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Model organisms in aging research: *Caenorhabditis elegans*

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Model organisms in aging research: *Caenorhabditis elegans*

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REVIEW
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Insights Into the Links Between Proteostasis and Aging From *C. elegans*

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Protein homeostasis (proteostasis) is maintained by a tightly regulated and interconnected network of biological pathways, preventing the accumulation and aggregation of damaged or misfolded proteins. Thus, the proteostasis network is essential to ensure organism longevity and health, while proteostasis failure contributes to the development of aging and age-related diseases that involve protein aggregation. The model organism *Caenorhabditis elegans* has proved invaluable for the study of proteostasis in the context of aging, longevity and disease, with a number of pivotal discoveries attributable to the use of this organism. In this review, we discuss prominent findings from *C. elegans* across the many key aspects of the proteostasis network, within the context of aging and disease. These studies collectively highlight numerous promising therapeutic targets, which may 1 day facilitate the development of interventions to delay aging and prevent age-associated diseases.

Keywords: proteostasis, *C. elegans*, protein translation, chaperones, ubiquitin-proteasome system, autophagy, protein aggragation, stress responses

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1 INTRODUCTION

The use of short-lived, rapidly reproducing, and easily modified model organisms has allowed us to undertake cause and effect studies for cellular and organismal aging on a massive scale. The nematode *Caenorhabditis elegans* in particular provides exceptional utility, as it possesses high genetic homology with humans (>70%), and conserved biological signaling pathways (S. Zhang et al., 2020). With the aid of *C. elegans* and other model organisms, we now know that many age-associated phenotypes do not depend on the chronological age of an organism, but instead depend on the accumulation of damage to the genome or proteome, and are defined by key signaling cascades such as the insulin/insulin-like growth factor (IGF-1) signalling (IIS) pathway (López-Otín et al., 2013). Identifying and exploiting biological networks or molecular targets that control organism aging and longevity has thus become the focus of research, with the long-term goal of translating these findings into therapeutic strategies. This is similarly true for the study of diseases arising from protein dysregulation, where *C. elegans* is an invaluable model towards the study of neurodegenerative proteinopathies, and often within the context of aging (Saez and Vilchez, 2014; Koyuncu et al., 2015).

Protein homeostasis (proteostasis) is crucial for organism longevity and health, and impairment to the proteostasis network is a hallmark of aging (López-Otín et al., 2013). This network principally functions to maintain proteome integrity, and is inclusive of the processes encompassing the translation and post-translational processing of newly-synthesized proteins, as well as those that control protein localization and degradation (Hipp et al., 2019). However, during aging this network

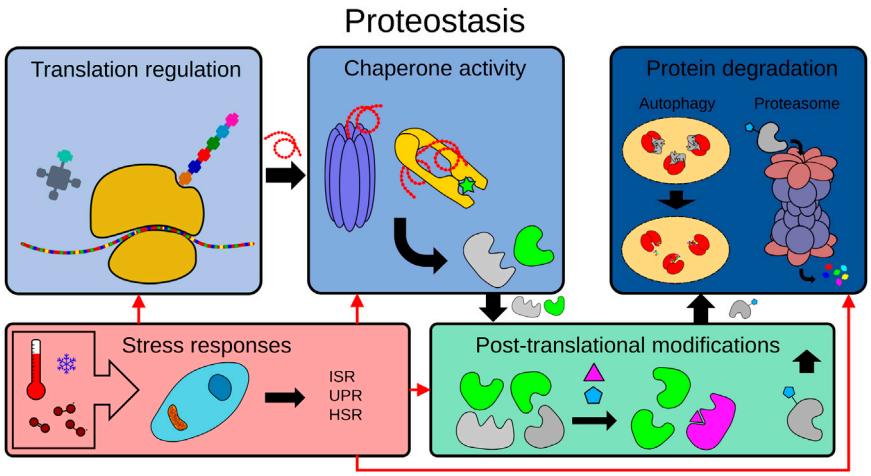

FIGURE 1 | The proteostasis network. To maintain proteome integrity, the proteostasis network is tightly regulated in healthy cells from translation to degradation. The proteostasis network includes protein translation, protein folding by chaperones, post-translational modifications (PTMs), protein degradation mechanisms (e.g. the ubiquitin-proteasome system (UPS), the autophagy-lysosome pathway) and the modulation of adaptive stress responses by physiological or environmental signals (e.g. ISR: integrated stress response, UPR: unfolded protein response, HSR: heat shock response.

becomes progressively impaired, and this drives the accumulation of misfolded, dysfunctional and aggregated proteins (Saez and Vilchez, 2014).

Here, we discuss how *C. elegans* has been used to understand and exploit the underlying mechanisms behind proteostasis in determining organismal longevity and aging. This will encompass discussing protein translation, folding and maintenance by chaperones, post-translational modifications, and the two main proteolytic mechanisms: the ubiquitin-proteasome (UPS) and the autophagy-lysosome pathway (**Figure 1**).

2 PROTEIN SYNTHESIS

Both the overall rate of protein synthesis and the fidelity of translation decreases with age. These two observations are distinct and reproducible across numerous studies and model organisms (Anisimova et al., 2018). However, genetic modulation to induce a decreased rate of protein synthesis has been shown to be generally lifespan-extending, while conversely, a decrease in translational accuracy is associated with aged and diseased phenotypes (Syntichaki et al., 2007b; Anisimova et al., 2018; Martinez-Miguel et al., 2021) (**Figure 2**). As such, these two parameters of protein synthesis are often studied independently from each other, and rely on different biological components and mechanisms for their observed effects on lifespan.

2.1 Translational Rates

Aging causes a functional decline in various components of the protein translation system, as well as age-related regulatory changes (Walther et al., 2015; Dhondt et al., 2017; Anisimova et al., 2018). The net effect is that the rate and the frequency of protein translation, and by extension protein synthesis, decreases with age, with many of these age-associated changes often having detrimental impacts on health and lifespan (Syntichaki et al., 2007b; Dhondt et al., 2017). This observed correlation has previously led to the speculation of a possible relationship between a decreased translation rate and the progression of age (Tavernarakis and Driscoll, 2002). However, contrary to this expectation, a number of studies have since shown that decreased protein synthesis increases lifespan in both normal aging and long-lived paradigms (Syntichaki et al., 2007b; Depuydt et al., 2016; Dhondt et al., 2017). Thus, studies regarding protein translational rates focus not only on the characterization and understanding of translation pathways and regulators in respect to health and lifespan, but also on how these pathways can be exploited for potential therapeutic benefit.

There are primarily two non-conflicting theories often put forward to rationalize the lifespan improvement observed with the inhibition of protein synthesis. The first is that a decrease in protein synthesis allows a greater allocation of metabolic energy towards cellular maintenance and repair. This is broadly in line with the principles of the disposable Soma theory of aging (Jin, 2010), which postulates that there is a fitness cost in the growth and development of an organism through the diversion of resources away from cellular maintenance (Wieser and Krumschnabel, 2001; Anisimova et al., 2018). The second theory is that decreased protein synthesis also broadly reduces the expression of aggregation-prone proteins, thereby partially alleviating the buildup of toxic aggregates and reducing the burden placed on aggregate-clearance pathways (Silva et al., 2011; Kim and Strange, 2013; Solis et al., 2018). From a molecular perspective, there are a wide array of causes behind this decrease in protein synthesis. This includes a broad decrease in transfer RNAs (tRNAs) availability, downregulation and functional impairment of ribosomes, as well as several hormonal and transcriptional changes (Gonskikh and Polacek, 2017).

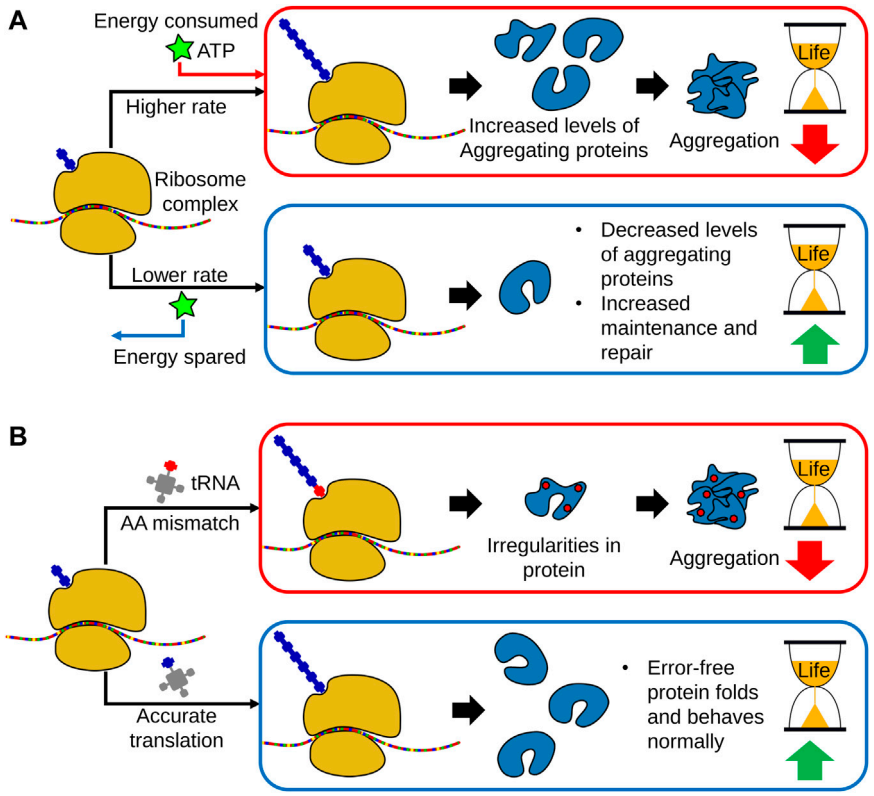

FIGURE 2 | The links between translational regulation and aging. **(A)** With age, global translational rates decrease in a broad range of organisms, indicating a link between loss of protein synthesis capacity and aging. However, translation is a highly energy consuming process and growing evidence indicates that a decline in protein synthesis allows a greater allocation of metabolic energy towards cellular maintenance and repair. Subsequently, a decrease in protein translation promotes proteostasis and leads to lifespan extension. Moreover, lowering protein translation also decreases the production of aggregation prone-proteins. **(B)** In addition to global translational rates, translational fidelity may also decline with age, leading to increased amino acid misincorporation, as well as erroneous start and stop codon readthrough. This results in the production of dysfunctional proteins and peptides, thereby increasing the accumulation of protein aggregates. As such, accurate translation is necessary for organismal homeostasis and long lifespan.

2.1.1 The Mechanistic Target of Rapamycin Pathway

One of the most crucial, and rate limiting, steps of translation is the initiation of translation by the mTOR pathway (Johnson et al., 2013). This pathway, which is itself regulated by the availability of metabolic energy and resources, controls various aspects of growth, development, metabolism and stress responses. As such, it possesses tight control over protein synthesis, controlling the initiation and inhibition of key genes. The two different mTOR complexes, mTORC1 and mTORC2, are the central regulatory components of this pathway, with each containing the mTOR kinase as the core functional unit (Johnson et al., 2013). Our understanding of this pathway and it is constituent components in the context of lifespan and aging, while far from complete, has been greatly bolstered by numerous studies utilizing *C. elegans*. We know that downregulation of the mTOR pathway leads to an extension of organismal lifespan, where, as discussed previously, this negative regulation leads to a reduction in protein translation. This has been demonstrated through RNA interference (RNAi) mediated knockdown of various *C. elegans* analogues of mTOR pathway components, such as the mTOR kinase (*let-363*), or the mTORC1 component raptor (*daf-15*), which was found to yield an increase in lifespan (Vellai et al., 2003; Jia et al., 2004). The pharmacological inhibition of mTOR activity by rapamycin similarly results in an extension of lifespan (Cornu et al., 2013). Caloric restriction also increases lifespan through the downregulation of mTOR activity. Likewise, reduced insulin signaling, achievable through calorie restriction, leads to the activation of glutamine synthase (GS), which then inhibits mTORC1 activity (van der Vos et al., 2012). Moreover, the insulin-regulated transcription factor DAF-16 (a FOXO analogue) negatively regulates transcription of *daf-15* and therefore the mTOR pathway (Jia et al., 2004). Interestingly, mTOR inhibition through caloric restriction also upregulates autophagy, another important component of the proteostasis network (Hansen et al., 2008; Tóth et al., 2008). Collectively, these studies in *C. elegans* demonstrate that the mTOR pathway is broadly essential for longevity regulation through cross-talk with other pathways, and also tightly regulates translation. However, they also highlight important caveats, where although inhibition of the mTORC1 complex can enhance lifespan, it is not without undesirable "side effects". This includes delayed development, metabolic

impairment, and decreased fertility (Schreiber et al., 2010; Zhang et al., 2019). Furthermore, some of these changes in metabolism and development may not be true "side effects", but are rather a direct outcome of lifespan extension arising from the fitness cost in diverting energy and resources away from the growth and development of organism towards maintenance (Blagosklonny, 2013; Maklakov and Chapman, 2019). Notably, recent studies have shown that neuronal mTORC1 activation promotes lifespan extension without delayed development (Zhang et al., 2019; Smith et al., 2021) and another study found that reduced translation in neurons, hypodermis or germline through inhibition of mTOR pathways improved survival rate, suggesting that mTOR inhibition regulates lifespan in a tissue-dependent manner (Howard et al., 2021). These studies in *C. elegans* thus not only advance our understanding of the mTOR pathway in organism development and proteostasis maintenance, but also provide important insights into the utility of the mTOR pathway as a potential therapeutic target in humans for combatting aging.

2.1.2 Translation Factors: Ribosomes, Elongation Factors, and Initiation Factors

Outside of the mTOR pathway, translation rate is determined by the combined interactions between the different components of the translational machinery. As such many studies have focused on individual components to assess their role and importance to translation in the context of aging. RNAi-mediated knockdown of components of either the small or large ribosomal subunits prolongs lifespan in *C. elegans* (Hansen et al., 2007). Moreover, depletion of the translational regulator S6K similarly extends lifespan while also decreasing age-associated protein aggregation (Hansen et al., 2007; Yee et al., 2021). Likewise, inhibition of distinct translation initiation factors also enhances *C. elegans* lifespan by up to 50% (Curran and Ruvkun, 2007). It has been shown that depletion of IFE-2, a worm orthologue of human eIF4E, increases lifespan through decreasing global protein synthesis (Syntichaki et al., 2007a). Interestingly, although IFE-2 availability has been known to decline with age, inhibition of this factor resulted in improvements to the lifespan and stress resistance of *C. elegans* (Hansen et al., 2007; Syntichaki et al., 2007a). Moreover, another study has shown that IFE-2 is highly sequestered in mRNA processing (P) bodies due to age and upon stress, and this sequestration decreases translation in somatic tissues (Rieckher et al., 2018). Depletion of another initiation factor, eIF4G/*ifg-1*, and deletion of two distinct subunits of eIF3 also increases lifespan through decreasing protein synthesis (Pan et al., 2007; Rogers et al., 2011; Cattie et al., 2016). Cumulatively, these studies support the principle that decreasing protein synthesis improves lifespan, furthermore they have identified key translation initiation factors that appear to be primary mediators of organismal longevity.

Indeed, translation initiation factors are central components of survival and stress responses. One such example is the integrated stress response (ISR), an important signaling pathway for the regulation of protein translation that relies on the phosphorylation of the translation initiation factor eIF2 (Derisbourg et al., 2021a). Interestingly, inhibition of the ISR through mutations to the eIF2-activating protein complex eIF2B promotes proteostasis and enhances lifespan (Derisbourg et al., 2021b). Similarly, preventing the phosphorylation of the eIF2α subunit of the eIF2 complex by either mutations or pharmacological inhibition increases lifespan. However, this extension was found to not be due to a decline in overall protein synthesis, but instead arises from variations in the translational efficiency of a subset of mRNAs (Derisbourg et al., 2021b). The downstream effects of the ISR have a crucial role in the proteostasis network for organism survival, and is discussed in-depth further in this review. Nonetheless, these studies indicate that decreasing protein synthesis for a subset of genes, rather than a global decrease to protein synthesis, may be sufficient to achieve an improvement to lifespan. Furthermore, such studies indicate that because these translational elements are involved in different stress responses, they can also influence organismal lifespan by mechanisms besides resource preservation arising from global protein synthesis inhibition.

Collectively, growing evidence supports that decreasing the rate of protein synthesis can have pro-longevity effects, with the majority of proposed mechanisms relying on the reduction of cellular burden, either metabolically or proteopathically. These insights have been made possible through the use of *C. elegans* as a model organism, and although comparatively slower, these key findings are starting to be reproduced in mammalian models (Essers et al., 2016; Thompson et al., 2016; Swovick et al., 2021).

2.2 Translation Fidelity

Although there is ongoing debate as to how significantly translation fidelity changes with age, cumulative evidence firmly indicates that the accurate synthesis of proteins defines organismal lifespan and is essential for organismal health (Tavernarakis and Driscoll, 2002; Anisimova et al., 2018; Ke et al., 2018; Francisco et al., 2020). Excluding genomic causes, these errors primarily arise due to inaccurate start and stop codon recognition by the ribosome, amino acid mismatch during the aminoacylation of tRNAs by aminoacyl-tRNA synthetases, and inaccurate aminoacyl-tRNA selection by the ribosomes. One study showed that the lifespan of *C. elegans* could be improved by increasing translational fidelity, through the use of pharmacological anti-aging treatments such as rapamycin, trametinib and torin 1 (Martinez-Miguel et al., 2021). This study further demonstrated that a fidelity-improving mutation to the ribosomal 40S subunit RPS23 could likewise improve lifespan primarily by decreasing erroneous stop-codon read-through, with this lifespan extension reproducible across multiple species, including *C. elegans*. Crucially, this mutation did not impact the rate of translation, allowing translational accuracy to be studied in a manner isolated from the effects on translation rate (Martinez-Miguel et al., 2021). Another study showed that knocking out *efk-1*, the *C. elegans*, orthologue of the elongation factor eEf2K, decreases translation fidelity and lifespan (Xie et al., 2019). Furthermore, depletion of multiple aminoacyl-tRNA synthetases (ARSs) including leucyl, arginyl, asparaginyl and methionyl ARSs similarly caused a decrease in

lifespan through higher levels of amino acid misincorporation (Xie et al., 2019). It has also become increasingly apparent that errors in protein translation may arise from faulty RNA splicing, where it has been shown that RNA splicing fidelity decreases during aging, and that this decline is ameliorated by caloric restriction (Heintz et al., 2017). In addition, the exposure to reagents such as cadmium lead to disruption in RNA splicing and contribute to aging (Wu et al., 2019).

Other studies in *C. elegans* have found that inhibition of some ARS can instead have pro-longevity effects. In these lines, RNAi-mediated knockdown of the tyrosine ARS *yars-2* is necessary for the longevity phenotype of *daf-2* mutant worms, a genetic model of reduced IIS signaling (Son et al., 2017). Similarly, another study showed that the leucine ARS *lars-1* activates the mTOR pathway, which as discussed previously, negatively impacts lifespan (Nakamura et al., 2016). These effects on lifespan appear to be independent of changes to translational fidelity, and are a relatively recent discovery. As such, much of the regulatory roles played by ARSs and tRNAs remains unclear. Indeed, despite evidence that the misaminoacylation of tRNAs can lead to diseases that involve protein aggregation, misaminoacylation has recently been acknowledged to play an important functional role in various cellular processes and stress responses (Schimmel, 2018). Although studies investigating the role of misaminoacylation in aging and disease with *C. elegans* remains sparse, there are an increasing number of studies using *C. elegans* to study how ARSs and the loss of tRNA into small tRNA-derived fragments contributes to aging and disease, with detailed studies and extensive reviews into this subject available elsewhere (Kato et al., 2011; Shin et al., 2021; Zhou et al., 2021).

From studies in *C. elegans*, there is now clear evidence that translational fidelity is important for the preservation and maintenance of proteome integrity, and by extension both longevity and healthspan. Moreover, these studies identified new sources of translational error, and have begun to clarify previously unknown regulatory roles of the different components of the translational machinery. These new findings may have significant consequences for therapeutic development, and as such, there is ample room for further research.

2.3 Protein Folding

The chaperome network is formed by chaperones and co-chaperones that have an integral role in enabling the assembly of proteins into a functional state (Brehme et al., 2014). As such, the chaperome network stabilizes folding intermediates of newly synthesized or unfolded proteins, and further prevents the denaturation or irreversible aggregation of many proteins (Heintz et al., 2017). Chaperones can be broadly classified as ATP dependent, or ATP independent (Mogk et al., 2018). ATP-dependent chaperones such as HSP70 are responsible for both protecting proteins against aggregation while also using the chemical energy provided by ATP to overcome thermodynamically unfavorable intermediates during folding. ATP-independent chaperones such as small heat shock chaperones, also called holdases, likewise bind to unfolded proteins to prevent their aggregation, but most are not thought to contribute to protein folding (Mogk et al., 2018).

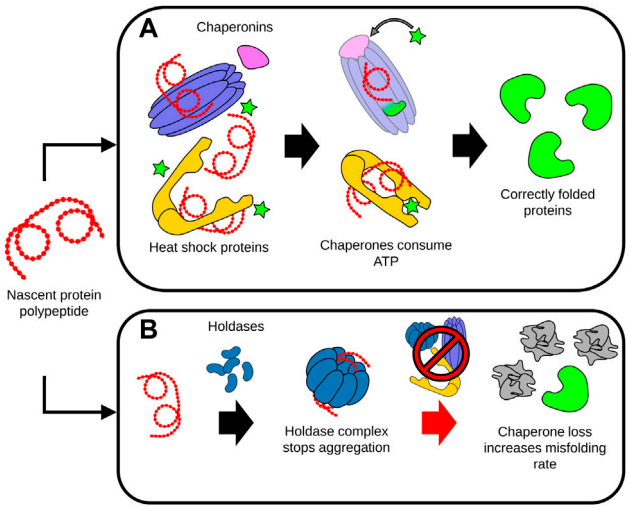

FIGURE 3 | Dysregulation of protein folding during aging. **(A)** Chaperones assist the proper folding, refolding and disaggregation of proteins. ATP-dependent chaperones such as chaperonins and heat shock proteins interact with protein polypeptides to stabilize folding intermediates. The energy provided by ATP enables the conformational support and subsequent release of the folded protein by the chaperone complexes. **(B)** Unfolded proteins or polypeptides can be sequestered by holdases. Holdase proteins assemble into higher-order complexes, capable of isolating and preventing the aggregation of the disordered unfolded proteins and peptides. During aging, deficits in chaperone levels and activity significantly increases the rate of protein misfolding, accelerating the accumulation of damaged, misfolded and aggregated proteins This accumulation of misfolded proteins can in turn overwhelm the capacity of the remaining chaperones to maintain proteostasis, leading to cell malfunction and death.

Due to their essential role, chaperones must be ever-present in cells for proteome maintenance and protein folding. However, with age, misfolded and aggregated proteins accumulate and exceed the stabilizing capacity of available chaperones, diverting many chaperones away from other crucial functions such as regulating the proper folding of nascent proteins (Labbadia and Morimoto, 2015) (**Figure 3**). In addition, there is evidence from studies in *C. elegans* showing that chaperone expression becomes increasingly impaired with age, further compounding the burden of insults to the proteome (Labbadia and Morimoto, 2015).

The overexpression of several chaperones, such as small heat shock proteins and members of Hsp70 family, decreases aggregate formation and extends lifespan in *C. elegans* (Yokoyama et al., 2002; Walker and Lithgow, 2003; Morley and Morimoto, 2004). Accordingly, the disruption of chaperone complexes can be deleterious to lifespan and health. Indeed, the TRiC/CCT complex, a chaperonin that promotes the folding of 10% of the proteome, is essential for regulation of longevity (Noormohammadi et al., 2016). Disruption in the assembly of TRiC/CCT complex causes cellular defects, while increasing TRiC/CCT assembly through the overexpression of the subunit CCT8 leads to an extended lifespan in *C. elegans* and reduces the neurotoxicity of aggregation-prone polyglutamine

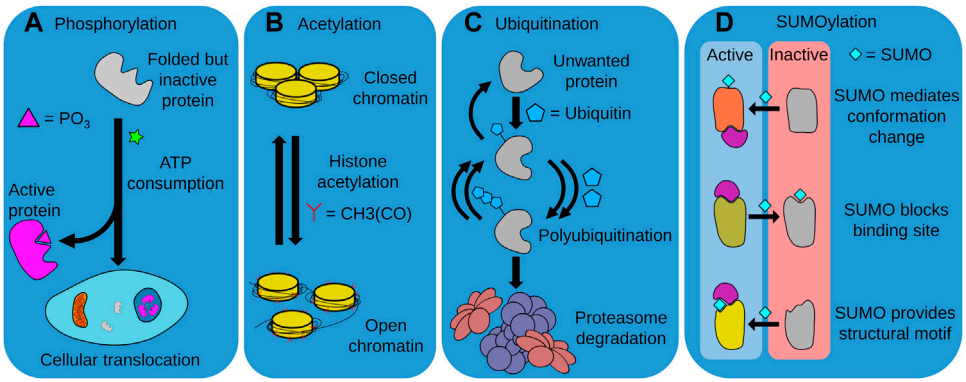

FIGURE 4 | Post-translational modifications (PTMs). Distinct PTMs such as phosphorylation, acetylation, ubiquitination, and SUMOylation modulate the activity, intracellular localization and degradation of numerous proteins, determining cellular function and organismal longevity. **(A)** The ATP-consuming process of phosphorylation is required for the activity of many proteins, either through providing a functional chemical moiety, or by allowing the protein to translocate to the required cellular compartment. **(B)** Acetylation is also required for the function of various proteins, and is particularly important for correct chromatin function. In this instance, histone acetylation is required for chromatin opening and access to DNA by the cell. **(C)** The ubiquitination of unwanted proteins marks them for recognition and degradation by the UPS. This often requires repeated units of ubiquitin to be successively added to a growing polyubiquitin chain. The balance between ubiquitination and deubiquitination can thus control and regulate the composition of the proteome. **(D)** SUMOylation can both activate or deactivate modified proteins. SUMOylation can trigger conformational changes that allow proteins to interact with their biological substrates, block binding sites to prevent substrate interaction, or act as a component of a structural motif to enable recognition of the modified protein.

(polyQ) (Noormohammadi et al., 2016). Accordingly, one paradigm is that protein aggregates are invariably deleterious to organism health and lifespan. However, this has been challenged by the proteomic analysis of long lived *daf-2* mutant worms, which found that long-lived adult *daf-2* worms possess a higher chaperone-associated aggregate load compared to wild-type worms (Walther et al., 2015). These aggregates were comparatively chaperone-rich, and were proposed to be a feature of a "protective aggregation response" that sequestered surplus and dysfunctional proteins in order to alleviate the burden to the proteostasis system. Interestingly, while there were no changes in Hsp70 and Hsp90 expression, small heat shock protein levels increased significantly, meaning this response prioritized sequestration and isolation of unfolded proteins, rather than repair and folding (Walther et al., 2015). Although there are several studies in other organisms that investigate the protective potential of aggregation (Saad et al., 2017), further work is required to fully characterize this potential stress response and how it impacts aging.

It has also become increasingly apparent that individual chaperones possess important regulatory functions outside protein stabilization and folding, and that these unique functions are not compensated for by other types of chaperones. For instance, RNAi-mediated knockdown of *daf-21*/Hsp90 chaperone in non-neuronal tissues decreases lifespan in both wild-type and long-lived *daf-2* worms. Notably, this lifespan attenuation revealed that Hsp90 ensures DAF-16 isoform A nuclear translocation and function, but this process does not rely on any of the protein stabilizing properties of Hsp90 (Somogyvári et al., 2018). Although there is much more to uncover in regards to other functions of individual chaperones, it is clear that chaperones are primary mediators of proteostasis and therefore lifespan. Studies in *C. elegans*, have begun to uncover, but also explore their role as the primary effector of numerous stress responses.

3 POST-TRANSLATIONAL MODIFICATIONS

The regulation of protein fate through post-translational modifications (PTMs) is a crucial mechanism by which proteostasis is maintained. PTMs achieve this through several mechanisms, including modulation of protein stability, activity, and degradation (Ito et al., 2021). Although there are numerous PTMs, in this review, we focus on the four most common PTMs thought to be involved with aging and longevity, i.e. phosphorylation, SUMOylation, acetylation, and ubiquitination (Walsh et al., 2005) (**Figure 4**).

3.1 Phosphorylation

The addition of a phosphoryl group from adenosine triphosphate (ATP) to serine, threonine, or tyrosine residues serves as the most common PTM for proteins (Santos and Lindner, 2017). Phosphorylation is often necessary to facilitate functional conformational changes or provide a chemical moiety required for catalytic activity and protein-protein interactions (Yaffe, 2002). Numerous studies have demonstrated that protein phosphorylation regulates longevity in *C. elegans* (Derisbourg et al., 2021b; Huang et al., 2018; Lin et al., 2001; Li et al., 2021). One well studied mechanism of longevity regulation is in how phosphorylation controls various aspects of the IIS pathway (Kenyon et al., 1993; Lin et al., 2001). The foremost example arises when insulin-like ligands bind to the insulin/IGF-1 receptor DAF-2, which initiates a phosphorylation cascade culminating in the phosphorylation of the transcription factor DAF-16. Like its analogue FOXO in humans, the

phosphorylation of DAF-16 results in its inactivation and retention in the cytoplasm, negatively regulating longevity by preventing the transcription of lifespan-extending effectors (Tatar et al., 2003). Targeted RNAi screens for different serine/threonine protein phosphatases to further study the role of the kinases in the IIS pathway have subsequently identified other novel longevity regulators, such as *pptr-1* (Padmanabhan et al., 2009). PPTR-1 negatively regulates the phosphorylation of AKT-1, preventing AKT-1 from inhibiting DAF-16. The modulation of *pptr-1* regulates a wide range of reduced IIS-related phenotypes including longevity, the activation of stress responses, and entry into the dauer state (Padmanabhan et al., 2009). A more recent study, similarly focused on IIS-dependent phosphorylation, demonstrated that there are 476 differentially regulated phosphosites in *daf-2* mutant worms (W.-J. Li et al., 2021). Their analysis also indicated that casein kinase 2 (CK2) negatively modulates longevity (Li et al., 2021).

These numerous studies thus cement phosphorylation as a fundamental component of the IIS pathway, however phosphorylation has also been found to play a crucial role beyond IIS. Phosphoproteomics analysis of *C. elegans* at two different temperatures (20 and 25°C) has revealed that phosphoprotein GTBP-1 modulates longevity at both temperatures, and promotes resistance to heat and oxidative stresses (Huang et al., 2018). This study also indicated that the kinases CK2, MAPK, and CAMK2 may similarly modulate aging through their kinase activity (Huang et al., 2018). Other studies have found that the phosphorylation of actin binding protein drebrin through the kinase ataxia-telangiectasia mutated (ATM) regulates lifespan and stress tolerance by improving the stability of drebrin and dynamics of actin remodeling (Kreis et al., 2019). Furthermore, phosphorylation of AMP-activated protein kinases has been found to be crucial for the regulation of cellular energy metabolism and cellular homeostasis, and by extension lifespan, across numerous studies (Hwang et al., 2014; Chang et al., 2017; Park et al., 2020). For example, one recent study demonstrates that the nuclear protein kinase vaccinia-related kinase (VRK-1) promotes lifespan extension through phosphorylation and activation of AMP-kinases (Park et al., 2020).

Thus, both preventing phosphorylation, as with DAF-16, or promoting phosphorylation, through phosphoproteins such as GTBP-1 or the phosphorylation of various AMP-kinases, can drastically increase lifespan, and firmly demonstrates that PTMs are key determinants of longevity.

3.2 Acetylation

Acetylation is the addition of an acetyl group to a nitrogen molecule of a target protein (Santos and Lindner, 2017). Numerous studies into the impact of protein acetylation on aging focus on age-associated changes in histone acetylation. During aging, histone acetylation is modulated, and is coupled to changes in metabolic activity and gene expression (Peleg et al., 2016). Sirtuin proteins are histone deacetylases, and act as important regulators of histone acetylation (Grabowska et al., 2017). Increased histone deacetylase activity of silent information regulator 2 (*Sir2*) extends lifespan (Tissenbaum and Guarente, 2001). Moreover, DAF-16 nuclear localization, which is essential for longevity and stress signal regulation, can be modulated by acetylation through the sirtuin SIR 2.4 protein (Chiang et al., 2012). Sirtuin activators such as oligonol also prolong lifespan in *C. elegans* infected with lethal *Vibrio* cholera (Park et al., 2016). Another study has indicated that early stage exposure to heat stress results in increased histone acetylation and helps the establishment of epigenetic "memory", leading to an extended stress response and longevity in *C. elegans* (Zhou et al., 2019). Crucially, proteomics analysis comparing young worms to aged worms revealed the accumulation of acylated proteins, particularly in mitochondria (Hong et al., 2016). The accumulation of acylated proteins in mitochondria has been further shown to cause mitochondrial dysfunction and contribute to aging (Hong et al., 2016).

Although the detrimental effects of acetylated protein accumulation remains poorly understood, these studies nonetheless show that epigenetic and metabolic regulation by acetylation modifications have an important role in longevity.

3.3 SUMOylation

Protein SUMOylation is the covalent attachment of a small ubiquitin like modifier (SUMO) to lysine residues of target proteins (K. A. Wilkinson and Henley, 2010). This SUMOylation then facilitates, or prevents, the interaction of the modified protein with its interaction partner. SUMOylation is involved in several biological processes including development, DNA damage stress-responses, and mitochondrial dynamics (Flotho and Melchior, 2013). These diverse roles of SUMOylation are well studied, in particular, its role in development (Broday et al., 2004; Zhang et al., 2004; Kaminsky et al., 2009; Pelisch et al., 2017). However, many recent studies also highlight the role of SUMOylation in the regulation of longevity (Moll et al., 2018; Princz et al., 2020). Of note is how reduced IIS can modulate protein SUMOylation, where IIS driven SUMOylation of the germline RNA binding protein CAR-1 was found to shorten lifespan in *C. elegans*. Conversely, the expression of a mutant CAR-1, one which cannot be SUMOylated, promotes enhanced proteostasis and lifespan extension (Moll et al., 2018). The role of SUMOylation in IIS was further expanded in a recent study showing that SUMOylation of DAF-16 regulates mitophagy and mitochondrial dynamics, affecting lifespan (Princz et al., 2020). Furthermore, the same study showed a tissue-specific dependency on SUMOylation, where the RNAi-mediated knockdown of the small ubiquitin-like modifier gene, *smo-1*, shortened lifespan, while the overexpression of *smo-1*, specifically in intestine tissue, was sufficient to extend lifespan (Princz et al., 2020). Moreover, SUMOylation also regulates essential biological processes for healthspan and lifespan (Lim et al., 2014; Baytek et al., 2021). SUMOylation modulates the UPRER in *C. elegans* by regulating calreticulin gene expression in an XBP-1-dependent manner (Lim et al., 2014). SUMOylation also regulates chromatin dynamics by regulating protein activity of the chromodomain factor MRG-1 (Baytek et al., 2021). SUMOylation targets have previously been identified through gene ontology analysis, with most of these SUMOlyated proteins playing a role in metabolism (Drabikowski et al., 2018), and

indeed evidence has been found that SUMOylation plays a role under calorie restricted conditions, by modulating the NHR-49 transcription factor (Drabikowski, 2020). These studies demonstrate that SUMOylation plays a decisive role in the proteostasis network for determining aging and longevity in *C. elegans*, in particular through modulation nodes of the IIS. However, as the majority of SUMOylation targets remain poorly characterized or understood, further research is necessary before the true impact of SUMOylation can be appreciated.

3.4 Ubiquitination

Ubiquitination is the covalent conjugation of ubiquitin, a small highly-conserved protein, to a lysine residue or N-terminal methionine of a target protein (Yau and Rape, 2016). Ubiquitination is a multistep reaction reliant on the coordination of E1, E2, and E3 enzymes (Hochstrasser, 2006). The process begins with ubiquitin-activating enzyme (E1), which activates ubiquitin through an ATP-dependent mechanism. The activated ubiquitin then transfers to the ubiquitin-conjugating enzyme (E2). Finally, a specific ubiquitin-protein ligase (E3) mediates the attachment of ubiquitin from the E2 enzyme to the target protein (Hochstrasser, 1996). Conversely, deubiquitinating enzymes (DUBs) can remove ubiquitin molecules and thereby unmark proteins (K. D. Wilkinson, 2000).

Ubiquitination can determine the fate of a given protein in several ways; it can mark it for degradation by the proteasome or autophagy pathways, regulate their activity, modulate protein-protein interactions and intracellular localization (Hershko and Ciechanover, 1998). Ubiquitination thus has a central regulatory role across a wide range of biological process, including signal transduction, transcriptional regulation, the DNA damage response, and the immune response (Hershko and Ciechanover, 1998). Furthermore, ubiquitination also regulates various stress responses and can also influence protein aggregation (Brehme et al., 2014) (Vilchez et al., 2014; Koyuncu et al., 2018). There is ample evidence that ubiquitination plays a crucial role in aging and longevity (Li et al., 2007; Powers et al., 2009; Kevei and Hoppe, 2014; Tawo et al., 2017; Koyuncu et al., 2021), however the underlying mechanisms are only now becoming clearer. The ubiquitination of a target protein is primarily achieved by the activity of E3 ubiquitin ligases, and can be reversed with deubiquitinase enzymes (Hochstrasser, 2006). We now understand that several of these E3 ligases and deubiquitinases modulate longevity (W. Li et al., 2007; Mehta et al., 2009). Several E3 ligases have been reported as regulators of the IIS pathway. For instance, the E3 ubiquitin ligase CHIP regulates the levels of DAF-2 through its ubiquitination and degradation, which modulates longevity (Tawo et al., 2017). Downregulation of RLE-1 E3 ligases results in less polyubiquitination of DAF-16, and increased DAF-16 transcriptional activation which results in extended lifespan (W. Li et al., 2007). The WWP-1 E3 ligases are required for the regulation of lifespan under caloric restricted conditions (Carrano et al., 2009). The lifespan regulation by WWP-1 depends on E3 Ubiquitin ligase activity and also interactions with the E2 ubiquitin conjugating enzyme UBC-18 (Carrano et al., 2009). Moreover, it has been shown the interactions of ubiquitin-selective chaperone CDC-48 and ATX-3 deubiquitinase modulate longevity through IIS (Kuhlbrodt et al., 2011). A decrease in both *cdc-48.1* and *atx-3* enhances their substrate stability and longevity by up to 50% (Kuhlbrodt et al., 2011). Recently, we have explored the role of ubiquitination in maintaining proteostasis and regulating longevity by analyzing system-wide ubiquitination changes that occur during aging (Koyuncu et al., 2021). Our findings revealed a global loss of ubiquitination during aging, which is ameliorated by longevity pathways, such as caloric restriction and reduced IIS (Koyuncu et al., 2021). This remodeling of ubiquitination patterns throughout the proteome is a result of elevated deubiquitinase activity. Remarkably, dysregulation of ubiquitination leads to the selective accumulation of various proteasome targets such as the intermediate filament, IFB-2 and EPS-8, a modulator of RAC signaling (Di Fiore and Scita, 2002; Geisler et al., 2019; Koyuncu et al., 2021). Accumulation of IFB-2 leads to loss of intestinal integrity while increased EPS-8 hyperactivates RAC signaling in muscle and neurons causing changes in actin cytoskeleton and hyperactivation of protein kinase JNK. Therefore, the dysregulation in the ubiquitination of structural and regulatory proteins across tissues contributes to aging features and regulate longevity (Koyuncu et al., 2021).

Ubiquitination thus has a much more significant role in aging than previously thought, and further research may allow us to therapeutically exploit parts of the ubiquitination network for anti-aging purposes.

5 PROTEIN DEGRADATION SYSTEMS

5.1 The Ubiquitin-Proteasome System

The UPS is the main system for selective degradation of proteins, determining the half-life of multiple regulatory proteins and controlling the clearance of damaged and unnecessary proteins (Pickart, 2001). Repeated addition of ubiquitin creates a polyubiquitin chain, marking the target protein for recognition and processing by the proteolytic machinery of the UPS, the 26S proteasome (Glickman and Ciechanover, 2002). A Lys48-linked polyubiquitin chain is the primary signal for recognition and degradation by the 26S proteasome. The 26S proteasome itself is composed of a 20S core catalytic particle and 19S regulatory particles (Saez and Vilchez, 2014).

