mitochondrial respiration, decreased energy production in the form of adenosine triphosphate (ATP), as well as widespread changes in metabolites associated with mitochondrial function (Lesnefsky and Hoppel, 2006; Houtkooper et al., 2011). Moreover, as a consequence of energy production, mitochondria also produce reactive oxygen species (ROS), which are formed during OXPHOS, causing oxidative damage to the mitochondria (Lesnefsky and Hoppel, 2006). ROS can contribute to damage to mitochondrial DNA (mtDNA), oxidation of mitochondrial proteins, less effective electron transport chain and impaired quality control in mitophagy (Bratic and Larsson, 2013; López-Otín et al., 2013). Yet, antioxidants do not provide lifespan benefits in model organisms through ROS scavenging, suggesting that ROS generation and oxidative stress do not themselves cause aging (Schulz et al., 2007). Instead, ROS have even been described as important signaling molecules in the cell, eliciting protective gene expression beneficial to longevity (Schulz et al., 2007). In line with this, a systematic review showed that there was no beneficial effect of antioxidant supplementation on the mortality in patients with various diseases (Bjelakovic et al., 2012). Recently, comparative analysis between mammalian species ranging in longevity identified a correlation between low levels of specific OXPHOS complex I proteins, ROS production, and lifespan in a species-specific manner (Mota-Martorell et al., 2020). Therefore, speciation may affect the role of ROS in aging and vice versa. In other words, ROS might have evolved different roles and physiologically normal levels depending on the environment of the species. These different selective pressures result in differences in the role of ROS.

While mitochondrial dysfunction shows detrimental effects at old age, it can also provide protection via hormetic stresses that promote longevity, especially when targeted early in life. For instance, reduction of nuclear encoded OXPHOS proteins via RNAi leads to extended life span in when the treatment is initiated in the larval stages of *C. elegans* (Dillin et al., 2002; Houtkooper et al., 2013). The proteins of the OXPHOS complexes are encoded by both nuclear DNA (nDNA) and mtDNA. Inhibiting mitochondrial translation and thus lowering synthesis of mtDNA-encoded OXPHOS proteins extends lifespan in *C. elegans*, and the link between mitochondrial translation and lifespan is conserved in natural populations of mice (Houtkooper et al., 2013). Inhibiting mitochondrial translation causes a mitonuclear protein imbalance that activates the protective mitochondrial unfolded protein response (UPR^{mt}). The UPR^{mt} is a stress response and one example of how dysfunctional mitochondria communicate to the nucleus where it activates mitochondrial chaperones and proteases, ROS detoxification enzymes, and mitochondrial protein import components (Nargund et al., 2015). In addition to UPR^{mt}, several other pathways exist to protect mitochondria from intrinsic or extrinsic damage, including mitoCPR, MAGIC, UPR^{am}, and mito-cytosolic translational balance (Molenaars et al., 2020a,b).

It is not only mitochondrial function that plays a role in aging, but also mitochondrial form. Mitochondria are constantly dynamically rearranging and degrading via the processes of fission, fusion and mitophagy. Mitochondria use these processes as quality control systems, degrading poorly-functional mitochondria on the go, and while specific proteins vary between model organisms, these processes are highly conserved (Liu et al., 2020a). Mitophagy involves the elimination of non-functional mitochondria, for instance upon their loss of membrane potential. Mitophagy is reduced throughout the aging process, and conversely, inducing mitophagy through genetic or pharmacological means leads to a longer lifespan

(Wu et al., 2013; Ryu et al., 2016). Aging and age-related diseases are often accompanied by a lack of mitochondrial fusion, which leads to fragmented mitochondria (Chen et al., 2007; Houtkooper et al., 2013; Sharma et al., 2019), and altering mitochondrial dynamics of fission and fusion can extend lifespan in worms (Weir et al., 2017; Liu et al., 2020b).

Each of these aspects of mitochondrial dysfunction plays a significant role in aging. We next look beyond mitochondrial dysfunction as an individual hallmark and explore how these mitochondrial processes and age-related deteriorations interrelate and communicate with the other hallmarks of aging.

INTEGRATING MITOCHONDRIAL FUNCTION WITH THE PRIMARY HALLMARKS OF AGING

The primary hallmarks of aging are defined as unequivocally deleterious to the cell. This means that proper functioning of these processes is important for the viability of the cell and the dysfunction that occurs with age leads to cellular damage. Mitochondrial dysfunction interacts with each of these primary hallmarks, thus leading to progression of the aging process.

Genomic Instability and Mitochondrial Dysfunction

During aging, DNA damage accumulates, for instance due to ROS or environmental sources such as radiation (Fakouri et al., 2019). When DNA damage occurs, the DNA damage repair (DDR) is triggered (Niedernhofer et al., 2018; Fakouri et al., 2019). DDR activates several pathways, such as apoptosis, repair, or senescence. Every day, over 10^4 mutations occur in the genome (Niedernhofer et al., 2018). These mutations can be repaired by several mechanisms such as base excision repair, nucleotide excision repair, interstrand crosslink repair, non-homologous end joining and homologous recombination (Niedernhofer et al., 2018). The capacity for DNA repair decreases with aging, which results in accumulations of mutations with age and genomic instability (Niedernhofer et al., 2018).

The increasing amounts of DNA damage occurring during aging lead to activation of poly (ADP-ribose) polymerase 1 (PARP1), which serves as a DNA damage sensor and activator of various target proteins involved in the DNA damage response (Gupte et al., 2017). PARP1 uses nicotinamide adenine dinucleotide (NAD^+) as a substrate in this process, and increased amounts of DNA damage therefore require large amounts of NAD^+, resulting in a depletion of the NAD^+ pool (Berger, 1985). This depletion of the NAD^+ pool contributes to mitochondrial dysfunction considering that NAD^+ acts as a co-factor to activate sirtuins. Sirtuins play important roles for mitochondrial function and longevity, including the activation the transcription factor peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α), which stimulates mitochondrial biogenesis and function, and NAD^+ depletion reduces this activation (Houtkooper et al., 2012). In line with this observation, preserving NAD^+ by inhibiting PARP activity leads to healthier cells. Possible detrimental effects. Indeed, mice with mutations in PARP1 or PARP2 or those treated with pharmacological PARP inhibitors showed improved mitochondrial function and organismal fitness (Bai et al., 2011; Pirinen et al., 2014), and worms with PARP inhibition had extended lifespan (Mouchiroud et al., 2013). However, it should also be taken into account that PARP is important for DNA repair and PARP inhibition can possibly lead to genomic instability and cancer. Therefore, it should be investigated what are the long-term side effects of PARP inhibition and how these can be prevented (Bai et al., 2011).

Another avenue in which mitochondrial dysfunction and genomic instability directly meet is through the mutation of mtDNA genes. Mutations in mtDNA is generally 10-fold higher than in nuclear DNA (Wang et al., 2005). mtDNA damage can have various downstream consequences related to aging, such as damage in neurons, increasing the risk on neurodegenerative diseases such as AD (Wang et al., 2005). Another way by which mitochondria might contribute to DNA maintenance is through their role in nucleotide synthesis. Indeed, mitochondrial dysfunction leads to impaired synthesis of nucleotides (Tufi et al., 2014), and although not formally demonstrated one might expect downstream consequences on telomere attrition and genome stability. To summarize, genome instability, for instance caused by endogenous or exogenous DNA damage sources can damage mtDNA or indirectly impair mitochondrial function through NAD+.

Telomere Attrition and Mitochondrial Dysfunction

All species must maintain the integrity of the ends of their chromosomes. How this is achieved varies greatly per species, for instance through (a) retrotransposons in *Drosophila melanogaster*, (b) having circular DNA and therefore no chromosome ends, such as with bacteria, or (c) possessing "telomeres" at the ends of chromosomes, such as in humans. When cells divide, telomeres become shorter, and when telomeres become too short, cells enter the state of senescence, which is an irreversible growth arrest. Cellular senescence is itself a hallmark of aging that contributes to age-related dysfunction and disease (López-Otín et al., 2013). Rapid telomere shortening can therefore coincide with an acceleration of aging and, indeed, studies in humans demonstrated that individuals with shorter telomeres have mortality rates twice as high as those with longer telomeres. Additionally, individuals with shorter telomeres have a significantly higher chance of having heart disease and age-related diseases compared to individuals with longer telomeres (Cawthon et al., 2003).

Mitochondria can directly contribute to an acceleration of telomere shorting, due to their production of ROS, which lead to DNA damage and telomere shortening (Liu et al., 2002). For example, cells treated with the protonophore carbonyl p-trifluoromethoxyphenylhydrazone (FCCP), a compound which depolarize the mitochondrial membrane potential

and disrupts ATP synthesis, have increased ROS production (Liu et al., 2002) as well as shorter telomeres compared to untreated cells. Both ROS levels and shorter telomeres were rescued by treating the cells with ROS scavenger molecules, indicating that ROS leads to the shortening of telomeres (Liu et al., 2002). Similarly, irradiating cells with UV-A caused an increased level of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a marker of DNA oxidation, as well as accelerated telomere shortening (Kawanishi and Oikawa, 2004).

The presence of critically short telomeres leads to the activation of the tumor suppressor gene p53 in mice (Sahin et al., 2011). P53 itself is known to inhibit PGC-1α/β, leading to the inhibition of mitochondrial biogenesis, OXPHOS, decreased mitochondrial mass, less ATP production and increased ROS production (Sahin et al., 2011). Therefore, initial damage to telomeres by mitochondrial ROS can cause a cycle of increasing damage, whereby PGC-1α inhibition further impairs mitochondrial function, creating additional ROS, which then contributes to further telomere damage. This is in line with the observations of telomere shortening, oxidative stress and aging in PGC-1α knock-out mice (Xiong et al., 2015). Generally, mitochondrial dysfunction can cause telomere attrition, which inhibits PGC-1α/β, resulting in further mitochondrial dysfunction. Moreover, future work might reveal more crosstalk links.

Epigenetics and Mitochondrial Dysfunction

Reversible modifications of DNA and chromosomes, called epigenetics, are another hallmark of aging (López-Otín et al., 2013). Out of all the epigenetic changes that occur, most is known regarding DNA methylation and histone modifications. DNA methylation occurs through the activity of DNA methyltransferases (DNMTs), on regions of cytosines followed by guanines (termed CpG islands). During aging, hypermethylation of these CpG regions occurs, while hypomethylation occurs outside of these CpG regions (Christensen et al., 2009). Histone modifications are also affected during aging. Trimethylation on the lysine 4 residue of histone 3 (H3K4me3) leads to transcriptional activation, while trimethylation of lysine 27 of histone 3 (H3K27me3) is a repressive mark. Knockdown of one of the complexes of the H3K4me3 methyltransferase results in a longer lifespan in *C. elegans* (Greer et al., 2010). Another epigenetic modification is through histone deacetylases (HDACs), removing acetyl groups on histones. HDACs regulate the chromatin structure and play a critical role in transcription regulation. HDAC inhibitor compounds and their effects on each of the hallmarks of aging have previously been reviewed (McIntyre et al., 2019). HDAC inhibitors can increase lifespan in yeast, worms and flies (Lee et al., 2012; McIntyre et al., 2019).

Mitochondrial dysfunction plays a role in determining epigenetics changes during aging. Increased ROS leads to changes in the methylome, inducing aspects of the epigenetic aging process (Kietzmann et al., 2017). ROS causes DNA lesions and the most common base lesion is 8-hydroxyguanine (8-OHdG), which is in fact used as a measure for the level of oxidative stress (Turk et al., 1995). 8-OHdG lesions in cells caused by ROS inhibit DNA methylation, in line with the hypothesis that mitochondrial dysfunction and ROS production may be a driver of altered DNA methylation observed during aging (Turk et al., 1995).

HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trapoxin A result in mitochondrial elongation, leading to healthy aging (Lee et al., 2012). In *C. elegans*, mitochondrial stress can lead to changes in chromatin structures such as H3K9 di-methylation (Tian et al., 2016). Normally these marks lead to silenced chromatin, however, some parts open up which lead to the expression of the mitochondrial unfolded protein response (UPR^{mt}), including activation of chaperones and quality control proteases (Tian et al., 2016). H3K27 demethylases, *jmjd-1.2* and *jmjd-1.3*, are activated when there is a perturbation in the ETC, which leads to the demethylation of H3K27 and UPR^{mt} activation, inducing longevity in *C. elegans* (Merkwirth et al., 2016).

Another study looked at a genome wide blood methylome profile, or DNA methylation age clock, in 656 individuals with the age between 19 and 101. Three methylation sites that change with age were found (Hannum et al., 2013). For instance, a missense mutation was found in the locus of GTP binding protein 10 (*GTPBP10*), located close to the gene *STEAP2*. The methylation state of *GTPBP10* also lowers the gene expression of *STEAP2* and influences the synthesis of iron and copper (Hannum et al., 2013), elements that are required for proper OXPHOS (Ohgami et al., 2006).

CpG methylation of 12 other genes were later associated with aging, and two of these genes encode proteins that affect mitochondrial function (D'Aquila et al., 2019). The genes ras-related protein 32 *(RAB32)* and mitochondrial rho GTPase 2 *(RHOT2)*, were hyper- and hypomethylated with age, respectively. Furthermore, *RAB32* mRNA expression was decreased with age and *RHOT2* expression was increased. *RAB32* encodes for a GTPase and is important for mitochondrial fission and fusion, mitophagy and apoptosis (Alto et al., 2002). *RHOT2* encodes for a protein in the outer mitochondrial membrane (OMM) and is important for the transport of molecules into the intermembrane space in the mitochondria, and also for mitochondrial fission and fusion (Fransson et al., 2006). These provide direct examples of how methylation state may influence mitochondrial biology during aging (D'Aquila et al., 2019).

To summarize, changes in mitochondrial biology with aging may result in increased ROS, which concomitantly alter epigenetic state at the DNA methylation level. Furthermore, DNA methylation as well as histone acetylation change during aging and impart alterations in gene expression of mitochondrial genes, creating a feedback loop of declining mitochondrial function.

Impaired Proteostasis and Mitochondrial Dysfunction

Mitochondrial proteostasis has been extensively linked to aging (Jensen and Jasper, 2014; Moehle et al., 2019). Several pathways of mitochondrial proteostasis exist that restore

mitochondrial function, including UPR^{am}, which is activated by mistargeted mitochondrial precursor proteins, and mitoCPR which is activated upon mitochondrial import stress and (Wrobel et al., 2015; Weidberg and Amon, 2018). While these proteostasis pathways were predominantly described in yeast, the most extensively described pathway, UPR^{mt}, was implicated in lifespan extension in worms, flies, and mice, suggesting a conserved role in the long-term maintenance of cellular homeostasis (Jovaisaite et al., 2014).

While the UPR^{mt} ensures proteostasis specifically for mitochondrial proteins as a relatively independent protein quality control system, cytosolic proteins are simultaneously synthesized, folded, maintained and degraded in the cytosol. Stress can cause the unfolding and aggregation of proteins, and cells have different mechanisms to maintain proteostasis: specifically molecular chaperones, stress-response transcription factors and protein degradation via autophagy (Hartl et al., 2011). Another study showed that in *C. elegans* the mitochondrial ETC is a central regulator of the age-related decline of cytosolic proteostasis (Labbadia et al., 2017). Many more of these cytosolic proteostasis mechanisms linked to longevity are highly interconnected with mitochondria (reviewed in D'Amico et al., 2017; Molenaars et al., 2020a).

For instance, slowing down the synthesis of new proteins, by knockdown of cytosolic ribosomal proteins, extends lifespan in the model organism *C. elegans* (Hansen et al., 2007; Pan et al., 2007). Linking this to mitochondria, slowing down cytosolic protein synthesis is one of the consequences of mitochondrial disturbances that extend lifespan in *C. elegans*. For instance, ROS generated from dysfunctional mitochondria activates GCN-2-dependent eIF2α phosphorylation, leading to reduced cytosolic protein synthesis (Baker et al., 2012). Moreover, when looking at translational efficiencies in *C. elegans* with dysfunctional mitochondria, mRNAs coding for elements of the translation machinery were decreased and those coding for the OXPHOS and autophagy pathways were increased (Molenaars et al., 2018). Additionally, in *C. elegans*, mitochondrial translation and dynamics can synergistically regulate lifespan. This lifespan extension was dependent on the autophagy and lysosome biogenesis regulator, *hlh-30* (*TFEB* in mammals) (Liu et al., 2020b), demonstrating a clear connection and communication between mitochondrial form and function with global cellular proteostasis.

Mitochondria are not only linked to cytosolic protein synthesis; accumulating evidence has shown that cytosolic proteins and aggregation-prone misfolded proteins can be translocated into the mitochondria. For instance, in yeast, cytosolic proteins prone to aggregation are imported into mitochondria for degradation which can be degraded via mitophagy (Ruan et al., 2017; Eldeeb and Fahlman, 2018).

In conclusion, both mitochondrial and cytosolic proteostasis, which can be linked via mito-cytosolic translational balance (Suhm et al., 2018; Molenaars et al., 2020b), are important for healthy aging. There is significant crosstalk between mitochondria and various aspects of proteostasis, both mitochondrial and cytosolic. At the same time there is a lot more to be discovered, especially with regard to the type of proteostatic response that is triggered depending on the context of the cellular or mitochondrial stress.

INTEGRATING MITOCHONDRIAL FUNCTION WITH THE ANTAGONISTIC HALLMARKS OF AGING

The antagonistic hallmarks of aging are hallmarks that can have beneficial or deleterious effects on the cell, depending on the level of intensity (López-Otín et al., 2013). When regulated properly, these hallmarks are beneficial or protective, but can be deleterious when levels are too high, or unregulated.

Deregulated Nutrient Sensing and Mitochondrial Dysfunction

Nutrient sensing pathways involve the detection and cellular adaptation to nutritional challenges, either scarcity or excess. The activity of nutrient sensing pathways changes with age. Some of the best described nutrient sensing pathways involved in aging are the insulin/IGF1 pathway, the mechanistic target of rapamycin (mTOR), the sirtuin pathway, and AMP-activated protein kinase (AMPK) pathway (Houtkooper et al., 2010). Insulin/IGF1 and mTOR are typically activated under conditions of nutrient excess, leading to the activation of anabolic responses including glucose uptake, protein synthesis and inhibition of autophagy. Inhibition of these pathways, for instance inhibition of mTOR complex 1 (mTORC1) with rapamycin leads to marked lifespan extension in model organisms, including mice (Harrison et al., 2009). AMPK and sirtuins are activated upon caloric restriction and lead to activation of various catabolic pathways as well as stress defense systems. Activation of AMPK and sirtuins leads to enhanced autophagy, mitochondrial biogenesis and stress response, culminating in prolonged lifespan (Pearson et al., 2008). Importantly, the anabolic and catabolic nutrient sensing pathways function in intricate networks with bidirectional molecular communication (Houtkooper et al., 2010).

It is evident that mitochondrial (dys)function and nutrient sensing are connected. Through translational and transcriptional mechanisms, mTOR can regulate mitochondrial biogenesis, functions and dynamics (Cunningham et al., 2007; Morita et al., 2013, 2017; Gandin et al., 2016). Translation of nDNA-encoded proteins of OXPHOS complex I and V and fission protein mitochondrial fission process 1 (MTFP1) are stimulated by mTORC1 (Morita et al., 2013, 2017). When mTORC1 and thus MTFP1 is inhibited, this will lead to fusion of mitochondria and survival of the cell. In addition, mTORC1 can stimulate transcription of mitochondrial genes of the ETC leading to sustain high ATP production in the cell (Morita et al., 2013). Another mechanism connecting mTOR with mitochondrial function involves mitophagy. Mitophagy is reduced during aging. In TSC2-null cells, which have high mTORC1 activity, the activity of mitophagy was reduced (Bartolomé et al., 2017). This resulted an accumulation of defective mitochondria, leading to a shorter lifespan (Palikaras et al., 2015; Bartolomé et al., 2017).

Mitochondrial biogenesis regulates intracellular energy metabolism, in response to decreased energy. One of the regulators of mitochondrial biogenesis is AMPK. Activation of AMPK leads to initiation of mitophagy, for instance by activating the autophagy activating kinase 1 (Ulk1) (Egan et al., 2011; Laker et al., 2017). Furthermore, AMPK is important in fission of mitochondria (Toyama et al., 2016). The induction of AMPK can increase lifespan of model organisms such as *C. elegans* by maintaining mitochondrial homeostasis through the regulation of mitochondrial dynamics (Weir et al., 2017). Besides this, excess in nutrients, for instance in glucose or amino acids, can also lead to mitochondrial dysfunction (Houtkooper et al., 2010). Collectively, there is a tight-knit network of interacting nutrient sensing pathways that govern the cellular metabolic state, in part through modulating mitochondrial function, and thereby influence aging.

Cellular Senescence and Mitochondrial Dysfunction

Cellular senescence is the transition to quiescence where cells cease dividing, and is characterized by the secretion of inflammatory signaling factors. This can be triggered by different mechanisms such as genomic instability or telomere attrition (McHugh and Gil, 2018). Senescent cells accumulate during aging in all tissues (Hudgins et al., 2018). This accumulation has an effect on cellular homeostasis. It increases inflammation, decreases tissue function and causes stem cell exhaustion, which all contribute to aging (López-Otín et al., 2013; McHugh and Gil, 2018).

One factor that can induce cellular senescence is oxidative stress. Indeed, when complex I, II and III are inhibited, by specific complex inhibitors, this will induce senescence (Yoon et al., 2003; Moiseeva et al., 2009). Similarly, when cells are treated with FCCP to depolarize the mitochondrial membrane potential, senescence is induced (Stöckl et al., 2006). All these findings indicate that defective OXPHOS leads to cellular senescence. Mitochondrial dynamics also play a role in cellular senescence. By knocking out a protein important for fission in mammalian cells, Fis1, mitochondria were elongated, which was accompanied by lower OXPHOS and higher ROS production, and this led to a significantly higher level of senescence (Lee et al., 2007). Another key aspect of senescence is the senescence-associated secretory phenotype (SASP), secreting pro-inflammatory cytokines, proteases and growth factors (Wiley et al., 2016). This secretory phenotype is activated upon mitochondrial dysfunction and depends on AMPK activation (Correia-Melo et al., 2016; Wiley et al., 2016). AMPK will phosphorylate the tumor suppressor gene p53 and this induces senescence (Wiley et al., 2016). These senescent cells will release the SASP, which amplifies the progression of senescence by inducing senescence in neighboring cells (Correia-Melo et al., 2016).

Together these results indicate that defects in OXPHOS, fission/fusion, high ROS production and altered mitochondrial biogenesis induce senescent cells and therefore the aging phenotype.

INTEGRATING MITOCHONDRIAL FUNCTION WITH THE INTEGRATIVE HALLMARKS OF AGING

Integrative hallmarks of aging are hallmarks that have a more direct effect on the tissue homeostasis and function.

Stem Cell Exhaustion and Mitochondrial Dysfunction

Stem cells can renew themselves and specific stem cells can give rise to a specific kind of tissue. However, aging reduces the renewal capability of stem cells (Ermolaeva et al., 2018). During aging, telomeres shorten and mutations occur in stem cells, which give rise to senescence of these stem cells. This stem cell exhaustion can, for instance, lead to neurodegenerative diseases or a decline in hematopoiesis which in turn leads to less production of adaptive immune responses (López-Otín et al., 2013; Ermolaeva et al., 2018). Though still a long way from application, it may eventually be possible to reprogram aged somatic stem cells into pluripotent stem cells as a way to prevent or reverse aspects of aging (Ahlqvist et al., 2015).

Stem cell exhaustion can also be caused by mitochondrial dysfunction. Increased ROS production in stem cells reduces the renewal of the stem cells in mice, which was rescued by antioxidant treatment (Jang and Sharkis, 2007). In line with that observation, mutator mice, which express proofreading defects in mitochondrial DNA polymerase gamma and thereby accumulate random mutations in mtDNA, present with an accelerated aging phenotype, demonstrated by graying fur, significant weight loss, osteoporosis and less ATP production in the heart when compared to wild type mice of the same age (Trifunovic et al., 2004). Moreover, these mice had both neural and hematopoietic stem cell dysfunction during their development. The neural stem cells in the mutator mice had decreased self-renewal capacity that was rescued by treating the cells with a ROS inhibitor (Ahlqvist et al., 2012), demonstrating that rescuing the mitochondrial dysfunction could rescue the stem cell dysfunction. Furthermore, lowering the mitochondrial activity in hematopoietic stem cell in mice by using carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), which chemically uncouples the electron transport chain, resulted in rapid stem cell differentiation (Vannini et al., 2016). These data suggest that mtDNA maintenance is important for healthy stem cells renewal.

Thus, mitochondrial dysfunction can cause stem cell exhaustion via several mechanisms. At the same time, this creates avenues for intervention since methods to improve mitochondrial function might improve stem cell renewal capacity and hence prevent its contribution to aging.

Altered Intercellular Communication and Mitochondrial Dysfunction

One example of altered intercellular communication is "inflamm-aging," which is the development of chronic inflammation in aged people (Ferrucci and Fabbri, 2018). This chronic inflammation

includes high levels of pro-inflammatory cytokines such as IL-1, IL-6, IFNα, transforming growth factor beta (TGFβ), and tumor necrosis factor (TNF) secreted by T and B cells (Ferrucci and Fabbri, 2018). Inflamm-aging is a risk factor for cancer, dementia and poor health status (Borrello et al., 2005; Gorelick, 2010; Leonardi et al., 2018). For instance, in many it was shown that the *RET/PTC* oncogene, a thyroid tumor, induces genes which are involved in inflammation, such as chemokines and cytokines (Borrello et al., 2005).

Mitochondria play an important role in altered intercellular communication. Upon inflammation, cells secrete signals to trigger an immune response. Damage associated molecular patterns (DAMPs) are danger signals released by cells if there is stress, apoptosis or necrosis. Mitochondria also secrete DAMPs, including molecules such as ATP, mtDNA and ROS (McGuire, 2019). For instance, it was reported that there is a high increase in mtDNA levels in plasma of people of 50+ years old with concomitant high levels of inflammatory cytokines (Pinti et al., 2014). Mitochondria can also trigger inflammatory responses via the mitochondrial antiviral signaling proteins (MAVS) (Koshiba et al., 2011). These proteins localize on the outer mitochondrial membrane, and phosphorylate interferon-regulatory factors 1/5/7 (IRF1/5/7) in an OXPHOS-dependent manner (Koshiba et al., 2011; Lazear et al., 2013). IRFs activate antiviral responses and this will lead to the production of type I interferon (IFN). Aged monocytes, however, have mitochondrial dysfunction including lower OXPHOS, but also lower IFR3/7. This results in lower IFN synthesis, and lower anti-viral response (Molony et al., 2017; Pence and Yarbro, 2018).

Alongside increased inflammation, the adaptive immune response declines with age. T-cells are important for the adaptive immune response to prevent infections (McGuire, 2019), but in the elderly the T-cell activity is reduced, making

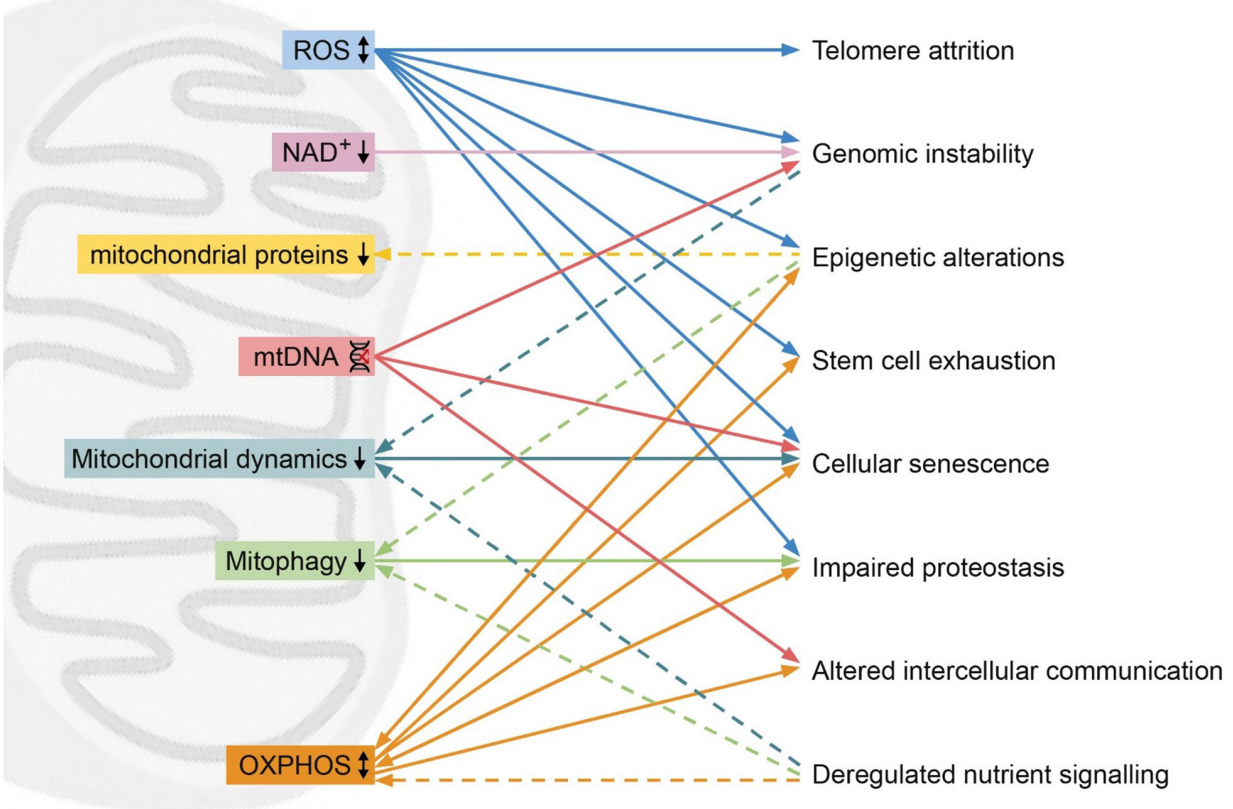

FIGURE 1 | Mitochondrial dysfunction (left) and its relation with other hallmarks (right). High levels of reactive oxygen species (ROS, blue) can lead to telomere attrition, genomic instability, epigenetic alternations, stem cell exhaustion, cellular senescence. On the other hand, ROS can also improve proteostasis. Low levels of NAD^+ (pink) can lead to genomic instability. Epigenetic alterations can lead to reduced mitochondrial proteins (yellow) such as STEAP2, RHOT2, and RAB32. Mitochondrial DNA (mtDNA) damage (red) can result in genomic instability, cellular senescence and altered intercellular communication. Hallmarks of aging genomic instability and deregulated nutrient sensing can lead to reduced mitochondrial dynamics (turquoise), while reduced mitochondrial dynamics induces cellular senescence. Reduced mitophagy (green) leads to impaired proteostasis, while epigenetic alterations and deregulated nutrient sensing induce reduced mitophagy. Oxidative phosphorylation (OXPHOS, orange) affects the hallmarks epigenetic alterations, stem cell exhaustion, cellular senescence, impaired proteostasis, altered intercellular communication and is in turn affected by deregulated nutrient sensing, epigenetic alterations and impaired proteostasis. Dashed line represents an effect of a hallmarks of aging on the mitochondrial dysfunction.

them more susceptible for diseases (Pieren et al., 2019). Interestingly, mice with T cells that were specifically deficient in a mitochondrial DNA–stabilizing protein exhibited multiple features associated with aging, including neurological, metabolic, muscular, and cardiovascular impairments (Desdín-Micó et al., 2020). The defective T cells initiated an early inflammatory program that induced premature senescence (Desdín-Micó et al., 2020). Another study showed that T cells from older subjects also had defects in autophagy and mitochondrial bioenergetics when compared to those of younger subjects, and that metformin alleviate aging-associated inflammation enhances autophagy and normalizes mitochondrial function to alleviate aging-associated inflammation (Bharath et al., 2020). T-cells with a cytochrome C oxidase 10 (*Cox10*), which is a part of OXPHOS complex IV, were infected with influenza (Tarasenko et al., 2017). These T-cells were not activated and were immunodeficient (Tarasenko et al., 2017), demonstrating that OXPHOS is important for T-cell activation.

To conclude, altered intercellular communication can cause mitochondrial dysfunction and vice versa, which contributes to age-related inflammation.

INTEGRATING THE HALLMARKS OF AGING ACROSS THE TREE OF LIFE

Since their classification, the nine hallmarks of aging have been used to describe and clarify the cellular and molecular processes involved in aging as a phenotype. However, these hallmarks are often thought of as discrete processes, as opposed to an intertwined system. In this review, we highlight the interrelation between the hallmarks of aging, with a focus on the mitochondria. Unifying mitochondrial factors such as decreased OXPHOS, increased ROS, reduced mitophagy, dysregulated fission or fusion, and mitochondrial proteostasis connect all of the hallmarks (**Figure 1**).

The majority of mechanistic aging research occurs in model organisms such as *C. elegans*, mice and *D. melanogaster*, and the interactions we describe are based on such traditional experimental models. However, resources such as the AnAge database demonstrate the dramatic variation in organism aging and longevity, which all can contribute to our understanding of these processes (Tacutu et al., 2018). The emergence of new model organisms provides further insight into aging mechanisms. The killifish (*Nothobranchius furzeri*) is a vertebrate, allowing for confirmation of findings in invertebrate organisms before moving to experiments in mammals. For example, the manipulation of the mitonuclear balance in assembly of the respiratory chain was shown to extend lifespan in *C. elegans* (Houtkooper et al., 2013). Recently, using comparative analysis of the genomes of various species within the killifish family, these genes were linked to evolution of lifespans in vertebrates as well (Sahm et al., 2017). These findings demonstrate not only the benefit of studying a wide variety of model organisms, but that comparative studies within the tree of life can also provide important insights into the mechanisms of aging.

However, the same reasons these organisms are often chosen as models can also make them less than suitable representations of the entirety of the phylogenetic tree. For instance, killifish, along with yeast, worms, flies, and mice, are all short-lived and fast-aging (Cohen, 2018). Extremely long-lived species, such as the naked mole rat and turtles, or seemingly immortal species, such as Hydra, can also provide valuable insights into molecular aging, such as the role of mitochondrial dysfunction plays.

The naked mole rat (*Heterocephalus glaber*) is well-known in the aging field as it boasts the most extreme longevity relative to its body size (Gorbunova et al., 2008). This extraordinary lifespan can potentially be connected to mitochondrial function. Naked mole rats exhibit remarkably less age-related changes in mitochondrial mass and efficiency, as well as lipid peroxidation (Buffenstein, 2008; Stoll et al., 2016). Particularly, OXPHOS complex IV expression and activity remains stable throughout lifespan (Stoll et al., 2016). When comparing the skeletal and heart muscle of naked mole rats to mice, one study found that the two species generated essentially equal quantities of mitochondrial H_2O_2 as a proxy of overall ROS metabolism. However, naked mole rats had a significantly greater capacity to consume ROS compared to mice (Munro et al., 2019). The naked mole rat therefore demonstrates a clear model for the aging benefits of maintaining mitochondrial function. Another example is birds. Birds have a relatively long lifespan considering their high metabolic activity and small body size, and it is shown that birds have less ROS production and longer telomeres (Hickey et al., 2012). Similarly, it has been noted that hypoxia in the freshwater turtle *Trachemys scripta elegans* inhibits mitochondrial respiration and ROS production, preventing oxidative damage (Bundgaard et al., 2019). These examples illustrate that different species have different ways to maintain mitochondrial function in the face of physiological challenges, and thereby sustain a relatively long and healthy lifespan.

The cnidarian Hydra (*hydra vulgaris*) is capable of continuous self-renewal and is therefore considered immortal (Schaible et al., 2015). This renewal is mainly due to the robust activity of three stem cell populations (Tomczyk et al., 2015). However, Hydra also have a well-described stress response system, including antioxidant processes (Dash et al., 2007). The FoxO transcription factor is expressed in stem cells, and reduction in FoxO levels negatively affect the proliferation of stem cells (Boehm et al., 2012). While there are few mitochondria-specific studies in Hydra, the interaction of these stem cells and FoxO regulation provide an example of interacting hallmarks in regulation of Hydra immortality. Therefore, interactions between hallmarks are not only present outside the classical aging model organisms, but also that all hallmarks connect together, not just within the lens of mitochondrial dysfunction.

CONCLUSION

Across the phylogenetic tree, the hallmarks of aging connect to and influence one another. Although we chose to focus our review on the hallmark of mitochondrial dysfunction, a clear pattern emerges that all hallmarks of aging influence one another.

For instance, cellular senescence can be induced by genomic instability or telomere attrition (Lidzbarsky et al., 2018) and epigenetic alternations can lead to genomic instability (Pal and Tyler, 2016). It is hence evident that the hallmarks of aging are not discrete entities as how are often presented, but instead operate in a large and tightly connected network. Targeting one factor of this network can result in affecting other hallmarks and thus influence the whole network of aging. Although this complicates our interpretation of anti-aging interventions and requires a more holistic approach, it also opens opportunities for treatment options that not only target one hallmark but in fact act on the entire, or at least a large section of the network. In relation to the phylogenetic tree of life, while the exact details of the hallmarks of aging may differ, the main commonality that unifies aging across all species is the fact that all their hallmarks interconnect. Taking the entirety of this network into account will benefit the aging research community, and ultimately allow for a greater understanding of the aging processes and the progression of age-related disease.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

Ahlqvist, K. J., Hämäläinen, R. H., Yatsuga, S., Uutela, M., Terzioglu, M., Götz, A., et al. (2012). Somatic progenitor cell vulnerability to mitochondrial DNA mutagenesis underlies progeroid phenotypes in polg mutator mice. *Cell Metab.* 15, 100–109. doi: 10.1016/j.cmet.2011.11.012

Ahlqvist, K. J., Suomalainen, A., and Hämäläinen, R. H. (2015). Stem cells, mitochondria and aging. *Biochim. Biophys. Acta Bioenerg.* 1847, 1380–1386. doi: 10.1016/j.bbabio.2015.05.014

Alto, N. M., Soderling, J., and Scott, J. D. (2002). Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. *J. Cell Biol.* 158, 659–668. doi: 10.1083/jcb.200204081 doi: 10.1083/jcb.200204081

Bai, P., Cantó, C., Oudart, H., Brunyánszki, A., Cen, Y., Thomas, C., et al. (2011). PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation. *Cell Metab.* 13, 461–468. doi: 10.1016/j.cmet.2011.03.004

Baker, B. M., Nargund, A. M., Sun, T., and Haynes, C. M. (2012). Protective coupling of mitochondrial function and protein synthesis via the eIF2α kinase GCN-2. *PLoS Genet.* 8:e1002760. doi: 10.1371/journal.pgen.1002760

Bartolomé, A., García-Aguilar, A., Asahara, S.-I., Kido, Y., Guillén, C., Pajvani, U. B., et al. (2017). MTORC1 regulates both general autophagy and mitophagy induction after oxidative phosphorylation uncoupling. *Mol. Cell. Biol.* 37:e00441-17. doi: 10.1128/mcb.00441-17

Bar-Ziv, R., Bolas, T., and Dillin, A. (2020). Systemic effects of mitochondrial stress. *EMBO Rep.* 21:e50094. doi: 10.15252/embr.202050094

Berger, N. A. (1985). Poly(ADP-Ribose) in the cellular response to DNA damage. *Radiat. Res.* 101:4. doi: 10.2307/3576299

Bharath, L. P., Agrawal, M., McCambridge, G., Nicholas, D. A., Hasturk, H., Liu, J., et al. (2020). Metformin enhances autophagy and normalizes mitochondrial function to alleviate aging-associated inflammation. *Cell Metab.* 32, 44–55.e6. doi: 10.1016/j.cmet.2020.04.015

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., and Gluud, C. (2012). Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst. Rev.* 3:CD007176. doi: 10.1002/14651858.cd007176.pub2

Boehm, A. M., Khalturin, K., Anton-Erxleben, F., Hemmrich, G., Klostermeier, U. C., Lopez-Quintero, J. A., et al. (2012). FoxO is a critical regulator of stem cell maintenance in immortal Hydra. *Proc. Natl. Acad. Sci. U.S.A.* 109, 19697–19702. doi: 10.1073/pnas.1209714109

Borrello, M. G., Alberti, L., Fischer, A., Degl'Innocenti, D., Ferrario, C., Gariboldi, M., et al. (2005). Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc. Natl. Acad. Sci. U.S.A.* 102, 14825–14830. doi: 10.1073/pnas.0503039102

Bratic, A., and Larsson, N.-G. (2013). The role of mitochondria in aging. *J. Clin. Invest.* 123, 951–957. doi: 10.1172/JCI64125

Buffenstein, R. (2005). The naked mole-rat: a new long-living model. *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 1369–1377. doi: 10.1093/gerona/60.11.1369

Buffenstein, R. (2008). Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 178, 439–445. doi: 10.1007/s00360-007-0237-5

Bundgaard, A., Qvortrup, K., Rasmussen, L. J., and Fago, A. (2019). Turtles maintain mitochondrial integrity but reduce mitochondrial respiratory capacity in the heart after cold acclimation and anoxia. *J. Exp. Biol.* 222:jeb200410. doi: 10.1242/jeb.200410

Cawthon, R. M., Smith, K. R., O'Brien, E., Sivatchenko, A., and Kerber, R. A. (2003). Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361, 393–395. doi: 10.1016/S0140-6736(03)12384-7

Chen, H., McCaffery, J. M., and Chan, D. C. (2007). Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell* 130, 548–562. doi: 10.1016/j.cell.2007.06.026

Christensen, B. C., Houseman, E. A., Marsit, C. J., Zheng, S., Wrensch, M. R., Wiemels, J. L., et al. (2009). Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet.* 5:e1000602. doi: 10.1371/journal.pgen.1000602

Cohen, A. A. (2018). Aging across the tree of life: the importance of a comparative perspective for the use of animal models in aging. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 2680–2689. doi: 10.1016/J.BBADIS.2017.05.028

Correia-Melo, C., Marques, F. D., Anderson, R., Hewitt, G., Hewitt, R., Cole, J., et al. (2016). Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J.* 35, 724–742. doi: 10.15252/embj.201592862

Cunningham, J. T., Rodgers, J. T., Arlow, D. H., Vazquez, F., Mootha, V. K., and Puigserver, P. (2007). mTOR controls mitochondrial oxidative function through a YY1-PGC-1α transcriptional complex. *Nature* 450, 736–740. doi: 10.1038/nature06322

D'Amico, D., Sorrentino, V., and Auwerx, J. (2017). Cytosolic proteostasis networks of the mitochondrial stress response. *Trends Biochem. Sci.* 42, 712–725. doi: 10.1016/j.tibs.2017.05.002

D'Aquila, P., Montesanto, A., De Rango, F., Guarasci, F., Passarino, G., and Bellizzi, D. (2019). Epigenetic signature: implications for mitochondrial quality control in human aging. *Aging* 11, 1240–1251. doi: 10.18632/aging.101832

Dash, B., Metz, R., Huebner, H. J., Porter, W., and Phillips, T. D. (2007). Molecular characterization of two superoxide dismutases from Hydra vulgaris. *Gene* 387, 93–108. doi: 10.1016/j.gene.2006.08.020

Desdín-Micó, G., Soto-Heredero, G., Aranda, J. F., Oller, J., Carrasco, E., Gabandé-Rodríguez, E., et al. (2020). T cells with dysfunctional mitochondria induce multimorbidity and premature senescence. *Science* 368, 1371–1376. doi: 10.1126/science.aax0860

Dillin, A., Hsu, A. L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A. G., et al. (2002). Rates of behavior and aging specified by mitochondrial function during development. *Science* 298, 2398–2401. doi: 10.1126/science.1077780

Egan, D. F., Shackelford, D. B., Mihaylova, M. M., Gelino, S., Kohnz, R. A., Mair, W., et al. (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331, 456–461. doi: 10.1126/science.1196371

Eldeeb, M. A., and Fahlman, R. P. (2018). Does too much MAGIC lead to mitophagy? *Trends Biochem. Sci.* 43, 485–487. doi: 10.1016/j.tibs.2018.04.008

Ermolaeva, M., Neri, F., Ori, A., and Rudolph, K. L. (2018). Cellular and epigenetic drivers of stem cell ageing. *Nat. Rev. Mol. Cell Biol.* 19, 594–610. doi: 10.1038/s41580-018-0020-3

Fakouri, N. B., Hou, Y., Demarest, T. G., Christiansen, L. S., Okur, M. N., Mohanty, J. G., et al. (2019). Toward understanding genomic instability, mitochondrial dysfunction and aging. *FEBS J.* 286, 1058–1073. doi: 10.1111/febs.14663

Ferrucci, L., and Fabbri, E. (2018). Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat. Rev. Cardiol.* 15, 505–522. doi: 10.1038/s41569-018-0064-2

Franceschi, C., Garagnani, P., Morsiani, C., Conte, M., Santoro, A., Grignolio, A., et al. (2018). The continuum of aging and age-related diseases: common mechanisms but different rates. *Front. Med.* 5:61. doi: 10.3389/fmed.2018.00061

Fransson, Å, Ruusala, A., and Aspenström, P. (2006). The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem. Biophys. Res. Commun.* 344, 500–510. doi: 10.1016/j.bbrc.2006.03.163

Gandin, V., Masvidal, L., Hulea, L., Gravel, S. P., Cargnello, M., McLaughlan, S., et al. (2016). NanoCAGE reveals 5' UTR features that define specific modes of translation of functionally related MTOR-sensitive mRNAs. *Genome Res.* 26, 636–648. doi: 10.1101/gr.197566.115

Gems, D., and Partridge, L. (2013). Genetics of longevity in model organisms: debates and paradigm shifts. *Annu. Rev. Physiol.* 75, 621–644. doi: 10.1146/annurev-physiol-030212-183712

Gorbunova, V., Bozzella, M. J., and Seluanov, A. (2008). Rodents for comparative aging studies: from mice to beavers. *Age* 30, 111–119. doi: 10.1007/s11357-008-9053-4

Gorelick, P. B. (2010). Role of inflammation in cognitive impairment: results of observational epidemiological studies and clinical trials. *Ann. N. Y. Acad. Sci.* 1207, 155–162. doi: 10.1111/j.1749-6632.2010.05726.x

Greer, E. L., Maures, T. J., Hauswirth, A. G., Green, E. M., Leeman, D. S., Maro, G. S., et al. (2010). Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans. *Nature* 466, 383–387. doi: 10.1038/nature09195

Gupte, R., Liu, Z., and Kraus, W. L. (2017). Parps and adp-ribosylation: recent advances linking molecular functions to biological outcomes. *Genes Dev.* 31, 101–126. doi: 10.1101/gad.291518.116

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* 144, 646–674. doi: 10.1016/j.cell.2011.02.013

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., et al. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol. Cell* 49, 359–367. doi: 10.1016/j.molcel.2012.10.016

Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S. J., and Kenyon, C. (2007). Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. *Aging Cell* 6, 95–110. doi: 10.1111/j.1474-9726.2006.00267.x

Harel, I., and Brunet, A. (2016). The African turquoise killifish: a model for exploring vertebrate aging and diseases in the fast lane. *Cold Spring Harb. Symp. Quant. Biol.* 80, 275–279. doi: 10.1101/sqb.2015.80.027524

Harrison, D. E., Strong, R., Sharp, Z. D., Nelson, J. F., Astle, C. M., Flurkey, K., et al. (2009). Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–395. doi: 10.1038/nature08221

Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular chaperones in protein folding and proteostasis. *Nature* 475, 324–332. doi: 10.1038/nature10317

Hickey, A. J. R., Jüllig, M., Aitken, J., Loomes, K., Hauber, M. E., and Phillips, A. R. J. (2012). Birds and longevity: does flight driven aerobicity provide an oxidative sink? *Ageing Res. Rev.* 11, 242–253. doi: 10.1016/j.arr.2011.12.002

Houtkooper, R. H., Argmann, C., Houten, S. M., Cantòo, C., Jeninga, E. H., Andreux, ṔA., et al. (2011). The metabolic footprint of aging in mice. *Sci. Rep.* 1:134. doi: 10.1038/srep00134

Houtkooper, R. H., Mouchiroud, L., Ryu, D., Moullan, N., Katsyuba, E., Knott, G., et al. (2013). Mitonuclear protein imbalance as a conserved longevity mechanism. *Nature* 497, 451–457. doi: 10.1038/nature12188

Houtkooper, R. H., Pirinen, E., and Auwerx, J. (2012). Sirtuins as regulators of metabolism and healthspan. *Nat. Rev. Mol. Cell Biol.* 13, 225–238. doi: 10.1038/nrm3293

Houtkooper, R. H., Williams, R. W., and Auwerx, J. (2010). Metabolic networks of longevity. *Cell* 142, 9–14. doi: 10.1016/j.cell.2010.06.029

Hudgins, A. D., Tazearslan, C., Tare, A., Zhu, Y., Huffman, D., and Suh, Y. (2018). Age- and tissue-specific expression of senescence biomarkers in mice. *Front. Genet.* 9:59. doi: 10.3389/fgene.2018.00059

Jang, Y.-Y., and Sharkis, S. J. (2007). A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. *Blood* 110, 3056–3063. doi: 10.1182/blood-2007-05-087759

Jensen, M. B., and Jasper, H. (2014). Mitochondrial proteostasis in the control of aging and longevity. *Cell Metab.* 20, 214–225. doi: 10.1016/j.cmet.2014.05.006

Jovaisaite, V., Mouchiroud, L., and Auwerx, J. (2014). The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. *J. Exp. Biol.* 217, 137–143. doi: 10.1242/jeb.090738

Kawanishi, S., and Oikawa, S. (2004). Mechanism of telomere shortening by oxidative stress. *Ann. N. Y. Acad. Sci.* 1019, 278–284. doi: 10.1196/annals.1297.047

Kietzmann, T., Petry, A., Shvetsova, A., Gerhold, J. M., and Görlach, A. (2017). The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. *Br. J. Pharmacol.* 174, 1533–1554. doi: 10.1111/bph.13792

Koshiba, T., Yasukawa, K., Yanagi, Y., and Kawabata, S. I. (2011). Mitochondrial membrane potential is required for MAVS-mediated antiviral signaling. *Sci. Signal.* 4:ra7. doi: 10.1126/scisignal.2001147

Labbadia, J., Brielmann, R. M., Neto, M. F., Lin, Y. F., Haynes, C. M., and Morimoto, R. I. (2017). Mitochondrial stress restores the heat shock response and prevents proteostasis collapse during aging. *Cell Rep.* 21, 1481–1494. doi: 10.1016/j.celrep.2017.10.038

Laker, R. C., Drake, J. C., Wilson, R. J., Lira, V. A., Lewellen, B. M., Ryall, K. A., et al. (2017). Ampk phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. *Nat. Commun.* 8, 1–13. doi: 10.1038/s41467-017-00520-9

Lazear, H. M., Lancaster, A., Wilkins, C., Suthar, M. S., Huang, A., Vick, S. C., et al. (2013). IRF-3, IRF-5, and IRF-7 coordinately regulate the type I IFN response in myeloid dendritic cells downstream of MAVS signaling. *PLoS Pathog.* 9:e1003118. doi: 10.1371/journal.ppat.1003118

Lee, J. S., Yoon, Y. G., Yoo, S. H., Jeong, N. Y., Jeong, S. H., Lee, S. Y., et al. (2012). Histone deacetylase inhibitors induce mitochondrial elongation. *J. Cell. Physiol.* 227, 2856–2869. doi: 10.1002/jcp.23027

Lee, S., Jeong, S.-Y., Lim, W.-C., Kim, S., Park, Y.-Y., Sun, X., et al. (2007). Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. *J. Biol. Chem.* 282, 22977–22983. doi: 10.1074/jbc.M700679200

Leonardi, G. C., Accardi, G., Monastero, R., Nicoletti, F., and Libra, M. (2018). Ageing: from inflammation to cancer. *Immun. Ageing* 15:1. doi: 10.1186/s12979-017-0112-5

Lesnefsky, E. J., and Hoppel, C. L. (2006). Oxidative phosphorylation and aging. *Ageing Res. Rev.* 5, 402–433. doi: 10.1016/j.arr.2006.04.001

Lidzbarsky, G., Gutman, D., Shekhidem, H. A., Sharvit, L., and Atzmon, G. (2018). Genomic instabilities, cellular senescence, and aging:

In vitro, in vivo and aging-like human syndromes. *Front. Med.* 5:104. doi: 10.3389/fmed.2018.00104

Liu, L., Trimarchi, J. R., Smith, P. J. S., and Keefe, D. L. (2002). Mitochondrial dysfunction leads to telomere attrition and genomic instability. *Aging Cell* 1, 40–46. doi: 10.1046/j.1474-9728.2002.00004.x

Liu, Y. J., McIntyre, R. L., Janssens, G. E., and Houtkooper, R. H. (2020a). Mitochondrial fission and fusion: a dynamic role in aging and potential target for age-related disease. *Mech. Ageing Dev.* 186:111212. doi: 10.1016/j.mad.2020.111212

Liu, Y. J., McIntyre, R. L., Janssens, G. E., Williams, E. G., Lan, J., van Weeghel, M., et al. (2020b). Mitochondrial translation and dynamics synergistically extend lifespan in C. elegans through HLH-30. *J. Cell Biol.* 219:e201907067. doi: 10.1083/jcb.201907067

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039

McGuire, P. J. (2019). Mitochondrial dysfunction and the aging immune system. *Biology* 8:26. doi: 10.3390/biology8020026

McHugh, D., and Gil, J. (2018). Senescence and aging: causes, consequences, and therapeutic avenues. *J. Cell Biol.* 217, 65–77. doi: 10.1083/jcb.201708092

McIntyre, R. L., Daniels, E. G., Molenaars, M., Houtkooper, R. H., and Janssens, G. E. (2019). From molecular promise to preclinical results: HDAC inhibitors in the race for healthy aging drugs. *EMBO Mol. Med.* 11:e9854. doi: 10.15252/emmm.201809854

Merkwirth, C., Jovaisaite, V., Durieux, J., Matilainen, O., Jordan, S. D., Quiros, P. M., et al. (2016). Two conserved histone demethylases regulate mitochondrial stress-induced longevity. *Cell* 165, 1209–1223. doi: 10.1016/j.cell.2016.04.012

Mitchell, S. J., Scheibye-Knudsen, M., Longo, D. L., and de Cabo, R. (2015). Animal models of aging research: implications for human aging and age-related diseases. *Annu. Rev. Anim. Biosci.* 3, 283–303. doi: 10.1146/annurev-animal-022114-110829

Moehle, E. A., Shen, K., and Dillin, A. (2019). Mitochondrial proteostasis in the context of cellular and organismal health and aging. *J. Biol. Chem.* 294, 5396–5407. doi: 10.1074/jbc.TM117.000893

Moiseeva, O., Bourdeau, V., Roux, A., Deschênes-Simard, X., and Ferbeyre, G. (2009). Mitochondrial dysfunction contributes to oncogene-induced senescence. *Mol. Cell. Biol.* 29, 4495–4507. doi: 10.1128/MCB.01868-08

Molenaars, M., Daniels, E. G., Meurs, A., Janssens, G. E., and Houtkooper, R. H. (2020a). Mitochondrial cross-compartmental signalling to maintain proteostasis and longevity. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 375:20190414. doi: 10.1098/rstb.2019.0414

Molenaars, M., Janssens, G. E., Santermans, T., Lezzerini, M., Jelier, R., MacInnes, A. W., et al. (2018). Mitochondrial ubiquinone–mediated longevity is marked by reduced cytoplasmic mRNA translation. *Life Sci. Alliance* 1:e201800082. doi: 10.26508/lsa.201800082

Molenaars, M., Janssens, G. E., Williams, E. G., Jongejan, A., Lan, J., Rabot, S., et al. (2020b). A conserved mito-cytosolic translational balance links two longevity pathways. *Cell Metab.* 31, 549–563.e7. doi: 10.1016/j.cmet.2020.01.011

Molony, R. D., Nguyen, J. T., Kong, Y., Montgomery, R. R., Shaw, A. C., and Iwasaki, A. (2017). Aging impairs both primary and secondary RIG-I signaling for interferon induction in human monocytes. *Sci. Signal.* 10:eaan2392. doi: 10.1126/scisignal.aan2392

Morita, M., Gravel, S. P., Chénard, V., Sikström, K., Zheng, L., Alain, T., et al. (2013). MTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. *Cell Metab.* 18, 698–711. doi: 10.1016/j.cmet.2013.10.001

Morita, M., Prudent, J., Basu, K., Goyon, V., Katsumura, S., Hulea, L., et al. (2017). mTOR controls mitochondrial dynamics and cell survival via MTFP1. *Mol. Cell* 67, 922–935.e5. doi: 10.1016/j.molcel.2017.08.013

Mota-Martorell, N., Jove, M., Pradas, I., Sanchez, I., Gómez, J., Naudi, A., et al. (2020). Low abundance of NDUFV2 and NDUFS4 subunits of the hydrophilic complex I domain and VDAC1 predicts mammalian longevity. *Redox Biol.* 34:101539. doi: 10.1016/j.redox.2020.101539

Mouchiroud, L., Houtkooper, R. H., Moullan, N., Katsyuba, E., Ryu, D., Cantó, C., et al. (2013). The NAD(+)/sirtuin pathway modulates longevity through activation of mitochondrial UPR and FOXO signaling. *Cell* 154, 430–441. doi: 10.1016/j.cell.2013.06.016

Munro, D., Baldy, C., Pamenter, M. E., and Treberg, J. R. (2019). The exceptional longevity of the naked mole-rat may be explained by mitochondrial antioxidant defenses. *Aging Cell* 18, 1–13. doi: 10.1111/acel.12916

Nargund, A. M., Fiorese, C. J., Pellegrino, M. W., Deng, P., and Haynes, C. M. (2015). Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPRmt. *Mol. Cell* 58, 123–133. doi: 10.1016/j.molcel.2015.02.008

Niedernhofer, L. J., Gurkar, A. U., Wang, Y., Vijg, J., Hoeijmakers, J. H. J., and Robbins, P. D. (2018). Nuclear genomic instability and aging. *Annu. Rev. Biochem.* 87, 295–322. doi: 10.1146/annurev-biochem-062917-012239

Oeppen, J., and Vaupel, J. W. (2002). Demography: broken limits to life expectancy. *Science* 296, 1029–1031. doi: 10.1126/science.1069675

Ohgami, R. S., Campagna, D. R., McDonald, A., and Fleming, M. D. (2006). The Steap proteins are metalloreductases. *Blood* 108, 1388–1394. doi: 10.1182/blood-2006-02-003681

Pal, S., and Tyler, J. K. (2016). Epigenetics and aging. *Sci. Adv.* 2:e1600584. doi: 10.1126/sciadv.1600584

Palikaras, K., Lionaki, E., and Tavernarakis, N. (2015). Coordination of mitophagy and mitochondrial biogenesis during ageing in C. elegans. *Nature* 521, 525–528. doi: 10.1038/nature14300

Pan, K. Z., Palter, J. E., Rogers, A. N., Olsen, A., Chen, D., Lithgow, G. J., et al. (2007). Inhibition of mRNA translation extends lifespan in Caenorhabditis elegans. *Aging Cell* 6, 111–119. doi: 10.1111/j.1474-9726.2006.00266.x

Pearson, K. J., Baur, J. A., Lewis, K. N., Peshkin, L., Price, N. L., Labinskyy, N., et al. (2008). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* 8, 157–168. doi: 10.1016/j.cmet.2008.06.011

Pence, B. D., and Yarbro, J. R. (2018). Aging impairs mitochondrial respiratory capacity in classical monocytes. *Exp. Gerontol.* 108, 112–117. doi: 10.1016/J.EXGER.2018.04.008

Pieren, D. K. J., Smits, N. A. M., van de Garde, M. D. B., and Guichelaar, T. (2019). Response kinetics reveal novel features of ageing in murine T cells. *Sci. Rep.* 9, 1–13. doi: 10.1038/s41598-019-42120-1

Pinti, M., Cevenini, E., Nasi, M., De Biasi, S., Salvioli, S., Monti, D., et al. (2014). Circulating mitochondrial DNA increases with age and is a familiar trait: implications for "inflamm-aging.". *Eur. J. Immunol.* 44, 1552–1562. doi: 10.1002/eji.201343921

Pirinen, E., Cantó, C., Jo, Y. S., Morato, L., Zhang, H., Menzies, K. J., et al. (2014). Pharmacological inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. *Cell Metab.* 19, 1034–1041. doi: 10.1016/j.cmet.2014.04.002

Rath, C. P. (2020). *Models, Molecules and Mechanisms in Biogerontology*. Cham: Springer. doi: 10.1007/978-981-32-9005-1

Ruan, L., Zhou, C., Jin, E., Kucharavy, A., Zhang, Y., Wen, Z., et al. (2017). Cytosolic proteostasis through importing of misfolded proteins into mitochondria. *Nature* 543, 443–446. doi: 10.1038/nature21695

Ryu, D., Mouchiroud, L., Andreux, P. A., Katsyuba, E., Moullan, N., Nicolet-Dit-Félix, A. A., et al. (2016). Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. *Nat. Med.* 22, 879–888. doi: 10.1038/nm.4132

Sahin, E., Colla, S., Liesa, M., Moslehi, J., Müller, F. L., Guo, M., et al. (2011). Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* 470, 359–365. doi: 10.1038/nature09787

Sahm, A., Bens, M., Platzer, M., and Cellerino, A. (2017). Parallel evolution of genes controlling mitonuclear balance in short-lived annual fishes. *Aging Cell* 16, 488–496. doi: 10.1111/acel.12577

Schaible, R., Scheuerlein, A., Dańko, M. J., Gampe, J., Martínez, D. E., and Vaupel, J. W. (2015). Constant mortality and fertility over age in Hydra. *Proc. Natl. Acad. Sci. U.S.A.* 112, 15701–15706. doi: 10.1073/pnas.1521002112

Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M., and Ristow, M. (2007). Glucose restriction extends caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* 6, 280–293. doi: 10.1016/J.CMET.2007.08.011

Sharma, A., Smith, H. J., Yao, P., and Mair, W. B. (2019). Causal roles of mitochondrial dynamics in longevity and healthy aging. *EMBO Rep.* 20:e48395. doi: 10.15252/embr.201948395

Stöckl, P., Hütter, E., Zwerschke, W., and Jansen-Dürr, P. (2006). Sustained inhibition of oxidative phosphorylation impairs cell proliferation and induces

premature senescence in human fibroblasts. *Exp. Gerontol.* 41, 674–682. doi: 10.1016/j.exger.2006.04.009

Stoll, E. A., Karapavlovic, N., Rosa, H., Woodmass, M., Rygiel, K., White, K., et al. (2016). Naked mole-rats maintain healthy skeletal muscle and Complex IV mitochondrial enzyme function into old age. *Aging* 8, 3468–3485. doi: 10.18632/aging.101140

Suhm, T., Kaimal, J. M., Dawitz, H., Peselj, C., Masser, A. E., Hanzén, S., et al. (2018). Mitochondrial translation efficiency controls cytoplasmic protein homeostasis. *Cell Metab.* 27, 1309–1322.e6. doi: 10.1016/j.cmet.2018.04.011

Sun, N., Youle, R. J., and Finkel, T. (2016). The mitochondrial basis of aging. *Mol. Cell* 61, 654–666. doi: 10.1016/j.molcel.2016.01.028

Tacutu, R., Thornton, D., Johnson, E., Budovsky, A., Barardo, D., Craig, T., et al. (2018). Human ageing genomic resources: new and updated databases. *Nucleic Acids Res.* 46, D1083–D1090. doi: 10.1093/nar/gkx1042

Tarasenko, T. N., Pacheco, S. E., Koenig, M. K., Gomez-Rodriguez, J., Kapnick, S. M., Diaz, F., et al. (2017). Cytochrome c oxidase activity is a metabolic checkpoint that regulates cell fate decisions during T cell activation and differentiation. *Cell Metab.* 25, 1254–1268.e7. doi: 10.1016/J.CMET.2017.05.007

Tian, Y., Garcia, G., Bian, Q., Steffen, K. K., Joe, L., Wolff, S., et al. (2016). Mitochondrial stress induces chromatin reorganization to promote longevity and UPRmt. *Cell* 165, 1197–1208. doi: 10.1016/j.cell.2016.04.011

Tomczyk, S., Fischer, K., Austad, S., and Galliot, B. (2015). Hydra, a powerful model for aging studies. *Invertebr. Reprod. Dev.* 59, 11–16. doi: 10.1080/07924259.2014.927805

Toyama, E. Q., Herzig, S., Courchet, J., Lewis, T. L., Losón, O. C., Hellberg, K., et al. (2016). AMP-activated protein kinase mediates mitochondrial fission in response to energy stress. *Science* 351, 275–281. doi: 10.1126/science.aab4138

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J. N., Rovio, A. T., Bruder, C. E., et al. (2004). Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429, 417–423. doi: 10.1038/nature02517

Tufi, R., Gandhi, S., De Castro, I. P., Lehmann, S., Angelova, P. R., Dinsdale, D., et al. (2014). Enhancing nucleotide metabolism protects against mitochondrial dysfunction and neurodegeneration in a PINK1 model of Parkinson's disease. *Nat. Cell Biol.* 16, 157–166. doi: 10.1038/ncb2901

Turk, P. W., Laayoun, A., Smith, S. S., and Weitzman, S. A. (1995). DNA adduct 8-hydroxyl-2'-deoxyguanosine (8-hydroxyguanine) affects function of human DNA methyltransferase. *Carcinogenesis* 16, 1253–1255. doi: 10.1093/carcin/16.5.1253

Vannini, N., Girotra, M., Naveiras, O., Nikitin, G., Campos, V., Giger, S., et al. (2016). Specification of haematopoietic stem cell fate via modulation of mitochondrial activity. *Nat. Commun.* 7:13125. doi: 10.1038/ncomms13125

Wang, J., Xiong, S., Xie, C., Markesbery, W. R., and Lovell, M. A. (2005). Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.* 93, 953–962. doi: 10.1111/j.1471-4159.2005.03053.x

Weidberg, H., and Amon, A. (2018). MitoCPR–A surveillance pathway that protects mitochondria in response to protein import stress. *Science* 360:eaan4146. doi: 10.1126/science.aan4146

Weir, H. J., Yao, P., Huynh, F. K., Escoubas, C. C., Goncalves, R. L., Burkewitz, K., et al. (2017). Dietary restriction and AMPK increase lifespan via mitochondrial network and peroxisome remodeling. *Cell Metab.* 26, 884–896.e5. doi: 10.1016/j.cmet.2017.09.024

Wiley, C. D., Velarde, M. C., Lecot, P., Liu, S., Sarnoski, E. A., Freund, A., et al. (2016). Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab.* 23, 303–314. doi: 10.1016/j.cmet.2015.11.011

Wrobel, L., Topf, U., Bragoszewski, P., Wiese, S., Sztolsztener, M. E., Oeljeklaus, S., et al. (2015). Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. *Nature* 524, 485–488. doi: 10.1038/nature14951

Wu, J. J., Liu, J., Chen, E. B., Wang, J. J., Cao, L., Narayan, N., et al. (2013). Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Rep.* 4, 913–920. doi: 10.1016/j.celrep.2013.07.030

Xiong, S., Patrushev, N., Forouzandeh, F., Hilenski, L., and Alexander, R. W. (2015). PGC-1α modulates telomere function and DNA damage in protecting against aging-related chronic diseases. *Cell Rep.* 12, 1391–1399. doi: 10.1016/j.celrep.2015.07.047

Yoon, Y. S., Byun, H. O., Cho, H., Kim, B. K., and Yoon, G. (2003). Complex II defect via down-regulation of iron-sulfur subunit induces mitochondrial dysfunction and cell cycle delay in iron chelation-induced Senescence-associated Growth Arrest. *J. Biol. Chem.* 278, 51577–51586. doi: 10.1074/jbc.M308489200

Zhang, H., Menzies, K. J., and Auwerx, J. (2018). The role of mitochondria in stem cell fate and aging. *Development* 145:dev143420. doi: 10.1242/dev.143420 doi: 10.1242/dev.143420

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REVIEW
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Beneficial and Detrimental Effects of Reactive Oxygen Species on Lifespan: A Comprehensive Review of Comparative and Experimental Studies

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Aging is the greatest risk factor for a multitude of diseases including cardiovascular disease, neurodegeneration and cancer. Despite decades of research dedicated to understanding aging, the mechanisms underlying the aging process remain incompletely understood. The widely-accepted free radical theory of aging (FRTA) proposes that the accumulation of oxidative damage caused by reactive oxygen species (ROS) is one of the primary causes of aging. To define the relationship between ROS and aging, there have been two main approaches: comparative studies that measure outcomes related to ROS across species with different lifespans, and experimental studies that modulate ROS levels within a single species using either a genetic or pharmacologic approach. Comparative studies have shown that levels of ROS and oxidative damage are inversely correlated with lifespan. While these studies in general support the FRTA, this type of experiment can only demonstrate correlation, not causation. Experimental studies involving the manipulation of ROS levels in model organisms have generally shown that interventions that increase ROS tend to decrease lifespan, while interventions that decrease ROS tend to increase lifespan. However, there are also multiple examples in which the opposite is observed: increasing ROS levels results in extended longevity, and decreasing ROS levels results in shortened lifespan. While these studies contradict the predictions of the FRTA, these experiments have been performed in a very limited number of species, all of which have a relatively short lifespan. Overall, the data suggest that the relationship between ROS and lifespan is complex, and that ROS can have both beneficial or detrimental effects on longevity depending on the species and conditions. Accordingly, the relationship between ROS and aging is difficult to generalize across the tree of life.

Keywords: aging, reactive oxygen species, free radical theory of aging, genetics, lifespan, antioxidants, model organisms

INTRODUCTION

Aging can be described as the gradual loss of fitness due to detrimental changes occurring at the cell and molecular level over time. It is characterized by dysregulation of cellular processes, accumulation of damaged materials and toxins, altered gene expression, and poor immune and stress responses (Partridge and Gems, 2002; Kennedy et al., 2014; DiLoreto and Murphy, 2015). While the changes that occur during the aging process have been well described, the mechanisms underlying aging remain poorly understood, and have thus been the subject of much research. One of the most widely accepted theories of aging, called the free radical theory of aging (FRTA), proposes that oxidative damage caused by reactive oxygen species (ROS) is the primary cause of aging. While there have been many studies examining the relationship between ROS and aging, this is still an area of much debate. In this work, we review comparative studies and studies involving experimental modulation that have investigated the role of ROS in aging across species. Overall, these studies demonstrate that the relationship between ROS and aging is complex, in that ROS can both increase or decrease lifespan depending on the experimental conditions.

Reactive Oxygen Species

Reactive oxygen species are highly reactive, oxygen containing molecules that are the result of an incomplete reduction of molecular oxygen in the cell (Santos et al., 2018). ROS can be free radicals, or molecules that have the capacity to generate free radicals. Free radicals consist of atoms or molecules with an unpaired electron in their outer shell causing them to be unstable and highly reactive, or in other words, prone to "stealing" electrons from other molecules (Pham-Huy et al., 2008). Free radicals are highly reactive and are therefore generally short-lived and often unable to leave the subcellular location where they are generated without first becoming reduced. Examples of ROS that are free radicals include the superoxide ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$), peroxyl ($RO_2^{\bullet -}$), hydroperoxyl ($HO_2^{\bullet}$), and alkoxyl radicals ($RO^{\bullet}$) (Winterbourn, 2008; Phaniendra et al., 2015). ROS that are not free radicals do not have unpaired electrons, and are often less reactive, thus allowing them to leave the subcellular location where they are generated as well as pass through membranes (Winterbourn, 2008). Examples of ROS that are not free radicals include hydrogen peroxide (H_2O_2), the hydroxide ion (OH^-) and organic peroxides (ROOH) (Phaniendra et al., 2015).

Despite being less reactive, ROS that are not free radicals are still incompletely reduced and thus can undergo redox reactions to produce free radicals as a result. For example, if hydrogen peroxide encounters a reduced transition metal ion such as ferrous iron (Fe^{2+}) or cuprous copper (Cu^+), the Fenton reaction will occur (**Figure 1**), resulting in the production of the hydroxyl radical which acts as a potent oxidant (Sutton and Winterbourn, 1989). The ability for ROS that are not free radicals, such as hydrogen peroxide, to move within the cell becomes important when considering the redox state for cell signaling in various subcellular locations as well as antioxidant distribution within the cell (Travasso et al., 2017; Di Marzo et al., 2018).

A $O_2 + e^- \rightarrow O_2^{\bullet -}$

B $O_2 + NADPH \rightarrow NADP^+ + H^+ + O_2^{\bullet -}$

C $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$

D $H_2O_2 + Cl^- \rightarrow H_2O + HOCl$

E $H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^- + Fe^{3+}$

FIGURE 1 | Chemical reactions that generate reactive oxygen species. **(A)** Superoxide is generated in the mitochondria when electrons leak out of the electron transport chain and reduce singlet oxygen. **(B)** Superoxide can also be generated in the cell when enzymes catalyze the transfer of an electron from NADPH to singlet oxygen, often during metabolism reactions. **(C)** Two superoxide molecules can be converted to hydrogen peroxide and oxygen by the superoxide dismutase enzymes. **(D)** Myeloperoxidase catalyzes the conversion of hydrogen peroxide and a chloride anion to hypochlorous acid which acts as a potent oxidizer in the respiratory burst. **(E)** When hydrogen peroxide encounters free ferrous iron within the cell, the Fenton reaction occurs, producing a hydroxyl radical.

In addition to ROS, organisms also generate reactive nitrogen species (RNS). Nitric oxide (NO) is produced from L-arginine by the enzyme nitric oxide synthase of which there are three forms: endothelial (eNOS), inducible (iNOS) and neuronal (nNOS). Nitric oxide can then react with superoxide to produce peroxynitrite ($ONOO^-$), which can directly damage cellular components, or further react to generate other types of RNS. As with ROS, there are both free radical forms of RNS [nitric oxide, nitric dioxide ($NO_2\cdot$), nitrate radical ($NO_3\cdot$)] and non-radical forms [peroxynitrite, nitrous acid (HNO_2), nitrite (NO_2-), nitrosyl cation (NO+), nitroxyl anion (NO−), peroxynitrous acid (ONOOH), dinitrogen trioxide (N_2O_3)]. Also similar to ROS, RNS can cause cellular damage called nitrosative damage, but also have functional roles in cellular signaling and pathogen defense. Little is known about the effects of RNS on longevity.

Oxidative Damage as a Biomarker of Aging

The tendency for free radicals to steal electrons in order to stabilize themselves can be problematic in cells, where they may cause oxidative damage to macromolecules such as DNA, proteins and lipids (Pham-Huy et al., 2008). When DNA is exposed to ROS, guanine is modified to 8-oxoguanine (**Figure 2A**), allowing it to pair with cytosine and adenine. This mutation can occur in both nuclear and mitochondrial DNA and can give rise to double stranded breaks (DSBs) in the DNA, leading to genomic instability (Kregel and Zhang, 2007). Proteins can be damaged when amino acid side chains and backbones, especially in thiol-containing cysteine and methionine residues, are oxidized by ROS (**Figure 2B**); this can result in structural changes to the protein that may lead to loss of function (Sohal, 2002) or be used for ROS-mediated signaling. Additionally, the exposure of lipids to ROS results in lipid peroxidation (**Figure 2C**), which gives rise to cell membrane damage and generates reactive by-products which can further damage the cell (Mylonas and Kouretas, 1999).

All of these types of oxidative damage have been shown to increase with advancing age including DNA damage (Massudi

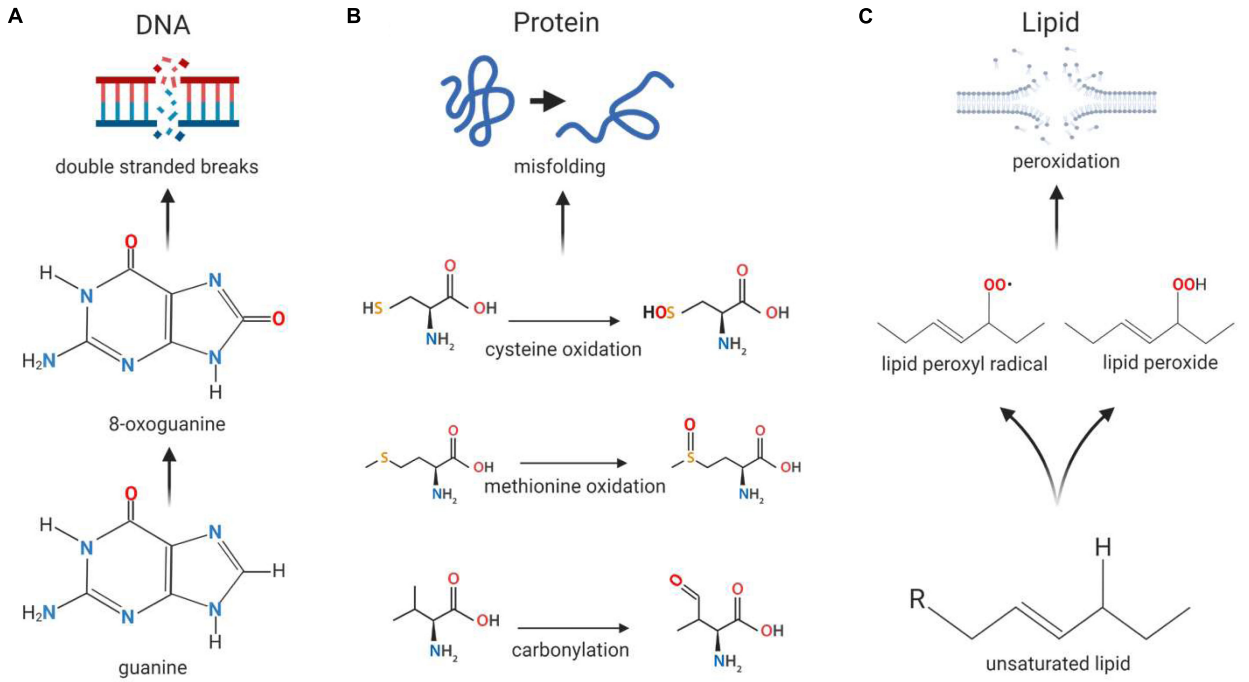

FIGURE 2 | Macromolecular damage caused by reactive oxygen species. Reactive oxygen species (ROS) can cause damage to the basic building blocks of the cell including DNA, protein and lipids. **(A)** DNA damage can occur in the form of double stranded breaks as a result of ROS-induced conversion of guanine to 8-oxoguanine. As 8-oxoguanine can be mis-paired with adenine, transversion mutations can occur, resulting in double stranded breaks after replication. **(B)** Proteins can become misfolded when exposed to ROS due to oxidation of amino acids cysteine and methionine, as well as carbonylation of the peptide backbone, resulting in changes to the molecular interactions that normally occur within the peptide. **(C)** Exposure to ROS can induce membrane damage when lipid peroxidation occurs as a result of the formation of lipid peroxides and lipid peroxide radicals, the latter of which can cause further oxidative damage to other lipids.

et al., 2012; Moskalev et al., 2013), protein carbonylation (Moskovitz and Oien, 2010; Jha and Rizvi, 2011), lipid peroxidation (Massudi et al., 2012) and damage to mitochondrial DNA (Park and Larsson, 2011). As a result, these outcome measures have been proposed as biomarkers of aging [reviewed elsewhere e.g. (Syslova et al., 2014; Liguori et al., 2018)].

The Free Radical Theory of Aging

The FRTA proposes that aging is caused by the accumulation of molecular damage caused by ROS that are generated by normal metabolism (Harman, 1956). The logic of the FRTA stems from the idea that ROS have the capacity to be highly damaging to cell components, as well as the idea that the mechanism of aging is likely to be an intrinsic process given that aging always occurs, regardless of differences in environment. The FRTA developed into the mitochondrial FRTA (MFRTA) which became a popular way to explain why the rate of aging and the maximum lifespan varies so significantly among species (Barja, 2013). The MFRTA specifies that the ROS that cause aging are produced by the mitochondria, and that the lifespan of an organism thereby depends on their rate of oxygen consumption by the mitochondrial respiratory chain, which is believed to be the main process that produces ROS. The theory fit with the idea that the mechanism of aging is intrinsic, given the constant endogenous production of ROS as a byproduct of a process essential to all known aerobic organisms.

Initially proposed by Harman (1956) and revised in Harman (1972), the FRTA has since been supported by observations on the association between ROS, oxidative damage and longevity. These observations include: (1) higher levels of ROS generation with increasing age (Sawada and Carlson, 1987; Sohal and Sohal, 1991; Sohal and Dubey, 1994; Bejma and Ji, 1999; Driver et al., 2000; Capel et al., 2005); (2) higher levels of oxidative damage with increasing age (Oliver et al., 1987; Fraga et al., 1990; Hamilton et al., 2001; Bokov et al., 2004; Navarro and Boveris, 2004; Muller et al., 2007; Halliwell, 2009); (3) gradual increase in mitochondrial dysfunction with age (Rockstein and Brandt, 1963; Trounce et al., 1989; Cooper et al., 1992; Genova et al., 2004; Green et al., 2011; Gouspillou et al., 2014; Sun et al., 2016); (4) an increase in ROS generation upon inhibition of components of the electron transport chain (Turrens and Boveris, 1980; Forman and Azzi, 1997; Kushnareva et al., 2002; Chen et al., 2003; Li et al., 2003; Fato et al., 2009); and (5) high levels of oxidative stress in several age-related diseases (Stohs, 1995; Berliner and Heinecke, 1996; Forsberg et al., 2001; Oberley, 2002; Lassègue and Griendling, 2004; Cutler, 2005; Chung et al., 2006; Pham-Huy et al., 2008).

In addition to support for the FRTA, observations refuting the theory have also accumulated (Yang et al., 2007; Van Raamsdonk and Hekimi, 2009; Yang and Hekimi, 2010; Van Raamsdonk and Hekimi, 2012; Wang et al., 2018), with recent findings ultimately leading to the formation of new models for the relationship between ROS, redox signaling, oxidative damage and lifespan

(Lapointe and Hekimi, 2010; Van Raamsdonk and Hekimi, 2010; Hekimi et al., 2011). Newer models for the role of ROS in the cell suggest that a mild elevation in ROS can be beneficial to an organism, perhaps through the activation of cellular stress response signaling pathways, while very low and very high levels can be detrimental to organisms (Hekimi et al., 2011; Sena and Chandel, 2012; Van Raamsdonk, 2015). These models incorporate the importance of the maintenance of adequate ROS levels for redox signaling components (ex. thiol-containing proteins) which may rely on ROS-mediated oxidation in order to activate desired cell survival pathways (Sundaresan et al., 1995; McCord, 2000; Woo et al., 2000; Dröge, 2002; Theopold, 2009; Flohé, 2010; Ray et al., 2012).

Sources of Reactive Oxygen Species in the Cell

Mitochondrial respiration is a major source of ROS within the cell. As electrons are transferred between the complexes of the electron transport chain, some of these electrons can "leak" out to react directly with oxygen to form superoxide (**Figures 1A** and **3**) (Kushnareva et al., 2002; Chen et al., 2003; Balaban et al., 2005). The majority of electron leakage is thought to occur as electrons are passed from Complex I or Complex II to Complex III via ubiquinone (Van Raamsdonk and Hekimi, 2010).

There are also important non-mitochondrial sources of ROS in the cell. For example, immune cells attack pathogens in the body by releasing ROS extracellularly or into the phagolysosome, a strategy termed the respiratory burst (**Figure 3**) (Beckman and Ames, 1998). The ROS used in this attack are generated by the membrane-bound phagocyte NADPH oxidase as well as the granule-localized myeloperoxidase (Nguyen et al., 2017). NADPH oxidase uses the electrons donated from NADPH to convert molecular oxygen to a superoxide anion (**Figure 1B**), while myeloperoxidase (MPO) converts hydrogen peroxide to hypochlorous acid (**Figure 1D**) (Nguyen et al., 2017). Importantly, NADPH oxidase homologs (NOX enzymes; **Figure 3**) expressed in other cell types also play important roles in the generation of ROS for signal transduction in pathways for growth, survival and apoptosis (Lambeth, 2020).

In fact, there are many ROS-generating enzymes that play essential roles in various physiological systems (**Figure 3**). Some examples include: the cytochrome P450 (CYP) family of enzymes, which produce ROS in the process of the detoxification and excretion of xenobiotics (McDonnell and Dang, 2013); xanthine oxidoreductase (XOR), which produces superoxide

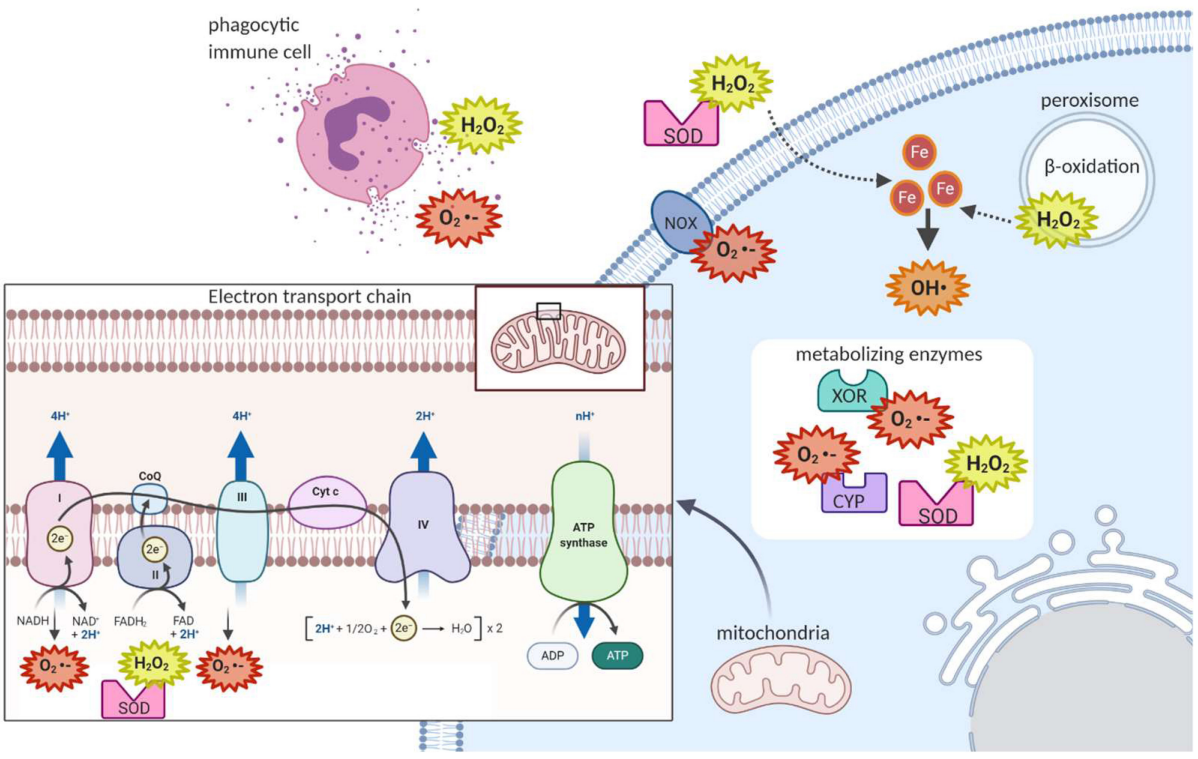

FIGURE 3 | Sites of reactive oxygen species generation in the cell. As electrons are being passed from Complex I or Complex II to Complex III via ubiquinone in the mitochondrial electron transport chain, some of these electrons can escape and react with oxygen to form superoxide. The enzymes superoxide dismutase can convert the superoxide to hydrogen peroxide, which can then exit the mitochondria. In the cytoplasm, metabolism reactions such as those of the cytochrome p family of enzymes (CYP) produce ROS. In the peroxisome, fatty acid beta-oxidation produces hydrogen peroxide. At the plasma membrane, NADPH Oxidase produces superoxide. Extracellularly, ROS can be released in processes such as the respiratory burst, where phagocytic immune cells release ROS to attack pathogens. Extracellular superoxide dismutase can then convert extracellular superoxide to hydrogen peroxide. Hydrogen peroxide, which can cross membranes, can be converted to the potent hydroxyl radical when in contact with cellular ferrous iron.

anions during the break down of purines to uric acid (Battelli et al., 2016); superoxide dismutase (SOD), which converts superoxide to hydrogen peroxide and oxygen (**Figure 1C**) (McCord and Fridovich, 1969); and monoamine oxidase, which breaks down the neurotransmitter dopamine after signaling occurs and produces hydrogen peroxide in neurons in the process (Shih et al., 1999).

Finally, an additional source of ROS comes from β-oxidation of fatty acids in the peroxisome, producing hydrogen peroxide which can then cross the plasma membrane of the peroxisome, to reach the cytoplasm (**Figure 3**) (Beckman and Ames, 1998). Along with NADPH oxidase-generated ROS, peroxisome-generated ROS are thought to contribute to regulation of the activity of NF-kB and mTORC1 which both play important roles in cell survival in response to stressors (Lismont et al., 2015).

Detoxification of Reactive Oxygen Species

Reactive oxygen species levels in the cell can be seen as a balance between levels of ROS generation and levels of ROS detoxification by antioxidants. Maintaining homeostatic levels of ROS as a result of this balance is important both for preventing oxidative damage as well as maintaining an appropriate redox environment for normal signaling pathways within the cell (Forman et al., 2014). Thus, just as ROS detoxification is required to avoid increases in ROS levels, antioxidant activity must be regulated to avoid over-detoxification and low levels of ROS. In response to an imbalanced redox environment, gene expression of antioxidant enzymes is triggered (Ma, 2013). Antioxidants scavenge ROS through various reactions which facilitate the reduction of ROS to less reactive and more stable forms (Matés et al., 1999). Enzymatic antioxidants are localized to various subcellular locations in order to be in proximity to sites of ROS generation. Non-enzymatic antioxidants can be endogenous or supplied by dietary nutrients (Matés et al., 1999).

Enzymatic Antioxidants

Superoxide dismutase

The superoxide dismutase (SOD) enzyme converts the highly reactive superoxide anion to a less reactive hydrogen peroxide. The enzyme catalyzes the transfer of electrons through two redox reactions using transition metals in its active site (Tainer et al., 1983). In the first reaction, superoxide is oxidized to molecular oxygen, leaving the transition metal in the active site reduced; in the second reaction, superoxide is reduced to hydrogen peroxide and the transition metal in the active site returns to its oxidized form (**Figure 4A**) (McCord and Fridovich, 1969). Humans have three different forms of SOD (**Figure 5**): SOD1 is located in the cytosol and its active site is Cu/Zn; SOD2 is located in the mitochondria and its active site is Mn; and SOD3 is located extracellularly with a Cu/Zn active site (Fukai and Ushio-Fukai, 2011).

Catalase

Catalase (CAT) catalyzes the conversion of hydrogen peroxide to water and molecular oxygen (**Figure 4B**) and thus plays an important role in dealing with hydrogen peroxide-producing reactions, including that of the SODs (Ighodaro and Akinloye, 2019). Its active site is composed of heme groups which facilitate the transfer of electrons. Catalase is primarily located in the peroxisomes (**Figure 5**), where hydrogen peroxide production can be high due to β-oxidation (Birben et al., 2012; Ighodaro and Akinloye, 2019).

Glutathione peroxidase

Glutathione peroxidase (GPx) functions in conjunction with catalase to reduce hydrogen peroxide levels in the cell. GPx is located in the cytoplasm and mitochondria, while catalase is mainly in peroxisomes (Baud et al., 2004; Ighodaro and Akinloye, 2019). While catalase mainly detoxifies hydrogen peroxide, GPx is also able to detoxify various peroxides, notably lipid peroxides (Ighodaro and Akinloye, 2019). In the reaction to reduce hydrogen peroxide (**Figure 4C**), the selenocysteine active site of GPx becomes oxidized. In order to return the active site to its functional state, two glutathione (GSH) molecules are needed (Flohé, 1985), resulting in the production of glutathione disulfide (GS-SG). Given that GSH is essential to maintain the activity of GPx, GS-SG must be reduced back to two molecules of GSH; this is catalyzed by glutathione reductase (GR), and uses electrons donated by NADPH (Flohé, 1985; Ursini et al., 1985). The GPx enzymes act in multiple subcellular compartments (**Figure 5**): GPX1 and GPX2 deal with hydrogen peroxide and organic peroxides in the cytosol, GPX3 acts as an extracellular antioxidant and GPX4, which can metabolize phospholipid hydroperoxides, is located in cell membranes and in mitochondria (Arthur, 2000).

Glutaredoxin and thioredoxin

The glutaredoxin (Grx) and thioredoxin (Trx) family of antioxidant enzymes act to protect thiol-containing proteins by repairing damage caused by exposure to oxidants. This system catalyzes the transfer of electrons to the disulfide bonds of target proteins, in order to convert the target protein back to its reduced form (Lee et al., 2013). This is done through a series of reactions (**Figures 6A,B**), which begin with the reduction of the target residue, and oxidation of the Grx or Trx active site, which consist of cysteine residues (Hanschmann et al., 2013). Each enzyme then needs to be reduced back to its active form: Grx is reduced by two GSH molecules, which are in turn oxidized to glutathione disulfide while thioredoxin is reduced by an enzyme called thioredoxin reductase (TrxR) (Hanschmann et al., 2013). TrxR then harnesses electrons donated by NADPH molecules to reduce its own thiol-containing active site back to its active state while glutathione disulfide is reduced back to two GSH molecules by the GR, which also harnesses electrons from NADPH (Lee et al., 2013). Importantly, both enzymes are recognized as playing important roles in redox signaling pathways within the cell, especially given their abundance throughout the cell (**Figure 5**). In mammalian cells, for example, TRX1 and GRX1 are both located in the cytosol, nucleus and can be secreted, TRX2 and GRX2 are located in the mitochondria, GRX3 is also located in the cytosol and nucleus and GRX5 is in the mitochondria (Hanschmann et al., 2013).

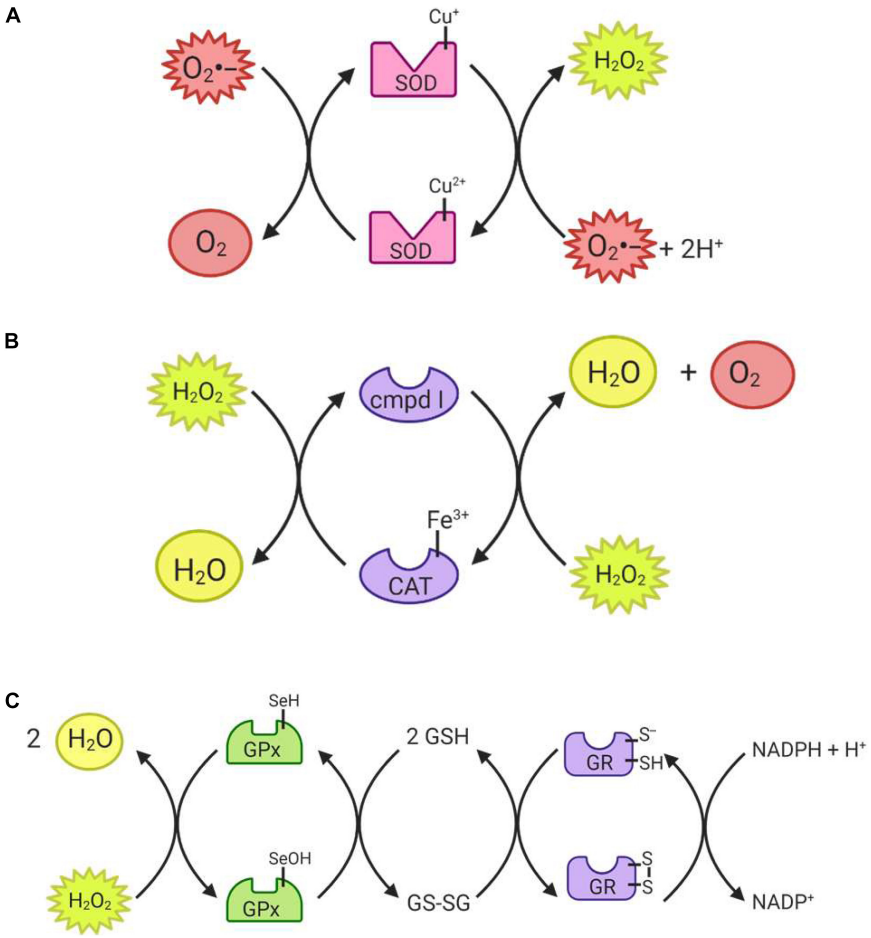

FIGURE 4 | Reactions catalyzed by antioxidant enzymes I. **(A)** Superoxide dismutase (SOD) uses either a reduced copper or manganese active site to reduce two superoxide radicals, producing hydrogen peroxide in the process. **(B)** Catalase (CAT) can convert hydrogen peroxide to water, and is itself converted to compound I in the process. In order to return to its active state, the enzyme must reduce another hydrogen peroxide molecule to water and oxygen. **(C)** Glutathione peroxidase (GPx) can reduce both hydrogen peroxide and organic peroxides using its selenocysteine active site. The enzyme then returns to its active state through reduction by glutathione (GSH). Glutathione reductase (GR) can then reduce glutathione disulfide (GSSG) back to GSH molecules.

Peroxiredoxin

The family of peroxiredoxin (Prx) enzymes are cysteine-containing enzymes that reduce peroxides, including organic peroxides (**Figure 6C**) (Perkins et al., 2015). Several Prx enzymes are located throughout the cell (**Figure 5**): in mammals, PRDX1 and PRDX2 are located in the cytosol and nucleus, PRDX3 in the mitochondria, PRDX4 in the endoplasmic reticulum, PRDX5 in peroxisomes, mitochondria and cytosol, and PRDX6 in the cytosol (Hanschmann et al., 2013; Rhee, 2016). Once the cysteine-containing active site reduces the target peroxide, Prx enzymes are returned to their active state when reduced by either Trx or GSH (Perkins et al., 2015).

Glutathione S-transferase

Glutathione *S*-transferases (GSTs) have been linked to various stress responses and are thought to help GPx in attenuating lipid peroxidation by reducing fatty acid hydroperoxides (Sharma et al., 2004). The enzyme does so by binding GSH and catalyzing the transfer of electrons to the target lipid, its active site consisting of a serine residue (Sharma et al., 2004; Dixon et al., 2011). In addition to preventing lipid peroxidation, GSTs may also help detoxify the end products of lipid peroxidation, which can be very harmful to the cell. There are many GST isozymes, most of which are in the cytosol, microsomes and plasma membrane (**Figure 5**) (Sharma et al., 2004).

Non-enzymatic Antioxidants

Glutathione

Appropriate glutathione (GSH) levels are essential for the activity of the GPx and Grx systems, where GSH acts to return each enzyme back to its active state (Flohé, 1985; Birben et al., 2012). In addition, GSH itself can act as an antioxidant using the sulfhydryl group to donate electrons in order to reduce and detoxify ROS (Birben et al., 2012). Levels of active GSH can be restored either by converting oxidized GSH back to its reduced state by GR and NADPH, or by dietary supplementation (Wu et al., 2004). GSH can be added to oxidized cysteine residues in proteins through the

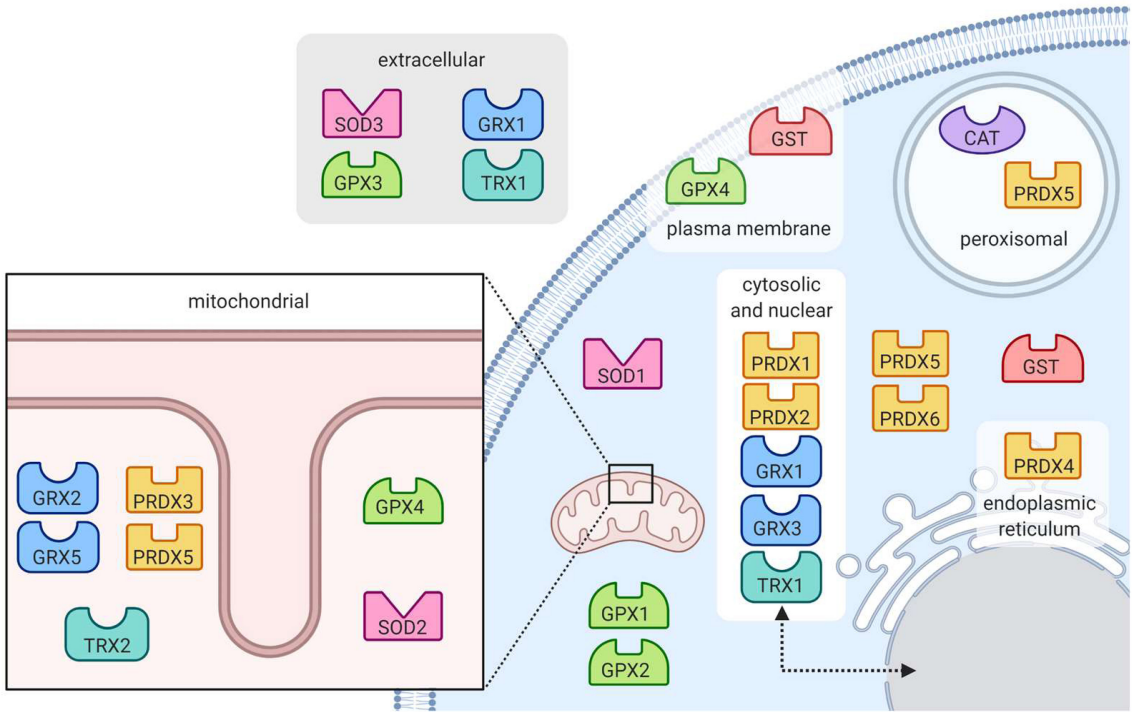

FIGURE 5 | Location of antioxidant enzymes within the cell. Superoxide dismutase enzymes (SOD) are found in the mitochondria, cytoplasm and extracellular space. Catalase (CAT) works to eradicate hydrogen peroxide in the peroxisome. Peroxide-reducing peroxiredoxins (PRX) are present throughout the cell, notably in peroxisomes. Also reducing peroxides, glutathione peroxidases (GPX) are present in the mitochondria and cytoplasm, at the plasma membrane and in the extracellular space. Protein-protecting glutaredoxins (GRX) and thioredoxins (TRX) are present in many subcellular locations including in the mitochondria, cytoplasm, nucleus, and extracellular space. Glutathione *s*-transferases (GST) are located in the cytoplasm and at the plasma membrane.

process of *S*-glutathionylation. Because this process is reversible and can modify protein function, glutathionylation can be used in intracellular signaling (Xiong et al., 2011). Interestingly, the levels of glutathione can be increased by the endogenously-produced signaling molecule hydrogen sulfide (H_2S) (Kimura et al., 2010), which has also been shown to increase lifespan (Miller and Roth, 2007).

N-acetyl cysteine

Not only is *N*-acetyl cysteine (NAC) a precursor molecule in the formation of GSH, it also has its own thiol group, allowing it to participate in redox reactions and donate its electrons for the detoxification of ROS and the protection of sulfhydryl-containing proteins from oxidative damage (Aldini et al., 2018). NAC levels are maintained by the consumption of cysteine in high protein foods (Salamon et al., 2019).

Vitamin C and vitamin E

Vitamin C (ascorbic acid) is a water soluble antioxidant that is present both intracellularly and extracellularly. It acts by scavenging oxygen free radicals in aqueous environments, and by providing a line of defense against oxidation of cholesterols (Frei, 1994). Vitamin E (alpha-, beta-, gamma-, delta-tocopherols, and alpha-, beta-, gamma-, delta-tocotrienols) is lipid soluble and present in cell membrane and lipoproteins, working to inhibit lipid peroxidation as a result of oxidative stress (Frei, 1994). Vitamin C can help convert Vitamin E back to it reduced form (Birben et al., 2012).

Repair of Oxidative Damage

While antioxidant systems work to balance ROS levels and prevent oxidative damage, the cell also employs various repair systems to reverse damaging oxidation of cell components. As described above, glutaredoxins and thioredoxins play an important role in the reduction of thiol-containing proteins. Additional enzymes work alongside thioredoxins such as thioredoxin-related protein of 14 kDa and methionine sulfide reductase to reduce cysteine and methionine residues, respectively, both of which are amino acids that are most susceptible to oxidation by ROS (Moskovitz et al., 2001; Pader et al., 2014). DNA damage repair mechanisms can be trigged by redox sensors which activate the expression of enzymes mediating base excision repair (BER), nucleotide excision repair (NER) and mismatch repair. The common DNA oxidation product, 8-oxoguanine, is repaired by 8-oxoguanine glycosylase (OGG1) via base excision (Van Houten et al., 2018).

In addition to mechanisms which work to reverse oxidative damage, quality control systems which prevent the over-accumulation of oxidatively damaged molecules and organelles have been described. Irreversibly oxidized proteins are targeted by unfolded protein response machinery and can be degraded via the proteasome in the cytosol or by proteases in the

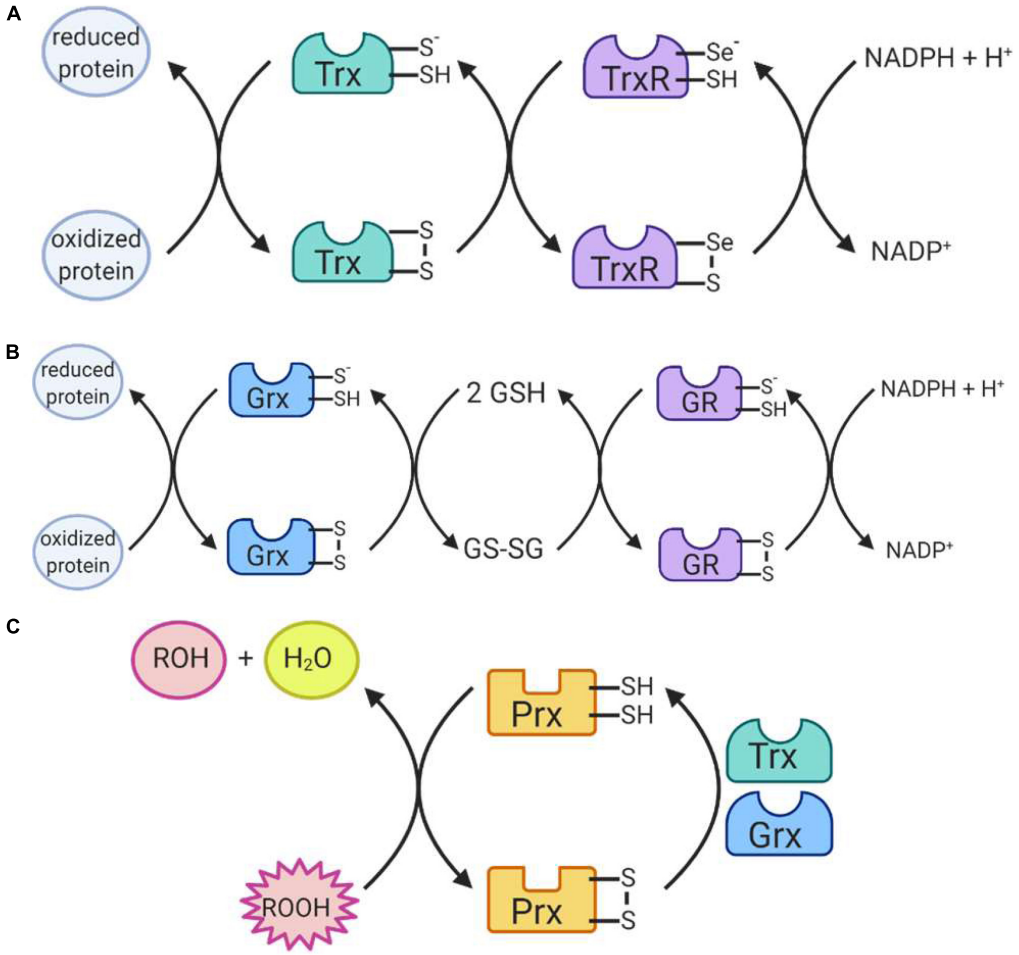

FIGURE 6 | Reactions catalyzed by antioxidant enzymes II. **(A)** Thioredoxin (Trx) uses its thiol containing active site to return proteins to a reduced state. Trx itself is then reduced by the selenium-containing active site of thioredoxin reductase (TrxR), which is in turn reduced by NADPH. **(B)** Glutaredoxin (Grx) also uses its thiol-containing active site to reduce proteins. The enzyme is then reduced by 2 glutathione (GSH) molecules, producing glutathione disulfide (GSSG). GSSG is reduced by the thiol-containing active site of glutathione reductase (GR) which is in turn reduced by NADPH. **(C)** Peroxiredoxins (Prx) work to reduce hydrogen peroxide and organic peroxides using its thiol-containing active site. The enzyme is itself then reduced by Trx or Grx to return to its active state.

mitochondria (Friguet, 2006; Lionaki and Tavernarakis, 2013). Furthermore, oxidative stress may trigger autophagy of peroxisomes (pexophagy) and mitochondria (mitophagy) allowing for the removal of dysfunctional and ROS-generating organelles, followed by the synthesis of new undamaged organelles (Lemasters, 2005).

Measuring Reactive Oxygen Species

While the short lifespan and high reactivity of ROS make them difficult to measure accurately, especially *in vivo*, a number of methods to measure ROS directly have been developed [for more comprehensive reviews of approaches to measure ROS see (Dikalov and Harrison, 2014; Kaludercic et al., 2014; Pavelescu, 2015; Griendling et al., 2016)]. Many of these approaches rely on molecules that emit fluorescence after they have been oxidized by ROS. Among the most utilized of these molecules are 2′,7′-dichlorodihydrofluorescein diacetate (DCF), dihydroethidium (DHE), and MitoSOX, which are believed to measure overall cellular ROS (Wang and Roper, 2014), cellular superoxide levels, and mitochondrial superoxide levels, respectively (Wang and Zou, 2018). Similarly, Amplex Red has been used extensively to measure hydrogen peroxide levels. However, there is much disagreement about the sensitivity and specificity of these approaches especially given the fact that these molecules can also participate in non-specific redox reactions, producing a different but similarly fluorescent adduct that can be hard to distinguish from the fluorescent products derived from reactions with ROS (Wang and Zou, 2018). Furthermore, these molecules are membrane permeable and can leak out of the cell, posing a problem when measuring cellular ROS in tissues (Wang and Roper, 2014).

Given the difficulties in directly measuring ROS levels, investigators often instead quantify ROS indirectly, either by assessing oxidative damage to cell components, or measuring survival under conditions of oxidative stress (Katerji et al., 2019).

Levels of oxidative damage have most commonly been assessed by measuring the levels of 8-oxoguanine residues in DNA, carbonylation of protein, or levels of malondialdehyde (MDA), an end product of lipid peroxidation (**Figure 2**) (Esterbauer and Cheeseman, 1990; Ock et al., 2012; Mesquita et al., 2014). Increased levels of oxidative damage are generally interpreted to indicate increased levels of ROS; however, this outcome could also result from decreased levels of antioxidant or repair enzymes. To quantify resistance to oxidative stress, animals can be treated with ROS-generating compounds such as paraquat, juglone, and menadione (Fukushima et al., 2002; Criddle et al., 2006; Loor et al., 2010; Anaissi-Afonso et al., 2018; Ahmad and Suzuki, 2019). Decreased resistance to oxidative stress may be an indication of elevated baseline levels of ROS; however, increased levels of ROS can also cause upregulation of antioxidant enzymes, and thus resistance to oxidative stress must be interpreted with great care.

More recently, genetically-encoded redox sensors have been developed to measure ROS *in vivo* without the spatial and temporal restrictions encountered with other ROS detection methods (Chiu et al., 2014; Kaludercic et al., 2014; Erard et al., 2018). These sensors involve the expression of a redox-sensitive protein that emits fluorescence after it encounters ROS. These sensors are minimally invasive, can be stably expressed in specific tissues, can be targeted to specific sub-cellular locations, and can be used for real-time ROS detection *in vivo* (Erard et al., 2018). Given that an increasing body of work shows that the specific conditions (e.g., timing, sub-cellular location, and levels) determine whether ROS have beneficial or detrimental effects, genetically-encoded redox sensors are expected to provide a highly useful tool for future studies on the role of ROS in aging and disease (Chiu et al., 2014; Erard et al., 2018).

ROLE OF ROS IN DETERMINING LONGEVITY: EVIDENCE BY ASSOCIATION

In order to study the relationship between ROS and aging, many groups have utilized a comparative biology approach in which the levels of ROS, or ROS-related outcomes, are measured across species with differing lifespans. In examining the relationship between lifespan and either ROS levels, antioxidant levels or oxidative damage, most studies have demonstrated a negative correlation. While this comparative data is generally supportive of the FRTA, exceptions and contradictions have also been observed.

ROS Production Is Negatively Correlated With Longevity

Across species with varying lifespan, data collected from comparative studies shows a negative correlation between the rate of mtROS production and lifespan (**Table 1**). Comparing the superoxide and hydrogen peroxide generation rates across seven mammalian species revealed an inverse relationship between ROS production rates and lifespan (Sohal et al., 1989; Ku et al., 1993). Furthermore, a similar study comparing five different species of dipteran flies supported these findings, showing that longer lifespan correlates with relatively low levels of ROS generation (Sohal et al., 1995). In these studies, measurement of superoxide production was carried out in submitochondrial particles by looking at the reduction of cytochrome *c* due to its interaction with superoxide, while hydrogen peroxide generation was measured fluorometrically by monitoring the oxidation of *p*-hydroxyphenylacetate (PHPA) coupled to the enzymatic reduction of H_2O_2 by horseradish peroxidase (Sohal et al., 1989, 1995; Ku et al., 1993).

Despite seemingly supportive evidence from these studies, it was found that metabolic rate also correlated with lifespan (Sohal et al., 1989; Ku et al., 1993). In order to isolate which factor is responsible for the observed results, the levels of mtROS production were compared between species with the same metabolic rate but different lifespans. While pigeons and rats are similar in size and have the same metabolic rate, pigeons live 35 years whereas rats only live 4 years. When superoxide and hydrogen peroxide generation rates where studied in the brain, heart, and kidney of these two animals, it was found that the pigeon had a significantly lower ROS generation rate (Ku and Sohal, 1993), again supporting a negative relationship between ROS production and lifespan.

While many studies have observed the same inverse relationship between lifespan and the rate of ROS generation, not all of these studies took into account possible confounding variables such as the effects of body mass and non-independence of different species due to phylogenetic relatedness (Page et al., 2010). To address these potential shortcomings, one study examined the rates of hydrogen peroxide generation by heart mitochondria isolated from pairs or groups of two classes of vertebrate homeotherms with similar body mass, but very different lifespan (Lambert et al., 2007). As with previous studies, the results showed a negative correlation between the rate of H_2O_2 generation and lifespan.

Although data from comparative studies supports the idea that lower rates of mtROS generation correlate with a greater lifespan across species, there are certain exceptions. For example, *Heterocephalus glabe* (the naked mole-rat) is the longest living rodent species known to date, with an lifespan upward of 30 years (Dammann, 2017), yet it produces similar levels of mtROS to mice, who have a lifespan of less than 4 years (Munro et al., 2019). This exception may result from the fact that naked mole-rats also have increased antioxidant defenses, which may compensate for their elevated levels of ROS (Munro et al., 2019). This highlights the importance of not considering ROS levels in a vacuum, as it is a fine balance between ROS production and ROS detoxification.

Levels of Antioxidants Can Be Negatively Correlated With Longevity

As with levels of ROS, multiple comparative studies have examined the relationship between antioxidants and lifespan. The

TABLE 1 | Levels of reactive oxygen species are negatively correlated with longevity in comparative studies.

ROS being measured	Experimental animals	Range of lifespans	Finding	References
H_2O_2, O_2^-	Flies	29.5–65.5 days	Negative correlation	Sohal et al., 1995
H_2O_2, O_2^-	Pigeons and rats	4.5–35 years	Negative correlation	Ku and Sohal, 1993
H_2O_2, O_2^-	Mammals	3.5–30 years	Negative correlation	Ku et al., 1993
H_2O_2	Mammals and birds	3.5–37.5 years	Negative correlation	Lambert et al., 2007
O_2^-	Mammals and flies	3 months–30 years	Negative correlation	Sohal et al., 1989

results from these studies are somewhat variable in that some studies show a negative correlation while others show a positive correlation, or no correlation (**Table 2**). To some extent the results are dependent on the specific antioxidant being measured and which tissue it is being measured in.

In a study examining antioxidant enzyme activity in six mammalian species, it was found that superoxide dismutase and catalase activity were positively correlated with lifespan, while glutathione concentration was negatively correlated with longevity (Sohal et al., 1990). A subsequent study examining antioxidant levels in liver tissue found that catalase, glutathione peroxidase and glutathione levels are negatively correlated with lifespan, while superoxide dismutase and glutathione reductase were not correlated with longevity (Lopez-Torres et al., 1993). Comparison of superoxide dismutase, catalase, glutathione peroxidases, and glutathione reductase between various amphibia, mammal and bird species in lung tissue revealed a negative correlation with lifespan (Pérez-Campo et al., 1994). In brain, heart and liver tissue of mammalian and avian species, CuZnSOD, glutathione peroxidase and glutathione reductase show no correlation with lifespan in any of the three tissues (Page et al., 2010). MnSOD and catalase were observed to be positively correlated with lifespan, however, only in brain tissue (Page et al., 2010).

Analysis of a magnitude of studies, encompassing a large variety of vertebrate species and comparing antioxidant levels in tissues from multiple organs, suggests that there is an overall negative correlation between antioxidants and lifespan. In a meta-analysis of 78 correlations between endogenous antioxidants and longevity, it was found that 72 were negative, one was positive and the rest were insignificant (Perez-Campo et al., 1998). While on the surface a negative correlation between antioxidant levels and lifespan appears to contradict the FRTA because high levels of antioxidants should result in low levels of ROS, another possible interpretation is that the levels of antioxidants are regulated in response to the levels of ROS that are present, such that high levels of antioxidants are in fact an indication of high levels of ROS generation. These two possibilities can be distinguished by measuring oxidative damage, which is determined by the balance between ROS and antioxidants.

Oxidative Damage Is Negatively Correlated With Longevity

Oxidative damage results when the levels of ROS generated exceed a cell's antioxidant capacity. In examining the relationship between oxidative damage and lifespan, comparative studies have generally observed a negative correlation (**Table 3**).

Reactive oxygen species can oxidatively damage many biomolecules such as DNA, protein and lipids (**Figure 2**). The two biomolecules which show the greatest correlation with lifespan are membrane fatty acids and mtDNA (Barja, 2002). mtDNA is located in close proximity to the site of mtROS production and is therefore highly susceptible to damage during aging, leading to large deletions over time (Kowald and Kirkwood, 2018). This accumulation of oxidative damage during the aging process implicated mtDNA as a potential regulator of lifespan. In support of this idea, oxidative damage to mtDNA in the heart and brain, estimated by levels of 8-hydroxy-2-deoxyguanosine (8-oxodG), negatively correlates with lifespan in mammals and birds (Barja and Herrero, 2000). Additionally, despite extensive oxidative damage occurring to nuclear DNA (nDNA) (Richter et al., 1988), the same correlation with lifespan is not observed (Barja, 2004). Since the rate of repair of 8-oxodG is rapid in both the mitochondria and the nucleus (Anson et al., 1998), increased 8-oxodG levels in the mitochondria are due to higher rates of oxidative attack, not an inefficient repair mechanism (Barja, 2000).

Studies looking at oxidative damage to mtDNA in bivalves and other marine species have also shown a negative correlation with lifespan. The longest living non-colonial animal, *Arctica islandica,* has a lifespan of up to 400 years and provides an excellent opportunity to study aging in distant taxonomic groups. Studies using *A. islandica* find low rates of ROS generation, low levels of oxidative damage to DNA and a lower mitochondrial membrane peroxidation index compared to short-lived bivalves in the same subclass – Heteroconchia (Blier et al., 2017). Additionally, comparison between *A. islandica* and a shorter lived bivalve from the same subclass, *Mercenaria mercenaria* (lifespan of 106 years), revealed increased oxidative stress resistance associated with the species' heightened longevity (Ungvari et al., 2011). Previous correlations between oxidative damage to mtDNA and lifespan, found in mammals and avian species, have further been corroborated in *Sebastes* (rockfish) (Hua et al., 2015).

The degree of fatty acid unsaturation in mitochondrial and cellular membranes is another parameter negatively correlated with both lifespan and oxidative stress. Unsaturated fatty acids possess extremely unstable electrons near the double bonds, making them highly susceptible to oxidative damage – a sensitivity that increases exponentially as a function of the number of double bonds per molecule (Barja, 2002). Studies have shown that the degree of fatty acid unsaturation is lower

TABLE 2 | Levels of antioxidants are negatively correlated with longevity in some comparative studies but not others.

Antioxidant being measured	Experimental animals	Range of lifespans	Finding	References
SOD, CAT, GPx, GSH	Mammals	3.5–30 years	Negative and positive correlations	Sohal et al., 1990
CAT, GPx, GSH	Mammals, amphibians, birds and trout	3.5–35 years	Negative correlation	Lopez-Torres et al., 1993
SOD, GRX	Mammals, amphibians, birds and trout	3.5–30 years	No correlation	Lopez-Torres et al., 1993
SOD, CAT, GPx, GRX	Amphibians, mammals and birds	4–35 years	Negative correlation	Pérez-Campo et al., 1994
SOD, CAT, GPx, GRX	Mammals and birds	3–122 years	No correlation	Page et al., 2010

SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH, glutathione; GRX, glutaredoxin.

TABLE 3 | Levels of oxidative damage are negatively correlated with longevity in comparative studies.

Type of oxidative damage	Experimental animals	Range of lifespans	Finding	References
mtDNA	Mammals and birds	3.5–46 years	Negative correlation	Barja and Herrero, 2000
DNA and Lipids	Bivalves	36–500 years	Negative correlation	Blier et al., 2017
Lipids	Mammals	3.5–46 years	Negative correlation	Pamplona et al., 2002
Lipids	Mice and birds	3.5–24 years	Negative correlation	Pamplona et al., 1999
Lipids	Rats and pigeons	4–35	Negative correlation	Pamplona et al., 1996

mtDNA, mitochondrial DNA.

in long-lived animals than in short-lived (Pamplona et al., 2002). This adaptation in long-lived animals may be advantageous as it decreases their sensitivity to lipid peroxidation, in turn protecting other molecules from lipoxidation-derived damage (Pamplona and Barja, 2007).

A study in *Drosophila melanogaster* found that flies that developed under larval crowding had a shorter lifespan and a greater proportion of unsaturated fatty acids (Moghadam et al., 2015). They found a significant negative correlation between lifespan and the peroxidation index. Similar results have been found in studies comparing unsaturated fatty acid content and lipid peroxidation levels between mammalian and avian species (Pamplona et al., 1996, 1999; Portero-Otín et al., 2001). Despite high metabolic rates, birds, on average, have an increased lifespan compared to mammals of a similar size. Fatty acid unsaturation and lipid peroxidation are shown to be lower in parakeets and canaries compared to the mouse, protecting their tissues and organelles against free radical-induced lipid peroxidation and possibly contributing to their slower rate of aging (Pamplona et al., 1999). Similar results are obtained in studies comparing liver mitochondria of rats and pigeons (Pamplona et al., 1996) and mammals (Portero-Otín et al., 2001). The increasing evidence supporting a causal role for membrane fatty acid composition has led to an expansion of the "oxidative stress" theory of aging, described by some as the "membrane pacemaker" theory of aging (Hulbert, 2005).

Bats have been shown to live much longer than is predicted by their size and metabolic rates. While these mammals can live up to 40 years, similarly sized mice rarely live longer than 4 years. In comparing oxidative damage between bats and mice, it was found that bats have decreased protein carbonylation (Salmon et al., 2009). Bats also show decreased ROS production compared to the much shorter lived mice and shrews, though only as a ratio of oxygen consumption (Brunet-Rossinni, 2004).

Despite strong evidence demonstrating a negative correlation between oxidative damage and lifespan, there are certain exceptions. The naked mole-rat produces high levels of mtROS and accumulates significant levels of oxidative damage (Saldmann et al., 2019) and yet has a lifespan 10-fold greater than that of a similarly sized laboratory mouse (Austad, 2018). In fact, naked mole rates accrue greater oxidative damage to lipids (2-fold), mtDNA (2-fold), nDNA (8-fold) and proteins (1.5- to 2-fold) than physiologically age-matched mice (Andziak et al., 2006). Similarly, long-lived strains of *Drosophila,* vampire bats and birds have also been observed to have high levels of oxidative damage (Buffenstein et al., 2008).

ROLE OF ROS IN DETERMINING LONGEVITY: EXPERIMENTAL MANIPULATION OF ROS LEVELS

While data from comparative studies provides a useful starting point for understanding the relationship between ROS and aging, these studies are only able to demonstrate correlation, not causation. In order to determine the extent to which ROS contribute to aging, it is necessary to experimentally manipulate the levels of ROS and measure the resulting effect on lifespan. In these experimental studies the levels of ROS have been increased indirectly by knocking out genes encoding antioxidant enzymes, or directly through exposure to a ROS-generating compound. Similarly, ROS levels have been decreased by overexpressing antioxidant enzymes, or treatment with an antioxidant compound. While these types of experiments allow researchers to make conclusions about the causative role of ROS in lifespan, these studies have only been performed in a limited number of model organisms, all of which are relatively short-lived. Nonetheless, experiments involving the manipulation of ROS levels have been important in demonstrating that ROS can have beneficial or detrimental effects on longevity.

Lifespan of Animal Models With Genetic Manipulations of Antioxidant Expression

In order to examine the effect of ROS on lifespan, researchers have generated transgenic and knockout animals that express increased or decreased levels of antioxidant enzymes and then quantified the resulting effect on lifespan. While increasing or decreasing levels of antioxidants would be predicted to decrease or increase ROS levels, it is important to measure the resulting effect on ROS levels to make firm conclusions about ROS and aging. In the case of disrupting antioxidant genes, it is possible that redundancy or a compensatory upregulation of other antioxidant genes leads to little or no change in ROS levels. In the case of overexpression of antioxidant genes, it is possible that the normal level of antioxidant gene expression is already in excess of the amount of ROS being produced such that increasing antioxidant levels has no effect.

Superoxide Dismutase

The effect of increasing or decreasing the levels of SOD on lifespan has been examined in yeast, worms, flies and mice. Overexpression of either cytoplasmic or mitochondrial SOD resulted in increased lifespan in yeast, worms and flies (**Table 4**) (Melov et al., 2000; Fabrizio et al., 2003; Harris et al., 2005; Doonan et al., 2008; Cabreiro et al., 2011). Interestingly, however, the increase in lifespan does not seem to be attributable to decreased oxidative damage, at least in worms, as worms overexpressing *sod-1* show increased levels of protein oxidation and hydrogen peroxide *in vivo* (Cabreiro et al., 2011), as well as increased sensitivity to paraquat (Doonan et al., 2008) compared to wild-type worms. In addition, the results in *Drosophila* are dependent on the strain background as overexpression of cytoplasmic SOD with catalase alone or in combination with mitochondrial SOD failed to increase lifespan in a relatively long-lived strain background (Orr et al., 2003). In contrast to the other model organisms, overexpression of cytoplasmic SOD, mitochondrial SOD or cytoplasmic and mitochondrial SOD together in mice has no effect on longevity (Huang et al., 2000; Pérez et al., 2009).

Deletion of cytoplasmic SOD decreases lifespan in yeast, worms, flies and mice (**Table 5**) (Phillips et al., 1989; Elchuri et al., 2005; Ünlü and Koç, 2007; Doonan et al., 2008). Disruption of mitochondrial SOD also decreases lifespan in yeast and flies (Duttaroy et al., 2003; Ünlü and Koç, 2007). In contrast, deletion of the mitochondrial *sod* gene, *sod-2,* results in a significant increase in lifespan in *Caenorhabditis elegans* (Van Raamsdonk and Hekimi, 2009). In mice, it has not been possible to examine the effect of knocking out *Sod2* on adult lifespan as *Sod2* knockout mice die early in development (Li et al., 1995; Lebovitz et al., 1996). However, the need for *Sod2* in early development can now be circumvented using an inducible *Sod2* knockdown mouse model (Cox et al., 2018). Examination of heterozygous

TABLE 4 | Overexpressing antioxidant enzymes can increase or decrease lifespan.

Antioxidant Enzyme	Location	Organism	Effect on lifespan	References
Superoxide dismutase	Cytoplasm (*SOD1*)	Yeast	Increase	Harris et al., 2005
	Cytoplasm (*sod-1*)	Worms	Increase	Doonan et al., 2008
	Cytoplasm (*Sod1*)	Flies	Increase	Sun and Tower, 1999
	Cytoplasm (*Sod1*)	Mice	No effect	Huang et al., 2000
	Mitochondria (*SOD2*)	Yeast	Increase	Fabrizio et al., 2003
	Mitochondria (*sod-2*)	Worms	Increase	Melov et al., 2000
	Mitochondria (*Sod2*)	Flies	Increase	Curtis et al., 2007
	Mitochondria (*Sod2*)	Mice	No effect	Pérez et al., 2009
Catalase	Peroxisome (*CTT1*)	Yeast	Decrease	Fabrizio et al., 2003
	Peroxisome (*Cat*)	Flies	Decrease	Sun and Tower, 1999
	Cytoplasm and peroxisome (*ctl-1,ctl-2,* and *ctl-3*)	Worms	No effect	Cabreiro et al., 2011
	Peroxisome (*Cat*)	Mice	No effect	Pérez et al., 2009
	Mitochondria (*Cat*)	Mice	Increase	Schriner et al., 2005
Peroxiredoxin	Cytoplasm (*TSA1*)	Yeast	Increase	Hanzen et al., 2016
	Cytoplasm (*Prx2*)	Flies	Increase	Lee et al., 2009
	Endoplasmic reticulum (*Prx4*)	Flies	Increase	Klichko et al., 2016
	Cytoplasm, nucleus and mitochondria (*Prx5*)	Flies	Increase	Radyuk et al., 2009
Thioredoxin	Cytoplasm and nucleus (*trx-1*)	Worms	Increase	Miranda-Vizuete et al., 2006
	Nucleus (*TrxT*)	Flies	Increase	Umeda-Kameyama et al., 2007
	Cytoplasm and nucleus (*Trx-1*)	Flies	Increase	Oberacker et al., 2018
	Cytoplasm and nucleus (*Trx1*)	Mice	Increase in males	Pérez et al., 2011
Thioredoxin reductase	Mitochondria (*Trxr2*)	Flies	Increase	Pickering et al., 2017
Glutaredoxin	Exogenous	Worms	Increase	Li et al., 2018
Glutathione *S*-transferase	Cytoplasm (*gst-10*)	Worms	Increase	Ayyadevara et al., 2005
	Cytoplasm (*gst-4*)	Worms	No effect	Leiers et al., 2003
	Cytoplasm (*GstS1*)	Flies	Increase	Simonsen et al., 2008

TABLE 5 | Deletion of genes encoding antioxidant enzymes can increase or decrease longevity.

Enzyme	Location	Organism	Effect on lifespan	References
Superoxide dismutase	Cytoplasm (*SOD1*)	Yeast	Decrease	Ünlü and Koç, 2007
	Cytoplasm (*sod-1*)	Worms	Decrease	Doonan et al., 2008
	Cytoplasm (*Sod1*)	Flies	Decrease	Phillips et al., 1989
	Cytoplasm (*Sod1*)	Mice	Decrease	Elchuri et al., 2005
	Mitochondria (*SOD2*)	Yeast	Decrease	Ünlü and Koç, 2007
	Mitochondria (*sod-2*)	Worms	Increase	Van Raamsdonk and Hekimi, 2009
	Mitochondria (*Sod2*)	Flies	Decrease	Duttaroy et al., 2003
	Mitochondria (*Sod2*)	Mice	Decrease	Li et al., 1995; Lebovitz et al., 1996
Catalase	Peroxisome (*CTA1*)	Yeast	Increase	Mesquita et al., 2010
	Peroxisome (*CTT1*)	Yeast	Increase	Mesquita et al., 2010
	Cytoplasm (*ctl-1*)	Worms	No effect	Petriv and Rachubinski, 2004
	Peroxisome (*ctl-2*)	Worms	Decrease	Petriv and Rachubinski, 2004
	Peroxisome (*catn1/catn4*)	Flies	No effect	Orr et al., 1992
	Peroxisome (*Cat*)	Mice	Decrease	Perez-Estrada et al., 2019
Glutathione peroxidase	Mitochondria (*GRX2*)	Yeast	Decrease	Managbanag et al., 2008
	Cytoplasm, nucleus and extracellular space (*gpx-1, gpx-2, gpx-6*, and *gpx-7*)	Worms	Decrease	Sakamoto et al., 2014
	Cytoplasm (*Gpx1*)	Mice	No effect	Zhang et al., 2009
	Mitochondria (*Gpx4*)	Mice	Increase	Ran et al., 2007
	Endoplasmic reticulum (*Gpx7*)	Mice	Decrease	Wei et al., 2012
Peroxiredoxin	Mitochondria (*PRX1*)	Yeast	Decrease	Molin et al., 2011
	Cytoplasm (*prdx-2*)	Worms	Decrease	Oláhová et al., 2008
	Mitochondria (*prdx-3*)	Worms	Decrease	Ha et al., 2006
	Cytoplasm, nucleus and mitochondria (*Prx5*)	Flies	Decrease	Radyuk et al., 2009
	Cytoplasm (*Prdx1*)	Mice	Decrease	Neumann et al., 2003
Thioredoxin	Cytoplasm (*TRX1*)	Yeast	Decrease	Laschober et al., 2010
	Cytoplasm (*trx-1*)	Worms	Decrease	Jee et al., 2005
	Mitochondria (*trx-2*)	Worms	No effect	Cacho-Valadez et al., 2012
	Mitochondria (*Trx2*)	Flies	Decrease	Svensson and Larsson, 2007
	Mitochondria (*Trx2*)	Mice	Decrease	Pérez et al., 2008
Thioredoxin reductase	Cytoplasm (*TRXR1*)	Yeast	Decrease	Picazo et al., 2018
	Cytoplasm (*trxr-1*)	Worms	No effect	Li et al., 2012
	Mitochondria (*trxr-2*)	Worms	No effect	Li et al., 2012
	Cytoplasm (*Trxr1*)	Flies	Decrease	Missirlis et al., 2001
Glutaredoxin	Cytoplasm (*GRX1*)	Yeast	Decrease	Liu et al., 2018
	Cytoplasm and mitochondria (*GRX2*)	Yeast	Decrease	Liu et al., 2018
	Mitochondria (*GRX5*)	Yeast	Decrease	Zadrag et al., 2008
Glutathione *S*-transferase	Cytoplasm (*gst-5*)	Worms	Decrease	Ayyadevara et al., 2007
	Cytoplasm (*gst-10*)	Worms	Decrease	Ayyadevara et al., 2007
	Microsome (*MGst1*)	Flies	Decrease	Toba and Aigaki, 2000
	Cytoplasm (*GstA4*)	Mice	Increase	Singh et al., 2010

Sod2+/− mice reveals normal longevity despite increased levels of oxidative damage (Van Remmen et al., 2003).

Catalase

In contrast to what was observed with SOD, overexpression of catalase resulted in decreased lifespan in both yeast and flies (**Table 4**) (Sun and Tower, 1999; Fabrizio et al., 2003), despite the fact that catalase overexpression increased resistance to oxidative stress (Sun and Tower, 1999). In worms, overexpression of all three catalase genes combined (*ctl-1, ctl-2,* and *ctl-3*) did not affect lifespan but resulted in a paradoxical increase in oxidative damage to proteins (Cabreiro et al., 2011). In mice, overexpression of catalase in the peroxisome, where catalase is normally found, did not affect longevity (Pérez et al., 2009), while overexpression of catalase targeted to the mitochondria did increase lifespan (Schriner et al., 2005).

Simultaneous overexpression of cytosolic SOD and catalase was initially reported to extend lifespan in *Drosophila* (Orr and Sohal, 1994), but when these experiments were replicated, there was no significant change in lifespan (Sun and Tower, 1999). Similar to the latter experiment, simultaneous overexpression of cytoplasmic SOD and catalase in mice did not affect

lifespan (Pérez et al., 2009). Simultaneous overexpression of mitochondrial SOD and catalase resulted in decreased lifespan in *Drosophila* (Bayne et al., 2005).

Inactivation of catalase in yeast resulted in increased lifespan, despite also increasing oxidative damage (**Table 5**) (Mesquita et al., 2010). In *C. elegans,* deletion of cytoplasmic catalase has no effect on lifespan, while loss of peroxisomal catalase decreases lifespan (Petriv and Rachubinski, 2004). In *Drosophila,* complete loss of catalase activity does not affect longevity (Orr et al., 1992). Finally, disruption of catalase in mice results in decreased lifespan (Perez-Estrada et al., 2019).

Glutathione Peroxidase

There have been only a limited number of studies examining the effects of glutathione peroxidase modulation on lifespan. Deletion of *gpx2* in yeast results in decreased lifespan (Managbanag et al., 2008). In *C. elegans,* simultaneous deletion of four intestinal *gpx* genes decreases lifespan, despite having no effect on resistance to oxidative stress (**Table 5**) (Sakamoto et al., 2014). In mice, deletion of *Gpx1*, one of the most abundant glutathione peroxidases, increases oxidative damage but does not significantly affect longevity, even when combined with a heterozygous mutation in *Sod2* (Zhang et al., 2009). Interestingly, heterozygous mice that have a 50% reduction in *Gpx4* levels have increased lifespan compared to wild-type mice (Ran et al., 2007). Finally, it has been shown that disruption of *Gpx7* in mice results in decreased lifespan, which is associated with increased oxidative stress (Wei et al., 2012). Interestingly, GPX7 lacks enzymatic activity and instead acts as a stress sensor (Chen et al., 2016).

Peroxiredoxin

Single copy overexpression of the yeast cytosolic peroxiredoxin gene, *tsa1*, is sufficient to increase lifespan (**Table 4**) (Hanzen et al., 2016). Overexpression of either *Prx2*(*Jafrac1*) or *Prx5* in flies also increases resistance to oxidative stress and lifespan (Lee et al., 2009; Radyuk et al., 2009). A moderate overexpression of *Prx4* reduces oxidative damage and extends lifespan in flies, but high levels of expression shortens longevity (Klichko et al., 2016).

Deletion of the yeast cytosolic peroxiredoxin, *tsa1*, decreases lifespan (**Table 5**) (Molin et al., 2011). While loss of *Tsa1* also increases sensitivity to hydrogen peroxide, this does not contribute to the shortening of lifespan, which instead is attributed to the role of *Tsa1* as an inhibitor of nutrient signaling via protein kinase A (Roger et al., 2020). Others have reported that disruption of all five yeast peroxiredoxin genes does not decrease lifespan (Zadrag et al., 2008). In *C. elegans*, deletion of the cytoplasmic peroxiredoxin, *prdx-2,* results in increased sensitivity to hydrogen peroxide and decreased lifespan (Oláhová et al., 2008). Interestingly, expression of PRDX-2 in the intestine of *prdx-2* deletion mutants eliminates sensitivity to hydrogen peroxide but fails to restore lifespan (Oláhová et al., 2008) suggesting that the decrease in lifespan may not be attributable to the increase in sensitivity to oxidative stress. As with *prdx-2,* knockdown of mitochondrial peroxiredoxin, *prdx-3*, in *C. elegans* using RNAi resulted in decreased lifespan (Ha et al., 2006). In *Drosophila,* deletion of *Prx5* (Radyuk et al., 2009) or *Prx3* and *Prx5* together (Orr et al., 2013) increases susceptibility to oxidative stress and decreases lifespan. Finally, deletion of *Prx1* in mice increases ROS production and oxidative damage and decreases lifespan (Neumann et al., 2003; Rani et al., 2012).

Thioredoxin and Thioredoxin Reductase

In *C. elegans,* overexpression of the cytoplasmic thioredoxin gene *trx-1* moderately increases lifespan (**Table 4**) (Miranda-Vizuete et al., 2006). Similarly, overexpression of *TrxT* in flies is also found to increase lifespan (Umeda-Kameyama et al., 2007). Downregulation of thioredoxin-interacting protein (TXNIP), a negative regulator or *Trx1*, increases resistance to oxidative stress and extends lifespan, while overexpression decreases lifespan (Oberacker et al., 2018). While one group reported that overexpressing mitochondrial thioredoxin reductase significantly increases lifespan in flies (Pickering et al., 2017), it has also been reported that overexpression of thioredoxin reductase in combination with CuZnSOD and catalase does not affect longevity (Orr et al., 2003). While an earlier study found that overexpression of human thioredoxin in mice resulted in decreased oxidative stress and increased lifespan (Mitsui et al., 2002), subsequent studies observed less beneficial or even detrimental effects. Overexpression of *Trx1* in mice was found to only have significant effects on lifespan in male, but not female, mice, and only increased the earlier part of lifespan (Pérez et al., 2011; Flores et al., 2018). Interestingly, simultaneous overexpression of *Trx1* and *Trx2* resulted in significantly decreased lifespan (Cunningham et al., 2018).

Deletion of the gene encoding thioredoxin in yeast, *trx1*, slightly reduces chronological lifespan (**Table 5**) (Laschober et al., 2010). Deletion of the yeast cytosolic thioredoxin reductase, *trr1*, also reduces chronological lifespan and increases sensitivity to oxidative stress (Picazo et al., 2018). In *C. elegans,* disruption of the cytoplasmic thioredoxin gene, *trx-1,* causes a significant decrease in lifespan (Jee et al., 2005), while deletion of the mitochondrial thioredoxin gene, *trx-2* has no effect (Cacho-Valadez et al., 2012). In contrast, disruption of the cytoplasmic (*trxr-1*) or mitochondrial (*trxr-2*) thioredoxin reductase genes, or both together, did not affect lifespan at the normal growing temperature of 20°C (Li et al., 2012). In flies, mutations affecting *Trx2* shortens lifespan and increases sensitivity to oxidative stress (Svensson and Larsson, 2007). Unlike *C. elegans*, mutations that reduce thioredoxin reductase activity in *Drosophila* cause a severe reduction in adult lifespan (Missirlis et al., 2001). In mice, a 50% reduction in expression of the mitochondrial thioredoxin gene, *Trx2*, increases ROS production and oxidative damage and slightly reduces lifespan (Pérez et al., 2008).

Glutaredoxin

Relatively few studies have examined the effect of glutaredoxins on longevity. Intake of recombinant buckwheat glutaredoxin in *C. elegans* increases lifespan and resistance to oxidative stress (**Table 4**) (Li et al., 2018). Deletion of either *grx1* or *grx2* in yeast shortens the chronological lifespan via increased ROS accumulation which subsequently activates the RAS/PKA signaling pathway and decreases stress resistance (**Table 5**) (Liu et al., 2018). Disruption of the mitochondrial glutaredoxin gene, *grx5,* also decreases lifespan in yeast (Zadrag et al., 2008).

Glutathione *S*-Transferase

Despite the fact that there are many different glutathione *S*-transferases (e.g., 57 in *C. elegans*), very few have been shown to influence lifespan, possibly due to functional redundancy with other glutathione *S*-transferases. In *C. elegans,* overexpression of *gst-4* increases resistance to oxidative stress but does not increase lifespan (Leiers et al., 2003), while overexpression of *gst-10* decreased oxidative damage (4-HNE) and increased lifespan (**Table 4**) (Ayyadevara et al., 2005). In *Drosophila*, overexpression of *GstS1* significantly increases mean lifespan and resistance to oxidative stress (Simonsen et al., 2008).

Decreasing the expression of either *gst-5* or *gst-10* causes decreased lifespan in *C. elegans* (**Table 5**) (Ayyadevara et al., 2007). In contrast, deletion of *gst-14* has the opposite effect of increasing lifespan, although its effects were only reported in mutants that have impaired mitochondrial function (Suthammarak et al., 2013). In *Drosophila*, deletion of microsomal glutathione *S*-transferase was found to reduce lifespan (Toba and Aigaki, 2000). In mice, deletion of *GstA4*, a glutathione *S*-transferase important for detoxification of the lipid peroxidation product 4-hydroxynonenal (4-HNE), extends mean lifespan (Singh et al., 2010).

In summary, genetic manipulations of antioxidant gene expression have varied effects on the lifespan of model organisms. While the general trend is that overexpression of antioxidant enzymes increases lifespan and disruption of antioxidant genes decreases lifespan, there are multiple examples in which no effect or the opposite effect is observed. The ability of antioxidant gene manipulation to significantly change lifespan is dependent on a number of factors including the species in which the manipulation is taking place, the specific antioxidant being altered, and the subcellular location in which the antioxidant is expressed.

Effect of Antioxidant Compounds on Lifespan

Treatment with antioxidant compounds is another approach to studying the relationship between ROS and lifespan. While this approach is an indirect way of modulating ROS levels, it can be applied to a wide range of species, including humans. Nonetheless, experiments using antioxidant compounds have generally been performed in short-lived species so that experiments can be completed in a feasible time frame. Because this approach is indirect, it is important to measure the effect of the antioxidant compound on the levels of ROS in order to fully interpret the results. In addition, when performing experiments with compounds, it is important to consider that most or all of these antioxidant compounds have other effects aside from their antioxidant activity that may contribute to or account for their effects on lifespan. Experiments using this approach have provided mixed results as to the effect of antioxidant supplementation on lifespan (**Table 6**).

N-Acetyl Cysteine

N-acetyl cysteine (NAC) is a scavenger of free radicals as well as a precursor of L-cysteine and a source of sulfhydryl groups (Karalija et al., 2012). It derives most of its antioxidant properties as a cysteine precursor, promoting the synthesis of glutathione. Treatment of *C. elegans* with NAC shows a U-shaped dose-response curve in which low concentrations of NAC can increase lifespan, while higher concentrations decrease lifespan (Oh et al., 2015; Desjardins et al., 2017). A similar pattern is observed in *Drosophila* where lower concentrations of NAC can increase lifespan, while higher concentrations shorten lifespan (Brack et al., 1997; Niraula and Kim, 2019). The effect of NAC treatment in mice is sex dependent as NAC was found to only increase lifespan in males, but not in females (Flurkey et al., 2010). It is important to note, however, that mice treated with NAC show decreases in body weight making it possible that the increase in lifespan is due to dietary restriction (Flurkey et al., 2010).

Vitamin C

Vitamin C is a hydrophilic antioxidant and a strong inhibitor of lipid peroxidation (Sadowska-Bartosz and Bartosz, 2014). Vitamin C treatment in yeast was found to increase survival in wild-type strains (Owsiak et al., 2010). Treatment of *C. elegans* with Vitamin C has also been shown to increase wild-type lifespan when delivered with liposomes (Shibamura et al., 2009) or when the *C. elegans* cuticle is made more permeable with a *bus-8* mutation (Desjardins et al., 2017). Vitamin C supplementation in *Drosophila* can extend the lifespan of wild-type flies, while high doses of Vitamin C can be toxic and shorten lifespan (Bahadorani et al., 2008). Vitamin C treatment in rodents has provided somewhat mixed results. Treating lab mice with Vitamin C resulted in a slight increase in lifespan (Massie et al., 1984). In contrast, Vitamin C treatment shortened the lifespan of wild-derived voles (Selman et al., 2013). Additionally, Vitamin C treatment in guinea-pigs had no effect on lifespan (Davies et al., 1977).

Vitamin E

Vitamin E is a lipophilic antioxidant that protects membranes from oxidative damage as well as regulating signal transduction and gene expression. Vitamin E supplementation in yeast is observed to shorten replicative lifespan (Lam et al., 2010) despite increasing the mean lifespan of four other single cell organisms (Ernst et al., 2013). Multiple groups have shown that Vitamin E treatment can increase lifespan in *C. elegans* (Zuckerman and Geist, 1983; Harrington and Harley, 1988), but interestingly this treatment was found to have no effect on superoxide levels (Ishii et al., 2004). While some groups have reported that Vitamin E treatment increases lifespan in *Drosophila,* others have observed little or no effect on longevity (Driver and Georgeou, 2003; Zou et al., 2007; Bahadorani et al., 2008). Vitamin E treatment in mice is largely observed to have no significant effect on lifespan (Lipman et al., 1998; Morley and Trainor, 2001) but was found to significantly increase lifespan in male mice in one study (Navarro et al., 2005). In contrast, Vitamin E supplementation was found to decrease lifespan in wild-derived voles (Selman et al., 2013).

Vitamin Supplementation Does Not Decrease Mortality in Humans

A number of randomized clinical trials have examined the effects of different antioxidants in humans, including Vitamin C,

TABLE 6 | Exposing animals to antioxidant compounds can increase or decrease lifespan.

Compound	Organism	Dose	Effect on lifespan	References
N-acetyl cysteine	Worms	9 mM	Increase	Oh et al., 2015
	Flies	1 mg/ml	Increase	Brack et al., 1997
	Mice	10 g/L	Increase in males	Flurkey et al., 2010
Vitamin C	Yeast	20 mM	Increase	Owsiak et al., 2010
	Worms	0.24 mg/liposome	Increase	Shibamura et al., 2009
	Flies	20 mM	Increase	Bahadorani et al., 2008
	Mice	57 mM	Increase	Massie et al., 1984
	Wild-derived voles	180 mg/kg	Decrease	Selman et al., 2013
	Guinea pigs	57 mM	No change	Davies et al., 1977
Vitamin E	Yeast	0.05 mM	Decrease	Lam et al., 2010
	Worms	0.2 mg/ml	Increase	Harrington and Harley, 1988
	Flies	20 μg/ml	Increase	Driver and Georgeou, 2003
	Mice	20–4,000 μg/g	No change	Morley and Trainor, 2001
	Wild-derived voles	550 mg/kg	Decrease	Selman et al., 2013

Vitamin E, Vitamin A, beta-carotene and selenium. A meta-analysis of 78 trials that included a total of 296,707 individuals showed no beneficial effect of any of the antioxidants for human longevity in control or disease populations (Bjelakovic et al., 2012, 2013). In fact, treatment with Vitamin E or beta carotene resulted in a higher risk of all-cause mortality. While the average length of supplementation was 3 years, these results suggest that decreasing ROS levels may not be beneficial for lifespan in people.

A MILD INCREASE IN LEVELS OF REACTIVE OXYGEN SPECIES CAN EXTEND LONGEVITY

Exposure to a Mild Dose of ROS-Generating Compounds Increases Lifespan

Another approach to examine the relationship between ROS and aging is to expose animals to compounds that act to generate ROS. Although this approach can be applied to any species, these experiments have only been performed in relatively short-lived species for practical reasons. While exposing animals to high levels of ROS-generating compounds is toxic, evidence from multiple species indicates that mildly increasing ROS levels with ROS-generating compounds can extend longevity (**Table 7**).

One of the first experiments to show that compounds that increase ROS levels can extend longevity involved treating *C. elegans* with 2-deoxy-D-glucose (Schulz et al., 2007). 2-deoxy-D-glucose acts as an inhibitor of glycolysis, leading to increased mitochondrial respiration, increased production of ROS and increased lifespan. Importantly, the increase in ROS levels was found to be required for the lifespan extension as the increase in longevity was prevented by treatment with antioxidants (NAC, Vitamin C or Vitamin E). Similarly, treatment of *C. elegans* with arsenite results in increased ROS and extended longevity, which is completely dependent on the increase in ROS, as treatment with antioxidants (NAC, BHA[butylated hydroxyanisole]) reverts lifespan to control (Schmeisser et al., 2013a). In *C. elegans,* inhibition of mitochondrial electron transport chain Complex I with rotenone causes an increase in mitochondrial ROS and extends lifespan (Schmeisser et al., 2013a). Metformin has also been shown to increase worm lifespan through a similar mechanism. It was found that metformin treatment led to an inhibition of Complex I, increased respiration, increased ROS and extended longevity, which was prevented by treatment with the antioxidant NAC (De Haes et al., 2014). It has also been shown that treating worms with either juglone or paraquat, which act to directly increase superoxide levels primarily in the mitochondria, extends longevity (Heidler et al., 2010; Yang and Hekimi, 2010).

The ability of ROS-generating compounds to extend lifespan is not limited to *C. elegans*, as treating yeast with menadione, which acts to generate mitochondrial ROS through redox cycling, was also shown to increase lifespan (Pan et al., 2011). Interestingly, menadione did not increase lifespan in *C. elegans* (Hunt et al., 2011; Urban et al., 2017). Similarly, treatment with paraquat or metformin failed to extend lifespan in *Drosophila* (Slack et al., 2012; Scialo et al., 2016). The reason why these ROS-generating compounds increase lifespan in some species but not others is currently unclear. Since these compounds have the potential to be toxic at higher doses, it is possible that the doses used were simply too high, or that other experimental parameters were not optimal for the compound to extend lifespan in the species showing no effect. It is also possible that there are differences between species in the ways that ROS can extend longevity. For example, it was proposed that compounds that affect Complex I, such as paraquat and metformin, don't increase lifespan in *Drosophila* because an intact Complex I may be necessary for lifespan extension in this organism (Scialo et al., 2016).

Importantly, ROS-generating compounds have also been shown to increase lifespan in mammals. Similar to 2-deoxy-D-glucose, D-glucosamine acts as an inhibitor of glycolysis leading to increased respiration and increased production of ROS. Treatment of worms or mice with D-glucosamine increases lifespan, and this increase in lifespan was shown to be dependent on elevated ROS as it

TABLE 7 | Examples of ROS-generating compounds that have been shown to increase lifespan.

Compound	Organism	Dose	Effect on lifespan	References
2-deoxy-D-glucose	Worms	5 mM	Increase	Schulz et al., 2007
Juglone	Worms	40 μM	Increase	Heidler et al., 2010
Paraquat	Worms	0.1 mM	Increase	Yang and Hekimi, 2010
Plumbagin	Worms	25 μM	Increase	Hunt et al., 2011
Menadione	Yeast	1 μM	Increase	Pan et al., 2011
Rotenone	Worms	100 nM	Increase	Schmeisser et al., 2013a
Arsenite	Worms	0.1 μM	Increase	Schmeisser et al., 2013b
Metformin	Worms	50 mM	Increase	De Haes et al., 2014
D-glucosamine	Worms	100 μM	Increase	Weimer et al., 2014
	Mice	10 g/kg	Increase	Weimer et al., 2014

TABLE 8 | Examples in which increased levels of ROS cause increased lifespan.

Organism	Intervention	Effect	Effect on lifespan	References
Yeast	Catalase deletion	Increase ROS	Increase	Mesquita et al., 2010
Worms	*sod-2* deletion	Decrease mitochondrial superoxide detoxification	Increase	Van Raamsdonk and Hekimi, 2009
	clk-1 mutation	Increase ROS	Increase	Schaar et al., 2015
	nuo-6 mutation	Increase mtROS	Increase	Yang and Hekimi, 2010
	isp-1 mutation	Increase mtROS	Increase	Yang and Hekimi, 2010
	daf-2 mutation	Increase ROS	Increase	Zarse et al., 2012
	glp-1 mutation	Increase ROS	Increase	Wei and Kenyon, 2016
	memo-1 mutation	Increase ROS	Increase	Ewald et al., 2017
	Knockdown of *ero-1*	Increase ROS	Increase	Hourihan et al., 2016
Flies	Expression of NDI1 (alternative component of ETC)	Increase mtROS	Increase	Scialo et al., 2016
	Muscle-specific knockdown of ND75 component on ETC complex I	Increase ROS	Increase	Owusu-Ansah et al., 2013
Mice	Mclk1 heterozygosity	Increase mtROS	Increase	Liu et al., 2005
	Decreasing body temperature	Increase mtROS	Increase	Ristow and Schmeisser, 2011

is prevented by treatment with antioxidants (NAC, BHA) (Weimer et al., 2014).

Genetic Mutations That Extend Longevity by Increasing Levels of Reactive Oxygen Species

Genetic mutations that lead to increased levels of ROS have been shown to have variable effects on lifespan: they can decrease lifespan, increase lifespan or have no effect (Van Raamsdonk and Hekimi, 2010; Van Raamsdonk et al., 2010). In this section, we will focus on genetic mutants in which increased ROS causes extended longevity, providing examples from yeast, worms, flies and mice (**Table 8**).

In yeast, it has been shown that disruption of genes encoding catalase (*cta1* or *ctt1*) increases the levels of intracellular ROS (hydrogen peroxide) and increases oxidative damage (protein carbonylation), but also increases lifespan (Mesquita et al., 2010). This shows that increasing ROS can increase lifespan, and that oxidative damage can be experimentally dissociated from lifespan.

In *C. elegans,* it has been shown that deletion of the mitochondrial *sod* gene, *sod-2,* increases lifespan, despite also resulting in increased oxidative damage (Van Raamsdonk and Hekimi, 2009). The increase in lifespan in *sod-2* mutant worms is dependent on elevated ROS, as treatment with antioxidants (Vitamin C, α-lipoic acid, or epigallocatechin gallate) reverts their lifespan toward wild-type. Three different mutations that affect mitochondrial function in *C. elegans, clk-1, nuo-6,* and *isp-1,* have all been shown to extend longevity despite increasing levels of ROS (Yang and Hekimi, 2010; Schaar et al., 2015; Dues et al., 2017). As with *sod-2* mutants, treatment of these strains with antioxidants decreases their lifespan, again demonstrating that the elevation in ROS levels in these strains is required for their longevity (Yang and Hekimi, 2010; Van Raamsdonk and Hekimi, 2012). Decreasing insulin-IGF1 signaling has been shown to increase lifespan in multiple species and to be associated with longevity in humans. In *C. elegans*, disruption of this pathway with a mutation in the insulin-IGF1 receptor gene, *daf-2,* results in increased levels of ROS, which are required for their long lifespan (Zarse et al., 2012). Similarly, inhibition of the germline has been shown to increase lifespan in *C. elegans* in a ROS-dependent manner: mutations in *glp-1* prevent germline development, increase ROS levels and extend longevity (Wei and Kenyon, 2016).

In *Drosophila,* expression of an alternative component of the mitochondrial electron transport chain called NDI1 causes increased production of ROS during mitochondrial electron transport. This increase in ROS is sufficient to extend the longevity of the fly, and is prevented by overexpression of catalase (Scialo et al., 2016). Also in *Drosophila,* it has been shown that muscle-specific knockdown of a subunit of electron transport chain Complex I called ND75 results in increased levels of ROS and increased lifespan (Owusu-Ansah et al., 2013). Overexpression of either catalase or glutathione peroxidase reverted the lifespan of ND75-knockdown flies to wild-type, thereby indicating that the increase in ROS is required for their long lifespan (Owusu-Ansah et al., 2013).

In mice, it has been shown that a heterozygous mutation in the mouse homolog of *clk-1, Mclk1,* results in increased levels of mitochondrial ROS, increased levels of oxidative damage and increased lifespan (Liu et al., 2005). Decreasing body temperature has been shown to increase ROS and to extend longevity in mice (Conti et al., 2006; Ristow and Schmeisser, 2011).

Combined, the experiments described above demonstrate that genetic mutations that lead to increased levels of ROS can extend longevity in yeast, worms, flies and mice. Importantly, in most of these experiments the lifespan-extending effect of the mutation was prevented by treatment with antioxidants, thereby indicating that the elevated levels of ROS are required for the observed lifespan extension.

ROS-Mediated Pro-survival Signaling

While the reactive nature of ROS can be perceived as making them dangerous to cells, it is also possible for cells to harness this property to use ROS as an efficient tool for intracellular signaling (de Magalhaes and Church, 2006). ROS have been shown to act as signaling molecules for a number of biological processes (Sena and Chandel, 2012; Reczek and Chandel, 2015; Sies and Jones, 2020), including development (Covarrubias et al., 2008) and survival (Groeger et al., 2009). ROS can oxidize cysteine residues within proteins leading to allosteric changes that alter that protein's function. Those cysteine residues can subsequently be reduced by thioredoxin or glutaredoxin to restore their original function or be further oxidized in a manner that is irreversible. In this way, ROS can activate kinase signaling pathways through inactivation of phosphatases or turn off kinase signaling pathways by inactivating kinases. Similarly, ROS can act directly on effector proteins to mediate their effects, such as inhibiting cell death proteins to promote survival. ROS involved in intracellular signaling can be generated by enzymes such as NADPH oxidases (Lambeth, 2004). In fact, there are over 40 enzymes that generate hydrogen peroxide or superoxide in human cells (Sies and Jones, 2020). In the context of signaling, the ROS generated from the electron transport chain may be a cell's way of communicating the status of the electron transport chain function to the rest of the cell.

The ability for redox-signaling proteins to switch between oxidized and reduced states is essential for many of the signaling pathways mentioned. The redox environment in the cell, as determined by levels of ROS and antioxidants, determines the balance between proteins in reduced and oxidized states, also known as the redox poise of a protein. Maintaining an optimal state of redox poise is important not only for signaling molecules that respond to changes within the redox environment, but also for optimal transfer of electrons in the electron transport chain. This notion that both over-reduction and over-oxidation of cell components can be detrimental to cell function supports the hypothesis that optimal levels of ROS and not simply low levels of ROS are required to maintain homeostasis (Allen, 2004).

The fact that ROS are involved in intracellular signaling and cause oxidative damage highlights the crucial importance of maintaining ROS at an optimal level, and provides a mechanism for how ROS act to extend longevity. There appears to be a "goldilocks" zone in which the beneficial effects of ROS-mediated signaling are maximized while minimizing the amount of oxidative damage caused by ROS (Alleman et al., 2014).

DISCUSSION

The FRTA provides a conceptual framework that is used to think about the relationship between ROS and aging, as well as specific predictions that can be experimentally tested. At its most basic level, the FRTA posits that aging is driven by the accumulation of ROS-induced macromolecular damage. If the FRTA is correct then (1) levels of oxidative damage should be negatively correlated with lifespan, (2) experimental manipulations that decrease oxidative damage should increase longevity, and (3) experimental manipulations that increase oxidative damage should shorten lifespan.

Comparative studies generally suggest that the levels of ROS and oxidative damage are negatively correlated with lifespan (**Table 1** and **Table 3**). While examples that do not follow this trend exist, such as the naked mole-rat, it is unclear if these examples are exceptions to the rule, or evidence that the rule is incorrect. The great strength of comparative studies is that they study a diverse set of species with lifespans ranging from days to hundreds of years. However, care must be taken when interpreting comparative data, as correlation cannot demonstrate causation. Thus, even though oxidative damage increases with age, this does not necessarily mean that it causes aging. It is also possible that different factors cause oxidative damage and aging but that both increase over time, or that the same factor causes both, but does so independently. In order to distinguish between these possibilities, it is necessary to experimentally modulate the proposed causative factor and measure the resulting effect on lifespan.

A number of approaches have been utilized to experimentally modulate the levels of ROS or oxidative damage to determine the effect of the intervention on longevity. In general, interventions aimed at decreasing ROS levels, including overexpression of antioxidant enzymes or treatment with antioxidant compounds, have been shown to increase lifespan, though there are also examples where no effect or a decrease in lifespan is observed (**Table 4**). While theoretically these interventions should decrease oxidative damage, this is not always the case. In some cases, interventions aimed at decreasing ROS resulted in increased oxidative damage and still increased lifespan, thereby indicating

that the underlying mechanism of lifespan extension with interventions that decrease ROS is not necessarily a reduction in oxidative damage. In general, interventions aimed at increasing ROS levels, primarily disruption of genes encoding antioxidant enzymes, have been shown to decrease lifespan (**Table 5**). Nonetheless, there are also examples in which deletion of genes encoding antioxidant enzymes has no effect or results in increased lifespan, despite increasing levels of oxidative damage.

Despite the fact that the bulk of evidence suggests that ROS are detrimental for longevity, a number of experimental studies have demonstrated that ROS can also increase lifespan. This has been demonstrated in yeast, worms, flies and mice and has been shown through both genetic manipulations and compounds that increase ROS levels (**Tables 7** and **8**). These experiments highlight the fact that ROS can be beneficial or detrimental with respect to longevity. The effect of ROS on lifespan is determined by a number of factors including which type of ROS it is (e.g., superoxide and hydrogen peroxide), which subcellular compartment it is in (e.g., mitochondria and cytoplasm), which tissue it is in (e.g., neurons and muscle), when during the life cycle it is increased (e.g., development and adulthood) and how much the ROS levels are increased. The mechanisms by which ROS increase lifespan are likely mediated by their ability to participate in ROS-mediated signaling to activate genetic pathways that increase survival (e.g., Senchuk et al., 2018; Wu et al., 2018).

There are two important caveats for experimental studies. First, genetic studies involving overexpression or deletion of antioxidant genes have only been performed in four well-characterized genetic model organisms: yeast, worms, flies and mice. It is unclear to what extent findings in these four species are reflective of the wide diversity of organisms across the tree of life. The advance of genome sequencing technologies and genetic engineering tools such as CRISPR-Cas9 will make it easier to manipulate gene expression in other organisms, thereby enabling researchers to determine if the results from these four organisms are representative of a wider range of species. Given that different outcomes have already been observed between these four species, it is uncertain if general patterns will emerge when studying a broader range of species.

The second major caveat of experimental studies, both genetic and pharmacological, is the fact that these studies have primarily been performed in short-lived species. In order to test the effect of a gene or compound on the lifespan of an organism, practicalities make it challenging to perform experiments in species that live longer than 5 years, which is the length of a typical grant and roughly the duration of a doctoral program. Thus, one cannot exclude the possibility that ROS affect lifespan differently in short-lived and long-lived species.

Perhaps one of the greatest limitations of both comparative and experimental studies examining the effect of ROS on lifespan is the need to measure ROS. Comparative studies require accurate measurement of ROS and oxidative damage to correlate with longevity. Experimental studies require precise measurement of ROS levels to determine whether the experimental manipulation is having the desired effect. However, the instability and reactivity of ROS make them extremely difficult to quantify. If they are not measured *in vivo,* in real-time, it is unclear whether the ROS levels being measured are truly reflective of the ROS levels in the organisms being tested, since ROS can react rapidly with cellular components, or be detoxified by antioxidants. Unfortunately, current methods to measure ROS are still limited by many confounding variables, such as limited specificity and sensitivity (Starkov, 2010; Deshwal et al., 2018). As experimental studies have demonstrated that the effect of ROS on lifespan is dependent on many factors, current approaches to measure ROS may be too blunt to distinguish between lifespan-extending ROS and lifespan-shortening ROS. This may be especially true with respect to the spatial localization of ROS within a cell. For example, elevated superoxide in the mitochondria can extend longevity through mitochondria-nucleus signaling, while increasing superoxide in other parts of the cell can shorten lifespan (Schaar et al., 2015).

Many of the studies reported here provide a limited view of the redox balance occurring in the organism being studied. Oxidative stress is a balance between ROS production and ROS detoxification by antioxidants, and this balance may change over time. However, most studies only measure one type of ROS, at one time point, in one tissue. A challenge for the field moving forward is to be able to measure and take into account all of these different factors that can influence how ROS affects longevity in a way that is experimentally feasible.

As a final note, it is important to consider the possible effects of observation bias. In cases where an experiment shows no effect on lifespan, it is unclear whether this finding is because the intervention does not affect lifespan, or because the experiment failed to demonstrate an effect of the intervention on lifespan. As a result, experiments in which the outcome shows that the intervention tested does not affect lifespan are difficult to interpret and are more likely not to be reported than experiments showing a positive or negative effect on lifespan.

CONCLUSION

Despite extensive research, the role of ROS in aging remains incompletely understood. While comparative studies have demonstrated a negative relationship between both ROS levels and oxidative damage, and lifespan, these correlations do not necessarily imply causation, and exceptions to these relationships have been observed. In experimental studies, it has generally been observed that manipulations that increase ROS decrease lifespan, while manipulations that decrease ROS increase lifespan. However, there are several examples in which a manipulation's effect on ROS levels or oxidative damage can be experimentally dissociated from its effect on lifespan. In addition, there are examples in which increasing ROS levels extends longevity, and in which decreasing ROS levels shortens lifespan. One of the greatest limitations to our understanding of the role of ROS in aging is our ability to measure ROS. There are many different types of ROS, and the exact tissue, subcellular location, timing and levels of ROS act to determine whether ROS increase or

decrease lifespan. With the currently available tools to measure ROS and the present limitations of comparative and experimental studies, it may not be possible to generalize the relationship between ROS and aging across the tree of life.

AUTHOR CONTRIBUTIONS

JV: conceptualization and supervision. HS and AT: investigation and writing – original draft. HS, AT, and JV: visualization and writing – review and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

Ahmad, T., and Suzuki, Y. J. (2019). Juglone in oxidative stress and cell signaling. *Antioxidants (Basel)* 8:91. doi: 10.3390/antiox8040091

Aldini, G., Altomare, A., Baron, G., Vistoli, G., Carini, M., Borsani, L., et al. (2018). N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic. Res.* 52, 751–762. doi: 10.1080/10715762.2018.1468564

Alleman, R. J., Katunga, L. A., Nelson, M. A., Brown, D. A., and Anderson, E. J. (2014). The "Goldilocks Zone" from a redox perspective-Adaptive vs. deleterious responses to oxidative stress in striated muscle. *Front. Physiol.* 5:358. doi: 10.3389/fphys.2014.00358

Allen, J. F. (2004). Chloroplast redox poise and signaling. *Encyclop. Biol. Chem.* 1, 438–445. doi: 10.1016/b0-12-443710-9/00111-3

Anaissi-Afonso, L., Oramas-Royo, S., Ayra-Plasencia, J., Martín-Rodríguez, P., García-Luis, J., Lorenzo-Castrillejo, I., et al. (2018). Lawsone, Juglone, and β-lapachone derivatives with enhanced mitochondrial-based toxicity. *ACS Chem. Biol.* 13, 1950–1957. doi: 10.1021/acschembio.8b00306

Andziak, B., O'connor, T. P., Qi, W., Dewaal, E. M., Pierce, A., Chaudhuri, A. R., et al. (2006). High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5, 463–471. doi: 10.1111/j.1474-9726.2006.00237.x

Anson, R. M., Croteau, D. L., Stierum, R. H., Filburn, C., Parsell, R., and Bohr, V. A. (1998). Homogenous repair of singlet oxygen-induced DNA damage in differentially transcribed regions and strands of human mitochondrial DNA. *Nucleic Acids Res.* 26, 662–668. doi: 10.1093/nar/26.2.662

Arthur, J. R. (2000). The glutathione peroxidases. *CMLS* 57, 1825–1835.

Austad, S. N. (2018). The comparative biology of mitochondrial function and the rate of aging. *Integr. Comp. Biol.* 58, 559–566. doi: 10.1093/icb/icy068

Ayyadevara, S., Dandapat, A., Singh, S. P., Siegel, E. R., Shmookler Reis, R. J., Zimniak, L., et al. (2007). Life span and stress resistance of *Caenorhabditis elegans* are differentially affected by glutathione transferases metabolizing 4-hydroxynon-2-enal. *Mech. Ageing Dev.* 128, 196–205. doi: 10.1016/j.mad.2006.11.025

Ayyadevara, S., Engle, M. R., Singh, S. P., Dandapat, A., Lichti, C. F., Benes, H., et al. (2005). Lifespan and stress resistance of *Caenorhabditis elegans* are increased by expression of glutathione transferases capable of metabolizing the lipid peroxidation product 4-hydroxynonenal. *Aging Cell* 4, 257–271. doi: 10.1111/j.1474-9726.2005.00168.x

Bahadorani, S., Bahadorani, P., Phillips, J. P., and Hilliker, A. J. (2008). The effects of vitamin supplementation on *Drosophila* life span under normoxia and under oxidative stress. *J. Gerontol. Ser. A* 63, 35–42. doi: 10.1093/gerona/63.1.35

Balaban, R. S., Nemoto, S., and Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell* 120, 483–495. doi: 10.1016/j.cell.2005.02.001

Barja, G. (2000). The flux of free radical attack through mitochondrial DNA is related to aging rate. *Aging Clin. Exp. Res.* 12, 342–355. doi: 10.1007/bf03339859

Barja, G. (2002). Rate of generation of oxidative stress-related damage and animal longevity. *Free Radic. Biol. Med.* 33, 1167–1172. doi: 10.1016/s0891-5849(02)00910-3

Barja, G. (2004). Free radicals and aging. *Trends Neurosci.* 27, 595–600.

Barja, G. (2013). Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. *Antioxid. Redox Signal.* 19, 1420–1445. doi: 10.1089/ars.2012.5148

Barja, G., and Herrero, A. (2000). Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J.* 14, 312–318. doi: 10.1096/fasebj.14.2.312

Battelli, M. G., Polito, L., Bortolotti, M., and Bolognesi, A. (2016). Xanthine oxidoreductase in drug metabolism: beyond a role as a detoxifying enzyme. *Curr. Med. Chem.* 23, 4027–4036. doi: 10.2174/0929867323666160725091915

Baud, O., Greene, A. E., Li, J., Wang, H., Volpe, J. J., and Rosenberg, P. A. (2004). Glutathione peroxidase-catalase cooperativity is required for resistance to hydrogen peroxide by mature rat oligodendrocytes. *J. Neurosci.* 24, 1531–1540. doi: 10.1523/jneurosci.3989-03.2004

Bayne, A. C., Mockett, R. J., Orr, W. C., and Sohal, R. S. (2005). Enhanced catabolism of mitochondrial superoxide/hydrogen peroxide and aging in transgenic *Drosophila*. *Biochem. J.* 391, 277–284. doi: 10.1042/bj20041872

Beckman, K. B., and Ames, B. N. (1998). The free radical theory of aging matures. *Physiol. Rev.* 78, 547–581. doi: 10.1152/physrev.1998.78.2.547

Bejma, J., and Ji, L. L. (1999). Aging and acute exercise enhance free radical generation in rat skeletal muscle. *J. Appl. Physiol. (1985)* 87, 465–470. doi: 10.1152/jappl.1999.87.1.465

Berliner, J. A., and Heinecke, J. W. (1996). The role of oxidized lipoproteins in atherogenesis. *Free Radic. Biol. Med.* 20, 707–727. doi: 10.1016/0891-5849(95)02173-6

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., and Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organ. J.* 5, 9–19.

Bjelakovic, G., Nikolova, D., and Gluud, C. (2013). Antioxidant supplements to prevent mortality. *JAMA* 310, 1178–1179. doi: 10.1001/jama.2013.277028

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., and Gluud, C. (2012). Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst. Rev.* 3, CD007176.

Blier, P. U., Abele, D., Munro, D., Degletagne, C., Rodriguez, E., and Hagen, T. (2017). What modulates animal longevity? Fast and slow aging in bivalves as a model for the study of lifespan. *Semin. Cell Dev. Biol.* 70, 130–140. doi: 10.1016/j.semcdb.2017.07.046

Bokov, A., Chaudhuri, A., and Richardson, A. (2004). The role of oxidative damage and stress in aging. *Mech. Ageing Dev.* 125, 811–826.

Brack, C., Bechter-Thüring, E., and Labuhn, M. (1997). N-acetylcysteine slows down ageing and increases the life span of *Drosophila melanogaster*. *Cell Mol. Life Sci.* 53, 960–966. doi: 10.1007/pl00013199

Brunet-Rossinni, A. K. (2004). Reduced free-radical production and extreme longevity in the little brown bat (*Myotis lucifugus*) versus two non-flying mammals. *Mech. Ageing Dev.* 125, 11–20. doi: 10.1016/j.mad.2003.09.003

Buffenstein, R., Edrey, Y. H., Yang, T., and Mele, J. (2008). The oxidative stress theory of aging: embattled or invincible? Insights from non-traditional model organisms. *Age* 30, 99–109. doi: 10.1007/s11357-008-9058-z

Cabreiro, F., Ackerman, D., Doonan, R., Araiz, C., Back, P., Papp, D., et al. (2011). Increased life span from overexpression of superoxide dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. *Free Radic. Biol. Med.* 51, 1575–1582. doi: 10.1016/j.freeradbiomed.2011.07.020

Cacho-Valadez, B., Munoz-Lobato, F., Pedrajas, J. R., Cabello, J., Fierro-Gonzalez, J. C., Navas, P., et al. (2012). The characterization of the *Caenorhabditis elegans* mitochondrial thioredoxin system uncovers an unexpected protective role of thioredoxin reductase 2 in beta-amyloid peptide toxicity. *Antioxid. Redox Signal.* 16, 1384–1400. doi: 10.1089/ars.2011.4265

Capel, F. R. V., Lioger, D., Diot, A., Rousset, P., Mirand, P. P., Boirie, Y., et al. (2005). Due to reverse electron transfer, mitochondrial H2O2 release increases with age in human vastus lateralis muscle although oxidative capacity is preserved. *Mech. Ageing Dev.* 126, 505–511. doi: 10.1016/j.mad.2004.11.001

Chen, Q., Vazquez, E. J., Moghaddas, S., Hoppel, C. L., and Lesnefsky, E. J. (2003). Production of reactive oxygen species by mitochondria: central role of complex III. *J. Biol. Chem.* 278, 36027–36031.

Chen, Y. I., Wei, P. C., Hsu, J. L., Su, F. Y., and Lee, W. H. (2016). NPGPx (GPx7): a novel oxidative stress sensor/transmitter with multiple roles in redox homeostasis. *Am. J. Transl. Res.* 8, 1626–1640.

Chiu, W. K., Towheed, A., and Palladino, M. J. (2014). Genetically encoded redox sensors. *Methods Enzymol.* 542, 263–287.

Chung, H. Y., Sung, B., Jung, K. J., Zou, Y., and Yu, B. P. (2006). The molecular inflammatory process in aging. *Antioxid. Redox Signal.* 8, 572–581. doi: 10.1089/ars.2006.8.572

Conti, B., Sanchez-Alavez, M., Winsky-Sommerer, R., Morale, M. C., Lucero, J., Brownell, S., et al. (2006). Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314, 825–828. doi: 10.1126/science.1132191

Cooper, J. M., Mann, V. M., and Schapira, A. H. (1992). Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. *J. Neurol. Sci.* 113, 91–98. doi: 10.1016/0022-510x(92)90270-u

Covarrubias, L., Hernandez-Garcia, D., Schnabel, D., Salas-Vidal, E., and Castro-Obregon, S. (2008). Function of reactive oxygen species during animal development: passive or active? *Dev. Biol.* 320, 1–11. doi: 10.1016/j.ydbio.2008.04.041

Cox, C. S., Mckay, S. E., Holmbeck, M. A., Christian, B. E., Scortea, A. C., Tsay, A. J., et al. (2018). Mitohormesis in mice via sustained basal activation of mitochondrial and antioxidant signaling. *Cell Metab.* 28, 776–786e5.

Criddle, D. N., Gillies, S., Baumgartner-Wilson, H. K., Jaffar, M., Chinje, E. C., Passmore, S., et al. (2006). Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. *J. Biol. Chem.* 281, 40485–40492. doi: 10.1074/jbc.m607704200

Cunningham, G. M., Flores, L. C., Roman, M. G., Cheng, C., Dube, S., Allen, C., et al. (2018). Thioredoxin overexpression in both the cytosol and mitochondria accelerates age-related disease and shortens lifespan in male C57BL/6 mice. *GeroScience* 40, 453–468. doi: 10.1007/s11357-018-0039-6

Curtis, C., Landis, G. N., Folk, D., Wehr, N. B., Hoe, N., Waskar, M., et al. (2007). Transcriptional profiling of MnSOD-mediated lifespan extension in *Drosophila* reveals a species-general network of aging and metabolic genes. *Genome Biol.* 8, R262.

Cutler, R. G. (2005). Oxidative stress profiling: part i. its potential importance in the optimization of human health. *Ann. N. Y. Acad. Sci.* 1055, 93–135. doi: 10.1196/annals.1323.027

Dammann, P. (2017). Slow aging in mammals-Lessons from African mole-rats and bats. *Semin. Cell Dev. Biol.* 70, 154–163. doi: 10.1016/j.semcdb.2017.07.006

Davies, J. E., Ellery, P. M., and Hughes, R. E. (1977). Dietary ascorbic acid and life span of guinea-pigs. *Exp. Gerontol.* 12, 215–216. doi: 10.1016/0531-5565(77)90008-0

De Haes, W., Frooninckx, L., Van Assche, R., Smolders, A., Depuydt, G., Billen, J., et al. (2014). Metformin promotes lifespan through mitohormesis via the peroxiredoxin PRDX-2. *Proc. Natl. Acad. Sci. U.S.A.* 111, E2501–E2509.

de Magalhaes, J. P., and Church, G. M. (2006). Cells discover fire: employing reactive oxygen species in development and consequences for aging. *Exp. Gerontol.* 41, 1–10. doi: 10.1016/j.exger.2005.09.002

Deshwal, S., Antonucci, S., Kaludercic, N., and Di Lisa, F. (2018). "Measurement of mitochondrial ROS formation," in *Mitochondrial Bioenergetics: Methods and Protocols*, eds C. M. Palmeira and A. J. Moreno (New York, NY: Springer), 403–418. doi: 10.1007/978-1-4939-7831-1_24

Desjardins, D., Cacho-Valadez, B., Liu, J.-L., Wang, Y., Yee, C., Bernard, K., et al. (2017). Antioxidants reveal an inverted U-shaped dose-response relationship between reactive oxygen species levels and the rate of aging in *Caenorhabditis elegans*. *Aging Cell* 16, 104–112. doi: 10.1111/acel.12528

Di Marzo, N., Chisci, E., and Giovannoni, R. (2018). The role of hydrogen peroxide in redox-dependent signaling: homeostatic and pathological responses in mammalian cells. *Cells* 7, 156. doi: 10.3390/cells7100156

Dikalov, S. I., and Harrison, D. G. (2014). Methods for detection of mitochondrial and cellular reactive oxygen species. *Antioxid. Redox Signal.* 20, 372–382. doi: 10.1089/ars.2012.4886

DiLoreto, R., and Murphy, C. T. (2015). The cell biology of aging. *Mol. Biol. Cell* 26, 4524–4531.

Dixon, D. P., Steel, P. G., and Edwards, R. (2011). Roles for glutathione transferases in antioxidant recycling. *Plant Signal. Behav.* 6, 1223–1227. doi: 10.4161/psb.6.8.16253

Doonan, R., Mcelwee, J. J., Matthijssens, F., Walker, G. A., Houthoofd, K., Back, P., et al. (2008). Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. *Genes Dev.* 22, 3236–3241. doi: 10.1101/gad.504808

Driver, A. S., Kodavanti, P. R., and Mundy, W. R. (2000). Age-related changes in reactive oxygen species production in rat brain homogenates. *Neurotoxicol. Teratol.* 22, 175–181. doi: 10.1016/s0892-0362(99)00069-0

Driver, C., and Georgeou, A. (2003). Variable effects of vitamin E on *Drosophila* longevity. *Biogerontology* 4, 91–95.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.* 82, 47–95. doi: 10.1152/physrev.00018.2001

Dues, D. J., Schaar, C. E., Johnson, B. K., Bowman, M. J., Winn, M. E., Senchuk, M. M., et al. (2017). Uncoupling of oxidative stress resistance and lifespan in long-lived isp-1 mitochondrial mutants in *Caenorhabditis elegans*. *Free Radic. Biol. Med.* 108, 362–373. doi: 10.1016/j.freeradbiomed.2017.04.004

Duttaroy, A., Paul, A., Kundu, M., and Belton, A. (2003). A Sod2 null mutation confers severely reduced adult life span in *Drosophila*. *Genetics* 165, 2295–2299.

Elchuri, S., Oberley, T. D., Qi, W., Eisenstein, R. S., Jackson Roberts, L., Van Remmen, H., et al. (2005). CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* 24, 367–380. doi: 10.1038/sj.onc.1208207

Erard, M., Dupre-Crochet, S., and Nusse, O. (2018). Biosensors for spatiotemporal detection of reactive oxygen species in cells and tissues. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 314, R667–R683.

Ernst, I. M., Pallauf, K., Bendall, J. K., Paulsen, L., Nikolai, S., Huebbe, P., et al. (2013). Vitamin E supplementation and lifespan in model organisms. *Ageing Res. Rev.* 12, 365–375. doi: 10.1016/j.arr.2012.10.002

Esterbauer, H., and Cheeseman, K. H. (1990). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* 186, 407–421. doi: 10.1016/0076-6879(90)86134-h

Ewald, C. Y., Hourihan, J. M., Bland, M. S., Obieglo, C., Katic, I., Moronetti Mazzeo, L. E., et al. (2017). NADPH oxidase-mediated redox signaling promotes oxidative stress resistance and longevity through memo-1 in *C. elegans*. *eLife* 6, e19493.

Fabrizio, P., Liou, L. L., Moy, V. N., Diaspro, A., Valentine, J. S., Gralla, E. B., et al. (2003). SOD2 functions downstream of Sch9 to extend longevity in yeast. *Genetics* 163, 35–46.

Fato, R., Bergamini, C., Bortolus, M., Maniero, A. L., Leoni, S., Ohnishi, T., et al. (2009). Differential effects of mitochondrial complex I inhibitors on production of reactive oxygen species. *Biochim. Biophys. Acta* 1787, 384–392. doi: 10.1016/j.bbabio.2008.11.003

Flohé, L. (1985). The glutathione peroxidase reaction: molecular basis of the antioxidant function of selenium in mammals. *Curr. Top. Cell. Regul.* 27, 473–478. doi: 10.1016/b978-0-12-152827-0.50047-5

Flohé, L. (2010). Changing paradigms in thiology from antioxidant defense toward redox regulation. *Methods Enzymol.* 473, 1–39. doi: 10.1016/s0076-6879(10)73001-9

Flores, L. C., Roman, M. G., Cunningham, G. M., Cheng, C., Dube, S., Allen, C., et al. (2018). Continuous overexpression of thioredoxin 1 enhances cancer development and does not extend maximum lifespan in male C57BL/6 mice. *Pathobiol. Aging Age Relat. Dis.* 8, 1533754. doi: 10.1080/20010001.2018.1533754

Flurkey, K., Astle, C. M., and Harrison, D. E. (2010). Life extension by diet restriction and N-Acetyl-L-Cysteine in genetically heterogeneous mice. *J. Gerontol. Ser. A* 65A, 1275–1284. doi: 10.1093/gerona/glq155

Forman, H. J., and Azzi, A. (1997). On the virtual existence of superoxide anions in mitochondria: thoughts regarding its role in pathophysiology. *FASEB J.* 11, 374–375. doi: 10.1096/fasebj.11.5.9141504

Forman, H. J., Ursini, F., and Maiorino, M. (2014). An overview of mechanisms of redox signaling. *J. Mol. Cell Cardiol.* 73, 2–9.

Forsberg, L., De Faire, U., and Morgenstern, R. (2001). Oxidative stress, human genetic variation, and disease. *Arch. Biochem. Biophys.* 389, 84–93. doi: 10.1006/abbi.2001.2295

Fraga, C. G., Shigenaga, M. K., Park, J. W., Degan, P., and Ames, B. N. (1990). Oxidative Damage to DNA During Aging: 8-hydroxy-2'-deoxyguanosine in Rat Organ DNA and Urine. *Proc. Natl. Acad. Sci. U.S.A.* 87, 4533–4537. doi: 10.1073/pnas.87.12.4533

Frei, B. (1994). Reactive oxygen species and antioxidant vitamins: mechanisms of action. *Am. J. Med.* 97, 5S–13S.

Friguet, B. (2006). Oxidized protein degradation and repair in ageing and oxidative stress. *FEBS Lett.* 580, 2910–2916. doi: 10.1016/j.febslet.2006.03.028

Fukai, T., and Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid. Redox Signal.* 15, 1583–1606. doi: 10.1089/ars.2011.3999

Fukushima, T., Tanaka, K., Lim, H., and Moriyama, M. (2002). Mechanism of cytotoxicity of paraquat. *Environ. Health Prev. Med.* 7, 89–94. doi: 10.1265/ehpm.2002.89

Genova, M. L., Pich, M. M., Bernacchia, A., Bianchi, C., Biondi, A., Bovina, C., et al. (2004). The mitochondrial production of reactive oxygen species in relation to aging and pathology. *Ann. N. Y. Acad. Sci.* 1011, 86–100. doi: 10.1007/978-3-662-41088-2_10

Gouspillou, G., Bourdel-Marchasson, I., Rouland, R., Calmettes, G., Biran, M., Deschodt-Arsac, V., et al. (2014). Mitochondrial energetics is impaired in vivo in aged skeletal muscle. *Aging Cell* 13, 39–48.

Green, D. R., Galluzzi, L., and Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 333, 1109–1112. doi: 10.1126/science.1201940

Griendling, K. K., Touyz, R. M., Zweier, J. L., Dikalov, S., Chilian, W., Chen, Y. R., et al. (2016). Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the american heart association. *Circ. Res.* 119, e39–e75.

Groeger, G., Quiney, C., and Cotter, T. G. (2009). Hydrogen peroxide as a cell-survival signaling molecule. *Antioxid. Redox Signal.* 11, 2655–2671. doi: 10.1089/ars.2009.2728

Ha, M. K., Soo Cho, J., Baik, O. R., Lee, K. H., Koo, H. S., and Chung, K. Y. (2006). Caenorhabditis elegans as a screening tool for the endothelial cell-derived putative aging-related proteins detected by proteomic analysis. *Proteomics* 6, 3339–3351. doi: 10.1002/pmic.200500395

Halliwell, B. (2009). The wanderings of a free radical. *Free Radic. Biol. Med.* 46, 531–542. doi: 10.1016/j.freeradbiomed.2008.11.008

Hamilton, M. L., Van Remmen, H., Drake, J. A., Yang, H., Guo, Z. M., Kewitt, K., et al. (2001). Does oxidative damage to dna increase with age? *Proc. Natl. Acad. Sci. U.S.A.* 98, 10469–10474. doi: 10.1073/pnas.171202698

Hanschmann, E. M., Godoy, J. R., Berndt, C., Hudemann, C., and Lillig, C. H. (2013). Thioredoxins, glutaredoxins, and peroxiredoxins–molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antioxid. Redox Signal.* 19, 1539–1605. doi: 10.1089/ars.2012.4599

Hanzen, S., Vielfort, K., Yang, J., Roger, F., Andersson, V., Zamarbide-Fores, S., et al. (2016). Lifespan control by redox-dependent recruitment of chaperones to misfolded proteins. *Cell* 166, 140–151. doi: 10.1016/j.cell.2016.05.006

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300. doi: 10.1093/geronj/11.3.298

Harman, D. (1972). The biological clock: the mitochondria? *J. Am. Geriatr. Soc.* 20, 145–147.

Harrington, L. A., and Harley, C. B. (1988). Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. *Mech. Ageing Dev.* 43, 71–78. doi: 10.1016/0047-6374(88)90098-x

Harris, N., Bachler, M., Costa, V., Mollapour, M., Moradas-Ferreira, P., and Piper, P. W. (2005). Overexpressed Sod1p acts either to reduce or to increase the lifespans and stress resistance of yeast, depending on whether it is Cu2+-deficient or an active Cu,Zn-superoxide dismutase. *Aging Cell* 4, 41–52. doi: 10.1111/j.1474-9726.2005.00142.x

Heidler, T., Hartwig, K., Daniel, H., and Wenzel, U. (2010). Caenorhabditis elegans lifespan extension caused by treatment with an orally active ROS-generator is dependent on DAF-16 and SIR-2.1. *Biogerontology* 11, 183–195. doi: 10.1007/s10522-009-9239-x

Hekimi, S., Lapointe, J., and Wen, Y. (2011). Taking a "good" look at free radicals in the aging process. *Trends Cell Biol.* 21, 569–576. doi: 10.1016/j.tcb.2011.06.008

Hourihan, J. M., Moronetti Mazzeo, L. E., Fernandez-Cardenas, L. P., and Blackwell, T. K. (2016). Cysteine sulfenylation directs IRE-1 to activate the SKN-1/Nrf2 antioxidant response. *Mol. Cell* 63, 553–566. doi: 10.1016/j.molcel.2016.07.019

Hua, X., Cowman, P., Warren, D., and Bromham, L. (2015). Longevity is linked to mitochondrial mutation rates in rockfish: a test using poisson regression. *Mol. Biol. Evol.* 32, 2633–2645. doi: 10.1093/molbev/msv137

Huang, T.-T., Carlson, E. J., Gillespie, A. M., Shi, Y., and Epstein, C. J. (2000). Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. *J. Gerontol. Biol. Sci. Med. Sci.* 55, B5.

Hulbert, A. J. (2005). On the importance of fatty acid composition of membranes for aging. *J. Theor. Biol.* 234, 277–288. doi: 10.1016/j.jtbi.2004.11.024

Hunt, P. R., Son, T. G., Wilson, M. A., Yu, Q. S., Wood, W. H., Zhang, Y., et al. (2011). Extension of lifespan in *C. elegans* by naphthoquinones that act through stress hormesis mechanisms. *PLoS One* 6:e21922. doi: 10.1371/journal.pone.0021922

Ighodaro, O. M., and Akinloye, O. A. (2019). First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J. Med.* 54, 287–293. doi: 10.1016/j.ajme.2017.09.001

Ishii, N., Senoo-Matsuda, N., Miyake, K., Yasuda, K., Ishii, T., Hartman, P. S., et al. (2004). Coenzyme Q10 can prolong C. elegans lifespan by lowering oxidative stress. *Mech. Ageing Dev.* 125, 41–46. doi: 10.1016/j.mad.2003.10.002

Jee, C., Vanoaica, L., Lee, J., Park, B. J., and Ahnn, J. (2005). Thioredoxin is related to life span regulation and oxidative stress response in *Caenorhabditis elegans*. *Genes Cells* 10, 1203–1210. doi: 10.1111/j.1365-2443.2005.00913.x

Jha, R., and Rizvi, S. I. (2011). Carbonyl formation in erythrocyte membrane proteins during aging in humans. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* 155, 39–42. doi: 10.5507/bp.2011.013

Kaludercic, N., Deshwal, S., and Di Lisa, F. (2014). Reactive oxygen species and redox compartmentalization. *Front. Physiol.* 5:285. doi: 10.3389/fphys.2014.00285

Karalija, A., Novikova, L. N., Kingham, P. J., Wiberg, M., and Novikov, L. N. (2012). Neuroprotective effects of N-Acetyl-Cysteine and Acetyl-L-Carnitine after spinal cord injury in adult rats. *PLoS One* 7:e41086. doi: 10.1371/journal.pone.0041086

Katerji, M., Filippova, M., and Duerksen-Hughes, P. (2019). Approaches and methods to measure oxidative stress in clinical samples: research applications in the cancer field. *Oxid. Med. Cell Longev.* 2019, 1279250.

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., et al. (2014). Aging: a common driver of chronic diseases and a target for novel interventions. *Cell* 159, 709–713.

Kimura, Y., Goto, Y., and Kimura, H. (2010). Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid. Redox Signal.* 12, 1–13. doi: 10.1089/ars.2008.2282

Klichko, V. I., Orr, W. C., and Radyuk, S. N. (2016). The role of peroxiredoxin 4 in inflammatory response and aging. *Biochim. Biophys. Acta* 1862, 265–273. doi: 10.1016/j.bbadis.2015.12.008

Kowald, A., and Kirkwood, T. B. L. (2018). Resolving the enigma of the clonal expansion of mtDNA deletions. *Genes (Basel)* 9, 126. doi: 10.3390/genes9030126

Kregel, K. C., and Zhang, H. J. (2007). An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am. J. Physiol.* 292, R18–R36.

Ku, H.-H., Brunk, U. T., and Sohal, R. S. (1993). Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. *Free Radic. Biol. Med.* 15, 621–627. doi: 10.1016/0891-5849(93)90165-q

Ku, H. H., and Sohal, R. S. (1993). Comparison of mitochondrial pro-oxidant generation and anti-oxidant defenses between rat and pigeon: possible basis of variation in longevity and metabolic potential. *Mech. Ageing Dev.* 72, 67–76. doi: 10.1016/0047-6374(93)90132-b

Kushnareva, Y., Murphy, A. N., and Andreyev, A. (2002). Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and NAD(P)+ oxidation-reduction state. *Biochem. J.* 368(Pt 2), 545–553. doi: 10.1042/bj20021121

Lam, Y. T., Stocker, R., and Dawes, I. W. (2010). The lipophilic antioxidants α-tocopherol and coenzyme Q10 reduce the replicative lifespan of Saccharomyces cerevisiae. *Free Radic. Biol. Med.* 49, 237–244. doi: 10.1016/j.freeradbiomed.2010.04.008

Lambert, A. J., Boysen, H. M., Buckingham, J. A., Yang, T., Podlutsky, A., Austad, S. N., et al. (2007). Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell* 6, 607–618. doi: 10.1111/j.1474-9726.2007.00312.x

Lambeth, J. D. (2004). NOX enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol.* 4, 181–189. doi: 10.1038/nri1312

Lambeth, L. D. (2020). NOX enzymes and the biology of reactive oxygen. *Nat. Rev. Immunol.* 4, 181–189.

Lapointe, J., and Hekimi, S. (2010). When a theory of aging ages badly. *Cell Mol. Life Sci.* 67, 1–8. doi: 10.1007/s00018-009-0138-8

Laschober, G. T., Ruli, D., Hofer, E., Muck, C., Carmona-Gutierrez, D., Ring, J., et al. (2010). Identification of evolutionarily conserved genetic regulators of cellular aging. *Aging Cell* 9, 1084–1097. doi: 10.1111/j.1474-9726.2010.00637.x

Lassègue, B., and Griendling, K. K. (2004). Reactive oxygen species in hypertension; an update. *Am. J. Hypertens.* 17, 852–860. doi: 10.1016/j.amjhyper.2004.02.004

Lebovitz, R. M., Zhang, H., Vogel, H., Cartwright, J. Jr., Dionne, L., Lu, N., et al. (1996). Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 93, 9782–9787. doi: 10.1073/pnas.93.18.9782

Lee, K. S., Iijima-Ando, K., Iijima, K., Lee, W. J., Lee, J. H., Yu, K., et al. (2009). JNK/FOXO-mediated neuronal expression of fly homologue of peroxiredoxin II reduces oxidative stress and extends life span. *J. Biol. Chem.* 284, 29454–29461. doi: 10.1074/jbc.m109.028027

Lee, S., Kim, S. M., and Lee, R. T. (2013). Thioredoxin and thioredoxin target proteins: from molecular mechanisms to functional significance. *Antioxid. Redox Signal.* 18, 1165–1207. doi: 10.1089/ars.2011.4322

Leiers, B., Kampkötter, A., Grevelding, C. G., Link, C. D., Johnson, T. E., and Henkle-Dührsen, K. (2003). A stress-responsive glutathione S-transferase confers resistance to oxidative stress in *Caenorhabditis elegans*. *Free Radic. Biol. Med.* 34, 1405–1415. doi: 10.1016/s0891-5849(03)00102-3

Lemasters, J. J. (2005). Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res.* 8, 3–5. doi: 10.1089/rej.2005.8.3

Li, F., Ma, X., Cui, X., Li, J., and Wang, Z. (2018). Recombinant buckwheat glutaredoxin intake increases lifespan and stress resistance via hsf-1 upregulation in *Caenorhabditis elegans*. *Exp. Gerontol.* 104, 86–97. doi: 10.1016/j.exger.2018.01.028

Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., Melendez, J. A., et al. (2003). Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J. Biol. Chem.* 278, 8516–8525. doi: 10.1074/jbc.m210432200

Li, W., Bandyopadhyay, J., Hwaang, H. S., Park, B.-J., Cho, J. H., Lee, J. I., et al. (2012). Two thioredoxin reductases, trxr-1 and trxr-2, have differential physiological roles in *Caenorhabditis elegans*. *Mol. Cells* 34, 209–218. doi: 10.1007/s10059-012-0155-6

Li, Y., Huang, T. T., Carlson, E. J., Melov, S., Ursell, P. C., Olson, J. L., et al. (1995). Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11, 376–381. doi: 10.1038/ng1295-376

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., et al. (2018). Oxidative stress, aging, and diseases. *Clin. Interv. Aging* 13, 757–772.

Lionaki, E., and Tavernarakis, N. (2013). Oxidative stress and mitochondrial protein quality control in aging. *J. Proteom.* 92, 181–194. doi: 10.1016/j.jprot.2013.03.022

Lipman, R., Bronson, R., Wu, D., Smith, D., Prior, R., Cao, G., et al. (1998). Disease incidence and longevity are unaltered by dietary antioxidant supplementation initiated during middle age in C57BL/6 mice. *Mech. Ageing Dev.* 103, 269–284. doi: 10.1016/s0047-6374(98)00048-7

Lismont, C., Nordgren, M., Van Veldhoven, P. P., and Fransen, M. (2015). Redox interplay between mitochondria and peroxisomes. *Front. Cell Dev. Biol.* 3:35. doi: 10.3389/fcell.2015.00035

Liu, X., Jiang, N., Hughes, B., Bigras, E., Shoubridge, E., and Hekimi, S. (2005). Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice. *Genes Dev.* 19, 2424–2434. doi: 10.1101/gad.1352905

Liu, Y., Yang, F., Li, S., Dai, J., and Deng, H. (2018). Glutaredoxin deletion shortens chronological life span in *Saccharomyces cerevisiae* via ROS-mediated Ras/PKA activation. *J. Proteome Res.* 17, 2318–2327. doi: 10.1021/acs.jproteome.8b00012

Loor, G., Kondapalli, J., Schriewer, J. M., Chandel, N. S., Vanden Hoek, T. L., and Schumacker, P. T. (2010). Menadione triggers cell death through ROS-dependent mechanisms involving PARP activation without requiring apoptosis. *Free Radic. Biol. Med.* 49, 1925–1936. doi: 10.1016/j.freeradbiomed.2010.09.021

Lopez-Torres, M., Perez-Campo, R., Rojas, C., Cadenas, S., and Barja, G. (1993). Maximum life span in vertebrates: relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, in vivo H2O2, and basal and maximum aerobic capacity. *Mech. Ageing Dev.* 70, 177–199. doi: 10.1016/0047-6374(93)90047-u

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annu. Rev. Pharmacol. Toxicol.* 53, 401–426. doi: 10.1146/annurev-pharmtox-011112-140320

Managbanag, J. R., Witten, T. M., Bonchev, D., Fox, L. A., Tsuchiya, M., Kennedy, B. K., et al. (2008). Shortest-path network analysis is a useful approach toward identifying genetic determinants of longevity. *PLoS One* 3:e3802. doi: 10.1371/journal.pone.0003802

Massie, H. R., Aiello, V. R., and Doherty, T. J. (1984). Dietary vitamin C improves the survival of mice. *Gerontology* 30, 371–375. doi: 10.1159/000212659

Massudi, H., Grant, R., Braidy, N., Guest, J., Farnsworth, B., and Guillemin, G. J. (2012). Age-associated changes in oxidative stress and NAD+ metabolism in human tissue. *PLoS One* 7:e42357. doi: 10.1371/journal.pone.0042357

Matés, J. M., Pérez-Gómez, C., and Núñez De Castro, I. (1999). Antioxidant enzymes and human diseases. *Clin. Biochem.* 32, 595–603. doi: 10.1016/s0009-9120(99)00075-2

McCord, J. M. (2000). The evolution of free radicals and oxidative stress. *Am. J. Med.* 108, 652–659.

McCord, J. M., and Fridovich, I. (1969). Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244, 6049–6055.

McDonnell, A. M., and Dang, C. H. (2013). Basic review of the cytochrome P450 system. *J. Adv. Pract. Oncol.* 4, 263–268.

Melov, S., Ravenscroft, J., Malik, S., Gill, M. S., Walker, D. W., Clayton, P. E., et al. (2000). Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 289, 1567–1569. doi: 10.1126/science.289.5484.1567

Mesquita, A., Weinberger, M., Silva, A., Sampaio-Marques, B., Almeida, B., Leão, C., et al. (2010). Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H_2O_2 and superoxide dismutase activity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15123–15128. doi: 10.1073/pnas.1004432107

Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., and Marcos, J. C. (2014). Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. *Anal. Biochem.* 458, 69–71. doi: 10.1016/j.ab.2014.04.034

Miller, D. L., and Roth, M. B. (2007). Hydrogen sulfide increases thermotolerance and lifespan in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20618–20622. doi: 10.1073/pnas.0710191104

Miranda-Vizuete, A., Fierro Gonzalez, J. C., Gahmon, G., Burghoorn, J., Navas, P., and Swoboda, P. (2006). Lifespan decrease in a *Caenorhabditis elegans* mutant lacking TRX-1, a thioredoxin expressed in ASJ sensory neurons. *FEBS Lett.* 580, 484–490. doi: 10.1016/j.febslet.2005.12.046

Missirlis, F., Phillips, J. P., and Jäckle, H. (2001). Cooperative action of antioxidant defense systems in *Drosophila*. *Curr. Biol.* 11, 1272–1277. doi: 10.1016/s0960-9822(01)00393-1

Mitsui, A., Hamuro, J., Nakamura, H., Kondo, N., Hirabayashi, Y., Ishizaki-Koizumi, S., et al. (2002). Overexpression of human thioredoxin in transgenic mice controls oxidative stress and life span. *Antioxid. Redox Signal.* 4, 693–696. doi: 10.1089/15230860260220201

Moghadam, N. N., Holmstrup, M., Manenti, T., and Loeschcke, V. (2015). Phospholipid fatty acid composition linking larval-density to lifespan of adult *Drosophila melanogaster*. *Exp. Gerontol.* 72, 177–183. doi: 10.1016/j.exger.2015.10.007

Molin, M., Yang, J., Hanzen, S., Toledano, M. B., Labarre, J., and Nystrom, T. (2011). Life span extension and H(2)O(2) resistance elicited by caloric restriction require the peroxiredoxin Tsa1 in *Saccharomyces cerevisiae*. *Mol. Cell* 43, 823–833. doi: 10.1016/j.molcel.2011.07.027

Morley, A. A., and Trainor, K. J. (2001). Lack of an effect of vitamin E on lifespan of mice. *Biogerontology* 2, 109–112.

Moskalev, A. A., Shaposhnikov, M. V., Plyusnina, E. N., Zhavoronkov, A., Budovsky, A., Yanai, H., et al. (2013). The role of DNA damage and repair in aging through the prism of Koch-like criteria. *Ageing Res. Rev.* 12, 661–684. doi: 10.1016/j.arr.2012.02.001

Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S., and Stadtman, E. R. (2001). Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc. Natl. Acad. Sci. U.S.A.* 98, 12920–12925. doi: 10.1073/pnas.231472998

Moskovitz, J., and Oien, D. B. (2010). Protein carbonyl and the methionine sulfoxide reductase system. *Antioxid. Redox Signal.* 12, 405–415. doi: 10.1089/ars.2009.2809

Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., and Van Remmen, H. (2007). Trends in oxidative aging theories. *Free Radic. Biol. Med.* 43, 477–503. doi: 10.1016/j.freeradbiomed.2007.03.034

Munro, D., Baldy, C., Pamenter, M. E., and Treberg, J. R. (2019). The exceptional longevity of the naked mole-rat may be explained by mitochondrial antioxidant defenses. *Aging Cell* 18, e12916. doi: 10.1111/acel.12916

Mylonas, C., and Kouretas, D. (1999). Lipid peroxidation and tissue damage. *In Vivo* 13, 295–309.

Navarro, A., and Boveris, A. (2004). Rat brain and liver mitochondria develop oxidative stress and lose enzymatic activities on aging. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R1244–R1249.

Navarro, A., Gómez, C., Sánchez-Pino, M.-J., González, H., Bández, M. J., Boveris, A. D., et al. (2005). Vitamin E at high doses improves survival, neurological performance, and brain mitochondrial function in aging male mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R1392–R1399.

Neumann, C. A., Krause, D. S., Carman, C. V., Das, S., Dubey, D. P., Abraham, J. L., et al. (2003). Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 424, 561–565. doi: 10.1038/nature01819

Nguyen, G. T., Green, E. R., and Mecsas, J. (2017). Neutrophils to the ROScue: mechanisms of NADPH oxidase activation and bacterial resistance. *Front. Cell Infect. Microbiol.* 7:373. doi: 10.3389/fcimb.2017.00373

Niraula, P., and Kim, M. S. (2019). N-Acetylcysteine extends lifespan of *Drosophila* via modulating ROS scavenger gene expression. *Biogerontology* 20, 533–543. doi: 10.1007/s10522-019-09815-4

Oberacker, T., Bajorat, J., Ziola, S., Schroeder, A., Röth, D., Kastl, L., et al. (2018). Enhanced expression of thioredoxin-interacting-protein regulates oxidative DNA damage and aging. *FEBS Lett.* 592, 2297–2307. doi: 10.1002/1873-3468.13156

Oberley, T. D. (2002). Oxidative damage and cancer. *Am. J. Pathol.* 160, 403–408.

Ock, C. Y., Kim, E. H., Choi, D. J., Lee, H. J., Hahm, K. B., and Chung, M. H. (2012). 8-Hydroxydeoxyguanosine: not mere biomarker for oxidative stress, but remedy for oxidative stress-implicated gastrointestinal diseases. *World J. Gastroenterol.* 18, 302–308. doi: 10.3748/wjg.v18.i4.302

Oh, S.-I., Park, J.-K., and Park, S.-K. (2015). Lifespan extension and increased resistance to environmental stressors by N-Acetyl-L-Cysteine in *Caenorhabditis elegans*. *Clinics* 70, 380–386. doi: 10.6061/clinics/2015(05)13

Oláhová, M., Taylor, S. R., Khazaipoul, S., Wang, J., Morgan, B. A., Matsumoto, K., et al. (2008). A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19839–19844. doi: 10.1073/pnas.0805507105

Oliver, C. N., Ahn, B. W., Moerman, E. J., Goldstein, S., and Stadtman, E. R. (1987). Age-related changes in oxidized proteins. *J. Biol. Chem.* 262, 5488–5491. doi: 10.1016/s0021-9258(18)45598-6

Orr, W. C., Arnold, L. A., and Sohal, R. S. (1992). Relationship between catalase activity, life span and some parameters associated with antioxidant defenses in *Drosophila melanogaster*. *Mech. Ageing Dev.* 63, 287–296. doi: 10.1016/0047-6374(92)90006-y

Orr, W. C., Mockett, R. J., Benes, J. J., and Sohal, R. S. (2003). Effects of overexpression of copper-zinc and manganese superoxide dismutases, catalase, and thioredoxin reductase genes on longevity in *Drosophila melanogaster*. *J. Biol. Chem.* 278, 26418–26422.

Orr, W. C., Radyuk, S. N., and Sohal, R. S. (2013). Involvement of redox state in the aging of *Drosophila melanogaster*. *Antioxid. Redox Signal.* 19, 788–803. doi: 10.1089/ars.2012.5002

Orr, W. C., and Sohal, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263, 1128–1130. doi: 10.1126/science.8108730

Owsiak, A., Bartosz, G., and Bilinski, T. (2010). Oxidative stress during aging of the yeast in a stationary culture and its attenuation by antioxidants. *Cell Biol. Int.* 34, 731–736. doi: 10.1042/cbi20100134

Owusu-Ansah, E., Song, W., and Perrimon, N. (2013). Muscle mitohormesis promotes longevity via systemic repression of insulin signaling. *Cell* 155, 699–712. doi: 10.1016/j.cell.2013.09.021

Pader, I., Sengupta, R., Cebula, M., Xu, J., Lundberg, J. O., Holmgren, A., et al. (2014). Thioredoxin-related protein of 14 kDa is an efficient L-cystine reductase and S-denitrosylase. *Proc. Natl. Acad. Sci. U.S.A.* 111, 6964–6969. doi: 10.1073/pnas.1317320111

Page, M. M., Richardson, J., Wiens, B. E., Tiedtke, E., Peters, C. W., Faure, P. A., et al. (2010). Antioxidant enzyme activities are not broadly correlated with longevity in 14 vertebrate endotherm species. *Age* 32, 255–270. doi: 10.1007/s11357-010-9131-2

Pamplona, R., and Barja, G. (2007). Highly resistant macromolecular components and low rate of generation of endogenous damage: two key traits of longevity. *Ageing Res. Rev.* 6, 189–210. doi: 10.1016/j.arr.2007.06.002

Pamplona, R., Barja, G., and Portero−Otín, M. (2002). Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous−longevity adaptation? *Ann. N. Y. Acad. Sci.* 959, 475–490. doi: 10.1111/j.1749-6632.2002.tb02118.x

Pamplona, R., Portero-Otín, M., Riba, D., Ledo, F., Gredilla, R., Herrero, A., et al. (1999). Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the canary and the parakeet than in the mouse. *Aging Clin. Exp. Res.* 11, 44–49. doi: 10.1007/bf03399636

Pamplona, R., Prat, J., Cadenas, S., Rojas, C., Pérez-Campo, R., López Torres, M., et al. (1996). Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and human case. *Mech. Ageing Dev.* 86, 53–66. doi: 10.1016/0047-6374(95)01673-2

Pan, Y., Schroeder, E. A., Ocampo, A., Barrientos, A., and Shadel, G. S. (2011). Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. *Cell Metab.* 13, 668–678. doi: 10.1016/j.cmet.2011.03.018

Park, C. B., and Larsson, N. G. (2011). Mitochondrial DNA mutations in disease and aging. *J. Cell Biol.* 193, 809–818.

Partridge, L., and Gems, D. (2002). Mechanisms of ageing: public or private? *Nat. Rev. Genet.* 3, 165–175.

Pavelescu, L. (2015). On reactive oxygen species measurement in living systems. *J. Med. Life* 8, 38–42.

Pérez, V. I., Cortez, L. A., Lew, C. M., Rodriguez, M., Webb, C. R., Van Remmen, H., et al. (2011). Thioredoxin 1 overexpression extends mainly the earlier part of life span in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 66, 1286–1299. doi: 10.1093/gerona/glr125

Pérez, V. I., Lew, C. M., Cortez, L. A., Webb, C. R., Rodriguez, M., Liu, Y., et al. (2008). Thioredoxin 2 haploinsufficiency in mice results in impaired mitochondrial function and increased oxidative stress. *Free Radic. Biol. Med.* 44, 882–892. doi: 10.1016/j.freeradbiomed.2007.11.018

Pérez, V. I., Van Remmen, H., Bokov, A., Epstein, C. J., Vijg, J., and Richardson, A. (2009). The overexpression of major antioxidant enzymes does not extend the lifespan of mice. *Aging Cell* 8, 73–75. doi: 10.1111/j.1474-9726.2008.00449.x

Perez-Campo, R., Lopez-Torres, M., Cadenas, S., Rojas, C., and Barja, G. (1998). The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J. Comp. Physiol. B* 168, 149–158. doi: 10.1007/s003600050131

Pérez-Campo, R., López-Torres, M., Rojas, C., Cadenas, S., and Barja, G. (1994). Longevity and antioxidant enzymes, non-enzymatic antioxidants and oxidative stress in the vertebrate lung: a comparative study. *J. Comp. Physiol. B* 163, 682–689. doi: 10.1007/bf00369520

Perez-Estrada, J. R., Hernandez-Garcia, D., Leyva-Castro, F., Ramos-Leon, J., Cuevas-Benitez, O., Diaz-Munoz, M., et al. (2019). Reduced lifespan of mice lacking catalase correlates with altered lipid metabolism without oxidative damage or premature aging. *Free Radic. Biol. Med.* 135, 102–115. doi: 10.1016/j.freeradbiomed.2019.02.016

Perkins, A., Nelson, K. J., Parsonage, D., Poole, L. B., and Karplus, P. A. (2015). Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. *Trends Biochem. Sci.* 40, 435–445. doi: 10.1016/j.tibs.2015.05.001

Petriv, O. I., and Rachubinski, R. A. (2004). Lack of peroxisomal catalase causes a progeric phenotype in *Caenorhabditis elegans*. *J. Biol. Chem.* 279, 19996–20001. doi: 10.1074/jbc.m400207200

Pham-Huy, L. A., He, H., and Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* 4, 89–96.

Phaniendra, A., Jestadi, D. B., and Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J. Clin. Biochem.* 30, 11–26. doi: 10.1007/s12291-014-0446-0

Phillips, J. P., Campbell, S. D., Michaud, D., Charbonneau, M., and Hilliker, A. J. (1989). Null mutation of copper/zinc superoxide dismutase in *Drosophila* confers hypersensitivity to paraquat and reduced longevity. *Proc. Natl. Acad. Sci. U.S.A.* 86, 2761–2765. doi: 10.1073/pnas.86.8.2761

Picazo, C., Matallana, E., and Aranda, A. (2018). Yeast thioredoxin reductase Trr1p controls TORC1-regulated processes. *Sci. Rep.* 8, 16500.

Pickering, A. M., Lehr, M., Gendron, C. M., Pletcher, S. D., and Miller, R. A. (2017). Mitochondrial thioredoxin reductase 2 is elevated in long-lived primate as well as rodent species and extends fly mean lifespan. *Aging Cell* 16, 683–692. doi: 10.1111/acel.12596

Portero-Otín, M., Josep Bellumunt, M., Cristina Ruiz, M., Barja, G., and Pamplona, R. (2001). Correlation of fatty acid unsaturation of the major liver mitochondrial phospholipid classes in mammals to their maximum life span potential. *Lipids* 36, 491–498. doi: 10.1007/s11745-001-0748-y

Radyuk, S. N., Michalak, K., Klichko, V. I., Benes, J., Rebrin, I., Sohal, R. S., et al. (2009). Peroxiredoxin 5 confers protection against oxidative stress and apoptosis and also promotes longevity in *Drosophila*. *Biochem. J.* 419, 437–445. doi: 10.1042/bj20082003

Ran, Q., Liang, H., Ikeno, Y., Qi, W., Prolla, T. A., Roberts, L. J., et al. (2007). Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. *J. Gerontol. Ser. A* 62, 932–942. doi: 10.1093/gerona/62.9.932

Rani, V., Neumann, C. A., Shao, C., and Tischfield, J. A. (2012). Prdx1 deficiency in mice promotes tissue specific loss of heterozygosity mediated by deficiency in DNA repair and increased oxidative stress. *Mutat. Res.* 735, 39–45. doi: 10.1016/j.mrfmmm.2012.04.004

Ray, P. D., Huang, B. W., and Tsuji, Y. (2012). Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal.* 24, 981–990. doi: 10.1016/j.cellsig.2012.01.008

Reczek, C. R., and Chandel, N. S. (2015). ROS-dependent signal transduction. *Curr. Opin. Cell Biol.* 33, 8–13. doi: 10.1016/j.ceb.2014.09.010

Rhee, S. G. (2016). Overview on Peroxiredoxin. *Mol Cells* 39, 1–5. doi: 10.14348/molcells.2016.2368

Richter, C., Park, J. W., and Ames, B. N. (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc. Natl. Acad. Sci. U.S.A.* 85, 6465–6467. doi: 10.1073/pnas.85.17.6465

Ristow, M., and Schmeisser, S. (2011). Extending life span by increasing oxidative stress. *Free Radic. Biol. Med.* 51, 327–336. doi: 10.1016/j.freeradbiomed.2011.05.010

Rockstein, M., and Brandt, K. F. (1963). Enzyme changes in flight muscle correlated with aging and flight ability in the male housefly. *Science* 139, 1049–1051. doi: 10.1126/science.139.3559.1049

Roger, F., Picazo, C., Reiter, W., Libiad, M., Asami, C., Hanzén, S., et al. (2020). Peroxiredoxin promotes longevity and H(2)O(2)-resistance in yeast through redox-modulation of protein kinase A. *eLife* 9, e60346.

Sadowska-Bartosz, I., and Bartosz, G. (2014). Effect of antioxidants supplementation on aging and longevity. *Biomed. Res. Int.* 2014, 404680.

Sakamoto, T., Maebayashi, K., Nakagawa, Y., and Imai, H. (2014). Deletion of the four phospholipid hydroperoxide glutathione peroxidase genes accelerates aging in *Caenorhabditis elegans*. *Genes Cells* 19, 778–792. doi: 10.1111/gtc.12175

Salamon, S., Kramar, B., Marolt, T. P., Poljšak, B., and Milisav, I. (2019). Medical and dietary uses of N-Acetylcysteine. *Antioxidants (Basel)* 8, 111. doi: 10.3390/antiox8050111

Saldmann, F., Viltard, M., Leroy, C., and Friedlander, G. (2019). The naked mole rat: a unique example of positive oxidative stress. *Oxid. Med. Cell Longev.* 2019, 4502819.

Salmon, A. B., Leonard, S., Masamsetti, V., Pierce, A., Podlutsky, A. J., Podlutskaya, N., et al. (2009). The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. *FASEB J.* 23, 2317–2326. doi: 10.1096/fj.08-122523

Santos, A. L., Sinha, S., and Lindner, A. B. (2018). The good, the bad, and the ugly of ROS: new insights on aging and aging-related diseases from eukaryotic and prokaryotic model organisms. *Oxid. Med. Cell. Longev.* 2018, 1941285.

Sawada, M., and Carlson, J. C. (1987). Changes in superoxide radical and lipid peroxide formation in the brain, heart and liver during the lifetime of the rat. *Mech. Ageing Dev.* 41, 125–137. doi: 10.1016/0047-6374(87)90057-1

Schaar, C. E., Dues, D. J., Spielbauer, K. K., Machiela, E., Cooper, J. F., Senchuk, M., et al. (2015). Mitochondrial and cytoplasmic ROS have opposing effects on lifespan. *PLoS Genet.* 11:e1004972. doi: 10.1371/journal.pgen.1004972

Schmeisser, S., Priebe, S., Groth, M., Monajembashi, S., Hemmerich, P., Guthke, R., et al. (2013a). Neuronal ROS signaling rather than AMPK/sirtuin-mediated energy sensing links dietary restriction to lifespan extension. *Mol. Metab.* 2, 92–102. doi: 10.1016/j.molmet.2013.02.002

Schmeisser, S., Schmeisser, K., Weimer, S., Groth, M., Priebe, S., Fazius, E., et al. (2013b). Mitochondrial hormesis links low-dose arsenite exposure to lifespan extension. *Aging Cell* 12, 508–517. doi: 10.1111/acel.12076

Schriner, S. E., Linford, N. J., Martin, G. M., Treuting, P., Ogburn, C. E., Emond, M., et al. (2005). Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308, 1909–1911. doi: 10.1126/science.1106653

Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M., and Ristow, M. (2007). Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* 6, 280–293. doi: 10.1016/j.cmet.2007.08.011

Scialo, F., Sriram, A., Fernandez-Ayala, D., Gubina, N., Lohmus, M., Nelson, G., et al. (2016). Mitochondrial ROS produced via reverse electron transport extend animal lifespan. *Cell Metab.* 23, 725–734. doi: 10.1016/j.cmet.2016.03.009

Selman, C., Mclaren, J. S., Collins, A. R., Duthie, G. G., and Speakman, J. R. (2013). Deleterious consequences of antioxidant supplementation on lifespan in a wild-derived mammal. *Biol. Lett.* 9, 20130432. doi: 10.1098/rsbl.2013.0432

Sena, L. A., and Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. *Mol. Cell* 48, 158–167. doi: 10.1016/j.molcel.2012.09.025

Senchuk, M. M., Dues, D. J., Schaar, C. E., Johnson, B. K., Madaj, Z. B., Bowman, M. J., et al. (2018). Activation of DAF-16/FOXO by reactive oxygen species contributes to longevity in long-lived mitochondrial mutants in Caenorhabditis elegans. *PLoS Genet.* 14:e1007268. doi: 10.1371/journal.pgen.1007268

Sharma, R., Yang, Y., Sharma, A., Awasthi, S., and Awasthi, Y. (2004). Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. *Antioxid. Redox Signal.* 6, 289–300. doi: 10.1089/152308604322899350

Shibamura, A., Ikeda, T., and Nishikawa, Y. (2009). A method for oral administration of hydrophilic substances to *Caenorhabditis elegans*: effects of oral supplementation with antioxidants on the nematode lifespan. *Mech. Ageing Dev.* 130, 652–655. doi: 10.1016/j.mad.2009.06.008

Shih, J. C., Chen, K., and Ridd, M. J. (1999). MONOAMINE OXIDASE: from genes to behavior. *Annu. Rev. Neurosci.* 22, 197–217. doi: 10.1146/annurev.neuro.22.1.197

Sies, H., and Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 21, 363–383. doi: 10.1038/s41580-020-0230-3

Simonsen, A., Cumming, R. C., Brech, A., Isakson, P., Schubert, D. R., and Finley, K. D. (2008). Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. *Autophagy* 4, 176–184. doi: 10.4161/auto.5269

Singh, S. P., Niemczyk, M., Saini, D., Sadovov, V., Zimniak, L., and Zimniak, P. (2010). Disruption of the mGsta4 gene increases life span of C57BL mice. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 14–23. doi: 10.1093/gerona/glp165

Slack, C., Foley, A., and Partridge, L. (2012). Activation of AMPK by the putative dietary restriction mimetic metformin is insufficient to extend lifespan in *Drosophila*. *PLoS One* 7:e47699. doi: 10.1371/journal.pone.0047699

Sohal, R. S. (2002). Role of oxidative stress and protein oxidation in the aging process. *Free Radic. Biol. Med.* 33, 37–44.

Sohal, R. S., and Dubey, A. (1994). Mitochondrial oxidative damage, hydrogen peroxide release, and aging. *Free Rad. Biol. Med.* 16, 621–626. doi: 10.1016/0891-5849(94)90062-0

Sohal, R. S., and Sohal, B. H. (1991). Hydrogen peroxide release by mitochondria increases during aging. *Mechan. Ageing Dev.* 57, 187–202. doi: 10.1016/0047-6374(91)90034-w

Sohal, R. S., Sohal, B. H., and Brunk, U. T. (1990). Relationship between antioxidant defenses and longevity in different mammalian species. *Mech Ageing Dev.* 53, 217–227. doi: 10.1016/0047-6374(90)90040-m

Sohal, R. S., Sohal, B. H., and Orr, W. C. (1995). Mitochondrial superoxide and hydrogen peroxide generation, protein oxidative damage, and longevity in different species of flies. *Free Radic. Biol. Med.* 19, 499–504. doi: 10.1016/0891-5849(95)00037-x

Sohal, R. S., Svensson, I., Sohal, B. H., and Brunk, U. T. (1989). Superoxide anion radical production in different animal species. *Mech. Ageing Dev.* 49, 129–135. doi: 10.1016/0047-6374(89)90096-1

Starkov, A. A. (2010). "Measurement of mitochondrial ROS production," in *Protein Misfolding and Cellular Stress in Disease and Aging: Concepts and Protocols*, eds P. Bross and N. Gregersen (Totowa, NJ: Humana Press), 245–255. doi: 10.1007/978-1-60761-756-3_16

Stohs, S. J. (1995). The role of free radicals in toxicity and disease. *J. Basic Clin. Physiol. Pharmacol.* 6, 205–228.

Sun, J., and Tower, J. (1999). FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol. Cell Biol.* 19, 216–228. doi: 10.1128/mcb.19.1.216

Sun, N., Youle, R. J., and Finkel, T. (2016). The mitochondrial basis of aging. *Mol. Cell* 61, 654–666. doi: 10.1016/j.molcel.2016.01.028

Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K., and Finkel, T. (1995). Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270, 296–299. doi: 10.1126/science.270.5234.296

Suthammarak, W., Somerlot, B. H., Opheim, E., Sedensky, M., and Morgan, P. G. (2013). Novel interactions between mitochondrial superoxide dismutases and the electron transport chain. *Aging Cell* 12, 1132–1140. doi: 10.1111/acel.12144

Sutton, H. C., and Winterbourn, C. C. (1989). On the participation of higher oxidation states of iron and copper in Fenton reactions. *Free Radic. Biol. Med.* 6, 53–60. doi: 10.1016/0891-5849(89)90160-3

Svensson, M. J., and Larsson, J. (2007). Thioredoxin-2 affects lifespan and oxidative stress in *Drosophila*. *Hereditas* 144, 25–32. doi: 10.1111/j.2007.0018-0661.01990.x

Syslova, K., Bohmova, A., Mikoska, M., Kuzma, M., Pelclova, D., and Kacer, P. (2014). Multimarker screening of oxidative stress in aging. *Oxid. Med. Cell Longev.* 2014:562860.

Tainer, J. A., Getzoff, E. D., Richardson, J. S., and Richardson, D. C. (1983). Structure and mechanism of copper, zinc superoxide dismutase. *Nature* 306, 284–287. doi: 10.1038/306284a0

Theopold, U. (2009). Developmental biology: a bad boy comes good. *Nature* 461, 486–487. doi: 10.1038/461486a

Toba, G., and Aigaki, T. (2000). Disruption of the microsomal glutathione S-transferase-like gene reduces life span of *Drosophila melanogaster*. *Gene* 253, 179–187. doi: 10.1016/S0378-1119(00)00246-8

Travasso, R. D., Sampaio Dos Aidos, F., Bayani, A., Abranches, P., and Salvador, A. (2017). Localized redox relays as a privileged mode of cytoplasmic hydrogen peroxide signaling. *Redox Biol.* 12, 233–245. doi: 10.1016/j.redox.2017.01.003

Trounce, I., Byrne, E., and Marzuki, S. (1989). Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet* 1, 637–639. doi: 10.1016/S0140-6736(89)92143-0

Turrens, J. F., and Boveris, A. (1980). Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem. J.* 191, 421–427. doi: 10.1042/bj1910421

Umeda-Kameyama, Y., Tsuda, M., Ohkura, C., Matsuo, T., Namba, Y., Ohuchi, Y., et al. (2007). Thioredoxin suppresses Parkin-associated endothelin receptor-like receptor-induced neurotoxicity and extends longevity in *Drosophila*. *J. Biol. Chem.* 282, 11180–11187. doi: 10.1074/jbc.M700937200

Ungvari, Z., Ridgway, I., Philipp, E. E. R., Campbell, C. M., Mcquary, P., Chow, T., et al. (2011). Extreme longevity is associated with increased resistance to oxidative stress in Arctica islandica, the longest-living non-colonial animal. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 66, 741–750.

Ünlü, E. S., and Koç, A. (2007). Effects of deleting mitochondrial antioxidant genes on life span. *Ann. N. Y. Acad. Sci.* 1100, 505–509. doi: 10.1196/annals.1395.055

Urban, N., Tsitsipatis, D., Hausig, F., Kreuzer, K., Erler, K., Stein, V., et al. (2017). Non-linear impact of glutathione depletion on *C. elegans* life span and stress resistance. *Redox Biol.* 11, 502–515.

Ursini, F., Maiorino, M., and Gregolin, C. (1985). The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. *Biochim. Biophys. Acta* 839, 62–70. doi: 10.1016/0304-4165(85)90182-5

Van Houten, B., Santa-Gonzalez, G. A., and Camargo, M. (2018). DNA repair after oxidative stress: current challenges. *Curr. Opin. Toxicol.* 7, 9–16. doi: 10.1016/j.cotox.2017.10.009

Van Raamsdonk, J. M. (2015). Levels and location are crucial in determining the effect of ROS on lifespan. *Worm* 4:e1094607. doi: 10.1080/21624054.2015.1094607

Van Raamsdonk, J. M., and Hekimi, S. (2009). Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 5:e1000361. doi: 10.1371/journal.pgen.1000361

Van Raamsdonk, J. M., and Hekimi, S. (2010). Reactive oxygen species and aging in caenorhabditis elegans: causal or casual relationship? *Antioxid Redox Signal* 13, 1911–1953. doi: 10.1089/ars.2010.3215

Van Raamsdonk, J. M., and Hekimi, S. (2012). Superoxide dismutase is dispensable for normal animal lifespan. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5785–5790. doi: 10.1073/pnas.1116158109

Van Raamsdonk, J. M., Meng, Y., Camp, D., Yang, W., Jia, X., Benard, C., et al. (2010). Decreased energy metabolism extends life span in *Caenorhabditis elegans* without reducing oxidative damage. *Genetics* 185, 559–571. doi: 10.1534/genetics.110.115378

Van Remmen, H., Ikeno, Y., Hamilton, M., Pahlavani, M., Wolf, N., Thorpe, S. R., et al. (2003). Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Phys. Genom.* 16, 29–37.

Wang, Q., and Zou, M. H. (2018). Measurement of reactive oxygen species (ROS) and mitochondrial ROS in AMPK knockout mice blood vessels. *Methods Mol. Biol.* 1732, 507–517. doi: 10.1007/978-1-4939-7598-3_32

Wang, X., and Roper, M. G. (2014). Measurement of DCF fluorescence as a measure of reactive oxygen species in murine islets of Langerhans. *Anal. Methods* 6, 3019–3024. doi: 10.1039/C4AY00288A

Wang, Y., Branicky, R., Noe, A., and Hekimi, S. (2018). Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. *J. Cell Biol.* 217, 1915–1928. doi: 10.1083/jcb.201708007

Wei, P. C., Hsieh, Y. H., Su, M. I., Jiang, X., Hsu, P. H., Lo, W. T., et al. (2012). Loss of the oxidative stress sensor NPGPx compromises GRP78 chaperone activity and induces systemic disease. *Mol. Cell* 48, 747–759.

Wei, Y., and Kenyon, C. (2016). Roles for ROS and hydrogen sulfide in the longevity response to germline loss in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 113, E2832–E2841. doi: 10.1073/pnas.1524727113

Weimer, S., Priebs, J., Kuhlow, D., Groth, M., Priebe, S., Mansfeld, J., et al. (2014). D-Glucosamine supplementation extends life span of nematodes and of ageing mice. *Nat. Commun.* 5:3563.

Winterbourn, C. C. (2008). Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem. Biol.* 4, 278–286. doi: 10.1038/nchembio.85

Woo, C. H., Eom, Y. W., Yoo, M. H., You, H. J., Han, H. J., Song, W. K., et al. (2000). Tumor necrosis factor-alpha generates reactive oxygen species via a cytosolic phospholipase A2-linked cascade. *J. Biol. Chem.* 275, 32357–32362. doi: 10.1074/jbc.M005638200

Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R., and Turner, N. D. (2004). Glutathione metabolism and its implications for health. *J. Nutr.* 134, 489–492.

Wu, Z., Senchuk, M. M., Dues, D. J., Johnson, B. K., Cooper, J. F., Lew, L., et al. (2018). Mitochondrial unfolded protein response transcription factor ATFS-1 promotes longevity in a long-lived mitochondrial mutant through activation of stress response pathways. *BMC Biol.* 16:147. doi: 10.1186/s12915-018-0615-3

Xiong, Y., Uys, J. D., Tew, K. D., and Townsend, D. M. (2011). S-glutathionylation: from molecular mechanisms to health outcomes. *Antioxid Redox Signal* 15, 233–270. doi: 10.1089/ars.2010.3540

Yang, W., and Hekimi, S. (2010). A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol.* 8:e1000556. doi: 10.1371/journal.pbio.1000556

Yang, W., Li, J., and Hekimi, S. (2007). A Measurable increase in oxidative damage due to reduction in superoxide detoxification fails to shorten the life span of long-lived mitochondrial mutants of *Caenorhabditis elegans*. *Genetics* 177, 2063–2074. doi: 10.1534/genetics.107.080788

Zadrag, R., Bartosz, G., and Bilinski, T. (2008). Is the yeast a relevant model for aging of multicellular organisms? An insight from the total lifespan of Saccharomyces cerevisiae. *Curr. Aging Sci.* 1, 159–165. doi: 10.2174/1874609810801030159

Zarse, K., Schmeisser, S., Groth, M., Priebe, S., Beuster, G., Kuhlow, D., et al. (2012). Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab.* 15, 451–465. doi: 10.1016/j.cmet.2012.02.013

Zhang, Y., Ikeno, Y., Qi, W., Chaudhuri, A., Li, Y., Bokov, A., et al. (2009). Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. *J. Gerontol. Ser. A* 64, 1212–1220.

Zou, S., Sinclair, J., Wilson, M. A., Carey, J. R., Liedo, P., Oropeza, A., et al. (2007). Comparative approaches to facilitate the discovery of prolongevity interventions: effects of tocopherols on lifespan of three invertebrate species. *Mech. Ageing Dev.* 128, 222–226.

Zuckerman, B. M., and Geist, M. A. (1983). Effects of vitamin E on the nematode *Caenorhabditis elegans*. *Age* 6, 1–4. doi: 10.1007/BF02431837

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Flavin-Containing Monooxygenases Are Conserved Regulators of Stress Resistance and Metabolism

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Flavin-Containing Monooxygenases are conserved xenobiotic-detoxifying enzymes. Recent studies have revealed endogenous functions of FMOs in regulating longevity in *Caenorhabditis elegans* and in regulating aspects of metabolism in mice. To explore the cellular mechanisms of FMO's endogenous function, here we demonstrate that all five functional mammalian FMOs may play similar endogenous roles to improve resistance to a wide range of toxic stresses in both kidney and liver cells. We further find that stress-activated c-Jun N-terminal kinase activity is enhanced in FMO-overexpressing cells, which may lead to increased survival under stress. Furthermore, FMO expression modulates cellular metabolic activity as measured by mitochondrial respiration, glycolysis, and metabolomics analyses. FMO expression augments mitochondrial respiration and significantly changes central carbon metabolism, including amino acid and energy metabolism pathways. Together, our findings demonstrate an important endogenous role for the FMO family in regulation of cellular stress resistance and major cellular metabolic activities including central carbon metabolism.

Keywords: mammalian cells, stress, metabolism, FMO, *C. elegans*, longevity

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INTRODUCTION

Flavin-containing monooxygenases (FMOs) are primarily studied as xenobiotic-metabolizing enzymes that oxygenate substrates by adding a molecule of oxygen to nitrogen, sulfur or other soft-nucleophilic atoms to increase the solubility and excretion of xenobiotics (Ziegler et al., 1971; Poulsen et al., 1976; Poulsen and Ziegler, 1979). The oxygenation activity of FMOs is highly efficient, since it does not require the presence of a substrate to start the catalytic cycle. Utilizing flavin adenine dinucleotide (FAD) as a prosthetic group, NADPH as a hydride donor, and oxygen as a co-substrate, a stable C4a-hydroperoxyflavin [(FADH-OOH) (NADP+)] is formed and is primed to oxygenate any soft nucleophile-containing substrate that can access the active site of FMO (Beaty and Ballou, 1981; Jones and Ballou, 1986; Poulsen and Ziegler, 1995). This uniquely efficient catalytic mechanism is in contrast to cytochrome P450 (CYP), the major xenobiotic-metabolizing monooxygenase, which requires the binding of substrate to activate oxygen for oxygenation (Capdevila et al., 1984; Lu et al., 1984).

Flavin-containing monooxygenases are ancient enzymes present in all phyla so far examined, and they are widely conserved from bacteria to vertebrates. There is a single ancestral FMO in

yeast (*Saccharomyces cerevisiae*), while there are multiple FMOs in nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), mice (*Mus musculus*), and humans (*Homo sapiens*). The additional *FMO* genes may exist due to gene duplication events in evolutionary response to new xenobiotics in the environment (Hao da et al., 2009). Human *FMO1*, *FMO2*, *FMO3*, *FMO4*, and *FMO6* are clustered on the region q24.3 on Chromosome 1 (Hernandez et al., 2004). Human *FMO6* does not encode a functional protein thus was identified as a pseudo gene (Hines et al., 2002; Hernandez et al., 2004). Human *FMO5* is about 26 Mb away and is located in the region 1q21.1 on Chromosome 1 (Hernandez et al., 2004). Similarly, mouse *Fmo1*, *Fmo2*, *Fmo3*, *Fmo4*, and *Fmo6* are clustered on Chromosome 1, and mouse *Fmo5* is located outside of the *Fmo* cluster on Chromosome 3 (Hernandez et al., 2004). Mouse *Fmo6* is homologous in sequence to human *FMO6* but needs further investigation to classify whether it is a pseudo gene (Hernandez et al., 2004). The *C. elegans* genome contains five *FMOs*, arbitrarily numbered as *fmo-1* to *fmo-5*, but they are not counterparts to individual *FMOs* with the same number in human and mouse (Petalcorin et al., 2005). Nematode *fmo-1 – fmo-5* are paralogous to each other, and homologous to all mouse and human FMOs, with mammalian *FMO5* containing the highest sequence identity (Petalcorin et al., 2005; Nicoll et al., 2019).

Flavin-containing monooxygenases show developmental and tissue-specific expression in different organisms. Human *FMO1* expression is silenced in the adult liver but present in the kidney and small intestine (Yeung et al., 2000). In contrast, mouse *Fmo1* is highly expressed in the adult liver with expression also detected in kidney, lung, adipose tissue, and brain (Janmohamed et al., 2004; Veeravalli et al., 2014). The majority of humans do not express functional *FMO2* because of a mutation that has introduced a premature stop codon (Dolphin et al., 1998; Veeramah et al., 2008). *Fmo2* is most expressed in the lungs of mice (Siddens et al., 2008). FMO3 and FMO5 are the major forms of FMOs in the liver of humans (Dolphin et al., 1996; Overby et al., 1997) and mice (Cherrington et al., 1998). FMO4 expression is very low in multiple tissues of both humans and mice, with relatively higher expression in the adult liver and kidney (Janmohamed et al., 2004; Zhang and Cashman, 2006). FMO5 is most highly expressed in the liver of humans and mice and also shows expression in the gastrointestinal tract in both organisms (Scott et al., 2017; Zhang et al., 2018). In *C. elegans*, *fmo-1*, *fmo-2*, and *fmo-5* are expressed in the intestine, which is thought to be equivalent to human kidney and small and large intestines; *fmo-3* and *fmo-4* are expressed in *C. elegans* hypodermis, which is equivalent to human liver and adipose tissue (Petalcorin et al., 2005; Kaletsky et al., 2018).

How FMOs act within physiological processes is largely unknown except for the role of FMO3 in the conversion of odorous trimethylamine to non-odorous trimethylamine N-oxide (Dolphin et al., 1997; Lang et al., 1998). Failure to oxygenate the volatile substrate trimethylamine (TMA) to the soluble product trimethylamine–N-oxide (TMAO) is caused by human FMO3 mutations and leads to trimethylaminuria, previously known as "Fish odor syndrome" (Dolphin et al., 1997). FMO1, 2, 4 knockouts also show that in male mice, where FMO3 expression in the liver is normally not detected, FMO1 also plays a key role in metabolizing TMA to TMAO (Veeravalli et al., 2018). However, the percentage of excreted TMAO was far less in male than in female mice regardless of *Fmo* genotype, supporting FMO3 as the major TMA oxygenating enzyme (Veeravalli et al., 2018). FMO3 liver-specific knockdown and transgenic overexpression also show that FMO3 is involved in cholesterol balance and glucose and lipid metabolism (Shih et al., 2015; Warrier et al., 2015). Studies from the Shephard group utilizing FMO knockout mice also reveal that two knockout mouse models (FMO1, 2, and 4 knockout and FMO5 knockout) are each leaner with higher whole-body energy expenditure, indicating that FMO1 and FMO5 regulate energy balance and promote metabolic efficiency (Veeravalli et al., 2014; Gonzalez Malagon et al., 2015; Scott et al., 2017). Additionally, in FMO5 knockout mice, key enzymes for carbohydrate, fatty acid metabolism, and glycolysis are down-regulated, leading the authors to report FMO5 as a regulator of metabolic aging (Gonzalez Malagon et al., 2015; Scott et al., 2017). These reports suggest that FMOs are key regulators of metabolism and physiology in mice.

Recent reports reveal that FMOs also play important roles in aging in *C. elegans* and possibly in mice. *C. elegans fmo-2* is a longevity gene that is induced and required in the worm intestine by at least two longevity signaling pathways, hypoxia and dietary restriction, for lifespan extension. Furthermore, overexpression of *fmo-2* is sufficient to extend lifespan, improve healthspan, and increase stress resistance in *C. elegans* (Leiser et al., 2015). All five mouse FMOs are reported to be upregulated in long-lived mouse models (Swindell, 2009; Steinbaugh et al., 2012). This evidence supports a role for FMOs as pro-longevity and beneficial for health in both lower organisms and vertebrates, potentially through a conserved endogenous function. In contrast, FMO5 knockout mice have shown improved glucose homeostasis, generally a positive long-term health measure (Scott et al., 2017).

Earlier studies have shown that long-lived *C. elegans* and *Drosophila* mutant strains are more resistant to multiple stresses, including oxidative stress, heat, and UV light (Larsen, 1993; Lithgow et al., 1995; Murakami and Johnson, 1996; Lin et al., 1998). More recently, the Miller group showed that skin-derived fibroblasts from long-lived mice are resistant to lethal effects of multiple stressors, including cadmium, hydrogen peroxide (H_2O_2), and UV-radiation (Murakami et al., 2003; Salmon et al., 2005; Harper et al., 2006). They went on to show that cells from a variety of rodents and birds possess a strong correlation between the maximum lifespan of the organism and the stress resistance of their cells (Harper et al., 2011). These reports support the hypothesis that increased stress resistance to various environmental insults contributes to longer lifespan in different species.

We hypothesized that, like in *C. elegans,* one or more mammalian FMOs would improve resistance to stress and would likely do so by modifying endogenous metabolism. To test this, we overexpressed mouse FMO1, FMO2, FMO3, FMO4, and FMO5 in two cell types in which FMOs are most highly expressed. We examine cellular stress resistance and metabolic changes, with the aim to demonstrate the endogenous metabolic functions of

FMOs and to determine whether there are differential effects when each FMO is expressed individually in the same cell line.

We demonstrate that increased FMO expression renders cells resistant to multiple stressors, including the heavy metals cadmium and arsenite, the free radical generator paraquat, UV-radiation, and the mitochondrial toxin rotenone. These results indicate that vertebrates with higher cellular levels of FMOs may be protected from damage. Through cellular metabolic activities and metabolomics analyses, we also show that FMOs significantly change cellular metabolism. It is imperative to test in the future whether regulation of metabolism by FMOs contribute to the stress resistance and potential benefits of FMOs in human health.

RESULTS

FMOs Improve Stress Resistance to Oxidative Stress in *C. elegans* and in Mammalian Cells

Recently, the crystal structure of three reconstructed, predicted ancestral FMO sequences were resolved, revealing that eight amino acids in the catalytic site, NADPH binding region, FAD binding region and the stabilization of the C4a-hydroperoxyflavin [(FADH-OOH) (NADP+)] intermediate are highly conserved among different FMOs (Nicoll et al., 2019). To explore the conservation of the catalytic active residues between *C. elegans* and mammalian FMOs, we aligned the regions containing these eight active residues (Asn62, Thr63, His151, Asn195, Arg224, His282, Gln373, and Ile378) among reconstructed ancestral mammalian FMO5, *C. elegans* FMO-2, and mouse FMO1–5 (**Figure 1A**). Full length amino acid alignment and sequence identities among *C. elegans* FMO-2 and mouse FMO1–5 are shown in **Supplementary Figures 1A,B**. Although the percent sequence identity is lower between *C. elegans* FMO-2 and mouse FMOs than within mouse FMOs, the catalytic residues of these proteins are much more highly conserved with only one catalytic residue difference (**Supplementary Figures 1A,B** and **Figure 1A**). This indicates that the enzymatic activity and function of *C. elegans* and mouse FMO could be conserved.

To test this possibility, we measured stress resistance in *C. elegans* and mammalian cell lines with FMO overexpression (**Figures 1B,D–G**). We previously reported that *C. elegans fmo-2* overexpression is sufficient to extend lifespan and improve resistance to proteotoxic stress induced by tunicamycin, heat, or dithiothreitol (DTT) (Leiser et al., 2015). To test whether this stress resistance is limited to just proteotoxic stress, we asked whether *fmo-2* expression also confers resistance to mitochondrial oxidative stress through the reactive oxygen species (ROS) inducer paraquat. The resulting data (**Figure 1B**) show that worms overexpressing *fmo-2* are more resistant to paraquat stress, consistent with the stress resistance phenotype being broad, and not specific to proteotoxic stress. This result, together with our previous findings, led to our interest in two primary questions: (1) whether mammalian FMOs confer similar benefits to mammals as nematode FMOs do to worms, and (2) which (if any) mammalian FMO is most likely functionally equivalent to nematode FMO-2?

To answer these questions, we utilized tissue culture to overexpress each of the five functional mouse FMOs individually in HEK293A kidney and HepG2 liver cells. The overexpression of different FMOs was confirmed by western blotting (**Figure 1C**) and quantitative PCR (**Supplementary Figures 2A,B**). Each FMO is overexpressed in its overexpressing cell lines (hereafter referred to as FMO-OE cells) in both HEK293A and HepG2. The expression pattern of endogenous FMO is consistent with the literature in that FMO1 is not expressed in adult liver cells (HepG2) and can be detected in kidney cells (HEK293A); FMO2 is not expressed both in liver and kidney cells; FMO3 is present in both kidney cells (HEK293A) and liver cells (HepG2); FMO4 is very low in kidney cells (HEK293A) but can be detected in liver cells (HepG2); FMO5 is highly expressed in liver cells (HepG2) but not detectable in kidney cells (HEK293A) (**Figure 1C**). With increasing doses of paraquat applied to FMO-OE cells or empty vector control cells, both HEK293A cells and HepG2 cells show the expected decreased survival rates (**Figure 1D** and **Supplementary Figures 3A,B**). Surprisingly, our results show that overexpression of FMO1, FMO2, FMO3, FMO4, and FMO5 each significantly and consistently increases cell survival in the tested dose responses to paraquat in both cell types (**Figure 1D** and **Supplementary Figures 3A,B**). We also compared the LD_{50} (Lethal Dose 50%) values, the dose of stress agent that lead to survival of 50% of the cells, for the FMO-OE and control cells (**Figure 1E**). FMO-OE cells were significantly more resistant to paraquat compared with control cells in both HEK293A and HepG2 (**Figure 1E**). The LD_{50}, *t*-test *P* values, and percentage of LD_{50} increases of each FMO-OE cell line compared to the control cell line are summarized in **Table 1A** for HEK293A and **Table 1B** for HepG2.

Next, we tested cell survival under cadmium, which indirectly produces ROS and induces oxidative stress. Cadmium is a heavy metal with extensive use in industry and has become a ubiquitous environmental toxicant. Cadmium accumulates mainly in the liver and kidney and causes lasting damage, in part due to its long half-life (Satarug et al., 2010). Similar to paraquat, we find that all five FMO-OE cell lines show improved stress resistance to cadmium in both HEK293A kidney cells and HepG2 liver cells (**Figure 1F** and **Supplementary Figures 3C,D**). LD_{50} values of FMO1–4 OE in HEK293A cells are significantly higher than the control cells (**Figure 1G**). FMO5-OE increases resistance so much that the survival rate of FMO5-OE cells is greater than 50% even at the highest dose of 64 μM, meaning LD_{50} cannot be accurately calculated (**Figure 1F**). In HepG2 cells, FMO-OE cells are significantly more resistant than the control cells in response to cadmium at individual doses (**Figure 1F** and **Supplementary Figure 3D**). The cadmium LD_{50} of FMO1, 2, 3, and 5 OE cells is increased compared to the control cells but did not reach statistical significance, while FMO4-OE is significant (**Figure 1G**). The values of LD_{50}, significance, and percentage of LD_{50} increases for FMO-OE and control cells in response to cadmium are listed in **Tables 1C,D**. We note that while each FMO-OE cell line exhibits improved resistance to cadmium and paraquat, we observed variability between the lines

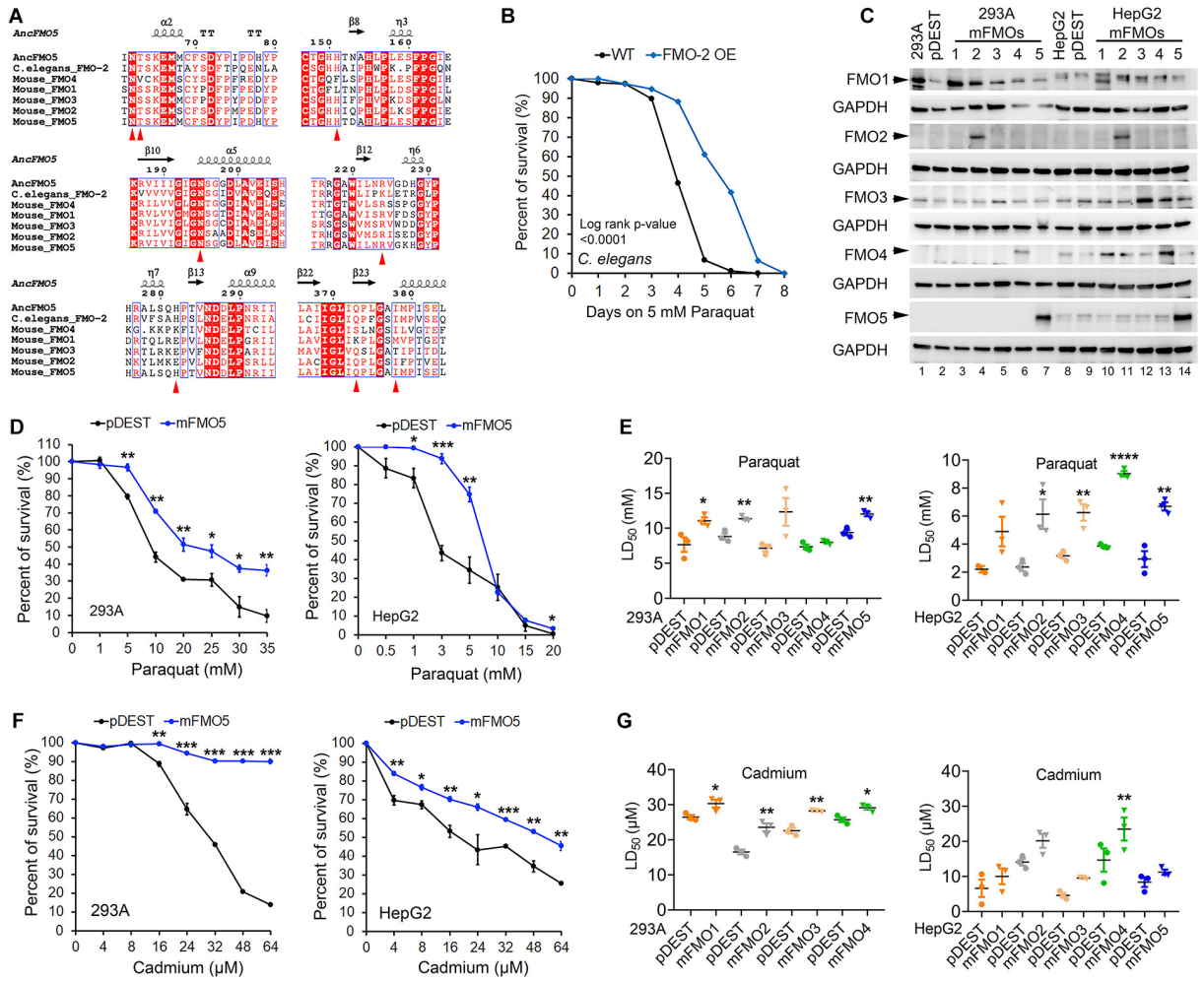

FIGURE 1 | *C. elegans* FMO-2 and mammalian FMOs improve stress resistance to oxidative stress. **(A)** Amino acid sequence alignment of FMOs across species with the regions containing the eight essential residues denoted as red arrowheads in the catalytic active site among reconstructed ancestral mammalian FMO5, *C. elegans* FMO-2, and mouse FMO1–5. **(B)** Wild-Type and FMO-2 overexpressing (FMO-2 OE) worm survival curves on paraquat stress. Each strain was placed on NGM (Nematode Growth Medium) containing 5 mM paraquat from the fourth larvae stage (L4) at Day 0. Survival was quantified every day until all worms were dead. The difference between the survival curves is denoted with the log-rank test *p*-value. **(C)** FMO1–5 protein levels in FMO1–5 OE and control cells of both HEK293A and HepG2. **(D)** FMO5 OE and control cell survival curves on paraquat stress in HEK293A and HepG2. HEK293A cells or HepG2 cells stably expressing FMO5 or empty vector pDEST were subjected to indicated increasing doses of paraquat. **(E)** LD_{50} values of FMO1–5 OE cells compared to the control cells on paraquat stress in HEK293A and HepG2. HEK293A cells or HepG2 cells stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing doses of paraquat. **(F)** FMO5 OE and control cell survival curves on cadmium stress in HEK 293A and HepG2. HEK293A cells or HepG2 cells stably expressing FMO5 or empty vector pDEST were subjected to indicated increasing doses of cadmium. **(G)** LD_{50} values of FMO-OE cells compared to the control cells on cadmium stress in HEK293A and HepG2. HEK293A cells or HepG2 cells stably expressing FMO or empty vector pDEST were subjected to indicated increasing doses of paraquat. Data represent mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001.

(e.g., FMO5 improved the cadmium stress resistance the most compared to other FMOs in HEK293A cells by more than 140.6% as shown in **Tables 1C,D**). We next applied a mitochondrial respiration chain complex I inhibitor, rotenone, and found that FMO-OE cells also exhibited increased survival in both cell types (**Supplementary Figures 5A,B**), albeit to a lesser extent. These results demonstrate that the role of FMOs in increasing resistance to oxidative stress is likely conserved from *C. elegans* to mammals. These data also show that, contrary to our initial hypothesis, all five mouse FMO-OE cell lines are similar to *C. elegans fmo-2* overexpressing worms in resisting stresses, and any mouse FMO could be functionally equivalent to *C. elegans fmo-2* in stress resistance.

FMOs Confer Resistance to Broad Stressors Including Arsenite and UV-Radiation

Fibroblasts isolated from long-lived organisms are resistant to multiple stressors compared to their shorter-lived evolutionary closely related species (Murakami et al., 2003; Salmon et al., 2005; Harper et al., 2006; Wang and Miller, 2012;

TABLE 1 | FMOs improve stress resistance to oxidative stress in HEK 293A and HepG2 cells.

A

Stress	Cell lines 293A	LD_{50} (mM) Mean ± SED	*t*-Test *P* Value	Increase, %
Paraquat	pDEST	7.66 ± 1.03	–	–
	mFMO1	11.1 ± 0.48	0.040	44.3%
Paraquat	pDEST	8.83 ± 0.46	–	–
	mFMO2	11.4 ± 0.22	0.008	28.6%
Paraquat	pDEST	7.15 ± 0.47	–	–
	mFMO3	12.3 ± 1.97	0.063	n.s.
Paraquat	pDEST	7.35 ± 0.34	–	–
	mFMO4	8.00 ± 0.18	0.165	n.s.
Paraquat	pDEST	9.38 ± 0.38	–	–
	mFMO5	12.0 ± 0.32	0.006	28.1%

B

Stress	Cell lines HepG2	LD_{50} (mM) Mean ± SED	*t*-Test *P* Value	Increase, %
Paraquat	pDEST	2.20 ± 0.24	–	–
	mFMO1	4.89 ± 1.05	0.145	n.s.
Paraquat	pDEST	2.36 ± 0.29	–	–
	mFMO2	6.13 ± 1.06	0.026	159.8%
Paraquat	pDEST	3.17 ± 0.20	–	–
	mFMO3	6.24 ± 0.59	0.008	97.2%
Paraquat	pDEST	3.85 ± 0.08	–	–
	mFMO4	9.03 ± 0.19	<0.0001	134.2%
Paraquat	pDEST	2.93 ± 0.57	–	–
	mFMO5	6.69 ± 0.29	0.004	128.6%

C

Stress	Cell lines 293A	LD_{50} (μM) Mean ± SED	*t*-Test *P* Value	Increase, %
Cadmium	pDEST	26.5 ± 0.61	–	–
	mFMO1	30.3 ± 1.11	0.038	14.6%
Cadmium	pDEST	16.5 ± 0.67	–	–
	mFMO2	23.6 ± 1.15	0.006	42.5%
Cadmium	pDEST	22.6 ± 0.82	–	–
	mFMO3	28.2 ± 0.12	0.002	24.9%
Cadmium	pDEST	25.7 ± 0.82	–	–
	mFMO4	29.1 ± 0.59	0.019	13.2%
Cadmium	pDEST	26.6 ± 0.60	–	–
	mFMO5	>64.0	–	>140.6%

D

Stress	Cell lines HepG2	LD_{50} (μM) Mean ± SED	*t*-Test *P* Value	Increase, %
Cadmium	pDEST	6.59 ± 2.49	–	–
	mFMO1	9.99 ± 2.24	0.367	n.s.
Cadmium	pDEST	14.1 ± 1.01	–	–
	mFMO2	20.1 ± 1.98	0.052	n.s.
Cadmium	pDEST	4.58 ± 0.67	–	–
	mFMO3	9.50 ± 0.15	0.002	107.4%
Cadmium	pDEST	14.7 ± 3.33	–	–
	mFMO4	23.5 ± 3.28	0.132	n.s.
Cadmium	pDEST	8.35 ± 1.37	–	–
	mFMO5	11.2 ± 0.75	0.142	n.s.

(A,B) *LD_{50}, t-test P values, and percentage of LD50 increase of FMO1–5 OE cell line compared to the control cell line on paraquat stress in HEK293A and HepG2. HEK293A cells* ***(A)*** *or HepG2 cells* ***(B)*** *stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing doses of paraquat.* ***(C,D)*** *LD_{50}, t-test P values, and percentage of LD_{50} increases of FMO1–5 OE cell line compared to the control cell line on cadmium stress in HEK293A and HepG2. HEK293A cells* ***(C)*** *or HepG2 cells* ***(D)*** *stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing doses of cadmium.*

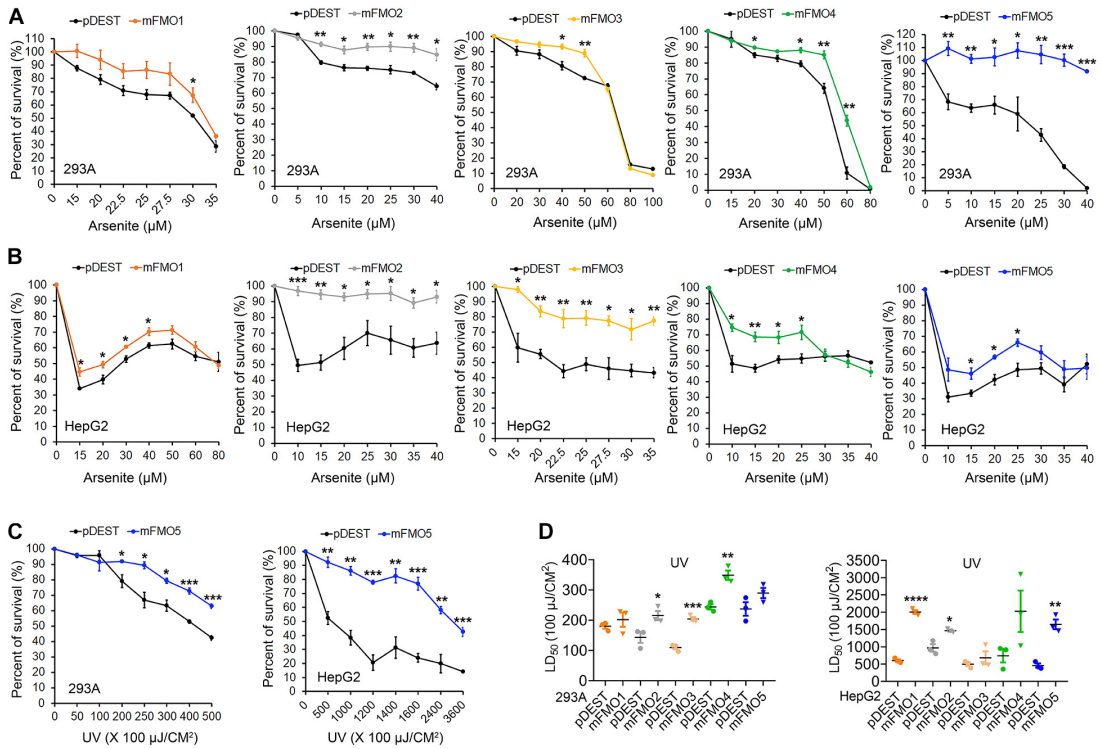

FIGURE 2 | FMOs improve stress resistance to broader stressors in mammalian cells. **(A,B)** FMO1–5 OE and control cell survival curves on arsenite stress in HEK293A and HepG2. HEK293A cells **(A)** or HepG2 cells **(B)** stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing doses of arsenite. **(C)** FMO5 OE and control cell survival curves on UV-radiation in HEK 293A and HepG2. HEK293A cells or HepG2 cells stably expressing FMO5 or empty vector pDEST were subjected to indicated increasing energies of UV-radiation. **(D)** LD_{50} values of FMO1–5 OE cells compared to the control cells on UV-radiation in HEK293A and HepG2. HEK293A cells or HepG2 cells stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing energies of UV-radiation. Data represent mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

Ozkurede and Miller, 2019). This broad stress resistance is a frequent marker of longevity, while resistance to specific stressors often marks more narrow evolutionary adaptations. Since FMOs are known to detoxify xenobiotics, we posited that while paraquat has not been ruled out as an FMO substrate, FMOs are unlikely to detoxify heavy metals, such as cadmium and arsenite, and could not reasonably block UV-radiation. Thus, to further test the hypothesis that FMOs have a conserved and broad role in stress resistance, we asked whether FMO-OE cells demonstrate resistance to the broad stressors arsenite and UV-radiation. Arsenite causes a variety of stress responses, including oxidative stress, heat-stress response, and cytoplasmic stress granule formation (Bernstam and Nriagu, 2000; Chen and Liu, 2017). Our data show that increased expression of each FMO significantly improves cell survival rates for at least one dose of arsenite in both HEK293A (**Figure 2A** and **Supplementary Figure 4A**) and HepG2 cells (**Figure 2B**), with FMO5 resisting arsenite most in HEK293A cells by more than 130.1% (**Table 2A**). We next tested cell survival under UV-radiation, which causes both protein and DNA damage through cross-linking. Consistently, each of the FMO-OE cell lines shows increased resistance to UV light compared to the control in both cell types (**Supplementary Figures 4B,C** and **Figures 2C,D**), with FMO5 expression again increasing resistance most, this time in HepG2 cells by 261% (**Tables 2B,C**). Together, our results indicate that exogenously expressing FMOs in kidney and liver cells render their stress resistance signatures similar to fibroblasts from long-lived organisms.

JNK Kinase Activity Is Increased in FMO-Overexpressing Cells Under Cadmium-Induced Oxidative Stress

We were next interested in the mechanism that FMOs act through to resist stress. A subfamily of mitogen-activated protein kinases (MAPKs) activated specifically by stress are the stress-activated protein kinases (SAPKs). c-Jun N-terminal kinases (JNKs), the enzymes that phosphorylate the transcriptional factor AP-1(c-Jun), and p38 kinases are the two best characterized SAPKs. These enzymes are responsive to multiple stressors including inflammatory cytokines, UV-radiation, and oxidative stress. The extracellular signal-regulated kinase (ERK), another member of MAPK, also responds to stress but is more commonly linked to growth factor stimuli and cell proliferation regulation (Cicenas et al., 2017; Corre et al., 2017). JNK, p38, and ERK are reportedly activated by cadmium (Chuang et al., 2000; Escobar Mdel et al., 2009; Zhao et al., 2015;

TABLE 2 | FMOs improve stress resistance to broader stressors in mammalian cells.

A

Stress	Cell lines 293A	LD_{50} (μM) Mean ± SED	*t*-Test *P* Value	Increase, %
Arsenite	pDEST	25.2 ± 0.13	–	–
	mFMO1	28.3 ± 0.98	0.033	12.6%
Arsenite	pDEST	>40	–	–
	mFMO2	>40	–	–
Arsenite	pDEST	58.5 ± 1.02	–	–
	mFMO3	62.4 ± 0.53	0.026	6.80%
Arsenite	pDEST	49.7 ± 1.65	–	–
	mFMO4	58.2 ± 0.70	0.009	17.1%
Arsenite	pDEST	17.4 ± 3.72	–	–
	mFMO5	>40	–	>130.1%

B

Stress	Cell lines 293A	LD_{50} (100 μJ/CM^2) Mean ± SED	*t*-Test *P* Value	Increase, %
UV	pDEST	180.0 ± 7.87	–	–
	mFMO1	201.9 ± 23.8	0.431	n.s.
UV	pDEST	143.1 ± 18.1	–	–
	mFMO2	215.4 ± 15.2	0.038	50.5%
UV	pDEST	109.6 ± 6.54	–	–
	mFMO3	204.1 ± 6.37	0.0005	86.2%
UV	pDEST	244.0 ± 8.81	–	–
	mFMO4	348.7 ± 15.4	0.004	42.9%
UV	pDEST	237.2 ± 22.4	–	–
	mFMO5	289.8 ± 16.9	0.134	n.s.

C

Stress	Cell lines HepG2	LD_{50} (100 μJ/CM^2) Mean ± SED	*t*-Test *P* Value	Increase, %
UV	pDEST	602.7 ± 37.6	–	–
	mFMO1	2006 ± 52.9	<0.0001	233%
UV	pDEST	966.6 ± 111	–	–
	mFMO2	1466 ± 28.5	0.012	51.7%
UV	pDEST	498.3 ± 55.4	–	–
	mFMO3	681.4 ± 188	0.403	n.s.
UV	pDEST	740.5 ± 193	–	–
	mFMO4	2027 ± 599	0.110	n.s.
UV	pDEST	456.9 ± 59.8	–	–
	mFMO5	1650 ± 140	0.001	261%

(A) *LD_{50}, t-test P values, and percentage of LD_{50} increases of FMO1–5 OE cell line compared to the control cell line on arsenite stress in HEK293A. HEK293A cells stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing doses of arsenite.* ***(B,C)*** *LD_{50}, t-test P values, and percentage of LD_{50} increases of FMO1–5 OE cell line compared to the control cell line on UV-radiation in HEK293A and HepG2. HEK293A cells* ***(B)*** *or HepG2 cells* ***(C)*** *stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing energies of UV-radiation.*

Tsai et al., 2016). Interestingly, JNK and p38 are also reportedly activated by paraquat, arsenite, UV, and rotenone (Derijard et al., 1994; Cavigelli et al., 1996; Newhouse et al., 2004; Peng et al., 2004; Klintworth et al., 2007) each of which FMO-OE cell lines resist as shown in **Figures 1, 2** and **Supplementary Figures 3–5**.

To explore whether FMO-OE cells resist stress through SAPKs, we utilized cadmium as a tool, largely because it is the most consistent and reproducible stressor among those we have tested. First, we determined the time point and dosage under which SAPKs are activated by cadmium in our system. The results show that 4 and 6 h treatments with 10 μM cadmium activated SAPKs, including JNK, p38, and ERK (**Figure 3A**). We used 4 h as the time point to assess cadmium-induced kinase activities because we wanted the earliest time point before damage also becomes a factor. We then asked whether JNK, p38, and ERK activities are changed in FMO-overexpressing cells compared to control cells under stress. The results show that only JNK activity is consistently increased in all five FMO-OE cell lines of HEK293A (**Figures 3B,C** and **Supplementary Figure 6A**) and

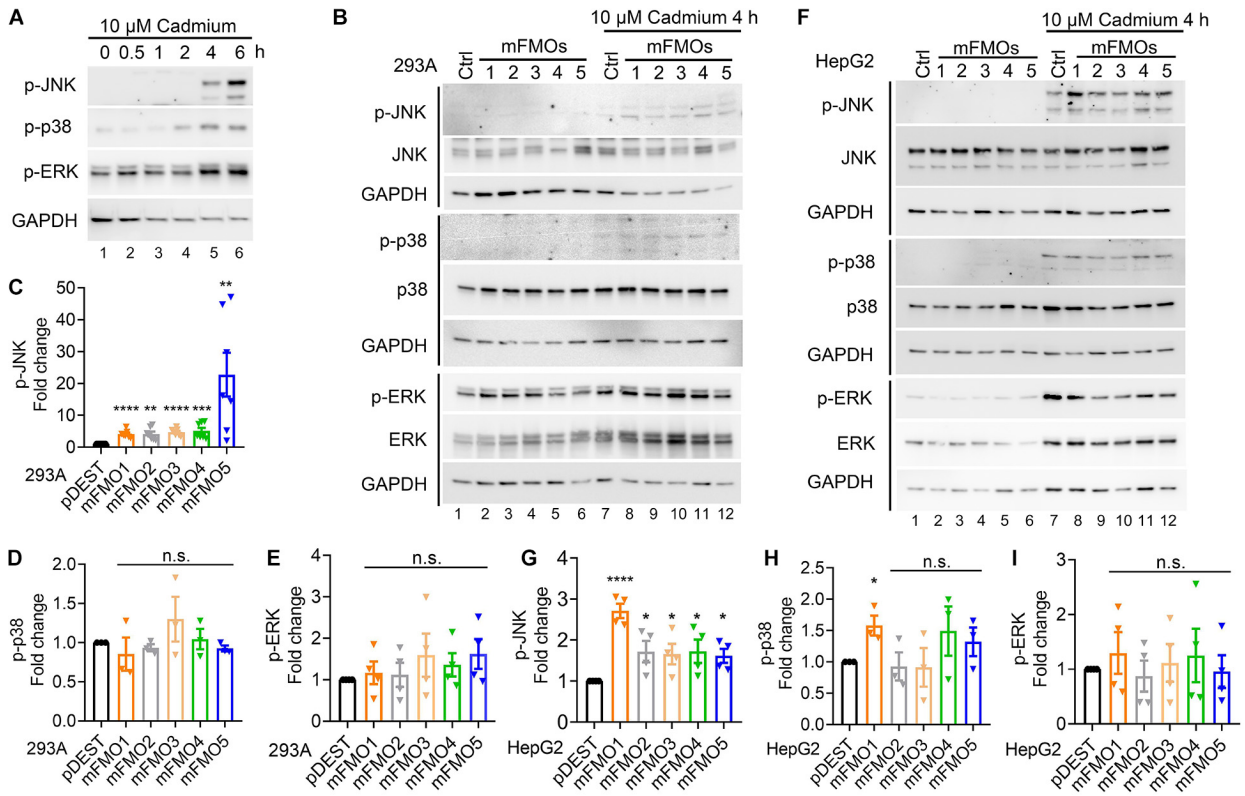

FIGURE 3 | JNK kinase activity is increased in FMO-overexpressing cells under cadmium-induced oxidative stress. **(A)** SAPK levels and phosphorylation including JNK, p38, and ERK after 10 μM cadmium treatment of indicated time. HEK293A cells were treated with cadmium, and the SAPK activities were measured with antibodies against phosphorylated SAPKs. **(B)** SAPKs levels and phosphorylation including JNK, p38, and ERK in FMO1–5 OE HEK293A cells and empty vector control cells after 10 μM Cadmium treatment for 4 h. Quantitation of the phosphorylated JNK **(C)**, p38 **(D)**, and ERK **(E)** after 10 μM Cadmium treatment for 4 h in FMO 1–5 OE HEK293A cells [lane 8–12 in **(B)**] compared to empty vector control HEK293A cells [lane 7 in **(B)**]. **(F)** SAPK levels and phosphorylation including JNK, p38, and ERK in FMO1–5 OE HepG2 cells and empty vector control cells after 10 μM Cadmium treatment for 4 h. Quantitation of the phosphorylated JNK **(G)**, p38 **(H)**, and ERK **(I)** after 10 μM Cadmium treatment for 4 h in FMO1–5 OE HepG2 cells [lane 8–12 in **(F)**] compared to empty vector control HepG2 cells [lane 7 in **(F)**]. Western blot band intensities were quantified by Image J. The phosphorylated bands were normalized to the corresponding unphosphorylated bands of each SAPK, and then were compared to the empty vector pDEST control as fold changes. Data represent mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

HepG2 (**Figures 3F,G** and **Supplementary Figure 6B**) under cadmium stress. p38 activity is significantly increased in HepG2 FMO1-OE cells (**Figure 3H**) but not in other FMO-OE cells (**Figures 3D,H**). ERK activity is largely not changed in all FMO-OE cells (**Figures 3E,I**). These data are consistent with JNK better responding and exerting protective mechanisms of cell survival when FMOs are overexpressed. Since we have shown that FMO-OE cells have similar stress resistance profiles to the fibroblasts from long-lived mice, the activation of JNKs in the FMO-OE cells is reminiscent of previous findings showing that increased JNK activity leads to increase tolerance for oxidative stress and increases lifespan dramatically in both *C. elegans* and *Drosophila* (Wang et al., 2003; Oh et al., 2005).

FMO Expression Modulates the Balance Between Mitochondrial Respiration and Glycolysis

Previous reports using FMO knockout mice suggest that mouse FMOs play physiological roles in endogenous metabolism (Veeravalli et al., 2014; Gonzalez Malagon et al., 2015; Shih et al., 2015; Warrier et al., 2015), including decreased expression of glycolytic enzymes in the liver of FMO5 KO mice (Gonzalez Malagon et al., 2015). However, implications are complicated due to the tissue specific, developmental and sex-differential expression of the different FMOs. In our stable FMO-OE cell lines, we can assess metabolic changes without the *in vivo* tissue-specific complications. Mitochondrial respiration and glycolysis are the two major energy producing pathways of the cell. Mitochondrial respiration can be roughly measured by the oxygen consumption rate (OCR) for ATP production. During glycolysis, glucose is broken down to lactic acid, and protons produced are exported to the extracellular media and can be measured as extracellular acidification rate (ECAR). Using the Seahorse XFe96 Analyzer, we tested the mitochondrial respiration and glycolytic activities of FMO-OE and control empty vector cell lines. As shown in **Figure 4A**, mitochondrial respiration is increased in all HEK293A FMO-OE cells. Basal respiration, ATP production, and maximal respiration are all increased in these kidney FMO-OE cells (**Figure 4B**). Similarly,

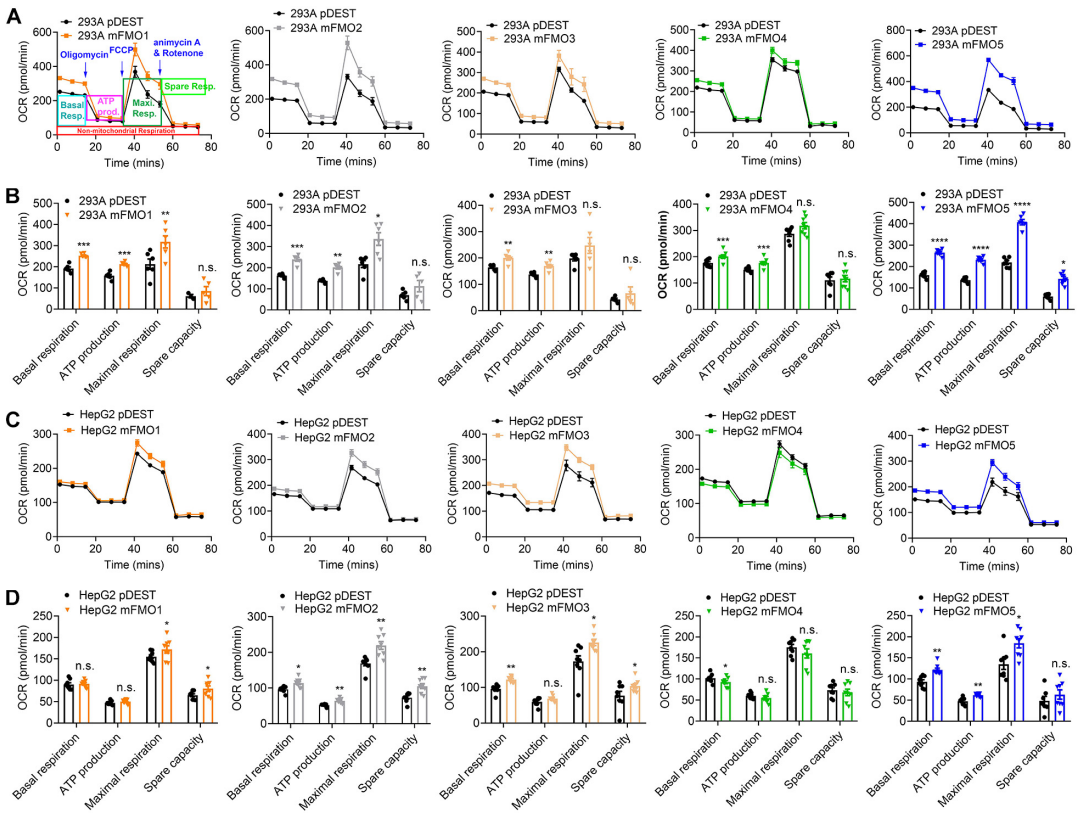

FIGURE 4 | FMO expression increases mitochondrial respiration. **(A)** Mitochondrial respiration measured by OCR (oxygen consumption rate) in FMO1–5 OE HEK293A cells and empty vector control cells. Mitochondrial respiration chain complex inhibitors Oligomycin, Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP), and rotenone/antimycin A were injected stepwise as indicated. As denoted in the first panel, basal respiration, ATP production, maximal respiration, and spare respiration can be calculated by the OCR level changes in response to the inhibitor injections. **(B)** Basal respiration, ATP production, maximal respiration, and spare capacity in FMO1–5 OE HEK293A cells and empty vector control cells. **(C)** Mitochondrial respiration in FMO1–5 OE HepG2 cells. **(D)** Basal respiration, ATP production, maximal respiration, and spare capacity in FMO1–5 OE HepG2 cells. Data represent mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

mitochondrial respiration is increased in FMO1, FMO2, FMO3, and FMO5 but not FMO4-OE HepG2 cells (**Figure 4C**). Maximal respiration is significantly improved in FMO1, FMO2, FMO3, and FMO5-OE HepG2 cells (**Figure 4D**). FMO2, FMO3, and FMO5 increase basal mitochondrial respiration (**Figure 4D**). ATP production is increased by FMO2 and FMO5 in HepG2 cells (**Figure 4D**). These results suggest that oxygen consumption through mitochondrial respiration is increased by FMO overexpression. In agreement with an increase in respiration, overall glycolytic activity as measured by ECAR shows a decreasing trend in HEK293A FMO-OE cells (**Figures 5A,B**). As quantified in **Figure 5B**, FMO3 significantly decreases glycolysis. Glycolytic capacity and glycolytic reserve are significantly decreased in FMO1, FMO2, and FMO5-OE HEK293A cells, while FMO3 and FMO4 show an insignificant decrease in glycolytic capacity and reserve (**Figure 5B**). In HepG2 cells, glycolytic activity is significantly decreased in all five HepG2 FMO-OE cells compared with vector controls (**Figure 5C**). As quantified in **Figure 5D**, glycolysis is decreased most significantly by FMO3 or FMO4 overexpression. Glycolytic capacity and glycolytic reserve are both significantly decreased in all five FMO-OE HepG2 cell lines (**Figure 5D**). These data are consistent with overexpression of FMOs shifting energy production to mitochondrial metabolism and away from carbohydrate metabolism.

FMOs Regulate Essential Amino Acid, Carbohydrate, and Energetic Pathways

To investigate perturbations in metabolism in the stably transfected cell lines, we employed untargeted metabolomics analysis to our FMO-OE cell lines and compared them to empty vector control cell lines. The results show differences in fundamental metabolism pathways, including amino acid, carbohydrate, and energetic pathways (**Figure 6A** and **Supplementary Figures 7–15**). Through human KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses, we find that there are no clear discriminations between FMOs, and the metabolic pathways are significantly shared by almost all FMO-OE cells. From the shared metabolic pathways, amino acid metabolism, and protein biosynthesis are major pathways modulated by FMOs. "Glycine, serine,

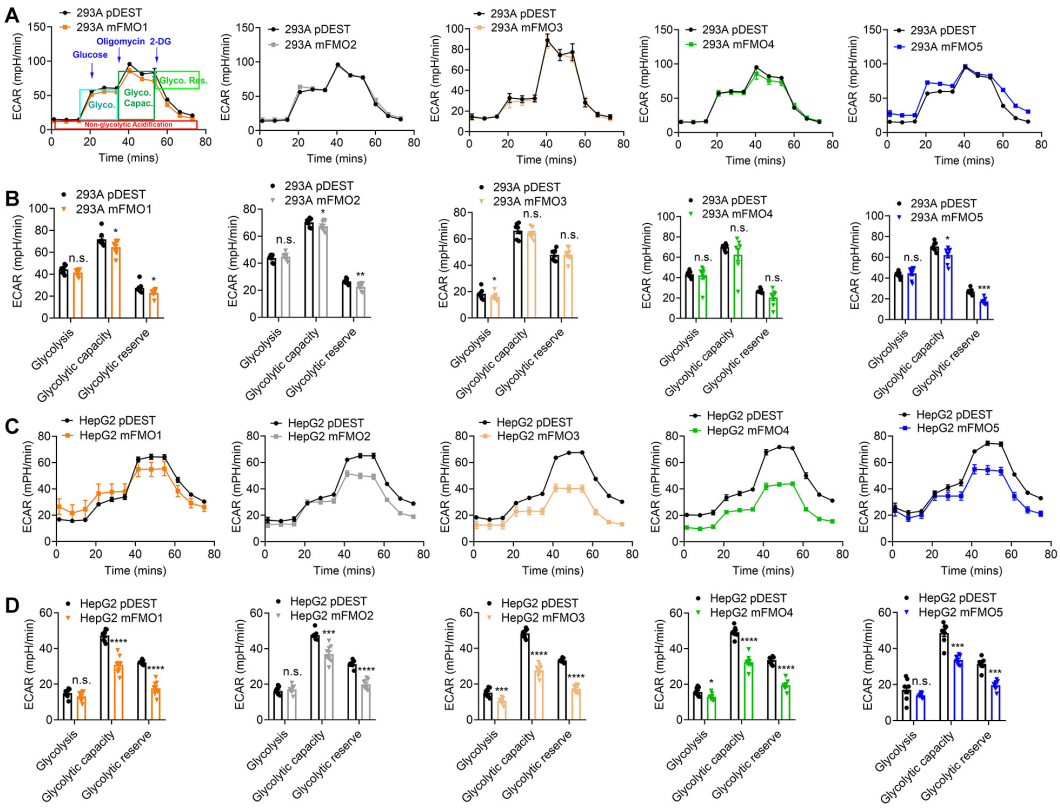

FIGURE 5 | FMO overexpression decreases overall glycolytic activity. **(A)** Overall glycolytic activity measured by ECAR (extracellular acidification rates) in HEK293A FMO1–5 OE cells and empty vector control cells. Sequential injections of glucose, oligomycin, and 2-DG were applied over time as indicated. As denoted in the first panel, glycolysis, glycolytic capacity, and glycolytic reserve can be calculated by the ECAR level changes in response to individual injections. **(B)** Glycolysis, glycolytic capacity, and glycolytic reserve in HEK293A FMO1–5 OE cells and empty vector control cells. **(C)** Overall glycolytic activity in FMO1–5 OE HepG2 cells. **(D)** Glycolysis, glycolytic capacity, and glycolytic reserve in FMO1–5 OE HepG2 cells and empty vector control cells. Data represent mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

and threonine metabolism; Cyanoamino acid metabolism; Aminoacyl-tRNA biosynthesis; Selenoamino acid metabolism; Taurine and hypotaurine metabolism; Cysteine and methionine metabolism; Arginine and proline metabolism; Lysine biosynthesis; Alanine, aspartate, and glutamate metabolism; Pantothenate and CoA biosynthesis; Vitamin B6 metabolism" are the 11 pathways that are most significantly modified and shared by three or more FMOs in FMO1–5 (Highlighted by bold text in **Figure 6A** and **Supplementary Figures 7–15**). The enrichment of metabolites and the significance of the top pathways in HEK293A FMO1-OE cells are shown in **Figure 6A** as representative results. The enriched metabolic pathways in HEK293A FMO1, FMO2, FMO3, FMO4, and FMO5-OE cells are shown in **Supplementary Figures 7–10** and **Table 3**.

The metabolic pathways significantly changed by FMO1–5 in HepG2 cells are shown in **Supplementary Figures 11–15** and **Table 3**. Taurine and hypotaurine metabolism is detected as modified by FMO1, FMO3 and FMO5 in HEK293A cells (**Figure 6A** and **Supplementary Figures 8, 10** and **Supplementary Table 1**). This is consistent with recent findings that FMO1 is a key enzyme for taurine biosynthesis through catalyzing the S-oxygenation of hypotaurine (Veeravalli et al., 2020). In addition to the common pathways shared by almost all FMOs, there are several pathways in the categories of carbohydrate, energy, lipid, and vitamin metabolisms shared by multiple FMOs (**Supplementary Table 1**). Interestingly, and consistently with our findings from seahorse metabolic measurements in **Figures 4, 5** that FMOs shift energy production to mitochondrial respiration from glycolysis, pathways in carbohydrate metabolism, and energy metabolism are significantly differentially regulated in FMO-OE cells (**Supplementary Table 1**). Carbohydrate metabolism pathways, including the pentose phosphate pathway (by FMO1), TCA cycle (by FMO3), ascorbate and aldarate metabolism (by FMO4), and butanoate metabolism (by FMO3 and FMO4), are all significantly modified (**Supplementary Table 1**). Energy metabolism pathways, including methane metabolism (by FMO3 and FMO5), sulfur metabolism (by FMO5), and nitrogen metabolism (by FMO5), are also altered in these cell lines (**Supplementary Table 1**). Metabolism of cofactors and vitamins is another aspect differentially regulated in FMO-OE cells, with significant changes in vitamin B6 metabolism (by FMO1, FMO2, FMO3, and FMO4), pantothenate and

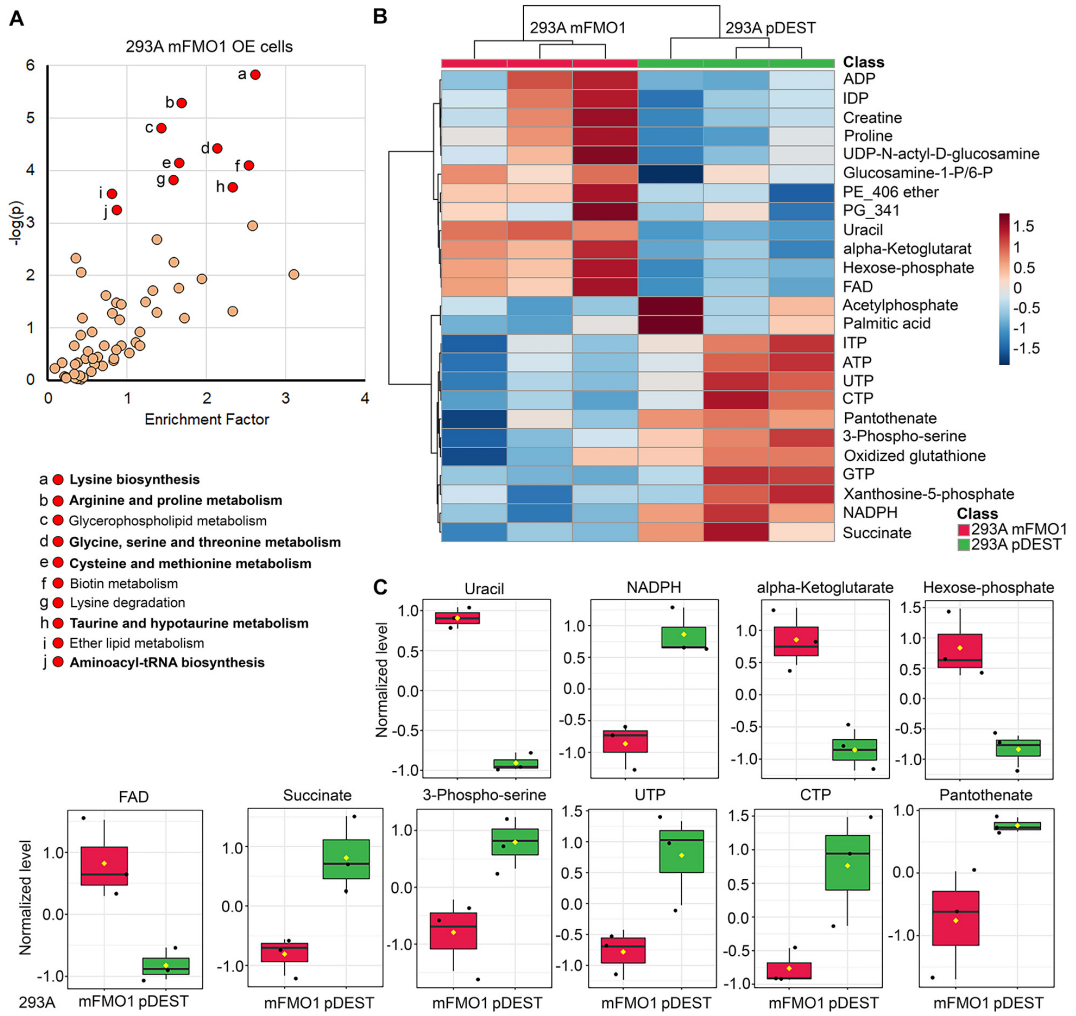

FIGURE 6 | FMOs regulate amino acid and energetic metabolic pathways. Untargeted metabolomics in FMO1–5 OE cells (**A**, see also **Supplementary Figures 7–15**) compared to empty vector control cells, and the significantly regulated metabolic pathways that are enriched. The abundance analyses of metabolites in central carbon metabolism in FMO1–5 OE cells compared to empty vector control cells (**B,C**, see also **Supplementary Figures 16–24**). **(A)** Metabolic pathways regulated by FMO1 are plotted by the enrichment factor (obtained by dividing "significant hits" by "expected hits" for each pathway) on the *x*-axis and –log of the *p*-value on the *y*-axis. Red indicates significantly changed pathways with $p < 0.001$. Shared significantly regulated metabolic pathways by more than three FMOs in FMO1–5 are indicated in bold text, including amino acid metabolism (Glycine, serine, and threonine metabolism; Cyanoamino acid metabolism; Aminoacyl-tRNA biosynthesis; Selenoamino acid metabolism; Taurine and hypotaurine metabolism; Cysteine and methionine metabolism; Arginine and proline metabolism; Lysine biosynthesis; Alanine, aspartate, and glutamate metabolism) and metabolism of cofactors and vitamins (Pantothenate and CoA biosynthesis; Vitamin B6 metabolism) (see also **Supplementary Figures 7–15**). **(B)** The levels of the top 25 changed metabolites between FMO1-OE cells and empty vector control cells are shown in heat map. **(C)** The levels of metabolites significantly regulated by FMO1 are shown in FMO1-OE cells and empty vector control cells.