The UPS declines after development in *C. elegans*, as observed by *in vivo* imaging strategy following the levels of chimeric green fluorescent protein fused to a non-cleavable ubiquitin moiety (Segref et al., 2011). Studies into the relationship between proteasome activity and longevity have shown that elevated UPS activity, mediated by elevated assembly or activity through induction of proteasome subunits, leads to an extension in lifespan (Vilchez et al., 2012; Chondrogianni et al., 2015). Reverting this age-related decrease in proteasome activity through the overexpression of 19S proteasome subunit *rpn-6.1* increased survival rate and heat stress resistance (Vilchez et al., 2012). Moreover, long-lived *glp-1* mutants, which lack a germline, have enhanced proteasome activity upon DAF-16

activation (Vilchez et al., 2012). Another study has shown that overexpression of the *psb-5* catalytic subunit of the 20S proteasome results in an extension of lifespan and resistance to oxidative stress in a DAF-16 dependent manner (Chondrogianni et al., 2015).

Similarly, epidermal growth factor (EGF) signaling has been reported to positively modulate proteasome activity by upregulating the expression of genes involved in the UPS such as Skp1-like protein SKR-5 (Liu et al., 2011). EGF signaling also regulates longevity by upregulating UPS activity, where animals lacking SKR-5 also have shorter lifespans (Liu et al., 2011). Other recent studies also support the role of proteasome activity on the regulation of lifespan, where defects in the import of mitochondrial proteins results in proteasome activation and lifespan extension (Sladowska et al., 2021).

These studies into the impact of proteasomal regulation on longevity highlights the importance of the UPS on aging and longevity. Thus, the proteasome can be an important target to find novel interventions to promote healthy aging.

5.2 Autophagy-Lysosome Pathway

Autophagy is an evolutionary conserved degradation pathway, in which cellular components including defective organelles and protein aggregates are sequestered in double-membrane vesicles and delivered to the lysosome for degradation (He and Klionsky, 2009). More than 30 proteins (encoded by *ATG* genes) are recruited at different steps of the autophagy process (Klionsky et al., 2016). There are three well-characterized types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). In macroautophagy, cytoplasmic components are first encapsulated into a double membrane-bound vesicle, called an autophagosome. This autophagosome then fuses with the lysosome to form an autolysosome, in which the sequestered cytoplasmic cargo is degraded with hydrolases, glycosidases, nucleotidases, lipases and proteases (Klionsky et al., 2016). In microautophagy, cytosolic cargo is delivered directly to the lysosome for degradation. In CMA, chaperones such as HSP70 promote the lysosomal degradation of targeted proteins (Glick et al., 2010).

Like the UPS, there is now substantial evidence that the autophagy-lysosome pathway is linked to the regulation of aging and age-related diseases. Dysfunctional autophagy during aging has been observed across a diverse range of species, including *C. elegans* (Aman et al., 2021). Genetic manipulation experiments of selective and non-selective autophagy pathway components have demonstrated an important role of autophagy in lifespan and healthspan (Tóth et al., 2008; Hansen et al., 2018; Kumsta et al., 2019). Either the knockdown or inactivating mutations of several autophagy components such as *bec-1* (orthologue of the mammalian APG6/VPS30/beclin1), *lgg-1, Igg-3, unc-51,* or *atg-7* leads to an accelerated aging phenotype and shortened lifespan (Tóth et al., 2008; Hansen et al., 2018). Moreover, several studies indicate that the upregulation of autophagy is mechanistically crucial for the extension of lifespan by different pro-longevity pathways (Meléndez et al., 2003; Hansen et al., 2008; Alberti et al., 2010; Gelino and Hansen, 2012). For instance, the RNAi-mediated downregulation of bec-1 suppresses the extension of lifespan in *daf-2* mutants (Meléndez et al., 2003). In addition, the knockdown of autophagy-regulating transcriptional factors such as *hlh-30* and *daf-16* shortens the lifespan of both wild type and *daf-2* mutant worms (Lin et al., 2018). It has been shown that worms exposed to dietary restriction have increased levels of the autophagy marker LGG-1 (the orthologue of ATG8) in their hypodermis, and require functional autophagy promoting genes for longevity (Mörck and Pilon, 2006; Hansen et al., 2008; Tóth et al., 2008).

Overexpression of the key regulator of autophagy *sqst-1/p62* induces autophagy in distinct tissues of *C. elegans*, leading to an extension of lifespan, and an overall improvement in organismal fitness (Kumsta et al., 2019). Overexpression of autophagy regulators such as AMPK enhances autophagy activity and extends lifespan (Hansen et al., 2018). Additionally, the induction of autophagy with different pharmacological agents such as spermidine, resveratrol, and metformin prolongs lifespan in *C. elegans* (Mariño et al., 2011; Gillespie et al., 2019). Pharmacological inhibition of XPO-1 similarly leads to increased autophagy and lifespan by nuclear enrichment of *HLH-30* in *C. elegans* (Silvestrini et al., 2018).

These studies consistently demonstrate that maintaining and upregulating autophagy can be beneficial for lifespan. Mechanistically, this longevity effects may arise from the persistent clearance of misfolded and aggregated proteins.

6 ADAPTIVE STRESS RESPONSE MECHANISMS

Alterations to the activity of various organelles and biological pathways in response to stress has been extensively studied (Schulz et al., 2007; Dilberger et al., 2019; Taylor and Hetz, 2020; Derisbourg et al., 2021b). However, these stress responses are now known to change with age, and also contribute to organismal longevity. Given the stabilizing effects of chaperones, they are a key component of many adaptive responses to environmental stressors that challenge cellular integrity (Hipp et al., 2019). However, these stress responses also include wide regulatory changes, including variations in gene expression, activation of proteolytic pathways, and more. The most common stress responses in the cell include the heat shock response (HSR), the ISR, the unfolded protein response of the endoplasmic reticulum (UPR^{ER}) and the UPR of the mitochondria (UPR^{mt}). Here we focus on recent studies examining the link between the different stress responses and longevity in *C. elegans*.

6.1 Heat Shock Response

Proteotoxic stress, such as heat stress, can upregulate the expression of many specialist chaperones as a part of the HSR. Moreover, binding of damaged and misfolded proteins to chaperones leads to the liberation of heat shock transcriptional factors (HSFs) from chaperone complexes, which further upregulates the transcription of additional chaperones. The chaperones upregulated by the HSR principally act to stabilize

and refold thermally denatured proteins, however the HSR also regulates a broad range of genes involved in normal aging, including the small heat shock protein *sip-1*, and *cyp-35B1*, a member of cytochrome P450 family (Hsu et al., 2003).

The HSR is mainly regulated by HSFs, specifically HSF-1 in *C. elegans* (Akerfelt et al., 2010). In worms, the activation of HSR by HSF-1 is also controlled by thermosensory neurons that sense temperature changes, in addition to the basal levels of HSF-1 present in chaperone complexes (Prahlad et al., 2008). Activation of HSF-1 induces the transcription of several chaperones, including HSP70, HSP90 family members, and small heat shock proteins (Hsu et al., 2003). There is substantial evidence that HSF-1 is also a regulator of aging, where HSR activation enhances longevity and stress tolerance by utilizing aspects of longevity-enhancing mechanisms similar to reduced IIS, caloric restriction and suppression of mTOR activity (Morley and Morimoto, 2004; Steinkraus et al., 2008; Seo et al., 2013; Kovács et al., 2019). Overexpression of *hsf-1* prolongs lifespan and decreases age-associated protein aggregation of disease-related proteins. Moreover, upregulation of HSF-1-target genes under unstressed conditions similarly extends longevity (Walker and Lithgow, 2003). Accordingly, RNAi-mediated knockdown of *hsf-1* shortens lifespan (Hsu et al., 2003; Morley and Morimoto, 2004). Interestingly, overexpression of a modified version of HSF-1, incapable of inducing the expression of HSPs, was found to also prolong lifespan (Baird et al., 2014). Thus HSF-1 can likely upregulate lifespan-extending genes outside what is considered the subset of HSR target genes. This is supported by other studies, where transcriptomics analysis revealed that several distinct longevity associated genes including *pha-4*, *lys-7* and *dod-3* are upregulated in HSF-1-dependent long-lived strains (Sural et al., 2019). Likewise, a recent study has also shown that mitochondrial stress can result in HSF-1 dephosphorylation, which induces the upregulation of lifespan extending holdases (Williams et al., 2020), further exemplifying the role of HSF-1 in mediating lifespan extending pathways. In addition, the upregulation of HSF-1 in neurons leads to the activation of DAF-16 in other tissues, making neuronal HSF-1 essential for longevity in a cell non-autonomous manner (Douglas et al., 2015).

Overall, studies in *C. elegans* have not only shown that the HSR is necessary for thermal protection of the proteome, but also for normal function under unstressed conditions. Moreover, HSF-1 is an essential modulator of aging and longevity through the activation of the HSR and other pathways.

6.2 Integrated Stress Response

The ISR is an important central stress response in eukaryotic cells, which is induced by a broad range of physiological and environmental changes (Pakos-Zebrucka et al., 2016). The ISR is primarily activated by the phosphorylation of a serine of eIF2*α*. This reaction is catalyzed by eIF2*α* kinases after stress stimuli such as viral infection, hypoxia and amino acid deprivation (Harding et al., 2003; Wek et al., 2006; García et al., 2007). The phosphorylation of eIF2*α* results in a broad decrease in protein translation, while increasing the translation of selected survival genes, such as activating transcription factor 4 (ATF4). If this adaptive response proves insufficient to counteract the stress, additional components of the ISR are activated to induce cell death, preventing potential cellular dysfunctions from impacting organismal health. After the stress stimulus disappears, or is mitigated, eIF2α is dephosphorylated, stopping the ISR and thereby allowing translation and other cellular process to return to normal levels (Novoa et al., 2003; Donnelly et al., 2013). In worms, there are two main eIF2α kinases; the general control nonderepressible 2 (GCN2) kinase, and the PKR-like endoplasmic reticulum kinase (PERK) (Derisbourg et al., 2021b). Previous studies have suggested that ISR is induced with age in different organisms (Derisbourg et al., 2021a), whereas enhanced ISR activation is already observed from early adulthood in *C. elegans* (Derisbourg et al., 2021b).

As discussed previously (**section 2.1.2**), although the decrease in protein synthesis induced by the ISR may be expected to be lifespan extending, the ISR is detrimental to lifespan in *C. elegans*. The cause behind this impairment to lifespan has only recently become to be understood. Through a large-scale mutagenesis screen, it was found that lifespan extending mutations to eIF2 inhibited the ISR, and these mutations relied on the putative kinase *kin-35*. Crucially, the lifespan extension mediated by kin-35 was found to be independent of any changes to protein synthesis (Derisbourg et al., 2021b). This finding indicates that the ISR may decrease lifespan due to the selective translation of key detrimental genes. Furthermore, contrary to previous studies that observed that knockouts of ISR kinases *gcn-2* and *pek-1* do not have impact on longevity, this study also showed that single inhibitory amino acid substitutions to GCN-2 and PEK-1 lead to lifespan extensions (Henis-Korenblit et al., 2010; Baker et al., 2012). There is still further investigation needed to fully understand the transcriptional changes caused by the ISR, and why this decreases lifespan in *C. elegans*.

The unfolded protein response of the endoplasmic reticulum (UPR^{ER}).

The endoplasmic reticulum (ER) houses and regulates many of the chaperones that aid protein folding, as well as many enzymes that are responsible for the maintenance of proteostasis (Schönthal, 2012). The protein folding capacity of the ER is monitored by the unfolded protein response (UPR^{ER}) signaling pathway. This pathway is conserved from yeasts to mammals, and is activated by the accumulation of unfolded and misfolded proteins in the ER lumen (Hipp et al., 2019). To maintain protein folding fidelity, the UPR^{ER} regulates mRNA translation to decrease the further accumulation of misfolded proteins, while also upregulating folding chaperones in the ER (Taylor and Hetz, 2020). In the metazoan ER, there are three identified activators for the different signaling pathway sub-branches of the UPR^{ER}, i.e. IRE1 (inositol-requiring enzyme), PERK (protein kinase RNA-like endoplasmic reticulum kinase), and ATF6 (activating transcription factor 6).

Activation of the UPR^{ER} by external stress declines with age, and this decline is associated with several age-related diseases (Taylor and Dillin, 2011). By studying the metazoan UPR^{ER}-component analogues in *C. elegans*, we have begun to understand what contributes to this decline in UPR^{ER} responsiveness, and how this impacts organismal aging. Genetic manipulation of the ER stress response has shown that the UPR^{ER} has an important

role in the modulation of lifespan and healthspan (Klionsky et al., 2016). Further studies in *C. elegans*, demonstrated that overexpression of the stress-activated transcriptional factor XBP-1 in neurons prolongs lifespan in a cell non-autonomous manner (Taylor and Dillin, 2013). In addition to neuronal UPR^{ER} activation, intestinal UPR^{ER} activation enhanced longevity through an increase in lipophagy (Imanikia et al., 2019; Daniele et al., 2020). A recent study further demonstrated that constitutive activation of the UPR^{ER} with XBP-1 in astrocyte-like glia prolongs lifespan and stress resistance (Frakes et al., 2020). Moreover, a wide range of longevity paradigms such as reduced insulin signaling and caloric restriction require the UPR^{ER} (Chen et al., 2009; Henis-Korenblit et al., 2010; Matai et al., 2019). Thus, the consensus between these studies is that activation of the UPR improves lifespan, and is most likely mediated through the increased presence of chaperones preventing aggregate formation, as well as some contribution from upregulation of various metabolic processes such as lipophagy.

Interestingly, although the UPR^{ER} is not activated in the long-lived *daf-2* mutant worms, an enhancement to lifespan through reduced insulin signaling requires the presence of IRE1α/XBP1 (Henis-Korenblit et al., 2010). Conversely, caloric restriction of worms induces a higher basal level of UPR^{ER} activity, and the increased lifespan phenotype requires the ER stress branch IRE-1 (Matai et al., 2019). This provides clear evidence that the IIS and UPR^{ER} are in some way linked by a common regulatory element, and that the UPR^{ER} contributes to the lifespan enhancement observed under calorie restricted conditions. However, the precise mechanism behind how caloric restriction, or the IIS pathway, influence or utilize the UPR^{ER} remains unclear.

It has been also shown that the activation of the hexosamine pathway, which leads to enhanced UPR^{ER} activity, increases lifespan through improvement of ER-associated protein degradation (Denzel et al., 2014). Another study has also reported that enhancing lifespan with vitamin D treatments requires IRE1α and XBP1 (Mark et al., 2016). Additionally, treatment with activators of the UPR^{ER}, such as tunicamycin, prolongs lifespan based on IRE1α branch of UPR^{ER} (Matai et al., 2019). Accordingly, mutations in IRE1α or XBP1 has been found to shorten lifespan (Taylor and Hetz, 2020). Collectively, these studies show that UPR^{ER} activation is necessary for both normal and enhanced organismal lifespan, that multiple regulatory and nutrient sensing pathways converge on the UPR^{ER}, and also that various components of the UPR show promise as therapeutic targets for anti-aging outcomes.

The unfolded protein response of the mitochondria (UPR^{mt}).

The mitochondria provides cellular energy and regulates a broad range of metabolic events (Dilberger et al., 2019). As a consequence of the process of oxidative phosphorylation (OXPHOS), mitochondria produce reactive oxygen species (ROS) (Sies and Cadenas, 1985). Elevated levels of ROS can lead to cellular damage, with such damage also called oxidative stress (Sies and Cadenas, 1985). Impairment of the mitochondria is associated with cellular dysfunction and is considered a hallmark of aging. Damage to mitochondrial integrity induces transcriptional responses, including the mitochondrial unfolded protein response (UPR^{mt}) which is regulated by mitochondrial-to-nuclear communication. The UPR^{mt} induces the recovery of mitochondrial networks through mitochondrial biogenesis and metabolic adaptations, promoting cell survival under stress conditions (Münch and Harper, 2016; Shpilka and Haynes, 2018). Whereas an acute stress in the mitochondria can lead to cell dysfunction and death, a reduced amount of mitochondrial stress can be beneficial for organismal longevity in a process known as mitohormesis. Mitohormesis also encompasses the activation of the UPR^{mt}.

In *C. elegans*, the UPR^{mt} is regulated by the transcription factor ATFS-1 and the co-factors DVE-1 and UBL-5, where ATFS-1 is activated by disruptions to mitochondrial proteostasis as well as ROS produced by OXPHOS. This activation leads to transcriptional regulation to regulate survival and mitochondrial stress through ATFS-1 (Shpilka and Haynes, 2018). It is important to note that although ATSF-1-regulated genes are upregulated in long-lived worms, chronic expression of ATFS-1 itself is not sufficient to extend lifespan (Soo et al., 2021). However, activation of the UPR^{mt} is known to positively regulate longevity in *C. elegans* (Dillin et al., 2002; Durieux et al., 2011; Ito et al., 2021). In these lines, RNAi-mediated knockdown of mitochondrial OXPHOS components such as complexes I, III and IV promotes longevity through activation of the UPR^{mt} (Feng et al., 2001; Dillin et al., 2002). Decreasing mitochondrial protein translation by knockdown of mitochondrial ribosomal protein S5 (*mrps-5*) likewise extends lifespan through enhanced UPR^{mt} (Houtkooper et al., 2013). Furthermore, changes in mitochondrial dynamics due to impaired fission or fusion has been shown to decrease mitochondrial translation, upregulating the UPR^{mt} and extending lifespan (Y. J. Liu et al., 2020).

Pharmacological agents can similarly extend lifespan through targeting activators and inhibitors of the UPR^{mt}. Antimycin, an inhibitor of mitochondrial ETC complex III, extends lifespan (Dillin et al., 2002). Likewise, Metolazone, a blocker of Na + -Cl– cotransporters, including NKCC-1, also prolongs lifespan by activating the UPR^{mt}. However how NKCC-1 activates the UPR^{mt} in *C. elegans* is still unclear (Ito et al., 2021). Another study has shown that NAD^+ level decreases with age in *C. elegans*, and restoration of NAD^+ levels with NAD + boosters increases *sir-2.1* (sirtuin homolog) activity, which in turn improves lifespan through activation of the UPR^{mt} (Mouchiroud et al., 2013). In addition, the mitochondrial chaperone prohibitin is an important part of the UPR^{mt} in longevity regulation (Gatsi et al., 2014). Interestingly, depletion of prohibitin, which induces UPR^{mt}, shortens lifespan in wild type worms whereas this depletion extends lifespan in metabolically compromised worms (Artal-Sanz and Tavernarakis, 2009; Gatsi et al., 2014).

The lifespan extension afforded by the UPR^{mt} relies, at least in part, on cell non-autonomous interactions between different tissues. It has been shown that neuronal induction of UPR^{mt} by the accumulation of expanded-polyQ aggregates leads to UPR^{mt} induction in the intestine, and depends on the neuronal release of serotonin and long-range Wnt signaling pathway (Berendzen et al., 2016; Q.; Zhang et al., 2018). In addition to the nervous system, other tissues can also communicate their proteostasis status and induce the UPR^{mt}

in distal tissues (Calculli et al., 2021). For instance, aggregation of the germ granule component PGL-1 triggers intracellular changes in the mitochondrial network of *C. elegans* germline cells. In turn, the germline releases long-range WNT ligands that induce an overactivation of the UPR^{mt} in somatic tissues, promoting somatic mitochondrial fragmentation and aggregation of proteins linked with age-related neurodegenerative diseases such amyotrophic lateral sclerosis and Huntington's (Calculli et al., 2021).

Beyond the UPR^{mt}, other factors could be involved in mitohormesis. The most prominent example is the contribution of ROS towards aging in respect to oxidative damage and regulatory roles. The Harman Free Radical Theory of Aging postulates that cellular aging is driven by the formation of mitochondrial ROS. These ROS induce a damage to distinct components of the cell, including DNA, the proteome, and the mitochondria itself. This has been challenged by studies in *C. elegans*, which have found that lifespan can be decoupled from oxidative damage or oxidant sensitivity. In fact, it has been proposed that low levels of ROS may be beneficial for longevity, where ROS could potentially act as signaling molecules that promote mitohormesis (Ristow and Schmeisser, 2014). For example, it has been reported that deletion of the mitochondrial superoxide dismutase *sod-2* increases ROS production, yet prolongs lifespan despite the increased levels of oxidative damaged proteins (Van Raamsdonk and Hekimi, 2009). Moreover, worms treated with low amounts of the oxidant reagent paraquat also live longer (Yang and Hekimi, 2010). One study showed that a mild reduction of respiration extends longevity through ROS mediated activation of the hypoxia-inducible factor HIF-1 (Lee et al., 2010). Glucose restriction also leads to the formation of ROS due to enhanced mitochondrial respiration, prolonging lifespan in *C. elegans* (Schulz et al., 2007). The knockdown of the hydroxylase *clk-1* was also found to enhance longevity despite elevated levels of ROS production (Lakowski and Hekimi, 1996; Lee et al., 2010) Together, these studies indicate that the role of ROS in mitohormesis and aging is less clear as previously thought, and further demonstrate possible roles of ROS as both signal molecules and cellular stress factors. Further studies are required to fully assess the contribution of ROS and oxidative damage towards organism health and longevity. Nevertheless, studies in *C. elegans* support that the UPR^{mt} can be a powerful promoter of longevity, and a promising therapeutic target for pharmacological intervention.

7 CONCLUSION

Ensuring proteome integrity requires tight regulation and crosstalk of distinct components of the proteostasis network from translation to degradation. However, with age, the burden of misfolded proteins exceeds the capacity of cells to maintain proper proteome integrity, leading to disruptions in cellular function. Cumulative evidence using *C. elegans* as a model organism has highlighted the important role of the proteostasis network in longevity regulation as well as the onset of age-related disease regulation. These studies in *C. elegans* have also provided invaluable information about the regulation of distinct proteostasis nodes at the organismal level, including their regulation by cell non-autonomous mechanisms that can be crucial to find novel therapeutic targets to delay age-related diseases in humans.

AUTHOR CONTRIBUTIONS

DV, WZ, and SK designed the review. WZ and SK wrote the initial draft. DV edited the manuscript.

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REFERENCES

Åkerfelt, M., Morimoto, R. I., and Sistonen, L. (2010). Heat Shock Factors: Integrators of Cell Stress, Development and Lifespan. *Nat. Rev. Mol. Cel Biol* 11 (8), 545–555. doi:10.1038/nrm2938

Alberti, A., Michelet, X., Djeddi, A., and Legouis, R. (2010). The Autophagosomal Protein LGG-2 Acts Synergistically with LGG-1 in Dauer Formation and Longevity in *C. elegans*. *Autophagy* 6 (5), 622–633. doi:10.4161/auto.6.5.12252

Aman, Y., Schmauck-Medina, T., Hansen, M., Morimoto, R. I., Simon, A. K., Bjedov, I., et al. (2021). Autophagy in Healthy Aging and Disease. *Nat. Aging* 1 (8), 634–650. doi:10.1038/s43587-021-00098-4

Anisimova, A. S., Alexandrov, A. I., Makarova, N. E., Gladyshev, V. N., and Dmitriev, S. E. (2018). Protein Synthesis and Quality Control in Aging. *Aging* 10 (12), 4269–4288. doi:10.18632/aging.101721

Artal-Sanz, M., and Tavernarakis, N. (2009). Prohibitin Couples Diapause Signalling to Mitochondrial Metabolism during Ageing in *C. elegans*. *Nature* 461 (7265), 793–797. doi:10.1038/nature08466

Baird, N. A., Douglas, P. M., Simic, M. S., Grant, A. R., Moresco, J. J., Wolff, S. C., et al. (2014). HSF-1-mediated Cytoskeletal Integrity Determines Thermotolerance and Life Span. *Science* 346 (6207), 360–363. doi:10.1126/science.1253168

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 (6), e1002760. doi:10.1371/journal.pgen.1002760

Baytek, G., Blume, A., Demirel, F. G., Bulut, S., Mertins, P., and Tursun, B. (2021). SUMOylation of the Chromodomain Factor MRG-1 in *C. elegans* Affects Chromatin-Regulatory Dynamics. *Biorxiv[Preprint]*, 1–21. doi:10.1101/2021.02.14.431134

Berendzen, K. M., Durieux, J., Shao, L.-W., Tian, Y., Kim, H.-E., Wolff, S., et al. (2016). Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis. *Cell* 166 (6), 1553–1563. e10. doi:10.1016/j.cell.2016.08.042

Blagosklonny, M. V. (2013). MTOR-driven Quasi-Programmed Aging as a Disposable Soma Theory: Blind Watchmaker vs. Intelligent Designer. *Cell Cycle* 12 (12), 1842–1847. doi:10.4161/cc.25062

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. *Cel Rep.* 9 (3), 1135–1150. doi:10.1016/j.celrep.2014.09.042

Broday, L., Kolotuev, I., Didier, C., Bhoumik, A., Gupta, B. P., Sternberg, P. W., et al. (2004). The Small Ubiquitin-like Modifier (SUMO) Is Required for Gonadal and Uterine-Vulval Morphogenesis in *Caenorhabditis elegans*. *Genes Dev.* 18 (19), 2380–2391. doi:10.1101/gad.1227104

Calculli, G., Lee, H. J., Shen, K., Pham, U., Herholz, M., Trifunovic, A., et al. (2021). Systemic Regulation of Mitochondria by Germline Proteostasis Prevents Protein Aggregation in the Soma of *C. elegans*. *Sci. Adv.* 7 (26), eabg3012. doi:10.1126/sciadv.abg3012

Carrano, A. C., Liu, Z., Dillin, A., and Hunter, T. (2009). A Conserved Ubiquitination Pathway Determines Longevity in Response to Diet Restriction. *Nature* 460 (7253), 396–399. doi:10.1038/nature08130

Cattie, D. J., Richardson, C. E., Reddy, K. C., Ness-Cohn, E. M., Droste, R., Thompson, M. K., et al. (2016). Mutations in Nonessential eIF3k and eIF3l Genes Confer Lifespan Extension and Enhanced Resistance to ER Stress in *Caenorhabditis elegans*. *Plos Genet.* 12 (9), e1006326. doi:10.1371/journal.pgen.1006326

Chang, H.-W., Pisano, S., Chaturbedi, A., Chen, J., Gordon, S., Baruah, A., et al. (2017). Transcription Factors CEP-1/p53 and CEH-23 Collaborate with AAK-2/AMPK to Modulate Longevity in *Caenorhabditis elegans*. *Aging Cell* 16 (4), 814–824. doi:10.1111/acel.12619

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 (5), e1000486. doi:10.1371/journal.pgen.1000486

Chiang, W.-C., Tishkoff, D. X., Yang, B., Wilson-Grady, J., Yu, X., Mazer, T., et al. (2012). *C. elegans* SIRT6/7 Homolog SIR-2.4 Promotes DAF-16 Relocalization and Function during Stress. *Plos Genet.* 8 (9), e1002948. doi:10.1371/journal.pgen.1002948

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 (2), 611–622. doi:10.1096/fj.14-252189

Cornu, M., Albert, V., and Hall, M. N. (2013). MTOR in Aging, Metabolism, and Cancer. *Curr. Opin. Genet. Develop.* 23 (1), 53–62. doi:10.1016/j.gde.2012.12.005

Curran, S. P., and Ruvkun, G. (2007). Lifespan Regulation by Evolutionarily Conserved Genes Essential for Viability. *Plos Genet.* 3 (4), e56. doi:10.1371/journal.pgen.0030056

Daniele, J. R., Higuchi-Sanabria, R., Durieux, J., Monshietehadi, S., Ramachandran, V., Tronnes, S. U., et al. (2020). UPR ER Promotes Lipophagy Independent of Chaperones to Extend Life Span. *Sci. Adv.* 6 (1). eaaz1441. doi:10.1126/sciadv.aaz1441

Denzel, M. S., Storm, N. J., Gutschmidt, A., Baddi, R., Hinze, Y., Jarosch, E., et al. (2014). Hexosamine Pathway Metabolites Enhance Protein Quality Control and Prolong Life. *Cell* 156 (6), 1167–1178. doi:10.1016/j.cell.2014.01.061

Depuydt, G., Shanmugam, N., Rasulova, M., Dhondt, I., and Braeckman, B. P. (2016). Increased Protein Stability and Decreased Protein Turnover in theCaenorhabditis elegansIns/IGF-1daf-2Mutant. *Gerona* 71 (12), 1553–1559. doi:10.1093/gerona/glv221

Derisbourg, M. J., Hartman, M. D., and Denzel, M. S. (2021a). Modulating the Integrated Stress Response to Slow Aging and Ameliorate Age-Related Pathology. *Nat. Aging* 1 (9), 760–768. doi:10.1038/s43587-021-00112-9

Derisbourg, M. J., Wester, L. E., Baddi, R., and Denzel, M. S. (2021b). Mutagenesis Screen Uncovers Lifespan Extension through Integrated Stress Response Inhibition without Reduced mRNA Translation. *Nat. Commun.* 12 (1), 1678. doi:10.1038/s41467-021-21743-x

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 (9), 1621–1633. doi:10.1074/mcp.RA117.000049

Di Fiore, P. P., and Scita, G. (2002). Eps8 in the Midst of GTPases. *Int. J. Biochem. Cel Biol.* 34 (10), 1178–1183. doi:10.1016/s1357-2725(02)00064-x

Dilberger, B., Baumanns, S., Schmitt, F., Schmiedl, T., Hardt, M., Wenzel, U., et al. (2019). Mitochondrial Oxidative Stress Impairs Energy Metabolism and Reduces Stress Resistance and Longevity ofC. Elegans. *Oxidative Med. Cell Longevity* 2019, 1–14. doi:10.1155/2019/6840540

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 (5602), 2398–2401. doi:10.1126/science.1077780

Donnelly, N., Gorman, A. M., Gupta, S., and Samali, A. (2013). The eIF2α Kinases: Their Structures and Functions. *Cell. Mol. Life Sci.* 70 (19), 3493–3511. doi:10.1007/s00018-012-1252-6

Douglas, P. M., Baird, N. A., Simic, M. S., Uhlein, S., McCormick, M. A., Wolff, S. C., et al. (2015). Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Longevity. *Cel Rep.* 12 (7), 1196–1204. doi:10.1016/j.celrep.2015.07.026

Drabikowski, K., Ferralli, J., Kistowski, M., Oledzki, J., Dadlez, M., and Chiquet-Ehrismann, R. (2018). Comprehensive List of SUMO Targets in *Caenorhabditis elegans* and its Implication for Evolutionary Conservation of SUMO Signaling. *Sci. Rep.* 8, 1139. doi:10.1038/s41598-018-19424-9

Drabikowski, K. (2020). Ubiquitin and SUMO Modifications in *Caenorhabditis elegans* Stress Response. *Curr. Issues Mol. Biol.* 35, 145–158. doi:10.21775/cimb.035.145

Durieux, J., Wolff, S., and Dillin, A. (2011). The Cell-Non-Autonomous Nature of Electron Transport Chain-Mediated Longevity. *Cell* 144 (1), 79–91. doi:10.1016/j.cell.2010.12.016

Essers, P., Tain, L. S., Nespital, T., Goncalves, J., Froehlich, J., and Partridge, L. (2016). Reduced Insulin/insulin-like Growth Factor Signaling Decreases Translation in Drosophila and Mice. *Sci. Rep.* 6 (1), 30290. doi:10.1038/srep30290

Feng, J., Bussière, F., and Hekimi, S. (2001). Mitochondrial Electron Transport is a Key Determinant of Life Span in Caenorhabditis Elegans. *Dev. Cell* 1 (5), 633–644. doi:10.1016/s1534-5807(01)00071-5

Flotho, A., and Melchior, F. (2013). Sumoylation: A Regulatory Protein Modification in Health and Disease. *Annu. Rev. Biochem.* 82, 357–385. doi:10.1146/annurev-biochem-061909-093311

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 (6476), 436–440. doi:10.1126/science.aaz6896

Francisco, S., Ferreira, M., Moura, G., Soares, A. R., and Santos, M. A. S. (2020). Does Proteostasis Get Lost in Translation? Implications for Protein Aggregation across the Lifespan. *Ageing Res. Rev.* 62, 101119. doi:10.1016/j.arr.2020.101119

García, M. A., Meurs, E. F., and Esteban, M. (2007). The dsRNA Protein Kinase PKR: Virus and Cell Control. *Biochimie* 89 (6–7), 799–811. doi:10.1016/j.biochi.2007.03.001

Gatsi, R., Schulze, B., Rodríguez-Palero, M. J., Hernando-Rodríguez, B., Baumeister, R., and Artal-Sanz, M. (2014). Prohibitin-Mediated Lifespan and Mitochondrial Stress Implicate SGK-1, Insulin/IGF and mTORC2 in *C. elegans*. *PLOS ONE* 9 (9), e107671. doi:10.1371/journal.pone.0107671

Geisler, F., Coch, R. A., Richardson, C., Goldberg, M., Denecke, B., Bossinger, O., et al. (2019). The Intestinal Intermediate Filament Network Responds to and Protects against Microbial Insults and Toxins. *Development (Cambridge, England)* 146 (2), dev169482. doi:10.1242/dev.169482

Gillespie, Z. E., Wang, C., Vadan, F., Yu, T. Y., Ausió, J., Kusalik, A., et al. (2019). Metformin Induces the AP-1 Transcription Factor Network in normal Dermal Fibroblasts. *Sci. Rep.* 9, 5369. doi:10.1038/s41598-019-41839-1

Glick, D., Barth, S., and Macleod, K. F. (2010). Autophagy: Cellular and Molecular Mechanisms. *J. Pathol.* 221 (1), 3–12. doi:10.1002/path.2697

Glickman, M. H., and Ciechanover, A. (2002). The Ubiquitin-Proteasome Proteolytic Pathway: Destruction for the Sake of Construction. *Physiol. Rev.* 82 (2), 373–428. doi:10.1152/physrev.00027.2001

Gonskikh, Y., and Polacek, N. (2017). Alterations of the Translation Apparatus during Aging and Stress Response. *Mech. Ageing Develop.* 168, 30–36. doi:10.1016/j.mad.2017.04.003

Grabowska, W., Sikora, E., and Bielak-Zmijewska, A. (2017). Sirtuins, a Promising Target in Slowing Down the Ageing Process. *Biogerontology* 18 (4), 447–476. doi:10.1007/s10522-017-9685-9

Hansen, M., Chandra, A., Mitic, L. L., Onken, B., Driscoll, M., and Kenyon, C. (2008). A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in *C. elegans*. *Plos Genet.* 4 (2), e24. doi:10.1371/journal.pgen.0040024

Hansen, M., and Hansen, M. (2012). Autophagy - an Emerging Anti-aging Mechanism? *J. Clin. Exp. Pathol.* s4, 006. doi:10.4172/2161-0681.s4-006

Hansen, M., Rubinsztein, D. C., and Walker, D. W. (2018). Autophagy as a Promoter of Longevity: Insights from Model Organisms. *Nat. Rev. Mol. Cel Biol* 19 (9), 579–593. doi:10.1038/s41580-018-0033-y

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 (1), 95–110. doi:10.1111/j.1474-9726.2006.00267.x

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., et al. (2003). An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress. *Mol. Cel* 11 (3), 619–633. doi:10.1016/s1097-2765(03)00105-9

He, C., and Klionsky, D. J. (2009). Regulation Mechanisms and Signaling Pathways of Autophagy. *Annu. Rev. Genet.* 43, 67–93. doi:10.1146/annurev-genet-102808-114910

Heintz, C., Doktor, T. K., Lanjuin, A., Escoubas, C. C., Zhang, Y., Weir, H. J., et al. (2017). Splicing Factor 1 Modulates Dietary Restriction and TORC1 Pathway Longevity in *C. elegans*. *Nature* 541 (7635), 102–106. doi:10.1038/nature20789

Henis-Korenblit, S., Zhang, P., Hansen, M., McCormick, M., Lee, S.-J., Cary, M., et al. (2010). Insulin/IGF-1 Signaling Mutants Reprogram ER Stress Response Regulators to Promote Longevity. *Proc. Natl. Acad. Sci.* 107 (21), 9730–9735. doi:10.1073/pnas.1002575107

Hershko, A., and Ciechanover, A. (1998). The Ubiquitin System. *Annu. Rev. Biochem.* 67, 425–479. doi:10.1146/annurev.biochem.67.1.425

Hipp, M. S., Kasturi, P., and Hartl, F. U. (2019). The Proteostasis Network and its Decline in Ageing. *Nat. Rev. Mol. Cel Biol* 20 (7), 421–435. doi:10.1038/s41580-019-0101-y

Hochstrasser, M. (2006). Lingering Mysteries of Ubiquitin-Chain Assembly. *Cell* 124 (1), 27–34. doi:10.1016/j.cell.2005.12.025

Hochstrasser, M. (1996). Ubiquitin-dependent Protein Degradation. *Annu. Rev. Genet.* 30, 405–439. doi:10.1146/annurev.genet.30.1.405

Hong, S. Y., Ng, L. T., Ng, L. F., Inoue, T., Tolwinski, N. S., Hagen, T., et al. (2016). The Role of Mitochondrial Non-enzymatic Protein Acylation in Ageing. *PLOS ONE* 11 (12), e0168752. doi:10.1371/journal.pone.0168752

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 (7450), 451–457. doi:10.1038/nature12188

Howard, A. C., Mir, D., Snow, S., Horrocks, J., Sayed, H., Ma, Z., et al. (2021). Anabolic Function Downstream of TOR Controls Trade-Offs between Longevity and Reproduction at the Level of Specific Tissues in *C. elegans*. *Front. Aging* 2, 47. doi:10.3389/fragi.2021.725068

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 (5622), 1142–1145. doi:10.1126/science.1083701

Huang, J., Wu, Z., Wang, J., and Zhang, X. (2018). Quantitative Phosphoproteomics Reveals GTBP-1 Regulating C.Elegans Lifespan at Different Environmental Temperatures. *Biochem. Biophysical Res. Commun.* 503 (3), 1962–1967. doi:10.1016/j.bbrc.2018.07.142

Hwang, A. B., Ryu, E.-A., Artan, M., Chang, H.-W., Kabir, M. H., Nam, H.-J., et al. (2014). Feedback Regulation via AMPK and HIF-1 Mediates ROS-dependent Longevity in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 111 (42), E4458–E4467. doi:10.1073/pnas.1411199111

Imanikia, S., Sheng, M., Castro, C., Griffin, J. L., and Taylor, R. C. (2019). XBP-1 Remodels Lipid Metabolism to Extend Longevity. *Cel Rep.* 28 (3), 581–589. e4. doi:10.1016/j.celrep.2019.06.057

Ito, A., Zhao, Q., Tanaka, Y., Yasui, M., Katayama, R., Sun, S., et al. (2021). Metolazone Upregulates Mitochondrial Chaperones and Extends Lifespan in *Caenorhabditis elegans*. *Biogerontology* 22 (1), 119–131. doi:10.1007/s10522-020-09907-6

Jia, K., Chen, D., and Riddle, D. L. (2004). The TOR Pathway Interacts with the Insulin Signaling Pathway to regulateC. Eleganslarval Development, Metabolism and Life Span. *metabolism Life spanDevelopment (Cambridge, England)* 131 (16), 3897–3906. doi:10.1242/dev.01255

Jin, K. (2010). Modern Biological Theories of Aging. *Aging Dis.* 1 (2), 72–74.