CoA biosynthesis (by FMO1, FMO2, FMO3, and FMO5), biotin metabolism (by FMO1 and FMO3), nicotinate and nicotinamide metabolism (by FMO3), and porphyrin and chlorophyll metabolism (by FMO4 and FMO5) (**Supplementary Table 1**). Lipid metabolism is also differentially regulated in FMO-OE cells, including glycerophospholipid metabolism (by FMO1 and FMO4), ether lipid metabolism (by FMO1 and FMO4), alpha-Linolenic acid metabolism (by FMO3), and the synthesis and degradation of ketone bodies pathway (by FMO3) (**Supplementary Table 1**). Together, these results from untargeted metabolomics analyses suggest that FMO expression significantly changes endogenous metabolism and regulates amino acid, carbohydrate, energy, vitamin, and lipid metabolism.

Central Carbon Metabolism Is Regulated by FMOs

Since carbon metabolism is a common feature of the metabolic pathways regulated by FMOs (**Figure 6A** and **Supplementary Figures 7–15**), and mitochondrial respiration and glycolytic activities are also modulated (**Figures 4, 5**), we next asked whether FMOs regulate central carbon metabolism. We targeted the abundance of 102 central carbon metabolites

TABLE 3 | FMOs regulate amino acid, carbohydrate, and energetic metabolic pathways.

	293A	HepG2
mFMO1	Lysine biosynthesis	Cyanoamino acid metabolism
	Arginine and proline metabolism	Pantothenate and CoA biosynthesis
	Glycerophospholipid metabolism	Pentose phosphate pathway
	Glycine, serine, and threonine metabolism	Selenoamino acid metabolism
	Cysteine and methionine metabolism	Vitamin B6 metabolism
mFMO2	Alanine, aspartate, and glutamate metabolism	Vitamin B6 metabolism
	Cyanoamino acid metabolism	Valine, leucine, and isoleucine biosynthesis
	Lysine biosynthesis	Aminoacyl-tRNA biosynthesis
	Pantothenate and CoA biosynthesis	Pentose phosphate pathway
	Vitamin B6 metabolism	Biotin metabolism
mFMO3	Pyrimidine metabolism	Alanine, aspartate and glutamate metabolism
	Synthesis and degradation of ketone bodies	Arginine and proline metabolism
	Glycine, serine, and threonine metabolism	Pantothenate and CoA biosynthesis
	Alanine, aspartate, and glutamate metabolism	Butanoate metabolism
	Taurine and hypotaurine metabolism	Cyanoamino acid metabolism
mFMO4	Ascorbate and aldarate metabolism	Ether lipid metabolism
	Vitamin B6 metabolism	Glycerophospholipid metabolism
	Porphyrin and chlorophyll metabolism	D-Glutamine and D-glutamate metabolism
	Alanine, aspartate, and glutamate metabolism	Arachidonic acid metabolism
	Butanoate metabolism	Citrate cycle (TCA cycle)
mFMO5	Glycine, serine, and threonine metabolism	Methane metabolism
	Cyanoamino acid metabolism	Cyanoamino acid metabolism
	Pantothenate and CoA biosynthesis	Glutathione metabolism
	Aminoacyl-tRNA biosynthesis	Pyrimidine metabolism
	Sulfur metabolism	Glycine, serine, and threonine metabolism

The top five metabolic pathways of FMO1–5 in HEK293A and HepG2 cells. Untargeted metabolomics in FMO1–5 OE cells compared to empty vector control cells, and the significantly regulated metabolic pathways that are enriched.

in FMO-OE cells compared to empty vector control cells. As shown by Principle Component Analysis (PCA), the clusters of three replicates of FMO-overexpressing or empty vector control HEK293A cells are distinctly separated for FMO1 (**Supplementary Figure 16A**), FMO2 (**Supplementary Figure 16B**), FMO3 (**Supplementary Figure 17A**), FMO4 (**Supplementary Figure 18A**), and FMO5 (**Supplementary Figure 19A**), suggesting FMO overexpression results in dramatic changes in central carbon metabolism in HEK293A cells. In HepG2 cells, the differences in central carbon metabolites regulations between FMO-OE cells and empty vector control cells are not as distinct as in HEK293A cells, with overlapping clusters between FMO1, FMO3, or FMO5 OE cells and empty vector control cells (**Supplementary Figures 20A–24A**). The levels of the top 25 changed metabolites between FMO-OE cells and empty vector control cells are shown in a heat map for FMO1 (**Figure 6B**), FMO2 (**Supplementary Figure 16C**), FMO3 (**Supplementary Figure 17B**), FMO4 (**Supplementary Figure 18B**), and FMO5 (**Supplementary Figure 19B**) in HEK293A cells, and FMO1–5 OE in HepG2 cells (**Supplementary Figures 20B–24B**). Among the individual metabolites that are significantly regulated by FMOs shown in **Figure 6C** and **Supplementary Figures 16D, 17C–24C** and **Supplementary Tables 2–11**, amino acids, including arginine (**Supplementary Figures 17C, 19C, 22C**), glutamine (**Supplementary Figures 21C, 24C**), methionine (**Supplementary Figures 21C, 23C**), tryptophan (**Supplementary Figures 21C, 23C**), citrulline (**Supplementary Figures 21C, 23C**), and phenylalanine (**Supplementary Figures 21C, 23C, 24C**), are downregulated by FMOs, consistent with the results from untargeted metabolomics that amino acid metabolism is the most significantly regulated pathway. The metabolites in energy production metabolism, including FAD, NADP, NADPH, ADP, AMP, ATP, phosphocreatine, succinate, and glycerol-3-phosphate, are also significantly regulated by multiple FMOs, with upregulation of FAD (**Figure 6C** and **Supplementary Figures 18C, 19C**), NADP (**Supplementary Figure 18C**), ADP (**Supplementary Figure 24C**) and AMP (**Supplementary Figure 18C**) and downregulation of NADPH (**Figure 6C** and **Supplementary Figure 19C**), ATP (**Supplementary Figure 19C**), phosphocreatine (**Supplementary Figures 19C–23C**), succinate (**Figure 6C** and **Supplementary Figure 24C**), and glycerol-3-phosphate (**Supplementary Figures 17C, 18C, 20C, 21C**). To summarize, our untargeted and targeted metabolomics analyses demonstrate that FMO expression significantly modifies endogenous metabolism, primarily in amino acid and energy metabolism.

DISCUSSION

In this study, we evaluated the possible benefits of mammalian FMOs by exploring and comparing the stress resistance and metabolic impacts of all FMOs in the same platform. Our results demonstrate that FMOs may all play similar endogenous functions to improve resistance to a broad range of stressors. Our metabolic analyses demonstrate that FMOs balance the cellular energy producing pathways between mitochondrial respiration and glycolysis, with enhanced activity of mitochondrial respiration and depressed glycolytic activity. Through metabolomics analyses, we reveal that amino acid, carbohydrate, and energy metabolism are the major regulated pathways by FMOs, which is further confirmed by the significant changes in the central carbon metabolites of amino acids and energy metabolism cofactors. Our study for the first

time elucidates the cellular functions and metabolic pathways regulated by all five FMOs in a cell culture system.

Mice are a useful model to study FMOs because they allow the examination of mammalian physiology in respect to the endogenous functions of FMOs, with all the intricacy and interactions of an intact animal. However, the tissue-specific, developmental, and gender differential expressions of different FMOs complicate the interpretation of results in mammals. For example, FMO1 is not expressed after birth in the human liver; FMO4 expression is low in both human and mouse; and FMO3 is not normally expressed in male mice liver but is highly expressed in female mice liver. The concern of gender differential expression of FMO3 also applies to mouse liver cells as it matters which gender the hepatocytes were originally isolated from Houseman et al. (2015). Taking these potential confounders into consideration, we focus here on the endogenous role of FMOs by overexpressing FMOs in mammalian cells. By overexpressing mouse FMO1, FMO2, FMO3, FMO4, or FMO5 in both liver and kidney cells, we successfully examined the cellular stress resistance and metabolic changes resulting from this expression. This allowed us to compare whether there were significant and/or differential effects of different FMOs in the same platform, without the complication of tissue-specific, developmental, and gender differential expression of different FMOs *in vivo*.

Stress resistance assays show that all five FMOs help resist a wide range of stressors, including paraquat, cadmium, rotenone, arsenite, and UV-radiation, with variations in the extent of stress resistance by different FMOs in HEK293A and HepG2 cells (**Figures 1, 2** and **Supplementary Figures 3–5**). Due to the tissue-specific expression of different FMOs as reported, the endogenous basal expression levels of different FMOs are different in the two cell types (HEK293A kidney cells and HepG2 liver cells) that we used (**Figure 1C**). Consistent with the report that human liver cells express high level of FMO5 (Zhang and Cashman, 2006), our data also show that endogenous FMO5 levels are lower in HEK293A than HepG2 cells (**Figure 1C**). This difference between these two cell types may contribute to some differences in their response to the same type of stress. For example, FMO5-OE HEK293A cells show a dramatic increase in stress resistance to cadmium and arsenite when compared to the control HEK293A cells where almost no endogenous FMO5 was detected (**Figures 1C,F, 2A** and **Tables 1C, 2A**). Conversely, the increase in stress resistance of FMO5-OE HepG2 cells to cadmium and arsenite is not as dramatic as in HEK293A cells, plausibly due to high level of endogenous FMO5 in HepG2 control cells (**Figures 1C,F, 2B** and **Table 1D**). All five FMOs increased stress resistance when overexpressed, regardless of whether they are normally expressed in the cell-type, consistent with FMOs sharing a common and redundant function in stress resistance. It is also interesting that FMO-OE cell lines resist such a wide range of stressors, resembling the stress resistance profile of fibroblasts from long-lived mice.

Further exploring the underlying mechanism of stress resistance through FMO expression, our results in **Figure 3** show that JNK activity is upregulated in FMO-OE cell lines under cadmium stress. This result suggests that SAPKs, which are responsive to multiple stresses, are more readily activated in FMO-OE cells and could lead to their improved stress resistance. Interestingly, SAPK activity is reported to closely correlate with longevity. JNK acts in parallel with the insulin-like signaling pathway and directly phosphorylates DAF-16/FOXO, the forkhead transcription factor, leading to lifespan extension in *C. elegans* (Oh et al., 2005). Another SAPK, p38, also acts in the insulin-like signaling pathway for lifespan extension in *C. elegans* (Troemel et al., 2006). ERK also promotes longevity through two pro-longevity transcriptional factors, SKN-1 and DAF-16, in *C. elegans* (Okuyama et al., 2010). Similar to FMOs, increased levels of ERK activity are also found in long-lived mice models, including Snell dwarf mice and caloric restricted mice (Ikeyama et al., 2002; Madsen et al., 2004). Future studies will need to further examine whether JNK loss-of-function mutations diminish the increased stress resistance in mammalian FMO-OE cells or if JNK is necessary for the lifespan extension by *fmo-2* overexpression in *C. elegans*. It is also not clear how JNK activity is upregulated by FMO-overexpression and what substrates and metabolites produced by FMOs might lead to the activation of JNK.

The primary mechanism of FMO activity is to oxygenate substrates containing soft-nucleophiles, such as nitrogen and sulfur. Our metabolic data do not test what the key endogenous substrates may be, but they will be of great interest based on their potential benefits. Our untargeted (**Figure 6A** and **Supplementary Figures 7–15**) and targeted metabolomics (**Figures 6B,C** and **Supplementary Figures 16–24**) analyses show that FMO expression significantly changes endogenous metabolism. The most regulated pathways of amino acid, carbohydrate, and energy metabolism indicate that FMOs may regulate the fundamental metabolism of the cell. Additional work will be necessary to identify the endogenous FMO substrates that are responsible for direct and/or indirect changes to broader metabolic pathways. It will be interesting to test possible FMO substrates from the set of metabolites identified in our untargeted and targeted metabolomics, using stress resistance in FMO-OE cells or lifespan extension by *fmo-2* in worms as a readout.

Together, despite tissue-specific, developmental and gender differential expression in mice and humans, when overexpressed individually in kidney or liver cells, all five FMOs provide similar stress resistance (**Figures 1,2** and **Supplementary Figures 3–5**) and share many primary pathways and metabolic changes (**Figure 6** and **Supplementary Figures 7–24** and **Supplementary Tables 1–11**). This redundancy and compensatory role of FMOs are reasonable because: (1) the recently resolved crystal structures of reconstructed ancestral FMO2, FMO3–6, and FMO5 showed that FMOs have a high degree of similarity in structure and also catalytic cavity (Nicoll et al., 2019); (2) this is in agreement with previous findings in mice showing that, although expressed in different tissues, FMO1, 2, and 4 knockout mice and FMO5 knockout mice display similar lean phenotypes and higher whole-body energy expenditure, while at different ages (Veeravalli et al., 2014; Gonzalez Malagon et al., 2015); and (3) resistance to multiple stressors conferred by overexpression of all five FMOs may indicate that FMOs share metabolic pathways to confer stress resistance. An interesting example of this is *FMO2*, which is non-functional in most humans due to a nonsense

mutation producing a truncated inactive protein (Dolphin et al., 1998; Whetstine et al., 2000; Veeramah et al., 2008). However, in our FMO2-OE cell lines where a full-length FMO2 was expressed, FMO2-OE has a similar stress resistance and metabolic profile as with other FMO-OE cells. This further indicates the redundancy and compensatory roles of FMOs. In line with this, it will be interesting to test other mammalian FMOs. Human *FMO6–11* exhibit characteristics of pseudogenes; however, the highly homologous mouse *Fmo6*, *Fmo9*, *Fmo12*, and *Fmo13* are predicted to produce polypeptides and thus may not be pseudogenes in mice (Hernandez et al., 2004). Further experimental analyses are needed to characterize them as functional FMOs; therefore, while we did not include these mouse FMOs in this study, they may be of interest. Overall, after years of research focusing primarily on the classic role of FMOs in metabolizing environmental chemicals and therapeutic drugs, we have shown new roles for FMOs in regulating cellular stress resistance and metabolism, which provides insights into an exciting new area of FMO research.

EXPERIMENTAL PROCEDURES

C. elegans Strain Maintenance and Paraquat Stress Assay

Standard procedures for *C. elegans* strain maintenance and handling were used. In detail, Wild-Type and FMO-2 OE strains were kept at 20°C in a temperature-controlled incubator on NGM (Nematode Growth Medium) with the food source of *Escherichia coli* OP50 seeded on top of NGM. For the paraquat stress assay, both strains were synchronized from eggs and grown to the fourth larvae stage (L4). For each replicate, 30 L4 worms of wild-type or FMO-2 OE were put on OP50-seeded NGM containing 5 mM paraquat. Three replicates of the same condition were included. Experimental animals were then scored every day and counted as dead when not responding to prodding under a dissection microscope.

Chemicals, Antibodies, Plasmids and Primers

Cadmium was purchased from HAMPTON RESEARCH (HR2-715, 1.0 M), and diluted to 10 mg/ml as stock solution. Paraquat (Methyl viologen dichloride hydrate) was purchased from Sigma (856177-1g), and dissolved fresh each day as a stock solution of 1 M. Sodium Arsenite Solution was purchased from HACH (104732-100 mL, 5 g/L). Rotenone was purchased from Sigma (R8857), and dissolved in DMSO as a stock solution of 100 mM. The following antibodies were used: mouse monoclonal antibody against FMO2 (Proteintech 67019-1-Ig) and polyclonal antibodies against FMO1 (Invitrogen PA5-95285), FMO3 (Abcam ab126711), FMO4 (Invitrogen PA5-79276), FMO5 (Proteintech 13699-1-AP), JNKs (Cell Signaling Technology 9252), phospho-SAPK/JNK (Thr183/Tyr185) (Cell Signaling Technology 9251), ERK1/2 (Cell Signaling Technology 4695), phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling Technology 9101), p38 (Cell Signaling Technology 9212), phospho-p38 MAPK (Thr180/Tyr182) (Cell Signaling Technology 9211), and GAPDH (Cell Signaling Technology 2118).

Mouse FMO1 (Accession No. U87456), FMO2 (Accession No. AF184981), and FMO3 (Accession No. NM_008030) were cloned from mouse liver cDNA (ZYAGEN) and inserted into Gateway™ pcDNA™-DEST47 Vector (Invitrogen 12281010). Mouse FMO5 (Accession No. U90535) cDNA was cloned from mouse FMO5 ORF clone purchased from ORIGENE and then inserted into Gateway™ pcDNA™-DEST47 Vector (Invitrogen 12281010). Mouse FMO4 (Accession No. NM_144878) in pcDNA 3.1 (+) was purchased from GenScript. All constructs were confirmed by DNA sequencing. These FMOs constructs were then transfected into HEK293A or HepG2 cells using TransIT®-LT1 (Mirus MIR2300).

Quantitative Real-Time PCR

Total RNA was extracted using RNeasy Mini Kit (QIAGEN) and 1 μg RNA was reverse transcribed to cDNA by Maxima™ H Minus cDNA Synthesis Master Mix (Invitrogen). qPR-PCR was performed with 1 μg of cDNA and SYBR™ Green PCR Master (Applied Biosystems). β-2-microglobulin was used as a housekeeping gene control for FMO mRNA level normalization. FMO mRNA expression in the FMO-OE cell line was compared to the empty vector pDEST control as fold change. The following qPCR primers were used to confirm the overexpression levels of FMOs in the stable FMO-OE cell lines: mFMO1 forward primer (5′-ACAGCCGACAGTATAAACATCCA-3′) and reverse primer (5′-CCCTCCAGTAGTGCTGAGGAACA-3′); mFMO2 forward primer (5′- AGTGGCCTAATCTCTCTGAAGT-3′) and reverse primer (5′-CATCGGGAAGTCACTGAAACAG-3′); mFMO3 forward primer (5′-ACTGGTGGTACACAAGGCAG-3′) and reverse primer (5′-ATGGTCCCATCCTCAAACACA-3′); mFMO4 forward primer (5′-GATTGGAGCTGGCGTAAGT G-3′) and reverse primer (5′-TGTCAGCAAACTTCCACAGTC-3′); mFMO5 forward primer (5′-GAGGGCTTGGAACCTGTCT G-3′) and reverse primer (5′-CACGGACTGGTAAATAC TGGC-3′).

Cell Culture and Stress Resistance Assay

HEK293A and HepG2 cells were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. 2 μg of FMO plasmid was transfected into HEK293A or HepG2 cells using TransIT®-LT1 Transfection Reagent (Mirus) according to the manufacturer's instructions. 48 h after transfection, the cells were then cultured with G418 sulfate Geneticin™ (Gibco10131035) at 500 μg/ml to obtain G418-resistant cell lines. The stably transfected cells were maintained in medium containing 500 μg/ml G418 throughout cultures. For stress resistance assay, cells were seeded to 96-well microplates with 25,000 trypsinized cells per well for HEK293A cells and 40,000 cells per well for HepG2 cells. Empty vector control cells and FMO-overexpressing cells were seeded in triplicate for each dose of stressor on the same plate. After 16–18 h overnight incubation in complete medium, the cells were incubated for 18–24 h in serum-free

DMEM supplemented with 2% bovine serum albumin (BSA) as described previously (Murakami et al., 2003). For stress treatments, cells were exposed to indicated range of doses for 6 h (cadmium and paraquat stressors) or 24 h (rotenone and arsenite stressors) in 2% BSA supplemented DMEM, and then incubated in fresh 2% BSA supplemented DMEM without stressor for 18 h, followed by measurements of cell survival by Cell Proliferation Reagent WST-1 (Sigma 5015944001). For UV stress, the medium of the cells in the 96-well microplates were changed to 100 μL phosphate-buffered saline (PBS) and then irradiated with UV light for indicated range of intensity ($\mu J/cm^2$). After radiation, cells were incubated in 2% BSA supplemented DMEM for 18 h and followed by WST-1 cell survival measurements.

Measurement of Mitochondrial Respiration and Glycolysis

Cells were seeded to Seahorse XF96 Cell Culture Microplates with 40,000 trypsinized cells per well for HEK293A cells or HepG2 cells. The mitochondrial respiration rate was measured by oxygen consumption rate (OCR) detected by the Agilent Seahorse XFe96 Analyzer at 37°C. Glycolysis was measured by protons extruded into the extracellular media that can be detected by the Agilent Seahorse XFe96 Analyzer as extracellular acidification rates (ECAR) at 37°C. For mitochondrial respiration measurements, the analyzer stepwise injects oligomycin (2 μM final concentration per well), Carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) (1 μM final concentration per well), and rotenone/antimycin A (0.5 μM final concentration per well). The first injection of oligomycin, the ATP synthase inhibitor, decreases OCR correlated to mitochondrial respiration associated with ATP production. The second injection of FCCP, an uncoupling agent that collapses the proton gradient and the mitochondrial membrane potential, frees the electron flow through the electron transport chain (ETC) so that the oxygen is maximally consumed by complex IV, generating maximal respiration. The difference between basal respiration and maximal respiration defines the spare respiratory capacity responding to energy demand. The final injection is mixture of rotenone, a complex I inhibitor, and antimycin A, a complex III inhibitor, which completely inhibits mitochondrial respiration and allows the calculation of non-mitochondrial respiration from processes outside the mitochondria. In the glycolysis measurements, the analyzer stepwise injects glucose (10 mM final concentration per well), oligomycin (2 μM final concentration per well), and 2-deoxy-glucose (2-DG) (50 mM final concentration per well). The cells were cultured in XF Glycolysis stress test assay medium without glucose before the injections. The first injection of glucose catabolizes it through glycolysis to pyruvate, producing ATP, NADH, water, and protons. The second injection of oligomycin, an ATP synthase inhibitor, inhibits mitochondrial ATP production and shifts the energy production to glycolysis, producing the cellular maximum glycolytic capacity. The difference between glycolysis and glycolytic capacity indicates the glycolytic reserve. The final injection of 2-DG, a glucose analog, inhibits the first step of glycolysis through competitive binding to glucose hexokinase, resulting collapse of ECAR produced in the glycolysis. The detailed procedure followed the manufacturer's instructions in XFe96 Training Manual[1].

Metabolomics Assay

Sample preparation was performed by rinsing the cells with cold 150 mM ammonium acetate for less than 5 s. After removing the rinse buffer, cells were snap frozen directly in the plates by pouring liquid nitrogen into the plates, and then stored in −80°C until extraction. Metabolites were then identified by mass spectrometry. Specifically, metabolites were extracted from the cells by addition of 500 μL of ice-cold 9:1 methanol: chloroform. The resulting suspension was immediately transferred to tubes and probe sonicated for 10 s with a Branson 450 Sonicator. The resulting homogenates supernatant was then transferred to autosampler vials for analysis. Hydrophilic interaction liquid chromatography-electrospray ionization mass spectrometry (HILIC-LC-ESI -MS) analysis was performed in negative ion mode using an Agilent 1200 LC system coupled to an Agilent 6220 time-of-flight mass spectrometer. For chromatography, the Phenomenex Luna NH2 column was used with dimensions of 150 mm × 1.0 mm ID, 0.07 mL/min flow rate, and 10 μL injection volume, with LC gradient and MS parameters as previously described (Thonusin et al., 2017). The resulting untargeted metabolomics data were analyzed using MetaboAnalyst 4.0[2] in the MS Peaks to Paths module. The resulting targeted metabolomics data were analyzed using MetaboAnalyst 4.0 in the Statistical Analysis module with median normalization, log transformation and auto scaling adjustments.

Calculation of LD_{50} and Statistical Analyses

Mean survival rate from triplicates was used to determine the LD_{50} for each biological replicate of FMO-OE or empty vector control cell line by "Non-linear Regression" and "Dose-response-inhibition" after normalization, using GraphPad Prism 8.0.0. Some of the survival curves in response to stressors did not reach a survival rate under 50% at the highest dose of the stressor. In this situation, LD50 cannot be accurately calculated, therefore, we have listed them as greater than the highest dose we tested in the summarizing tables and showed the dose-dependent survival curves in the figures. Student *t*-test analysis was used to calculate *p*-values for comparisons between FMOs and empty vector control in stress assays, western blots, and seahorse assays. Log-rank test was used to derive *p*-value for survival curves comparison.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

[1] www.agilent.com

[2] http://metaboanalyst.ca

AUTHOR CONTRIBUTIONS

SL conceived the research. SL and SH designed the experiments, analyzed the data, and wrote the manuscript. SH performed the experiments. MH performed the sequence alignments. CD cloned FMOs genes and constructed FMOs plasmids. CE performed the metabolomics assay. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.630188/full#supplementary-material

Supplementary Figure 1 | Amino acid sequence alignment and sequence identities among reconstructed ancestral mammalian FMO5, *C. elegans* FMO-2, and mouse FMO1–5. **(A)** Full length amino acid alignment of reconstructed ancestral mammalian FMO5 with *C. elegans* and mouse FMOs. Eight essential residues in the catalytic active site were denoted as red arrow heads. **(B)** The percent identity among reconstructed ancestral mammalian FMO5, *C. elegans* FMO-2, and mouse FMO1–5 based on alignment using Clustal Omega.

Supplementary Figure 2 | Expression of FMO1–5 in HEK293A and HepG2 cells. FMO1–5 mRNA levels in HEK293A FMO-OE cells **(A)** and HepG2 FMO-OE cells **(B)** were examined by quantitative PCR. Relative ratios of FMOs mRNA levels between FMO-OE and empty vector control pDEST cells are shown.

Supplementary Figure 3 | FMOs improve stress resistance to oxidative stress in HEK 293A and HepG2 cells. **(A,B)** FMO1–4 OE and control cells survival curves on paraquat stress in HEK293A and HepG2. HEK293A cells **(A)** or HepG2 cells **(B)** stably expressing FMO1–4 or empty vector pDEST were subjected to indicated increasing doses of paraquat. **(C,D)** FMO1–4 OE and control cells survival curves on cadmium stress in HEK 293A and HepG2. HEK293A cells **(C)** or HepG2 cells **(D)** stably expressing FMO1–4 or empty vector pDEST were subjected to indicated increasing doses of cadmium. Data represent mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Supplementary Figure 4 | FMOs improve stress resistance to broader stressors in mammalian cells. **(A)** LD_{50} values of FMO1, FMO3, or FMO4 OE and control cells on arsenite stress in HEK293A. HEK293A cells stably expressing FMO or empty vector pDEST were subjected to indicated increasing doses of arsenite. **(B,C)** FMO1-4 OE and control cells survival curves on UV-radiation in HEK 293A and HepG2. HEK293A cells **(B)** or HepG2 cells **(C)** stably expressing FMO1-4 or empty vector pDEST were subjected to indicated increasing UV-radiation. Data represent mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Supplementary Figure 5 | FMOs improve stress resistance to rotenone in HEK 293A and HepG2 cells. FMO1–5 OE cells and control cells survival curves on rotenone stress in HEK293A and HepG2. HEK293A cells **(A)** or HepG2 cells **(B)** stably expressing FMO1–5 or empty vector pDEST were subjected to indicated increasing doses of rotenone. Data represent mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Supplementary Figure 6 | JNK kinase activity is increased in FMO-overexpressing cells under cadmium-induced oxidative stress. SAPKs levels and phosphorylation including JNK, p38, and ERK in FMO1–5 OE HEK293A cells **(A)** or HepG2 cells **(B)** and empty vector control cells after 10 μM Cadmium treatment for 4 h.

Supplementary Figures 7–15 | FMOs regulate amino acid, carbohydrate, and energetic metabolic pathways. Untargeted metabolomics in FMO1–5 OE cells compared to empty vector control cells, and the significantly regulated metabolic pathways are enriched (see also **Figure 6A**). **(A)** Metabolic pathways regulated by FMO1 (**Supplementary Figure 7A**), FMO2 (**Supplementary Figure 7B**), FMO3 (**Supplementary Figure 8A**), FMO4 (**Supplementary Figure 9A**) or FMO5 (**Supplementary Figure 10A**) in HEK293A cells and FMO1 (**Supplementary Figure 11A**), FMO2 (**Supplementary Figure 12A**), FMO3 (**Supplementary Figure 13A**), FMO4 (**Supplementary Figure 14A**), or FMO5 (**Supplementary Figure 15A**) in HepG2 cells are ranked according to the *p*-values from pathway enrichment analyses. Red indicates the most significantly changed pathways with $p < 0.001$. **(B)** Metabolic pathways regulated by FMO2 (**Supplementary Figure 7C**), FMO3 (**Supplementary Figure 8B**), FMO4 (**Supplementary Figure 9B**), or FMO5 (**Supplementary Figure 10B**) in HEK293A cells and FMO1 (**Supplementary Figure 11B**), FMO2 (**Supplementary Figure 12B**), FMO3 (**Supplementary Figure 13B**), FMO4 (**Supplementary Figure 14B**), or FMO5 (**Supplementary Figure 15B**) in HepG2 cells are plotted by the enrichment factor (obtained by dividing "significant hits" by "expected hits" for each pathway) on the *x*-axis and −log of the *p*-value on the *y*-axis. Red indicates significantly changed pathways with $p < 0.001$. Shared significantly regulated metabolic pathways by more than 3 FMOs in FMO1–5 are indicated by bold text.

Supplementary Figures 16–24 | Central carbon metabolism is regulated by FMOs. The abundance analyses of metabolites in central carbon metabolism in FMO-OE cells compared to empty vector control cells (see also **Figure 6**). **(A)** Principle Component Analysis (PCA) plot of the clusters of three replicates of FMO1-OE (**Supplementary Figure 16A**), FMO2-OE (**Supplementary Figure 16B**), FMO3-OE (**Supplementary Figure 17A**), FMO4-OE (**Supplementary Figure 18A**), or FMO5-OE (**Supplementary Figure 19A**) and empty vector control of HEK293A cells and FMO1-OE (**Supplementary Figure 20A**), FMO2-OE (**Supplementary Figure 21A**), FMO3-OE (**Supplementary Figure 22A**), FMO4-OE (**Supplementary Figure 23A**), or FMO5-OE (**Supplementary Figure 24A**) and empty vector control of HepG2 cells. **(B)** The levels of the top 25 changed metabolites between FMO-OE cells and empty vector control cells are shown in heat map (**Supplementary Figures 16C, 17B–24B**). **(C)** The levels of metabolites significantly regulated by FMOs are compared between FMO-OE cells and empty vector control pDEST cells (**Supplementary Figures 16D, 17C–24C**).

Supplementary Table 1 | Significant pathways in all FMOs in HEK293A and HepG2 cells from the untargeted metabolomics analyses. "Metabolism pathways" shows the significant pathways. "Categories of pathways" shows the category that the metabolic pathway is classified into according to KEGG (Kyoto Encyclopedia of Genes and Genomes) PATHWAY Database. Numbers shown under each FMO of HEK293A or HepG2 cells are the values of Fisher's exact test (FET) which is used to assess the significance of pathways from MetaboAnalyst 4.0 in the MS Peaks to Paths module. Only pathways with a significant FET < 0.05 are shown in this table.

Supplementary Tables 2–11 | Significantly regulated metabolites by FMOs from the targeted metabolomics analyses. The significantly changed metabolites between FMO1–5 OE and empty vector control in HEK293A cells (**Supplementary Tables 2–6**) and HepG2 cells (**Supplementary Tables 7–11**) are shown. "*p* value" shows the *p*-value of each metabolite from MetaboAnalyst 4.0 in the Statistical Analysis module by *t*-test with a threshold of 0.05.

REFERENCES

Beaty, N. B., and Ballou, D. P. (1981). The oxidative half-reaction of liver microsomal FAD-containing monooxygenase. *J. Biol. Chem.* 256, 4619–4625. doi: 10.1016/s0021-9258(19)69480-9

Bernstam, L., and Nriagu, J. (2000). Molecular aspects of arsenic stress. *J. Toxicol. Environ. Health B Crit. Rev.* 3, 293–322. doi: 10.1080/109374000436355

Capdevila, J., Saeki, Y., and Falck, J. R. (1984). The mechanistic plurality of cytochrome P-450 and its biological ramifications. *Xenobiotica* 14, 105–118. doi: 10.3109/00498258409151401

Cavigelli, M., Li, W. W., Lin, A., Su, B., Yoshioka, K., and Karin, M. (1996). The tumor promoter arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase. *EMBO J.* 15, 6269–6279. doi: 10.1002/j.1460-2075.1996.tb01017.x

Chen, L., and Liu, B. (2017). Relationships between stress granules, oxidative stress, and neurodegenerative diseases. *Oxid. Med. Cell Longev.* 2017:1809592.

Cherrington, N. J., Cao, Y., Cherrington, J. W., Rose, R. L., and Hodgson, E. (1998). Physiological factors affecting protein expression of flavin-containing monooxygenases 1, 3 and 5. *Xenobiotica* 28, 673–682. doi: 10.1080/004982598239254

Chuang, S. M., Wang, I. C., and Yang, J. L. (2000). Roles of JNK, p38 and ERK mitogen-activated protein kinases in the growth inhibition and apoptosis induced by cadmium. *Carcinogenesis* 21, 1423–1432. doi: 10.1093/carcin/21.5.423

Cicenas, J., Zalyte, E., Rimkus, A., Dapkus, D., Noreika, R., and Urbonavicius, S. (2017). JNK, p38, ERK, and SGK1 inhibitors in cancer. *Cancers* 10:1. doi: 10.3390/cancers10010001

Corre, I., Paris, F., and Huot, J. (2017). The p38 pathway, a major pleiotropic cascade that transduces stress and metastatic signals in endothelial cells. *Oncotarget* 8, 55684–55714. doi: 10.18632/oncotarget.18264

Derijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., et al. (1994). JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76, 1025–1037. doi: 10.1016/0092-8674(94)90380-8

Dolphin, C. T., Beckett, D. J., Janmohamed, A., Cullingford, T. E., Smith, R. L., Shephard, E. A., et al. (1998). The flavin-containing monooxygenase 2 gene (FMO2) of humans, but not of other primates, encodes a truncated, nonfunctional protein. *J. Biol. Chem.* 273, 30599–30607. doi: 10.1074/jbc.273.46.30599

Dolphin, C. T., Cullingford, T. E., Shephard, E. A., Smith, R. L., and Phillips, I. R. (1996). Differential developmental and tissue-specific regulation of expression of the genes encoding three members of the flavin-containing monooxygenase family of man, FMO1, FMO3 and FM04. *Eur. J. Biochem.* 235, 683–689. doi: 10.1111/j.1432-1033.1996.00683.x

Dolphin, C. T., Janmohamed, A., Smith, R. L., Shephard, E. A., and Phillips, I. R. (1997). Missense mutation in flavin-containing mono-oxygenase 3 gene, FMO3, underlies fish-odour syndrome. *Nat. Genet.* 17, 491–494. doi: 10.1038/ng1297-491

Escobar Mdel, C., Souza, V., Bucio, L., Hernandez, E., Gomez-Quiroz, L. E., and Gutierrez Ruiz, M. C. (2009). MAPK activation is involved in cadmium-induced Hsp70 expression in HepG2 cells. *Toxicol. Mech. Methods* 19, 503–509. doi: 10.3109/15376510903325670

Gonzalez Malagon, S. G., Melidoni, A. N., Hernandez, D., Omar, B. A., Houseman, L., Veeravalli, S., et al. (2015). The phenotype of a knockout mouse identifies flavin-containing monooxygenase 5 (FMO5) as a regulator of metabolic ageing. *Biochem. Pharmacol.* 96, 267–277. doi: 10.1016/j.bcp.2015.05.013

Hao da, C., Chen, S. L., Mu, J., and Xiao, P. G. (2009). Molecular phylogeny, long-term evolution, and functional divergence of flavin-containing monooxygenases. *Genetica* 137, 173–187. doi: 10.1007/s10709-009-9382-y

Harper, J. M., Salmon, A. B., Chang, Y., Bonkowski, M., Bartke, A., and Miller, R. A. (2006). Stress resistance and aging: influence of genes and nutrition. *Mech. Ageing Dev.* 127, 687–694. doi: 10.1016/j.mad.2006.04.002

Harper, J. M., Wang, M., Galecki, A. T., Ro, J., Williams, J. B., and Miller, R. A. (2011). Fibroblasts from long-lived bird species are resistant to multiple forms of stress. *J. Exp. Biol.* 214, 1902–1910. doi: 10.1242/jeb.054643

Hernandez, D., Janmohamed, A., Chandan, P., Phillips, I. R., and Shephard, E. A. (2004). Organization and evolution of the flavin-containing monooxygenase genes of human and mouse: identification of novel gene and pseudogene clusters. *Pharmacogenetics* 14, 117–130. doi: 10.1097/00008571-200402000-00006

Hines, R. N., Hopp, K. A., Franco, J., Saeian, K., and Begun, F. P. (2002). Alternative processing of the human FMO6 gene renders transcripts incapable of encoding a functional flavin-containing monooxygenase. *Mol. Pharmacol.* 62, 320–325. doi: 10.1124/mol.62.2.320

Houseman, L., Edwards, M., Phillips, I. R., and Shephard, E. A. (2015). Isolation and culture of mouse hepatocytes: gender-specific gene expression responses to chemical treatments. *Methods Mol. Biol.* 1250, 3–12. doi: 10.1007/978-1-4939-2074-7_1

Ikeyama, S., Kokkonen, G., Shack, S., Wang, X. T., and Holbrook, N. J. (2002). Loss in oxidative stress tolerance with aging linked to reduced extracellular signal-regulated kinase and Akt kinase activities. *FASEB J.* 16, 114–116.

Janmohamed, A., Hernandez, D., Phillips, I. R., and Shephard, E. A. (2004). Cell-, tissue-, sex- and developmental stage-specific expression of mouse flavin-containing monooxygenases (Fmos). *Biochem. Pharmacol.* 68, 73–83. doi: 10.1016/j.bcp.2004.02.036

Jones, K. C., and Ballou, D. P. (1986). Reactions of the 4a-hydroperoxide of liver microsomal flavin-containing monooxygenase with nucleophilic and electrophilic substrates. *J. Biol. Chem.* 261, 2553–2559. doi: 10.1016/s0021-9258(17)35823-4

Kaletsky, R., Yao, V., Williams, A., Runnels, A. M., Tadych, A., Zhou, S., et al. (2018). Transcriptome analysis of adult *Caenorhabditis elegans* cells reveals tissue-specific gene and isoform expression. *PLoS Genet.* 14:e1007559. doi: 10.1371/journal.pgen.1007559

Klintworth, H., Newhouse, K., Li, T., Choi, W. S., Faigle, R., and Xia, Z. (2007). Activation of c-Jun N-terminal protein kinase is a common mechanism underlying paraquat- and rotenone-induced dopaminergic cell apoptosis. *Toxicol. Sci.* 97, 149–162. doi: 10.1093/toxsci/kfm029

Lang, D. H., Yeung, C. K., Peter, R. M., Ibarra, C., Gasser, R., Itagaki, K., et al. (1998). Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. *Biochem. Pharmacol.* 56, 1005–1012. doi: 10.1016/s0006-2952(98)00218-4

Larsen, P. L. (1993). Aging and resistance to oxidative damage in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 90, 8905–8909. doi: 10.1073/pnas.90.19.8905

Leiser, S. F., Miller, H., Rossner, R., Fletcher, M., Leonard, A., Primitivo, M., et al. (2015). Cell nonautonomous activation of flavin-containing monooxygenase promotes longevity and health span. *Science* 350, 1375–1378. doi: 10.1126/science.aac9257

Lin, Y. J., Seroude, L., and Benzer, S. (1998). Extended life-span and stress resistance in the *Drosophila* mutant methuselah. *Science* 282, 943–946. doi: 10.1126/science.282.5390.943

Lithgow, G. J., White, T. M., Melov, S., and Johnson, T. E. (1995). Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7540–7544. doi: 10.1073/pnas.92.16.7540

Lu, A. Y., Harada, N., and Miwa, G. T. (1984). Rate-limiting steps in cytochrome P-450-catalysed reactions: studies on isotope effects in the O-de-ethylation of 7-ethoxycoumarin. *Xenobiotica* 14, 19–26. doi: 10.3109/00498258409151396

Madsen, M. A., Hsieh, C. C., Boylston, W. H., Flurkey, K., Harrison, D., and Papaconstantinou, J. (2004). Altered oxidative stress response of the long-lived Snell dwarf mouse. *Biochem. Biophys. Res. Commun.* 318, 998–1005. doi: 10.1016/j.bbrc.2004.04.126

Murakami, S., and Johnson, T. E. (1996). A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*. *Genetics* 143, 1207–1218. doi: 10.1093/genetics/143.3.1207

Murakami, S., Salmon, A., and Miller, R. A. (2003). Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J.* 17, 1565–1566. doi: 10.1096/fj.02-1092fje

Newhouse, K., Hsuan, S. L., Chang, S. H., Cai, B., Wang, Y., and Xia, Z. (2004). Rotenone-induced apoptosis is mediated by p38 and JNK MAP kinases in human dopaminergic SH-SY5Y cells. *Toxicol. Sci.* 79, 137–146. doi: 10.1093/toxsci/kfh089

Nicoll, C. R., Bailleul, G., Fiorentini, F., Mascotti, M. L., Fraaije, M. W., and Mattevi, A. (2019). Ancestral-sequence reconstruction unveils the structural

basis of function in mammalian FMOs. *Nat. Struct. Mol. Biol.* 27, 14–24. doi: 10.1038/s41594-019-0347-2

Oh, S. W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R. J., and Tissenbaum, H. A. (2005). JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc. Natl. Acad. Sci. U.S.A.* 102, 4494–4499. doi: 10.1073/pnas.0500749102

Okuyama, T., Inoue, H., Ookuma, S., Satoh, T., Kano, K., Honjoh, S., et al. (2010). The ERK-MAPK pathway regulates longevity through SKN-1 and insulin-like signaling in *Caenorhabditis elegans*. *J. Biol. Chem.* 285, 30274–30281. doi: 10.1074/jbc.m110.146274

Overby, L. H., Carver, G. C., and Philpot, R. M. (1997). Quantitation and kinetic properties of hepatic microsomal and recombinant flavin-containing monooxygenases 3 and 5 from humans. *Chem. Biol. Interact.* 106, 29–45. doi: 10.1016/s0009-2797(97)00055-0

Ozkurede, U., and Miller, R. A. (2019). Improved mitochondrial stress response in long-lived Snell dwarf mice. *Aging Cell* 18:e13030.

Peng, J., Mao, X. O., Stevenson, F. F., Hsu, M., and Andersen, J. K. (2004). The herbicide paraquat induces dopaminergic nigral apoptosis through sustained activation of the JNK pathway. *J. Biol. Chem.* 279, 32626–32632. doi: 10.1074/jbc.m404596200

Petalcorin, M. I., Joshua, G. W., Agapow, P. M., and Dolphin, C. T. (2005). The fmo genes of *Caenorhabditis elegans* and *C. briggsae*: characterisation, gene expression and comparative genomic analysis. *Gene* 346, 83–96. doi: 10.1016/j.gene.2004.09.021

Poulsen, L. L., Masters, B. S., and Ziegler, D. M. (1976). Mechanism of 2-naphthylamine oxidation catalysed by pig liver microsomes. *Xenobiotica* 6, 481–498. doi: 10.3109/00498257609151661

Poulsen, L. L., and Ziegler, D. M. (1979). The liver microsomal FAD-containing monooxygenase. Spectral characterization and kinetic studies. *J. Biol. Chem.* 254, 6449–6455. doi: 10.1016/s0021-9258(18)50388-4

Poulsen, L. L., and Ziegler, D. M. (1995). Multisubstrate flavin-containing monooxygenases: applications of mechanism to specificity. *Chem. Biol. Interact.* 96, 57–73. doi: 10.1016/0009-2797(94)03583-t

Salmon, A. B., Murakami, S., Bartke, A., Kopchick, J., Yasumura, K., and Miller, R. A. (2005). Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress. *Am. J. Physiol. Endocrinol. Metab.* 289, E23–E29.

Satarug, S., Garrett, S. H., Sens, M. A., and Sens, D. A. (2010). Cadmium, environmental exposure, and health outcomes. *Environ. Health Perspect.* 118, 182–190. doi: 10.1289/ehp.0901234

Scott, F., Gonzalez Malagon, S. G., O'Brien, B. A., Fennema, D., Veeravalli, S., Coveney, C. R., et al. (2017). Identification of flavin-containing monooxygenase 5 (FMO5) as a regulator of glucose homeostasis and a potential sensor of gut bacteria. *Drug Metab. Dispos.* 45, 982–989. doi: 10.1124/dmd.117.076612

Shih, D. M., Wang, Z., Lee, R., Meng, Y., Che, N., Charugundla, S., et al. (2015). Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. *J. Lipid Res.* 56, 22–37. doi: 10.1194/jlr.m051680

Siddens, L. K., Henderson, M. C., Vandyke, J. E., Williams, D. E., and Krueger, S. K. (2008). Characterization of mouse flavin-containing monooxygenase transcript levels in lung and liver, and activity of expressed isoforms. *Biochem. Pharmacol.* 75, 570–579. doi: 10.1016/j.bcp.2007.09.006

Steinbaugh, M. J., Sun, L. Y., Bartke, A., and Miller, R. A. (2012). Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. *Am. J. Physiol. Endocrinol. Metab.* 303, E488–E495.

Swindell, W. R. (2009). Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. *BMC Genomics* 10:585. doi: 10.1186/1471-2164-10-585

Thonusin, C., IglayReger, H. B., Soni, T., Rothberg, A. E., Burant, C. F., and Evans, C. R. (2017). Evaluation of intensity drift correction strategies using MetaboDrift, a normalization tool for multi-batch metabolomics data. *J. Chromatogr. A* 1523, 265–274. doi: 10.1016/j.chroma.2017.09.023

Troemel, E. R., Chu, S. W., Reinke, V., Lee, S. S., Ausubel, F. M., and Kim, D. H. (2006). p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet.* 2:e183. doi: 10.1371/journal.pgen.0020183

Tsai, J. S., Chao, C. H., and Lin, L. Y. (2016). Cadmium activates multiple signaling pathways that coordinately stimulate Akt activity to enhance c-Myc mRNA stability. *PLoS One* 11:e0147011. doi: 10.1371/journal.pone.0147011

Veeramah, K. R., Thomas, M. G., Weale, M. E., Zeitlyn, D., Tarekegn, A., Bekele, E., et al. (2008). The potentially deleterious functional variant flavin-containing monooxygenase 2*1 is at high frequency throughout sub-Saharan Africa. *Pharmacogenet. Genomics* 18, 877–886. doi: 10.1097/fpc.0b013e3283097311

Veeravalli, S., Karu, K., Scott, F., Fennema, D., Phillips, I. R., and Shephard, E. A. (2018). Effect of flavin-containing monooxygenase genotype, mouse strain, and gender on trimethylamine n-oxide production, plasma cholesterol concentration, and an index of atherosclerosis. *Drug Metab. Dispos.* 46, 20–25. doi: 10.1124/dmd.117.077636

Veeravalli, S., Omar, B. A., Houseman, L., Hancock, M., Gonzalez Malagon, S. G., Scott, F., et al. (2014). The phenotype of a flavin-containing monooyxgenase knockout mouse implicates the drug-metabolizing enzyme FMO1 as a novel regulator of energy balance. *Biochem. Pharmacol.* 90, 88–95. doi: 10.1016/j.bcp.2014.04.007

Veeravalli, S., Phillips, I. R., Freire, R. T., Varshavi, D., Everett, J. R., and Shephard, E. A. (2020). Flavin-containing monooxygenase 1 catalyzes the production of taurine from hypotaurine. *Drug Metab. Dispos.* 48, 378–385. doi: 10.1124/dmd.119.089995

Wang, M., and Miller, R. A. (2012). Fibroblasts from long-lived mutant mice exhibit increased autophagy and lower TOR activity after nutrient deprivation or oxidative stress. *Aging Cell* 11, 668–674. doi: 10.1111/j.1474-9726.2012.00833.x

Wang, M. C., Bohmann, D., and Jasper, H. (2003). JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. *Dev. Cell* 5, 811–816. doi: 10.1016/s1534-5807(03)00323-x

Warrier, M., Shih, D. M., Burrows, A. C., Ferguson, D., Gromovsky, A. D., Brown, A. L., et al. (2015). The TMAO-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. *Cell Rep.* 10, 326–338. doi: 10.1016/j.celrep.2014.12.036

Whetstine, J. R., Yueh, M. F., McCarver, D. G., Williams, D. E., Park, C. S., Kang, J. H., et al. (2000). Ethnic differences in human flavin-containing monooxygenase 2 (FMO2) polymorphisms: detection of expressed protein in African-Americans. *Toxicol. Appl. Pharmacol.* 168, 216–224. doi: 10.1006/taap.2000.9050

Yeung, C. K., Lang, D. H., Thummel, K. E., and Rettie, A. E. (2000). Immunoquantitation of FMO1 in human liver, kidney, and intestine. *Drug Metab. Dispos.* 28, 1107–1111. doi: 10.3109/00498259109039550

Zhang, J., and Cashman, J. R. (2006). Quantitative analysis of FMO gene mRNA levels in human tissues. *Drug Metab. Dispos.* 34, 19–26. doi: 10.1124/dmd.105.006171

Zhang, T., Yang, P., Wei, J., Li, W., Zhong, J., Chen, H., et al. (2018). Overexpression of flavin-containing monooxygenase 5 predicts poor prognosis in patients with colorectal cancer. *Oncol. Lett.* 15, 3923–3927.

Zhao, H., Liu, W., Wang, Y., Dai, N., Gu, J., Yuan, Y., et al. (2015). Cadmium induces apoptosis in primary rat osteoblasts through caspase and mitogen-activated protein kinase pathways. *J. Vet. Sci.* 16, 297–306. doi: 10.4142/jvs.2015.16.3.297

Ziegler, D. M., Poulsen, L. L., and McKee, E. M. (1971). Interaction of primary amines with a mixed-function amine oxidase isolated from pig liver microsomes. *Xenobiotica* 1, 523–531. doi: 10.3109/00498257109041521

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REVIEW
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Regulation of Age-Related Protein Toxicity

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Proteome damage plays a major role in aging and age-related neurodegenerative diseases. Under healthy conditions, molecular quality control mechanisms prevent toxic protein misfolding and aggregation. These mechanisms include molecular chaperones for protein folding, spatial compartmentalization for sequestration, and degradation pathways for the removal of harmful proteins. These mechanisms decline with age, resulting in the accumulation of aggregation-prone proteins that are harmful to cells. In the past decades, a variety of fast- and slow-aging model organisms have been used to investigate the biological mechanisms that accelerate or prevent such protein toxicity. In this review, we describe the most important mechanisms that are required for maintaining a healthy proteome. We describe how these mechanisms decline during aging and lead to toxic protein misassembly, aggregation, and amyloid formation. In addition, we discuss how optimized protein homeostasis mechanisms in long-living animals contribute to prolonging their lifespan. This knowledge might help us to develop interventions in the protein homeostasis network that delay aging and age-related pathologies.

Keywords: protein homeostasis, protein quality control, aggregation, phase separation, amyloid, aging

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INTRODUCTION

Declining protein homeostasis is a major cause of age-related diseases (Koga et al., 2011; López-Otín et al., 2013; Stroo et al., 2017). Tight regulation of protein homeostasis is required to maintain a stable proteome. Regulatory mechanisms include correct protein folding and removal of proteins that are no longer functional or required (Hipp et al., 2019). The ability of the protein homeostasis system to stabilize native proteins declines with age, resulting in protein misassembly, aggregation and cellular toxicity (reviewed in Hipp et al., 2019). Many forms of neurodegenerative diseases are age-dependent and develop in parallel to a decline in protein homeostasis pathways (reviewed in Klaips et al., 2018; Hipp et al., 2019). Recent studies have focused on age-related changes in protein homeostasis and have identified remarkable differences in the protein homeostasis systems of long-living species and their closely related short-living species (Gruber et al., 2015; Rodriguez et al., 2016; Du et al., 2020; Lagunas-Rangel, 2020). Although most protein homeostasis pathways are generally the same, differences in expression and function of certain protein homeostasis components may contribute to longevity and healthy aging.

Most of what we know about protein homeostasis and aging has come from studies in *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Mus musculus*. These models are useful for studying aging and age-related diseases because they are easy to maintain and have short lifespans (i.e., they age quickly) (Valenzano et al., 2017). In addition,

they are well characterized and their genomes have been fully sequenced (C. elegans Sequencing Consortium, 1998; Adams et al., 2000; Waterston et al., 2002; Engel et al., 2014). Since a decline in protein homeostasis has been proposed to cause aging, researchers have looked for ways to optimize protein homeostasis to prevent or delay the development of age-related diseases. Long-living *C. elegans*, *S. cerevisiae*, *D. melanogaster* and *M. musculus* mutant models help us understand which mechanisms are important for longevity (Kenyon et al., 1993; Clancy et al., 2001; Brown-Borg and Bartke, 2012; Muid et al., 2019). However, examining those species that have naturally evolved as long-living might provide additional clues about the mechanisms of healthy aging (Cohen, 2018). For example, long-living animal species like bivalve mollusks, naked mole-rats, and bats have developed mechanisms to reduce reactive oxygen species (ROS) production and to protect their proteomes from unfolding (Brunet-Rossinni, 2004; Gruber et al., 2015; Treaster et al., 2015). Increased chaperone production, autophagy, and proteasome activity further prolong the lifespan of these long-living species (Pérez et al., 2009; Rodriguez et al., 2014, 2016).

In this review, we discuss the most important protein homeostasis mechanisms for a healthy proteome. We summarize the current knowledge on factors and pathways that play a role in mammalian protein homeostasis and how changes in protein homeostasis can contribute to aging. We also discuss what we can learn from protein homeostasis machineries in short- and long-living animal species. These lessons could suggest interventions for improving protein homeostasis in humans to prevent or delay the onset of age-related diseases.

PROTEIN HOMEOSTASIS DECLINES WITH AGING

Protein Synthesis

The protein homeostasis system regulates protein function from the moment a protein is synthesized to when it is degraded or secreted. A strict balance between protein synthesis, folding, and degradation is needed to maintain the protein levels required for normal cellular function without overwhelming the protein quality control machinery. The number of proteins that can be synthesized depends on several factors, including the availability of mRNA transcripts and ribosomes for protein translation (Walther et al., 2015; Hipp et al., 2019). The presence and activity of translation initiation (eIF) and elongation factors (eEF) (e.g., eIF2α, eIF4E and eEF2) additionally determine the rate of protein synthesis (Papadopoli et al., 2019; Xie et al., 2019; Anisimova et al., 2020). For example, phosphorylation of eIF2α inhibits protein synthesis, and multiple studies have shown that a reduction in protein translation improves health and extends lifespan (Hansen et al., 2007; Pan et al., 2007; Pakos-Zebrucka et al., 2016; Xie et al., 2019). Other initiation and elongation factors are regulated by the mechanistic target of rapamycin complex 1 (mTORC1)-signaling pathway (Papadopoli et al., 2019; Xie et al., 2019). mTORC1 is an important regulator of protein synthesis and mediates, for example, the phosphorylation eIF4E-binding proteins (4E-BPs) that control the activity of the translation initiation factor eIF4E (Papadopoli et al., 2019). In addition, mTORC1 mediates translation accuracy by controlling eEF2 kinase (Xie et al., 2019).

During aging, protein synthesis rates decline (Dhondt et al., 2017; Yang et al., 2019). Inhibition of protein translation could be a protective mechanism to reduce the burden on protein quality control machineries and to restore protein homeostasis (Hipp et al., 2019). This decline may in part result from a reduction in ribosome abundance during aging (Walther et al., 2015). On the other hand, however, mTOR activity increases with increasing age, which results in increased protein synthesis rates, and reduced expression of chaperones, autophagy and proteasome activity (Yang et al., 2012; Papadopoli et al., 2019). With increasing age, imbalances in protein homeostasis can therefore lead to chronic stress conditions, reduced phosphorylation and lack of inhibition of factors involved in protein synthesis (Ben-Zvi et al., 2009; Taylor, 2016).

Molecular Chaperones

After a polypeptide chain is synthesized, it undergoes structural conversions before being folded into its stable native state (Hartl, 2017). Many proteins need this stable 3D-structure to function properly, and correct folding is largely determined by the amino acid sequence of the protein (Anfinsen, 1973). Intra-molecular amino acid interactions like hydrogen bonds, disulfide bonds, electrostatic interactions, and hydrophobic interactions guide proteins toward their native conformation (Longo and Blaber, 2016). These intra-molecular forces are usually sufficient to fold short polypeptide chains, but ATP-dependent molecular chaperones are needed to fold larger globular proteins into their native conformation (**Figure 1**).

The main molecular chaperone families that are involved in *de novo* folding of newly synthesized proteins are the heat-shock proteins (Hsps) 70 and 90, and chaperonins. The most universal chaperone is Hsp70, which has multiple functions in protein homeostasis, and is involved in nascent protein folding at the ribosome, in post-translational refolding of aggregation-prone proteins in the cytosol, and in re-solubilization of aggregates (**Figure 1**) (reviewed in Matthias P Mayer and Gierasch, 2019). Once bound to Hsp70, substrate proteins are stabilized and ready for folding or refolding with the help of co-chaperones (e.g., Hsp40, Hsp90) or chaperonins (Hsp60) (Kim et al., 2013; Genest et al., 2015, 2019). ATP-dependent chaperones protect against protein aggregation by promoting the correct folding of unfolded or aggregation-prone proteins. In contrast, small heat shock proteins (sHsps) are ATP-independent chaperones that bind and hold onto unfolded protein species (reviewed in Mogk et al., 2018). During cellular stress, sHsps keep unfolded proteins in a refolding-competent state so that refolding can be initiated upon stress relief (Cashikar et al., 2005; Escusa-Toret et al., 2013; Ungelenk et al., 2016; Shen et al., 2019). sHsps cannot refold aggregation-prone proteins to their native state. For refolding, they require the assistance of ATP-dependent chaperones, like Hsp70. Under proteotoxic stress conditions chaperones levels are increased by cellular stress response pathways, such as the heat shock response. This cytosolic stress response, is regulated by heat

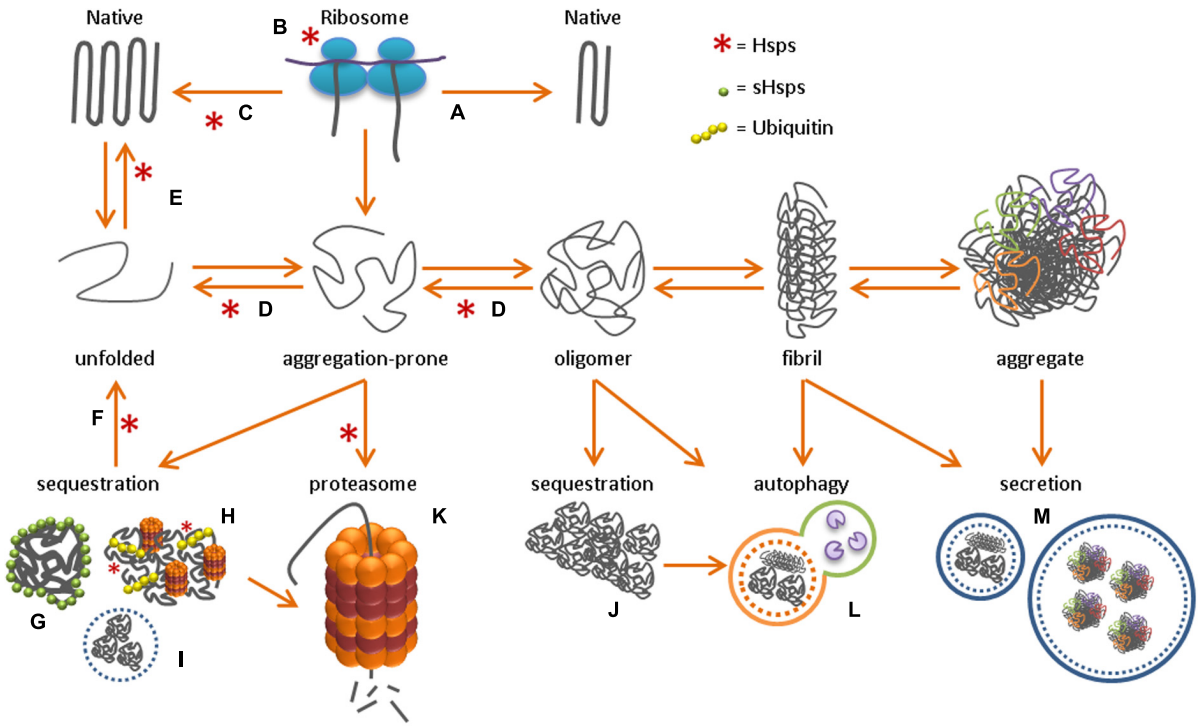

FIGURE 1 | Protein homeostasis network After the synthesis of a new polypeptide chain, the folding of a protein toward its native conformation can either directly occur through intermolecular interactions **(A)**, or with the help of molecular chaperones. Chaperones can either promote correct folding co-translationally at the ribosome **(B)** or post-translationally in the cytosol **(C)**. Upon toxic stress conditions, protein misfolding toward aggregation-prone species and oligomers can occur, followed by fibril formation and the formation of insoluble protein aggregates. To protect the cell from these toxic protein species, active unfolding **(D)**, re-folding **(E)** and re-solubilization of aggregates through disaggregation **(F)** occurs, with the help of molecular chaperones, while sHsps perform their function as 'holdase' in sHSP oligomers **(G)**. Alternatively, misfolded protein species can be sequestered in, for example, the JUNQ **(H)**, nucleolus **(I)** or aggresome **(J)**. Alternatively, proteins can be degraded by the proteasome **(K)** or through autophagy **(L)**, or secreted into the extracellular environment **(M)**.

shock factors (HSFs), of which HSF1 is the best characterized (Chaudhury et al., 2021).

Chaperones are also important when the number of misfolded proteins increase with aging. However, studies in C. elegans showed that the ability to activate the heat shock response reduces with increasing age (Ben-Zvi et al., 2009; Shemesh et al., 2013; Brehme et al., 2014; Labbadia and Morimoto, 2015). This decline in ability occurs early in adulthood, at the onset of oocyte biomass production, and seems a consequences of a reduced expression of the H3K27 demethylase jmjd-3.1 (Shemesh et al., 2013; Labbadia and Morimoto, 2015). A decline in the ability to induce chaperone expression with increasing age, was also found in senescent human lung fibroblasts (Sabath et al., 2020). In contrast, Walther and colleagues did not observe major changes in Hsp70 and Hsp90 expression in C. elegans throughout life, while sHsp expression even increased dramatically during aging (Walther et al., 2015). Most sHsps accumulated in aggregates during aging, indicating that cells actively sequester their proteins to cope with the increase in aggregation-prone proteins (Walther et al., 2015). In addition, the number of sHsp-associated inclusions strongly increased with aging in the long-living daf-2 C. elegans mutant, which suggests that sequestration is a protective mechanism (Walther et al., 2015). These findings agree with previous results in human tissue samples, where increased expression of sHsp genes was observed in the aging brain, and elevated sHsp levels were detected in skeletal muscles of aged individuals (Yamaguchi et al., 2007; Brehme et al., 2014).

Unfolded Protein Responses in the ER

Another important stress response pathway that regulates protein-folding is the unfolded protein response in the endoplasmic reticulum (ER), the UPR^{ER} pathway (Taylor, 2016; Frakes and Dillin, 2017). The ER creates a tightly regulated environment for folding, processing, and secretion of newly synthesized secretory and membrane proteins. The ER detects and responds to any imbalances in protein homeostasis, such as hypoxia, nutrient deprivation and excessive protein oxidation (Martínez et al., 2017). The three most important factors responsible for the UPR^{ER} are the transmembrane sensors inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6), and PKR-like ER kinase (PERK) (Frakes and Dillin, 2017). During non-stressful physiological conditions, these sensors are quiescent through interaction with the ER chaperone binding immunoglobulin protein (BiP). When the number of unfolded or aggregation-prone proteins increases, BiP is recruited and titrated away from the stress sensors, which activates the UPR^{ER} (Bertolotti et al., 2000; Carrara et al.,

2015). In addition, unfolded or aggregation-prone proteins can directly bind to the UPR^{ER} sensors and activate the UPR^{ER} (Gardner and Walter, 2011).

Activation of different UPR^{ER} components has been associated with lifespan extension, and results in reduced protein synthesis and increased expression of chaperones and factors contributing to proteasomal degradation (Taylor and Dillin, 2013; Luis et al., 2016; Martínez et al., 2017). Upon UPR^{ER} activation, the transcription factor X-box binding protein 1 (Xbp1) promotes transcription of genes encoding for chaperones and factors that promote ER-associated degradation. In neurons, overexpression of Xbp1 can prevent the decline in ability to induce the UPR^{ER} with aging (Frakes et al., 2020). At the same time, enhanced lipid biogenesis increases protein folding and protein degradation in the ER (Chalmers et al., 2017; Frakes and Dillin, 2017). However, the ability to induce the UPR^{ER} and its downstream targets decline with increasing age (Sabath et al., 2020; Taylor and Hetz, 2020). In addition, absolute UPR^{ER}-induced chaperone levels decrease during aging and the UPR^{ER}-regulated chaperones that are still present, accumulate increasing amounts of oxidative damage (Taylor, 2016). The role of UPR^{ER} in aging has recently been extensively reviewed by Taylor and Hetz (Taylor and Hetz, 2020).

Unfolded Protein Responses in the Mitochondria

Another important unfolded protein response mechanism is the mitochondrial UPR (UPR^{mt}). The UPR^{mt} is activated in response to different kinds of stressors. Examples include excessive amounts of reactive oxygen species (ROS) or impaired import of mitochondrial proteins due to damaged protein accumulation (Nargund et al., 2012; Fiorese et al., 2016; Shpilka and Haynes, 2018). Activation of the UPR^{mt} is required for repair of the mitochondrial network and maintenance of the mitochondrial function for the cell (Shpilka and Haynes, 2018). Mild, temporary mitochondrial stress is beneficial as it maintains protein homeostasis through upregulation of HSF-1 (Labbadia et al., 2017).

Chronic activation of the UPR^{mt}, however, which also occurs during aging, gradually impairs mitochondrial ATP production, and increases electron leakage (Shpilka and Haynes, 2018). Aging has therefore been associated with increased levels of ROS. A chronic increase in ROS production causes oxidative stress and contributes to the accumulation of DNA damage (e.g., mutations and chromosomal aneuploidies), RNA damage, and further mitochondrial damage (Korovila et al., 2017). All of which contribute to the increase in aggregation-prone proteins with aging (Faggioli et al., 2012; Forsberg et al., 2012; Liu et al., 2020). Furthermore, proteins can be directly damaged by oxidation, which induces structural changes and makes proteins more aggregation-prone (Serebryany et al., 2016; Lévy et al., 2019). Especially the amino acid cysteine (Cys) is susceptible due to the presence of a nucleophilic thiol-group, but also the amino acids tryptophan, tyrosine, methionine and histidine are prone for oxidation (Lévy et al., 2019). In addition, oxidation can alter the side-chain charges of amino acids, which affects native folding and repulsion between proteins (known as colloidal stability) (Samantha S. Strickler et al., 2006; Gribenko and Makhatadze, 2007; Beerten et al., 2012; De Baets et al., 2014). Altogether, age-related chronic mitochondrial stress results in the accumulation of ROS and damaged proteins, and in a reduction in ATP, which further contributes to the decline in mitochondrial function and results in an imbalance in protein homeostasis (Korovila et al., 2017; Shpilka and Haynes, 2018).

Regulated Sequestration and Disaggregation

Aggregation-prone proteins or prematurely terminated proteins (defective ribosomal products) can be stored in compartments such as the juxtanuclear quality control compartment (JUNQ) or membraneless nuclear bodies (Kaganovich et al., 2008; Mediani et al., 2019). In addition, functional amyloids assemble into storage sites termed amyloid (A)-bodies, to store proteins under stressful conditions. The formation of these storage sites might be a physiological mechanism to immobilize proteins and allow the cell to become dormant (Audas et al., 2016). Once the stressor is released, proteins in the A-bodies disaggregate back to a soluble state with the help of molecular chaperones. Several specialized protein quality control sites have been identified in the mammalian cell (reviewed in Sontag et al., 2017), including the JUNQ, the perivacuolar compartment (aggresome, equivalent to the insoluble protein deposit, IPOD, in yeast), and the nucleolus (Kaganovich et al., 2008; Frottin et al., 2019). Sorting of aggregation-prone cytosolic proteins to these distinct compartments depends on chaperone binding and ubiquitination. Soluble aggregation-prone proteins that are recognized by the protein quality control machinery are ubiquitinated and subsequently transported to the JUNQ (**Figure 1**; Sontag et al., 2017). The JUNQ contains disaggregating chaperones and 26S proteasomes, which increase the efficiency for refolding or degradation of aggregation-prone proteins (Kaganovich et al., 2008). However, if the quality control machinery is overwhelmed or impaired, aggregation-prone proteins might continue to accumulate. These bigger assemblies are usually directed to aggresomes, which terminally sequester small protein aggregates, including amyloidogenic aggregates (Johnston et al., 1998; **Figure 1**).

The role of the above-mentioned sequestration compartments in aging remains unclear. Studies indicate that soluble aggregation-prone proteins and oligomers have a pathological role in neurodegenerative diseases like Alzheimer's disease and Huntington's disease (Mucke et al., 2000; Arrasate et al., 2004; Iulita et al., 2014). The aggregation of these soluble proteins was therefore suggested as a protective mechanism to prevent cytotoxicity (Arrasate et al., 2004; Cohen et al., 2006; Hong et al., 2014). Indeed, hyperaggregation of amyloid-beta was associated with delayed age-related proteotoxicity of soluble amyloid-beta, upon reduction of insulin/insulin growth factor (IGF) signaling in an Alzheimer's mouse model (Cohen et al., 2009). As mentioned before, also the upregulation of sHsp

inclusions has been associated with lifespan extension in worms (Walther et al., 2015). However, how the formation of storage compartments affect aging remains unclear. While sequestration of aggregation-prone proteins might seem beneficial initially, these temporary storage sites might become permanent insoluble aggregates during chronic stress conditions such as aging. Cells may not be able to tolerate these large aggregates as they might sequester functional proteins, release aggregation-prone species back to the cellular environment, or interfere with cellular processes (Morley et al., 2002; Olzscha et al., 2011; Mogk et al., 2018).

Phase Separation and Liquid Droplet Formation

Proteins can also be compartmentalized by membraneless liquid-like organelles, which regulate cellular processes rather than store aggregation-prone and prematurely terminated proteins (Shin et al., 2017; Mediani et al., 2019). Well-known examples of membraneless compartments are P-bodies and stress granules in the cytoplasm, or Cajal bodies in the nucleus. They are normally characterized by their spherical composition and dynamic properties, and are therefore also known as liquid droplets (Brangwynne et al., 2009). These liquid-like compartments are formed by liquid-liquid phase separation (LLPS). Through LLPS, a compartment with a higher molecular concentration than its surrounding is formed. LLPS can be regulated by distinct proteins, including multivalent proteins (Li et al., 2012) and intrinsically disordered proteins (Kato et al., 2012; Uversky, 2017). Intrinsically disordered proteins (also known as natively unfolded proteins) have an amino acid sequence that does not favor folding into a 3D structure by itself. Most, but not all unfolded proteins go through a folding-upon-binding transition as soon as the protein binds to its physiological ligand (Bonetti et al., 2018; Fuxreiter, 2018). While low complexity domains are required for LLPS, interactions between RNA and RNA-recognition motifs further contribute to the assembly of liquid-like droplets (**Figure 2A**) (Teixeira et al., 2005; Molliex et al., 2015; Zhang et al., 2015). Liquid-like compartments have been implicated in several cellular processes, including organization and regulation of proteins in the cytosol, and the controlled release of sequestered molecules from cellular compartments (Boisvert et al., 2007; Kroschwald et al., 2015; Molliex et al., 2015; Wheeler et al., 2016; Banani et al., 2017; Shin and Brangwynne, 2017). Compartments with regulatory functions include the nucleolus and stress granules. In the event of stress, they regulate signaling molecules, mRNA, and transcription/translation complexes to prevent off-target interactions with other molecules. In addition, studies have suggested a role for liquid droplet formation in the nucleation and polymerization of actin and tubulin bundles (Banjade and Rosen, 2014; Hernández-Vega et al., 2017).

The dynamics of membrane-less compartments with high concentrations of proteins need to be tightly regulated and efficient protein quality control usually prevents the transition of stress granules toward a more solid state (Lechler et al., 2017). However, such phase transitions can occur if protein quality control mechanisms are impaired during aging, which can reduce the dynamic properties of liquid-like compartments and turn them into solid structures (Seguin et al., 2014). Also, the number of stress granules that are formed increases with aging (Lechler et al., 2017). Liquid-to-solid phase transitions occur more often with increasing age, potentially due to the presence of increased amounts of damaged proteins that may be recruited to membrane-less compartments and seed the formation of aggregates (Lechler et al., 2017; Hipp et al., 2019). Stress granules and other membrane-less compartments are enriched in RNA-binding and disordered proteins. Although there are fewer aggregation-prone regions in disordered proteins, their aggregation has been associated with aging and age-related (neurodegenerative) diseases (Linding et al., 2004). Changes in pH, protein concentration, salt concentration, or temperature can affect the viscosity of granules and transform liquid droplets into hydrogels or insoluble amyloid aggregates (**Figure 2B**; Alberti and Hyman, 2016; Mateju et al., 2017; Peskett et al., 2018). Also mutations in or adjacent to the low complexity domain of RNA-binding proteins could change the biophysical properties of liquid droplets and accelerate liquid-to-solid phase transitions (Patel et al., 2015; Gopal et al., 2017). Stress granules could therefore act as nucleation sites for pathological aggregates. Several neurodegenerative diseases have been associated with the aggregation of RNA-binding proteins, including TAR DNA binding protein of 43 kDa (TDP-43) and fused in sarcoma (FUS) (Patel et al., 2015; Gopal et al., 2017; Peskett et al., 2018; Ray et al., 2020).

Cellular Factors That Enhance Protein Aggregation and Toxicity

Protein homeostasis prevents toxic formation of protein aggregates. In recent years, however, a range of factors have been identified that can promote misfolding and aggregation of various disease-related proteins. For example, cytoplasmic polyphosphate (polyP) polymers, glucosaminoglycans like heparin, nucleic acids, and metal cations have been found to modify the aggregation of Tau, alpha-synuclein, prion protein, and amyloid-beta, respectively (Uversky et al., 2001; Jeffrey A. Cohlberg et al., 2002; Nandi et al., 2002; Yugay et al., 2016; Wickramasinghe et al., 2019). Although the exact mechanisms remain unresolved, studies suggest that interaction of polyP with Tau and metal cations with alpha-synuclein could alter the native protein conformation, increasing the probability of disease-related aggregation (Uversky et al., 2001; Wickramasinghe et al., 2019).

A group of proteins which were described for their aggregation promoting effects, called Modifiers of Aggregation (MOAGs), were originally identified in a chemical mutagenesis screen in a *C. elegans* model of neurodegenerative diseases. In this screen, MOAG-2 and MOAG-4 promoted polyglutamine aggregation (Van Ham et al., 2010; Sin et al., 2017). MOAG-2 (also known as *lin-26*-related gene 3 [*lir-3*]) was first identified as an aggregation-promoting factor that catalyzed the sequestration and toxicity of polyglutamine in large insoluble aggregates (Sin et al., 2017). However, this sequestration mechanism seemed to be a

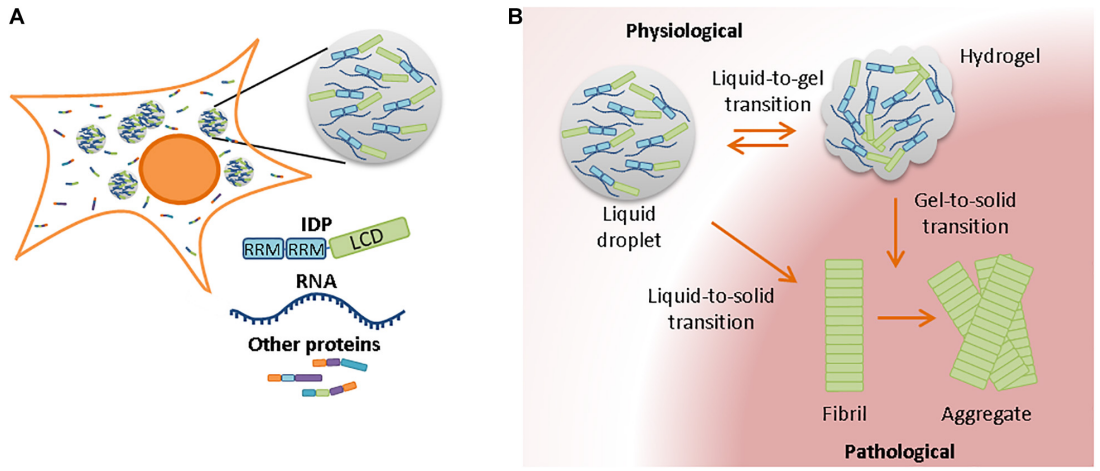

FIGURE 2 | Membrane-less compartments in the cell and their involvement in aggregation with aging **(A)** Liquid-liquid phase separation requires often, but not always, the presence of RNA and RNA-binding proteins. RNA-binding proteins contain RNA-recognition motives and a low-complexity domain in their protein sequences. **(B)** Due to the high concentration of proteins in liquid droplets, a strict protein homeostasis is required to prevent phase transitions from liquid-like compartments to less dynamic states. Although transition from hydrogel to liquid droplet is in some cases still possible, liquid-to-solid and gel-to-solid transitions normally result in the formation of pathological fibrils and insoluble aggregates.

consequence of MOAG-2/LIR-3 mislocalization from the nucleus to the cytosol in the presence of polyglutamine, rather than a protective compartmentalization mechanism in the presence of aggregation-prone proteins. In the absence of polyglutamine in wild-type *C. elegans*, MOAG-2/LIR-3 regulated the transcription of small non-coding RNAs (Sin et al., 2017).

Moag-4 encodes a small protein with unknown function that is evolutionarily highly conserved. MOAG-4 is a highly dynamic, intrinsically disordered protein that forms transient alpha-helical secondary structures but not tertiary conformations (Yoshimura et al., 2017). It was shown to act cell-autonomously and independent from quality control mechanisms such as chaperones or proteasomes (Van Ham et al., 2010). Also, its human orthologs, small EDRK-rich factors (SERF)1a and SERF2 were found to enhance off-pathway structural conversions for several unrelated amyloidogenic proteins (Van Ham et al., 2010; Falsone et al., 2012). MOAG-4, SERF1a, and SERF2 promote aggregation of proteins into compact aggregation intermediates that eventually become large, insoluble aggregates. SERF promotes aggregation through direct and transient interactions. Electrostatic interactions between MOAG-4/SERF1a and alpha-synuclein accelerate the formation of alpha-synuclein fibrils (Falsone et al., 2012; Yoshimura et al., 2017; Merle et al., 2019). This aggregation-promoting effect of SERF on alpha-synuclein was recently suggested to be a toxic side effect, mediated by an interaction between SERF1a and the negatively charged C-terminus of alpha-synuclein (Meyer et al., 2019). This study suggested that SERF1a acts as an RNA chaperone in the formation of liquid-like RNA organelles. Under stressful conditions, RNA and alpha-synuclein may compete for SERF binding, possibly favoring an interaction between alpha-synuclein and SERF that accelerates amyloid formation (Meyer et al., 2019). Such stressful conditions could arise during aging as protein homeostasis declines. Under these conditions, SERF-like proteins might turn into toxic factors and threaten the proteome. Nevertheless, the exact function of SERF in the proteome remains elusive.

Protein Degradation

Numerous mechanisms in the cell regulate protein degradation and secretion. These mechanisms are necessary to maintain physiological protein concentrations, clear the cell from proteins that are no longer required, and to avoid toxic accumulation of non-native proteins. Proteins are eliminated via three main pathways: proteasomal degradation, autophagy, and extracellular secretion (**Figure 1**).

Proteasome-mediated degradation is regulated by the ubiquitin-proteasome system (UPS), which is the primary route for eliminating non-native monomeric proteins. The autophagy-lysosome pathway (ALP) removes larger proteins, aggregates, or dysfunctional organelles (reviewed in Dikic, 2017). Proteins are directed to the UPS and ALP by chaperones and additional co-factors with ubiquitin ligase activity. The proteasome regulatory particle specifically recognizes ubiquitinated substrates and directs them to the proteasome core particle for degradation (Lander et al., 2012). With the help of ATP-dependent chaperones, the substrate proteins are unfolded and digested into peptides of 2–24 residues by protease enzymes.

The best characterized type of autophagy is macroautophagy, which is the degradation pathway for large components like cellular organelles and protein aggregates. Macroautophagy is mediated by a large family of autophagy-related proteins (ATG proteins) (Noda and Inagaki, 2015). Upon encapsulation of the substrate material, the autophagosome is formed and transported along microtubules to fuse together with lysosomes and form an autolysosome (Yu et al., 2018; **Figure 1**). Protease enzymes in the lysosome then degrade the encapsulated material. Another autophagy pathway, known as endosomal microautophagy in

mammals, involves the bulk or selective degradation of cytosolic proteins by endosomes (reviewed in Tekirdag and Cuervo, 2018). For bulk degradation, cytosolic substrates are directly trapped in late endosomes. For selective degradation, the heat shock cognate 70 (Hsc70) protein is required (Chiang and Dice, 1988; Sahu et al., 2011). Another type of chaperone-mediated autophagy in mammals involves the direct targeting of substrate proteins to the lysosome by Hsc70. Hsc70 regulates the delivery of the substrate protein to the lysosome by interacting with the lysosome-associated membrane protein 2A (Cuervo and Dice, 1996; Salvador et al., 2000). The substrate protein is then unfolded and translocated to the lysosome for degradation.

Protein degradation relieves the cell from protein overload and provides amino acids for further protein synthesis (Suraweera et al., 2012; Dikic, 2017). These processes are important for maintaining a healthy proteome. In *C. elegans*, an age-related decline in proteasomal function and autophagy was observed (Cuervo and Dice, 2000; Paisán-Ruíz et al., 2004; Tonoki et al., 2009; Brehme et al., 2014; Martinez-Lopez et al., 2015; Cho et al., 2018). The imbalance between production and clearance of misfolded proteins correlates with aging and ultimately results in protein supersaturation and aggregation (Ciryam et al., 2013, 2015). The proteostasis network tries to restore these imbalances by upregulating components of the ubiquitin proteasome system (Chondrogianni et al., 2015; Walther et al., 2015). This proteasomal upregulation has been associated with an increased lifespan in *C. elegans* (Chondrogianni et al., 2015).

PROTEIN HOMEOSTASIS IN SHORT- AND LONG-LIVING ANIMAL SPECIES

Several interventions have been shown to promote health and extend lifespan in model organisms, including upregulation and overexpression of different protein homeostasis components, such as HSF1, Hsp16, 19S proteasomal subunits, and selective autophagy receptors (Hsu et al., 2003; Walker and Lithgow, 2003; Morley and Morimoto, 2004; Vilchez et al., 2012b; Kumsta et al., 2019) (**Figure 3**). Most of this research was conducted in *C. elegans*, yeast and mouse models, however, understanding how improved protein homeostasis mechanisms contribute to the long lifespans of naturally evolved long-living animal species might additionally help us understand the mechanisms that are important in healthy aging.

Oxidative Stress Defense and Proteome Protection Contribute to Lifespan

Levels of oxidative stress and antioxidants have been correlated with life-expectancy in the 'oxidative stress hypothesis of aging' (Harman, 1956; Csiszar et al., 2007; Shi et al., 2013). In accordance with this hypothesis, long-living *daf-2* mutant *C. elegans* models maintain lower oxidative stress levels during their transition to adulthood than short-living *daf-16* mutant strains do (Knoefler et al., 2012). ROS levels have also been correlated with lifespan in several long-living animals, including the bivalve mollusk species *Arctica islandica*, and the little brown bat species *Myotis lucifugus* (Brunet-Rossinni, 2004; Ungvari et al., 2011, 2013; Gruber et al., 2015). In both animal species, ROS production is low (Brunet-Rossinni, 2004; Ungvari et al., 2011; Gruber et al., 2015). *A. islandica* is an ocean quahog that can live for over 500 years, and the age can be determined by counting the annual growth rings in the shell. In addition to low ROS production, *A. islandica* has shown increased resistance to (mitochondrial) oxidative stressors and most genotoxic stressors compared with shorter-living bivalve species (Salmon et al., 2009; Ungvari et al., 2011, 2013). Especially remarkable is the low level of antioxidant response upon acute stress exposure (Ungvari et al., 2011). In *C. elegans* the antioxidant enzyme superoxide dismutase (SOD) is not required for lifespan regulation, although it is necessary to be able to cope with acute stressors (Van Raamsdonk and Hekimi, 2009, 2012). In *A. islandica*, the exact mechanisms for the remarkable resistance against oxidative stressors remains unknown, but the resistance to genotoxic stressors indicates optimal defense pathways such as strong DNA repair mechanisms, as has also been proposed in several long-living mammals, including some long-living mouse species, muroid rodents, bats and primates (Salmon et al., 2008; Ungvari et al., 2008, 2013; A. Podlutsky et al., 2008). Low ROS levels and resistance to genome damage likely reduce protein damage, but to what extent this contributes to the exceptional long lifespan of *A. islandica* remains to be determined.

In *C. elegans*, significant changes in relative proteins abundance and solubility of the proteome have been reported with increasing age (David et al., 2010; Reis-Rodrigues et al., 2012; Walther et al., 2015). An imbalance between the production and clearance of insoluble proteins correlates positively with aging and ultimately results in protein supersaturation and subsequent protein aggregation (Ciryam et al., 2013, 2015). The formation of insoluble protein aggregates in turn further promotes aging (Reis-Rodrigues et al., 2012; Huang et al., 2019). *A. islandica* has an improved ability to protect its proteome for unfolding stressors (Treaster et al., 2014, 2015; Gruber et al., 2015). Although the optimal living temperature for *A. islandica* ranges between 5 and 15°C, most of its proteins stayed soluble even under extreme temperatures of 100°C (Treaster et al., 2014). In addition, *A. islandica* maintained 45% of its GAPDH activity in muscle tissue in the presence of 6M urea, indicating superior proteome protection (Treaster et al., 2015). The strong resistance to protein unfolding stressors in *A. islandica* could indicate a prominent role for molecular chaperones in stabilizing protein structures. However, ATP-dependent chaperones and small heat shock proteins could not be identified as responsible factors for the effective protein homeostasis in *A. islandica* in this study (Treaster et al., 2015). Which factors exactly are responsible for the stabilization of the proteome in *A. islandica* remain elusive.

Unique Mechanisms Promote the Health and Lifespan of Naked Mole-Rats

In contrast to *A. Islandica* and *M. lucifugus*, oxidative stress levels are higher in naked mole-rats in comparison with physiologically age-matched mice (Andziak et al., 2005, 2006; Pérez et al., 2009). Naked mole-rats can live for more than 30 years; they

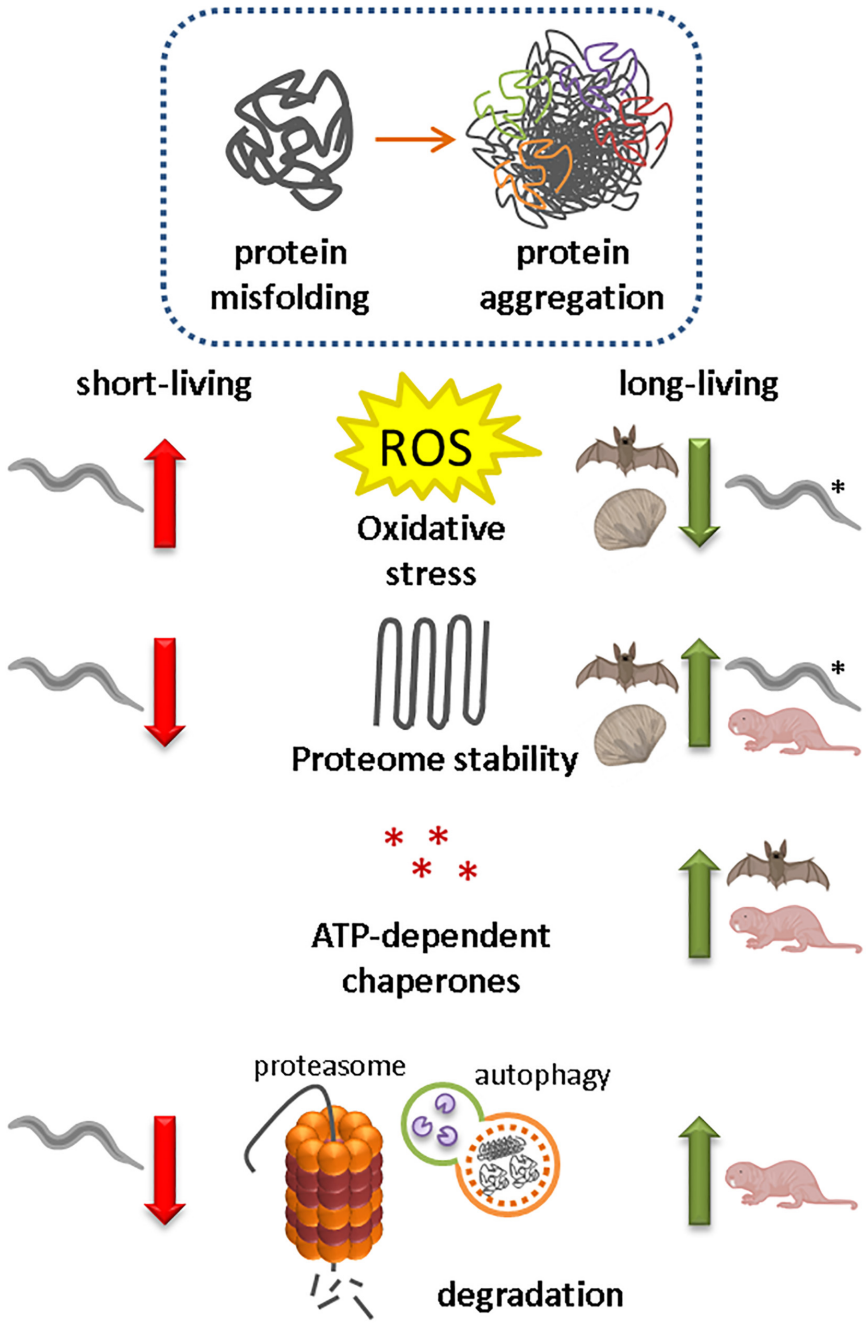

FIGURE 3 | Protein homeostasis capacity in animal species correlates with lifespan A decline in protein homeostasis due to increased oxidative stress, reduced proteome stability, reduced expression and activity of ATP-dependent chaperones and reduced protein degradation, has been associated with protein misfolding and the formation of insoluble protein aggregates. Naturally evolved long-living animal species (bivalve mollusk *Arctica islandica*, some bat species and naked mole-rats) have reduced their ROS production, increased their proteome stability and/or optimized protein folding and degradation pathways for a longer and healthier life. Also long-living *daf-2* mutant *Caenorhabditis elegans* models (*) have lower oxidative stress levels.

have negligible senescence and are resistant to cancer and other age-related diseases (Buffenstein, 2008; Edrey et al., 2011, 2013; Azpurua et al., 2013; Tian et al., 2013). Surprisingly, despite their elevated oxidative stress levels, activities of Cu/Zn superoxide dismutase, Mn superoxide dismutase, catalase and cellular glutathione peroxidase are not higher in naked mole-rats than in mice, and levels do not change with aging (Andziak et al., 2005, 2006). This has also been observed in certain long-living bird and bat species, and contradicts the oxidative stress hypothesis of aging (Andziak et al., 2006; Munshi-South and Wilkinson, 2010). High oxidative stress levels are expected to increase damage to DNA and lipids, and increase production

of misfolded proteins. Indeed, damage to DNA, lipids, and proteins was higher in naked mole-rats than in mice (Andziak et al., 2006). The lack of elevated antioxidant levels indicates that other mechanisms are responsible for the long lifespan and resistance to aging in these animals. A recent study indicates the contribution of elevated expression of peroxiredoxin 1 (PRDX1) and thioredoxin reductase 1 (TXNRD1) in the liver of naked mole-rats to their long lifespan (Heinze et al., 2018). *PRDX1* and *TXNRD1* are known for their ROS buffering capacities and their ability to promote protein homeostasis (Heinze et al., 2018). *PRDX1* and *TXNRD1* are targets of the transcription factor erythroid2-related factor 2 NFE2L2, which regulates the transcription of cytoprotective factors, and activation of NFE2L2 correlates with life expectancy (Malhotra et al., 2010; Lewis et al., 2015). Another mechanism could be the unique split-ribosome structure and accuracy of these ribosomes, which significantly improve the translational fidelity in these animals. This mechanism might, despite higher protein damage levels, prevent supersaturation of aggregation-prone proteins in naked mole-rats (Azpurua et al., 2013).

Just like the proteome of *A. islandica,* the proteomes of naked mole-rats, Mexican free-tailed bats and cave myotis bats are very resistant to unfolding stressors like urea (Pérez et al., 2009; Salmon et al., 2009). In naked mole-rats, however, increased chaperone levels do seem to play a role in the protection of the proteome against unfolding stressors. ATP-dependent chaperone levels were elevated under normal and heat shock conditions in cultured fibroblasts from naked mole-rats compared with cells cultured from short-living counterparts (Rodriguez et al., 2014; Pride et al., 2015). These elevated chaperone levels were also observed in cells from other long-living animal species, including sugar gliders, the Australian black flying fox and the cave nectar bat (Rodriguez et al., 2014, 2016; Pride et al., 2015; Chionh et al., 2019).

The importance of protein degradation for protein homeostasis is reflected in the elevated macroautophagy rate and proteasomal activity in the improved protein homeostasis network of naked mole-rats (Pérez et al., 2009; Rodriguez et al., 2014, 2016; Triplett et al., 2015). Particularly interesting is the stress-resistance of naked mole-rat proteasomes compared with those of other species. Proteasomes of naked mole-rats retained their activity after treatment with increasing concentrations of different proteasome competitive inhibitors, while mouse proteasomes lost all activity after exposure to low concentrations of the same inhibitors (Rodriguez et al., 2014). Interestingly, the proteasomes of naked mole-rats lost their resistance to proteasomal inhibitors when resuspended in proteasome-depleted mouse cytosolic extracts (Rodriguez et al., 2014). Conversely, enhanced resistance and increased levels of proteasomal activity were observed for mouse, yeast, and human proteasomes that were resuspended in cytosolic extracts of naked mole-rats. This indicates that factors specifically present in the cytosol of naked mole-rats are responsible for the improved proteolytic resistance and activity. Although the exact composition of this cytosolic factor remains unknown, inhibition of Hsp72 and its co-chaperone Hsp40 reduced the activity of proteasomes, indicating that these factors contribute to resistance to proteasomal stressors in naked mole rats (Rodriguez et al., 2014). This is particularly interesting as no Hsp has previously been described to specifically promote proteasome activity and to protect proteasomes from proteasome-specific inhibitors. The contribution of an active proteolytic system to healthy aging has also been proposed in humans. The expression of proteasomal components is reduced in aged individuals, whereas expression in centenarians was found to be similar to the expression levels in much younger individuals (Chondrogianni et al., 2000). Increased levels of proteasomal subunits could contribute to a more efficient degradation of (oxidized) proteins and therefore to a longer and healthier life.

An improved protein homeostasis in long-living animal species does not seem to depend on a single pathway, but rather on a combination of multiple optimized pathways (**Figure 3**). Each animal species has its own combination of mechanisms that protect its proteome from aging. The proteomes of long-living animal species have been optimized for living under specific environmental conditions. For example, reduced ROS production seems beneficial, but not all animals can regulate this. Bats and birds have to cope with high metabolic rates during flight (Munshi-South and Wilkinson, 2010), and naked mole-rats might have to deal with high concentrations of heavy metals in the soil they are living in (Rodriguez et al., 2016). Whereas some bats and the bivalve mollusk *A. islandica* might have optimized their DNA repair mechanisms and/or antioxidant levels to prevent protein damage (Brunet-Rossinni, 2004; Podlutsky et al., 2005; Wilhelm Filho et al., 2007; Ungvari et al., 2013; Huang et al., 2020), naked mole-rats have improved their refolding and degradation capacities to deal with damaged proteins (Pérez et al., 2009; Rodriguez et al., 2014, 2016; Pride et al., 2015; Triplett et al., 2015). Some animal species have evolved unique mechanisms to prolong their health and lifespan, as for example the cytosolic factor that promotes proteasome activity and resistance to proteasomal stressors in naked mole-rats (Rodriguez et al., 2014). Long-living species generally seem to have evolved an improved protein structure, strong DNA repair mechanisms, and more stable proteomes. Multiple studies indicate that additional, yet unknown mechanisms may increase protein homeostasis and longevity. For example, better resistance to protein unfolding could be explained by unknown chaperones that prevent protein unfolding or the existence of more stable protein conformations (Pérez et al., 2009; Salmon et al., 2009). Animal models that are capable of regeneration like the flatworm *Macrostomum lignano* or immortal cell lines might also extend our knowledge about the role of protein homeostasis in longevity (Vilchez et al., 2012a; Noormohammadi et al., 2016; Mouton et al., 2018). Recent studies have revealed that genes that are associated with increased longevity in other organisms are naturally upregulated with age in the long-living, regeneration-capable flatworm *M. lignano* (Mouton et al., 2018). Further identification of genes that are upregulated in this animal model and their role in protein homeostasis pathways could contribute to our knowledge about how protein homeostasis affects aging. In addition, enhanced expression of the CCT8 subunit of the chaperonin TRiC/CCT complex was shown to play an important role in proteome stability in human pluripotent stem cells. Upon

differentiation, CCT8 levels decrease and differentiated cells become more susceptible to a decline in protein homeostasis and aging (Noormohammadi et al., 2016).

CONCLUSION

To prevent or delay age-related diseases, we need to understand the underlying mechanisms that are involved in aging and disease. Understanding the differences in protein homeostasis between closely related animal species with different lifespans is a useful way of acquiring knowledge about the mechanisms of aging. A healthy, long life is clearly not dependent on the optimization of a single pathway. The multiple adaptations in the naked mole-rat which increase its resistance to cancer and neurodegenerative diseases are a clear example of this. The use of naturally long-living animal species like bivalve mollusks, bats, and naked mole-rats may also uncover ways to improve health and prolong lifespan. Further research of long-living animal models may therefore contribute to uncover the pathways that are involved in preventing amyloid formation and toxic sequestration of aggregation-prone proteins. These studies might help us understand these pathways and allow us to develop strategies to suppress age-related protein toxicity.

AUTHOR CONTRIBUTIONS

AP wrote the manuscript with the contribution and input from EN and the reviewers. Both authors contributed to the article and approved the submitted version.

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REFERENCES

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., et al. (2000). The genome sequence of *Drosophila melanogaster*. *Science* 287, 2185–2195. doi: 10.1126/science.287.5461.2185

Alberti, S., and Hyman, A. A. (2016). Are aberrant phase transitions a driver of cellular aging? *Bioessays* 38, 959–968. doi: 10.1002/bies.201600042

Andziak, B., O'Connor, T. P., and Buffenstein, R. (2005). Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. *Mech. Ageing Dev.* 126, 1206–1212. doi: 10.1016/j.mad.2005.06.009

Andziak, B., O'Connor, T. P., Qi, W., DeWaal, E. M., Pierce, A., Chaudhuri, A. R., et al. (2006). High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5, 463–471. doi: 10.1111/j.1474-9726.2006.00237.x

Anfinsen, C. B. (1973). Principles that govern the folding of protein chains. *Science* 181, 223–230.

Anisimova, A. S., Meerson, M. B., Gerashchenko, M. V., Kulakovskiy, I. V., Dmitriev, S. E., and Gladyshev, V. N. (2020). Multifaceted deregulation of gene expression and protein synthesis with age. *Proc. Natl. Acad. Sci. U.S.A.* 117, 15581–15590. doi: 10.1073/pnas.2001788117

Arrasate, M., Mitra, S., Schweitzer, E. S., Segal, M. R., and Finkbeiner, S. (2004). Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431, 805–810. doi: 10.1038/nature02998

Audas, T. E., Audas, D. E., Jacob, M. D., Ho, J. J. D., Khacho, M., Wang, M., et al. (2016). Adaptation to stressors by systemic protein amyloidogenesis. *Dev. Cell* 39, 155–168. doi: 10.1016/j.devcel.2016.09.002

Azpurua, J., Ke, Z., Chen, I. X., Zhang, Q., Ermolenko, D. N., Zhang, Z. D., et al. (2013). Naked mole-rat has increased translational fidelity compared with the mouse, as well as a unique 28S ribosomal RNA cleavage. *Proc. Natl. Acad. Sci. U.S.A.* 110, 17350–17355. doi: 10.1073/pnas.1313473110

Banani, S. F., Lee, H. O., Hyman, A. A., and Rosen, M. K. (2017). Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* 18, 285–298. doi: 10.1038/nrm.2017.7

Banjade, S., and Rosen, M. K. (2014). Phase transitions of multivalent proteins can promote clustering of membrane receptors. *eLife* 3:e04123. doi: 10.7554/eLife.04123

Beerten, J., Schymkowitz, J., and Rousseau, F. (2012). Aggregation prone regions and gatekeeping residues in protein sequences. *Curr. Top. Med. Chem.* 12, 2470–2478.

Ben-Zvi, A., Miller, E. A., and Morimoto, R. I. (2009). Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14914–14919. doi: 10.1073/pnas.0902882106

Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P., and Ron, D. (2000). Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* 2, 326–332. doi: 10.1038/35014014

Boisvert, F.-M., van Koningsbruggen, S., Navascués, J., and Lamond, A. I. (2007). The multifunctional nucleolus. *Nat. Rev. Mol. Cell Biol.* 8, 574–585. doi: 10.1038/nrm2184

Bonetti, D., Troilo, F., Brunori, M., Longhi, S., and Gianni, S. (2018). How robust is the mechanism of folding-upon-binding for an intrinsically disordered protein? *Biophys. J.* 114, 1889–1894. doi: 10.1016/j.bpj.2018.03.017

Brangwynne, C. P., Eckmann, C. R., Courson, D. S., Rybarska, A., Hoege, C., Gharakhani, J., et al. (2009). Germline P granules are liquid droplets that localize by controlled dissolution/condensation. *Science* 324, 1729–1732. doi: 10.1126/science.1172046

Brehme, M., Voisine, C., Rolland, T., Wachi, S., Soper, J. H., Zhu, Y., et al. (2014). A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. *Cell Rep.* 9, 1135–1150. doi: 10.1016/j.celrep.2014.09.042

Brown-Borg, H. M., and Bartke, A. (2012). GH and IGF1: roles in energy metabolism of long-living GH mutant mice. *J. Gerontol. A. Biol. Sci. Med. Sci.* 67, 652–660. doi: 10.1093/gerona/gls086

Brunet-Rossinni, A. K. (2004). Reduced free-radical production and extreme longevity in the little brown bat (*Myotis lucifugus*) versus two non-flying mammals. *Mech. Ageing Dev.* 125, 11–20. doi: 10.1016/j.mad.2003.09.003

Buffenstein, R. (2008). Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. *J. Comp. Physiol. B* 178, 439–445. doi: 10.1007/s00360-007-0237-5

C. elegans Sequencing Consortium (1998). Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282, 2012–2018. doi: 10.1126/science.282.5396.2012

Carrara, M., Prischi, F., Nowak, P. R., Kopp, M. C., and Ali, M. M. U. (2015). Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling. *eLife* 2015:e03522. doi: 10.7554/eLife.03522

Cashikar, A. G., Duennwald, M., and Lindquist, S. L. (2005). A chaperone pathway in protein disaggregation. *Hsp26* alters the nature of protein aggregates to facilitate reactivation by Hsp104. *J. Biol. Chem.* 280, 23869–23875. doi: 10.1074/jbc.M502854200

Chalmers, F., van Lith, M., Sweeney, B., Cain, K., and Bulleid, N. J. (2017). Inhibition of IRE1α-mediated XBP1 mRNA cleavage by XBP1 reveals a novel regulatory process during the unfolded protein response. *Wellcome Open Res.* 2:36. doi: 10.12688/wellcomeopenres.11764.2

Chaudhury, S., Keegan, B. M., and Blagg, B. S. J. (2021). The role and therapeutic potential of Hsp90, Hsp70, and smaller heat shock proteins in peripheral and central neuropathies. *Med. Res. Rev.* 41, 202–222. doi: 10.1002/med.21729

Chiang, H. L., and Dice, J. F. (1988). Peptide sequences that target proteins for enhanced degradation during serum withdrawal. *J. Biol. Chem.* 263, 6797–6805.

Chionh, Y. T., Cui, J., Koh, J., Mendenhall, I. H., Ng, J. H. J., Low, D., et al. (2019). High basal heat-shock protein expression in bats confers resistance to cellular heat/oxidative stress. *Cell Stress Chaperones* 24, 835–849. doi: 10.1007/s12192-019-01013-y

Cho, B. A., Yoo, S.-K., Song, Y. S., Kim, S.-J., Lee, K. E., Shong, M., et al. (2018). Transcriptome network analysis reveals aging-related mitochondrial and proteasomal dysfunction and immune activation in human thyroid. *Thyroid* 28, 656–666. doi: 10.1089/thy.2017.0359

Chondrogianni, N., Georgila, K., Kourtis, N., Tavernarakis, N., and Gonos, E. S. (2015). 20S proteasome activation promotes life span extension and resistance to proteotoxicity in *Caenorhabditis elegans*. *FASEB J.* 29, 611–622. doi: 10.1096/fj.14-252189

Chondrogianni, N., Petropoulos, I., Franceschi, C., Friguet, B., and Gonos, E. S. (2000). Fibroblast cultures from healthy centenarians have an active proteasome. *Exp. Gerontol.* 35, 721–728. doi: 10.1016/s0531-5565(00)00137-6

Ciryam, P., Kundra, R., Morimoto, R. I., Dobson, C. M., and Vendruscolo, M. (2015). Supersaturation is a major driving force for protein aggregation in neurodegenerative diseases. *Trends Pharmacol. Sci.* 36, 72–77. doi: 10.1016/j.tips.2014.12.004

Ciryam, P., Tartaglia, G. G., Morimoto, R. I., Dobson, C. M., and Vendruscolo, M. (2013). Widespread aggregation and neurodegenerative diseases are associated with supersaturated proteins. *Cell Rep.* 5, 781–790. doi: 10.1016/j.celrep.2013.09.043

Clancy, D. J., Gems, D., Harshman, L. G., Oldham, S., Stocker, H., Hafen, E., et al. (2001). Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292, 104–106. doi: 10.1126/science.1057991

Cohen, A. A. (2018). Aging across the tree of life: the importance of a comparative perspective for the use of animal models in aging. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864, 2680–2689. doi: 10.1016/j.bbadis.2017.05.028

Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W., and Dillin, A. (2006). Opposing activities protect against age-onset proteotoxicity. *Science* 313, 1604–1610. doi: 10.1126/science.1124646

Cohen, E., Paulsson, J. F., Blinder, P., Burstyn-Cohen, T., Du, D., Estepa, G., et al. (2009). Reduced IGF-1 signaling delays age-associated proteotoxicity in mice. *Cell* 139, 1157–1169. doi: 10.1016/j.cell.2009.11.014

Cohlberg, J. A., Li, J., Uversky, V. N., and Fink, A. L. (2002). Heparin and other glycosaminoglycans stimulate the formation of amyloid fibrils from α-synuclein in vitro†. *Biochemistry* 41, 1502–1511. doi: 10.1021/BI011711S

Csiszar, A., Labinskyy, N., Zhao, X., Hu, F., Serpillon, S., Huang, Z., et al. (2007). Vascular superoxide and hydrogen peroxide production and oxidative stress resistance in two closely related rodent species with disparate longevity. *Aging Cell* 6, 783–797. doi: 10.1111/j.1474-9726.2007.00339.x

Cuervo, A. M., and Dice, J. F. (1996). A receptor for the selective uptake and degradation of proteins by lysosomes. *Science* 273, 501–503. doi: 10.1126/science.273.5274.501

Cuervo, A. M., and Dice, J. F. (2000). Age-related decline in chaperone-mediated autophagy. *J. Biol. Chem.* 275, 31505–31513. doi: 10.1074/jbc.M002102200

David, D. C., Ollikainen, N., Trinidad, J. C., Cary, M. P., Burlingame, A. L., and Kenyon, C. (2010). Widespread protein aggregation as an inherent part of aging in *C. elegans*. *PLoS Biol.* 8:e1000450. doi: 10.1371/journal.pbio.1000450

De Baets, G., Van Durme, J., Rousseau, F., and Schymkowitz, J. (2014). A genome-wide sequence-structure analysis suggests aggregation gatekeepers constitute an evolutionary constrained functional class. *J. Mol. Biol.* 426, 2405–2412.

Dhondt, I., Petyuk, V. A., Bauer, S., Brewer, H. M., Smith, R. D., Depuydt, G., et al. (2017). Changes of protein turnover in aging *Caenorhabditis elegans*. *Mol. Cell. Proteomics* 16, 1621–1633. doi: 10.1074/mcp.RA117.000049

Dikic, I. (2017). Proteasomal and autophagic degradation systems. *Annu. Rev. Biochem.* 86, 193–224. doi: 10.1146/annurev-biochem-061516-044908

Du, Z., Chakrabarti, S., Kulaberoglu, Y., Smith, E. S. J., Dobson, C. M., Itzhaki, L. S., et al. (2020). Probing the unfolded protein response in long-lived naked mole-rats. *Biochem. Biophys. Res. Commun.* 529, 1151–1157. doi: 10.1016/j.bbrc.2020.06.118

Edrey, Y. H., Hanes, M., Pinto, M., Mele, J., and Buffenstein, R. (2011). Successful aging and sustained good health in the naked mole rat: a long-lived mammalian model for biogerontology and biomedical research. *ILAR J.* 52, 41–53. doi: 10.1093/ilar.52.1.41

Edrey, Y. H., Medina, D. X., Gaczynska, M., Osmulski, P. A., Oddo, S., Caccamo, A., et al. (2013). Amyloid beta and the longest-lived rodent: the naked mole-rat as a model for natural protection from Alzheimer's disease. *Neurobiol. Aging* 34, 2352–2360. doi: 10.1016/j.neurobiolaging.2013.03.032

Engel, S. R., Dietrich, F. S., Fisk, D. G., Binkley, G., Balakrishnan, R., Costanzo, M. C., et al. (2014). The reference genome sequence of *Saccharomyces cerevisiae*: then and now. *G3 Genes Genomes Genet.* 4, 389–398. doi: 10.1534/g3.113.008995

Escusa-Toret, S., Vonk, W. I. M., and Frydman, J. (2013). Spatial sequestration of misfolded proteins by a dynamic chaperone pathway enhances cellular fitness during stress. *Nat. Cell Biol.* 15, 1231–1243. doi: 10.1038/ncb2838

Faggioli, F., Wang, T., Vijg, J., and Montagna, C. (2012). Chromosome-specific accumulation of aneuploidy in the aging mouse brain. *Hum. Mol. Genet.* 21, 5246–5253. doi: 10.1093/hmg/dds375

Falsone, S. F., Meyer, N. H., Schrank, E., Leitinger, G., Pham, C. L. L., Fodero-Tavoletti, M. T., et al. (2012). SERF protein is a direct modifier of amyloid fiber assembly. *Cell Rep.* 2, 358–371. doi: 10.1016/j.celrep.2012.06.012

Fiorese, C. J., Schulz, A. M., Lin, Y. F., Rosin, N., Pellegrino, M. W., and Haynes, C. M. (2016). The transcription factor ATF5 mediates a mammalian mitochondrial UPR. *Curr. Biol.* 26, 2037–2043. doi: 10.1016/j.cub.2016.06.002

Forsberg, L. A., Rasi, C., Razzaghian, H. R., Pakalapati, G., Waite, L., Thilbeault, K. S., et al. (2012). Age-related somatic structural changes in the nuclear genome of human blood cells. *Am. J. Hum. Genet.* 90, 217–228. doi: 10.1016/j.ajhg.2011.12.009

Frakes, A. E., and Dillin, A. (2017). The UPR ER: sensor and coordinator of organismal homeostasis. *Mol. Cell* 66, 761–771. doi: 10.1016/j.molcel.2017.05.031

Frakes, A. E., Metcalf, M. G., Tronnes, S. U., Bar-Ziv, R., Durieux, J., Gildea, H. K., et al. (2020). Four glial cells regulate ER stress resistance and longevity via neuropeptide signaling in *C. Elegans*. *Science* 367, 436–440. doi: 10.1126/science.aaz6896

Frottin, F., Schueder, F., Tiwary, S., Gupta, R., Körner, R., Schlichthaerle, T., et al. (2019). The nucleolus functions as a phase-separated protein quality control compartment. *Science* 365, 342–347. doi: 10.1126/science.aaw9157

Fuxreiter, M. (2018). Fuzziness in protein interactions-a historical perspective. *J. Mol. Biol.* 430, 2278–2287. doi: 10.1016/j.jmb.2018.02.015

Gardner, B. M., and Walter, P. (2011). Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Science* 333, 1891–1894. doi: 10.1126/science.1209126

Genest, O., Hoskins, J. R., Kravats, A. N., Doyle, S. M., and Wickner, S. (2015). Hsp70 and Hsp90 of *E. coli* directly interact for collaboration in protein remodeling. *J. Mol. Biol.* 427, 3877–3889. doi: 10.1016/j.jmb.2015.10.010

Genest, O., Wickner, S., and Doyle, S. M. (2019). Hsp90 and Hsp70 chaperones: collaborators in protein remodeling. *J. Biol. Chem.* 294, 2109–2120. doi: 10.1074/JBC.REV118.002806

Gopal, P. P., Nirschl, J. J., Klinman, E., and Holzbaurb, E. L. F. (2017). Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons. *Proc. Natl. Acad. Sci. U.S.A.* 114, E2466–E2475. doi: 10.1073/pnas.1614462114

Gribenko, A. V., and Makhatadze, G. I. (2007). Role of the charge–charge interactions in defining stability and halophilicity of the CspB proteins. *J. Mol. Biol.* 366, 842–856. doi: 10.1016/j.jmb.2006.11.061

Gruber, H., Wessels, W., Boynton, P., Xu, J., Wohlgemuth, S., Leeuwenburgh, C., et al. (2015). Age-related cellular changes in the long-lived bivalve *A. islandica*. *Age* 37:90. doi: 10.1007/s11357-015-9831-8

Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S. J., and Kenyon, C. (2007). Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. *Aging Cell* 6, 95–110. doi: 10.1111/j.1474-9726.2006.00267.x

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300. doi: 10.1093/geronj/11.3.298

Hartl, F. U. (2017). Protein misfolding diseases. *Annu. Rev. Biochem.* 86, 21–26. doi: 10.1146/annurev-biochem-061516-044518

Heinze, I., Bens, M., Calzia, E., Holtze, S., Dakhovnik, O., Sahm, A., et al. (2018). Species comparison of liver proteomes reveals links to naked mole-rat longevity and human aging. *BMC Biol.* 16:82. doi: 10.1186/s12915-018-0547-y

Hernández-Vega, A., Braun, M., Scharrel, L., Jahnel, M., Wegmann, S., Hyman, B. T., et al. (2017). Local nucleation of microtubule bundles through tubulin concentration into a condensed tau phase. *Cell Rep.* 20, 2304–2312. doi: 10.1016/j.celrep.2017.08.042

Hipp, M. S., Kasturi, P., and Hartl, F. U. (2019). The proteostasis network and its decline in ageing. *Nat. Rev. Mol. Cell Biol.* 20, 421–435. doi: 10.1038/s41580-019-0101-y

Hong, S., Ostaszewski, B. L., Yang, T., O'Malley, T. T., Jin, M., Yanagisawa, K., et al. (2014). Soluble Aβ oligomers are rapidly sequestered from brain ISF in vivo and bind GM1 ganglioside on cellular membranes. *Neuron* 82, 308–319. doi: 10.1016/j.neuron.2014.02.027

Hsu, A.-L., Murphy, C. T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300, 1142–1145. doi: 10.1126/science.1083701

Huang, C., Wagner-Valladolid, S., Stephens, A. D., Jung, R., Poudel, C., Sinnige, T., et al. (2019). Intrinsically aggregation-prone proteins form amyloid-like aggregates and contribute to tissue aging in *Caenorhabditis elegans*. *eLife* 8:e43059. doi: 10.7554/eLife.43059

Huang, Z., Whelan, C. V., Dechmann, D., and Teeling, E. C. (2020). Genetic variation between long-lived versus short-lived bats illuminates the molecular signatures of longevity. *Aging* 12, 15962–15977. doi: 10.18632/aging.103725

Iulita, M. F., Allard, S., Richter, L., Munter, L.-M., Ducatenzeiler, A., Weise, C., et al. (2014). Intracellular Aβ pathology and early cognitive impairments in a transgenic rat overexpressing human amyloid precursor protein: a multidimensional study. *Acta Neuropathol. Commun.* 2:61. doi: 10.1186/2051-5960-2-61

Johnston, J. A., Ward, C. L., and Kopito, R. R. (1998). Aggresomes: a cellular response to misfolded proteins. *J. Cell Biol.* 143, 1883–1898. doi: 10.1083/jcb.143.7.1883

Kaganovich, D., Kopito, R., and Frydman, J. (2008). Misfolded proteins partition between two distinct quality control compartments. *Nature* 454, 1088–1095. doi: 10.1038/nature07195

Kato, M., Han, T. W., Xie, S., Shi, K., Du, X., Wu, L. C., et al. (2012). Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell* 149, 753–767. doi: 10.1016/j.cell.2012.04.017

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366, 461–464. doi: 10.1038/366461a0

Kim, Y. E., Hipp, M. S., Bracher, A., Hayer-Hartl, M., and Ulrich Hartl, F. (2013). Molecular chaperone functions in protein folding and proteostasis. *Annu. Rev. Biochem.* 82, 323–355. doi: 10.1146/annurev-biochem-060208-092442

Klaips, C. L., Jayaraj, G. G., and Hartl, F. U. (2018). Pathways of cellular proteostasis in aging and disease. *J. Cell Biol.* 217, 51–63. doi: 10.1083/jcb.201709072

Knoefler, D., Thamsen, M., Koniczek, M., Niemuth, N. J., Diederich, A.-K., and Jakob, U. (2012). Quantitative in vivo redox sensors uncover oxidative stress as an early event in life. *Mol. Cell* 47, 767–776. doi: 10.1016/j.molcel.2012.06.016

Koga, H., Kaushik, S., and Cuervo, A. M. (2011). Protein homeostasis and aging: the importance of exquisite quality control. *Ageing Res. Rev.* 10, 205–215. doi: 10.1016/j.arr.2010.02.001

Korovila, I., Hugo, M., Castro, J. P., Weber, D., Höhn, A., Grune, T., et al. (2017). Proteostasis, oxidative stress and aging. *Redox Biol.* 13, 550–567. doi: 10.1016/j.redox.2017.07.008

Kroschwald, S., Maharana, S., Mateju, D., Malinovska, L., Nüske, E., Poser, I., et al. (2015). Promiscuous interactions and protein disaggregases determine the material state of stress-inducible RNP granules. *eLife* 4:e06807. doi: 10.7554/eLife.06807

Kumsta, C., Chang, J. T., Lee, R., Tan, E. P., Yang, Y., Loureiro, R., et al. (2019). The autophagy receptor p62/SQST-1 promotes proteostasis and longevity in *C. elegans* by inducing autophagy. *Nat. Commun.* 10:5648. doi: 10.1038/s41467-019-13540-4

Labbadia, J., Brielmann, R. M., Neto, M. F., Lin, Y. F., Haynes, C. M., and Morimoto, R. I. (2017). Mitochondrial stress restores the heat shock response and prevents proteostasis collapse during aging. *Cell Rep.* 21, 1481–1494. doi: 10.1016/j.celrep.2017.10.038

Labbadia, J., and Morimoto, R. I. (2015). Repression of the heat shock response is a programmed event at the onset of reproduction. *Mol. Cell* 59, 639–650. doi: 10.1016/j.molcel.2015.06.027

Lagunas-Rangel, F. A. (2020). Why do bats live so long?—Possible molecular mechanisms. *Biogerontology* 21, 1–11. doi: 10.1007/s10522-019-09840-3

Lander, G. C., Estrin, E., Matyskiela, M. E., Bashore, C., Nogales, E., and Martin, A. (2012). Complete subunit architecture of the proteasome regulatory particle. *Nature* 482, 186–191. doi: 10.1038/nature10774

Lechler, M. C., Crawford, E. D., Groh, N., Widmaier, K., Jung, R., Kirstein, J., et al. (2017). Reduced insulin/IGF-1 signaling restores the dynamic properties of key stress granule proteins during aging. *Cell Rep.* 18, 454–467. doi: 10.1016/j.celrep.2016.12.033

Lévy, E., El Banna, N., Baïlle, D., Heneman-Masurel, A., Truchet, S., Rezaei, H., et al. (2019). Causative links between protein aggregation and oxidative stress: a review. *Int. J. Mol. Sci.* 20:3896. doi: 10.3390/ijms20163896

Lewis, K. N., Wason, E., Edrey, Y. H., Kristan, D. M., Nevo, E., and Buffenstein, R. (2015). Regulation of Nrf2 signaling and longevity in naturally long-lived rodents. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3722–3727. doi: 10.1073/pnas.1417566112

Li, P., Banjade, S., Cheng, H.-C., Kim, S., Chen, B., Guo, L., et al. (2012). Phase transitions in the assembly of multivalent signalling proteins. *Nature* 483, 336–340. doi: 10.1038/nature10879

Linding, R., Schymkowitz, J., Rousseau, F., Diella, F., and Serrano, L. (2004). A comparative study of the relationship between protein structure and beta-aggregation in globular and intrinsically disordered proteins. *J. Mol. Biol.* 342, 345–353. doi: 10.1016/j.jmb.2004.06.088

Liu, Z., Chen, X., Li, Z., Ye, W., Ding, H., Li, P., et al. (2020). Role of RNA oxidation in neurodegenerative diseases. *Int. J. Mol. Sci.* 21, 1–14. doi: 10.3390/ijms21145022

Longo, L. M., and Blaber, M. (2016). "Proteins: folding, misfolding, disordered proteins, and related diseases," in *Encyclopedia of Cell Biology*, eds R. A. Bradshaw and P. Stahl (Amsterdam: Elsevier Inc.), 108–114.

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039

Luis, N. M., Wang, L., Ortega, M., Deng, H., Katewa, S. D., Li, P. W.-L., et al. (2016). Intestinal IRE1 is required for increased triglyceride metabolism and longer lifespan under dietary restriction. *Cell Rep.* 17, 1207–1216. doi: 10.1016/j.celrep.2016.10.003

Malhotra, D., Portales-Casamar, E., Singh, A., Srivastava, S., Arenillas, D., Happel, C., et al. (2010). Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through chip-seq profiling and network analysis. *Nucleic Acids Res.* 38, 5718–5734. doi: 10.1093/nar/gkq212

Martínez, G., Duran-Aniotz, C., Cabral-Miranda, F., Vivar, J. P., and Hetz, C. (2017). Endoplasmic reticulum proteostasis impairment in aging. *Aging Cell* 16, 615–623. doi: 10.1111/acel.12599

Martinez-Lopez, N., Athonvarangkul, D., and Singh, R. (2015). "Autophagy and aging," in *Advances in Experimental Medicine and Biology*, eds W. E. Crusio, H. Dong, J. D. Lambris, H. H. Radeke, and N. Rezaei (Amsterdam: Elsevier), 73–87.

Mateju, D., Franzmann, T. M., Patel, A., Kopach, A., Boczek, E. E., Maharana, S., et al. (2017). An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function. *EMBO J.* 36:1669. doi: 10.15252/EMBJ.201695957

Mayer, M. P., and Gierasch, L. M. (2019). Recent advances in the structural and mechanistic aspects of Hsp70 molecular chaperones. *J. Biol. Chem.* 294, 2085–2097. doi: 10.1074/jbc.REV118.002810

Mediani, L., Guillén-Boixet, J., Vinet, J., Franzmann, T. M., Bigi, I., Mateju, D., et al. (2019). Defective ribosomal products challenge nuclear function by impairing nuclear condensate dynamics and immobilizing ubiquitin. *EMBO J.* 38:e101341. doi: 10.15252/embj.2018101341

Merle, D. A., Witternigg, A., Tam-Amersdorfer, C., Hartlmüller, C., Spreitzer, E., Schrank, E., et al. (2019). Increased aggregation tendency of alpha-synuclein in a fully disordered protein complex. *J. Mol. Biol.* 431, 2581–2598. doi: 10.1016/j.jmb.2019.04.031

Meyer, N. H., Dellago, H., Tam-Amersdorfer, C., Merle, D. A., Parlato, R., Gesslbauer, B., et al. (2019). Structural fuzziness of the RNA-organizing protein SERF1a determines a toxic gain-of-interaction. *bioRxiv* [Preprint]. doi: 10.1101/713511

Mogk, A., Bukau, B., and Kampinga, H. H. (2018). Cellular handling of protein aggregates by disaggregation machines. *Mol. Cell* 69, 214–226. doi: 10.1016/j.molcel.2018.01.004

Molliex, A., Temirov, J., Lee, J., Coughlin, M., Kanagaraj, A. P., Kim, H. J., et al. (2015). Phase separation by low complexity domains promotes stress granule assembly and drives pathological fibrillization. *Cell* 163, 123–133. doi: 10.1016/j.cell.2015.09.015

Morley, J. F., Brignull, H. R., Weyers, J. J., and Morimoto, R. I. (2002). The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 10417–10422. doi: 10.1073/pnas.152161099

Morley, J. F., and Morimoto, R. I. (2004). Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol. Biol. Cell* 15, 657–664. doi: 10.1091/mbc.e03-07-0532

Mouton, S., Grudniewska, M., Glazenburg, L., Guryev, V., and Berezikov, E. (2018). Resilience to aging in the regeneration-capable flatworm *Macrostomum lignano*. *Aging Cell* 17:e12739. doi: 10.1111/acel.12739

Mucke, L., Masliah, E., Yu, G. Q., Mallory, M., Rockenstein, E. M., Tatsuno, G., et al. (2000). High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* 20, 4050–4058.

Muid, K. A., Kimyon, Ö, Reza, S. H., Karakaya, H. C., and Koc, A. (2019). Characterization of long living yeast deletion mutants that lack mitochondrial metabolism genes DSS1, PPA2 and AFG3. *Gene* 706, 172–180. doi: 10.1016/j.gene.2019.05.001

Munshi-South, J., and Wilkinson, G. S. (2010). Bats and birds: exceptional longevity despite high metabolic rates. *Ageing Res. Rev.* 9, 12–19. doi: 10.1016/j.arr.2009.07.006

Nandi, P. K., Leclerc, E., Nicole, J.-C., and Takahashi, M. (2002). DNA-induced partial unfolding of prion protein leads to its polymerisation to amyloid. *J. Mol. Biol.* 322, 153–161. doi: 10.1016/s0022-2836(02)00750-7

Nargund, A. M., Pellegrino, M. W., Fiorese, C. J., Baker, B. M., and Haynes, C. M. (2012). Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science* 337, 587–590. doi: 10.1126/science.1223560

Noda, N. N., and Inagaki, F. (2015). Mechanisms of autophagy. *Annu. Rev. Biophys.* 44, 101–122. doi: 10.1146/annurev-biophys-060414-034248

Noormohammadi, A., Khodakarami, A., Gutierrez-Garcia, R., Lee, H. J., Koyuncu, S., König, T., et al. (2016). Somatic increase of CCT8 mimics proteostasis of human pluripotent stem cells and extends *C. elegans* lifespan. *Nat. Commun.* 7:13649. doi: 10.1038/ncomms13649

Olzscha, H., Schermann, S. M., Woerner, A. C., Pinkert, S., Hecht, M. H., Tartaglia, G. G., et al. (2011). Amyloid-like aggregates sequester numerous metastable proteins with essential cellular functions. *Cell* 144, 67–78. doi: 10.1016/j.cell.2010.11.050

Paisán-Ruíz, C., Jain, S., Evans, E. W., Gilks, W. P., Simón, J., van der Brug, M., et al. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* 44, 595–600. doi: 10.1016/j.neuron.2004.10.023

Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., and Gorman, A. M. (2016). The integrated stress response. *EMBO Rep.* 17, 1374–1395. doi: 10.15252/embr.201642195

Pan, K. Z., Palter, J. E., Rogers, A. N., Olsen, A., Chen, D., Lithgow, G. J., et al. (2007). Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* 6, 111–119. doi: 10.1111/j.1474-9726.2006.00266.x

Papadopoli, D., Boulay, K., Kazak, L., Pollak, M., Mallette, F., Topisirovic, I., et al. (2019). mTOR as a central regulator of lifespan and aging. *F1000Research* 8:F1000 Faculty Rev-998. doi: 10.12688/f1000research.17196.1

Patel, A., Lee, H. O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M. Y., et al. (2015). A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162, 1066–1077. doi: 10.1016/j.cell.2015.07.047

Pérez, V. I., Buffenstein, R., Masamsetti, V., Leonard, S., Salmon, A. B., Mele, J., et al. (2009). Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3059–3064. doi: 10.1073/pnas.0809620106

Peskett, T. R., Rau, F., O'Driscoll, J., Patani, R., Lowe, A. R., and Saibil, H. R. (2018). A liquid to solid phase transition underlying pathological huntingtin exon1 aggregation. *Mol. Cell* 70, 588.e6–601.e6. doi: 10.1016/j.molcel.2018.04.007

Podlutsky, A., Podlutskaya, N., Bakri, I., Csiszar, A., Ungvari, Z., and Austad, S. (2008). Comparative analysis of DNA repair pathways in mammals. *FASEB J.* 22:1239.32. doi: 10.1096/FASEBJ.22.1_SUPPLEMENT.1239.32

Podlutsky, A. J., Khritankov, A. M., Ovodov, N. D., and Austad, S. N. (2005). A new field record for bat longevity. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 60, 1366–1368. doi: 10.1093/gerona/60.11.1366

Pride, H., Yu, Z., Sunchu, B., Mochnick, J., Coles, A., Zhang, Y., et al. (2015). Long-lived species have improved proteostasis compared to phylogenetically-related shorter-lived species. *Biochem. Biophys. Res. Commun.* 457, 669–675. doi: 10.1016/j.bbrc.2015.01.046

Ray, S., Singh, N., Kumar, R., Patel, K., Pandey, S., Datta, D., et al. (2020). α-Synuclein aggregation nucleates through liquid-liquid phase separation. *Nat. Chem.* 12, 705–716. doi: 10.1038/s41557-020-0465-9

Reis-Rodrigues, P., Czerwieniec, G., Peters, T. W., Evani, U. S., Alavez, S., Gaman, E. A., et al. (2012). Proteomic analysis of age-dependent changes in protein solubility identifies genes that modulate lifespan. *Aging Cell* 11, 120–127. doi: 10.1111/j.1474-9726.2011.00765.x

Rodriguez, K. A., Osmulski, P. A., Pierce, A., Weintraub, S. T., Gaczynska, M., and Buffenstein, R. (2014). A cytosolic protein factor from the naked mole-rat activates proteasomes of other species and protects these from inhibition. *Biochim. Biophys. Acta* 1842, 2060–2072. doi: 10.1016/j.bbadis.2014.07.005

Rodriguez, K. A., Valentine, J. M., Kramer, D. A., Gelfond, J. A., Kristan, D. M., Nevo, E., et al. (2016). Determinants of rodent longevity in the chaperone-protein degradation network. *Cell Stress Chaperones* 21, 453–466. doi: 10.1007/s12192-016-0672-x

Sabath, N., Levy-Adam, F., Younis, A., Rozales, K., Meller, A., Hadar, S., et al. (2020). Cellular proteostasis decline in human senescence. *Proc. Natl. Acad. Sci. U.S.A.* 117, 202018138. doi: 10.1073/pnas.2018138117

Sahu, R., Kaushik, S., Clement, C. C., Cannizzo, E. S., Scharf, B., Follenzi, A., et al. (2011). Microautophagy of cytosolic proteins by late endosomes. *Dev. Cell* 20, 131–139. doi: 10.1016/j.devcel.2010.12.003

Salmon, A. B., Leonard, S., Masamsetti, V., Pierce, A., Podlutsky, A. J., Podlutskaya, N., et al. (2009). The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. *FASEB J.* 23, 2317–2326. doi: 10.1096/fj.08-122523

Salmon, A. B., Ljungman, M., and Miller, R. A. (2008). Cells from long-lived mutant mice exhibit enhanced repair of ultraviolet lesions. *J. Gerontol. A. Biol. Sci. Med. Sci.* 63, 219–231. doi: 10.1093/gerona/63.3.219

Salvador, N., Aguado, C., Horst, M., and Knecht, E. (2000). Import of a cytosolic protein into lysosomes by chaperone-mediated autophagy depends on its folding state. *J. Biol. Chem.* 275, 27447–27456. doi: 10.1074/jbc.M001394200

Seguin, S. J., Morelli, F. F., Vinet, J., Amore, D., De Biasi, S., Poletti, A., et al. (2014). Inhibition of autophagy, lysosome and VCP function impairs stress granule assembly. *Cell Death. Diff.* 21, 1838–1851. doi: 10.1038/cdd.2014.103

Serebryany, E., Woodard, J. C., Adkar, B. V., Shabab, M., King, J. A., and Shakhnovich, E. I. (2016). An internal disulfide locks a misfolded aggregation-prone intermediate in cataract-linked mutants of human γD-crystallin. *J. Biol. Chem.* 291, 19172–19183. doi: 10.1074/jbc.M116.735977

Shemesh, N., Shai, N., and Ben-Zvi, A. (2013). Germline stem cell arrest inhibits the collapse of somatic proteostasis early in *Caenorhabditis elegans* adulthood. *Aging Cell* 12, 814–822. doi: 10.1111/acel.12110

Shen, K., Gamerdinger, M., Chan, R., Gense, K., Martin, E. M., Sachs, N., et al. (2019). Dual role of ribosome-binding domain of NAC as a potent suppressor of protein aggregation and aging-related proteinopathies. *Mol. Cell* 74, 729.e7–741.e7. doi: 10.1016/j.molcel.2019.03.012

Shi, Y., Pulliam, D. A., Liu, Y., Hamilton, R. T., Jernigan, A. L., Bhattacharya, A., et al. (2013). Reduced mitochondrial ROS, enhanced antioxidant defense, and distinct age-related changes in oxidative damage in muscles of long-lived *Peromyscus leucopus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R343–R355. doi: 10.1152/ajpregu.00139.2012

Shin, Y., Berry, J., Pannucci, N., Haataja, M. P., Toettcher, J. E., and Brangwynne, C. P. (2017). Spatiotemporal control of intracellular phase transitions using light-activated optodroplets. *Cell* 168, 159.e14–171.e14. doi: 10.1016/j.cell.2016.11.054

Shin, Y., and Brangwynne, C. P. (2017). Liquid phase condensation in cell physiology and disease. *Science* 357:eaaf4382. doi: 10.1126/science.aaf4382

Shpilka, T., and Haynes, C. M. (2018). The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. *Nat. Rev. Mol. Cell Biol.* 19, 109–120. doi: 10.1038/nrm.2017.110

Sin, O., de Jong, T., Mata-Cabana, A., Kudron, M., Zaini, M. A., Aprile, F. A., et al. (2017). Identification of an RNA polymerase III regulator linked to disease-associated protein aggregation. *Mol. Cell* 65, 1096.e6–1108.e6. doi: 10.1016/j.molcel.2017.02.022

Sontag, E. M., Samant, R. S., and Frydman, J. (2017). Mechanisms and functions of spatial protein quality control. *Annu. Rev. Biochem.* 86, 97–122. doi: 10.1146/annurev-biochem-060815-014616

Strickler, S. S., Gribenko, A. V., Gribenko, A. V., Keiffer, T. R., Tomlinson, J., Reihle, T., et al. (2006). Protein stability and surface electrostatics: a charged relationship†. *Biochemistry* 45, 2761–2766. doi: 10.1021/BI0600143

Stroo, E., Koopman, M., Nollen, E. A. A., and Mata-Cabana, A. (2017). Cellular regulation of amyloid formation in aging and disease. *Front. Neurosci.* 11:64. doi: 10.3389/fnins.2017.00064

Suraweera, A., Münch, C., Hanssum, A., and Bertolotti, A. (2012). Failure of amino acid homeostasis causes cell death following proteasome inhibition. *Mol. Cell* 48, 242–253. doi: 10.1016/j.molcel.2012.08.003

Taylor, R. C. (2016). Aging and the UPR(ER). *Brain Res.* 1648, 588–593. doi: 10.1016/j.brainres.2016.04.017

Taylor, R. C., and Dillin, A. (2013). XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. *Cell* 153, 1435–1447. doi: 10.1016/j.cell.2013.05.042

Taylor, R. C., and Hetz, C. (2020). Mastering organismal aging through the endoplasmic reticulum proteostasis network. *Aging Cell* 19:e13265. doi: 10.1111/acel.13265

Teixeira, D., Sheth, U., Valencia-Sanchez, M. A., Brengues, M., and Parker, R. (2005). Processing bodies require RNA for assembly and contain nontranslating mRNAs. *RNA* 11, 371–382. doi: 10.1261/rna.7258505

Tekirdag, K., and Cuervo, A. M. (2018). Chaperone-mediated autophagy and endosomal microautophagy: jointed by a chaperone. *J. Biol. Chem.* 293:5414. doi: 10.1074/JBC.R117.818237

Tian, X., Azpurua, J., Hine, C., Vaidya, A., Myakishev-Rempel, M., Ablaeva, J., et al. (2013). High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* 499, 346–349. doi: 10.1038/nature12234

Tonoki, A., Kuranaga, E., Tomioka, T., Hamazaki, J., Murata, S., Tanaka, K., et al. (2009). Genetic evidence linking age-dependent attenuation of the 26S proteasome with the aging process. *Mol. Cell. Biol.* 29, 1095–1106. doi: 10.1128/MCB.01227-08

Treaster, S. B., Chaudhuri, A. R., and Austad, S. N. (2015). Longevity and GAPDH stability in bivalves and mammals: a convenient marker for comparative gerontology and proteostasis. *PLoS One* 10:e0143680. doi: 10.1371/journal.pone.0143680

Treaster, S. B., Ridgway, I. D., Richardson, C. A., Gaspar, M. B., Chaudhuri, A. R., and Austad, S. N. (2014). Superior proteome stability in the longest lived animal. *Age* 36:9597. doi: 10.1007/s11357-013-9597-9

Triplett, J. C., Tramutola, A., Swomley, A., Kirk, J., Grimes, K., Lewis, K., et al. (2015). Age-related changes in the proteostasis network in the brain of the naked mole-rat: implications promoting healthy longevity. *Biochim. Biophys. Acta* 1852, 2213–2224. doi: 10.1016/j.bbadis.2015.08.002

Ungelenk, S., Moayed, F., Ho, C.-T., Grousl, T., Scharf, A., Mashaghi, A., et al. (2016). Small heat shock proteins sequester misfolding proteins in near-native conformation for cellular protection and efficient refolding. *Nat. Commun.* 7:13673. doi: 10.1038/ncomms13673

Ungvari, Z., Krasnikov, B. F., Csiszar, A., Labinskyy, N., Mukhopadhyay, P., Pacher, P., et al. (2008). Testing hypotheses of aging in long-lived mice of the genus Peromyscus: association between longevity and mitochondrial stress resistance, ROS detoxification pathways, and DNA repair efficiency. *Age* 30, 121–133. doi: 10.1007/s11357-008-9059-y

Ungvari, Z., Ridgway, I., Philipp, E. E. R., Campbell, C. M., McQuary, P., Chow, T., et al. (2011). Extreme longevity is associated with increased resistance to oxidative stress in Arctica islandica, the longest-living non-colonial animal. *J. Gerontol. A. Biol. Sci. Med. Sci.* 66, 741–750. doi: 10.1093/gerona/glr044

Ungvari, Z., Sosnowska, D., Mason, J. B., Gruber, H., Lee, S. W., Schwartz, T. S., et al. (2013). Resistance to genotoxic stresses in Arctica islandica, the longest living noncolonial animal: is extreme longevity associated with a multistress resistance phenotype? *J. Gerontol. A. Biol. Sci. Med. Sci.* 68, 521–529. doi: 10.1093/gerona/gls193

Uversky, V. N. (2017). Intrinsically disordered proteins in overcrowded milieu: membrane-less organelles, phase separation, and intrinsic disorder. *Curr. Opin. Struct. Biol.* 44, 18–30. doi: 10.1016/j.sbi.2016.10.015

Uversky, V. N., Li, J., and Fink, A. L. (2001). Metal-triggered structural transformations, aggregation, and fibrillation of human alpha-synuclein. A possible molecular NK between Parkinson's disease and heavy metal exposure. *J. Biol. Chem.* 276, 44284–44296. doi: 10.1074/jbc.M105343200

Valenzano, D. R., Aboobaker, A., Seluanov, A., and Gorbunova, V. (2017). Non-canonical aging model systems and why we need them. *EMBO J.* 36, 959–963. doi: 10.15252/embj.201796837

Van Ham, T. J., Holmberg, M. A., Van Der Goot, A. T., Teuling, E., Garcia-Arencibia, M., Kim, H.-E., et al. (2010). Identification of MOAG-4/SERF as a regulator of age-related proteotoxicity. *Cell* 142, 601–612. doi: 10.1016/j.cell.2010.07.020

Van Raamsdonk, J. M., and Hekimi, S. (2009). Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 5:e1000361. doi: 10.1371/journal.pgen.1000361

Van Raamsdonk, J. M., and Hekimi, S. (2012). Superoxide dismutase is dispensable for normal animal lifespan. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5785–5790. doi: 10.1073/pnas.1116158109

Vilchez, D., Boyer, L., Morantte, I., Lutz, M., Merkwirth, C., Joyce, D., et al. (2012a). Increased proteasome activity in human embryonic stem cells is regulated by PSMD11. *Nature* 489, 304–308. doi: 10.1038/nature11468

Vilchez, D., Morantte, I., Liu, Z., Douglas, P. M., Merkwirth, C., Rodrigues, A. P. C., et al. (2012b). RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions. *Nature* 489, 263–268. doi: 10.1038/nature11315

Walker, G. A., and Lithgow, G. J. (2003). Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. *Aging Cell* 2, 131–139. doi: 10.1046/j.1474-9728.2003.00045.x

Walther, D. M., Kasturi, P., Zheng, M., Pinkert, S., Vecchi, G., Ciryam, P., et al. (2015). Widespread proteome remodeling and aggregation in aging *C. elegans*. *Cell* 161, 919–932. doi: 10.1016/j.cell.2015.03.032

Waterston, R. H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J. F., Agarwal, P., et al. (2002). Initial sequencing and comparative analysis of the mouse genome. *Nature* 420, 520–562. doi: 10.1038/nature01262

Wheeler, J. R., Matheny, T., Jain, S., Abrisch, R., and Parker, R. (2016). Distinct stages in stress granule assembly and disassembly. *eLife* 5:e18413. doi: 10.7554/eLife.18413

Wickramasinghe, S. P., Lempart, J., Merens, H. E., Murphy, J., Huettemann, P., Jakob, U., et al. (2019). Polyphosphate initiates tau aggregation through intra- and intermolecular scaffolding. *Biophys. J.* 117, 717–728. doi: 10.1016/j.bpj.2019.07.028

Wilhelm Filho, D., Althoff, S. L., Dafré, A. L., and Boveris, A. (2007). Antioxidant defenses, longevity and ecophysiology of South American bats. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 146, 214–220. doi: 10.1016/j.cbpc.2006.11.015

Xie, J., de Souza Alves, V., von der Haar, T., O'Keefe, L., Lenchine, R. V., Jensen, K. B., et al. (2019). Regulation of the elongation phase of protein synthesis enhances translation accuracy and modulates lifespan. *Curr. Biol.* 29, 737.e5–749.e5. doi: 10.1016/j.cub.2019.01.029

Yamaguchi, T., Arai, H., Katayama, N., Ishikawa, T., Kikumoto, K., and Atomi, Y. (2007). Age-related increase of insoluble, phosphorylated small heat shock proteins in human skeletal muscle. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 62, 481–489. doi: 10.1093/gerona/62.5.481

Yang, L., Cao, Y., Zhao, J., Fang, Y., Liu, N., and Zhang, Y. (2019). Multidimensional proteomics identifies declines in protein homeostasis and mitochondria as early signals for normal aging and age-associated disease in *Drosophila*. *Mol. Cell. Proteomics* 18, 2078–2088. doi: 10.1074/mcp.RA119.001621

Yang, S. B., Tien, A. C., Boddupalli, G., Xu, A. W., Jan, Y. N., and Jan, L. Y. (2012). Rapamycin ameliorates age-dependent obesity associated with increased mTOR signaling in hypothalamic POMC neurons. *Neuron* 75, 425–436. doi: 10.1016/j.neuron.2012.03.043

Yoshimura, Y., Holmberg, M. A., Kukic, P., Andersen, C. B., Mata-Cabana, A., Falsone, S. F., et al. (2017). MOAG-4 promotes the aggregation of α-synuclein

by competing with self-protective electrostatic interactions. *J. Biol. Chem.* 292, 8269–8278. doi: 10.1074/jbc.M116.764886

Yu, L., Chen, Y., and Tooze, S. A. (2018). Autophagy pathway: cellular and molecular mechanisms. *Autophagy* 14, 207–215. doi: 10.1080/15548627.2017.1378838

Yugay, D., Goronzy, D. P., Kawakami, L. M., Claridge, S. A., Song, T.-B., Yan, Z., et al. (2016). Copper ion binding site in β-amyloid peptide. *Nano Lett.* 16, 6282–6289. doi: 10.1021/acs.nanolett.6b02590

Zhang, H., Elbaum-Garfinkle, S., Langdon, E. M., Taylor, N., Occhipinti, P., Bridges, A. A., et al. (2015). RNA controls PolyQ protein phase transitions. *Mol. Cell.* 60, 220–230. doi: 10.1016/j.molcel.2015.09.017

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No Evidence for Trade-Offs Between Lifespan, Fecundity, and Basal Metabolic Rate Mediated by Liver Fatty Acid Composition in Birds

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The fatty acid composition of biological membranes has been hypothesised to be a key molecular adaptation associated with the evolution of metabolic rates, ageing, and life span – the basis of the membrane pacemaker hypothesis (MPH). MPH proposes that highly unsaturated membranes enhance cellular metabolic processes while being more prone to oxidative damage, thereby increasing the rates of metabolism and ageing. MPH could, therefore, provide a mechanistic explanation for trade-offs between longevity, fecundity, and metabolic rates, predicting that short-lived species with fast metabolic rates and higher fecundity would have greater levels of membrane unsaturation. However, previous comparative studies testing MPH provide mixed evidence regarding the direction of covariation between fatty acid unsaturation and life span or metabolic rate. Moreover, some empirical studies suggest that an n-3/n-6 PUFA ratio or the fatty acid chain length, rather than the overall unsaturation, could be the key traits coevolving with life span. In this study, we tested the coevolution of liver fatty acid composition with maximum life span, annual fecundity, and basal metabolic rate (BMR), using a recently published data set comprising liver fatty acid composition of 106 avian species. While statistically controlling for the confounding effects of body mass and phylogeny, we found no support for long life span evolving with low fatty acid unsaturation and only very weak support for fatty acid unsaturation acting as a pacemaker of BMR. Moreover, our analysis provided no evidence for the previously reported links between life span and n-3 PUFA/total PUFA or MUFA proportion. Our results rather suggest that long life span evolves with long-chain fatty acids irrespective of their degree of unsaturation as life span was positively associated with at least one long-chain fatty acid of each type (i.e., SFA, MUFA, n-6 PUFA, and n-3 PUFA). Importantly, maximum life span, annual fecundity, and BMR were associated with different fatty acids or fatty acid indices, indicating that longevity, fecundity, and BMR coevolve with different aspects of fatty acid composition. Therefore, in addition to posing significant challenges to MPH, our results imply that fatty acid composition does not pose an evolutionary constraint underpinning life-history trade-offs at the molecular level.