Johnson, S. C., Rabinovitch, P. S., and Kaeberlein, M. (2013). MTOR Is a Key Modulator of Ageing and Age-Related Disease. *Nature* 493 (7432), 338–345. doi:10.1038/nature11861

Kaminsky, R., Denison, C., Bening-Abu-Shach, U., Chisholm, A. D., Gygi, S. P., and Broday, L. (2009). SUMO Regulates the Assembly and Function of a Cytoplasmic Intermediate Filament Protein in *C. elegans*. *Develop. Cel* 17 (5), 724–735. doi:10.1016/j.devcel.2009.10.005

Kato, M., Chen, X., Inukai, S., Zhao, H., and Slack, F. J. (2011). "Age-associated Changes in Expression of Small, Noncoding RNAs, Including microRNAs, in *C. elegans*,". Editor C. elegans, 17, 1804–1820. doi:10.1261/rna.2714411

Ke, Z., Seluanov, A., and Gorbunova, V. (2018). Accurate Translation Is Important for Longevity. *Aging* 10 (3), 297–298. doi:10.18632/aging.101398

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

Kevei, É., and Hoppe, T. (2014). Ubiquitin Sets the Timer: Impacts on Aging and Longevity. *Nat. Struct. Mol. Biol.* 21 (4), 290–292. doi:10.1038/nsmb.2806

Kim, H., and Strange, K. (2013). Changes in Translation Rate Modulate Stress-Induced Damage of Diverse Proteins. *Am. J. Physiology-Cell Physiol.* 305 (12), C1257–C1264. doi:10.1152/ajpcell.00176.2013

Klionsky, D. J., Abdelmohsen, K., Abe, A., Abedin, M. J., Abeliovich, H., Acevedo Arozena, A., et al. (2016). Guidelines for the Use and Interpretation of Assays for Monitoring Autophagy (3rd Edition). *Autophagy* 12 (1), 1–222. doi:10.1080/15548627.2015.1100356

Kovács, D., Sigmond, T., Hotzi, B., Bohár, B., Fazekas, D., Deák, V., et al. (2019). HSF1Base: A Comprehensive Database of HSF1 (Heat Shock Factor 1) Target Genes. *Int. J. Mol. Sci.* 20 (22), E5815. doi:10.3390/ijms20225815

Koyuncu, S., Irmak, D., Saez, I., and Vilchez, D. (2015). Defining the General Principles of Stem Cell Aging: Lessons from Organismal Models. *Curr. Stem Cel Rep.* 1 (3), 162–169. doi:10.1007/s40778-015-0017-1

Koyuncu, S., Loureiro, R., Lee, H. J., Wagle, P., Krueger, M., and Vilchez, D. (2021). Rewiring of the Ubiquitinated Proteome Determines Ageing in *C. elegans*. *Nature* 596 (7871), 285–290. doi:10.1038/s41586-021-03781-z

Koyuncu, S., Saez, I., Lee, H. J., Gutierrez-Garcia, R., Pokrzywa, W., Fatima, A., et al. (2018). The Ubiquitin Ligase UBR5 Suppresses Proteostasis Collapse in Pluripotent Stem Cells from Huntington's Disease Patients. *Nat. Commun.* 9 (1), 2886. doi:10.1038/s41467-018-05320-3

Kreis, P., Gallrein, C., Rojas-Puente, E., Mack, T. G. A., Kroon, C., Dinkel, V., et al. (2019). ATM Phosphorylation of the Actin-Binding Protein Drebrin Controls Oxidation Stress-Resistance in Mammalian Neurons and *C. elegans*. *Nat. Commun.* 10 (1), 486. doi:10.1038/s41467-019-08420-w

Kuhlbrodt, K., Janiesch, P. C., Kevei, É., Segref, A., Barikbin, R., and Hoppe, T. (2011). The Machado-Joseph Disease Deubiquitylase ATX-3 Couples Longevity and Proteostasis. *Nat. Cel Biol.* 13 (3), 273–281. doi:10.1038/ncb2200

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 (1), 5648. doi:10.1038/s41467-019-13540-4

Labbadia, J., and Morimoto, R. I. (2015). The Biology of Proteostasis in Aging and Disease. *Annu. Rev. Biochem.* 84, 435–464. doi:10.1146/annurev-biochem-060614-033955

Lakowski, B., and Hekimi, S. (1996). Determination of Life-Span in *Caenorhabditis elegans* by Four Clock Genes. *Science (New York, N.Y.)* 272 (5264), 1010–1013. doi:10.1126/science.272.5264.1010

Lee, S.-J., Hwang, A. B., and Kenyon, C. (2010). Inhibition of Respiration Extends *C. elegans* Life Span via Reactive Oxygen Species that Increase HIF-1 Activity. *Curr. Biol. CB* 20 (23), 2131–2136. doi:10.1016/j.cub.2010.10.057

Li, W.-J., Wang, C.-W., Tao, L., Yan, Y.-H., Zhang, M.-J., Liu, Z.-X., et al. (2021). Insulin Signaling Regulates Longevity through Protein Phosphorylation in *Caenorhabditis elegans*. *Nat. Commun.* 12 (1), 4568. doi:10.1038/s41467-021-24816-z

Li, W., Gao, B., Lee, S.-M., Bennett, K., and Fang, D. (2007). RLE-1, an E3 Ubiquitin Ligase, Regulates *C. elegans* Aging by Catalyzing DAF-16 Polyubiquitination. *Develop. Cel* 12 (2), 235–246. doi:10.1016/j.devcel.2006.12.002

Lim, Y., Lee, D., Kalichamy, K., Hong, S.-E., Michalak, M., Ahnn, J., et al. (2014). Sumoylation Regulates ER Stress Response by Modulating Calreticulin Gene Expression in XBP-1-dependent Mode in *Caenorhabditis elegans*. *Int. J. Biochem. Cel Biol.* 53, 399–408. doi:10.1016/j.biocel.2014.06.005

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 (2), 139–145. doi:10.1038/88850

Lin, X.-X., Sen, I., Janssens, G. E., Zhou, X., Fonslow, B. R., Edgar, D., et al. (2018). DAF-16/FOXO and HLH-30/TFEB Function as Combinatorial Transcription Factors to Promote Stress Resistance and Longevity. *Nat. Commun.* 9 (1), 4400. doi:10.1038/s41467-018-06624-0

Liu, G., Rogers, J., Murphy, C. T., and Rongo, C. (2011). EGF Signalling Activates the Ubiquitin Proteasome System to Modulate *C. elegans* Lifespan. *EMBO J.* 30 (15), 2990–3003. doi:10.1038/emboj.2011.195

Liu, Y. J., McIntyre, R. L., Janssens, G. E., Williams, E. G., Lan, J., van Weeghel, M., et al. (2020). Mitochondrial Translation and Dynamics Synergistically Extend Lifespan in *C. elegans* through HLH-30. *J. Cel Biol.* 219 (6), 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 (6), 1194–1217. doi:10.1016/j.cell.2013.05.039

Maklakov, A. A., and Chapman, T. (2019). Evolution of Ageing as a Tangle of Trade-Offs: Energy versus Function. *Proc. Biol. Sci.* 286 (1911), 20191604. doi:10.1098/rspb.2019.1604

Mariño, G., Morselli, E., Bennetzen, M. V., Eisenberg, T., Megalou, E., Schroeder, S., et al. (2011). Longevity-relevant Regulation of Autophagy at the Level of the Acetylproteome. *Autophagy* 7 (6), 647–649. doi:10.4161/auto.7.6.15191

Mark, K. A., Dumas, K. J., Bhaumik, D., Schilling, B., Davis, S., Oron, T. R., et al. (2016). Vitamin D Promotes Protein Homeostasis and Longevity via the Stress Response Pathway Genes Skn-1, Ire-1, and Xbp-1. *Cel Rep.* 17 (5), 1227–1237. doi:10.1016/j.celrep.2016.09.086

Martinez-Miguel, V. E., Lujan, C., Espie–Caullet, T., Martinez-Martinez, D., Moore, S., Backes, C., et al. (2021). Increased Fidelity of Protein Synthesis Extends Lifespan. *Cel Metab.* 33 (11), 2288–2300. e12. doi:10.1016/j.cmet.2021.08.017

Matai, L., Sarkar, G. C., Chamoli, M., Malik, Y., Kumar, S. S., Rautela, U., et al. (2019). Dietary Restriction Improves Proteostasis and Increases Life Span through Endoplasmic Reticulum Hormesis. *Proc. Natl. Acad. Sci.* 116 (35), 17383–17392. doi:10.1073/pnas.1900055116

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,". Editor C. elegans, 324, 1196–1198. doi:10.1126/science.1173507*Science (New York, N.Y.)*5931

Meléndez, A., Tallóczy, Z., Seaman, M., Eskelinen, E.-L., Hall, D. H., and Levine, B. (2003). Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in *C. elegans*. *Science (New York, N.Y.)* 301 (5638), 1387–1391. doi:10.1126/science.1087782

Mogk, A., Bukau, B., and Kampinga, H. H. (2018). Cellular Handling of Protein Aggregates by Disaggregation Machines. *Mol. Cel* 69 (2), 214–226. doi:10.1016/j.molcel.2018.01.004

Moll, L., Roitenberg, N., Bejerano-Sagie, M., Boocholez, H., Carvalhal Marques, F., Volovik, Y., et al. (2018). The Insulin/IGF Signaling Cascade Modulates SUMOylation to Regulate Aging and Proteostasis in Caenorhabditis Elegans. *ELife* 7, e38635. doi:10.7554/eLife.38635

Mörck, C., and Pilon, M. (2006). *C. elegans* Feeding Defective Mutants Have Shorter Body Lengths and Increased Autophagy. *BMC Develop. Biol.* 6, 39. doi:10.1186/1471-213X-6-39

Morley, J. F., and Morimoto, R. I. (2004). Regulation of Longevity in *Caenorhabditis elegans* by Heat Shock Factor and Molecular Chaperones. *Mol. Biol. Cel* 15 (2), 657–664. doi:10.1091/mbc.E03-07-0532

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 (2), 430–441. doi:10.1016/j.cell.2013.06.016

Münch, C., and Harper, J. W. (2016). Mitochondrial Unfolded Protein Response Controls Matrix Pre-RNA Processing and Translatio. *Nature* 534 (7609), 710–713. doi:10.1038/nature18302

Nakamura, S., Karalay, Ö., Jäger, P. S., Horikawa, M., Klein, C., Nakamura, K., et al. (2016). Mondo Complexes Regulate TFEB via TOR Inhibition to Promote Longevity in Response to Gonadal Signals. *Nat. Commun.* 7, 10944. doi:10.1038/ncomms10944

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 (1), 13649. doi:10.1038/ncomms13649

Novoa, I., Zhang, Y., Zeng, H., Jungreis, R., Harding, H. P., and Ron, D. (2003). Stress-induced Gene Expression Requires Programmed Recovery from Translational Repression. *EMBO J.* 22 (5), 1180–1187. doi:10.1093/emboj/cdg112

Padmanabhan, S., Mukhopadhyay, A., Narasimhan, S. D., Tesz, G., Czech, M. P., and Tissenbaum, H. A. (2009). A PP2A Regulatory Subunit Regulates *C. elegans* Insulin/IGF-1 Signaling by Modulating AKT-1 Phosphorylation. *Cell* 136 (5), 939–951. doi:10.1016/j.cell.2009.01.025

Pakos-Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., and Gorman, A. M. (2016). The Integrated Stress Response. *EMBO Rep.* 17 (10), 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 (1), 111–119. doi:10.1111/j.1474-9726.2006.00266.x

Park, S.-K., Seong, R.-K., Kim, J.-A., Son, S.-J., Kim, Y., Yokozawa, T., et al. (2016). Oligonol Promotes Anti-aging Pathways via Modulation of SIRT1-AMPK-Autophagy Pathway. *Nutr. Res. Pract.* 10 (1), 3–10. doi:10.4162/nrp.2016.10.1.3

Park, S., Artan, M., Han, S. H., Park, H.-E. H., Jung, Y., Hwang, A. B., et al. (2020). VRK-1 Extends Life Span by Activation of AMPK via Phosphorylation. *Sci. Adv.* 6 (27), eaaw7824. doi:10.1126/sciadv.aaw7824

Peleg, S., Feller, C., Ladurner, A. G., and Imhof, A. (2016). The Metabolic Impact on Histone Acetylation and Transcription in Ageing. *Trends Biochem. Sci.* 41 (8), 700–711. doi:10.1016/j.tibs.2016.05.008

Pelisch, F., Tammsalu, T., Wang, B., Jaffray, E. G., Gartner, A., and Hay, R. T. (2017). A SUMO-dependent Protein Network Regulates Chromosome Congression during Oocyte Meiosis. *Mol. Cel* 65 (1), 66–77. doi:10.1016/j.molcel.2016.11.001

Pickart, C. M. (2001). Mechanisms Underlying Ubiquitination. *Annu. Rev. Biochem.* 70 (1), 503–533. doi:10.1146/annurev.biochem.70.1.503

Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W., and Balch, W. E. (2009). Biological and Chemical Approaches to Diseases of Proteostasis Deficiency. *Annu. Rev. Biochem.* 78, 959–991. doi:10.1146/annurev.biochem.052308.114844

Prahlad, V., Cornelius, T., and Morimoto, R. I. (2008). Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons. *Science (New York, N.Y.)* 320 (5877), 811–814. doi:10.1126/science.1156093

Princz, A., Pelisch, F., and Tavernarakis, N. (2020). SUMO Promotes Longevity and Maintains Mitochondrial Homeostasis During Ageing in Caenorhabditis Elegans. *Sci. Rep.* 10 (1), 15513. doi:10.1038/s41598-020-72637-9

Rieckher, M., Markaki, M., Princz, A., Schumacher, B., and Tavernarakis, N. (2018). Maintenance of Proteostasis by P Body-Mediated Regulation of eIF4E Availability during Aging in *Caenorhabditis elegans*. *Cel Rep.* 25 (1), 199–211. e6. doi:10.1016/j.celrep.2018.09.009

Ristow, M., and Schmeisser, K. (2014). Mitohormesis: Promoting Health and Lifespan by Increased Levels of Reactive Oxygen Species (ROS). *Dose-Response* 12 (2), 288–341. doi:10.2203/dose-response.13-035.Ristow

Rogers, A. N., Chen, D., McColl, G., Czerwieniec, G., Felkey, K., Gibson, B. W., et al. (2011). Life Span Extension via eIF4G Inhibition Is Mediated by Posttranscriptional Remodeling of Stress Response Gene Expression in *C. elegans*. *Cel Metab.* 14 (1), 55–66. doi:10.1016/j.cmet.2011.05.010

Saad, S., Cereghetti, G., Feng, Y., Picotti, P., Peter, M., and Dechant, R. (2017). Reversible Protein Aggregation Is a Protective Mechanism to Ensure Cell Cycle Restart after Stress. *Nat. Cel Biol.* 19 (10), 1202–1213. doi:10.1038/ncb3600

Saez, I., and Vilchez, D. (2014). The Mechanistic Links between Proteasome Activity, Aging and Age-Related Diseases. *Curr. Genomics* 15 (1), 38–51. doi:10.2174/1389202915011403061133344

Santos, A. L., and Lindner, A. B. (2017). Protein Posttranslational Modifications: Roles in Aging and Age-Related Disease. *Oxidative Med. Cell Longevity* 2017, 5716409. doi:10.1155/2017/5716409

Schimmel, P. (2018). The Emerging Complexity of the tRNA World: Mammalian tRNAs beyond Protein Synthesis. *Nat. Rev. Mol. Cel Biol.* 19 (1), 45–58. doi:10.1038/nrm.2017.77

Schreiber, M. A., Pierce-Shimomura, J. T., Chan, S., Parry, D., and McIntire, S. L. (2010). Manipulation of Behavioral Decline in *Caenorhabditis elegans* with the Rag GTPase Raga-1. *PLoS Genet.* 6 (5), e1000972. doi:10.1371/journal.pgen.1000972

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. *Cel Metab.* 6 (4), 280–293. doi:10.1016/j.cmet.2007.08.011

Schönthal, A. H. (2012). Endoplasmic Reticulum Stress: Its Role in Disease and Novel Prospects for Therapy. *Scientifica* 2012, 857516. doi:10.6064/2012/857516

Segref, A., Torres, S., and Hoppe, T. (2011). A Screenable *In Vivo* Assay to Study Proteostasis Networks in *Caenorhabditis elegans*. *Genetics* 187 (4), 1235–1240. doi:10.1534/genetics.111.126797

Seo, K., Choi, E., Lee, D., Jeong, D.-E., Jang, S. K., and Lee, S.-J. (2013). Heat Shock Factor 1 Mediates the Longevity Conferred by Inhibition of TOR and insulin/IGF-1 Signaling Pathways in *C. elegans*. *Aging Cell* 12 (6), 1073–1081. doi:10.1111/acel.12140

Shin, G., Koo, H. J., Seo, M., Lee, S.-J. V., Nam, H. G., and Jung, G. Y. (2021). Transfer RNA-Derived Fragments in Aging *Caenorhabditis elegans* Originate from Abundant Homologous Gene Copies. *Scientific Rep.* 11 (1), 12304. doi:10.1038/s41598-021-91724-z

Shpilka, T., and Haynes, C. M. (2018). The Mitochondrial UPR: Mechanisms, Physiological Functions and Implications in Ageing. *Nat. Rev. Mol. Cel Biol.* 19 (2), 109–120. doi:10.1038/nrm.2017.110

Sies, H., and Cadenas, E. (1985). Oxidative Stress: Damage to Intact Cells and Organs. *Philosophical Transactions of the Royal Society of London.* Series B. *Biol. Sci.* 311 (1152), 617–631. doi:10.1098/rstb.1985.0168

Silva, M. C., Fox, S., Beam, M., Thakkar, H., Amaral, M. D., and Morimoto, R. I. (2011). A Genetic Screening Strategy Identifies Novel Regulators of the Proteostasis Network. *PLoS Genet.* 7 (12), e1002438. doi:10.1371/journal.pgen.1002438

Silvestrini, M. J., Johnson, J. R., Kumar, A. V., Thakurta, T. G., Blais, K., Neill, Z. A., et al. (2018). Nuclear Export Inhibition Enhances HLH-30/TFEB Activity, Autophagy, and Lifespan. *Cel Rep.* 23 (7), 1915–1921. doi:10.1016/j.celrep.2018.04.063

Sladowska, M., Turek, M., Kim, M.-J., Drabikowski, K., Mussulini, B. H. M., Mohanraj, K., et al. (2021). Proteasome Activity Contributes to Pro-survival Response upon Mild Mitochondrial Stress in *Caenorhabditis elegans*. *PLOS Biol.* 19 (7), e3001302. doi:10.1371/journal.pbio.3001302

Smith, H. J., Lanjuin, A., Sharma, A., Prabhakar, A., Tabakovic, E., Sehgal, R., et al. (2021). Neuronal mTORC1 Inhibition Promotes Longevity without Suppressing Anabolic Growth and Reproduction in *C. elegans*. doi:10.1101/2021.08.12.456148

Solis, G. M., Kardakaris, R., Valentine, E. R., Bar-Peled, L., Chen, A. L., Blewett, M. M., et al. (2018). Translation Attenuation by Minocycline Enhances Longevity and Proteostasis in Old post-stress-responsive Organisms. *ELife* 7, e40314. doi:10.7554/eLife.40314

Somogyvári, M., Gecse, E., and Sőti, C. (2018). DAF-21/Hsp90 Is Required for *C. elegans* Longevity by Ensuring DAF-16/FOXO Isoform A Function. *Scientific Rep.* 8 (1), 12048. doi:10.1038/s41598-018-30592-6

Son, H. G., Seo, M., Ham, S., Hwang, W., Lee, D., An, S. W. A., et al. (2017). RNA Surveillance via Nonsense-Mediated mRNA Decay Is Crucial for Longevity in daf-2/insulin/IGF-1 Mutant *C. elegans*. *Nat. Commun.* 8, 14749. doi:10.1038/ncomms14749

Soo, S. K., Traa, A., Rudich, P. D., Mistry, M., and Raamsdonk, J. M. V. (2021). Activation of Mitochondrial Unfolded Protein Response Protects against Multiple Exogenous Stressors. *Life Sci. Alliance* 4 (12). doi:10.26508/lsa.202101182

Steinkraus, K. A., Smith, E. D., Davis, C., Carr, D., Pendergrass, W. R., Sutphin, G. L., et al. (2008). Dietary Restriction Suppresses Proteotoxicity and Enhances Longevity by an Hsf-1-dependent Mechanism in *Caenorhabditis elegans*. *Aging Cell* 7 (3), 394–404. doi:10.1111/j.1474-9726.2008.00385.x

Sural, S., Lu, T.-C., Jung, S. A., and Hsu, A.-L. (2019). HSB-1 Inhibition and HSF-1 Overexpression Trigger Overlapping Transcriptional Changes to Promote Longevity in *Caenorhabditis elegans*. *G3: Genes|Genomes|Genetics* 9 (5), 1679–1692. doi:10.1534/g3.119.400044

Swovick, K., Firsanov, D., Welle, K. A., Hryhorenko, J. R., Wise, J. P., George, C., et al. (2021). Interspecies Differences in Proteome Turnover Kinetics Are Correlated with Life Spans and Energetic Demands. *Mol. Cell Proteomics* 20, 100041. doi:10.1074/mcp.RA120.002301

Syntichaki, P., Troulinaki, K., and Tavernarakis, N. (2007a). EIF4E Function in Somatic Cells Modulates Ageing in *Caenorhabditis elegans*. *Nature* 445 (7130), 922–926. doi:10.1038/nature05603

Syntichaki, P., Troulinaki, K., and Tavernarakis, N. (2007b). Protein Synthesis Is a Novel Determinant of Aging in *Caenorhabditis elegans*. *Ann. N.Y Acad. Sci.* 1119, 289–295. doi:10.1196/annals.1404.001

Tatar, M., Bartke, A., and Antebi, A. (2003). The Endocrine Regulation of Aging by Insulin-like Signals. *Science (New York, N.Y.)* 299 (5611), 1346–1351. doi:10.1126/science.1081447

Tavernarakis, N., and Driscoll, M. (2002). Caloric Restriction and Lifespan: A Role for Protein Turnover? *Mech. Ageing Develop.* 123 (2–3), 215–229. doi:10.1016/s0047-6374(01)00341-4

Tawo, R., Pokrzywa, W., Kevei, É., Akyuz, M. E., Balaji, V., Adrian, S., et al. (2017). The Ubiquitin Ligase CHIP Integrates Proteostasis and Aging by Regulation of Insulin Receptor Turnover. *Cell* 169 (3), 470–482. e13. doi:10.1016/j.cell.2017.04.003

Taylor, R. C., and Dillin, A. (2011). Aging as an Event of Proteostasis Collapse. *Cold Spring Harbor Perspect. Biol.* 3 (5), a004440. doi:10.1101/cshperspect.a004440

Taylor, R. C., and Dillin, A. (2013). XBP-1 Is a Cell-Nonautonomous Regulator of Stress Resistance and Longevity. *Cell* 153 (7), 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 (11), e13265. doi:10.1111/acel.13265

Thompson, A. C. S., Bruss, M. D., Price, J. C., Khambatta, C. F., Holmes, W. E., Colangelo, M., et al. (2016). Reduced *In Vivo* Hepatic Proteome Replacement Rates but Not Cell Proliferation Rates Predict Maximum Lifespan Extension in Mice. *Aging Cell* 15 (1), 118–127. doi:10.1111/acel.12414

Tissenbaum, H. A., and Guarente, L. (2001). Increased Dosage of a Sir-2 Gene Extends Lifespan in *Caenorhabditis elegans*. *Nature* 410 (6825), 227–230. doi:10.1038/35065638

Tóth, M. L., Sigmond, T., Borsos, E., Barna, J., Erdélyi, P., Takács-Vellai, K., et al. (2008). Longevity Pathways Converge on Autophagy Genes to Regulate Life Span in *Caenorhabditis elegans*. *Autophagy* 4 (3), 330–338. doi:10.4161/auto.5618

van der Vos, K. E., Eliasson, P., Proikas-Cezanne, T., Vervoort, S. J., van Boxtel, R., Putker, M., et al. (2012). Modulation of Glutamine Metabolism by the PI(3)K-PKB-FOXO Network Regulates Autophagy. *Nat. Cel Biol.* 14 (8), 829–837. doi:10.1038/ncb2536

Van Raamsdonk, J. M., and Hekimi, S. (2009). Deletion of the Mitochondrial Superoxide Dismutase Sod-2 Extends Lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 5 (2), e1000361. doi:10.1371/journal.pgen.1000361

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A. L., Orosz, L., and Müller, F. (2003). Genetics: Influence of TOR Kinase on Lifespan in *C. elegans*. *Nature* 426 (6967), 620. doi:10.1038/426620a

Vilchez, D., Morantte, I., Liu, Z., Douglas, P. M., Merkwirth, C., Rodrigues, A. P. C., et al. (2012). RPN-6 Determines *C. elegans* Longevity under Proteotoxic Stress Conditions. *Nature* 489 (7415), 263–268. doi:10.1038/nature11315

Vilchez, D., Saez, I., and Dillin, A. (2014). The Role of Protein Clearance Mechanisms in Organismal Ageing and Age-Related Diseases. *Nat. Commun.* 5, 5659. doi:10.1038/ncomms6659

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 (2), 131–139. doi:10.1046/j.1474-9728.2003.00045.x

Walsh, C. T., Garneau-Tsodikova, S., and Gatto, G. J., Jr. (2005). Protein Posttranslational Modifications: The Chemistry of Proteome Diversifications. *Angew. Chem. Int. Edition* 44 (45), 7342–7372. doi:10.1002/anie.200501023

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 (4), 919–932. doi:10.1016/j.cell.2015.03.032

Wek, R. C., Jiang, H.-Y., and Anthony, T. G. (2006). Coping with Stress: EIF2 Kinases and Translational Control. *Biochem. Soc. Trans.* 34 (Pt 1), 7–11. doi:10.1042/BST20060007

Wieser, W., and Krumschnabel, G. (2001). Hierarchies of ATP-Consuming Processes: Direct Compared with Indirect Measurements, and Comparative Aspects. *Biochem. J.* 355 (Pt 2), 389–395. doi:10.1042/0264-6021:3550389

Wilkinson, K. A., and Henley, J. M. (2010). Mechanisms, Regulation and Consequences of Protein SUMOylation. *Biochem. J.* 428 (2), 133–145. doi:10.1042/BJ20100158

Wilkinson, K. D. (2000). Ubiquitination and Deubiquitination: Targeting of Proteins for Degradation by the Proteasome. *Semin. Cel Develop. Biol.* 11 (3), 141–148. doi:10.1006/scdb.2000.0164

Williams, R., Laskovs, M., Williams, R. I., Mahadevan, A., and Labbadia, J. (2020). A Mitochondrial Stress-specific Form of HSF1 Protects against Age-Related Proteostasis Collapse. *Develop. Cel* 54 (6), 758–772. e5. doi:10.1016/j.devcel.2020.06.038

Wu, C.-W., Wimberly, K., Pietras, A., Dodd, W., Atlas, M. B., and Choe, K. P. (2019). RNA Processing Errors Triggered by Cadmium and Integrator Complex Disruption Are Signals for Environmental Stress. *BMC Biol.* 17 (1), 56. doi:10.1186/s12915-019-0675-z

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. CB* 29 (5), 737–749. e5. doi:10.1016/j.cub.2019.01.029

Yaffe, M. B. (2002). Phosphotyrosine-binding Domains in Signal Transduction. *Nat. Rev. Mol. Cel Biol.* 3 (3), 177–186. doi:10.1038/nrm759

Yang, W., and Hekimi, S. (2010). A Mitochondrial Superoxide Signal Triggers Increased longevity in Caenorhabditis Elegans. *PLoS Biol.* 8 (12), e1000556. doi:10.1371/journal.pbio.1000556

Yau, R., and Rape, M. (2016). The Increasing Complexity of the Ubiquitin Code. *Nat. Cel Biol.* 18 (6), 579–586. doi:10.1038/ncb3358

Yee, Z., Lim, S. H. Y., Ng, L. F., and Gruber, J. (2021). Inhibition of mTOR Decreases Insoluble Proteins burden by Reducing Translation in *C. elegans*. *Biogerontology* 22 (1), 101–118. doi:10.1007/s10522-020-09906-7

Yokoyama, K., Fukumoto, K., Murakami, T., Harada, S., Hosono, R., Wadhwa, R., et al. (2002). Extended Longevity of *Caenorhabditis elegans* by Knocking in Extra Copies of hsp70F, a Homolog of Mot-2 (mortalin)/mthsp70/Grp75. *FEBS Lett.* 516 (1–3), 53–57. doi:10.1016/s0014-5793(02)02470-5

Zhang, H., Smolen, G. A., Palmer, R., Christoforou, A., van den Heuvel, S., and Haber, D. A. (2004). SUMO Modification Is Required for *In Vivo* Hox Gene Regulation by the *Caenorhabditis elegans* Polycomb Group Protein SOP-2. *Nat. Genet.* 36 (5), 507–511. doi:10.1038/ng1336

Zhang, Q., Wu, X., Chen, P., Liu, L., Xin, N., Tian, Y., et al. (2018). The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-Autonomously by Retromer-dependent Wnt Signaling. *Cell* 174 (4), 870–883. e17. doi:10.1016/j.cell.2018.06.029

Zhang, S., Li, F., Zhou, T., Wang, G., and Li, Z. (2020). *Caenorhabditis elegans* as a Useful Model for Studying Aging Mutations. *Front. Endocrinol.* 11, 554994. doi:10.3389/fendo.2020.554994

Zhang, Y., Lanjuin, A., Chowdhury, S. R., Mistry, M., Silva-García, C. G., Weir, H. J., et al. (2019). Neuronal TORC1 Modulates Longevity via AMPK and Cell Nonautonomous Regulation of Mitochondrial Dynamics in *C. elegans*. *ELife* 8, e49158. doi:10.7554/eLife.49158

Zhou, L., He, B., Deng, J., Pang, S., and Tang, H. (2019). Histone Acetylation Promotes Long-Lasting Defense Responses and Longevity Following Early Life Heat Stress. *PLOS Genet.* 15 (4), e1008122. doi:10.1371/journal.pgen.1008122

Zhou, Z., Sun, B., Yu, D., and Bian, M. (2021). Roles of tRNA Metabolism in Aging and Lifespan. *Cel Death Dis.* 12 (6), 548. doi:10.1038/s41419-021-03838-x

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Hijacking Cellular Stress Responses to Promote Lifespan

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Organisms are constantly exposed to stress both from the external environment and internally within the cell. To maintain cellular homeostasis under different environmental and physiological conditions, cell have adapted various stress response signaling pathways, such as the heat shock response (HSR), unfolded protein responses of the mitochondria (UPR^{MT}), and the unfolded protein response of the endoplasmic reticulum (UPR^{ER}). As cells grow older, all cellular stress responses have been shown to deteriorate, which is a major cause for the physiological consequences of aging and the development of numerous age-associated diseases. In contrast, elevated stress responses are often associated with lifespan extension and amelioration of degenerative diseases in different model organisms, including *C. elegans*. Activating cellular stress response pathways could be considered as an effective intervention to alleviate the burden of aging by restoring function of essential damage-clearing machinery, including the ubiquitin-proteosome system, chaperones, and autophagy. Here, we provide an overview of newly emerging concepts of these stress response pathways in healthy aging and longevity with a focus on the model organism, *C. elegans*.

Keywords: mitochondria, endoplasmic reticulum, heat-shock, stress, aging, *C. elegans*

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INTRODUCTION

Rejuvenation of human life by reducing the damaging effects of aging is one of the trending focuses of current biological research across the globe. Aging is an obvious physiological condition for all living organisms on this planet. It has been designated as a cumulative impairment of different cellular events, which are mainly associated with the maintenance of cellular homeostasis. This frailty condition, broadly influenced by various stressors or genetic factors, leads to the development of major disease conditions including diabetes, neurodegenerative disorders, cardiovascular disorders, and cancer, all of which together increases the vulnerability to death (Franceschi et al., 2018). In 2014, Carlos López-Otín et al. has characterized nine major factors as the hallmark of aging: genomic instability, telomere attrition, epigenetic alterations, and loss of protein homeostasis (proteostasis) categorized as the primary hallmarks of aging; stem cell exhaustion and altered intercellular communication referred to as integrative hallmarks; and–mitochondrial dysfunction, deregulated nutrient sensing, and cellular senescence categorized as antagonistic hallmarks (López-Otín et al., 2013).

Stress, generated from different endogenous or exogenous sources can disturb homeostasis across cellular compartments, contribute to cellular dysfunction, and impact the aging process (Li et al., 2021). As such, living organisms have acquired different unique cellular signaling pathways through evolution to cope with the constant exposure of different internal or external stresses (Kirkwood and Austad 2000). These responses generally include the activation of a widespread transcriptional program to promote genes important for restoring cellular homeostasis. These include the heat-

shock response (HSR) and organelle-specific stress response pathways like the endoplasmic reticulum unfolded protein response (UPR^{ER}) and the mitochondrial unfolded protein response (UPR^{MT}), which have been studied in different organisms, including *Caenorhabditis elegans* (Kenyon 2010; Kourtis and Tavernarakis 2011). Often, it is the breakdown or dysregulation of these important cellular quality control and stress response machineries that are causative of aging. For example, in neurodegenerative disorders like Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD), misfolded protein accumulation caused by compromised activation of stress responses lead to decreased health. In addition, dysfunction of different organelles such as the mitochondria and endoplasmic reticulum under these conditions can also facilitate neurodegenerative diseases and metabolic disorders (Ozcan and Tabas 2012; Hartl 2017; Colla 2019; Misrani et al., 2021).

Using *C. elegans* as a model system, research over the last few decades has established the importance of the activation of cellular stress response pathways in healthy life. Stimulation of stress response pathways can restore cellular homeostasis and reduce the risk of the manifestation of age-related diseases, so lengthening life span. In this context, short exposure to mild stress can be beneficial to organismal health and longevity. Termed hormesis, early exposure to stress can activate critical cellular stress responses that can mitigate the accumulation of damage at advanced age (Rattan 2004). Many of these pathways, which include the HSR, UPR^{MT}, and UPR^{ER} have originally been identified in *C. elegans*, again highlighting the strength of this model in stress biology. In this review, we sum up our latest understanding on the significance of successful stress response pathways in longevity, distinctly emphasizing the model organism *C. elegans*.

The Cytosolic Heat-Shock Response

In 1962 an accidental finding by an Italian scientist Ferruccio Ritossa opens a new window in biological research. He observed a different and unique puffing pattern in the polytene chromosome of the salivary cells of the fruit fly, *Drosophila* busckii under an elevated temperature condition (Ritossa 1962; Ritossa 1996). That unknown chromosomal puff was later identified as the active transcriptional region for the synthesis of a special group of proteins known as heat shock proteins, which maintain proteostasis in a cell by facilitating protein stabilization, refolding of misfolded protein structure, protein translocation, and degradation of toxic protein aggregates (Tissiéres et al., 1974; Lindquist and Craig 1988; Anckar and Sistonen 2011). These heat shock proteins are conserved across living organisms and considered as the principal functional unit for the HSR. Heat shock proteins are widely grouped into two families based on their molecular weight: First, the large ATP-dependent heat shock proteins of molecular mass between 40 and 105 kDa, including the major chaperone proteins, HSP70, HSP90, etc., and second, the small heat shock proteins of molecular mass between 8 and 25 kDa, which includes HSP27, HSP25, ubiquitin, and others (Jee 2016).

Most protein chaperones share similarities in their function. However, they differ in respect to their cellular localization, substrate specificity, and mechanistic details. The HSP70 family make up the most abundant chaperone proteins present in the cell. The cytosolic HSP70 has several homologs, which are present in different subcellular compartments including the heat shock cognate 70 (HSC70) present in the cytosol along with HSP70 and GRP78/BiP present in the endoplasmic reticulum (Radons 2016). HSP90 is another ATP-dependent chaperone protein that is constitutively expressed in cells and found in different cellular compartments like the cytosol, mitochondria, endoplasmic reticulum, nucleus etc. HSP90 shows the specificity to bind with a large number of misfolded proteins and provide support to refold to their functional state (Schopf et al., 2017). In contrast to the ATP-dependent chaperone proteins, small heat shock proteins function in an ATP-independent manner. These small heat shock proteins such as HSP27 and αβ-crystallin show induced expression in response to stress. During proteotoxic stress, they bind with misfolded client proteins and block further misfolding and/or the formation of misfolded protein aggregates until the clients are delivered to repairing chaperones like HSP70 and HSP40 (Bakthisaran et al., 18542015; Schopf et al., 2017). In addition to protein refolding, many molecular chaperones also participate in proteasomal degradation. For example, chaperone molecules can couple with ubiquitin ligase proteins that facilitate the polyubiquitination of misfolded proteins and prepare them for proteasomal degradation.

HSF1 and the Heat Shock Response

Heat shock proteins are synthesized in a large amount immediately after sensing stress, such as elevated temperature, hypoxia, exposure to heavy metals, etc. This universally conserved cellular stress response is orchestrated by a key transcription factor known as heat shock factor 1 or simply HSF1 (Anckar and Sistonen 2011). In invertebrates, the heat shock factor is encoded by a single gene, whereas in vertebrates, four major heat shock factor isoforms, HSF1, HSF2, HSF3, and HSF4 have evolved. In the case of both vertebrates and invertebrates, HSF1 plays the major decisive role in the activation of the HSR while the other isoforms that exist only in vertebrates are less studied and found to show some unique and sometimes tissue-specific functions. Interestingly, while HSF1, HSF2, and HSF4 have been found in all vertebrates, HSF3 was specifically observed in avian species only (Nakai et al., 1995). HSF2 plays an important role in female fertility, spermatogenesis, and early development, whereas HSF4 participates in eye lens development (Schuetz et al., 1991; Nakai et al., 1997).

HSF1 is made of 529 amino acids and its structure consists of four major structural/functional domains: the DNA binding domain (DBD), oligomerization domain, regulatory domain (RD), and transactivation domain (TAD) (**Figure 1A**). The DBD is present at the amino-terminal end of HSF1 and forms a helix turn helix structure. Through this domain, HSF1 binds with its specific DNA sequence and that association is stabilized by the interaction between the amphipathic helical region and the hydrophobic DNA pocket (Neudegger et al., 2016). Next to the

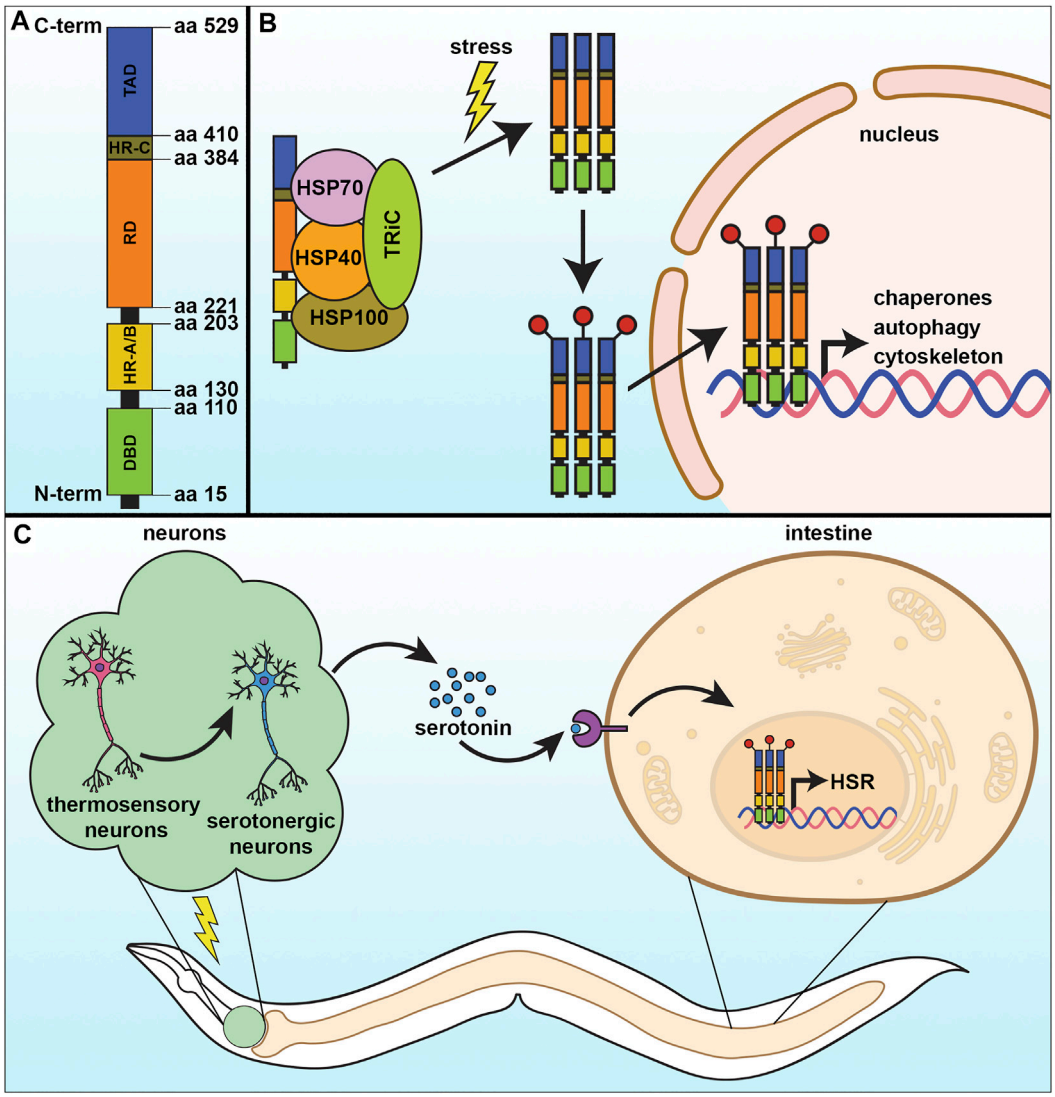

FIGURE 1 | The cytosolic heat-shock response. **(A)** The heat-shock response (HSR) is regulated by the transcription factor, HSF-1, which contains four major structural domains: the DNA-binding domain (DBD), regulatory domain (RD), trans-activating domain (TAD), and the oligomerization domain divided into HR-A and HR-B. **(B)** Under basal conditions, HSF-1 is bound by regulatory proteins that prevent its activation. Upon stress, these proteins are released from HSF-1 as they are titrated away to serve as molecular chaperones to damaged proteins, allowing HSF-1 to trimerize and serve as a transcription factor to activate genes important to mitigate damage and increase survival. **(C)** The HSR can be communicated in a non-autonomous manner, whereby thermosensory and serotonergic neurons can communicate to peripheral tissue, including the intestine, to activate a systemic HSR and promote organismal health and lifespan.