Keywords: membrane pacemaker hypothesis, life-history trade-offs, pace-of-life syndromes, membrane unsaturation, evolution of longevity, ageing, aging, senescence

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INTRODUCTION

The fatty acid (FA) composition of biological membranes has a strong influence on many cellular processes (Grecco et al., 2011). This fact has sparked the development of hypotheses that propose that the membrane FA composition is a key molecular adaptation underpinning the evolution of metabolic rates, ageing, and life span (Hulbert and Else, 1999; Pamplona et al., 2002; Hulbert, 2010). The most prominent of these hypotheses is the membrane pacemaker hypothesis (MPH), which proposes that the level of membrane FA unsaturation could act as a pacemaker of metabolism and ageing, thereby determining species-specific life span (Hulbert and Else, 1999; Hulbert, 2008). More specifically, MPH proposes two interacting molecular mechanisms linking membrane FA composition to the rates of metabolism and ageing.

First, the incorporation of unsaturated FA (i.e., those containing at least one double bond) into biological membranes enhances membrane fluidity (Jaureguiberry et al., 2014) and the activity of membrane proteins, which may enable higher cellular and organismal metabolic rates (Hulbert, 2008). Mechanistically, the increase in fluidity with unsaturation is attributed to the presence of *cis* double bonds that bend the acyl chains of unsaturated FA, resulting in greater interchain distances and, consequently, in the decrease in van der Waals force of intermolecular attraction (Hulbert and Else, 1999). The addition of polyunsaturated FA (PUFA) also modifies the spatial distribution of lipid rafts (i.e., the relatively ordered and rigid membrane lipid regions), pushing them closer to each other and stabilising raft-associated proteins (Shaikh et al., 2015). Both membrane fluidity and the effect of PUFA on the spatial distribution of lipid rafts may enhance the activity of membrane proteins important for energetic metabolism, such as glucose transporter proteins (Weijers, 2016) and Na^+/K^+ ATPases (Lingwood et al., 2005; Welker et al., 2007), respectively. These effects at the molecular level may eventually lead to higher metabolic rates at the cellular and organismal level (Hulbert, 2008). Higher metabolic rates are often associated with increased generation of free radicals, which induce oxidative damage to vital cellular components (Perez-Campo et al., 1998; da Costa et al., 2016). Hence, in addition to enhanced metabolic rates, higher membrane unsaturation can increase the rate of senescence according to MPH because the accumulation of oxidative damage has been proposed as a major cause of senescence by the free-radical theory of ageing (Barja, 2013).

Second, owing to the presence of highly reactive hydrogen atoms in their double bonds, unsaturated FA are known to be more prone to oxidative damage by free radicals and other reactive oxygen species (ROS; Holman, 1954; Hulbert, 2005). Based on this knowledge, MPH suggests that the level of membrane unsaturation influences senescence rate not only through increased ROS generation accompanying fast metabolic rates but also through susceptibility of unsaturated membranes to oxidative damage (Pamplona et al., 2002; Pamplona and Barja, 2011).

The appeal of MPH lies in its potential to provide a simple mechanistic explanation of the central paradigm of the life-history theory, namely the physiological trade-off between metabolic rate and fecundity on one side and the rate of senescence and longevity on the other (Stearns, 1989, 1992; Ricklefs and Wikelski, 2002). In other words, MPH implies that membrane unsaturation may be a major molecular mechanism constraining the evolution of life histories (or pace of life) along the fast–slow continuum with highly fecund, short-lived species on one end and long-lived species with low fecundity on the opposite end (Williams et al., 2010; Jimenez et al., 2014; Gaillard et al., 2016).

However, the causal role of ROS in senescence has been questioned (Lapointe and Hekimi, 2010; Speakman and Selman, 2011), challenging both the free-radical theory of ageing and MPH although such challenges have been dismissed as misconceptions by defenders of those two hypotheses (Barja, 2013) or may have resulted from methodological pitfalls (Munro and Pamenter, 2019). Moreover, the empirical support for MPH has been mixed so far, showing negative, no, or positive correlation between membrane unsaturation and life span or metabolic rate although studies focused on life span provide relatively more support for MPH (reviewed in Calhoon et al., 2015; Jové et al., 2020). It is also worth noting that many early studies supporting MPH are based on limited sample sizes (often comparing one short-lived mammal with one longer-lived avian species) or do not control for the confounding effects of body mass and phylogenetic non-independence of different species (Naudí et al., 2013; Valencak and Azzu, 2014; Calhoon et al., 2015; Bozek et al., 2017; Jové et al., 2020). Statistical control for the confounding effect of body mass is particularly important as the observed relationships could potentially arise due to the correlation of membrane unsaturation with body mass while not being associated with longevity directly (Speakman, 2005b; Valencak and Azzu, 2014). When controlling for body mass and phylogenetic autocorrelation, comparative studies using sufficient numbers of mammalian or avian species found no (Valencak and Ruf, 2007) or even a positive (Galván et al., 2015) relationship between FA unsaturation and maximum life span.

Several lines of experimental evidence further suggest that the association between FA unsaturation and susceptibility to oxidative stress may not be as straightforward as commonly assumed. For example, the peroxidation rate of FA in an aqueous environment may not reflect their degree of unsaturation (Visioli et al., 1998). In cell cultures and plants, PUFA have even been reported to efficiently scavenge ROS and protect other biomolecules from oxidative damage (Richard et al., 2008; Mène-Saffrané et al., 2009). The ROS-scavenging effect was positively related to the degree of PUFA unsaturation with n-3 PUFA being the most effective ones. Such observations led to the hypothesis that membranes rich in PUFA may act as structural antioxidants (Richard et al., 2008; Mène-Saffrané et al., 2009; Farmer and Mueller, 2013; Schmid-Siegert et al., 2016). Such an antioxidant function of PUFA could provide

Abbreviations: ACL, average chain length; AI, anti-inflammatory index; DBI, double bond index; DHA, docosahexaenoic acid; MPH, membrane pacemaker hypothesis; PI, peroxidizability index.

a possible explanation for the positive relationship between maximum life span and the degree of liver FA unsaturation reported by a recent phylogenetic comparative study on 107 bird species (Galván et al., 2015). However, despite the mixed support for MPH and the emergence of alternative hypotheses about PUFA properties (Richard et al., 2008; Mène-Saffrané et al., 2009; Farmer and Mueller, 2013; Schmid-Siegert et al., 2016), it is still widely assumed that long-lived species evolve membranes with low FA unsaturation (Schroeder and Brunet, 2015; Johnson and Stolzing, 2019; Papsdorf and Brunet, 2019; Jové et al., 2020).

To further complicate matters, a phylogenetic comparative study on 42 mammalian species found a negative relationship between maximum life span and n-3/n-6 PUFA ratio (Valencak and Ruf, 2007), which contrasts with the experimental evidence purporting antioxidant properties to n-3 PUFA. In addition, the length of the FA carbon chain could be a key trait coevolving with longevity as suggested by a recent comparative study on birds, which reported a positive relationship between life span and length of liver FA (Galván et al., 2015). However, such a positive relationship is in contrast with a mammalian comparative study, showing lower concentrations of long-chained FA in the blood plasma of long-lived species (Jové et al., 2013). It is unclear whether such contrasting results arise from physiological differences between taxa or because of different choices of tissues or statistical approaches among the studies. Consequently, the importance of membrane FA composition for life span or metabolic rate evolution remains an unresolved question (Calhoon et al., 2015).

Comparative studies may provide important insights into physiological and biochemical mechanisms modulating longevity and metabolic rates, considering the great variation in life-histories among various species even when body mass is accounted for Munro and Pamenter (2019). Here, we combine recently published data on liver FA of 106 European bird species (Galván et al., 2015) with published data on life-history traits (Fransson et al., 2017; Storchová and Hořák, 2018) and basal metabolic rate (BMR; McNab, 2009) to examine the links between FA composition and maximum life span, annual fecundity, or BMR. We include annual fecundity because reproduction is a highly energy-demanding process associated with elevated metabolic rates (Drent and Daan, 1980). To our knowledge, the coevolution of fecundity with FA composition has never been tested before even though it logically follows from the hypothesis that FA unsaturation should act as a pacemaker for metabolism and, hence, pace of life. The relationship between FA composition and maximum life span was analysed in the original study (Galván et al., 2015). The authors suggest that long life span evolves with higher proportions of long-chain and monounsaturated FA (MUFA), lower proportion of PUFA, and, surprisingly, higher overall level of unsaturation. They further observed that long life span was related to lower anti-inflammatory index, which is a ratio of anti-inflammatory FA to pre-inflammatory arachidonic acid. Nevertheless, Galván et al. (2015) relate life span to the first component of partial least squares regression, which, in addition to FA indexes, also include the effect of body mass and phylogeny. Hence, it is unclear to what extent the results of the original study may be affected by those confounding effects.

We employ Bayesian hierarchical (mixed effect) models, which allow us to perform beta regression within a phylogenetic context (Douma and Weedon, 2019) while controlling for the confounding effects of body mass and phylogeny. Beta regression is the preferred method to analyse proportional data, such as the proportions of FA because the commonly applied Gaussian regression of logit or square root–transformed data may yield biased estimates. The potential for such a bias is particularly high when the proportions are close to zero or one (Douma and Weedon, 2019) as is the case with many FA (Galván et al., 2015). Because differences in FA metabolism may evolve between sedentary and migratory species – either resulting from metabolic adaptations to long-distance flight (Jenni and Jenni-Eiermann, 1998; Guglielmo, 2010) or due to wintering in different environments (Calhoon et al., 2014; Jimenez et al., 2014) – we further include migratory behaviour as a covariate in the models. Additionally, sedentary and migratory species breeding in the temperate zone are also known to differ in their life-histories (Mönkkönen, 1992; Soriano-Redondo et al., 2020) and physiological components of pace of life (McNab, 2009; Gavrilov, 2014; Tomasek et al., 2019).

While statistically controlling for interspecific variation in body mass, migratory behaviour, and phylogeny, we aimed (i) to test whether maximum life span, annual fecundity, and BMR are associated with the aspects of FA composition proposed previously (i.e., FA unsaturation, n-3 PUFA/total PUFA proportion, average chain length, and anti-inflammatory index) and (ii) to carry out a detailed analysis exploring the associations between life-history traits and individual fatty acids.

MATERIALS AND METHODS

FA Data

We obtained data on the total liver FA composition of 106 European bird species encompassing 15 taxonomic orders from Galván et al. (2015). The original data set consisted of 107 species, but we excluded common cuckoo (*Cuculus canorus*), which is an obligate brood parasite with indefinable clutch size and complete lack of parental care. The authors utilised liver tissues of free-living birds collected in Denmark to extract total lipids, followed by conversion of FA to FA methyl esters and quantification by gas chromatography. The molar proportions of individual FA were given as the mean values per species.

In addition to the proportions of individual FA, we further used the following set of FA indices available in the original data set: the proportions of saturated FA (SFA), MUFA, and PUFA; the proportion of n-3 PUFA to total PUFA (hereafter, n3 PUFA/total PUFA); average chain length (ACL); double bond index (DBI); peroxidizability index (PI); and anti-inflammatory index (AI). We calculated n-3 PUFA/total PUFA as an alternative to n-3/n6 PUFA ratio as analysing ratios in regression models is challenging (Sollberger and Ehlert, 2016). ACL indicates the average length of the FA carbon chain based on the proportional representation of individual FA. DBI gives the

mean number of double bonds per 100 fatty acids. PI reflects the susceptibility of fatty acids to oxidative damage and is calculated as follows: PI = [(0.025 × Σmol% monoenoic) + (1 × Σmol% dienoic) + (2 × Σmol% trienoic) + (4 × Σmol% tetraenoic) + (6 × Σmol% pentaenoic) + (8 × Σmol% hexaenoic)] (Holman, 1954; Galván et al., 2015). AI is the percentage ratio of FA with anti-inflammatory properties (20:3n-6, 20:5n-3, 22:6n-3) to pre-inflammatory arachidonic acid (20:4n-6) (Galván et al., 2015).

Life-History and BMR Data

To control for the confounding effect of body mass (Speakman, 2005b), we used data on body mass from the original FA data set (Galván et al., 2015). We obtained maximum life span estimates predominantly from EURING (Fransson et al., 2017). Only for the water pipit (*Anthus spinoletta*), which had no maximum life span estimate in EURING, we used the corresponding record from AnAge longevity database (de Magalhães and Costa, 2009). We also extracted the number of recoveries of each species from EURING as capture effort is positively correlated with estimates of species maximum life span (Møller, 2008; Tomasek et al., 2019). To calculate annual fecundity (number of eggs laid per year), we utilised data on clutch size and number of clutches per year from Storchová and Hořák (2018).

Data on BMR came from the data set compiled by McNab (2009) except for the white-throated dipper (*Cinclus cinclus*) for which the value was obtained from a separate study (Bryant and Newton, 1994). BMR values were reported in kJ/h and adhered to the standard set of conditions necessary for metabolic rate measurements to be regarded as BMR (McKechnie and Wolf, 2004; McNab, 2009).

Migration Distance

Migration distance was calculated as a distance in thousands of kilometres between the centroid of the Jutland part of Denmark (original sampling location of data used in Galván et al., 2015) and the centroid of non-breeding distribution of each species using the haversine formula. Map data were obtained from BirdLife International and Handbook of the Birds of the World (2018). Centroids were calculated based on species distributions limited to Europe and Africa, using the ArcGIS 10.3 software (Esri, Redlands, CA, United States). The migration distance was set to zero in sedentary species.

Phylogenetic Data

Species phylogenetic non-independence is a form of autocorrelation due to shared ancestry (some species are more closely related to each other than to other species) and needs to be controlled for statistically to avoid biased parameter estimates (Speakman, 2005b). To control for the effect of phylogenetic non-independence, we used the most complete molecular phylogeny of extant bird species to date[1] based on the phylogenetic hypotheses of Hackett et al. (2008) and Jetz et al. (2012). For the 106 species included in our study, we downloaded 10,000 phylogenetic trees and generated a consensus tree using the TreeAnnotator tool implemented in the BEAST 2.6.3 software (Bouckaert et al., 2019).

[1] BirdTree.org

Statistical Analysis

We analysed the data using Bayesian hierarchical models implemented in the brms package (Bürkner, 2018) in R 4.0.3 software (R Core Team, 2020). We used this approach rather than the phylogenetic least squares because it allowed us to control for species-level phylogenetic non-independence while applying beta regression, which is the preferred method for continuous proportional data (Douma and Weedon, 2019). In addition, Bayesian inference does not need corrections for multiple testing because it estimates the probability of an effect of interest given the prior belief and the observed data and, unlike the frequentist framework, does not make assumptions about the distribution of *p* values in theoretical repetitions of an experiment (Berry and Hochberg, 1999; Gelman et al., 2012; Sjölander and Vansteelandt, 2019). Nevertheless, we follow the suggestion of Sjölander and Vansteelandt (2019) and adjust for multiple testing informally by discussing the effects while considering their dependence (**Figure 2**) and relative support (**Figure 3**).

In the beta regression models, we fitted proportions of the individual FA, SFA, MUFA, PUFA, or n-3 PUFA/total PUFA as response variables. In addition, we used Gaussian regression to model non-proportional response variables, namely ACL, DBI, PI, and log-transformed AI.

We fitted four types of regression models differing in the fixed-effect structure. First, to estimate the effect of body mass, we fitted simple phylogenetic regression models with body mass as the sole predictor (**Supplementary Table 1**). Second, to analyse the association of FA composition with life span, the models contained maximum life span as the focal predictor (**Supplementary Table 2**). These models further included body mass, number of recoveries, and migration distance among the predictors to control for their effects. Third, to analyse the association of FA composition with fecundity, we fitted models with annual fecundity, body mass, and migration distance as predictors (**Supplementary Table 3**). The fourth set of models included total BMR along with body mass as the predictors (**Supplementary Table 4**). We log-transformed body mass, annual fecundity, BMR, and the number of recoveries before fitting them in the models. We also standardised (*z*-transformed) each predictor and non-proportional response variable (ACL, DBI, PI, log-transformed AI) by subtracting its mean from the raw values and dividing them by standard deviation of the variable. Phylogeny was included in all the models as a random effect (Garamszegi, 2014). We used default priors calculated by the brms R package and ran the models in 25 chains, each with 6,000 iterations, 2,000 burn-in, and thinning set to 10. Potential scale reduction factor was <1.01 in all cases, indicating good model convergence (Gelman and Rubin, 1992).

We present resulting estimates as posterior means together with their equal-tailed 95% credible intervals based on quantiles (Bürkner, 2017). In the Bayesian framework, the 95% credible interval represents a range of values that, given the observed data, contain the true effect value with 95% probability. We consider

the support for an effect to be significant if 95% credible intervals do not contain zero (Hespanhol et al., 2019).

RESULTS

Interspecific Variation in Liver FA Composition

The abundance and interspecific variation in liver FA is visualised in **Figure 1**. The most abundant liver FA according to their median values were palmitic acid (C16:0; 23.3%), stearic acid (C18:0; 22.2%), oleic acid (C18:1n9; 21.1%), linoleic acid (C18:2n6; 9.3%), arachidonic acid (C20:4n6; 8.5%), docosahexaenoic acid (C22:6n3; 3.5%), and palmitoleic acid (C16:1n7; 1.3%). Medians of all the other FA were lower than 1%.

Index of overall FA unsaturation (DBI) and PI were strongly positively correlated to ACL and PUFA (**Figure 2**). At the level of individual FA, DBI, and PI showed the strongest positive correlations with C20:4n6 and C22:6n3 and the strongest negative correlation with C16:0. ACL was most strongly associated with C20:4n6. Besides this, C18:0 and all the FA with 20 or more carbons in their chains contributed to ACL as all of them showed positive correlations with ACL. In contrast, ACL was negatively correlated with C14:0, C16:0, C16:1n7, and C18:1n9. Moreover, the pattern of pairwise correlations between individual FA suggests that ACL is the primary axis of variation. We only show correlations of residuals from a phylogenetic generalised least squares regression on body mass in **Figure 2** because there was no difference between correlations based on raw values. This indicates that the correlation structure of liver FA is independent of body mass and phylogeny.

Scaling of Liver FA Composition With Body Mass

With regards to the FA indices, only DBI and ACL exhibited a positive covariation with body mass (**Figure 3**; **Supplementary Table 1**). Body mass was not significantly associated with most individual FA. Only C14:0 showed a negative association with body mass, whereas C20:1n9, C20:4n6, and C22:4n6 showed a negative association.

Liver FA Composition and Life Span

In contradiction to MPH, our analysis did not reveal a significant relationship between SFA, MUFA, PUFA, DBI, or PI and maximum life span while controlling for confounding effects of body mass, migratory behaviour, and phylogeny (**Supplementary Table 2**; **Figure 3**). Such a result indicates that species life span is not determined by FA unsaturation. There was also no association of life span with n-3 PUFA/total PUFA or anti-inflammatory index. The only integrative component of FA composition that did correlate in a significant manner with maximum life span was ACL (**Figure 3**).

At the level of individual FA, long life span was associated with low levels of C18:3n3 and high levels of C20:1n9, C22:0, C22:4n6, and C22:6n3. There was also weak support for a positive covariation of maximum life span with 20:4n6, but the 95% credible intervals of the estimate marginally contained zero (**Figure 3**).

Liver FA Composition and Annual Fecundity

Our analysis did not reveal a significant correlation between any of the FA composition indices and annual fecundity. The only individual FA that were associated with annual fecundity were C16:1n7 and C20:3n6, both exhibiting positive association (**Figure 3**; **Supplementary Table 3**).

Liver FA Composition and BMR

Basal metabolic rate was not significantly associated with any of the indices of the liver FA composition although there were marginally non-significant trends toward a positive covariation with PUFA, DBI, and PI and a negative covariation with MUFA. Among the individual FA, only C18:1n9 showed a significant association in the negative direction (**Figure 3**; **Supplementary Table 4**).

DISCUSSION

Our comparative analysis does not support the MPH as a whole because none of the key indices of FA unsaturation (DBI, PI, SFA, MUFA, and PUFA) covaried with maximum life span, BMR, or annual fecundity when controlled for the confounding effects of body mass, migration behaviour and phylogeny. Contrary to the previous analysis of the same data set (Galván et al., 2015), our results do not support the conclusion that long life span evolves with high MUFA and low PUFA content, which is commonly considered to be one of the mechanisms underpinning negative covariation between unsaturation and longevity (Schroeder and Brunet, 2015; Johnson and Stolzing, 2019; Papsdorf and Brunet, 2019; Jové et al., 2020). Moreover, maximum life span tended to positively covary, although non-significantly, with DBI and PI in our study, providing strong evidence against the prediction that long-lived species evolve membranes with low FA unsaturation.

Although the early comparative studies support a negative relationship between FA unsaturation and longevity, those studies are mostly based on only a low number of species, sometimes comparing one short-lived mammal species with one longer-lived bird species, and do not control for the confounding effects of body mass and phylogenetic non-independence (Naudí et al., 2013; Valencak and Azzu, 2014; Calhoon et al., 2015; Bozek et al., 2017; Jové et al., 2020). Modern phylogenetic comparative studies utilising larger sample sizes and controlling for body mass and phylogeny found no (Valencak and Ruf, 2007) or even positive (Galván et al., 2015) association between FA unsaturation and maximum life span. Similarly, the positive associations between FA unsaturation and BMR or calcium ATPase in muscles have not been supported in phylogenetic comparative studies on mammals and fish, respectively (Valencak and Ruf, 2007; Gonzalez et al., 2015). Moreover, one of the fundamental assumptions of MPH coming from the original rate-of-living theory (Pearl, 1928) – i.e., the negative correlation between metabolic rates and life span – has been refuted, at least

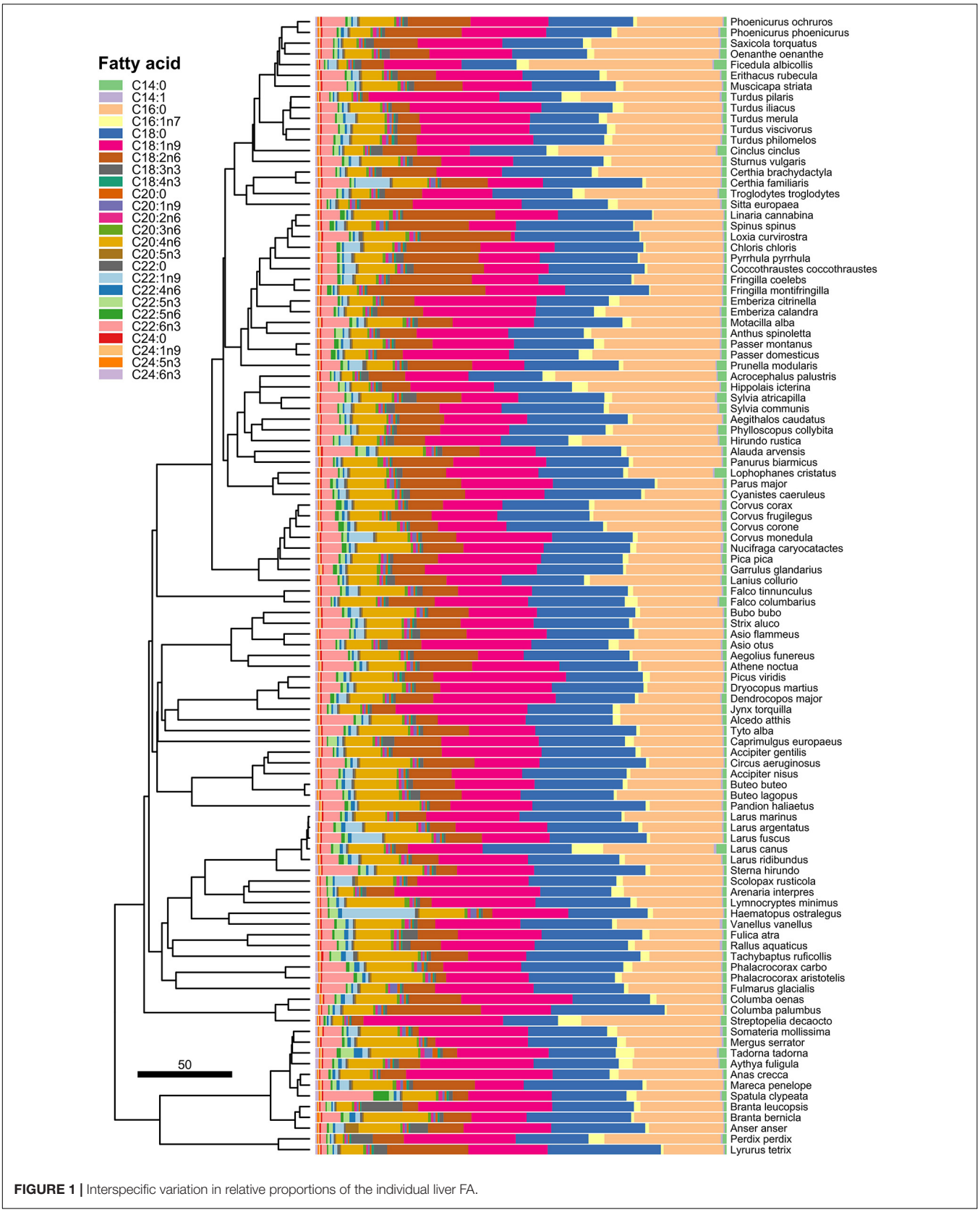

FIGURE 1 | Interspecific variation in relative proportions of the individual liver FA.

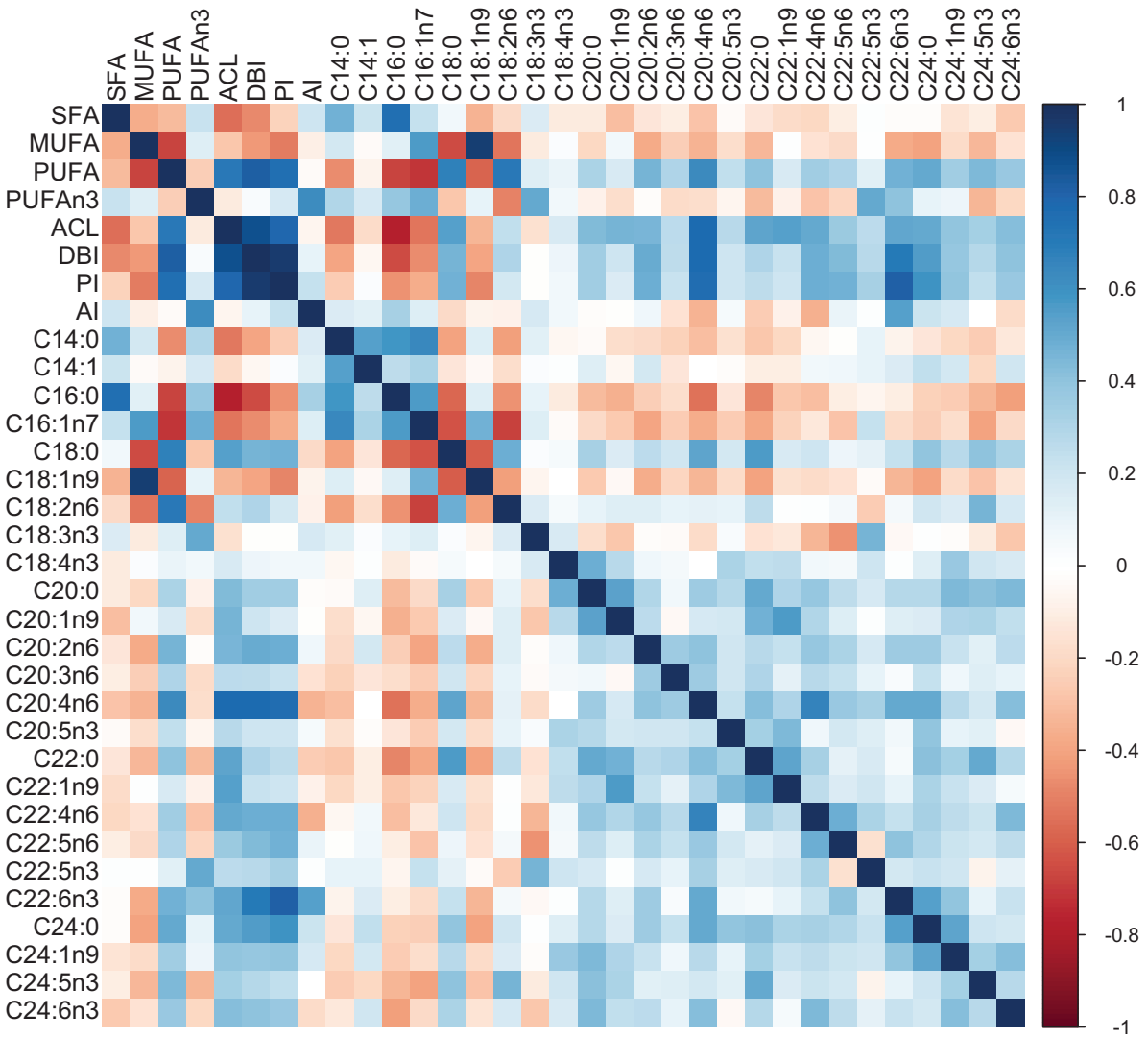

FIGURE 2 | Pairwise correlations between residuals of FA indices and individual FA. Correlations were calculated using residuals from phylogenetic generalised least squares regression on body mass. The proportional variables were logit-transformed, and AI was log-transformed before the analysis. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, DBI – double bond index, PI – peroxidizability index, ACL – average chain length, AI – anti-inflammatory index.

at the interspecific level, by large comparative studies controlling for body mass and phylogeny (Speakman, 2005a; de Magalhães et al., 2007; Furness and Speakman, 2008; Møller, 2008). Our results fit into such emerging comparative evidence against MPH, specifically against the prediction that evolution of long life span is underpinned by low FA unsaturation. Nevertheless, marginally non-significant positive association between PI and BMR may provide some, although very weak, support for the second prediction of MPH, namely that higher FA unsaturation enhances metabolic rates (Hulbert and Else, 1999).

It is important to note, however, that the total liver lipids analysed in our study and that by Galván et al. (2015) also comprise lipids other than membrane phospholipids, such as triglycerides derived from the diet. Hence, caution should be exercised when interpreting the observed patterns as reflecting variation in membrane FA composition. However, it seems reasonable to assume that phospholipid FA contribute considerably to the patterns because phospholipids represent the majority of lipids in the avian liver (Grande and Prigge, 1970; Galván et al., 2015). Moreover, a phylogenetic analysis comparing the composition of muscle phospholipids across mammals similarly reported a lack of an association between life span and FA unsaturation or the contents of SFA, MUFA, and PUFA (Valencak and Ruf, 2007), suggesting that the absence of such relationships in our study is probably not due to a confounding effect of dietary triglycerides.

Our analysis does not support several conclusions of the original study analysing the same data set (Galván et al., 2015),

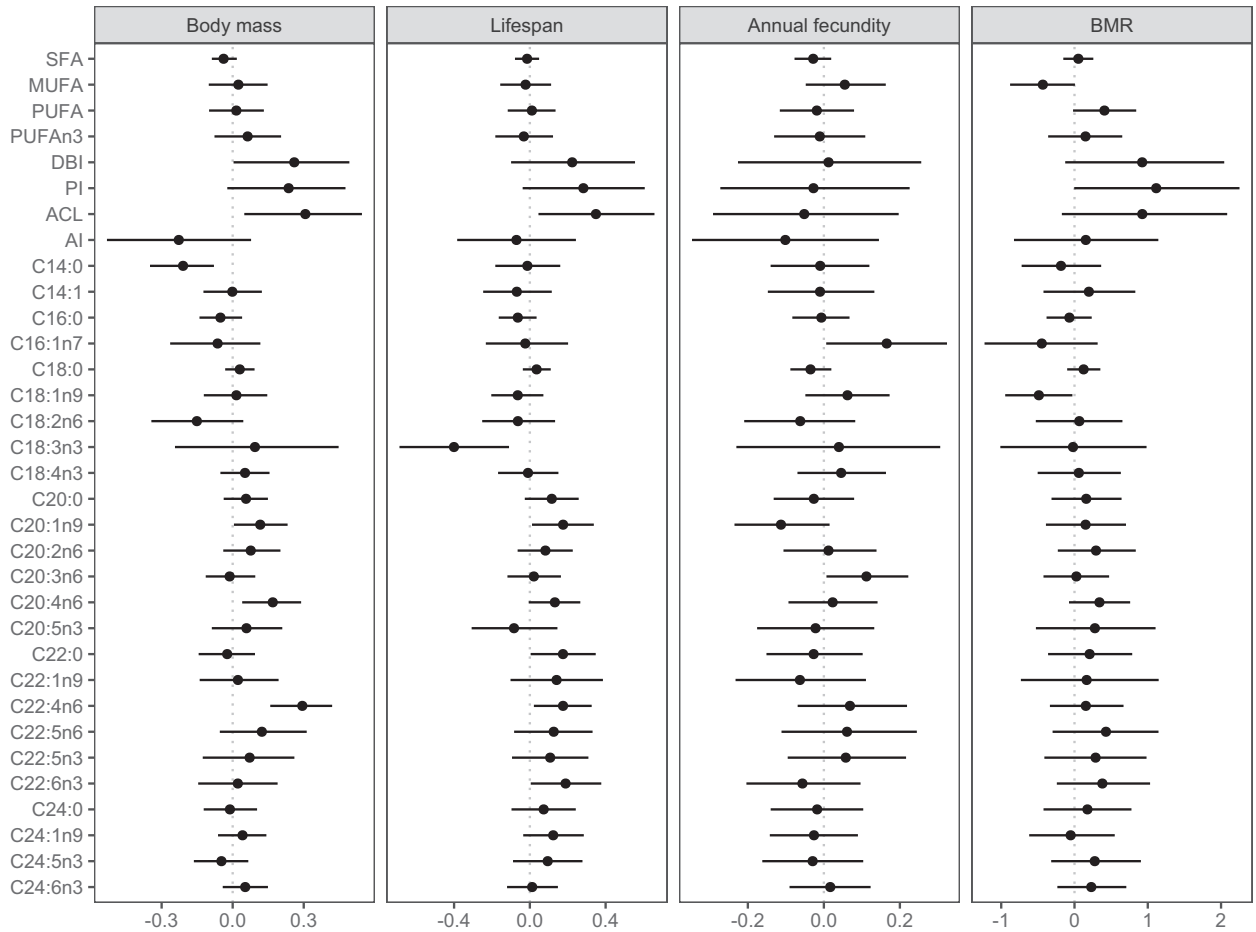

FIGURE 3 | Effects of body mass, life span, annual fecundity, and BMR on liver FA composition. Shown are average marginal effects from Bayesian phylogenetic models and their 95% credible intervals. The 95% credible interval represents a range of values that, given the observed data, contain the true effect value with 95% probability. We consider the effect to be significantly supported if the 95% credible interval do not contain zero. Effect of body mass is from a model with body mass as a sole fixed-effect predictor. The effect of life span was controlled for body mass, number of recoveries, and migration distance. The effect of annual fecundity was controlled for body mass and migration distance. The effect of BMR was controlled for body mass. Body mass, life span, number of recoveries, annual fecundity, BMR, and anti-inflammatory index were log-transformed. All the non-proportional response and predictor variables were standardised by *z*-transformation. Beta regression and Gaussian regression were used for proportional and non-proportional data, respectively. SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, DBI – double bond index, PI – peroxidizability index, ACL – average chain length, AI – anti-inflammatory index.

namely associations of maximum life span with proportions of MUFA and PUFA, anti-inflammatory index, or several individual FA, including C18:1n9, C18:2n6, C20:3n6, C22:1n9, and C22:5n6. Instead, we identified some other FA to be associated with maximum life span (C18:3n3, C20:4n6, C22:0, and C22:6n3). Such a discrepancy is most probably down to the different statistical approaches employed. The conclusion of the original study was based on the significant contributions of those indices and FA to the life span–predicting component of partial least square regression; however, that component integrated variation shared by all the FA indices, body mass, and phylogeny. It is, therefore, unclear as to what extent MUFA covaried with maximum life span directly. Because the conventional phylogenetic regression model used in our study is more straightforward to interpret, the discrepancies between the studies suggest potential pitfalls of the interpretation of individual predictors in partial least squares regression. Another factor potentially contributing to such discrepant conclusions could be that the original study used square-root transformation of proportional data, which may lead to biased estimates (Douma and Weedon, 2019). In contrast, we used beta regression in our study, which is the preferred statistical method for proportional data (Douma and Weedon, 2019).

In accordance with the conclusions of the original study (Galván et al., 2015), our results show a positive covariation between maximum life span and ACL, supporting the role of FA chain length in life span evolution. Considering the differences in statistical approaches explained above, our analysis provides an improved interpretation of the positive longevity–ACL relationship as being independent of the confounding effects of body mass and phylogeny. Our analysis of individual FA further indicates that this covariation was mainly driven by long

life span being associated with low levels of α-linolenic acid (C18:3n3) and high levels of gondoic acid (C20:1n9), behenic acid (C22:0), adrenic acid (C22:4n6), and docosahexaenoic acid (DHA; C22:6n3). Nevertheless, except for the eicosapentaenoic acid (C20:5n3), all the other FA with 20 or more carbons in their chains probably contributed to the longevity–ACL relationship because their models show positive regression coefficients of life span although with 95% credible intervals containing zero. Taken together, these results suggest that longevity does not evolve with any particular type of FA as life span was positively associated with at least one long-chain FA of each type (i.e., SFA, MUFA, n-6 PUFA, and n-3 PUFA).

Fatty acid unsaturation was positively associated with maximum life span in the original study (Galván et al., 2015). Here, we observed a similar, although non-significant, trend. On one hand, such a positive covariation between life span and unsaturation is consistent with the hypothesis that membranes rich in long-chain PUFA function as structural antioxidants (Richard et al., 2008; Mène-Saffrané et al., 2009; Farmer and Mueller, 2013; Schmid-Siegert et al., 2016). On the other hand, the fact that all the types of long-chain FA, irrespective of their unsaturation, covaried positively with life span in our analysis indicates that unsaturation is not the key trait in life span evolution. Moreover, ACL showed a much stronger association with life span than DBI or PI in our study, suggesting that the positive covariation of unsaturation indices with life span more likely reflects a tight relationship between fatty acid unsaturation and chain length (**Figure 2**).

Another feature of FA composition proposed to be linked to life span evolution is the n-3/n-6 PUFA ratio (Valencak and Ruf, 2007). Specifically, long life span is reported to evolve with relatively smaller proportions of n-3 PUFA in muscle phospholipids across mammals (Valencak and Ruf, 2007). It is further suggested that a low level of DHA, a highly unsaturated n-3 PUFA, is particularly important for the evolution of long life span due to its high susceptibility to peroxidation (Pamplona et al., 1998; Hulbert, 2003). Our results do not support the importance of n-3/n-6 PUFA ratio, however, as the n-3 PUFA/total PUFA did not show any association with maximum life span. Moreover, DHA was significantly positively related to maximum life span in our analysis, which is contrary to the proposed negative correlation (Pamplona et al., 1998; Hulbert, 2003). The positive association between DHA and life span is intriguing and has not been observed in previous comparative studies, including the original study analysing the same data set using partial least squares analysis (Valencak and Ruf, 2007; Galván et al., 2015). Such a pattern provides other evidence against MPH. It is rather consistent with the hypothesis that membranes with long-chain PUFA act as structural antioxidants (Richard et al., 2008; Mène-Saffrané et al., 2009; Farmer and Mueller, 2013; Schmid-Siegert et al., 2016). As discussed, however, the positive association of DHA with life span may merely reflect the overall importance of long-chain FA irrespective of their unsaturation.

Docosahexaenoic acid was further proposed as an important pacemaker of metabolic rate (Hulbert and Else, 1999; Hulbert, 2003) based on its observed enhancing effect on the molecular activity of Na^+/K^+-ATPase (Turner et al., 2003). Our results do not support such a role of DHA at the interspecific level in birds as DHA was not significantly associated with BMR. This is in accordance with the findings of a previous comparative study in mammals, which also reported no relation between DHA and BMR (Valencak and Ruf, 2007). Hence, DHA is probably not an important molecular adaptation underpinning metabolic rate evolution.

Nevertheless, the main factor hypothesised to underpin a variation in metabolic rates is membrane unsaturation (Hulbert and Else, 1999). Our findings provide weak support for this hypothesis, revealing marginally non-significant positive covariation of BMR with DBI, PI, and PUFA and negative covariation with MUFA. These patterns contrast with the results reported by the Valencak and Ruf (2007) comparative study on mammals, which found no link between BMR and DBI, PUFA, or MUFA and even a negative association of BMR with PI. However, the high level of MUFA in species with low BMR observed in our analysis is in accordance with the recent study in dogs, demonstrating a decrease in cellular metabolic rate following an enrichment of fibroblasts with MUFA (Jimenez et al., 2020). It is important to note, however, that 95% credible intervals of our estimates marginally contained zero, and we performed a high number of comparisons in our analysis. Clearly, more phylogenetic comparative studies focused on FA composition of phospholipids are needed to validate the hypothesised macroevolutionary link between membrane unsaturation and metabolic rates.

In conclusion, our analysis presents significant challenges to the current hypotheses about the role of FA composition in life span and life-history evolution. We found no support for membrane unsaturation, MUFA/PUFA ratio, or n-3/n-6 PUFA ratio playing an important role in life span evolution as commonly assumed based on MPH (Hulbert, 2003; Schroeder and Brunet, 2015; Johnson and Stolzing, 2019; Papsdorf and Brunet, 2019; Jové et al., 2020). Our results rather support the importance of FA chain length for life span evolution as proposed by the previous study (Galván et al., 2015). Our contribution to such a conclusion lies in a clear demonstration that the positive longevity–ACL relationship is independent of body mass and phylogeny. We only found weak support for the second MPH prediction, namely that high membrane unsaturation promotes high metabolic rates, thereby functioning as a pacemaker of metabolic rates; however, the weakness of the effect prevents any firm conclusion in this regard. We also identified specific types of FA associated with maximum life span, annual fecundity, and BMR evolution that may represent promising avenues for future research. A striking finding of our study is that life span, fecundity, and BMR are each associated with different FA or FA indices. Not even a single FA showed significant association with more than one life-history trait or BMR. Such a pattern indicates that maximum life span, annual fecundity, and BMR coevolve with different aspects of liver FA composition. Such differences imply that liver FA composition does not pose molecular constraints underpinning life-history trade-offs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because publicly available data were only used in this study.

AUTHOR CONTRIBUTIONS

OT conceived and designed the study with input from TA. SK, OK, and OT collected and processed the data. SK and OT analysed data with input from TA. SK and OT wrote the manuscript with input from TA and OK. All authors approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.638501/full#supplementary-material

REFERENCES

Barja, G. (2013). Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. *Antioxid. Redox Signal.* 19, 1420–1445. doi: 10.1089/ars.2012.5148

Berry, D. A., and Hochberg, Y. (1999). Bayesian perspectives on multiple comparisons. *J. Statist. Plan. Infer.* 82, 215–227. doi: 10.1016/S0378-3758(99)00044-0

BirdLife International and Handbook of the Birds of the World (2018). *Bird Species Distribution Maps of the World. Version 2018.1*. Available online at: http://datazone.birdlife.org/species/requestdis (accessed November 29, 2019).

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., et al. (2019). BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* 15:e1006650. doi: 10.1371/journal.pcbi.1006650

Bozek, K., Khrameeva, E. E., Reznick, J., Omerbašić, D., Bennett, N. C., Lewin, G. R., et al. (2017). Lipidome determinants of maximal lifespan in mammals. *Sci. Rep.* 7:5. doi: 10.1038/s41598-017-00037-7

Bryant, D. M., and Newton, A. V. (1994). Metabolic costs of dominance in dippers, *Cinclus cinclus*. *Anim. Behav.* 48, 447–455. doi: 10.1006/anbe.1994.1258

Bürkner, P.-C. (2017). brms: an R package for Bayesian multilevel models using Stan. *J. Stat. Soft.* 80, 1–28. doi: 10.18637/jss.v080.i01

Bürkner, P.-C. (2018). Advanced Bayesian multilevel modeling with the R package brms. *R. J.* 10:395. doi: 10.32614/RJ-2018-017

Calhoon, E. A., Jimenez, A. G., Harper, J. M., Jurkowitz, M. S., and Williams, J. B. (2014). Linkages between mitochondrial lipids and life history in temperate and tropical birds. *Physiol. Biochem. Zool.* 87, 265–275. doi: 10.1086/674696

Calhoon, E. A., Ro, J., and Williams, J. B. (2015). Perspectives on the membrane fatty acid unsaturation/pacemaker hypotheses of metabolism and aging. *Chem. Phys. Lipids* 191, 48–60. doi: 10.1016/j.chemphyslip.2015.08.008

da Costa, J. P., Vitorino, R., Silva, G. M., Vogel, C., Duarte, A. C., and Rocha-Santos, T. (2016). A synopsis on aging—Theories, mechanisms and future prospects. *Age. Res. Rev.* 29, 90–112. doi: 10.1016/j.arr.2016.06.005

de Magalhães, J. P., and Costa, J. (2009). A database of vertebrate longevity records and their relation to other life-history traits. *J. Evol. Biol.* 22, 1770–1774. doi: 10.1111/j.1420-9101.2009.01783.x

de Magalhães, J. P., Costa, J., and Church, G. M. (2007). An analysis of the relationship between metabolism, developmental schedules, and longevity using phylogenetic independent contrasts. *J. Gerontol. A Biol. Sci. Med.* 62, 149–160. doi: 10.1093/gerona/62.2.149

Douma, J. C., and Weedon, J. T. (2019). Analysing continuous proportions in ecology and evolution: a practical introduction to beta and Dirichlet regression. *Methods Ecol. Evol.* 10, 1412–1430. doi: 10.1111/2041-210X.13234

Drent, R. H., and Daan, S. (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea* 38–90, 225–252. doi: 10.5253/arde.v68.p225

Farmer, E. E., and Mueller, M. J. (2013). ROS-mediated lipid peroxidation and RES-activated signaling. *Annu. Rev. Plant Physiol.* 64, 429–450. doi: 10.1146/annurev-arplant-050312-120132

Fransson, T., Jansson, L., Kolehmainen, T., Kroon, C., and Wenninger, T. (2017). *EURING List of Longevity Records for European Birds*. Available online at: https://euring.org/data-and-codes/longevity-list (accessed November 24, 2018).

Furness, L. J., and Speakman, J. R. (2008). Energetics and longevity in birds. *Age* 30, 75–87. doi: 10.1007/s11357-008-9054-3

Gaillard, J.-M., Lemaître, J.-F., Berger, V., Bonenfant, C., Devillard, S., Douhard, M., et al. (2016). "Life histories, axes of variation in," in *Encyclopedia of Evolutionary Biology*, Vol. 2, ed. R. M. Kliman (Amsterdam: Elsevier), 312–323. doi: 10.1016/B978-0-12-800049-6.00085-8

Galván, I., Naudí, A., Erritzŕe, J., Møller, A. P., Barja, G., and Pamplona, R. (2015). Long lifespans have evolved with long and monounsaturated fatty acids in birds. *Evolution* 69, 2776–2784. doi: 10.1111/evo.12754

Garamszegi, L. Z. (2014). "Uncertainties due to within-species variation in comparative studies: measurement errors and statistical weights," in *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*, ed. L. Z. Garamszegi (Heidelberg: Springer), 157–199.

Gavrilov, V. M. (2014). Ecological and scaling analysis of the energy expenditure of rest, activity, flight, and evaporative water loss in passeriformes and non-passeriformes in relation to seasonal migrations and to the occupation of boreal stations in high and moderate latitudes. *Q. Rev. Biol.* 89, 107–150. doi: 10.1086/676046

Gelman, A., Hill, J., and Yajima, M. (2012). Why we (usually) don't have to worry about multiple comparisons. *J. Res. Educ. Effect.* 5, 189–211. doi: 10.1080/19345747.2011.618213

Gelman, A., and Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statist. Sci.* 7, 457–472. doi: 10.1214/ss/1177011136

Gonzalez, A., Pagé, B., and Weber, J.-M. (2015). Membranes as a possible pacemaker of metabolism in cypriniform fish: does phylogeny matter? *J. Exp. Biol.* 218, 2563–2572. doi: 10.1242/jeb.117630

Grande, F., and Prigge, W. F. (1970). Glucagon infusion, plasma FFA and triglycerides, blood sugar, and liver lipids in birds. *Am. J. Physiol.* 218, 1406–1411. doi: 10.1152/ajplegacy.1970.218.5.1406

Grecco, H. E., Schmick, M., and Bastiaens, P. I. H. (2011). Signaling from the living plasma membrane. *Cell* 144, 897–909. doi: 10.1016/j.cell.2011.01.029

Guglielmo, C. G. (2010). Move that fatty acid: fuel selection and transport in migratory birds and bats. *Integr. Comp. Biol.* 50, 336–345. doi: 10.1093/icb/icq097

Hackett, S. J., Kimball, R. T., Reddy, S., Bowie, R. C. K., Braun, E. L., Braun, M. J., et al. (2008). A phylogenomic study of birds reveals their evolutionary history. *Science* 320, 1763–1768. doi: 10.1126/science.1157704

Hespanhol, L., Vallio, C. S., Costa, L. M., and Saragiotto, B. T. (2019). Understanding and interpreting confidence and credible intervals around effect estimates. *Braz. J. Phys. Ther.* 23, 290–301. doi: 10.1016/j.bjpt.2018.12.006

Holman, R. T. (1954). Autoxidation of fats and related substances. *Prog. Chem. Fats Other Lipids* 2, 51–98. doi: 10.1016/0079-6832(54)90004-X

Hulbert, A. (2003). Life, death and membrane bilayers. *J. Exp. Biol.* 206, 2303–2311. doi: 10.1242/jeb.00399

Hulbert, A. J. (2005). On the importance of fatty acid composition of membranes for aging. *J. Theor. Biol.* 234, 277–288. doi: 10.1016/j.jtbi.2004.11.024

Hulbert, A. J. (2008). The links between membrane composition, metabolic rate and lifespan. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 150, 196–203. doi: 10.1016/j.cbpa.2006.05.014

Hulbert, A. J. (2010). Metabolism and longevity: is there a role for membrane fatty acids? *Integr. Comp. Biol.* 50, 808–817. doi: 10.1093/icb/icq007

Hulbert, A. J., and Else, P. L. (1999). Membranes as possible pacemakers of metabolism. *J. Theor. Biol.* 199, 257–274. doi: 10.1006/jtbi.1999.0955

Jaureguiberry, M. S., Tricerri, M. A., Sanchez, S. A., Finarelli, G. S., Montanaro, M. A., Prieto, E. D., et al. (2014). Role of plasma membrane lipid composition on cellular homeostasis: learning from cell line models expressing fatty acid desaturases. *Acta Biochim. Biophys. Sin.* 46, 273–282. doi: 10.1093/abbs/gmt155

Jenni, L., and Jenni-Eiermann, S. (1998). Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* 29, 521–528. doi: 10.2307/3677171

Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., and Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature* 491, 444–448. doi: 10.1038/nature11631

Jimenez, A. G., Cooper-Mullin, C., Calhoon, E. A., and Williams, J. B. (2014). Physiological underpinnings associated with differences in pace of life and metabolic rate in north temperate and neotropical birds. *J. Comp. Physiol. B* 184, 545–561. doi: 10.1007/s00360-014-0825-0

Jimenez, A. G., Winward, J. D., Walsh, K. E., and Champagne, A. M. (2020). Effects of membrane fatty acid composition on cellular metabolism and oxidative stress in dermal fibroblasts from small and large breed dogs. *J. Exp. Biol.* 223:jeb221804. doi: 10.1242/jeb.221804

Johnson, A. A., and Stolzing, A. (2019). The role of lipid metabolism in aging, lifespan regulation, and age-related disease. *Aging Cell* 18:e13048. doi: 10.1111/acel.13048

Jové, M., Mota-Martorell, N., Pradas, I., Galo-Licona, J. D., Martín-Gari, M., and Obis, È (2020). The lipidome fingerprint of longevity. *Molecules* 25:4343. doi: 10.3390/molecules25184343

Jové, M., Naudí, A., Aledo, J. C., Cabré, R., Ayala, V., Portero-Otin, M., et al. (2013). Plasma long-chain free fatty acids predict mammalian longevity. *Sci. Rep.* 3:sre03346. doi: 10.1038/srep03346

Lapointe, J., and Hekimi, S. (2010). When a theory of aging ages badly. *Cell. Mol. Life Sci.* 67, 1–8. doi: 10.1007/s00018-009-0138-8

Lingwood, D., Harauz, G., and Ballantyne, J. S. (2005). Regulation of fish gill Na(+)-K(+)-ATPase by selective sulfatide-enriched raft partitioning during seawater adaptation. *J. Biol. Chem.* 280, 36545–36550. doi: 10.1074/jbc.M506670200

McKechnie, A. E., and Wolf, B. O. (2004). The allometry of avian basal metabolic rate: good predictions need good data. *Physiol. Biochem. Zool.* 77, 502–521. doi: 10.1086/383511

McNab, B. K. (2009). Ecological factors affect the level and scaling of avian BMR. *Comp. Biochem. Physiol. A* 152, 22–45. doi: 10.1016/j.cbpa.2008.08.021

Mène-Saffrané, L., Dubugnon, L., Chételat, A., Stolz, S., Gouhier-Darimont, C., and Farmer, E. E. (2009). Nonenzymatic oxidation of trienoic fatty acids contributes to reactive oxygen species management in Arabidopsis. *J. Biol. Chem.* 284, 1702–1708. doi: 10.1074/jbc.M807114200

Møller, A. P. (2008). Relative longevity and field metabolic rate in birds. *J. Evol. Biol.* 21, 1379–1386. doi: 10.1111/j.1420-9101.2008.01556.x

Mönkkönen, M. (1992). Life history traits of Palaearctic and Nearctic migrant passerines. *Ornis Fennica* 69, 161–172.

Munro, D., and Pamenter, M. E. (2019). Comparative studies of mitochondrial reactive oxygen species in animal longevity: technical pitfalls and possibilities. *Aging Cell* 18:e13009. doi: 10.1111/acel.13009

Naudí, A., Jové, M., Ayala, V., Portero-Otín, M., Barja, G., and Pamplona, R. (2013). Membrane lipid unsaturation as physiological adaptation to animal longevity. *Front. Physiol.* 4:372. doi: 10.3389/fphys.2013.00372

Pamplona, R., and Barja, G. (2011). An evolutionary comparative scan for longevity-related oxidative stress resistance mechanisms in homeotherms. *Biogerontology* 12:409. doi: 10.1007/s10522-011-9348-1

Pamplona, R., Barja, G., and Portero-Otín, M. (2002). Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous-longevity adaptation? *Ann. N. Y. Acad. Sci.* 959, 475–490. doi: 10.1111/j.1749-6632.2002.tb02118.x

Pamplona, R., Portero-Otín, M., Riba, D., Ruiz, C., Prat, J., Bellmunt, M. J., et al. (1998). Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *J. Lipid Res.* 39, 1989–1994.

Papsdorf, K., and Brunet, A. (2019). Linking lipid metabolism to chromatin regulation in aging. *Trends Cell Biol.* 29, 97–116. doi: 10.1016/j.tcb.2018.09.004

Pearl, R. (1928). *The Rate of Living*. London: University of London Press.

Perez-Campo, R., Lopez-Torres, M., Cadenas, S., Rojas, C., and Barja, G. (1998). The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 168, 149–158.

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Richard, D., Kefi, K., Barbe, U., Bausero, P., and Visioli, F. (2008). Polyunsaturated fatty acids as antioxidants. *Pharmacol. Res.* 57, 451–455. doi: 10.1016/j.phrs.2008.05.002

Ricklefs, R. E., and Wikelski, M. (2002). The physiology/life-history nexus. *Trends. Ecol. Evol.* 17, 462–468. doi: 10.1016/S0169-5347(02)02578-8

Schmid-Siegert, E., Stepushenko, O., Glauser, G., and Farmer, E. E. (2016). Membranes as structural antioxidants. Recycling of malondialdehyde to its source in oxidation-sensitive chloroplast fatty acids. *J. Biol. Chem.* 291, 13005–13013. doi: 10.1074/jbc.M116.729921

Schroeder, E. A., and Brunet, A. (2015). Lipid profiles and signals for long life. *Trends Endocrinol. Metab.* 26, 589–592. doi: 10.1016/j.tem.2015.08.007

Shaikh, S. R., Kinnun, J. J., Leng, X., Williams, J. A., and Wassall, S. R. (2015). How polyunsaturated fatty acids modify molecular organization in membranes: insight from NMR studies of model systems. *Biochim. Biophys. Acta Biomembr.* 1848, 211–219. doi: 10.1016/j.bbamem.2014.04.020

Sjölander, A., and Vansteelandt, S. (2019). Frequentist versus Bayesian approaches to multiple testing. *Eur. J. Epidemiol.* 34, 809–821. doi: 10.1007/s10654-019-00517-2

Sollberger, S., and Ehlert, U. (2016). How to use and interpret hormone ratios. *Psychoneuroendocrinology* 63, 385–397. doi: 10.1016/j.psyneuen.2015.09.031

Soriano-Redondo, A., Gutiérrez, J. S., Hodgson, D., and Bearhop, S. (2020). Migrant birds and mammals live faster than residents. *Nat. Commun.* 11:5719. doi: 10.1038/s41467-020-19256-0

Speakman, J. R. (2005a). Body size, energy metabolism and lifespan. *J. Exp. Biol.* 208, 1717–1730. doi: 10.1242/jeb.01556

Speakman, J. R. (2005b). Correlations between physiology and lifespan - two widely ignored problems with comparative studies. *Aging Cell* 4, 167–175. doi: 10.1111/j.1474-9726.2005.00162.x

Speakman, J. R., and Selman, C. (2011). The free-radical damage theory: accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *Bioessays* 33, 255–259. doi: 10.1002/bies.201000132

Stearns, S. (1989). Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259–268.

Stearns, S. C. (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.

Storchová, L., and Hořák, D. (2018). Life-history characteristics of European birds. *Glob. Ecol. Biogeogr.* 27, 400–406. doi: 10.1111/geb.12709

Tomasek, O., Bobek, L., Kralova, T., Adamkova, M., and Albrecht, T. (2019). Fuel for the pace of life: baseline blood glucose concentration co-evolves with life-history traits in songbirds. *Funct. Ecol.* 33, 239–249. doi: 10.1111/1365-2435.13238

Turner, N., Else, P. L., and Hulbert, A. J. (2003). Docosahexaenoic acid (DHA) content of membranes determines molecular activity of the sodium pump: implications for disease states and metabolism. *Naturwissenschaften* 90, 521–523. doi: 10.1007/s00114-003-0470-z

Valencak, T. G., and Azzu, V. (2014). Making heads or tails of mitochondrial membranes in longevity and aging: a role for comparative studies. *Longev. Healthspan* 3:3. doi: 10.1186/2046-2395-3-3

Valencak, T. G., and Ruf, T. (2007). N-3 polyunsaturated fatty acids impair lifespan but have no role for metabolism. *Aging Cell* 6, 15–25. doi: 10.1111/j.1474-9726.2006.00257.x

Visioli, F., Colombo, C., and Galli, C. (1998). Oxidation of individual fatty acids yields different profiles of oxidation markers. *Biochem. Biophys. Res. Commun.* 245, 487–489. doi: 10.1006/bbrc.1998.8463

Weijers, R. N. M. (2016). Membrane flexibility, free fatty acids, and the onset of vascular and neurological lesions in type 2 diabetes. *J. Diabetes Metab. Disord.* 15:13. doi: 10.1186/s40200-016-0235-9

Welker, P., Geist, B., Frühauf, J.-H., Salanova, M., Groneberg, D. A., Krause, E., et al. (2007). Role of lipid rafts in membrane delivery of renal epithelial Na+-K+-ATPase, thick ascending limb. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R1328–R1337. doi: 10.1152/ajpregu.00166.2006

Williams, J. B., Miller, R. A., Harper, J. M., and Wiersma, P. (2010). Functional linkages for the pace of life, life-history, and environment in birds. *Integr. Comp. Biol.* 50, 855–868. doi: 10.1093/icb/icq024

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Do Early-Life Conditions Drive Variation in Senescence of Female Bighorn Sheep?

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The rate of senescence may vary among individuals of a species according to individual life histories and environmental conditions. According to the principle of allocation, changes in mortality driven by environmental conditions influence how organisms allocate resources among costly functions. In several vertebrates, environmental conditions during early life impose trade-offs in allocation between early reproduction and maintenance. The effects of conditions experienced during early life on senescence, however, remain poorly documented in wild populations. We examined how several early-life environmental conditions affected reproductive and survival senescence in wild bighorn sheep. We found long-term effects of high population density at birth, precipitations during the winter before birth, and temperature during the winter following birth that decreased survival after 7 years of age. High temperature during the first summer and autumn of life and high Pacific decadal oscillation decreased reproductive success at old ages. However, harsh early-life environment did not influence the rate of senescence in either survival or reproduction. Contrary to our expectation, we found no trade-off between reproductive allocation prior to senescence and senescence. Our results do show that early-life environmental conditions are important drivers of later survival and reproductive success and contribute to intra-specific variation in late-life fitness, but not aging patterns. These conditions should therefore be considered when studying the mechanisms of senescence and the determinants of variation in both survival and reproductive senescence at older ages.

Keywords: early-life, senescence, environmental conditions, long-term effects, life history, mortality

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INTRODUCTION

Demographic senescence is a decrease in survival and reproductive performance with age due to progressive decline in physiological functions (Kirkwood and Austad, 2000). The rate of senescence varies between species (Jones et al., 2014) but also among individuals of the same population. According to the disposable soma theory, senescence occurs because of trade-offs in the allocation of resources to maintenance and repair (Kirkwood, 1977; Monaghan et al., 2008). This theory proposes an evolutionary explanation of aging, based on the fact that resources are limited (Kirkwood and Austad, 2000). Organisms need resources for maintenance and repair, but also for other costly functions directly linked to fitness such as reproduction or growth.

Because resources are limited, allocation to life history traits involves trade-offs, and selection is expected to favor allocation strategies that maximize fitness (Cole, 1954; Stearns, 1989). Allocating resources to reproduction or growth rather than to maintenance and repair early in life should lead to faster senescence. For example, large breeds of dogs grow faster and exhibit higher mortality than smaller breeds that have not been artificially selected for rapid growth (Kraus et al., 2013). Thus, allocation strategies determine resource availability for different physiological functions and drive cell functioning and deterioration, ultimately affecting life histories including the rate of senescence.

Trade-offs between reproduction and maintenance and the optimal allocation between these traits will depend substantially on resource availability (Baudisch and Vaupel, 2012). For example, female mammals age more rapidly in the wild than in captivity (Lemaître et al., 2013). The consequences of resource limitation, however, can be complex. Dietary restriction can slow senescence in laboratory organisms (Nakagawa et al., 2012). In wild mammals and birds, some vital rates may be sensitive than others, as shown by stronger effects of birth environment on the rates of senescence in survival than in reproduction (Cooper and Kruuk, 2018).

Several studies show that early-life environmental conditions can have long-term effects on senescence in vertebrates (Lindström, 1999; Cooper and Kruuk, 2018; Spagopoulou et al., 2020). For example, poor early environmental conditions were linked with catch-up growth later in life, leading to shorter lifespan in three-spined sticklebacks (*Gasterosteus aculeatus*; Lee et al., 2013, 2016). Female red deer (*Cervus elaphus*) and bighorn sheep (*Ovis canadensis*) born at high population density show, respectively, faster rates of senescence and reduced reproduction and survival compared to those born when intraspecific competition is weak (Nussey et al., 2007; Pigeon et al., 2017). Early-life environment may also have long-term effects on human health (Bateson et al., 2004; Hanson and Gluckman, 2014). These studies emphasized that other environmental factors, including food availability, population density and other stressors, may affect the rate of senescence. Variation in early-life environmental conditions experienced by different cohorts may therefore generate heterogeneity in life-histories and variation in senescence rates between individuals within a population. These differences in life-history patterns reflect underlying trade-offs in soma allocation and are therefore key to better understand both the evolutionary and ecological causes of ageing and its diversity.

Most previous studies on the effect of early environmental conditions on aging considered only one or a few environmental drivers (e.g., Lindström, 1999; Brakefield et al., 2005; Nussey et al., 2007; Pigeon et al., 2017). Studies considering multiple drivers of senescence are scarce and few have investigated of the effects of early-life environment on older age classes in the wild (Rose, 1991; Hammers et al., 2013). Here, we tested whether early environmental conditions, quantified with several environmental variables, or higher reproductive allocation at young ages affected survival and reproduction after the onset of senescence in wild bighorn ewes. We expected that harsh birth environment (high density and high temperature) and high reproductive allocation would lower survival and reproductive success at old age and increase the rates of demographic senescence.

MATERIALS AND METHODS

Study Population

Since 1972, the bighorn sheep population on Ram Mountain (Alberta, Canada, 52° N 115° W, elevation: 1080–2170 m) has been monitored each year between late May and September, the period from birth to weaning of lambs (Jorgenson et al., 1993). Nearly all individuals were marked with colored collars (ewes) or ear tags (lambs and rams). Yearly resighting probability for females was more than 99% (Jorgenson et al., 1997), thus survival was known with precision. Each year, reproduction was monitored by udder inspection at capture and observations of females suckling lambs. We focused on cohorts born between 1973 and 2004, for which all individuals had a known year of death. We restricted analyses to females because males do not provide parental care and their reproductive effort was not quantified. As we were interested in how early environment affected survival and reproductive success at old ages, we considered females past the onset of senescence. We used data for 140 females from 30 cohorts whose age at death ranged from 7 to 19 years (646 female-years) to investigate survival senescence, which begins at age seven in this population (Jorgenson et al., 1997). Female reproductive senescence begins after 11 years of age (Bérubé et al., 1999; Martin and Festa-Bianchet, 2011; **Supplementary Figure 1**). We therefore restricted analysis of the rate of reproductive senescence to 63 females monitored when 11 years of age or more (205 female-years from 24 cohorts). Females can give birth to one lamb per year. We define successful reproduction as survival of the lamb to late September, the approximate age of weaning (Festa-Bianchet, 1988).

Early-Life Environment

We examined the effects of weather and population density in the year of birth (**Table 1**). Data on precipitations (rainfall plus water equivalent of snowfall in mm) and temperature (in °C) were obtained from the Environment Canada meteorological station at Nordegg (52°47′ N, 116°08′ W, elevation: 1,333 m; 25 km from Ram Mountain). These two measures were considered for the winter before birth (early gestation, December to March), spring (late gestation, April and May), summer (birth to weaning, mid-June to September), autumn (mid-September to November) and first winter (December to March). Over these periods, we averaged temperatures and calculated the sum of precipitations. Between 1973 and 2004, temperature increased in summer only (**Supplementary Table 1**). Precipitation did not show any detectable trends in any season (**Supplementary Table 1**). Temperature and precipitation affect individual mass changes in this population, making them relevant variables to investigate resource allocation trade-offs (Douhard et al., 2018). We also used the annual mean of the Pacific Decadal Oscillation (PDO), which is negatively related to horn growth (Douhard et al., 2017). This climatic index measures shifts between decades of warm and dry

TABLE 1 | Variables included in models of bighorn sheep female survival and reproductive success at Ram Mountain, Alberta, Canada.

Variables	Description	Unit	Mean	SD	Min	Max
Age	Age	years	9.66	2.43	7	19
Spring temperature	Mean temperature during spring preceding birth	°C	3.80	1.46	0.92	6.33
Spring precipitations	Total amount of precipitations during spring preceding birth	mm	104.35	44.03	37.50	229.20
Fall temperature	Mean temperature in autumn following birth	°C	0.08	2.05	−5.33	3.57
Fall precipitations	Total amount of precipitations in autumn following birth	mm	74.77	27.04	30.90	139.80
Summer temperature	Mean temperature during summer of birth	°C	11.19	0.75	9.87	13.08
Summer precipitations	Total amount of precipitations during summer of birth	mm	295.10	96.56	138.40	484.50
Temperature winter before	Mean temperature during winter preceding birth	°C	−7.97	2.43	−12.43	−2.86
Precipitations winter before	Total amount of precipitations during winter preceding birth	mm	84.08	31.23	29.50	158.50
Temperature winter after	Mean temperature during winter following birth	°C	−7.87	2.48	−12.43	−2.86
Precipitations winter after	Total amount of precipitations during winter following birth	mm	84.33	31.23	29.50	158.50
PDO (annual)	Annual mean of the Pacific Decadal Oscillation index	°C	0.32	0.76	−1.10	1.82
Density	Number of females in the population in June		51.81	24.62	18	103
Reproductive allocation	Number of lambs weaned before survival senescence (7 years)		2.61	1.21	0	5
	Number of lambs weaned before reproductive senescence (11 years)		5.64	1.8	1	9

winters, and decades of cooler winters with more precipitation in the Canadian Rocky Mountains (Trenberth and Hurrell, 1994; Whitfield et al., 2010). PDO values were obtained from http://research.jisao.washington.edu/pdo/PDO.latest. Annual PDO did not show any detectable trends over the study period. Population density was estimated by the number of adult females in June each year (Jorgenson et al., 1997). High population density increases competition, and thus corresponds to unfavorable environmental conditions (Nussey et al., 2007; Pigeon et al., 2017). Density peaked at 103 females in 1992 and decreased to a minimum of 18 in 2004. Correlations between environmental variables are in **Supplementary Figure 2**. Allocation of resources to reproduction is expected to reduce allocation to somatic maintenance and accelerate senescence (Kirkwood and Rose, 1991). We quantified reproductive allocation as the number of lambs weaned prior to the onset of senescence (7 and 11 years of age for survival and reproduction, respectively).

Statistical Analyses

To investigate the effect of early environment on reproductive success and survival, we performed generalized linear mixed-effects model using the glmer function in the lme4 R package. Survival was a binomial variable representing whether an individual seen in a given year was seen the following year or not. Reproductive success was defined as whether or not a female weaned a lamb in a given year. We quantified the rate of senescence as a linear decline of survival and reproductive success with age after the age of onset. To investigate the effect of environment during early life on senescence rates, we tested the effect of the interaction between age and each early-life environmental variable. Each model therefore included age, one of the environmental variables in early life (temperature and precipitation for each season, PDO, population density, and reproductive allocation) and its interaction with age. Models including only additive effects of age and early-life environment were also tested to investigate if effect of early life persisted at the oldest ages. We report parameter estimates from the additive models when the interaction was non-significant. Environmental variables were tested one at a time to avoid multi-collinearity (**Supplementary Figure 4**). All environmental variables were centered and scaled. To account for selective disappearance of frail individuals, models for reproduction also included longevity (van de Pol and Verhulst, 2006). We tested the contribution of selective disappearance to rates of senescence by comparing the parameter estimates for age in the best model with and without longevity included (Hayward et al., 2013). Individual identity, cohort and year were also included as random variables.

RESULTS

As expected, all models showed a decline in survival and reproductive success with age (**Supplementary Tables 2, 3** and **Figures 1A**, **2A**). Early environmental conditions affected reproduction and survival of senescent females (**Supplementary Table 2**). Heavy precipitations during the winter preceding birth and high temperatures during the winter following birth decreased survival, independently of age (**Table 2**; **Supplementary Table 2**, and **Figures 1B,C**, respectively). Similarly, high population density at birth decreased survival (**Table 2** and **Figure 1D**). However, we found no effect of early-life environment on the rate of actuarial senescence.

For reproductive success, three models showed a detectable effect of early environments (**Table 3** and **Supplementary Table 3**). The variables retained were temperature during summer of birth, temperature the autumn after birth and PDO. Weaning success was 70% for females born in the coldest summers but declined to 25% for those born in the hottest summers (**Figure 3A**). Similarly, high autumn temperature and PDO in the year of birth decreased late-life reproductive success (**Figures 3B,C**). Despite the persistent effects of early-life environment on female reproductive success, we found no evidence that it influenced the rate of reproductive senescence (**Supplementary Table 3**). When selective disappearance was

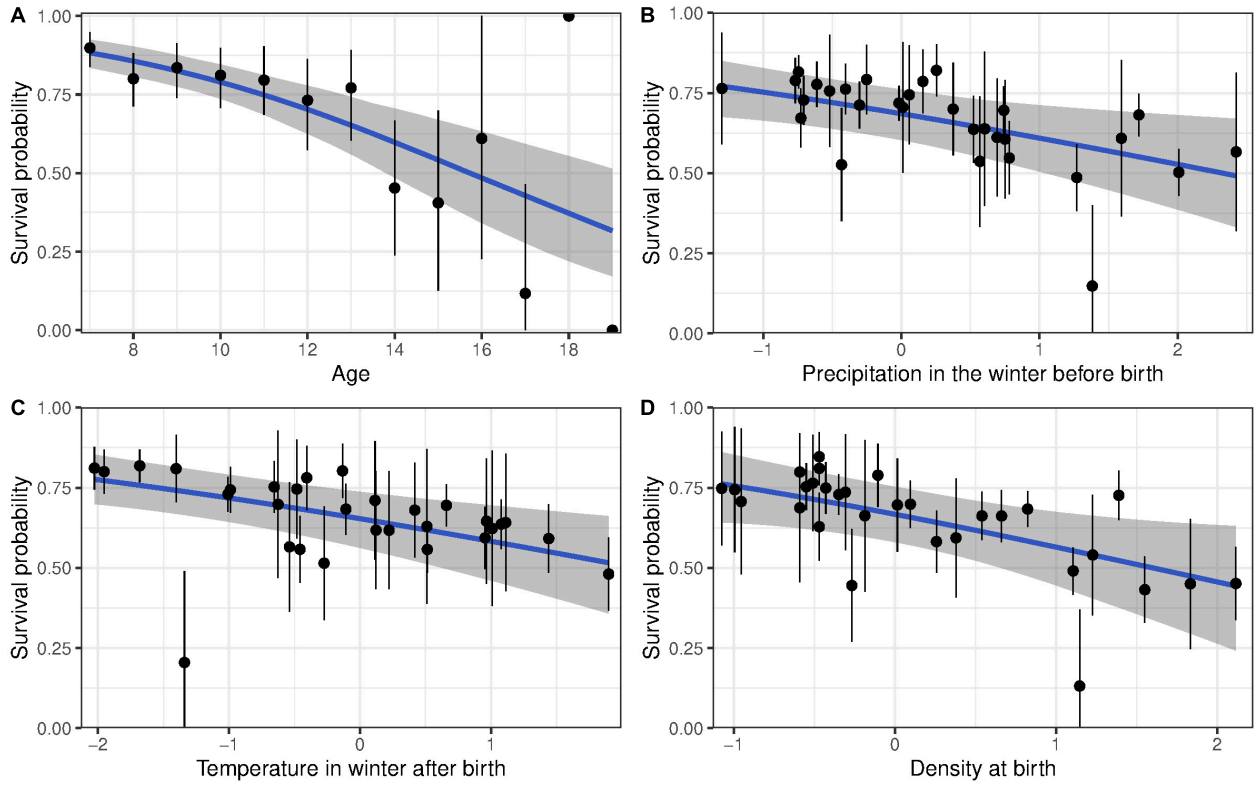

FIGURE 1 | Survival probability of bighorn sheep females born at Ram Mountain, Canada, 1973–2004. **(A)** Shows the age-related decline in survival for females aged 7 years and older, adjusted for current and birth environmental conditions. **(B–D)** show survival of females adjusted to age 13 according to **(B)** precipitation in the winter before birth, **(C)** temperature in the first winter of life, **(D)** population density at birth. The blue line shows predicted survival with associated 95% CI given a linear decline in survival with age. The points in panel **(A)** show average (±95% CI) age-specific survival while points in panels **(B–D)** show each cohort's survival (±se) adjusted to age 13.

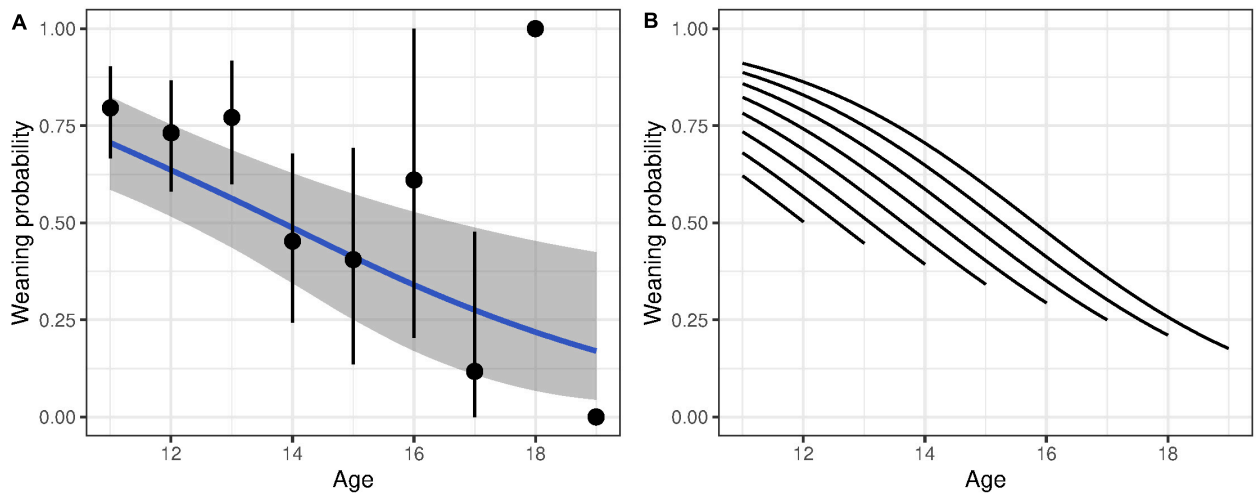

FIGURE 2 | Probability of weaning a lamb as a function of age for bighorn females. **(A)** Shows age-specific probability without accounting for longevity. The blue line shows predicted weaning probability with associated 95% CI given a linear decline in weaning probability with age. The points show average age-specific values (±95% CI). **(B)** Shows the change in weaning success with age decomposed into the effect from within-individual change with age and from selective disappearance. Each line shows the average within-individual change with age of individuals grouped by longevity.

TABLE 2 | Estimated coefficients, standard errors (SE) and corresponding *P*-values of the three generalized linear mixed-effects models showing how survival at old ages is affected by age, precipitations during the winter before preceding birth and temperature during the winter following birth for bighorn sheep females born at Ram Mountain, Canada, from 1973 to 2004, and by population density at birth.

Model	Variables	Coefficient	SE	*P*-value
1	(Intercept)	3.615	0.592	<0.001
	Age	−0.218	0.060	<0.001
	Precipitations (winter before)	−0.334	0.136	0.014
2	(Intercept)	3.485	0.529	<0.001
	Age	−0.219	0.053	<0.001
	Temperature (winter after)	−0.300	0.102	0.003
3	(Intercept)	3.907	0.537	<0.001
	Age	−0.247	0.051	<0.001
	Density	−0.435	0.175	0.013

TABLE 3 | Estimated coefficients, standard errors (SE) and corresponding *P*-values of the three generalized linear mixed-effects models showing how reproductive success at old ages is affected by age and by fall temperature, summer temperature and Pacific Decadal Oscillation (PDO) for bighorn sheep females born at Ram Mountain, Canada, from 1973 to 2003.

Model	Variables	Coefficient	SE	*P*-value
1	(Intercept)	2.937	1.477	0.047
	Age	−0.495	0.131	0.000
	Fall temperature	−0.407	0.183	0.026
	Longevity	0.243	0.112	0.029
2	(Intercept)	2.335	1.451	0.108
	Age	−0.486	0.129	0.000
	Summer temperature	−0.612	0.270	0.023
	Longevity	0.274	0.112	0.014
3	(Intercept)	3.295	1.481	0.026
	Age	−0.505	0.133	0.000
	PDO (annual)	−0.569	0.222	0.010
	Longevity	0.241	0.113	0.033

accounted for, the decline in age-specific reproductive success of old females was considerably stronger. Estimates of the rate of reproductive senescence were more pronounced by 30% after accounting for longevity, from −0.35 (95% CI = −0.59, −0.15) to −0.51 [95% CI = −0.78, 0.26 (**Figure 2B**)]. Selective disappearance likely affects some cohorts more than others, as we found significant relationship between several birth environment variables and longevity (**Supplementary Table 4**).

DISCUSSION

Our results show that early-life environmental conditions of bighorn females correlate with survival and reproductive success late in life. However, early-life environmental conditions influenced survival and reproductive success independently of age for older females. Individuals that experienced high population density at birth, high precipitations during their winter *in utero* and high temperature during their first winter of life had lower survival when aged 7 years and older compared to individuals born under more favorable environmental conditions. In addition, females that experienced high temperature during their first summer and autumn as well as high PDO showed lower reproductive success late in life.

Numerous studies reported a link between early-life environment and rates of reproductive but not survival senescence (reviewed by Cooper and Kruuk, 2018). Similarly, we found no evidence that early-life environment affected the rate of survival senescence. However, unlike studies on red deer (Nussey et al., 2007) or red squirrel (*Tamiasciurus hudsonicus*; Descamps et al., 2008), we also found no effect of early-life environment on the rate of reproductive senescence in old females. Bouwhuis et al. (2010), also found that early-life density and food abundance affected the average reproductive success but not the rate of reproductive senescence in great tits (*Parus major*). In Mountain goats (*Oreamnos americanus*), early-life conditions also affected average late-life survival and reproduction without affecting rates of senescence (Panagakis et al., 2017). These mixed results may be due to low sample size at old age, but also to a failure to account for the shape of senescence (Ronget and Gaillard, 2020). Across species, senescence in survival and reproduction show a variety of shapes (Jones et al., 2014; Lemaître et al., 2020), but the impact of early life on the shape of senescence within species remains poorly known. **Figure 4** illustrates conceptually how changes in onset and rate of senescence may be shaped by difference in early-life conditions. Compared to individuals born in good conditions (**Figure 1A**), challenging environmental conditions early in life could increase the rate of senescence or advance its onset (**Figure 4**b,c, respectively). For example, Hammers et al. (2013) found that early-life environment reduced survival probabilities of Seychelles warblers (*Acrocephalus sechellensis*) independently of age for individuals over 6 years and suggested that this was caused by an earlier onset of senescence (**Figure 4**c). Alternatively, performance over the entire lifespan may be lower for individuals that develop in poor environments, without changing the rate or onset of senescence (**Figure 4**d). Individuals may also compensate for a poor start through catch-up growth (Marcil-Ferland et al., 2013) or delayed primiparity (Pigeon and Pelletier, 2018), leading to late-life performances similar to those of individuals that experience favorable early environments (**Figure 4**a). Distinguishing between these non-mutually exclusive patterns of senescence, however, is impossible if studies focus only on old individuals, which many do, including this study. Non-linear analysis of the entire life of individuals with different early-life environment will be required to disentangle the alternative. That analysis, however, will be challenging because of the small number of individuals per cohort, especially for cohorts born during unfavorable years that have low early survival. Mixture models may provide a solution to this challenge by grouping similar individuals together independently of cohort (Hamel et al., 2017).

The effect of early-life environment on senescence rates may be further obscured by selective disappearance of frail individuals

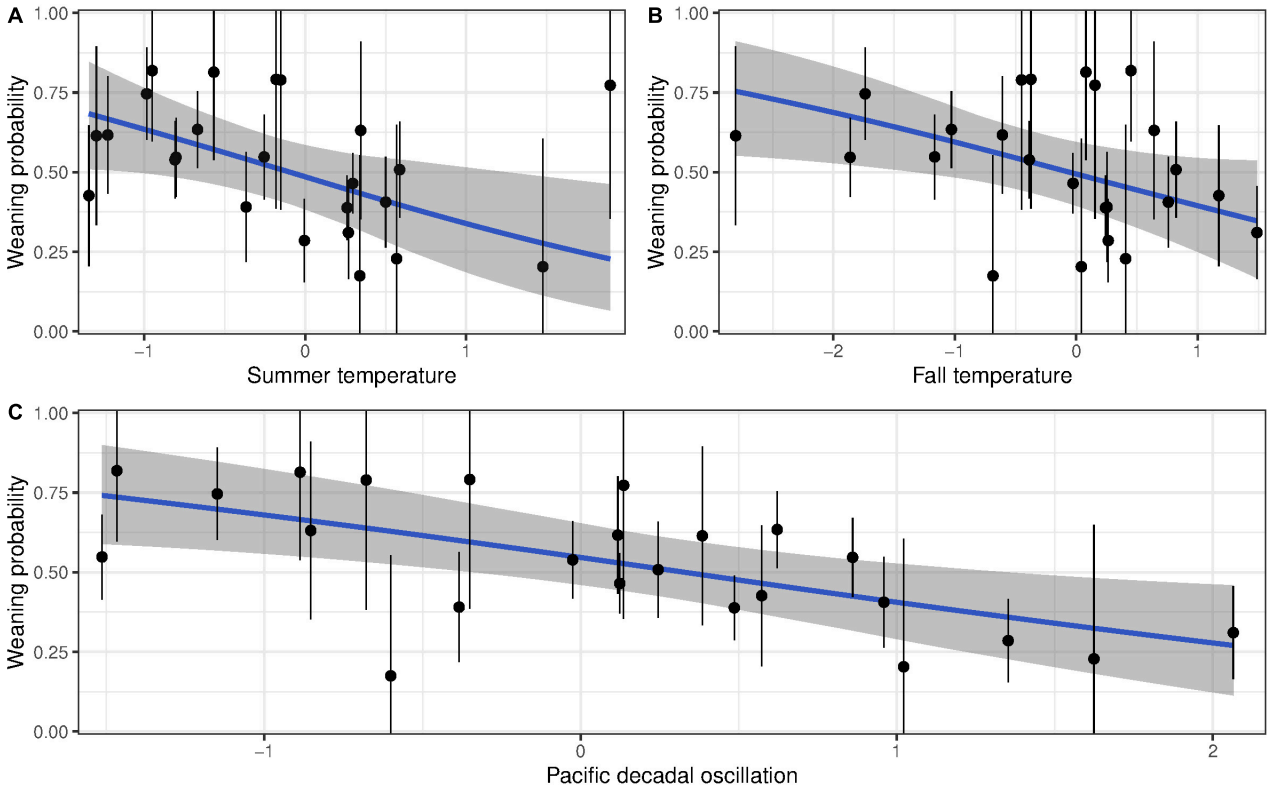

FIGURE 3 | Probability of weaning a lamb for bighorn females born at Ram Mountain, Canada, 1973–2003 according to birth **(A)** summer temperature, **(B)** autumn temperature, and **(C)** Pacific Decadal Oscillation (blue line). Points show the average weaning success (±se) of each cohort adjusted to age 13 years.

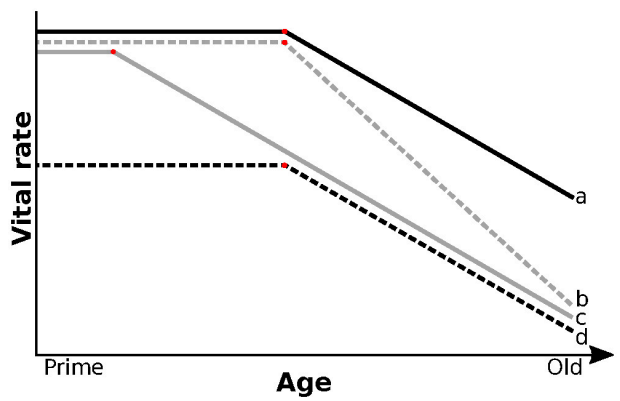

FIGURE 4 | Poor environmental conditions during early life could have different effects on late-life performance. Solid black line (a) illustrates individuals born in good environment. When faced with challenging early life, senescence may show a higher rate (gray dashed: b) or an earlier onset (gray solid, c). Harsh early-life environment may also lower performance independently of age (black dashed; d). In wild populations, patterns of age specific change in performance may be a combination of the above.

(van de Pol and Verhulst, 2006). In bighorn females, large body mass in prime age is associated with greater longevity (Bérubé et al., 1999) and mass is influenced by early-life environment (Pigeon and Pelletier, 2018). Frail females could also die as young adults and only those robust enough to withstand the negative effect of high precipitations in the year of birth may survive to older ages. Even among older females, selective disappearance plays an important role, decreasing the apparent rates of senescence by 30%. That disappearance is not random in relation to birth environment, as individuals born in harsh environments (**Supplementary Table 4**) die at an earlier age. The effects of selective disappearance on vital rates are of similar magnitude to those reported for Soay sheep (*Ovis aries*; Hayward et al., 2013). Despite its importance, this selective effect is probably under-estimated in our study because only 14% of females survive from the time they are marked to the onset of reproductive senescence at 11 years (**Supplementary Figure 3**). Selection against frail individuals early in life is therefore most likely excluded from our estimate.

Our study suggests that a poor start can leave a permanent signature on fitness despite large variation in subsequent environmental conditions (Pigeon and Pelletier, 2018), although we did not investigate the mechanisms behind these patterns. Rapid development early in life, including catch-up growth (Marcil-Ferland et al., 2013), often has negative long-term effects. Several physiological mechanisms causing long-term effects have been proposed, including epigenetic programming (Heijmans et al., 2008; Bar-Sadeh et al., 2020). Stress during early life has also been associated with shorter telomeres and mitochondrial

inefficiency (Casagrande et al., 2020; but see Brown et al., 2021). Drosophila (*Drosophila melanogaster*) reared on a poor diet show a higher rate of germline stem cell decline due to changes in insulin signaling (Hsu and Drummond-Barbosa, 2009), which is linked to reproductive senescence (Quesada-Candela et al., 2021). Linking physiological process with late-life fitness in natural conditions will be critical to understand the mechanisms of senescence.

The disposable soma hypothesis predicts that allocation of resources to reproduction rather than to somatic maintenance should lead to faster senescence (Kirkwood and Rose, 1991). Detection of such tradeoffs is common, but not universal (Lemaître et al., 2015). Unlike red deer (Nussey et al., 2007) and common guillemots (*Uria aalge*; Reed et al., 2008), in bighorn sheep we found no evidence that increased reproductive allocation decreases survival of old females or increases their rate of actuarial senescence. On the contrary, we found a trend for positive covariance between early reproductive allocation and late-life survival, confirming an earlier (Bérubé et al., 1999). This result is consistent with studies of other long-lived capital breeders like elephant seals (*Mirounga leonina*; Oosthuizen et al., 2021) and mountain goats (Panagakis et al., 2017) that did not detect early-late life trade-offs. Strong individual heterogeneity can limit our ability to detect life-history trade-offs through phenotypic correlations (Hamel et al., 2010). The exact source of this heterogeneity, however, remains to be explored and may be unrelated to early-life environment. While the effect of early-life environmental conditions on allocation trade-offs and their consequence on senescence has received much attention (Lemaître et al., 2015), fewer studies have considered allocation throughout the lifespan. In long-lived species, events occurring at maturity may also play a more important role (Baudisch and Vaupel, 2012). Heterogeneity may also stem from factors other than resource scarcity. For example, in baboons (*Papio spp.*), early social environment, which varies greatly between individuals, plays a predominant role (Alberts, 2019). Thus, variation in social context experienced by different cohorts or individuals can, generates heterogeneity in life-history, even within a single population. Future studies should therefore consider a broader view of the drivers of variation in senescence patterns, and not only early-life resource scarcity. Since senescence is the result of resource allocation decisions made to face stressors through life, individual experiences, at birth but also later in life, may be just as important.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal-handling procedures were approved by the Animal Care Committee of the University of Sherbrooke (protocol 2020-2707).

AUTHOR CONTRIBUTIONS

GP and JL performed the analyses. GP, JL, MF-B, and FP contributed to the data collection, conception, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.637692/full#supplementary-material

REFERENCES

Alberts, S. C. (2019). Social influences on survival and reproduction: insights from a long-term study of wild baboons. *J. Anim. Ecol.* 88, 47–66. doi: 10.1111/1365-2656.12887

Bar-Sadeh, B., Rudnizky, S., Pnueli, L., Bentley, G. R., Stöger, R., Kaplan, A., et al. (2020). Unravelling the role of epigenetics in reproductive adaptations to early-life environment. *Nat. Rev. Endocrinol.* 16, 519–533. doi: 10.1038/s41574-020-0370-8

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R. A., et al. (2004). Developmental plasticity and human health. *Nature* 430, 419–421. doi: 10.1038/nature02725

Baudisch, A., and Vaupel, J. W. (2012). Getting to the root of aging. *Science* 338, 618–619. doi: 10.1126/science.1226467

Bérubé, C. H., Festa-Bianchet, M., and Jorgenson, J. T. (1999). Individual differences, longevity, and reproductive senescence in bighorn ewes. *Ecology* 80, 2555–2565.

Bouwhuis, S., Charmantier, A., Verhulst, S., and Sheldon, B. C. (2010). Individual variation in rates of senescence: natal origin effects and disposable soma in a wild bird population: Individual variation in rates of senescence. *J. Anim. Ecol.* 79, 1251–1261. doi: 10.1111/j.1365-2656.2010.01730.x

Brakefield, P. M., Gems, D., Cowen, T., Christensen, K., Grubeck-Loebenstein, B., Keller, L., et al. (2005). What are the effects of maternal and pre-adult environments on ageing in humans, and are there lessons from animal models? *Mech. Age. Dev.* 126, 431–438. doi: 10.1016/j.mad.2004.07.013

Brown, T., Dugdale, H., Spurgin, L., Komdeur, J., Burke, T., and Richardson, D. (2021). Causes and consequences of telomere lengthening in a wild vertebrate population. [Preprints]. doi: 10.22541/au.161408541.15345829/v1

Casagrande, S., Stier, A., Monaghan, P., Loveland, J. L., Boner, W., Lupi, S., et al. (2020). Increased glucocorticoid concentrations in early life cause mitochondrial inefficiency and short telomeres. *J. Exp. Biol.* 223:jeb222513. doi: 10.1242/jeb.222513

Cole, L. C. (1954). The population consequences of life history phenomena. *Q. Rev. Biol.* 36, 103–137.

Cooper, E. B., and Kruuk, L. E. B. (2018). Ageing with a silver-spoon: a meta-analysis of the effect of developmental environment on senescence. *Evol. Lett.* 2, 460–471. doi: 10.1002/evl3.79

Descamps, S., Boutin, S., Berteaux, D., and Gaillard, J.-M. (2008). Age-specific variation in survival, reproductive success and offspring quality in red squirrels: evidence of senescence. *Oikos* 117, 1406–1416. doi: 10.1111/j.0030-1299.2008.16545.x

Douhard, M., Guillemette, S., Festa-Bianchet, M., and Pelletier, F. (2018). Drivers and demographic consequences of seasonal mass changes in an alpine ungulate. *Ecology* 99, 724–734. doi: 10.1002/ecy.2141

Douhard, M., Pigeon, G., Festa-Bianchet, M., Coltman, D. W., Guillemette, S., and Pelletier, F. (2017). Environmental and evolutionary effects on horn growth of male bighorn sheep. *Oikos* 126, 1031–1041. doi: 10.1111/oik.03799

Festa-Bianchet, M. (1988). Birthdate and survival in bighorn lambs (*Ovis canadensis*). *J. Zool.* 214, 653–661. doi: 10.1111/j.1469-7998.1988.tb03764.x

Hamel, S., Gaillard, J.-M., Yoccoz, N. G., Loison, A., Bonenfant, C., and Descamps, S. (2010). Fitness costs of reproduction depend on life speed: empirical evidence from mammalian populations: fitness costs of reproduction in mammals. *Ecol. Lett.* 13, 915–935. doi: 10.1111/j.1461-0248.2010.01478.x

Hamel, S., Yoccoz, N. G., and Gaillard, J.-M. (2017). Assessing variation in life-history tactics within a population using mixture regression models: a practical guide for evolutionary ecologists: mixture regression models and life-history tactics. *Biol. Rev.* 92, 754–775. doi: 10.1111/brv.12254

Hammers, M., Richardson, D. S., Burke, T., and Komdeur, J. (2013). The impact of reproductive investment and early-life environmental conditions on senescence: support for the disposable soma hypothesis. *J. Evol. Biol.* 26, 1999–2007. doi: 10.1111/jeb.12204

Hanson, M. A., and Gluckman, P. D. (2014). Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol. Rev.* 94, 1027–1076. doi: 10.1152/physrev.00029.2013

Hayward, A. D., Wilson, A. J., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J. M., and Kruuk, L. E. B. (2013). Reproductive senescence in female Soay sheep: variation across traits and contributions of individual ageing and selective disappearance. *Funct. Ecol.* 27, 184–195. doi: 10.1111/1365-2435.12029

Heijmans, B. T., Tobi, E. W., Stein, A. D., Putter, H., Blauw, G. J., Susser, E. S., et al. (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17046–17049. doi: 10.1073/pnas.0806560105

Hsu, H.-J., and Drummond-Barbosa, D. (2009). Insulin levels control female germline stem cell maintenance via the niche in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 1117–1121. doi: 10.1073/pnas.0809144106

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of ageing across the tree of life. *Nature* 505, 169–173. doi: 10.1038/nature12789

Jorgenson, J. T., Festa-Bianchet, M., Gaillard, J.-M., and Wishart, W. D. (1997). Effects of age, sex, disease, and density on survival of bighorn sheep. *Ecology* 78, 1019–1032.

Jorgenson, J. T., Festa-Bianchet, M., and Wishart, W. D. (1993). Harvesting bighorn ewes: consequences for population size and trophy ram production. *J. Wildl. Manag.* 57, 429–435. doi: 10.2307/3809267

Kirkwood, T. B. L. (1977). Evolution of ageing. *Nature* 270, 301–304. doi: 10.1038/270301a0

Kirkwood, T. B. L., and Austad, S. N. (2000). Why do we age? *Nature* 408, 233–238. doi: 10.1038/35041682

Kirkwood, T. B. L., and Rose, M. R. (1991). Evolution of senescence: late survival sacrificed for reproduction. *Phil. Trans. R. Soc. Lond. B* 332, 15–24. doi: 10.1098/rstb.1991.0028

Kraus, C., Pavard, S., and Promislow, D. E. L. (2013). The size–life span trade-off decomposed: why large dogs die young. *Am. Nat.* 181, 492–505. doi: 10.1086/669665

Lee, W., Monaghan, P., and Metcalfe, N. B. (2016). Perturbations in growth trajectory due to early diet affect age-related deterioration in performance. *Funct. Ecol.* 30, 625–635. doi: 10.1111/1365-2435.12538

Lee, W.-S., Monaghan, P., and Metcalfe, N. B. (2013). Experimental demonstration of the growth rate–lifespan trade-off. *Proc. R. Soc. B* 280:20122370. doi: 10.1098/rspb.2012.2370

Lemaître, J.-F., Berger, V., Bonenfant, C., Douhard, M., Gamelon, M., Plard, F., et al. (2015). Early-late life trade-offs and the evolution of ageing in the wild. *Proc. R. Soc. B* 282:20150209. doi: 10.1098/rspb.2015.0209

Lemaître, J.-F., Gaillard, J.-M., Lackey, L. B., Clauss, M., and Müller, D. W. H. (2013). Comparing free-ranging and captive populations reveals intra-specific variation in aging rates in large herbivores. *Exp. Gerontol.* 48, 162–167. doi: 10.1016/j.exger.2012.12.004

Lemaître, J.-F., Ronget, V., and Gaillard, J.-M. (2020). Female reproductive senescence across mammals: a high diversity of patterns modulated by life history and mating traits. *Mech. Age. Dev.* 192:111377. doi: 10.1016/j.mad.2020.111377

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends Ecol. Evol.* 14, 343–348. doi: 10.1016/S0169-5347(99)01639-0

Marcil-Ferland, D., Festa-Bianchet, M., Martin, A. M., and Pelletier, F. (2013). Despite catch-up, prolonged growth has detrimental fitness consequences in a long-lived vertebrate. *Am. Nat.* 182, 775–785. doi: 10.1086/673534

Martin, J. G. A., and Festa-Bianchet, M. (2011). Age-independent and age-dependent decreases in reproduction of females: age-independent and age-dependent senescence. *Ecol. Lett.* 14, 576–581. doi: 10.1111/j.1461-0248.2011.01621.x

Monaghan, P., Charmantier, A., Nussey, D. H., and Ricklefs, R. E. (2008). The evolutionary ecology of senescence. *Funct. Ecol.* 22, 371–378. doi: 10.1111/j.1365-2435.2008.01418.x

Nakagawa, S., Lagisz, M., Hector, K. L., and Spencer, H. G. (2012). Comparative and meta-analytic insights into life extension via dietary restriction: dietary restriction and longevity: meta-analysis. *Aging Cell* 11, 401–409. doi: 10.1111/j.1474-9726.2012.00798.x

Nussey, D. H., Kruuk, L. E. B., Morris, A., and Clutton-Brock, T. H. (2007). Environmental conditions in early life influence ageing rates in a wild population of red deer. *Curr. Biol.* 17, R1000–R1001. doi: 10.1016/j.cub.2007.10.005

Oosthuizen, W. C., Péron, G., Pradel, R., Bester, M. N., and de Bruyn, P. J. N. (2021). Positive early-late life-history trait correlations in elephant seals. *Ecology* 102:e03288. doi: 10.1002/ecy.3288

Panagakis, A., Hamel, S., and Côté, S. D. (2017). Influence of early reproductive success on longevity and late reproductive success in an alpine ungulate. *Am. Nat.* 189, 667–683. doi: 10.1086/691388

Pigeon, G., Festa-Bianchet, M., and Pelletier, F. (2017). Long-term fitness consequences of early environment in a long-lived ungulate. *Proc. R. Soc. B* 284:20170222. doi: 10.1098/rspb.2017.0222

Pigeon, G., and Pelletier, F. (2018). Direct and indirect effects of early-life environment on lifetime fitness of bighorn ewes. *Proc. R. Soc. B* 285:20171935. doi: 10.1098/rspb.2017.1935

Quesada-Candela, C., Loose, J., Ghazi, A., and Yanowitz, J. L. (2021). Molecular basis of reproductive senescence: insights from model organisms. *J. Assist. Reprod. Genet.* 38, 17–32. doi: 10.1007/s10815-020-01959-4

Reed, T. E., Kruuk, L. E. B., Wanless, S., Frederiksen, M., Cunningham, E. J. A., and Harris, M. P. (2008). Reproductive senescence in a long-lived seabird: rates of decline in late-Life performance are associated with varying costs of early reproduction. *Am. Nat.* 171, E89–E101. doi: 10.1086/524957

Ronget, V., and Gaillard, J. (2020). Assessing ageing patterns for comparative analyses of mortality curves: going beyond the use of maximum longevity. *Funct. Ecol.* 34, 65–75. doi: 10.1111/1365-2435.13474

Rose, M. R. (1991). *Evolutionary Biology of Aging*. Oxford: Oxford University Press.

Spagopoulou, F., Teplitsky, C., Chantepie, S., Lind, M. I., Gustafsson, L., and Maklakov, A. A. (2020). Silver-spoon upbringing improves early-life fitness but promotes reproductive ageing in a wild bird. *Ecol. Lett.* 23, 994–1002. doi: 10.1111/ele.13501

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259–268. doi: 10.2307/2389364

Trenberth, K. E., and Hurrell, J. W. (1994). Decadal atmosphere-ocean variations in the Pacific. *Clim. Dyn.* 9, 303–319. doi: 10.1007/BF00204745

van de Pol, M., and Verhulst, S. (2006). Age-dependent traits: a new statistical model to separate within- and between-individual effects. *Am. Nat.* 167, 766–773. doi: 10.1086/503331

Whitfield, P. H., Moore, R. D., Fleming, S. W., and Zawadzki, A. (2010). Pacific decadal oscillation and the hydroclimatology of western Canada—review and prospects. *Can. Water Resour. J.* 35, 1–28. doi: 10.4296/cwrj3501001

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Salamander Insights Into Ageing and Rejuvenation

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Exhibiting extreme regenerative abilities which extend to complex organs and entire limbs, salamanders have long served as research models for understanding the basis of vertebrate regeneration. Yet these organisms display additional noteworthy traits, namely extraordinary longevity, indefinite regenerative potential and apparent lack of traditional signs of age-related decay or "negligible senescence." Here, I examine existing studies addressing these features, highlight outstanding questions, and argue that salamanders constitute valuable models for addressing the nature of organismal senescence and the interplay between regeneration and ageing.

Keywords: axolotl, newt, cellular senescence, negligible senescence, regeneration, cancer

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INTRODUCTION

Salamanders, or urodele amphibians, stand out among vertebrates due to their ability to regenerate extensive sections of their body plan including ocular tissues, jaws, lungs, sections of their heart and brain, spinal cord, and entire limbs throughout their lives (Brockes and Kumar, 2008; Cox et al., 2019). As the evolutionarily closest organisms to humans capable of complex regeneration, salamanders constitute valuable models for regenerative biology studies. In particular, the axolotl—*Ambystoma mexicanum*—and the Iberian ribbed newt—*Pleurodeles waltl*—are two laboratory-tractable systems whose adoption has exponentially grown in recent years due to the ease of captive breeding and rearing (Khattak et al., 2014; Joven et al., 2015), efficient transgenesis and genome editing methods (Khattak et al., 2013b; Hayashi et al., 2014; Fei et al., 2018; Cai et al., 2019), availability of genomic and transcriptomic information (Elewa et al., 2017; Nowoshilow et al., 2018; Smith et al., 2019), and advanced imaging techniques (Masselink and Tanaka, 2020; Subiran Adrados et al., 2020; **Box 1**). Newts present a conventional salamander life cycle, undergoing metamorphosis and becoming fully developed adults with reduced to imperceptible continuous growth. In contrast, axolotls are neotenic organisms, exhibiting larval traits throughout their lives and indefinite growth. While occasional differences in regenerative capacity (Suetsugu-Maki et al., 2012) and mechanisms (Tanaka, 2016; Tanaka et al., 2016) exist, both species are capable of extensive organ and appendage regeneration following important clade-conserved principles. Particularly, salamander regeneration is associated with an unusual ability to regulate the plasticity of the differentiated state. Instead of relying exclusively on stem cells, the progenitors for the new structure are often obtained through limited reprogramming—dedifferentiation and transdifferentiation—of mature, differentiated adult cells (Tanaka and Reddien, 2011; Yun et al., 2013, 2014). In the context of the axolotl limb, the connective tissue cells at the stump dedifferentiate to form the various connective tissue derivatives of the new structure (Gerber et al., 2018). In newts, muscle regeneration relies on progenitors derived from dedifferentiation of mature muscle fibres (Lo et al., 1993; Tanaka et al., 2016), while the lens of the eye is regenerated *de novo*

BOX 1 | Experimental toolbox for salamander models*.

- **Germline transgenesis.** Tools for germline transgenesis are available for both axolotls and Iberian ribbed newts, based on I-*Sce*I meganuclease and Tol2 transposon technologies, including the CRE/LoxP system for tissue and time dependent control of gene expression (Khattak et al., 2013a; Hayashi and Takeuchi, 2015).
- **Genome assembly and CRISPR-mediated gene editing.** The recent sequencing and assembly of the 32-Gb axolotl genome (Nowoshilow et al., 2018; Smith et al., 2019) and the 20-Gb *P. waltl* genome (Elewa et al., 2017) provides a rich platform for investigations into the molecular basis of biological phenomena. Together with TALEN and CRISPR/Cas9-mediated gene editing (Khattak et al., 2013a; Hayashi et al., 2014; Fei et al., 2018; Cai et al., 2019), it is possible to assess candidate genes for functional analysis.
- **Somatic gene delivery methods.** Various technologies are available for gene delivery to salamander cells and tissues (Echeverri and Tanaka, 2003; Yun et al., 2013), including electroporation and viral transfection methods (Khattak et al., 2013a; Whited et al., 2013; Oliveira et al., 2018).
- **Cell and tissue transplantation.** The amenability of salamanders to cell and tissue transplantation combined with transgenic technologies (Kragl et al., 2009; Kragl and Tanaka, 2009; Lopez et al., 2014; Yun et al., 2015), has been informative toward understanding key aspects of development and regeneration.
- **Advanced imaging.** Many salamander tissues are optically transparent, and highly suited to live imaging. Further, several optical clearing methods have been adapted to the salamander system, enabling volumetric quantitative imaging (Duerr et al., 2020; Masselink and Tanaka, 2020; Pinheiro et al., 2020; Subiran Adrados et al., 2020).
- **Chemical screenings.** Due to their size and skin mediated compound exchange, salamanders can be used for moderate-throughput screening of pharmaceutical compounds (Ponomareva et al., 2015).

*Adapted from Yu and Yun (2020).

through transdifferentiation of pigmented epithelial cells of the dorsal iris (Henry and Tsonis, 2010). Reversals of the differentiated state for the generation of regenerative progenitors are also common in other vertebrates capable of complex regeneration, such as zebrafish (Jopling et al., 2010; Knopf et al., 2011), yet rarely observed in mammals. In this connection, the existence of roadblocks to dedifferentiation has been proposed to underlie the limited regenerative potential found in mammalian systems (Pajcini et al., 2010; Yun et al., 2013).

INDEFINITE REGENERATIVE CAPACITY

A salient feature of salamander regeneration is its resilience. Urodele regenerative capacity does not decline with time, and most studies suggest it is not impaired by repetitive regeneration events (Yun, 2015). A landmark study by Eguchi et al. (2011) tracked the process of lens regeneration over 16 years in Japanese newts, removing the lens from the same animals 18 times and allowing them to undergo regeneration. Remarkably, the resulting lenses were structurally identical to the original ones and expressed similar levels of lens-specific genes. Subsequent analysis revealed that the transcriptomes of young and old (19-times regenerated) lenses are nearly indistinguishable (Sousounis et al., 2015), showcasing the robustness of newt lens regeneration. Of note, by the end of the study the specimens were at least 30 years old, representing a geriatric population in this species (Eguchi et al., 2011). This provides an interesting contrast to the declines in regenerative capacities observed in most vertebrate contexts (Yun, 2015). Additional studies indicate that repetitive amputations do not affect tail regenerative potential in the newt *Triturus carnifex*, as examined over a 10 year period with up to nine tail regeneration cycles (Margotta et al., 2002; Margotta, 2008), nor that of the axolotl limb, challenged by five regeneration rounds during 3 years (Yun et al., 2015). In this connection, a recent study observed increasing rates of incomplete or failed regeneration after 3 regenerative cycles in the axolotl (Bryant et al., 2017). This interesting observation was based on studies using American axolotl strains, whereas similar studies in European strains have not shown the described regenerative impairment. It is thus conceivable that the phenotypic differences stem from a diverse genetic background, something which should be addressed by further studies. Taken together, the evidence to date suggests that the ability of urodeles to regenerate complex structures does not decline with time or serial regeneration cycles. In mammals, loss of regenerative potential with ageing has been largely attributed to the ageing of stem cell populations and/or their niche (Yun, 2015). Whether the prevalence of dedifferentiation as a regenerative mechanism in salamanders is linked to the indefinite nature of their regenerative potential remains an outstanding question.

EXTREME LIFESPANS

Beyond their remarkable regenerative abilities, salamanders exhibit extraordinary longevity (Sousounis et al., 2014), constituting lifespan outliers with respect to organismal size (**Figure 1**). Among animal species, there is a notable correlation between body mass and lifespan, with larger animals living longer. Yet, salamanders break this rule by several orders of magnitude. For example, axolotls—average mass: 60–110 g—live over 20 years (CRTD colony and (Warburg, 2007)), *P. walt* newts—average mass: 25 g—live up to 20 years in the wild (Warburg, 2007; Tacutu et al., 2018), Japanese newts—*Cynops pyrrhogaster*, average mass: 8 g—have a 25 year lifespan (Sousounis et al., 2015), spotted salamanders—*Ambystoma maculatum*; average mass: 13 g—reach 30 years of age, and cave olms—*Proteus anguinus*; average mass: 17 g—can surpass 100 years (Voituron et al., 2011; Tacutu et al., 2018). Indeed, they match and in some cases exceed the lifespan/body mass ratios found in other well-known outliers such as the naked mole rat (Ruby et al., 2018) and Brandt's bat (Seim et al., 2013). This is even more remarkable given that most salamander longevity data derive from specimens in the wild (Warburg, 2007), where animals are exposed to environmental challenges, predation, pathogens, and food source fluctuations. The establishment of research colonies for certain species, enabling breeding and rearing of individuals under controlled conditions, has contributed to the acquisition of more accurate lifespan measurements. Unsurprisingly, in most cases these surpass the estimates obtained from wild specimens, as in the extreme case of *P. anguinus*, whose lifespan fluctuates from 15 years in the

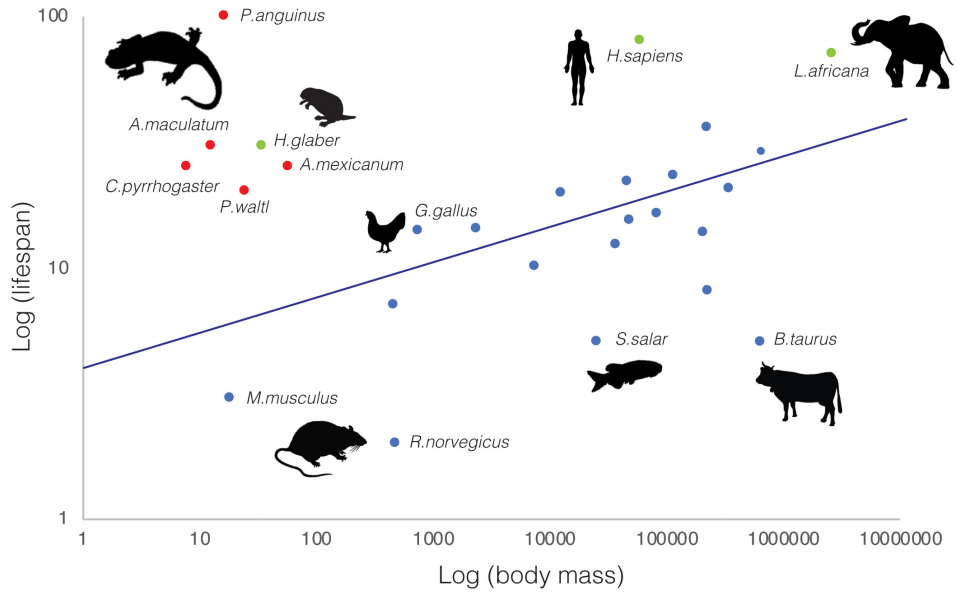

FIGURE 1 | Salamanders are lifespan outliers. Relationship between average body mass (g) and lifespan (years) for selected salamander (red) and representative vertebrate species (blue). Additional upper-end lifespan outliers (naked mole rat—*Heterocephalus glaber*-, African bush elephant—*Loxodonta africana*—and human—*Homo sapiens*—) are indicated in green. Animal silhouettes (not drawn to scale) represent the vertebrate clades to which the selected representative species belong to. Data was obtained from various sources, including ADW Animal diversity web, AnAge (Tacutu et al., 2018) and Amniote Life History Database (Myhrvold et al., 2015).

wild to a predicted maximum exceeding 100 years in lab cave conditions established in the 1950's (Voituron et al., 2011; Tacutu et al., 2018). Thus, salamanders are not only lifespan outliers, but also in many cases their longevity may be underestimated.

NEGLIGIBLE SENESCENCE

While the underlying basis of their exceptional longevity remains unknown, salamanders exhibit an uncommon resistance to ageing. Although few studies have addressed this topic, these, together with evidence from captive records in zoos and laboratories, suggest that a number of urodele species do not display the traditional signs of physiological decay that accompany mammalian ageing and are thus considered organisms of "negligible senescence" (Finch, 1990; Margotta et al., 2002; Cayuela et al., 2019). This phenomenon, also observed in other vertebrates such as turtles, rockfish and naked mole rats (Finch, 2009), is intrinsically linked to a defiance of the Gompertz-Makeham law of mortality (Gompertz, 1825; Makeham, 1860), which states that death risk increases exponentially as an organism ages. Indeed, a recent study involving three salamander species (*Lyciasalamandra fazilae, Salamandra salamandra,* and *Salamandra perspicillata*), indicates that their mortality rate is stable and weakly affected by age, in keeping with them exhibiting negligible senescence (Cayuela et al., 2019). This observation raises several important yet outstanding questions, including whether salamanders manifest cellular hallmarks of ageing as defined in mammalian contexts (Lopez-Otin et al., 2013), whether they age at the molecular level, what principles govern ageing—or lack of—in these organisms and, in particular, what is the role played by their extreme regenerative abilities in this process.

As a salamander grows older, changes in its tissues do occur. A number of these have been reported for the axolotl, including increase in size, progressive replacement of skeletal cartilage by bone, reduced locomotion and thickening of the dermal layer (Vieira et al., 2020). However, these changes are likely associated with the species' traits—as in the case of size-, and organism's maturation—in the case of the skeleton, dermis and locomotion—rather than ageing. In addition, a reduction in the rate of limb regeneration through time has been observed for the axolotl (Vieira et al., 2020). Yet, this can be interpreted as a consequence of the continuous growth that characterises this species, as the regeneration rate is proportional to the size of the structure being regenerated. Furthermore, time-related declines in regeneration rate are not observed in salamander species with limited adult growth (e.g., *Notophthalmus viridescens*). An additional change that may occur in axolotls as they age is a decline in fertility. This notion, based on anecdotal reports of mating success, including in our colony, does not extend to other salamander species, nor is conserved across the amphibian clade (Jones et al., 2014).

Long lifespans combined with a lack of ageing biomarkers have so far precluded the determination of biological age in urodeles. While this issue has seldom been studied, time-related expression changes have been reported for aged tail and iris cells from c. 30 year old newts (Sousounis et al., 2015). Some of these changes are consistent with features of molecular ageing as observed in mammalian contexts. Namely, aged tail and iris

samples displayed a downregulation of electron transport chain genes when compared to their young counterparts, indicating that these tissues could undergo molecular ageing (Sousounis et al., 2015). Nevertheless, further research should determine to what extent salamander tissues age at the molecular level.

CANCER RESISTANCE

Molecular ageing aside, no clear manifestations of age-related physiological declines or pathologies have been reported in urodeles to date. On the contrary, they exhibit a very low incidence of cancer, one of the most prevalent age-related pathologies. Neoplastic growth is rarely observed among salamander species, as documented in newts and axolotls (Ingram, 1971; Tsonis, 1983). Further, treatment with carcinogens can result in neoplasm induction but only at higher concentrations and longer treatment periods than those required to elicit malignant transformations in mammalian settings (Tsonis, 1983). Evidence also indicates that regenerating tissues such as limbs are particularly resistant to tumourigenesis (Tsonis and Eguchi, 1981). Indeed, malignant outgrowths show regression, incorporation to regenerating tissues or induction of axis duplications and accessory limbs, yet they do not persist as tumours in the regenerated structure (Breedis, 1952; Ingram, 1971). This is surprising as, paradoxically, the process of regeneration shares many similarities with tumour development, including downregulation of tumour suppressors (e.g., p.53, Yun et al., 2013, 2014), upregulation of oncogenes (e.g., c-myc, Maki et al., 2009), and extensive cell proliferation (Subiran Adrados et al., 2020). In line with Waddington's individuation field hypothesis (Waddington, 1935) it is possible that, in a regenerative context, active patterning and differentiation mechanisms influence cell behaviour away from neoplasia. However, this is a notion that merits further consideration.

SALAMANDERS AND THE HALLMARKS OF AGEING

When looking for factors that may account for the absence of age-related declines in urodeles, it is worth considering whether and how hallmarks of ageing are manifested in these organisms. One such hallmark is cellular senescence, which in the recent years has emerged as a driver of several age-related disorders. Senescent cells are induced by various forms of cellular stress such as DNA damage, telomere shortening, oxidative challenges and oncogene activation (Gorgoulis et al., 2019). In response to these stimuli, these cells undergo a permanent cell cycle arrest and acquire a characteristic phenotype which includes the ability to secrete a repertoire of growth factors, matrix remodelling proteins and modulators of inflammation and immunity (Walters and Yun, 2020). Senescent cells play physiological roles in a number of contexts, including development (Munoz-Espin et al., 2013; Storer et al., 2013; Davaapil et al., 2017), wound healing (Jun and Lau, 2010; Demaria et al., 2014; Ritschka et al., 2017) and tissue repair processes (Yun et al., 2015; Sarig et al., 2019; Da Silva-Alvarez et al., 2020), in both mammals and salamanders. However, in mice and humans, they accumulate in various tissues as the organism ages, resulting in an imbalance in the inflammatory response and the promotion of age-related disorders such as sarcopenia, atherosclerosis, subcutaneal fat loss, osteoarthritis and neurodegeneration (van Deursen, 2014; Gorgoulis et al., 2019). Importantly, this role is causal, as the elimination of senescent cells attenuates age-related decay and leads to significant lifespan extension in mice (Baker et al., 2011, 2016). Moreover, it has been recently suggested that an age-related slow-down in senescent cell turnover could be a major contributing factor to the Gompertz law of mortality (Karin et al., 2019), which several salamander species defy. Relevant to this suggestion, we have observed that axolotls and newts (up to 10 years old) do not accumulate senescent cells in their tissues—e.g., liver, spleen, heart, limbs—as they age (Yun et al., 2015). Further, we have now extended these observations for axolotls up to 20 years old, and have not observed senescent cell accumulation. While the mechanistic reasons for this phenomenon remain elusive, it is notable that salamanders have a very rapid and efficient immune-dependent mechanism for senescent cell clearance, which may account for the lack of senescent cell accumulation (Yun et al., 2015; Walters and Yun, 2020). It is also possible that avoidance of replicative senescence, a form of senescent cell arrest triggered by telomere shortening, also plays a role in this context. While this process has not been studied in urodeles, observations suggest that salamander cells do not exhibit replicative senescence in culture (Ferretti and Brockes, 1988). Given the strong correlation between telomere shortening rate and the lifespan of a species (Whittemore et al., 2019), this is a topic worthy of further research efforts. In addition, another factor that could contribute to a lack of senescent cell accumulation in salamanders is the existence of well-geared mechanisms of genome maintenance. Although still a poorly developed area, recent studies suggest that axolotls employ robust DNA damage response mechanisms to promote proper cell cycle progression upon injury (Sousounis et al., 2020), which may restrict excessive generation of senescent cells in regenerative contexts. This is an interesting notion in light of evidence suggesting that other species of negligible senescence, such as the naked mole rat, exhibit efficient DNA repair mechanisms (Tian et al., 2017). In addition, it is possible that their large genomes (Elewa et al., 2017; Nowoshilow et al., 2018; Smith et al., 2019) provide an additional level of protection against mutagenic challenges, as the presence of extensive non-coding, non-regulatory areas would help ease the mutagenic burden (Poetsch et al., 2018). Nevertheless, it is yet unclear if the mechanisms of genome maintenance in salamander cells are more efficient than those found in their mammalian counterparts both in regenerative and homeostatic contexts, whether salamander cells are more resistant to certain types of genome challenges, and how well does this explain their limited senescent cell accumulation and resistance to age-related decay.

Another important hallmark with a well-documented impact on ageing and longevity is metabolic dysregulation. In particular, deregulated nutrient sensing is one of the most common age-related traits, with the insulin–insulin growth factor 1

(IGF1) signalling pathway constituting the most conserved age-controlling mechanism in evolution (Lopez-Otin et al., 2013; Fontana and Partridge, 2015; Partridge et al., 2018). This pathway mediates, partially through its control of the age-implicated mTOR complexes, the beneficial effects of dietary restriction on longevity from mice through worms to flies. Genetic mutations leading to a reduction in the functions of insulin receptor, IGF1 or mTOR result in lifespan extension (Fontana and Partridge, 2015). Further, mTOR inhibition through rapamycin leads to significant longevity increases, one of the most robust pharmacological interventions to promote lifespan extension in mammalian contexts (Fontana and Partridge, 2015). While little is known of how salamanders regulate nutrient sensing and its connection to their longevity, it is noteworthy that salamanders, as ectotherms, have inherently high levels of metabolic plasticity including thermal acclimation and hibernation/aestivation cycles, which may facilitate achieving metabolic states—at least temporarily—similar to those conducing to lifespan extension in mammals. Nevertheless, it is also worth noting that the aforementioned molecular regulators of anti-ageing are also involved in regenerative processes (Lund-Ricard et al., 2020). Particularly, IGF1 and mTOR inhibition suppress blastema formation during zebrafish fin regeneration (Hirose et al., 2014). In axolotls, mTORC1, a key complex whose downregulation promotes mammalian longevity, is implicated in mediating a systemic response to injury (Johnson et al., 2018). It would thus be of interest to understand the activity balance of this molecular axis in connection to both ageing and regeneration.

ON THE LINK BETWEEN REGENERATION AND AGEING

Together, the aforementioned observations raise a critical question, namely what is the link between longevity, lack of age-related decay and extreme regenerative abilities such as those found in salamanders? Could regeneration, in particular the limited reprogramming used by these organisms, elicit a process akin to tissue rejuvenation? Again, almost no studies to date have tackled this question. Anecdotal observations indicate that in axolotls the regenerated skin is structurally distinct from that of the original tissue, exhibiting greater thickness and dermal connective tissue (McCusker and Gardiner, 2011), suggesting a phenomenon akin to rejuvenation. Further, it has been proposed that the activation of developmental pathways in the regenerative context would lead to the generation of new tissues of equivalent age to those that arise immediately upon development (McCusker and Gardiner, 2011). While this idea has not been formally addressed, the study by Sousounis et al. has offered initial insights into this problem. By comparing gene expression signatures of lenses that had undergone repeated regeneration cycles to those of the original lenses, the authors observed that the regenerated lens transcriptome resembled the original one and thus appeared not to have aged. In contrast, old structures that never regenerated, such as the tail or the iris—the source of progenitors for the lens—exhibited more noticeable time-related changes (Sousounis et al., 2015). Unfortunately, this study did not include a comparison with old lenses that had never undergone regeneration, therefore its conclusions are based on the effect of time on other tissue populations. Consequently, the similarities in gene-expression between original and regenerated lenses could be explained by a rejuvenation effect, but also by an inherent resistance of the lens itself to the passing of time that is not found in tail or iris tissues. Whilst this remains an open question, this study constitutes a first attempt to address the link between the limited reprogramming associated to lens regeneration and rejuvenation. This is particularly interesting in light of recent findings suggesting that reprogramming may revert aging through epigenomic mechanisms (Lu et al., 2020). Challenging the link between regeneration and rejuvenation further will require establishing reliable aging biomarkers in salamanders, capable of accurate determinations of tissue age, and leveraging systems approaches to determine the molecular changes that occur with time from individual cells to entire structures and how these are affected by regenerative processes. Lastly, this would also benefit from comparative genomic approaches, taking advantage of the available repertoire of models of regeneration and ageing (Valenzano et al., 2017).

CONCLUDING REMARKS

Salamanders offer a wealth of interesting biology, from their seemingly endless regenerative abilities to their extreme lifespans and resistance to cancer and age-related decay. Thanks to recent technological advances in transgenesis, gene editing tools and fully assembled genomes, models such as the axolotl and the Iberian ribbed newt provide an opportunity to unravel the cellular and molecular basis of these remarkable traits. Together, the resulting insights will help us further understand the nature of regeneration, ageing and their interconnection, central to the development of rejuvenation strategies of clinical relevance.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MHY conceived and wrote this manuscript.

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REFERENCES

Baker, D. J., Childs, B. G., Durik, M., Wijers, M. E., Sieben, C. J., Zhong, J., et al. (2016). Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* 530, 184–189. doi: 10.1038/nature16932nature16932

Baker, D. J., Wijshake, T., Tchkonia, T., LeBrasseur, N. K., Childs, B. G., van de Sluis, B., et al. (2011). Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 479, 232–236. doi: 10.1038/nature10600

Breedis, C. (1952). Induction of accessory limbs and of sarcoma in the Newt (*Triturus viridescens*) with carcinogenic substances. *Cancer Res.* 12, 861–866.

Brockes, J. P., and Kumar, A. (2008). Comparative aspects of animal regeneration. *Annu. Rev. Cell Dev. Biol.* 24, 525–549. doi: 10.1146/annurev.cellbio

Bryant, D. M., Sousounis, K., Payzin-Dogru, D., Bryant, S., Sandoval, A. G. W., Martinez Fernandez, J., et al. (2017). Identification of regenerative roadblocks via repeat deployment of limb regeneration in axolotls. *NPJ Regen. Med.* 2:30. doi: 10.1038/s41536-017-0034-z

Cai, H., Peng, Z., Ren, R., and Wang, H. (2019). Efficient gene disruption via base editing induced stop in newt *Pleurodeles waltl*. *Genes* 10:837. doi: 10.3390/genes10110837

Cayuela, H., Olgun, K., Angelini, C., Uzum, N., Peyronel, O., Miaud, C., et al. (2019). Slow life-history strategies are associated with negligible actuarial senescence in western Palaearctic salamanders. *Proc. Biol. Sci.* 286:20191498. doi: 10.1098/rspb.2019.1498

Cox, B. D., Yun, M. H., and Poss, K. D. (2019). Can laboratory model systems instruct human limb regeneration? *Development* 146:dev181016. doi: 10.1242/dev.181016

Da Silva-Alvarez, S., Guerra-Varela, J., Sobrido-Camean, D., Quelle, A., Barreiro-Iglesias, A., Sanchez, L., et al. (2020). Cell senescence contributes to tissue regeneration in zebrafish. *Aging Cell* 19:e13052. doi: 10.1111/acel.13052

Davaapil, H., Brockes, J. P., and Yun, M. H. (2017). Conserved and novel functions of programmed cellular senescence during vertebrate development. *Development* 144, 106–114. doi: 10.1242/dev.138222

Demaria, M., Ohtani, N., Youssef, S. A., Rodier, F., Toussaint, W., Mitchell, J. R., et al. (2014). An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev. Cell* 31, 722–733. doi: 10.1016/j.devcel.2014.11.012S1534-5807(14)00729-1

Duerr, T. J., Comellas, E., Jeon, E. K., Farkas, J. E., Joetzjer, M., Garnier, J., et al. (2020). 3D visualization of macromolecule synthesis. *Elife* 9:e60354. doi: 10.7554/eLife.60354

Echeverri, K., and Tanaka, E. M. (2003). Electroporation as a tool to study in vivo spinal cord regeneration. *Dev. Dyn.* 226, 418–425. doi: 10.1002/dvdy.10238

Eguchi, G., Eguchi, Y., Nakamura, K., Yadav, M. C., Millan, J. L., and Tsonis, P. A. (2011). Regenerative capacity in newts is not altered by repeated regeneration and ageing. *Nat. Commun.* 2:384. doi: 10.1038/ncomms1389ncomms1389

Elewa, A., Wang, H., Talavera-Lopez, C., Joven, A., Brito, G., Kumar, A., et al. (2017). Reading and editing the *Pleurodeles waltl* genome reveals novel features of tetrapod regeneration. *Nat. Commun.* 8:2286. doi: 10.1038/s41467-017-01964-9

Fei, J. F., Lou, W. P., Knapp, D., Murawala, P., Gerber, T., Taniguchi, Y., et al. (2018). Application and optimization of CRISPR-Cas9-mediated genome engineering in axolotl (*Ambystoma mexicanum*). *Nat. Protoc.* 13, 2908–2943. doi: 10.1038/s41596-018-0071-0

Ferretti, P., and Brockes, J. P. (1988). Culture of newt cells from different tissues and their expression of a regeneration-associated antigen. *J. Exp. Zool.* 247, 77–91. doi: 10.1002/jez.1402470111

Finch, C. E. (1990). *Longevity, Senescence, and the Genome*. Chicago, IL: The University of Chicago Press.

Finch, C. E. (2009). Update on slow aging and negligible senescence–a mini-review. *Gerontology* 55, 307–313. doi: 10.1159/000215589

Fontana, L., and Partridge, L. (2015). Promoting health and longevity through diet: from model organisms to humans. *Cell* 161, 106–118. doi: 10.1016/j.cell.2015.02.020

Gerber, T., Murawala, P., Knapp, D., Masselink, W., Schuez, M., Hermann, S., et al. (2018). Single-cell analysis uncovers convergence of cell identities during axolotl limb regeneration. *Science* 362:eaaq0681. doi: 10.1126/science.aaq0681

Gompertz, B. (1825). On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philos. Trans. R. Soc. Lond.* 115:513. doi: 10.1098/rstl.1825.0026

Gorgoulis, V., Adams, P. D., Alimonti, A., Bennett, D. C., Bischof, O., Bishop, C., et al. (2019). Cellular senescence: defining a path forward. *Cell* 179, 813–827. doi: 10.1016/j.cell.2019.10.005

Hayashi, T., Sakamoto, K., Sakuma, T., Yokotani, N., Inoue, T., Kawaguchi, E., et al. (2014). Transcription activator-like effector nucleases efficiently disrupt the target gene in Iberian ribbed newts (*Pleurodeles waltl*), an experimental model animal for regeneration. *Dev. Growth Differ.* 56, 115–121. doi: 10.1111/dgd.12103

Hayashi, T., and Takeuchi, T. (2015). Gene manipulation for regenerative studies using the Iberian ribbed newt, Pleurodeles waltl. *Methods Mol. Biol.* 1290, 297–305. doi: 10.1007/978-1-4939-2495-0_23

Henry, J. J., and Tsonis, P. A. (2010). Molecular and cellular aspects of amphibian lens regeneration. *Prog. Retin. Eye Res.* 29, 543–555. doi: 10.1016/j.preteyeres.2010.07.002

Hirose, K., Shiomi, T., Hozumi, S., and Kikuchi, Y. (2014). Mechanistic target of rapamycin complex 1 signaling regulates cell proliferation, cell survival, and differentiation in regenerating zebrafish fins. *BMC Dev. Biol.* 14:42. doi: 10.1186/s12861-014-0042-9

Ingram, A. J. (1971). The reactions to carcinogens in the axolotl (*Ambystoma mexicanum*) in relation to the "regeneration field control" hypothesis. *J. Embryol. Exp. Morphol.* 26, 425–441. doi: 10.1242/dev.26.3.425

Johnson, K., Bateman, J., DiTommaso, T., Wong, A. Y., and Whited, J. L. (2018). Systemic cell cycle activation is induced following complex tissue injury in axolotl. *Dev. Biol.* 433, 461–472. doi: 10.1016/j.ydbio.2017.07.010

Jones, O. R., Scheuerlein, A., Salguero-Gomez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of ageing across the tree of life. *Nature* 505, 169–173. doi: 10.1038/nature12789

Jopling, C., Sleep, E., Raya, M., Marti, M., Raya, A., and Izpisua Belmonte, J. C. (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* 464, 606–609. doi: 10.1038/nature08899

Joven, A., Kirkham, M., and Simon, A. (2015). Husbandry of Spanish ribbed newts (*Pleurodeles waltl*). *Methods Mol. Biol.* 1290, 47–70. doi: 10.1007/978-1-4939-2495-0_4

Jun, J. I., and Lau, L. F. (2010). Cellular senescence controls fibrosis in wound healing. *Aging* 2, 627–631. doi: 10.18632/aging.100201

Karin, O., Agrawal, A., Porat, Z., Krizhanovsky, V., and Alon, U. (2019). Senescent cell turnover slows with age providing an explanation for the Gompertz law. *Nat. Commun.* 10:5495. doi: 10.1038/s41467-019-13192-4

Khattak, S., Murawala, P., Andreas, H., Kappert, V., Schuez, M., Sandoval-Guzman, T., et al. (2014). Optimized axolotl (*Ambystoma mexicanum*) husbandry, breeding, metamorphosis, transgenesis and tamoxifen-mediated recombination. *Nat. Protoc.* 9, 529–540. doi: 10.1038/nprot.2014.040

Khattak, S., Sandoval-Guzman, T., Stanke, N., Protze, S., Tanaka, E. M., and Lindemann, D. (2013a). Foamy virus for efficient gene transfer in regeneration studies. *BMC Dev. Biol.* 13:17. doi: 10.1186/1471-213X-13-17

Khattak, S., Schuez, M., Richter, T., Knapp, D., Haigo, S. L., Sandoval-Guzman, T., et al. (2013b). Germline transgenic methods for tracking cells and testing gene function during regeneration in the axolotl. *Stem Cell Rep.* 1, 90–103. doi: 10.1016/j.stemcr.2013.03.002

Knopf, F., Hammond, C., Chekuru, A., Kurth, T., Hans, S., Weber, C. W., et al. (2011). Bone regenerates via dedifferentiation of osteoblasts in the zebrafish fin. *Dev. Cell* 20, 713–724. doi: 10.1016/j.devcel.2011.04.014

Kragl, M., Knapp, D., Nacu, E., Khattak, S., Maden, M., Epperlein, H. H., et al. (2009). Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature* 460, 60–65. doi: 10.1038/nature08152

Kragl, M., and Tanaka, E. M. (2009). Grafting axolotl (*Ambystoma mexicanum*) limb skin and cartilage from GFP+ donors to normal hosts. *Cold Spring Harb. Protoc.* 2009. doi: 10.1101/pdb.prot5266

Lo, D. C., Allen, F., and Brockes, J. P. (1993). Reversal of muscle differentiation during urodele limb regeneration. *Proc. Natl. Acad. Sci. U.S.A.* 90, 7230–7234. doi: 10.1073/pnas.90.15.7230

Lopez, D., Lin, L., Monaghan, J. R., Cogle, C. R., Bova, F. J., Maden, M., et al. (2014). Mapping hematopoiesis in a fully regenerative vertebrate: the axolotl. *Blood* 124, 1232–1241. doi: 10.1182/blood-2013-09-526970

Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039S0092-8674(13)00645-4

Lu, Y., Brommer, B., Tian, X., Krishnan, A., Meer, M., Wang, C., et al. (2020). Reprogramming to recover youthful epigenetic information and restore vision. *Nature* 588, 124–129. doi: 10.1038/s41586-020-2975-4

Lund-Ricard, Y., Cormier, P., Morales, J., and Boutet, A. (2020). mTOR signaling at the crossroad between metazoan regeneration and human diseases. *Int. J. Mol. Sci.* 21:2718. doi: 10.3390/ijms21082718

Makeham, W. M. (1860). On the law of mortality and construction of annuity tables. *Assur. Mag. J. Inst. Actuar.* 8, 301–310. doi: 10.1017/s204616580000126x

Maki, N., Suetsugu-Maki, R., Tarui, H., Agata, K., Del Rio-Tsonis, K., and Tsonis, P. A. (2009). Expression of stem cell pluripotency factors during regeneration in newts. *Dev. Dyn.* 238, 1613–1616. doi: 10.1002/dvdy.21959

Margotta, V. (2008). Further amputations of the tail in adult *Triturus carnifex*: contribution to the study on the nature of regenerated spinal cord. *Ital. J. Anat. Embryol.* 113, 167–186.

Margotta, V., Filoni, S., Merante, A., and Chimenti, C. (2002). Analysis of morphogenetic potential of caudal spinal cord in *Triturus carnifex* adults (*Urodele amphibians*) subjected to repeated tail amputations. *Ital. J. Anat. Embryol.* 107, 127–144.