DBD, the alpha-helix rich oligomerization domain is situated through which one HSF1 molecule interacts with another (Peteranderl et al., 1999). That oligomerization domain is further divided into two subdomains HR-A and HR-B. A separate and similar domain HR-C is present between the transactivation domain and the regulatory domain that helps to inhibit random oligomerization of HSF1 (Anckar and Sistonen 2011; Rabindran et al., 1993). The regulatory domain, present alongside the oligomerization domain (between HR-A/HR-B and HR-C) controls the transactivation of HSF1 and is the site for regulatory post-translational modifications, such as phosphorylation, acetylation/deacetylation, and sumoylation (Anckar and Sistonen 2011). Ultimately at the C-terminus of the HSF1 protein is the TAD, which facilitates the transcriptional activation of its target genes. However, this is the most structurally unknown part of the protein to date. In normal growth conditions, monomeric HSF1 exists as an inactive form in the cytoplasm in association with a complex of some regulatory proteins such as HSP70, HSP90, and TRiC. These molecular chaperones act as a negative regulator for HSF1 function (Anckar and Sistonen 2011; Shi et al., 1998; Neef et al., 2014). In response to stress, the monomeric HSF1 is released from its inhibitory complex and trimerizes, allowing for a series of post-translational modifications which help in its nuclear translocation and conversion to an active DNA binding component (**Figure 1B**). Those post-translational modifications

include phosphorylation, sumoylation, acetylation, and deacetylation, which are vital to the activation-attenuation cycle of HSF1 (Anckar and Sistonen 2011; Dai 2018). HSF1 binds to a conserved pentameric sequence motif termed the heat shock element or HSE (5′nGAAn3′) at the promoter of its target genes, which include the heat shock proteins, autophagy, actin, and innate immunity (Anckar and Sistonen 2011).

Heat Shock Response and Aging

As a critical player in cellular stress response, HSF1 function can have a direct impact on physiological health. However, like most quality control mechanisms, HSF1 activity, and the HSR deteriorate during the aging process. Specifically, the HSR in *C. elegans* exhibits decreased transcriptional output early in adulthood (Labbadia and Morimoto 2015). Shockingly, there is a precipitous drop in the induction of HSR genes (including *hsp-70* and *hsp-16.11*) in response to thermal stress within a narrow 4-h window that coincides with the start of egg-laying. Interestingly, this collapse of the HSR was not due to the decreased binding ability of HSF-1, but rather due to profound changes to the chromatin landscape due to the overt decline in expression of the histone demethylase *jmjd-3.1*. This decreased functional output of the HSR has direct physiological ramifications, as animals exhibit an age-dependent decrease in thermotolerance and an increase in protein aggregation load (Morley et al., 2002; Ben-Zvi et al., 2009). The repression of HSR can be compensated by increased expression of *hsf-1* to diminish the burden of proteotoxicity at advanced age to improve lifespan (Morley and Morimoto 2004; Ben-Zvi et al., 2009; Shemesh et al., 2013). Specifically, overexpression of *hsf-1* can significantly extend lifespan (Hsu et al., 2003; Baird et al., 2014), whereas downregulation shortens lifespan and results in premature aging phenotypes (Garigan et al., 2002). Knockdown of the downstream targets of HSF-1, including the heat shock proteins, HSP70 and HSP90 also decreases lifespan (Morley and Morimoto 2004), whereas long-lived mutants including *daf-2* and *age-1* show increased expression of these chaperones (Hsu et al., 2003; McElwee et al., 2003; Murphy et al., 2003), providing further evidence that HSF-1-mediated stress resilience is important for longevity. In fact, longevity can actually be predicted by the expression of a single target of HSF-1, *hsp-16.2* (Mendenhall et al., 2012). Importantly, HSF-1 has a beneficial effect on multiple tissue types, as overexpression solely in neurons, the body wall muscle, or intestinal cells was sufficient to promote lifespan extension (Morley and Morimoto 2004). Finally, *hsf-1* has a temporal requirement in lifespan extension, whereby its expression during development impacts lifespan, whereas overexpression later in adulthood has no effect (Volovik et al., 2012).

Beyond just an ectopic expression of *hsf-1*, activation of the HSR through exposure to low levels of thermal stress have also been shown to impact longevity (Butov et al., 2001). Termed hormesis, this described the phenomenon whereby low-grade exposure to stress can be beneficial to organismal health by activating critical stress response pathways. While these beneficial effects of hormesis were primarily ascribed to heatshock proteins and DAF-16 (insulin growth factor) signaling (Cypser and Johnson 2002; Hsu et al., 2003; Morley and Morimoto 2004; Rattan 2005), it is becoming increasingly clear that heatshock proteins are not the only effector molecules. Based on several genome-scale studies, it has been recognized that HSF1 has a much broader functional spectrum that can control numerous molecular events associated with cellular quality control pathways, including ubiquitin-mediated proteasomal degradation, autophagy, and the maintenance of organelles including mitochondria, ER, and the actin cytoskeleton (Barna et al., 2018). In fact, hypomorphic mutations of *hsf-1* and perturbations of some HSR chaperones had no impact on thermotolerance (McColl et al., 2010; Kourtis et al., 2012). One study even found that overexpression of *hsf-1* with a truncation in the C-terminal transactivating domain dramatically extended lifespan, despite having no impact on the induction of heat-shock proteins (Baird et al., 2014). Instead, this variant of HSF-1 increased the expression of *pat-10*, a troponin-like calcium-binding protein that promoted actin cytoskeletal maintenance and function. This increase in cytoskeletal integrity at advanced age directly impacted organismal health and promoted longevity. Similar to the induction of heat-shock proteins (Morley and Morimoto 2004), HSF-1's beneficial impact on the cytoskeleton independently affected multiple cell types to promote lifespan (Higuchi-Sanabria et al., 2018a).

HSF-1 has also been shown to function both alongside and as a regulator of autophagy. It can bind directly to the promoter of the autophagy component, ATG7, and induce its expression, which is independent of the canonical function of HSF1 (Desai et al., 2013). Furthermore, it has been also reported that casein kinase 1 phosphorylates the essential autophagy receptor SQSTM1/p62 to lift up the selective autophagic clearance in an HSF1 dependent manner (Watanabe et al., 2017). Induction and activation of the SQSTM1/p62 receptor is sufficient to induce autophagy in distinct tissues and for longevity (Kumsta et al., 2019). A recent study in *C. elegans* has explored the induction of HSF-1 by hormetic heat stress exposure, which can clear the accumulation of PolyQ aggregates and contributes to enhanced stress resistance and extended lifespan (Kumsta et al., 2017). Similarly, overexpression of *hsf-1* is sufficient to induce autophagy and induction of autophagy is required for the beneficial effects of HSF-1. However, a conflicting study found that HSF-1 can actually prevent autophagy by promoting HSP70 expression, which can inhibit starvation and rapamycin-induced autophagy (Dokladny et al., 2013). These seemingly contradicting results may be due to differences in thermal stress conditions. For example, it is entirely possible that under conditions of acute stress, HSF-1 can coordinate heat-shock proteins and chaperones to mitigate damage by protein remodeling. In contrast, under chronic stress when damage exceeds the repair capacity of chaperones, autophagy is essential. Regardless, it is clear that a proper balance between HSR and autophagy is essential for proper maintenance of homeostasis. Indeed, HSR and autophagy can be delicately balanced and coordinated under specific environmental stress and metabolic cues via the homeodomain interacting protein kinase, HPK-1 (Das et al., 2017). Specifically, HPK-1 sits at the center of a dual

proteostatic network whereby it is essential for the induction of autophagy gene expression in response to dietary restriction and inactivation of mTOR, but also inhibits sumoylation of HSF-1 to promote its transcriptional activity upon thermal stress.

Studies about the cross-talk between the proteostatic network and immune response of an organism revealed that long-lived *C. elegans* mutants showed higher resistance to bacterial pathogens along with increased longevity (Garsin et al., 2003). In fact, one of the primary causes of death in C. elelgans is due to pathogenic invasion (Zhao et al., 2017), suggesting that factors that there may be heavy overlap between factors that increase lifespan and immunity. Thus, it is not surprising that HSF-1 has also been implicated in immune response. Specifically in *C. elegans*, HSP90 and the small heat shock proteins are important for the development of immunity. Interestingly, this HSF-1-mediated defense response did not require p38 MAPK, but recruits the DAF16 pathway for the development of multi-pathogen resistance (Singh and Aballay 2006). Similar to autophagy, this beneficial effect of the HSR is not limited to *hsf-1* overexpression and hormetic heat-shock can stimulate the immune response to promote resistance to pathogen exposure (Prithika et al., 2016). In higher eukaryotes with more complex immune systems, the function of induced heat shock proteins is not only limited to ameliorating inflammatory damage, but also encourage the production of anti-inflammatory cytokines (van Eden et al., 2005). HSF1 has been found as a transcriptional activator for the expression of interleukin 10 (IL10) which inhibits the bacterial lipopolysaccharide-mediated production of pro-inflammatory cytokines like TNFα, IL-12, IL-1b, IFNg (Zhang et al., 2012). Furthermore, the inflammatory stress-dependent and the HSF1 mediated induction of heat shock proteins plays an essential role in preparing peptides for proper antigen presentation by $CD8^+$ T lymphocytes (Binder and Srivastava 2005).

Neuronal Transmission of the Heat Shock Response

In metazoans, the HSR can also be activated non-autonomously irrespective of the presence of thermal stress. It has been reported in rats for the first time by Fawcett et al. that HSF1 activation can be governed by the nervous system in the absence of environmental stress. That study revealed that controlled stress conditions release adrenocorticotropin from the pituitary gland of Wistar rats that facilitate HSF1 trimerization and increase its DNA binding activity, which consequently induces HSP70 expression in adrenal tissue (Fawcett et al., 1994). Later, a similar mechanism involving the nervous system in proteostatic regulation has been observed in *C. elegans*, where the HSR of somatic cells was found to be controlled in a cell non-autonomous manner by two thermosensory AFD neurons, GCY-3, and TTX-3 (**Figure 1C**) (Prahlad et al., 2008a). Optogenetic stimulation of the AFD neurons communicates with two serotonergic neurons ADF and NSM that releases serotonin, which was sufficient to activate HSF1 in another cell. In the receiving cell, the serotonin receptor SER-1 drove increased synthesis of chaperone proteins. They firmly established that activation of serotonergic neurons is sufficient to reduce a load of misfolded protein accumulation in *C. elegans* (Tatum et al., 2015). To survive in nature an organism needs to have the ability to react rapidly to environmental challenges. Thus, it is not surprising that organisms with a developed nervous system have neurons which can sense stress and relay a signal to other tissue. Indeed, neurons can also sense an olfactory experience to prime the function of HSF1 to provide a rapid and stronger response to subsequent exposure to any proteotoxic agents (Ooi and Prahlad 2017). Stimulation of the olfactory neurons by the odorants produced by the toxic bacterium *P. aeruginosa* can enhance the pathogen-avoiding ability in *C. elegans* in an HSF-1 and serotonin-signaling dependent manner.

Importantly, one study found that overexpression of *hsf-1* in neurons was sufficient to drive increased HSR and DAF-16/FOXO activity in peripheral tissue (Douglas et al., 2015). This combined activation of HSF-1 and DAF-16 targets was sufficient to increase thermotolerance and lifespan, which was similar to the increase in lifespan found in animals with reduced insulin signaling (Son et al., 2018). In addition, neuronal *hsf-1* was sufficient to drive protection of the actin cytoskeleton in multiple cell types during aging, including the muscle, hypodermis, and intestine, which was equally important for the increased longevity of these animals. This is curious considering the previously identified involvement of integrins in actin function (Tharp et al., 2021) and HSF-1-mediated depletion of integrin-linked kinase in increased stress resistance and longevity in *C. elegans* (Kumsta et al., 2014). These data beg the question of whether non-autonomous HSF-1 signaling could potentially utilize similar integrin signaling mechanisms to drive longevity. Finally, neuronal *hsf-1* signaling also directly impacts fat metabolism and results in extensive fat remodeling. Specifically, neuronal *hsf-1* results in decreased expression of the fat desaturases *fat-6/fat-7*, while activating the expression of catabolic lysosomal lipases, which shifts the fatty acid composition of the plasma membrane to a more saturated state. This change in fat mimics those downstream of exposure to thermal stress, and is sufficient to drive long-term survival of animals at elevated temperature (Chauve et al., 2021).

Beyond nonautonomous signaling from neurons to periphery, a recent study has found that serotonin can actually transmit HSF-1 signaling to future progeny (Das et al., 2020). Serotonin, released from the maternal neurons can ensure higher longevity and stress resilience in their future offspring through HSF-1 activation in germ cells. Specifically, serotonin signaling promotes protein kinase A (PKA)-dependent modification of HSF-1, increasing the occupancy of RNA Pol II and HSF1 at the promoter of various protective genes, including molecular chaperones which are the targets of HSF1 in response to even minimum heat stress. In addition, HSF-1 promotes the recruitment of a chromatin remodeler FACT (Facilitates Chromatin Transcription) to alter histone dynamics to initiate transcription. This mechanistic pathway is also conserved in mammalian cells. Overall, these studies highlight the numerous pathways HSF-1 modulates to impact organismal health and lifespan.

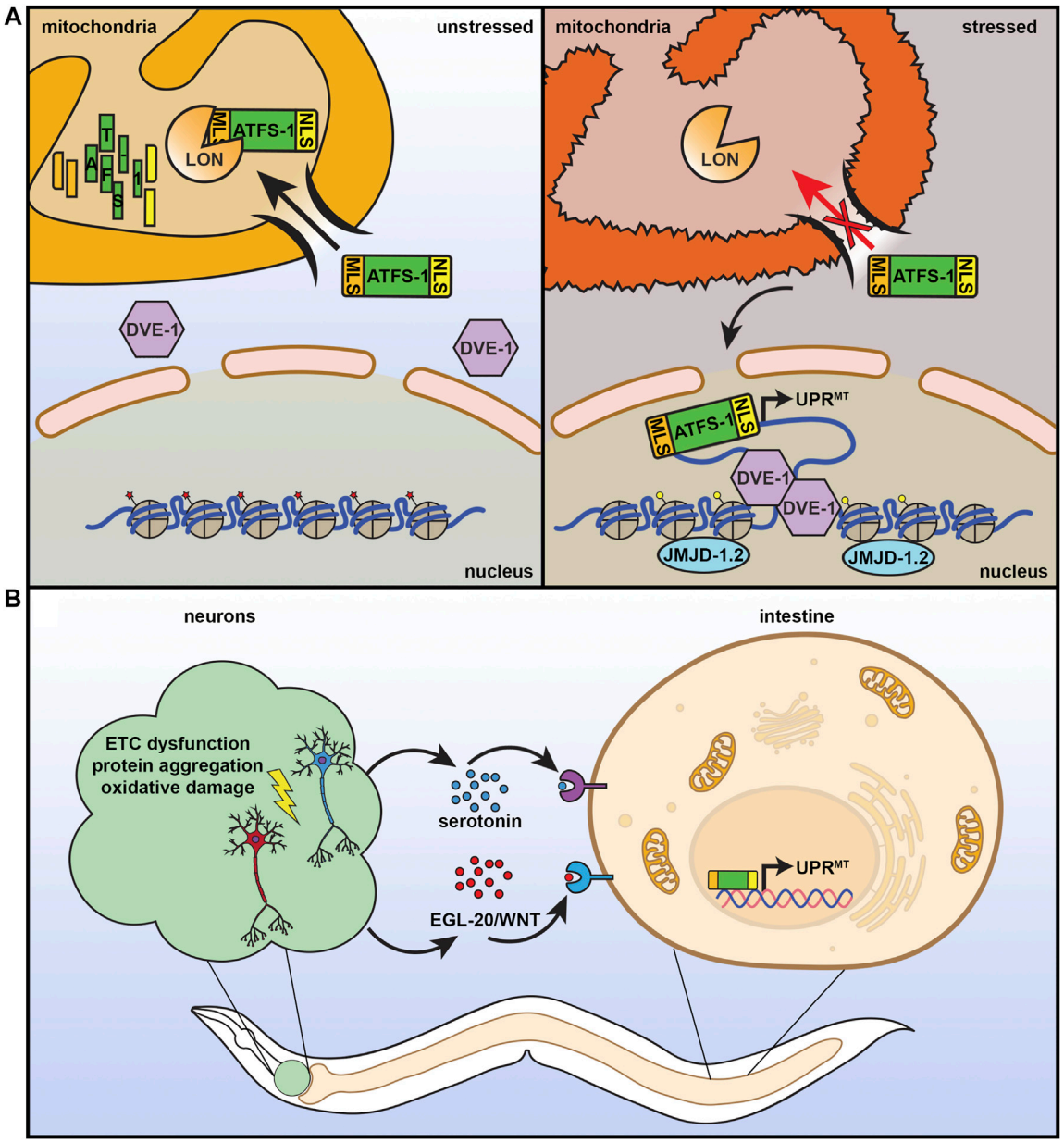

FIGURE 2 | The mitochondrial unfolded protein response. **(A)** ATFS-1 is a unique protein that can serve as a sensor for mitochondrial health and fitness. It contains both a nuclear localization signal (NLS) and a mitochondrial localization signal (MLS). Under basal, unstressed conditions, ATFS-1 is imported into the mitochondria where it is degraded by LON protease. Under conditions of mitochondrial stress or damage, mitochondrial import decreases, allowing ATFS-1 to instead accumulate in the mitochondria where it can activate UPR^{MT} with additional transcriptional regulators including DVE-1 and chromatin regulator JMJD-1.2. **(B)** Similar to the HSR, UPR^{MT} can also be communicated in a nonautonomous manner. Neurons that experience mitochondrial stress can signal to the peripheral tissue, including the intestine, through serotonin and WNT signaling to result in systemic activation of UPR^{MT}, increased stress resilience, and increased lifespan.

Perspectives and Concluding Remarks

Beyond its clear role in regulating the HSR, HSF-1 has now been ascribed to several other equally important processes, including autophagy, immune response, and maintenance of the cytoskeleton. Considering its numerous functions, one intriguing question is how this single transcription factor can coordinate such diverse processes, and whether exposure to specific types of stressors can titrate HSF-1 to promoters of appropriate genes. For example, upon deletion of its C-terminal domain, HSF-1 can no longer induce heat-shock proteins, but strongly upregulates genes involved in cytoskeletal maintenance (Baird et al., 2014). It is possible that the C-terminal domain contains an important residue that allows for coordination with other transcriptional regulators recruits HSF-1 to heat-shock proteins, and loss of this domain causes increased accumulation of HSF-1 to cytosolic targets. Still to be

understood is the identity of these potential cofactors that would allow HSF-1 to be targeted to specific gene loci.

Another intriguing question is how all the functional roles of HSF-1 are coordinated. To date, separate studies have shown that the induction of chaperones (Morley and Morimoto 2004), cytoskeletal function (Higuchi-Sanabria et al., 2018b), and autophagy (Kumsta et al., 2017) are all necessary for the beneficial effects of HSF-1 activation, such that perturbing any individually can completely abrogate HSF-1-mediated longevity. And while studies with the C-terminal deletion of HSF-1 showed that cytoskeletal regulation can be separated from chaperone induction (Baird et al., 2014), whether the beneficial effects of this HSF-1 variant requires autophagy has yet to be identified. Importantly, all these processes have been shown to be induced upon exposure to heat-shock, suggesting that the majority of HSF-1 targets are simultaneously induced, rather than being separable. Thus, are these seemingly divergent responses actually distinct mechanisms, or all parts of the same pathway? Indeed, all of these pathways do converge into a similar goal: increased proteostasis through protein folding by chaperones, clearance of damaged proteins through autophagy, and even the actin cytoskeleton has clear implications in protein homeostasis (Gross and Kinzy 2007).

The Mitochondrial Unfolded Protein Response

Mitochondrial fitness and function are inarguably significant to cell viability, due to their numerous functions, including energy production, regulating apoptotic and necrotic cell death, storing calcium and amino acids, lipid oxidation, and heat production. Mitochondrial dysfunction is linked to aging and several age-related diseases, including Alzheimer's disease, Parkinson's, and metabolic syndrome (Higuchi-Sanabria et al., 2018c; Moehle et al., 2019). This means that proper functioning of mitochondria is essential for a healthy physiological state. However, almost counterintuitively, a growing number of studies have shown that perturbations to mitochondrial function can actually lead to lifespan extension (Dillin et al., 2002; Liu et al., 2005; Owusu-Ansah et al., 2013; Yee et al., 2014). The primary reason for these seemingly contradictory observations is that impairment of mitochondria triggers the unfolded protein response of the mitochondria (UPR^{MT}), which results in beneficial effects on organismal health (Durieux et al., 2011).

Considering the importance of mitochondria to cellular health, it is not surprising that cells have adapted many quality control mechanisms to protect mitochondrial function, including the UPR^{MT}. UPR^{MT} is robustly activated by a variety of sources of mitochondrial stress: stoichiometric imbalance between proteins coded in the nuclear or mitochondrial genome (Couvillion et al., 2016; Houtkooper et al., 2013; Molenaars et al., 2020), impaired electron transport chain (ETC) function (Khalimonchuk et al., 2007; Zara et al., 2007; He et al., 2018), mitochondrial protein aggregation (Hallberg et al., 1993; Dubaquié et al., 1998), defects in mitochondrial import (Rolland et al., 2019; Xin et al., 2020), loss of mitochondrial membrane potential (Tharp et al., 2021), or disrupting mitochondrial translation (Nolden et al., 2005) and DNA replication (Kenny and Germain 2017). This process is best understood in *C. elegans*, where UPR^{MT} is regulated by several transcriptional regulators (**Figure 2A**). One specific transcription factor, ATFS-1, serves as a signal between the mitochondria and the nucleus. ATFS-1 contains both a nuclear localization sequence and a mitochondrial signal sequence and is preferentially imported into the mitochondria where it is subsequently degraded by mitochondrial Lon proteases. When mitochondrial import is compromised, such as under conditions of stress, ATFS-1 import is reduced and can accumulate in the nucleus to activate UPR^{MT} (Nargund et al., 2012). Several other transcriptional regulators work either in concert with, or independent of, ATFS-1 to regulate UPR^{MT}, including the transcription factor DVE-1 and its ubiquitin-like cofactor UBL-5 (Benedetti et al., 2006; Haynes et al., 2007). Nuclear localization and transcriptional activation by DVE-1 is further promoted by the histone methyltransferase MET-2 and its cofactor LIN-65, which also induce epigenetic changes required for transmission of mitochondrial stress signaling between cells and through generations (Tian et al., 2016). Finally, histone demethylases JMJD-1.2 and JMJD-3.1 remodel chromatin to facilitate access to promoters of numerous UPR^{MT} target genes (Merkwirth et al., 2016).

At the genetic level, UPR^{MT} is characterized by a coordinated activation of genes essential for restoring mitochondrial fitness and function. Mitochondrial chaperones, such as Hsp70 and Hsp60/10 can refold misfolded proteins (Castro et al., 2018) and proteases, such as LONP and ClpP can degrade misfolded proteins (Wang et al., 1993) to alleviate proteotoxic stress. Importantly, proteins involved in mitochondrial import are also upregulated to ensure proper import of essential protein homeostatic machinery (Xin et al., 2020). UPR^{MT} targets are not only limited to protein homeostasis, as genes involved in immunity (Pellegrino et al., 2014), autophagy (Haeussler et al., 2020), and xenobiotic stress (Nargund et al., 2012; Nargund et al., 2015) are also induced. Moreover, direct applications of stress to different mitochondrial processes with drugs results in vast differences in transcriptional response (Quirós et al., 2017), thus highlighting the complexities of mitochondrial stress signaling and regulation (refer to (Bar-Ziv et al., 2020a) for a more thorough review). Here, we focus primarily on the impact of UPR^{MT} on aging in the model organism, *C. elegans*.

UPR^{MT} and Aging: Mitochondrial Hormesis Through Inhibition of the Electron Transport Chain

The UPR^{MT} was first used to describe a transcriptional response whereby loss of the mitochondrial genome led to increased expression of mitochondrial chaperones. This process was originally identified in mammalian cells and was named after its presumed effects on mitochondrial protein folding (Martinus et al., 1996). Further studies identified additional targets for UPR^{MT} activation, which include ROS detoxification, mitochondrial import, and glycolysis (Zhao et al., 2002). Since then, it has been heavily studied in various model systems, most notably in *C. elegans*, as a beneficial response to stress. In this model coined "mitohormesis", exposure to low grade stress to the mitochondria results in the induction of an adaptive program that

can benefit both lifespan and healthspan (Ristow and Zarse 2010). In *C. elegans*, the phenomenon of mitohormesis was first identified in a large-scale RNAi screen of genes found on chromosome I. In this study, RNAi knockdown of three components of the mitochondrial ETC, mitochondrial ATP synthase (*atp-1*), NADH/ubiquinone oxidoreductase (*nuo-2*), and cytochrome c reductase (*cco-1*/*cox-5b*) all resulted in reduced body size and extended lifespan (Dillin et al., 2002). A similar RNAi screen of nearly all the genes on chromosomes I and II consistently found that knockdown of a large number of mitochondrial genes extended lifespan, including cytochrome C oxidase VIIc (*cox-7c*), mitochondrial ribosomal protein L47 (*mrpl-47*), and the mitochondrial solute carrier family 25 member 32 (*slc-25A32*) (Lee et al., 2003). This study also performed a classical forward genetic screen and identified a mitochondrial leucyl-tRNA synthetase (*lrs-2*), which similarly extends lifespan when disrupted. Since then, multiple perturbations to mitochondrial function have demonstrated a positive impact on lifespan: mitochondrial ribosomal protein knockdown (Houtkooper et al., 2013), perturbing mitochondrial dynamics in favor of mitochondrial fusion (Chaudhari and Kipreos 2017), mitochondrial genome impairment (Tsang and Lemire 2002), and many others reviewed in (Munkácsy and Rea 2014).

An intriguing phenomenon in *C. elegans* is that there is a specific temporal requirement during development to enact the positive benefits of mitohormesis. That is, animals exposed to RNAi knockdown of ETC components during development exhibited lifespan extension, even if normal levels of ETC component expression were restored at adulthood (Li et al., 2021). Conversely, reducing levels of ETC components in adulthood had no impact on lifespan. These data suggested that a long-lasting signature exists to elicit lifespan extension following mitochondrial dysfunction. This specific signal is initiated during the L3/L4 larval stage of development (Dillin et al., 2002; Rea et al., 2007). Interestingly, this phenomenon also had a dose-dependent effect. Titrating the RNAi of five genes encoding mitochondrial proteins *atp-3*, *nuo-2*, *isp-1*, *cco-1*, and *frh-1* showed that there was a consistent three-phase lifespan response: low levels of knockdown had no effect, but as expression reduced, lifespan extension lengthened until at the highest level of knockdown, lifespan actually shortened for some conditions (Rea et al., 2007).

UPR^{MT} and Aging: Chromatin Changes Result in Long-Term Effects

The most obvious response to mitochondrial stress is the massive and persistent restructuring of gene expression, which is a hallmark of UPR^{MT} activation. In *C. elegans*, this process is mediated by the converted effort of the transcriptional regulators ATFS-1, DVE-1, and UBL-5 (Benedetti et al., 2006; Haynes et al., 2007; Nargund et al., 2012). However, the existence of a long-term hormesis suggested that a more permanent signature must exist upon exposure to mitochondrial stress. One study identified the methyltransferase MET-2 and a nuclear cofactor LIN-65 are required for UPR^{MT} activation and lifespan extension found in animals with exposure to mitochondrial stress (*cco-1* knockdown or exposure to aggregation prone polyQ described further below) (Tian et al., 2016). Specifically, 1,264 of the 1,312 genes differentially expressed under *cco-1* knockdown were dependent on the functional activity of either MET-2 or LIN-65. During mitochondrial stress, MET-2 produces H3K9me1/2. Nuclear localization of LIN-65 then deposits H3K9me2 subunits onto the chromatin, titrating DVE-1 to loose regions of the chromatin and allowing for sustained ATFS-1 localization at these loci. Thus, loss of any of these factors under stress could result in decreased UPR^{MT} activity and loss of lifespan extension.

A complementary study identified another chromatin modifier as a critical regulator of UPR^{MT}-induced longevity (Merkwirth et al., 2016). This study performed a screen to identify genes required for the lifespan extension found in *cco-1* knockdown animals and identified the gene encoding the histone demethylase, *jmjd-1.2*. Knockdown of *jmjd-1.2* decreased lifespan and suppressed cco-1 induced lifespan extension, similar to *met-2* and *lin-65*. Importantly, overexpression of *jmjd-1.2* was sufficient to induce UPR^{MT} and promote longevity. This was the first study that showed that hyperactivation of UPR^{MT} in the absence of stress was sufficient to extend lifespan. Because JMJD-1.2 functions downstream of mitochondrial stress, its overexpression bypasses the need to cause damage to the mitochondria to induce UPR^{MT}.

UPR^{MT} and Aging: A Conundrum and Paradox

An unaddressed conundrum is that the targets of UPR^{MT} include a large number of chaperones and other protein processing components that must be imported into the mitochondria to serve their function to restore organellar homeostasis. However, the dogma for UPR^{MT} activation is that decreased mitochondrial membrane potential drives the mitochondria-to-nuclear signaling by preferential localization of ATFS-1 to the nucleus due to failed entry in the mitochondria. How then do proteins get imported into the mitochondria when membrane potential is compromised? In an exciting piece of work, this paradox was addressed by finding that the induction of UPR^{MT} can actually increase mitochondrial import, despite a loss of membrane potential (Xin et al., 2020). The efficiency of import is increased by upregulating components of the mitochondrial import machinery, including *timm-17*, *timm-23*, *tomm-20*, *tomm-22*, and *tomm-40*. In addition, because ATFS-1 has a weak MTS, it continues to fail to enter the mitochondria and can continue to drive UPR^{MT} induction until mitochondrial function is fully restored.

Interestingly, lifespan was never reportedly increased in response to knockdown of complex II subunits (Ichimiya et al., 2002; Rea et al., 2007; Kuang and Ebert 2012). In addition, a screen for novel regulators of UPR^{MT} identified 19 genes that when knocked down induce UPR^{MT} but showed no correlation for lifespan extension. In fact, 6 out of the 19 RNAi conditions actually decreased lifespan, while 3 showed no

significant effect (Bennett et al., 2014). Notably, all the genes that decreased lifespan were important for mitochondrial import, which a previous study has implicated as necessary for any beneficial effects of UPR^{MT} (Xin et al., 2020). Thus, it is entirely possible that when mitochondrial import is reduced, there is robust activation of UPR^{MT} without a beneficial impact on organismal health, which would likely require import of newly synthesized mitochondrial protein homeostasis machinery. However, a striking finding in the study was that the lifespan extension found in several conditions of mitochondrial stress induction (RNAi knockdown of transaldolase *tald-1*, mitochondrial transmembrane protein *letm-1*, *cco-1*, and *isp-1*) were all independent of *atfs-1*. Moreover, constitutive activation of *atfs-1* did not improve lifespan, but instead shortened it, despite measurable activation of UPR^{MT} (Bennett et al., 2014), although it is unclear whether atfs-1 activation may cause unexpected and detrimental consequences to mitochondrial quality (Shpilka et al., 2021). In another study, loss of *atfs-1* during adulthood did not affect lifespan extension of *clk-1*, *isp-1*, or *nuo-6* loss of function animals (Wu et al., 2018), consistent with previous findings that the beneficial impact of mitochondrial stress may be important only during development (Durieux et al., 2011). However, deletion of *atfs-1* during development resulted in defects in growth in *clk-1* and *isp-1* loss of function animals, although it did suppress lifespan of *nuo-6* mutants. Overall, these conflicting data argued against the longstanding model that activation of UPR^{MT} improves longevity.

Taken together, it is clear and consistent across numerous studies that mitohormesis is evident: exposure to mitochondrial dysfunction can indeed activate a protective pathway that can promote lifespan extension. However, whether this protective pathway can be fully ascribed to the currently defined model of UPR^{MT} is still up for debate. The response to mitochondrial stress in human cells is complex and context-dependent, as diverse drugs that target different components of the mitochondria show little overlap in transcriptional response (Quirós et al., 2017). Similarly, it is likely that activation of UPR^{MT} in *C. elegans* is equally context dependent, and a broader definition of UPR^{MT} beyond induction of a small subset of chaperones (e.g., *hsp-6* and *hsp-60*) is necessary. Perhaps a more thorough investigation of specific UPR^{MT} targets downstream of multiple transcriptional regulators–and not just ATFS-1–is essential to understand the nuances between seemingly "UPR^{MT}" dependent and independent mechanisms of lifespan extension downstream of mitochondrial dysfunction. For example, many other pathways including activation of hypoxia inducible factor HIF-1 (Kourtis et al., 2012), homeobox protein CEH-24 (Walter et al., 2011), AMP-activated protein kinase AAK-2 (Curtis et al., 2006), and the p53 homolog CEP-1 (Ventura et al., 2009); and the integrated stress response (ISR) critical for responding to mitochondrial stress in higher mammals (reviewed in (Anderson and Haynes 2020)) have all been implicated in longevity in response to mitochondrial stress in *C. elegans*. In addition, there are also gene-diet interactions that alter the impact of UPR^{MT} on longevity (Amin et al., 2020). Thus, in its current state, it is clear that a single mechanism cannot describe all the mitochondrial longevity paradigms and more expansive studies are required to better understand this complex phenomenon.

Neuronal Transmission of UPR^{MT}

Under conditions of stress, multicellular organisms must coordinate a systemic response across diverse cell types, all with their own unique energetic and metabolic demands. Mitochondrial number, activity, protein composition, morphology, and mtDNA contents can all vary across different tissues (Leary et al., 1998), and thus responses to mitochondrial stress are unique in each cell type. In *C. elegans*, exposure to mitochondrial stress specifically in the intestine or neurons results in a significant increase in lifespan, whereas exposure of muscle cells to stress had the opposite effect and shortened lifespan (Durieux et al., 2011), highlighting the significance of context-specific benefits of UPR^{MT} activation. Importantly, applying mitochondrial stress to neurons was sufficient to induce systemic activation of UPR^{MT} due to a neuron-to-periphery cell non-autonomous response, which was critical for lifespan extension (**Figure 2B**) (Leary et al., 1998). This early paradigm of non-autonomous UPR^{MT} involved a "Mjolnor hammer" approach where perturbation of ETC function via *cco-1* knockdown caused severe mitochondrial stress in neurons. However, since then, more physiologically relevant stressors confirmed these findings where overexpression of polyQ40 (and larger polyQ repeats) (Berendzen et al., 2016) or expression of ROS-producing KillerRed fluorescent protein (Shao et al., 2016) specifically in neurons robustly induced UPR^{MT} in peripheral cells. Interestingly, overexpression of amyloid *ß* associated with Alzheimer's disease or mutant TDP-43 associated with amyotrophic lateral sclerosis in neurons failed to induce peripheral UPR^{MT} activation, suggesting that this neuronal stress signaling paradigm was also context specific (Shao et al., 2016). Importantly, neurons can transmit UPR^{MT} signals to the periphery in the absence of stress, as overexpression of *jmjd-1.2* solely in neurons was also able to induce this non-autonomous UPR^{MT} signal and was sufficient to promote longevity (Merkwirth et al., 2016).

The phenomenon whereby neurons can transmit stress signals to the rest of the body is not unique to UPR^{MT}, and has also been described for UPR^{ER} (Taylor and Dillin 2013) and the HSR (Prahlad et al., 2008b; Higuchi-Sanabria et al., 2018b) (see individual sections), although the mechanisms driving these responses seem to be distinct. While UPR^{MT} (Berendzen et al., 2016), UPR^{ER} (Higuchi-Sanabria et al., 2020), and HSR (Tatum et al., 2015) all seem to utilize serotonergic circuits, Wnt signaling was also identified to be critical for transmitting the UPR^{MT} signal (Zhang et al., 2018). Specifically, knockdown of *mig-14*, *dpy-23*, and *mig-1*, critical components in receiving and internalizing Wnt signals completely abolished UPR^{MT} activation downstream of neuronal polyQ40 expression. Most importantly, overexpression of the gene encoding the secreted Wnt ligand *egl-20* specifically in neurons was sufficient to drive systemic UPR^{MT} activation and extend lifespan. Collectively, these findings have led to the conclusion that there exist not one, but two secreted mitokines from neurons that drive

mitochondrial stress signaling, serotonin and Wnt. Perhaps most intriguing was the finding that this induced systemic UPR^{MT} downstream of neuronal mitochondrial perturbations were transmitted to offspring over multiple generations in *C. elegans*. Specifically, neuronal expression of polyQ40 resulted in a transgenerational induction of UPR^{MT} that was observed more than 50 generations out upon loss of the neuronal polyQ40 transgene. This transmission of UPR^{MT} to offspring was through a Wnt-dependent elevation of mtDNA levels across generations (Zhang et al., 2021).

The capacity of cells to transmit mitochondrial stress signals to other cells is not a unique feature of neurons. In fact, germline-specific loss of the cytochrome c ortholog, *cyc-2.1* initiates a non-autonomous response that activates UPR^{MT} and AMPK in the intestine, which results in a robust lifespan extension. Importantly, this lifespan extension was dependent on ATFS-1, which drives DRP-1-mediated mitochondrial fragmentation (Lan et al., 2019). Decreased protein homeostasis in the germline–defined in this context as increased aggregation of PGL-1, an RNA-binding protein involved in P granule formation in the germline–also resulted in a non-autonomous activation of UPR^{MT} in the soma, providing evidence that this germline-to-soma transmission of UPR^{MT} could be a general response to germline stress. Increased PGL-1 aggregation resulted in a significant decrease in mitochondrial protein levels in the germline, which resulted in a Wnt-dependent transmission of mitochondrial stress signals to the soma. Specifically, PGL-1 aggregation in the germline resulted in an EGL-20 (Wnt ligand) and MIG-1 (Wnt receptor)-dependent mitochondrial fragmentation and UPR^{MT} induction in the soma (Calculli et al., 2021). However, it is still unclear whether this somatic UPR^{MT} induction is beneficial to organismal health and lifespan.

Perspectives and Concluding Remarks

Perhaps the most controversial point of UPR^{MT} on aging is whether activation of UPR^{MT} directly correlates with aging. As described above, knockdown of complex II genes never exhibited lifespan extension, while other methods to induce UPR^{MT} have inconsistent impacts on lifespan (Bennett et al., 2014). The most obvious hypothesis is that there are varying degrees of mitochondrial stress, whereby only those conditions that induce sufficient mitochondrial stress to activate UPR^{MT} without causing irreversible damage can promote hormesis and extend lifespan. In contrast, causing too much damage would be detrimental, regardless of whether UPR^{MT} is activated or not. Another plausible explanation is that the methods to measure UPR^{MT} activation are not sufficient: most studies rely on artificial transcriptional reporters, such as the overexpression of the *hsp-6p::GFP* reporter. As an overexpressed system, it is possible that *hsp-6p::GFP* exaggerates the intensity of UPR^{MT} activation, which is clear in some reports where western blots and qPCR show markedly lower *hsp-6* induction than the reporter (Berendzen et al., 2016). Thus, it is possible that a more thorough investigation of UPR^{MT} activation (e.g., survey of more gene targets, measurements of nuclear localization of UPR^{MT} regulators like DVE-1::GFP, measurements of chromatin compaction, etc.) is essential for understanding the true impact of UPR^{MT} on longevity.

Additionally, it would be of great interest to determine whether other cell types can initiate non-autonomous mitochondrial stress signatures. In flies, mitochondrial perturbations through dysfunction of complex I specifically in muscle results also results in impairment of mitochondrial function in the body fat (Song et al., 2017). Moreover, in mice, muscle-specific deletion of a critical autophagy gene Atf7 results in increased secretion of Fgf21, which increased resistance of these animals to diet-induced obesity (Kim et al., 2013). Importantly, Fgf21 serves as an endocrine signal to elicit ATF3-, ATF-4, and ATF-5 dependent ISR and UPR^{MT} (Forsström et al., 2019). Thus, it is entirely possible that other cell types in *C. elegans* also have the capacity to promote systemic UPR^{MT} through non-autonomous signaling.

Finally, while we survey the impact of UPR^{MT} on longevity in *C. elegans*, it is of critical importance to put into perspective the translatability of these findings to mammalian systems. One report argued that ATF5, the predicted mammalian homologue of ATFS-1, is critical for mitochondrial quality control (Fiorese et al., 2016). However, large-scale -omics-based approaches showed that the major responses to various drugs that target mitochondrial processes involved ATF4, which activates the ISR (Quirós et al., 2017). Furthermore, studies of human cells in more physiologically similar matrices (softer, 400 Pa hydrogels compared to the ~3 GPa of polystyrene) highlighted the major involvement of HSF1 and NRF2 in mitochondrial homeostasis in cancer cells (Tharp et al., 2021). Thus, it is still unclear how UPR^{MT} is regulated in mammalian systems, how ISR, UPR^{MT}, HSF1, and NRF2 either simultaneously or independently coordinate aspects of mitochondrial homeostasis, and ultimately, what impact–if any–these mechanisms have on mammalian longevity.

The Endoplasmic Reticulum Unfolded Protein Response

The endoplasmic reticulum (ER) is a complex multi-faceted organelle. While maintaining a contiguous membrane with the nucleus, the ER functions in protein folding, lipid synthesis, lipid droplet formation, and calcium storage (Fagone and Jackowski 2009; Benham 2012; Pol et al., 2014; Raffaello et al., 2016). The ER must also coordinate these responsibilities with other cellular organelles to traffic transmembrane proteins, lipids, and calcium. Therefore, a functional ER is essential to maintaining a healthy cellular status. Perturbations in ER protein quality control have been implicated in age-related diseases like Alzheimer's disease and Parkinson's disease (Hartl 2017), while dysregulation of lipid synthesis has been associated with diabetes and cardiovascular disease (Chaurasia and Summers 2015; Sletten et al., 2018). Activation of the unfolded protein response of the ER (UPR^{ER}), a transcriptional program activated upon exposure to ER stress, is often correlated with the development of these diseases as well as cancer (Clarke et al., 2014; Zhang et al., 2017). Interestingly, studies have also shown that genetic activation of the most conserved branch of the UPR^{ER} can instead result in

health benefits and an increased lifespan (Taylor and Dillin 2013; Frakes et al., 2020). These opposing phenotypes highlight the complexity and importance of understanding the role that the UPR^{ER} plays in disease and aging.

Similar to the mammalian ER, the *C. elegans* UPR^{ER} is composed of three signaling branches, each branch signaling from a unique transmembrane sensor (**Figure 3A**) (Shen et al., 2001). Initially discovered through a genetic screen for mutants that failed to induce an Unfolded Protein Response Element (UPRE) reporter in yeast, the most conserved of the these UPR^{ER} sensors is inositol-requiring enzyme 1 (*ire-1*) (Cox et al., 1993; Aragón et al., 2009). The IRE-1 protein is composed of an N-terminal ER luminal domain that is linked by a single-pass transmembrane domain to its cytosolic portion. The cytosolic side of IRE-1 contains both a kinase domain and an RNAse domain (Adams et al., 2019). In unstressed conditions, IRE-1 is bound to the chaperone HSP-4 (HSP70/BiP) at the luminal domain, which maintains IRE-1 as a monomer (Amin-Wetzel et al., 2017). Upon unfolded protein stress, HSP-4 is titrated away from IRE-1, freeing the luminal domain to directly bind unfolded proteins and dimerize (Zhou et al., 2006). Dimerized IRE-1 undergoes autophosphorylation, which activates its RNAse domain (Prischi et al., 2014), resulting in non-canonical splicing of the *xbp-1* mRNA to allow synthesis of the active transcription factor, XBP-1s (Calfon et al., 2002). XBP-1s can then enter the nucleus and induce expression of genes aimed at mitigating the stress on the ER, including those involved in protein quality control, secretion, and lipid metabolism (Shen et al., 2005).

In addition to the induction of ER homeostasis genes through activating *xbp-1s*, IRE-1 also plays a functional role in the degradation of mRNAs in a process known as Regulated Ire1 Dependent Decay (RIDD) (Tam et al., 2014). Targets of RIDD generally include proteins with signal peptides and transmembrane domains, or secretory proteins whose decreased translation is expected to reduce the protein folding burden of the ER (Hollien and Weissman 2006; Lee et al., 2011; Tsuru et al., 2013). Mammalian IRE-1 can also interact with TNF Receptor Associated Factor 2 (TRAF2) and activate the c-Jun N-terminal kinase (JNK) pathway (Urano et al., 2000). Activation of JNK signaling can promote cell death, while inhibiting downstream activation of JNK can promote cell survival under ER stress (Nishitoh et al., 2002; Verma and Datta 2010).

The UPR^{ER} sensors of other two branches of the UPR^{ER} are the protein kinase R (PKR)-like endoplasmic reticulum kinase (*pek-1*) and the activating transcription factor 6 (*atf-6*). Similar to IRE-1, both PEK-1 and ATF-6 contain a luminal domain that binds unfolded proteins, a transmembrane domain, and a cytosolic domain. However, they differ in the functions of the cytosolic domain (Schröder and Kaufman 2005). While PEK-1 also contains a kinase domain on its cytosolic side, it does not possess RNAse function (Cui et al., 2011). Instead, activation of PEK-1 results in the phosphorylation of the alpha subunit of eukaryotic initiation factor (*eif-2A*) to reduce global translation, but favors translation of the transcription factor ATF-4 to induce expression of genes to aid in mitigation of the ER stress (Han et al., 2013; Ma and Hendershot 2003; B'chir et al., 2013). The final branch of the UPR^{ER} has an entirely different mechanism of action and involves the cytosolic domain of the ATF-6 protein, which contains a leucine zipper family transcription factor (Schröder and Kaufman 2005). Upon the accumulation of unfolded proteins, the luminal domain of ATF-6 loses its association with ER-resident chaperones, which results in its translocation to the Golgi (Stauffer et al., 2020) where it is further processed (Haze et al., 1999; Ye et al., 2000). The ATF-6 transcription factor then enters the nucleus to promote expression of UPR^{ER} target genes (Shoulders et al., 2013; Stauffer et al., 2020).

UPR^{ER} During Stress

Like in most organisms, the IRE-1 branch of the UPR^{ER} is the most heavily studied branch in *C. elegans*. The IRE-1 branch is primarily responsible for the induction of genes encoding ER resident chaperones, including *hsp-4* and *hsp-3* (Shen et al., 2001). As such, the transcriptional reporter for *hsp-4* has become one of the most common and robust methods of monitoring UPR^{ER} activation within the nematode (Calfon et al., 2002; Bar-Ziv et al., 2020b). However, it is becoming increasingly clear that chaperone induction and protein homeostasis are not the only targets of UPR^{ER}, as other critical processes are also induced including immune response, lysosomal function and autophagy, and lipid homeostasis. The UPR^{ER} also plays an important role in development in *C. elegans* and the simultaneous loss of multiple branches results in developmental arrest of animals (Shen et al., 2005). Importantly, the IRE-1 branch of UPR^{ER} is critical for the exit from cellular quiescence (Roux et al., 2016). Newly hatched nematodes that enter L1 arrest due to the absence of food can exit this quiescent state to resume normal development and a normal lifespan upon the introduction of food. Larvae that maintain the L1 arrest for extended periods require ire-1 to recover from this state and resume development. The mRNA degradation function of IRE-1 has also been shown to regulate cellular fate decisions in *C. elegans*. Activation of IRE-1 within tumorous germline cells stimulates differentiation that halts progression of the tumor growth (Levi-Ferber et al., 2015; Levi-Ferber et al., 2021). This function is also conserved in mammals, *drosophila*, and yeast, where the UPR^{ER} has also been found to be required for normal development of various cell types (Reimold et al., 2001; Jung et al., 2016; Xu et al., 2016; Hillary and FitzGerald 2018; Kroeger et al., 2018).

Dysregulation of the UPR^{ER} is a common feature in many diseases, including neurodegeneration, metabolic disease, and cancer, and has been shown to decline in function during aging. In *C. elegans*, the capacity to induce IRE-1 and XBP-1 mediated UPR^{ER} fundamentally declines during aging, which results in increased sensitivity to ER stress (Taylor and Dillin 2013). A similar phenomenon is seen in aged mice where expression of ER quality control genes show marked decline in the brain (Naidoo et al., 2008; Nuss et al., 2008). The loss of UPR^{ER} function during aging can lead to the accumulation of damaged and aggregated proteins, which results in the physiological consequences of aging including increased proteotoxicity and cell death (Estébanez et al., 2018).

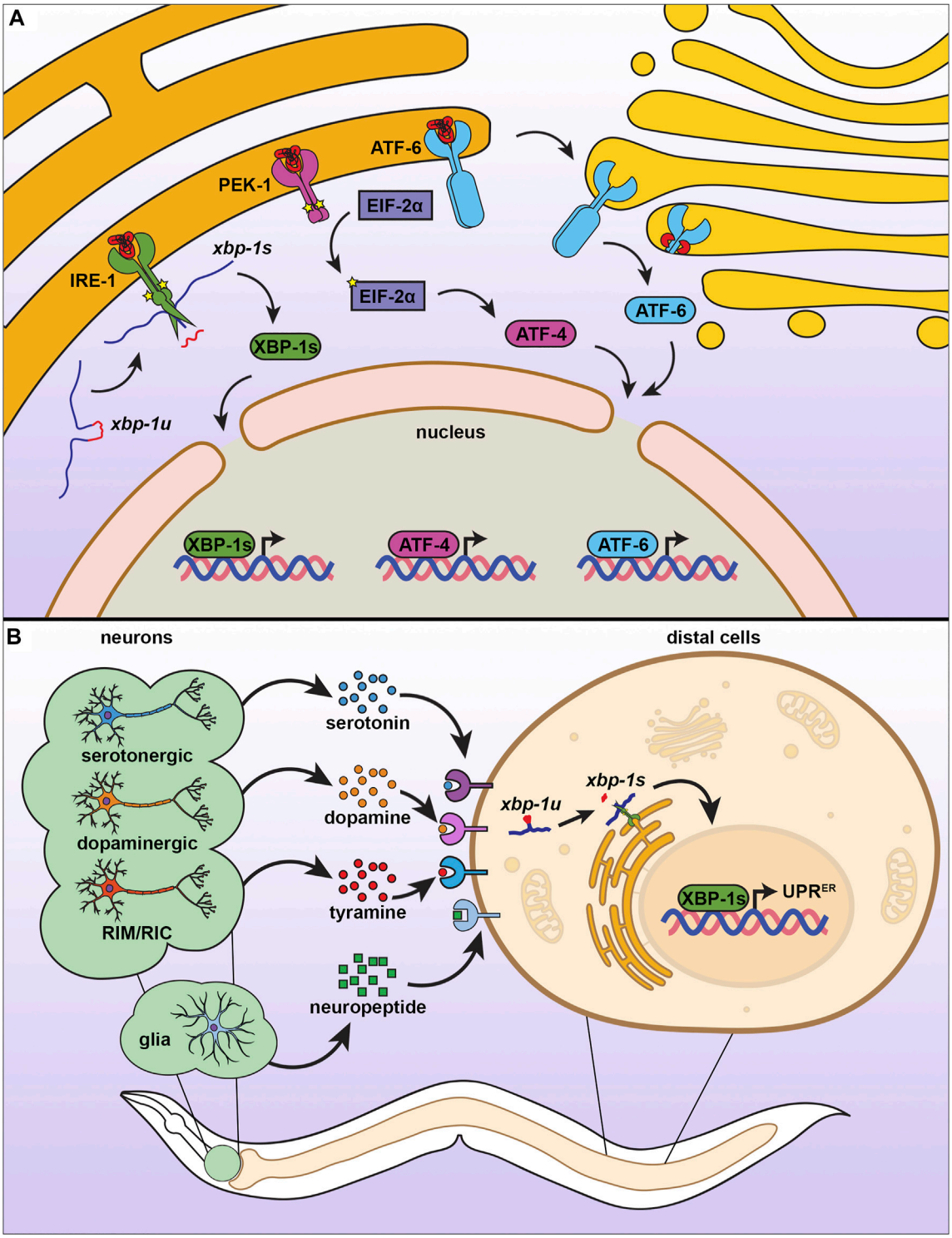

FIGURE 3 | The endoplasmic reticulum unfolded protein response. **(A)** There are three branches of UPR^{ER}, each consisting of an ER transmembrane protein with different mechanisms of action. When IRE-1 senses misfolded proteins, it homodimerizes, autophosphorylates, and promotes splicing of *xbp-1u* to *xbp-1s*, which can then be translated into the transcription factor XBP-1s, which activates UPR^{ER}. Similar to IRE-1, PEK-1 undergoes oligomerization, which induces eIF2α phosphorylation and activation of ATF4 to inhibit global translation. ATF6 is activated by proteolytic cleavage under ER stress, which causes translocation to the Golgi for further processing, resulting in a transcriptionally active ATF6 that promotes UPR^{ER}. **(B)** The UPR^{ER} is communicated in a nonautonomous manner through multiple neuronal subtypes and through glial cells. Serotonergic, dopaminergic, and RIM/RIC neurons signal to peripheral cells using specific neurotransmitters, whereas glial cells communicate through neuropeptide signaling. While the exact identify of receptors involved have not yet been fully characterized, distal cells respond by promoting IRE-1-dependent splicing of xbp-1s to induce the UPR^{ER}.

Although the UPR^{ER} is an adaptive and beneficial mechanism involved in clearing and mitigating damage under conditions of stress, sustained and unresolved UPR^{ER} can result in the activation of apoptosis. As such, chronic and irreversible UPR^{ER} is actually a hallmark for several disease, including neurodegeneration. Unresolved ER stress can activate pro-apoptotic machinery, including the C/EBP homologous protein CHOP, which downregulates anti-apoptotic factors, like B-cell lymphoma 2 (BCL2) (Chauve et al., 2021). Under certain conditions, ER stress can also promote MAPK signaling, including ERK1/2, which can be a pro-cancer signal, which makes inhibiting UPR^{ER} a potential therapeutic intervention for these cancers (Jiang et al., 2007; Beck et al., 2013). In addition, activation of the UPR^{ER} can also promote an inflammatory signaling cascade, including cytokine release, which can be correlated with common inflammatory disease including diabetes, atherosclerosis, and inflammatory bowel disease (Zhang and Zhang 2012; Coleman and Haller 2019). These studies suggest a potentially negative role for UPR^{ER} in organismal health and disease pathology. Indeed, studies in *C. elegans* have also revealed a potentially negative role for the UPR^{ER}, whereby whole-organism overexpression of xbp-1s is not beneficial to longevity and muscle-specific overexpression actually reduces lifespan (Taylor and Dillin 2013). Thus, while it is clear that the connection between UPR^{ER} and longevity are complex, myriad studies in *C. elegans* are in agreement that UPR^{ER} in the nervous system can be highly beneficial to organismal health. Thus, we focus on how UPR^{ER} function can impact the aging process in *C. elegans*, which primarily involves non-autonomous communication of ER stress signals through neural cells (**Figure 3B**).

UPR^{ER} and Aging: Neuronal Transmission of UPR^{ER}

Multicellular organisms coordinate systemic changes for development and metabolism through secretion of factors from the nervous system (e.g., neurotransmitter, hormones). Indeed, perceived ER stress in neural cells results in secretion of signals to orchestrate an organismal response improve ER homeostasis, ER stress resilience, and longevity. Specifically, overexpression of the spliced *xbp-1s* transcription factor within neurons or amphid sheath glia of *C. elegans* results in activation of the UPR^{ER} not only within the neurons and glia themselves, but also within distal intestinal cells (Taylor and Dillin 2013; Frakes et al., 2020). This non-autonomous induction of the UPR^{ER} results in increased stress resilience and longevity. Interestingly, the mechanisms whereby neurons and glia coordinate this organism-wide response differ: non-autonomous signaling through neurons is dependent on the release of small clear vesicles (SCVs) through UNC-13, such that loss of *unc-13* results in elimination of the beneficial effects of neuronal *xbp-1s* overexpression (Taylor and Dillin 2013); in contrast, non-autonomous signaling through glia are dependent on release of neuropeptides through the release of dense core vesicles mediated by UNC-31 and neuropeptide processing by EGL-3 (Frakes et al., 2020). The distinction between the two origins of the UPR^{ER} signal is further highlighted by the partially additive increase in lifespan that is observed when *xbp-1s* is overexpressed in both types of neural cells. As neurons and glia have a well-established relationship, it would be likely that both paradigms share some form of communication or mechanism to extend lifespan, though current research has not yet clarified whether direct communication between neurons and glia exist in this paradigm.

In its original study, the beneficial effects of neuronal UPR^{ER} were ascribed to the upregulation of chaperones, which resulted in increased protein homeostasis and ER stress resilience. Since then, further studies found that neuronal UPR^{ER} also improved lipid metabolism. Lipid staining and lipidomic analysis revealed a decrease in neutral lipid stores, while concurrently showing an increase in monounsaturated fatty acids, including oleic acid (Imanikia et al., 2019a). In fact, supplementation of oleic acid was sufficient to increase the lifespan of wild-type and *xbp-1* deficient animals but did not further extend the lifespan of xbp-1s overexpressing animals, providing further evidence that the beneficial effects of neuronal *xbp-1s* could be at least partially ascribed to changes in lipid profiles. Interestingly, oleic acid supplementation also provided protection against the effects of proteotoxic polyQ40 and α-Aß proteins, suggesting that improving lipid metabolism in the ER could also impact protein homeostasis, though the mechanism whereby this happens is still unexplored (Imanikia et al., 2019a). Importantly, this study ascribed the primary mechanism of lipid remodeling to the desaturases FAT-6 and FAT-7, which metabolized neutral lipid stores into oleic acid to promote organismal health.

The contribution of lipid metabolism to the beneficial effects of non-autonomous UPR^{ER} phenotypes was further indicated by the expansion of lysosomes and increased lipophagy found in these animals (Imanikia et al., 2019b; Daniele et al., 2020a). Neuronal *xbp-1s* overexpression resulted in a significant expansion of lysosomes and increased expression of lysosomal lipases. Changes to lysosomal activity were essential for the beneficial effects of neuronal *xbp-1s* as knockdown of the *C. elegans* homolog to mammalian TFEB, *hlh-30*, was sufficient to suppress the lifespan extension of these animals (Imanikia et al., 2019b). This is in direct agreement with another study that found that HPL-2, a chromatin modifying protein, promotes autophagy to increase ER stress resilience (Kozlowski et al., 2014). Further, transcriptional profiling of worms deficient in phosphatidylcholine (PC) synthesis–which causes ER stress through lipid dysregulation–also induced autophagy in an IRE-1/XB-1-dependent manner (Koh et al., 2018). This is highly similar to a process previously described in yeast, where inhibition of PC biosynthesis activates microlipophagy downstream of UPR^{ER} (Vevea et al., 2015). Indeed, in *C. elegans*, promoting lipophagy by overexpression of *ehbp-1*, a core component of the conserved RME-1/RAB-10/EHBP-1 lipophagy complex, was sufficient to drive lipid remodeling and lifespan extension (Daniele et al., 2020a). Importantly, this beneficial effect of lipophagy downstream of neuronal UPR^{ER} was independent from its canonical role in protein homeostasis through chaperones.

A subsequent study found that the chaperone induction and altered lipid metabolism downstream of non-autonomous UPR^{ER} signaling was in part due to signaling from distinct subsets of neurons (Higuchi-Sanabria et al., 2020). Overexpression of xbp-1s within dopaminergic neurons was sufficient to drive EHBP-1 regulated lipid metabolism, while overexpression in serotonergic neurons induced expression of ER chaperones to promote protein homeostasis. Both sources of non-autonomous UPR^{ER} signals independently promoted organismal health and lifespan, and their effects were observed to be additive, suggesting an intricate model whereby neurons can differ in their capacity to elicit a peripheral response through unique stress signals. Indeed, a separate genetic screen to identify neurotransmitters involved in neuronal UPR^{ER} signals failed to identify dopamine or serotonin signaling, and instead found that tyramine synthesis was essential for transmitting UPR^{ER} from neurons to the intestine (Özbey et al., 2020). Overexpression of *xbp-1s* within the tyraminergic neurons was sufficient to induce the UPR^{ER} within the intestine and stimulate changes to the animal's feeding behavior and brood size. While dopaminergic, serotonergic, and tyraminergic neurons have now been shown to contribute to the non-autonomous UPR^{ER}, whether these neurons directly communicate with each other or whether other subtypes or specific neurons may also contribute to–or potentially hinder–the longevity phenotypes of the non-autonomous UPR^{ER} remains to be known.

Neuronal transmission of an ER stress response is not limited to the genetic models observed in *C. elegans*. When Xbp1s is overexpressed in POMC neurons of mice, a similar non-autonomous activation of UPR^{ER} exists in peripheral cells and beneficially impacts metabolic physiology (e.g., improved glucose homeostasis, increased insulin sensitivity, and protection against high-fat diet induced obesity) (Williams et al., 2014). Importantly, a similar process can occur with naturally occurring stimuli whereby sensory perception of food can result in activation of POMC neurons to activate UPR^{ER} within the liver to prepare for incoming nourishment (Brandt et al., 2018). This induction resulted in increased lipid synthesis and remodeling of the ER, likely to prime the liver for its roles in processing the nutrients. However, how this nonautonomous signaling impacts aging or longevity has not been confirmed, still making a conclusive correlation between UPR^{ER} activity and lifespan difficult.

Perspectives and Concluding Remarks

As aptly named, the historical function ascribed to the UPR^{ER} is to promote protein homeostasis, though it has become increasingly clear that the UPR^{ER} regulates many critical functions outside of protein quality control, including autophagy and lipid homeostasis as described briefly above. One important question is how these functional roles overlap. For example, it isn't difficult to ascertain how increased lysosomal function can promote autophagy to clear damaged proteins, ultimately resulting in increased protein homeostasis. However, increased lipid metabolism downstream of nonautonomous UPR^{ER} signals also resulted in increased clearance of protein aggregates (Imanikia et al., 2019a) and increased resistance to protein misfolding stress (Higuchi-Sanabria et al., 2020). It is entirely possible that improved lipid metabolism simply increases general organismal health, making animals more resilient to all sources of stress or increases the resources available for mitigating damage. Alternatively, it is possible that improved lipid homeostasis can actually have direct impacts on protein homeostasis. For example, increased lipid metabolism can increase secretory capacity of the ER (Daniele et al., 2020b), and it is possible that damaged proteins can be secreted to external environments, such as the pseudocoelom in C.*elegans*, where it will cause less damage.

Beyond the beneficial roles of the UPR^{ER}, unresolved UPR^{ER} signaling can be detrimental. For example, we briefly discussed how chronic UPR^{ER} can cause apoptosis in mammalian cells, and overexpression of HAC1s, the *S. cerevisiae* homolog of *xbp-1*, can perturb cell cycle progression (Sopko et al., 2006). However, promoting UPR^{ER} in *C. elegans* seems to promote organismal health and extend lifespan, which can be due to the post-mitotic nature of the adult worm, which is not deterred by cell cycle or apoptosis machinery. Indeed, ectopic activation of UPR^{ER} has negative consequences in the germline, the few actively dividing cells of the adult worm, whereby neuronal UPR^{ER} causes decreased fecundity and brood size (Özbey et al., 2020). However, this differentiation between actively dividing versus post-mitotic cells still does not explain the negative impact of *xbp-1s* overexpression in muscle cells of *C. elegans* (Taylor and Dillin 2013), as these cells are also post-mitotic. One plausible explanation is that increased UPR^{ER} causes lipid depletion, which may be detrimental to the highly energy-demanding muscle cells.

A final thought that we find important to mention is to understand the multi-faceted response of UPR^{ER}. With the increasing number of mechanistic pathways being ascribed to UPR^{ER} activation, how then does a cell coordinate these downstream pathways? For example, serotonergic and dopaminergic signals elicit two different responses in peripheral cells: induction of chaperones and lipid regulatory enzymes, respectively. How does one transcription factor, XBP-1s, coordinate two distinct responses? It is possible that other cofactors titrate XBP-1s to its appropriate targets; for example, TFEB/HLH-30 is required for the changes to lysosomal genes downstream of XBP-1s activation, suggesting that interaction of TFEB/XBP-1s is important for lysosomal genes. Still to be identified are what other XBP-1s interactors exist to titrate XBP-1s specifically to other target pathways, including lipid regulation, ERAD, or chaperones.

Impact of Cellular Stress Responses on Other Compartments

Perhaps one historically overlooked issue in the field of stress biology is in its often single focus: generally, stress responses are studied with a focus on the single organelle that it impacts. However, it is becoming increasingly clear that activation or perturbation of stress responses elicit pleiotropic changes that alter multiple systems.

Mitochondria-ER Interactions During Stress

The functional state of the mitochondria directly impacts the state of other organelles, and thus it is unsurprising that UPR^{MT} function has ramifications on other cellular processes. For example, ER-mitochondria contact sites (ERMCS) allow for the exchange of proteins, metabolites, ions, and lipids between the organelles and are critical in maintaining cellular homeostasis (Xu et al., 2020). In HeLa cells, mitochondrial stress induced by doxycycline exposure can increase ERMCS (Lopez-Crisosto et al., 2021), potentially through UPR^{MT} activation. Increased ERMCS can also result in creased lifespan in *drosophila* (Garrido-Maraver et al., 2020), opening up the possibility that UPR^{MT} activation may potentially increase lifespan through effects on ERMCS. One important function of ERMCS is CA^{2+} homeostasis regulated by ER-to-mitochondria CA^{2+} transfer. In *C. elegans*, the UPR^{ER} regulator, ATF-6 regulates lifespan through modulation of ER-mitochondria calcium transport. As *C. elegans* age, ATF-6 activity increases, resulting in activation of UPR^{ER} and subsequent increase in the calreticulin, CRT-1. Increased levels of CRT-1 result in aberrant accumulation of CA^{2+} ions in the ER. Interestingly, perturbations in *atf-6* result in decreased CRT-1, calcium efflux through the inositol triphosphate receptor, ITR-1, and increased mitochondrial CA^{2+} import, resulting in increased metabolic activity of mitochondria and extension of lifespan (Burkewitz et al., 2020).

Impact of Mitochondrial Quality Control on the Heat Shock Response

Considering the close communication between the mitochondria and ER and the impact of the UPR^{ER} on mitochondrial health, it would be of great interest to determine the overlapping functions of all stress responses and how each impact the other. For example, induction of the UPR^{MT} has direct impact on the HSR. HSF-1 function declines during the aging process (Trivedi and Jurivich 2020) and the HSR can become dysfunctional as early as the second day of reproductive capacity in *C. elegans* (Labbadia and Morimoto 2015). However, one study found that low levels of mitochondrial stress through perturbation of a cytochrome C oxidase subunit, *cox-6c*, can increase functional capacity of the HSR at late age and has a positive impact on stress resilience and longevity. Specifically, activation of both the UPR^{MT} and HSR are both equally responsible for the increase in organismal health and lifespan found in animals exposed to mitochondrial stress (Labbadia et al., 2017). Interestingly, knockdown of *hsp-6* (the gene encoding the primary mitochondrial chaperone mtHSP70) also resulted in activation of the HSR. In fact, microarray analysis of animals with *hsp-6* knockdown found 187 genes differentially expressed, with 66 being known targets of HSF-1 and DVE-1, highlighting the distinct and important overlap between UPR^{MT} and HSR. Further investigation of these overlapping genes found that many genes were involved in lipid metabolism. Subsequent metabolomics identified that this mitochondria-to-cytosolic stress response (MCSR) was dependent on an overall increase in fatty acid levels. Specifically, increased cardiolipin and inhibition of ceramide synthesis were sufficient to drive MCSR induction. Most importantly, blocking fatty acid oxidation to increase fats using the drug, perhexiline, was sufficient to drive MCSR activation in both worms and human cells (Kim et al., 2016). This study has powerful translational potential, with perhexiline serving as a potential therapeutic intervention for aging and protein aggregation disorders, including Huntington's, Alzheimer's, and ALS. Finally, HSF-1 has been shown to directly impact mtDNA gene expression through elevated histone H4 levels. Specifically, knockdown of the conserved heat shock factor binding protein, *hsb-1* results in increased H4 levels early in development, which alters chromatin state of mtDNA, decreases expression of mtDNA-encoded genes, reduces mitochondrial respiratory capacity, and promotes lifespan in a UPR^{MT}-dependent manner (Sural et al., 2020).

These studies continued to bridge the functional role of the mitochondria to other organelles, particularly with the surprising finding that HSF-1, the transcriptional regulator originally ascribed to HSR, now being directly influenced by UPR^{MT}. HSF-1 has also been shown to directly impact the actin cytoskeleton (Baird et al., 2014; Higuchi-Sanabria et al., 2018b), and many studies have already highlighted the importance of actin as serving as the scaffold for–and regulating the dynamics of–the mitochondria (Fehrenbacher et al., 2004; Boldogh and Pon 2006; Higuchi et al., 2013; Korobova et al., 2013; Manor et al., 2015). A large-scale cross-organism screen combining the power of CRISPR-Cas9 screening in human cells (Shalem et al., 2014) and RNAi screening in *C. elegans* identified EPS-8 as a novel link between actin and mitochondrial homeostasis (Moehle et al., 2021). Specifically, knockdown of *eps-8* resulted in hyperstabilization of actin through the integrin, PAT-3. This increased stability of actin resulted in fragmentation of mitochondria and a beneficial activation of the UPR^{MT}, such that these animals exhibited increased lifespan. These findings are conserved in human cells where hyperactive integrin signaling can hyperstabilize actin filaments, resulting in increased stress resilience through activation of UPR^{MT} through HSF1 and ATF5 (Tharp et al., 2021). These studies beg the question of what impact–if any–UPR^{MT} and mitochondrial dysfunction can have on the actin cytoskeleton. While one would expect that mitochondrial dysfunction and decreased energetics may negatively influence the actin cytoskeleton, the activation of the HSR and increased function of HSF-1 during mitochondrial stress may actually positively impact actin health and function.

Overlapping Functions of Heat Shock Response and UPR^{ER}

Similar to the overlapping functions of UPR^{MT} and HSR, the UPR^{ER} also shows some overlap. In fact, nine stress response genes are induced by both UPR^{ER} and HSR (Liu and Chang 2008), including the ER chaperone, HSP-4/BiP (Kohno et al., 1993) and the COPII cargo receptor, Erv29 (Caldwell et al., 2001). Importantly, the activation of the HSR via a constitutively active HSF1 rescued the growth defect of UPR^{ER} deficient cells. In addition, induction of the UPR^{ER} via heat-shock in mammalian cells can activate canonical XBP1 targets (Heldens et al., 2011).

While these studies were not performed in *C. elegans*, overexpression of *xbp-1s* in *C. elegans* did seem to negatively impact the expression of key HSF-1 targets, including *hsp-16.2* and *hsp-70* (Taylor and Dillin 2013), clearly indicating that UPR^{ER} function has at least some indirect impact on HSR. More thorough analysis is certainly necessary to determine the direct ramifications of UPR^{ER} on HSR and vice versa during the aging process in *C. elegans*.

CONCLUDING REMARKS

Overall, it is becoming increasingly clear that overlapping functions exist between stress responses and that each response can impact other organelles. For example, UPR^{MT} has direct ramifications on overall health and fitness of an organism–not just on the mitochondria, and not just on the single cell type exposed to mitochondrial stress. The same is true for the UPR^{ER} and the HSR, highlighting the critical importance of studying the cross-regulation of stress responses, and moving away from studying these quality control mechanisms in a black box of specificity. One intriguing question is to understand how all quality control machineries are regulated as a whole. For example, under conditions of competing needs, does there exist a preference for a specific stress response? While it is clear that the HSR is a critical response to thermal stress, heat can also damage the mitochondria and ER, so how does the cell prioritize HSR over UPR^{ER}/UPR^{MT} activation? Under these more "generalized" stresses, how does the cell preferentially activate one response over the other? Even further, if multiple stress responses are activated, would this have a synergistic effect producing a hyper long-lived animal? Or would there be a detrimental effect when activating too many stress responses? Future studies must continue to focus on the direct and indirect effects of one stress response on all organelles and take into consideration the effects of organelle-to-organelle signaling and even cell-to-cell signaling in the cross communication and cross regulation of stress responses.

AUTHOR CONTRIBUTIONS

ND prepared the introduction and heat-shock response sections, GG prepared the UPR^{ER} section and all figures, and RHS prepared the UPR^{MT} and conclusion sections. ND, GG, and RHS reviewed and edited all sections of the manuscript.

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REFERENCES

Adams, C. J., Kopp, M. C., Larburu, N., Nowak, P. R., and Ali, M. M. U. (2019). Structure and Molecular Mechanism of ER Stress Signaling by the Unfolded Protein Response Signal Activator IRE1. *Front. Mol. Biosci.* 6, 1. doi:10.3389/fmolb.2019.00011

Amin, M. R., Mahmud, S. A., Dowgielewicz, J. L., Sapkota, M., and Pellegrino, M. W. (2020). A Novel Gene-Diet Interaction Promotes Organismal Lifespan and Host protection during Infection via the Mitochondrial UPR. *Plos Genet.* 16, e1009234. doi:10.1371/journal.pgen.1009234

Amin-Wetzel, N., Saunders, R. A., Kamphuis, M. J., Rato, C., Preissler, S., Harding, H. P., et al. (2017). A J-Protein Co-chaperone Recruits BiP to Monomerize IRE1 and Repress the Unfolded Protein Response. *Cell* 171, 1625e13–1637. doi:10.1016/j.cell.2017.10.040

Anckar, J., and Sistonen, L. (2011). Regulation of HSF1 Function in the Heat Stress Response: Implications in Aging and Disease. *Annu. Rev. Biochem.* 80, 1089–1115. doi:10.1146/annurev-biochem-060809-095203

Anderson, N. S., and Haynes, C. M. (2020). Folding the Mitochondrial UPR into the Integrated Stress Response. *Trends Cell Biol.* 30, 428–439. doi:10.1016/j.tcb.2020.03.001

Aragón, T., van Anken, E., Pincus, D., Serafimova, I. M., Korennykh, A. V., Rubio, C. A., et al. (2009). Messenger RNA Targeting to Endoplasmic Reticulum Stress Signalling Sites. *Nature* 457, 736–740. doi:10.1038/nature07641

Baird, N. A., Douglas, P. M., Simic, M. S., Grant, A. R., Moresco, J. J., Wolff, S. C., et al. (2014). HSF-1-mediated Cytoskeletal Integrity Determines Thermotolerance and Life Span. *Science* 346, 360–363. doi:10.1126/science.1253168

Bakthisaran, R., Tangirala, R., and Rao, Ch. M. (18542015). Small Heat Shock Proteins: Role in Cellular Functions and Pathology. *Biochim. Biophys. Acta* 1854, 291–319. doi:10.1016/j.bbapap.2014.12.019

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

Bar-Ziv, R., Frakes, A. E., Higuchi-Sanabria, R., Bolas, T., Frankino, P. A., Gildea, H. K., et al. (2020). Measurements of Physiological Stress Responses in C. Elegans. *JoVE* 159, 1. doi:10.3791/61001

Barna, J., Csermely, P., and Vellai, T. (2018). Roles of Heat Shock Factor 1 beyond the Heat Shock Response. *Cell. Mol. Life Sci.* 75, 2897–2916. doi:10.1007/s00018-018-2836-6

B'chir, W., Maurin, A.-C., Carraro, V., Averous, J., Jousse, C., Muranishi, Y., et al. (2013). The eIF2α/ATF4 Pathway Is Essential for Stress-Induced Autophagy Gene Expression. *Nucleic Acids Res.* 41, 7683–7699.