Masselink, W., and Tanaka, E. M. (2020). Toward whole tissue imaging of axolotl regeneration. *Dev. Dyn.* doi: 10.1002/dvdy.282

McCusker, C., and Gardiner, D. M. (2011). The axolotl model for regeneration and aging research: a mini-review. *Gerontology* 57, 565–571. doi: 10.1159/000323761

Munoz-Espin, D., Canamero, M., Maraver, A., Gomez-Lopez, G., Contreras, J., Murillo-Cuesta, S., et al. (2013). Programmed cell senescence during mammalian embryonic development. *Cell* 155, 1104–1118. doi: 10.1016/j.cell.2013.10.019S0092-8674(13)01295-6

Myhrvold, M. P., Baldridge, E., Chan, B., Sivam, D., Freeman, D. L., and Ernest, S. K. M. (2015). An amniote life-history database to perform comparative analyses with birds, mammals, and reptiles. *Ecology* 96:3109. doi: 10.1890/15-0846r.1

Nowoshilow, S., Schloissnig, S., Fei, J. F., Dahl, A., Pang, A. W. C., Pippel, M., et al. (2018). The axolotl genome and the evolution of key tissue formation regulators. *Nature* 554, 50–55. doi: 10.1038/nature25458

Oliveira, C. R., Lemaitre, R., Murawala, P., Tazaki, A., Drechsel, D. N., and Tanaka, E. M. (2018). Pseudotyped baculovirus is an effective gene expression tool for studying molecular function during axolotl limb regeneration. *Dev. Biol.* 433, 262–275. doi: 10.1016/j.ydbio.2017.10.008

Pajcini, K. V., Corbel, S. Y., Sage, J., Pomerantz, J. H., and Blau, H. M. (2010). Transient inactivation of Rb and ARF yields regenerative cells from postmitotic mammalian muscle. *Cell Stem Cell* 7, 198–213. doi: 10.1016/j.stem.2010.05.022

Partridge, L., Deelen, J., and Slagboom, P. E. (2018). Facing up to the global challenges of ageing. *Nature* 561, 45–56. doi: 10.1038/s41586-018-0457-8

Pinheiro, T., Mayor, I., Edwards, S., Joven, A., Kantzer, C. G., Kirkham, M., et al. (2020). CUBIC-f: an optimized clearing method for cell tracing and evaluation of neurite density in the salamander brain. *J. Neurosci. Methods* 348:109002. doi: 10.1016/j.jneumeth.2020.109002

Poetsch, A. R., Boulton, S. J., and Luscombe, N. M. (2018). Genomic landscape of oxidative DNA damage and repair reveals regioselective protection from mutagenesis. *Genome Biol.* 19:215. doi: 10.1186/s13059-018-1582-2

Ponomareva, L. V., Athippozhy, A., Thorson, J. S., and Voss, S. R. (2015). Using *Ambystoma mexicanum* (Mexican axolotl) embryos, chemical genetics, and microarray analysis to identify signaling pathways associated with tissue regeneration. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 178, 128–135. doi: 10.1016/j.cbpc.2015.06.004

Ritschka, B., Storer, M., Mas, A., Heinzmann, F., Ortells, M. C., Morton, J. P., et al. (2017). The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev.* 31, 172–183. doi: 10.1101/gad.290635.116

Ruby, J. G., Smith, M., and Buffenstein, R. (2018). Naked mole-rat mortality rates defy gompertzian laws by not increasing with age. *Elife* 7:e31157. doi: 10.7554/eLife.31157

Sarig, R., Rimmer, R., Bassat, E., Zhang, L., Umansky, K. B., Lendengolts, D., et al. (2019). Transient p53-mediated regenerative senescence in the injured heart. *Circulation* 139, 2491–2494. doi: 10.1161/CIRCULATIONAHA.119.040125

Seim, I., Fang, X., Xiong, Z., Lobanov, A. V., Huang, Z., Ma, S., et al. (2013). Genome analysis reveals insights into physiology and longevity of the Brandt's bat *Myotis brandtii*. *Nat. Commun.* 4:2212. doi: 10.1038/ncomms3212

Smith, J. J., Timoshevskaya, N., Timoshevskiy, V. A., Keinath, M. C., Hardy, D., and Voss, S. R. (2019). A chromosome-scale assembly of the axolotl genome. *Genome Res.* 29, 317–324. doi: 10.1101/gr.241901.118

Sousounis, K., Baddour, J. A., and Tsonis, P. A. (2014). Aging and regeneration in vertebrates. *Curr. Top. Dev. Biol.* 108, 217–246. doi: 10.1016/B978-0-12-391498-9.00008-5B978-0-12-391498-9.00008-5

Sousounis, K., Bryant, D. M., Martinez Fernandez, J., Eddy, S. S., Tsai, S. L., Gundberg, G. C., et al. (2020). Eya2 promotes cell cycle progression by regulating DNA damage response during vertebrate limb regeneration. *Elife* 9:e51217. doi: 10.7554/eLife.51217

Sousounis, K., Qi, F., Yadav, M. C., Millan, J. L., Toyama, F., Chiba, C., et al. (2015). A robust transcriptional program in newts undergoing multiple events of lens regeneration throughout their lifespan. *Elife* 4:e09594. doi: 10.7554/eLife.09594

Storer, M., Mas, A., Robert-Moreno, A., Pecoraro, M., Ortells, M. C., Di Giacomo, V., et al. (2013). Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell* 155, 1119–1130. doi: 10.1016/j.cell.2013.10.041S0092-8674(13)01359-7

Subiran Adrados, C., Yu, Q., Bolanos Castro, L. A., Rodriguez Cabrera, L. A., and Yun, M. (2020). Salamander-Eci: an optical clearing protocol for the three-dimensional exploration of regeneration. *Dev. Dyn.* doi: 10.1002/dvdy.264

Suetsugu-Maki, R., Maki, N., Nakamura, K., Sumanas, S., Zhu, J., Del Rio-Tsonis, K., et al. (2012). Lens regeneration in axolotl: new evidence of developmental plasticity. *BMC Biol.* 10:103. doi: 10.1186/1741-7007-10-103

Tacutu, R., Thornton, D., Johnson, E., Budovsky, A., Barardo, D., Craig, T., et al. (2018). Human ageing genomic resources: new and updated databases. *Nucleic Acids Res.* 46, D1083–D1090. doi: 10.1093/nar/gkx1042

Tanaka, E. M. (2016). The molecular and cellular choreography of appendage regeneration. *Cell* 165, 1598–1608. doi: 10.1016/j.cell.2016.05.038

Tanaka, E. M., and Reddien, P. W. (2011). The cellular basis for animal regeneration. *Dev. Cell.* 21, 172–185. doi: 10.1016/j.devcel.2011.06.016

Tanaka, H. V., Ng, N. C. Y., Yang Yu, Z., Casco-Robles, M. M., Maruo, F., Tsonis, P. A., et al. (2016). A developmentally regulated switch from stem cells to dedifferentiation for limb muscle regeneration in newts. *Nat. Commun.* 7:11069. doi: 10.1038/ncomms11069

Tian, X., Seluanov, A., and Gorbunova, V. (2017). Molecular mechanisms determining lifespan in short- and long-lived species. *Trends Endocrinol. Metab.* 28, 722–734. doi: 10.1016/j.tem.2017.07.004

Tsonis, P. A. (1983). Effects of carcinogens on regenerating and non-regenerating limbs in amphibia (review). *Anticancer Res.* 3, 195–202.

Tsonis, P. A., and Eguchi, G. (1981). Carcinogens on regeneration. effects of N-methyl-N'-nitro-N-nitrosoguanidine and 4-nitroquinoline-1-oxide on limb regeneration in adult newts. *Differentiation* 20, 52–60. doi: 10.1111/j.1432-0436.1981.tb01155.x

Valenzano, D. R., Aboobaker, A., Seluanov, A., and Gorbunova, V. (2017). Non-canonical aging model systems and why we need them. *EMBO J.* 36, 959–963. doi: 10.15252/embj.201796837

van Deursen, J. M. (2014). The role of senescent cells in ageing. *Nature* 509, 439–446. doi: 10.1038/nature13193nature13193

Vieira, W. A., Wells, K. M., and McCusker, C. D. (2020). Advancements to the axolotl model for regeneration and aging. *Gerontology* 66, 212–222. doi: 10.1159/000504294

Voituron, Y., de Fraipont, M., Issartel, J., Guillaume, O., and Clobert, J. (2011). Extreme lifespan of the human fish (*Proteus anguinus*): a challenge for ageing mechanisms. *Biol. Lett.* 7, 105–107. doi: 10.1098/rsbl.2010.0539

Waddington, C. H. (1935). Cancer and the theory of organisers. *Nature* 135:606. doi: 10.1038/135606a0

Walters, H. E., and Yun, M. H. (2020). Rising from the ashes: cellular senescence in regeneration. *Curr. Opin. Genet. Dev.* 64, 94–100. doi: 10.1016/j.gde.2020.06.002

Warburg, M. R. (2007). Longevity in *Salamandra infraimmaculata* from Israel with a partial review of life expectancy in urodeles. *Salamandra* 43, 21–34.

Whited, J. L., Tsai, S. L., Beier, K. T., White, J. N., Piekarski, N., Hanken, J., et al. (2013). Pseudotyped retroviruses for infecting axolotl in vivo and in vitro. *Development* 140, 1137–1146. doi: 10.1242/dev.087734

Whittemore, K., Vera, E., Martinez-Nevado, E., Sanpera, C., and Blasco, M. A. (2019). Telomere shortening rate predicts species life span. *Proc. Natl. Acad. Sci. U.S.A.* 116, 15122–15127. doi: 10.1073/pnas.1902452116

Yu, Q. H., and Yun, M. H. (2020). Interconnection between cellular senescence, regeneration and ageing in salamanders. *Healthy Ageing Long* 11, 43–62. doi: 10.1007/978-3-030-44903-2_3

Yun, M. H. (2015). Changes in regenerative capacity through lifespan. *Int. J. Mol. Sci.* 16, 25392–25432. doi: 10.3390/ijms161025392ijms161025392

Yun, M. H., Davaapil, H., and Brockes, J. P. (2015). Recurrent turnover of senescent cells during regeneration of a complex structure. *Elife* 4:e05505. doi: 10.7554/eLife.05505

Yun, M. H., Gates, P. B., and Brockes, J. P. (2013). Regulation of p53 is critical for vertebrate limb regeneration. *Proc. Natl. Acad. Sci. U.S.A.* 110, 17392–17397. doi: 10.1073/pnas.1310519110

Yun, M. H., Gates, P. B., and Brockes, J. P. (2014). Sustained ERK activation underlies reprogramming in regeneration-competent salamander cells and distinguishes them from their mammalian counterparts. *Stem Cell Rep.* 3, 15–23. doi: 10.1016/j.stemcr.2014.05.009

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MINI REVIEW
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Eusociality and Senescence: Neuroprotection and Physiological Resilience to Aging in Insect and Mammalian Systems

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Are eusociality and extraordinary aging polyphenisms evolutionarily coupled? The remarkable disparity in longevity between social insect queens and sterile workers—decades vs. months, respectively—has long been recognized. In mammals, the lifespan of eusocial naked mole rats is extremely long—roughly 10 times greater than that of mice. Is this robustness to senescence associated with social evolution and shared mechanisms of developmental timing, neuroprotection, antioxidant defenses, and neurophysiology? Focusing on brain senescence, we examine correlates and consequences of aging across two divergent eusocial clades and how they differ from solitary taxa. Chronological age and physiological indicators of neural deterioration, including DNA damage or cell death, appear to be decoupled in eusocial insects. In some species, brain cell death does not increase with worker age and DNA damage occurs at similar rates between queens and workers. In comparison, naked mole rats exhibit characteristics of neonatal mice such as protracted development that may offer protection from aging and environmental stressors. Antioxidant defenses appear to be regulated differently across taxa, suggesting independent adaptations to life history and environment. Eusocial insects and naked mole rats appear to have evolved different mechanisms that lead to similar senescence-resistant phenotypes. Careful selection of comparison taxa and further exploration of the role of metabolism in aging can reveal mechanisms that preserve brain functionality and physiological resilience in eusocial species.

Keywords: hymenoptera, termite, naked mole rat, lifespan, antioxidant, neurodegeneration, metabolism, polyethism

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INTRODUCTION

Eusocial animals, characterized by reproductive division of labor, cooperative brood care, and overlap of generations, can have extraordinary lifespans. Eusocial hymenopteran (ants, bees, and wasps) queens may live 100 times longer than solitary insects. In some eusocial species there is a similar lifespan differential between queens and workers (Keller and Genoud, 1997;

Kramer and Schaible, 2013). Naked mole-rats (NMRs), exemplar eusocial mammals, also have highly extended lifespans compared to solitary rodents (Buffenstein, 2008) and do not exhibit typical age-associated increases in mortality (Ruby et al., 2018). These clades present opportunities to examine molecular and physiological processes underlying differential longevity, their degree of conservation, and relationships to social evolution (Lucas and Keller, 2020). Aging resilience is often manifest as a lack of deterioration in neural, reproductive, or immune function (Buffenstein, 2008; Finch, 2009; Stenvinkel and Shiels, 2019). Robustness to senescence in eusocial taxa may be associated with adaptations involving damage-repair mechanisms, neuronal protection, neurometabolic efficiency, and fossorial ecology, among other factors. Social development—age-related changes in behavioral role or task performance—may also be involved. Here we focus on senescence in eusocial insects and NMRs to identify commonalities in aging phenotypes, examine anti-aging mechanisms, and suggest future research.

CASTE DETERMINATION AND AGING

Reproductive caste is determined nutritionally in honeybees, by nutrition and social factors in many wasps (O'Donnell, 1998; Berens et al., 2015), and by social, environmental, and genetic factors in ants (Schwander et al., 2010). In most cases, caste is determined early in development and remains fixed for life, although in a few taxa, trajectories are more plastic. In the ant *Harpagnathos salatator*, workers can facultatively become reproductive upon queen loss (Liebig et al., 2000; Peeters et al., 2000). Similarly, queens are replaced after dominance contests in NMRs (Clarke and Faulkes, 1997). Cape honeybee workers may become egg-laying pseudoqueens and increase their lifespan to 5 months (Rueppell et al., 2016). Therefore, social insects have plastic developmental trajectories of senescence. Changes in gene expression (Gospocic et al., 2017; Shields et al., 2018) can drive differentiation toward reproductive competence to influence lifespan. Reproductive status and longer lifespans correlate with a reduction in physiological aging and limited senescence in the brain (Finch, 2009; Parker, 2010; Giraldo and Traniello, 2014; Giraldo et al., 2016).

NMRs, which differ from mice in many physiological and biochemical markers of aging (Buffenstein, 2008), have a 30+ year lifespan and significantly delayed senescence (Lee et al., 2020). Although early studies reported lifespans between queens and subordinates were similar (Sherman and Jarvis, 2002), a more recent and comprehensive study revealed that NMR breeders, like many eusocial insects, have lower age-associated mortality than non-breeders (Ruby et al., 2018). Similarly, reproductives of the eusocial Damaraland mole rat are longer lived than non-breeders (Schmidt et al., 2013), suggesting convergence of aging phenotypes. However, longevity may not correlate with eusociality *per se* (Lucas and Keller, 2020) and selection can differ among species. The subterranean life of NMRs has been suggested to drive long lifespan (Healy et al., 2014), although it appears to have no effect across mammals after accounting for sociality (Healy, 2015).

DEVELOPMENTAL TIMING AND NEOTENY

What does it mean to live a long time? Measuring lifespans simply as the interval between birth or hatching and death ignores significant variation across solitary and eusocial taxa in the timing and significance of developmental events related to senescence. Ant queen larval stages are relatively short and adults may live decades (Hölldobler and Wilson, 1990). Immature periodical cicadas and mayflies live for years, but adults live only days (Britain, 1990; Grant, 2005). Variation in lifespan and developmental timing is also evident across mammals (Healy, 2015; Healy et al., 2019). However, the relationship between developmental timing and aging in eusocial systems is unclear.

Eusociality may be associated with extended developmental periods and the retention of juvenile traits into adulthood (neoteny or pedomorphy; Orr et al., 2016) that may affect lifespan. Brain development in NMRs occurs at a significantly different pace than in mice. Although similar in size, NMRs are born with larger, more slowly developing brains. In eusocial insects, major developmental transitions occur from egg to adult and during age-related behavioral development within adulthood (Whitfield et al., 2003; Seid and Traniello, 2006). Eusocial insect neoteny could therefore be evident in delayed pupation or altered rates of adult maturation and behavioral development relative to solitary taxa. Indeed, ant species with shorter egg-to-eclosion development may have longer latencies to the onset of foraging, suggesting a tradeoff between development and behavioral maturation (Muscedere, 2011). Also, in the ant *Pheidole dentata,* newly eclosed minor workers possess undeveloped mandibular muscles (Muscedere et al., 2011) and small task repertoires lacking efficiency (Muscedere et al., 2009). Such morphological and behavioral immaturity would likely be costly in adult solitary insects. *P. dentata* minor worker brains collaterally undergo significant age-related changes in size, monoamine titer, and synaptic structure as they mature behaviorally (Seid and Traniello, 2005; Seid et al., 2005; Muscedere and Traniello, 2012; Muscedere et al., 2012, 2013). Whether worker "neoteny" is altriciality or accelerated larval and pupal maturation, the process appears to enable workers to eclose earlier as undeveloped adults. The influence of pace of development on aging and longevity remain unexplored.

The adaptive nature of developmental patterns and their distribution across eusocial species that vary in ecology and life histories is unstudied. Division of labor in NMR colonies among non-breeders can be influenced by age, with older individuals generally performing less work, but factors such as body size and rank can interact in non-linear ways (Gilbert et al., 2020). In NMRs, neoteny may be an adaptation to living in hypoxic burrows: neonatal mammal brains often have higher hypoxia tolerance than adult brains (Larson and Park, 2009). This could apply to some subterranean eusocial insects if their nests are hypoxic. Reductions in extrinsic mortality risk in eusocial animals arising from their well-defended nests could also select for neoteny, in addition to longer lifespans in general

(c.f. Keller and Genoud, 1997), allowing relatively undeveloped individuals to safely labor (Muscedere, 2011).

NEUROBIOLOGICAL RESILIENCE

Neural markers of social insect senescence suggest chronological age and neural deterioration, including DNA damage or cell death, are decoupled. Brain cell death does not increase with age in minor workers of the ant *Pheidole dentata* (Giraldo et al., 2016) and synaptic complexes in higher-order processing centers (mushroom bodies) do not change over up to 68% of their 140-day laboratory lifespan, suggesting a lack of neurodegeneration. Worker lifespan in the field is likely to be significantly shorter, rendering aging inconsequential in nature. *Drosophila melanogaster* exhibit apoptosis in muscle and adipose tissue, but do not show programmed brain cell death beyond neural remodeling post-eclosion (Zheng et al., 2005), although the antennal lobes and ellipsoid body, which are critical for olfaction and spatial orientation, respectively, exhibit elevated caspase-3-like activity (DEVDase) in older flies (Chihara et al., 2014). Antennal lobe apoptosis appears to be restricted to specific classes of olfactory receptor neurons causally related to age-related declines in olfaction (Chihara et al., 2014). In social insects, DNA damage occurs at similar rates between queens and workers (Lucas et al., 2017), but DNA repair gene expression is higher in the former (Lucas et al., 2016). Although honeybees exhibit generally low levels of oxidative damage in the brain, foragers show higher levels of protein carbonylation in the optic lobes than chronologically older overwintering bees (Seehuus et al., 2006a). Although chronological age and associative learning are not correlated in honeybees (Rueppell et al., 2007), increased foraging is generally associated with memory-associated declines (Behrends et al., 2007). Examination of brain compartment-specific changes in protein abundances suggest neural senescence may not be regulated at the level of the whole-brain (Wolschin et al., 2010). Together, these studies suggest that despite very different aging phenotypes, eusocial and solitary insects could share common mechanisms conferring aging resilience in neural tissue.

NMRs exhibit neuroprotective characteristics of neonatal mice such as protracted development (Buffenstein et al., 2020). Perhaps due to a focus on comparisons with mice, little research has explored whether queen and worker brains differ in neural markers of aging. In one study, transcriptomes of breeding NMRs were enriched for aging-related genes compared to their non-breeding littermates, although not in the brain regions measured (Bens et al., 2018). Unlike most mammals, few genes were found to be differentially expressed between 4 and 20-year-old NMRs, particularly in the brain (Kim et al., 2011), suggesting maintenance of aging-resistance throughout life. Alternative splicing, in which a single gene leads to different functional isoforms, has been suggested as an adaptive stress response and seems to be upregulated in NMR brains relative to mice (Lee et al., 2020). *In situ* comparison of NMRs and mice indicate that NMR neurons are more resistant to acid-induced cell-death (Husson and Smith, 2018).

Eusocial brains may also be robust to injury or disease. For example, unilateral antennectomy in newly eclosed *P. dentata* workers led to a significant reduction in ipsilateral antennal lobe volume but had few other neurobiological effects, and performance of most tasks remained unaffected, suggesting developmental neural resilience to damage at least early in adult life (Waxman et al., 2017). This is consistent with studies in solitary insects that demonstrated marked robustness to injury. Unilaterally antennectomized cockroaches are able to successfully track odor plumes (Lockey and Willis, 2015). By removing the distal segments of the antennae in hawkmoths during the beginning of the pupal stage, researchers removed sensory neurons, resulting in abnormal antennal lobe development; nevertheless, adults were able to successfully find the source of the odor plume in a wind tunnel (Willis et al., 1995). These findings suggest resilience in both solitary and social insects.

The NMR immune system appears well-adapted to combat bacterial infections (Hilton et al., 2019) but vulnerable to viruses (Artwohl et al., 2009), although the impact on general immunosurvellience has yet to be elucidated (Hilton et al., 2019). Immune-function genes appear to be upregulated in some bees and termites but not ants, suggesting a lack of universal pathways in social insect immune responses (Korb et al., 2021). Whole-body transcriptomic analysis of workers, queens, and kings of the termite *Reticulitermes speratus* show strong up-regulation of DNA repair genes in mature kings, indicating that DNA repair may be an important component of aging resilience (Tasaki et al., 2018). BRCA1, involved in DNA repair and antioxidant signaling among other functions, was highly expressed in mature kings. Mature queens and kings showed different levels of expression (Tasaki et al., 2018). This system, and hymenopterans, enable sex-related differences in reproductive longevity to be examined.

Glia may offer neuroprotection. Aging *D. melanogaster* and *H. saltator* workers show decreases in gene expression for transcripts that characterize ensheathing glia (Sheng et al., 2020), which in *Drosophila* respond to injury (Doherty et al., 2009; Kato et al., 2011, 2018) and decline in function with increasing age (Purice et al., 2016). However, age-matched *H. saltator* reproductives exhibit a higher proportion of these glia, suggesting a neuroprotective role. Old honey bee foragers exhibit lower levels of two glial metabolic enzymes, glutamine synthase and glycogen phophorylase, perhaps driven by declining protein expression and/or glial cell loss (Shah et al., 2018).

The association of eusociality and neurobiological resilience is unclear, although a general *lack* of resilience has historically been implied. Exposing workers to disease, injury, or carbon dioxide induces early foraging, as expected from manipulations that shorten lifespan and reduce worker residual value (Moroń et al., 2008; Tofilski, 2009). Old ant workers have been hypothesized to be "disposable" (Porter and Jorgensen, 1981), and in some species workers have been characterized as "cheap" and replaceable (Wilson, 2003). The costs and benefits of portioning risky tasks by age depends on aging rates and whether age-related mortality varies with task performance (Tofilski, 2002). Improved task performance by mature older workers may instead alter the colony-level fitness costs of worker maintenance or replacement,

selecting for robustness, neural resilience, and extended lifespans. A rigorous test of these hypotheses would require controlling for phylogeny and body size.

ANTIOXIDANTS AND AGING IN EUSOCIAL SPECIES

Harman (1956) first hypothesized that reactive oxygen species (ROS) produced as byproducts of cellular metabolism result in accumulated molecular and cellular damage and ultimately degradation and death. This free radical theory of aging has been tested, with inconsistent results (Ashok and Ali, 1999; Ziegler et al., 2015; Grimm and Eckert, 2017). A key limitation of ROS theory in its most simplistic form is that it fails to explain the wide variation in lifespans across animals (Keller and Genoud, 1997; Rose et al., 2002). Nevertheless, as one of multiple potential aging mechanisms, experimental studies have examined ROS and antioxidant systems across taxa.

Insect fat body produces and processes hemolymph proteins and hormones implicated in aging (Amdam et al., 2004; Corona et al., 2007; Smedal et al., 2009). In *Drosophila,* expression of the antioxidant-related gene *catalase* (CAT) declines with age (Klichko et al., 2004), and overexpression of CAT and another antioxidant gene, *superoxide dismutase* (SOD), lowers levels of protein carbonylation (a measure of oxidative damage) and extends lifespan (Orr and Sohal, 1994). The pattern in eusocial insects, however, varies. Senescence-associated changes in brain and fat body gene expression in young and old queens in the ant *Temnothorax rugatulus* are highly tissue-specific (Negroni et al., 2019), but old queens exhibit higher levels of SOD and CAT. Queen termites, *Reticulitermes speratus,* also show lower levels of oxidative damage and CAT upregulation (Tasaki et al., 2017). CAT activity is higher in *R. speratus* compared to solitary insects and eusocial hymenopteran queens (Tasaki et al., 2017). In contrast, levels of most antioxidant genes in the brains of honey bee queens decline with age, whereas levels in worker brains remain constant or increase (Corona et al., 2005). Queen mRNA levels of these genes were often lower than in workers at least 1 week old, although this does not suggest honeybee queens lack pro-longevity repair mechanisms. Honey bee queens showed an upregulation of antioxidant activity in the fat bodies and trophocytes, accompanying an increase in ROS (Hsieh and Hsu, 2013). In contrast, ROS levels decline with age in worker honey bees, but antioxidant levels are constant or increase (Hsu and Hsieh, 2014). These studies suggest that ROS and antioxidant activity may involve tissue-specific regulation. Honeybee workers are protected from oxidative stress by the yolk precursor vitellogenin, an additional ROS protection mechanism (Seehuus et al., 2006b). Alternatively, *Lasius niger* queens invest in DNA and protein repair rather than antioxidants (Lucas et al., 2016). Oxidative damage can also be induced by stressful environmental conditions in honeybees (Simone-Finstrom et al., 2016), but does not necessarily result from lifespan reducing stress (Rueppell et al., 2017). In the reproductively plastic termite *Crypotermes secundus*, colonies maintained at constant temperature counterintuitively showed higher stress responses, lower survival, and reduced reproductive output than those at variable temperatures (Rau and Korb, 2021). Changes in gene expression were similar between queens and pseudergates ("false workers"), perhaps due to their reproductive plasticity (Rau and Korb, 2021). A comparative transcriptomic analysis of termite, bee, and ant species identified a few genes associated with increasing age and/or caste but no consistent patterns across taxa (Korb et al., 2021). ROS systems are complex, and manipulations of multiple systems will be necessary to uncover underlying genetic mechanisms of aging (De Verges and Nehring, 2016). Although some social insect queens exhibit higher levels of antioxidants, as expected given their long lifespans, this mechanism does not appear to be necessary in other species. Indeed, a recent comparative analysis of levels of oxidative damage and antioxidant genes in some ant, bee, and termite species found a marked lack of consistency (Kramer et al., 2021).

Like eusocial insects, the extreme longevity of the naked mole-rat does not completely align with the oxidative stress theory of aging (Lewis et al., 2013). ROS production—specifically in heart tissue—is similar to that observed in shorter-lived mice (Lambert et al., 2007; Munro et al., 2019), and equivocal findings have been reported for antioxidant defenses that are not only tissue and cell-site specific but also specific to different antioxidants (Andziak et al., 2005; Munro et al., 2019; Viltard et al., 2019; Takasugi et al., 2020). Notably, NMRs appear to sustain high levels of oxidative damage (Andziak et al., 2005, 2006; Pérez et al., 2009; De Waal et al., 2013; Lewis et al., 2013). However, NMRs exhibit higher levels of mitochondrial consumption of hydrogen peroxide in skeletal muscle and the heart than mice, suggesting improved ROS scavenging (Munro et al., 2019), and kidney protein function is maintained in NMRs despite high levels of protein carbonylation (De Waal et al., 2013). Levels of mitochondria-bound hexokinases, which can prevent ROS formation during cellular respiration, decline in many tissues including the brain in mice but are maintained for minimally a decade in NMRs (Vyssokikh et al., 2020). Membrane phosopholipids are more resistant to oxidation in NMRs than mice in many tissues, although their brains do not differ (Hulbert et al., 2006). These seemingly confusing findings suggests that the long lifespan of NMRs is not simply a result of less production or more scavenging of ROS. Instead, high levels of ROS observed early in life in long-lived vertebrates such as NMRs, birds, and bats could be adaptive, priming lifespan management of ROS (Saldmann et al., 2019). Direct comparisons with eusocial insects are hampered by methodological differences, but will be important to explore variation across taxa in ROS and antioxidant production as well as management of ROS-related damage.

AGE, LONGEVITY, AND BRAIN METABOLISM

Eusocial insect worker and reproductive castes differ in metabolism, and metabolic pathways may correlate with aging

phenotypes (Corona et al., 2007; Ihle et al., 2019; Haroon, Ma et al., 2020). Caloric restriction has a positive effect on lifespan and senescence in humans (Most et al., 2017), some genetic strains of mice (Weindruch, 1992; Liao et al., 2010) and *Drosophila* (Burger et al., 2010), suggesting that by lowering metabolic activity cells form fewer injurious metabolites (Speakman and Mitchell, 2011). Nevertheless, caloric restriction does not extend lifespan in all species (Speakman and Mitchell, 2011) and can even reduce longevity (Kaitala, 1991; Kirk, 2001; Liao et al., 2010). Resveratrol, which mimics the effects of caloric restriction, extended lifespan in worker honeybees while reducing food consumption (Rascón et al., 2012).

How does brain metabolism scale with longevity? Comparison of brain investment and energetic demands in ant species that differ in social complexity suggest tradeoffs between increased brain size and metabolism (Kamhi et al., 2016). A plastic shift in energetic investment appears to occur in the ant *Harpegnathos saltator*, in which gamergates that are experimentally reverted to foragers increase investment in their brains and decrease investment in the gonads, reversing patterns found in naturally occurring gamergates and workers (Penick et al., 2021). Reverted honey bee foragers did not exhibit brain shrinkage in the mushroom bodies after 5 days (Fahrbach et al., 2003), although the short time scale may not have been sufficient to see effects similar to *Harpegnathos* (Penick et al., 2021). These studies suggest that social insects may be able to modulate their brain volumes adaptively, although limits to this plasticity likely exist.

In honeybees and *Drosophila*, experimentally inhibiting oxidative phosphorylation pathways leads to increased aggression (Li-Byarlay et al., 2014). These effects can be socially modulated in honey bees (Li-Byarlay et al., 2014), potentially impacting aging and life history if it alters social roles. NMRs exhibit a point mutation in a neuronal potassium chloride cotransporter that lowers the energy costs of GABAergic signaling in low oxygen environments (Zions et al., 2020), intriguingly suggesting an interaction between brain metabolism and environment. Interactions between abiotic conditions and aging related genes in termites (Rau and Korb, 2021) suggest that metabolism-environment interactions could exist in many taxa. In old honey bee queens, oenocytes and trophocytes do not show declines in mitochondrial energy metabolism compared to young queens (Hsu and Lu, 2015). In contrast, workers experience age-related declines in energy-related molecules (Hsu and Chuang, 2014) and cellular metabolism (Lu et al., 2017).

FUTURE EXPLORATIONS OF AGING AMONG EUSOCIAL TAXA

Caste and Species Comparisons

Understanding age-related changes in neurobiology and behavior in long-lived ant queens requires knowing queen age. Fire ant queens (6–7 year lifespan) store sperm from a single insemination for life, allowing sperm counts to reliably estimate queen age (Tschinkel, 1987). Queens collected from established colonies can be aged and brains analyzed for neuroanatomical changes, synaptic structure, neurochemistry, and gene expression. By contrasting patterns between queens and workers, the influence of colony reproductive phenotype—monogyne or polygyne—can be determined to assess the role of social structure on aging. This single trait has been evolutionary labile in social insects and has multiple impacts on social phenotypes (Hölldobler and Wilson, 1977), including longer lifespans in monogynous than polygynous queens (Keller and Genoud, 1997).

Caste theory (Oster and Wilson, 1978; Wilson, 2003) can be applied to the neurobiology and physiology of aging, and the hypothesis that workers are "cheap or disposable" can be tested by estimating brain production and/or maintenance costs to understand the physiological underpinnings of minimal or discontinued investment. Additionally, neuroanatomical analyses in species capable of facultative switching between worker and reproductive castes can examine whether the transition to a long-lived reproductive activates neurobiological and physiological mechanisms that protect now reproductively competent individuals from senescence. Comparisons between taxa in which workers can assume a reproductive role—including termites and NMRs—will reveal whether independent evolution of reproductive plasticity involves common pro-longevity mechanisms.

Most aging comparisons with NMRs involve studies of other taxa. Mutant dwarf mice with growth hormone mutations exhibit pedomorphic traits and have at least 50% longer lifespans than wild-type mice, and fewer aging-associated diseases (Buffenstein et al., 2020). Experiments that present NMR non-breeders, breeders, and dwarf mice of different ages with environmental toxins or thermal stress could help separate the roles of sociality and reproductive status in aging.

Metabolism and Aging

Brain metabolism is little explored in eusocial and solitary species (Neville et al., 2018; Coto and Traniello, 2021). Is energy use plastically regulated across caste and lifespan? Do queens and workers differ in brain metabolic scaling? Do subterranean social insects experience hypoxic or hypercapnic conditions and if so, have they evolved NMR-like neurometabolic adaptations to these environments? Queens of the termite *Reticulitermes speratus* reproduce in hypoxic, hypercapnic chambers, conditions that enhance their reproductive output (Tasaki et al., 2020), although the generality of this result is unclear. These questions should be comparatively addressed across eusocial and long-lived solitary species. Additionally, despite relatively long-lived NMR workers, gene expression differences between breeders and non-breeders exist in skin and gonads, tied to reproductive maturation and in some cases aging (Bens et al., 2018). Future studies could explore how reproduction and energy use affect brain metabolism that go beyond volumetric measurements in social insects. Although plastic changes in brain volume, as in *Harpegnathos saltator* (Penick et al., 2021) hint at metabolic tradeoffs, methods that can quantify brain metabolism (e.g., Neville et al., 2018) will allow researchers to directly test whether volume changes correlate directly with tissue energy consumption and how energy use may change across the lifespan.

CONCLUSION

Eusocial insects and eusocial mammals share aging phenotypes despite phylogenetic divergence, but different mechanisms appear to have evolved to facilitate delayed aging in their nervous systems. The striking difference in worker and reproductive lifespan in eusocial insects is less pronounced in NMRs, further suggesting the evolution of multiple pathways to achieve long and healthy lifespans in eusocial taxa. To enable precise comparisons between vertebrate and invertebrate taxa, similar methodologies, such as quantifying levels of homologous biochemical markers of aging or comparisons among divergent taxa must be applied. Integration of theories of aging and development in eusocial and solitary species will enhance our understanding of how social organization shapes aging phenotypes and mechanisms that promote longevity.

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YG, MM, and JT wrote the manuscript. YG and JT provided the funding. All authors contributed to the article and approved the submitted version.

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REFERENCES

Amdam, G. V., Simões, Z. L. P., Hagen, A., Norberg, K., Schrøder, K., Mikkelsen, Ø, et al. (2004). Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. *Exp. Gerontol.* 39, 767–773. doi: 10.1016/j.exger.2004.02.010

Andziak, B., O'Connor, T. P., and Buffenstein, R. (2005). Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. *Mech. Ageing Dev.* 126, 1206–1212. doi: 10.1016/j.mad.2005.06.009

Andziak, B., O'Connor, T. P., Qi, W., Dewaal, E. M., Pierce, A., Chaudhuri, A. R., et al. (2006). High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5, 463–471. doi: 10.1111/j.1474-9726.2006.00237.x

Artwohl, J., Ball-Kell, S., Valyi-Nagy, T., Wilson, S. P., Lu, Y., and Park, T. J. (2009). Extreme susceptibility of african naked mole rats (*Heterocephalus glaber*) to experimental infection with herpes simplex virus type 1. *Comp. Med.* 59, 83–90.

Ashok, B. T., and Ali, R. (1999). The aging paradox: Free radical theory of aging. *Exp. Gerontol.* 34, 293–303. doi: 10.1016/S0531-5565(99)00005-4

Behrends, A., Scheiner, R., Baker, N., and Amdam, G. V. (2007). Cognitive aging is linked to social role in honey bees (*Apis mellifera*). *Exp. Gerontol.* 42, 1146–1153. doi: 10.1016/j.exger.2007.09.003

Bens, M., Szafranski, K., Holtze, S., Sahm, A., Groth, M., Kestler, H. A., et al. (2018). Naked mole-rat transcriptome signatures of socially-suppressed sexual maturation and links of reproduction to aging. *BMC Biol.* 16:77. doi: 10.1101/221333

Berens, A. J., Hunt, J. H., and Toth, A. L. (2015). Nourishment level affects caste-related gene expression in *Polistes* wasps. *BMC Genomics* 16:1–12. doi: 10.1186/s12864-015-1410-y

Britain, J. E. (1990). "Life History of Ephemeroptera and Plecoptera," in *Mayflies and Stoneflies: Life Histories and Biology*, ed. I. C. Campbell (Dordrecht: Springer), 1–12.

Buffenstein, R. (2008). Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. *J. Comp. Physiol. B.* 178, 439–445. doi: 10.1007/s00360-007-0237-5

Buffenstein, R., Lewis, K. N., Gibney, P. A., Narayan, V., Grimes, K. M., Smith, M., et al. (2020). Probing pedomorphy and prolonged lifespan in naked mole-rats and dwarf mice. *Physiology* 35, 96–111. doi: 10.1152/physiol.00032.2019

Burger, J. M. S., Buechel, S. D., and Kawecki, T. J. (2010). Dietary restriction affects lifespan but not cognitive aging in *Drosophila melanogaster*. *Aging Cell* 9, 327–335. doi: 10.1111/j.1474-9726.2010.00560.x

Chihara, T., Kitabayashi, A., Morimoto, M., Takeuchi, K., Masuyama, K., Tonoki, A., et al. (2014). Caspase inhibition in select olfactory neurons restores innate attraction behavior in aged *Drosophila*. *PLoS Genet.* 10:1004437. doi: 10.1371/journal.pgen.1004437

Clarke, F. M., and Faulkes, C. G. (1997). Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber*. *Proc. R. Soc. B Biol. Sci.* 264, 993–1000. doi: 10.1098/rspb.1997.0137

Corona, M., Hughes, K. A., Weaver, D. B., and Robinson, G. E. (2005). Gene expression patterns associated with queen honey bee longevity. *Mech. Ageing Dev.* 126, 1230–1238. doi: 10.1016/j.mad.2005.07.004

Corona, M., Velarde, R. A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K. A., et al. (2007). Vitellogenin, juvenile hormone, insulin signaling and queen honey bee longevity. *Proc. Natl. Acad. Sci.* 104, 7128–7133. doi: 10.1073/pnas.0701909104

Coto, Z. N., and Traniello, J. F. A. (2021). Brain size, metabolism, and social evolution. *Front. Physiol.* 12:612865. doi: 10.3389/fphys.2021.612865

De Verges, J., and Nehring, V. (2016). A critical look at proximate causes of social insect senescence: Damage accumulation or hyperfunction? *Curr. Opin. Insect Sci.* 16, 69–75. doi: 10.1016/j.cois.2016.05.003

De Waal, E. M., Liang, H., Pierce, A., Hamilton, R. T., Buffenstein, R., and Chaudhuri, A. R. (2013). Elevated protein carbonylation and oxidative stress do not affect protein structure and function in the long-living naked-mole rat: A proteomic approach. *Biochem. Biophys. Res. Commun.* 434, 815–819. doi: 10.1016/j.bbrc.2013.04.019

Doherty, J., Logan, M. A., Taşdemir, ÖE., and Freeman, M. R. (2009). Ensheathing glia function as phagocytes in the adult *Drosophila* brain. *J. Neurosci.* 29, 4768–4781. doi: 10.1523/JNEUROSCI.5951-08.2009

Fahrbach, S. E., Farris, S. M., Sullivan, J. P., and Robinson, G. E. (2003). Limits on volume changes in the mushroom bodies of the honey bee brain. *J. Neurobiol.* 57, 141–151. doi: 10.1002/neu.10256

Finch, C. E. (2009). Update on slow aging and negligible senescence–a mini-review. *Gerontology* 55, 307–313. doi: 10.1159/000215589

Gilbert, J. D., Rossiter, S. J., and Faulkes, C. G. (2020). The relationship between individual phenotype and the division of labour in naked mole-rats: It's complicated. *PeerJ.* 8:9891. doi: 10.7717/peerj.9891

Giraldo, Y. M., Kamhi, J. F., Fourcassié, V., Moreau, M., Robson, S. K. A., Rusakov, A., et al. (2016). Lifespan behavioural and neural resilience in a social insect. *Proc. R. Soc. B Biol. Sci.* 283, 1–9. doi: 10.1098/rspb.2015.2603

Giraldo, Y. M., and Traniello, J. F. A. (2014). Worker senescence and the sociobiology of aging in ants. *Behav. Ecol. Sociobiol.* 68, 1901–1919. doi: 10.1007/s00265-014-1826-4

Gospocic, J., Shields, E. J., Glastad, K. M., Lin, Y., Penick, C. A., Yan, H., et al. (2017). The neuropeptide orazonin controls social behavior and caste identity in ants. *Cell* 170, 748.e–759.e. doi: 10.1016/j.cell.2017.07.014

Grant, P. R. (2005). The priming of periodical cicada life cycles. *Trends Ecol. Evol.* 20, 169–174. doi: 10.1016/j.tree.2005.01.016

Grimm, A., and Eckert, A. (2017). Brain aging and neurodegeneration: from a mitochondrial point of view. *J. Neurochem.* 143, 418–431. doi: 10.1111/jnc.14037

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300.

Haroon, Ma, X. M., Li, Y. X., Zhang, H. X., Liu, Q., Su, X. H., et al. (2020). Transcriptomic evidence that insulin signalling pathway regulates the ageing

of subterranean termite castes. *Sci. Rep.* 10, 1–13. doi: 10.1038/s41598-020-64890-9

Healy, K. (2015). Eusociality but not fossoriality drives longevity in small mammals. *Proc. R. Soc. B Biol. Sci.* 282, 2–3. doi: 10.1098/rspb.2014.2917

Healy, K., Ezard, T. H. G., Jones, O. R., Salguero-Gómez, R., and Buckley, Y. M. (2019). Animal life history is shaped by the pace of life and the distribution of age-specific mortality and reproduction. *Nat. Ecol. Evol.* 3, 1217–1224. doi: 10.1038/s41559-019-0938-7

Healy, K., Guillerme, T., Finlay, S., Kane, A., Kelly, S. B. A., McClean, D., et al. (2014). Ecology and mode-of-life explain lifespan variation in birds and mammals. *Proc. R. Soc. B Biol. Sci.* 281:298. doi: 10.1098/rspb.2014.0298

Hilton, H. G., Rubinstein, N. D., Janki, P., Ireland, A. T., Bernstein, N., Wright, K. M., et al. (2019). Single-cell transcriptomics of the naked mole-rat reveals unexpected features of mammalian immunity. *PLoS Biol.* 17:e3000528. doi: 10.1101/597195

Hölldobler, B., and Wilson, E. O. (1977). The number of queens: An important trait in ant evolution. *Naturwissenschaften* 64, 8–15. doi: 10.1007/BF00439886

Hölldobler, B., and Wilson, E. O. (1990). *The Ants*. Cambridge, MA: Belknap Press of Harvard University Press.

Hsieh, Y. S., and Hsu, C. Y. (2013). Oxidative stress and anti-oxidant enzyme activities in the trophocytes and fat cells of queen honeybees (Apis mellifera). *Rejuvenation Res.* 16, 295–303. doi: 10.1089/rej.2013.1420

Hsu, C. Y., and Chuang, Y. L. (2014). Changes in energy-regulated molecules in the trophocytes and fat cells of young and old worker honeybees (Apis mellifera). *J. Geront. Ser. A Biol. Sci. Med. Sci.* 69, 955–964. doi: 10.1093/gerona/glt163

Hsu, C. Y., and Hsieh, Y. S. (2014). Oxidative stress decreases in the trophocytes and fat cells of worker honeybees during aging. *Biogerontology* 15, 129–137. doi: 10.1007/s10522-013-9485-9

Hsu, C. Y., and Lu, C. Y. (2015). Mitochondrial energy utilization maintains young status in the trophocytes and oenocytes of old queen honeybees. *Apidologie* 46, 583–594. doi: 10.1007/s13592-015-0348-z

Hulbert, A. J., Faulks, S. C., and Buffenstein, R. (2006). Oxidation-resistant membrane phospholipids can explain longevity differences among the longest-living rodents and similarly-sized mice. *J. Geront. Ser. A Biol. Sci. Med. Sci.* 61, 1009–1018. doi: 10.1093/gerona/61.10.1009

Husson, Z., and Smith, E. S. J. (2018). Naked mole-rat cortical neurons are resistant to acid-induced cell death. *Mol. Brain* 11, 1–10. doi: 10.1186/s13041-018-0369-4

Ihle, K. E., Mutti, N. S., Kaftanoglu, O., and Amdam, G. V. (2019). Insulin receptor substrate gene knockdown accelerates behavioural maturation and shortens lifespan in honeybee workers. *Insects* 10:10110390. doi: 10.3390/insects10110390

Kaitala, A. (1991). Phenotypic plasticity in reproductive behaviour of waterstriders: Trade-offs between reproduction and longevity during food stress. *Funct. Ecol.* 5:12. doi: 10.2307/2389551

Kamhi, J. F., Gronenberg, W., Robson, S. K. A., and Traniello, J. F. A. (2016). Social complexity influences brain investment and neural operation costs in ants. *Proc. R. Soc. B Biol. Sci.* 283:1949. doi: 10.1098/rspb.2016.1949

Kato, K., Forero, M. G., Fenton, J. C., and Hidalgo, A. (2011). The glial regenerative response to central nervous system injury is enabled by pros-notch and pros-NFκB feedback. *PLoS Biol.* 9:e1001133. doi: 10.1371/journal.pbio.1001133

Kato, K., Losada-Perez, M., and Hidalgo, A. (2018). Gene network underlying the glial regenerative response to central nervous system injury. *Dev. Dyn.* 247, 85–93. doi: 10.1002/dvdy.24565

Keller, L., and Genoud, M. (1997). Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature* 389, 3–6.

Kim, E. B., Fang, X., Fushan, A. A., Huang, Z., Lobanov, A. V., Han, L., et al. (2011). Genome sequencing reveals insights into physiology and longevity of the naked mole rat. *Nature* 479, 223–227. doi: 10.1038/nature10533

Kirk, K. L. (2001). Dietary restriction and aging: Comparative tests of evolutionary hypotheses. *J. Geront. Ser. A Biol. Sci. Med. Sci.* 56, 123–129. doi: 10.1093/gerona/56.3.B123

Klichko, V. I., Radyuk, S. N., and Orr, W. C. (2004). Profiling catalase gene expression in *Drosophila melanogaster* during development and aging. *Arch. Insect Biochem. Physiol.* 56, 34–50. doi: 10.1002/arch.10142

Korb, J., Meusemann, K., Aumer, D., Bernadou, A., Elsner, D., Feldmeyer, B., et al. (2021). Comparative transcriptomic analysis of the mechanisms underpinning ageing and fecundity in social insects. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 376, 20190728. doi: 10.1098/rstb.2019.0728

Kramer, B. H., Nehring, V., Buttstedt, A., Heinze, J., Korb, J., Libbrecht, R., et al. (2021). Oxidative stress and senescence in social insects: a significant but inconsistent link? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 376, 20190732. doi: 10.1098/rstb.2019.0732

Kramer, B. H., and Schaible, R. (2013). Life span evolution in eusocial workers-A theoretical approach to understanding the effects of extrinsic mortality in a hierarchical system. *PLoS One* 8:0061813. doi: 10.1371/journal.pone.0061813

Lambert, A. J., Boysen, H. M., Buckingham, J. A., Yang, T., Podlutsky, A., Austad, S. N., et al. (2007). Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell* 6, 607–618. doi: 10.1111/j.1474-9726.2007.00312.x

Larson, J., and Park, T. J. (2009). Extreme hypoxia tolerance of naked mole-rat brain. *Neuroreport* 20, 1634–1637. doi: 10.1097/WNR.0b013e32833370cf

Lee, B. P., Smith, M., Buffenstein, R., and Harries, L. W. (2020). Negligible senescence in naked mole rats may be a consequence of well-maintained splicing regulation. *GeroScience* 42, 633–651. doi: 10.1007/s11357-019-00150-7

Lewis, K. N., Andziak, B., Yang, T., and Buffenstein, R. (2013). The naked mole-rat response to oxidative stress: Just deal with it. *Antioxidants Redox Signal.* 19, 1388–1399. doi: 10.1089/ars.2012.4911

Li-Byarlay, H., Rittschof, C. C., Massey, J. H., Pittendrigh, B. R., and Robinson, G. E. (2014). Socially responsive effects of brain oxidative metabolism on aggression. *Proc. Natl. Acad. Sci. U. S. A* 2014:1412306111. doi: 10.1073/pnas.1412306111

Liao, C. Y., Rikke, B. A., Johnson, T. E., Diaz, V., and Nelson, J. F. (2010). Genetic variation in the murine lifespan response to dietary restriction: From life extension to life shortening. *Aging Cell* 9, 92–95. doi: 10.1111/j.1474-9726.2009.00533.x

Liebig, J., Peeters, C., Oldham, N. J., Markstädter, C., and Hölldobler, B. (2000). Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant Harpegnathos saltator? *Proc. Natl. Acad. Sci. U. S. A.* 97, 4124–4131. doi: 10.1073/pnas.97.8.4124

Lockey, J. K., and Willis, M. A. (2015). One antenna, two antennae, big antennae, small: Total antennae length, not bilateral symmetry, predicts odor-tracking performance in the American cockroach Periplaneta americana. *J. Exp. Biol.* 218, 2156–2165. doi: 10.1242/jeb.117721

Lu, C. Y., Chuang, Y. L., and Hsu, C. Y. (2017). Aging results in a decline in cellular energy metabolism in the trophocytes and oenocytes of worker honeybees (Apis mellifera). *Apidologie* 48, 761–775. doi: 10.1007/s13592-017-0521-7

Lucas, E. R., Augustyniak, M., Kędziorski, A., and Keller, L. (2017). Lifespan differences between queens and workers are not explained by rates of molecular damage. *Exp. Gerontol.* 92, 1–6. doi: 10.1016/j.exger.2017.03.008

Lucas, E. R., and Keller, L. (2020). The co-evolution of longevity and social life. *Funct. Ecol.* 34, 76–87. doi: 10.1111/1365-2435.13445

Lucas, E. R., Privman, E., and Keller, L. (2016). Higher expression of somatic repair genes in long-lived ant queens than workers. *Aging* 8, 1940–1951. doi: 10.18632/aging.101027

Moroń, D., Witek, M., and Woyciechowski, M. (2008). Division of labour among workers with different life expectancy in the ant Myrmica scabrinodis. *Anim. Behav.* 75, 345–350. doi: 10.1016/j.anbehav.2007.06.005

Most, J., Tosti, V., Redman, L. M., and Fontana, L. (2017). Calorie restriction in humans: An update. *Ageing Res. Rev.* 39, 36–45. doi: 10.1016/j.arr.2016.08.005

Munro, D., Baldy, C., Pamenter, M. E., and Treberg, J. R. (2019). The exceptional longevity of the naked mole-rat may be explained by mitochondrial antioxidant defenses. *Aging Cell* 18, 1–13. doi: 10.1111/acel.12916

Muscedere, M. L. (2011). *Social Organization, Development, and Functional Neuroplasticity in the Ant Genus Pheidole*. Boston, MA: Boston University.

Muscedere, M. L., Djermoun, A., and Traniello, J. F. A. (2013). Brood-care experience, nursing performance, and neural development in the ant *Pheidole dentata*. *Behav. Ecol. Sociobiol.* 67, 775–784. doi: 10.1007/s00265-013-1501-1

Muscedere, M. L., Johnson, N., Gillis, B. C., Kamhi, J. F., and Traniello, J. F. A. (2012). Serotonin modulates worker responsiveness to trail pheromone in the ant *Pheidole dentata*. *J. Comp. Physiol. A Neuroethol. Sensory, Neural, Behav. Physiol.* 198, 219–227. doi: 10.1007/s00359-011-0701-2

Muscedere, M. L., and Traniello, J. F. A. (2012). Division of labor in the hyperdiverse ant genus *Pheidole* is associated with distinct subcaste- and age-related patterns of worker brain organization. *PLoS One* 7:e31618. doi: 10.1371/journal.pone.0031618

Muscedere, M. L., Traniello, J. F. A., and Gronenberg, W. (2011). Coming of age in an ant colony: cephalic muscle maturation accompanies behavioral development in *Pheidole dentata*. *Naturwissenschaften* 98, 783–793. doi: 10.1007/s00114-011-0828-6

Muscedere, M. L., Willey, T. A., and Traniello, J. F. A. (2009). Age and task efficiency in the ant *Pheidole dentata*: young minor workers are not specialist nurses. *Anim. Behav.* 77, 911–918. doi: 10.1016/j.anbehav.2008.12.018

Negroni, M. A., Foitzik, S., and Feldmeyer, B. (2019). Long-lived *Temnothorax* ant queens switch from investment in immunity to antioxidant production with age. *Sci. Rep.* 9, 1–10. doi: 10.1038/s41598-019-43796-1

Neville, K. E., Bosse, T. L., Klekos, M., Mills, J. F., Weicksel, S. E., Waters, J. S., et al. (2018). A novel ex vivo method for measuring whole brain metabolism in model systems. *J. Neurosci. Methods* 296, 32–43. doi: 10.1016/j.jneumeth.2017.12.020

O'Donnell, S. (1998). Reproductive caste determination in eusocial wasps (Hymenoptera: Vespidae). *Annu. Rev. Entomol.* 43, 323–346. doi: 10.1146/annurev.ento.43.1.323

Orr, M. E., Garbarino, V. R., Salinas, A., and Buffenstein, R. (2016). Extended postnatal brain development in the longest-lived rodent: Prolonged maintenance of neotenous traits in the naked mole-rat brain. *Front. Neurosci.* 10:1–17. doi: 10.3389/fnins.2016.00504

Orr, W. C., and Sohal, R. S. (1994). Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* 263, 1128–1130. doi: 10.1126/science.8108730

Oster, G. F., and Wilson, E. O. (1978). *Caste and Ecology in the Social Insects*. Princeton, N.J: Princeton University Press.

Parker, J. D. (2010). What are social insects telling us about aging? *Myrmecolog. News* 13, 103–110.

Peeters, C., Liebig, J., and Hölldobler, B. (2000). Sexual reproduction by both queens and workers in the ponerine ant *Harpegnathos saltator*. *Insectes Soc.* 47, 325–332. doi: 10.1007/PL00001724

Penick, C. A., Ghaninia, M., Haight, K. L., Opachaloemphan, C., Yan, H., Reinberg, D., et al. (2021). Reversible plasticity in brain size, behaviour and physiology characterizes caste transitions in a socially flexible ant (Harpegnathos saltator). *Proc. R. Soc. B Biol. Sci.* 288:2021.0141.

Pérez, V. I., Buffenstein, R., Masamsetti, V., Leonard, S., Salmon, A. B., Mele, J., et al. (2009). Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. *Proc. Natl. Acad. Sci. U. S. A.* 106, 3059–3064. doi: 10.1073/pnas.0809620106

Porter, S. D., and Jorgensen, C. D. (1981). Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? *Behav. Ecol. Sociobiol.* 9, 247–256. doi: 10.1007/BF00299879

Purice, M. D., Speese, S. D., and Logan, M. A. (2016). Delayed glial clearance of degenerating axons in aged *Drosophila* is due to reduced PI3K/Draper activity. *Nat. Commun.* 7:12871. doi: 10.1038/ncomms12871

Rascón, B., Hubbard, B. P., Sinclair, D. A., and Amdam, G. V. (2012). The lifespan extension effects of resveratrol are conserved in the honey bee and may be driven by a mechanism related to caloric restriction. *Aging* 4, 499–508. doi: 10.18632/aging.100474

Rau, V., and Korb, J. (2021). The effect of environmental stress on ageing in a termite species with low social complexity. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 376:20190739. doi: 10.1098/rstb.2019.0739

Rose, M. R., Drapeau, M. D., Yazdi, P. G., Shah, K. H., Moise, D. B., Thakar, R. R., et al. (2002). Evolution of late-life mortality in *Drosophila melanogaster*. *Evolution* 56, 1982–1991.

Ruby, J. G., Smith, M., and Buffenstein, R. (2018). Naked mole-rat mortality rates defy Gompertzian laws by not increasing with age. *Elife* 7:e31157. doi: 10.7554/eLife.47047

Rueppell, O., Aumer, D., and Moritz, R. F. A. (2016). Ties between aging plasticity and reproductive physiology in honey bees . *Curr. Opin. Insect Sci.* 16, 64–68. doi: 10.1016/j.cois.2016.05.009.Ties

Rueppell, O., Christine, S., Mulcrone, C., and Groves, L. (2007). Aging without functional senescence in honey bee workers. *Curr. Biol.* 17, R274–R275. doi: 10.1016/j.cub.2007.02.015

Rueppell, O., Yousefi, B., Collazo, J., and Smith, D. (2017). Early life stress affects mortality rate more than social behavior, gene expression or oxidative damage in honey bee workers. *Exp. Gerontol.* 90, 19–25. doi: 10.1016/j.exger.2017.01.015

Saldmann, F., Viltard, M., Leroy, C., and Friedlander, G. (2019). The naked mole rat: A unique example of positive oxidative stress. *Oxid. Med. Cell. Longev.* 2019:4502819. doi: 10.1155/2019/4502819

Schmidt, C. M., Jarvis, J. U. M., and Bennett, N. C. (2013). The long-lived queen: reproduction and longevity in female eusocial Damaraland mole-rats (*Fukomys damarensis*). *African Zool.* 48, 193–196. doi: 10.1080/15627020.2013.11407583

Schwander, T., Lo, N., Beekman, M., Oldroyd, B. P., and Keller, L. (2010). Nature versus nurture in social insect caste differentiation. *Trends Ecol. Evol.* 25, 275–282. doi: 10.1016/j.tree.2009.12.001

Seehuus, S.-C., Krekling, T., and Amdam, G. V. (2006a). Cellular senescence in honey bee brain is largely independent of chronological age. *Exp. Gerontol.* 41, 1117–1125. doi: 10.1016/j.exger.2006.08.004

Seehuus, S.-C., Norberg, K., Gimsa, U., Krekling, T., and Amdam, G. V. (2006b). Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proc. Natl. Acad. Sci. U. S. A.* 103, 962–967. doi: 10.1073/pnas.0502681103

Seid, M. A., Harris, K. M., and Traniello, J. F. A. (2005). Age-related changes in the number and structure of synapses in the lip region of the mushroom bodies in the ant *Pheidole dentata*. *J. Comp. Neurol.* 488, 269–277. doi: 10.1002/cne.20545

Seid, M. A., and Traniello, J. F. A. (2005). Age-related changes in biogenic amines in individual brains of the ant *Pheidole dentata*. *Naturwissenschaften* 92, 198–201. doi: 10.1007/s00114-005-0610-8

Seid, M. A., and Traniello, J. F. A. (2006). Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants. *Behav. Ecol. Sociobiol.* 60, 631–644. doi: 10.1007/s00265-006-0207-z

Shah, A. K., Kreibich, C. D., Amdam, G. V., and Münch, D. (2018). Metabolic enzymes in glial cells of the honeybee brain and their associations with aging, starvation and food response. *PLoS One* 13:1–22. doi: 10.1371/journal.pone.0198322

Sheng, L., Shields, E. J., Gospocic, J., Glastad, K. M., Ratchasanmuang, P., Berger, S. L., et al. (2020). Social reprogramming in ants induces longevity-associated glia remodeling. *Sci. Adv.* 6:eaba9869. doi: 10.1126/sciadv.aba9869

Sherman, P. W., and Jarvis, J. U. M. (2002). Extraordinary life spans of naked mole-rats (*Heterocephalus glaber*). *J. Zool.* 258, 307–311. doi: 10.1017/S0952836902001437

Shields, E. J., Sheng, L., Weiner, A. K., Garcia, B. A., and Bonasio, R. (2018). High-quality genome assemblies reveal long non-coding RNAs expressed in ant brains. *Cell Rep.* 23, 3078–3090. doi: 10.1016/j.celrep.2018.05.014

Simone-Finstrom, M., Li-Byarlay, H., Huang, M. H., Strand, M. K., Rueppell, O., and Tarpy, D. R. (2016). Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees. *Sci. Rep.* 6, 1–10. doi: 10.1038/srep32023

Smedal, B., Brynem, M., Kreibich, C. D., and Amdam, G. V. (2009). Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*). *J. Exp. Biol.* 212, 3795–3801. doi: 10.1242/jeb.035063

Speakman, J. R., and Mitchell, S. E. (2011). Caloric restriction. *Mol. Aspects Med.* 32, 159–221. doi: 10.1016/j.mam.2011.07.001

Stenvinkel, P., and Shiels, P. G. (2019). Long-lived animals with negligible senescence: Clues for ageing research. *Biochem. Soc. Trans.* 47, 1157–1164. doi: 10.1042/BST20190105

Takasugi, M., Firsanov, D., Tombline, G., Ning, H., Ablaeva, J., Seluanov, A., et al. (2020). Naked mole-rat very-high-molecular-mass hyaluronan exhibits superior cytoprotective properties. *Nat. Commun.* 11, 1–10. doi: 10.1038/s41467-020-16050-w

Tasaki, E., Kobayashi, K., Matsuura, K., and Iuchi, Y. (2017). An efficient antioxidant system in a longlived termite queen. *PLoS One* 12:1–16. doi: 10.1371/journal.pone.0167412

Tasaki, E., Komagata, Y., Inagaki, T., and Matsuura, K. (2020). Reproduction deep inside wood: A low O2 and high CO2 environment promotes egg production by termite queens. *Biol. Lett.* 16:0049. doi: 10.1098/rsbl.2020.0049

Tasaki, E., Mitaka, Y., Nozaki, T., Kobayashi, K., Matsuura, K., and Iuchi, Y. (2018). High expression of the breast cancer susceptibility gene BRCA1 in long-lived termite kings. *Aging* 10, 2668–2683. doi: 10.18632/aging.101578

Tofilski, A. (2002). Influence of age polyethism on longevity of workers in social insects. *Behav. Ecol. Sociobiol.* 51, 234–237. doi: 10.1007/s00265-001-0429-z

Tofilski, A. (2009). Shorter-lived workers start foraging earlier. *Insectes Soc.* 56, 359–366. doi: 10.1007/s00040-009-0031-3

Tschinkel, W. R. (1987). Fire ant queen longevity and age: estimation by sperm depletion. *Ann. Entomol. Soc. Am.* 80, 263–266.

Viltard, M., Durand, S., Pérez-Lanzón, M., Aprahamian, F., Lefevre, D., Leroy, C., et al. (2019). The metabolomic signature of extreme longevity: Naked mole rats versus mice. *Aging* 11, 4783–4800. doi: 10.18632/aging.102116

Vyssokikh, M. Y., Holtze, S., Averina, O. A., Lyamzaev, K. G., Panteleeva, A. A., Marey, M. V., et al. (2020). Mild depolarization of the inner mitochondrial membrane is a crucial component of an anti-aging program. *Proc. Natl. Acad. Sci. U. S. A.* 117, 6491–6501. doi: 10.1073/pnas.1916414117

Waxman, H. K., Muscedere, M. L., and Traniello, J. F. A. (2017). Behavioral performance and neural systems are robust to sensory injury in workers of the ant *Pheidole dentata*. *Brain. Behav. Evol.* 89, 195–208. doi: 10.1159/000470899

Weindruch, R. (1992). Effect of caloric restriction on age-associated cancers. *Exp. Gerontol.* 27, 575–581. doi: 10.1016/0531-5565(92)90012-O

Whitfield, C. W., Cziko, A. M., and Robinson, G. E. (2003). Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302, 296–299. doi: 10.1126/science.1086807

Willis, M. A., Butler, M. A., and Tolbert, L. P. (1995). Normal glomerular organization of the antennal lobes is not necessary for odor-modulated flight in female moths. *J. Comp. Physiol. A* 176, 205–216. doi: 10.1007/BF00239923

Wilson, E. O. (2003). *Pheidole in the New World: a dominant, hyperdiverse ant genus*. Cambridge, MA: Harvard University Press.

Wolschin, F., Münch, D., and Amdam, G. V. (2010). Structural and proteomic analyses reveal regional brain differences during honeybee aging. *J. Exp. Biol.* 2010, 4027–4032. doi: 10.1242/jeb.033845

Zheng, J., Edelman, S. W., Tharmarajah, G., Walker, D. W., Pletcher, S. D., and Seroude, L. (2005). Differential patterns of apoptosis in response to aging in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 102, 12083–12088.

Ziegler, D. V., Wiley, C. D., and Velarde, M. C. (2015). Mitochondrial effectors of cellular senescence: Beyond the free radical theory of aging. *Aging Cell* 14, 1–7. doi: 10.1111/acel.12287

Zions, M., Meehan, E. F., Kress, M. E., Thevalingam, D., Jenkins, E. C., Kaila, K., et al. (2020). Nest carbon dioxide masks GABA-dependent seizure susceptibility in the naked mole-rat. *Curr. Biol.* 30, 2068.e–2077.e. doi: 10.1016/j.cub.2020.03.071

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REVIEW
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Senescence in Bacteria and Its Underlying Mechanisms

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Bacteria have been thought to flee senescence by dividing into two identical daughter cells, but this notion of immortality has changed over the last two decades. Asymmetry between the resulting daughter cells after binary fission is revealed in physiological function, cell growth, and survival probabilities and is expected from theoretical understanding. Since the discovery of senescence in morphologically identical but physiologically asymmetric dividing bacteria, the mechanisms of bacteria aging have been explored across levels of biological organization. Quantitative investigations are heavily biased toward *Escherichia coli* and on the role of inclusion bodies—clusters of misfolded proteins. Despite intensive efforts to date, it is not evident if and how inclusion bodies, a phenotype linked to the loss of proteostasis and one of the consequences of a chain of reactions triggered by reactive oxygen species, contribute to senescence in bacteria. Recent findings in bacteria question that inclusion bodies are only deleterious, illustrated by fitness advantages of cells holding inclusion bodies under varying environmental conditions. The contributions of other hallmarks of aging, identified for metazoans, remain elusive. For instance, genomic instability appears to be age independent, epigenetic alterations might be little age specific, and other hallmarks do not play a major role in bacteria systems. What is surprising is that, on the one hand, classical senescence patterns, such as an early exponential increase in mortality followed by late age mortality plateaus, are found, but, on the other hand, identifying mechanisms that link to these patterns is challenging. Senescence patterns are sensitive to environmental conditions and to genetic background, even within species, which suggests diverse evolutionary selective forces on senescence that go beyond generalized expectations of classical evolutionary theories of aging. Given the molecular tool kits available in bacteria, the high control of experimental conditions, the high-throughput data collection using microfluidic systems, and the ease of life cell imaging of fluorescently marked transcription, translation, and proteomic dynamics, in combination with the simple demographics of growth, division, and mortality of bacteria, make the challenges surprising. The diversity of mechanisms and patterns revealed and their environmental dependencies not only present challenges but also open exciting opportunities for the discovery and deeper understanding of aging and its mechanisms, maybe beyond bacteria and aging.

Keywords: aging, *E. coli*, asymmetry, inclusion bodies, protein clusters, proteomic instability, evolutionary demography, biodemography

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INTRODUCTION

Studying senescence—the decline of function with age—in bacteria has been a dichotomous field, divided into population aging on the one hand and senescence at the single-cell level on the other hand. Classically, senescence in bacteria has been concerned with population aging, the decline in viability with age of lineages, strains, or populations. An early emphasis has been on populations under stressful and suboptimal conditions, such as disinfectants. Bacteria populations exposed to disinfectants decline exponentially with time in their densities or in the numbers of viable populations (Chick, 1908). Different concentrations of disinfectants, disinfectant types, strain types, or bacteria types alter the pace of the decline, but the approximate exponential decline remains conserved (Chick, 1908). These findings are interesting, because an exponential decline implies that the force of mortality, the rate at which populations go extinct or the number of bacteria in a population decreases, is constant, i.e., no senescence is found. The implied non-senescence inspired reflections on mechanisms: if a cumulative deleterious effect of the disinfectant exists, the mortality rate should increase with time; alternatively, if the disinfectant has a selective effect acting on heterogeneity among cells in resisting the disinfectant, the mortality rate should decrease (Yule, 1910). An equilibrium balancing these two effects could theoretically lead to non-senescence, but such precise balancing seems unlikely. More than 100 years later, similar questions on mechanisms of aging remain: how do aging factors accumulate and how do differential selective effects influence senescence? The questions and mechanisms of aging studied have diversified, but nine hallmarks of aging have been carved out: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. We will consider these hallmarks in the following sections in the light of bacteria aging. We discuss conflicting results and some surprising challenges we are confronted with when studying bacteria aging.

INDIVIDUAL HETEROGENEITY AND SENESCENCE

Concepts of differential selection acting on heterogeneity among individuals and accumulating aging factors that influence physiological function and mortality, as found in the early studies on exposing bacteria to disinfectants, remain central in the study of senescence in bacteria and beyond. For instance, senescence patterns at the population level, such as late age mortality plateaus, are found in bacteria populations, but also metazoans including *Caenorhabditis elegans*, humans, and other organisms (Vaupel et al., 1998; Jones et al., 2014; Steiner et al., 2019). Such plateaus can arise out of selection acting on random accumulation of an aging factor, which generate heterogeneity in accumulated damage, or such plateaus arise out of differential selection on heterogeneity determined at birth (Vaupel and Yashin, 1985; Weitz and Fraser, 2001). Such heterogeneity determined at birth or gained throughout life has not only become of interest to basic aging research but also to applied questions of increased antibiotic resistance in healthcare. Differential selection becomes apparent for bacteria cells that can persist high doses of antibiotics. Such persistence is achieved without developing genetically fixed resistance. The current debate centers around the question whether cells are born as persister cells or switch in a stochastic or induced manner to such states during their lives, and findings suggest that these options are not mutually exclusive but differ in their frequency (Balaban et al., 2013; Brauner et al., 2017). Despite inferring on bacteria aging mechanisms from population level studies, deeper insights can be gained from single-cell investigations, because such investigations allow for comparison and heterogeneity in life courses among cells. For the remainder of this article, such single-cell senescence is our focus.

CELL SENESCENCE IN BACTERIA

Historically, little interest has been given to senescence at the single-cell level in bacteria, because morphologically symmetric dividing bacteria were thought to be immortal and flee senescence (Williams, 1957; Partridge and Barton, 1993; Moger-Reischer and Lennon, 2019). The argument goes as follows: a mother cell divides into two identical daughter cells, which themselves are identical to the mother, and only asymmetric reproduction, such as a soma and a germline, would allow for senescence as postulated in the disposable soma theory of aging (Kirkwood, 1977). Over the last two decades, this notion of non-senescence and immortality in bacteria has been overhauled. Reproductive senescence has been shown for *Caulobacter crescentus*, a morphologically asymmetric dividing bacterium (Ackermann et al., 2003). Similar results were shown for morphologically symmetrical dividing *Escherichia coli* bacteria, by studying microcolonies up to the seventh cell division in Petri dishes (Stewart et al., 2005). Stewart et al. (2005) introduced the concept of an old pole cell and a new pole cell for the rod-shaped *E. coli* bacteria. The cells built at each fission at the axial center plane a new cell wall that becomes the new pole, whereas the distal, retained cell wall defines the old pole. The old pole cell wall is retained, and therefore, the age of a cell can be determined by the age of the old pole; hence, not all old pole cells have the same age (**Figure 1**). Analogies to mothers and daughters for old pole daughter cells and new pole daughter cells have been made, respectively (Wang et al., 2010; Steiner et al., 2019). The finding of Stewart et al. (2005) that the apparent morphologically symmetric dividing *E. coli* bacteria are functionally asymmetric has stirred much empirical and theoretical interest in bacterial senescence. Empirical studies performed in microfluidic devices, mainly so-called mother machines, challenged the finding of reproductive senescence in bacteria by showing that no reduced growth rates were observed between the 10th and up to the 200th division of a cell when measured in microfluidic devices (Wang et al., 2010). Despite the lack of reproductive senescence, that is, no reduction in growth rates was found in microfluidic devices, increased filamentation rates and chronological senescence,

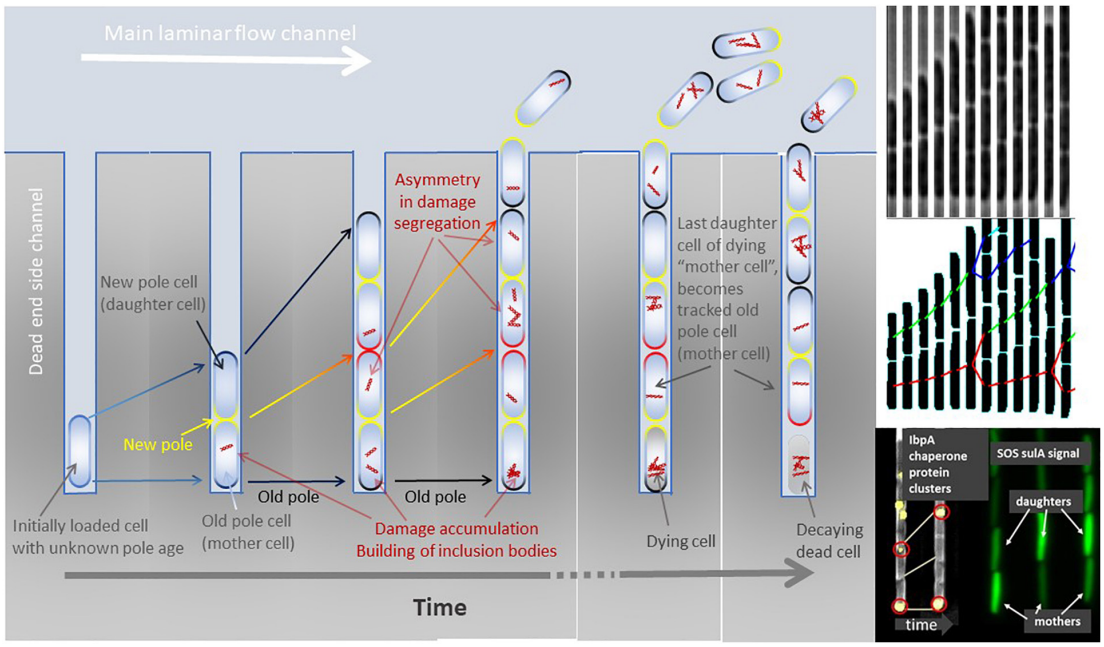

FIGURE 1 | Left panel: Cartoon of a mother machine channel across time. The initial loaded cell (left most channel in the panel) is of unknown cell wall age and polarity. After its first division, a new cell wall is built at the mid cell planar plane and becomes the new pole (yellow). The opposite cell poles define the old poles (dark). Pole age increases with time and division (yellow new pole, red poles built at previous division, black poles built at least two divisions ago). Over multiple divisions, the cell with the oldest pole remains at the bottom of the dead-end side channel and can be tracked until it dies, decays, and finally dissolves. Other cells than the bottom-most cell are pushed out of the side channels and washed away by the laminar flow. Damage, illustrated by red intracellular structures, accumulates throughout the life of a cell and can be purged by asymmetric division. Damage repair and recycling is not visualized here. Once the bottom-most old pole cell (mother cell) dies, its last produced daughter cell becomes the new cell that can be tracked throughout its lifespan. **Right top and middle panels:** Time sliced growth and division of bacteria cells growing in a dead-end side channel of a mother machine (phase contrast images top) and its lineage tracking with divisions after image analysis (**middle panel**). **Right bottom panel:** Examples of asymmetric division of fluorescently marked protein clusters (IbpA chaperones) and asymmetric segregation of transcription factor signal related to SOS stress response (sulA) among old pole (mother) and new pole (daughter) cells.

determined by increased mortality with age, were detected (Wang et al., 2010). Filamentation is the continued growth of a cell without division and is a stress response, indicating that stress responses might play a central role in bacteria senescence, as suggested for eucaryotic cells (Bi and Lutkenhaus, 1993). The link of aging to stress in bacteria is strengthened by the link between the rate of aging and the RpoS pathways, a general stress response (Yang et al., 2019). RpoS activity inhibits cell division under nutrient-limiting conditions, making such linkage physiologically plausible (Mauri and Klumpp, 2014).

In attempts to understand the discrepancies among studies that found or did not find reproductive senescence, i.e., a decline in growth rates with age, Rang et al. (2012) concluded that senescence in *E. coli* occurs only when an extrinsic stressor is present, a finding similar to fission yeast where senescence under stressful but not benign conditions has been observed (Coelho et al., 2013). In yeast, reproductive senescence is mainly measured as the budding rate, the division time among budding events, or the number of budded daughter cells before budding ceases. Stressful conditions—in *E. coli* studies that found reduced growth rates, i.e., reproductive senescence—could arise from fluorescence imaging techniques, including production costs of fluorescent proteins, genetic costs associated with expressing fluorescent proteins, stress incurred by exposing cells to high energy light required for exciting fluorescent proteins, or potentially toxic substances that are used to detect cell death (e.g., propidium iodide) or that prevent cell adhesion and clustering (e.g., Tween 20). Costs are paid through damage that leads to reduced growth rates—reproductive senescence—or even increased mortality—chronological senescence. Recent studies on individual *E. coli* showed that even without extrinsic stressors, reproductive senescence occurs (Łapińska et al., 2019) and thereby contrasts the findings of another recent study that did not find such reproductive senescence without extrinsic stressors (Proenca et al., 2019). These studies used different K-12 wild-type-derived strains (BW25113 and MG1655, respectively), but were similar in growth conditions both using similar microfluidic devices and grew cells in rich media: lysogeny broth. The study that did not find reproductive senescence even used low concentrations of Tween 20, which might impose some extrinsic stress. Furthermore, under extrinsic stress, reproductive senescence as well as chronological senescence has been or has not been detected (Wang et al., 2010; Vedel et al., 2016; Łapińska et al., 2019; Proenca et al., 2019; Steiner et al., 2019). Among these studies, differences exist in duration (hours to several days), culture media (minimal medium M9, or rich medium LB), fluorescence excitation (none, to short excitation 200 ms each 4 min), temperature (mainly 37°C but up to 42°C),

and strain types [most on MG1655 or BW25113, **Table 1**, but see also Wang et al. (2010) and Jouvet et al. (2018)]. It is apparent that morphologically similar dividing bacteria, such as *E. coli*, senesce and show functional asymmetry between daughter cells after fission under most conditions; single studies suggest that optimal environments might prevent senescence, but conflicting results exist.

ASYMMETRY IN DAMAGE DISTRIBUTION AND SENESCENCE

The empirical findings on bacterial senescence were closely accompanied by mathematical models, showing how functional asymmetry turns out to be crucial for allowing partial rejuvenation of some cells to prevent population aging (Ackermann et al., 2007). Three key assumptions have been made: first, cells will accumulate damage, or any other aging factor, that reduces function with increasing age, an assumption well in line with assumed causes of aging such as oxidative processes, including DNA oxidation or other effects of reactive oxygen species (Santos et al., 2018); second, damage repair mechanisms can only slow but not prevent damage accumulation—if damage would not accumulate, cells could flee senescence (Clegg et al., 2014); and third, asymmetry in damage distribution at cell fission allows partial purging of damage in one cell by increasing damage load in the other cell (**Figure 2**; Ackermann et al., 2007). This third assumption of selective segregation has also been termed exogenous repair (Bell and Cambridge University Press, 1988) and is required if the first two assumptions hold. If damage would accumulate within cells (assumptions 1 and 2) and could not be purged through asymmetric divisions (assumption 3), population aging would result and populations would go extinct (Ackermann et al., 2007). High mortality and fission rates can be beneficial by purging at higher frequency damage through asymmetric division to the extent that dying cells are used as "garbage" dumps to rid large fractions of accumulated damage. Under conditions of high turnover, increased asymmetry at fission in damage load is expected, with the option of selective death of highly damaged cells (Watve et al., 2006; Evans and Steinsaltz, 2007). Contrasting these predictions, benign conditions have been associated with reduced asymmetry (Vedel et al., 2016). Considering most assumptions of these models, extreme damage segregation might not lead to maximum population growth rates, that is, perfect symmetry or complete asymmetry appears to be only favored under simplistic assumptions. Model predictions range between complete asymmetry, intermediate asymmetry, or no asymmetry (Watve et al., 2006; Ackermann et al., 2007; Evans and Steinsaltz, 2007; Erjavec et al., 2008; Chao, 2010; Rashidi et al., 2012; Clegg et al., 2014; Moger-Reischer and Lennon, 2019; Blitvić and Fernandez, 2020). This implies for populations segregating the accumulating damage in an optimal asymmetric fashion, whose lines of descendants that continuously inherit the larger fraction of damage go extinct after some variable time, while the population—consisting of many lines, including those that inherit small fractions of damage—remains viable.

Damage accumulation, damage segregation at cell fission, and damage-dependent selective mortality are balanced in a way that damage distribution stabilizes across the population level, but not within the individual cell. Within cells, damage load can be highly dynamic, dependent on individual damage aggregation and damage segregation (Weitz and Fraser, 2001; Steiner et al., 2019). As in other optimization models, findings depend on assumed costs. In bacteria, senescence cost is associated with cell growth, damage repair, damage accumulation, asymmetry, and mortality. Various models predict non-senescence, for instance, when repair rates exceed damage rates and thereby any accumulation of damage is prevented (Clegg et al., 2014). This model contrasts with the second key assumption mentioned above that repair rates cannot exceed damage rates. Other models that do not predict senescence but predict asymmetry in segregation are based on modified population genetic models (Rang et al., 2011, 2012; Proenca et al., 2018, 2019). In those models, one-cell lineage, the one corresponding to the lineage repeatedly inheriting the maternal old pole cell, attracts to one equilibrium growth state that defines lower fitness (lower cell division rates) and a second equilibrium attractor growth state with slightly higher fitness (higher cell division rates) that corresponds to the lineage that sequentially receives the maternal new pole. Rejuvenation occurs in the new pole lineage and any damage that accumulates between cell divisions is transmitted to the old pole lineage. In these old pole lineages, damage does not accumulate over multiple divisions. Cells exist between the new pole lineage and the old pole lineage attractor states, but empirical observations suggest that these cells converge to the respective attractor state within three divisions. The crux of these models is that the amount of accumulating damage between two divisions cannot exceed the dilution effect achieved through cell growth and fission; otherwise, the old pole lineage would age and go extinct (Evans and Steinsaltz, 2007). Also, under such assumptions, the growth rate (fitness) of the two types of lineages is not equal since faster dividing lineages will grow faster and therefore contribute to larger fractions to the overall population. This fitness difference due to differences in generation times results in non-stable equilibria at the population level, though at the single-cell lineage level, equilibria might be observed (Blitvić and Fernandez, 2020). The model predicting two growth equilibria is supported by empirical data under benign conditions where no senescence, with respect to cell growth, has been observed (Proenca et al., 2018, 2019), though other studies performed under conditions that mirrored largely the study conditions of Proenca et al. (2019) revealed senescence by illustrating declining growth rates (or increased doubling times) with age (Łapińska et al., 2019). Non-senescence seems a rare event and most studies detected senescence in at least some traits, but in those studies, some level of extrinsic stressors, such as increased temperature (high energy), light exposure to excite fluorescein, potentially toxic chemicals that are used to determine cell death (propidium iodide) or prevent cell agglomeration, and biofilm forming (e.g., Tween 20), cannot be ruled out (Wang et al., 2010; Winkler et al., 2010; Steiner et al., 2019). To conclude on theoretical approaches, all models motivate their assumptions based on empirical findings, and the diversity of

TABLE 1 | Examples of single-cell studies on aging using molecular targets.

Aim	Device	Promoter/ molecular target	Function of target (on protein)	Main findings	Strain	Experimental conditions	References
Heat shock-induced PA	Agar plate	Dnak (Hsp70) (disaggregation)	Refolding	PA at poles	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Repair deficiency	Mother machine	ΔdnaK	Knockout quality and repair	Old pole reduced growth	K-12 *E. coli* wt BW25113	LB 37°C	Proenca et al., 2019
Heat shock-induced PA	Agar plate	DnaJ (disaggregation)	Refolding	PA at poles	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Heat shock-induced PA	Agar plate	ClpB (Hsp104) (disaggregation)	Refolding, quality control	PA at poles. Old pole with PA grow slow	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Repair deficiency	Mother machine	ΔclpB	Knockout quality and repair	Old pole reduced growth	K-12 *E. coli* wt BW25113	LB 37°C	Proenca et al., 2019
Heat shock-induced PA	Agar plate	GroEL–GroES (disaggregation)	Refolding	No relocation	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Heat shock-induced PA	Agar plate	Lon (disaggregation)	Degradation	No relocation	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Heat shock-induced PA	Agar plate	ClpX	Degradation	No relocation	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Heat shock-induced PA	Agar plate	ClpP	Degradation	No relocation	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Heat shock-induced PA	Agar plate	HslU	Degradation	No relocation	wt K-12 *E. coli* MC4100	LB/45°C for 20 min, then 30°C	Winkler et al., 2010
Spontaneous PA	Agar plates	IbpA (sHsp)	Sequestration	PA at old pole. Old pole reduced growth	*E. coli* wt MG1655	LB 37°C	Lindner et al., 2008
Heat shock-induced PA	Agar plate	IbpA (sHsp)	Sequestration	Reduced growth independent of PA. PA increased stress tolerance	*E. coli* wt MG1655	LB/47°C for 15 min, then 37°C	Govers et al., 2018
Localizing PA	Mother machine	IbpA	Sequestration	PA located at old poles; reduced growth	K-12 *E. coli* wt MG1655	LB 37°C	Proenca et al., 2019
Glucose accumulation	Mother machine	2-NBDG, (ThT) staining amyloid aggregates	Glucose uptake	Old pole grows slow; slow glucose accumulation. No PA. Aging not linked to PA	*E. coli* wt BW21113	LB 37°C M9 glucose accumulation	Łapińska et al., 2019
Protein expression old and new pole	Agar plates	mut3b	General protein expression	Old daughter less protein expression. Old pole lineages higher asymmetry	*E. coli* K12 wt NCM3722	M9	Shi et al., 2020
Translation errors and mutations	Mother machine	MutL	DNA mismatch repair	Age-independent rate of mutations	*E. coli* wt MG1655 and mutH strain	LB 37°C	Robert et al., 2018

The examples listed here are on E. coli strains and have a focus on protein aggregates. Many of the studies used in addition to the fluorescently labeled target also knockout strains of these molecular targets to gain a deeper mechanistic understanding. Either experiments were performed in a microfluidic device (mother machine) or colony growth was tracked on an agar plate.
PA, protein aggregates; wt, wild type; LB, Luria–Bertani.

theoretical predictions reflects on the inconsistencies of empirical results (**Figure 2**). Despite these inconsistencies, the models help to understand changing optima for asymmetry dependent on environmental conditions, as suggested by empirical findings. Unfortunately, senescence and asymmetry in simple organisms such as bacteria are not as simple as one might hope. Perfect rejuvenation, where cells are left without any damage, as realized for metazoans, might not be optimal for unicellular organisms (Evans and Steinsaltz, 2007; Moger-Reischer and Lennon, 2019). Even though these theoretical models rely on partly fundamental different methodological approaches, such as branching processes models, super processes models, structured matrix models, or classical differential equation models, they all agree in their prediction of asymmetry as found in all empirical studies.

MECHANISMS OF AGING

The potential for revealing aging mechanisms in bacteria is considered substantial, since various bacteria species—*E. coli* as a foremost example—are model systems for molecular exploration (Stewart et al., 2005). In addition, their somewhat simpler biology suggests them as model systems to link aging phenotypes

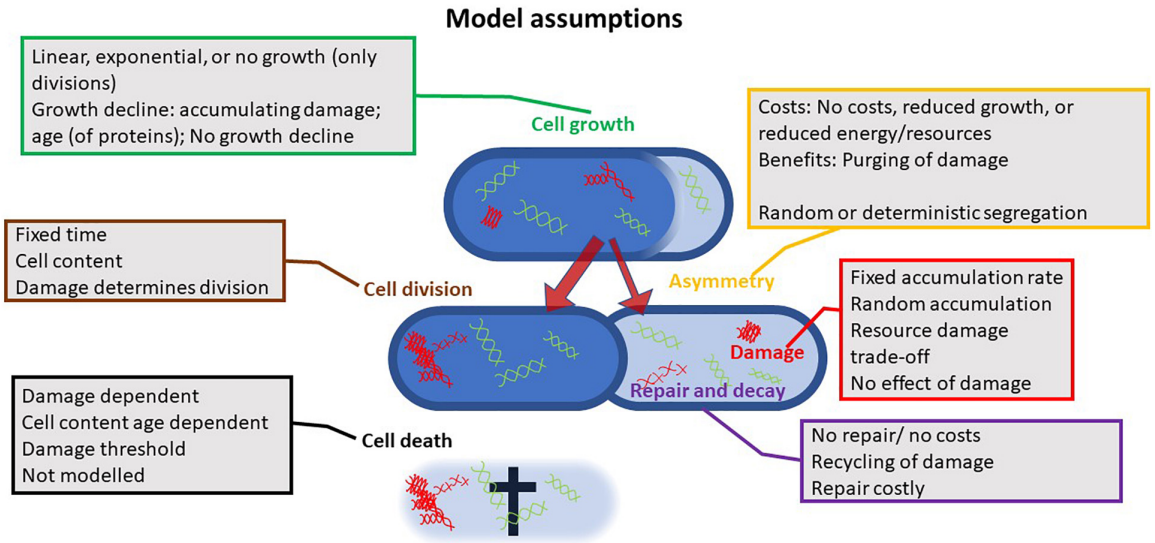

FIGURE 2 | Illustration of the assumptions different models rely on; all models investigated the effects of asymmetry at cell fission. The multitude of assumptions and combinations thereof highlights how contrasting findings can be explained. Up to six parameters have been considered in such models: growth or the increase in active proteins, cell division, damage accumulation or the rate at which active proteins are transformed into passive proteins, the asymmetry at fission in damage or passive proteins, the decay of damage or the repair for passive to active proteins, and cell death. Differences in assumed cost functions, efficiencies of repair, and effect of damage further increase the diversity of outcomes.

and mechanisms. Considering the nine hallmarks of aging defined for metazoans—genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication—bacteria, as unicellular organisms, do not require distinction between organismal senescence and cellular senescence, as various other hallmarks of aging can be excluded in bacteria (López-Otín et al., 2013). Bacteria do not have stem cells; therefore, stem cell exhaustion does not need to be considered. Most bacteria lack telomeres; therefore, telomere attrition requires no consideration. Bacteria lack mitochondria; therefore, no increased mitochondrial dysfunction with age can influence senescence—though oxidative stress-related mechanisms show similarities between metazoans and bacteria (Imlay, 2013; Janikiewicz et al., 2018).

Altered intercellular communication with increasing age, another hallmark of metazoan aging, is little explored in bacteria. In metazoans, such age-specific intercellular communication has been described in the context of chronic low-grade inflammation (López-Otín et al., 2013; Zhang et al., 2013). In a single-celled organism, intercellular communication might be expected to be less important, but that seems not to be the case. In the context of aging, at least, intercellular communication is no prerequisite for detecting senescence in bacteria. Most aging research in bacteria at the single-cell level is done using microfluidic devices, but despite preventing most intercellular communication in microfluidic chips, chronological or reproductive senescence is found (Wang et al., 2010; Łapińska et al., 2019; Steiner et al., 2019). Obviously, observing senescence under conditions that prevent intercellular communication is not sufficient to rule out the potential impact of altered communication at old age for senescence. Still, some evidence of limited influence of altered intercellular communication at old ages comes from studies on microcolonies grown on Petri dishes, where intercellular communication is allowed. Senescence patterns show similarities between microcolony studies and studies done in microfluidic devices, with the former being limited in time depth (up to 7–10 divisions) (Stewart et al., 2005; Wang et al., 2010; Winkler et al., 2010; Łapińska et al., 2019; Steiner et al., 2019). Intercellular communication is intensively explored in biofilm studies, with aging biofilms showing altered bacteria behavior for, e.g., *Bacillus subtilis* (Bartolini et al., 2019). Whether the changed behaviors of cells in aging biofilms are due to nutrient depletion, crowding, quorum sensing, or altered intercellular communication is not yet known. In senescing biofilms, cells show general stress response, like other aging phenotypes, but senescing biofilms rather mirror settings of population aging than single-cell senescence. The complexity of biofilms confronts us, in a very general way, with challenges that might suggest a simpler approach, such as controlled intercellular communication among single cells in microfluidic devices to explore potential altered intracellular communication with age in bacteria (Gupta et al., 2020).

Age-specific nutrient sensing, another hallmark of metazoan aging, is maybe most prominently known for the TOR and insulin/IGF-1 pathway in metazoans, but these pathways seem not to function in bacteria. Bacteria use for nutrient sensing various known mechanisms, but they appear to be distinct from metazoans and other organisms (Chantranupong et al., 2015; Bhattacharyya et al., 2018; Alvarado et al., 2020). Known bacteria sensing mechanisms involve for instance PII (sensing of glutamine) or various chemoreceptors, e.g., Tar, Tsr, Tap, Trg, and

Aer, but their age specificity is little explored, not to mention at the single-cell level. At least, the described molecular mechanisms involved in nutrient sensing pave the way for age-specific exploration. Indications that dietary restriction can reduce mortality in *E. coli* are an encouraging sign of physiological and environmental integration for senescence, and what role altered nutrient sensing might play remains to be determined.

What remains of the metazoan hallmarks of aging for bacteria are genomic instability, loss of proteostasis, and epigenetic alterations. These three hallmarks belong, with telomere attrition, to the four primary hallmarks that are stated as undeniably negative in their effects. Most work on the mechanisms of bacteria senescence has been on the loss of proteostasis, with a focus on asymmetric division of inclusion bodies, but very insightful findings on mutation rates explored at the single-cell level along the age axis exist, while epigenetic explorations are less explicitly studied.

Genomic Instability and Senescence

Genomic instability in bacteria is substantial, foremost evidenced by the exceptional evolvability of bacteria populations (Darmon and Leach, 2014). Despite genomic instability being intensively studied in bacteria, exploring aging-related questions on genomic instability at the single-cell level is rare. Bacteria still provide exciting opportunities for exploring age-specific genomic instability. Genomic instability is generally associated with either the frequency of occurrence of DNA damage, or epigenetic or mutational reduction in expression of DNA repair genes. In short, the mutations originating from replication errors depend on both the rate at which errors occur and to what rate they can be repaired, and for senescence, whether these rates change with age. Quantifying such rates at the single-cell level in a high-throughput manner has only recently become feasible. Using fluorescently labeled MutL mismatch repair proteins that form fluorescent foci, replication errors can be visualized and thereby quantified in live cells at the single-cell level (Elez et al., 2010; **Table 1**). Comparing wild-type strains, holding a functional repair system, to strains with an inactive mismatch repair (MutL deficient), allows quantifying both mutation rates and repair accuracy (Robert et al., 2018). Replication errors occur at a constant rate across ages and followed a Poisson distribution. Such a pattern of Poisson distributed errors suggests that events occur independently of each other, i.e., replication errors that resulted in mutations occur at a constant rate across life and, hence, independent of age. This averaged constancy in mutation rates over age does not prevent variation within one and the next division of a cell. Replication error rate within a single-cell cycle varies by a factor of ∼3 and is associated with the number of replication forks, i.e., replication error correlates with the size of the cell (Robert et al., 2018). Slowly growing cells, which might be biologically older with more accumulated damage (accumulated mutations), did not show higher mutation rates for the remainder of their lives. We need to caution that these findings relate to mutation rates for replication errors and remind ourselves that stress-induced mutagenesis acts mainly at the level of error repair, and there might be age-related changes in repair efficiencies. Furthermore, benign environments reduce stress-induced mutagenesis, which agrees with the observation of non-reproductive senescence in these fascinating studies on mutation dynamics (Robert et al., 2018).

Comparing mutation rates for WT strains and repair-deficient mutants at ∼0.0022 and ∼0.32 mutations/h, respectively, shows that most mutations that occur during replication are efficiently repaired (1 out of 145 mutations slips the repair) (Robert et al., 2018). Such high rates of repair are not surprising given the effort bacteria invest to detect errors; *E. coli* cells produce every 10 min enough base excision repair enzyme to scan the entire chromosome (Lee and Wallace, 2017). Concerns that the application of heterologous fluorescence markers and high energy excitation light could increase mutation rates and aging can partly be relaxed, as the mutation rates quantified at the single-cell level using these methods of potential concern mirror estimates based on whole-genome sequencing (Lee et al., 2012). Mutations that slipped repair did not lead to reduced growth and no evidence of senescence with respect to growth was revealed. However, for cells with deficient repair and therefore increased number of mutations, the mean growth rate slightly decreased. These repair-deficient cells either reduced growth in a stepwise manner, suggesting that single mutations caused growth reduction or ceased growth completely and finally died (Robert et al., 2018). At the single-cell level, heavily deleterious mutations are found but are rare (∼0.3% of all mutations). Heavily deleterious mutations could hence only account for the senescence of a few cells, not enough to drive senescence patterns across cells in a population; most non-lethal mutations had small fitness effects. These precisely quantified numbers at the single-cell level, in combination with the findings that mutations occur at a constant rate and that current and future mutations are independent of the accumulation of previous mutations, strongly suggest a minor role of genomic instability for senescence in *E. coli*. Together, no evidence is revealed for increased genomic instability with age, and rare non-lethal mutations with stronger deleterious effects are not sufficient to shape observed senescence patterns at the population level, despite them being inherited to their daughter cells. In yeast cells, genomic destabilization has been shown, and this instability has been associated with mitochondrial fragmentation and dysfunction that showed age-specific patterns (Moger-Reischer and Lennon, 2019).

Loss of Proteostasis

Mechanistic aging research in bacteria centers around loss of proteostasis, with an emphasis on studying inclusion bodies. Inclusion bodies consist primarily of aggregated damaged and misfolded proteins; for additional details on inclusion bodies, we refer to an excellent recent review (Schramm et al., 2019). Inclusion bodies, i.e., aggregates, appear in response to metabolic activity associated with cell growth and extrinsic or intrinsic stresses, including antibiotics, temperature, osmolarity, ionic strength, pH, heavy metals, hypochlorous acids, and macromolecular crowding. The depletion of intracellular ATP seems also be linked to the emergence and disappearance of aggregates (Pu et al., 2019). Important for senescence, aggregates increase in number and size with cell age (Maisonneuve et al., 2008). Emerging small aggregates

cluster to larger aggregates that vary in size; heat shock-induced aggregates are estimated to be composed of 2,400–16,500 protein molecules, and approximately 1.5–3% of all proteins in the cytosol are found in such aggregates after heat shock (Winkler et al., 2010). Aggregate emergence, growth, and size can be visualized using fluorescently labeled chaperones involved in quality control, degrading, refolding, and recycling misfolded or damaged proteins. Chaperones include, e.g., DnaK, DnaJ, GrpE, GroEL–GroES, ClpB, ClpX, ClpP, HslU, IbpA, and IbpB to name a few (**Table 1**), and several of these chaperones are also found in metazoans, including humans (Hartl and Hayer-Hartl, 2002). At emergence, aggregates are distributed relatively randomly within the cell, but then move toward the old pole of the cell over multiple-cell fission events (Lindner et al., 2008; Proenca et al., 2019). Contrasting to yeast and cells of metazoans, where within the cell, movement of inclusion bodies is energetically costly (Nyström, 2005), in bacteria, this movement is a passive—non-energy requiring—process potentially driven by crowding of DNA in the center of the cell where the new cell poles are built (Winkler et al., 2010; Coquel et al., 2013, but also see Rokney et al., 2009). Note that aggregation is non-energy demanding, while disaggregation, i.e., the chaperone activity itself, is energy demanding (Winkler et al., 2010, again see Rokney et al., 2009 for conflicting results).

Inclusion bodies became a focus of mechanistic aging research in bacteria for their assumed universal deleterious effects and them being one of the end products in a chain of reactions triggered by reactive oxygen species (Sabate et al., 2010; Moger-Reischer and Lennon, 2019). Interest increased when it was shown that inclusion bodies segregated asymmetrically with inclusion bodies being frequently located at the old pole of the cell after a few divisions and the discovery of correlated reduced growth of old poled cells (Lindner et al., 2008; Winkler et al., 2010). Beyond old pole cells showing reduced growth and division rates with increasing age, the amount of misfolded proteins correlates with the number of dead cells in bulk populations (Maisonneuve et al., 2008). Detailed analysis revealed that new pole cells that lost polar aggregates through asymmetric division grew significantly faster than new pole cells that still held aggregates (Winkler et al., 2010). Recently, more and more mechanistic understanding is gained, for instance, under benign conditions where no senescence is observed, an equilibrium in asymmetry and cell elongation can be found in repair mutants that lack specific repair chaperones (ΔclpB, ΔdnaK), but when ΔdnaK is knocked out, new daughter lineages remain at their equilibrium, while old daughter lineages show increased mortality (Proenca et al., 2019). Therefore, an intact repair capacity is not conditional for observing non-senescence with respect to growth and division rates. Growth differences among old pole and new pole cells also correlate with differences in physiology, with old pole cells accumulating glucose at a slower rate (Łapińska et al., 2019). Lower protein expression is observed in older daughter cells compared with young daughters, and old daughters produced daughters that were more asymmetric in protein expression (40%) compared with young daughters (10%) (Shi et al., 2020). Also, strains possessing the ability to create minicells from the pole regions, and thereby ejecting aggregates, grew faster after such ejection (Rang et al., 2018). All these findings support that protein aggregates contribute to physiological heterogeneity among cells (Mortier et al., 2019). Despite these encouraging findings, to date, an unambiguous causal relationship between senescence and inclusion bodies has not been established. A causal relationship of inclusion bodies and senescence is rather questioned, including a lack of correlation between cell growth rate and fluorescent concentration for cells holding aggregates (Govers et al., 2018).

Under benign conditions, no or few inclusion bodies occur (Łapińska et al., 2019). The prevention of building observable protein clusters supports the assumption that misfolded proteins can be immediately repaired or recycled under certain conditions (Clegg et al., 2014), but the notion that no senescence has to be observed (Rang et al., 2012) does not hold. Even under benign conditions where no inclusion bodies could be detected, senescence was still observed (Łapińska et al., 2019). Therefore, stressful conditions or inclusion bodies are not a prerequisite of senescence. Furthermore, inclusion bodies might not be universally deleterious in their effect, but rather a stress response induced by extrinsic factors including experimental conditions such as exposure to high energy light, heat shock, peroxide, and antibiotics (Govers et al., 2018). When exposed to heat shocks, cells with inclusion bodies showed higher adaptive potential and fitness to further heat shocks, exposure to antibiotics, or reactive oxygen species (Govers et al., 2018). This fitness advantage might be gained by inclusion bodies providing a reservoir to buffer negative fitness effects of stochastic environments (Baig et al., 2014b). The argument of inclusion bodies providing a reservoir to buffer severe conditions is supported by observations of a small number of inclusion bodies under stressful conditions and the decomposition of inclusion bodies to the extreme of complete disappearance (Baig et al., 2014a; Govers et al., 2018). More evidence against strict deleterious effects of inclusion bodies comes from studies on the interaction between DNA damage and mistranslation of proteins. Mistranslation enhances survival under changing and stressful conditions, shown by high mistranslation increasing the phenotypic tolerance and genetical resistance under DNA damage, as well as survival under temperature stress. Decreasing the basal mistranslation rate, however, reduces cell survival (Samhita et al., 2020). Such findings strengthen the interconnectedness among damage at the DNA level and damage at the protein level, both processes that have been argued to be the central mechanisms of aging.

The pace at which inclusion bodies can be decomposed also depends on the type of agglomerates as well as on the degree to which inclusion bodies are harmful. Agglomerates that contain many proteins for which misfolding has been triggered by heat shock or cells entering stationary growth phase (as in the experiments by Govers et al. (2018), described above) are easier decomposed than agglomerates being triggered by reactive oxygen species (ROS) and reactive chlorine species (RCS). These latter processes damage proteins in a covalent way and thereby make the proteins irreversibly misfolded (Schramm et al., 2019). When inclusion bodies consist of, and are induced by, overexpression of proteins (e.g., amyloidogenic CsgA protein; Marcoleta et al., 2019), these inclusion bodies are

mostly cordial to the host cell, and a fraction of the overexpressed proteins can maintain their activity in the aggregated state (De Marco et al., 2019). Such differences in decomposition properties and harmfulness can help to understand what role inclusion bodies play for senescence—to date, differentiation among inclusion body types has rarely been considered in aging studies, and the potential positive effects of the types of inclusion bodies are little explored. This differentiation might allow for generalization across taxa, since prokaryotic cells differ in the biochemical makeup of damage triggered by ROS compared with eukaryotic cells, where ROS-induced senescence processes are characterized by imbalanced ROS production and ROS neutralization (Ksiazek, 2010). To date, the linkage between inclusion bodies, protein aggregates, and cellular senescence remains elusive and questioned with various mortality pathways [e.g., *mazEF* triggered cell death in *E. coli* or *skf* and *sdp* operons in *Bacillus* (Erental et al., 2012)] that are independent of asymmetric segregation (Baig et al., 2014b).

Various studies argue that inclusion bodies are attached and anchored to the old pole cell wall after the agglomerate has moved to this pole (Proenca et al., 2019; Shi et al., 2020). Such findings contrast with substantial stochastic influences in segregating inclusion bodies when cells are only tracked over a few divisions (Winkler et al., 2010; Ni et al., 2012). Attachment to the cell wall of inclusion bodies, damage, or any other aging factors that influence mortality would also be difficult to align with findings comparing new pole daughter cells that arise from old mother cells and those that arise from young mothers. Despite the two types of new pole cells having exactly the same pole age, new pole daughter cells arising from old mothers are born at an older biological age and differ substantially in senescence compared with new born daughters arising from young mothers (Steiner et al., 2019). Therefore, the age of the cell wall itself is not determining the lifespan or growth rate of a cell, but the inherited cytosol content should (Steiner et al., 2019). The difference in senescence among the two types of young daughter cells also suggests that aging factors accumulate over time, and on average, larger fractions of this aging factor are transmitted to daughter cells. Such transmission should not lead to two growth equilibria among old and young daughter lineages, as suggested by Rang et al. (2011), but rather to the distribution of cells with different aging factors. Aging factors generally tend to increase with age, and selection is assumed to be dependent on accumulated aging factor among cells. Theoretical understanding and empirical findings on senescence patterns suggest that both the accumulation of the aging factor within the cell and the asymmetric segregation at cell fission are determined by stochastic processes (Steiner et al., 2019). Young daughters of older mothers have higher mortality rates from birth onwards compared with young daughter cells of young mothers (Lansing effect), but the lifespan of young daughters is uncorrelated to the lifespan of their young or old mothers. If mother cells increase their aging factor with age and would inherit a defined fraction of this aging factor to their daughters, one would expect a correlation in lifespan among mother and daughter cells, a pattern not found (Steiner et al., 2019). Also, if mothers would differ in their rate of accumulation and inherit a low accumulation rate, a correlation in lifespan would be expected, again, a pattern not found. Taken together, we conclude that aging factors are likely not anchored to the cell wall; in average, they accumulate with age, but this accumulation differs among individuals, and asymmetric segregation at cell fission among the old pole and new pole cell holds a large stochastic component.

Epigenetic Alterations

Non-genetically determined heterogeneity in mortality among cells has been shown when cells have been exposed to normally bactericidal concentrations of antibiotics (Brauner et al., 2017). The non-genetic heterogeneity in phenotypes, including gene expression, growth rate, and mortality, can be increased by exposing cells to sublethal levels of antibiotics (Ni et al., 2012). More importantly, for aging and epigenetics, these characteristics can be traced back to asymmetric division events of cell lineages occurring much before the exposure to the stressor (Ni et al., 2012). This predisposition effect might be enhanced by positive feedback loops. Cells that show, prior to stress exposure, higher RpoH transcription activity—an indicator of higher investment in maintenance—are more likely to divide into cells exhibiting higher stress response and increased mortality, but a reliable link to senescence through, for instance, inclusion bodies has not been established (Ni et al., 2012). Cells inheriting the ancestral protein aggregate were more stress resistant compared with sister cells that did not inherit any protein aggregate, which could provide leads to age-related patterns of protein clustering, but such relationship could not yet be established (Govers et al., 2018). One challenge for establishing relationships among senescence- and epigenetic age-related alterations is the substantial stochastic variation that overrides such signal (Ni et al., 2012; Steiner et al., 2019). To our knowledge, no clear age-specific epigenetic alterations have yet been shown, but efforts and tailored studies seem to be lacking; future discoveries will provide a more reliable answer to this question.

ENVIRONMENTAL DEPENDENCIES OF SENESCENCE

The demographic rates and the functional traits senescence is mainly quantified with are environmental dependent. In consequence of this environmental sensitivity of the functional traits, senescence itself is environmental dependent. Light excitation, for instance, can influence senescence patterns of growth rates, survival rates, numbers and growth of inclusion bodies, filamentation rates, and many other physiological and biochemical characteristics associated with senescence (Wang et al., 2010; Winkler et al., 2010; Łapińska et al., 2019; Proenca et al., 2019; Steiner et al., 2019). High caloric environments trigger higher cell division asymmetry and increased frequency of senescent cells (Baig et al., 2014a). Such environmental sensitivity challenges us in identifying general senescence patterns and aging mechanisms, but at the same time opens opportunities for comparative exploration of mechanisms. Evidence for decoupling reproductive senescence—reduced cell growth and

division rate with age—and chronological senescence—increased mortality rates with age—is found in various studies conducted under different media (Wang et al., 2010; Steiner et al., 2019), and though under severe starvation, a trade-off between chronological senescence and growth has been shown (Yang et al., 2019). Benign conditions can prevent senescence but such non-senescence has not been found in all studies (Proenca et al., 2018, 2019; Łapińska et al., 2019). Preliminary data on *E. coli* suggest that scaling of chronological senescence along a temperature gradient, but altered shapes of senescence patterns when nutrients—glucose availability—are changed, results similar to those found in *C. elegans* (Stroustrup et al., 2016; Jouvet and Steiner, unpublished). Assuming a role of asymmetry for senescence, not only environmental conditions allow for altering senescence by altering the level of asymmetry among old and new poled cells, but also variance in asymmetry can be changed. Stochastic variance in asymmetry is enhanced under high light conditions, while the average fraction characteristic of the asymmetry is not altered (Proenca et al., 2019). Differences in variance might not necessarily alter senescence patterns, as other studies found that asymmetric segregation of protein aggregates at cell fission was independent of nutrient conditions (Baig et al., 2014a). Somewhat contrasting, different nutrient conditions triggered different selective forces on asymmetric cell division as explored in evolution experiments (Lele et al., 2011). Asymmetry in cell division might not only be environmentally but also partly genetically controlled. Nutrient-sensitive senescence is expected as the rate of aging is linked to stress response (RpoS) pathways (Yang et al., 2019), with RpoS activity inhibiting growth and nutrient assimilation (Mauri and Klumpp, 2014). More systematic exploration of environmental impact on senescence is needed. To date, we do not even have an understanding of the influence of basic conditions such as starting experiments with stationary phase cells or exponentially growing cells (Winkler et al., 2010; Proenca et al., 2019). It is evident that environmental conditions can scale and alter the process of senescence within cells, though scaling and altering senescence is not limited to environmental conditions, as evidenced by differences among *E. coli* strains exhibiting different senescence patterns under highly controlled environmental conditions (Jouvet et al., 2018).

PERSPECTIVES

The major challenge for aging research according to the influential review on hallmarks of aging is "to dissect the interconnectedness between the candidate hallmarks and their relative contribution to aging" (López-Otín et al., 2013). Even though the nine hallmarks have a focus on metazoan aging, they should generally be applicable and show many similarities to prokaryotic senescence, including the importance of asymmetry for rejuvenation and aging, as well as basic senescence patterns such as an exponential increase in mortality earlier in life followed by a late age mortality plateau (Moger-Reischer and Lennon, 2019; Steiner et al., 2019).

The hope, to establish bacteria as simple model systems for aging, roots in bacteria being molecularly well explored, similar to other model systems that have shed new light on aging questions, including yeast and *C. elegans* (Stewart et al., 2005; Moger-Reischer and Lennon, 2019). Despite fascinating studies and approaches, the potential for gaining a much improved mechanistic understanding of senescence—including costs and roots of repair, maintenance, and longevity—has not yet been exploited to its full extent. Many bacteria studies approaching aging mechanisms from a molecular angle miss adequate time depth and are limited to a few divisions, while other studies, approaching bacteria aging from a demographic, ecological, or evolutionary perspective, fail to exploit the potential the deep molecular knowledge bacteria systems offer. Bridging the gap between organismic aging research and molecular mechanism remains elusive, which might explain why even to date no conclusive evidence for causal mechanisms of aging exists in bacteria. Bridging the molecular and organismic approach might help to establish bacteria as a model organism for aging.

Quantifying Mechanisms

Another challenge to dissect the interconnectedness between the candidate hallmarks of aging is a qualitative rather than quantitative evaluation of (molecular) mechanistic influences on senescence. Studying populations in bulk cultures, even when deeper molecular knowledge is applied, makes it challenging to quantitatively separate senescence at the individual and population level, similar to the challenges of dissecting population and individual senescence known from the early explorations on disinfectants (Chick, 1908; Yule, 1910). Qualitative evaluation does not dissect among mutually non-exclusive mechanisms. An example can be given by the manifold influences of ROS on senescence. ROS-triggered processes might be involved in various hallmarks of aging, including DNA oxidation and dysregulation, and loss of proteostasis where ROS might trigger inclusion bodies. Without a quantification, separating out these processes for fitness at different levels is not possible, and such quantification requires, in large, an evaluation at the individual level. There are specific conditions, such as stationary phase populations, for which it was shown that when incubated in the absence of oxygen, they show significantly extended lifespans compared with populations grown in the presence of oxygen (Dukan and Nyström, 1998). These findings link ROS and oxidative stress to stationary phase-associated senescence, but even at stationary phase, a small turnover of cells exists, making it difficult to exactly quantify contributions. On the other extreme of resource availability, under very benign conditions, even when oxygen is available and exponential growth is achieved, bacteria cells often switch to anoxic and somewhat wasteful metabolism (Basan et al., 2015), but extensions of lifespan under such benign conditions remain controversial (Baig et al., 2014a; Proenca et al., 2019). Preliminary evidence exists for calorie restriction to act in bacteria as it has been shown for yeast (Taormina and Mirisola, 2014; Yang et al., 2019). As in eukaryotes for bacteria, the positive role of ROS, e.g., as an important signaling and regulating factor and not only as a damaging agent, is not well understood (Santos et al., 2018), as well as its role for bacterial senescence. Aside of the abovementioned results on increased

lifespan of stationary phase populations under anoxic conditions, reproductively arrested populations of *E. coli* show increased population resistance to external oxidative stress and high levels of oxidative defense proteins (Maiese et al., 2009; Kwak et al., 2015). At the same time, such growth-arrested *E. coli* populations displayed high levels of damaged proteins (Dukan and Nyström, 1998, 1999) and did not show well-correlated responses between respiratory activity, protein oxidation, and lifespan (Ballesteros et al., 2001). Taking these findings together, as in yeast and eukaryotes, the importance of ROS for longevity is likely multifold and not unidirectional, likely condition dependent, and far from clear, and only a thorough quantitative evaluation at the single-cell level will allow to dissect this multifold influence (Santos et al., 2018).

The challenge—through such quantification—to identify aging factors and causal mechanisms of senescence in the first place, and their interconnectedness in the second place, is heightened by the strong stochastic aspects of senescence (Steinsaltz et al., 2019). Bacteria research has been at the forefront to quantify stochastic processes, and substantial stochastic characteristics have been shown, from transcription and translation, to the demographic fate of cells (Elowitz et al., 2002; Raj and van Oudenaarden, 2008; Jouvet et al., 2018; Steiner et al., 2019; Sampaio and Dunlop, 2020). Using fluorescent labeling of transcription and translation processes at the single-cell level has proven a powerful method for quantifying stochastic processes—methods that mirror those used for aging research on bacteria. Interest on stochastic characteristics and their functional role has risen recently and is no longer simply considered noise (Steiner and Tuljapurkar, 2012; Proenca et al., 2019). Dissecting the stochastic aspects of senescence might be needed to dissect the interconnected deterministic aspects of senescence as stated as the major challenge for the hallmarks of aging (López-Otín et al., 2013). Stochastic aspects might be similarly interconnected and scaled across levels of biological organization with feedback or snowball effects leading to self-enforcing effects or stochastic buffering across levels of organization, but we are only in the infancies to understand such interactions in stochasticity. Bacteria provide the experimental conditions for such dissection through high control of the genetic background and the environment, the tracking of trait dynamics at all levels of biological organization, and the collection of high-throughput data over a short time required for quantifying stochastic aspects.

The challenges ahead can be surmised through cross-linkage between protein synthesis and DNA repair, where global mistranslation increases cell survival under stress. Linkage becomes evident through similarities between genomic instability and protein misfolding where protein aggregation and self-assembly can be observed in proteins that regulate the DNA damage response (Xie and Jarosz, 2018). Dissecting and verifying links will remain an exciting though challenging task in bacteria, which may not be as challenging as for more complex metazoan systems. Bacteria illustrate how observations over short times, suggesting deterministic aspects of senescence, can easily be overwritten by stochastic variation when longer time frames are considered (Ni et al., 2012). Sister cells show for a brief time after cell division similar phenotypes with respect to heat shock survival, but then rapidly transition to stochastic patterns, likely due to a combination of multiple stochastic cellular processes (Govers et al., 2017). Lasting effects can still sometimes be observed: mothers that divide more slowly have daughters that divide more slowly, presumably due to larger transmission of damage (Proenca et al., 2018), but longevity does not seem to be heritable, and long-lived mothers do not have predictable longer- or shorter-lived daughters (Steiner et al., 2019). When stochastic aspects override deterministic ones, evolution, or at least the pace of evolutionary processes, is altered, and therefore, understanding stochastic influences in a quantitative way is required to answer the question on why senescence evolved in the first place (Steiner and Tuljapurkar, 2012).

Differentiate Cause and Consequence of Senescence

The molecular toolbox is well filled for bacteria, and the ability to manipulate bacteria strains will help to differentiate among what causes senescence and what is simply a consequence of senescence. One reason why bridging molecular mechanism and organismic aging research has been challenging is the interdisciplinarity and required cross talk among labs coming from different disciplines. Recent and most promising approaches combine fluorescently labeled molecular mechanisms such as chaperones, tracking their fluorescently labeled expression dynamics in thousands of single cells throughout their lives using microfluidic devices, followed by image and data analysis, and best accompanied by theoretical understanding *via* mathematical modeling. Such expertise in molecular biology, biophysics, computational biology, statistics, and mathematics is rarely combined in a single lab. Fortunately, integration of fluorescent markers is fairly standard for most molecular labs working on model bacteria systems (Datsenko and Wanner, 2000); the number of microfluidic setups such as the mother machine increases rapidly within and beyond biophysics labs (Wang et al., 2010; Kaiser et al., 2016; Steiner et al., 2019; Sampaio and Dunlop, 2020); the recently developed deep learning-based tools overcome the bottleneck for efficient and accurate image analysis (Lugagne et al., 2020; Ollion and Ollion, 2020); and the interest on theoretical explorations and deeper analysis of precise, high-throughput, and high-quality data remains high as evidenced by previous empirical studies that have sparked numerous theoretical models (Watve et al., 2006; Ackermann et al., 2007; Evans and Steinsaltz, 2007; Erjavec et al., 2008; Chao, 2010; Rashidi et al., 2012; Clegg et al., 2014; Robert et al., 2018; Moger-Reischer and Lennon, 2019; Steiner et al., 2019; Blitvić and Fernandez, 2020). Putting these pieces together requires joint efforts, cross talk, and collaborations that have not yet been widely established. Framework programs that foster establishing such interdisciplinary consortia are popular these days, but bacteria aging has not yet profited heavily of such programs.

Aside from utilizing the molecular toolbox, experimental evolution studies on bacteria open unique insights into aging mechanisms, their role for fitness, and the fundamental question of why senescence has evolved. When taking the

evolutionary perspective on bacteria studies, we need to remind ourselves that bacteria studies in the lab are far from natural conditions. Benign conditions with high division rates of 20–30 min as observed in *E. coli* are attractive to collect large quantities of data in a short time and can reveal surprising results including non- (or negligible) senescence (Proenca et al., 2018, 2019). However, bacteria in the wild have 2–50 times longer doubling time than in the lab, which illustrates that bacteria in their natural settings spend most of their life under resource-limiting growth conditions (Gefen et al., 2014; Moger-Reischer and Lennon, 2019). Without such limiting conditions, we would face a Darwinian demon and the universe would be filled with *E. coli* or similar fast dividing cells in less than a week. When considering evolutionary aspects of senescence, the fitness effect of chronological senescence, at least under laboratory conditions, is negligible, because growth differences, which determine division rates and generation times (the time to reach the average number of offspring a cell produces over its lifespan), determine fitness, while longevity does not influence fitness to an important extent. Even in such a system, where mortality might be less important than reproduction, one might reveal deeply rooted evolutionary forces shaping senescence patterns, and it is surprising to find classical patterns such as mortality plateaus in bacteria. Whether these patterns are shaped by similar forces than in metazoans remains to be determined.

Beyond the molecular toolbox and the unique opportunities experimental evolution studies in bacteria offer, comparative approaches among bacteria can provide better mechanistic understanding since bacteria differ in their strategies for instance with respect to inclusion body inheritance; some inherit inclusion bodies asymmetrically, whereas others show symmetric inheritance; some show deterministic inheritance, while others show highly stochastic inheritance (Schramm et al., 2019). Most aging research in bacteria has focused on *E. coli*, but similarities in aging mechanisms to other bacteria species exist. Aggregate inheritance in *Mycobacterium smegmatis* correlates with growth and mortality, and stress levels correlate with asymmetry in distributing aggregates between daughter cells (Vaubourgeix et al., 2015). Despite some bacteria systems starting to establish themselves as model organisms for aging, overall exploration of senescence in bacteria is limited to very few species, including *B. subtilis* (Veening et al., 2008), *Mycobacterium* spp. (Aldridge et al., 2012), or *Methylobacterium extorquens* (Bergmiller and Ackermann, 2011). This list is by no means exhaustive, but still most bacteria have not been explored for senescence. The short list of bacteria species investigated has revealed unique aspects of asymmetry and senescence, illustrating the potential for discovery and inspiration on aging research beyond bacteria.

CONCLUSION

Bacteria show chronological and reproductive senescence like metazoans. The detailed investigations into mechanisms showed that aging factors being contained in the cytosol and asymmetric segregation of inclusion bodies are widely observed, even though understanding the role inclusion bodies play for senescence remains ambiguous. The diversity of empirical findings, the dependencies on environmental conditions, and the genetic background highlight the complexity of senescence. Novel insights on aging might come from bacteria studies, for instance, questioning the notion that inclusion bodies have unambiguously negative effects (Baig et al., 2014a; Govers et al., 2018). The highly controlled conditions of bacteria experiments and the large quantity of data collected at the individual level revealed the strong influences of stochastic processes on aging. If similar stochastic influences would be found in more complex metazoan systems, the dissection of the interconnectedness among the hallmarks and their contributions to aging will be challenging. The exciting technical opportunities that bacteria offer, in combination with the molecular knowledge on bacteria, will provide a deeper mechanistic understanding of bacteria senescence, but these opportunities need to be explored more systematically across environmental conditions and genetic backgrounds and in a highly quantitative way at the individual level. To date, despite their large representation in the tree of life (Hug et al., 2016), only few bacteria species have been explicitly investigated for senescence (Florea, 2017). To what degree understanding of bacteria processes of aging will be insightful for metazoan aging remains to be seen. Improved survival under antibiotic exposure of cells that hold inclusion bodies might not only shed light on senescence in bacteria but might also have medical relevance for antibiotic treatment in humans. The complex influence of inclusion bodies for bacteria aging can be insightful for the development of age-related deleterious diseases in humans such as Alzheimer's, Parkinson's, or type II diabetes (Sabate et al., 2010), because protein aggregates correlate with the development of such deleterious diseases. Understanding age-related aggregation processes, self-enforcing feedback loops, or snowball-like-triggered deleterious effects might inspire research on such age-related deleterious diseases, but at the same time, important differences in the emergence, biochemical makeup, and decomposing of inclusion bodies among bacteria and metazoan cells exist (Mogk et al., 2018; Nillegoda et al., 2018), and such differences might even provide greater insights. Without a shift from qualitative understanding of the mechanisms involved in senescence to a quantitative dissection of their influences, we will not gain a deeper understanding of the mechanisms and the evolution of senescence. In the end, we might get held up in discussions that were well known for the classical evolutionary theories of senescence two decades ago, where the two main mutually non-exclusive theories—antagonistic pleiotropy theory and mutation accumulation theory (Medawar, 1952; Williams, 1957)—were contrasted against each other. Both antagonist pleiotropic effects and mutation accumulation likely influence senescence in bacteria, but what their respective contributions are remains elusive. Illustrating that transcriptional error rates and mutation rates are age independent (Robert et al., 2018) is one example of

such qualitative investigation, though such understanding of age-independent mutation rates does not reveal a quantitative understanding of mutation accumulation. Quantitative investigation at the individual level will likely be the crux for dissecting the interconnectedness and identifying the contributions of the identified hallmarks of aging in bacteria and hopefully beyond.

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REFERENCES

Ackermann, M., Chao, L., Bergstrom, C. T., and Doebeli, M. (2007). On the evolutionary origin of aging. *Aging Cell* 6, 235–244. doi: 10.1111/j.1474-9726.2007.00281.x

Ackermann, M., Stearns, S. C., and Jenal, U. (2003). Senescence in a bacterium with asymmetric division. *Science (New York, N.Y.)* 300:1920. doi: 10.1126/science.1083532

Aldridge, B. B., Fernandez-Suarez, M., Heller, D., Ambravaneswaran, V., Irimia, D., Toner, M., et al. (2012). Asymmetry and aging of mycobacterial cells lead to variable growth and antibiotic susceptibility. *Science (New York, N.Y.)* 335, 100–104. doi: 10.1126/science.1216166

Alvarado, A., Behrens, W., and Josenhans, C. (2020). Protein activity sensing in bacteria in regulating metabolism and motility. *Front. Microbiol.* 10:3055. doi: 10.3389/fmicb.2019.03055

Baig, U. I., Bhadbhade, B. J., Mariyam, D., and Watve, M. G. (2014a). Protein aggregation in E. coli: short term and long term effects of nutrient density. *PLoS One* 9:e107445. doi: 10.1371/journal.pone.0107445

Baig, U. I., Bhadbhade, B. J., and Watve, M. G. (2014b). Evolution of aging and death: what insights bacteria can provide. *Q. Rev. Biol.* 89, 209–223. doi: 10.1086/677572

Balaban, N. Q., Gerdes, K., Lewis, K., and McKinney, J. D. (2013). A problem of persistence: still more questions than answers? *Nat. Rev. Microbiol.* 11, 587–591. doi: 10.1038/nrmicro3076

Ballesteros, M., Fredriksson, Å, Henriksson, J., and Nyström, T. (2001). Bacterial senescence: protein oxidation in non-proliferating cells is dictated by the accuracy of the ribosomes. *EMBO J.* 20, 5280–5289. doi: 10.1093/emboj/20.18.5280

Bartolini, M., Cogliati, S., Vileta, D., Bauman, C., Rateni, L., Leñini, C., et al. (2019). Regulation of biofilm aging and dispersal in Bacillus subtilis by the alternative sigma factor SigB. *J. Bacteriol.* 201, e473–e18.

Basan, M., Hui, S., Okano, H., Zhang, Z., Shen, Y., Williamson, J. R., et al. (2015). Overflow metabolism in *Escherichia coli* results from efficient proteome allocation. *Nature* 528, 99–104. doi: 10.1038/nature15765

Bell, G., and Cambridge University Press (1988). *Sex and Death in Protozoa: The History of An Obsession*. Cambridge: Cambridge University Press.

Bergmiller, T., and Ackermann, M. (2011). Pole age affects cell size and the timing of cell division in Methylobacterium extorquens AM1. *J. Bacteriol.* 193, 5216–5221. doi: 10.1128/jb.00329-11

Bhattacharyya, N. I, Nkumama, N., Newland-Smith, Z., Lin, L. Y., Yin, W., Cullen, R. E., et al. (2018). An aspartate-specific solute-binding protein regulates protein kinase G activity to control glutamate metabolism in mycobacteria. *mBio* 9:e931–e18.

Bi, E., and Lutkenhaus, J. (1993). Cell division inhibitors SulA and MinCD prevent formation of the FtsZ ring. *J. Bacteriol.* 175, 1118–1125. doi: 10.1128/jb.175.4.1118-1125.1993

Blitvić, N., and Fernandez, V. I. (2020). Aging a little: on the optimality of limited senescence in *Escherichia coli*. *J. Theor. Biol.* 502, 110331. doi: 10.1016/j.jtbi.2020.110331

Brauner, A., Shoresh, N., Fridman, O., and Balaban, N. Q. (2017). An experimental framework for quantifying bacterial tolerance. *Biophys. J.* 112, 2664–2671. doi: 10.1016/j.bpj.2017.05.014

Chantranupong, L., Wolfson, R. L., and Sabatini, D. M. (2015). Nutrient-sensing mechanisms across evolution. *Cell* 161, 67–83. doi: 10.1016/j.cell.2015.02.041

Chao, L. (2010). A model for damage load and its implications for the evolution of bacterial aging. *PLoS Genet.* 6:e1001076. doi: 10.1371/journal.pgen.1001076

Chick, H. (1908). An investigation of the laws of disinfection. *J. Hygiene* 8, 92–158. doi: 10.1017/s0022172400006987

Clegg, R. J., Dyson, R. J., and Kreft, J.-U. (2014). Repair rather than segregation of damage is the optimal unicellular aging strategy. *BMC Biol.* 12:52. doi: 10.1186/s12915-014-0052-x

Coelho, M., Dereli, A., Haese, A., Kühn, S., Malinovska, L., DeSantis, M. E., et al. (2013). Fission yeast does not age under favorable conditions, but does so after stress. *Curr. Biol.* 23, 1844–1852. doi: 10.1016/j.cub.2013.07.084

Coquel, A.-S., Jacob, J.-P., Primet, M., Demarez, A., Dimiccoli, M., Julou, T., et al. (2013). Localization of protein aggregation in *Escherichia coli* is governed by diffusion and nucleoid macromolecular crowding effect. *PLoS Comp. Biol.* 9:e1003038. doi: 10.1371/journal.pcbi.1003038

Darmon, E., and Leach, D. R. F. (2014). Bacterial genome instability. *Microbiol. Mol. Biol. Rev.* 78, 1–39. doi: 10.1128/mmbr.00035-13

Datsenko, K. A., and Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6640–6645. doi: 10.1073/pnas.120163297

De Marco, A., Ferrer-Miralles, N., Garcia-Fruitós, E., Mitraki, A., Peternel, S., Rinas, U., et al. (2019). Bacterial inclusion bodies are industrially exploitable amyloids. *FEMS Microbiol. Rev.* 43, 53–72. doi: 10.1093/femsre/fuy038

Dukan, S., and Nyström, T. (1998). Bacterial senescence: Stasis results in increased and differential oxidation of cytoplasmic proteins leading to developmental induction of the heat shock regulon. *Genes Dev.* 12, 3431–3441. doi: 10.1101/gad.12.21.3431

Dukan, S., and Nyström, T. (1999). Oxidative stress defense and deterioration of growth-arrested *Escherichia coli* cells. *J. Biol. Chem.* 274, 26027–26032. doi: 10.1074/jbc.274.37.26027

Elez, M., Murray, A. W., Bi, L. J., Zhang, X. E., Matic, I., and Radman, M. (2010). Seeing mutations in living cells. *Curr. Biol.* 20, 1432–1437. doi: 10.1016/j.cub.2010.06.071

Elowitz, M. B., Levine, A. J., Siggia, E. D., and Swain, P. S. (2002). Stochastic gene expression in a single cell. *Science* 297, 1183–1186. doi: 10.1126/science.1070919

Erental, A., Sharon, I., and Engelberg-Kulka, H. (2012). Two programmed cell death systems in *Escherichia coli*: an apoptotic-like death is inhibited by the mazef-mediated death pathway. *PLoS Biol.* 10:e1001281. doi: 10.1371/journal.pbio.1001281

Erjavec, N., Cvijovic, M., Klipp, E., and Nyström, T. (2008). Selective benefits of damage partitioning in unicellular systems and its effects on aging. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18764–18769. doi: 10.1073/pnas.0804550105

Evans, S. N., and Steinsaltz, D. (2007). Damage segregation at fissioning may increase growth rates: a superprocess model. *Theor. Population Biol.* 71, 473–490. doi: 10.1016/j.tpb.2007.02.004

Florea, M. (2017). Aging and immortality in unicellular species. *Mech. Ageing Dev.* 167, 5–15. doi: 10.1016/j.mad.2017.08.006

Gefen, O., Fridman, O., Ronin, I., and Balaban, N. Q. (2014). Direct observation of single stationary-phase bacteria reveals a surprisingly long period of constant

protein production activity. *Proc. Natl. Acad. Sci. U.S.A.* 111, 556–561. doi: 10.1073/pnas.1314114111

Govers, S. K., Adam, A., Blockeel, H., and Aertsen, A. (2017). Rapid phenotypic individualization of bacterial sister cells. *Sci. Rep.* 7, 1–9. doi: 10.1038/s41598-017-08660-0

Govers, S. K., Mortier, J., Adam, A., and Aertsen, A. (2018). Protein aggregates encode epigenetic memory of stressful encounters in individual *Escherichia coli* cells. *PLoS Biol.* 16:e2003853. doi: 10.1371/journal.pbio.2003853

Gupta, S., Ross, T. D., Gomez, M. M., Grant, J. L., Romero, P. A., and Venturelli, O. S. (2020). Investigating the dynamics of microbial consortia in spatially structured environments. *Nat. Commun.* 11, 1–15. doi: 10.1007/978-981-13-1840-5_1

Hartl, F. U., and Hayer-Hartl, M. (2002). Protein folding. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295, 1852–1858. doi: 10.1126/science.1068408

Hug, L. A., Baker, B. J., Anantharaman, K., Brown, C. T., Probst, A. J., Castelle, C. J., et al. (2016). A new view of the tree of life. *Nat. Microbiol.* 1:16048.

Imlay, J. A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium. *Nat. Rev. Microbiol.* 11, 443–454. doi: 10.1038/nrmicro3032

Janikiewicz, J., Szymański, J., Malinska, D., Patalas-Krawczyk, P., Michalska, B., Duszyński, J., et al. (2018). Mitochondria-associated membranes in aging and senescence: structure, function, and dynamics. *Cell Death Dis.* 9:332.

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of ageing across the tree of life. *Nature* 505, 169–173. doi: 10.1038/nature12789

Jouvet, L., Rodríguez-Rojas, A., and Steiner, U. K. (2018). Demographic variability and heterogeneity among individuals within and among clonal bacteria strains. *Oikos* 127, 728–737. doi: 10.1111/oik.04292

Kaiser, M., Jug, F., Silander, O., Deshpande, S., Pfohl, T., Julou, T., et al. (2016). Tracking single-cell gene regulation in dynamically controlled environments using an integrated microfluidic and computational setup. *bioRxiv [Preprint]* doi: 10.1101/076224

Kirkwood, T. B. L. (1977). Evolution of ageing. *Nature* 270, 301–304.

Ksiazek, K. (2010). Bacterial aging: from mechanistic basis to evolutionary perspective. *Cell. Mol. Life Sci.* 67, 3131–3137. doi: 10.1007/s00018-010-0417-4

Kwak, J. Y., Ham, H. J., Kim, C. M., and Hwang, E. S. (2015). Nicotinamide exerts antioxidative effects on senescent cells. *Mol. Cells* 38, 229–235. doi: 10.14348/molcells.2015.2253

Łapińska, U., Glover, G., Capilla-Lasheras, P., Young, A. J., and Pagliara, S. (2019). Bacterial ageing in the absence of external stressors. *Philos. Trans. R. Soc. B Biol. Sci.* 374:20180442. doi: 10.1098/rstb.2018.0442

Lee, A. J., and Wallace, S. S. (2017). Hide and seek: how do DNA glycosylases locate oxidatively damaged DNA bases amidst a sea of undamaged bases? *Free Radical Biol. Med.* 107, 170–178. doi: 10.1016/j.freeradbiomed.2016.11.024

Lee, H., Popodi, E., Tang, H., and Foster, P. L. (2012). Rate and molecular spectrum of spontaneous mutations in the bacterium *Escherichia coli* as determined by whole-genome sequencing. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2774–E2783.

Lele, U. N., Baig, U. I., and Watve, M. G. (2011). Phenotypic plasticity and effects of selection on cell division symmetry in *Escherichia coli*. *PLoS One* 6:e14516. doi: 10.1371/journal.pone.0014516

Lindner, A. B., Madden, R., Demarez, A., Stewart, E. J., and Taddei, F. (2008). Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3076–3081. doi: 10.1073/pnas.0708931105

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging. *Cell* 153, 1194–1217.

Lugagne, J.-B., Lin, H., and Dunlop, M. J. (2020). DeLTA: automated cell segmentation, tracking, and lineage reconstruction using deep learning. *PLoS Comp. Biol.* 16:e1007673. doi: 10.1371/journal.pcbi.1007673

Maiese, K., Chong, Z. Z., Hou, J., and Shang, C. (2009). The vitamin nicotinamide: Translating nutrition into clinical care. *Molecules* 14, 3446–3485. doi: 10.3390/molecules14093446

Maisonneuve, E., Ezraty, B., and Dukan, S. (2008). Protein aggregates: an aging factor involved in cell death. *J. Bacteriol.* 190, 6070–6075. doi: 10.1128/jb.00736-08

Marcoleta, A., Wien, F., Arluison, V., Lagos, R., and Giraldo, R. (2019). *Bacterial Amyloids. eLS*. Chichester: Wiley.

Mauri, M., and Klumpp, S. (2014). A model for sigma factor competition in bacterial cells. *PLoS Comp. Biol.* 10:e1003845. doi: 10.1371/journal.pcbi.1003845

Medawar, P. B. (1952). "An unsolved problem of biology," in *The Uniqueness of the Individual*, ed. H. K. Lewis (London: Routledge), 27.

Moger-Reischer, R. Z., and Lennon, J. T. (2019). Microbial ageing and longevity. *Nat. Rev. Microbiol.* 17, 679–690. doi: 10.1038/s41579-019-0253-y

Mogk, A., Bukau, B., and Kampinga, H. H. (2018). Cellular handling of protein aggregates by disaggregation machines. *Mol. Cell.* 69, 214–226. doi: 10.1016/j.molcel.2018.01.004

Mortier, J., Tadesse, W., Govers, S. K., and Aertsen, A. (2019). Stress-induced protein aggregates shape population heterogeneity in bacteria. *Curr. Genet.* 65, 865–869. doi: 10.1007/s00294-019-00947-1

Ni, M., Decrulle, A. L., Fontaine, F., Demarez, A., Taddei, F., and Lindner, A. B. (2012). Pre-disposition and epigenetics govern variation in bacterial survival upon stress. *PLoS Genet.* 8:e1003148. doi: 10.1371/journal.pgen.1003148

Nillegoda, N. B., Wentink, A. S., and Bukau, B. (2018). Protein disaggregation in multicellular organisms. *Trends Biochem. Sci.* 43, 285–300. doi: 10.1016/j.tibs.2018.02.003

Nyström, T. (2005). Role of oxidative carbonylation in protein quality control and senescence. *EMBO J.* 24, 1311–1317. doi: 10.1038/sj.emboj.7600599

Ollion, J., and Ollion, C. (2020). "DistNet: deep tracking by displacement regression: application to bacteria growing in the *Mother Machine*," in *Proceedings of the Medical Image Computing and Computer Assisted Intervention – MICCAI 2020*, Vol. Vol. 12265, (Berlin: Springer Science and Business Media), 215–225. doi: 10.1007/978-3-030-59722-1_21

Partridge, L., and Barton, N. H. (1993). Optimality, mutation and the evolution of ageing. *Nature* 362, 305–311. doi: 10.1038/362305a0

Proenca, A. M., Rang, C. U., Buetz, C., Shi, C., and Chao, L. (2018). Age structure landscapes emerge from the equilibrium between aging and rejuvenation in bacterial populations. *Nat. Commun.* 9:3722.

Proenca, A. M., Rang, C. U., Qiu, A., Shi, C., and Chao, L. (2019). Cell aging preserves cellular immortality in the presence of lethal levels of damage. *PLoS Biol.* 17:e3000266. doi: 10.1371/journal.pbio.3000266

Pu, Y., Li, Y., Jin, X., Tian, T., Ma, Q., Zhao, Z., et al. (2019). ATP-dependent dynamic protein aggregation regulates bacterial dormancy depth critical for antibiotic tolerance. *Mol. Cell* 73, 143–156.e4.

Raj, A., and van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* 135, 216–226. doi: 10.1016/j.cell.2008.09.050

Rang, C. U., Peng, A. Y., and Chao, L. (2011). Temporal dynamics of bacterial aging and rejuvenation. *Curr. Biol.* 21, 1813–1816. doi: 10.1016/j.cub.2011.09.018

Rang, C. U., Peng, A. Y., Poon, A. F., and Chao, L. (2012). Ageing in *Escherichia coli* requires damage by an extrinsic agent. *Microbiology (Reading, England)* 158, 1553–1559. doi: 10.1099/mic.0.057240-0

Rang, C. U., Proenca, A. M., Buetz, C., Shi, C., and Chao, L. (2018). Minicells as a damage disposal mechanism in Escherichia coli. *mSphere* 3,e00428–18. doi: 10.1128/mSphere.00428-18

Rashidi, A., Kirkwood, T. B. L., and Shanley, D. P. (2012). Evolution of asymmetric damage segregation: a modelling approach. *Sub Cell. Biochem.* 57, 315–330.

Robert, L., Ollion, J., Robert, J., Song, X., Matic, I., and Elez, M. (2018). Mutation dynamics and fitness effects followed in single cells. *Science* 359, 1283–1286. doi: 10.1126/science.aan0797

Rokney, A., Shagan, M., Kessel, M., Smith, Y., Rosenshine, I., and Oppenheim, A. B. (2009). E. coli transports aggregated proteins to the poles by a specific and energy-dependent process. *J. Mol. Biol.* 392, 589–601. doi: 10.1016/j.jmb.2009.07.009

Sabate, R., De Groot, N. S., and Ventura, S. (2010). Protein folding and aggregation in bacteria. *Cell Mol. Life Sci.* 67, 2695–2715.

Samhita, L., Raval, P. K., and Agashe, D. (2020). Global mistranslation increases cell survival under stress in *Escherichia coli*. *PLoS Genet.* 16:e1008654. doi: 10.1371/journal.pgen.1008654

Sampaio, N. M. V., and Dunlop, M. J. (2020). Functional roles of microbial cell-to-cell heterogeneity and emerging technologies for analysis and control. *Curr. Opin. Microbiol.* 57, 87–94. doi: 10.1016/j.mib.2020.08.002

Santos, A. L., Sinha, S., and Lindner, A. B. (2018). The good, the bad, and the ugly of ROS: new insights on aging and aging-related diseases from eukaryotic and prokaryotic model organisms. *Oxid. Med. Cell. Longev.* 2018:1941285.

Schramm, F. D., Schroeder, K., and Jonas, K. (2019). Protein aggregation in bacteria. *FEMS Microbiol. Rev.* 44, 54–72.

Shi, C., Chao, L., Proenca, A. M., Qiu, A., Chao, J., and Rang, C. U. (2020). Allocation of gene products to daughter cells is determined by the age of the mother in single *Escherichia coli* cells. *Proc. R. Soc. B Biol. Sci.* 287:20200569. doi: 10.1098/rspb.2020.0569

Steiner, U. K., Lenart, A., Ni, M., Chen, P., Song, X., Taddei, F., et al. (2019). Two stochastic processes shape diverse senescence patterns in a single-cell organism. *Evolution* 73, 847–857. doi: 10.1111/evo.13708

Steiner, U. K., and Tuljapurkar, S. (2012). Neutral theory for life histories and individual variability in fitness components. *Proc. Natl. Acad. Sci. U.S.A.* 109, 4684–4689. doi: 10.1073/pnas.1018096109

Steinsaltz, D., Christodoulou, M., Cohen, A., and Steiner, U. (2019). "Chance events in aging," in *Encyclopedia of Biomedical Gerontology*, ed. S. I. S. Rattan (Cambridge, MA: Academic Press), 386–394.

Stewart, E. J., Madden, R., Paul, G., and Taddei, F. (2005). Aging and death in an organism that reproduces by morphologically symmetric division. *PLoS Biol.* 3:e45. doi: 10.1371/journal.pbio.0030045

Stroustrup, N., Anthony, W. E., Nash, Z. M., Gowda, V., Gomez, A. I, ópez-Moyado, F. L., et al. (2016). The temporal scaling of Caenorhabditis elegans ageing. *Nature* 530, 103–107. doi: 10.1038/nature16550

Taormina, G., and Mirisola, M. G. (2014). Calorie restriction in mammals and simple model organisms. *BioMed. Res. Int.* 2014:308690.

Vaubourgeix, J., Lin, G., Dhar, N., Chenouard, N., Jiang, X., Botella, H., et al. (2015). Stressed mycobacteria use the chaperone ClpB to sequester irreversibly oxidized proteins asymmetrically within and between cells. *Cell Host. Microbe* 17, 178–190. doi: 10.1016/j.chom.2014.12.008

Vaupel, J. W., Carey, J. R., Christensen, K., Johnson, T. E., Yashin, A. I., Holm, N. V. I, et al. (1998). Biodemographic trajectories of longevity. *Science* 280, 855–860. doi: 10.1126/science.280.5365.855

Vaupel, J. W., and Yashin, A. I. (1985). Heterogeneity's ruses: some surprising effects of selection on population dynamics. *Am. Stat.* 39, 176–185. doi: 10.1080/00031305.1985.10479424

Vedel, S., Nunns, H., Košmrlj, A., Semsey, S., and Trusina, A. (2016). Asymmetric damage segregation constitutes an emergent population-level stress response. *Cell Syst.* 3, 187–198. doi: 10.1016/j.cels.2016.06.008

Veening, J.-W., Stewart, E. J., Berngruber, T. W., Taddei, F., Kuipers, O. P., and Hamoen, L. W. (2008). Bet-hedging and epigenetic inheritance in bacterial cell development. *Proc. Natl. Acad. Sci. U.S.A.* 105, 4393–4398. doi: 10.1073/pnas.0700463105

Wang, P., Robert, L., Pelletier, J., Dang, W. L., Taddei, F., Wright, A., et al. (2010). Robust growth of *Escherichia coli*. *Curr. Biol. CB* 20, 1099–1103.