Beck, D., Niessner, H., Smalley, K. S., Flaherty, K., Paraiso, K. H., Busch, C., et al. (2013). Vemurafenib Potently Induces Endoplasmic Reticulum Stress-Mediated Apoptosis in BRAFV600E Melanoma Cells. *Sci. Signal.* 6, ra7. doi:10.1126/scisignal.2003057

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.* 106, 14914–14919. doi:10.1073/pnas.0902882106

Benedetti, C., Haynes, C. M., Yang, Y., Harding, H. P., and Ron, D. (2006). Ubiquitin-like Protein 5 Positively Regulates Chaperone Gene Expression in the Mitochondrial Unfolded Protein Response. *Genetics* 174, 229–239. doi:10.1534/genetics.106.061580

Benham, A. M. (2012). Protein Secretion and the Endoplasmic Reticulum. *Cold Spring Harbor Perspect. Biol.* 4, a012872. doi:10.1101/cshperspect.a012872

Bennett, C. F., Vander Wende, H., Simko, M., Klum, S., Barfield, S., Choi, H., et al. (2014). Activation of the Mitochondrial Unfolded Protein Response Does Not Predict Longevity in *Caenorhabditis elegans*. *Nat. Commun.* 5, 3483. doi:10.1038/ncomms4483

Berendzen, K. M., Durieux, J., Shao, L.-W., Tian, Y., Kim, H.-E., Wolff, S., et al. (2016). Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis. *Cell* 166, 1553–1563. doi:10.1016/j.cell.2016.08.042

Binder, R. J., and Srivastava, P. K. (2005). Peptides Chaperoned by Heat-Shock Proteins Are a Necessary and Sufficient Source of Antigen in the Cross-Priming of CD8+ T Cells. *Nat. Immunol.* 6, 593–599. doi:10.1038/ni1201

Boldogh, I. R., and Pon, L. A. (2006). Interactions of Mitochondria with the Actin Cytoskeleton. *Biochim. Biophys. Acta (Bba) - Mol. Cell Res.* 1763, 450–462. doi:10.1016/j.bbamcr.2006.02.014

Brandt, C., Nolte, H., Henschke, S., Engström Ruud, L., Awazawa, M., Morgan, D. A., et al. (2018). Food Perception Primes Hepatic ER Homeostasis via Melanocortin-dependent Control of mTOR Activation. *Cell* 175, 1321–1335. e20. doi:10.1016/j.cell.2018.10.015

Burkewitz, K., Feng, G., Dutta, S., Kelley, C. A., Steinbaugh, M., Cram, E. J., et al. (2020). Atf-6 Regulates Lifespan through ER-Mitochondrial Calcium Homeostasis. *Cell Rep.* 32, 108125. doi:10.1016/j.celrep.2020.108125

Butov, A., Johnson, T., Cypser, J., Sannikov, I., Volkov, M., Sehl, M., et al. (2001). Hormesis and Debilitation Effects in Stress Experiments Using the Nematode Worm *Caenorhabditis elegans*: the Model of Balance between Cell Damage and HSP Levels. *Exp. Gerontol.* 37, 57–66. doi:10.1016/s0531-5565(01)00161-9

Calculli, G., Lee, H. J., Shen, K., Pham, U., Herholz, M., Trifunovic, A., et al. (2021). Systemic Regulation of Mitochondria by Germline Proteostasis Prevents Protein Aggregation in the Soma of *C. elegans*. *Sci. Adv.* 7, eabg3012. doi:10.1126/sciadv.abg3012

Caldwell, S. R., Hill, K. J., and Cooper, A. A. (2001). Degradation of Endoplasmic Reticulum (ER) Quality Control Substrates Requires Transport between the ER and Golgi. *J. Biol. Chem.* 276, 23296–23303. doi:10.1074/jbc.m102962200

Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., et al. (2002). IRE1 Couples Endoplasmic Reticulum Load to Secretory Capacity by Processing the XBP-1 mRNA. *Nature* 415, 92–96. doi:10.1038/415092a

Castro, J. P., Wardelmann, K., Grune, T., and Kleinridders, A. (2018). Mitochondrial Chaperones in the Brain: Safeguarding Brain Health and Metabolism? *Front. Endocrinol.* 9, 196. doi:10.3389/fendo.2018.00196

Chaudhari, S. N., and Kipreos, E. T. (2017). Increased Mitochondrial Fusion Allows the Survival of Older Animals in Diverse *C. elegans* Longevity Pathways. *Nat. Commun.* 8, 182. doi:10.1038/s41467-017-00274-4

Chaurasia, B., and Summers, S. A. (2015). Ceramides - Lipotoxic Inducers of Metabolic Disorders. *Trends Endocrinol. Metab.* 26, 538–550. doi:10.1016/j.tem.2015.07.006

Chauve, L., Hodge, F., Murdoch, S., Masoudzadeh, F., Mann, H.-J., Lopez-Clavijo, A. F., et al. (2021). Neuronal HSF-1 Coordinates the Propagation of Fat Desaturation across Tissues to Enable Adaptation to High Temperatures in *C. elegans*. *Plos Biol.* 19, e3001431. doi:10.1371/journal.pbio.3001431

Clarke, H. J., Chambers, J. E., Liniker, E., and Marciniak, S. J. (2014). Endoplasmic Reticulum Stress in Malignancy. *Cancer Cell* 25, 563–573. doi:10.1016/j.ccr.2014.03.015

Coleman, O. I., and Haller, D. (2019). ER Stress and the UPR in Shaping Intestinal Tissue Homeostasis and Immunity. *Front. Immunol.* 10, 1. doi:10.3389/fimmu.2019.02825

Colla, E. (2019). Linking the Endoplasmic Reticulum to Parkinson's Disease and Alpha-Synucleinopathy. *Front. Neurosci.* 13. doi:10.3389/fnins.2019.00560

Couvillion, M. T., Soto, I. C., Shipkovenska, G., and Churchman, L. S. (2016). Synchronized Mitochondrial and Cytosolic Translation Programs. *Nature* 533, 499–503. doi:10.1038/nature18015

Cox, J. S., Shamu, C. E., and Walter, P. (1993). Transcriptional Induction of Genes Encoding Endoplasmic Reticulum Resident Proteins Requires a Transmembrane Protein Kinase. *Cell* 73, 1197–1206. doi:10.1016/0092-8674(93)90648-a

Cui, W., Li, J., Ron, D., and Sha, B. (2011). The Structure of the PERK Kinase Domain Suggests the Mechanism for its Activation. *Acta Crystallogr. D Biol. Cryst.* 67, 423–428. doi:10.1107/s0907444911006445

Curtis, R., O'Connor, G., and DiStefano, P. S. (2006). Aging Networks inCaenorhabditis Elegans: AMP-Activated Protein Kinase (Aak-2) Links Multiple Aging and Metabolism Pathways. *Aging Cell* 5, 119–126. doi:10.1111/j.1474-9726.2006.00205.x

Cypser, J. R., and Johnson, T. E. (2002). Multiple Stressors in *Caenorhabditis elegans* Induce Stress Hormesis and Extended Longevity. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 57, B109–B114. doi:10.1093/gerona/57.3.b109

Dai, C. (2018). The Heat-Shock, or HSF1-Mediated Proteotoxic Stress, Response in Cancer: from Proteomic Stability to Oncogenesis. *Phil. Trans. R. Soc. B* 373, 20160525. doi:10.1098/rstb.2016.0525

Daniele, J. R., Higuchi-Sanabria, R., Durieux, J., Monshietehadi, S., Ramachandran, V., Tronnes, S. U., et al. (2020). UPRER Promotes Lipophagy Independent of Chaperones to Extend Life Span. *Sci. Adv.* 6, eaaz1441. doi:10.1126/sciadv.aaz1441

Daniele, J. R., Higuchi-Sanabria, R., Durieux, J., Monshietehadi, S., Ramachandran, V., Tronnes, S. U., et al. (2020). UPR ER Promotes Lipophagy Independent of Chaperones to Extend Life Span. *Sci. Adv.* 6, 1. doi:10.1126/sciadv.aaz1441

Das, R., Melo, J. A., Thondamal, M., Morton, E. A., Cornwell, A. B., Crick, B., et al. (2017). The Homeodomain-Interacting Protein Kinase HPK-1 Preserves Protein Homeostasis and Longevity through Master Regulatory Control of the HSF-1 Chaperone Network and TORC1-Restricted Autophagy in *Caenorhabditis elegans*. *Plos Genet.* 13, e1007038. doi:10.1371/journal.pgen.1007038

Das, S., Ooi, F. K., Cruz Corchado, J., Fuller, L. C., Weiner, J. A., and Prahlad, V. (2020). Serotonin Signaling by Maternal Neurons upon Stress Ensures Progeny Survival. *Elife* 9, e55246. doi:10.7554/eLife.55246

Desai, S., Liu, Z., Yao, J., Patel, N., Chen, J., Wu, Y., et al. (2013). Heat Shock Factor 1 (HSF1) Controls Chemoresistance and Autophagy through Transcriptional Regulation of Autophagy-Related Protein 7 (ATG7). *J. Biol. Chem.* 288, 9165–9176. doi:10.1074/jbc.m112.422071

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

Dokladny, K., Zuhl, M. N., Mandell, M., Bhattacharya, D., Schneider, S., Deretic, V., et al. (2013). Regulatory Coordination between Two Major Intracellular Homeostatic Systems. *J. Biol. Chem.* 288, 14959–14972. doi:10.1074/jbc.m113.462408

Douglas, P. M., Baird, N. A., Simic, M. S., Uhlein, S., McCormick, M. A., Wolff, S. C., et al. (2015). Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Longevity. *Cell Rep.* 12, 1196–1204. doi:10.1016/j.celrep.2015.07.026

Dubaquié, Y., Looser, R., Fünfschilling, U., Jenö, P., and Rospert, S. (1998). Identification of Invivo Substrates of the Yeast Mitochondrial Chaperonins Reveals Overlapping but Non-identical Requirement for Hsp60 and Hsp10. *EMBO J.* 17, 5868–5876. doi:10.1093/emboj/17.20.5868

Durieux, J., Wolff, S., and Dillin, A. (2011). The Cell-Non-Autonomous Nature of Electron Transport Chain-Mediated Longevity. *Cell* 144, 79–91. doi:10.1016/j.cell.2010.12.016

Estébanez, B., de Paz, J. A., Cuevas, M. J., and González-Gallego, J. (2018). Endoplasmic Reticulum Unfolded Protein Response, Aging and Exercise: An Update. *Front. Physiol.* 9. doi:10.3389/fphys.2018.01744

Fagone, P., and Jackowski, S. (2009). Membrane Phospholipid Synthesis and Endoplasmic Reticulum Function. *J. Lipid Res.* 50, S311–S316. doi:10.1194/jlr.r800049-jlr200

Fawcett, T. W., Sylvester, S. L., Sarge, K. D., Morimoto, R. I., and Holbrook, N. J. (1994). Effects of Neurohormonal Stress and Aging on the Activation of Mammalian Heat Shock Factor 1. *J. Biol. Chem.* 269, 32272–32278. doi:10.1016/s0021-9258(18)31631-4

Fehrenbacher, K. L., Yang, H.-C., Gay, A. C., Huckaba, T. M., and Pon, L. A. (2004). Live Cell Imaging of Mitochondrial Movement along Actin Cables in Budding Yeast. *Curr. Biol.* 14, 1996–2004. doi:10.1016/j.cub.2004.11.004

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

Forsström, S., Jackson, C. B., Carroll, C. J., Kuronen, M., Pirinen, E., Pradhan, S., et al. (2019). Fibroblast Growth Factor 21 Drives Dynamics of Local and Systemic Stress Responses in Mitochondrial Myopathy with mtDNA Deletions. *Cell Metab.* 30, 1040–1054. e7. doi:10.1016/j.cmet.2019.08.019

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

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

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

Garrido-Maraver, J., Loh, S. H. Y., and Martins, L. M. (2020). Forcing Contacts between Mitochondria and the Endoplasmic Reticulum Extends Lifespan in a Drosophila Model of Alzheimer's Disease. *Biol. Open* 9, bio047530. doi:10.1242/bio.047530

Garsin, D. A., Villanueva, J. M., Begun, J., Kim, D. H., Sifri, C. D., Calderwood, S. B., et al. (2003). Long-lived *C. elegans* Daf-2 Mutants Are Resistant to Bacterial Pathogens. *Science* 300, 1921. doi:10.1126/science.1080147

Gross, S. R., and Kinzy, T. G. (2007). Improper Organization of the Actin Cytoskeleton Affects Protein Synthesis at Initiation. *Mol. Cell Biol* 27, 1974–1989. doi:10.1128/mcb.00832-06

Haeussler, S., Köhler, F., Witting, M., Premm, M. F., Rolland, S. G., Fischer, C., et al. (2020). Autophagy Compensates for Defects in Mitochondrial Dynamics. *Plos Genet.* 16, e1008638. doi:10.1371/journal.pgen.1008638

Hallberg, E. M., Shu, Y., and Hallberg, R. L. (1993). Loss of Mitochondrial Hsp60 Function: Nonequivalent Effects on Matrix-Targeted and Intermembrane-Targeted Proteins. *Mol. Cel. Biol.* 13, 3050–3057. doi:10.1128/mcb.13.5.3050

Han, J., Back, S. H., Hur, J., Lin, Y.-H., Gildersleeve, R., Shan, J., et al. (2013). ER-stress-induced Transcriptional Regulation Increases Protein Synthesis Leading to Cell Death. *Nat. Cell Biol* 15, 481–490. doi:10.1038/ncb2738

Hartl, F. U. (2017). Protein Misfolding Diseases. *Annu. Rev. Biochem.* 86, 21–26. doi:10.1146/annurev-biochem-061516-044518

Haynes, C. M., Petrova, K., Benedetti, C., Yang, Y., and Ron, D. (2007). ClpP Mediates Activation of a Mitochondrial Unfolded Protein Response in *C. elegans*. *Dev. Cell* 13, 467–480. doi:10.1016/j.devcel.2007.07.016

Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999). Mammalian Transcription Factor ATF6 Is Synthesized as a Transmembrane Protein and Activated by Proteolysis in Response to Endoplasmic Reticulum Stress. *MBoC* 10, 3787–3799. doi:10.1091/mbc.10.11.3787

He, J., Ford, H. C., Carroll, J., Douglas, C., Gonzales, E., Ding, S., et al. (2018). Assembly of the Membrane Domain of ATP Synthase in Human Mitochondria. *Proc. Natl. Acad. Sci. USA* 115, 2988–2993. doi:10.1073/pnas.1722086115

Heldens, L., Hensen, S. M. M., Onnekink, C., van Genesen, S. T., Dirks, R. P., and Lubsen, N. H. (2011). An Atypical Unfolded Protein Response in Heat Shocked Cells. *PLoS ONE* 6, e23512. doi:10.1371/journal.pone.0023512

Higuchi, R., Vevea, J. D., Swayne, T. C., Chojnowski, R., Hill, V., Boldogh, I. R., et al. (2013). Actin Dynamics Affect Mitochondrial Quality Control and Aging in Budding Yeast. *Curr. Biol.* 23, 2417–2422. doi:10.1016/j.cub.2013.10.022

Higuchi-Sanabria, R., Durieux, J., Kelet, N., Homentcovschi, S., de Los Rios Rogers, M., Monshietehadi, S., et al. (2020). Divergent Nodes of Non-autonomous UPRER Signaling through Serotonergic and Dopaminergic Neurons. *Cell Rep.* 33, 108489. doi:10.1016/j.celrep.2020.108489

Higuchi-Sanabria, R., Frankino, P. A., Paul, J. W., Tronnes, S. U., and Dillin, A. (2018). A Futile Battle? Protein Quality Control and the Stress of Aging. *Dev. Cell* 44, 139–163. doi:10.1016/j.devcel.2017.12.020

Higuchi-Sanabria, R., Paul, J. W., 3rd, Durieux, J., Benitez, C., Frankino, P. A., Tronnes, S. U., et al. (2018). Spatial Regulation of the Actin Cytoskeleton by HSF-1 during Aging. *MBoC* 29, 2522–2527. doi:10.1091/mbc.e18-06-0362

Higuchi-Sanabria, R., Paul, J. W., Durieux, J., Benitez, C., Frankino, P. A., Tronnes, S. U., et al. (2018). Spatial Regulation of the Actin Cytoskeleton by HSF-1 during Aging. *MBoC* 29, 2522–2527. doi:10.1091/mbc.e18-06-0362

Hillary, R. F., and FitzGerald, U. (2018). A Lifetime of Stress: ATF6 in Development and Homeostasis. *J. Biomed. Sci.* 25, 48. doi:10.1186/s12929-018-0453-1

Hollien, J., and Weissman, J. S. (2006). Decay of Endoplasmic Reticulum-Localized mRNAs during the Unfolded Protein Response. *Science* 313, 104–107. doi:10.1126/science.1129631

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

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

Ichimiya, H., Huet, R. G., Hartman, P., Amino, H., Kita, K., and Ishii, N. (2002). Complex II Inactivation Is Lethal in the Nematode *Caenorhabditis elegans*. *Mitochondrion* 2, 191–198. doi:10.1016/s1567-7249(02)00069-7

Imanikia, S., Özbey, N. P., Krueger, C., Casanueva, M. O., and Taylor, R. C. (2019). Neuronal XBP-1 Activates Intestinal Lysosomes to Improve Proteostasis in *C. elegans*. *Curr. Biol.* 29, 2322–2338. e7. doi:10.1016/j.cub.2019.06.031

Imanikia, S., Sheng, M., Castro, C., Griffin, J. L., and Taylor, R. C. (2019). XBP-1 Remodels Lipid Metabolism to Extend Longevity. *Cell Rep.* 28, 581e4–589. doi:10.1016/j.celrep.2019.06.057

Jee, H. (2016). Size Dependent Classification of Heat Shock Proteins: a Mini-Review. *J. Exerc. Rehabil.* 12, 255–259. doi:10.12965/jer.1632642.321

Jiang, C. C., Chen, L. H., Gillespie, S., Wang, Y. F., Kiejda, K. A., Zhang, X. D., et al. (2007). Inhibition of MEK Sensitizes Human Melanoma Cells to Endoplasmic Reticulum Stress-Induced Apoptosis. *Cancer Res.* 67, 9750–9761. doi:10.1158/0008-5472.can-07-2047

Jung, K.-W., So, Y.-S., and Bahn, Y.-S. (2016). Unique Roles of the Unfolded Protein Response Pathway in Fungal Development and Differentiation. *Sci. Rep.* 6, 33413. doi:10.1038/srep33413

Kenny, T. C., and Germain, D. (2017). mtDNA, Metastasis, and the Mitochondrial Unfolded Protein Response (UPRmt). *Front. Cell Dev. Biol.* 5, 37. doi:10.3389/fcell.2017.00037

Kenyon, C. J. (2010). The Genetics of Ageing. *Nature* 464, 504–512. doi:10.1038/nature08980

Khalimonchuk, O., Bird, A., and Winge, D. R. (2007). Evidence for a Pro-oxidant Intermediate in the Assembly of Cytochrome Oxidase. *J. Biol. Chem.* 282, 17442–17449. doi:10.1074/jbc.m702379200

Kim, H.-E., Grant, A. R., Simic, M. S., Kohnz, R. A., Nomura, D. K., Durieux, J., et al. (2016). Lipid Biosynthesis Coordinates a Mitochondrial-To-Cytosolic Stress Response. *Cell* 166, 1539–1552. e16. doi:10.1016/j.cell.2016.08.027

Kim, K. H., Jeong, Y. T., Oh, H., Kim, S. H., Cho, J. M., Kim, Y.-N., et al. (2013). Autophagy Deficiency Leads to protection from Obesity and Insulin Resistance by Inducing Fgf21 as a Mitokine. *Nat. Med.* 19, 83–92. doi:10.1038/nm.3014

Kirkwood, T. B. L., and Austad, S. N. (2000). Why Do We Age? *Nature* 408, 233–238. doi:10.1038/35041682

Koh, J. H., Wang, L., Beaudoin-Chabot, C., and Thibault, G. (2018). Lipid Bilayer Stress-Activated IRE-1 Modulates Autophagy during Endoplasmic Reticulum Stress. *J. Cel. Sci.* 131, 1. doi:10.1242/jcs.217992

Kohno, K., Normington, K., Sambrook, J., Gething, M. J., and Mori, K. (1993). The Promoter Region of the Yeast KAR2 (BiP) Gene Contains a Regulatory Domain that Responds to the Presence of Unfolded Proteins in the Endoplasmic Reticulum. *Mol. Cel. Biol.* 13, 877–890. doi:10.1128/mcb.13.2.877-890.1993

Korobova, F., Ramabhadran, V., and Higgs, H. N. (2013). An Actin-dependent Step in Mitochondrial Fission Mediated by the ER-Associated Formin INF2. *Science* 339, 464–467. doi:10.1126/science.1228360

Kourtis, N., Nikoletopoulou, V., and Tavernarakis, N. (2012). Small Heat-Shock Proteins Protect from Heat-Stroke-Associated Neurodegeneration. *Nature* 490, 213–218. doi:10.1038/nature11417

Kourtis, N., and Tavernarakis, N. (2011). Cellular Stress Response Pathways and Ageing: Intricate Molecular Relationships. *EMBO J.* 30, 2520–2531. doi:10.1038/emboj.2011.162

Kozlowski, L., Garvis, S., Bedet, C., and Palladino, F. (2014). The *Caenorhabditis elegans* HP1 Family Protein HPL-2 Maintains ER Homeostasis through the UPR and Hormesis. *Proc. Natl. Acad. Sci.* 111, 5956–5961. doi:10.1073/pnas.1321698111

Kroeger, H., Grimsey, N., Paxman, R., Chiang, W. C., Plate, L., Jones, Y., et al. (2018). The Unfolded Protein Response Regulator ATF6 Promotes Mesodermal Differentiation. *Sci. Signal.* 11, eaan5785. doi:10.1126/scisignal.aan5785

Kuang, J., and Ebert, P. R. (2012). The Failure to Extend Lifespan via Disruption of Complex II Is Linked to Preservation of Dynamic Control of Energy Metabolism. *Mitochondrion* 12, 280–287. doi:10.1016/j.mito.2011.10.003

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

Kumsta, C., Chang, J. T., Schmalz, J., and Hansen, M. (2017). Hormetic Heat Stress and HSF-1 Induce Autophagy to Improve Survival and Proteostasis in *C. elegans*. *Nat. Commun.* 8, 14337. doi:10.1038/ncomms14337

Kumsta, C., Ching, T.-T., Nishimura, M., Davis, A. E., Gelino, S., Catan, H. H., et al. (2014). Integrin-linked Kinase Modulates Longevity and Thermotolerance inC. Elegansthrough Neuronal Control of HSF-1. *Aging cell* 13, 419–430. doi:10.1111/acel.12189

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. Cel.* 59, 639–650. doi:10.1016/j.molcel.2015.06.027

Lan, J., Rollins, J. A., Zang, X., Wu, D., Zou, L., Wang, Z., et al. (2019). Translational Regulation of Non-autonomous Mitochondrial Stress Response Promotes Longevity. *Cell Rep.* 28, 1050–1062. e6. doi:10.1016/j.celrep.2019.06.078

Leary, S. C., Battersby, B. J., Hansford, R. G., and Moyes, C. D. (1998). Interactions between Bioenergetics and Mitochondrial Biogenesis. *Biochim. Biophys. Acta (Bba) - Bioenerg.* 1365, 522–530. doi:10.1016/s0005-2728(98)00105-4

Lee, A.-H., Heidtman, K., Hotamisligil, G. S., and Glimcher, L. H. (2011). Dual and Opposing Roles of the Unfolded Protein Response Regulated by IRE1 and XBP1 in Proinsulin Processing and Insulin Secretion. *Proc. Natl. Acad. Sci.* 108, 8885–8890. doi:10.1073/pnas.1105564108

Lee, S. S., Lee, R. Y. N., Fraser, A. G., Kamath, R. S., Ahringer, J., and Ruvkun, G. (2003). A Systematic RNAi Screen Identifies a Critical Role for Mitochondria in *C. elegans* Longevity. *Nat. Genet.* 33, 40–48. doi:10.1038/ng1056

Levi-Ferber, M., Gian, H., Dudkevich, R., and Henis-Korenblit, S. (2015). Transdifferentiation Mediated Tumor Suppression by the Endoplasmic Reticulum Stress Sensor IRE-1 in *C. elegans*. *eLife* 4, e08005. doi:10.7554/eLife.08005

Levi-Ferber, M., Shalash, R., Le-Thomas, A., Salzberg, Y., Shurgi, M., Benichou, J. I., et al. (2021). Neuronal Regulated Ire-1-dependent mRNA Decay Controls Germline Differentiation in *Caenorhabditis elegans*. *eLife* 10, e65644. doi:10.7554/elife.65644

Li, Z., Zhang, Z., Ren, Y., Wang, Y., Fang, J., Yue, H., et al. (2021). Aging and Age-related Diseases: from Mechanisms to Therapeutic Strategies. *Biogerontology* 22, 165–187. doi:10.1007/s10522-021-09910-5

Lindquist, S., and Craig, E. A. (1988). The Heat-Shock Proteins. *Annu. Rev. Genet.* 22, 631–677. doi:10.1146/annurev.ge.22.120188.003215

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., and Chang, A. (2008). Heat Shock Response Relieves ER Stress. *EMBO J.* 27, 1049–1059. doi:10.1038/emboj.2008.42

Lopez-Crisosto, C., Díaz-Vegas, A., Castro, P. F., Rothermel, B. A., Bravo-Sagua, R., and Lavandero, S. (2021). Endoplasmic Reticulum–mitochondria Coupling Increases during Doxycycline-Induced Mitochondrial Stress in HeLa Cells. *Cell Death Dis* 12, 1–12. doi:10.1038/s41419-021-03945-9

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

Ma, Y., and Hendershot, L. M. (2003). Delineation of a Negative Feedback Regulatory Loop that Controls Protein Translation during Endoplasmic Reticulum Stress. *J. Biol. Chem.* 278, 34864–34873. doi:10.1074/jbc.m301107200

Manor, U., Bartholomew, S., Golani, G., Christenson, E., Kozlov, M., Higgs, H., et al. (2015). A Mitochondria-Anchored Isoform of the Actin-Nucleating Spire Protein Regulates Mitochondrial Division. *eLife* 4, e08828. doi:10.7554/eLife.08828

Martinus, R. D., Garth, G. P., Webster, T. L., Cartwright, P., Naylor, D. J., Høj, P. B., et al. (1996). Selective Induction of Mitochondrial Chaperones in Response to Loss of the Mitochondrial Genome. *Eur. J. Biochem.* 240, 98–103. doi:10.1111/j.1432-1033.1996.0098h.x

McColl, G., Rogers, A. N., Alavez, S., Hubbard, A. E., Melov, S., Link, C. D., et al. (2010). Insulin-like Signaling Determines Survival during Stress via Posttranscriptional Mechanisms in *C. elegans*. *Cell Metab.* 12, 260–272. doi:10.1016/j.cmet.2010.08.004

McElwee, J., Bubb, K., and Thomas, J. H. (2003). Transcriptional Outputs of the *Caenorhabditis elegans* Forkhead Protein DAF-16. *Aging cell* 2, 111–121. doi:10.1046/j.1474-9728.2003.00043.x

Mendenhall, A. R., Tedesco, P. M., Taylor, L. D., Lowe, A., Cypser, J. R., and Johnson, T. E. (2012). Expression of a Single-Copy hsp-16.2 Reporter Predicts Life Span. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 67, 726–733. doi:10.1093/gerona/glr225

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

Misrani, A., Tabassum, S., and Yang, L. (2021). Mitochondrial Dysfunction and Oxidative Stress in Alzheimer's Disease. *Front. Aging Neurosci.* 13, 18. doi:10.3389/fnagi.2021.617588

Moehle, E. A., Higuchi-Sanabria, R., Tsui, C. K., Homentcovschi, S., Tharp, K. M., Zhang, H., et al. (2021). Cross-species Screening Platforms Identify EPS-8 as a Critical Link for Mitochondrial Stress and Actin Stabilization. *Sci. Adv.* 7, eabj6818. doi:10.1126/sciadv.abj6818

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

Molenaars, M., Janssens, G. E., Williams, E. G., Jongejan, A., Lan, J., Rabot, S., et al. (2020). A Conserved Mito-Cytosolic Translational Balance Links Two Longevity Pathways. *Cell Metab.* 31, 549e7–563. doi:10.1016/j.cmet.2020.01.011

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.* 99, 10417–10422. doi:10.1073/pnas.152161099

Morley, J. F., and Morimoto, R. I. (2004). Regulation of Longevity inCaenorhabditis Elegansby Heat Shock Factor and Molecular Chaperones. *MBoC* 15, 657–664. doi:10.1091/mbc.e03-07-0532

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

Murphy, C. T., McCarroll, S. A., Bargmann, C. I., Fraser, A., Kamath, R. S., Ahringer, J., et al. (2003). Genes that Act Downstream of DAF-16 to Influence the Lifespan of *Caenorhabditis elegans*. *Nature* 424, 277–283. doi:10.1038/nature01789

Naidoo, N., Ferber, M., Master, M., Zhu, Y., and Pack, A. I. (2008). Aging Impairs the Unfolded Protein Response to Sleep Deprivation and Leads to Proapoptotic Signaling. *J. Neurosci.* 28, 6539–6548. doi:10.1523/jneurosci.5685-07.2008

Nakai, A., Kawazoe, Y., Tanabe, M., Nagata, K., and Morimoto, R. I. (1995). The DNA-Binding Properties of Two Heat Shock Factors, HSF1 and HSF3, Are Induced in the Avian Erythroblast Cell Line HD6. *Mol. Cell Biol* 15, 5268–5278. doi:10.1128/mcb.15.10.5268

Nakai, A., Tanabe, M., Kawazoe, Y., Inazawa, J., Morimoto, R. I., and Nagata, K. (1997). HSF4, a New Member of the Human Heat Shock Factor Family Which Lacks Properties of a Transcriptional Activator. *Mol. Cell Biol* 17, 469–481. doi:10.1128/mcb.17.1.469

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

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

Neef, D. W., Jaeger, A. M., Gomez-Pastor, R., Willmund, F., Frydman, J., and Thiele, D. J. (2014). A Direct Regulatory Interaction between Chaperonin TRiC and Stress-Responsive Transcription Factor HSF1. *Cell Rep.* 9, 955–966. doi:10.1016/j.celrep.2014.09.056

Neudegger, T., Verghese, J., Hayer-Hartl, M., Hartl, F. U., and Bracher, A. (2016). Structure of Human Heat-Shock Transcription Factor 1 in Complex with DNA. *Nat. Struct. Mol. Biol.* 23, 140–146. doi:10.1038/nsmb.3149

Nishitoh, H., Matsuzawa, A., Tobiume, K., Saegusa, K., Takeda, K., Inoue, K., et al. (2002). ASK1 Is Essential for Endoplasmic Reticulum Stress-Induced Neuronal Cell Death Triggered by Expanded Polyglutamine Repeats. *Genes Dev.* 16, 1345–1355. doi:10.1101/gad.992302

Nolden, M., Ehses, S., Koppen, M., Bernacchia, A., Rugarli, E. I., and Langer, T. (2005). The M-AAA Protease Defective in Hereditary Spastic Paraplegia Controls Ribosome Assembly in Mitochondria. *Cell* 123, 277–289. doi:10.1016/j.cell.2005.08.003

Nuss, J. E., Choksi, K. B., DeFord, J. H., and Papaconstantinou, J. (2008). Decreased Enzyme Activities of Chaperones PDI and BiP in Aged Mouse Livers. *Biochem. Biophysical Res. Commun.* 365, 355–361. doi:10.1016/j.bbrc.2007.10.194

Ooi, F. K., and Prahlad, V. (2017). Olfactory Experience Primes the Heat Shock Transcription Factor HSF-1 to Enhance the Expression of Molecular

Chaperones in *C. elegans*. *Sci. Signal.* 10, eaan4893. doi:10.1126/scisignal.aan4893

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

Özbey, N. P., Imanikia, S., Krueger, C., Hardege, I., Morud, J., Sheng, M., et al. (2020). Tyramine Acts Downstream of Neuronal XBP-1s to Coordinate Inter-tissue UPRER Activation and Behavior in *C. elegans*. *Dev. Cell* 55, 754–770. e6. doi:10.1016/j.devcel.2020.10.024

Ozcan, L., and Tabas, I. (2012). Role of Endoplasmic Reticulum Stress in Metabolic Disease and Other Disorders. *Annu. Rev. Med.* 63, 317–328. doi:10.1146/annurev-med-043010-144749

Pellegrino, M. W., Nargund, A. M., Kirienko, N. V., Gillis, R., Fiorese, C. J., and Haynes, C. M. (2014). Mitochondrial UPR-Regulated Innate Immunity Provides Resistance to Pathogen Infection. *Nature* 516, 414–417. doi:10.1038/nature13818

Peteranderl, R., Rabenstein, M., Shin, Y.-K., Liu, C. W., Wemmer, D. E., King, D. S., et al. (1999). Biochemical and Biophysical Characterization of the Trimerization Domain from the Heat Shock Transcription Factor. *Biochemistry* 38, 3559–3569. doi:10.1021/bi981774j

Pol, A., Gross, S. P., and Parton, R. G. (2014). Biogenesis of the Multifunctional Lipid Droplet: Lipids, Proteins, and Sites. *J. Cell Biol* 204, 635–646. doi:10.1083/jcb.201311051

Prahlad, V., Cornelius, T., and Morimoto, R. I. (2008). Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons. *Science* 320, 811–814. doi:10.1126/science.1156093

Prahlad, V., Cornelius, T., and Morimoto, R. I. (2008). Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons. *Science* 320, 811–814. doi:10.1126/science.1156093

Prischi, F., Nowak, P. R., Carrara, M., and Ali, M. M. U. (2014). Phosphoregulation of Ire1 RNase Splicing Activity. *Nat. Commun.* 5, 3554. doi:10.1038/ncomms4554

Prithika, U., Deepa, V., and Balamurugan, K. (2016). External Induction of Heat Shock Stimulates the Immune Response and Longevity of *Caenorhabditis elegans* towards Pathogen Exposure. *Innate Immun.* 22, 466–478. doi:10.1177/1753425916654557

Quirós, P. M., Prado, M. A., Zamboni, N., D'Amico, D., Williams, R. W., Finley, D., et al. (2017). Multi-omics Analysis Identifies ATF4 as a Key Regulator of the Mitochondrial Stress Response in Mammals. *J. Cell Biol* 216, 2027–2045. doi:10.1083/jcb.201702058

Rabindran, S. K., Haroun, R. I., Clos, J., Wisniewski, J., and Wu, C. (1993). Regulation of Heat Shock Factor Trimer Formation: Role of a Conserved Leucine Zipper. *Science* 259, 230–234. doi:10.1126/science.8421783

Radons, J. (2016). The Human HSP70 Family of Chaperones: where Do We Stand? *Cell Stress and Chaperones* 21, 379–404. doi:10.1007/s12192-016-0676-6

Raffaello, A., Mammucari, C., Gherardi, G., and Rizzuto, R. (2016). Calcium at the Center of Cell Signaling: Interplay between Endoplasmic Reticulum, Mitochondria, and Lysosomes. *Trends Biochem. Sci.* 41, 1035–1049. doi:10.1016/j.tibs.2016.09.001

Rattan, S. I. (2004). Aging Intervention, Prevention, and Therapy through Hormesis. *J. Gerontol. A. Biol. Sci. Med. Sci.* 59, 705–709. doi:10.1093/gerona/59.7.b705

Rattan, S. I. S. (2005). Hormetic Modulation of Aging and Longevity by Mild Heat Stress. *Dose-Response* 3 (4), dose–response. doi:10.2203/dose-response.003.04.008

Rea, S. L., Ventura, N., and Johnson, T. E. (2007). Relationship between Mitochondrial Electron Transport Chain Dysfunction, Development, and Life Extension in *Caenorhabditis elegans*. *Plos Biol.* 5, e259. doi:10.1371/journal.pbio.0050259

Reimold, A. M., Iwakoshi, N. N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravallese, E. M., et al. (2001). Plasma Cell Differentiation Requires the Transcription Factor XBP-1. *Nature* 412, 300–307. doi:10.1038/35085509

Ristow, M., and Zarse, K. (2010). How Increased Oxidative Stress Promotes Longevity and Metabolic Health: The Concept of Mitochondrial Hormesis (Mitohormesis). *Exp. Gerontol.* 45, 410–418. doi:10.1016/j.exger.2010.03.014

Ritossa, F. (1962). A New Puffing Pattern Induced by Temperature Shock and DNP in drosophila. *Experientia* 18, 571–573. doi:10.1007/bf02172188

Ritossa, F. (1996). Discovery of the Heat Shock Response. *Cell Stress Chaper* 1, 97–98. doi:10.1379/1466-1268(1996)001<0097:dothsr>2.3.co;2

Rolland, S. G., Schneid, S., Schwarz, M., Rackles, E., Fischer, C., Haeussler, S., et al. (2019). Compromised Mitochondrial Protein Import Acts as a Signal for UPRmt. *Cell Rep.* 28, 1659e5–1669. doi:10.1016/j.celrep.2019.07.049

Roux, A. E., Langhans, K., Huynh, W., and Kenyon, C. (2016). Reversible Age-Related Phenotypes Induced during Larval Quiescence in *C. elegans*. *Cell Metab.* 23, 1113–1126. doi:10.1016/j.cmet.2016.05.024

Schopf, F. H., Biebl, M. M., and Buchner, J. (2017). The HSP90 Chaperone Machinery. *Nat. Rev. Mol. Cell Biol* 18, 345–360. doi:10.1038/nrm.2017.20

Schröder, M., and Kaufman, R. J. (2005). The Mammalian Unfolded Protein Response. *Annu. Rev. Biochem.* 74, 739–789. doi:10.1146/annurev.biochem.73.011303.074134

Schuetz, T. J., Gallo, G. J., Sheldon, L., Tempst, P., and Kingston, R. E. (1991). Isolation of a cDNA for HSF2: Evidence for Two Heat Shock Factor Genes in Humans. *Proc. Natl. Acad. Sci.* 88, 6911–6915. doi:10.1073/pnas.88.16.6911

Shalem, O., Sanjana, N. E., Hartenian, E., Shi, X., Scott, D. A., Mikkelsen, T. S., et al. (2014). Genome-scale CRISPR-Cas9 Knockout Screening in Human Cells. *Science* 343, 84–87. doi:10.1126/science.1247005

Shao, L.-W., Niu, R., and Liu, Y. (2016). Neuropeptide Signals Cell Non-autonomous Mitochondrial Unfolded Protein Response. *Cell Res* 26, 1182–1196. doi:10.1038/cr.2016.118

Shemesh, N., Shai, N., and Ben-Zvi, A. (2013). Germline Stem Cell Arrest Inhibits the Collapse of Somatic Proteostasis Early inCaenorhabditis Elegansadulthood. *Aging cell* 12, 814–822. doi:10.1111/acel.12110

Shen, X., Ellis, R. E., Lee, K., Liu, C.-Y., Yang, K., Solomon, A., et al. (2001). Complementary Signaling Pathways Regulate the Unfolded Protein Response and Are Required for *C. elegans* Development. *Cell* 107, 893–903. doi:10.1016/s0092-8674(01)00612-2

Shen, X., Ellis, R. E., Sakaki, K., and Kaufman, R. J. (2005). Genetic Interactions Due to Constitutive and Inducible Gene Regulation Mediated by the Unfolded Protein Response in *C. elegans*. *Plos Genet.* 1, e37. doi:10.1371/journal.pgen.0010037

Shi, Y., Mosser, D. D., and Morimoto, R. I. (1998). Molecularchaperones as HSF1-specific Transcriptional Repressors. *Genes Dev.* 12, 654–666. doi:10.1101/gad.12.5.654

Shoulders, M. D., Ryno, L. M., Genereux, J. C., Moresco, J. J., Tu, P. G., Wu, C., et al. (2013). Stress-Independent Activation of XBP1s And/or ATF6 Reveals Three Functionally Diverse ER Proteostasis Environments. *Cell Rep.* 3, 1279–1292. doi:10.1016/j.celrep.2013.03.024

Shpilka, T., Du, Y., Yang, Q., Melber, A., Uma Naresh, N., Lavelle, J., et al. (2021). UPRmt Scales Mitochondrial Network Expansion with Protein Synthesis via Mitochondrial Import in *Caenorhabditis elegans*. *Nat. Commun.* 12, 479. doi:10.1038/s41467-020-20784-y

Singh, V., and Aballay, A. (2006). Heat-shock Transcription Factor (HSF)-1 Pathway Required for *Caenorhabditis elegans* Immunity. *Proc. Natl. Acad. Sci.* 103, 13092–13097. doi:10.1073/pnas.0604050103

Sletten, A. C., Peterson, L. R., and Schaffer, J. E. (2018). Manifestations and Mechanisms of Myocardial Lipotoxicity in Obesity. *J. Intern. Med.* 284, 478–491. doi:10.1111/joim.12728

Son, H. G., Seo, K., Seo, M., Park, S., Ham, S., An, S. W. A., et al. (2018). Prefoldin 6 Mediates Longevity Response from Heat Shock Factor 1 to FOXO in *C. elegans*. *Genes Dev.* 32, 1562–1575. doi:10.1101/gad.317362.118

Song, W., Owusu-Ansah, E., Hu, Y., Cheng, D., Ni, X., Zirin, J., et al. (2017). Activin Signaling Mediates Muscle-To-Adipose Communication in a Mitochondria Dysfunction-Associated Obesity Model. *Proc. Natl. Acad. Sci. USA* 114, 8596–8601. doi:10.1073/pnas.1708037114

Sopko, R., Huang, D., Preston, N., Chua, G., Papp, B., Kafadar, K., et al. (2006). Mapping Pathways and Phenotypes by Systematic Gene Overexpression. *Mol. Cell* 21, 319–330. doi:10.1016/j.molcel.2005.12.011

Stauffer, W. T., Arrieta, A., Blackwood, E. A., and Glembotski, C. C. (2020). Sledgehammer to Scalpel: Broad Challenges to the Heart and Other Tissues Yield Specific Cellular Responses via Transcriptional Regulation of the ER-Stress Master Regulator ATF6α. *Ijms* 21, 1134. doi:10.3390/ijms21031134

Sural, S., Liang, C. Y., Wang, F. Y., Ching, T. T., and Hsu, A. L. (2020). HSB-1/HSF-1 Pathway Modulates Histone H4 in Mitochondria to Control mtDNA Transcription and Longevity. *Sci. Adv.* 6, eaaz4452. doi:10.1126/sciadv.aaz4452

Tam, A. B., Koong, A. C., and Niwa, M. (2014). Ire1 Has Distinct Catalytic Mechanisms for XBP1/HAC1 Splicing and RIDD. *Cell Rep.* 9, 850–858. doi:10.1016/j.celrep.2014.09.016

Tatum, M. C., Ooi, F. K., Chikka, M. R., Chauve, L., Martinez-Velazquez, L. A., Steinbusch, H. W. M., et al. (2015). Neuronal Serotonin Release Triggers the Heat Shock Response in *C. elegans* in the Absence of Temperature Increase. *Curr. Biol.* 25, 163–174. doi:10.1016/j.cub.2014.11.040

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

Tharp, K. M., Higuchi-Sanabria, R., Timblin, G. A., Ford, B., Garzon-Coral, C., Schneider, C., et al. (2021). Adhesion-mediated Mechanosignaling Forces Mitohormesis. *Cell Metab* 33, 1322–1341. doi:10.1016/j.cmet.2021.04.017

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 UPR Mt. *Cell* 165, 1197–1208. doi:10.1016/j.cell.2016.04.011

Tissiéres, A., Mitchell, H. K., and Tracy, U. M. (1974). Protein Synthesis in Salivary Glands of *Drosophila melanogaster*: Relation to Chromosome Puffs. *J. Mol. Biol.* 84, 389–398. doi:10.1016/0022-2836(74)90447-1

Trivedi, R., and Jurivich, D. A. (2020). A Molecular Perspective on Age-dependent Changes to the Heat Shock axis. *Exp. Gerontol.* 137, 110969. doi:10.1016/j.exger.2020.110969

Tsang, W. Y., and Lemire, B. D. (2002). Mitochondrial Genome Content Is Regulated during Nematode Development. *Biochem. Biophysical Res. Commun.* 291, 8–16. doi:10.1006/bbrc.2002.6394

Tsuru, A., Fujimoto, N., Takahashi, S., Saito, M., Nakamura, D., Iwano, M., et al. (2013). Negative Feedback by IRE1 Optimizes Mucin Production in Goblet Cells. *Proc. Natl. Acad. Sci.* 110, 2864–2869. doi:10.1073/pnas.1212484110

Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., et al. (2000). Coupling of Stress in the ER to Activation of JNK Protein Kinases by Transmembrane Protein Kinase IRE1. *Science* 287, 664–666. doi:10.1126/science.287.5453.664

van Eden, W., van der Zee, R., and Prakken, B. (2005). Heat-shock Proteins Induce T-Cell Regulation of Chronic Inflammation. *Nat. Rev. Immunol.* 5, 318–330. doi:10.1038/nri1593

Ventura, N., Rea, S. L., Schiavi, A., Torgovnick, A., Testi, R., and Johnson, T. E. (2009). p53/CEP-1 Increases or Decreases Lifespan, Depending on Level of Mitochondrial Bioenergetic Stress. *Aging Cell* 8, 380–393. doi:10.1111/j.1474-9726.2009.00482.x

Verma, G., and Datta, M. (2010). IL-1β Induces ER Stress in a JNK Dependent Manner that Determines Cell Death in Human Pancreatic Epithelial MIA PaCa-2 Cells. *Apoptosis* 15, 864–876. doi:10.1007/s10495-010-0498-4

Vevea, J. D., Garcia, E. J., Chan, R. B., Zhou, B., Schultz, M., Di Paolo, G., et al. (2015). Role for Lipid Droplet Biogenesis and Microlipophagy in Adaptation to Lipid Imbalance in Yeast. *Dev. Cell* 35, 584–599. doi:10.1016/j.devcel.2015.11.010

Volovik, Y., Maman, M., Dubnikov, T., Bejerano-Sagie, M., Joyce, D., Kapernick, E. A., et al. (2012). Temporal Requirements of Heat Shock Factor-1 for Longevity Assurance. *Aging Cell* 11, 491–499. doi:10.1111/j.1474-9726.2012.00811.x

Walter, L., Baruah, A., Chang, H.-W., Pace, H. M., and Lee, S. S. (2011). The Homeobox Protein CEH-23 Mediates Prolonged Longevity in Response to Impaired Mitochondrial Electron Transport Chain in *C. elegans*. *Plos Biol.* 9, e1001084. doi:10.1371/journal.pbio.1001084

Wang, N., Gottesman, S., Willingham, M. C., Gottesman, M. M., and Maurizi, M. R. (1993). A Human Mitochondrial ATP-dependent Protease that Is Highly Homologous to Bacterial Lon Protease. *Pnas* 90, 11247–11251. doi:10.1073/pnas.90.23.11247

Watanabe, Y., Tsujimura, A., Taguchi, K., and Tanaka, M. (2017). HSF1 Stress Response Pathway Regulates Autophagy Receptor SQSTM1/p62-Associated Proteostasis. *Autophagy* 13, 133–148. doi:10.1080/15548627.2016.1248018

Williams, K. W., Liu, T., Kong, X., Fukuda, M., Deng, Y., Berglund, E. D., et al. (2014). Xbp1s in Pomc Neurons Connects ER Stress with Energy Balance and Glucose Homeostasis. *Cell Metab.* 20, 471–482. doi:10.1016/j.cmet.2014.06.002

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

Xin, N., Durieux, J., Yang, C., Wolff, S., Kim, H.-E., and Dillin, A. (2020). *The UPRmt Preserves Mitochondrial Import to Extend Lifespan*. New York, NY: Cold Spring Harbor Laboratory. doi:10.1101/2020.07.01.182980

Xu, L., Wang, X., and Tong, C. (2020). Endoplasmic Reticulum-Mitochondria Contact Sites and Neurodegeneration. *Front. Cell Dev. Biol.* 8, 428. doi:10.3389/fcell.2020.00428

Xu, Z., Chikka, M. R., Xia, H., and Ready, D. F. (2016). Ire1 Supports normal ER Differentiation in Developing Drosophila Photoreceptors. *J. Cell Sci* 129, 921–929. doi:10.1242/jcs.180406

Ye, J., Rawson, R. B., Komuro, R., Chen, X., Davé, U. P., Prywes, R., et al. (2000). ER Stress Induces Cleavage of Membrane-Bound ATF6 by the Same Proteases that Process SREBPs. *Mol. Cell* 6, 1355–1364. doi:10.1016/s1097-2765(00)00133-7

Yee, C., Yang, W., and Hekimi, S. (2014). The Intrinsic Apoptosis Pathway Mediates the Pro-longevity Response to Mitochondrial ROS in *C. elegans*. *Cell* 157, 897–909. doi:10.1016/j.cell.2014.02.055

Zara, V., Conte, L., and Trumpower, B. L. (2007). Identification and Characterization of Cytochrome Bc1 Subcomplexes in Mitochondria from Yeast with Single and Double Deletions of Genes Encoding Cytochrome Bc1 Subunits. *FEBS J.* 274, 4526–4539. doi:10.1111/j.1742-4658.2007.05982.x

Zhang, C., Syed, T. W., Liu, R., and Yu, J. (2017). Role of Endoplasmic Reticulum Stress, Autophagy, and Inflammation in Cardiovascular Disease. *Front. Cardiovasc. Med.* 4, 1. doi:10.3389/fcvm.2017.00029

Zhang, H., Zhang, L., Yu, F., Liu, Y., Liang, Q., Deng, G., et al. (2012). HSF1 Is a Transcriptional Activator of IL-10 Gene Expression in RAW264.7 Macrophages. *Inflammation* 35, 1558–1566. doi:10.1007/s10753-012-9471-4

Zhang, Q., Wang, Z., Zhang, W., Wen, Q., Li, X., Zhou, J., et al. (2021). The Memory of Neuronal Mitochondrial Stress Is Inherited Transgenerationally via Elevated Mitochondrial DNA Levels. *Nat. Cell Biol* 23, 870–880. doi:10.1038/s41556-021-00724-8

Zhang, Q., Wu, X., Chen, P., Liu, L., Xin, N., Tian, Y., et al. (2018). The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-Autonomously by Retromer-dependent Wnt Signaling. *Cell* 174, 870e17–883. doi:10.1016/j.cell.2018.06.029

Zhang, X., and Zhang, K. (2012). Endoplasmic Reticulum Stress-Associated Lipid Droplet Formation and Type II Diabetes. *Biochem. Res. Int.* 2012, 1–5. doi:10.1155/2012/247275

Zhao, Q., Wang, J., Levichkin, I. V., Stasinopoulos, S., Ryan, M. T., and Hoogenraad, N. J. (2002). A Mitochondrial Specific Stress Response in Mammalian Cells. *EMBO J.* 21, 4411–4419. doi:10.1093/emboj/cdf445

Zhao, Y., Gilliat, A. F., Ziehm, M., Turmaine, M., Wang, H., Ezcurra, M., et al. (2017). Two Forms of Death in Ageing *Caenorhabditis elegans*. *Nat. Commun.* 8, 15458. doi:10.1038/ncomms15458

Zhou, J., Liu, C. Y., Back, S. H., Clark, R. L., Peisach, D., Xu, Z., et al. (2006). The crystal Structure of Human IRE1 Luminal Domain Reveals a Conserved Dimerization Interface Required for Activation of the Unfolded Protein Response. *Proc. Natl. Acad. Sci.* 103, 14343–14348. doi:10.1073/pnas.0606480103

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Temperature-Dependent Regulation of Proteostasis and Longevity

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Temperature is an important environmental condition that determines the physiology and behavior of all organisms. Animals use different response strategies to adapt and survive fluctuations in ambient temperature. The hermaphrodite *Caenorhabditis elegans* has a well-studied neuronal network consisting of 302 neurons. The bilateral AFD neurons are the primary thermosensory neurons in the nematode. In addition to regulating thermosensitivity, AFD neurons also coordinate cellular stress responses through systemic mechanisms involving neuroendocrine signaling. Recent studies have examined the effects of temperature on altering various signaling pathways through specific gene expression programs that promote stress resistance and longevity. These studies challenge the proposed theories of temperature-dependent regulation of aging as a passive thermodynamic process. Instead, they provide evidence that aging is a well-defined genetic program. Loss of protein homeostasis (proteostasis) is one of the key hallmarks of aging. Indeed, proteostasis pathways, such as the heat shock response and aggregation of metastable proteins, are also controlled by thermosensory neurons in *C. elegans*. Prolonged heat stress is thought to play a critical role in the development of neurodegenerative protein misfolding diseases in humans. This review presents the latest evidence on how temperature coordinates proteostasis and aging. It also discusses how studies of poikilothermic organisms can be applied to vertebrates and provides new therapeutic strategies for human disease.

Keywords: temperature, proteostasis, protein aggregation, longevity, *Caenorhabditis elegans*, thermosensation, AFD neuron, cell non-autonomous signaling

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INTRODUCTION

"Homeostasis"—a term coined by Walter Cannon—is the self-regulating dynamic process by which an organism maintains internal stability in response to external conditions (Billman, 2020). Regulation of core body temperature (thermoregulation) is one of the ways to maintain this balance. Homeotherms (warm-blooded animals) such as mammals and birds keep a constant core temperature via hypothalamic regulation of heat production and dissipation. In contrast, poikilotherms (cold-blooded animals) such as flies, nematodes, amphibians, and reptiles lack this thermoregulatory ability. As a result, the core temperature of poikilothermic organisms fluctuates with ambient temperature changes (Tabarean et al., 2010). Another necessary means of maintaining a constant internal milieu is to preserve the integrity of cellular macromolecules such as proteins and maintain a balanced cellular proteome. Protein homeostasis (proteostasis) is promoted by a network of cellular quality control pathways (Kaushik and Cuervo, 2015), including molecular chaperones,

the ubiquitin–proteasome system (UPS), and the autophagy machinery (Chen et al., 2011). Regulated synthesis of polypeptides involves proper protein folding by molecular chaperones, whereas the selective degradation of damaged proteins is mediated by the UPS and the autophagy machinery (Hoppe and Cohen, 2020).

Endogenous and exogenous challenges constantly threaten the integrity of the organismal proteome and affect longevity (Gumeni et al., 2017). Cells cope with cellular stress by adopting sophisticated protective measures. For example, to combat heat stress, organisms have an ancient, highly conserved genetic program termed the heat shock response (HSR) (Morimoto, 1998). An abnormal rise in temperature that triggers protein misfolding and aggregation activates the heat shock transcription factor-1 (HSF-1) in the cytoplasm. Subsequently, HSF-1 converts from an inactive monomer to an active trimeric form and activates expression of heat shock proteins (HSPs), including molecular chaperones (Anckar and Sistonen, 2011). The suppression of HSR in early adulthood renders organisms susceptible to various environmental stressors and eventually leads to breakdown of proteostasis (Ben-Zvi et al., 2009; Labbadia and Morimoto, 2015). The capacity of the proteostasis network (PN) decreases with age, leading to the accumulation of damaged proteins and chronic age-related diseases (Balchin et al., 2016). This review summarizes how proteostasis and longevity are modulated by changes in environmental temperature, focusing on studies in *Caenorhabditis elegans*.

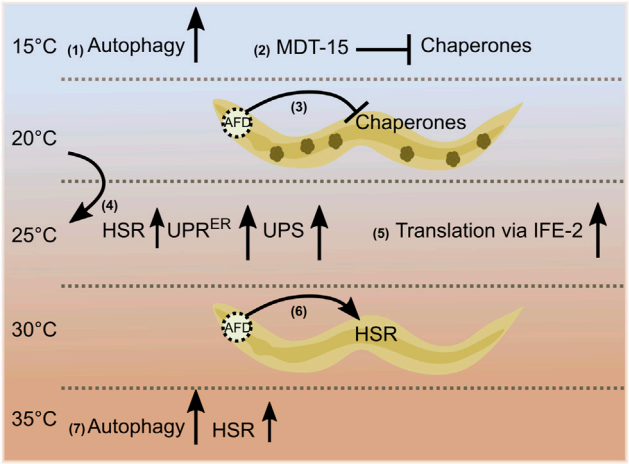

FIGURE 1 | Temperature-dependent regulation of proteostasis. Autophagy and chaperone levels are affected at low temperatures via fatty acid signaling (1), (2). A wild-type thermosensory circuit attenuates protein folding during chronic stress; small dark green structures inside the worm indicate protein aggregates (3). A 1-day transfer of late larval staged *C. elegans* from 20 to 25°C increases the heat shock response [HSR (mildly, small arrow)], the unfolded protein response in the endoplasmic reticulum (UPR^{ER}), and the activity of the ubiquitin/proteasome-system (UPS) in the intestine (4). The translational efficiency of selective mRNAs (*msh-4/him-14*, *msh-5*) increases at 25°C via an eIF4E family protein, IFE-2 (5). AFD thermosensory neuron cells non-autonomously regulate the heat shock response [acute heat shock (HS) of 30°C for 15 min] (6). Hormetic heat shock of 1 h at 36°C induces autophagy and selective HSR (7).

THE INFLUENCE OF TEMPERATURE ON PROTEOSTASIS

The soil-dwelling nematode *C. elegans* is a poikilothermic organism that must constantly adapt its physiology to the changing temperature conditions in its natural habitat (Mendenhall et al., 2017). The worm perceives environmental temperature by the thermosensory neurons named AFD, AWC, ASI, and ASJ (Ohta et al., 2014). The interneurons AIY and AIZ receive thermal inputs from upstream thermosensory neurons, which are further integrated by the RIA interneurons (Kimata et al., 2012). The bilateral AFD neuron is the primary thermosensory neuron that senses ambient temperatures to regulate animal behavior (Goodman and Sengupta, 2018). Thermosensation by AFD and AIY neurons has been associated with organismal proteostasis, particularly in the regulation of the HSR. The heat shock response has always been considered a cell-autonomous response triggered by accumulation of damaged proteins. Surprisingly, a pioneering study by the Morimoto laboratory shows that thermosensory neurons control the heat shock response of somatic tissues. Consequently, worms lacking these neurons exhibit reduced thermotolerance when exposed to increased temperature (Prahlad et al., 2008). The temperature-dependent activation of AFD neurons triggers the HSR in distal tissues by serotonin signaling (Tatum et al., 2015). Reduced HSR in thermosensory mutants suggests a potential increase in protein misfolding. However, the data prove otherwise. At physiological temperatures (20°C), thermosensory mutants suppress protein aggregation and toxicity in multiple tissues. This counterintuitive result shows that the neuronal circuitry based on the thermosensory AFD neurons differentiates between acute heat stress and chronic protein misfolding stress (Prahlad and Morimoto, 2011).

Several interesting observations have been made in recent years despite the lack of mechanistic insight into the effects of temperature on proteostasis. For example, when late larval stages of *C. elegans* grown at 20°C are exposed to 25°C for 1 day, several stress responses are regulated. The unfolded protein response in the endoplasmic reticulum (UPR^{ER}) is strongly activated, whereas HSR increases only transiently after a 1-day exposure to 25°C. Surprisingly, a change in ambient temperature from 20 to 25°C can affect selective protein degradation via the UPS. UPS activity increases in the intestine but not in muscle cells, suggesting tissue-specific regulation of protein degradation (Pispa et al., 2020). Increasing temperature conditions also modulates mRNA translation. One of the five eukaryotic initiation factor (eIF)-4E proteins in *C. elegans*, IFE-2, increases the translational efficiency of certain mRNAs at 25°C (Song et al., 2010). Furthermore, hormetic heat shock of 1 h at 36°C induces autophagy and selective HSR in adult worms, associated with decreased protein aggregation (Kumsta et al., 2017). These studies suggest that *C. elegans* respond to elevated temperature conditions not only by triggering HSR but also other branches of the PN, including translation, protein degradation, and UPR.

Low temperatures regulate proteostasis pathways via modulation of lipid homeostasis. Culturing worms at 15˚C promotes autophagy through signaling mediated by the adiponectin receptor PAQR-2. PAQR-2 increases fatty acid desaturase FAT-7, which triggers the biosynthesis of polyunsaturated fatty acids, namely γ-linolenic acid and arachidonic acid, to induce autophagy (Chen et al., 2019). Mediator complex subunit—mediator 15 (MDT-15/MED15) regulates the expression of *fat-7* at lower temperatures. Decreasing MDT-15 levels at 15˚C increases protein aggregates and cytosolic chaperone expression via HSF-1 as an adaptive response (Dongyeop Lee et al., 2019). Alternatively, gene ontology analysis shows that genes regulated by cold warming—exposure to cold shock (4˚C) followed by recovery at normal temperatures (20˚C)—are involved in biological processes such as autophagy and proteostasis (Jiang et al., 2018). These studies highlight the importance of different temperature conditions in regulating proteome dynamics (**Figure 1**). The maintenance of proteostasis is essential for healthy aging, and its impairment is considered one of the critical hallmarks of aging (López-Otín et al., 2013). In the following section, studies on the influence of temperature on longevity are described in detail.

MODULATION OF ORGANISMAL LONGEVITY BY TEMPERATURE

Poikilothermic animals have shorter lifespans at higher temperatures than at lower temperatures (Lamb, 1968). *C. elegans*, for example, has a mean lifespan of 15.2 ± 0.5 (mean lifespan ± SEM provided) and 26.1 ± 0.6 days at 25 and 15˚C, respectively (Lee and Kenyon, 2009). Pioneering studies led to the postulation of two theories, rate-of-living theory and threshold theory, to explain the effects of temperature on lifespan. Pearl's rate-of-living theory attempts to determine lifespan as a simple rate-limiting response and proposes that the rate of aging increases at higher temperatures (Shaw and Bercaw, 1962). Alternatively, the threshold theory introduces two phases that determine lifespan—aging and dying. It states that the rate of aging is independent of temperature, while the rate of dying depends on temperature (Smith, 1963). Interestingly, the effect of temperature on lifespan has been considered mainly as a passive thermodynamic process in the aforementioned scenarios. However, several studies on poikilothermic organisms, including *C. elegans*, provide considerable evidence to the contrary.

Sensory perception of thermal signals via thermosensory neurons plays a critical role in regulating *C. elegans* lifespan. AFD thermosensory neurons control lifespan at warm temperatures via a steroid signaling pathway. If AFD neuron function is knocked down from early development by genetic mutation or laser ablation, lifespan shortens at 25˚C but not at 15˚C. Thermosensation via these neurons contributes to inhibition of the nuclear hormone receptor (NHR) DAF-12 via DAF-9/Cytochrome P450 (Lee and Kenyon, 2009). Neuronal synaptic transmission may also affect longevity at 25˚C (He et al., 2009). The cyclic AMP (cAMP)-responsive element binding protein, CRH-1/CREB, increases expression of the FMRFamide-like neuropeptide FLP-6 in AFD neurons, promoting adult lifespan. Increased expression of FLP-6 increases DAF-9/sterol signaling in AIY neurons and downregulates insulin signaling in the gut to regulate longevity (Chen et al., 2016). Heat-sensitive ASJ neurons, when ablated, extend lifespan at 25˚C. ASJ neurons act via UNC-31-dependent release of two neuropeptides, INS-6 and DAF-28, to inhibit the FOXO transcription factor DAF-16 in the gut (Zhang et al., 2018). STR-2, a G-protein-coupled receptor expressed in AWC^{ON} and ASI neurons, controls lifespan at 20˚C and higher temperatures. STR-2 fine-tunes neutral lipid levels in non-neuronal tissues to adapt to higher temperatures to maintain lifespan (Dixit et al., 2020). Systemic temperature signaling at culture temperatures (17–23˚C) occurs via HSF-1 activity in non-neuronal cells such as the gut or muscle. Downstream signaling modifies the thermotaxis circuit via the nuclear hormone receptor NHR-69-mediated estrogen signaling (Sugi et al., 2011). HSF-1 not only regulates thermotaxic behavioral performance but also contributes to extending animal lifespan at warm temperatures. Pioneering work by Lee and Kenyon showed that a reduction in HSF-1 function further shortened lifespan at 22.5˚C. Consistent with this, recent data show that overexpression of *hsf-1* in neurons protects animals at 25˚C and extends their lifespan (Chauve et al., 2021). Overexpression of the proteasomal subunit *rpn-6.1* extends lifespan independently of HSF-1 in a DAF-16-dependent manner at 25˚C (Vilchez et al., 2012). Temperature experiences under different growth conditions can lead to different outcomes via the same central player. For example, the co-chaperone DAF-41/p23 modulates lifespan in different ways at warm and cold temperatures, as *daf-41* mutants live longer at higher temperatures but are short-lived at cold temperatures (Horikawa et al., 2015).

Temperature sensing across different tissues and lipid signaling interact to regulate lifespan at cold temperatures. Low temperatures significantly extend *C. elegans* lifespan via the cold-sensitive transient receptor potential (TRP) channel, TRPA-1. TRPA-1 is a non-selective cation channel that is also permeable to calcium. Expression of TRPA-1 in neurons or intestine promotes DAF-16 activity via a genetic program involving a calcium-sensitive kinase, PKC-2, and a DAF-16 kinase, SGK-1. Intriguingly, this study shows that the intestine, which is a non-excitable tissue in worms, functions as a cold receptor (Xiao et al., 2013). TRPA-1 acts in the cold-sensitive IL1 sensory neurons. Glutamatergic and serotoninergic signals from IL1 and NSM neurons, respectively, activate a pro-longevity cascade. This neuroendocrine signaling regulates DAF-16 function in intestinal cells (Zhang et al., 2018). Moreover, temperature signaling from IL1 and AFD neurons maintains germline proliferation and delays germline stem cell (GSC) exhaustion. Prostaglandin E2 (PGE2) signals from adult GSCs communicate with the gut to produce hydrogen sulfide (H_2S). Thus, germline and somatic tissues contribute to cold-induced longevity (Hyun Ju Lee et al., 2019). PAQR-2, MDT-15, and azelaic acid (AzA) support longevity at low temperatures through fatty acid–mediated signaling (Chen et al., 2019; Dongyeop Lee

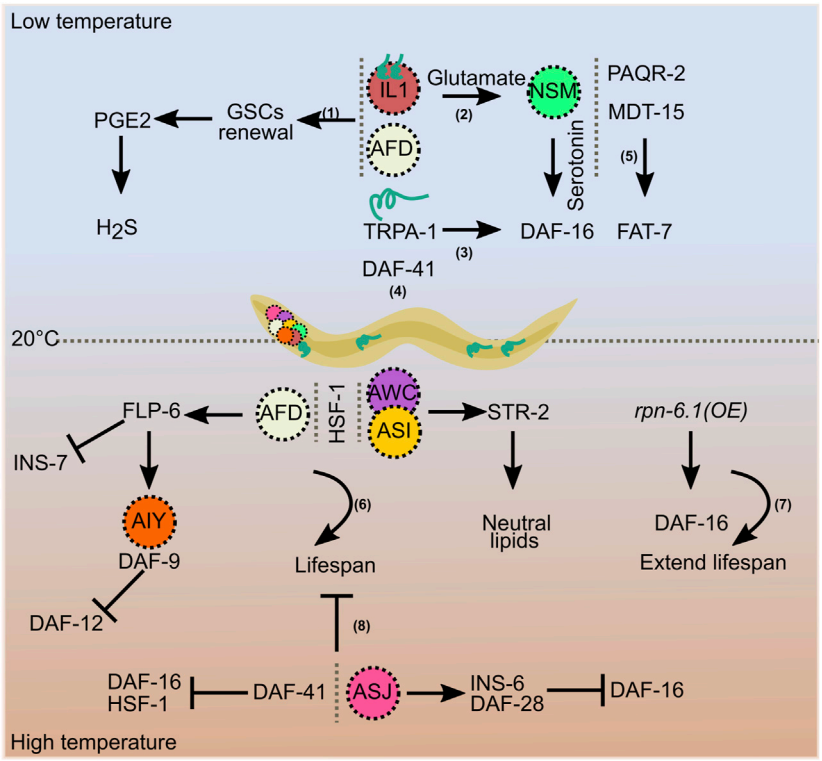

FIGURE 2 | Temperature-dependent regulation of longevity. Increased lifespan at low temperatures is regulated by signals involving germline stem cells (1), AFD and IL1 neurons (1 and 2), TRPA-1 (3), the co-chaperone DAF-41 (4), and fatty acids (5). AFD, AWC, and ASI neurons, and HSF-1 maintain lifespan at higher temperatures (6), overexpression of *rpn-6.1* prolongs it (7), and ASJ neurons and DAF-41 shorten lifespan at higher temperatures (8).

et al., 2019; Bai et al., 2021). Although these different factors regulating life expectancy in warm and cold temperatures have been identified, it remains unclear how they communicate with each other and coordinate their functions. Extensive studies have helped to identify the main players that regulate longevity at cold and warm temperatures. Cultivation at low temperatures is beneficial, while warm temperatures affect longevity. However, this relationship does not always hold. When *C. elegans* is exposed to high temperatures during early developmental stages, adult lifespan increases (Zhang et al., 2015). This transient heat stress during early life activates long-lasting defense responses via histone acetyltransferase CBP-1 and the chromatin remodeling complex SWI/SNF, which promote longevity (Zhou et al., 2019). These studies show that the temperature-dependent effects on aging are a well-regulated event controlled by genetic and epigenetic factors (**Figure 2**).

DISCUSSION

The evidence discussed thus far reveals a common theme in the temperature-dependent regulation of proteostasis and longevity. Environmental signals such as temperature influence organismal physiology via non-autonomous cell signaling mechanisms. Neurons act as receptors for thermal information and send signals mediated by small molecules to distal tissues. Insulin-like peptides, FMRFamide-like peptides, biogenic amines, and neurotransmitters are critical for triggering downstream responses.

Studies in poikilothermic animals have undoubtedly improved our understanding of temperature-dependent effects on organismal survival. However, many questions remain unanswered, particularly regarding the regulation of proteostasis. AFD thermosensory neurons and associated neuroendocrine signaling are well studied with respect to HSR. Surprisingly, there is little evidence of molecular players controlling other proteostasis pathways in response to temperature. In particular, it is unclear how temperature affects the UPS, the autophagy machinery, the unfolded protein response, and the genetic components involved. In addition to AFD, AWC, ASI, and ASJ neurons, the intestine act as temperature sensors in *C. elegans*. How thermosensation mediates proteostasis via these neurons and the gut remains to be elucidated. A crucial step would be to analyze how cell-specific thermosensory receptors and circuits control organismal proteostasis. *C. elegans* adapts to different environmental temperatures by calibrating unsaturated fatty acid levels to maintain optimal membrane fluidity (Ma et al., 2015). The role of fatty acids in regulating distinct proteostasis pathways under different temperature conditions is still unexplored. Further studies are needed to clarify these crucial issues.

TEMPERATURE—A POTENTIAL THERAPEUTIC INTERVENTION?

There are few studies demonstrating the effects of temperature on proteostasis and homeotherm longevity. However, some basic principles remain. Temperature determines, in part, the effects of birth time on human fetal development and longevity. In particular, an increase in ambient temperature at birth has detrimental effects (Flouris et al., 2009). The central thermostat in the preoptic area controls core body temperature (CBT) in homeotherms. Transgenic mice overexpressing the mitochondrial membrane protein uncoupling protein 2 (UCP2) in hypocretin neurons (Hcrt-UCP2 mice) exhibit increased hypothalamic temperature and reduced CBT. A modest but sustained reduction in CBT increases the life expectancy of Hcrt-UCP2 mice (Conti et al., 2006). Further studies have also shown that gonad-dependent differences in CBT affect life expectancy in a sex-specific manner (Sanchez-Alavez et al., 2011). In addition, selective temperature conditions may alter the pathophysiology of age-related diseases. Aggregation of damaged proteins contributes to neurodegenerative diseases. A recent review suggested heat therapy that promotes chaperone expression as a potential treatment strategy (Hunt et al., 2020). Further evidence suggests that sauna bathing—a passive heat therapy—reduces the risk of death from cardiovascular disease (Laukkanen et al., 2018). Swimming in cold water (temperature <5°C) is also beneficial for experienced healthy individuals when practiced with caution (Knechtle et al., 2020). Cold-shock proteins such as RNA-binding motif protein 3, RBM3, are essential for maintaining synapses in laboratory mouse models of neurodegenerative diseases. These results suggest a potential role for therapeutic human hypothermia in achieving neuroprotective effects (Peretti et al., 2015). In-depth analyses in higher-order animals may help to exploit the benefits of temperature in improving organismal health. On a positive note, previous results from a poikilothermic animal may serve as a springboard for exploring this potential therapeutic area.

AUTHOR CONTRIBUTIONS

KV and TH conceived, wrote, and critically revised the article. KV designed all figures.

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REFERENCES

Anckar, J., and Sistonen, L. (2011). Regulation of HSF1 Function in the Heat Stress Response: Implications in Aging and Disease. *Annu. Rev. Biochem.* 80, 1089–1115. doi:10.1146/annurev-biochem-060809-095203

Bai, J., Farias-Pereira, R., Jang, M., Zhang, Y., Lee, S. M., Kim, Y.-S., et al. (2021). Azelaic Acid Promotes *Caenorhabditis elegans* Longevity at Low Temperature via an Increase in Fatty Acid Desaturation. *Pharm. Res.* 38, 15–26. doi:10.1007/s11095-020-02975-w

Balchin, D., Hayer-Hartl, M., and Hartl, F. U. (2016). *In Vivo* aspects of Protein Folding and Quality Control. *Science* 353, aac4354. doi:10.1126/science.aac4354

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.* 106, 14914–14919. doi:10.1073/pnas.0902882106

Billman, G. E. (2020). Homeostasis: The Underappreciated and Far Too Often Ignored Central Organizing Principle of Physiology. *Front. Physiol.* 11, 1–12. doi:10.3389/fphys.2020.00200

Chauve, L., Hodge, F., Murdoch, S., Masoudzadeh, F., Mann, H.-J., Lopez-Clavijo, A. F., et al. (2021). Neuronal HSF-1 Coordinates the Propagation of Fat Desaturation across Tissues to Enable Adaptation to High Temperatures in *C. elegans*. *PLoS Biol.* 19, e3001431. doi:10.1371/journal.pbio.3001431

Chen, B., Retzlaff, M., Roos, T., and Frydman, J. (2011). Cellular Strategies of Protein Quality Control. *Cold Spring Harbor. Perspect. Biol.* 3, a004374. doi:10.1101/cshperspect.a004374

Chen, Y.-C., Chen, H.-J., Tseng, W.-C., Hsu, J.-M., Huang, T.-T., Chen, C.-H., et al. (2016). A *C. elegans* Thermosensory Circuit Regulates Longevity through Crh-1/CREB-dependent Flp-6 Neuropeptide Signaling. *Develop. Cel* 39, 209–223. doi:10.1016/j.devcel.2016.08.021

Chen, Y.-L., Tao, J., Zhao, P.-J., Tang, W., Xu, J.-P., Zhang, K.-Q., et al. (2019). Adiponectin Receptor PAQR-2 Signaling Senses Low Temperature to Promote *C. elegans* Longevity by Regulating Autophagy. *Nat. Commun.* 10, 2602. doi:10.1038/s41467-019-10475-8

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

Dixit, A., Sandhu, A., Modi, S., Shashikanth, M., Koushika, S. P., Watts, J. L., et al. (2020). Neuronal Control of Lipid Metabolism by STR-2 G Protein-coupled Receptor Promotes Longevity in *Caenorhabditis elegans*. *Aging Cell* 19, 1–14. doi:10.1111/acel.13160

Lee, D., An, S. W. A., Jung, Y., Yamaoka, Y., Ryu, Y., Goh, G. Y. S., et al. (2019). MDT-15/MED15 Permits Longevity at Low Temperature via Enhancing Lipidostasis and Proteostasis. *PLoS Biol.* 17, e3000415–28. doi:10.1371/journal.pbio.3000415

Flouris, A. D., Spiropoulos, Y., Sakellariou, G. J., and Koutedakis, Y. (2009). Effect of Seasonal Programming on Fetal Development and Longevity: Links with Environmental Temperature. *Am. J. Hum. Biol.* 21, 214–216. doi:10.1002/ajhb.20818

Goodman, M. B., and Sengupta, P. (2018). The Extraordinary AFD Thermosensor of *C. elegans*. *Pflugers Arch. Eur. J. Physiol.* 470, 839–849. doi:10.1007/s00424-017-2089-5

Gumeni, S., Evangelakou, Z., Gorgoulis, V., and Trougakos, I. (2017). Proteome Stability as a Key Factor of Genome Integrity. *Int. J. Mol. Sci.* 18, 2036. doi:10.3390/ijms18102036

He, K.-W., Shen, L.-L., Zhou, W.-W., and Wang, D.-Y. (2009). Regulation of Aging by Unc-13 and Sbt-1 in *Caenorhabditis elegans* Is Temperature-dependent. *Neurosci. Bull.* 25, 335–342. doi:10.1007/s12264-009-6123-2

Hoppe, T., and Cohen, E. (2020). Organismal Protein Homeostasis Mechanisms. *Genetics* 215, 889–901. doi:10.1534/genetics.120.301283

Horikawa, M., Sural, S., Hsu, A.-L., and Antebi, A. (2015). Co-chaperone P23 Regulates *C. elegans* Lifespan in Response to Temperature. *PLoS Genet.* 11, e1005023. doi:10.1371/journal.pgen.1005023

Hunt, A. P., Minett, G. M., Gibson, O. R., Kerr, G. K., and Stewart, I. B. (2020). Could Heat Therapy Be an Effective Treatment for Alzheimer's and Parkinson's Diseases? A Narrative Review. *Front. Physiol.* 10, 1–14. doi:10.3389/fphys.2019.01556

Lee, H. J., Noormohammadi, A., Koyuncu, S., Calculli, G., Simic, M. S., Herholz, M., et al. (2019). Prostaglandin Signals from Adult Germline Stem Cells Delay Somatic Ageing of *Caenorhabditis elegans*. *Nat. Metab.* 1, 790–810. doi:10.1038/s42255-019-0097-9

Jiang, W., Wei, Y., Long, Y., Owen, A., Wang, B., Wu, X., et al. (2018). A Genetic Program Mediates Cold-Warming Response and Promotes Stress-Induced Phenoptosis in *C. elegans*. *Elife* 7, 1–19. doi:10.7554/eLife.35037

Kaushik, S., and Cuervo, A. M. (2015). Proteostasis and Aging. *Nat. Med.* 21, 1406–1415. doi:10.1038/nm.4001

Kimata, T., Sasakura, H., Ohnishi, N., Nishio, N., and Mori, I. (2012). Thermotaxis of *C. elegans* as a Model for Temperature Perception, Neural Information Processing and Neural Plasticity. *Worm* 1, 31–41. doi:10.4161/worm.19504

Knechtle, B., Waśkiewicz, Z., Sousa, C. V., Hill, L., and Nikolaidis, P. T. (2020). Cold Water Swimming-Benefits and Risks: A Narrative Review. *Int. J. Environ. Res. Public Health* 17, 8984–9020. doi:10.3390/ijerph17238984

Kumsta, C., Chang, J. T., Schmalz, J., and Hansen, M. (2017). Hormetic Heat Stress and HSF-1 Induce Autophagy to Improve Survival and Proteostasis in C. Elegans. *Nat. Commun.* 8, 14337. doi:10.1038/ncomms14337

Labbadia, J., and Morimoto, R. I. (2015). Repression of the Heat Shock Response Is a Programmed Event at the Onset of Reproduction. *Mol. Cel* 59, 639–650. doi:10.1016/j.molcel.2015.06.027

Lamb, M. J. (1968). Temperature and Lifespan in drosophila. *Nature* 220, 808–809. doi:10.1038/220808a0

Laukkanen, T., Kunutsor, S. K., Khan, H., Willeit, P., Zaccardi, F., and Laukkanen, J. A. (2018). Sauna Bathing Is Associated with Reduced Cardiovascular Mortality and Improves Risk Prediction in Men and Women: A Prospective Cohort Study. *BMC Med.* 16, 1–14. doi:10.1186/s12916-018-1198-0

Lee, S.-J., and Kenyon, C. (2009). Regulation of the Longevity Response to Temperature by Thermosensory Neurons in *Caenorhabditis elegans*. *Curr. Biol.* 19, 715–722. doi:10.1016/j.cub.2009.03.041

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

Ma, D. K., Li, Z., Lu, A. Y., Sun, F., Chen, S., Rothe, M., et al. (2015). Acyl-CoA Dehydrogenase Drives Heat Adaptation by Sequestering Fatty Acids. *Cell* 161, 1152–1163. doi:10.1016/j.cell.2015.04.026

Mendenhall, A., Crane, M. M., Leiser, S., Sutphin, G., Tedesco, P. M., Kaeberlein, M., et al. (2017). Environmental Canalization of Life Span and Gene Expression in *Caenorhabditis elegans*. *J. Gerontol. Ser. A. Biol. Sci. Med. Sci.* 72, 1033–1037. doi:10.1093/gerona/glx017

Morimoto, R. I. (1998). Regulation of the Heat Shock Transcriptional Response: Cross Talk between a Family of Heat Shock Factors, Molecular Chaperones, and Negative Regulators. *Genes Dev.* 12, 3788–3796. doi:10.1101/GAD.12.24.3788

Ohta, A., Ujisawa, T., Sonoda, S., and Kuhara, A. (2014). Light and Pheromone-Sensing Neurons Regulates Cold Habituation through Insulin Signalling in *Caenorhabditis elegans*. *Nat. Commun.* 5, 8–9. doi:10.1038/ncomms5412

Peretti, D., Bastide, A., Radford, H., Verity, N., Molloy, C., Martin, M. G., et al. (2015). RBM3 Mediates Structural Plasticity and Protective Effects of Cooling in Neurodegeneration. *Nature* 518, 236–239. doi:10.1038/nature14142

Pispa, J., Matilainen, O., and Holmberg, C. I. (2020). Tissue-specific Effects of Temperature on Proteasome Function. *Cell Stress Chaperones* 25, 563–572. doi:10.1007/s12192-020-01107-y

Prahlad, V., and Morimoto, R. I. (2011). Neuronal Circuitry Regulates the Response of *Caenorhabditis elegans* to Misfolded Proteins. *Proc. Natl. Acad. Sci.* 108, 14204–14209. doi:10.1073/pnas.1106557108

Prahlad, V., Cornelius, T., and Morimoto, R. I. (2008). Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons. *Science* 320, 811–814. doi:10.1126/science.1156093

Sanchez-Alavez, M., Alboni, S., and Conti, B. (2011). Sex- and Age-specific Differences in Core Body Temperature of C57Bl/6 Mice. *Age* 33, 89–99. doi:10.1007/s11357-010-9164-6

Shaw, R. F., and Bercaw, B. L. (1962). Temperature and Life-Span in Poikilothermous Animals. *Nature* 196, 454–457. doi:10.1038/196454a0

Smith, J. M. (1963). Temperature and the Rate of Ageing in Poikilotherms. *Nature* 199, 400–402. doi:10.1038/199400a0

Song, A., Labella, S., Korneeva, N. L., Keiper, B. D., Aamodt, E. J., Zetka, M., et al. (2010). A *C. elegans* eIF4E-Family Member Upregulates Translation at Elevated Temperatures of mRNAs Encoding MSH-5 and other Meiotic Crossover Proteins. *J. Cel Sci.* 123, 2228–2237. doi:10.1242/jcs.063107

Sugi, T., Nishida, Y., and Mori, I. (2011). Regulation of Behavioral Plasticity by Systemic Temperature Signaling in *Caenorhabditis elegans*. *Nat. Neurosci.* 14, 984–992. doi:10.1038/nn.2854

Tabarean, I., Morrison, B., Marcondes, M. C., Bartfai, T., and Conti, B. (2010). Hypothalamic and Dietary Control of Temperature-Mediated Longevity. *Ageing Res. Rev.* 9, 41–50. doi:10.1016/j.arr.2009.07.004

Tatum, M. C., Ooi, F. K., Chikka, M. R., Chauve, L., Martinez-Velazquez, L. A., Steinbusch, H. W. M., et al. (2015). Neuronal Serotonin Release Triggers the Heat Shock Response in *C. elegans* in the Absence of Temperature Increase. *Curr. Biol.* 25, 163–174. doi:10.1016/j.cub.2014.11.040

Vilchez, D., Morantte, I., Liu, Z., Douglas, P. M., Merkwirth, C., Rodrigues, A. P. C., et al. (2012). RPN-6 Determines *C. elegans* Longevity under Proteotoxic Stress Conditions. *Nature* 489, 263–268. doi:10.1038/nature11315

Xiao, R., Zhang, B., Dong, Y., Gong, J., Xu, T., Liu, J., et al. (2013). A Genetic Program Promotes *C. elegans* Longevity at Cold Temperatures via a Thermosensitive TRP Channel. *Cell* 152, 806–817. doi:10.1016/j.cell.2013.01.020

Zhang, B., Xiao, R., Ronan, E. A., He, Y., Hsu, A.-L., Liu, J., et al. (2015). Environmental Temperature Differentially Modulates *C. elegans* Longevity through a Thermosensitive TRP Channel. *Cel Rep.* 11, 1414–1424. doi:10.1016/j.celrep.2015.04.066

Zhang, B., Gong, J., Zhang, W., Xiao, R., Liu, J., and Xu, X. Z. S. (2018). Brain-gut Communications via Distinct Neuroendocrine Signals Bidirectionally Regulate Longevity in *C. elegans*. *Genes Dev.* 32, 258–270. doi:10.1101/gad.309625.117

Zhou, L., He, B., Deng, J., Pang, S., and Tang, H. (2019). Histone Acetylation Promotes Long-Lasting Defense Responses and Longevity Following Early Life Heat Stress. *PLoS Genet.* 15, e1008122. doi:10.1371/journal.pgen.1008122

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REVIEW
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New Roles for MicroRNAs in Old Worms

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The use of *Caenorhabditis elegans* as a model organism in aging research has been integral to our understanding of genes and pathways involved in this process. Several well-conserved signaling pathways that respond to insulin signaling, diet, and assaults to proteostasis have defined roles in controlling lifespan. New evidence shows that microRNAs (miRNAs) play prominent roles in regulating these pathways. In some cases, key aging-related genes have been established as direct targets of specific miRNAs. However, the precise functions of other miRNAs and their protein cofactors in promoting or antagonizing longevity still need to be determined. Here, we highlight recently uncovered roles of miRNAs in common aging pathways, as well as new techniques for the ongoing discovery of miRNA functions in aging *C. elegans*.