Watve, M., Parab, S., Jogdand, P., and Keni, S. (2006). Aging may be a conditional strategic choice and not an inevitable outcome for bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 103, 14831–14835. doi: 10.1073/pnas.0606499103

Weitz, J., and Fraser, H. (2001). Explaining mortality rate plateaus. *Proc. Natl. Acad. Sci. U.S.A.* 98, 15383–15386. doi: 10.1073/pnas.261228098

Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11:398. doi: 10.2307/2406060

Winkler, J., Seybert, A., König, L., Pruggnaller, S., Haselmann, U., Sourjik, V., et al. (2010). Quantitative and spatio-temporal features of protein aggregation in *Escherichia coli* and consequences on protein quality control and cellular ageing. *EMBO J.* 29, 910–923. doi: 10.1038/emboj.2009.412

Xie, J. L., and Jarosz, D. F. (2018). Mutations, protein homeostasis, and epigenetic control of genome integrity. *DNA Rep.* 71, 23–32. doi: 10.1016/j.dnarep.2018.08.004

Yang, Y., Santos, A. L., Xu, L., Lotton, C., Taddei, F., and Lindner, A. B. (2019). Temporal scaling of aging as an adaptive strategy of *Escherichia coli*. *Sci. Adv.* 5:2069.

Yule, G. U. (1910). On the distribution of deaths with age when the causes of death act cumulatively, and similar frequency distributions. *J. R. Stat. Soc.* 73:26. doi: 10.2307/2340011

Zhang, G., Li, J., Purkayastha, S., Tang, Y., Zhang, H., Yin, Y., et al. (2013). Hypothalamic programming of systemic ageing involving IKK-β, NF-κB and GnRH. *Nature* 497, 211–216. doi: 10.1038/nature12143

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BRIEF RESEARCH REPORT
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Interactions Between Genes From Aging Pathways May Influence Human Lifespan and Improve Animal to Human Translation

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A major goal of aging research is identifying genetic targets that could be used to slow or reverse aging – changes in the body and extend limits of human lifespan. However, majority of genes that showed the anti-aging and pro-survival effects in animal models were not replicated in humans, with few exceptions. Potential reasons for this lack of translation include a highly conditional character of genetic influence on lifespan, and its heterogeneity, meaning that better survival may be result of not only activity of individual genes, but also gene–environment and gene–gene interactions, among other factors. In this paper, we explored associations of genetic interactions with human lifespan. We selected candidate genes from well-known aging pathways (IGF1/FOXO growth signaling, P53/P16 apoptosis/senescence, and mTOR/SK6 autophagy and survival) that jointly decide on outcomes of cell responses to stress and damage, and so could be prone to interactions. We estimated associations of pairwise statistical epistasis between SNPs in these genes with survival to age 85+ in the Atherosclerosis Risk in Communities study, and found significant (FDR < 0.05) effects of interactions between SNPs in *IGF1R*, *TGFBR2*, and *BCL2* on survival 85+. We validated these findings in the Cardiovascular Health Study sample, with $P < 0.05$, using survival to age 85+, and to the 90th percentile, as outcomes. Our results show that interactions between SNPs in genes from the aging pathways influence survival more significantly than individual SNPs in the same genes, which may contribute to heterogeneity of lifespan, and to lack of animal to human translation in aging research.

Keywords: aging pathways, animal to human translation, heterogeneity of longevity, genetic interactions, statistical epistasis, stress response, human lifespan, aging genes

INTRODUCTION

Many genes and their products have individually been found to significantly influence aging and survival traits in experimental models, including yeast, nematodes, flies, and mice. Genes for growth hormone and IGF1 receptors, FOXO transcription factors, target of rapamycin, p16, klotho, sirtuins, and some others, were repeatedly featured in experimental studies of aging and

lifespan extension [e.g., Johnson et al., 2002, 2013; Braeckman and Vanfleteren, 2007; Kenyon, 2010; Pavlatou et al., 2016; Uno and Nishida, 2016; Bartke and Quainoo, 2018; Singh et al., 2019; Tian et al., 2019; also reviewed in Ukraintseva et al. (2021)]. However, the majority of such genes have not been consistently replicated in humans, with few exceptions such as, e.g., *FOXO3* and *KL* (Arking et al., 2005; Willcox et al., 2008; Zeng et al., 2010; Nygaard et al., 2013, 2014; Soerensen et al., 2016; Donlon et al., 2017; Revelas et al., 2018; Morris et al., 2019).

The potential reasons for this lack of animal to human translation may include a highly conditional character of genetic influence on lifespan, and heterogeneity of longevity. The former refers to the possibility of different (or even antagonistic) influence of the same genetic variant on survival in different species/strains, or in the same species in different age groups and environments (de Magalhães, 2014; Ukraintseva et al., 2016). The latter (heterogeneity) implies that the high chances of survival to extreme age could be achieved through different genetic pathways, individual genes, additive polygenic effects, genetic interactions (G × G), and gene–environment interactions (G × E), among other factors (**Figure 1**).

A careful look at genes (and their products) that have been consistently featured in experimental aging research reveals that the majority of such genes belong to just a few well-known "aging pathways" that regulate outcomes of the cell responses to stress and damage, such as (using human orthologs names):

- IGF1/AKT/FOXO3 – mediated cell survival, growth, and DNA repair;
- TP53/P21/P16 – mediated apoptosis, growth arrest, senescence, and autophagy;
- mTOR/S6K – mediated autophagy, cell survival, and growth.

These pathways closely biologically interact and work in concert to decide on outcomes of cell responses to stress and damage, such as apoptosis, senescence, growth, division, autophagy, and repair, which may impact tissue resilience and, in turn, organismal survival and longevity (Levine et al., 2006; Feng et al., 2007; Tran et al., 2014; Bitto et al., 2015; Werner et al., 2016; Ukraintseva et al., 2021; **Figure 2**). We, therefore, propose that the interplay between genes in these pathways may influence human lifespan.

In this paper, we selected candidate genes that belong to these aging-related pathways *and* have also been featured in experimental aging research, and investigated the effects of interactions (statistical epistasis) between SNPs in these genes on survival in human data.

MATERIALS AND METHODS

Data

We used existing human data collected in the Atherosclerosis Risk in Communities (ARIC) and the Cardiovascular Health Study (CHS). The ARIC cohort of 15,792 participants, aged 45–64 at baseline received an extensive examination at 5 clinic visits conducted between 1987 and 2013, and yearly follow-ups assessing health status by telephone. By the time of last exam, almost all participants were older than 65. The CHS original cohort of 5,201 participants aged 65+ at baseline in 1989–1990 has been followed with annual clinic examinations till 1999, and then for another 20 years (2000-present) with twice yearly telephone contacts and additional examination in 2005–2006. Genetic, phenotypic, and survival information collected in these studies has been provided to us through the NIH supported portal dbGaP upon request for controlled access. We used version 5 of the ARIC data (dbGaP accession phs000280), and version 7 of the CHS data (dbGaP accession phs000287), including the genotyping data from the Candidate Gene Association Resource (CARe). The CARe covers ∼49K SNPs in ∼2,100 candidate health-related genes (with increased coverage in CVD-related loci) genotyped on the Illumina ITMAT-Broad-CARe (IBC) chip designed to be inclusive of the intronic, exonic, untranslated regions (UTRs) and ∼5 kb of the promoter regions (Keating et al., 2008; Musunuru et al., 2010). The IBC chip utilizes a tagging approach to capture the genetic diversity across these candidate genes, informed by GWAS, expression quantitative trait loci, pathway-based approaches and comprehensive literature searching, prioritized based on consensus by the investigators, as described in detail in Keating et al. (2008). Using the same array (IBC chip) in different CARe studies, including ARIC and CHS, aims to facilitate harmonization and replication of gene–phenotype associations across the studies (Musunuru et al., 2010). We used ARIC as the discovery dataset, and CHS as the validation set because the total number of SNPs available in the candidate genes of interest was lower in ARIC than in CHS after quality control (QC) (863 vs. 1,053), and the ARIC sample also included more Black participants (**Table 1A**).

Candidate Genes

To explore the possibility that genes from the major aging pathways (IGF1/AKT/FOXO3, TP53/P21/P16, and mTOR/S6K mediated) may influence human lifespan as result of their interplay rather than independently, we selected the set of candidate genes (**Table 1B**) that belong to these pathways *and* have also been featured in aging research, as genes or their products (Braeckman and Vanfleteren, 2007; Feng et al., 2007; Tsai et al., 2008; Ghosh et al., 2010; Kenyon, 2010; Johnson et al., 2013; Nojima et al., 2013; Ortega-Molina and Serrano, 2013; Tran et al., 2014; Cetrullo et al., 2015; Pavlatou et al., 2016; Uno and Nishida, 2016; Yuan et al., 2016; Donlon et al., 2017; Bartke and Quainoo, 2018; Morris et al., 2019; Singh et al., 2019; Blasiak et al., 2020; Zhang et al., 2020; Tabibzadeh, 2021). Majority of these genes are involved in cell/tissue responses to stress and damage that can contribute to the body's ability to recover (resilience) and through this to its ability to survive to the oldest old age (Ukraintseva et al., 2021). For the epistasis analysis, we selected 863 SNPs located in these genes based on the list of the SNPs genotyped on the IBC chip and available in both ARIC and CHS CARe data after QC (**Table 1A** and **Supplementary Table 2**).

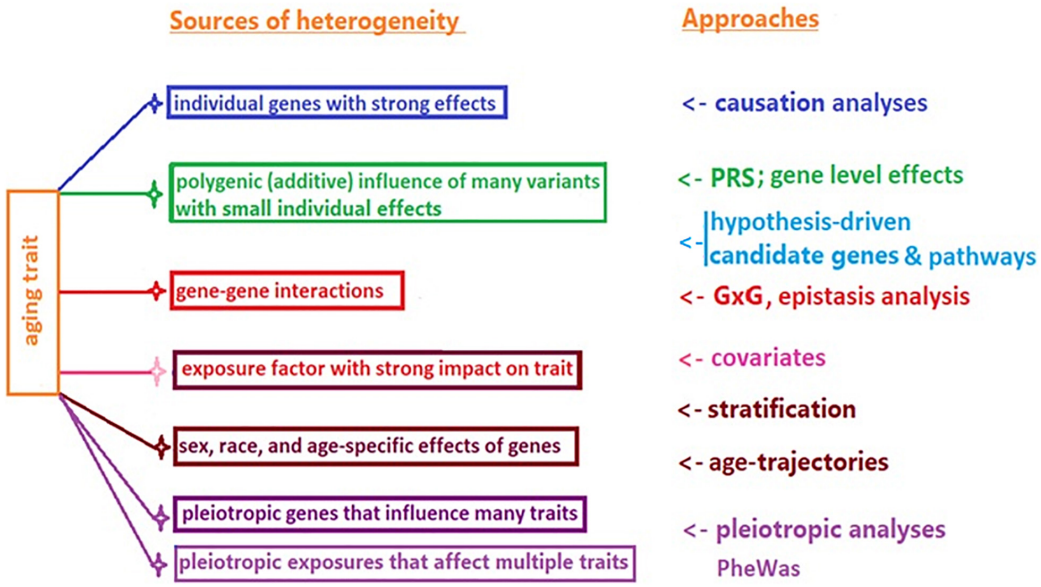

FIGURE 1 | Common sources of heterogeneity of aging and longevity related traits, and approaches to their study.

Phenotypes

As the main outcome phenotype, we used a binary survival trait: *survived to age 85+ (1) vs. died before age 85 (0)*. The choice of this phenotype was motivated by our working hypothesis that the age around 85 may be a turning point in the course of aging, characterized by trade-off-like changes in the effects of certain risk factors on survival (Ukraintseva et al., 2016). Our earlier studies suggested that risks of many major diseases start to decline or level-off (after prior increase) around that age (e.g., Ukraintseva and Yashin, 2003; Akushevich et al., 2012), which could be due to selection, under-diagnosis, or the aging itself changing the effects of respective risk factors antagonistically, so that they may negatively affect health and survival chances before the age 85 but become somewhat protective afterwards (Ukraintseva et al., 2016). So, identifying the genetic factors that can influence survival at ages 85+ vs. 85− was of particular interest to us.

We also included an additional phenotype of survival in the CHS data analysis, using the age cut-off based on survival to the 90th percentile (corresponding to 10% of the longest lived in the US population). Our discovery dataset (ARIC) is not suitable for investigating such outcome because it includes only a few people older than 90. However, the validation dataset (CHS) contains a considerably larger sample of individuals aged 90+ (600), allowing to include the "90th percentile" survival outcome in the analysis. We used population life tables from the US Centers for Disease Control and Prevention[1] to determine the ages corresponding to the survival to the 90th percentile, by sex and race: 95 years for Black and White females, 91 years for Black males, and 93 years for White males. These ages were used to construct respective phenotypes of survival (**Table 1C**) and include them in the validation analysis in the CHS, using genes that were discovered in ARIC (see section "Results").

Statistical Analysis

The QC procedures were based on Anderson et al. (2010) and Marees et al. (2018), and were performed before the epistasis analysis. We removed duplicates, people who failed in sex check, individuals with >5% missing SNPs, and SNPs with the genotyping rate lower than 95% and minor allele frequency (MAF) lower than 1%. In addition, SNPs that failed the Hardy–Weinberg test (P-value $< 10^{-10}$) were also excluded. Study sample and numbers of individuals and SNPs available for the analysis are shown in **Table 1A** and in **Supplementary Tables 1.1, 1.2**.

Using all genotyped SNPs available after QC in the selected candidate genes (**Supplementary Table 2**), we estimated associations of a pairwise SNP × SNP epistasis with survival 85+ in ARIC data (discovery set) and validated the findings in CHS data, using the binary phenotypes of survival to the age 85+ and to the 90th percentile as outcomes (**Table 1C**; see section "Phenotypes" for detail). For this analysis, we used INTERSNP software for statistical epistasis (Herold et al., 2009), and logistic regression model with covariates, including: birth cohort z-score, education level (0 – below high school, 1 – high school, 2 – above high school), and smoking status (1 – ever smoked, 0 – never smoked). We also included the first two principal components to control for possible population stratification. All analyses were stratified by sex (males and females) and race (Blacks and Whites). Also, to address a common concern in a high dimensional study that small sample sizes in some groups may lead to zero data points in contingency table cells and to increase in type I errors, we applied an additional selection criterion in INTERSNP, requiring the sum of the four genotype

[1]https://www.cdc.gov/nchs/data/nvsr/nvsr68/nvsr68_04-508.pdf

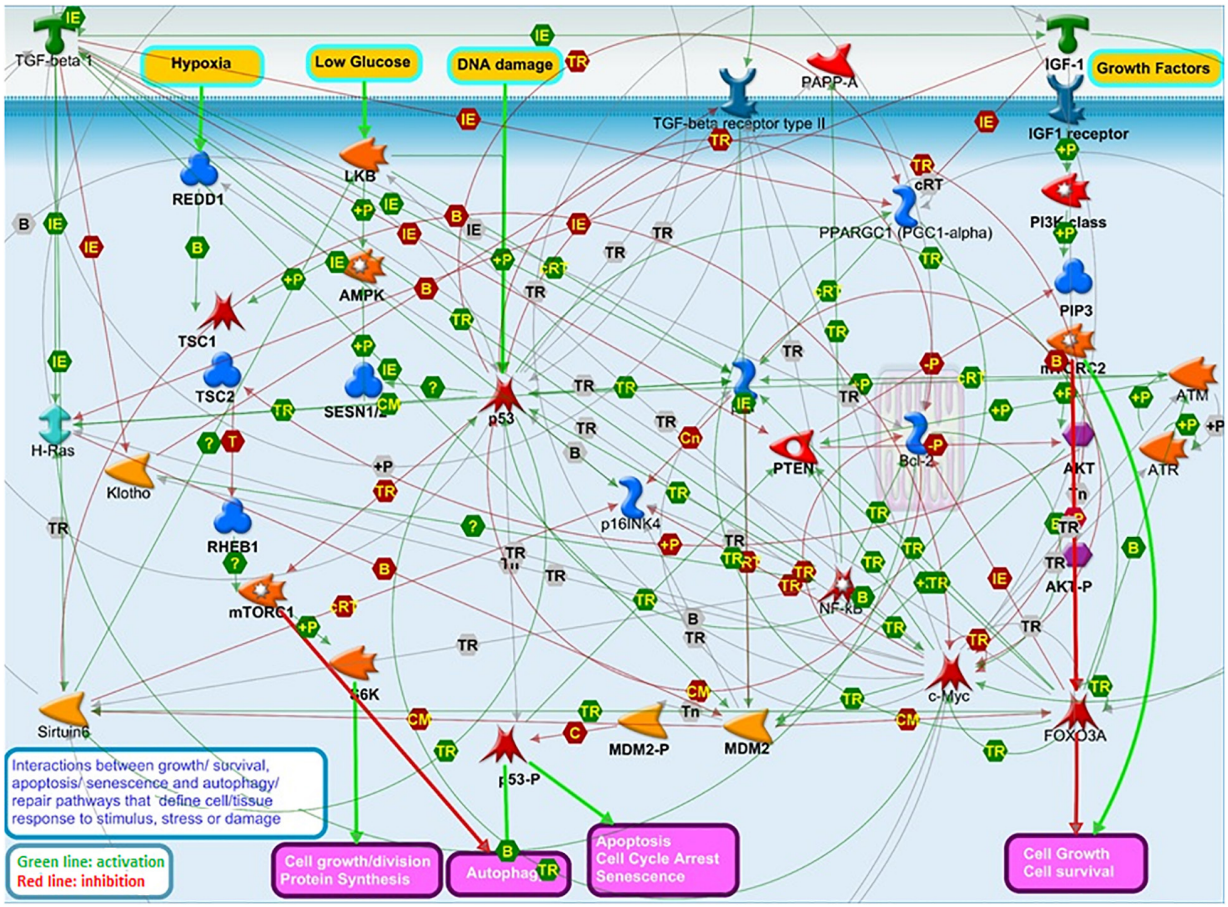

FIGURE 2 | The interplay among "aging" pathways that have been a major focus of aging research over the last decades: IGF1/AKT/FOXO3 growth signaling, TP53/P21/P16 apoptosis and senescence, and mTOR/S6K autophagy and survival pathways. This Figure illustrates the complexity of the interactions between these pathways regulating cell responses to stress and damage, which may in turn impact tissue resilience, and, ultimately, influence organismal survival and lifespan. This Figure was prepared using MetaCore Pathway Map Creator (PMC) tool (Dubovenko et al., 2017) from *Clarivate Analytics*. The PMC allows to connect gene products selected by the user in a picture showing the molecular interactions occurring between members of respective cellular processes and pathways. The latter are defined, annotated, and manually curated by *Clarivate Analytics* scientists based on the up-to-date literature and pathway libraries. Main protein classes shown on **Figure 2** are annotated in **Supplementary Table 3**.

combinations containing minor alleles (MA) (2MA–2MA, 2MA–1MA, 1MA–2MA, 1MA–1MA) to be at least 20, to ensure that there are sufficient numbers of individuals in the analysis in each race and sex group. We also estimated and compared the effects of individual SNPs in the selected candidate genes with the effects of the SNP × SNP interactions on the same survival outcomes.

RESULTS

Survival to Age 85+

We first estimated associations of the interactions between SNPs in the candidate genes shown in **Table 1B** with *survival to age 85+* in the discovery set (ARIC), and selected results corrected for multiple comparisons (FDR < 0.05) to reduce chances of false positive findings. Of those, we selected the SNP pairs that also influenced *survival to age 85+* in the validation set (CHS) with at least conventional significance (*P*-value < 0.05). These results are shown in **Table 2**. One can see that the interaction between rs939626 (*IGF1R*) and rs3773663 (*TGFBR2*) is significantly (*P*-value = 2.1E−06; FDR < 0.05) associated with *survival to age 85+* in White women in ARIC, which is confirmed in CHS with conventional significance (*P*-value = 0.03) and the same direction of the effect. The associations of individual SNPs in these same genes with *survival to age 85+* didn't reach a significance at the 0.05 level in this data (**Table 2**, *P*-v1 and *P*-v2 columns).

Our analysis suggests that the double-carriers of major alleles of the two SNPs (i.e., individuals carrying both rs939626 AA *and* rs3773663 GG variants) have a higher probability to live to age 85+, compared to the carriers of other allelic combinations of these SNPs. Additional exploration using the Genotype-Tissue Expression (GTEx) eQTL Calculator[2] revealed that, for

[2]https://gtexportal.org/home/testyourown

TABLE 1 | Study sample, candidate genes, and outcome phenotypes.

Dataset	Males	Females	Black (%)	White (%)	SNPs (within candidate genes ±1 kb)
A. Study sample by sex, race, and number of SNPs, after QC					
CHS	2,201	2,955	15.2	84.1	1,053
ARIC	5,962	7,350	26	74	863 (overlapped with SNPs in ARIC)
B. Selected candidate genes from the aging-related pathways that have been featured in experimental research (human ortholog names, by the HUGO Gene Nomenclature Committee)					
IGF1/AKT/FOXO3 growth signaling:					
AKT1, ATM, FOXO1, FOXO3, GHR, HIF1A, IGF1, IGF1R, PIK3CA, PIK3CB;					
TP53/P21/P16 apoptosis/senescence:					
BAX, BCL2, CDK4, CDK6, CDKN1A (P21), CDKN2A (P16), CDKN2B (P15), FAS, TP53;					
mTOR/S6K mediated autophagy/survival:					
AMPK subunits (PRKAA1, PRKAA2, PRKAB2, PRKAG1, PRKAG2), SIRT1, RPS6KB1 (S6K), TSC2;					
Genes broadly involved in the cross-talk between the aging pathways:					
KL, MYC, NFKB1, NFKB2, PPARGC1A, PTEN, TGFB1, TGFBR2.					
C. Outcome phenotypes					
Survival 85+ (in ARIC and CHS):					
Survived to age 85+ (1) vs. died before age 85 (0)					
Survival to the 90th percentile (in CHS only, see section "Phenotypes" for detail):					
Survived to *age corresponding to 10% of the longest lived (1) vs. died before that age (0)					

**The ages corresponding to 10% of the longest lived, based on sex- and race-specific life tables for the US population: Black and White women – 95 years; Black men – 91 years; White men – 93 years.*

TABLE 2 | Results of associations of interactions between SNPs in selected candidate genes from aging pathways (**Table 1B**) with survival to age 85+ in ARIC and CHS CARe.

Data	Ch1	SNP1	MAF1	*P*-v1	Gene1	Ch2	SNP2	MAF2	*P*-v2	Gene2	Race	S	OR	*P*-value	*N*
ARIC	3	rs3773663	A(0.42)	0.14	TGFBR2	15	rs939626	G(0.47)	0.07	IGF1R	W	F	0.26	*2.1E−06	1,430
CHS	3	rs3773663	A(0.41)	0.09	TGFBR2	15	rs939626	G(0.45)	0.12	IGF1R	W	F	0.75	0.03	1,519
ARIC	15	rs11247378	T(0.13)	0.96	IGF1R	18	rs956572	A(0.25)	0.73	BCL2	B	F	0.10	*3.9E−07	810
ARIC	15	rs11247380	A(0.28)	0.37	IGF1R	18	rs956572	A(0.25)	0.73	BCL2	B	F	0.35	5.5E−04	811
CHS	15	rs11247380	A(0.27)	0.32	IGF1R	18	rs956572	A(0.25)	0.20	BCL2	B	F	0.33	0.007	276

Ch1 and Ch2, chromosome numbers for SNP1 and SNP2, respectively; SNP1 and SNP2, dbSNP Reference SNP numbers. All SNPs shown in this table are located in introns of corresponding genes. Note that the SNPs rs11247378 and rs11247380 are in LD in African Americans (D′ = 0.69; R^2 = 0.13), according to LDlink (https://ldlink.nci.nih.gov/?tab=ldpair); MAF1 and MAF2, minor allele (and its frequency) for SNP1 and SNP2, respectively; P-v1 and P-v2, P-values for associations of individual SNPs with survival 85+, for SNP1 and SNP2, respectively; Gene1 and Gene2, gene names by the HUGO Gene Nomenclature Committee; Race: B, Black; W, White; S, sex; F, female; OR, odds ratio; P-value, unadjusted P-value for the association of SNP1 × SNP2 interaction with survival 85+. Asterisk before the P-value indicates results with FDR < 0.05 in discovery set (ARIC). N, number of people included in the analysis.

each of these SNPs, being homozygous for major allele may result in a slightly (though not significantly) lower average expression of respective gene (IGF1R or TGFBR2). Although this tendency does not allow us to make a reliable interpretation, it, potentially, may point to benefits of downregulation of respective pathways for achieving the longer life. To further explore this, more data about the effects of these SNPs on gene expression is needed, which could become available in the future. We do not describe here the effects of the epistatic *interaction* between rs939626 and rs3773663 on gene/protein expression, since this information is not yet available in relevant bases. It is important to note, however, that the detection of statistical interaction between the two loci does not necessarily mean the existence of direct biochemical interaction between respective genetic products (see also the section "Discussion").

A significant (*P*-value = 3.9E−07; FDR < 0.05) association with *survival to age 85+* was found for the interaction between rs956572 (*BCL2*) and rs11247378 (*IGF1R*) in Black women in ARIC. This was supported by the result of interaction analysis between rs956572 and rs11247380 in Black women in both ARIC and CHS, with conventional *P*-values (**Table 2**). However, rs11247380 and rs11247378 (*IGF1R*) are in only moderate LD (D′ = 0.69; R^2 = 0.13), therefore, the effect of the interaction between rs956572 and rs11247378 or rs11247380 on *survival 85+* should be viewed as suggestive and has to be confirmed in further research.

The results in **Table 2** also point to a potentially major role of the IGF1R gene in the non-additive multigenic regulation of lifespan in both Blacks and Whites. However, significant (FDR < 0.05) associations with *survival 85+* were observed for the interactions that included different SNPs in IGF1R gene in Blacks and Whites. Such differences may be caused by, e.g., differences between Blacks and Whites in MAF and LD structures, or in histories of exposures to environmental and living conditions, among other factors.

Survival to the 90th Percentile

Next, we selected all 355 SNPs available in data for the top interacting genes that influenced *survival 85+* in the first step (i.e., TGFBR2, IGF1R, and BCL2, from **Table 2**), and estimated associations of the interactions between SNPs in these genes with *survival to the 90th percentile* (see **Table 1C** and section "Phenotypes") in the CHS validation set. The strongest effect on *survival to the 90th percentile* in the CHS was observed for the interaction between rs4955189 (TGFBR2) and rs8034284 (IGF1R) in White males (*P*-value = 4.9E−06; FDR = 0.029). According to LDlink,[3] rs4955189 is in moderate LD ($D' = 0.51$; $R^2 = 0.22$; Whites-CEU) with rs3773663 (TGFBR2) from **Table 2**, which means that the top G × G effects on *survival 85+* and *survival to the 90th percentile* include correlated SNPs.

Notably, the *P*-value for the interaction between rs4955189 (TGFBR2) and rs8034284 (IGF1R) in the total sample (males and females combined) was less significant (7.5E−05) than that for White males (4.9E−06) despite the fact that the total sample was larger than that of the White males (2,235 vs. 1,141). This indicates that using a more restrictive model with sex as a covariate may not always benefit the analysis, if the associations differ by sex. Some experimental studies demonstrated sexually dimorphic effects of IGF1R (e.g., Corrochano et al., 2014; Xu et al., 2014), which may provide an additional support to the sex-stratified analysis of this gene.

We also found that the interaction between rs4955189 (TGFBR2) and rs7167580 (IGF1R) influenced *survival to the 90th percentile* in White males, as well as in the total CHS sample, with conventional significance (*P*-values = 0.009 and 0.003, respectively). This result may additionally support the interaction between rs3773663 (TGFBR2) and rs939626 (IGF1R) found in the discovery stage (**Table 2**), because rs7167580 is in moderate LD ($D' = 1$; $R^2 = 0.35$; Whites-CEU) with rs939626 (IGF1R).

DISCUSSION

Despite decades of aging research, the role of genetic interactions (G × G) in heterogeneity of human lifespan, and in animal to human translation, remains not fully understood. Several studies supported the involvement of G × G in human longevity (e.g., Zeng et al., 2010; Deelen et al., 2013; Fuku et al., 2017; Dato et al., 2018). Some focused specifically on the interactions between FOXO3 and other genes (Zeng et al., 2010; Fuku et al., 2017) and on relevant biology, such as DNA damage response (Tsai et al., 2008). Other researchers (Deelen et al., 2013) applied the pathway-based geneset approach to evaluating the joint effect of SNPs in genes from aging pathways on longevity in humans, and yielded results suggesting a major impact of the genetic variation in the IGF1 signaling pathway.

Dato et al. (2018) investigated the synergic SNP × SNP interactions in nonagenarians compared with controls aged 46–55 years, using tagging SNPs in 140 genes belonging to three candidate pathways (insulin/insulin-like growth signaling, DNA repair, and pro/antioxidant ones). They found the most significant interactions (FDR < 0.0001) between rs12437963 (IGF1R) and rs6067484 (PTPN1), as well as between rs2078486 (TP53) and two other genes (Dato et al., 2018). Results of our study seem to be in line with their findings, despite the difference in approach to the epistasis analysis between the two studies [INTERSNP in our case, and the multidimensional reduction (MDR) approach in Dato et al. (2018)]. In our study, we found a significant (FDR < 0.05) interaction between rs939626 and rs3773663 (**Table 2**), and it turns out that rs939626 is in LD (albeit a modest one: $D' = 0.79$; $R^2 = 0.10$) with rs12437963, the SNP in IGF1R gene that was involved in the top significant SNP × SNP interaction in the Dato et al. (2018). The fact that the top SNPs found in the two studies are correlated may additionally support the results of both these studies.

A central role of the communication between the P53–IGF1–AKT–mTOR pathways in regulating the cell growth, proliferation, and death, was suggested by Levine et al. (2006) about 15 years ago. Specifically, the authors pointed to a major significance of the interplay between cell survival and apoptosis in human lifespan. Results of our study support this view. They suggest that the interactions between IGF1R and TGFBR2, as well as BCL2, may influence human lifespan. IGF1R is growth factor receptor, which can promote cell survival acting as anti-apoptotic agent or inductor of proliferation. TGFBR2 is also a growth factor receptor, which may show growth inhibitory effect, depending on context. BCL2 gene codes for a mitochondrial anti-apoptotic protein, which can promote cell survival, including that of cancer cells, by inhibiting the apoptosis induced by oxidative stress. Current evidence suggests that these genes, and respective proteins, can be concurrently up/down-regulated to influence cell survival, apoptosis, and proliferation (Mayeenuddin et al., 2010; Basu et al., 2012; Valenciano et al., 2012; Alsina-Sanchis et al., 2016), which might contribute to their propensity to the epistatic interactions.

It is important to stress, however, that detection of the association of genetic interactions with complex traits using statistical methods (a.k.a. statistical epistasis) does not necessarily mean that products of respective genes interact directly biologically. Statistical analysis can capture genetic interactions mediated by molecular products of many other genes. The data from experimental studies may bring additional light on possible players in such mediation. Evaluating their role in human survival could be a next step in the clarification of multigenic mechanism of lifespan regulation in humans.

It is also worth mentioning that the SNP rs956572 (BCL2) that interacted with SNPs in IGF1R gene in our analysis (**Table 2**), on itself has been intensively studied for more than a decade, and appears to be broadly involved in aging and AD-related traits (e.g., Salvadore et al., 2009; Uemura et al., 2011; Liu et al., 2013; Chang et al., 2018), which might contribute to its propensity to genetic interactions that influence human lifespan. One should note that individual associations of this SNP with survival did not reach statistical significance in our analysis (*P*-v2 in **Table 2**).

[3]https://ldlink.nci.nih.gov/?tab=ldpair

In summary, the results of this study suggest that interactions between genes from the aging-related pathways may influence survival in humans more significantly than individual polymorphisms in the same genes. The fact that IGF1R, TGFBR2, and BCL2 genes appear among the top results of the interaction analysis may point to a major role of the interplay between cell survival and apoptosis in determining human lifespan. The G × G interactions may contribute to the lack of animal to human translation in genetics of aging because the landscape of such interactions, and their role in the structure of heterogeneity of lifespan may differ across species and strains.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data were made available for the secondary analyses relevant to this article *via* controlled access provided by dbGaP, NIH supported resource. We do not own this data. Requests to access these datasets should be directed to: https://www.ncbi.nlm.nih.gov/gap/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Duke University IRB. Note that in this article, we analyzed only already existing genetic and phenotypic information previously collected in CHS and ARIC. These data were de-identified by providers before release based on their expert opinion, which is in line with Expert Determination method of the data de-identification recommended by the Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule (https://www.hhs.gov/hipaa/for-professionals/privacy/special-topics/de-identification/index.html#standard). The data were made available for the secondary analyses relevant to this article through dbGaP, NIH supported online data base. dbGaP, however, requires local IRB approval in order to grant access to the data. Duke University IRB approval was, therefore, obtained before the start of the analyses. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SU conceived and designed the study, wrote the manuscript, and provided interpretation of the results, with input from other co-authors. MD prepared the data for analysis, with input from DW, OB, and GG. MD analyzed the data, with input from DW, OB, and KA. MD and KA wrote sections of the manuscript. DW, KA, AY, IA, APY, and AK contributed to selection and discussion of methods used in the manuscript. KA, MD, and AY contributed to interpretation of the results. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.692020/full#supplementary-material

Supplementary Table 1.1 | Total numbers of individuals and SNPs available in data, before and after QC.

Supplementary Table 1.2 | Numbers of individuals used in the analyses, by age, sex, race, covariates, and comorbidity status.

Supplementary Table 2 | SNPs in the candidate genes available for this study after QC.

Supplementary Table 3 | Main protein/metabolite classes shown in **Figure 2** in the text.

REFERENCES

Akushevich, I., Kravchenko, J., Ukraintseva, S., Arbeev, K., and Yashin, A. I. (2012). Age patterns of incidence of geriatric disease in the U.S. elderly population: medicare-based analysis. *J. Am. Geriatr. Soc.* 60, 323–327. doi: 10.1111/j.1532-5415.2011.03786.x

Alsina-Sanchis, E., Figueras, A., Lahiguera, Á, Vidal, A., Casanovas, O., Graupera, M., et al. (2016). The TGFβ pathway stimulates ovarian cancer cell proliferation

by increasing IGF1R levels. *Int. J. Cancer* 139, 1894–1903. doi: 10.1002/ijc.30233

Anderson, C. A., Pettersson, F. H., Clarke, G. M., Cardon, L. R., Morris, A. P., and Zondervan, K. T. (2010). Data quality control in genetic case-control association studies. *Nat. Protoc.* 5, 1564–1573. doi: 10.1038/nprot.2010.116

Arking, D. E., Atzmon, G., Arking, A., Barzilai, N., and Dietz, H. C. (2005). Association between a functional variant of the KLOTHO gene and high-density lipoprotein cholesterol, blood pressure, stroke, and longevity. *Circ. Res.* 96, 412–418. doi: 10.1161/01.RES.0000157171.04054.30

Bartke, A., and Quainoo, N. (2018). Impact of growth hormone-related mutations on mammalian aging. *Front. Genet.* 9:586. doi: 10.3389/fgene.2018.00586

Basu, S., Rajakaruna, S., and Menko, A. S. (2012). Insulin-like growth factor receptor-1 and nuclear factor κB are crucial survival signals that regulate caspase-3-mediated lens epithelial cell differentiation initiation. *J. Biol. Chem.* 287, 8384–8397. doi: 10.1074/jbc.M112.341586

Bitto, A., Wang, A. M., Bennett, C. F., and Kaeberlein, M. (2015). Biochemical genetic pathways that modulate aging in multiple species. *Cold Spring Harb. Perspect. Med.* 5:a025114. doi: 10.1101/cshperspect.a025114

Blasiak, J., Pawlowska, E., Sobczuk, A., Szczepanska, J., and Kaarniranta, K. (2020). The aging stress response and its implication for AMD pathogenesis. *Int. J. Mol. Sci.* 21:8840. doi: 10.3390/ijms21228840

Braeckman, B. P., and Vanfleteren, J. R. (2007). Genetic control of longevity in C. elegans. *Exp. Gerontol.* 42, 90–98. doi: 10.1016/j.exger.2006.04.010

Cetrullo, S., D'Adamo, S., Tantini, B., Borzi, R. M., and Flamigni, F. (2015). mTOR, AMPK, and Sirt1: key players in metabolic stress management. *Crit. Rev. Eukaryot. Gene Expr.* 25, 59–75. doi: 10.1615/critreveukaryotgeneexpr.2015012975

Chang, C. C., Chang, Y. T., Huang, C. W., Tsai, S. J., Hsu, S. W., Huang, S. H., et al. (2018). Associations of Bcl-2 rs956572 genotype groups in the structural covariance network in early-stage Alzheimer's disease. *Alzheimers Res. Ther.* 10:17. doi: 10.1186/s13195-018-0344-4

Corrochano, S., Renna, M., Osborne, G., Carter, S., Stewart, M., May, J., et al. (2014). Reducing Igf-1r levels leads to paradoxical and sexually dimorphic effects in HD mice. *PLoS One* 9:e105595. doi: 10.1371/journal.pone.0105595

Dato, S., Soerensen, M., De Rango, F., Rose, G., Christensen, K., Christiansen, L., et al. (2018). The genetic component of human longevity: new insights from the analysis of pathway-based SNP-SNP interactions. *Aging Cell* 17:e12755. doi: 10.1111/acel.12755

de Magalhães, J. P. (2014). Why genes extending lifespan in model organisms have not been consistently associated with human longevity and what it means to translation research. *Cell Cycle* 13, 2671–2673. doi: 10.4161/15384101.2014.950151

Deelen, J., Uh, H. W., Monajemi, R., van Heemst, D., Thijssen, P. E., Böhringer, S., et al. (2013). Gene set analysis of GWAS data for human longevity highlights the relevance of the insulin/IGF-1 signaling and telomere maintenance pathways. *Age (Dordr.)* 35, 235–249. doi: 10.1007/s11357-011-9340-3

Donlon, T. A., Willcox, B. J., and Morris, B. J. (2017). FOXO3 cell resilience gene neighborhood. *Aging (Albany N. Y.)* 9, 2467–2468. doi: 10.18632/aging.101349

Dubovenko, A., Nikolsky, Y., Rakhmatulin, E., and Nikolskaya, T. (2017). Functional analysis of OMICs data and small molecule compounds in an integrated "Knowledge-Based" platform. *Methods Mol. Biol.* 1613, 101–124. doi: 10.1007/978-1-4939-7027-8_6

Feng, Z., Hu, W., de Stanchina, E., Teresky, A. K., Jin, S., Lowe, S., et al. (2007). The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. *Cancer Res.* 67, 3043–3053. doi: 10.1158/0008-5472

Fuku, N., Díaz-Peña, R., Arai, Y., Abe, Y., Zempo, H., Naito, H., et al. (2017). Epistasis, physical capacity-related genes and exceptional longevity: FNDC5 gene interactions with candidate genes FOXOA3 and APOE. *BMC Genomics* 18(Suppl. 8):803. doi: 10.1186/s12864-017-4194-4

Ghosh, H. S., McBurney, M., and Robbins, P. D. (2010). SIRT1 negatively regulates the mammalian target of rapamycin. *PLoS One* 5:e9199. doi: 10.1371/journal.pone.0009199

Herold, C., Steffens, M., Brockschmidt, F. F., Baur, M. P., and Becker, T. (2009). INTERSNP: genome-wide interaction analysis guided by a priori information. *Bioinformatics* 25, 3275–3281. doi: 10.1093/bioinformatics/btp596

Johnson, S. C., Rabinovitch, P. S., and Kaeberlein, M. (2013). mTOR is a key modulator of ageing and age-related disease. *Nature* 493, 338–345. doi: 10.1038/nature11861

Johnson, T. E., Henderson, S., Murakami, S., de Castro, E., de Castro, S. H., Cypser, J., et al. (2002). Longevity genes in the nematode *Caenorhabditis elegans* also mediate increased resistance to stress and prevent disease. *J. Inherit. Metab. Dis.* 25, 197–206. doi: 10.1023/a:1015677828407

Keating, B. J., Tischfield, S., Murray, S. S., Bhangale, T., Price, T. S., Glessner, J. T., et al. (2008). Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. *PLoS One* 3:e3583. doi: 10.1371/journal.pone.0003583

Kenyon, C. J. (2010). The genetics of ageing. *Nature* 464, 504–512. doi: 10.1038/nature08980

Levine, A. J., Feng, Z., Mak, T. W., You, H., and Jin, S. (2006). Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. *Genes Dev.* 20, 267–275. doi: 10.1101/gad.1363206

Liu, M. E., Huang, C. C., Hwang, J. P., Yang, A. C., Tu, P. C., Yeh, H. L., et al. (2013). Effect of Bcl-2 rs956572 SNP on regional gray matter volumes and cognitive function in elderly males without dementia. *Age (Dordr.)* 35, 343–352. doi: 10.1007/s11357-011-9367-5

Marees, A. T., de Kluiver, H., Stringer, S., Vorspan, F., Curis, E., Marie-Claire, C., et al. (2018). A tutorial on conducting genome-wide association studies: quality control and statistical analysis. *Int. J. Methods Psychiatr. Res.* 27:e1608. doi: 10.1002/mpr.1608

Mayeenuddin, L. H., Yu, Y., Kang, Z., Helman, L. J., and Cao, L. (2010). Insulin-like growth factor 1 receptor antibody induces rhabdomyosarcoma cell death via a process involving AKT and Bcl-x(L). *Oncogene* 29, 6367–6377. doi: 10.1038/onc.2010.364

Morris, B. J., Willcox, B. J., and Donlon, T. A. (2019). Genetic and epigenetic regulation of human aging and longevity. *Biochim. Biophys. Acta Mol. Basis Dis.* 1865, 1718–1744. doi: 10.1016/j.bbadis.2018.08.039

Musunuru, K., Lettre, G., Young, T., Farlow, D. N., Pirruccello, J. P., Ejebe, K. G., et al. (2010). NHLBI candidate gene association resource. Candidate gene association resource (CARe): design, methods, and proof of concept. *Circ. Cardiovasc. Genet.* 3, 267–275. doi: 10.1161/CIRCGENETICS.109.882696

Nojima, A., Yamashita, M., Yoshida, Y., Shimizu, I., Ichimiya, H., Kamimura, N., et al. (2013). Haploinsufficiency of akt1 prolongs the lifespan of mice. *PLoS One* 8:e69178. doi: 10.1371/journal.pone.0069178

Nygaard, M., Lindahl-Jacobsen, R., Soerensen, M., Mengel-From, J., Andersen-Ranberg, K., Jeune, B., et al. (2014). Birth cohort differences in the prevalence of longevity-associated variants in APOE and FOXO3A in Danish long-lived individuals. *Exp. Gerontol.* 57, 41–46. doi: 10.1016/j.exger.2014.04.018

Nygaard, M., Soerensen, M., Flachsbart, F., Mengel-From, J., Tan, Q., Schreiber, S., et al. (2013). AKT1 fails to replicate as a longevity-associated gene in Danish and German nonagenarians and centenarians. *Eur. J. Hum. Genet.* 21, 574–577. doi: 10.1038/ejhg.2012.196

Ortega-Molina, A., and Serrano, M. (2013). PTEN in cancer, metabolism, and aging. *Trends Endocrinol. Metab.* 24, 184–189. doi: 10.1016/j.tem.2012.11.002

Pavlatou, M. G., Remaley, A. T., and Gold, P. W. (2016). Klotho: a humeral mediator in CSF and plasma that influences longevity and susceptibility to multiple complex disorders, including depression. *Transl. Psychiatry* 6:e876. doi: 10.1038/tp.2016.135

Revelas, M., Thalamuthu, A., Oldmeadow, C., Evans, T. J., Armstrong, N. J., Kwok, J. B., et al. (2018). Review and meta-analysis of genetic polymorphisms associated with exceptional human longevity. *Mech. Ageing Dev.* 175, 24–34. doi: 10.1016/j.mad.2018.06.002

Salvadore, G., Nugent, A. C., Chen, G., Akula, N., Yuan, P., Cannon, D. M., et al. (2009). Bcl-2 polymorphism influences gray matter volume in the ventral striatum in healthy humans. *Biol. Psychiatry* 66, 804–807. doi: 10.1016/j.biopsych.2009.05.025

Singh, P. P., Demmitt, B. A., Nath, R. D., and Brunet, A. (2019). The genetics of aging: a vertebrate perspective. *Cell* 177, 200–220. doi: 10.1016/j.cell.2019.02.038

Soerensen, M., Nygaard, M., Debrabant, B., Mengel-From, J., Dato, S., Thinggaard, M., et al. (2016). No association between variation in longevity candidate genes and aging-related phenotypes in oldest-old danes. *Exp. Gerontol.* 78, 57–61. doi: 10.1016/j.exger.2016.03.001

Tabibzadeh, S. (2021). Signaling pathways and effectors of aging. *Front. Biosci. (Landmark Ed.)* 26:50–96. doi: 10.2741/4889

Tian, X., Firsanov, D., Zhang, Z., Cheng, Y., Luo, L., Tombline, G., et al. (2019). SIRT6 is responsible for more efficient DNA double-strand break repair in long-lived species. *Cell* 177, 622–638.e22. doi: 10.1016/j.cell.2019.03.043

Tran, D., Bergholz, J., Zhang, H., He, H., Wang, Y., Zhang, Y., et al. (2014). Insulin-like growth factor-1 regulates the SIRT1-p53 pathway in cellular senescence. *Aging Cell* 13, 669–678. doi: 10.1111/acel.12219

Tsai, W. B., Chung, Y. M., Takahashi, Y., Xu, Z., and Hu, M. C. (2008). Functional interaction between FOXO3a and ATM regulates DNA damage response. Nat Cell Biol. 10(4):460-7. *Erratum Nat. Cell Biol.* 11:1387.

Uemura, T., Green, M., Corson, T. W., Perova, T., Li, P. P., and Warsh, J. J. (2011). Bcl-2 SNP rs956572 associates with disrupted intracellular calcium homeostasis in bipolar I disorder. *Bipolar Disord.* 13, 41–51. doi: 10.1111/j.1399-5618.2011.00897.x

Ukraintseva, S., Arbeev, K., Duan, M., Akushevich, I., Kulminski, A., Stallard, E., et al. (2021). Decline in biological resilience as key manifestation of aging: potential mechanisms and role in health and longevity. *Mech. Ageing Dev.* 194:111418. doi: 10.1016/j.mad.2020.111418

Ukraintseva, S., Yashin, A., Arbeev, K., Kulminski, A., Akushevich, I., Wu, D., et al. (2016). Puzzling role of genetic risk factors in human longevity: "risk alleles" as pro-longevity variants. *Biogerontology* 17, 109–127. doi: 10.1007/s10522-015-9600-1

Ukraintseva, S. V., and Yashin, A. I. (2003). Individual aging and cancer risk: how are they related? *Demogr. Res.* 9, 163–196. doi: 10.4054/DEMRES.2003.9.8

Uno, M., and Nishida, E. (2016). Lifespan-regulating genes in *C. elegans*. *NPJ Aging Mech. Dis.* 2:16010. doi: 10.1038/npjamd.2016.10

Valenciano, A., Henríquez-Hernández, L. A., Moreno, M., Lloret, M., and Lara, P. C. (2012). Role of IGF-1 receptor in radiation response. *Transl. Oncol.* 5, 1–9. doi: 10.1593/tlo.11265

Werner, H., Sarfstein, R., LeRoith, D., and Bruchim, I. (2016). Insulin-like growth factor 1 signaling axis meets p53 genome protection pathways. *Front. Oncol.* 6:159. doi: 10.3389/fonc.2016.00159

Willcox, B. J., Donlon, T. A., He, Q., Chen, R., Grove, J. S., Yano, K., et al. (2008). FOXO3A genotype is strongly associated with human longevity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 13987–13992. doi: 10.1073/pnas.0801030105

Xu, J., Gontier, G., Chaker, Z., Lacube, P., Dupont, J., and Holzenberger, M. (2014). Longevity effect of IGF-1R(+/-) mutation depends on genetic background-specific receptor activation. *Aging Cell* 13, 19–28. doi: 10.1111/acel.12145

Yuan, Y., Cruzat, V. F., Newsholme, P., Cheng, J., Chen, Y., and Lu, Y. (2016). Regulation of SIRT1 in aging: roles in mitochondrial function and biogenesis. *Mech. Ageing Dev.* 155, 10–21. doi: 10.1016/j.mad.2016.02.003

Zeng, Y., Cheng, L., Chen, H., Cao, H., Hauser, E. R., Liu, Y., et al. (2010). Effects of FOXO genotypes on longevity: a biodemographic analysis. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 1285–1299. doi: 10.1093/gerona/glq156

Zhang, Z. D., Milman, S., Lin, J. R., Wierbowski, S., Yu, H., Barzilai, N., et al. (2020). Genetics of extreme human longevity to guide drug discovery for healthy ageing. *Nat. Metab.* 2, 663–672. doi: 10.1038/s42255-020-0247-0

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Reproductive Suicide: Similar Mechanisms of Aging in *C. elegans* and Pacific Salmon

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In some species of salmon, reproductive maturity triggers the development of massive pathology resulting from reproductive effort, leading to rapid post-reproductive death. Such reproductive death, which occurs in many semelparous organisms (with a single bout of reproduction), can be prevented by blocking reproductive maturation, and this can increase lifespan dramatically. Reproductive death is often viewed as distinct from senescence in iteroparous organisms (with multiple bouts of reproduction) such as humans. Here we review the evidence that reproductive death occurs in *C. elegans* and discuss what this means for its use as a model organism to study aging. Inhibiting insulin/IGF-1 signaling and germline removal suppresses reproductive death and greatly extends lifespan in *C. elegans*, but can also extend lifespan to a small extent in iteroparous organisms. We argue that mechanisms of senescence operative in reproductive death exist in a less catastrophic form in iteroparous organisms, particularly those that involve costly resource reallocation, and exhibit endocrine-regulated plasticity. Thus, mechanisms of senescence in semelparous organisms (including plants) and iteroparous ones form an etiological continuum. Therefore understanding mechanisms of reproductive death in *C. elegans* can teach us about some mechanisms of senescence that are operative in iteroparous organisms.

Keywords: aging, *C. elegans*, programmatic aging, reproductive death, semelparity, senescent pathology

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INTRODUCTION: *C. ELEGANS* AS A MODEL FOR UNDERSTANDING HUMAN AGING

In its later stages, aging (senescence) manifests as an array of pathologies whose large number and complexity makes understanding its initial causes difficult. For this reason, simple animal models with the possibility of fully understanding senescence, such as *Caenorhabditis elegans*, are invaluable. Studies of this free-living nematode have yielded many insights into biological mechanisms of aging. These include acceleration of aging by insulin/IGF-1 signaling (IIS), germline signaling, mitochondrial function, loss of protein folding homeostasis, but not oxidative damage, and modulation of aging by steroid hormones and epigenetic changes (Greer et al., 2010; Kenyon, 2010; Van Raamsdonk and Hekimi, 2010; Antebi, 2013; Labbadia and Morimoto, 2014; Munkácsy and Rea, 2014).

The extent to which the primary causes of aging in *C. elegans* are the same or different to those in humans will only become clear once both are fully understood. However, it is already evident

that *C. elegans* and mammals share some but not all senescent etiologies. For example, in mammals stem cell exhaustion (Shaw et al., 2010; Conboy and Rando, 2012) and accumulation of senescent cells (van Deursen, 2014) (*sensu* Hayflick; note that there are two distinct meanings of the word *senescence*) contribute to senescence in the broad sense. By contrast, in adult *C. elegans* somatic cells are post-mitotic, and cellular senescence (*sensu* Hayflick) does not seem to occur. By contrast, interventions reducing insulin/IGF-1 or mTOR (mechanistic target of rapamycin) signaling or supporting protein folding homeostasis protect against aging in *C. elegans* and mammals (Zhang and Cuervo, 2008; Kenyon, 2010; Labbadia and Morimoto, 2014). Moreover, interventions causing loss of antioxidant defense or mitochondrial impairment which cause death in mammals can increase lifespan in *C. elegans* (Rea, 2005; Van Raamsdonk and Hekimi, 2009).

We recently proposed that two forms of programmatic aging are major determinants of *C. elegans* lifespan: adaptive death, which promotes fitness (i.e., provides a fitness benefit) in a manner similar to apoptosis (Lohr et al., 2019; Galimov and Gems, 2020, 2021), and reproductive death (Kern et al., 2020, 2021). In this essay, we explore further the possibility that *C. elegans* undergoes semelparous reproductive death by comparing it with other organisms known to undergo reproductive death. We then discuss the implications of reproductive death in *C. elegans*, and argue that some mechanisms of senescence are operative in both semelparous and iteroparous organisms.

ANTAGONISTIC PLEIOTROPY AND PROGRAMMATIC MECHANISMS AS CONSERVED CAUSES OF AGING

The predominant causes of aging are the ultimate, evolutionary processes that generate proximate biological mechanisms that cause senescent pathology (Flatt and Schmidt, 2009). One evolutionary cause of aging that is shared between *C. elegans* and humans is antagonistic pleiotropy (AP). Here gene variants that provide a fitness benefit in early life can be favored by natural selection, even where as a side effect they promote pathology in later life (Williams, 1957). How AP acts in terms of proximate mechanisms to cause aging remains unclear.

A traditional interpretation is that trade-offs promoting senescence involve physiological costs in terms of reduced allocation of resources to somatic maintenance (Kirkwood and Rose, 1991), but there are also other possibilities. For example, a different type of AP mechanism altogether, suggested in a hypothetical example by George Williams himself, is continued wild-type gene action in late life with pathogenic effects (Williams, 1957). A more recent elaboration of this idea, drawn in particular from the effects of mTOR, is that late-life action of regulators of growth and reproduction results in futile and pathogenic execution of complex biological programs (de Magalhães and Church, 2005; Blagosklonny, 2006). Because the term *program* implies the presence of a function, while such late-life action is futile, Blagosklonny introduced the term *quasi-program*; in other words, programmed in the mechanistic sense but not the adaptive sense (Galimov et al., 2019). More broadly, one may accurately describe proximate mechanisms of this type as *programmatic* (de Magalhães and Church, 2005; Maklakov and Chapman, 2019). As a primary mechanism of aging, this form of AP is distinct from damage accumulation and, in the case of IIS/mTOR for example, results not from a passive loss of function (or wearing out), but rather active gene function, or *hyperfunction* (Blagosklonny, 2008) (see Glossary for definition of key terms).

Our recent studies of several major *C. elegans* senescent pathologies imply that they originate predominantly from hyperfunction rather than molecular damage (Gems and de la Guardia, 2013; de la Guardia et al., 2016; Ezcurra et al., 2018; Wang et al., 2018b; Sornda et al., 2019). For example, physiological apoptosis (PA) in the hermaphrodite germline supports nascent oocyte growth, and apparently futile run-on of PA contributes to gonad atrophy and fragmentation (**Figure 1A**; de la Guardia et al., 2016). In another example, activation of embryogenetic functions in unfertilized oocytes in the uterus leads to extreme polyploidy, cellular hypertrophy and teratoma-like tumors (**Figure 1A**; McGee et al., 2012; Wang et al., 2018a,b). In both cases, quasi-programs promoted by wild-type gene action contribute to the development of major senescent pathology.

As a further example, during hermaphrodite aging large pools of material that appears oily when viewed using Nomarski microscopy accumulate in the body cavity (**Figure 1B**), and contain vitellogenin (yolk protein) and lipid (Garigan et al., 2002; Herndon et al., 2002; McGee et al., 2011; Yi et al., 2014; Chen et al., 2016; Ezcurra et al., 2018). Such pseudocoelomic lipoprotein pools (PLPs) represent a form of senescent steatosis (Palikaras et al., 2017; Ezcurra et al., 2018). Moreover, levels of vitellogenins increase dramatically, reaching up to sevenfold of that seen in young adults (Depina et al., 2011; Ezcurra et al., 2018; Sornda et al., 2019). Given that this accumulation occurs in post-reproductive hermaphrodites, it appears to be the result of futile, open faucet-type run-on of yolk synthesis, or a vitellogenic quasi-program (Herndon et al., 2002; Gems and de la Guardia, 2013; Ezcurra et al., 2018).

The *C. elegans* intestine is the largest somatic organ and serves multiple functions, including those played by the liver and adipose tissue in vertebrates (McGhee, 2007). It is a site of action of genes affecting lifespan (Lin et al., 2001; Libina et al., 2003; Venz et al., 2021). During aging in *C. elegans* hermaphrodites, the intestine undergoes major atrophy, losing most of its volume (**Figure 1B**; Garigan et al., 2002; McGee et al., 2011; Ezcurra et al., 2018). The intestine is the site of yolk synthesis for oocyte provision (Kimble and Sharrock, 1983), and consumption of intestinal biomass to support continued yolk export is a cause of intestinal atrophy (Ezcurra et al., 2018; Sornda et al., 2019). Loss of function of genes supporting autophagy inhibits both intestinal atrophy and PLP accumulation, suggesting that autophagy facilitates gut-to-yolk biomass conversion, and that futile run-on of vitellogenesis promotes intestinal atrophy (Ezcurra et al., 2018; Sornda et al., 2019).

These proximate, pathogenetic mechanisms are distinct from molecular damage accumulation, traditionally viewed as the

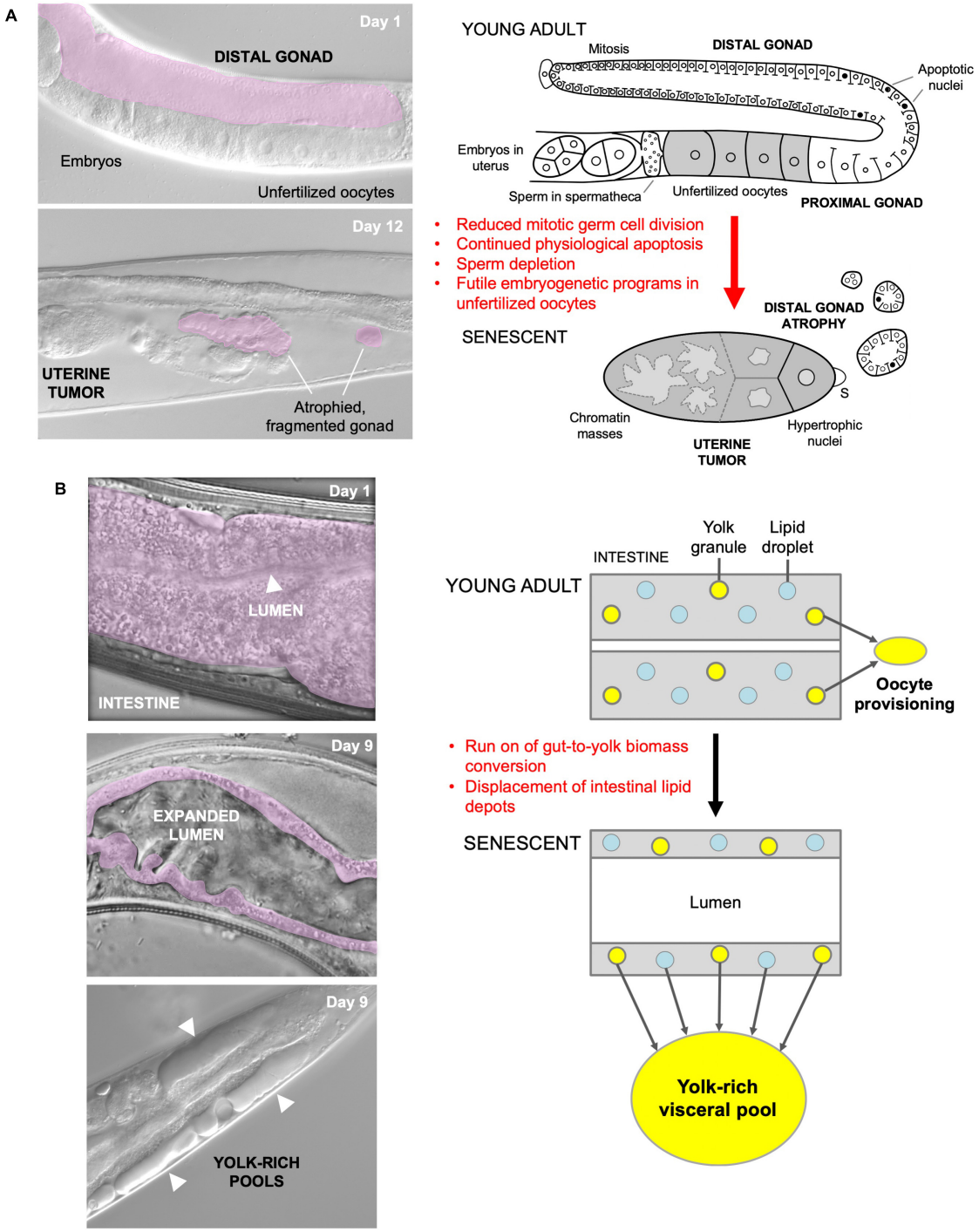

FIGURE 1 | Quasi-programs as a cause of *C. elegans* hermaphrodite senescence. **(A)** Distal gonad atrophy and teratoma-like uterine tumor formation. Left: appearance of pathologies under Nomarski microscopy; distal gonad marked in pink. Right: proposed pathophysiology involving quasi-programs. In the young adult oocytes are generated by proliferation of mitotic germline stem cells which then enter meiosis, and then in most cases undergo physiological apoptosis (PA) to generate cytoplasm to fill expanding oocytes (Gumienny et al., 1999; Jaramillo-Lambert et al., 2007; Wolke et al., 2007). Subsequently, declining stem cell division (conceivably adaptive) (Kocsisova et al., 2019) and run-on of PA promotes distal gonad atrophy and fragmentation (de la Guardia et al., 2016). Unfertilized oocytes fail to complete meiosis, enter the uterus and develop into teratoma-like tumors containing massively polyploid chromatin masses (Golden et al., 2007) which appears to result, as in mammalian ovarian teratomas, from embryonic quasi-programs (Wang et al., 2018a,b). **(B)** Left, intestinal atrophy and yolk-rich visceral pool accumulation. Right, hypothesis for etiology of both pathologies: a vitellogenic quasi-program, where remobilization of intestinal biomass into yolk continues in a futile fashion (Ezcurra et al., 2018; Benedetto and Gems, 2019).

predominant cause of aging; however, this does not rule out a contributory role for molecular damage in aging in general.

YOLK VENTING SUGGESTS THAT *C. ELEGANS* COULD BE SEMELPAROUS

The interpretation of late-life yolk production as quasi-programmed is based on the reasonable assumption that it is futile, but is it really? Could later yolk accumulation somehow promote fitness? Our recent study of the phenomenon of yolk venting supports the latter possibility (Kern et al., 2021). Beginning at the end of egg laying, hermaphrodites vent substantial amounts of liquid rich in vitellogenins and lipid through the vulva and into their local vicinity. Notably, consumption by larvae of this vented yolky substance, present either as free pools or within unfertilized oocytes, can promote larval growth and increase fertility (Kern et al., 2021). This suggests a later function for vented yolky fluid similar to that of milk (we suggest the term *yolk milk*). Feeding of milk-like fluid by mothers to offspring has been observed before in various other invertebrates, such as the Pacific beetle cockroach, *Diploptera punctata* (Marchal et al., 2013) and the tsetse fly (*Glossina* spp.) (Benoit et al., 2015). Such behavior exemplifies the wider phenomenon of trophallaxis, the social transfer of nutrient fluids between individuals, particularly in the context of parental care. Trophallaxis also encompasses fluid exchanges between social insects and mammalian nursing (LeBoeuf, 2017). In *C. elegans*, mutation of the *daf-2* insulin/IGF-1 receptor, which greatly extends lifespan, also suppresses venting of both yolk and unfertilized oocytes (Kenyon et al., 1993; Gems et al., 1998; Kern et al., 2021). Function as a vector for trophallactic fluid (**Figure 2A**) could provide an answer to the long-standing mystery of why adult hermaphrodites lay more than their own volume in unfertilized oocytes (Ward and Carrel, 1979).

If late-life yolk production provides a fitness benefit, then yolk steatosis and intestinal atrophy are not the result of a vitellogenic quasi-program. Instead, intestinal atrophy results from a life history trade-off involving physiological costs (**Figure 2B**). As previously defined, physiological costs can be either direct (e.g., the energy or nutrient requirements of reproduction) or indirect (Zera and Harshman, 2001; Speakman, 2008). Indirect costs include consequential costs, where harm occurs unavoidably as a consequence of the reproductive event, for example bone loss in mammals due to calcium remobilization during lactation (Speakman, 2008). In that example and in intestinal involution to support yolk milk production in *C. elegans* (Ezcurra et al., 2018), an active, programmed process of resource reallocation promotes fitness; however, intestinal atrophy itself is a pathological side effect and does not promote fitness. A further, formal possibility here is that gut-to-yolk resource reallocation includes resources diverted from cellular processes that protect against molecular damage.

The existence of yolk milk venting as a means of resource transfer from post-reproductive mothers to larval kin could also resolve another puzzle, relating to the overall pattern of senescent pathogenesis in *C. elegans*. In humans, age-related diseases appear late in life after an extended period of optimal health (Niccoli and Partridge, 2012). However, in *C. elegans* hermaphrodites, development of senescent pathologies begins within days of reproductive maturity (Ezcurra et al., 2018), and involves a level of destructive severity (including massive organ hypertrophy, atrophy, and disintegration) that is not typical of senescence in higher animals (Garigan et al., 2002; Herndon et al., 2002; McGee et al., 2011, 2012; de la Guardia et al., 2016). By contrast, in wild-type males, these pathologies are not seen (de la Guardia et al., 2016; Ezcurra et al., 2018). This pattern of rapid and severe pathological change affecting organs linked to reproduction [the nervous system is relatively well preserved in aging *C. elegans* (Herndon et al., 2002)] is reminiscent of semelparous organisms that undergo programmed reproductive death. Previously, the apparent absence of any fitness benefit to which these destructive changes could be linked as a cost argued against the idea that *C. elegans* is semelparous. However, with the discovery of "lactation" in *C. elegans*, it now appears more likely that this organism is semelparous. To explore this possibility, let us next consider semelparity in more detail.

SEMELPARITY AND REPRODUCTIVE DEATH

Comparer, c'est comprendre. Charles de Gaulle

Life histories may be broadly classified according to reproductive schedule, where semelparous species reproduce once and iteroparous species more than once (Cole, 1954; Finch, 1990b); but more precisely, semelparity and iteroparity represent two ends of a continuum of parity (Hughes, 2017). Reproduction in semelparous species can lead to rapid, post-reproductive death (reproductive death) by various mechanisms, usually coupled to very high levels of reproductive effort and investment which leads rapidly to severe pathology (Finch, 1990b). Though semelparous organisms do not necessarily undergo reproductive death, the term *semelparous* is sometimes used to denote semelparity with reproductive death; for convenience, we will often follow that usage here. In many semelparous organisms, rapid senescence is triggered by sexual maturation and under hormonal control. This form of reproductive death can be prevented, for example by surgical removal of organs that direct physiological changes that lead to death or by removing environmental cues, and this can result in increases in lifespan of a large magnitude (as detailed below).

The biology of animal semelparity has been explored in more detail in vertebrates than invertebrates. Semelparity in vertebrates is rare, but found in some fish (e.g., salmon, lampreys, eels), and a few reptiles (e.g., the aspic viper) (Bonnet, 2011) and marsupial mammals. Semelparity in Pacific salmon such as *Oncorhynchus nerka* has been studied in some detail. Semelparous salmon are usually anadromous, migrating from the sea to spawn in fresh water. When swimming up river they undergo marked anatomical changes where testes and ovaries grow dramatically, plasma vitellogenin levels rise (von der Decken, 1992), and males develop secondary sexual characteristics, including growth of the

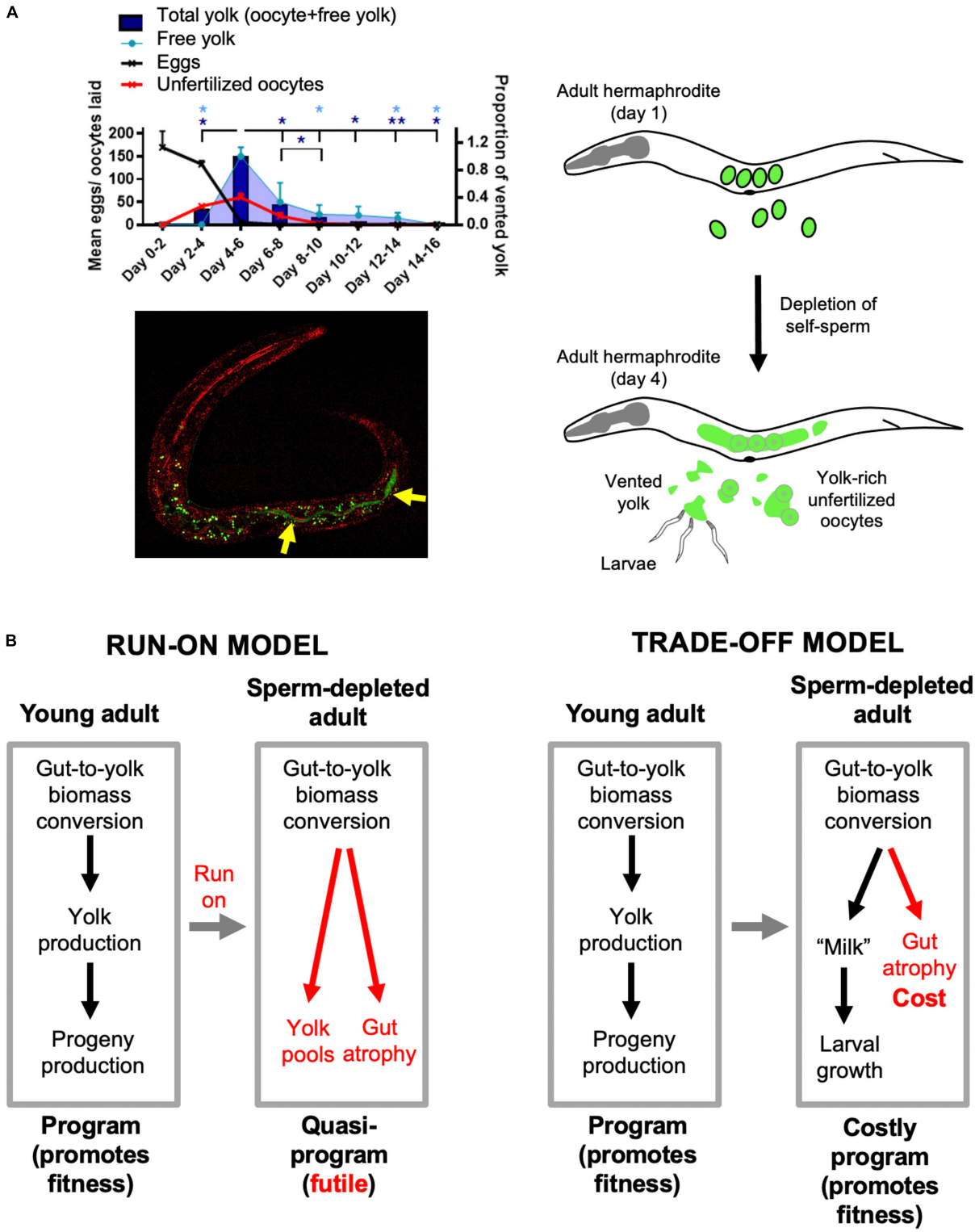

FIGURE 2 | Lactation by *C. elegans* hermaphrodites, and its implications. **(A)** Trophallaxis ("milk" provision) by *C. elegans*. Top left: schedule of production of eggs, unfertilized oocytes and vented yolk by wild-type *C. elegans* hermaphrodites (20°C); $^{*}p < 0.05$, $^{**}p < 0.01$, one-way ANOVA. Bottom left: L1 larva with ingested yolk in intestinal lumen (reproduced from Kern et al., 2021). Green: yolk marked with VIT-2:GFP (arrows); green dots are autofluorescent gut granules. Red, reflective confocal microscopy to highlight intestinal lumen (intestinal cell apices). Right: scheme showing transition from egg laying to yolk (milk) venting after hermaphrodite self-sperm depletion. **(B)** Implications: two interpretations of origins of intestinal atrophy. Left: After sperm depletion the program for yolk synthesis runs on to become a futile quasi-program (Ezcurra et al., 2018). Right: after sperm depletion the program for yolk production becomes a costly program supporting lactation (Kern et al., 2021).

beak to form the hook, and hump development (Quinn and Foote, 1994; **Figure 3A**). These changes are triggered by gonadal steroids, leading to increased corticosteroid production (Hane and Robertson, 1959; Mcquillan et al., 2003), which mobilizes energy to support reproduction but also impairs immune defense mechanisms, in a manner that resembles Cushing's disease in humans (hyper-adrenocorticism). As in *C. elegans* hermaphrodites, a range of severe, deteriorative pathologies rapidly develop, here affecting the liver, kidney, spleen, heart, thymus, and digestive tract (Robertson et al., 1961; Finch, 1990b). Death occurs a week or two after spawning (Carruth et al., 2002).

Reproductive death is also seen in lampreys, jawless fish of the class Agnatha, such as the European river lamprey *Lampetra fluviatilis* (**Figure 3B**). Lampreys pass through larval and non-reproductive juvenile stages of variable duration before undergoing sexual maturation and spawning, usually after around 4–8 years. Prior to spawning in fresh water they cease feeding, and before and during sexual maturation undergo major anatomical changes including atrophy of many somatic organs, such as the body wall (including muscle), intestine and liver (but not the heart), and organism-wide loss of protein, glycogen, and fat, which supports both gonadal growth (including vitellogenesis by the liver) and swimming (Bentley and Follett, 1965; Larsen, 1969, 1980; Mewes et al., 2002). Atrophy of the intestine is particularly marked (Larsen, 1965, 1969; Higashi et al., 2005; **Figure 3B**), reminiscent of *C. elegans* hermaphrodites (Ezcurra et al., 2018), but this occurs prior to sexual maturation, where the main source of remobilized resources is the body wall (Larsen, 1980). Death occurs shortly after spawning (a few days or weeks) (Larsen, 1980). Intestinal atrophy during sexual maturation has also been documented in eel species (genus *Anguila*) (Pankhurst and Sorensen, 1984).

A number of dasyurid marsupials of the genera *Antechinus*, *Phascogale,* and *Dasykaluta* exhibit reproductive death (Braithwaite and Lee, 1979; Hayes et al., 2019). For example, males of the mouse-like brown antechinus *A. stuartii* enter the breeding season at around the end of their first year of life, and most die within 2–3 weeks of reproductive maturity (Woolley, 1966). As in semelparous salmon, a major driver of pathology is hypercorticism associated with adrenal hyperplasia, which causes the males to become ill and die, e.g., due to infection and gastrointestinal hemorrhage (Barker et al., 1978; Bradley et al., 1980). Reproductive death is seen particularly in plants, as detailed below (**Figure 3C**).

A common feature of semelparous species is an extended pre-reproductive stage, with death following rapidly after reproductive maturation. For example, eels of the genus *Anguilla* typically spawn and die at 6–12 years of age (Tesch, 1977), and the bamboo *Phyllostachys bambusoides* flowers and dies after as much as 120 years (Janzen, 1976; Soderstrom and Calderon, 1979). As previously noted (Finch, 1990b, p. 118), *C. elegans* shows this pattern: diapausal dauer larvae can survive for up to 90 days, whereas after recovery from dauer and attainment of adulthood, death occurs within 2–3 weeks (Klass and Hirsh, 1976; Klass, 1977).

In conclusion, the pattern of pathological anatomical change seen *C. elegans* hermaphrodites resembles that seen in reproductive death, particularly in semelparous fish. Next we explore the similarities between *C. elegans* and semelparous organisms in terms of the possible proximate mechanisms of aging involved.

DESTRUCTIVE RESOURCE REALLOCATION IN REPRODUCTIVE DEATH

Our working hypothesis is that *C. elegans* reproductive death results, at least partly, from the costs of consequential indirect physiological trade-offs, including one in which intestinal biomass is consumed to generate trophallactic fluid (yolk milk) that nourishes larval kin (Speakman, 2008; Ezcurra et al., 2018; Kern et al., 2021). Broadly, this is a type of process where biological structures at one site (the source) are broken down and converted into structures at another location, or into activity (the sink). As has been said: "The massive translocation of resources at the time of reproduction is fundamental to the biology of semelparous species" (Young and Augspurger, 1991). While providing a fitness benefit at the sink, source organs can be impaired, e.g., due to atrophy (**Figure 4A**). The nature of reproductive effort supported at the sink can involve increased gonadal development, gamete production (including vitellogenesis) or lactation, or enhanced performance (e.g., courtship, mating). For example, in semelparous salmon, muscle catabolism to generate nutrients supports gonadal and gamete development, and the effort of swimming upstream, but also causes muscle atrophy (von der Decken, 1992). Similarly, in eels and lampreys atrophy of muscle is coupled to gonad growth and sustained swimming, and in eels skeletal breakdown releases calcium and phosphate for transfer to gonads (Larsen, 1980; Freese et al., 2019). Again, during their brief breeding season male *A. stuartii* cease feeding, and glucose availability is increased by gluconeogenesis promoted by elevated plasma corticosteroid levels, which both provides energy to support their extended copulatory exertions (increased performance; *A. stuartii* will copulate for up to 8 h continuously) and causes lethal immune deficiency (Naylor et al., 2008).

The Role of Autophagy in Source-to-Sink Biomass Conversion

Source-to-sink biomass conversion implies the occurrence of bulk autolysis of biomass in the source tissue. This suggests a role of enzymatic degradation, which usually occurs within acidic compartments within the cell, including lysosomes in animals, and the vacuole in fungi and plants (**Figure 4A**). In animals, the major, regulated intracellular mechanism of bulk autolysis is autophagy (specifically macroautophagy). In *C. elegans*, inhibition of autophagy inhibits both intestinal atrophy and yolk steatosis (Ezcurra et al., 2018). The implied role of autophagy as a promoter of senescent pathology is somewhat unexpected given previous evidence that autophagy is important in maintaining homeostasis and protecting against senescent decline (Gelino and Hansen, 2012). Plausibly, physiological costs

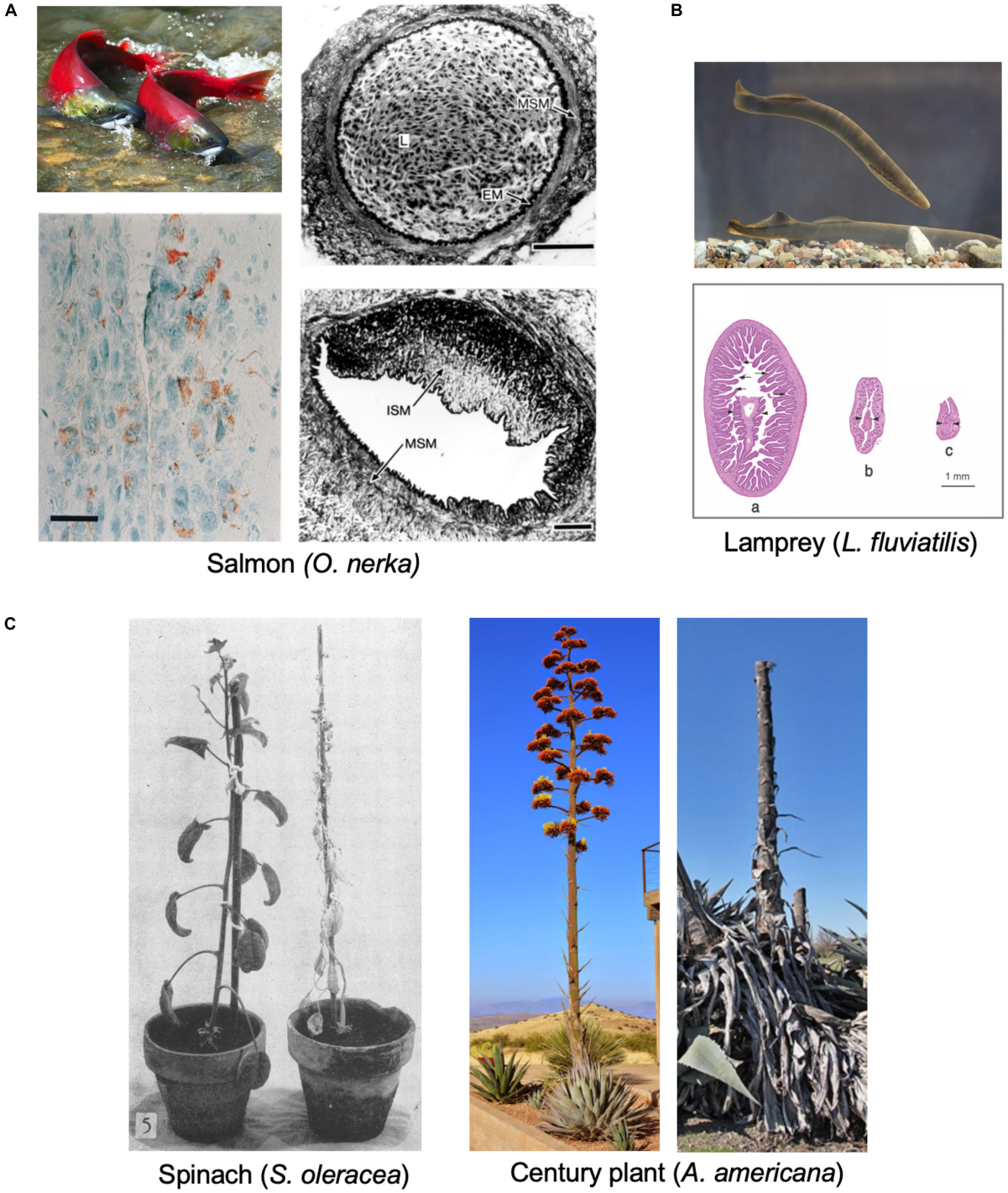

FIGURE 3 | Examples of semelparous organisms and their senescent pathologies. **(A)** Pacific salmon *O. nerka*. Top left: sexually mature adults (photo courtesy of Georgia Strait Alliance, www.georgiastrait.org© Olga Vasik—Adobe Stock). Bottom left: Immunoreactivity to $A\beta_{1-42}$ antibody in the brain of spawning kokanee salmon (Maldonado et al., 2000), c.f. amyloid plaques associated with Alzheimer's disease. Bar, 20 μm. Right: cross section of normal coronary artery (top); L, lumen, filled with nucleated red blood cells; MSM, medial layer of vascular smooth muscle; EM, elastic membrane; ISM; or (bottom) from mature adult with severe arteriosclerotic lesion, containing mainly intimal smooth muscle cells (ISM) (Farrell, 2002). Bars, 50 μm. **(B)** Lamprey (genus *Lampetra*). Top, European river lamprey (*L. fluviatilis*) (photo by Tiit Hunt, distributed under a CC BY-SA 3.0 license). Bottom: stages of intestinal atrophy during spawning in *L. japonica*. Diameters of (a) 3.9 mm, (b) 1.5 mm (b), and (c) 1 mm. Arrows, intestinal villi; arrowheads, typhosole (internal intestinal fold) (Higashi et al., 2005). **(C)** Examples of reproductive death in semelparous plants. Left, *Spinacia oleracea* 45 days after full bloom; reproductive death (right) has been suppressed by flower removal (left) (Leopold et al., 1959) (© American Society of Plant Biologists, reprinted with permission). Right, *Agave americana* during and after flowering (photos by Gerhard Bock, reproduced with permission). Century plants typically live 10–30 years, and death follows rapidly after a single massive reproductive event.

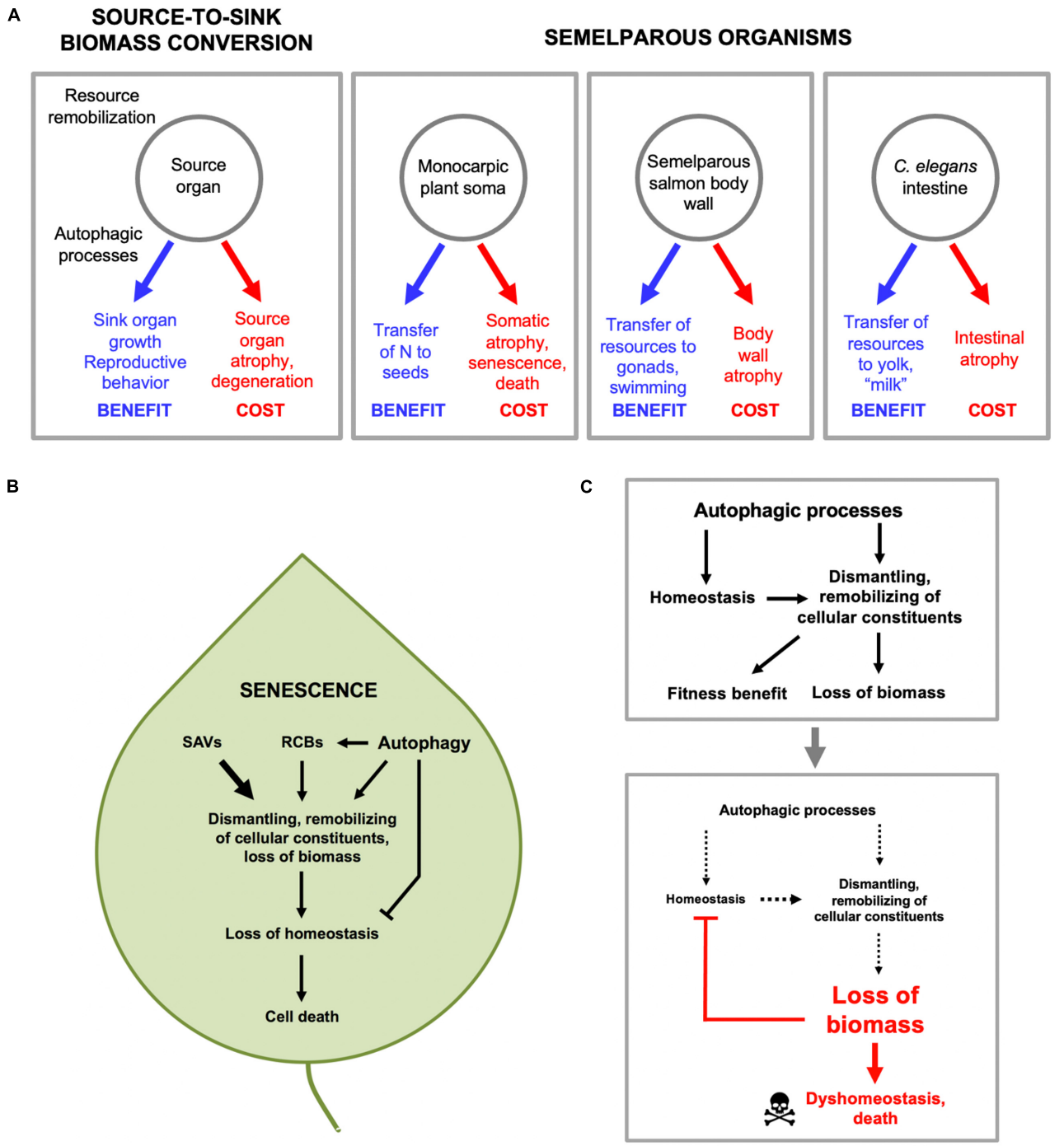

FIGURE 4 | Source-to-sink biomass conversion and physiological costs that cause pathology. **(A)** General form of source-to-sink biomass conversion (left) and three examples. In each case remobilization of resources lead to fitness benefits by supporting reproductive processes, but leads to atrophy and eventual pathology in source organs. **(B)** Autophagic processes and senescence in plants. Material from other organelles, particularly chloroplasts, is transported to the vacuole in several ways, including autophagosomes. First, via autophagosomes, double membrane-bound vesicles as found in animal and fungal autophagy pathways (Marshall and Vierstra, 2018). Second, via double membrane-bound rubisco-containing bodies (RCBs; rubisco is the most abundant stromal protein in chloroplasts) which contain fragments of chloroplast proteins (Chiba et al., 2003), and whose transport to the vacuole is dependent on genes of the autophagy pathway (Ishida et al., 2008; Wada et al., 2009). Third, via senescence-associated vacuoles (SAVs) which are single membrane bound and which, unlike autophagosomes, contain high levels of protease activity (Martinez et al., 2008). **(C)** Autophagic processes protect in order to destroy (demolition engineer principle). A hypothesis based on recent progress in understanding the role of autophagy in plant leaf senescence (Avila-Ospina et al., 2014) (with thanks to Prof. Céline Masclaux-Daubresse). Top: by maintaining homeostasis during the systematic destruction of the cell, autophagic processes aid in its destruction. Bottom: eventually the cell is dismantled to the point that even autophagic processes cannot be sustained, and homeostasis collapses, leading to death.

due to biomass conversion are more severe in semelparous than iteroparous organisms, such that a major role for autophagic processes in pathogenesis is a special feature of semelparity. Very little is known about the role of autophagy in reproductive death in animals. In lampreys, breakdown of intestinal biomass occurs in part in the stellate cells beneath the intestinal epithelium. In *L. japonica* there is some evidence that biomass breakdown (visible as loss of collagen fibrils) occurs by a process of phagocytosis and lysosomal proteolysis (Higashi et al., 2005). Intestinal atrophy in lampreys occurs largely prior to vitellogenesis, which occurs in the liver (Larsen, 1980), so lampreys differ from *C. elegans* here.

Destructive Resource Reallocation and Senescence in Plants

Much more is known about the biology of source-to-sink biomass conversion in plants, in the context of semelparity (in plants, monocarpy), and also leaf senescence (Young and Augspurger, 1991; Davies and Gan, 2012; Avila-Ospina et al., 2014). One reason is that semelparity is much more common among plants than animals. Another is that understanding the biology of biomass conversion is useful for crop improvement. This knowledge includes a detailed understanding of the proteolytic machinery involved in autolysis (including autophagy) in source tissues that provides useful insight into semelparous pathophysiology.

In deciduous trees in autumn, leaf senescence occurs during which leaf biomass is broken down and remobilized (particularly nitrogen), and transported via the phloem to support tree survival, resulting in leaf death. In many monocarpic angiosperms, the entire soma is broken down during flowering and fruiting, largely to support seed production (Schippers et al., 2015; Diaz-Mendoza et al., 2016). In perennial polycarps the entire plant above ground may die off to support growth and survival of the subterranean bulb. In each case, somatic biomass is transferred from source to sink organs (Davies and Gan, 2012). For example, in wheat and rice grains up to 90% of the nitrogen content is derived from the senescence of somatic tissues (Diaz-Mendoza et al., 2016).

Senescence-associated biomass conversion in plants is driven by action of a variety of proteases acting in different cellular compartments, but the final destination is mainly the large, acidic central vacuole (Avila-Ospina et al., 2014). This is functionally related to the lysosome of animal cells, e.g., as a major site of proteolysis by acid proteases. Material from other organelles, particularly chloroplasts, is transported to the vacuole in several ways, including autophagosomes (**Figure 4B**). Thus, in plants as in *C. elegans* gut-to-yolk biomass conversion, autophagy and autophagy-related processes promote senescence.

If autophagy promotes plant senescence, then inhibiting autophagy should retard senescence, as seen in *C. elegans* intestinal senescence (Ezcurra et al., 2018; Benedetto and Gems, 2019). The effects of inhibition of autophagy on plant senescence are complex but, interestingly, support the view that autophagy promotes the earlier stages of senescence but protects against its later stages. For example, in *Arabidopsis thaliana* loss of expression of genes encoding proteins involved in autophagy (*atg5, atg9,* or *atg18a*) inhibits the decline with advancing age in amino acid, protein and RNA content in plant rosettes (Guiboileau et al., 2013; Havè et al., 2018). Loss of *atg5* in plants subjected to mild (but not severe) stress suppresses leaf senescence (Sakuraba et al., 2014). Moreover, *atg* mutants are hypersensitive to N and C starvation, and deficient in N redistribution into seeds, not only in *A. thaliana* but also in maize and rice (Tang and Bassham, 2018). Furthermore, global expression of *atg* genes increases in the later stages of leaf senescence in many plant species, though in *A. thaliana* leaf senescence this occurs after N mobilization is well underway (Avila-Ospina et al., 2014; Tang and Bassham, 2018). Overall, this supports the view that autophagy promotes resource remobilization during senescence leading to loss of somatic biomass.

Autophagic Processes Maintain Homeostasis While They Destroy the Cell

Overall, studies of autophagy in plant senescence reveal its double-edged role in resource reallocation processes that lead to death. Here autophagy contributes to nutrient recycling and remobilization during leaf senescence, but also helps maintain homeostasis in the cell while it is being dismantled (Avila-Ospina et al., 2014). Thus, in the absence of the classic autophagy pathway, the destructive action of other autophagy-related processes (**Figure 4B**) would lead more rapidly to leaf dyshomeostasis and death. In other words, autophagy promotes senescence by facilitating resource reallocation, but also protects against it by maintaining homeostasis. However, given that sustaining homeostasis aids resource reallocation, this protective role of autophagy is ultimately destructive (**Figure 4C**), and analogous to the action of demolition engineers preparing a building for destruction, who work to maintain its structural integrity while stripping out reusable materials. Thus, in this context, autophagy protects in order to destroy.

Leaf senescence provides a lucid illustration of the relationship between the ordered, programmed process by which the plant cell is dismantled, and the resulting homeostatic collapse leading to death. The entire senescence process is pathological (at least with respect to the leaf). Though the leaf loses functionality from the outset of senescence (e.g., photosynthetic), only in its later stages does loss of homeostasis contribute to pathogenesis. The same is the case for many diseases, where the initial impact of etiology may not cause dyshomeostasis, as in early stages of cancer development, or viral infections.

According to the demolition engineer principle outlined above, a general feature of source and sink biomass conversion processes that lead eventually to death is that cells, tissues, and organisms need to remain alive and functioning to be able to efficiently dismantle themselves. For example, during leaf senescence, chloroplasts are broken down early on but mitochondria remain intact and functional until the final stages of senescence (Peterson and Huffaker, 1975; Diaz-Mendoza et al., 2016). Similarly, in *C. elegans*, the intestine and distal gonad undergo atrophy in early adulthood but the nervous

system remains intact into late life (Herndon et al., 2002; Ezcurra et al., 2018). Again, in sexually mature lampreys multiple organs (including the intestine and liver) undergo severe atrophy, but the heart is protected (Bentley and Follett, 1965; Larsen, 1980).

The ordered sequential nature of the destruction of organelles, cells and organs in semelparous organisms contrasts with aging in iteroparous organisms, such as mice or humans, where incidence of aging-related diseases varies greatly between individuals (Finch, 1990b; Austad, 2004). For example while mammalian cancers vary in type and incidence, all aging *C. elegans* hermaphrodites develop teratoma-like uterine tumors (Wang et al., 2018b).

Source-to-Sink Biomass Conversion Is Not Disposable Soma

There is a superficial resemblance between biomass conversion and another mechanism proposed to underlie trade-offs between reproduction and lifespan, but they are not the same. The disposable soma theory proposes that stochastic molecular damage causes aging, and that aging rate is determined by the level of resource investment into somatic maintenance mechanisms that prevent that damage (Kirkwood, 1977, 2005; **Figure 5A**). By contrast, in biomass conversion mechanisms source tissues and organs are actively dismantled in the process of promoting function at the sink (**Figure 5B**). While it is true that this can involve utilization of somatic tissues in a disposable fashion, this is not the same as the disposable soma theory as set out. The primary etiology is programmatic, not stochastic damage.

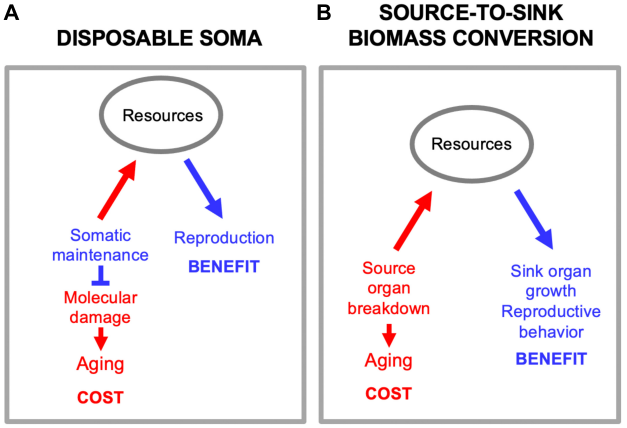

FIGURE 5 | Source-to-sink biomass conversion is not disposable soma. **(A)** Disposable soma. Here a primary cause of aging is stochastic molecular damage accumulation, prevented by somatic maintenance processes. Diversion of resources from somatic maintenance to reproduction provides a reproductive fitness benefit, but allows molecular damage to accumulation, causing aging. **(B)** Source-to-sink biomass conversion. Here a primary cause of aging is programmatic, active self-destruction of organs to release resources for reproduction.

PREVENTION OF REPRODUCTIVE DEATH CAN GREATLY EXTEND LIFESPAN

In *C. elegans* hermaphrodites, removal of the germline leaving the somatic gonad intact increases mean lifespan by some 60% (Hsin and Kenyon, 1999). One possibility is that this is due to suppression of reproductive death, which in other semelparous organisms increases lifespan substantially.

Life Extension by Suppression of Reproductive Death

Reproductive death in semelparous species is actively promoted by hormonal factors, for example corticosteroids in *A. stuartii* and salmon of the genus *Oncorhynchus*, and abscisic acid in monocarpic plants. Blocking production of such factors, e.g., by surgical removal of their source or by behavioral manipulation, can suppress reproductive death. As one would expect, this can cause large increases in lifespan. For example, in the salmon *O. nerka* castration before spawning prevented hypercorticism and increased maximum lifespan from 4.8 to 8.5 years (Robertson, 1961). It has also been suggested that parasitic mollusc larvae can suppress reproductive death and extend lifespan in Atlantic salmon (*Salmo salar*) (Ziuganov, 2005). Moreover, gonadectomy or hypophysectomy (removal of the pituitary gland equivalent) in the lamprey *L. fluviatilis* prior to sexual maturation inhibited body wall mobilization and intestinal atrophy (Larsen, 1974, 1980; Pickering, 1976) and instead of dying shortly after spawning, hypophysectomized animals survived for up to 11 months (Larsen, 1965; reviewed in Larsen, 1980).

In the eel *Anguila anguila* the bulk of pre-adult growth occurs in rivers, and after 6–12 years sexually mature adults make sea runs to spawn and die in the Sargasso Sea (Finch, 1990b). Prevention of the sea run and spawning can increase eel lifespan substantially. For example, one eel kept in a well in Denmark lived for 55 years (at least a 3.5-fold increase in lifespan) (Tesch, 1977), while another maintained in a Swedish aquarium lived for 88 years (at least a sevenfold increase in lifespan) (Vladykov, 1956).

Looking beyond fish, in the octopus *O. hummelincki* removal of the optic gland just after spawning in females increased lifespan measured from onset of egg-laying by up to 5.4-fold (maximum lifespan, from 51 to 277 days) (Wodinsky, 1977). Reproductive death in *A. stuartii* can be prevented either by capture and cage maintenance prior to mating or by castration. If males are captured prior to mating and maintained in the lab they can survive for 3 years or more (Woolley, 1966; Olsen, 1971; Bradley et al., 1980). Removal of reproductive structures can also inhibit senescence in monocarpic plants; for example, removal of flowers prior to pollination increased mean lifespan in soybean plants (*Glycine max*) from 119 to 179 days after sowing (+ 50.4%) (Leopold et al., 1959). The standard view is that, in these instances, extension of lifespan results not from retardation of aging but from prevention of reproductive death (but see below).

Removal of the germline can also increase lifespan in iteroparous species. For example, in *Drosophila subobscura* the *grandchildless* mutation, which causes germline loss, increased life expectancy (from day 10) by 15.1% (Maynard Smith, 1958). In *Drosophila melanogaster* loss of germ cells from late development or early adulthood extended median lifespan in both sexes by 21.0–50.0% (Flatt et al., 2008), but absence of the germline throughout life shortened female lifespan

TABLE 1 | Magnitude of increases in lifespan after gonadectomy or behavioral interventions that prevent reproductive death.

				Lifespan[a]			
Species, genotype	**Sex**	**Intervention**	**Conditions, strain**	**Control**	**Treated**	**% change**	**References**
C. elegans							
+	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	19.4 d	31.8 d	+63.9	Hsin and Kenyon, 1999
daf-2(e1370)	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	43.2 d	75.7 d	+75.2	Hsin and Kenyon, 1999
+	Hermaphrodite	Germline ablation (laser)	20°C, monoxenic liquid	16.8 d	35.0 d	+108	McCulloch, 2003
daf-2(e1368), daf-2 RNAi	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	51.0 d	124.1 d	+143	Arantes-Oliveira et al., 2003
***Caenorhabditis* species**							
C. elegans	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	16.7 d	35 d	+109.4	Kern et al., 2020
C. inopinata	Female	Germline ablation (laser)	20°C, agar plates	23.5 d	30.7 d	+30.6	Kern et al., 2020
C. tropicalis	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	18.8 d	35.9 d	+91	Kern et al., 2020
C. wallacei	Female	Germline ablation (laser)	20°C, agar plates	28.7 d	33.9 d	+18.5	Kern et al., 2020
C. briggsae	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	17.1 d	31 d	+81.5	Kern et al., 2020
C. nigoni	Female	Germline ablation (laser)	20°C, agar plates	29.7 d	34 d	+14.5	Kern et al., 2020
***Pristionchus* species**							
P. pacificus	Hermaphrodite	Germline ablation (laser)	20°C, agar plates	24.7 d	40.5 d	+64	Kern et al., 2020
P. exspectatus	Female	Germline ablation (laser)	20°C, agar plates	43.1 d	44.3 d	+2.7	Kern et al., 2020
Semelparous (with reproductive death)							
Glycine max (soy bean)	Monoecious	Flower removal		119 d	179 d	+50.4	Leopold et al., 1959
O. hummelincki (octopus)	Female	Optic gland removal		51 d	277 d	+443	Wodinsky, 1977
A. anguila (eel)	Unknown	Prevention of sea run	Fresh water	*9 y*[b]	55 y	+511	Tesch, 1977
A. anguila (eel)	Unknown	Prevention of sea run	Fresh water	*9 y*[b]	88 y	+877	Vladykov, 1956
O. nerka (salmon)	Both sexes	Castration		4.8 y	8.5 y	+77.0	Robertson, 1961
A. stuarti (marsupial)	Male	Lab capture prior to mating		1 y	3 y	+200	Olsen, 1971
Iteroparous							
D. subobscura	Female	*grandchildless* mutation	20°C, virgin	58.7 d[c]	67.6 d[c]	+15.1[d]	Maynard Smith, 1958
D. melanogaster	Female	*germ cell-less* mutation	25°C, virgin	*44 d*	*38 d*	−13.6	Barnes et al., 2006
D. melanogaster	Female	*tudor* mutation	25°C, virgin	*71 d*	*57 d*	−19.7	Barnes et al., 2006
D. melanogaster	Female	*bag of marbles* over-expression	25°C	*32,28 d*	*42 d*	+31.3, 50.0	Flatt et al., 2008
D. melanogaster	Male	*bag of marbles* over-expression	25°C	*38,36 d*	*46 d*	+21.0, 27.8	Flatt et al., 2008
R. microptera (grasshopper)	Female	Ovariectomy	28°C	*167 d*	*205 d*	+22.7	Hatle et al., 2008
R. microptera (grasshopper)	Female	Ovariectomy	32°C, 24°C	*245 d*	*285 d*	+16.3	Drewry et al., 2011
M. musculus (mouse)	Female	Ovariectomy before puberty	CBA/J	599 d	540 d	−9.8	Cargill et al., 2003
R. norwegicus (rat)	Male	Castration at birth	Inbred Lewis	454 d	521 d	+14.7	Talbert and Hamilton, 1965
R. norwegicus (rat)	Male	Castration just before puberty	Osborne-Mendel Yale	615 d	651 d	+5.8	Asdell et al., 1967
R. norwegicus (rat)	Female	Ovariectomy just before puberty	Osborne-Mendel Yale	742 d	669 d	−9.8	Asdell et al., 1967
R. norwegicus (rat)	Male	Castration just before puberty	Norway albino	727 d	817 d	+21.7	Drori and Folman, 1976
F. catus (cat)	Male	Castration		4.9 y	8.2 y	+67.3	Hamilton et al., 1969
F. catus (cat)	Female	Spayed		6.8 y	8.4 y	+23.5	Hamilton et al., 1969
F. catus (cat)	Both sexes	Gonadectomy		*11.0 y*	*15.0 y*	+36.3	O'Neill et al., 2015
C. lupus familiaris (dog)	Both sexes	Gonadectomy		7.9 y	9.4 y	+18.9	Hoffman et al., 2013
H. sapiens	Male	Castration		*55.7 y*	*69.3 y*	+24.4	Hamilton and Mestler, 1969
H. sapiens	Female	Oophorectomy		*65.2 y*	*65.2 y*	+0	Hamilton and Mestler, 1969
H. sapiens	Male	Castration		50.9 y	70.0 y	+37.5	Min et al., 2012
H. sapiens	Male	Castration		55.6 y	70.0 y	+25.8	Min et al., 2012

[a] *Mean lifespan (median lifespan, italics; maximum lifespan, underlined). d, days. y, years.*
[b] *Eels normally live 6–12 y; the median value is taken here.*
[c] *Life expectancy at age 10 days.*
[d] *It might be significant that the strain of D. subobscura used in this study mated only once, in contrast to D. melanogaster which can remate multiple times (Partridge and Sibly, 1991).*
Also included here are behavioral interventions that prevent reproductive death. It is notable that the magnitude of reported experimentally induced increases in lifespan, expressed in terms of proportional increase in lifespan, are generally greater in semelparous than iteroparous organisms.

(Barnes et al., 2006). Ovariectomy also increased median lifespan in grasshoppers by 16.3 or 22.7% (Hatle et al., 2008; Drewry et al., 2011).

In many mammals castration increases male lifespan while ovariectomy decreases female lifespan. For example, in studies of rats, castration increased male lifespan (Talbert and Hamilton, 1965; Asdell et al., 1967; Drori and Folman, 1976), but ovariectomy reduced it (Asdell et al., 1967) (though in some of these studies effects did not reach statistical significance). Ovariectomy also reduced survival in mice (Cargill et al., 2003; Benedusi et al., 2015). Castration also extended lifespan in male bank voles (Gipps and Jewell, 1979) and in male feral sheep (Jewell, 1997). Similarly, in humans there is some limited evidence of castration increasing lifespan in men (Hamilton and Mestler, 1969; Min et al., 2012), and more robust evidence that ovariectomy shortens lifespan in women (Rocca et al., 2006; Shoupe et al., 2007; Parker et al., 2009). By contrast gonadectomy increased lifespan in both sexes of domestic cats (Hamilton, 1965; Hamilton et al., 1969; O'Neill et al., 2015) and dogs (particularly in bitches) (Michell, 1999; Hoffman et al., 2013, 2018; O'Neill et al., 2013).

Thus, although germline removal can increase lifespan in both semelparous and iteroparous species, the effects on lifespan are typically far larger and less condition dependent in the former (**Table 1**), consistent with prevention of reproductive death rather than of the far more modest reproductive costs typical of iteroparous organisms.

Suppression of Reproductive Death by Germline Ablation in *C. elegans*

Could germline ablation in *C. elegans* hermaphrodites extend lifespan by preventing reproductive death? Supporting this, intestinal atrophy is suppressed by germline removal (Ezcurra et al., 2018; Kern et al., 2020). Moreover, the DAF-16/FOXO transcription factor is required for both life extension (Hsin and Kenyon, 1999) and suppression of intestinal atrophy (Ezcurra et al., 2018) by germline removal.

The striking senescent changes in anatomy seen in hermaphrodites are largely absent from males (de la Guardia et al., 2016; Ezcurra et al., 2018), suggesting that they do not undergo reproductive death. Consistent with this, a study of individually cultured nematodes in monoxenic liquid culture found that germline ablation by laser microsurgery increased lifespan in wild-type hermaphrodites but not males (McCulloch, 2003). Moreover, individually cultured wild-type males are longer lived than hermaphrodites (Gems and Riddle, 2000; McCulloch and Gems, 2007).

We recently examined the pattern of senescent pathology in two additional *Caenorhabditis* species that are, like *C. elegans*, androdioecious (with hermaphrodites and males), *C. briggsae* and *C. tropicalis*, and found them to be similar to *C. elegans*, suggesting the occurrence of reproductive death in these species too (Kern et al., 2020). The majority of *Caenorhabditis* species are gonochoristic (with females and males), and *C. elegans*, *C. briggsae* and *C. tropicalis* represent three independent occurrences of the evolution of androdioecy (Kiontke et al., 2011). Gonochoristic sibling species of these three androdioecious species are, respectively, *C. inopinata*, *C. nigoni,* and *C. wallacei*. Notably, in females (unmated) of these three species the senescent degeneration seen in hermaphrodites does not occur. Moreover, for all three sibling species pairs, hermaphrodites vent yolk and lay unfertilized oocytes, while females do not. However, senescent degeneration was seen in females after mating (Kern et al., 2020). Taken together, these results suggest that after the appearance of hermaphroditism in each instance, reproductive death evolved from being facultative (mating induced) to constitutive. A possible adaptive significance of this change is that females but not hermaphrodites need to await an encounter with a male before commencing reproduction.

Semelparity in *C. elegans* implies a cost of reproduction. It was previously noted that prevention of self-fertilization by means of mutations that impair sperm function does not increase lifespan (Klass, 1977; Kenyon et al., 1993); i.e., the effort of egg production, fertilization and egg laying does not shorten life. This implies that the costly lactational program is active and generates life-shortening pathology whether or not fertilization takes place.

The occurrence of constitutive reproductive death in *Caenorhabditis* hermaphrodites but not females is supported by several further observations. First, for all three sibling species pairs, the females (unmated) are longer lived than the hermaphrodites (Amrit et al., 2010; Kern et al., 2020). In the case of *C. elegans* and its sibling species *C. inopinata*, the latter is longer lived only when the two species are compared in the presence of antibiotics, suggesting greater susceptibility of *C. inopinata* to life-shortening infection by the bacterial food source (Woodruff et al., 2019; Kern et al., 2020).

Combining several of these observations suggests the following scenario: that hermaphrodites but not females undergo reproductive death constitutively, triggered by signals from the germline, leading to shorter lifespan in hermaphrodites. Consistent with this model, germline ablation causes large increases in lifespan in hermaphrodites but not females (**Table 1**), and abrogates the greater lifespan of females. Moreover, germline ablation suppresses intestinal atrophy in all hermaphroditic species (Kern et al., 2020; see **Figure 6** for schematic summary).

Taken together, these observations imply that extension of lifespan by germline ablation in *C. elegans* is due to suppression of semelparous reproductive death, as seen e.g., in Pacific salmon.

Does Reduced Insulin/IGF-1 Signaling Suppress Reproductive Death?

While the discovery of single gene mutations that alter lifespan in *C. elegans* was important, what generated particular excitement was the large magnitude of increases in lifespan observed, particularly from reductions in insulin/IGF-1 signaling (IIS). The largest effects have been observed in mutants defective in the *daf-2* insulin/IGF-1 receptor and the *age-1* phosphatidyinositol 3-kinase (PI3K) catalytic subunit (Kenyon, 2010) with up to 10-fold increases in mean and maximum lifespan recorded

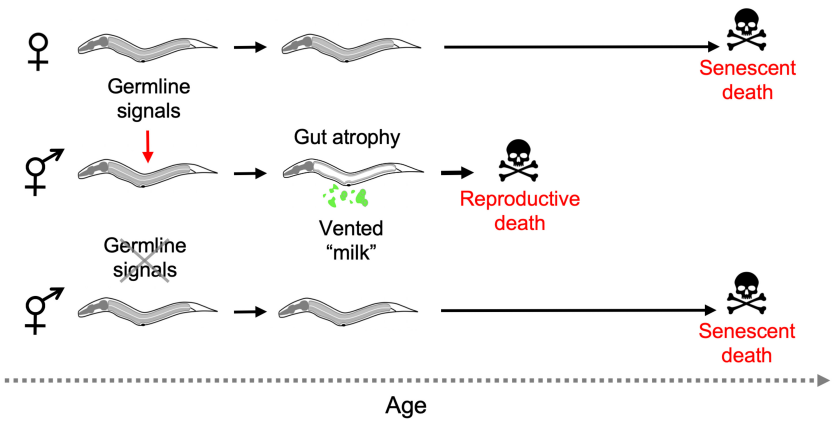

FIGURE 6 | Aging and death in *Caenorhabditis* females and hermaphrodites (simplified working model). In the absence of mating only hermaphrodites exhibit reproductive death, and this is triggered during reproductive maturation by signals from the germline. Removal of the germline by laser microsurgery blocks reproductive death, and markedly extends lifespan in hermaphrodites, removing the difference in lifespan between hermaphrodites and females. Germline ablation only modestly increases female lifespan (not depicted) (Kern et al., 2020).

(Ayyadevara et al., 2008). Could these large effects on lifespan reflect suppression of reproductive death, at least in part?

There is some evidence that IIS promotes reproductive death. Mutation of *daf-2* can suppress the dramatic morphological changes accompanying *C. elegans* hermaphrodite senescence (Garigan et al., 2002; Luo et al., 2010; McGee et al., 2012; Ezcurra et al., 2018). IIS also promotes vitellogenesis (Depina et al., 2011; Ezcurra et al., 2018) and venting of yolk milk and laying of yolk-replete oocytes (Gems et al., 1998; Kern et al., 2020). As already mentioned, effects on lifespan of both *daf-2* and germline removal require the DAF-16 FOXO transcription factor (Kenyon et al., 1993; Hsin and Kenyon, 1999) and in both cases its action in the intestine is important (Lin et al., 2001; Libina et al., 2003).

But other observations argue against the idea that reduced IIS extends lifespan simply by blocking reproductive death. First, germline ablation increases lifespan in *daf-2* mutants, seemingly more so than in wild type (+ ∼140% vs. + ∼60%) (Hsin and Kenyon, 1999; Arantes-Oliveira et al., 2002). Second, mutation of *daf-2* increases lifespan in males (Gems and Riddle, 2000; McCulloch and Gems, 2007; Hotzi et al., 2018), though they appear not to exhibit reproductive death. Thus, the relationship of IIS to germline signaling on the one hand and reproductive death on the other remains to be resolved. One possibility is that the two pathways act to some extent in parallel to promote reproductive death, while IIS also impacts lifespan via additional pathway-specific mechanisms, e.g., related to its role in dauer diapause (Kenyon et al., 1993), or to adaptive death (see below).

A CONTINUUM BETWEEN SEMELPAROUS AND ITEROPAROUS AGING

In this review, we have made the case that *C. elegans* undergo semelparous reproductive death; 12 items of evidence supporting this hypothesis are listed in **Table 2**.

Is *C. elegans* a Good Model Organism for Understanding Aging?

Caleb Finch said of semelparous dasyurid marsupials: "Their escape from 'natural death' under optimum conditions and their capacity to more than double their natural lifespan caution against overemphasizing lifespan and mortality rates as a basic index of cellular 'aging'." (Finch, 1990b, p. 95). Is this warning also applicable to *C. elegans*? If *C. elegans* is semelparous, such that the mechanisms controlling its lifespan are more akin to those in monocarpic plants than in humans, what does this mean for its use as a model organism for studying aging? A great deal of research has been carried out on *C. elegans* aging during the last 40 years; a PubMed search conducted on 20th July 2021 for articles including the terms "elegans" and "aging" identified 4,351 items. Are these studies in fact largely about reproductive death rather than aging?

For *C. elegans* researchers: don't panic. In the remainder of this essay, we propose a new perspective according to which *C. elegans* is a good model system for studying aging, despite its semelparity. Our key points are as follows. We have argued that *C. elegans* exhibits rapid senescence triggered by sexual maturation and coupled to reproductive effort, as seen in many other semelparous organisms. We postulate: (1) that this form of senescence involves exaggerated versions of mechanisms that are operative in iteroparous organisms, from which they evolved. (2) That such regulated mechanisms of senescence have a much larger effect on lifespan in semelparous organisms than iteroparous organisms. (3) That if such regulated mechanisms are blocked, pathologies that then become life limiting involve a wider spectrum of etiologies—both programmatic [e.g., involving antagonistic pleiotropy (AP) enacted in diverse ways] and stochastic (e.g., molecular damage accumulation, mechanical senescence). According to this view, a virtue of *C. elegans* is that one major form of senescent etiology (programmatic) plays a predominant role in aging, making it more experimentally tractable. This is also an argument for the potential value to

TABLE 2 | Features of *C. elegans* consistent with semelparous reproductive death.

(1) *C. elegans* hermaphrodites exhibit early, massive pathology affecting organs linked to reproduction (Garigan et al., 2002; Herndon et al., 2002; Ezcurra et al., 2018).
(2) Gut-to-yolk biomass conversion appears to be part of a suicidal reproductive effort that promotes fitness by feeding trophallactic fluid to larval kin (Kern et al., 2021).
(3) Blocking hermaphrodite reproductive maturation (e.g., by germline ablation) suppresses development of such pathologies, and leads to increases in lifespan of a large magnitude (Hsin and Kenyon, 1999; Ezcurra et al., 2018; Kern et al., 2020).
(4) Germline removal in wild-type males, which do not exhibit semelparity-like pathology, does not increase lifespan (McCulloch, 2003).
(5) *Caenorhabditis* hermaphrodites, which exhibit senescent transformation, are shorter lived than (unmated) *Caenorhabditis* females, which do not, consistent with reproductive death in the former only (Kern et al., 2020).
(6) *Caenorhabditis* hermaphrodites vent yolk and lay unfertilized oocytes in large numbers, while *Caenorhabditis* females do not (Kern et al., 2020).
(7) Germline removal in unmated *Caenorhabditis* females, which do not exhibit semelparity-like pathology, produces much smaller increases in lifespan than in *Caenorhabditis* hermaphrodites (Kern et al., 2020).
(8) Germline removal removes the difference in lifespan between *Caenorhabditis* females and hermaphrodites (Kern et al., 2020).
(9) *C. elegans* senescent transformation involves source-to-sink type resource remobilization, as seen in semelparous animals and plants (Ezcurra et al., 2018; Kern et al., 2021).
(10) Autophagic processes that enable biomass conversion and resource remobilization contribute to senescent pathogenesis in *C. elegans* as in semelparous organisms (particularly plants) (Ezcurra et al., 2018; Benedetto and Gems, 2019).
(11) Semelparous senescence occurs earlier in cell compartments or organs that are non-essential for survival and behavior (e.g., the *C. elegans* intestine) (Ezcurra et al., 2018) than in essential organs (e.g., the *C. elegans* nervous system) (Herndon et al., 2002).
(12) Semelparous species often have an extended pre-reproductive stage, followed by a very brief reproductive stage (c.f. the dauer stage in *C. elegans*) (Klass and Hirsh, 1976).

understanding animal aging of studying senescence in other semelparous species, including plant models such as *A. thaliana*.

A Continuum of Semelparity and Iteroparity

Mechanisms in sexual maturation-triggered reproductive death are likely to be related to the subtler mechanisms operative in iteroparous species, consistent with the existing continuum between iteroparity and semelparity (Hughes, 2017). A plausible scenario is that semelparous etiologies evolved by amplification of mechanisms operative in iteroparous ancestors. This resulted in exaggerated and life-limiting senescent pathologies resulting from relatively simple causes. If this were true then semelparous vertebrates should show age-related diseases similar to those seen in iteroparous ones. In fact, this is the case in Pacific salmon, one of the few semelparous vertebrates in which senescent pathologies have been studied. For example in spawning *O. tshawytscha* the coronary arteries, among others, exhibit endothelial cell hyper-proliferation (**Figure 3A**; Robertson et al., 1961; Farrell, 2002), reminiscent of human coronary artery disease, though lipid and calcium deposits typical of mammalian atheromas are not seen (Robertson et al., 1961; House and Benditt, 1981). Furthermore, starting at sexual maturity kokanee salmon develop amyloid deposits in multiple regions of the brain, similar to those occurring in Alzheimer's disease in humans (**Figure 3A**; Maldonado et al., 2000, 2002). This is one of the few examples of Alzheimer-like cytopathology found in wild vertebrates under natural conditions. The pathology includes extracellular amyloid plaques that are immunoreactive with anti-$A\beta_{1-42}$ antibodies. The distribution of amyloid deposition is similar to that of glucocorticoid receptors, suggesting that elevated glucocorticoids may cause this Alzheimer-like pathology (Maldonado et al., 2000). Similarly, thymic involution is promoted by sex steroid-induced glucocorticoid production in both spawning salmon and in mammals (Chen et al., 2010). It is also possible that IGF-1 (cf IIS) promotes reproductive death in salmon, e.g., through effects on gonadal development (Allard and Duan, 2011). Notably, many of the pathological changes that occur rapidly in spawning salmon also occur in later life in castrated salmon, i.e., reproductive death resembles accelerated aging (Robertson and Wexler, 1962).

One broad difference between semelparous etiologies and the iteroparous etiologies from which they evolved is that while the former are irreversible the latter can be reversible. For example, intestinal atrophy in adult *C. elegans* hermaphrodites or spawning lampreys appears to be irreversible, whereas loss of muscle during starvation or bone during lactation is reversible (Speakman, 2008). In summary, the nature of the diseases of aging in Pacific salmon supports the existence of a continuum between semelparous and iteroparous species in terms of senescent pathophysiology.

Quasi-Programs vs. Costly Programs as Ubiquitous Causes of Senescence

As a broad approximation, in terms of primary mechanisms, senescence has been viewed either as a passive process of stochastic damage and breakdown (loss of function), or as an active process driven by late-life effects of gene action (hyperfunction) (Harman, 1956; Blagosklonny, 2006; Gems and Partridge, 2013; Gladyshev, 2013; Shore and Ruvkun, 2013). In semelparous organisms, the mechanisms that give rise to senescent pathogenesis (such as those involving resource reallocation) are clearly active, programmed processes; here pathology is generated as a by-product of functions that promote fitness, but is not itself advantageous (Williams, 1957). In iteroparous organisms (including most mammals) senescent pathologies can result, at least in part, from programmatic mechanisms such as futile quasi-programs.

To understand the relevance of aging in *C. elegans* to that in iteroparous organisms we need to ask: What is the

relationship between reproductive death and the quasi-program (hyperfunction) theory? Here the growing understanding of senescent pathophysiology in *C. elegans* is instructive. Several major senescent pathologies, including intestinal atrophy, yolk accumulation and teratoma-like uterine tumors, have been interpreted as resulting from hyperfunction rather than molecular damage, and from run-on type quasi-programs (Herndon et al., 2002; Ezcurra et al., 2018; Wang et al., 2018b). However, the recent discovery that vented yolk promotes larval growth implies that yolk synthesis in sperm-depleted mothers is not, in fact, futile at all, but instead promotes fitness through resource reallocation from mothers to larval kin (Kern et al., 2021).

Thus, late-life yolk production and the intestinal atrophy to which it is coupled does not conform to Blagosklonny's definition of a quasi-program (futile program continuation). Instead it involves a physiological trade-off where intestinal senescence is a cost. However, in both cases, the mechanisms involved are programmatic, rather than relating to damage and maintenance.

Another difference between these two cases is the relative timing of benefits and costs. In the first account, a program that promotes fitness in early life becomes a harmful quasi-program in later life. By contrast, in the case of intestinal atrophy coupled to yolk production, benefit and harm are generated simultaneously.

Insofar as the term *program* implies complex function *and* promotion of fitness (Lohr et al., 2019), *C. elegans* intestinal resource reallocation may be referred to as a *costly program*. By contrast, development of uterine teratomas is the result of a quasi-program (Wang et al., 2018a,b; **Figure 7A**), since a fitness benefit from having tumors is difficult to envisage.

To create an integrated conceptual framework we propose the following new account: that in both cases (quasi-programs and costly programs), pathology results primarily from hyperfunction rather than loss of function. In costly programs hyperfunction exists with respect to the pathology (e.g., intestinal atrophy) but not the benefit (yolk milk production) (**Figure 7A**). Similarly, the process of N remobilization from leaves is hyperfunctional as far as leaf health is concerned, but not seed provisioning. Thus, precise use of the term hyperfunction requires reference to the entity that it injures (cell, tissue, organ, organism).

According to this account, in iteroparous organisms resource reallocation can involve costly programs where the debts can be repaid, as in lactation-associated bone loss or starvation-induced muscle atrophy. Thus, *C. elegans* reproductive death, like mammalian aging, involves both quasi-programs and costly programs (**Figure 7A**). Understanding *C. elegans* aging should therefore provide fundamental insights into the pathophysiology of human senescence.

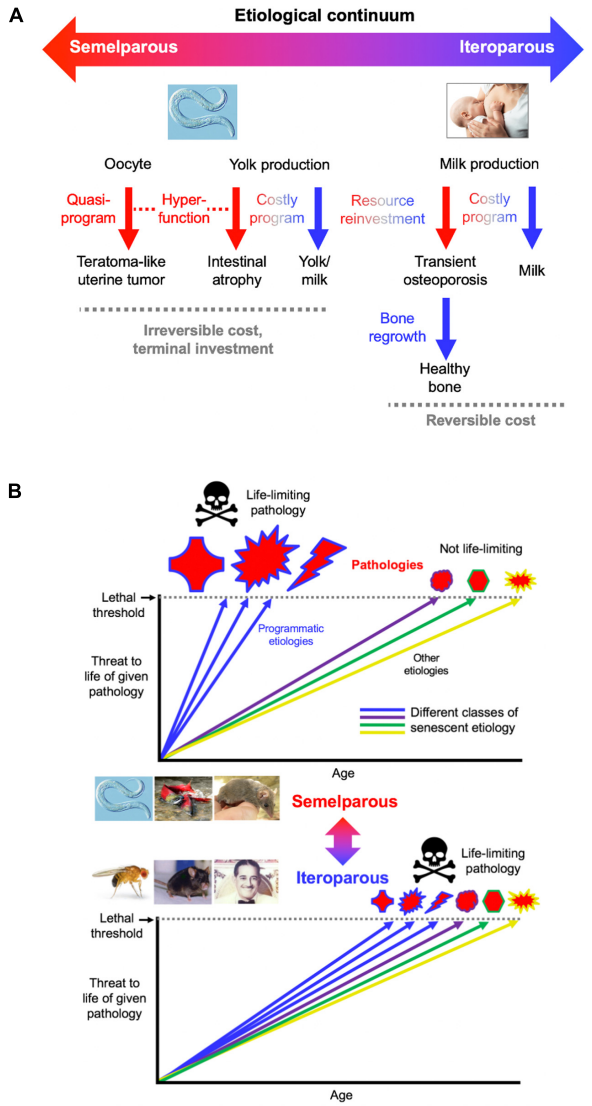

FIGURE 7 | Conceptual models of aging in semelparous and iteroparous organisms. **(A)** Programmatic mechanisms of aging in semelparous and iteroparous organisms. These include costly programs and quasi-programs. A broad prediction is that costly programs contribute more to disease during reproductive death, and quasi-programs more in iteroparous aging. **(B)** Difference in senescent pathogenesis in semelparous and iteroparous organisms. The figure shows the degree of harmfulness of a range of pathologies with different types of etiology (indicated by different colors). Top, reproductive death. Here exaggeration of programmatic mechanisms leads to rapid development of gross pathologies leading to death. Bottom, typical animal senescence (iteroparous species). Here many more types of etiology contribute to life-limiting pathology, to which programmatic etiologies contribute to some degree, and senescence is more multifactorial. Preventing programmatic pathophysiology that causes reproductive death causes very large increases in lifespan, giving a false impression that the entire aging process has been suppressed. All images reproduced with permission.

Neuroendocrine Promotion of Semelparous and Iteroparous Aging

Further evidence of conservation of mechanisms of aging between *C. elegans* and iteroparous species (e.g., *Drosophila*, rodents) is that insulin/IGF-1 and mTOR signaling promote aging in both (Kenyon, 2010; Weichhart, 2018). However, in *C. elegans* the magnitude (in relative terms) of life extension resulting from reduced IIS is typically far greater than in iteroparous species; for example, mutational reduction of PI3K increases median lifespan by up to ∼10-fold in *C. elegans* but

only ∼1.07- and ∼1.02-fold in *Drosophila* and mice, respectively (Ayyadevara et al., 2008; Slack et al., 2011; Foukas et al., 2013). This is consistent with the idea that programmatic etiologies occur in both semelparous and iteroparous species, are amplified in the former, and promoted by IIS in both. According to this view, although the large magnitude of the effect of IIS on lifespan in *C. elegans* reflects suppression of reproductive death, the etiology of senescent pathology in reproductive death is fundamentally similar to that of some senescent pathologies that contribute to late-life mortality in iteroparous species (including humans) (**Figure 7B**).

Such effects of IIS on aging are part of a broader neuroendocrine and steroid hormone signaling network affecting growth, reproduction and lifespan in both semelparous and iteroparous organisms (Finch, 1990a; Partridge and Gems, 2002; Gáliková et al., 2011; Atwood et al., 2017; Bartke, 2019). For example, in *C. elegans* sensory neurons exert IIS-mediated effects on lifespan (Apfeld and Kenyon, 1999), and germline effects on lifespan are mediated by steroid signaling (Antebi, 2013). In octopus the optic gland, equivalent to the pituitary gland, promotes vitellogenesis and reproductive death (Wodinsky, 1977). Reproductive death in salmon and *Antechinus* is driven by adrenohypercorticism (Barker et al., 1978; Mcquillan et al., 2003). In amphibians, reptiles and birds, vitellogenesis is promoted by growth hormone (GH) and estrogen (Dolphin et al., 1971; Wallace, 1985). In mammals pituitary GH acts through IGF-1 to promote gonadal growth and reproduction (Chandrashekar et al., 2004) and of course female reproductive function is regulated by estrogen. Taken together this, again, supports the view that reproductive death evolves by exaggeration of mechanisms (here endocrine) operative in iteroparous species.

Reproductive Death and Adaptive Death

Besides semelparous reproductive death and iteroparous senescence, another mode of life-limiting mechanism is programmed adaptive death. Here genetically determined mechanisms that actively cause death have evolved by natural selection because earlier death provides an inclusive fitness benefit, in a manner analogous to programmed cell death in metazoan organisms. Adaptive death is not expected to evolve in organisms with outbred, dispersed populations (e.g., most animal species), but can occur in those existing as compact (viscous) colonies of clonal individuals, such as the yeast *Saccharomyces cerevisiae* and possibly *C. elegans* too (Galimov et al., 2019; Lohr et al., 2019; Galimov and Gems, 2021). Evolutionary theory predicts that adaptive death can more readily evolve in the presence of semelparity, which could explain the apparent

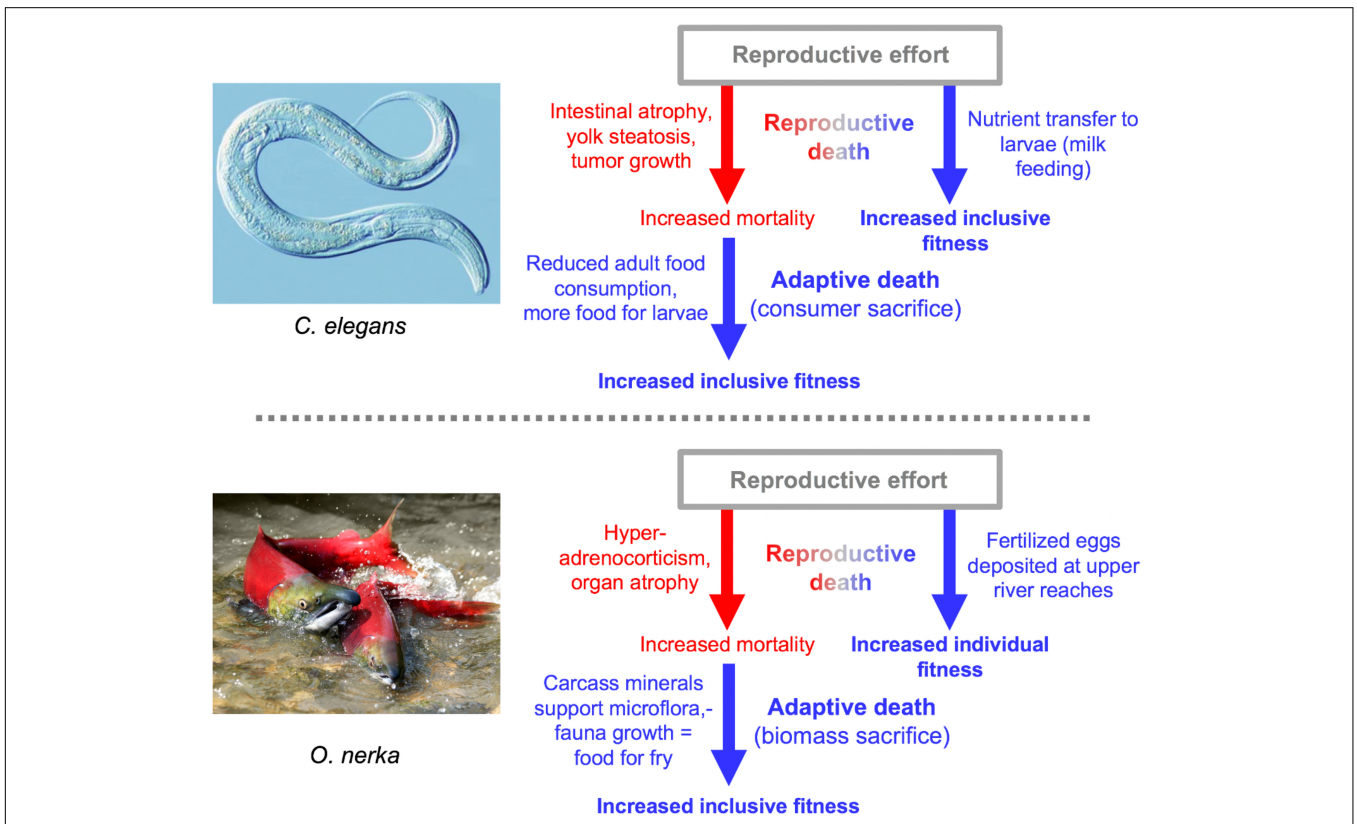

FIGURE 8 | Double death: Reproductive death and adaptive death combine to promote fitness. In both cases there is evidence for the existence of adaptive death, but its existence has not been definitively proven (Galimov and Gems, 2021). The two examples of double death differ in that adaptive death in *C. elegans* involves consumer sacrifice (removing a consumer to increase food availability for kin) while in *O. nerka* it involves biomass sacrifice (dying to facilitate resource remobilization) (Lohr et al., 2019).

presence of both in *C. elegans* and Pacific salmon (Galimov and Gems, 2021; **Figure 8**).

PERSPECTIVES

This essay presents an altered picture both of *C. elegans* as a model for aging research, and of aging more broadly. These changes imply some gains to the field, but also one grievous loss. The gains include an understanding that *C. elegans* is semelparous, and that the mechanisms involved in semelparous aging are a programmatic subset of those involved in iteroparous aging. This implies that *C. elegans* is an excellent model for studying programmatic mechanisms of senescence in a conveniently exaggerated and relatively pure form. Programmatic mechanisms potentially contribute to many diseases of human aging, for example those promoted by senescent cells, which are at least partly caused by quasi-programs (Blagosklonny, 2006). It also suggests that suppression of such mechanisms could unmask and bring into play more of the determinants of lifespan that are operative in iteroparous species. Recognition of the continuum between mechanisms of semelparous and iteroparous aging also removes a spurious separation between the biology of animal aging and plant senescence; from henceforth, scientists studying plant senescence ought to receive more invitations to biogerontology meetings.

Regarding the loss. Aging is now the main cause of disease and death worldwide, and yet its underlying mechanisms remain unclear. The discovery over three decades ago that single gene mutations can greatly increase lifespan in *C. elegans* (Klass, 1983; Friedman and Johnson, 1988; Kenyon et al., 1993) had extraordinary implications. First, the large increases in lifespan suggested the existence of core mechanisms underlying the entire aging process. Second, they implied that these mechanisms could be manipulated to slow down aging. Third, given that *C. elegans* is a highly tractable model organism, it suggested that it ought to be relatively easy to define these core mechanisms of aging. What is often exciting about studies in model organism biogerontology is the possibility that they bring us closer to a knowledge of these mysterious central mechanisms of aging, whose discovery promised to make possible extraordinary things in terms of slowing human aging and extending lifespan. The interpretations in this essay in some sense explain away the mystique of *C. elegans* life extension. We suggest that these large increases in lifespan could reflect suppression of reproductive death. This involves suppression of grossly exaggerated versions of programmatic mechanisms that are only one cause of aging in iteroparous organisms. More seriously, it also suggests that increases in lifespan achieved in iteroparous organisms may also reflect action on weaker programmatic determinants of senescence that are only a minor subset of the determinants of aging. This would imply relatively limited plasticity in aging in iteroparous organisms. Thus, the new picture that we present is, arguably, more realistic but less magical.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

DG wrote the manuscript, with contributions from CK, JN, and ME. All authors contributed to the article and approved the submitted version.

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REFERENCES

Allard, J. B., and Duan, C. (2011). Comparative endocrinology of aging and longevity regulation. *Front. Endocrinol.* 2:75. doi: 10.3389/fendo.2011.00075

Amrit, F. R. G., Boehnisch, C. M. L., and May, R. C. (2010). Phenotypic covariance of longevity, immunity and stress resistance in the *Caenorhabditis nematodes*. *PLoS One* 5:e9978. doi: 10.1371/journal.pone.0009978

Antebi, A. (2013). Steroid regulation of *C. elegans* diapause, developmental timing, and longevity. *Curr. Top. Dev. Biol.* 105, 181–212. doi: 10.1016/b978-0-12-396968-2.00007-5

Apfeld, J., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* 402, 804–809. doi: 10.1038/45544

Arantes-Oliveira, N., Apfeld, J., Dillin, A., and Kenyon, C. (2002). Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* 295, 502–505. doi: 10.1126/science.1065768

Arantes-Oliveira, N., Berman, J. R., and Kenyon, C. (2003). Healthy animals with extreme longevity. *Science* 302:611. doi: 10.1126/science.1089169

Asdell, S. A., Doornenbal, H., Joshi, S. R., and Sperling, G. A. (1967). The effects of sex steroid hormones upon longevity in rats. *J. Reprod. Fertil.* 14, 113–120. doi: 10.1530/jrf.0.0140113

Atwood, C. S., Hayashi, K., Meethal, S. V., Gonzales, T., and Bowen, R. L. (2017). Does the degree of endocrine dyscrasia post-reproduction dictate post-reproductive lifespan? lessons from semelparous and iteroparous species. *Geroscience* 39, 103–116. doi: 10.1007/s11357-016-9955-5

Austad, S. N. (2004). Is aging programed? *Aging Cell* 3, 249–251. doi: 10.1111/j.1474-9728.2004.00112.x

Avila-Ospina, L., Moison, M., Yoshimoto, K., and Masclaux-Daubresse, C. (2014). Autophagy, plant senescence, and nutrient recycling. *J. Exp. Botany* 65, 3799–3811. doi: 10.1093/jxb/eru039

Ayyadevara, S., Alla, R., Thaden, J. J., and Shmookler Reis, R. J. (2008). Remarkable longevity and stress resistance of nematode PI3K-null mutants. *Aging Cell* 7, 13–22. doi: 10.1111/j.1474-9726.2007.00348.x

Barker, I. K., Beveridge, I., Bradley, A. J., and Lee, A. K. (1978). Observations on spontaneous stress-related mortality among males of the dasyurid marsupial *Antechinus stuartii* Macleay. *Aust. J. Zool.* 26, 435–447. doi: 10.1071/zo9780435

Barnes, A. I., Boone, J. M., Jacobson, J., Partridge, L., and Chapman, T. (2006). No extension of lifespan by ablation of germ line in *Drosophila*. *Proc. R. Soc. Lond. B Biol. Sci.* 273, 939–947. doi: 10.1098/rspb.2005.3388

Bartke, A. (2019). Growth hormone and aging: updated review. *World J. Mens Health* 37, 19–30. doi: 10.5534/wjmh.180018

Benedetto, A., and Gems, D. (2019). Autophagy promotes visceral aging in wild-type *C. elegans*. *Autophagy* 15, 731–732. doi: 10.1080/15548627.2019.1569919

Benedusi, V., Martini, E., Kallikourdis, M., Villa, A., Meda, C., and Maggi, A. (2015). Ovariectomy shortens the life span of female mice. *Oncotarget* 6, 10801–10811. doi: 10.18632/oncotarget.2984

Benoit, J., Attardo, G., Baumann, A., Michalkova, V., and Aksoy, S. (2015). Adenotrophic viviparity in tsetse flies: potential for population control and as an insect model for lactation. *Annu. Rev. Entomol.* 60, 351–371. doi: 10.1146/annurev-ento-010814-020834

Bentley, P. J., and Follett, B. K. (1965). Fat and carbohydrate reserves in the river lamprey during spawning migration. *Life Sci.* 4, 2003–2007. doi: 10.1016/0024-3205(65)90058-5

Blagosklonny, M. V. (2006). Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* 5, 2087–2102. doi: 10.4161/cc.5.18.3288

Blagosklonny, M. V. (2008). Aging: ROS or TOR. *Cell Cycle* 7, 3344–3354.

Bonnet, X. (2011). "The evolution of semelparity," in *Reproductive Biology and Phylogeny of Snakes*, eds R. Aldridge and D. Sever (Boca Raton, FL: CRC Press), 645–672. doi: 10.1201/b10879-18

Bradley, A. J., Mcdonald, I. R., and Lee, A. K. (1980). Stress and mortality in a small marsupial (*Antechinus stuartii*. Macleay). *Gen. Comp. Endocrinol.* 40, 188–200. doi: 10.1016/0016-6480(80)90122-7

Braithwaite, R. W., and Lee, A. K. (1979). A mammalian example of semelparity. *Am. Nat.* 113, 151–155. doi: 10.1086/283372

Cargill, S. L., Carey, J. R., Muller, H. G., and Anderson, G. (2003). Age of ovary determines remaining life expectancy in old ovariectomized mice. *Aging Cell* 2, 185–190. doi: 10.1046/j.1474-9728.2003.00049.x

Carruth, L. L., Jones, R. E., and Norris, D. O. (2002). Cortisol and pacific salmon: a new look at the role of stress hormones in olfaction and home-stream migration. *Integ. Comp. Biol.* 42, 574–581. doi: 10.1093/icb/42.3.574

Chandrashekar, V., Zaczek, D., and Bartke, A. (2004). The consequences of altered somatotropic system on reproduction. *Biol. Reprod.* 71, 17–27. doi: 10.1095/biolreprod.103.027060

Chen, W. W., Yi, Y. H., Chien, C. H., Hsiung, K. C., Ma, T. H., Lin, Y. C., et al. (2016). Specific polyunsaturated fatty acids modulate lipid delivery and oocyte development in *C. elegans* revealed by molecular-selective label-free imaging. *Sci. Rep.* 6:32021.

Chen, Y., Qiao, S., Tuckermann, J., Okret, S., and Jondal, M. (2010). Thymus-derived glucocorticoids mediate androgen effects on thymocyte homeostasis. *FASEB J.* 24, 5043–5051. doi: 10.1096/fj.10.168724

Chiba, A., Ishida, H., Nishizawa, N. K., Makino, A., and Mae, T. (2003). Exclusion of ribulose-1,5-bisphosphate carboxylase/oxygenase from chloroplasts by specific bodies in naturally senescing leaves of wheat. *Plant Cell Physiol.* 44, 914–921. doi: 10.1093/pcp/pcg118

Cole, L. C. (1954). The population consequences of life history phenomena. *Q. Rev. Biol.* 29, 103–137. doi: 10.1086/400074

Conboy, I. M., and Rando, T. A. (2012). Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches. *Cell Cycle* 11, 2260–2267. doi: 10.4161/cc.20437

Davies, P. J., and Gan, S. (2012). Towards an integrated view of monocarpic plant senescence. *Russian J. Plant Physiol.* 59, 467–478. doi: 10.1134/s102144371204005x

de la Guardia, Y., Gilliat, A. F., Hellberg, J., Rennert, P., Cabreiro, F., and Gems, D. (2016). Run-on of germline apoptosis promotes gonad senescence in *C. elegans*. *Oncotarget* 7, 39082–39096. doi: 10.18632/oncotarget.9681

de Magalhães, J. P., and Church, G. M. (2005). Genomes optimize reproduction: aging as a consequence of the developmental program. *Physiology* 20, 252–259. doi: 10.1152/physiol.00010.2005

Depina, A. S., Iser, W. B., Park, S. S., Maudsley, S., Wilson, M. A., and Wolkow, C. A. (2011). Regulation of *Caenorhabditis elegans* vitellogenesis by DAF-2/IIS through separable transcriptional and posttranscriptional mechanisms. *BMC Physiol.* 11:11. doi: 10.1186/1472-6793-11-11

Diaz-Mendoza, M., Velasco-Arroyo, B., Santamaria, M. E., Gonzaìlez-Melendi, P., Martinez, M., and Diaz, I. (2016). Plant senescence and proteolysis: two processes with one destiny. *Genetics Mol. Biol.* 39, 329–338. doi: 10.1590/1678-4685-gmb-2016-0015

Dolphin, P. J., Ansari, A. Q., Lazier, C. B., Munday, K. A., and Akhtar, M. (1971). Studies on the induction and biosynthesis of vitellogenin, an oestrogen-induced glycolipophosphoprotein. *Biochem. J.* 124, 751–758. doi: 10.1042/bj1240751

Drewry, M. D., Williams, J. M., and Hatle, J. D. (2011). Life-extending dietary restriction and ovariectomy result in similar feeding rates but different physiologic responses in grasshoppers. *Exp. Gerontol.* 46, 781–786. doi: 10.1016/j.exger.2011.06.003

Drori, D., and Folman, Y. (1976). Environmental effects on longevity in the male rat: exercise, mating, castration and restricted feeding. *Exp. Gerontol.* 11, 25–32. doi: 10.1016/0531-5565(76)90007-3

Ezcurra, M., Benedetto, A., Sornda, T., Gilliat, A. F., Au, C., Zhang, Q., et al. (2018). *C. elegans* eats its own intestine to make yolk leading to multiple senescent pathologies. *Curr. Biol.* 28, 2544–2556. doi: 10.1016/j.cub.2018.06.035

Farrell, A. P. (2002). Coronary arteriosclerosis in salmon: growing old or growing fast? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 132, 723–735. doi: 10.1016/s1095-6433(02)00126-5

Finch, C. E. (1990a). *Longevity, Senescence and the Genome*. Chicago, IL: University of Chicago Press.

Finch, C. E. (Ed.). (1990b). "Rapid senescence and sudden death," in *Longevity, Senescence and the Genome* (Chicago, IL: University of Chicago Press), 43–119.

Flatt, T., Min, K. J., D'alterio, C., Villa-Cuesta, E., Cumbers, J., Lehmann, R., et al. (2008). Drosophila germ-line modulation of insulin signaling and lifespan. *Proc. Natl. Acad. Sci. U S A.* 105, 6368–6373. doi: 10.1073/pnas.0709128105

Flatt, T., and Schmidt, P. S. (2009). Integrating evolutionary and molecular genetics of aging. *Biochim. Biophys. Acta* 1790, 951–962. doi: 10.1016/j.bbagen.2009.07.010

Foukas, L. C., Bilanges, B., Bettedi, L., Pearce, W., Ali, K., Sancho, S., et al. (2013). Long-term p110alpha PI3K inactivation exerts a beneficial effect on metabolism. *EMBO Mol. Med.* 5, 563–571. doi: 10.1002/emmm.201201953

Freese, M., Yokota Rizzo, L., Pohlmann, J.-D., Marohn, L., Witten, P. E., Gremse, F., et al. (2019). Bone resorption and body reorganization during maturation induce maternal transfer of toxic metals in anguillid eels. *Proc. Natl. Acad. Sci. U S A.* 116, 11339–11344. doi: 10.1073/pnas.1817738116

Friedman, D. B., and Johnson, T. E. (1988). A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118, 75–86. doi: 10.1093/genetics/118.1.75

Gáliková, M., Klepsatel, P., Senti, G., and Flatt, T. (2011). Steroid hormone regulation of *C. elegans* and *Drosophila* aging and life history. *Exp. Gerontol.* 46, 141–147. doi: 10.1016/j.exger.2010.08.021

Galimov, E. R., and Gems, D. (2020). Shorter life and reduced fecundity can increase colony fitness in virtual *C. elegans*. *Aging Cell* 19:e13141.

Galimov, E. R., and Gems, D. (2021). Death happy: adaptive death and its evolution by kin selection in organisms with colonial ecology. *Philos. Trans. R. Soc. B* 376:20190730. doi: 10.1098/rstb.2019.0730

Galimov, E. R., Lohr, J. N., and Gems, D. (2019). When and how can death be an adaptation? *Biochemistry (Moscow)* 84, 1433–1437. doi: 10.1134/s0006297919120010

Garigan, D., Hsu, A. L., Fraser, A. G., Kamath, R. S., Ahringer, J., and Kenyon, C. (2002). Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* 161, 1101–1112. doi: 10.1093/genetics/161.3.1101

Gelino, S., and Hansen, M. (2012). Autophagy - an emerging anti-aging mechanism. *J. Clin. Exp. Pathol.* 4:006.

Gems, D., and de la Guardia, Y. (2013). Alternative perspectives on aging in *C. elegans*: reactive oxygen species or hyperfunction? *Antioxid. Redox Signal.* 19, 321–329. doi: 10.1089/ars.2012.4840

Gems, D., and Partridge, L. (2013). Genetics of longevity in model organisms: debates and paradigm shifts. *Annu. Rev. Physiol.* 75, 621–644. doi: 10.1146/annurev-physiol-030212-183712

Gems, D., and Riddle, D. L. (2000). Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* 154, 1597–1610. doi: 10.1093/genetics/154.4.1597

Gems, D., Sutton, A. J., Sundermeyer, M. L., Larsen, P. L., Albert, P. S., King, K. V., et al. (1998). Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* 150, 129–155. doi: 10.1093/genetics/150.1.129

Gipps, J. H. W., and Jewell, P. A. (1979). Maintaining populations of bank voles, *Clethrionomys glareolus*, in large outdoor enclosures, and measuring the response of population variables to the castration of males. *J. Anim. Ecol.* 48, 535–555. doi: 10.2307/4179

Gladyshev, V. N. (2013). The origin of aging: imperfectness-driven non-random damage defines the aging process and control of lifespan. *Trends Genet.* 29, 506–512. doi: 10.1016/j.tig.2013.05.004

Golden, T. R., Beckman, K. B., Lee, A. H., Dudek, N., Hubbard, A., Samper, E., et al. (2007). Dramatic age-related changes in nuclear and genome copy number in the nematode *Caenorhabditis elegans*. *Aging Cell* 6, 179–188. doi: 10.1111/j.1474-9726.2007.00273.x

Greer, E. L., Maures, T. J., Hauswirth, A. G., Green, E. M., Leeman, D. S., Maro, G. S., et al. (2010). Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in *C. elegans*. *Nature* 466, 383–387. doi: 10.1038/nature09195

Guiboileau, A., Avila-Ospina, L., Yoshimoto, K., Soulay, F., Azzopardi, M., Marmagne, A., et al. (2013). Physiological and metabolic consequences of autophagy deficiency for the management of nitrogen and protein resources in *Arabidopsis* leaves depending on nitrate availability. *New Phytol.* 199, 683–694. doi: 10.1111/nph.12307

Gumienny, T. L., Lambie, E., Hartwieg, E., Horvitz, H. R., and Hengartner, M. O. (1999). Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. *Development* 126, 1011–1022. doi: 10.1242/dev.126.5.1011

Hamilton, J. B. (1965). Relationship of castration, spaying, and sex to survival and duration of life in domestic cats. *J. Gerontol.* 20, 96–104. doi: 10.1093/geronj/20.1.96

Hamilton, J. B., Hamilton, R. S., and Mestler, G. E. (1969). Duration of life and causes of death in domestic cats: influence of sex, gonadectomy, and inbreeding. *J. Gerontol.* 24, 427–437. doi: 10.1093/geronj/24.4.427

Hamilton, J. B., and Mestler, G. E. (1969). Mortality and survival: comparison of eunuchs with intact men in a mentally retarded population. *J. Gerontol.* 24, 395–411. doi: 10.1093/geronj/24.4.395

Hane, S., and Robertson, O. H. (1959). Changes in plasma 17-hydroxycorticosteroids accompanying sexual maturation and spawning of the Pacific salmon (*Oncorhynchus tschawytscha*) and rainbow trout (*Salmo gairdnerii*). *Proc. Natl. Acad. Sci. U S A.* 45, 886–893. doi: 10.1073/pnas.45.6.886

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300. doi: 10.1093/geronj/11.3.298

Hatle, J. D., Paterson, C. S., Jawaid, I., Lentz, C., Wells, S. M., and Fronstin, R. B. (2008). Protein accumulation underlying lifespan extension via ovariectomy in grasshoppers is consistent with the disposable soma hypothesis but is not due to dietary restriction. *Exp. Gerontol.* 43, 900–908. doi: 10.1016/j.exger.2008.08.005

Haveì, M., Balliau, T., Cottyn-Boitte, B., DeìRond, E., Cueff, G., Soulay, F., et al. (2018). Increases in activity of proteasome and papain-like cysteine protease in *Arabidopsis* autophagy mutants: back-up compensatory effect or cell-death promoting effect? *J. Exp. Botany* 69, 1369–1385. doi: 10.1093/jxb/erx482

Hayes, G. L. T., Simmons, L. W., Dugand, R. J., Mills, H. R., Roberts, J. D., Tomkins, J. L., et al. (2019). Male semelparity and multiple paternity confirmed in an arid-zone dasyurid. *J. Zool.* 308, 266–273. doi: 10.1111/jzo.12672

Herndon, L. A., Schmeissner, P. J., Dudaronek, J. M., Brown, P. A., Listner, K. M., Sakano, Y., et al. (2002). Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature* 419, 808–814. doi: 10.1038/nature01135

Higashi, N., Wake, K., Sato, M., Kojima, N., Imai, K., and Senoo, H. (2005). Degradation of extracellular matrix by extrahepatic stellate cells in the intestine of the lamprey, Lampetra japonica. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 285, 668–675. doi: 10.1002/ar.a.20200

Hoffman, J. M., Creevy, K. E., and Promislow, D. E. L. (2013). Reproductive capability is associated with lifespan and cause of death in companion dogs. *PLoS One* 8:e61082. doi: 10.1371/journal.pone.0061082

Hoffman, J. M., O'Neill, D. G., Creevy, K. E., and Austad, S. N. (2018). Do female dogs age differently than male dogs? *J. Gerontol. A Biol. Sci. Med. Sci.* 73, 150–156. doi: 10.1093/gerona/glx061

Hotzi, B., Kosztelnik, M., Hargitai, B., Takács-Vellai, K., Barna, J., Bördén, K., et al. (2018). Sex-specific regulation of aging in *Caenorhabditis elegans*. *Aging Cell* 17:e12724. doi: 10.1111/acel.12724

House, E. W., and Benditt, E. P. (1981). The ultrastructure of spontaneous coronary arterial lesions in steelhead trout (*Salmo gairdneri*). *Am. J. Pathol.* 104, 250–257.

Hsin, H., and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* 399, 362–366. doi: 10.1038/20694

Hughes, P. W. (2017). Between semelparity and iteroparity: empirical evidence for a continuum of modes of parity. *Ecol. Evol.* 7, 8232–8261. doi: 10.1002/ece3.3341

Ishida, H., Yoshimoto, K., Izumi, M., Reisen, D., Yano, Y., Makino, A., et al. (2008). Mobilization of Rubisco and stroma- localized fluorescent proteins of chloroplasts to the vacuole by an ATG gene-dependent autophagic process. *Plant Physiol.* 148, 142–155. doi: 10.1104/pp.108.122770

Janzen, D. H. (1976). Why bamboos wait so long to flower. *Ann. Rev. Ecol. Syst.* 7, 347–391. doi: 10.1146/annurev.es.07.110176.002023

Jaramillo-Lambert, A., Ellefson, M., Villeneuve, A. M., and Engebrecht, J. (2007). Differential timing of S phases, X chromosome replication, and meiotic prophase in the *C. elegans* germ line. *Dev. Biol.* 308, 206–221. doi: 10.1016/j.ydbio.2007.05.019

Jewell, P. (1997). Survival and behaviour of castrated Soay sheep (*Ovis aries*) in a feral island population on Hirta, St. Kilda, Scotland. *J. Zool.* 243, 623–636. doi: 10.1111/j.1469-7998.1997.tb02806.x

Kenyon, C. (2010). The genetics of ageing. *Nature* 464, 504–512.

Kenyon, C., Chang, J., Gensch, E., Rudener, A., and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366, 461–464. doi: 10.1038/366461a0

Kern, C. C., Srivastava, S., Ezcurra, M., Hui, N., Townsend, S., Maczik, D., et al. (2020). *C. elegans* hermaphrodites undergo semelparous reproductive death. *BioRxiv* (Preprint). doi: 10.1101/2020.1111.1116.384255

Kern, C. C., Townsend, S., Salzmann, A., Rendell, N., Taylor, G., Comisel, R. M., et al. (2021). *C. elegans* feed yolk to their young in a form of primitive lactation. *Nat. Commun.*

Kimble, J., and Sharrock, W. J. (1983). Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. *Dev. Biol.* 96, 189–196. doi: 10.1016/0012-1606(83)90322-6

Kiontke, K., FeìLix, M.-A., Ailion, M., Rockman, M., Braendle, C., PeìNigault, J.-B., et al. (2011). A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evol. Biol.* 11:339. doi: 10.1186/1471-2148-11-339

Kirkwood, T. B. (2005). Understanding the odd science of aging. *Cell* 120, 437–447. doi: 10.1016/j.cell.2005.01.027

Kirkwood, T. B. L. (1977). Evolution of ageing. *Nature* 270, 301–304.

Kirkwood, T. B. L., and Rose, M. R. (1991). Evolution of senescence: late survival sacrificed for reproduction. *Phil. Trans. R. Soc. London* 332, 15–24. doi: 10.1098/rstb.1991.0028

Klass, M. R. (1977). Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech. Ageing Dev.* 6, 413–429. doi: 10.1016/0047-6374(77)90043-4

Klass, M. R. (1983). A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. *Mech. Ageing Dev.* 22, 279–286. doi: 10.1016/0047-6374(83)90082-9

Klass, M. R., and Hirsh, D. I. (1976). Nonaging developmental variant of *C. elegans*. *Nature* 260, 523–525. doi: 10.1038/260523a0

Kocsisova, Z., Kornfeld, K., and Schedl, T. (2019). Rapid population-wide declines in stem cell number and activity during reproductive aging in *C. elegans*. *Development* 146:dev173195.

Labbadia, J., and Morimoto, R. I. (2014). Proteostasis and longevity: when does aging really begin? *F1000Prime Rep* 6:7.

Larsen, L. O. (1965). Effects of hypophysectomy in the cyclostome, *Lampetra fluviatilis* (l.) gray. *Gen. Comp. Endocrinol.* 5, 16–30. doi: 10.1016/0016-6480(65)90064-x

Larsen, L. O. (1969). Effects of hypophysectomy before and during sexual maturation in the cyclostome, *Lampetra fluviatilis* (L.) Gray. *Gen. Comp. Endocrinol.* 12, 200–208. doi: 10.1016/0016-6480(69)90192-0

Larsen, L. O. (1974). Effects of testosterone and oestradiol on gonadectomized and intact male and female river lampreys (*Lampetra fluviatilis* (L.) Gray). *Gen. Comp. Endocrinol.* 24, 305–313. doi: 10.1016/0016-6480(74)90184-1

Larsen, L. O. (1980). Physiology of adult lampreys, with special regard to natural starvation, reproduction, and death after spawning. *Can. J. Fish. Aquat. Sci.* 37, 1762–1779. doi: 10.1139/f80-221

LeBoeuf, A. C. (2017). Trophallaxis. *Curr. Biol.* 27, R1299–R1300.

Leopold, A. C., Niedergang-Kamien, E., and Janick, J. (1959). Experimental modification of plant senescence. *Plant Physiol.* 34, 570–573. doi: 10.1104/pp.34.5.570

Libina, N., Berman, J. R., and Kenyon, C. (2003). Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell* 115, 489–502. doi: 10.1016/s0092-8674(03)00889-4

Lin, K., Hsin, H., Libina, N., and Kenyon, C. (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat. Genet.* 28, 139–145. doi: 10.1038/88850

Lohr, J., Galimov, E. R., and Gems, D. (2019). Does senescence promote fitness in *Caenorhabditis elegans* by causing death? *Ageing Res. Rev.* 50, 58–71. doi: 10.1016/j.arr.2019.01.008

Luo, S., Kleemann, G. A., Ashraf, J. M., Shaw, W. M., and Murphy, C. T. (2010). TGF-beta and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. *Cell* 143, 299–312. doi: 10.1016/j.cell.2010.09.013

Maklakov, A. A., and Chapman, T. (2019). Evolution of ageing as a tangle of trade-offs: energy versus function. *Proc. Biol. Sci.* 286:20191604. doi: 10.1098/rspb.2019.1604

Maldonado, T. A., Jones, R. E., and Norris, D. O. (2000). Distribution of beta-amyloid and amyloid precursor protein in the brain of spawning (senescent) salmon: a natural, brain-aging model. *Brain Res.* 858, 237–251. doi: 10.1016/s0006-8993(99)02328-8

Maldonado, T. A., Jones, R. E., and Norris, D. O. (2002). Intraneuronal amyloid precursor protein (APP) and appearance of extracellular beta-amyloid peptide (abeta) in the brain of aging kokanee salmon. *J. Neurobiol.* 53, 11–20. doi: 10.1002/neu.10086

Marchal, E., Hult, E., Huang, J., Stay, B., and Tobe, S. (2013). Diploptera punctata as a model for studying the endocrinology of arthropod reproduction and development. *Gen. Comp. Endocrinol.* 188, 85–93. doi: 10.1016/j.ygcen.2013.04.018

Marshall, R. S., and Vierstra, R. D. (2018). Autophagy: the master of bulk and selective recycling. *Annu. Rev. Plant Biol.* 69, 173–208. doi: 10.1146/annurev-arplant-042817-040606

Martinez, D. E., Costa, M. L., and Guiamet, J. J. (2008). Senescence-associated degradation of chloroplast proteins inside and outside the organelle. *Plant Biol.* 10, 15–22. doi: 10.1111/j.1438-8677.2008.00089.x

Maynard Smith, J. (1958). The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura*. *J. Exp. Biol.* 35, 832–842. doi: 10.1242/jeb.35.4.832

McCulloch, D. (2003). *Sex Differences in Ageing in the Nematode Caenorhabditis elegans*. London: University College London.

McCulloch, D., and Gems, D. (2007). Sex-specific effects of the DAF-12 steroid receptor on aging in *Caenorhabditis elegans*. *Ann. N Y Acad. Sci.* 1119, 253–259. doi: 10.1196/annals.1404.018

McGee, M. D., Day, N., Graham, J., and Melov, S. (2012). cep-1/p53-dependent dysplastic pathology of the aging *C. elegans* gonad. *Aging* 4, 256–269. doi: 10.18632/aging.100448

McGee, M. D., Weber, D., Day, N., Vitelli, C., Crippen, D., Herndon, L. A., et al. (2011). Loss of intestinal nuclei and intestinal integrity in aging *C. elegans*. *Aging Cell* 10, 699–710. doi: 10.1111/j.1474-9726.2011.00713.x

McGhee, J. D. (2007). The *C. elegans* intestine. *WormBook* 1–36. doi: 10.1007/978-3-319-23534-9_1

Mcquillan, H. J., Lokman, P. M., and Young, G. (2003). Effects of sex steroids, sex, and sexual maturity on cortisol production: an in vitro comparison of chinook salmon and rainbow trout interrenals. *Gen. Comp. Endocrinol.* 133, 154–163. doi: 10.1016/s0016-6480(03)00163-1

Mewes, K. R., Latz, M., Golla, H., and Fischer, A. (2002). Vitellogenin from female and estradiol-stimulated male river lampreys (*Lampetra fluviatilis* L.). *J. Exp. Zool.* 292, 52–72. doi: 10.1002/jez.1142

Michell, A. R. (1999). Longevity of British breeds of dog and its relationships with sex, size, cardiovascular variables and disease. *Vet. Rec.* 145, 625–629. doi: 10.1136/vr.145.22.625

Min, K.-J., Lee, C.-K., and Park, H.-N. (2012). The lifespan of Korean eunuchs. *Curr. Biol.* 22, R792–R793.

Munkácsy, E., and Rea, S. L. (2014). The paradox of mitochondrial dysfunction and extended longevity. *Exp. Gerontol.* 56, 221–233. doi: 10.1016/j.exger.2014.03.016

Naylor, R., Richardson, S. J., and Mcallan, B. M. (2008). Boom and bust: a review of the physiology of the marsupial genus Antechinus. *J. Comp. Physiol. B* 178, 545–562. doi: 10.1007/s00360-007-0250-8

Niccoli, T., and Partridge, L. (2012). Ageing as a risk factor for disease. *Curr. Biol.* 22, R741–R752.

Olsen, P. D. (1971). Differential mortality of *Antechinus stuartii* (Macleay): nitrogen balance and somatic changes. *Aust. J. Zool.* 19, 347–353. doi: 10.1071/zo9710347

O'Neill, D. G., Church, D. B., Mcgreevy, P. D., Thomson, P. C., and Brodbelt, D. C. (2013). Longevity and mortality of owned dogs in England. *Vet. J.* 198, 638–643. doi: 10.1016/j.tvjl.2013.09.020

O'Neill, D. G., Church, D. B., Mcgreevy, P. D., Thomson, P. C., and Brodbelt, D. C. (2015). Longevity and mortality of cats attending primary care veterinary practices in England. *J. Feline Med. Surg.* 17, 125–133. doi: 10.1177/1098612x14536176

Palikaras, K., Mari, M., Petanidou, B., Pasparaki, A., Filippidis, G., and Tavernarakis, N. (2017). Ectopic fat deposition contributes to age-associated pathology in *Caenorhabditis elegans*. *J. Lipid Res.* 58, 72–80. doi: 10.1194/jlr.m069385

Pankhurst, N. W., and Sorensen, P. W. (1984). Degeneration of the alimentary tract in sexually maturing European *Anguilla anguilla* (L.) and *American eels Anguilla rostrata* (LeSueur). *Can. J. Zool.* 62, 1143–1149. doi: 10.1139/z84-165

Parker, W. H., Broder, M. S., Chang, E., Feskanich, D., Farquhar, C., Liu, Z., et al. (2009). Ovarian conservation at the time of hysterectomy and long- term health outcomes in the nurses' health study. *Obstet. Gynecol.* 113, 1027–1037. doi: 10.1097/aog.0b013e3181a11c64

Partridge, L., and Gems, D. (2002). Mechanisms of ageing: public or private? *Nat. Rev. Genet.* 3, 165–175.

Partridge, L., and Sibly, R. (1991). Constraints in the evolution of life histories. *Phil. Trans. Roy. Soc. Lond. B.* 332, 3–13.

Peterson, L. W., and Huffaker, R. C. (1975). Loss of ribulose 1,5-diphosphate carboxylase in proteolytic activity during senescence of detached primary barley leaves. *Plant Physiol.* 55, 1009–1015. doi: 10.1104/pp.55.6.1009

Pickering, A. D. (1976). Stimulation of intestinal degeneration by oestradiol and testosterone implantation in the migrating river lamprey, *Lampetra fluviatilis* L. *Gen. Comp. Endocrinol.* 30, 340–346. doi: 10.1016/0016-6480(76)90085-x

Quinn, T. P., and Foote, C. J. (1994). The effects of body size and sexual dimorphism on the reproductive behaviour of sockeye salmon, *Oncorhynchus nerka*. *Anim. Behav.* 48, 751–761. doi: 10.1006/anbe.1994.1300

Rea, S. L. (2005). Metabolism in the *Caenorhabditis elegans* Mit mutants. *Exp. Gerontol.* 40, 841–849. doi: 10.1016/j.exger.2005.06.015

Robertson, O. H. (1961). Prolongation of the life span of kokanee salmon (*Oncorhynchus nerka* kennerlyi) by castration before the beginning of gonad development. *Proc. Natl. Acad. Sci. U S A.* 47, 609–621. doi: 10.1073/pnas.47.4.609

Robertson, O. H., and Wexler, B. C. (1962). Histological changes in the organs and tissues of senile castrated kokanee salmon (*Oncorhynchus nerka* kennerlyi). *Gen. Comp. Endocrinol.* 2, 458–472. doi: 10.1016/0016-6480(62)90044-8

Robertson, O. H., Wexler, B. C., and Miller, B. F. (1961). Degenerative changes in the cardiovascular system of the spawning Pacific salmon (*Oncorhynchus tshawytscha*). *Circ. Res.* 9, 826–834. doi: 10.1161/01.res.9.4.826

Rocca, W., Grossardt, B., De Andrade, M., Malkasian, G., and Melton, L. J. (2006). Survival patterns after oophorectomy in premenopausal women: a population-based cohort study. *Lancet Oncol.* 7, 821–828. doi: 10.1016/s1470-2045(06)70869-5

Sakuraba, Y., Lee, S.-H., Kim, Y.-S., Park, O., Hörtensteiner, S., and Paek, N.-C. (2014). Delayed degradation of chlorophylls and photosynthetic proteins in *Arabidopsis* autophagy mutants during stress- induced leaf yellowing. *J. Exp. Botany* 65, 3915–3925. doi: 10.1093/jxb/eru008

Schippers, J., Schmidt, R., Wagstaff, C., and Jing, H.-C. (2015). Living to die and dying to live: the survival strategy behind leaf senescence. *Plant Physiol.* 169, 914–930. doi: 10.1104/pp.15.00498

Shaw, A. C., Joshi, S., Greenwood, H., Panda, A., and Lord, J. M. (2010). Aging of the innate immune system. *Curr. Opin. Immunol.* 22, 507–513.

Shore, D. E., and Ruvkun, G. (2013). A cytoprotective perspective on longevity regulation. *Trends Cell. Biol.* 23, 409–420. doi: 10.1016/j.tcb.2013.04.007

Shoupe, D., Parker, W. H., Broder, M. S., Liu, Z., Farquhar, C., and Berek, J. S. (2007). Elective oophorectomy for benign gynecological disorders. *Menopause* 14, 580–585. doi: 10.1097/gme.0b013e31803c56a4

Slack, C., Giannakou, M. E., Foley, A., Goss, M., and Partridge, L. (2011). dFOXO-independent effects of reduced insulin-like signaling in Drosophila. *Aging Cell* 10, 735–748. doi: 10.1111/j.1474-9726.2011.00707.x

Soderstrom, T. R., and Calderon, C. E. (1979). A commentary on the bamboos (Poaceae: Bambusoideae). *Biotropica* 11, 161–172. doi: 10.2307/2388036

Sornda, T., Ezcurra, M., Kern, C., Galimov, E. R., Au, C., De La Guardia, Y., et al. (2019). Production of YP170 vitellogenins promotes intestinal senescence in *C. elegans*. *J. Gerontol. A* 74, 1180–1188. doi: 10.1093/gerona/glz067

Speakman, J. R. (2008). The physiological costs of reproduction in small mammals. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 375–398. doi: 10.1098/rstb.2007.2145

Talbert, G. B., and Hamilton, J. B. (1965). Duration of life in Lewis strain of rats after gonadectomy at birth and at other ages. *J. Gerontol.* 20, 489–491.

Tang, J., and Bassham, D. C. (2018). Autophagy in crop plants: what's new beyond *Arabidopsis*? *Open Biol.* 8:180162. doi: 10.1098/rsob.180162

Tesch, F.-W. (1977). *The Eel*. London: Chapman and Hall.

van Deursen, J. M. (2014). The role of senescent cells in ageing. *Nature* 509, 439–446. doi: 10.1038/nature13193

Van Raamsdonk, J. M., and Hekimi, S. (2009). Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. *PLoS Genet* 5:e1000361. doi: 10.1371/journal.pgen.1000361

Van Raamsdonk, J. M., and Hekimi, S. (2010). Reactive oxygen species and aging in *Caenorhabditis elegans*: causal or casual relationship? *Antioxid. Redox Signal.* 13, 1911–1953. doi: 10.1089/ars.2010.3215

Venz, R., Pekec, T., Katic, I., Ciosk, R., and Ewald, C. Y. (2021). End-of-life targeted auxin-mediated degradation of DAF-2 Insulin/IGF-1 receptor promotes longevity free from growth-related pathologies. *BioRxiv* [Preprint] doi: 10.1101/2021.1105.1131.446422

Vladykov, V. D. (1956). *The eel*. Quebec: Dept. de Pêcheries Province du Québec.

von der Decken, A. (1992). Physiological changes in skeletal muscle by maturation-spawning of non-migrating female atlantic salmon, Salmo salar. *Comp. Biochem. Physiol.* 101B, 299–301. doi: 10.1016/0305-0491(92)90002-9

Wada, S., Ishida, H., Izumi, M., Yoshimoto, K., Ohsumi, Y., Mae, T., et al. (2009). Autophagy plays a role in chloroplast degradation during senescence in individually darkened leaves. *Plant Physiol.* 149, 885–893. doi: 10.1104/pp.108.130013

Wallace, R. A. (1985). "Vitellogenesis and oocyte growth in nonmammalian vertebrates," in *Developmental Biology*, ed. L. W. Browder (New York, NY: Plenum Press), 127–177. doi: 10.1007/978-1-4615-6814-8_3

Wang, H., Zhang, Z., and Gems, D. (2018a). Monsters in the uterus: teratoma-like tumors in senescent *C. elegans* result from a parthenogenetic quasi-program. *Aging* 10, 1188–1189. doi: 10.18632/aging.101486

Wang, H., Zhao, Y., Ezcurra, M., Benedetto, A., Gilliat, A., Hellberg, J., et al. (2018b). A parthenogenetic quasi-program causes teratoma-like tumors during aging in wild-type *C. elegans*. *NPJ Aging Mech. Dis.* 4:6. doi: 10.1038/s41514-018-0025-3

Ward, S., and Carrel, J. S. (1979). Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. *Dev. Biol.* 73, 304–321. doi: 10.1016/0012-1606(79)90069-1

Weichhart, T. (2018). mTOR as regulator of lifespan, aging, and cellular senescence: a mini-review. *Gerontology* 64, 127–134. doi: 10.1159/000484629

Williams, G. C. (1957). Pleiotropy, natural selection and the evolution of senescence. *Evolution* 11, 398–411. doi: 10.2307/2406060

Wodinsky, J. (1977). Hormonal inhibition of feeding and death in octopus: control by optic gland secretion. *Science* 198, 948–951. doi: 10.1126/science.198.4320.948

Wolke, U., Jezuit, E. A., and Priess, J. R. (2007). Actin-dependent cytoplasmic streaming in *C. elegans* oogenesis. *Development* 134, 2227–2236. doi: 10.1242/dev.004952

Woodruff, G. C., Johnson, E., and Phillips, P. C. (2019). A large close relative of *C. elegans* is slow-developing but not long-lived. *BMC Evol. Biol.* 19:74. doi: 10.1186/s12862-019-1388-1

Woolley, P. (1966). "Reproduction in *Antechinus spp*. and other dasyurid marsupials," in *Proceedings of the Comparative Biology of Reproduction in Mammals. 15th Symposium of the Zoology Society, London* (New York, NY: Academic Press), 281–294.

Yi, Y.-H., Chien, C.-H., Chen, W.-W., Ma, T.-H., Liu, K.-Y., Chang, Y.-S., et al. (2014). Lipid droplet pattern and nondroplet-like structure in two fat mutants of *Caenorhabditis elegans* revealed by coherent anti-Stokes Raman scattering microscopy. *J. Biomed. Optics* 19:011011. doi: 10.1117/1.jbo.19.1.011011

Young, T. P., and Augspurger, C. K. (1991). Ecology and evolution of long-lived semelparous plants. *TREE* 6, 285–289. doi: 10.1016/0169-5347(91)90006-j

Zera, A. J., and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* 32, 95–126. doi: 10.1146/annurev.ecolsys.32.081501.114006

Zhang, C., and Cuervo, A. M. (2008). Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat. Med.* 14, 959–965. doi: 10.1038/nm.1851

Ziuganov, V. V. (2005). A paradox of parasite prolonging the life of its host. Pearl mussel can disable the accelerated senescence program in salmon. *Biol. Bull.* 32, 360–365. doi: 10.1007/s10525-005-0112-4

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GLOSSARY

Adaptive death: Synonymous with programmed organismal death. Here death of an individual is a selected trait, providing a direct benefit in terms of inclusive or group fitness (Lohr et al., 2019).
Aging: In the context of this article, synonymous with senescence, i.e., the deteriorative changes that become progressively worse with advancing age, leading to multiple pathologies and death. We argue here that reproductive death is a form of rapid aging, since it involves mechanisms that also contribute to iteroparous aging. From this perspective, leaf senescence may be viewed as a form of aging.
Androdioecious: Where adults are male or hermaphrodite (as opposed, in the context of this essay, to male or female).
Antagonistic pleiotropy *(AP):* Where action of a given gene leads to both fitness benefits and fitness costs. If the latter occur later in life and are therefore subject to weaker selection, such a gene may be favored by natural selection, and promote aging (Williams, 1957).
Costly program *(New term):* A biological program that simultaneously causes fitness benefits, and costs in terms of pathological changes to tissues or organs where the program is executed. One form of programmatic mechanism involving hyperfunction by which AP causes senescence (cf. quasi-program).
Demolition engineer principle *(New term):* Dual function of autophagic processes during resource remobilization, to both effect destructive turnover of cellular components, and maintain cellular homeostasis.
Disposable soma: Theory proposing that natural selection favors investment of limited resources into reproduction rather than somatic maintenance, accelerating damage accumulation and, therefore, senescence (Kirkwood, 1977).
Fitness: The measure of how well a given species is able to survive and reproduce.
Gonochoristic: Where adults are male or female (as opposed, in the context of this essay, to male or hermaphrodite).
Hyperfunction: Where wild-type gene function actively leads to senescent pathology, as opposed to passive random damage or wear and tear (Blagosklonny, 2006).
Iteroparous: Where multiple reproductive cycles can occur over the course of a lifetime.
Programmed aging: Senescence caused by a relatively ordered series of biological processes that promotes fitness via inclusive fitness or group fitness.
Programmatic aging: Where complex biological processes contributes to senescence, but not necessarily to fitness (cf. quasi-programs, costly programs).
Quasi-programmed aging: Senescence caused by a relatively ordered series of biological processes that does not promote fitness; may occur due to futile run-on of wild-type programs that promote fitness earlier in life (Blagosklonny, 2006).
Reproductive death: A form of suicidal reproductive effort found in some semelparous species (e.g., Pacific salmon, monocarpic plants). Here, reproductive maturity triggers the rapid development of lethal pathologies and fast senescence coupled to reproductive success (Finch, 1990b).
Resource reallocation: Where the building blocks of life (e.g., amino acids, lipids, carbohydrates) are transferred from one site (e.g., tissue, organ) to another, in a manner that typically involves breakdown (e.g., autophagic) of source biomass.
Run-on: Futile continuation of gene function or processes in later life, leading to pathology (de la Guardia et al., 2016) (cf. quasi-program).
Semelparous: Organisms with a single reproductive episode before death. Also used to denote semelparity with reproductive death.
Senescence: The overall process of deterioration with age or the resulting pathological condition (not to be confused with *cellular senescence*, which is a particular form of cell cycle arrest affecting some vertebrate cell types). Although *aging* has several meanings, in the biological context it is usually synonymous with senescence.
Source-to-sink biomass conversion: Resource remobilization where autophagic processes break down cellular constituents in one tissue/organ to provide resources for another (cf. costly program).

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REVIEW
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Lifespan Extension in Long-Lived Vertebrates Rooted in Ecological Adaptation

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Contemporary studies on aging and longevity have largely overlooked the role that adaptation plays in lifespan variation across species. Emerging evidence indicates that the genetic signals of extended lifespan may be maintained by natural selection, suggesting that longevity could be a product of organismal adaptation. The mechanisms of adaptation in long-lived animals are believed to account for the modification of physiological function. Here, we first review recent progress in comparative biology of long-lived animals, together with the emergence of adaptive genetic factors that control longevity and disease resistance. We then propose that hitchhiking of adaptive genetic changes is the basis for lifespan changes and suggest ways to test this evolutionary model. As individual adaptive or adaptation-linked mutations/substitutions generate specific forms of longevity effects, the cumulative beneficial effect is largely nonrandom and is indirectly favored by natural selection. We consider this concept in light of other proposed theories of aging and integrate these disparate ideas into an adaptive evolutionary model, highlighting strategies in decoding genetic factors of lifespan control.

Keywords: longevity, adaptive-hitchhiking, natural selection, aging, evolution theory

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INTRODUCTION

The aging process, also known as senescence, involves the gradual decline in vitality as a result of deterioration of physiological and biochemical functions, which subsequently leads to increased morbidity and mortality. Most of the organisms age, but some age slower than others (Finch, 1998), driving the fundamental question what underlying factors contribute to variation in aging processes across species, as well as to potential interventions to extend healthspan in humans (Reichard, 2016). Current research is hinged on the early theories of aging (Medawar, 1952; Harman, 1956; Williams, 1957; Hamilton, 1966) with supporting empirical evidence (Luckinbill et al., 1984; Austad, 1993; Shattuck and Williams, 2010). It is now well established that the mechanisms that control aging processes are under genetic regulation (Guarente and Kenyon, 2000; de Magalhães et al., 2007) and that these genetic regulators are constrained by environmental factors (Clare and Luckinbill, 1985; Williams and Day, 2003; Jobson et al., 2010). However, right from the onset, since the days of Charles Darwin, the evolvability of longevity, which means a duration of aging, has remained contentious, evolving alongside the concept of evolution (Gavrilov and Gavrilova, 2002). In the context of evolution by natural selection, longevity, but not aging, should be beneficial to species propagation; therefore, aging should have faded out to give way for

immortal species. This is in contrast to the prevailing evidence that, while vertebrates eventually age and die, various species with genetic proximity, such as rodents, may have widely varying lifespans (Gorbunova et al., 2008). Disagreement still exists regarding the mechanisms that determine the variability of species' lifespan across the tree of life (Jones et al., 2014), just as the process of aging remains controversial (Goldsmith, 2004; Kirkwood, 2005; Blagosklonny, 2013).

Despite the role that earlier evolutionary theories have played in experimental research relating to animal aging, these theories are delimited by the wide scope of aging and lifespan patterns observed under different environmental conditions across the tree of life (Jones et al., 2014). Subsequent models and empirical evidence have highlighted the shortcomings and shown that some of the general assumptions represent a drastically simplified version of a complex phenomenon (Abrams, 1993; Reznick et al., 2004; Caswell, 2007; Wensink et al., 2017; Moorad et al., 2019). For example, the classical evolutionary theories have assumed that lifespan evolves in response to the rate of extrinsic mortality, such that rapid senescence evolves under high extrinsic mortality. Conversely, it was reported that an increase in random extrinsic mortality does not evolve rapid senescence (Reznick et al., 2004; Wensink et al., 2014). More so, contrary to the assumptions on tradeoff (Williams, 1957; Kirkwood and Rose, 1991), the classical theories could not explain the unabated fecundity rate coupled with negligible senescence in turtles, tuatara, and other species (Cohen et al., 2020), and why some long-lived species show no sign of progressive aging as predicted by these theories (Vaupel et al., 2004; Finch, 2009; Ruby et al., 2018). There are indications that senescence, although rampant among complex species like mammals, might not be a universal phenomenon among all extant organisms (Jones et al., 2014). The paucity of empirical evidence to support the presence of biological tradeoff (Kirkwood and Holliday, 1979) between somatic maintenance and reproductive fitness indicates that tradeoffs cannot be the sole driver of aging or lifespan (Cohen et al., 2020). Another piece of evidence that does not support the disposable soma theory is found in human females that invest higher resources in reproduction yet outlive their male counterparts (Blagosklonny, 2010).

Interestingly at the moment, there is no explicit *a priori* theory to explain how natural selection works during evolution to extend lifespan, even though various studies on extremely long-lived animals try to uncover anti-aging mechanisms by searching for relevant genes and pathways under positive selection. In this regard, new hypotheses and models are required to better integrate and explain the findings. Here, we review adaptive responses that may drive longevity and propose an adaptive hitchhike model as a possible mechanism through which longevity evolves in the wild. We posit that lifespan extension is earned from adaptive changes embedded in animal genomes that control all life functions. Basic longevity criteria such as enhanced immune functions, efficient DNA response and repair, tight control of the cell cycle, and genome maintenance are shared among all long-lived species and are under adaptive regulation, but the major contributing factors are species-specific adaptations to their respective ecological niche, especially under extreme environments.

CROSSTALK BETWEEN ADAPTATION AND LIFESPAN EXTENSION

The ability of an organism to survive in a specific ecosystem as a result of changes to its behavioral, physiological, morphological, and genetic response is called adaptation. Ecological adaptation strongly underlies lifespan extension in lineages and species where longevity has been observed despite differences in species ecosystem, morphology, and complexity. Predictably, all long-lived species have low extrinsic mortality due to the nature of their habitat or have evolved mechanisms to evade predators and imminent dangers (Harvey and Purvis, 1999). For example, while bats and birds have evolved powered flight to search for food and escape from predators and unfavorable weather conditions, thereby reducing extrinsic mortality (Munshi-South and Wilkinson, 2010), mole-rats are subterranean and have even fewer predators (Lewis and Buffenstein, 2016), reptiles have a scaly shell to guard against predators and hazardous environments, and species like elephants, cetaceans, and other large animals have few predators due to their body size. Humans are highly intelligent and thereby have also developed a protected environment that contributes to lifespan extension (Austad, 1997). However, as much as this ability is expected to contribute to lowering extrinsic mortality, it neither explains the variation observed in lifespan or mechanisms through which lifespan is regulated.

Long-lived species are now known to exhibit efficient adaptive responses in essential pathways that contribute to lifespan with evidence of enhanced genome maintenance, DNA damage response, and repair attributing to their longevity (Gorbunova et al., 2014; Tian et al., 2019). Thanks to affordable genome sequencing, the availability of genome data revealed widespread adaptation in the genomes of long-lived species where positive selection, rare sequence variants, and genome duplication contributed to ecological adaptation, the evolution of body size, and disease resistance (Abegglen et al., 2015; Huang et al., 2019; Zhou et al., 2020). Although mechanisms of extended lifespan of these species are currently unclear, emerging evidence from genomic analyses points to the important role of species adaptation in longevity (**Figure 1**). This section reviews organismal adaptations across different taxa and highlights how natural selection contributed to the longevity of these species through adaptive responses to different ecological niches.

Hypoxic Adaptation Potentially Promotes Longevity

While various environments may support basic adaptive survival, some ecosystems have stringent features that limit the species that could survive in such habitats; examples include hot springs, the Arctic, and high mountains. Organisms that live in an environment with limited oxygen (hypoxic conditions), for instance, face persistent challenges of hypoxia and must develop physiological features that would aid in their survival in such a

challenging environment. Evolutionary adaptations have largely converged across these species to address oxygen demand either by optimizing oxygen uptake or reducing oxygen requirement for metabolism (Pamenter et al., 2020). Hypoxia-driven adaptation involves selection acting on pathways such as central metabolism, cellular respiration, hemoglobin-mediated oxygen transport, and hypoxia-inducible factor pathways (Simonson, 2015; Ding et al., 2018), whereas other adaptive mechanisms in these species could be attributed to species physiology, ecological differences, lifestyle, and adaptive fitness (Pamenter et al., 2020).

The Tibetan population on the Tibet plateau is an example of a human population that has adapted to hypoxia conditions at high altitudes (Beall et al., 2010); conversely, naked mole-rats are adapted to hypoxic conditions in a subterranean niche (Fang et al., 2014b), while species such as turtles and cetaceans (e.g., bowhead whale) have adapted to intermittent hypoxia conditions (Keane et al., 2015; Tian et al., 2016). Hypoxic adaptation is believed to contribute to longevity in these organisms as incidences of hypoxia have been reported to extend lifespan from invertebrates to vertebrate animals (Mehta et al., 2009; Boretto et al., 2018). Hypoxia-inducible factor (HIF-1) is the chief mediator of hypoxia-response pathways that are usually activated in low-oxygen conditions, and modulation of this pathway and HIF-1 has resulted in varying degrees of longevity (Leiser et al., 2013). HIF-1 has, however, been strongly linked to different types of tumor progression and its constitutive expression mediates intra-tumoral hypoxia (Zhong et al., 1999; Semenza, 2010). Nevertheless, HIF-1 and the hypoxia-response pathway have been touted as a viable intervention for lifespan extension as demonstrated in *Caenorhabditis elegans* (Mehta et al., 2009; Leiser et al., 2013). In a recent study, mice exposed to chronic hypoxia [11.8% atmospheric (atm) O_2] for 32 days demonstrated gene expression patterns that were similar to known longevity interventions including calorie restriction (Tyshkovskiy et al., 2019); this suggests that the mechanisms of lifespan control might have converged on related genes and pathways and exhibit a similar pattern of behavior when modulated by longevity interventions (Tyshkovskiy et al., 2019). Hypoxia is likely able to counteract and redeem the detrimental effects associated with aging-related pathways. As recently discovered, centenarians on the Tibet Plateau have a longer lifespan compared with any other region in China (Li et al., 2017). Genetic variation indicating rapid evolution, was significantly higher in aging-related genes compared to other genes in Tibetans than in the Han population; this study further found a significant negative association between expressed genes under hypoxia and during aging (Li et al., 2017). Similar to hypoxia-induced longevity in model organisms, hypoxia conditions upregulate longevity-associated genes that are usually downregulated during aging (Kim et al., 2011; Li et al., 2017). As expected, hypoxia-related genes and pathways are under strong selection on the Tibet plateau (Yi et al., 2010; Xu et al., 2011; Basang et al., 2015). The events leading to the acquisition of this unique adaptation are sketchy, but it has been speculated that adaptation to high altitude among Tibetans might have been transferred through introgression from the now-extinct *Homo denisova* (Denisovans) that inhabited the plateau during the mid-Pleistocene, circa 50,000 years ago (Huerta-Sánchez et al., 2014; Chen et al., 2019).

In the naked mole-rat (NMR), adaptation to underground hypoxia has been one of the central focuses in studying molecular mechanisms that support its longevity. Naked mole-rats and other subterranean mole-rats with extended lifespan share a similar pattern of adaptive constraint in their genomes and physiological architecture that have aided adaptation to underground burrows in contrast to non-subterranean mammals (Bennett and Faulkes, 2000; LaVinka et al., 2009). For instance, sweeping changes were observed in the amino acid residues of arginase 1 (ARG1), which functions in the urea cycle, and increased ARG1 activity has been linked to certain pathologies including human cancer (Fang et al., 2014b; Caldwell et al., 2018). Higher expression of hypoxia-related genes, DNA repair, and globin proteins was observed in all subterranean mole-rats as well as highland inhabitants that were evaluated for hypoxia adaptation (Fang et al., 2014a,b). Hypoxia adaptation induces hypothermia and hypo-metabolism, which are physiological hallmarks similar to hibernating species and CR animals, and with links to extended lifespan (Lewis et al., 2018). Likewise, mice exposed to a model of NMR natural environment (hypoxic–hypercapnic environment) exhibit a significant decrease in both body temperature and metabolic rate as observed in CR and mutant mice (Conti et al., 2006; Tolstun et al., 2020). There were no significant expression changes in stress-related genes, while also displaying accelerated wound healing (Tolstun et al., 2020).

Experimental manipulation of adaptive mechanisms in key aging-related pathways has led to similar lifespan extension, although sometimes with negative pleiotropic or epistasis effects in model organisms (Mehta et al., 2009). Hypoxia studies in the rotifer *Brachionus manjavacas*, an aquatic invertebrate suitable for aging studies, demonstrated a mean lifespan extension of 107% when continuously exposed to 1.6% atm. Hypoxic conditions in this organism increased reproductive success by twofold and conferred cytoprotection against stress (Snell et al., 2019). This observation supports previous studies on rotifer communities and their adaptation to high salinity and low oxygen (Esparcia et al., 1989). In *C. elegans*, lifespan extension was reported in both wild and mutant animals exposed to 1% atm. O_2 (Honda et al., 1993), while in Drosophila, adult lifespan was extended under moderate hypoxia conditions (10 kPa) (Rascón and Harrison, 2010). Hypoxia-induced stress in *C. elegans* has been linked to lifespan extension *via* the modulation of the HIF-1 pathway (Leiser et al., 2013), and loss of function of VHL-1 (von Hippel–Lindau 1), which negatively regulates HIF-1, resulted in a significant increase in lifespan in *C. elegans* (Mehta et al., 2009). Under different dietary restriction regimens, however, chronic hypoxia and HIF1 activity caused a reduction in lifespan in Drosophila and *C. elegans*, respectively (Vigne and Frelin, 2006; Chen et al., 2009). As a caveat, the hypoxic response in hypoxia-sensitive species such as many model organisms would be expected to differ from those of hypoxia-tolerant organisms adapted to a hypoxic habitat in the wild. Yet, a formal phylogenetic analysis is needed to evaluate the pro-longevity response of hypoxia. Altogether, these observations highlight the importance of HIF-1 and the hypoxia-response

pathway to modulate lifespan. We infer from the efficient cancer resistance mechanisms in the NMR (Seluanov et al., 2018) that adaptation to hypoxic conditions contributes to the suppression of the pro-cancer activities of HIF-1 and the hypoxia-response pathway, thereby extending lifespan. Overall, hypoxia adaptation contributes to longevity, although in a complex manner (Chen et al., 2009; Zhou et al., 2011; Leiser et al., 2013), as observed in the Tibetan population, mole-rats, and various model organisms.

Aquatic Lifestyle-Associated Adaptation and Lifespan Extension

Similar to the hypoxic terrestrial environment, the Arctic and Antarctic regions are highly unfavorable for many organisms but house some of the longest-lived vertebrates on Earth. The Arctic is characterized by extremely low temperatures, salinity, and limited resources, which greatly influence the type of animals that could adapt to such a challenging environment (Gradinger, 2001). Animals in this region have evolved physiological, behavioral, and physical adaptations to cope with this extreme condition (Blix, 2016). Species such as bowhead whales (*Balaena mysticetus*, BHW) and Greenland sharks (*Somniosus microcephalus*) are among the longest-lived vertebrates with a maximum lifespan of over 200 years, with the latter speculated to be capable of reaching an age above 300 years (George et al., 1999; Nielsen et al., 2016). These species have been poorly studied for their longevity trait, but adaptation, mainly driven by temperature, diet, and metabolism, has contributed to an extended lifespan in cetaceans (Keane et al., 2015).

Large body size in these species evolved alongside enhanced cancer defense mechanisms, efficient tumor suppressors, translating to a lifetime of low cancer risk; this indicates a strong interplay between molecular mechanisms that underlie tumor suppression, large body size, and longevity (Caulin and Maley, 2011; Caulin et al., 2015; Nunney et al., 2015; Wensink, 2016). This large body size in cetaceans is maintained under a strong aquatic selection driven by thermoregulation, feeding efficiency, and metabolic rate (Gearty et al., 2018; Goldbogen et al., 2019). BHWs are filter feeders with access to abundant small prey and lipid-rich diet; they possess exceptionally high deposits of adipose tissue (blubber) and high leptin accumulation (Ball et al., 2017; Jiménez-Cortegana et al., 2021). This could partly explain the evolution of extreme large body size and low cancer in this species (Goldbogen et al., 2019). Both Greenland sharks and BHW experience intermittent episodes of hypoxia during deep diving, and adaptation to such low oxygen conditions has been attributed to extended lifespan in terrestrial species. The Greenland shark, just like all other long-lived species, lacks exceptional antioxidant protection (Costantini et al., 2017) but has an extremely low metabolic rate, as well as low daily energy demands (Ste-Marie et al., 2020). BHW genome encompasses adaptive traits that support extended lifespan and has been perpetuated through gene duplication and selection, while also pruning diseases and cancer susceptibility genes through pseudogenization and total gene loss. This aligns with recent reports of positive selection and widespread gene duplication in tumor suppressor genes in cetaceans; the large body size in this clade appears to correlate with strong selection in tumor suppression pathways (Tollis et al., 2019; Tejada-Martinez et al., 2021). These genes are implicated in a wide range of tumor types, metabolism, cell proliferation, and pathways believed to regulate animal longevity.

Evidence from genome sequencing and comparative genome analyses revealed several unique amino acid replacements in the bowhead whale genome that are associated with ecological adaptations, disease resistance, and cancer (Keane et al., 2015). For instance, *ERCC1* and *HDAC1* genes possess unique amino acid residues, while *PCNA* might have undergone possible selection in bowhead whales; these genes are linked to DNA repair and chromatin structure regulation pathways, respectively (Gillet and Schärer, 2006). More so, a lineage-specific mutation was observed in uncoupling protein 1 (*UCP1*) gene in the bowhead whale that is likely to contribute to molecular adaptation in thermoregulation and energy metabolism as previously observed in the naked mole-rat (Kim et al., 2011; Keane et al., 2015). Several genes that mediate in metabolism and hypoxia-tolerance pathways, including insulin and mTOR, are under adaptive selection in cetacean lineages (Tian et al., 2016; Nam et al., 2017; Derous et al., 2021); these are essential pathways for extended lifespan and play important roles in metabolism and ecological adaptation.

Compared with other mammals, differentially expressed genes (DEGs) in the bowhead whale liver support enhanced gluconeogenesis as an adaptive mechanism to high lipid diet (Berge et al., 2012). Downregulation of *GRB14* gene impacts insulin/IGF1 signaling pathway function and also serves as a shield against diet-related chronic diseases, while higher expression of *CITED2* gene mimics that of calorie restriction (Carré et al., 2008; Sakai et al., 2012), suggesting that these genes contribute to lifespan regulation through lipid and glucose metabolism (Wang et al., 2014). Liver DEGs also support elevated tolerance to hypoxia and enhanced DNA repair that could be considered adaptive mechanisms to the Arctic habitat (Seim et al., 2014). Furthermore, 53 DEGs were observed in the kidney, whose functions involve maintenance of genome integrity and tumor suppression, which may confer protection against age-related changes in the BHW. Unique amino acid changes in several proteins were also identified as well as evidence of positive selection in essential genes with roles in cancer, e.g., *MTUS1*, *GSK3A*, *PRUNE*, and *CYFIP1* (Seim et al., 2014; Ma and Gladyshev, 2017). Overall, longevity of these aquatic species is coherent with ecological adaptation driven by selective constraints acting on body size, genome, and species physiology.

Metabolic Adjustment and Lifespan Extension

Aves are a successful class of vertebrates with ubiquitous longevity traits and an average life expectancy that supersedes that of most terrestrial mammals of comparable size (although few exceptions exist). According to the evolutionary theory of aging, their ability to fly shielded them from non-aging-related mortality (Pomeroy, 1990), hence, leading to longevity (Austad and Fischer, 1991). There seems to be no clear-cut adaptive mechanism that could

be solely responsible for a long lifespan of birds, but the ability to fly and glide, and arboreal living in this class and other classes of vertebrates is believed to contribute to their longevity (Partridge and Barton, 1993; Holmes and Austad, 1994; Moorad and Promislow, 2010; Shattuck and Williams, 2010), although cytoprotective adaptation appears to protect birds from tissue damage due to metabolic waste (Holmes et al., 2001). Powered flight supports longevity of extant flying birds by aiding quick escape from predators, disease, famine, and other environmental hazards, just as in the case of birds, which evolved biochemical adaptations to cope with intense metabolic rate during flight and also repress the damaging consequences of metabolic by-products (Munshi-South and Wilkinson, 2010). Longevity attributes in birds include social organization and cognitive abilities as seen in parrots (Wirthlin et al., 2018), and slow senescence alongside high reproductive success at an advanced age (Partridge and Barton, 1993). Hummingbirds, parrots, seabirds, and songbirds are a few of the species that have independently evolved longevity through unique and shared adaptive traits (Holmes and Austad, 1995).

Similar to birds, bats are capable of powered flight and may be considered the longest living mammals when adjusted by body mass; the maximum lifespan of bats can be 3.5 times higher than their non-flying mammalian counterparts (Wilkinson and South, 2002; Wilkinson and Adams, 2019). With powered flight and being the only mammals that can do this, bats have been able to reduce extrinsic mortality by flying away from hazardous environments and predators (Pomeroy, 1990; Partridge and Barton, 1993). Other adaptations that contribute to bat longevity include hibernation, cave roosting, and torpor (Jürgens and Prothero, 1987; Wilkinson and South, 2002). Comparative studies revealed that extreme longevity has evolved among bats at least four times, and these four groups include the *Myotis*, horseshoe bats (*Rhinolophus*), long-eared bats (*Plecotus*), and vampire bats (*Desmodus rotundus*). All these lineages also possess the ability to hibernate, except for vampire bats that can undergo torpor at feeding intervals (McNab, 1969; Wilkinson and Adams, 2019). Vampire bats also have cooperative social behavior and a food sharing pattern that reduces the likelihood of starvation of roost mates that are unable to secure blood meals (Carter and Wilkinson, 2013; Carter et al., 2017). The role of hibernation in longevity in chiropterans has been controversial. While Herreid (1964) reported no observable differences in longevity between hibernating and non-hibernating bats, Wilkinson and South (2002) reported a significant increase in lifespan upon hibernation. However, both hibernating and non-hibernating species can live much longer than other mammals of similar body size (Wilkinson and Adams, 2019). Hibernation reduces the risk of mortality by predation and the risk of starvation; it also lowers metabolic rate and decreases body temperature (Nagel and Nagel, 1991; Wilkinson and South, 2002; Wilkinson and Adams, 2019). A reduction in the metabolic rate during hibernation is also expected to reduce the accumulation of metabolic waste that could induce cellular damage. Furthermore, physiological manifestations of hibernation in bats are similar to those produced by calorie restriction in rodents, which include a decrease in blood glucose and insulin, reduced glycolytic enzyme activity, increased protein synthesis, and strong antioxidant defenses (Walford and Spindler, 1997; Masoro, 2000).

Flight is the main reason for a high metabolic rate in bats and birds (Munshi-South and Wilkinson, 2010). In itself, flight is an efficient but costly strategy compared with other forms of locomotion in vertebrates (Norberg, 2012). Flight requires an extremely high mass-adjusted metabolic rate that is three to five times higher than in non-flying mammals, making flight a high energy-demanding trait (Speakman and Thomas, 2003). With this high metabolic rate, it may be expected that bats and birds would exhibit higher mutation rates and accordingly be short lived (Goyns, 2002; Ruf and Geiser, 2015). The ability of some bat species to support daily reduction in metabolic rate, i.e., enter the state of torpor, could greatly contribute to bat longevity. Small-bodied bat species are mostly found in the temperate zone and can maintain a near-absolute (99%) reduction in metabolic rate in a low-energy torpor state (Ruf and Geiser, 2015). Bats also possess the ability to conserve energy expended by lowering heart rates from a high 900 beats per minute during flight to as low as 200 beats per minute in a resting state, thus, reducing daily energy consumption by 10% (O'Mara et al., 2017). Reactive oxygen species-induced oxidative damage due to high metabolic rate might be a contributory factor to aging, but it has failed to explain longevity in birds and bats (Brunet-Rossinni, 2004; Montgomery et al., 2012; Xia and Møller, 2018).

Unique segmental deletion and reduced intergenic regions in birds, and also in bats, support gene proximity in the genomes, aiding in rapid expression and regulation of discrete sets of genes needed for metabolism during flight (Zhang and Edwards, 2012). Furthermore, the possibility of gene coevolution, as well as co-regulation, exists between power flight and metabolism in birds as multiple genes linked with each mechanism were found to be under positive selection (Zhang et al., 2014). The mechanisms by which birds and bats evade the deleterious effect, which is the price of a high metabolic rate is not fully understood. NRF2 gene mediates antioxidant response in metazoans by activating cascades of genes responsible for cytoprotection and metabolic functions (Yamamoto et al., 2018). In the absence of oxidative stress, KEAP1 targets NRF2 for degradation and maintains a low expression level of NRF2 in the cell. Interestingly, KEAP1 gene, which is conserved across metazoans, has undergone fragmentation in the lineage leading to the Neoaves (modern birds). KEAP1 loss of function means that Neoaves NRF2 cannot be targeted for degradation, leading to its constitutive expression in birds. This is thought to be an adaptive mechanism that shields cells and tissues from ROS-induced damage during increased metabolism (Castiglione et al., 2020). Similarly, overexpression of NRF2 and knock-down of KEAP1 increased the lifespan in *C. elegans* and *Drosophila*, respectively (Sykiotis and Bohmann, 2008; Tullet et al., 2017), while xenobiotic-metabolizing enzymes, which are downstream targets of NRF2, were upregulated following CR intervention in mice (Tyshkovskiy et al., 2019). Several genes and pathways associated with DNA damage and repair, cell cycle regulation, and metabolic pathways were found to be under selection pressure in parrots, red-crowned cranes, and other long-lived birds (Lee et al., 2020). Interestingly, *SOD3* gene was found to be under positive selection in parrots, red-crowned crane, ostrich, and other long-lived birds (Wirthlin et al.,

2018; Lee et al., 2020) suggesting that birds may have evolved a similar adaptive response to combat the cost of high metabolic rates. However, regardless of species-specific metabolic rates, animals with extended longevity usually have a low generation rate of ROS in the mitochondria (Barja, 1999).

Temperature-Driven Changes and Lifespan Extension

Reptiles are generally long-lived, comprising species that exhibit negligible senescence in the wild, and have adapted to a range of environments. Tuatara (*Sphenodon punctatus*) is a species of reptiles with extreme longevity coupled with reproductive success that could live far beyond 100 years. This may be the species with the lowest growth rate among reptiles (Cree, 2014). The optimal body temperature of the tuatara is between 16 and 21°C, and the animal remains active at temperatures below 5°C. This unusual temperature adaptation is under the control of the thermoregulatory genes called transient receptor potential (TRP) ion channels (Nilius and Owsianik, 2011). Thirty-seven gene copies of TRP were identified in the tuatara, many of which are under positive selection (Gemmell et al., 2020). Also, the tuatara has 26 selenoprotein genes, one more than humans, and 4 copies of selenocysteine-specific tRNA; these genes are responsible for redox regulation and support other functions. Adaptation to extremely low temperatures, protection against oxidative damage and disease resistance appeared to contribute to tuatara's longevity (Gemmell et al., 2020).

Turtles are another reptile species that have been studied for negligible senescence, and accumulating empirical evidence revealed their longevity, with an unabated fecundity rate at an advanced age. Species such as Blanding's turtle (*Emydoidea blandingii*), three-toed box turtle (*Terrapene Carolina triunguis*), and the painted turtles (*Chrysemys picta*) have been reported for their reproductive success even at advanced ages (Congdon et al., 2001, 2003; Miller, 2001). Longevity in turtles encompasses adaptive mechanisms against extreme conditions more than any terrestrial vertebrate and could barely be compared with naked mole rats. For instance, the pond slider (*Trachemys scripta*) can survive harsh cold weather conditions in complete absence of oxygen and can rely entirely on anaerobic glycolysis for many weeks (Lutz et al., 2003). Brain mitochondria of *Trachemys scripta* after 2 weeks of exposure to anoxia exhibit elevated tolerance to chronic anoxia, indicating that turtles evolved efficient endogenous mechanisms to support a switch between normoxia and anoxia conditions to regulate metabolic functions (Pamenter et al., 2016; Bundgaard et al., 2019). *Trachemys scripta* deploy elevated levels of cytoprotective proteins in the tissue, are highly stress-resistant, and maintain hypometabolism (Willmore and Storey, 1997; Krivoruchko and Storey, 2010); more so, turtles maintain a higher brain concentration of ascorbic acid that doubles what is present in the mammalian brain cortex (Rice et al., 1995). These extraordinary mechanisms of anoxia tolerance possibly play a crucial role in the species longevity as it is a common phenomenon for such animals to acquire adaptive traits to overcome ecological constraints (Söti and Csermely, 2007).

Thermoregulation is an integral part of animal metabolism. However, adaptive mechanisms that modulate thermoregulation and metabolism in elephants are not yet understood, neither they have been assessed for a possible link to elephant longevity and aging. However, what we know is that elephants live in a hot climate where the environmental temperature often exceeds the body temperature (Mole et al., 2016). With sweat glands lacking, one of the heat loss mechanisms in this species is the adaptive behavior of flapping large ears, but the genetic mechanism of heat transfer is poorly understood (Wright and Luck, 1984; Robertshaw, 2006). Nevertheless, elephants might have evolved adaptive temperature-sensing mechanisms to cope with habitat thermodynamics through ion channel TRPM8, also known as transient receptor potential melastatin 8 that takes part in temperature-gating mechanisms. A single point mutation at site 919 within the pore domain of TRPM8 of the elephant has been suggested to either be a modulatory site for temperature sensors or include synchronously dispersed temperature-sensing residues (Yang et al., 2020). Previous studies have identified diurnal heat storage mechanisms that are responsible for the regulation of daily heat fluctuation as a major thermoregulatory mechanism in large animals including elephants. This mechanism, also known as heterothermy, is common among large desert animals such as the camel and Arabian oryx, and it is an adaptive strategy allowing conservation of both energy and water in a hot and dry climate (Elder and Rodgers, 1975; Ostrowski and Williams, 2006; Weissenböck et al., 2012). Similar heterothermic behavior is an adaptive trait in bat lineages where the *leptin* gene that plays an essential role in thermogenesis and energy metabolism is under positive selection (Yuan et al., 2011). However, unlike bats, whether adaptive thermoregulation in elephants has a role in aging and extended lifespan is an open question. Evidence of adaptive evolution was detected in the electron transfer chain (ETC) complex in the African elephants; two genes that encode NADH dehydrogenase and ATP synthase, and function in the oxidative phosphorylation pathway (OXPHOS) are under positive selection (Finch et al., 2014). Oxidative phosphorylation genes are responsible for energy metabolism, ATP generation, and heat production that are required for cellular activities.

Evolutionary events that led to gene duplication of *TP53* gene have been described in elephants; African elephants have 19 extra copies of *TP53* retrogenes (pseudogenes) (*TP53RTG1-19*) with evidence of expression (Abegglen et al., 2015; Sulak et al., 2016). It was suggested that these pseudogenes support lower cancer occurrence in these species, although more research is needed to establish whether this is the case (Caulin and Maley, 2011; Abegglen et al., 2015). One possible explanation is the protagonist pleiotropy, wherein longevity traits could be driven by selection against cancer-related damage (de Grey, 2007). Thus, one of the factors contributing to extended lifespan in the elephant may be the strong selection acting on tumor suppressor genes. Beyond the canonical anticancer activities of *TP53* protein, cancer-unrelated functions of this protein have not been investigated in elephants. *TP53* takes part in several biological processes that include stress response, senescence, cell cycle regulation, insulin homeostasis, and cellular metabolism (Krstic et al., 2018). Owing

to a wide range of functions of *TP53*, and its emerging role in cellular metabolism (Ranjan and Iwakuma, 2018), it is plausible to hypothesize that p53 duplication could have an adaptive role in elephant metabolism, thermoregulation, and habitat adaptation.

THE ADAPTIVE-HITCHHIKE MODEL

Although we discussed a number of genetic adaptions in the wild that contribute to lifespan extension, population genetics postulates that genetic changes are hardly to be fixed if such genetic changes do not increase fitness during long-term evolution. Nevertheless, we posit that strong selection acts to maintain these changes, leading to long lifespans in living organisms. We propose that extended lifespan is not by itself under selection but rather an epiphenomenon (by-product) of species adaptation, a phenomenon we have termed here the adaptive hitchhike model. First, the model implies that a new pleiotropic mutation, with one of its effects being extended lifespan, could be favored by natural selection due to its advantage to some other trait and therefore becomes fixed. Second, the model also applies to new pro-longevity mutations that occur at sites closely (or functionally) linked with the allelic sites under selection; if a new pro-longevity mutation arises at a site that is linked to an adapted genome region, natural selection may cause an increase in allele frequency and fix this pro-longevity mutation through linkage and allelic associations. Therefore, the adaptive-hitchhike model suggests that the selective constraint acting on the genomic region associated with adaptation and fitness is largely responsible for non-random beneficial pro-longevity effects. For example, patterns of selective sweep across loci of close proximities were reported for adaptation to altitude among the Tibetan population (Yi et al., 2010), and a further association was found between longevity and hypoxia response in this same population (Li et al., 2017). In other cases, natural selection might act on an already existing but neutral mutation through a sweeping selection; therefore, if neutral alleles responsible for lifespan extension are close enough to other alleles under selection, the chances of recombination are slim, and together, they become fixed in the population. This model could be mainly summarized in the following ways (**Figure 2**): (1) Some adaptive genetic changes could have dual functions, i.e., adaptive and longevity effects. (2) A pro-longevity mutation could come under selection and become fixed through direct selection or linkage and allelic association. (3) In the same way that a pro-longevity mutation could become fixed, a geronic (pro-aging) mutation could also become fixed and lead to a shorter lifespan. (4) In the case where environmental pressure is relaxed, pro-longevity effects may be lost. Therefore, our adaptive-hitchhike model of longevity of animals could be tested by (a) identifying pro-longevity effects of genetic changes that respond to adaptation and (b) detecting signals of linkage disequilibrium (LD) between adaptive and longevity variations. The novelty of this model is that it gives a key role to such nucleotide substitutions and loci with dual functions. Functional evaluation and validation of adaptive nucleotide substitutions with the pro-longevity potential could provide answers to the century-long questions surrounding the evolvability of animal lifespan.

RELATIONSHIP OF THE MODEL TO CURRENT UNDERSTANDING OF AGING

Programmed Aging and Quasi-Programmed Aging

Programmed cellular aging, by analogy to *mitoptosis*, *apoptosis*, and *organoptosis*, which work at the level of mitochondria, cells, and tissues, respectively, involves an event of self-elimination (Skulachev and Longo, 2005). The quasi-programmed model on the other hand posits that damage (aging or senescence) is the later life unintentional consequence of early developmental programs because these programs would not turn off when development is completed. The argument is that the same pathways that drive soma development during early life are responsible for aging and cellular senescence; this argument is supported by an example of the mTOR/insulin signaling pathway that supports development but contributes to accelerated aging and could be downregulated to extend the lifespan (Blagosklonny, 2006, 2013; Wu et al., 2013). The quasi-programmed model partially explains the antagonistic pleiotropy theory of aging such wherein the consequences of later-age hyperfunction stem from early developmental processes, and therefore, the aging process appears programmed (but is not really programmed, i.e., there are no genes that evolved with the goal to cause aging). Both models emphasize the genetic determinism of the aging process, wherein programmed aging and quasi-programmed models argue about purposeful and indirect effects, respectively. We posit that longevity is a by-product of adaptation through hitchhiking. We adopted a population genetic approach to explain the ability of natural selection to modulate lifespan in animals.

Damage-Centric and Imperfectness Model of Aging

These series of theories emphasize that the basis of aging is accumulated molecular damage (Medawar, 1952; Harman, 1956). For example, reactive oxygen species (ROS), free radicals, and other oxidants lead to damage to DNA, proteins, and other molecules in the cell. ROS and other metabolic wastes are deleterious products generated at varying amounts that mostly correspond to the metabolic activities and energy expenditure of organisms. High metabolic rate and energy expenditure, as seen in flight, should produce enough damage capable of driving rapid senescence and leading to short lifespan. This hypothesis does not hold for birds and bats. Despite having a metabolic rate, about twofold more than mammals of comparable size, birds with active flight do not show signs of rapid senescence and have a maximum lifespan that triples that of similar-sized mammals reared in captivity (Holmes and Austad, 1994; Munshi-South and Wilkinson, 2010). Birds appear to have evolve mechanisms to protect against the deleterious effect of high metabolism. ROS and other free

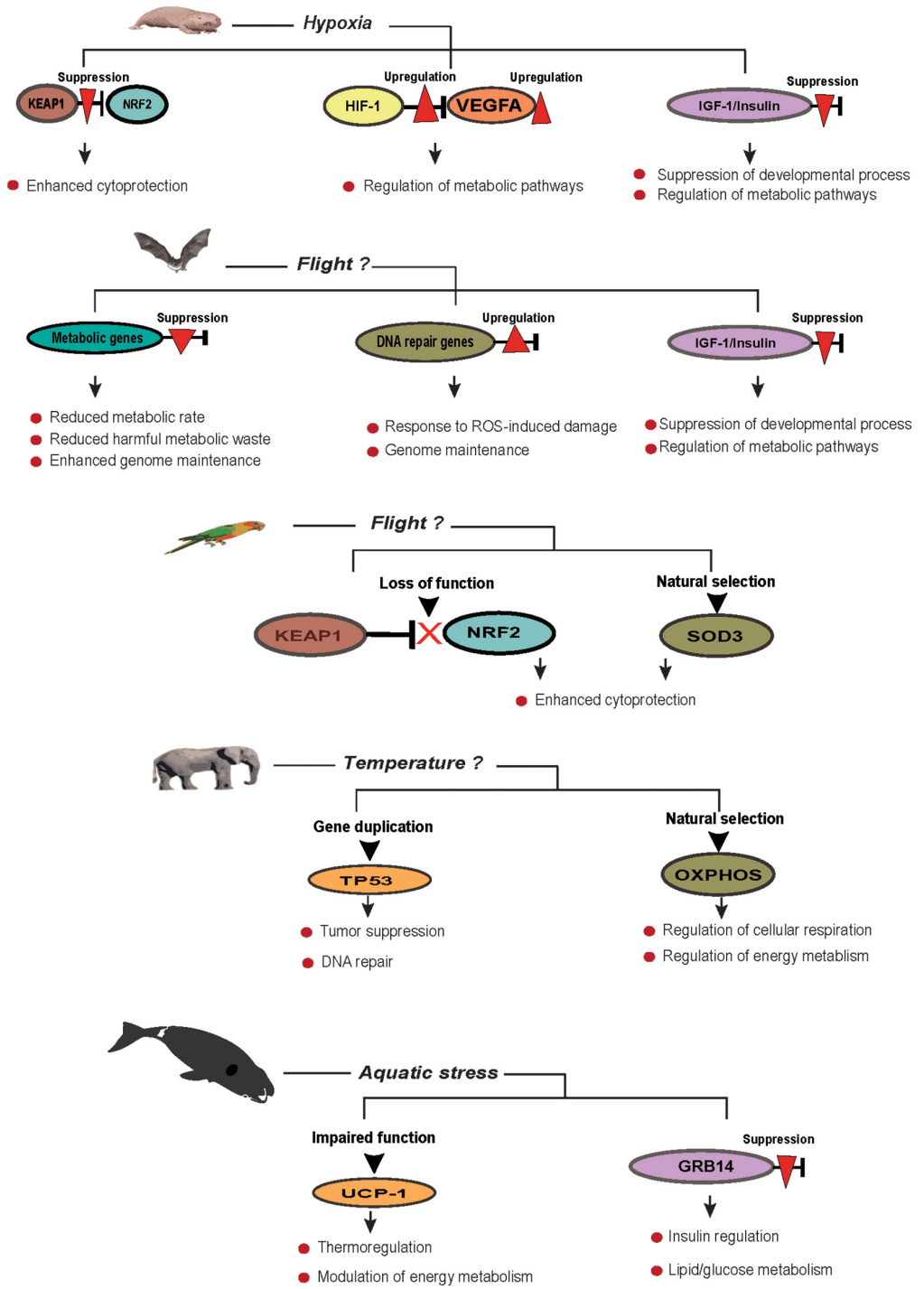

FIGURE 1 | Environmental and morphological contributions to an extended lifespan.

radicals undoubtedly induce damage and oxidative stress, but this, by itself, is not sufficient to drive aging. Consistent with this idea, there is no evidence of some special antioxidant defense, as would have been expected, in extremely long-lived animals compared with short-lived species (Andziak et al., 2006). It is reasonable to assume that this oxidative damage, nevertheless, contributes to the overall aging progress (Shi et al., 2019).

The imperfectness model, however, offers a mechanism that describes aging as a consequence of largely non-random damage that accrues due to the imperfect nature of biological systems (Gladyshev, 2013). An organism encompasses

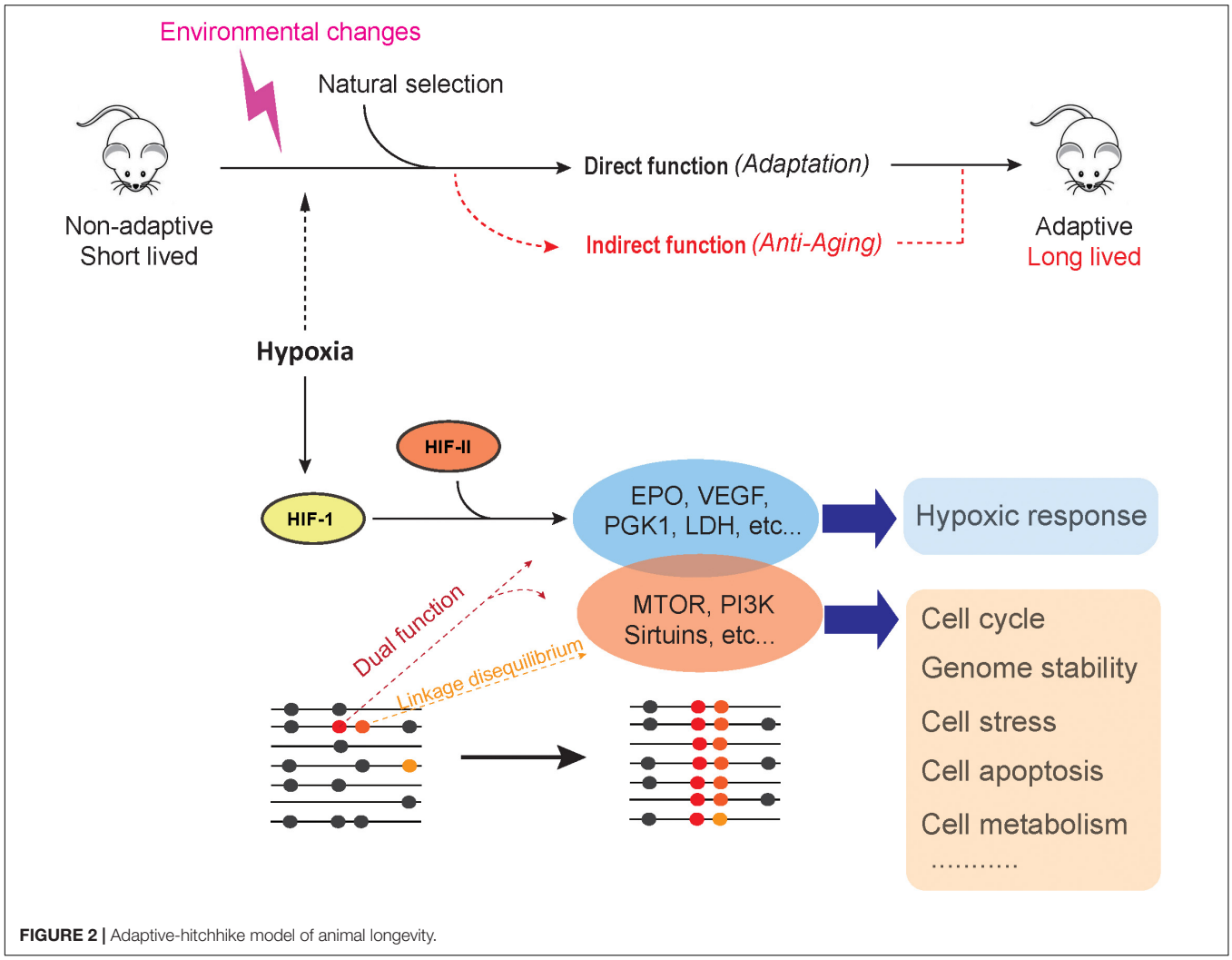

FIGURE 2 | Adaptive-hitchhike model of animal longevity.

different organs and cell types, the genome, proteins, signaling networks, macro-molecules, as well as the rest of the cellular machinery, and collectively, they carry out the physiological and metabolic activities required for optimal system function. These physiological functions unavoidably produce numerous forms of damage collectively known as the deleteriome (Gladyshev, 2016). When this damage is generated in organisms with fully differentiated non-renewable cells and structures, it will accumulate and cause aging. As the processes that generate these damages are under genetic regulation, different species will generate damage according to their genetic programs. In that sense, there is a nice connection between the deleteriome model of aging and the adaptive hitchhiking model. Indeed, control of lifespan involves explanations about the cause of variation in lifespan across the clades of organisms and how it can be fixed. All organisms generate damage and also develop mechanisms that protect against this damage. The regulation of biological processes and pathways in extremely long-lived animals (Moreno Santillán et al., 2021; Wilkinson et al., 2021) shows patterns of adaptation that are distinct from those of short-lived animals, and this is reflected in the damage that they produce (Bundgaard et al., 2019; Kacprzyk et al., 2021). Accordingly, modulation of related pathways in short-lived species is known to extend the lifespan (Leiser et al., 2013). Therefore, similar to the deleteriome model, wherein cumulative damage varies across taxa and contributes to variation in the aging process, we posit that control of lifespan is adaptive and driven by genetic heterogeneity.

Evolutionary Theory of Aging

Antagonistic pleiotropy and the disposable soma theories of aging are related in that they emphasize a tradeoff between reproductive fitness and extended lifespan. Intrinsically, tradeoffs are rampant in the wild and are usually biased toward species fecundity, which is expected, especially in a volatile environment or when self-perpetuation becomes increasingly difficult (Reznick et al., 2006). This bias has been described among vertebrates (Marshall et al., 2017) and invertebrates (Snell and King, 1977), both in the wild and under controlled laboratory environments. More so, antagonistic pleiotropic phenomenon seems to be pervasive in the genome (Austad and Hoffman, 2018). One such example is the mTOR and insulin signaling pathways that are responsible for

early-life development but contribute to the aging process. Whereas our model is not antagonistic, it could be likened to pleiotropy in that selective pressure acting at a locus influences both the adaptability and longevity of an organism. Moreover, our model does not assume that tradeoff should exist between reproduction and longevity as no genetic evidence of resource allocation and redistribution exists between somatic maintenance and fecundity; instead, we posit that longevity hitchhike along with adaptation, which, in turn, can contribute to overall reproductive success. It should be noted, however, that both models may apply to regulate lifespan.

The Pleiotropy Model of Aging and Cancer

The pleiotropy phenomenon is widespread in living organisms whereby multiple phenotypic traits could be attributed to a single locus. The protagonist pleiotropy of chromosomal damage model proposed two major forms of damage resulting from spontaneous DNA mutations; such damages could lead either to cancer or non-cancer pathologies (de Grey, 2007). It posits that the stronger the evolutionary pressure to prevent cancer-related deaths is the less likely the aging, non-cancer-related pathologies are. Thus, the selection force against the onset of cancer through the DNA maintenance machinery directly prevents non-cancer forms of aging. This is a cancer-resistance mechanism that indirectly promotes longevity; it has actually been shown by several studies that an underlying tradeoff exists between cancer and aging, such as the canonical p53 pathway (Tyner et al., 2002; Yashin et al., 2009). In line with our hitchhiking model, however, selection could act on non-cancer-related loci to extend lifespan due to selection pressure at the loci with linkage disequilibrium. Hence, it is highly likely that evolutionary constraints acting to prevent cancer also act against aging-related pathologies. However, the source of selection that extends lifespan or causes slow aging is not expected to originate solely from the selection signal that prevents cancer-causing mutations, but it is more likely that ecological adaptation and genetic heterogeneity play significant roles as well. Natural selection is, by itself, restrained by the randomness of DNA mutations, gene variants, and the pleiotropic interaction that exists between loci or genes, which is the basis of the antagonistic pleiotropy hypothesis (that evolution could adopt a pleiotropic trade-off of longevity for reproductive fitness). Under the strict rule of antagonistic pleiotropy, it is unlikely that lifespan extension would be achieved in the certainty that post-reproduction would be marked by deleterious manifestation of mutations. However, under positive pleiotropy, selection for early-life fitness could contribute to post-reproductive fitness and extend lifespan (Maklakov et al., 2015). Therefore, it is probable that a mutation could attain fixation that confers early life fitness and extends lifespan without tradeoff as observed in Drosophila and nematodes (Chen and Maklakov, 2012; Khazaeli and Curtsinger, 2013; Kimber and Chippindale, 2013). Altogether, we infer that pleiotropy is widespread in the genome and plays an important role in moderating species fitness through selection.

CONCLUDING COMMENTS

Exceptionally long-lived mammals and other vertebrate species might have acquired longevity traits over millions of years; they have independently overcome ecological constraints through adaptive mechanisms that concomitantly promote their longevity. The pattern of the aging process and longevity observed across the tree of life varies greatly and is largely maintained by the force of natural selection acting on species genome under given environment. Adaptive evolution is a non-random process that is dependent on random mutations in the genome; this could explain the importance of suitable genetic variants to drive lifespan extension, which might not be compatible with fitness or adaptability in short-lived species. Our model, in the context of population genetic framework, explains the mechanism through which extended lifespan evolved and is maintained in long-lived animals. The hitchhike model is an approach that is centered around animal adaptation driven by natural selection. We anticipate that the adaptive hitchhike model would generate many studies that would identify genomic elements under natural selection that drive extended lifespan in target species.

AUTHOR CONTRIBUTIONS

XZ conceived the idea. OO drafted the manuscript. XZ, VNG, and OO edited the draft. All authors approved the final manuscript.

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REFERENCES

Abegglen, L. M., Caulin, A. F., Chan, A., Lee, K., Robinson, R., Campbell, M. S., et al. (2015). Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. *JAMA* 314, 1850–1860. doi: 10.1001/jama.2015.13134

Abrams, P. A. (1993). Does increased mortality favor the evolution of more rapid senescence? *Evolution* 47, 877–887. doi: 10.1111/j.1558-5646.1993.tb01241.x

Andziak, B., O'Connor, T. P., Qi, W., DeWaal, E. M., Pierce, A., Chaudhuri, A. R., et al. (2006). High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5, 463–471. doi: 10.1111/j.1474-9726.2006.00237.x

Austad, S. N. (1993). Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). *J. Zool.* 229, 695–708.

Austad, S. N. (1997). Comparative aging and life histories in mammals. *Exp. Gerontol.* 32, 23–38. doi: 10.1016/s0531-5565(96)00059-9

Austad, S. N., and Fischer, K. E. (1991). Mammalian aging, metabolism, and ecology: evidence from the bats and marsupials. *J. Gerontol.* 46, B47–B53. doi: 10.1093/geronj/46.2.b47

Austad, S. N., and Hoffman, J. M. (2018). Is antagonistic pleiotropy ubiquitous in aging biology? *Evol. Med. Public Health* 2018, 287–294. doi: 10.1093/emph/eoy033

Ball, H. C., Londraville, R. L., Prokop, J. W., George, J. C., Suydam, R. S., Vinyard, C., et al. (2017). Beyond thermoregulation: metabolic function of cetacean blubber in migrating bowhead and beluga whales. *J. Comp. Physiol. B* 187, 235–252. doi: 10.1007/s00360-016-1029-6

Barja, G. (1999). Mitochondrial oxygen radical generation and leak: sites of production in states 4 and 3, organ specificity, and relation to aging and longevity. *J. Bioenerg. Biomembr.* 31, 347–366. doi: 10.1023/a:1005427919188

Basang, Z., Wang, B., Li, L., Yang, L., Liu, L., Cui, C., et al. (2015). HIF2A variants were associated with different levels of high-altitude hypoxia among native tibetans. *PLoS One* 10:e0137956. doi: 10.1371/journal.pone.0137956

Beall, C. M., Cavalleri, G. L., Deng, L., Elston, R. C., Gao, Y., Knight, J., et al. (2010). Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11459–11464. doi: 10.1073/pnas.1002443107

Bennett, N. C., and Faulkes, C. G. (2000). *African Mole-Rats: Ecology and Eusociality*. Cambridge: Cambridge University Press.

Berge, J., Gabrielsen, T. M., Moline, M., and Renaud, P. E. (2012). Evolution of the Arctic Calanus complex: an Arctic marine avocado? *J. Plankton Res.* 34, 191–195. doi: 10.1093/plankt/fbr103

Blagosklonny, M. V. (2006). Aging and immortality: quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* 5, 2087–2102. doi: 10.4161/cc.5.18.3288

Blagosklonny, M. V. (2010). Why the disposable soma theory cannot explain why women live longer and why we age. *Aging* 2, 884–887. doi: 10.18632/aging.100253

Blagosklonny, M. V. (2013). Aging is not programmed: genetic pseudo-program is a shadow of developmental growth. *Cell Cycle* 12, 3736–3742. doi: 10.4161/cc.27188

Blix, A. S. (2016). Adaptations to polar life in mammals and birds. *J. Exp. Biol.* 219(Pt 8), 1093–1105. doi: 10.1242/jeb.120477

Boretto, J. M., Cabezas-Cartes, F., and Ibargüengoytía, N. R. (2018). Slow life histories in lizards living in the highlands of the Andes Mountains. *J. Comp. Physiol. B* 188, 491–503. doi: 10.1007/s00360-017-1136-z

Brunet-Rossinni, A. K. (2004). Reduced free-radical production and extreme longevity in the little brown bat (*Myotis lucifugus*) versus two non-flying mammals. *Mech. Ageing Dev.* 125, 11–20. doi: 10.1016/j.mad.2003.09.003

Bundgaard, A., James, A. M., Gruszczyk, A. V., Martin, J., Murphy, M. P., and Fago, A. (2019). Metabolic adaptations during extreme anoxia in the turtle heart and their implications for ischemia-reperfusion injury. *Sci. Rep.* 9:2850. doi: 10.1038/s41598-019-39836-5

Caldwell, R. W., Rodriguez, P. C., Toque, H. A., Narayanan, S. P., and Caldwell, R. B. (2018). Arginase: a multifaceted enzyme important in health and disease. *Physiol. Rev.* 98, 641–665. doi: 10.1152/physrev.00037.2016

Carré, N., Caüzac, M., Girard, J., and Burnol, A. F. (2008). Dual effect of the adapter growth factor receptor-bound protein 14 (grb14) on insulin action in primary hepatocytes. *Endocrinology* 149, 3109–3117. doi: 10.1210/en.2007-1196

Carter, G. G., and Wilkinson, G. S. (2013). Food sharing in vampire bats: reciprocal help predicts donations more than relatedness or harassment. *Proc. R. Soc. B Biol. Sci.* 280:20122573. doi: 10.1098/rspb.2012.2573

Carter, G. G., Farine, D. R., and Wilkinson, G. S. (2017). Social bet-hedging in vampire bats. *Biol. Lett.* 13:20170112. doi: 10.1098/rsbl.2017.0112

Castiglione, G. M., Xu, Z., Zhou, L., and Duh, E. J. (2020). Adaptation of the master antioxidant response connects metabolism, lifespan and feather development pathways in birds. *Nat. Commun.* 11:2476. doi: 10.1038/s41467-020-16129-4

Caswell, H. (2007). Extrinsic mortality and the evolution of senescence. *Trends Ecol. Evol.* 22, 173–174. doi: 10.1016/j.tree.2007.01.006

Caulin, A. F., and Maley, C. C. (2011). Peto's paradox: evolution's prescription for cancer prevention. *Trends Ecol. Evol.* 26, 175–182. doi: 10.1016/j.tree.2011.01.002

Caulin, A. F., Graham, T. A., Wang, L. S., and Maley, C. C. (2015). Solutions to Peto's paradox revealed by mathematical modelling and cross-species cancer gene analysis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370:20140222. doi: 10.1098/rstb.2014.0222

Chen, D., Thomas, E. L., and Kapahi, P. (2009). HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in *Caenorhabditis elegans*. *PLoS Genet.* 5:e1000486. doi: 10.1371/journal.pgen.1000486

Chen, F., Welker, F., Shen, C. C., Bailey, S. E., Bergmann, I., Davis, S., et al. (2019). A late middle pleistocene denisovan mandible from the Tibetan Plateau. *Nature* 569, 409–412. doi: 10.1038/s41586-019-1139-x

Chen, H. Y., and Maklakov, A. A. (2012). Longer life span evolves under high rates of condition-dependent mortality. *Curr. Biol.* 22, 2140–2143. doi: 10.1016/j.cub.2012.09.021

Clare, M. J., and Luckinbill, L. S. (1985). The effects of gene-environment interaction on the expression of longevity. *Heredity* 55(Pt 1), 19–26. doi: 10.1038/hdy.1985.67

Cohen, A. A., Coste, C. F., Li, X. Y., Bourg, S., and Pavard, S. (2020). Are trade−offs really the key drivers of ageing and life span? *Funct. Ecol.* 34, 153–166. doi: 10.1111/1365-2435.13444

Congdon, J. D., Nagle, R. D., Kinney, O. M., and van Loben Sels, R. C. (2001). Hypotheses of aging in a long-lived vertebrate, Blanding's turtle (*Emydoidea blandingii*). *Exp. Gerontol.* 36, 813–827. doi: 10.1016/s0531-5565(00)00242-4

Congdon, J. D., Nagle, R. D., Kinney, O. M., van Loben Sels, R. C., Quinter, T., and Tinkle, D. W. (2003). Testing hypotheses of aging in long-lived painted turtles (*Chrysemys picta*). *Exp. Gerontol.* 38, 765–772. doi: 10.1016/s0531-5565(03)00106-22

Conti, B., Sanchez-Alavez, M., Winsky-Sommerer, R., Morale, M. C., Lucero, J., Brownell, S., et al. (2006). Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314, 825–828. doi: 10.1126/science.1132191

Costantini, D., Smith, S., Killen, S. S., Nielsen, J., and Steffensen, J. F. (2017). The Greenland shark: a new challenge for the oxidative stress theory of ageing? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 203, 227–232. doi: 10.1016/j.cbpa.2016.09.026

Cree, A. (2014). *Tuatara: Biology and Conservation of a Venerable Survivor*. Canterbury: Canterbury University Press.

de Grey, A. D. (2007). Protagonistic pleiotropy: why cancer may be the only pathogenic effect of accumulating nuclear mutations and epimutations in aging. *Mech. Ageing Dev.* 128, 456–459. doi: 10.1016/j.mad.2007.05.005

de Magalhães, J. P., Costa, J., and Church, G. M. (2007). An analysis of the relationship between metabolism, developmental schedules, and longevity using phylogenetic independent contrasts. *J. Gerontol. A Biol. Sci. Med. Sci.* 62, 149–160. doi: 10.1093/gerona/62.2.149

Derous, D., Sahu, J., Douglas, A., Lusseau, D., and Wenzel, M. (2021). Comparative genomics of cetartiodactyla: energy metabolism underpins the transition to an aquatic lifestyle. *Conserv. Physiol.* 9:coaa136. doi: 10.1093/conphys/coaa136

Ding, D., Liu, G., Hou, L., Gui, W., Chen, B., and Kang, L. (2018). Genetic variation in PTPN1 contributes to metabolic adaptation to high-altitude hypoxia in Tibetan migratory locusts. *Nat. Commun.* 9:4991. doi: 10.1038/s41467-018-07529-8

Elder, W. H., and Rodgers, D. H. (1975). Body temperature in the African elephant as related to ambient temperature. *Mammalia* 39, 395–400.

Esparcia, A., Miracle, M. R., and Serra, M. (1989). "*Brachionus plicatilis* tolerance to low oxygen concentrations," in *Rotifer Symposium V*, (Dordrecht: Springer), 331–337.

Fang, X., Seim, I., Huang, Z., Gerashchenko, M. V., Xiong, Z., Turanov, A. A., et al. (2014b). Adaptations to a subterranean environment and longevity revealed by the analysis of mole rat genomes. *Cell Rep.* 8, 1354–1364. doi: 10.1016/j.celrep.2014.07.030

Fang, X., Nevo, E., Han, L., Levanon, E. Y., Zhao, J., Avivi, A., et al. (2014a). Genome-wide adaptive complexes to underground stresses in blind mole rats Spalax. *Nat. Commun.* 5:3966. doi: 10.1038/ncomms4966

Finch, C. E. (1998). Variations in senescence and longevity include the possibility of negligible senescence. *J. Gerontol. A Biol. Sci. Med. Sci.* 53, B235–B239. doi: 10.1093/gerona/53a.4.b235

Finch, C. E. (2009). Update on slow aging and negligible senescence–a mini-review. *Gerontology* 55, 307–313. doi: 10.1159/000215589

Finch, T. M., Zhao, N., Korkin, D., Frederick, K. H., and Eggert, L. S. (2014). Evidence of positive selection in mitochondrial complexes I and V of the African elephant. *PLoS One* 9:e92587. doi: 10.1371/journal.pone.009 2587

Gavrilov, L. A., and Gavrilova, N. S. (2002). Evolutionary theories of aging and longevity. *Sci. World J.* 2, 339–356. doi: 10.1100/tsw.2002.96

Gearty, W., McClain, C. R., and Payne, J. L. (2018). Energetic tradeoffs control the size distribution of aquatic mammals. *Proc. Natl. Acad. Sci. U.S.A.* 115, 4194–4199. doi: 10.1073/pnas.1712629115

Gemmell, N. J., Rutherford, K., Prost, S., Tollis, M., Winter, D., Macey, J. R., et al. (2020). The tuatara genome reveals ancient features of amniote evolution. *Nature* 584, 403–409. doi: 10.1038/s41586-020-2 561-9

George, J. C., Bada, J., Zeh, J., Scott, L., Brown, S. E., O'Hara, T., et al. (1999). Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. *Can. J. Zool.* 77, 571–580. doi: 10.1080/00139157.2016.113 4020

Gillet, L. C., and Schärer, O. D. (2006). Molecular mechanisms of mammalian global genome nucleotide excision repair. *Chem. Rev.* 106, 253–276. doi: 10. 1021/cr040483f

Gladyshev, V. N. (2013). The origin of aging: imperfectness-driven non-random damage defines the aging process and control of lifespan. *Trends Genet.* 29, 506–512. doi: 10.1016/j.tig.2013.05.004

Gladyshev, V. N. (2016). Aging: progressive decline in fitness due to the rising deleteriome adjusted by genetic, environmental, and stochastic processes. *Aging Cell* 15, 594–602. doi: 10.1111/acel.12480

Goldbogen, J. A., Cade, D. E., Wisniewska, D. M., Potvin, J., Segre, P. S., Savoca, M. S., et al. (2019). Why whales are big but not bigger: physiological drivers and ecological limits in the age of ocean giants. *Science* 366, 1367–1372. doi: 10.1126/science.aax9044

Goldsmith, T. C. (2004). Aging as an evolved characteristic – Weismann's theory reconsidered. *Med. Hypotheses* 62, 304–308. doi: 10.1016/S0306-9877(03)0 0337-2

Gorbunova, V., Bozzella, M. J., and Seluanov, A. (2008). Rodents for comparative aging studies: from mice to beavers. *Age* 30, 111–119. doi: 10.1007/s11357-008-9053-4

Gorbunova, V., Seluanov, A., Zhang, Z., Gladyshev, V. N., and Vijg, J. (2014). Comparative genetics of longevity and cancer: insights from long-lived rodents. *Nat. Rev. Genet.* 15, 531–540. doi: 10.1038/nrg3728

Goyns, M. H. (2002). Genes, telomeres and mammalian ageing. *Mech. Ageing Dev.* 123, 791–799. doi: 10.1016/s0047-6374(01)00424-9

Gradinger, R. R. (2001). Adaptation of Arctic and Antarctic ice metazoan to their habitat. *Zoology* 104, 339–345. doi: 10.1078/0944-2006-00039

Guarente, L., and Kenyon, C. (2000). Genetic pathways that regulate ageing in model organisms. *Nature* 408, 255–262. doi: 10.1038/35041700

Hamilton, W. D. (1966). The moulding of senescence by natural selection. *J. Theor. Biol.* 12, 12–45. doi: 10.1016/0022-5193(66)90184-6

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* 11, 298–300. doi: 10.1093/geronj/11.3.298

Harvey, P. H., and Purvis, A. (1999). "Understanding the ecological and evolutionary reasons for life history variation: mammals as a case study," in *Advanced Ecological Theory: Principles and Applications*, ed. J. McGlade (Hoboken, NJ: Wiley), 232–248.

Herreid, C. F. II (1964). Bat longevity and metabolic rate. *Exp. Gerontol.* 1, 1–9.

Holmes, D. J., and Austad, S. N. (1994). Fly now, die later: life-history correlates of gliding and flying in mammals. *J. Mammal.* 75, 224–226. doi: 10.2307/138 2255

Holmes, D. J., and Austad, S. N. (1995). Birds as animal models for the comparative biology of aging: a prospectus. *J. Gerontol. A Biol. Sci. Med. Sci.* 50, B59–B66. doi: 10.1093/gerona/50a.2.b59

Holmes, D. J., Flückiger, R., and Austad, S. N. (2001). Comparative biology of aging in birds: an update. *Exp. Gerontol.* 36, 869–883. doi: 10.1016/s0531-5565(00) 00247-3

Honda, S., Ishii, N., Suzuki, K., and Matsuo, M. (1993). Oxygen-dependent perturbation of life span and aging rate in the nematode. *J. Gerontol.* 48, B57–B61. doi: 10.1093/geronj/48.2.b57

Huang, Z., Whelan, C. V., Foley, N. M., Jebb, D., Touzalin, F., Petit, E. J., et al. (2019). Longitudinal comparative transcriptomics reveals unique mechanisms underlying extended healthspan in bats. *Nat. Ecol. Evol.* 3, 1110–1120. doi: 10.1038/s41559-019-0913-3

Huerta-Sánchez, E., Jin, X., Asan, Bianba, Z., Peter, B. M., Vinckenbosch, N., et al. (2014). Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. *Nature* 512, 194–197. doi: 10.1038/nature1 3408

Jiménez-Cortegana, C., López-Saavedra, A., Sánchez-Jiménez, F., Pérez-Pérez, A., Castiñeiras, J., Virizuela-Echaburu, J. A., et al. (2021). Leptin, both bad and good actor in cancer. *Biomolecules* 11:913. doi: 10.3390/biom110 60913

Jobson, R. W., Nabholz, B., and Galtier, N. (2010). An evolutionary genome scan for longevity-related natural selection in mammals. *Mol. Biol. Evol.* 27, 840–847. doi: 10.1093/molbev/msp293

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of ageing across the tree of life. *Nature* 505, 169–173. doi: 10.1038/nature12789

Jürgens, K. D., and Prothero, J. (1987). Scaling of maximal lifespan in bats. *Comp. Biochem. Physiol. A Comp. Physiol.* 88:361. doi: 10.1016/0300-9629(87)90 498-1

Kacprzyk, J., Locatelli, A. G., Hughes, G. M., Huang, Z., Clarke, M., Gorbunova, V., et al. (2021). Evolution of mammalian longevity: age-related increase in autophagy in bats compared to other mammals. *Aging* 13, 7998–8025. doi: 10.18632/aging.202852

Keane, M., Semeiks, J., Webb, A. E., Li, Y. I., Quesada, V., Craig, T., et al. (2015). Insights into the evolution of longevity from the bowhead whale genome. *Cell Rep.* 10, 112–122. doi: 10.1016/j.celrep.2014. 12.008

Khazaeli, A. A., and Curtsinger, J. W. (2013). Pleiotropy and life history evolution in *Drosophila melanogaster*: uncoupling life span and early fecundity. *J. Gerontol. A Biol. Sci. Med. Sci.* 68, 546–553. doi: 10.1093/gerona/gls226

Kim, E. B., Fang, X., Fushan, A. A., Huang, Z., Lobanov, A. V., Han, L., et al. (2011). Genome sequencing reveals insights into physiology and longevity of the naked mole rat. *Nature* 479, 223–227. doi: 10.1038/nature1 0533

Kimber, C. M., and Chippindale, A. K. (2013). Mutation, condition, and the maintenance of extended lifespan in *Drosophila*. *Curr. Biol.* 23, 2283–2287. doi: 10.1016/j.cub.2013.09.049

Kirkwood, T. B. (2005). Understanding the odd science of aging. *Cell* 120, 437–447. doi: 10.1016/j.cell.2005.01.027

Kirkwood, T. B., and Holliday, R. (1979). The evolution of ageing and longevity. *Proc. R. Soc. Lond. B Biol. Sci.* 205, 531–546. doi: 10.1098/rspb.1979.0083

Kirkwood, T. B., and Rose, M. R. (1991). Evolution of senescence: late survival sacrificed for reproduction. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 332, 15–24. doi: 10.1098/rstb.1991.0028

Krivoruchko, A., and Storey, K. B. (2010). Forever young: mechanisms of natural anoxia tolerance and potential links to longevity. *Oxid. Med. Cell. Longev.* 3, 186–198. doi: 10.4161/oxim.3.3.12356

Krstic, J., Reinisch, I., Schupp, M., Schulz, T. J., and Prokesch, A. (2018). p53 functions in adipose tissue metabolism and homeostasis. *Int. J. Mol. Sci.* 19:2622. doi: 10.3390/ijms19092622

LaVinka, P. C., Brand, A., Landau, V. J., Wirtshafter, D., and Park, T. J. (2009). Extreme tolerance to ammonia fumes in African naked mole-rats: animals that naturally lack neuropeptides from trigeminal chemosensory nerve fibers. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 195, 419–427. doi: 10.1007/s00359-009-0420-0

Lee, H., Kim, J., Weber, J. A., Chung, O., Cho, Y. S., Jho, S., et al. (2020). Whole genome analysis of the red-crowned crane provides insight into avian longevity. *Mol. Cells* 43, 86–95. doi: 10.14348/molcells.2019.0190

Leiser, S. F., Fletcher, M., Begun, A., and Kaeberlein, M. (2013). Life-span extension from hypoxia in *Caenorhabditis elegans* requires both HIF-1 and DAF-16 and

is antagonized by SKN-1. *J. Gerontol. A Biol. Sci. Med. Sci.* 68, 1135–1144. doi: 10.1093/gerona/glt016

Lewis, K. N., and Buffenstein, R. (2016). "The naked mole-rat: a resilient rodent model of aging, longevity, and healthspan," in *Handbook of the Biology of Aging*, eds M. R. Kaeberlein and G. M. Martin (Cambridge, MA: Academic Press), 179–204.

Lewis, K. N., Rubinstein, N. D., and Buffenstein, R. (2018). A window into extreme longevity; the circulating metabolomic signature of the naked mole-rat, a mammal that shows negligible senescence. *GeroScience* 40, 105–121. doi: 10.1007/s11357-018-0014-2

Li, Y., Wang, M. S., Otecko, N. O., Wang, W., Shi, P., Wu, D. D., et al. (2017). Hypoxia potentially promotes Tibetan longevity. *Cell Res.* 27, 302–305. doi: 10.1038/cr.2016.105

Luckinbill, L. S., Arking, R., Clare, M. J., Cirocco, W. C., and Buck, S. A. (1984). Selection for delayed senescence in *Drosophila melanogaster*. *Evolution* 38, 996–1003. doi: 10.1111/j.1558-5646.1984.tb00369.x

Lutz, P. L., Nilsson, G. E., and Prentice, H. M. (2003). *The Brain Without Oxygen: Causes of Failure-Physiological and Molecular Mechanisms for Survival*. Berlin: Springer Science & Business Media.

Ma, S., and Gladyshev, V. N. (2017). Molecular signatures of longevity: insights from cross-species comparative studies. *Semin. Cell Dev. Biol.* 70, 190–203. doi: 10.1016/j.semcdb.2017.08.007

Maklakov, A. A., Rowe, L., and Friberg, U. (2015). Why organisms age: evolution of senescence under positive pleiotropy? *Bioassay* 37, 802–807. doi: 10.1002/bies.201500025

Marshall, H. H., Vitikainen, E. I., Mwanguhya, F., Businge, R., Kyabulima, S., Hares, M. C., et al. (2017). Lifetime fitness consequences of early-life ecological hardship in a wild mammal population. *Ecol. Evol.* 7, 1712–1724. doi: 10.1002/ece3.2747

Masoro, E. J. (2000). Caloric restriction and aging: an update. *Exp. Gerontol.* 35, 299–305. doi: 10.1016/s0531-5565(00)00084-x

McNab, B. K. (1969). The economics of temperature regulation in neutropical bats. *Comp. Biochem. Physiol.* 31, 227–268. doi: 10.1016/0010-406X(69)91651-

Medawar, P. B. (1952). *An Unsolved Problem of Biology*. London: Published for the college by HK Lewis.

Mehta, R., Steinkraus, K. A., Sutphin, G. L., Ramos, F. J., Shamieh, L. S., Huh, A., et al. (2009). Proteasomal regulation of the hypoxic response modulates aging in C. elegans. *Science* 324, 1196–1198. doi: 10.1126/science.1173507

Miller, J. K. (2001). Escaping senescence: demographic data from the three-toed box turtle (*Terrapene carolina* triunguis). *Exp. Gerontol.* 36, 829–832. doi: 10.1016/s0531-5565(00)00243-6

Mole, M. A., Rodrigues DÁraujo, S., van Aarde, R. J., Mitchell, D., and Fuller, A. (2016). Coping with heat: behavioural and physiological responses of savanna elephants in their natural habitat. *Conserv. Physiol.* 4:cow044. doi: 10.1093/conphys/cow044

Montgomery, M. K., Buttemer, W. A., and Hulbert, A. J. (2012). Does the oxidative stress theory of aging explain longevity differences in birds? II. Antioxidant systems and oxidative damage. *Exp. Gerontol.* 47, 211–222. doi: 10.1016/j.exger.2011.11.014

Moorad, J. A., and Promislow, D. E. (2010). Evolution: aging up a tree? *Curr. Biol.* 20, R406–R408. doi: 10.1016/j.cub.2010.03.016

Moorad, J., Promislow, D., and Silvertown, J. (2019). Evolutionary ecology of senescence and a reassessment of Williams' 'extrinsic mortality' hypothesis. *Trends Ecol. Evol.* 34, 519–530. doi: 10.1016/j.tree.2019.02.006

Moreno Santillán, D. D., Lama, T. M., Gutierrez Guerrero, Y. T., Brown, A. M., Donat, P., Zhao, H., et al. (2021). Large-scale genome sampling reveals unique immunity and metabolic adaptations in bats. *Mol. Ecol.* 1–19. doi: 10.1111/mec.16027

Munshi-South, J., and Wilkinson, G. S. (2010). Bats and birds: exceptional longevity despite high metabolic rates. *Ageing Res. Rev.* 9, 12–19. doi: 10.1016/j.arr.2009.07.006

Nagel, A., and Nagel, R. (1991). How do bats choose optimal temperatures for hibernation? *Comp. Biochem. Physiol. A* 99, 323–326. doi: 10.1016/0300-9629(91)90008-Z

Nam, K., Lee, K. W., Chung, O., Yim, H. S., Cha, S. S., Lee, S. W., et al. (2017). Analysis of the FGF gene family provides insights into aquatic adaptation in cetaceans. *Sci. Rep.* 7:40233. doi: 10.1038/srep40233

Nielsen, J., Hedeholm, R. B., Heinemeier, J., Bushnell, P. G., Christiansen, J. S., Olsen, J., et al. (2016). Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). *Science* 353, 702–704. doi: 10.1126/science.aaf1703

Nilius, B., and Owsianik, G. (2011). The transient receptor potential family of ion channels. *Genome Biol.* 12:218. doi: 10.1186/gb-2011-12-3-218

Norberg, U. M. (2012). *Vertebrate Flight: Mechanics, Physiology, Morphology, Ecology and Evolution*, Vol. 27. Berlin: Springer Science & Business Media.

Nunney, L., Maley, C. C., Breen, M., Hochberg, M. E., and Schiffman, J. D. (2015). Peto's paradox and the promise of comparative oncology. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370:20140177. doi: 10.1098/rstb.2014.0177

O'Mara, M. T., Wikelski, M., Voigt, C. C., Ter Maat, A., Pollock, H. S., Burness, G., et al. (2017). Cyclic bouts of extreme bradycardia counteract the high metabolism of frugivorous bats. *eLife* 6:e26686. doi: 10.7554/eLife.26686

Ostrowski, S., and Williams, J. B. (2006). Heterothermy of free-living Arabian sand gazelles (*Gazella subgutturosa* marica) in a desert environment. *J. Exp. Biol.* 209(Pt 8), 1421–1429. doi: 10.1242/jeb.02151

Pamenter, M. E., Gomez, C. R., Richards, J. G., and Milsom, W. K. (2016). Mitochondrial responses to prolonged anoxia in brain of red-eared slider turtles. *Biol. Lett.* 12:20150797. doi: 10.1098/rsbl.2015.0797

Pamenter, M. E., Hall, J. E., Tanabe, Y., and Simonson, T. S. (2020). Cross-species insights into genomic adaptations to hypoxia. *Front. Genet.* 11:743. doi: 10.3389/fgene.2020.00743

Partridge, L., and Barton, N. H. (1993). Optimality, mutation and the evolution of ageing. *Nature* 362, 305–311. doi: 10.1038/362305a0

Pomeroy, D. (1990). Why fly? The possible benefits for lower mortality. *Biol. J. Linn. Soc.* 40, 53–65. doi: 10.1111/j.1095-8312.1990.tb00534.x

Ranjan, A., and Iwakuma, T. (2018). Emerging non-canonical functions and regulation of p53. *Int. J. Mol. Sci.* 19:1015. doi: 10.3390/ijms19041015

Rascón, B., and Harrison, J. F. (2010). Lifespan and oxidative stress show a non-linear response to atmospheric oxygen in *Drosophila*. *J. Exp. Biol.* 213(Pt 20), 3441–3448. doi: 10.1242/jeb.044867

Reichard, M. (2016). Evolutionary ecology of aging: time to reconcile field and laboratory research. *Ecol. Evol.* 6, 2988–3000. doi: 10.1002/ece3.2093

Reznick, D. N., Bryant, M. J., Roff, D., Ghalambor, C. K., and Ghalambor, D. E. (2004). Effect of extrinsic mortality on the evolution of senescence in guppies. *Nature* 431, 1095–1099. doi: 10.1038/nature02936

Reznick, D., Bryant, M., and Holmes, D. (2006). The evolution of senescence and post-reproductive lifespan in guppies (*Poecilia reticulata*). *PLoS Biol.* 4:e7. doi: 10.1371/journal.pbio.0040007

Rice, M. E., Lee, E. J., and Choy, Y. (1995). High levels of ascorbic acid, not glutathione, in the CNS of anoxia-tolerant reptiles contrasted with levels in anoxia-intolerant species. *J. Neurochem.* 64, 1790–1799. doi: 10.1046/j.1471-4159.1995.64041790.x

Robertshaw, D. (2006). Mechanisms for the control of respiratory evaporative heat loss in panting animals. *J. Appl. Physiol.* 101, 664–668. doi: 10.1152/japplphysiol.01380.2005

Ruby, J. G., Smith, M., and Buffenstein, R. (2018). Naked mole-rat mortality rates defy gompertzian laws by not increasing with age. *eLife* 7:e31157. doi: 10.7554/eLife.31157

Ruf, T., and Geiser, F. (2015). Daily torpor and hibernation in birds and mammals. *Biol. Rev. Cambridge Philos. Soc.* 90, 891–926. doi: 10.1111/brv.12137

Sakai, M., Matsumoto, M., Tujimura, T., Yongheng, C., Noguchi, T., Inagaki, K., et al. (2012). CITED2 links hormonal signaling to PGC-1α acetylation in the regulation of gluconeogenesis. *Nat. Med.* 18, 612–617. doi: 10.1038/nm.2691

Seim, I., Ma, S., Zhou, X., Gerashchenko, M. V., Lee, S. G., Suydam, R., et al. (2014). The transcriptome of the bowhead whale *Balaena mysticetus* reveals adaptations of the longest-lived mammal. *Aging* 6, 879–899. doi: 10.18632/aging.100699

Seluanov, A., Gladyshev, V. N., Vijg, J., and Gorbunova, V. (2018). Mechanisms of cancer resistance in long-lived mammals. *Nat. Rev. Cancer* 18, 433–441. doi: 10.1038/s41568-018-0004-9

Semenza, G. L. (2010). Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 29, 625–634. doi: 10.1038/onc.2009.441

Shattuck, M. R., and Williams, S. A. (2010). Arboreality has allowed for the evolution of increased longevity in mammals. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4635–4639. doi: 10.1073/pnas.0911439107

Shi, Y., Fan, S., Wu, M., Zuo, Z., Li, X., Jiang, L., et al. (2019). YTHDF1 links hypoxia adaptation and non-small cell lung cancer progression. *Nat. Commun.* 10:4892.

Simonson, T. S. (2015). Altitude adaptation: a glimpse through various lenses. *High Alt. Med. Biol.* 16, 125–137. doi: 10.1089/ham.2015.0033

Skulachev, V. P., and Longo, V. D. (2005). Aging as a mitochondria-mediated atavistic program: can aging be switched off? *Ann. N. Y. Acad. Sci.* 1057, 145–164. doi: 10.1196/annals.1356.009

Snell, T. W., Johnston, R. K., and Jones, B. L. (2019). Hypoxia extends lifespan of *Brachionus manjavacas* (Rotifera). *Limnetica* 38, 159–166. doi: 10.1111/fwb.13440

Snell, T., and King, C. (1977). Lifespan and fecundity patterns in rotifers: the cost of reproduction. *Evolution* 31, 882–890. doi: 10.2307/2407451

Söti, C., and Csermely, P. (2007). Protein stress and stress proteins: implications in aging and disease. *J. Biosci.* 32, 511–515. doi: 10.1007/s12038-007-0050-z

Speakman, J. R., and Thomas, D. W. (2003). "Physiological ecology and energetics of bats," in *Bat Ecology*, eds T. H. Kunz and M. B. Fenton (Chicago, IL: University of Chicago Press), 430–490.

Ste-Marie, E., Watanabe, Y. Y., Semmens, J. M., Marcoux, M., and Hussey, N. E. (2020). A first look at the metabolic rate of Greenland sharks (*Somniosus microcephalus*) in the Canadian Arctic. *Sci. Rep.* 10:19297. doi: 10.1038/s41598-020-76371-0

Sulak, M., Fong, L., Mika, K., Chigurupati, S., Yon, L., Mongan, N. P., et al. (2016). TP53 copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants. *eLife* 5:e11994. doi: 10.7554/eLife.11994

Sykiotis, G. P., and Bohmann, D. (2008). Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Dev. Cell* 14, 76–85. doi: 10.1016/j.devcel.2007.12.002

Tejada-Martinez, D., de Magalhães, J. P., and Opazo, J. C. (2021). Positive selection and gene duplications in tumour suppressor genes reveal clues about how cetaceans resist cancer. *Proc. R. Soc. B Biol. Sci.* 288:20202592. doi: 10.1098/rspb.2020.2592

Tian, R., Wang, Z., Niu, X., Zhou, K., Xu, S., and Yang, G. (2016). Evolutionary genetics of hypoxia tolerance in cetaceans during diving. *Genome Biol. Evol.* 8, 827–839. doi: 10.1093/gbe/evw037

Tian, X., Firsanov, D., Zhang, Z., Cheng, Y., Luo, L., Tombline, G., et al. (2019). SIRT6 is responsible for more efficient DNA double-strand break repair in long-lived species. *Cell* 177, 622–638.e22. doi: 10.1016/j.cell.2019.03.043

Tollis, M., Robbins, J., Webb, A. E., Kuderna, L., Caulin, A. F., Garcia, J. D., et al. (2019). Return to the sea, get huge, beat cancer: an analysis of cetacean genomes including an assembly for the humpback whale (*Megaptera novaeangliae*). *Mol. Biol. Evol.* 36, 1746–1763. doi: 10.1093/molbev/msz099

Tolstun, D. A., Knyazer, A., Tushynska, T. V., Dubiley, T. A., Bezrukov, V. V., Fraifeld, V. E., et al. (2020). Metabolic remodelling of mice by hypoxic-hypercapnic environment: imitating the naked mole-rat. *Biogerontology* 21, 143–153. doi: 10.1007/s10522-019-09848-9

Tullet, J., Green, J. W., Au, C., Benedetto, A., Thompson, M. A., Clark, E., et al. (2017). The SKN-1/Nrf2 transcription factor can protect against oxidative stress and increase lifespan in C. elegans by distinct mechanisms. *Aging Cell* 16, 1191–1194. doi: 10.1111/acel.12627

Tyner, S. D., Venkatachalam, S., Choi, J., Jones, S., Ghebranious, N., Igelmann, H., et al. (2002). p53 mutant mice that display early ageing-associated phenotypes. *Nature* 415, 45–53. doi: 10.1038/415045a

Tyshkovskiy, A., Bozaykut, P., Borodinova, A. A., Gerashchenko, M. V., Ables, G. P., Garratt, M., et al. (2019). Identification and application of gene expression signatures associated with lifespan extension. *Cell Metab.* 30, 573–593.e8. doi: 10.1016/j.cmet.2019.06.018

Vaupel, J. W., Baudisch, A., Dölling, M., Roach, D. A., and Gampe, J. (2004). The case for negative senescence. *Theor. Popul. Biol.* 65, 339–351. doi: 10.1016/j.tpb.2003.12.003

Vigne, P., and Frelin, C. (2006). A low protein diet increases the hypoxic tolerance in *Drosophila*. *PLoS One* 1:e56. doi: 10.1371/journal.pone.0000056

Walford, R. L., and Spindler, S. R. (1997). The response to calorie restriction in mammals shows features also common to hibernation: a cross-adaptation hypothesis. *J. Gerontol. A Biol. Sci. Med. Sci.* 52, B179–B183. doi: 10.1093/gerona/52a.4.b179

Wang, L., Karpac, J., and Jasper, H. (2014). Promoting longevity by maintaining metabolic and proliferative homeostasis. *J. Exp. Biol.* 217(Pt 1), 109–118. doi: 10.1242/jeb.089920

Weissenböck, N. M., Arnold, W., and Ruf, T. (2012). Taking the heat: thermoregulation in Asian elephants under different climatic conditions. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 182, 311–319. doi: 10.1007/s00360-011-0609-8

Wensink, M. J. (2016). Size, longevity and cancer: age structure. *Proc. R. Soc. B Biol. Sci.* 283:20161510. doi: 10.1098/rspb.2016.1510

Wensink, M. J., Caswell, H., and Baudisch, A. (2017). The rarity of survival to old age does not drive the evolution of senescence. *Evol. Biol.* 44, 5–10. doi: 10.1007/s11692-016-9385-4

Wensink, M. J., Wrycza, T. F., and Baudisch, A. (2014). Interaction mortality: senescence may have evolved because it increases lifespan. *PLoS One* 9:e109638. doi: 10.1371/journal.pone.0109638

Wilkinson, G. S., Adams, D. M., Haghani, A., Lu, A. T., Zoller, J., Breeze, C. E., et al. (2021). DNA methylation predicts age and provides insight into exceptional longevity of bats. *Nat. Commun.* 12:1615. doi: 10.1038/s41467-021-21900-2

Wilkinson, G. S., and Adams, D. M. (2019). Recurrent evolution of extreme longevity in bats. *Biol. Lett.* 15:20180860. doi: 10.1098/rsbl.2018.0860

Wilkinson, G. S., and South, J. M. (2002). Life history, ecology and longevity in bats. *Aging Cell* 1, 124–131. doi: 10.1046/j.1474-9728.2002.00020.x

Williams, G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 11, 398–411.

Williams, P. D., and Day, T. (2003). Antagonistic pleiotropy, mortality source interactions, and the evolutionary theory of senescence. *Evolution* 57, 1478–1488. doi: 10.1111/j.0014-3820.2003.tb00356

Willmore, W. G., and Storey, K. B. (1997). Antioxidant systems and anoxia tolerance in a freshwater turtle *Trachemys scripta* elegans. *Mol. Cell. Biochem.* 170, 177–185. doi: 10.1023/a:1006817806010

Wirthlin, M., Lima, N., Guedes, R., Soares, A., Almeida, L., Cavaleiro, N. P., et al. (2018). Parrot genomes and the evolution of heightened longevity and cognition. *Curr. Biol.* 28, 4001–4008.e7. doi: 10.1016/j.cub.2018.10.050

Wright, P. G., and Luck, C. P. (1984). Do elephants need to sweat? *S. Afr. J. Zool.* 19, 270–274. doi: 10.1080/02541858.1984.11447892

Wu, J. J., Liu, J., Chen, E. B., Wang, J. J., Cao, L., Narayan, N., et al. (2013). Increased mammalian lifespan and a segmental and tissue-specific slowing of aging after genetic reduction of mTOR expression. *Cell Rep.* 4, 913–920. doi: 10.1016/j.celrep.2013.07.030

Xia, C., and Møller, A. P. (2018). Long-lived birds suffer less from oxidative stress. *Avian Res.* 9, 1–7. doi: 10.1186/s40657-018-0133-6

Xu, S., Li, S., Yang, Y., Tan, J., Lou, H., Jin, W., et al. (2011). A genome-wide search for signals of high-altitude adaptation in Tibetans. *Mol. Biol. Evol.* 28, 1003–1011. doi: 10.1093/molbev/msq277

Yamamoto, M., Kensler, T. W., and Motohashi, H. (2018). The KEAP1-NRF2 system: a Thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiol. Rev.* 98, 1169–1203. doi: 10.1152/physrev.00023.2017

Yang, S., Lu, X., Wang, Y., Xu, L., Chen, X., Yang, F., et al. (2020). A paradigm of thermal adaptation in penguins and elephants by tuning cold activation in TRPM8. *Proc. Natl. Acad. Sci. U.S.A.* 117, 8633–8638. doi: 10.1073/pnas.1922714117

Yashin, A. I., Ukraintseva, S. V., Akushevich, I. V., Arbeev, K. G., Kulminski, A., and Akushevich, L. (2009). Trade-off between cancer and aging: what role do other diseases play? Evidence from experimental and human population studies. *Mech. Ageing Dev.* 130, 98–104. doi: 10.1016/j.mad.2008.03.006

Yi, X., Liang, Y., Huerta-Sanchez, E., Jin, X., Cuo, Z. X., Pool, J. E., et al. (2010). Sequencing of 50 human exomes reveals adaptation to high altitude. *Science* 329, 75–78. doi: 10.1126/science.1190371

Yuan, L., Zhao, X., Lin, B., Rossiter, S. J., He, L., Zuo, X., et al. (2011). Adaptive evolution of leptin in heterothermic bats. *PLoS One* 6:e27189. doi: 10.1371/journal.pone.0027189

Zhang, G., Li, C., Li, Q., Li, B., Larkin, D. M., Lee, C., et al. (2014). Comparative genomics reveals insights into avian genome evolution and adaptation. *Science* 346, 1311–1320. doi: 10.1126/science.1251385

Zhang, Q., and Edwards, S. V. (2012). The evolution of intron size in amniotes: a role for powered flight? *Genome Biol. Evol.* 4, 1033–1043. doi: 10.1093/gbe/evs070

Zhong, H., De Marzo, A. M., Laughner, E., Lim, M., Hilton, D. A., Zagzag, D., et al. (1999). Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. *Cancer Res.* 59, 5830–5835.

Zhou, D., Udpa, N., Gersten, M., Visk, D. W., Bashir, A., Xue, J., et al. (2011). Experimental selection of hypoxia-tolerant *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2349–2354. doi: 10.1073/pnas.1010643108

Zhou, X., Dou, Q., Fan, G., Zhang, Q., Sanderford, M., Kaya, A., et al. (2020). Beaver and naked mole rat genomes reveal common paths to longevity. *Cell Rep.* 32:107949. doi: 10.1016/j.celrep.2020.107949

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Androgen Elevation Accelerates Reproductive Senescence in Three-Spined Stickleback

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Costs of reproduction shape the life-history evolution of investment in current and future reproduction and thereby aging. Androgens have been proposed to regulate the physiology governing these investments. Furthermore, androgens are hypothesized to play a central role in carotenoid-dependent sexual signaling, regulating how much carotenoids are diverted to ornamentation and away from somatic maintenance, increasing oxidative stress, and accelerating aging. We investigated these relationships in male three-spined stickleback in which we elevated 11-ketotestosterone and supplied vitamin E, an antioxidant, in a 2 × 2 design. Androgen elevation shortened the time stickleback maintained reproductive activities. We suspect that this effect is caused by 11-ketotestosterone stimulating investment in current reproduction, but we detected no evidence for this in our measurements of reproductive effort: nest building, body composition, and breeding coloration. Carotenoid-dependent coloration was even slightly decreased by 11-ketotestosterone elevation and was left unaffected by vitamin E. Red coloration correlated with life expectancy and reproductive capacity in a quadratic manner, suggesting overinvestment of the individuals exhibiting the reddest bellies. In contrast, blue iris color showed a negative relationship with survival, suggesting physiological costs of producing this aspect of nuptial coloration. In conclusion, our results support the hypothesis that androgens regulate investment in current versus future reproduction, yet the precise mechanisms remain elusive. The quadratic relationships between sexual signal expression and aspects of quality have wider consequences for how we view sexual selection on ornamentation and its relationship with aging.

Keywords: aging, fecundity, carotenoid, stickleback, signaling, sexual hormone, sexual selection

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INTRODUCTION

Organisms evolve to optimize allocation of resources between different physiological processes to maximize fitness. Such resource-based trade-offs are central to life-history theory (Stearns, 1989) and have been adopted throughout biology, including the biology of aging (Maklakov and Chapman, 2019). Questions concerning, for example, the optimal arrival time at breeding sites (Kokko, 1999), litter size (Dijkstra et al., 1990; Sikes et al., 1998), foraging effort (Abrams, 1991), dietary restriction

(McCracken et al., 2020a), and prey choice (Rutten et al., 2006) can all be explained in a framework of fitness costs and benefits. A central or arguably ultimate cost in life history and the biology of aging is the cost of reproduction (Reznick et al., 2000; Lemaître et al., 2015). If there are no costs of producing offspring, why not simply produce more offspring to increase fitness?

The most direct test of these "costs of reproduction" is to increase reproductive effort experimentally and measure the long-term fitness consequences for the parents. In birds, this approach, by manipulating clutch or brood size, has been used many times. Yet, despite there being some undisputed demonstrations (Daan et al., 1990; Tinbergen and Daan, 1990) of costs of reproduction, a recent meta-analysis of 29 studies (Santos and Nakagawa, 2012) found that parental effort was associated with survival only in males, but not in females, with notably small overall effect sizes. Possibly, the fitness costs of parental effort are not traded off exclusively against future survival but also to future reproduction. Alternatively, the costs of an increased brood size are mainly paid by the offspring. This notion is supported by the finding that, in general, animals will not increase parental effort to such a degree that it fully compensates for the extra provisioning and care required by the offspring added, reducing offspring quality (Dijkstra et al., 1990; Simons et al., 2011). Indeed, the combined effects of brood enlargement studies on current reproduction, future reproduction, parental survival, and offspring fitness can reduce the total sum of fitness gained (Smith et al., 1989; Dijkstra et al., 1990; Tinbergen and Daan, 1990). Thus, whether the relationship between survival and investment in reproduction is causal and central in shaping the aging phenotype of animals remains less clear than current theory predicts.

In *Drosophila*, more suitable for artificial selection experiments than vertebrates, a genetic relationship between reproduction and survival has been demonstrated (Flatt, 2011). Surprisingly, however, there are multiple examples of long-lived mutants or experimental manipulations in *Drosophila* and *Caenorhabditis elegans* that extend lifespan without reduced or even increased fecundity (Flatt, 2011; McCracken et al., 2020b; Yamamoto et al., 2020; Lind et al., 2021). Note that these observations are made in the laboratory environment, and thus might not occur in the wild (Briga and Verhulst, 2015). Costs of reproduction therefore remain plausible but may be difficult to demonstrate or detect for different reasons. Physiological costs of reproduction may be context specific (Simons et al., 2014a), could be difficult to measure, or might be compensated or temporally dynamic.

Costs are also central to sexual signaling theory (Kotiaho, 2001; Számadó, 2011), and the cost of sexual traits for acquiring fertilizations should be viewed as part of the cost of reproduction (Höglund et al., 1998). According to the handicap hypothesis, mate choice for traits signaling male quality is only evolutionary stable when the trait bears costs, precluding cheating. Behavioral punishment (Számadó, 2011), energetic investment (Grafen, 1990; Candolin, 1999), mechanistic constraints (Emlen et al., 2012), and specific resource investment (Simons et al., 2014b) are mechanisms mediating the fitness cost of sexual signals. This diversity in the nature of costs may hamper detection of costs of sexual signals (Svensson and Wong, 2011), also because they may not be apparent in a laboratory setting where behavioral punishment is not possible, or because food is supplied *ad libitum*.

In general, all investment into reproduction, sexual signaling, and otherwise should be viewed as investment into current reproduction (Höglund et al., 1998), which is predicted to trade off with somatic maintenance and repair. It is this central trade-off that is persistently viewed as central in the biology of aging field, and forms the basis of the disposable soma theory of aging (Kirkwood, 2002). Investing in reproduction at the cost of somatic maintenance is usually optimal, because extrinsic mortality (mortality that cannot be fully intrinsically controlled) is almost never zero and investment into the soma is lost at death by an extrinsic cause (Williams, 1957). Any physiological investment that increases reproductive success is thus expected to reduce future reproduction either *via* accelerated mortality or *via* reproductive senescence.

Given this connection between any physiological cost paid to enhance current reproductive success at the expense of future reproductive success, we may expect physiological regulators balancing these investment decisions. Hormones, and in particular sex hormones, have gathered considerable attention in this regard (Hau, 2007; Regan et al., 2019; Bell, 2020; Garratt et al., 2020; Sugrue et al., 2021). In sexual signaling studies, testosterone specifically has attracted considerable attention for several reasons. First, the immunocompetence handicap hypothesis postulates that testosterone both suppresses the immune system and enhances expression of sexual traits and behavior (Folstad and Karter, 1992; Sheldon and Verhulst, 1996). Yet, evidence for direct immunosuppressive effects of testosterone is limited (Roberts et al., 2004), but see Newhouse and Vernasco (2020). In contrast, however, the reverse does hold, immune activation suppresses plasma testosterone, suggesting that testosterone plays a role in the trade-off between reproduction and somatic maintenance (Boonekamp et al., 2008). Second, testosterone has been shown to elevate carotenoid-based coloration (Blas et al., 2006; Kurtz et al., 2007; Perez-Rodriguez et al., 2008; Khalil et al., 2020), thereby possibly mediating the trade-off between current reproductive effort and oxidative stress (Schantz et al., 1999), induced by allocating carotenoids, an antioxidant [but see Koch et al. (2018) and Koch and Hill (2018)], away from maintenance towards sexual signaling (Peters, 2007; Svensson and Wong, 2011; Simons et al., 2012a). The link between oxidative stress and carotenoid signaling has gained further support by the increases in coloration observed when antioxidants are supplemented, such as vitamin E in stickleback (*Gasterosteus aculeatus*) (Pike et al., 2007a) and in gulls (*Larus michahellis*) (Pérez et al., 2008), but see Karu et al. (2008) and Giraudeau et al. (2013). A trade-off concerning oxidative stress can also link testosterone to immune suppression, given that higher levels of oxidative stress are hypothesized to negatively affect immunity (Kurtz et al., 2007; Peters, 2007; Costantini and Møller, 2009), but see Casagrande et al. (2012) and Newhouse and Vernasco (2020). Third, testosterone has also been suggested to increase metabolic rate but evidence is mixed (Buttemer et al.,

2008; Holtmann et al., 2017), yet could possibly enhance food intake and thereby carotenoid intake or growth of bodily extremities used as ornaments (Mougeot et al., 2004).

These multiple relationships between testosterone, physiology, and sexual signaling hamper the interpretation of negative findings on the trade-off between current and future reproductive success as costs may come about in physiological aspects other than those under study. Moreover, which and how physiology is altered by elevated testosterone may vary over time or cost may come to expression only later in life. Long-term experiments of the physiological consequences of elevated testosterone are therefore required.

In several of such longer-term studies, testosterone has been shown to induce costs. Experimental elevation of testosterone in adult male brown-headed cowbirds (*Molothrus ater*) showed reduced return rates, and this has been explained by experiencing higher rates of aggression, because testosterone-implanted individuals also showed more signs of injury likely incurred during fighting (Dufty, 1989). Male testosterone-implanted mountain spiny lizards (*Sceloporus jarrovi*) also show reduced survival, but this effect is negated by food supplementation, suggesting an energetic cost (Marler and Moore, 1991). Experimental elevation in males of another lizard species (*Psammodromus algirus*) also reduced survival and increased ectoparasitic infestation (Salvador et al., 1996). In birds, survival of dark-eyed juncos (*Junco hyemalis*) (Reed et al., 2006), red grouse (*Lagopus lagopus scoticus*) (Moss et al., 1994; Mougeot et al., 2005; Redpath et al., 2006), and red-legged partridges (*Alectoris rufa*) (Alonso-Alvarez et al., 2020) was also lowered in individuals in which testosterone was experimentally elevated. Return rates of testosterone-implanted greater prairie-chicken cocks (*Tympanunchus cupido*) males were also lower although not significantly so (Augustine et al., 2011).

These studies suggest that testosterone elevation indeed has long-term survival costs. However, in several of these studies, elevations of testosterone were in the pharmacological range and/or were maintained after the breeding season in which the hormone is not elevated. So, to what extent elevated exposure to testosterone reduces survival or future reproduction under more natural conditions is not clear. In addition, effects on reproductive senescence and mechanistic links to carotenoid-dependent sexual signal expression were not directly investigated in these studies. Here, we test whether (11-keto)-testosterone (the most biologically active androgen in most teleost fish) modulates the trade-off between current and future reproduction in three-spined stickleback. Reproductive behaviors and sexual coloration are absent in castrated stickleback, but can readily be restored by 11-ketoandrostenedione, which is rapidly converted to 11-ketotestosterone (Borg and Mayer, 1995). Male sticklebacks produce nests from algae and plant material, glued together with "spiggin," produced in their kidneys in response to 11-ketotestosterone (Jakobsson et al., 1996; Jakobsson et al., 1999). Using elaborate courtship, males attract gravid females to their nest to spawn and care for the offspring, and they repeat this nesting cycle multiple times in a single breeding season (Wootton and Robert, 1984). During their breeding season, stickleback exhibit a carotenoid-dependent trait, their reddish belly (Wedekind et al., 1998), which is subject to female choice (Künzler and Bakker, 2001; Pike et al., 2007b) and shows senescence (a decrease in functioning attributed to aging) within one breeding season (Kim et al., 2016). Males with redder bellies were previously found to have longer lifespans, and carotenoid supplementation extends lifespan and the time reproductive effort can be maintained (Pike et al., 2007b). Stickleback populations can either inhabit fresh water throughout the year or migrate to sea and back to breed in spring (anadromous populations).

The subjects of this study are wild-caught individuals on migration from sea to their breeding grounds. This population has been reported to be annual (van Mullem and van der Vlugt, 1964) (including more contemporary information from the Dutch Water Board). Size distribution data from caught stickleback in fishways, estuaries (including fish included in this study), and at the freshwater breeding grounds at the start of the breeding season showed a single peak in the distribution. Notably returning fish to sea also indicated a single peak of juvenile fish on their return migration (personal communication with and reports from the Dutch Water Board). Size density distributions are the most practical indication of the age distribution of populations of small fish. All indications from the limited data (from peer-reviewed sources and ecological reports) available on this population are therefore that the ecology of this population is an anadromous population that has an annual breeding lifecycle in freshwater. Hence, reproductive activities during a single breeding season likely determine lifetime reproductive effort. We hypothesize that 11-ketotestosterone elevation increases investment in current reproduction, e.g., nest building and sexual coloration, at the cost of maintaining the soma and thereby future reproduction.

METHODS

Animals

Anadromous three-spined stickleback were caught using a lift net at the locks of Noordpolderzijl, the Netherlands (53°25′56″N, 6°34′ 59″E). Small leaks of fresh water through the locks attract stickleback into the estuary when they start migration toward fresh water early spring. Fish were transported to the laboratory (<25 km away) by car in aerated buckets filled with water from the estuary. In our aquarium facility, fresh water was added to adjust the fish to fresh water conditions across several days. Groups of fish were housed together in large glass aquaria (>60 × 30 × 30 cm, L × H × W).

Setup

At the start of the experiment, individual males (N = 237) were housed in individual plastic tanks (27.5 × 17.5 × 17 cm, L × H × W, Ferplast geolarge), covered with a see-through plastic lid, containing a plastic plant (in the front of the tank, Tetra Plantastics Ambulia *Limnophila heterophylla*, 11–15 cm) and a pressure air (provided *via* connected tubing by a Resun LP-100 air-pump)-operated filter (at the back of the tank, Europet Bernina). One side adjacent to another tank was blinded with

white adhesive plastic that precluded any visual contact between the fish. The tanks were set in eight vertically connected steel cabinets each containing six rows of shelves. Treatments were distributed across the cabinets balanced evenly for row and column and fish started the experiment distributed across 5 days balanced for treatment to divide the time required for husbandry and measurements. The room was air-conditioned to keep water temperatures at 18°C. Lighting, LD 16:8, corresponding to the daylength at the height of summer in the Netherlands, started when males were put in individual tanks, and was provided by fluorescent tubes (OSRAM Cool White, L40W/640SA) placed on the ceiling in front of the cabinets.

Fish were fed every morning with defrosted red bloodworms, (*Chironomus*, 3F Frozen Fish Food) in portions of ±0.25 g using a plastic pipette. If after 15 min of the first portion an individual fish had finished all the provided food, it received another portion, to achieve near *ad libitum* feeding without detrimental effects of overfeeding on water quality. At the end of each day (at least 1 h after the first feeding round), excess food was removed from each individual tank using a plastic syringe. To stimulate sexual behavior, gravid females in a plastic see-through jar were shown to individual males each day for 5 min. Each week, all water of each individual tank was changed, excluding the water retained in the small filter compartment.

Males were provided with 400 threads of green polyester threads (0.840 g of ±6-cm-long threads) (Barber, 2001; Rushbrook et al., 2008) placed behind the artificial plant and a petri dish (placed in the back of the aquarium) filled with white aquarium sand as nesting material. Each week, the fish received new nesting material and had to rebuild their nest. Nests were examined each day, and if completed (judged by the presence of a tunnel in the nest) and if all material was used in the nest, a portion of extra green polyester threads (0.150 g) was added to the aquarium behind the artificial plant. This protocol allowed us to calculate a metric of nesting intensity as a composite of nest material used and speed of nest completion. This metric was expressed as the amount of days nest material was added minus the days it took to complete the nest.

The ability to build nests we used as a proxy of reproductive capacity given that without a nest a male stickleback cannot produce offspring, when sneaking of fertilizations that sometimes happen in specific populations is ignored. Our experimental protocol therefore did not assess paternal care at any point. We purposefully studied the consequences of androgens on the reproductive phase in which males display and attract females, as we were interested in trade-offs with carotenoid-dependent signaling. The photoperiod was changed to a short photoperiod (LD 8:16) after 160 days, when less than 10% of the individuals were showing nest building behavior. At that point, nest material provision and female stimulation ceased and individuals were subsequently only monitored for survival.

Injections and Circulating 11 kt Concentrations

After 1 week of acclimatization, individual males received two intraperitoneal injections (using 0.3-ml syringes, Becton Dickinson, Micro-Fine) of molten cocoa butter (14 µl/g fish body mass). The cocoa butter was injected at a temperature of 37°C (and solidified quickly in the fish kept in water of 18°C) and was loaded with 11-keto-androstenedione (in suspension, 4 mg/ml, based on a pilot study described below; Sigma Aldrich) and/or with vitamin E (dissolved, 226 mg/ml, α-tocopherol, Sigma Aldrich) or with nothing. With these combinations, we created a balanced 2 × 2 design of 11-keto-androstenedione and vitamin E. Our rationale for choosing vitamin E as a supplement was to study the suggested androgen-regulated relationship between carotenoid-dependent signaling and oxidative stress (Peters, 2007). To this end, we wanted to supplement an antioxidant, rather than pigmentary carotenoids with disputed antioxidant potential (Simons et al., 2012b), and vitamin E has previously been shown to increase the red belly of the stickleback (Pike et al., 2007a). In fish, 11-keto-androstenedione is rapidly converted to 11-ketotestosterone and similar methods have been used in three-spined stickleback previously to elevate 11-ketotestosterone concentrations (Páll et al., 2002; Kurtz et al., 2007), the main androgen in most male teleost fish (Borg et al., 1993; Borg, 1994; Borg and Mayer, 1995).

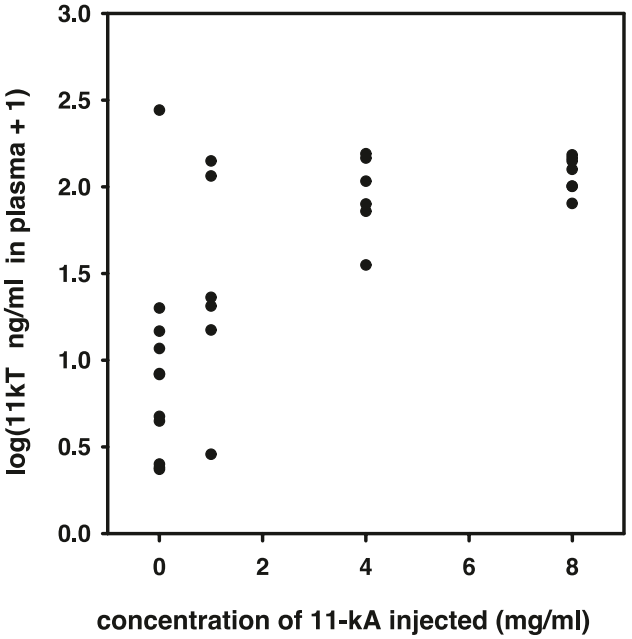

FIGURE 1 | Results of the pilot experiment to determine resulting plasma concentrations of 11-ketotestosterone after treatment with different dosages of 11-keto-androstenedione.

Prior to the main experiment, but in the same spring, we carried out a pilot experiment to determine the appropriate dose of 11-keto-androstenedione. We injected, as described above, concentrations of 1, 4, and 8 mg/ml 11-keto-androstenedione and subsequently obtained plasma 5 days later. 11-Ketotestosterone levels in plasma were determined by radioimmunoassay (RIA) and were found to be elevated strongly with the 4 mg/ml dose, without any apparent further increase at the highest dose [0 vs. 4, $\chi(1)^2 = 7.4$, $p = 0.007$; 4 vs. 8, $\chi(1)^2 = 0.62$, $p = 0.43$; **Figure 1**].

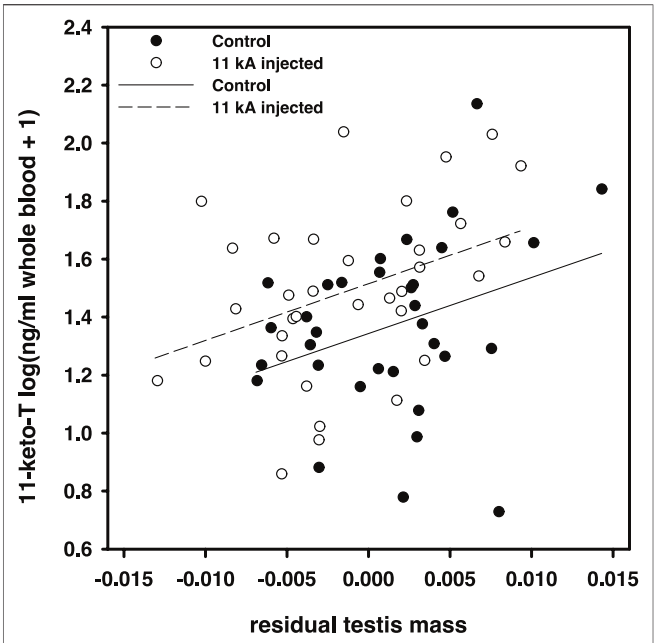

FIGURE 2 | 11-ketotestosterone plasma concentrations in the main experiment regressed against residual testis mass (against body mass). Open dots and dashed regression line, 11-keto-androstenedione treated individuals; closed dots and solid line, controls.

At 2, 3, 4, and 5 weeks after the injections, random subsets of individuals of the main experiment were sacrificed for blood collection balanced for treatment. The fish were killed by a blow to the head, the tail was cut just posterior of the anus, and blood was collected from the caudal vein using heparinized glass capillaries. Blood was kept on ice until plasma was obtained *via* centrifugation (850 RCF for 7 min). Hematocrit of the individual samples was measured from the centrifuged capillaries with a digital caliper (Mitutoyo, to the nearest 10 μm). Plasma was stored in −80°C prior to analyses.

Individual fish were weighed prior to blood taking and, after blood taking, the mass of the testes, liver, kidney, and spleen were determined. 11-Keto testosterone levels were determined *via* radioimmunoassay (RIA) as previously described (Sebire et al., 2007). After thawing, the plasma samples were centrifuged at 13,000 rpm for 3 min at 4°C and then 5 μl was transferred into a 1.5-ml Eppendorf tube. Distilled water (100 μl) and ethyl acetate (1 ml) were added to the tube and vortexed for a few seconds. The samples were again centrifuged (13,000 rpm for 3 min at 4°C) and the bottom of the tube was subsequently placed briefly into liquid nitrogen. The organic phase was separated from the frozen aqueous phase into a glass tube. This extraction was repeated a second time. The ethyl acetate extracts were dried under a nitrogen stream at 45°C and redissolved in 500 μl of RIA buffer prior to analysis by RIA.

Within the actual experiment, 11-keto-androstenedione treatment increased 11-ketotestosterone levels but relatively mildly [$F(1,64) = 5.97$, $p = 0.017$; **Figure 3**]. Note that these samples were taken later in the season (relative to the time of injection) than in the pilot study (**Figure 1**), which may explain the lower elevation measured. However, we did not detect an effect of time at which plasma was collected in this dataset [$F(1,61) = 0.18$, $p = 0.91$] which suggests that the mild elevation in 11-ketotestosterone our treatment induced was maintained for at least 5 weeks, yet levels prior to 2 weeks were probably higher, judging from the elevation measured in the pilot study. Furthermore, we only detected a significant effect of our treatment if we took natural variation of testis size into account, which covaried positively with 11-ketotestosterone plasma levels [$F(1,64) = 9.20$, $p = 0.004$; **Figure 2**]. Compared to the population variance in 11-ketotestosterone, independent of variance attributable to testis size, our experimental treatment with 11-keto-androstenedione resulted in an elevation of 0.63 standard deviation of 11-ketotestosterone. So, the 11-ketotestosterone level of a given fish in the 11-keto-androstenedione-treated group was elevated beyond its own endogenous production as determined by its testis size. This rationale is in line with the lack of feedback of circulating 11-ketotestosterone concentrations on its production in this species, implicating that experimental elevation of the hormone does not impact on its endogenous production. For example, removal of one testis halves 11-ketotestosterone levels (Hellqvist et al., 2002). No effects of vitamin E treatment on 11-ketotestosterone levels were detected ($p > 0.77$). Variation in 11-ketotestosterone did not correlate with maximum or average blue or red breeding coloration prior to sacrificing ($p > 0.64$ and $p > 0.10$, respectively).

Breeding Coloration

Weekly measurements of coloration were made using digital photography (Sony α-200) with fixed camera setting and in a controlled lighting environment. Fish were placed in a small glass container with a piece of foam at the back to restrain the fish to the front of the glass with its body side. The glass was fitted within a holder to allow it to be placed in a fully darkened box slightly tilted to avoid reflections in the glass. The camera was attached to this setup and a lightproof fabric was wrapped around the camera to avoid any outside light entering the box. A white-LED ring light (Sony HVL-RLAM) was placed on the lens of the camera and lit the box.

As digital cameras do not respond linearly to light, and hence are biased in measuring properties of light reflectance (e.g., color) (Stevens et al., 2007; Pike, 2011), we calibrated (Stigell et al., 2007) our camera with a large set of color patches (Munsell Glossy Edition, with known reflectance from the Joensuu Spectral Database) under the same lighting and fixed camera settings. Such an approach allows for an accurate representation of reflectance spectra from RGB values extracted from digital pictures (Stigell et al., 2007; Simons et al., 2012c; Simons et al., 2016). Fish were extracted from the pictures automatically using thresholding, cluster analysis, and alpha shapes in Matlab. The reddest and bluish part (consisting largely of blue iris coloration) of the individual fish were selected using image segmentation *via* thresholding of chroma from the simulated spectra per pixel (**Figure 3**). If all pixels fell below this threshold, this data point was excluded from the analysis, which happened in 8% of the cases within the analyses of blue coloration and in 0% of the cases where we analyzed red pigmentation. From these selected

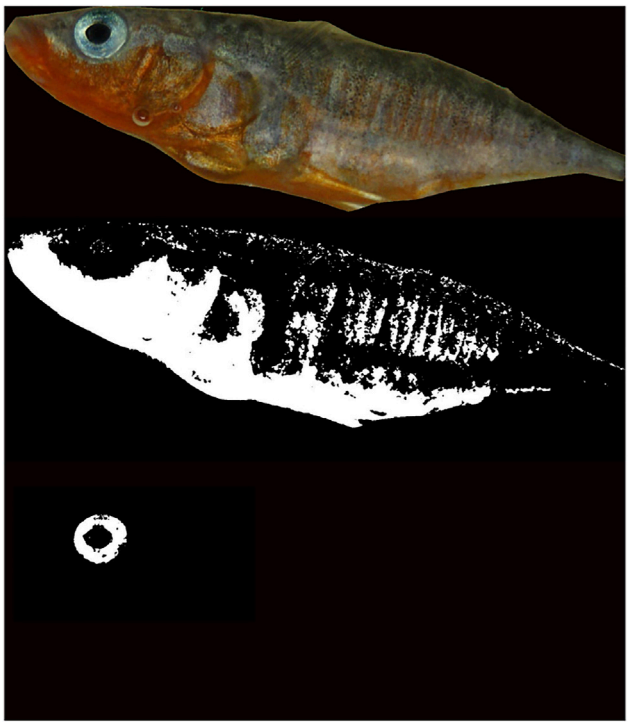

FIGURE 3 | Example of the automatic selection and thresholding used to obtain red and blue chroma of the nuptial coloration of the stickleback. Top shows the individual stickleback extracted from the picture, the middle part shows the selected pixels (in white) above the red chroma threshold, and the bottom part shows the extraction of the blue iris, also using chroma thresholding.

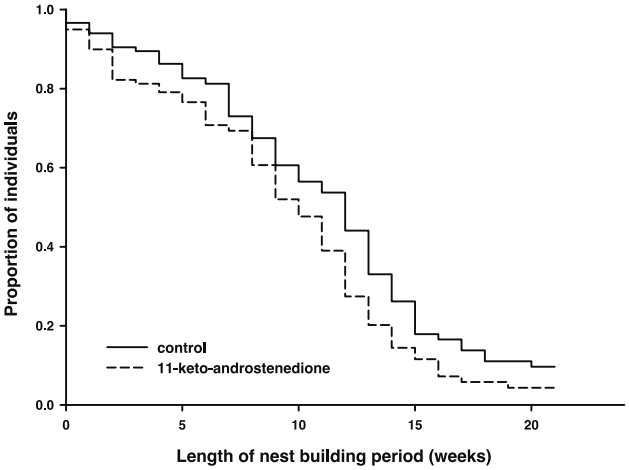

FIGURE 4 | Period (in weeks) during which reproductive activities could be maintained was reduced in individuals in which 11-ketotestosterone was elevated (dashed line) compared to controls (closed line).

patches, we calculated an average simulated spectra to estimate the chroma of the blue (summed reflectance between 420 and 540 nm divided by total reflectance between 420 and 740 nm) and red (summed reflectance between 620 and 740 nm divided by total reflectance between 420 and 740 nm) breeding coloration for each individual fish. The red and blue coloration, with the latter relatively understudied, of three-spined stickleback determine sexual attractiveness and are associated with aspects of quality (Milinski and Bakker, 1990; Künzler and Bakker, 2001; Rush et al., 2003; Pike et al., 2007b; Flamarique et al., 2013).

Statistical Analyses

All individual measures on the fish were analyzed with general linear models or mixed models. If multiple measures from a fish were included, a random intercept for each fish was included in the model to correct for pseudo-replication. Note, the time variable ("week") in the experiment was included as a categorical variable in the mixed models, as ignoring or misestimating random slopes in mixed models leads to a large inflation of type I error (Schielzeth and Forstmeier, 2009). Interpretation of patterns over time were based on least square means and their associated standard errors as provided in the figures. Model selection was based on backward selection of a model containing the interactions of the two treatment variables with the week of the experiment and mass at the start of the experiment. Breeding coloration was solely analyzed in fish that were still breeding (buildings nests), because breeding coloration fades quickly after the termination of reproductive activities. Reproductive senescence, as measured by the period that reproductive activities (i.e., nest building) were maintained, was analyzed using time to event Cox proportional hazard models ("coxph") (Therneau and Grambsch, 2000). These models used right-hand censoring for the sacrificed animals, those that died an accidental death, or those that were still building nests when the experiment was terminated (21 weeks after the injections). All analyses were performed in SAS JMP 7.0 and R. Sample sizes differ between the analyses that span across the breeding season, due to a decline in the number of eligible fish (i.e., surviving and in breeding state). Hence, degrees of freedom of each analysis provide information on the underlying sample size. No violations of the assumption of a Gaussian distribution were detected in the dependent variables and residuals of the parametric models.

RESULTS

Reproductive and Mortality Senescence

11-Keto-androstenedione treatment accelerated reproductive senescence. The period that individuals maintained their breeding activities (week of last complete nest minus week of first complete nest) was shorter after androgen treatment (logHR = 0.35 ± 0.16, $p = 0.034$, **Figure 4**), whereas the interaction with the main effect of vitamin E treatment was not significant ($p > 0.22$) and therefore removed from the model. This effect did not arise from 11-keto-androstenedione-treated animals starting with nest building sooner (rank test, $W = 6{,}985.5$, $p = 0.45$). A small part of this effect can be attributed to lower survival in the 11-keto-androstenedione treated fish. When analyzed across all data available for this study, there is a small decrease in survival that is far from significant (logHR = 0.05 ± 0.17, $p = 0.75$;

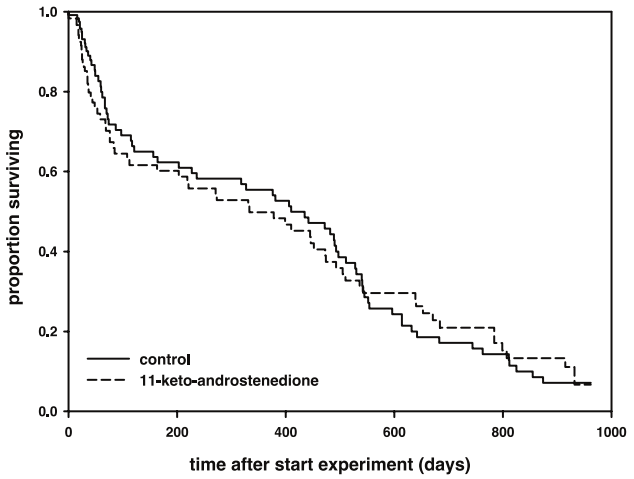

FIGURE 5 | Survival (in days after the start of the experiment) plotted across the whole follow-up period, separated for 11-keto-androstenedione-treated individuals (dashed) and controls (solid). The breeding season from which data on coloration and nest building are presented lasted for 160 days of long photoperiod after the start of the experiment.

TABLE 1 | Effects of treatment with 11-keto-androstenedione (11 kA) or vitamin E on organ mass (including mass at sacrificing as covariate) and mass at sacrificing and mass change (including mass at injection as covariate).

	Treatment	
	11 kA	**Vitamin E**
Testes	–0.0017 (0.0012)	–0.0010 (0.0012)
p	0.18	0.41
Liver	–0.0033 (0.0056)	–0.014 (0.0054)
p	0.56	0.011
Kidney	–0.0049 (0.0036)	–0.0044 (0.0036)
p	0.18	0.23
Spleen	–0.00037 (0.00097)	–0.0017 (0.00095)
p	0.71	0.07
Mass at sacrifice	–0.0053 (0.10)	–0.052 (0.10)
p	0.96	0.61
Mass change	–0.0072 (0.044)	–0.094 (0.044)
p	0.87	0.036

Estimates are given of the treatment effects with their standard errors, within parentheses. Sample size is between 76 and 79 individual fish due to missing data. Note that this sample size is higher than the sample sizes that we could use for the 11-ketotestosterone analyses, because of failures in collecting or processing blood.

Figure 5). Yet, when we analyzed data of the breeding season only, or up to the point of which we are sure 11-ketotestosterone is elevated (the last point at which animals were sacrificed for this purpose) the reduced survival in the 11-keto-androstenedione group these effects were stronger, but remained non-significant (logHR = 0.24 ± 0.25, $p = 0.33$, logHR = 0.53 ± 0.38, $p = 0.16$, respectively). No effects of vitamin E were detected in any of these models ($p > 0.24$).

Body Composition

Individual mass at sacrificing covaried positively with all four organ masses ($p < 0.0002$) and was therefore included in the models

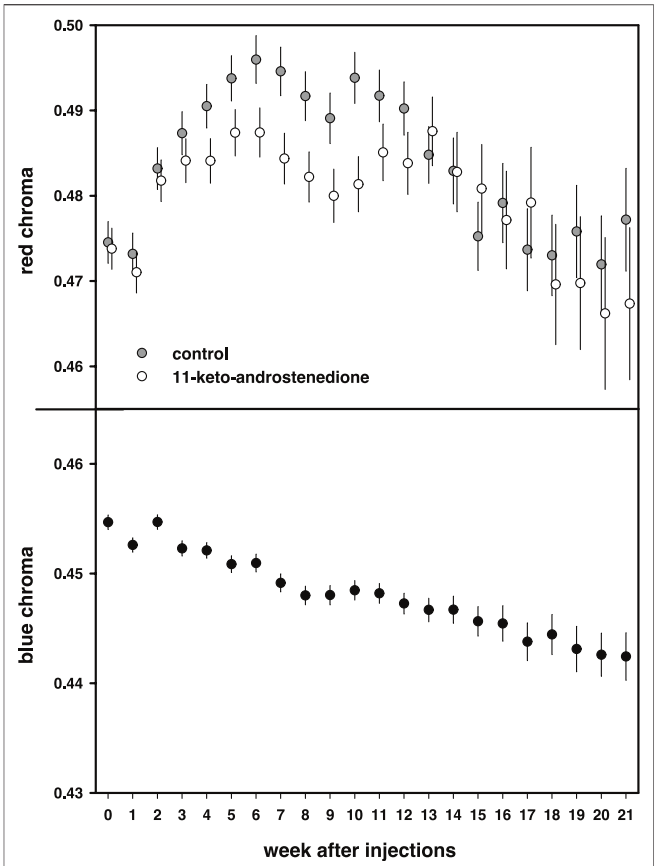

FIGURE 6 | Breeding coloration plotted against the weeks after injections. Red chroma of the belly is plotted in the top panel and first increases during the breeding season to subsequently decline after an optimum. 11-Keto-androstenedione-treated animals have slightly less concentrated coloration of their bellies before and after the period of the optimum. The bottom panel shows blue iris coloration, which steadily declines during the breeding season irrespective of treatment.

testing for treatment effects. 11-Keto-androstenedione treatment and vitamin E treatment did not interact for any of the organ masses ($p > 0.14$) and the week at which the individual was killed did not contribute significantly either ($p > 0.08$). Therefore, we tested the effect of 11-keto-androstenedione and vitamin E treatment separately on the masses of the testes, liver, kidney, and spleen, and also on mass at sacrificing with mass at the start of treatment included as covariate (**Table 1**). No effects of 11-keto-androstenedione treatment were detected. Vitamin E treatment reduced mass-specific liver and spleen mass, and also mass change from mass at sacrificing (**Table 1**). Note that most of these effects are likely driven by mass loss compared to mass at injection (mass at injection was balanced but slightly higher in vitamin E treated fish, estimate: 0.013 ± 0.11, $p = 0.23$). When mass at sacrificing was removed from the models, all associations with vitamin E and organ masses were reduced to non-significant trends, $p > 0.07$.

Coloration

Red coloration increased during the first part of the breeding season and declined at the end [week: $F(21,1663) = 14.8$, $p <$

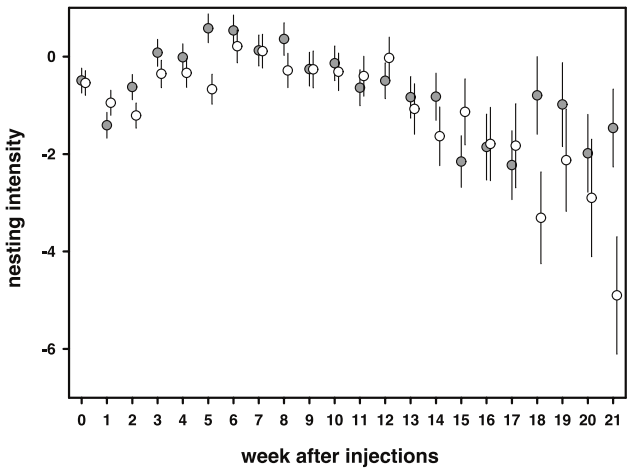

FIGURE 7 | Nesting intensity [amount of days extra material was added minus the time needed to complete a nest (days)] first increased during the breeding season and then declined. 11-Keto-androstenedione (open dots)-treated individuals showed in general reduced nesting intensity during the start of the breeding season.

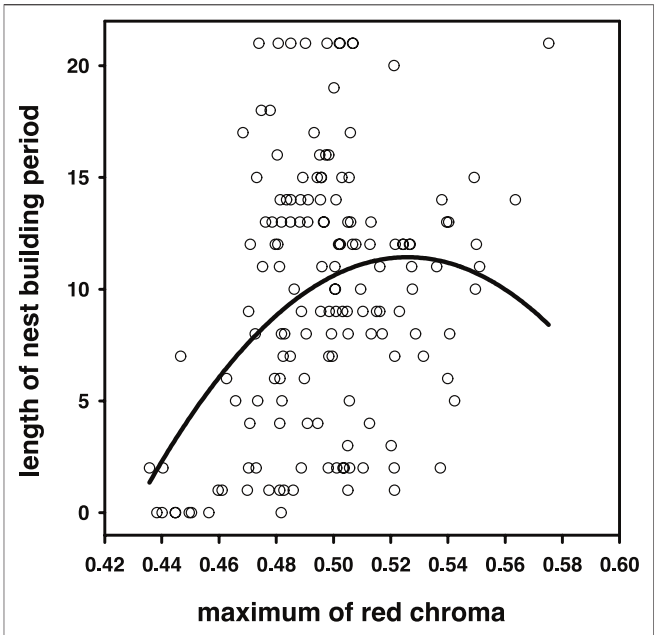

FIGURE 8 | Higher maximum red chroma achieved during the breeding season signaled higher reproductive capacity. However, the significance of the quadratic term suggests that in the reddest individuals, this relationship levels off or may even become negative.

0.0001]. This increase was lower in the androgen-treated fish, resulting in lower red coloration during the middle of the breeding season, but this effect did not reach statistical significance [11 kA × week: $F(21,1663) = 1.46$, $p = 0.08$; **Figure 6**]. In the models, we also included mass at the start of the experiment, which covaried positively with the intensity of the red breeding coloration [estimate: 0.010 ± 0.003, $F(1,220.2) = 10.90$, $p = 0.001$]. In these models, we did not detect any effects or interactions with vitamin E treatment ($p > 0.70$).

Blue coloration decreased during the breeding season [$F(21,1536) = 13.8$, $p < 0.0001$; **Figure 6**] but no effects or interactions with either 11-keto-androstenedione or vitamin E treatment were detected ($p > 0.72$) and no relationship with mass was detected [estimate: 0.0017 ± 0.001, $F(1,220.8) = 2.64$, $p = 0.11$].

Nest Building Behavior

Nesting intensity, the amount of times extra material was added minus the time needed to complete a nest, increased at the start of the breeding season and gradually declined towards the end of the season [week: $F(21,1609) = 6.18$, $p < 0.0001$, **Figure 7**]. 11-Keto-androstenedione-treated individuals showed reduced nesting intensity at the beginning and end of the breeding season [week × 11 kA: $F(21,1609) = 1.60$, $p = 0.042$; **Figure 7**]. No interactions with vitamin E treatment were detected ($p > 0.47$) and mass was positively related to nesting intensity [estimate = 0.94 ± 0.25, $F(1,203.8) = 13.6$, $p = 0.0003$].

Relationships Between the Intensity of Breeding Coloration With Reproductive Senescence and Lifespan

Average and maximum chroma (which correlated strongly, $r = 0.94$, $N = 155$, $p < 0.0001$) achieved during the breeding season correlated positively with the time individuals could maintain their nesting activity (average: $r = 0.17$, $p = 0.036$; maximum $r = 0.29$, $p = 0.0002$, $N = 155$; **Figure 8**). For maximum blue coloration, we also detected a trend of a positive relationship ($r = 0.15$, $p = 0.06$, $N = 155$, but not for average $r = -0.12$, $p = 0.12$, $N = 155$). Note that from these analyses, we excluded individuals that were sacrificed for the 11-ketotestosterone analyses, because these animals are censored with respect to the averaged and maximum chroma they could have achieved. Because quadratic relationships with sexual signal expression and longevity have been reported previously, we also tested for quadratic effects and detected a quadratic effect in the relationship between maximum red chroma and breeding period [$F(1,152) = 7.73$, $p = 0.006$; **Figure 8**, quadratic relationships with max blue chroma were not significant $p > 0.9$]. Note that this quadratic relationship with breeding period (and also lifespan below) and maximum chroma cannot be attributable to regression to the mean. The maximum is predicted to increase with the number of sampling points, related to breeding period (and lifespan) in our setup, and in this respect, the linear slope of maximum chroma may be biased upward, rather than downward.

Lifespan was also positively related to average [logHR: −3.29 ± 4.06, $\chi(1)^2 = 0.67$, $p = 0.42$] and maximum red chroma [logHR: −9.20 ± 4.00, $\chi(1)^2 = 5.50$, $p = 0.022$]. For maximum red chroma, we also detected a quadratic relationship (logHR estimates: linear: −290 ± 90.7, $p = 0.001$; quadratic 279 ± 89.8, $p < 0.002$). Higher maximum blue chroma tended to be associated with lower survival (logHR: 16.64 ± 10.90, $p = 0.13$). When maximum blue chroma was added to the quadratic maximum red chroma model, both the linear term and the quadratic term of

red chroma remained significant ($p < 0.008$), and maximum blue chroma was significantly positively related to mortality hazard (logHR: 27.7 ± 12.3, $p = 0.024$). Note that maximum red chroma and maximum blue chroma only loosely correlate ($r = 0.35$, $p < 0.001$) (Rush et al., 2003); multicollinearity biasing the model estimates is therefore unlikely.

DISCUSSION

Current and Future Reproduction

11-Ketotestosterone elevation decreased our proxy for future reproductive success, the length that reproductive activities are maintained. This suggests that androgens are involved in the regulation of the trade-off between current and future reproduction. However, we do not find any evidence for positive effects on our proxies for current reproduction after 11-ketotestosterone elevation. On the contrary, if anything, red breeding coloration and nesting vigor are lowered in the 11-ketotestosterone elevated animals. Negative consequences of our treatment because of high pharmacological dosing are unlikely because our treatment resulted in only a mild increase in 11-ketotestosterone. Therefore, investment induced by 11-ketotestosterone in other aspects of physiology that we did not measure may have generated the costs that resulted in the reduction in future reproductive success we observed. These costs could be related to various aspects of reproduction such as sperm production, spiggin production, and sexual behavior. It is probably unlikely that these costs involve direct increased metabolic demand, because mass and body composition were left unaffected by 11-ketotestosterone elevation. Alternatively, there might be direct costs of higher levels of 11-ketotestosterone imposed by unknown physiological constraints, thereby reducing future reproduction.

Androgens, Vitamin E, and Carotenoid-Dependent Coloration

The hypothesized involvement of androgens in the trade-offs concerning carotenoid-dependent coloration (Alonso-Alvarez et al., 2007; Peters, 2007) is not supported by our findings. Elevation of 11-ketotestosterone did not increase red breeding coloration, but rather decreased it. In addition, variation in 11-ketotestosterone levels was not related to breeding coloration, in line with an earlier smaller study (Wright et al., 2016). Furthermore, another study on stickleback red breeding coloration and 11-ketotestosterone (Kurtz et al., 2007) found that if individuals had spent 4 (higher 11-ketotestosterone level) compared to 6 weeks (lower 11-ketotestosterone levels) in breeding conditions prior to measurement of circulating 11-ketotestosterone levels, a correlation with breeding coloration was not apparent. Our results and previous ones might thus suggest that variation in 11-ketotestosterone levels in full breeding condition does not determine investment in breeding coloration but may still regulate a suite of other behaviors or aspects of physiology related to current reproduction.

Our results also do not support the mechanistic explanation for the hypothesized trade-off between oxidative stress and carotenoid allocation to sexual coloration. Vitamin E treatment did not increase coloration. This is contrary to an earlier report in stickleback showing that breeding coloration increased under a diet of a combination of vitamin C and E (Pike et al., 2007a). Similarly, the reduction in time that 11-keto-androstenedione-treated fish could maintain their reproductive activities is unlikely to be attributable to oxidative stress costs, because we detected no interactions with the vitamin E treatment. These conclusions all assume that our methodology of injecting vitamin E resulted in elevated levels of vitamin E in our fish. No pellets were lost by fish and were observed in all fish that were sacrificed and died a natural death. We must assume that vitamin E was therefore available to the fish for uptake. However, we prioritized analyzing the plasma samples for 11-ketotestosterone analyses as they were too small to also measure vitamin E. Treatment with vitamin E led to slightly lower body and organ mass, which could indicate a loss rather than a gain in body condition. Our conclusions are therefore dependent on the only partially supported assumption that vitamin E injection led to increased bioavailable vitamin E.

Senescence of Nuptial Coloration and Associations With Survival

Variation in red breeding coloration was positively related to longevity and the time nesting activities could be maintained. This is in concordance with an earlier study on sticklebacks of a smaller sample size ($N = 32$) that investigated the relationship between redness and longevity (Pike et al., 2007b). Sexual ornament expression is in general found to be positively related to survival (Jennions et al., 2001), yet examples for carotenoid-dependent coloration are relatively scarce and dominated by studies on birds (Hill, 1991; Hõrak et al., 2001; Figuerola and Carlos Senar, 2007; Simons et al., 2012d; Simons et al., 2016; Cantarero et al., 2019). Interestingly, we detected a quadratic relationship of redness with survival and the length that breeding can be maintained, suggesting that, at a certain point, carotenoid-dependent breeding coloration does not signal quality but may be related to reduced survival and breeding capacity. It is possible that this is a common pattern for carotenoid-dependent signals or even sexual signals in general. Serins (*Serinus serinus*) with intermediate carotenoid-derived brightness have higher survival (Figuerola and Carlos Senar, 2007), and in the zebra finch (*Taeniopygia guttata*), we detected a similar quadratic relationship of their carotenoid-based bill coloration and longevity, when controlling for terminal declines (Simons et al., 2012d; Simons et al., 2016). Together, these findings indicate that the reddest mates potentially overinvest in their ornament to attract females. Choice for these males (Milinski and Bakker, 1990; Künzler and Bakker, 2001; Pike et al., 2007b) might still provide the direct benefits of producing sexy sons, or high signaling males might signal different aspects of quality, perhaps related more to current rather than future reproduction. For example, redness of the stickleback belly is related to functional fertility (Pike et al., 2010) and unexpectedly higher somatic and germline damage was detected in males with the most red signals (Kim and Velando, 2020).

Compared to the carotenoid-based red belly of the stickleback, their iridescent blue iris has been studied less, but has been suggested to be a decisive feature during female mate choice (Rush et al., 2003; Flamarique et al., 2013). Males may maintain a red belly for the purpose of enhancing blue iris contrast (Rush et al., 2003; Flamarique et al., 2013). Surprisingly we find that chroma of the blue iris reduces over the breeding season and that red breeding coloration is not at its maximum when the blue iris is. There is thus a mismatch between these two signals that does not result in the highest possible contrast, which would be perceived as most attractive to females. Moreover, the maximum intensity of the blue iris was negatively related to survival, which can suggest that there are costs to maintain iris color. The iridescent blue iris of the stickleback is formed by endogenously produced pigments (Frischknecht, 1993). The associated costs or detailed information on its physiology is currently lacking and warrant future study.

CONCLUSION

11-Ketotestosterone elevation reduced future reproductive success. Although we did not detect benefits to current reproduction, it is plausible that 11-ketotestosterone regulates aspects of the trade-off between current and future reproduction. It remains to be determined what these aspects are. We did not detect any evidence for the proposed regulatory role of androgens in the carotenoid trade-off between somatic maintenance and sexual signaling. We did find, however, that carotenoid-dependent coloration signals reproductive capacity and longevity. In the reddest individuals, however, this relationship is diminished and even turns negative. These quadratic relationships between sexual signal expression and aspects of quality (Candolin, 1999; Simons et al., 2016), not unique to the stickleback, have important consequences for how we view sexual selection on ornamentation in general.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the DEC University of Groningen.

AUTHOR CONTRIBUTIONS

MJPS conducted the experiments, analyzed the data, and wrote the manuscript. MS conducted laboratory analyses. SV and TG supervised the project.

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REFERENCES

Abrams, P. A. (1991). The Fitness Costs of Senescence: The Evolutionary Importance of Events in Early Adult Life. *Evol. Ecol.* 5, 343–360. doi:10.1007/bf02214152

Alonso-Alvarez, C., Bertrand, S., Faivre, B., Chastel, O., and Sorci, G. (2007). Testosterone and Oxidative Stress: The Oxidation Handicap Hypothesis. *Proc. R. Soc. B.* 274, 819–825. doi:10.1098/rspb.2006.3764

Alonso-Alvarez, C., Cantarero, A., Romero-Haro, A. Á., Chastel, O., and Pérez-Rodríguez, L. (2020). Life-Long Testosterone and Antiandrogen Treatments Affect the Survival and Reproduction of Captive Male Red-Legged Partridges (*Alectoris rufa*). *Behav. Ecol. Sociobiol.* 74, 98. doi:10.1007/s00265-020-02878-1

Augustine, Jacqueline, K., Millspaugh, Joshua, J., Sandercock, and Brett, K. (2011). "Chapter Fourteen. Testosterone Mediates Mating Success in Greater Prairie-Chickens," in *Ecology, Conservation, and Management of Grouse: Published for the Cooper Ornithological Society*. Editors K. S. Brett, Kathy Martin, and Gernot Segelbacher. (Berkeley: University of California Press), 195–208. doi:10.1525/9780520950573-016

Barber, I. (2001). Nests as Ornaments: Revealing Construction by Male Sticklebacks. *Behav. Ecol.* 12, 390–396. doi:10.1093/beheco/12.4.390

Bell, A. M. (2020). Individual Variation and the Challenge Hypothesis. *Horm. Behav.* 123, 104549. doi:10.1016/j.yhbeh.2019.06.013

Blas, J., Perez-Rodriguez, L., Bortolotti, G. R., Vinuela, J., and Marchant, T. A. (2006). Testosterone Increases Bioavailability of Carotenoids: Insights into the Honesty of Sexual Signaling. *Proc. Natl. Acad. Sci.* 103, 18633–18637. doi:10.1073/pnas.0609189103

Boonekamp, J. J., Ros, A. H. F., and Verhulst, S. (2008). Immune Activation Suppresses Plasma Testosterone Level: A Meta-Analysis. *Biol. Lett.* 4, 741–744. doi:10.1098/rsbl.2008.0347

Borg, B. (1994). Androgens in Teleost Fishes. *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol.* 109, 219–245. doi:10.1016/0742-8413(94)00063-g

Borg, B., Antonopoulou, E., Andersson, E., Carlberg, T., and Mayer, I. (1993). Effectiveness of Several Androgens in Stimulating Kidney Hypertrophy, a Secondary Sexual Character, in Castrated Male Three-Spined Sticklebacks, *Gasterosteus aculeatus*. *Can. J. Zool.* 71, 2327–2329. doi:10.1139/z93-326

Borg, B., and Mayer, I. (1995). Androgens and Behaviour in the Three-Spined Stickleback. *Behaviour* 132, 1025–1035. doi:10.1163/156853995x00432

Briga, M., and Verhulst, S. (2015). What Can Long-Lived Mutants Tell Us about Mechanisms Causing Aging and Lifespan Variation in Natural Environments? *Exp. Gerontol.* 71, 21–26. doi:10.1016/j.exger.2015.09.002

Buttemer, W. A., Warne, S., Bech, C., and Astheimer, L. B. (2008). Testosterone Effects on Avian Basal Metabolic Rate and Aerobic Performance: Facts and

Artefacts. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 150, 204–210. doi:10.1016/j.cbpa.2006.06.047

Candolin, U. (1999). The Relationship between Signal Quality and Physical Condition: Is Sexual Signalling Honest in the Three-Spined Stickleback? *Anim. Behav.* 58, 1261–1267. doi:10.1006/anbe.1999.1259

Cantarero, A., Pérez-Rodríguez, L., Romero-Haro, A. Á., Chastel, O., and Alonso-Alvarez, C. (2019). Carotenoid-based Coloration Predicts Both Longevity and Lifetime Fecundity in Male Birds, but Testosterone Disrupts Signal Reliability. *PLoS One* 14, e0221436. doi:10.1371/journal.pone.0221436

Casagrande, S., Costantini, D., Dell'Omo, G., Tagliavini, J., and Groothuis, T. G. G. (2012). Differential Effects of Testosterone Metabolites Oestradiol and Dihydrotestosterone on Oxidative Stress and Carotenoid-Dependent Colour Expression in a Bird. *Behav. Ecol. Sociobiol.* 66, 1319–1331. doi:10.1007/s00265-012-1387-3

Costantini, D., and Møller, A. P. (2009). Does Immune Response Cause Oxidative Stress in Birds? A Meta-Analysis. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 153, 339–344. doi:10.1016/j.cbpa.2009.03.010

Daan, S., Dijkstra, C., and Tinbergen, J. M. (1990). Family Planning in the Kestrel (*Falco tinnunculus*): The Ultimate Control of Covariation of Laying Date and Clutch Size. *Behaviour* 114, 83–116. doi:10.1163/156853990x00077

Dijkstra, C., Bult, A., Bijlsma, S., Daan, S., Meijer, T., and Zijlstra, M. (1990). Brood Size Manipulations in the Kestrel (*Falco tinnunculus*): Effects on Offspring and Parent Survival. *J. Anim. Ecol.* 59, 269. doi:10.2307/5172

Dufty, A. M. (1989). Testosterone and Survival: A Cost of Aggressiveness. *Horm. Behav.* 23, 185–193. doi:10.1016/0018-506x(89)90059-7

Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I., and Lavine, L. C. (2012). A Mechanism of Extreme Growth and Reliable Signaling in Sexually Selected Ornaments and Weapons. *Science* 337, 860–864. doi:10.1126/science.1224286

Figuerola, J., and Carlos Senar, J. (2007). Serins with Intermediate Brightness Have a Higher Survival in the Wild. *Oikos* 116, 636–641. doi:10.1111/j.0030-1299.2007.14719.x

Flamarique, I. N., Bergstrom, C., Cheng, C. L., and Reimchen, T. E. (2013). Role of the Iridescent Eye in Stickleback Female Mate Choice. *J. Exp. Biol.* 216, 2806–2812. doi:10.1242/jeb.084889

Flatt, T. (2011). Survival Costs of Reproduction in Drosophila. *Exp. Gerontol.* 46, 369–375. doi:10.1016/j.exger.2010.10.008

Folstad, I., and Karter, A. J. (1992). Parasites, Bright Males, and the Immunocompetence Handicap. *The Am. Naturalist* 139, 603–622. doi:10.1086/285346

Frischknecht, M. (1993). The Breeding Colouration of Male Three-Spined Sticklebacks (*Gasterosteus aculeatus*) as an Indicator of Energy Investment in Vigour. *Evol. Ecol.* 7, 439–450. doi:10.1007/bf01237640

Garratt, M., Try, H., Smiley, K. O., Grattan, D. R., and Brooks, R. C. (2020). Mating in the Absence of Fertilization Promotes a Growth-Reproduction versus Lifespan Trade-Off in Female Mice. *Proc. Natl. Acad. Sci. U.S.A.* 117, 15748–15754. doi:10.1073/pnas.2003159117

Giraudeau, M., Sweazea, K., Butler, M. W., and McGraw, K. J. (2013). Effects of Carotenoid and Vitamin E Supplementation on Oxidative Stress and Plumage Coloration in House Finches (*Haemorhous mexicanus*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 166, 406–413. doi:10.1016/j.cbpa.2013.07.014

Grafen, A. (1990). Biological Signals as Handicaps. *J. Theor. Biol.* 144, 517–546. doi:10.1016/s0022-5193(05)80088-8

Hau, M. (2007). Regulation of Male Traits by Testosterone: Implications for the Evolution of Vertebrate Life Histories. *BioEssays* 29, 133–144. doi:10.1002/bies.20524

Hellqvist, A., Mayer, I., and Borg, B. (2002). Effects of Hemi-Castration on Plasma Steroid Levels in Two Teleost Fishes; the Three-Spined Stickleback, *Gasterosteus aculeatus*, and the Atlantic salmon, *Salmo salar*. *Fish. Physiol. Biochem.* 26, 107–110. doi:10.1023/A:1025435625551

Hill, G. E. (1991). Plumage Coloration Is a Sexually Selected Indicator of Male Quality. *Nature* 350, 337–339. doi:10.1038/350337a0

Höglund, J., Sheldon, B. C., and Hoglund, J. (1998). The Cost of Reproduction and Sexual Selection. *Oikos* 83, 478, doi:10.2307/3546675

Holtmann, B., Lagisz, M., and Nakagawa, S. (2017). Metabolic Rates, and Not Hormone Levels, are a Likely Mediator of Between-individual Differences in Behaviour: A Meta-Analysis. *Funct. Ecol.* 31, 685–696. doi:10.1111/1365-2435.12779

Hōrak, P., Ots, I., Vellau, H., Spottiswoode, C., and Pape Møller, A. (2001). Carotenoid-based Plumage Coloration Reflects Hemoparasite Infection and Local Survival in Breeding Great Tits. *Oecologia* 126, 166–173. doi:10.1007/s004420000513

Jakobsson, S., Borg, B., Haux, C., and Hyllner, S. J. (1999). An 11-ketotestosterone Induced Kidney-Secreted Protein: the Nest Building Glue from Male Three-Spined Stickleback, *Gasterosteus aculeatus*. *Fish. Physiol. Biochem.* 20, 79–85. doi:10.1023/a:1007776016610

Jakobsson, S., Mayer, I., Schulz, R. W., Blankenstein, M. A., and Borg, B. (1996). Specific Binding of 11-ketotestosterone in an Androgen Target Organ, the Kidney of the Male Three-Spined Stickleback, *Gasterosteus aculeatus*. *Fish. Physiol. Biochem.* 15, 459–467. doi:10.1007/bf01874920

Jennions, M. D., Moller, A. P., and Petrie, M. (2001). Sexually Selected Traits and Adult Survival: a Meta-Analysis. *Q. Rev. Biol.* 76, 3–36. doi:10.1086/393743

Karu, U., Saks, L., and Hōrak, P. (2008). Carotenoid-based Plumage Coloration Is Not Affected by Vitamin E Supplementation in Male Greenfinches. *Ecol. Res.* 23, 931–935. doi:10.1007/s11284-007-0457-x

Khalil, S., Welklin, J. F., McGraw, K. J., Boersma, J., Schwabl, H., Webster, M. S., et al. (2020). Testosterone Regulates CYP2J19-Linked Carotenoid Signal Expression in Male Red-Backed Fairywrens (*Malurus melanocephalus*). *Proc. Biol. Sci.* 287, 20201687. doi:10.1098/rspb.2020.1687

Kim, S. Y., Metcalfe, N. B., and Velando, A. (2016). A Benign Juvenile Environment Reduces the Strength of Antagonistic Pleiotropy and Genetic Variation in the Rate of Senescence. *J. Anim. Ecol.* 85, 705–714. doi:10.1111/1365-2656.12468

Kim, S. Y., and Velando, A. (2020). Attractive Male Sticklebacks Carry More Oxidative DNA Damage in the Soma and Germline. *J. Evol. Biol.* 33, 121–126. doi:10.1111/jeb.13552

Kirkwood, T. B. L. (2002). Evolution of Ageing. *Mech. Ageing Dev.* 123, 737–745. doi:10.1016/s0047-6374(01)00419-5

Koch, R. E., Kavazis, A. N., Hasselquist, D., Hood, W. R., Zhang, Y., Toomey, M. B., et al. (2018). No Evidence that Carotenoid Pigments Boost Either Immune or Antioxidant Defenses in a Songbird. *Nat. Commun.* 9, 491–497. doi:10.1038/s41467-018-02974-x

Koch, R. E., and Hill, G. E. (2018). Do Carotenoid-Based Ornaments Entail Resource Trade-offs? an Evaluation of Theory and Data. *Funct. Ecol.* 32, 1908–1920. doi:10.1111/1365-2435.13122

Kokko, H. (1999). Competition for Early Arrival in Migratory Birds. *J. Anim. Ecol.* 68, 940–950. doi:10.1046/j.1365-2656.1999.00343.x

Kotiaho, J. S. (2001). Costs of Sexual Traits: A Mismatch Between Theoretical Considerations and Empirical Evidence. *Biol. Rev.* 76, 365–376. doi:10.1017/s1464793101005711

Künzler, R., and Bakker, T. C. M. (2001). Female Preferences for Single and Combined Traits in Computer Animated Stickleback Males. *Behav. Ecol.* 12, 681–685. doi:10.1093/beheco/12.6.681

Kurtz, J., Kalbe, M., Langefors, Å, Mayer, I., Milinski, M., and Hasselquist, D. (2007). An Experimental Test of the Immunocompetence Handicap Hypothesis in a Teleost Fish: 11-Ketotestosterone Suppresses Innate Immunity in Three-Spined Sticklebacks. *Am. Nat.* 170, 509–519. doi:10.1086/521316

Lemaître, J. F., Berger, V., Bonenfant, C., Douhard, M., Gamelon, M., Plard, F., et al. (2015). Early-late Life Trade-Offs and the Evolution of Ageing in the Wild. *Proc. R. Soc. B Biol. Sci.* 282, 20150209. doi:10.1098/rspb.2015.0209

Lind, M. I., Carlsson, H., Duxbury, E. M. L., Ivimey-Cook, E., and Maklakov, A. A. (2021). Cost-free Lifespan Extension via Optimization of Gene Expression in Adulthood Aligns with the Developmental Theory of Ageing. *Proc. R. Soc. B Biol. Sci.* 288, 20201728. doi:10.1098/rspb.2020.1728

Maklakov, A. A., and Chapman, T. (2019). Evolution of Ageing as a Tangle of Trade-Offs: Energy versus Function. *Proc. Biol. Sci.* 286, 20191604. doi:10.1098/rspb.2019.1604

Marler, C. A., and Moore, M. C. (1991). Supplementary Feeding Compensates for Testosterone-Induced Costs of Aggression in Male Mountain Spiny Lizards, Sceloporus Jarrovi. *Anim. Behav.* 42, 209–219. doi:10.1016/s0003-3472(05)80552-4

McCracken, A. W., Adams, G., Hartshorne, L., Tatar, M., and Simons, M. J. P. (2020). The Hidden Costs of Dietary Restriction: Implications for its Evolutionary and Mechanistic Origins. *Sci. Adv.* 6, eaay3047. doi:10.1126/sciadv.aay3047

McCracken, A. W., Buckle, E., and Simons, M. J. P. (2020). The Relationship between Longevity and Diet is Genotype Dependent and Sensitive to Desiccation in *Drosophila melanogaster*. *J. Exp. Biol.* 223, jeb230185. doi:10.1242/jeb.230185

Milinski, M., and Bakker, T. C. M. (1990). Female Sticklebacks Use Male Coloration in Mate Choice and Hence Avoid Parasitized Males. *Nature* 344, 330–333. doi:10.1038/344330a0

Moss, R., Parr, R., and Lambin, X. (1994). Effects of Testosterone on Breeding Density, Breeding success and Survival of Red Grouse. *Proc. R. Soc. Lond. B* 258, 175–180. doi:10.1098/rspb.1994.0159

Mougeot, F., Irvine, J. R., Seivwright, L., Redpath, S. M., and Piertney, S. (2004). Testosterone, Immunocompetence, and Honest Sexual Signaling in Male Red Grouse. *Behav. Ecol.* 15, 930–937. doi:10.1093/beheco/arh087

Mougeot, F., Piertney, S. B., Leckie, F., Evans, S., Moss, R., Redpath, S. M., et al. (2005). Experimentally Increased Aggressiveness Reduces Population Kin Structure and Subsequent Recruitment in Red Grouse Lagopus Lagopus Scoticus. *J. Anim. Ecol.* 74, 488–497. doi:10.1111/j.1365-2656.2005.00947.x

Newhouse, D. J., and Vernasco, B. J. (2020). Developing a Transcriptomic Framework for Testing Testosterone-Mediated Handicap Hypotheses. *Gen. Comp. Endocrinol.* 298, 113577. doi:10.1016/j.ygcen.2020.113577

Páll, M. K., Mayer, I., and Borg, B. (2002). Androgen and Behavior in the Male Three-Spined Stickleback, *Gasterosteus aculeatus* I. - Changes in 11-ketotestosterone Levels during the Nesting Cycle. *Horm. Behav.* 41, 377–383. doi:10.1006/hbeh.2002.1777

Pérez, C., Lores, M., and Velando, A. (2008). Availability of Nonpigmentary Antioxidant Affects Red Coloration in Gulls. *Behav. Ecol.* 19, 967–973. doi:10.1093/beheco/arn053

Perez-Rodriguez, L., Mougeot, F., Alonso-Alvarez, C., Blas, J., Viñuela, J., and Bortolotti, G. R. (2008). Cell-mediated Immune Activation Rapidly Decreases Plasma Carotenoids but Does Not Affect Oxidative Stress in Red-Legged Partridges (*Alectoris rufa*). *J. Exp. Biol.* 211, 2155–2161. doi:10.1242/jeb.017178

Peters, A. (2007). Testosterone and Carotenoids: An Integrated View of Trade-Offs between Immunity and Sexual Signalling. *BioEssays* 29, 427–430. doi:10.1002/bies.20563

Pike, T. W., Blount, J. D., Bjerkeng, B., Lindström, J., and Metcalfe, N. B. (2007). Carotenoids, Oxidative Stress and Female Mating Preference for Longer Lived Males. *Proc. R. Soc. B.* 274, 1591–1596. doi:10.1098/rspb.2007.0317

Pike, T. W., Blount, J. D., Lindström, J., and Metcalfe, N. B. (2007). Availability of Non-Carotenoid Antioxidants Affects the Expression of a Carotenoid-Based Sexual Ornament. *Biol. Lett.* 3, 353–356. doi:10.1098/rsbl.2007.0072

Pike, T. W., Blount, J. D., Lindström, J., and Metcalfe, N. B. (2010). Dietary Carotenoid Availability, Sexual Signalling and Functional Fertility in Sticklebacks. *Biol. Lett.* 6, 191–193. doi:10.1098/rsbl.2009.0815

Pike, T. W. (2011). Using Digital Cameras to Investigate Animal Colouration: Estimating Sensor Sensitivity Functions. *Behav. Ecol. Sociobiol.* 65, 849–858. doi:10.1007/s00265-010-1097-7

Redpath, S. M., Mougeot, F., Leckie, F. M., and Evans, S. A. (2006). The Effects of Autumn Testosterone on Survival and Productivity in Red Grouse, Lagopus Lagopus Scoticus. *Anim. Behav.* 71, 1297–1305. doi:10.1016/j.anbehav.2005.08.012

Reed, W. L., Clark, M. E., Parker, P. G., Raouf, S. A., Arguedas, N., Monk, D. S., et al. (2006). Physiological Effects on Demography: A Long-Term Experimental Study of Testosterone's Effects on Fitness. *Am. Nat.* 167, 667–683. doi:10.1086/503054

Regan, J. C., Froy, H., Walling, C. A., Moatt, J. P., and Nussey, D. H. (2019). Dietary Restriction and Insulin-like Signalling Pathways as Adaptive Plasticity: A Synthesis and Re-evaluation. *Funct. Ecol.* 34, 107–128. doi:10.1111/1365-2435.13418

Reznick, D., Nunney, L., and Tessier, A. (2000). Big Houses, Big Cars, Superfleas and the Costs of Reproduction. *Trends Ecol. Evol.* 15, 421–425. doi:10.1016/s0169-5347(00)01941-8

Roberts, M. L., Buchanan, K. L., and Evans, M. R. (2004). Testing the Immunocompetence Handicap Hypothesis: A Review of the Evidence. *Anim. Behav.* 68, 227–239. doi:10.1016/j.anbehav.2004.05.001

Rush, V., Abney, M., McKinnon, J., and Sargent, R. C. (2003). Reflectance Spectra from Free-Swimming Sticklebacks (Gasterosteus): Social Context and Eye-Jaw Contrast. *Behaviour* 140, 1003–1019. doi:10.1163/156853903322589614

Rushbrook, B. J., Dingemanse, N. J., and Barber, I. (2008). Repeatability in Nest Construction by Male Three-Spined Sticklebacks. *Anim. Behav.* 75, 547–553. doi:10.1016/j.anbehav.2007.06.011

Rutten, A. L., Oosterbeek, K., Ens, B. J., and Verhulst, S. (2006). Optimal Foraging on Perilous Prey: Risk of Bill Damage Reduces Optimal Prey Size in Oystercatchers. *Behav. Ecol.* 17, 297–302. doi:10.1093/beheco/arj029

Salvador, A., Veiga, J. P., Martin, J., Lopez, P., Abelenda, M., and Puertac, M. (1996). The Cost of Producing a Sexual Signal: Testosterone Increases the Susceptibility of Male Lizards to Ectoparasitic Infestation. *Behav. Ecol.* 7, 145–150. doi:10.1093/beheco/7.2.145

Santos, E. S. A., and Nakagawa, S. (2012). The Costs of Parental Care: A Meta-Analysis of the Trade-Off between Parental Effort and Survival in Birds. *J. Evol. Biol.* 25, 1911–1917. doi:10.1111/j.1420-9101.2012.02569.x

Schantz, T. v., Bensch, S., Grahn, M., Hasselquist, D., and Wittzell, H. (1999). Good Genes, Oxidative Stress and Condition-Dependent Sexual Signals. *Proc. R. Soc. Lond. B* 266, 1–12. doi:10.1098/rspb.1999.0597

Schielzeth, H., and Forstmeier, W. (2009). Conclusions Beyond Support: Overconfident Estimates in Mixed Models. *Behav. Ecol.* 20, 416–420. doi:10.1093/beheco/arn145

Sebire, M., Katsiadaki, I., and Scott, A. P. (2007). Non-Invasive Measurement of 11-ketotestosterone, Cortisol and Androstenedione in Male Three-Spined Stickleback (*Gasterosteus aculeatus*). *Gen. Comp. Endocrinol.* 152, 30–38. doi:10.1016/j.ygcen.2007.02.009

Sheldon, B. C., and Verhulst, S. (1996). Ecological Immunology: Costly Parasite Defences and Trade-Offs in Evolutionary Ecology. *Trends Ecol. Evol.* 11, 317–321. doi:10.1016/0169-5347(96)10039-2

Sikes, R. S., Ylönen, H., and Ylonen, H. (1998). Considerations of Optimal Litter Size in Mammals. *Oikos* 83, 452. doi:10.2307/3546673

Simons, M. J., Briga, M., Koetsier, E., Folkertsma, R., Wubs, M. D., Dijkstra, C., et al. (2012). Bill Redness Is Positively Associated with Reproduction and Survival in Male and Female Zebra Finches. *PLoS One* 7, e40721. doi:10.1371/journal.pone.0040721

Simons, M. J., Briga, M., and Verhulst, S. (2016). Stabilizing Survival Selection on Presenescent Expression of a Sexual Ornament Followed by a Terminal Decline. *J. Evol. Biol.* 29, 1368–1378. doi:10.1111/jeb.12877

Simons, M. J., Cohen, A. A., and Verhulst, S. (2012). What Does Carotenoid-dependent Coloration Tell? Plasma Carotenoid Level Signals Immunocompetence and Oxidative Stress State in Birds-A Meta-Analysis. *PLoS One* 7, e43088. doi:10.1371/journal.pone.0043088

Simons, M. J., Cohen, A. A., and Verhulst, S. (2012). What Does Carotenoid-dependent Coloration Tell? Plasma Carotenoid Level Signals Immunocompetence and Oxidative Stress State in Birds-A Meta-Analysis. *PLoS One* 7, e43088. doi:10.1371/journal.pone.0043088

Simons, M. J., Maia, R., Leenknegt, B., and Verhulst, S. (2014). Carotenoid-dependent Signals and the Evolution of Plasma Carotenoid Levels in Birds. *Am. Nat.* 184, 741–751. doi:10.1086/678402

Simons, M. J. P., Briga, M., Koetsier, E., Folkertsma, R., Wubs, M. D., Dijkstra, C., et al. (2012). Bill Redness is Positively Associated with Reproduction and Survival in Male and Female Zebra Finches. *PLoS One* 7, e40721. doi:10.1371/journal.pone.0040721

Simons, M. J. P., Briga, M., Leenknegt, B., and Verhulst, S. (2014). Context-dependent Effects of Carotenoid Supplementation on Reproduction in Zebra Finches. *Behav. Ecol.* 25. doi:10.1093/beheco/aru062

Simons, M. J. P., Reimert, I., van der Vinne, V., Hambly, C., Vaanholt, L. M., Speakman, J. R., et al. (2011). Ambient Temperature Shapes Reproductive Output During Pregnancy and Lactation in the Common Vole (*Microtus arvalis*): A Test of the Heat Dissipation Limit Theory. *J. Exp. Biol.* 214, 38–49. doi:10.1242/jeb.044230

Smith, H. G., Kallander, H., and Nilsson, J.-A. (1989). The Trade-off Between Offspring Number and Quality in the Great Tit *Parus major*. *J. Anim. Ecol.* 58, 383–401. doi:10.2307/4837

Stearns, S. C. (1989). Trade-offs in Life-History Evolution. *Funct. Ecol.* 3, 259–268. doi:10.2307/2389364

Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C., and Troscianko, T. S. (2007). Using Digital Photography to Study Animal Coloration. *Biol. J. Linn. Soc.* 90, 211–237. doi:10.1111/j.1095-8312.2007.00725.x

Stigell, P., Miyata, K., and Hauta-Kasari, M. (2007). Wiener Estimation Method in Estimating of Spectral Reflectance from RGB Images. *Pattern Recognit. Image Anal.* 17, 233–242. doi:10.1134/s1054661807020101

Sugrue, V. J., Zoller, J. A., Narayan, P., Lu, A. T., Ortega-Recalde, O. J., Grant, M. J., et al. (2021). Castration Delays Epigenetic Aging and Feminizes DNA Methylation at Androgen-Regulated Loci. *Elife* 10, e64932. doi:10.7554/eLife.64932

Svensson, P. A., and Wong, B. B. M. (2011). Carotenoid-based Signals in Behavioural Ecology: A Review. *Behaviour* 148, 131–189. doi:10.1163/000579510x548673

Számadó, S. (2011). The Cost of Honesty and the Fallacy of the Handicap Principle. *Anim. Behav.* 81, 3–10. doi:10.1016/j.anbehav.2010.08.022

Therneau, T. M., and Grambsch, P. M. (2000). *Modeling Survival Data: Extending the Cox Model*. Germany: Springer New York. doi:10.1007/978-1-4757-3294-8

Tinbergen, J. M., and Daan, S. (1990). Family Planning in the Great Tit (Parus Major): Optimal Clutch Size as Integration of Parent and Offspring Fitness. *Behaviour* 114, 161–190. doi:10.1163/156853990x00103

van Mullem, P. J., and van der Vlugt, J. C. (1964). On the Age, Growth and Migration of the Anadromous Stickleback *Gasterosteus aculeatus* L. Investigated in Mixed Populations. *Arch. Néerl Zool* 16, 111–139. doi:10.1163/036551664x00031

Wedekind, C., Meyer, P., Frischknecht, M., Niggli, U. A., and Pfander, H. (1998). Different Carotenoids and Potential Information Content of Red Coloration of Male Tree-Spined Sticklebacks. *J. Chem. Ecol.* 24, 787–801. doi:10.1023/a:1022365315836

Williams, G. C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. *Evolution* 11, 398. doi:10.2307/2406060

Wootton, R. J., and Robert, J. (1984). *A Functional Biology of Sticklebacks*. Springer, 265.

Wright, D. S., Yong, L., Pierotti, M. E., and Mckinnon, J. S. (2016). Male Red Throat Coloration, Pelvic Spine Coloration, and Courtship Behaviours in Threespine Stickleback. *Evol. Ecol. Res.* 17 (3), 407–418.

Yamamoto, R., Palmer, M., Koski, H., Curtis-Joseph, N., and Tatar, M. (2020). Mapping Drosophila Insulin Receptor Structure to the Regulation of Aging Through Analysis of Amino Acid Substitutions. *bioRxiv*. doi:10.1101/2020.06.30.180505

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REVIEW
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The Danaid Theory of Aging

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The classical evolutionary theories of aging suggest that aging evolves due to insufficient selective pressure against it. In these theories, declining selection pressure with age leads to aging through genes or resource allocations, implying that aging could potentially be stalled were genes, resource allocation, or selection pressure somewhat different. While these classical evolutionary theories are undeniably part of a description of the evolution of aging, they do not explain the diversity of aging patterns, and they do not constitute the only possible evolutionary explanation. Without denying selection pressure a role in the evolution of aging, we argue that the origin and diversity of aging should also be sought in the nature and evolution of organisms that are, from their very physiological make up, unmaintainable. Drawing on advances in developmental biology, genetics, biochemistry, and complex systems theory since the classical theories emerged, we propose a fresh evolutionary-mechanistic theory of aging, the Danaid theory. We argue that, in complex forms of life like humans, various restrictions on maintenance and repair may be inherent, and we show how such restrictions are laid out during development. We further argue that there is systematic variation in these constraints across taxa, and that this is a crucial factor determining variation in aging and lifespan across the tree of life. Accordingly, the core challenge for the field going forward is to map and understand the mosaic of constraints, trade-offs, chance events, and selective pressures that shape aging in diverse ways across diverse taxa.

Keywords: evolution, aging, development, theory, constraint, maintenance, senescence

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INTRODUCTION

An evolutionary theory of aging should answer two key questions. First, why could aging evolve, given that, all else being equal, an individual's fitness should be maximized by living as long as possible? Second, why do patterns of aging vary across the tree of life the way they do (Omotoso et al., 2021)? The classical evolutionary theories of aging have long provided a convincing answer to the first question (Medawar, 1952; Williams, 1957; Hamilton, 1966; Kirkwood, 1977). However, as we learn more about the diversity of aging patterns across the tree of life and the diversity of mechanisms, it is increasingly clear that the classical theories do not provide a sufficient answer to the second question. Additionally, other answers to the first question are possible. Here, we propose a novel theory, The Danaid Theory of aging, that builds on existing theory, links mechanisms with evolution, and can simultaneously answer both questions. It integrates the previous theories with a modern understanding of development, aging biology, complex systems, and genetic control, contextualizing when previous theories may be key drivers, and when other forces may dominate the evolution of aging and lifespan.

The Danaid theory suggests that there are taxon-specific constraints on the ability of organisms to maintain themselves indefinitely, often arising from the inherently complex systems nature of

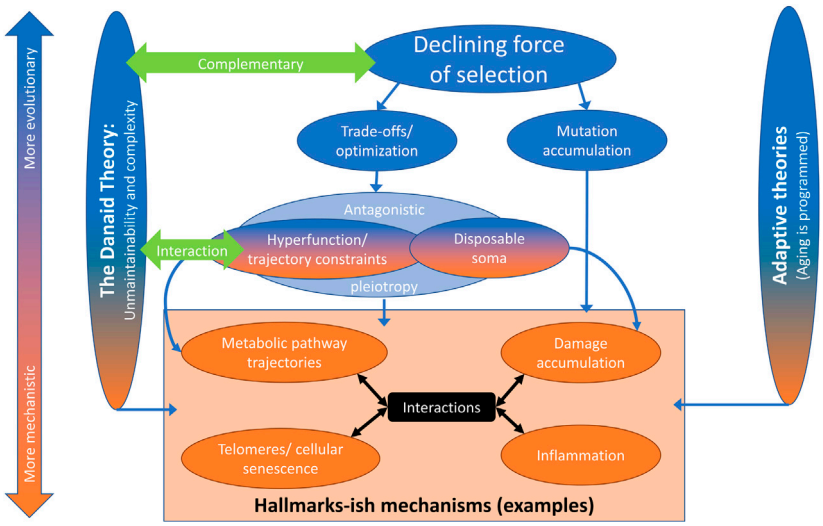

FIGURE 1 | A taxonomy of aging theories. Aging can be viewed from a more mechanistic or more evolutionary angle (vertical direction). Most approaches consider elements of both. Aging can also be viewed from more adaptive or less adaptive angles (horizontal direction). The programmed theories ascribe a direct function to aging; they are the most adaptive. The current theoretical framework, i.e. the various theories in the middle, consider aging a phenomenon that follows from evolutionary pressures, but is not as such selected for. The Danaid theory rather sees aging in part as the result of the physiological layout of organisms, with only limited malleability through selection.

organisms. This is complementary to a declining force of natural selection with age, but does not depend on it. Accordingly, we propose a framework in which specific types of mechanistic constraints complement the declining force of selection to explain the diversity of aging patterns, showing that evolutionary and mechanistic theories are inextricably intertwined. We start by establishing some preliminaries—a taxonomy of aging theories, definitions of aging, etc. Readers who wish to dive straight into the subject matter may wish to proceed directly to *Systemic Constraints on Physiology and Evolution*. We then address the necessary elements of evolutionary theory, followed by a consideration of how systemic constraints could influence aging, and a discussion of how such constraints may emerge from the complex nature of life. Finally, we consider how this theory interacts with our knowledge about the diversity of aging processes across taxa.

PRELIMINARIES

A Taxonomy of Aging Theories

While the classical theories of aging are often listed as mutation accumulation (Medawar, 1952), antagonistic pleiotropy (Williams, 1957), and the disposable soma theory (Kirkwood, 1977) (in large part to pay hommage to three landmark papers in the field), we believe there is now a clear consensus for a new way to think about these classical theories, as shown in **Figure 1**. This taxonomy of theories is not meant to be exhaustive, but does recognize other, more recent theoretical advances. On the one hand, there are the programmed/adaptive theories of aging (Goldsmith, 2012; Libertini, 2015; Mitteldorf, 2018). Though they remain popular with molecular biologists and some physicists, they have been debunked by evolutionary biologists, including ourselves and others (Austad, 2004; Cohen, 2015; Kowald and Kirkwood, 2016). We do not consider them futher.

The classical theories of aging all stem from a single principle, the declining force of selection with age ("selection shadow"): because future events cannot affect past reproduction, as organisms reproduce selection lessens with progressing age (Hamilton, 1966; Wensink et al., 2017a). Within this broad principle, there are trade-off/optimality theories (Stearns, 1989; Parker and Smith, 1990; Partridge and Barton, 1993) and mutation accumulation (Medawar, 1952). Trade-off/optimality theories hold that aging is a byproduct of maximizing fitness, generally through trade-offs between fertility/reproduction/condition early in life and the ability of the organism to maintain itself indefinitely. Mutation accumulation does not invoke an advantage linked to aging, but quite simply posits that weak selection against late-acting deleterious mutations increases the load of mutations with late-life-specific effects.

Within optimality theories, antagonistic pleiotropy (Williams, 1957) posits a genetic mechanism whereby a single allele might have constrasting effects on fitness early versus late in life. This is a special case; the broader principles of optimality/trade-offs can be expressed through multiple genes with contrasting effects, for example (Parker and Smith, 1990; Partridge and Barton, 1993). The disposable soma theory (Kirkwood, 1977) is another special case of trade-offs/optimality, wherein the major mechanistic manifestation is through trade-offs in resource allocation. The hyperfunction theory (Blagosklonny, 2012; Maklakov and Chapman, 2019) classically posits that processes that start earlier in life continue with, or set the organism on a

trajectory to, aging further down the road. In this type of trade-off, excessive late function is a price paid for appropriate early development, and alternative trajectories would show slower aging with lower early function. Slightly more broadly, we consider the hyperfunction theory a special case of a general principle: biological processes over the course of an organism's lifespan are generally hard to time precisely, particularly after development, and thus result more from trajectories than from precise temporal optimization (Cohen, 2004; Kirkwood and Melov, 2011; Wensink, 2013), much as artillery gunners can adjust the direction and angle of a cannon, but lack control over the cannonball after it has been fired.

Antagonistic pleiotropy as depicted in **Figure 1** largely but not completely covers both the disposable soma and hyperfunction theories. The lack of complete coverage acknowledges the potential for non-genetic mechanisms, or for mechanisms that are related to genetics in substantially more complex ways than typically considered under antagonistic pleiotropy (effects that are high-dimensionally epistatic as well as contingent on environment). The adaptive hitchhike hypothesis posits that slow aging is a byproduct of other adaptations (Omotoso et al., 2021), and thus is also consistent with optimality approaches. All of these theories, as well as our Danaid theory and adaptive theories, can then be related to purely mechanistic theories of aging, such as those contained in the Hallmarks framework (López-Otín et al., 2013). For example, it has been proposed that the apparently programmed nature of cellular senescence supports adaptive explanations for aging (Milewski, 2010). While we disagree with this contention, the nature of the mechanisms of aging can inform our evaluation of the various evolutionary theories.

Proximate Versus Ultimate Theories of Aging?

Descriptions of the mechanisms of aging are usually considered proximate explanations, as opposed to ultimate evolutionary explanations (Gems and Partridge, 2013). An evolutionary explanation would give the "what" and "why"; the mechanisms would provide the details of the "how". However, evolutionary models of aging consistently show that essentially any outcome can occur depending on the proximate mechanisms that constrain the range of possible evolutionary outcomes (Baudisch, 2008; Wensink et al., 2014a; Wensink et al., 2014b): depending on constraints, evolution can produce senescence, no senescence, or negative senescence, as well as variation within these categories. While studying mechanisms without evolution indeed means studying proximate but not ultimate explanations of aging, the reverse is not true: studying evolution without mechanisms does not yield an ultimate explanation, but rather no explanation at all. Although "nothing in biology makes sense except in the light of evolution" (Dobzhansky, 1973), nothing in evolution makes sense without the mechanisms. An ultimate theory of aging, if it exists, is found at the intersection of evolutionary and mechanistic forces. Any convincing evolutionary theory of aging must incorporate not only what is known about comparative demography and natural selection, but also what is known about mechanisms and their distribution across taxa.

Most scientists appear to agree with the above, and see existing evolutionary theories of aging as "solving the paradox": how could aging evolve given its apparently detrimental effect on fitness? The classical theories of aging do provide a plausible solution to the paradox. Yet as soon as we want to do more than solving the paradox, the mechanisms start to matter.

Even those who attempted to just solve the paradox seemed to feel that mechanisms mattered. Hamilton (Hamilton, 1966) sought to explain senescence through calculating the effect on Darwinian fitness of a change in mortality or fecundity at an isolated age. From the decline in the magnitudes of these sensitivities with age Hamilton inferred that senescence is inevitable. Yet he also gave a mechanistic justification:

> "Consider four hypothetical genes in man, (. . .) age-limited in the following way: each gives complete immunity against some lethal disease but only for one particular year of life. Suppose the first gives immunity for the first year, the second for the fifteenth, the third for the thirtieth, and the fourth for the forty-fifth. What are the relative selective advantages of these genes?"

There is no description of how this would work mechanistically, just "a gene". While we appreciate that Hamilton merely wished to justify the presentation of a set of mathematical results, later developments force us to take a broader perspective (Kirkwood, 1977; Noble, 2013; Wensink, 2013; Noble et al., 2014), focusing on the way an organism is built and how it functions, which depends on more than DNA alone. No gene gives immunity for one specific year of life.

The disposable soma theory, taking a thermodynamics perspective, comes closer to actual mechanisms. Still, nowadays its pure focus on trade-offs, in particular resource trade-offs, seems too narrow in scope (Cohen et al., 2019), in particular given that the co-existence of multiple trade-offs can change the outcome expected under individual trade-offs (Cohen et al., 2017), further discussed in subsection *Allocation Theory*.

In short, develomental biology and the role of DNA, for example, are seen in a much different light now compared to several decades ago. In this paper we continue the search for a theory of the evolution of aging more firmly rooted in the mechanisms of organismal physiology, informed where possible by accepted principles from other disciplines, e.g. physics and chemistry, in the tradition of D'Arcy Wentworth Thompson's *On Growth and Form* (Thompson, 2014). In particular, we suggest that multiple complex constraints evolve for reasons largely unrelated to lifespan, but nonetheless shape the relative "maintainability" of various taxa, and thereby their lifespans (**Table 1**).

What Is Aging?

Despite our clear intuition for what aging is, there are major disagreements among researchers as to its nature and definition (Cohen et al., 2020a), and there are important arguments against the idea of aging as a unified biological phenomenon (Cohen et al., 2020b). A demographic definition such as "monotonic

TABLE 1 | A comparison of evolutionary(-mechanistic) aging theories.

	Adaptive theories	Classical theories	The danaid theory
Role of natural selection	Aging is selected for	Aging is a byproduct of declining selection with age	Aging is inevitable, at least in some taxa, though selection could change its rate
Role of constraints	Constraints? What constraints?	Of course there are constraints, now let's talk about something interesting	Constraints vary across taxa and are key to understanding the interplay between mechanisms and selection
Integration of mechanisms	Mechanisms suggest adaptation	Mechanisms are generally incidental, but many support trade-offs	The evolution of aging can't really be understood without considering mechanisms and their variation across taxa
Role of trade-offs	Might affect how much aging is adaptive?	Core to DS and AP theories	Importance is highly variable across taxa
Relationships to other theories	Rejects other theories	Depends on which one, but generally consider themselves sufficient together	Incorporates classical theories as a partial but not global explanation
Explanation of taxonomic diversity in aging	Not on the radar	Not considered beyond basic trade-offs and life-history continua	Considers explanation of taxonomic diversity a core task of an aging theory
Role of development	Both development and aging are programmed and can be fine-tuned independently by selection; not therefore necessary to consider development to understand aging	Crucial for the hyperfunction theory; early-late trade-offs also consistent with AP	Considers taxon-specific developmental programs as potential key constraints on how aging can evolve
Aging as damage	Damage is a byproduct of programs to age	Damage is often considered crucial, but is not an inevitable conclusion of the theories	Damage is insufficient to understand aging. Unclear whether damage is cause, consequence, or both; this may vary across taxa
Role for complex systems	Not much	Not much	The nature of organisms as complex systems is a key contributor to taxon-specific constraints
Predictions	Support generally comes from simulations rather than empirical research; testable predictions needed	Substantial support for predictions in certain examples, but the universality of the explanations is questionable	Few broad generalizations expected, so specific predictions are hard. Empirical patterns consistent or inconsistent with the theory, such as concordance between patterns of aging and selection pressure, will nonetheless provide tests

Notes: MA: mutation accumulation; AP: antagonistic pleiotropy; DS: disposable soma

increases in age-specific mortality" includes cases where the mechanisms behind the demographic patterns have little to do with traditional concepts of aging biology. For example, fish and tree mortality are size dependent, a phenomenon largely unrelated to traditional aging mechanisms like declines in tissue function. Additionally, it is hard to know from demographic data what mechanistic aging might look like were we able to keep enough individuals alive longer in a protected environment. A more mechanistic definition, such as "age-related declines in organismal function due to the hallmarks of aging" [with the hallmarks described as in (López-Otín et al., 2013)], runs the risk of missing mechanisms that have yet to be discovered, or that play a role in other taxa, since the hallmarks were tailored to mammals. Indeed, there is every reason to expect a diversity of aging mechanisms across taxa, across individuals, and across environments (Cohen, 2018), presumably following a power-law distribution (**Figure 2**). An evolutionary definition such as "declines in age-specific fitness" might seem appropriate in this context, but poses the challenge of being hard to measure in many contexts, and hard to relate to mechanisms. We here focus on aging as a progressive and intrinsic decline in physiological function, i.e. an organism's ability to maintain homeostasis and respond to its environment. We nonetheless integrate information from different sources, including demographic, and do our best to acknowledge that these shifting data types may not always reflect the same underlying phenomenon of aging (Cohen et al., 2020b).

A popular idea is that aging is caused by the accumulation of somatic damage with age (Kirkwood and Austad, 2000; López-Otín et al., 2013). While appealing, this notion requires a clear definition of damage, which is problematic. López-Otín et al. (López-Otín et al., 2013) list nine "hallmarks of aging": genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Hallmarks like epigenetic alterations and telomere attrition consist of clear, spatial aberrations we would associate with the traditional meaning of

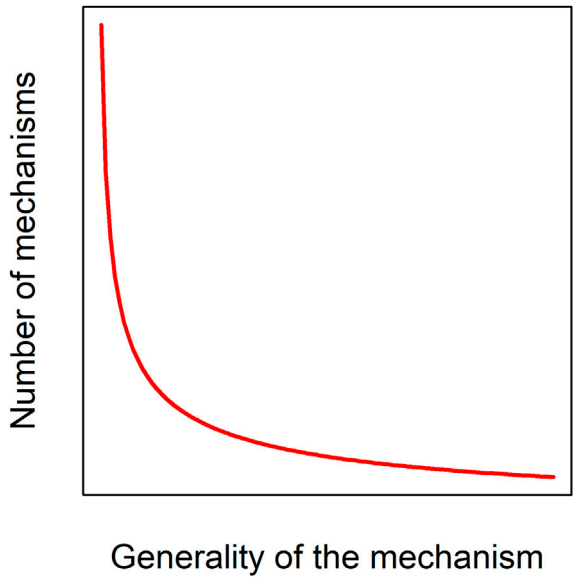

FIGURE 2 | Aging mechanisms likely follow a power-law distribution. Only a small number of mechanisms or pathways, such as DNA damage, are likely to have large, consistent effects across many species, individuals, and environments; a much larger number could have effects that are specific to a given taxon, genetic background, or environmental background.

damage. But other hallmarks, such as altered intercellular communication, deregulated nutrient sensing and mitochondrial dysfunction, have a less clear-cut spatial representation. What if a perfectly undamaged protein is in the wrong place? What if nutrient sensing is disturbed due to the expression of the wrong gene? If all aberrations such as these are classified as damage, then everything deleterious is damage and the definition becomes tautological.

Because many theories of aging do refer to damage accumulation in some way—for example, energy investment to damage repair in the disposable soma theory (Kirkwood, 1977; Kirkwood and Austad, 2000)—a definition of damage is important. The statement "aging is caused by cumulative damage" is meaningful only when adhering to a limited, three-dimensional definition of damage, which allows for alternative and/or complementary hypotheses. Malfunction may or may not have its origin in three-dimensional damage, and there is some evidence that the accumulation of damage with age may be correlation without causation (Doonan et al., 2008; Gems and Doonan, 2009). Alternatives include hyperfunction, such as unchecked growth through hormonal pathways (Blagosklonny, 2009; Blagosklonny, 2012), or other malfunctioning that does not reduce to damage in its traditional sense. For these reasons, we define damage here as a structural, physical, three-dimensional change: This definition would include DNA mutations, protein misfolding, wing damage, and tooth wear, but would exclude more general information loss, depletion of reserves, communication or regulatory errors, etc. There is increasing recognition of non-damage-based mechanisms (Gems and de Magalhães, 2021), and some are described below.

The Germ-Soma Distinction and Asymmetric Division

A long-recognized element in aging theory is the germ-soma distinction (Weismann et al., 1891; Pen and Flatt, 2021). A division of labor exists between the cells of multicellular organisms, such that the germ cells have the task and capability to form future generations, while the somatic cells form the body of one organism, in one generation alone.

A germ-soma distinction would appear a necessary but not a sufficient condition for aging to occur. If all cells aged, life would stop, so a minimum of one cell should be precluded from aging, to serve as the basis for a next generation: the germ cell. The somatic part of the body may age, which is entirely compatible with the continuation of life, but not necessarily.

Indeed, Turke (Turke, 2013) argues that a germ-soma distinction need not be *between cells*. In unicellular organisms, there can be *regions* that are insulated from adversities, which serve as the germ regions for next generations, while other regions take a soma role, and are pared away through asymmetrical cell division. The capability of building a perfectly healthy organism should be maintained somewhere, whether or not that be in a separate cell. This manifests particularly in the asymmetric division of some single-celled organisms such as *Escherichia coli* (Jouvet et al., 2018) and yeast (*Saccharomyces cerevisiae*) (He et al., 2018).

Contrary to what might be predicted, however, not only do some organisms with germ-soma distinctions show no apparent aging (Congdon et al., 2003; Sauer et al., 2021), but some organisms without the distinction do age, at least demographically (many plants, for example) (Jones et al., 2014), which might be due to factors other than cellular biology and physiology (size, length of time a plant has its roots in the same soil). This remains speculation however; all we can say for the moment is that the germ-soma distinction does not seem to be a decisive predictor of aging at the comparative level, at least based on demographic data.

Allocation Theory

Allocation theory is one popular framework for evolutionary thinking on aging (Kirkwood, 1977; Kirkwood and Austad, 2000; Baudisch, 2008). Like any household, company or country, organisms allocate scarce resources to competing functions, such as growth, somatic maintenance and reproduction. Resources invested in one function cannot be invested in another. There is thus expected to be strong selection to optimize resource allocation and the extent to which maintenance would be required if aging was to be halted (Wensink et al., 2012). Allocation theory has sometimes been framed in the light of a germ-soma distinction, since such a distinction presents the problem of the allocation of resource to germ (reproduction) versus soma (survival) (Kirkwood, 1977; Kirkwood and Holliday, 1979; Kirkwood and Rose, 1991).

There is little doubt that life has trade-offs to solve (Stearns, 1989), yet there are several potential problems in attempting to explain all aging phenomena in this framework. First, if there are other systemic constraints, for instance on damage detection, then it is not directly obvious how these constraints could be framed in terms of an allocation problem. Some might suggest that then resources should be allocated to damage detection, which keeps the argument within the realm of trade-offs. However, the problem cannot be reduced to this level of simplicity (see also *Systemic Constraints on Physiology and Evolution* and *Unmaintainability as an Emergent Property of the Complexity of Life*). Failing to detect damage (Hoogstraten et al., 2008) means that no allocation to repair could be made, let alone be optimized.

Second, even if aging did depend on resource allocation, there is a risk of missing the point: what drives the costs and benefits in the allocation model? Suppose that repair is simply impossible through other constraints. This is easily expressed in a resource allocation model: the gain of allocating resources to repair is zero, and the model will predict that no resources are spent on repair. But this is neither surprising nor interesting. What is interesting is the set of constraints driving the model. This principle would apply under a wide range of scenarios in which the constraint on optimal allocation is substantially larger than the range in which the allocation trade-off operates.

Both these points have been realized and subsumed in a broader field of optimality theory (Parker and Smith, 1990; Partridge and Barton, 1993). Scientists versed in optimality theory recognize that there are broader constraints to solve, and are well aware that model assumptions drive model outcomes. Yet we are wary of optimality theory becoming a *posthoc* justification, where constraints have to be assumed to create a certain model behavior or biological observation, rather than the other way round. Although few scientists would disagree that different model assumptions lead to different outcomes, this recognition is rarely sufficiently incorporated into the use of models. Since different model assumptions lead to different outcomes as diverse as senescence and negative senescence, then what are the predictions of a broader theory?

Furthermore, allocation theory is rooted in (classical) equilibrium thermodynamics: it is typically stated that entropy tends to increase in closed systems, but that organisms are open systems that take resources from their environment to oppose the entropy increase. This is true, but the newer field of non-equilibrium thermodynamics, which is about the organization of complex systems under a gradient (Schneider and Kay, 1994), states that non-equilibrium systems auto-organize so as to resist gradients as well as possible, which means that they reduce the gradient as much as possible at the local level. Within a specific range of gradient, but not outside, complex systems spontaneously assume the organization that best dissipates the gradient (Kondepudi et al., 2015). As energy flows such that gradients dissipate, then how allocable are resources? And how is this allocation achieved? The finding that ~25% of available energy is dissipated largely unused (Brand, 2000) suggests that the practical problem of keeping biochemical gradients within a certain range may sometimes be more important than finding an appropriate allocation of the available energy. It would be recommendable for the future of allocation theory to investigate the extent to which resources can actually be allocated under specific, targeted physiological control.

Fourth, there is increasing reason to doubt the importance of trade-offs in shaping interspecific evolutionary patterns, particularly in lifespan and aging (Cohen et al., 2019; Maklakov and Chapman, 2019). Trade-offs act not only on a single resource (energy), but on multiple resources simultaneously. When multiple resources trade-off simultaneously, the force of the trade-off tends to weaken and the outcome of selection becomes less predictable (Cohen et al., 2017). This is because the efficiency of different resources for different tasks is expected to vary, opening up optimization possibilities that diminish the force of the trade-off. Also, by arguing that trade-offs have near-universal power to shape life histories, classical theory implicitly or explicitly supposes linear shapes to trade-offs. As an example, the classic van Noordwijk and de Jong paper shows a figure with linear trade-offs, and uses covariances (which are most appropriate for linear relationships) (van Noordwijk and de Jong, 1986). Such linearity may rarely be the case, with the implication that trade-offs may only operate strongly in limited regions of trait space, i.e., where the slope is intermediate and both traits can thus be optimized at a reasonable cost to the other (Bourg et al., 2019). Empirical research also shows that predicted trade-offs do not always manifest under experimental conditions (Rose, 1984).

Trade-off theory seems to predict a canalized set of pathways along which selection can move a species. It need not be perfectly canalized, but in a complex organism where genetic changes can affect multiple phenotypes with high-dimensional pleiotropy and epistasis, coherent evolution without canalization would be unmanageable, and certain regulatory networks suggest this kind of canalization (Csete and Doyle, 2004; Cohen et al., 2012). While such canalized trade-offs appear to exist in some contexts (Dantzer and Swanson, 2012), there are also clear counter-examples. For example, short lifespan has evolved repeatedly in wild killifish not through a limited set of pathways, but apparently from relaxed selection on housekeeping genes leading to a diverse array of fast-aging phenotypes (Cui et al., 2019). That is, under strong selection to develop and reproduce quickly, and with little selection for longevity, short lifespan appears to evolve differently in different lineages, with each one losing a unique set of housekeeping genes due to mutation, i.e., consistent with mutation accumulation rather than trade-offs/optimality (**Figure 1**). In this case, short lifespan is not due to a cost of reproduction, but a simple failure to maintain selection for maintenance.

Lastly, many trade-offs force higher-level constraints, such as when decreasing one cause of mortality increases another, leading to a constraint on mortality more globally (Pavard et al., 2019). Generally, various potential causes of death exist and, depending on their age patterns, one or the other wins out (Wensink, 2016; Wensink et al., 2017b). Causes of death are themselves the result of interactions between component causes that may be correlated (Wensink et al., 2014c), and statistical modelling suggests these

interactions give rise to the typical Gompertz mortality pattern (Levy and Levin, 2014).

While there can be no doubt that trade-offs exist and may sometimes influence life histories, the ensemble of factors discussed imply that resource allocation trade-offs are not by themselves a sufficiently powerful or general explanation for the evolution of aging, and must be integrated with numerous other principles or forces (Cohen et al., 2019; Maklakov and Chapman, 2019).

Development, Differentiation, and Totipotency

A promising mechanistic line of thought in aging—that aging is due to an extension of development, and to constraints arising from developmental processes and cell differentiation (Magalhães, 2012)—may also have relevance for the evolution of aging. Building on the idea of the germ-soma distinction, it has been suggested that the very developmental process in multicellular organisms, during which cells differentiate away from totipotency, is sufficient to explain why aging occurs, as this limits repair (Turke, 2013). This idea is supported by the observation that induced neurons generated directly from human fibroblasts have an 'old' epigenetic and metabolic profile, whereas induced neurons from fibroblast-derived induced pluripotent stem cells are "young" (Mertens et al., 2015). Differentiation does seem to matter, and passing through a "pluspotent" ("more potent") state seems to allow for rejuvenation.

However, this seems more of a mechanistic problem than an evolutionary-conceptual one: there could be mechanisms to keep totipotent cells scattered across an organism which evolution simply has not yet happened on. If there are clear evolutionary benefits to totipotency, then why would an organism not retain a few totipotent cells to be able to form perfectly healthy new tissue? There may well be evolutionary advantages of such a solution, as a full-grown adult has much less to fear than immature offspring.

Another objection is that totipotency is not necessary for repair or replacement. As long as cells capable of forming a kidney are preserved, the kidney can be regenerated. As long as cells capable of forming the heart are preserved, the heart can be regenerated. It is not required that one cell can make both a heart and a kidney. Pluripotency suffices, and again the question is whether such is mechanistically achievable. While we agree with the hunch that complexity and differentiation away from totipotency play some role in aging, we argue below that it is not the loss of totipotent cells that limits the ability to regenerate and repair, but constraints at the higher level of the blueprint of an organism as a whole.

The Nature of Evolutionary Explanations

Evolution by natural selection consists of 1) variation in 2) heritable traits that 3) affect propagation (survival and reproduction). Variation in a trait may be quantitative (e.g. size of a tail) or qualitative (e.g. red versus white tail). When organisms are observed to have some trait A rather than an alternative trait B, there are three potential explanations for this observation. First, variation could have existed but evolved away; some organisms had trait A, while others had trait B, but trait A was better adapted than B, the frequency of trait A increased at a cost to B, and now only trait A can be observed. These are the explanations typically investigated in evolutionary research. Let these be ***Type I*** explanations. The second possibility is that an amount of variation could in principle exist, but it so happens that it has never come about (related to the idea of evolutionary lag (Smith, 1978) but on longer time scales). Since natural selection never acted on trait B, we observe only trait A. Let these be ***Type II*** explanations. Third, variation in a trait could be impossible because of biophysical or biochemical limitations. In this case no alternative to trait A could possibly exist—there is a constraint (Smith, 1978; Gould, 1980). Let these be ***Type III*** explanations. (Plasticity can also be considered a trait in this framework.)

The original evolutionary theories of aging are Type I explanations. They start from a (hypothetical) non-aging phenotype and argue how the aging phenotype could invade (Medawar, 1952; Williams, 1957; Hamilton, 1966; Kirkwood, 1977), as in Kirkwood's *mobbit* (Kirkwood, 1999). This implies that human-like complex organisms might evolve without aging if only a limited number of genes were different (mutation accumulation and antagonistic pleiotropy), or if the available energy were allocated differently (the disposable soma theory). One of the main purposes of the present paper is to argue the alternative: *there are some complex organisms such as humans for which no variation could possibly exist such that they do not age.* This is the evolution of unmaintainability, a Type III explanation.

SYSTEMIC CONSTRAINTS ON PHYSIOLOGY AND EVOLUTION

Genes, biochemistry, and physiology act in a world governed by the laws of physics and chemistry (Thompson, 2014). The product of a gene, whether that be a protein, microRNA, or any other substance, not only has to obey these laws, but also has to fit in the overall cellular metabolism, which may severely restrict DNA action (de Lorenzo, 2014; de Lorenzo, 2015). Likewise, there are no genes that invert gravity. If the laws of physics and chemistry do not change over time, which seems a reasonable assumption, then they are the same for parent and offspring. If these laws leave their traces in organismal physiology, then not all similarities between parent and offspring are heritable. It is such constraints that give rise to Type III evolutionary explanations.

Beyond the constraints of chemistry and physics, there exists a series of more specific biological constraints, most notably phylogenetic inertia, interoperability, and developmental constraints.

Phylogenetic Inertia

Genomes do not evolve *de novo*, but rather through small modifications to existing genomes. Adaptation must happen through continuous change in which all intermediate forms are viable in their current environment. Accordingly, the

history of a lineage as reflected in its genome may impose major constraints on the phenotypic space that is accessible through natural selection (Blomberg and Garland, 2002). For example, no mammals have the remarkable regenerative capacities that starfish do. This likely reflects a moment in the history of the mammalian lineage at which the flexibility to evolve regeneration was sacrificed for other properties of development; while it might be theoretically possible to imagine a series of genetic changes that would restore extreme regeneration, this will not happen in nature.

Interoperability

An organism is finely tuned, integrating countless molecules affecting numerous systems that together coordinate homeostasis at levels ranging from sub-cellular to organismal. By necessity, these systems interact, meaning that changes to one system can affect others. This presents a major constraint on the ability to optimize components piecemeal. For example, the inflammatory cytokine interleukin-6 plays important roles in acute inflammatory response to an infection (Helfgott et al., 1989), in the senescence-associated secretory phenotype of senescent cells (Laberge et al., 2012), and in the chronic low-grade inflammation ("inflamm-aging") that plays a role in many chronic diseases (Cohen et al., 2018). Its implication in pathways is different in each of these roles, and so any mutation that affected one role would also be under selection for its impacts on the other roles. Interoperability manifests itself hierarchically (cells need to interoperate with tissues and molecules, for example), across similar levels of organization (for example between organs), and environmentally (organisms need phenotypes that function under different conditions). Accordingly, the interoperability constraint affects nearly everything, often in ways that are very specific to a given species' biology and ecology.

Developmental Constraints

Adult phenotypes must be arrived at through a series of steps during development. Only phenotypes that can be arrived at by tweaking the developmental process are feasible. Phylogenetic inertia and interoperability constraints pertain also to the process of development, linking these constraints.

These three types of constraints can be quite forbidding, so much so that they might appear to give rise to Type III evolutionary explanations. They do however test the difference between Type II and Type III explanations, as we could imagine that in a much different evolutionary history, constraints could have been different. In any defined physiological and genetic setting however, these are hard, Type III constraints. For example, arthropod size is constrained by the ability to deliver oxygen to tissues as surface area-to-volume ratio decreases; this constraint was circumvented in tetrapods by the evolution of the lung. But within any given arthropod species, the size constraint can be considered Type III for all practical purposes.

When do systemic constraints arise? The design and construction of a complex organism versus products designed by humans are fundamentally distinct. In the latter case, there is a blueprint to which the engineer can refer at any moment, and the detailed instructions which the engineer tries to follow as closely as possible. In the former case, the information consists in procedural instructions without reference to a greater scheme of things: "There is (. . .) information in a fertilized egg (in genes, in molecular structures, and in spatial variations in the concentrations of particular chemicals), but this has no simple relationship with how the final built body will look. (Rather) the information has the effect of controlling the sequence of events that will follow." (Davies, 2014).

The cues that lead to developmental phenomena are provided by developing tissue, against the background of the environment the embryo finds itself in: for mammals the mother's womb. This means that repair capacity, at least in as much as it relies on *re-making* components, critically depends on the presence of conditions that may no longer exist in an adult organism. Informational cues, physical space, and accommodating complexities that were instrumental in the formation of a tissue may no longer be present in an adult organism. It is not without reason that the switching on and off of genes during development is so carefully timed: the moment these genes are active may be the only moment that expression has the desired effect (Davies, 2014).

Just being an adult changes things, too. Take the formation of the brain. A newborn human hardly relies on its higher brain functions for survival. Higher brain functions develop because brain cells are created in overabundance and make connections in overabundance. Nature then tests these connections and confirms or discards them during synaptic pruning. Those connections (and cells) that are useful are retained and reinforced, while the other connections are removed (Davies, 2014). In an adult organism, the circuits formed during pruning have become vital for its survival and cannot be repaired by repeating the same procedure; the organism would be helpless prey. For these and other reasons, the result of development is an organism with a physiology that restricts repair. Complex organisms such as humans have laid layer upon layer of complexity, inhibiting repair and leading to the loss of simple regenerative capacity of tissues.

UNMAINTAINABILITY AS AN EMERGENT PROPERTY OF THE COMPLEXITY OF LIFE

Even if we remain, for the moment, within the realm of aging caused by insufficient repair of damage in the classical, three-dimensional sense, after development repair may be severely limited by the wholesale physiological organization of an organism, due not only to the already mentioned constraints on resources, but also to the following.

Information. Any repair process requires guidance as to the desired state of the organism (Kirkwood, 1981). If repair amounts to replacement of a damaged molecule, then it is clear where that information could come from: new molecules are synthesized using the pathway involved in regular synthesis, while old ones are discarded. But it is not always that straightforward. In insects, the mother copies spatial information about her own body into

the eggs as gradients in concentrations of molecules [(Davies, 2014), notice the non-DNA inheritance]. The Bauplan of the offspring is derived from these gradients. If the body of a mature insect is sufficiently damaged, the information on the desired state is irretrievable: repair is limited.

Diminishing Marginal Returns. If a dirty floor is swept, the first pass with the broom will get up most but not all of the dirt; the second pass will get most of what is remaining, but much less than the first pass, and so forth. At a certain point, one decides to stop sweeping because the amount of additional dirt swept up is not worth the effort. This principle of diminishing marginal returns applies equally well to many repair and maintenance processes. For example, the energetic costs of repair become relatively higher the rarer damage types are (requiring more brooms and more sweeps), and thus the greater the investment required (Gems and McElwee, 2005). Diminishing marginal returns can also interact with other constraints, such as damage detection below.

Damage Detection. In addition to "knowing" the desired state, the repair machinery should also be able to detect a deviation from the desired state. (We are grateful to Diana van Heemst for pointing this out.) This could be by comparison to a blueprint, the sensing of a disturbed function, or through the local release of cytokines. Often the detection mechanism will only indicate that damage exists, without conveying its exact nature, limiting repair.

A Compatible Physical and Chemical Environment. A repair process needs to access the site of injury and to operate there, which may be limited by the function of the tissue to be repaired. Humans have to counter gravity and other forces, and structures that handle those forces (bones, tendons, actin filaments) cannot be (re)moved, or can be (re)moved only to a very limited extent. Similarly, repair machinery that deals with arterial damage should be able to operate in the sheer stress caused by the blood flow. Likewise, any repair machinery may need a specific chemical environment that interferes with the normal functioning of the damaged tissue. Repair in an artery takes place in an overall environment with a pH of 7.41; altering this pH would interfere with the blood's function of carrying oxygen, as oxygen dissociates from hemoglobin in an acidic environment (Bern et al., 2004). Furthermore, sending a cell into apoptosis and the communication of the apoptotic cell with its surroundings may interfere with normal cellular communications (Suzanne and Steller, 2013).

The evolution of the repair process. Even if a repair process could exist uninhibited by the constraints above, how would it come about?

Evolution works with the variation available as Gould proposed with the concept of exaptation (Gould and Vrba, 1982), and as Jacob expressed in his concept of "evolutionary tinkering" (Jacob, 1977). An example of tinkering is found in the brain, which consists of neurons that shape the electric connectivity of the brain, and supporting cells that insulate and feed the neurons, or have an immune function. Microglia are the supporting cells that perform the immune function, but their role encompasses much more than immunity (Marin and Kipnis, 2013). They secrete tumor necrosis factor alpha (TNF-α) into the synapse, where neurons communicate, which affects neuronal potentiation (Jebelli et al., 2015) and neuron network stability (Stellwagen and Malenka, 2006). Thus, it seems that a cell whose primary, historical function was immunity uses a molecular substance, TNF-α, whose primary, historical original function was immune regulation, to improve the function of the brain. Microglia (and TNF-α) were present in the brain because of their immune function, but because TNF-α happened to affect neuronal activity, a novel function evolved. This scenario is unconfirmed but arguably more likely than the emergence, through pure random variation, of some hypothetical cell type that similarly adjusts neuronal activity (Gladyshev, 2016). A comparable principle likely applies to many repair processes: they may have been adapted from other functions rather than being tailored to a specific type of damage. Accordingly, repair is unlikely to be perfect.

Straightforward exchange of components may be feasible in some organisms, but not others (Schaible et al., 2014), and not all types of repair consist in routine replacement. For instance, the arterial fatty streak or the extra-cellular plaques in Alzheimer's disease have no physiological function, but are (by-) products that should not be there in the first place. They should be removed rather than replaced, which requires a separate, new repair mechanism, or a complete reconstruction of an entire new organ. It is unclear how this could be achieved mechanistically, and even less clear which evolutionary-historical path could lead to such invention.

MOVING BEYOND DAMAGE

Going beyond the boundaries of damage being the cause of aging complicates matters further still. It is now well understood that life is a complex system in the formal sense: composed of multiple interacting elements with feedback loops, a hierarchical structure, non-linear dynamics, and emergent properties (Kitano, 2002). Emergent properties are properties of a system that are not evident by considering lower-level components individually or additively. For example, blood pressure is an emergent property of the circulatory system that cannot be understood as a simple property of cells in the vasculature. Consideration of an organism as a complex system can have a radical impact on the questions we ask: it is not sufficient to decompose an organism into constituent pieces, without asking how the pieces all interact as an ensemble to generate function.

Joining a complex systems perspective on biology with the above considerations on constraints and unmaintainability, a new hypothesis emerges for how unmaintainability (and thus aging) could evolve. A key property of many complex systems is robustness (Kitano, 2007; Kriete, 2013), defined here as the capacity to maintain stability of key aspects of a system in the face of perturbations and challenges, and thus an approximate converse to fragility. This is particularly crucial for biological systems, which, in order to survive and reproduce, must maintain dynamic equilibrium in the face of ever-changing conditions. Low robustness is not necessarily concomitant with unmaintainability or aging—for example, a lack of robustness

to a heat shock or to starvation could result in abrupt death, regardless of age. Nonetheless, most biological networks are highly redundant and buffered (Nijhout et al., 2017), explaining why so few genes are lethal when knocked out, and may not even produce noticeable effects under normal conditions. In this context, when the tolerance of the system is exceeded (i.e., insufficient robustness), the consequence is likely not death, but an adverse change to network state. This can manifest either as a shift to an alternative state that is less functional or less robust itself, or as a cascading series of failures that are not immediately fatal but set the organism on a downward spiral. For example, the vertebrate corticosteroid-based stress response creates a cascade of changes (blood pressure, kidney function, etc.) that are generally adaptive short-term, but when the tolerance of the system is exceeded by a chronic stress, these same changes impact many known aging mechanisms (telomeres, inflammation, oxidative stress, etc.) (McEwen, 1998; Zalli et al., 2014; Mocayar Marón et al., 2019; Sterling, 2020).

Theoretical work on robustness in complex systems, particularly in highly optimized tolerance systems such as living organisms, has shown that robustness is usually achieved with certain trade-offs: robustness to one type of perturbation may come at the cost of increased susceptibility to another, or may be achieved with increased resource investment, or a decrease in functionality, etc. (Zhou et al., 2005; Kriete, 2013). As evolution explores the space of robust potential phenotypes, constraints such as those outlined above pose further limits on the portions of phenotypic space that are accessible, and thus impose additional limits on maintainability. The robustness of the regulatory networks that maintain dynamic equilibrium within organisms over time is thus expected to be imperfect (Cohen, 2016). When a sufficient number of vulnerabilities exist in the system, it becomes unmaintainable, and aging results.

On the surface, this explanation may seem to agree with classic resource allocation and trade-off theory (and indeed it is complementary), but there are several important distinctions. First, classical trade-off theory would seem to imply that, with sufficient resource investment, or with different genes, aging might be avoided altogether. This is not predicted here: the nature of robustness is to be imperfect, and resource allocation is only sometimes expected to be the important factor with which robustness trades off. Infinite resources would not likely stave off aging much under this hypothesis, nor would a different set of genes. A thought experiment illustrates this. Imagine Earth is colonized by a dominant alien species that attempts to breed humans to become immortal. Only lineages stemming from the longest-lived individuals are allowed to persist, thereby weakening the selection shadow on the current human lifescale and largely counteracting mutation accumulation. Classical theory posits no force limiting evolution toward immortality, whereas the Danaid theory predicts that human lifespan would evolve asymptotically toward a limit defined by unmaintainability and systemic constraints: with the existing physiological *Bauplan*, aging is not avoidable.

Second, aging need not depend on any specific mechanism or pathway, or even any clearly demarcated set of pathways. It is the system that is robust (or not), the system that dysregulates (or not). Aging could emerge even in the absence of clear-cut mechanisms (though mechanisms are not precluded). Third, classical trade-off theory predicts a single fast-slow continuum of life histories across the tree of life, which is clearly not observed. A complex systems perspective on robustness implies that the specific physiology of each taxon will determine the landscape and strength of the trade-offs involved. Hence, some taxa [e.g. mammals (Gaillard et al., 2016)] would display universal aging, a fast-slow continuum, and clear trade-offs, whereas others might show very different patterns, ranging from apparent lack of aging (e.g., some sharks, some turtles) to taxonomically fine-scale variation in the presence of aging and the strength of trade-offs (e.g. ray-finned fish).

The theory we propose portrays aging as an arc or trajectory. Similar trajectories are known in development/morphogenesis (Davies, 2014), and are increasingly recognized in the immune system (Franceschi et al., 2017; Fulop et al., 2017) and in neurobiological and cognitive trajectories (Suzanne and Steller, 2013). We propose that such arcs are present in a wide array of aspects of organismal biology and physiology. Homeostatic networks may change with age (Dansereau et al., 2019), as may the functions of various organs, not because they are degrading or failing but because the changes are the natural consequence of the arc of that system, and of its integration with all the other systems following their arcs. The idea that all aspects of aging are deleterious may reflect a cultural bias (Cohen et al., 2020c), and many aspects may indeed be adaptive (Le Couteur and Simpson, 2011). For example, mammalian sarcopenia is widely seen as a major aging-related pathology, but it can also be conceived of as a mechanism to reduce energy requirements, and thus potentially adaptive in the broader context of the arc of the organism, despite its adverse consequences on muscle function. The Bauplan of an organism is the unit on which the arc takes its course, and unmaintainability is an emergent property of the multiple trajectories evolving concomitantly within that Bauplan; this may explain why aging is so different in organisms without a fixed Bauplan.

We thus argue that the aging process can be linked to the complexity of life: that it can be an emergent property of life, with unmaintainability generated by the numerous competing demands on the organism and/or its regulatory complexity across the arc of development and adulthood. This does not mean that all complex organisms will necessarily age, nor that all systemic constraints require complexity, but rather that complexity can in some cases be a driving force for aging at the interface of evolutionary and mechanistic levels. Additonally, complexity is not the only potential reason aging evolves, nor the only potential mechanism: clearly some simpler forms of life (e.g. yeast, *Caenhorabditis elegans*) do age, whereas some more complex forms (e.g. some vertebrates) do not. It is time to move beyond the attempt for silver-bullet explanations of aging. Multiple forces interact, with different strengths in different taxa, to generate patterns of aging.

At first glance, it might appear that the Danaid theory (**Table 1**) is simply a special case of the declining force of selection. At some point, organisms evolved greater complexity or other traits that made them less maintainable/more susceptible to aging; natural selection nevertheless favored these more-aging-susceptible variants, making it appear that the declining force of selection must always be upstream. However, this ignores two key points. First, it is not necessarily the case that complexity will shorten lifespan. It is fully possible that aspects of complexity could evolve and become fixed in a lineage under conditions where they have no impact on lifespan, or even improve lifespan (Wensink et al., 2014b), and that any lifespan-limiting impacts could become apparent only in daughter lineages with specific environmental or physiological conditions. The evolutionary origins of the complexity/unmaintainabilty are thus not crucial to consider. Second, as noted, we are not solely concerned with explaining why aging first evolved, but also why it differs as it does across taxa. Regardless of how complexity and unmaintainability were originally selected for, we argue that once present they have continuing impacts on how aging evolves subsequently (including the way it may disappear or arise anew in a lineage), and are thus germane to an evolutionary theory of aging but distinct from the declining force of selection. For example, it would appear that some organisms of moderate to high complexity do not age, at least within observable/relevant timescales (minimally hydra, but likely many vertebrates too (Congdon et al., 2003; Jones et al., 2014; Sauer et al., 2021)). How might that complexity affect the subsequent evolution of aging and lifespan in their descendant lineages?

THE COMPARATIVE BIOLOGY AND EVOLUTION OF UNMAINTAINABILITY

Classical theories rely on the selection shadow to explain aging. With some latitude for how the different sub-theories might interact, the prediction is thus that selection shadow should correlate tightly with aging rate. While this may indeed be the case within certain clades (mammals or mammalian orders, for example), it is almost certainly not the case across wider swaths of taxa.

Information on the comparative biology of aging comes from two types of sources: demographic data across a broad array of species (Jones et al., 2014; Salguero-Gómez et al., 2014; Salguero-Gómez et al., 2016; Scheuerlein et al., 2017), and experimental/lab work on mechanisms in diverse species. The former has the advantage of covering many more species and more branches of the tree of life, but the disadvantage of an inability to disentangle physiological aging mechanisms from other mechanisms that cause aging-like signatures in demography. Negative senescence due to factors such as size might partially cancel out mortality increases or fecundity decreases due to physiological aging, but, if aging is inexorable and leads to inevitable death, at some point the physiological aging should become demographically apparent if observation time suffices.

The picture painted by demographic studies is as follows: Aging appears to be present in all birds and all mammals. Potential exceptions such as naked mole-rats (Ruby et al., 2018) may not have been followed long enough; we remain agnostic. There are some taxa that appear not to age, or where lack of aging is widespread, such as sharks, crocodilians, and turtles (Finch, 1994; Jones et al., 2014). In many taxa, aging is more of a patchwork: in ray-finned fishes and plants, there are clear examples of species that appear not to age, and clear examples of species that age very quickly, often without a clear taxonomic pattern, though data are not yet complete enough to thoroughly evaluate phylogenetic signal. There are many examples of unusual aging patterns or lack of aging in invertebrates and plants with clonal reproduction (Shefferson et al., 2017); mechanistic studies generally do not contradict these findings, and provide better resolution for the physiology. Note, for example, that plants can handle polyploidy and aneuploidy, and even use it to their advantage (Tudge, 2006), while mammals cannot. Within mammals, exceptional lifespans of naked mole-rats, blind mole-rats, and bowhead whales appear related to specific adaptations, unique in each species, to avoid certain aging mechanisms (Gorbunova et al., 2012; Takasugi et al., 2020; Cooper and Gorbunova, 2021). It is unclear whether the adaptations discovered represent ways to constrain the most-limiting aging mechanisms in each species, or are a small subset of a large number of adaptations to limit a large number of mechanisms. Current evidence suggests, but does not definitively prove, that these adaptations can succeed in slowing but not stopping aging in mammals. Broadly, for both mammals and birds, correlations between lifespan and body size/metabolic rate/reproductive rate do suggest some role for trade-offs in structuring lifespan, though the associations are weak enough for other factors to play important roles.

Indeed, there appear to be a variety of factors that explain unusual aging patterns in different species, rather than a single unifying explanation. The exceptional lifespan of the ocean quahog, *Arctica islandica*, (record: 507 years) seems to be the extreme of a continuum. Various clam species with different lifespans differ continuously in their ability to avoid protein aggregation and loss of proteostasis (Ridgway et al., 2011; Ungvari et al., 2011; Treaster et al., 2015). The lack of aging in hydra appears to be dependent on both species and environment (Martínez, 1998; Martínez and Bridge, 2012). In killifish, as noted above, short lifespan appears to evolve repeatedly but through different mechanisms: breakdown in housekeeping genes across the genome as selection relaxes on the ability to maintain physiology for more than several months (Cui et al., 2019). In taxa such as lepidoptera and bees, wear-and-tear on wings may be a major factor limiting lifespan (Rueppell, 2009), and is probably not closely related to the conserved genetic pathways that appear limiting in yeast, fruit flies, nematodes, and mammals.

This portrait of comparative aging suggests that there is no single explanation that will concisely summarize everything. There are conserved genetic pathways, and these do modulate aging in some species, but not all. In some taxa, trade-offs have a large role, in others a weaker role. There are few if any universal mechanisms, but in at least some species controlling the key

known mechanisms is part of the reason they can evolve exceptional lifespans. Some constraints, while by definition inevitable, may nonetheless be modulated. For example, it is likely impossible to eliminate DNA damage completely, but it can be drastically reduced with appropriate investments in protection and repair. (By contrast, cellular senescence is a programmed pathway, and could presumably be eliminated completely by one or several key mutations. Cellular senescence is thus best thought of as an adaptation, for example to reduce cancer risk and for pruning in brain development.) Both adaptations and modulable constraints may be subject to trade-offs.

In this context, we argue that unmaintainability is the missing piece of the puzzle that allows us to make at least a little sense of this patchwork—for example, why mammals show a slow-fast life history continuum (Gaillard et al., 2016), but sharks do not. For any given taxon, the fundamental question it faces in the evolution of lifespan is, what are the constraints, i.e., what factors will limit lifespan regardless of selection and physiological adaptation? The answer to this question will be fundamentally different for a hydra, a butterfly, a fruit fly, a pine tree, a shark, and a mammal.

Above, we have given examples of the types of constraints that may apply, both generally and in regards to aging. One that deserves particular attention is the type of constraint arising from the structure of the homeostatic networks specific to each taxon. For example, in mammals, high levels of glucose are toxic and generate advanced glycosylation end products, which appear to contribute to aging; in birds, similar glucose levels are tolerated, and indeed necessary to sustain the metabolism associated with flight (Holmes et al., 2001). Thus, despite numerous similarities between bird and mammalian metabolic networks, there are also fundamental differences that generate regulatory weak points for each taxon. Metabolic syndrome and inflamm-aging, for example, appear to be mammalian phenomena. In other words, the regulatory networks that have evolved in each taxon have unique strengths and weaknesses; in some cases, these weaknesses will create ways the system is unmaintainable, and thus represent constraints on the evolution of lifespan.

Accordingly, the model we propose is that each taxon has a unique set of unmodulable constraints, modulable constraints, and adaptations that may affect aging. The constraints may often be related to the structure of regulatory networks and how this interacts with the taxon's ecology/niche (e.g., the need of a bird to migrate, insectivorous diet, etc.). Within the broad framework set by the constraints, trade-offs and drift/stochasticity then operate to produce the specific aging patterns of each species. Note that we discuss taxon-specific constraints rather than species-specific constraints because in some cases the relevant variation is mostly at higher levels. For example, it would appear that there is relatively little variation in the constraints within mammals, or within birds (the basic physiological and biochemical architecture being relatively similar). In contrast, the constraints may be different from one hydra species to another, though it is hard to say for certain (Martínez and Bridge, 2012).

The constraints arising in a given taxon may have their origins in relatively random factors early in the taxon's evolution, which have subsequently become fixed. For example, early mammals had a much more limited set of ecological niches for a long time and were presumably relatively short-lived; it would be unsurprising that their physiological architecture evolved containing constraints that imposed important limits on the subsequent evolution of lifespan as the taxon diversified. Differences in constraints across taxa create a patchwork of how unmaintainable each taxon is. Within this broader framework, trade-offs and other forces can operate to adjust aging. In some sense, this is a rather unsatisfying answer and explanation: the explicit prediction is that local, hard-to-predict and hard-to-identify factors are responsible for the diversity of aging patterns across the tree of life, and thus there is no broad theory that will explain the evolution of aging more generally. On the other hand, ours is a coherent explanation for why a simpler theory should probably never have been expected to be sufficient, and is consistent with the vast bulk of what is known about aging at many levels: genetic, molecular, physiological, demographic, and evolutionary.

SUMMARY AND THE NEXT STEPS

In Greek mythology, the Danaids were the 50 daughters of Danaus. They were supposed to marry the 50 sons of Aegyptus, but all but one of them killed their husbands on their wedding night. For this, they were condemned to spend eternity carrying water in a perforated vessel or sieve, and became a metaphor for futility. We call our theory the "Danaid theory of aging" because we propose that organismal biology and physiology are like the leaky vessels of the Danaids, unable to hold life in them eternally due to constraints in their basic structure. This metaphor creates a clear contrast with programmed theories of aging (which would imply that the Danaids would pour water out of the vessels intentionally for some reason), and with classical evolutionary theories relying on trade-offs and selection pressure (which might imply that the Danaids simply did not pay enough attention to avoid sloshing and spilling the water, or that they chose to accept some spillage because they wanted to run while carrying the water and accepted the consequences). We also add a nuance to the metaphor: some vessels (organismal physiologies) are leakier than others (and a few might even be completely unperforated), making them quite variable in their ability to hold water (life).

In summary, the Danaid theory of aging proposes that, in many taxa, aging is an inevitable consequence of how life has been constructed in that taxon, for reasons (constraints) largely independent of selection on aging and lifespan. Aging and lifespan are then further modified within each taxon by optimization and mutation accumulation as predicted based on the declining force of selection with age. The underlying constraints are expected to vary across taxa in ways that may appear arbitrary, reflecting developmental and regulatory requirements as well as the vagaries of chance across the ancestral lineage.

A famous quotation goes

> "It is indeed remarkable that after a seemingly miraculous feat of morphogenesis a complex metazoan should be unable to perform the much simpler task of merely maintaining what is already formed." (Williams, 1957).

Often still seen as the core question that an evolutionary theory of aging should seek to answer, this misses the point. Why would maintenance be "much simpler" and "mere"? Cues present during morphogenesis may no longer be there. Development may have closed repair options. And complex regulatory networks may simply slide out of equilibrium through repetitive perturbations, without a clear way of putting them back. Hence, the quote might just as well read:

> "Clearly, after the miraculous feat of morphogenesis many complex metazoans are unable to perform the virtually impossible task of maintaining what was formed."

But even more, we noted in Section *Systemic Constraints on Physiology and Evolution* that evolution can only pick up options that it stumbles across (Jacob, 1977). The process of morphogenesis already exists in any organism for historical reasons (Turke, 2013): Whether organisms age or not, they die. Hence, whether or not chronologically old organisms are also biologically old, the necessity to reproduce exists. It is not at all impossible, therefore, that in the light of the restrictions on repair discussed here, nature patches for the short term, while in the long term it applies one all-encompassing repair mechanism: the creation of a new organism. Hence, an even more apt variation on the quote above could read:

> "Rather than performing the virtually impossible task of maintaining itself, the complex metazoan repeats the miraculous but proven feat of morphogenesis."

We are not the first to mention the role of constraints in shaping the evolution of aging. The hyperfunction theory does so more specifically (Blagosklonny, 2012; Gems and de la Guardia, 2013), and trade-offs themselves are a form of constraint (Cohen et al., 2019). However, we feel that the broad picture that persists in the literature is that constraints are an afterthought, a detail that can be ignored, or worse: invoked whenever standard explanations fail. We argue that thinking carefully about what constraints are present can fundamentally alter how we understand the evolution of the aging process, and that such efforts have rarely been undertaken.

As we have emphasized, the exact mechanisms of aging, and hence its evolution, are expected to vary greatly from taxon to taxon, which complicates the task of making predictions. But across the tree of life, reproduction remains the ultimate repair. Some organisms also seem capable of keeping their own body in perfect shape, while others do not. In finding a new theory of the evolution of aging the central question thus becomes:

> "Why is it that some organisms (species) seem incapable of doing inside their body what they are perfectly capable of doing outside their body: to create a perfectly healthy organism?"

To answer that question, we need to map the diversity of mechanisms and pathways across the tree of life. Such efforts are beginning, including in this Research Topic, but the task is daunting because of the variety of potential mechanisms and the large number that may be highly specific. Efforts to study mechanisms in unusual species (ocean quahogs (Ungvari et al., 2011), naked mole-rats (Tian et al., 2013), etc.) have already proven highly fruitful; the next challenge is to identify unusual mechanisms and quantify their importance. This could lead to eventual empirical tests of this theory, and to further theory building. If unusual or non-canonical aging mechanisms are rarely if ever important for a species' demography, they may be negligible, in contradiction to our theory. If the Hallmarks of aging are shown to be not just present in most/all species, but sufficient explanations, and modulable, our theory would be disproven. Conversely, our theory will be supported if increasing data show variation in aging mechanisms/constraints in different taxa (Omotoso et al., 2021), often at variance with the forces of selection, and if we continue to uncover a larger and larger role for the breakdown in homeostatic mechanisms or other basic constraints in shaping the aging process. It is time for theory on the evolution of aging to incorporate what is known about development, physiology, genetics, and comparative biology, and to acknowledge explicitly that constraints could result in aging even in the absence of the selection shadow, and thus even in the absence of the classical theories.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to this work, which grew out of an extensive exchange of ideas that the authors had through video meetings and an original draft prepared by MW. This draft was materially modified and expanded with ideas from AC and carefully reworked several times by both authors.

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REFERENCES

Austad, S. N. (2004). Rebuttal to Bredesen: 'The Non-existent Aging Program: How Does it Work?'. *Aging Cell* 3 (5), 253–254. doi:10.1111/j.1474-9728.2004.00119.x

Baudisch, A. (2008). *Inevitable Aging?*

Bern, R. M., Levy, M. N., Koeppen, B. M., and Stanton, B. A. (2004). *Physiology*. 5th edn. St. Louis, Missouri: Mosby.

Blagosklonny, M. V. (2012). Answering the Ultimate Question "What Is the Proximal Cause of Aging?". *Aging* 4 (12), 861–877. doi:10.18632/aging.100525

Blagosklonny, M. V. (2009). TOR-driven Aging: Speeding Car without Brakes. *Cell Cycle* 8 (24), 4055–4059. doi:10.4161/cc.8.24.10310

Blomberg, S. P., and Garland, T. (2002). Tempo and Mode in Evolution: Phylogenetic Inertia, Adaptation and Comparative Methods. *J. Evol. Biol.* 15 (6), 899–910. doi:10.1046/j.1420-9101.2002.00472.x

Bourg, S., Jacob, L., Menu, F., and Rajon, E. (2019). Hormonal Pleiotropy and the Evolution of Allocation Trade-offs. *Evolution* 73 (4), 661–674. doi:10.1111/evo.13693

Brand, M. D. (2000). Uncoupling to Survive? the Role of Mitochondrial Inefficiency in Ageing. *Exp. Gerontol.* 35 (6-7), 811–820. doi:10.1016/s0531-5565(00)00135-2

Cohen, A. A. (2018). Aging across the Tree of Life: The Importance of a Comparative Perspective for the Use of Animal Models in Aging. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864 (9 Pt A), 2680–2689. doi:10.1016/j.bbadis.2017.05.028

Cohen, A. A., Bandeen-Roche, K., Morissette-Thomas, V., and Fulop, T. (2018). *A Robust Characterization of Inflammaging and Other Immune Processes through Multivariate Analysis of Cytokines from Longitudinal Studies. Handbook on Immunosenescence: Basic Understanding and Clinical Applications*. Doderecht: Springer Science+ Business Media BV. doi:10.1007/978-3-319-64597-1_120-1

Cohen, A. A. (2004). Female post-reproductive Lifespan: a General Mammalian Trait. *Biol. Rev. Camb Philos. Soc.* 79 (4), 733–750. doi:10.1017/s1464793103006432

Cohen, A. A. (2016). Complex Systems Dynamics in Aging: New Evidence, Continuing Questions. *Biogerontology* 17 (1), 205–220. doi:10.1007/s10522-015-9584-x

Cohen, A. A., Coste, C. F. D., Li, X. Y., Bourg, S., Pavard, S., and Gaillard, J. M. (2019). Are Trade-offs Really the Key Drivers of Ageing and Life Span? *Funct. Ecol.* 34 (1), 153–166. doi:10.1111/1365-2435.13444

Cohen, A. A., Isaksson, C., and Salguero-Gómez, R. (2017). Co-existence of Multiple Trade-Off Currencies Shapes Evolutionary Outcomes. *PLoS One* 12 (12), e0189124. doi:10.1371/journal.pone.0189124

Cohen, A. A., Kennedy, B. K., Anglas, U., Bronikowski, A. M., Deelen, J., Dufour, F., et al. (2020). Lack of Consensus on an Aging Biology Paradigm? A Global Survey Reveals an Agreement to Disagree, and the Need for an Interdisciplinary Framework. *Mech. Ageing Development* 191, 111316. doi:10.1016/j.mad.2020.111316

Cohen, A. A., Legault, V., and Fülöp, T. (2020). What if There's No Such Thing as "aging"? *Mech. Ageing Development* 192, 111344. doi:10.1016/j.mad.2020.111344

Cohen, A. A., Levasseur, M., Raina, P., Fried, L. P., and Fülöp, T. (2020). Is Aging Biology Ageist? *J. Gerontol. A. Biol. Sci. Med. Sci.* 75 (9), 1653–1655. doi:10.1093/gerona/glz190

Cohen, A. A., Martin, L. B., Wingfield, J. C., McWilliams, S. R., and Dunne, J. A. (2012). Physiological Regulatory Networks: Ecological Roles and Evolutionary Constraints. *Trends Ecol. Evol.* 27 (8), 428–435. doi:10.1016/j.tree.2012.04.008

Cohen, A. (2015). Physiological and Comparative Evidence Fails to Confirm an Adaptive Role for Aging in Evolution. *Cas* 8, 14–23. doi:10.2174/1874609808666150422124332

Congdon, J. D., Nagle, R. D., Kinney, O. M., van Loben Sels, R. C., Quinter, T., and Tinkle, D. W. (2003). Testing Hypotheses of Aging in Long-Lived Painted Turtles (*Chrysemys picta*). *Exp. Gerontol.* 38 (7), 765–772. doi:10.1016/s0531-5565(03)00106-2

Cooper, L. N., and Gorbunova, V. (2021). Molecular Insights into Anatomy and Physiology. *The Bowhead Whale*, 299–307. doi:10.1016/b978-0-12-818969-6.00020-0

Csete, M., and Doyle, J. (2004). Bow Ties, Metabolism and Disease. *Trends Biotechnol.* 22 (9), 446–450. doi:10.1016/j.tibtech.2004.07.007

Cui, R., Medeiros, T., Willemsen, D., Iasi, L. N. M., Collier, G. E., Graef, M., et al. (2019). Relaxed Selection Limits Lifespan by Increasing Mutation Load. *Cell* 178 (2), 385–399. doi:10.1016/j.cell.2019.06.004

Dansereau, G., Wey, T. W., Legault, V., Brunet, M. A., Kemnitz, J. W., Ferrucci, L., et al. (2019). Conservation of Physiological Dysregulation Signatures of Aging across Primates. *Aging Cell* 18 (2), e12925. doi:10.1111/acel.12925

Dantzer, B., and Swanson, E. M. (2012). Mediation of Vertebrate Life Histories *via* Insulin-like Growth Factor-1. *Biol. Rev. Camb Philos. Soc.* 87 (2), 414–429. doi:10.1111/j.1469-185x.2011.00204.x

Davies, J. (2014). *Life Unfolding: How the Human Body Created Itself*. Oxford University Press.

de Lorenzo, V. (2014). From Theselfish Genetoselfish Metabolism: Revisiting the central Dogma. *Bioessays* 36 (3), 226–235. doi:10.1002/bies.201300153

de Lorenzo, V. (2015). It's the Metabolism, Stupid!. *Environ. Microbiol. Rep.* 7 (1), 18–19. doi:10.1111/1758-2229.12223

Dobzhansky, T. (1973). Nothing in Biology Makes Sense except in the Light of Evolution. *Am. Biol. Teach.* 35 (3), 125–129. doi:10.2307/4444260

Doonan, R., McElwee, J. J., Matthijssens, F., Walker, G. A., Houthoofd, K., Back, P., et al. (2008). Against the Oxidative Damage Theory of Aging: Superoxide Dismutases Protect against Oxidative Stress but Have Little or No Effect on Life Span in *Caenorhabditis elegans*. *Genes Dev.* 22 (23), 3236–3241. doi:10.1101/gad.504808

Finch, C. E. (1994). *Longevity, Senescence and the Genome*. University of Chicago Press.

Franceschi, C., Salvioli, S., Garagnani, P., de Eguileor, M., Monti, D., and Capri, M. (2017). Immunobiography and the Heterogeneity of Immune Responses in the Elderly: A Focus on Inflammaging and Trained Immunity. *Front. Immunol.* 8, 982. doi:10.3389/fimmu.2017.00982

Fulop, T., Larbi, A., Dupuis, G., Le Page, A., Frost, E. H., Cohen, A. A., et al. (2017). Immunosenescence and Inflamm-Aging as Two Sides of the Same Coin: Friends or Foes? *Front. Immunol.* 8, 1960. doi:10.3389/fimmu.2017.01960

Gaillard, J. M., Lemaître, J. F., Berger, V., Bonenfant, C., Devillard, S., Douhard, M., et al. (2016). *Axes of Variation in Life Histories*. Lyon: University of Lyon, 312–323.

Gems, D., and de Magalhães, J. P. (2021). The Hoverfly and the Wasp: A Critique of the Hallmarks of Aging as a Paradigm. Preprints

Gems, D., and de la Guardia, Y. (2013). Alternative Perspectives on Aging in *Caenorhabditis elegans*: Reactive Oxygen Species or Hyperfunction? *Antioxid. Redox Signaling* 19 (3), 321–329. doi:10.1089/ars.2012.4840

Gems, D., and Doonan, R. (2009). Antioxidant Defense and Aging inC. Elegans: Is the Oxidative Damage Theory of Aging Wrong? *Cell Cycle* 8 (11), 1681–1687. doi:10.4161/cc.8.11.8595

Gems, D., and McElwee, J. J. (2005). Broad Spectrum Detoxification: the Major Longevity Assurance Process Regulated by insulin/IGF-1 Signaling? *Mech. Ageing Development* 126 (3), 381–387. doi:10.1016/j.mad.2004.09.001

Gems, D., and Partridge, L. (2013). Genetics of Longevity in Model Organisms: Debates and Paradigm Shifts. *Annu. Rev. Physiol.* 75, 621–644. doi:10.1146/annurev-physiol-030212-183712

Gladyshev, V. N. (2016). Aging: Progressive Decline in Fitness Due to the Rising Deleteriome Adjusted by Genetic, Environmental, and Stochastic Processes. *Aging Cell* 15 (4), 594–602. doi:10.1111/acel.12480

Goldsmith, T. C. (2012). On the Programmed/non-Programmed Aging Controversy. *Biochem. Mosc.* 77 (7), 729–732. doi:10.1134/s000629791207005x

Gorbunova, V., Hine, C., Tian, X., Ablaeva, J., Gudkov, A. V., Nevo, E., et al. (2012). Cancer Resistance in the Blind Mole Rat Is Mediated by Concerted Necrotic Cell Death Mechanism. *Proc. Natl. Acad. Sci.* 109 (47), 19392–19396. doi:10.1073/pnas.1217211109

Gould, S. J. (1980). The Evolutionary Biology of Constraint. *Daedalus* 109 (2), 39–52.

Gould, S. J., and Vrba, E. S. (1982). Exaptation-A Missing Term in the Science of Form. *Paleobiology* 8, 4–15. doi:10.1017/s0094837300004310

Hamilton, W. D. (1966). The Moulding of Senescence by Natural Selection. *J. Theor. Biol.* 12 (1), 12–45. doi:10.1016/0022-5193(66)90184-6

He, C., Zhou, C., and Kennedy, B. K. (2018). The Yeast Replicative Aging Model. *Biochim. Biophys. Acta Mol. Basis Dis.* 1864 (9 Pt A), 2690–2696. doi:10.1016/j.bbadis.2018.02.023

Helfgott, D. C., Tatter, S. B., Santhanam, U., Clarick, R. H., Bhardwaj, N., May, L. T., et al. (1989). Multiple Forms of IFN-Beta 2/IL-6 in Serum and Body Fluids during Acute Bacterial Infection. *J. Immunol.* 142 (3), 948–953.

Holmes, D. J., Flückiger, R., and Austad, S. N. (2001). Comparative Biology of Aging in Birds: an Update. *Exp. Gerontol.* 36 (4-6), 869–883. doi:10.1016/s0531-5565(00)00247-3

Hoogstraten, D., Bergink, S., Ng, J. M., Verbiest, V. H., Luijsterburg, M. S., Geverts, B., et al. (2008). Versatile DNA Damage Detection by the Global Genome Nucleotide Excision Repair Protein XPC. *J. Cel Sci* 121 (Pt 17), 2850–2859. doi:10.1242/jcs.031708

Jacob, F. (1977). Evolution and Tinkering. *Science* 196, 1161–1166. doi:10.1126/science.860134

Jebelli, J., Su, W., Hopkins, S., Pocock, J., and Garden, G. A. (2015). Glia: Guardians, Gluttons, or Guides for the Maintenance of Neuronal Connectivity? *Ann. N.Y. Acad. Sci.* 1351, 1–10. doi:10.1111/nyas.12711

Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al. (2014). Diversity of Ageing across the Tree of Life. *Nature* 505 (7482), 169–173. doi:10.1038/nature12789

Jouvet, L., Rodríguez-Rojas, A., and Steiner, U. K. (2018). Demographic Variability and Heterogeneity Among Individuals within and Among Clonal Bacteria Strains. *Oikos* 127 (5), 728–737. doi:10.1111/oik.04292

Kirkwood, T. B., and Holliday, R. (1979). The Evolution of Ageing and Longevity. *Proc. R. Soc. Lond. B Biol. Sci.* 205 (1161), 531–546. doi:10.1098/rspb.1979.0083

Kirkwood, T. B., and Rose, M. R. (1991). Evolution of Senescence: Late Survival Sacrificed for Reproduction. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 332 (1262), 15–24. doi:10.1098/rstb.1991.0028

Kirkwood, T. B. L. (1981). "Repair and its Evolution: Survival versus Reproduction," in *Physiological Ecology: An Evolutionary Approach to Resource Use*. Editor P. Townsend CRaC (Blackwell Scientific), 165–189.

Kirkwood, T. B. L. (1999). *Time of Our Lives: The Science of Human Aging*. Oxford University Press on Demand.

Kirkwood, T. B. L., and Austad, S. N. (2000). Why Do We Age? *Nature* 408 (6809), 233–238. doi:10.1038/35041682

Kirkwood, T. B. L. (1977). Evolution of Ageing. *Nature* 270 (5635), 301–304. doi:10.1038/270301a0

Kirkwood, T. B. L., and Melov, S. (2011). On the Programmed/non-Programmed Nature of Ageing within the Life History. *Curr. Biol.* 21 (18), R701–R707. doi:10.1016/j.cub.2011.07.020

Kitano, H. (2002). Systems Biology: a Brief Overview. *Science* 295 (5560), 1662–1664. doi:10.1126/science.1069492

Kitano, H. (2007). Towards a Theory of Biological Robustness. *Mol. Syst. Biol.* 3, 137. doi:10.1038/msb4100179

Kondepudi, D., Kay, B., and Dixon, J. (2015). End-directed Evolution and the Emergence of Energy-Seeking Behavior in a Complex System. *Phys. Rev. E Stat. Nonlin Soft Matter Phys.* 91 (5), 050902. doi:10.1103/PhysRevE.91.050902

Kowald, A., and Kirkwood, T. B. L. (2016). Can Aging Be Programmed? A Critical Literature Review. *Aging Cell* 15 (6), 986–998. doi:10.1111/acel.12510

Kriete, A. (2013). Robustness and Aging-A Systems-Level Perspective. *Biosystems* 112 (1), 37–48. doi:10.1016/j.biosystems.2013.03.014

Laberge, R.-M., Zhou, L., Sarantos, M. R., Rodier, F., Freund, A., de Keizer, P. L. J., et al. (2012). Glucocorticoids Suppress Selected Components of the Senescence-Associated Secretory Phenotype. *Aging Cell* 11 (4), 569–578. doi:10.1111/j.1474-9726.2012.00818.x

Le Couteur, D. G., and Simpson, S. J. (2011). Adaptive Senectitude: the Prolongevity Effects of Aging. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 66A (2), 179–182. doi:10.1093/gerona/glq171

Levy, G., and Levin, B. (2014). *The Biostatistics of Aging: From Gompertzian Mortality to an index of Aging-Relatedness*. Hoboken, New Jersey: Wiley.

Libertini, G. (2015). Non-programmed versus Programmed Aging Paradigm. *Cas* 8, 56–68. doi:10.2174/1874609808666150422111623

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The Hallmarks of Aging. *Cell* 153 (6), 1194–1217. doi:10.1016/j.cell.2013.05.039

Magalhães, J. P. (2012). Programmatic Features of Aging Originating in Development: Aging Mechanisms beyond Molecular Damage? *FASEB j.* 26 (12), 4821–4826. doi:10.1096/fj.12-210872

Maklakov, A. A., and Chapman, T. (2019). Evolution of Ageing as a Tangle of Trade-Offs: Energy versus Function. *Proc. R. Soc. B.* 286 (1911), 20191604. doi:10.1098/rspb.2019.1604

Marin, I., and Kipnis, J. (2013). Learning and Memory . . . and the Immune System. *Learn. Mem.* 20 (10), 601–606. doi:10.1101/lm.028357.112

Martínez, D. E., and Bridge, D. (2012). Hydra, the Everlasting Embryo, Confronts Aging. *Int. J. Dev. Biol.* 56 (6-8), 479–487. doi:10.1387/ijdb.113461dm

Martı´nez, D. E. (1998). Mortality Patterns Suggest Lack of Senescence in Hydra. *Exp. Gerontol.* 33 (3), 217–225.

McEwen, B. S. (1998). Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Ann. N. Y Acad. Sci.* 840, 33–44. doi:10.1111/j.1749-6632.1998.tb09546.x

Medawar, P. (1952). *An Unsolved Problem in Biology*. London: Lewis & Co.

Mertens, J., Paquola, A. C. M., Ku, M., Hatch, E., Böhnke, L., Ladjevardi, S., et al. (2015). Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects. *Cell Stem Cell* 17 (6), 705–718. doi:10.1016/j.stem.2015.09.001

Milewski, L. A. K. (2010). The Evolution of Ageing. *Biosci. Horizons* 3 (1), 77–84. doi:10.1093/biohorizons/hzq001

Mitteldorf, J. (2018). *Can. Aging Be Programmed? Biochem. (Mosc)* 83 (12), 1524–1533. doi:10.1134/s0006297918120106

Mocayar Marón, F. J., Ferder, L., Saraví, F. D., and Manucha, W. (2019). Hypertension Linked to Allostatic Load: from Psychosocial Stress to Inflammation and Mitochondrial Dysfunction. *Stress* 22 (2), 169–181. doi:10.1080/10253890.2018.1542683

Nijhout, H. F., Sadre-Marandi, F., Best, J., and Reed, M. C. (2017). Systems Biology of Phenotypic Robustness and Plasticity. *Integr. Comp. Biol.* 57 (2), 171–184. doi:10.1093/icb/icx076

Noble, D., Jablonka, E., Joyner, M. J., Müller, G. B., and Omholt, S. W. (2014). Evolution Evolves: Physiology Returns to centre Stage. *J. Physiol.* 592 (11), 2237–2244. doi:10.1113/jphysiol.2014.273151

Noble, D. (2013). Physiology Is Rocking the Foundations of Evolutionary Biology. *Exp. Physiol.* 98 (8), 1235–1243. doi:10.1113/expphysiol.2012.071134

Omotoso, O., Gladyshev, V., and Zhou, X. (2021). Lifespan Extension in Long-Lived Vertebrates Rooted in Ecological Adaptation. *Front Cel Dev Biol - Signaling.* 9, 704966. doi:10.3389/fcell.2021.704966

Parker, G. A., and Smith, J. M. (1990). Optimality Theory in Evolutionary Biology. *Nature* 348 (6296), 27–33. doi:10.1038/348027a0

Partridge, L., and Barton, N. H. (1993). Optimally, Mutation and the Evolution of Ageing. *Nature* 362, 305–311. doi:10.1038/362305a0

Pavard, S., and Metcalf, C. J. E. (2019). "Trade-offs between Causes of Mortality in Life History Evolution: the Case of Cancers," in *Human Evolutionary Demography*. Editor O. B. R. L. R. Sear (Open Book Publishers). In Press.

Pen, I., and Flatt, T. (2021). Asymmetry, Division of Labour and the Evolution of Ageing in Multicellular Organisms. *Phil. Trans. R. Soc. B* 376 (1823), 20190729. doi:10.1098/rstb.2019.0729

Ridgway, I. D., Richardson, C. A., and Austad, S. N. (2011). Maximum Shell Size, Growth Rate, and Maturation Age Correlate with Longevity in Bivalve Molluscs. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 66A (2), 183–190. doi:10.1093/gerona/glq172

Rose, M. R. (1984). Laboratory Evolution of Postponed Senescence in *Drosophila melanogaster*. *Evolution* 38 (5), 1004–1010. doi:10.1111/j.1558-5646.1984.tb00370.x

R. P. Shefferson, O. R. Jones, and R. Salguero-Gómez (Editors) (2017). *The Evolution of Senescence in the Tree of Life* (Cambridge University Press).

Ruby, J. G., Smith, M., and Buffenstein, R. (2018). Naked Mole-Rat Mortality Rates Defy Gompertzian Laws by Not Increasing with Age. *Elife* 7. doi:10.7554/eLife.31157

Rueppell, O. (2009). *Aging of Social Insects. Organization of Insect Societies: From Genome to Sociocomplexity2009*, 51–73.

Salguero-Gómez, R., Jones, O. R., Archer, C. R., Buckley, Y. M., Che-Castaldo, J., Caswell, H., et al. (2014). The Compadre Plant Matrix Database: an Open Online Repository for Plant Demography. *J. Ecol.* 103 (1), 202–218.

Salguero-Gómez, R., Jones, O. R., Archer, C. R., Bein, C., de Buhr, H., Farack, C., et al. (2016). COMADRE: a Global Data Base of Animal Demography. *J. Anim. Ecol.* 85 (2), 371–384. doi:10.1111/1365-2656.12482

Sauer, D. J., Heidinger, B. J., Kittilson, J. D., Lackmann, A. R., and Clark, M. E. (2021). No Evidence of Physiological Declines with Age in an Extremely Long-Lived Fish. *Sci. Rep.* 11 (1), 9065. doi:10.1038/s41598-021-88626-5

Schaible, R., Sussman, M., and Kramer, B. H. (2014). Aging and Potential for Self-Renewal: Hydra Living in the Age of Aging - A Mini-Review. *Gerontology* 60 (6), 548–556. doi:10.1159/000360397

Scheuerlein, A., Vieregg, D., and Prast, D. (2017). *DATLife Database. in: Max Planck*. Germany: Institut für demografische Forschung R.

Schneider, E. D., and Kay, J. J. (1994). Life as a Manifestation of the Second Law of Thermodynamics. *Math. Computer Model.* 19 (6-8), 25–48. doi:10.1016/0895-7177(94)90188-0

Smith, J. M. (1978). Optimization Theory in Evolution. *Annu. Rev. Ecol. Syst.* 9 (1), 31–56. doi:10.1146/annurev.es.09.110178.000335

Stearns, S. C. (1989). Trade-Offs in Life-History Evolution. *Funct. Ecol.* 3 (3). doi:10.2307/2389364

Stellwagen, D., and Malenka, R. C. (2006). Synaptic Scaling Mediated by Glial TNF-α. *Nature* 440 (7087), 1054–1059. doi:10.1038/nature04671

Sterling, P. (2020). *What Is Health? Allostasis and the Evolution of Human Design*. MIT Press.

Suzanne, M., and Steller, H. (2013). Shaping Organisms with Apoptosis. *Cell Death Differ* 20 (5), 669–675. doi:10.1038/cdd.2013.11

Takasugi, M., Firsanov, D., Tombline, G., Ning, H., Ablaeva, J., Seluanov, A., et al. (2020). Naked Mole-Rat Very-High-Molecular-Mass Hyaluronan Exhibits superior Cytoprotective Properties. *Nat. Commun.* 11 (1), 2376. doi:10.1038/s41467-020-16050-w

Thompson, D. (2014). in *On Growth and Form*. Editor J. Bonner (Cambridge, UK: Cambridge University Press). doi:10.1017/CBO9781107325852

Tian, X., Azpurua, J., Hine, C., Vaidya, A., Myakishev-Rempel, M., Ablaeva, J., et al. (2013). High-molecular-mass Hyaluronan Mediates the Cancer Resistance of the Naked Mole Rat. *Nature* 499 (7458), 346–349. doi:10.1038/nature12234

Treaster, S. B., Chaudhuri, A. R., and Austad, S. N. (2015). Longevity and GAPDH Stability in Bivalves and Mammals: A Convenient Marker for Comparative Gerontology and Proteostasis. *PLoS One* 10 (11), e0143680. doi:10.1371/journal.pone.0143680

Tudge, C. (2006). *The Secret Life of Trees*. London, England: Penguin Books.

Turke, P. W. (2013). Making Young from Old: How Is Sex Designed to Help? *Evol. Biol.* 40 (4), 471–479. doi:10.1007/s11692-013-9236-5

Ungvari, Z., Ridgway, I., Philipp, E. E. R., Campbell, C. M., McQuary, P., Chow, T., et al. (2011). Extreme Longevity Is Associated with Increased Resistance to Oxidative Stress in Arctica Islandica, the Longest-Living Non-colonial Animal. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 66A (7), 741–750. doi:10.1093/gerona/glr044

van Noordwijk, A. J., and de Jong, G. (1986). Acquisition and Allocation of Resources: Their Influence on Variation in Life History Tactics. *The Am. Naturalist* 128 (1), 137–142. doi:10.1086/284547

Weismann, A. (1891). in *Weismann on Heredity*. Editors E. B. S. S. Poulton and A. E. Shipley (Oxford University Press), 23–42.

Wensink, M. (2013). Age-specificity and the Evolution of Senescence: a Discussion. *Biogerontology* 14 (1), 99–105. doi:10.1007/s10522-012-9410-7

Wensink, M. J. (2016). Size, Longevity and Cancer: Age Structure. *Proc. Biol. Sci.* 283. doi:10.1098/rspb.2016.1510

Wensink, M. J., Caswell, H., and Baudisch, A. (2017). The Rarity of Survival to Old Age Does Not Drive the Evolution of Senescence. *Evol. Biol.* 44 (1), 5–10. doi:10.1007/s11692-016-9385-4

Wensink, M. J., van Heemst, D., Rozing, M. P., and Westendorp, R. G. J. (2012). The Maintenance gap: a New Theoretical Perspective on the Evolution of Aging. *Biogerontology* 13 (2), 197–201. doi:10.1007/s10522-011-9362-3

Wensink, M. J., Vaupel, J. W., and Christensen, K. (2017). Stem Cell Divisions Per Se Do Not Cause Cancer. *Epidemiology* 28 (4), e35–e37. doi:10.1097/ede.0000000000000612

Wensink, M. J., Wrycza, T. F., and Baudisch, A. (2014). Interaction Mortality: Senescence May Have Evolved Because it Increases Lifespan. *PLoS One* 9 (10), e109638. doi:10.1371/journal.pone.0109638

Wensink, M. J., Wrycza, T. F., and Baudisch, A. (2014). No Senescence Despite Declining Selection Pressure: Hamilton's Result in Broader Perspective. *J. Theor. Biol.* 347, 176–181. doi:10.1016/j.jtbi.2013.11.016

Wensink, M., Westendorp, R. G. J., and Baudisch, A. (2014). The Causal Pie Model: an Epidemiological Method Applied to Evolutionary Biology and Ecology. *Ecol. Evol.* 4 (10), 1924–1930. doi:10.1002/ece3.1074

Williams, G. C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. *Evolution* 11 (4). doi:10.2307/2406060

Zalli, A., Carvalho, L. A., Lin, J., Hamer, M., Erusalimsky, J. D., Blackburn, E. H., et al. (2014). Shorter Telomeres with High Telomerase Activity Are Associated with Raised Allostatic Load and Impoverished Psychosocial Resources. *Proc. Natl. Acad. Sci.* 111 (12), 4519–4524. doi:10.1073/pnas.1322145111

Zhou, T., Carlson, J. M., and Doyle, J. (2005). Evolutionary Dynamics and Highly Optimized Tolerance. *J. Theor. Biol.* 236 (4), 438–447. doi:10.1016/j.jtbi.2005.03.023

Conflict of Interest: AAC is founder and CEO at Oken.

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