Keywords: microRNA, *C. elegans*, aging, insulin signaling, dietary restriction, autophagy, proteostasis.

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INTRODUCTION

Since the discovery of long-lived *Caenorhabditis elegans* genetic mutants over 30 years ago, these nematodes have become an important tool in understanding the aging process (Mack et al., 2018). Their relatively short lifespan (~18–20 days), genetic manipulability, and consistent display of the hallmarks of aging make them an advantageous model organism for uncovering the molecular contributions to aging (Mack et al., 2018; Son et al., 2019; Zhang et al., 2020). Through the work of numerous groups, a few key modes of regulating longevity have been found in *C. elegans,* including changes in insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS), target of rapamycin (TOR) signaling, AMP-activated protein kinase (AMPK) signaling, autophagy, and diet (Kenyon, 2010; Uno and Nishida, 2016; Zhang et al., 2020). The broad conservation of the genes in these pathways suggests that new insights may be pertinent to mammalian aging (Bitto et al., 2015; Khan et al., 2019). Though, unsurprisingly, recent findings highlight the complexity of translating results from aging model organisms to human gerontology (Ukraintseva et al., 2021).

As the central players of these aging pathways are found, understanding how their expression is regulated emerges as a key problem. This review will focus on a prominent class of post-transcriptional regulators called microRNAs (miRNAs). miRNAs are short (~22 nucleotides), non-coding RNAs that target messenger RNAs (mRNAs) for degradation or translational repression through sequence-specificity (**Figure 1**) (Bartel, 2018). To regulate their targets, mature miRNAs need to be loaded into an Argonaute (AGO) protein to form the miRNA-induced silencing complex (miRISC). Once bound to a target mRNA via base-pairing interactions with the miRNA, miRISC recruits RNA degradation factors and translational repressors that down-regulate expression of the targeted mRNA through a variety of mechanisms, some of which are not yet well-understood. In *C. elegans*, there are more than 25 AGOs, but AGO-Like Gene 1 (ALG-1), and ALG-2 are the main AGOs dedicated to the miRNA pathway (Youngman and Claycomb, 2014). These two effector proteins are developmentally redundant and losing both AGOs results in embryonic lethality (Grishok et al., 2001; Vasquez-

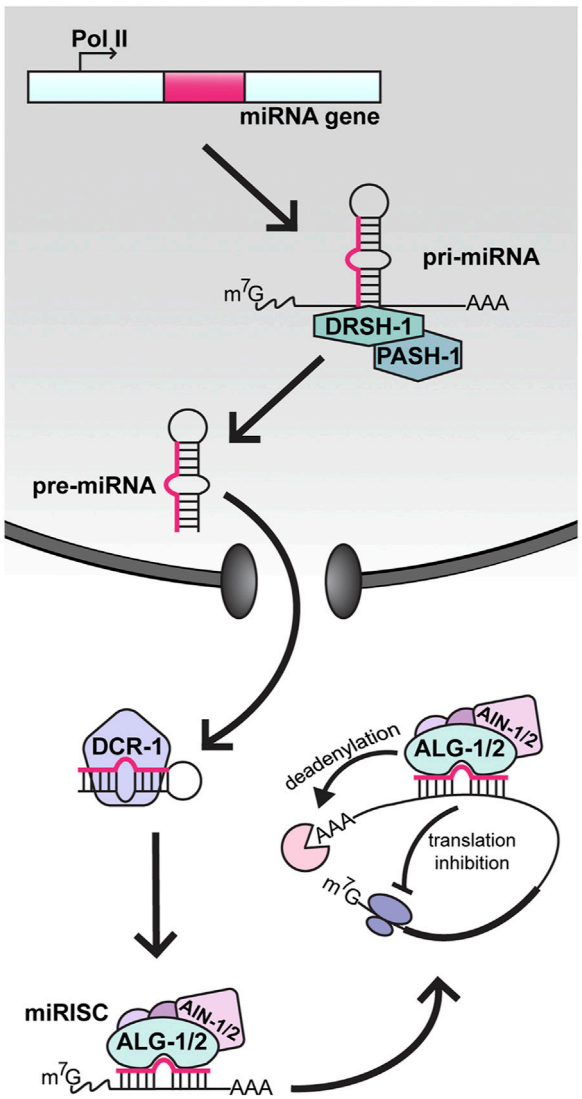

FIGURE 1 | MicroRNA biogenesis and function in *C. elegans*. In the nucleus, miRNA genes are transcribed by RNA polymerase II (Pol II) into long primary transcripts (pri-miRNAs). Pri-miRNAs are cleaved by Drosha (DRSH-1) in complex with the RNA binding protein Pasha (PASH-1). The resulting precursor miRNA (pre-miRNA) hairpin is then transported to the cytoplasm, where processing by Dicer (DCR-1) produces a double stranded heteroduplex consisting of the mature miRNA and the passenger strand. The mature miRNA is loaded into an Argonaute protein (ALG-1 or ALG-2), forming the core microRNA-Induced Silencing Complex (miRISC). Through partial base-pairing, the miRNA positions miRISC on a target messenger RNA (mRNA) and the recruitment of AIN-1/2, along with other factors, causes repression of the target *via* deadenylation and/or inhibition of translation.

Rifo et al., 2012). There are well over 100 different miRNA genes in the *C. elegans* genome (Kozomara et al., 2019), and specific miRNAs have been shown to impact important biological processes, ranging from developmental timing (Lee et al., 1993) and neuronal patterning (Johnston and Hobert, 2003) to stress recovery (Pagliuso et al., 2021) and innate immunity (Zhi et al., 2017).

The first reported miRNA with a role in aging in any organism was also the very first discovered miRNA, *C. elegans lin-4* (cell lineage) (Lee et al., 1993; Boehm and Slack, 2005). The *lin-4* gene was originally identified as a key regulator of temporal identity during early larval development (Chalfie et al., 1981; Ambros and Horvitz, 1984). Pioneering work in the Ambros and Ruvkun labs revealed that *lin-4* encodes a tiny non-coding RNA that binds the 3′ UTR of the mRNA encoding the transcription factor *lin-14*, repressing its expression (Lee et al., 1993; Wightman et al., 1993). Down-regulation of *lin-14* by lin-4 miRNA is critical for transition from first to later larval cell fates in several tissues (Olsen and Ambros, 1999). Curiously, the expression of *lin-4* and *lin-14* remains detectable into adulthood, leading Dr. Frank Slack's group to hypothesize that the pair may have roles beyond development. In support of this idea, they observed that losing the function of *lin-4* significantly reduces lifespan, whereas decreasing *lin-14* expression extends lifespan (Boehm and Slack, 2005). Additionally, they were able to show that this extension is dependent on two transcription factors in the IIS pathway, DAF-16 (dauer formation) and HSF-1 (heat shock factor) (**Figure 2**). Since then, many more miRNAs have been shown to influence the rate of aging through the regulation of established longevity pathways, and we recommend the following reviews for more in-depth summaries: Kinser and Pincus, 2020; Kim and Lee, 2019; Ambros and Ruvkun, 2018; Uno and Nishida, 2016; Garg and Cohen, 2014; Inukai and Slack, 2013; Smith-Vikos and Slack, 2012; Jung and Suh, 2012. Building upon these works, this review emphasizes recent findings, and methods specifically relating to the miRNA pathway in aging *C. elegans*.

OPPOSING MIRNA EFFECTS ON AGING THROUGH THE INSULIN/IGF-1 SIGNALING PATHWAY

As the first pathway to show significant lifespan extension by genetic manipulation of its core components (Friedman and Johnson, 1988; Kenyon et al., 1993), the IIS pathway has frequently been the center of aging studies. The IIS pathway links nutrient levels to longevity through a phosphorylation cascade activated by insulin-like peptides binding to a transmembrane receptor, DAF-2, which eventually results in the phosphorylation of the Forkhead box O (FOXO) transcription factor DAF-16 (Murphy and Hu, 2013). This phosphorylation restricts DAF-16 from entering the nucleus and accessing its transcriptional targets. Reduced insulin signaling and underphosphorylation of DAF-16 results in its nuclear translocation and transcriptional activation of genes that promote longevity and stress resistance. Consequently, partial loss of function mutations in *daf-2* can double the lifespan of *C. elegans* in a way that is entirely dependent on DAF-16, while losing the function of *daf-16* can substantially reduce lifespan (Kenyon et al., 1993).

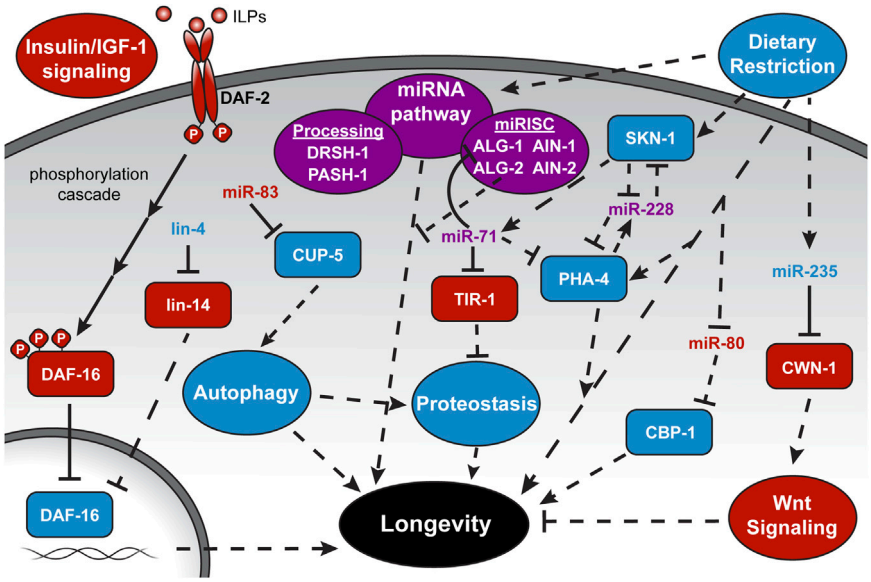

FIGURE 2 | MicroRNA regulation of longevity-associated pathways. Depicted is the complex interplay among miRNAs, targets, and longevity pathways highlighted in this review. More comprehensive schematics and discussion of individual pathways can be found in the recommended reviews in the introduction. ILPs, Insulin-like Peptides, and full gene names are provided at their first mention in the text. Solid lines represent direct interactions. Dashed lines represent indirect or unknown interactions. Pathway names are within ovals, proteins are within rectangles, and miRNAs stand alone. A pathway or factor with a blue background or text color is considered "lifespan-promoting," while red represents "lifespan-antagonizing," and purple is ambiguous within the context depicted.

Considering the broad requirement for the miRNA pathway in *C. elegans* embryogenesis and larval development (Alberti and Cochella, 2017), a general loss of miRNAs or their ability to function would be expected to negatively impact lifespan. Indeed, depletion of mature miRNA levels by inactivation of the PASH-1 miRNA processing factor or down-regulation of both *alg-1* and *alg-2* together in adult *C. elegans* shortens lifespan (**Figure 2**) (Lehrbach et al., 2012; Aalto et al., 2018). However, a more complicated role for the miRNA Argonautes was uncovered when they were individually tested for roles in aging. Aalto and colleagues found that loss of *alg-1* shortens while loss of *alg-2* extends lifespan (Aalto et al., 2018). These longevity phenotypes are independent of the roles of *alg-1* and *alg-2* in development, as their depletion by RNAi at the onset of adulthood also results in opposite lifespan effects (Aalto et al., 2018). These aging phenotypes are consistent with the differential mis-regulation of many genes under the transcriptional control of DAF-16. In *alg-2* mutants, over 30% of the up-regulated genes are considered DAF-16 targets and the extended lifespan of these mutants is dependent on *daf-16* activity (Aalto et al., 2018). In contrast, genes regulated by DAF-16 tend to be down-regulated in *alg-1* mutants, and loss of *alg-1* reduces the *daf-2* extended lifespan. Furthermore, loss of both *alg-1* and *daf-16* together shortened lifespan further than either mutant alone, suggesting that *alg-1* influences other modes of longevity regulation in addition to the IIS pathway. This broader role for *alg-1* is consistent with the conclusion that *pash-1* acts in parallel to the IIS, converging on downstream aging-associated genes (Lehrbach et al., 2012). Identification of the specific miRNAs and targets that alter lifespan when mis-regulated is needed to better understand the role of the general miRNA machinery in controlling longevity in *C. elegans*.

One miRNA that has been repeatedly implicated in promoting longevity through the IIS pathway is miR-71. Attention was originally drawn to miR-71 because it was one of the few up-regulated miRNAs in aging *C. elegans* (De Lencastre et al., 2010). A functional role for *miR-71* was established with the demonstration that genetic loss of *mir-71* shortens lifespan and overexpression of miR-71 extends it through pathways dependent on *daf-16* (De Lencastre et al., 2010; Boulias and Horvitz, 2012). Additionally, gene regulatory network analysis places miR-71 as a highly connected node among aging-associated miRNAs, transcription factors, and miRNA biogenesis genes (Inukai et al., 2018). Surprisingly, a direct target of miR-71 in adult *C. elegans* seems to be *alg-1* (**Figure 2**). The *alg-1* 3′UTR contains two miR-71 binding sites that confer miR-71 dependent repression of *alg-1* in adult animals (Broughton et al., 2016; Inukai et al., 2018). Disruption of this regulatory loop, where miR-71 represses the expression of an Argonaute needed for miRNA function, has broad downstream consequences that might explain how loss of *alg-1* as well as its negative regulator, *miR-71,* both result in similar shortened lifespan phenotypes. Since Argonaute proteins stabilize bound miRNAs, higher levels of ALG-1 in the absence of miR-71 results in global increases in miRNA abundance and, likely as a consequence, changes in protein coding gene expression (Inukai et al., 2018). Studies focused on how variations in miRNA and mRNA abundance in *miR-71* or *alg-1* mutants impact lifespan will further elucidate this intriguing miRNA feedback loop.

UP-REGULATION OF MIRNA PATHWAY FACTORS PROMOTES LONGEVITY INDUCED BY DIETARY RESTRICTION

Altering the caloric intake of an organism is another mode of regulating longevity. Dietary restriction (DR) by limiting food consumption without malnutrition delays aging and age-related diseases in many organisms (Fontana and Partridge, 2015). In *C. elegans*, DR can extend lifespan by up to 70% depending on the regimen, whether it's chronic, intermittent, or specific nutrient deficient fasting (Honjoh et al., 2009; Kaeberlein et al., 2006; Lee et al., 2006). Different regimens seem to mediate this extension through distinct pathways, requiring downstream effectors from IIS, TOR (target of rapamycin), and AMPK (adenosine monophosphate-activated protein kinase) pathways (Honjoh et al., 2009; Greer et al., 2007). Given the broad role of the miRNA pathway in gene regulation, it is perhaps not surprising that core miRNA factors are required for DR-induced longevity. An intermittent fasting (IF) protocol, where adult *C. elegans* were alternatively fed and starved every 2–3 days, was found to induce widespread transcriptional changes that promote longevity (Uno et al., 2013). Notably, expression of the miRNA processing enzyme DRSH-1 (drosha), as well as miRISC components ALG-1, ALG-2, AIN-1 (alg-1 interacting protein), and AIN-2 were observed to be up-regulated in response to IF (**Figure 2**) (Kogure et al., 2017). Increased levels of miRNA pathway components may contribute to IF-induced longevity, as the loss of DRSH-1 function entirely inhibits and loss of ALG-1, ALG-2, or AIN-1 partially suppress the lifespan extension. While the levels of several miRNAs also changed in response to IF (Kogure et al., 2017), a direct connection between the miRNA pathway and target genes that underlie the lifespan extension caused by IF is yet to be uncovered.

As another mode of DR, limiting caloric intake by bacterial food dilution extends lifespan by ~40% and also induces changes in the expression of specific miRNAs (Rollins, 2019; Smith-Vikos et al., 2014; Xu et al., 2019). Down-regulation of two miRNAs, miR-58 and miR-80, may be associated with enhanced translation of specific transcripts, as target sites for miR-58 and miR-80 were enriched in the 3′UTRs of mRNAs that increase in polysome association during DR (Rollins, 2019). Several studies have now shown that expression of miR-80 is regulated by food (Vora et al., 2013; Kogure et al., 2017; Rollins, 2019). Moreover, down-regulation of miR-80 upon food deprivation reflects the DR-constitutive state of *miR-80* loss of function mutants (Vora et al., 2013). The deletion of *mir-80* results in an extended lifespan and healthspan in a pathway that is dependent on the histone acetyltransferase transcriptional coactivator homolog, CBP-1 (CREB-binding protein) (**Figure 2**) (Vora et al., 2013). While *cbp-1* has potential miR-80 target sites in its 5′UTR and coding sequence, more work is needed to establish whether these non-canonical sites mediate direct repression by miR-80.

DR-induced longevity by reduced caloric intake also depends on specific miRNAs. MiR-71 and miR-228 are up-regulated at the onset of DR in adult *C. elegans,* and loss of these miRNAs prohibits lifespan extension by DR (Smith-Vikos et al., 2014). It has been proposed that miR-71 and miR-228 modulate lifespan together through a feedback loop with transcription factors PHA-4 (pharynx development) and SKN-1 (skinhead), which play integral roles in DR-induced longevity (Bishop and Guarente, 2007; Panowski et al., 2007). How PHA-1 and SKN-1 regulate the expression of these miRNAs and if they themselves are direct targets of these miRNAs are outstanding questions.

Focus on another DR-induced miRNA, miR-235, has revealed a mechanism for promoting longevity by suppressing an antagonistic pleiotropic pathway. Antagonistic pleiotropy refers to properties of a gene that enhance fitness early in life and negatively impact viability at post reproductive stages (Austad and Hoffman, 2018). An example is the Wnt-signaling pathway, which is essential for proper development and metabolism (Clevers, 2006; Sethi and Vidal-Puig, 2010) but also can induce various age-related pathologies when inappropriately active in adults (Ng et al., 2019). Xu et al., found that Wnt-signaling in calorie restricted adults is subdued by the induction of miR-235, and loss of this miRNA abolishes DR-mediated lifespan extension (Xu et al., 2019). A direct target of miR-235 seems to be the Wnt ligand *cwn-1* (*C. elegans* Wnt family), which has a single miR-235 binding site in its 3′UTR. Interestingly, increased expression of miR-235 and suppression of Wnt-signaling was only observed in adults and not in younger stages of animals under DR regimens. Thus, there is temporal control over the ability of miR-235 to respond to DR and modulate Wnt signaling to promote longevity. This apparent switch-like feature prevents premature repression of Wnt signaling early in development. This work provides a foundation for exploring the role of homologs of miR-235 (called miR-92 in mammals) and Wnt-signaling genes in regulating longevity in higher organisms under different nutrient states.

CROSS-TISSUE REGULATION OF AUTOPHAGY AND PROTEOSTASIS BY MIRNAS

Autophagy is a broadly conserved cellular mechanism for eliminating damaged proteins and organelles and recycling their components (Mizushima, 2007). Autophagy provides a source of nutrients for cellular functions during stress and helps clear misfolded proteins and old organelles from cells (Levine and Kroemer, 2008). Protein homeostasis (proteostasis) depends on autophagy and the ubiquitin-proteosome system (UPS), and these activities decline with age (Sarkis et al., 1988; Taylor and Dillin, 2011; Chang et al., 2017). Accordingly, pathways that impact lifespan, such as IIS, nutrient sensing through TOR, and dietary restriction, and converge onto autophagy to regulate aging (Bareja et al., 2019). Given its central role in multiple longevity pathways, a better understanding of how autophagy genes are regulated may reveal mechanisms for slowing deactivation of this pathway in aging and promoting healthy longevity.

To identify a basis for the decline in autophagy in older *C. elegans,* Zhou et al., 2019, examined transcriptome changes in aging adults. From this, miR-83 stood out as an up-regulated

miRNA with target sites in the autophagy-related gene, *cup-5* (coelomocyte uptake-defective) (Zhou et al., 2019). Loss of *miR-83* results in an extended lifespan and enhanced autophagy and proteostasis through derepression of *cup-5* in adults. Surprisingly, age-associated up-regulation of miR-83 seems to only occur in the intestine, yet repression of *cup-5* and the resulting dysregulation of autophagy was observed in intestine as well as in body wall muscle (BWM) cells. The cell non-autonomous regulation of *cup-5* is achieved by transport of miR-83 from the intestine to BWM cells. This remarkable finding establishes a specific role for the miRNA pathway in regulating autophagy during aging, and also presents a compelling example of a secreted miRNA having a biologically relevant function outside of its origin cell. It also questions the purpose of expressing a miRNA that negatively regulates autophagy and, hence, longevity. The answer, again, likely relates to antagonistic pleiotropy (Austad and Hoffman, 2018). Early in life, miR-83 is needed for proper germline development and fertility, and its post-reproductive effects are not subject to selection. The homolog of miR-83, called miR-29 in mammals, has the potential to target autophagy pathway genes (Zhou et al., 2019), raising the possibility that age-related changes in autophagic capacity might also be regulated by the miRNA pathway in humans.

In addition to autophagy, the other major pathway for maintaining proteostasis, UPS, is also under miRNA control. It was recently shown that short lived *miR-71* mutants are defective in ubiquitin-dependent protein turnover (Finger et al., 2019). Reduced lifespan and UPS activity both seem to be due to up-regulation of the miR-71 target *tir-1* (Toll and Interleukin 1 Receptor domain protein), as these phenotypes are largely suppressed by loss of *tir-1* and copied in strains expressing a version of *tir-1* that lacks the miR-71 3′UTR binding sites (Finger et al., 2019). In another example of cross-tissue effects of a miRNA, repression of *tir-1* by miR-71 occurs in AWC olfactory neurons, leading to release of neuropeptides that stimulate UPS activity in the intestine. Additionally, food odor was found to promote proteostasis and longevity through miR-71 mediated regulation of *tir-1* (Finger et al., 2019). The proposal that odor controls the ability of a miRNA to regulate its target offers a new mechanism linking environmental cues to the regulation of programs, such as UPS, that promote longevity.

NEW METHODS FOR ELUCIDATING MIRNA EXPRESSION AND FUNCTION DURING AGING

Cell non-autonomous signaling as a modifier of aging has been explored in various model organisms (Miller et al., 2020), but the broad impact of miRNAs on this phenomenon is just beginning to be recognized. Considering the newly identified cross-tissue roles of miR-71 and miR-83 in aging *C. elegans* (Finger et al., 2019; Zhou et al., 2019), it will be critical to determine where a miRNA of interest is expressed, where its targets are being regulated, and which tissues depend on this regulation to control longevity. In the first large scale effort, spatio-temporal control of miRNA expression was studied using reporters consisting of the miRNA promoter fused to GFP (Martinez et al., 2008). Most of the miRNA promoters that were tested showed tissue-specificity, with less than 5% having ubiquitous somatic expression. This tool offers useful information regarding the transcriptional domain of a miRNA gene, with the caveat that regulatory elements might have been excluded in the reporter design. Furthermore, it does not necessarily show the relative levels of mature, active miRNA or where it exerts regulation.

More recently, systematic efforts to isolate and sequence miRNAs from individual tissues have been developed to gain cellular-level resolution of mature miRNA localization. A new technology, microRNome by methylation-dependent sequencing (mime-seq), utilizes expression of a plant-specific methyltransferase, HEN1 (Hua enhancer), in individual tissues to methylate mature miRNAs, which can then be chemoselectively cloned and sequenced (Alberti et al., 2018). This method produces the miRNA profile of whichever cells express HEN1, and it is sensitive enough to reveal the miRNome of just two sensory neurons. Mime-seq is easier and less disruptive than previous tissue-specific isolation methods, like fluorescence-activated cell sorting, laser-capture microdissection, or immunoprecipitation, and has significantly higher yield. Though it detects miRNAs present in a particular tissue, they may not all be active miRNAs, as evidenced by the detection of miRNA passenger strands. To focus on active miRNAs, tissue-restricted miRISC components can be immunoprecipitated. This method was originally developed using tissue specific expression of the miRISC cofactor, AIN-2 (Kudlow et al., 2012). As AIN-2 is generally required for target regulation by miRISC, the isolation of complexes containing this factor are likely to select for functional miRNAs (Zhang et al., 2007). More recently, a spatio-temporal map of active miRNAs was constructed via immunoprecipitation of epitope-tagged ALG-1 or ALG-2 expressed in intestine, body wall muscle, or the nervous system (Brosnan et al., 2021). Preferential association of miRNAs with ALG-1 or ALG-2 globally in adult *C. elegans* had been uncovered (Aalto et al., 2018), but now this relationship can be viewed with a more nuanced, cell-specific focus. Results from these methods can also be compared to expression data from reporters driven by miRNA promoters to potentially reveal miRNAs, like miR-83, that are synthesized in one tissue and secreted to another to regulate specific targets. Using these applications in adults will shape our spatial understanding of aging-associated miRNAs.

With a higher-resolution picture of the miRNome comes the challenge of identifying direct targets of these miRNAs. Methods that use RNA immunoprecipitation (RIP), cross-linking immunoprecipitation with high-throughput sequencing (CLIP-seq), and individual-nucleotide resolution CLIP (iCLIP) of miRISC components (ALG-1, ALG-2, AIN-1, or AIN-2) have been used to detect miRNA targets genome-wide in larval stage *C. elegans* (Zhang et al., 2007; Zisoulis et al., 2010; Grosswendt et al., 2014; Broughton et al., 2016). Occasionally, sequence reads from some of these techniques correspond to a miRNA ligated to its target site (Grosswendt et al., 2014; Broughton et al., 2016). These rare chimeric sequences provide evidence of *in vivo* miRNA-

TABLE 1 | Summary of the methods for elucidating miRNA expression and function that are reviewed in the text.

Areas of miRNA discovery	Experimental methods	Additional information provided	References
Defining miRNA expression	miRNA promoter-GFP fusions	Shows only where miRNA genes are expressed	Martinez et al. (2008)
	mime-seq	Shows mature miRNA localization	Alberti et al. (2018)
	Immunoprecipitation of miRISC factors	Shows active miRNA localization	Kudlow et al. (2012) Brosnan et al. (2021)
Identifying miRNA targets	RIP, CLIP seq, iCLIP	Gives mRNAs associated with miRISC components; chimeric reads give direct miRNA-target interactions	Zhang et al. (2007) Zisoulis Yeo, (2010) Grosswendt et al. (2014) Broughton et al. (2016)
	CRISPR-based 3′UTR mutagenesis screen	Tests functionality of miRNA binding sites on target mRNA expression	Froehlich et al. (2021)
Determining miRNA target functions	GFP-targeting nanobodies	Spatial control of target protein degradation	Wang et al. (2017)
	AID system	Spatiotemporal control of target protein degradation	Zhang et al. (2015) Ashley et al. (2021) Negishi et al. (2021) Hills-Muckey et al. (2021)

target site interactions that are not confined to computational predictions. Improved methods that enrich for chimeras formed by RNA isolated from tissue-restricted miRISC components in aging *C. elegans* will go far to define specific targets and miRNAs that impact organismal longevity.

As exemplified by several of the studies highlighted here, advances in genome editing provide efficient and precise tools for validating miRNA targets and verifying their relevance to aging (Friedland et al., 2013). CRISPR-Cas9 can be used to knock out individual miRNAs and mutate potential miRNA binding sites in the 3′UTRs of target mRNAs. Using these genetic manipulations together can corroborate evidence that mis-regulation of a specific miRNA target results in a modification of longevity, or any phenotype of interest. Recently, this technology has been used as the basis of a large-scale mutagenesis screen to analyze the regulatory sequences of 3′UTRs (Froehlich et al., 2021). As a proof of concept, hundreds of small deletions were generated along the 3′UTR of *lin-41*, a gene known to be targeted by miRNA *let-7* (lethal) (Reinhart et al., 2000; Vella et al., 2004; Ecsedi et al., 2015). The screen succeeded in identifying the two previously-characterized *let-7* binding sites as important regulatory elements in the *lin-41* 3′UTR. Applying this strategy to aging-related genes may pinpoint specific 3′UTR sequences subject to regulation by miRNAs, as well as aid in untangling the complexity of multiple miRNAs targeting a single gene.

Advances in tissue-specific depletion of a gene of interest provide new means to better understand how a mis-regulated miRNA target contributes to aging phenotypes. Two methods for removal of a specific protein from a particular cell type that utilize GFP-targeted nanobodies or an auxin-inducible degron have been recently developed for *C. elegans* (Zhang et al., 2015; Wang et al., 2017). In the first system, a GFP-targeting nanobody fused to a ubiquitin ligase adaptor is driven by a tissue-specific promoter, which results in degradation of any GFP-tagged proteins expressed in the same tissue (Wang et al., 2017). Thus, spatial but not temporal control is available, as this system drives constitutive depletion of a protein of interest from an individual tissue. Removal of a protein from a specific tissue at a desired time can be achieved with the auxin-inducible degradation (AID) system (Zhang et al., 2015). Tissue-specific expression of the plant Transport Inhibitor Response 1 (TIR1) protein allows for spatially restricted degradation of a protein of interest that is tagged with a degron, which is only recognized by the TIR1-ubiquitin ligase complex in the presence of the plant hormone auxin (Nishimura et al., 2009). This allows researchers to trigger tissue-specific degradation of a degron-tagged protein at any life stage. While caution should be taken to recognize potential unintended effects of the degron tag, TIR1, or the presence of auxin (Schiksnis et al., 2020; Bhoi et al., 2021; Loose and Ghazi, 2021), improvements to the AID system for *C. elegans* promise to make this a powerful tool for studying the role of miRNA targets in aging, while bypassing their potential requirement during development (Ashley et al., 2021; Hills-Muckey et al., 2021; Negishi et al., 2021). A summary of the experimental methods described in this section are outlined in **Table 1**.

CONCLUDING REMARKS

As a model organism for uncovering roles for the miRNA pathway in aging, *C. elegans* offer a favorable balance of simple and complex. Besides their ease of maintenance and brief lifespan, established molecular tools and worm-specific resources make hypothesis generation and experimental set-up straightforward. Though *C. elegans* have limited anatomical features compared to mammals, there is now compelling evidence that miRNAs coordinate aging across major tissues in worms. Given the conservation of the miRNA pathway, we

expect that specific miRNAs will also have cell non-autonomous functions that modulate longevity in humans. The studies highlighted here contribute not only to a better understanding of the role of the miRNA pathway in aging, but also of mechanisms controlling miRNA expression and targeting, irrespective of the conservation of individual components. Further advances in the field will depend on identifying direct targets of miRNAs that regulate aging, potentially in a spatio-temporal manner. As these regulatory networks are solidified, searching for upstream factors that regulate the aging-associated miRNAs themselves will deepen our understanding of the programming changes that underlie differences in miRNA function in development versus adulthood. With a legacy of leading the discovery of miRNAs and conserved longevity pathways, we anticipate that *C. elegans* will continue to be at the forefront of research into miRNA-mediated regulation of lifespan and inspire new approaches to improve healthspan and treat age-related diseases in humans.

AUTHOR CONTRIBUTIONS

Original draft preparation: CE. Review and editing: CE and AP. Visualization: CE. Supervision: AP. Funding acquisition: AP.

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REFERENCES

Aalto, A. P., Nicastro, I. A., Broughton, J. P., Chipman, L. B., Schreiner, W. P., Chen, J. S., et al. (2018). Opposing Roles of MicroRNA Argonautes during *Caenorhabditis elegans* Aging. *PLoS Genet.* 14 (6): e1007379. doi:10.1371/journal.pgen.1007379

Alberti, C., and Cochella, L. (2017). A Framework for Understanding the Roles of MiRNAs in Animal Development. *Development* 144 (14), 2548–2559. doi:10.1242/dev.146613

Alberti, C., Manzenreither, R. A., Sowemimo, I., Burkard, T. R., Wang, J., Mahofsky, K., et al. (2018). Cell-Type Specific Sequencing of MicroRNAs from Complex Animal Tissues. *Nat. Methods* 15 (4), 283–289. doi:10.1038/nmeth.4610

Ambros, V., and Ruvkun, G. (2018). Recent Molecular Genetic Explorations of *Caenorhabditis elegans* MicroRNAs. *Genetics* 209 (3), 651–673. doi:10.1534/genetics.118.300291

Ambros, V., and Horvitz, H. R. (1984). Heterochronic Mutants of the Nematode *Caenorhabditis elegans*. *Science* 226 (4673), 409–416. doi:10.1126/science.6494891

Ashley, G. E., Duong, T., Levenson, M. T., Martinez, M. A. Q., Johnson, L. C., Hibshman, J. D., et al. (2021). An Expanded Auxin-Inducible Degron Toolkit for *Caenorhabditis elegans*. *Genetics* 217 (3). doi:10.1093/genetics/iyab006

Austad, S. N., and Hoffman, J. M. (2018). Is Antagonistic Pleiotropy Ubiquitous in Aging Biology? *Evol. Med. Public Health* 2018 (1), 287–294. doi:10.1093/emph/eoy033

Bareja, A., Lee, D. E., and White, J. P. (2019). Maximizing Longevity and Healthspan: Multiple Approaches All Converging on Autophagy. *Front. Cell Develop. Biol.* 7, 183. doi:10.3389/fcell.2019.00183

Bartel, D. P. (2018). Metazoan MicroRNAs. *Cell* 173 (1), 20–51. doi:10.1016/j.cell.2018.03.006

Bhoi, A., Palladino, F., and Fabrizio, P. (2021). Auxin Confers Protection against ER Stress in *Caenorhabditis elegans*. *Biol. Open* 10 (2). doi:10.1242/bio.057992

Bishop, N. A., and Guarente, L. (2007). Two Neurons Mediate Diet-Restriction-Induced Longevity in *C. elegans*. *Nature* 447 (7144), 545–549. doi:10.1038/nature05904

Bitto, A., Wang, A. M., Bennett, C. F., and Kaeberlein, M. (2015). Biochemical Genetic Pathways that Modulate Aging in Multiple Species. *Cold Spring Harbor Perspect. Med.* 5 (11). doi:10.1101/cshperspect.a025114

Boehm, M., and Slack, F. (2005). Physiology: A Developmental Timing MicroRNA and its Target Regulate Life Span in *C. elegans*. *Science* 310 (5756), 1954–1957. doi:10.1126/science.1115596

Boulias, K., and Horvitz, H. R. (2012). The *C. elegans* MicroRNA *Mir-71* Acts in Neurons to Promote Germline-Mediated Longevity through Regulation of DAF-16/FOXO. *Cel. Metab.* 15 (4), 439–450. doi:10.1016/j.cmet.2012.02.014

Brosnan, C. A., Palmer, A. J., and Zuryn, S. (2021). Cell-Type-Specific Profiling of Loaded MiRNAs from *Caenorhabditis elegans* Reveals Spatial and Temporal Flexibility in Argonaute Loading. *Nat. Commun.* 12 (1), 1–16. doi:10.1038/s41467-021-22503-7

Broughton, J. P., Lovci, M. T., Huang, J. L., Yeo, G. W., and Pasquinelli, A. E. (2016). Pairing beyond the Seed Supports MicroRNA Targeting Specificity. *Mol. Cel* 64 (2), 320–333. doi:10.1016/j.molcel.2016.09.004

Chalfie, M., Horvitz, H. R., and Sulston, J. E. (1981). Mutations that Lead to Reiterations in the Cell Lineages of *C. elegans*. *Cell* 24 (1), 59–69. doi:10.1016/0092-8674(81)90501-8

Chang, J. T., Kumsta, C., Hellman, A. B., Adams, L. M., and Hansen, M. (2017). Spatiotemporal Regulation of Autophagy during *Caenorhabditis elegans* Aging. *ELife* 6, e18459. doi:10.7554/eLife.18459

Clevers, H. (2006). Wnt/β-Catenin Signaling in Development and Disease. *Cell* 127 (3), 469–480. doi:10.1016/j.cell.2006.10.018

De Lencastre, A., Pincus, Z., Zhou, K., Kato, M., Lee, S. S., and Slack, F. J. (2010). MicroRNAs Both Promote and Antagonize Longevity in *C. elegans*. *Curr. Biol.* 20 (24), 2159–2168. doi:10.1016/j.cub.2010.11.015

Ecsedi, M., Rausch, M., and Großhans, H. (2015). The *Let-7* MicroRNA Directs Vulval Development through a Single Target. *Develop. Cel.* 32 (3), 335–344. doi:10.1016/j.devcel.2014.12.018

Finger, F., Ottens, F., Springhorn, A., Drexel, T., Proksch, L., Metz, S., et al. (2019). Olfaction Regulates Organismal Proteostasis and Longevity via MicroRNA-dependent Signalling. *Nat. Metab.* 1 (3), 350–359. doi:10.1038/s42255-019-0033-z

Fontana, L., and Partridge, L. (2015). Promoting Health and Longevity through Diet: From Model Organisms to Humans. *Cell* 161 (1), 106–118. doi:10.1016/j.cell.2015.02.020

Friedland, A. E., Tzur, Y. B., Esvelt, K. M., Colaiácovo, M. P., Church, G. M., and Calarco, J. A. (2013). Heritable Genome Editing in *C. elegans* via a CRISPR-Cas9 System. *Nat. Methods* 10 (8), 741–743. doi:10.1038/nmeth.2532

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 (1), 75–86. doi:10.1093/genetics/118.1.75

Froehlich, J. J., Uyar, B., Herzog, M., Theil, K., Glažar, P., Akalin, A., et al. (2021). "Parallel Genetics of Regulatory Sequences Using Scalable Genome Editing *In Vivo*. *Cell Rep.* 35 (2), 1089988. doi:10.1016/j.celrep.2021.108988

Garg, D., and Cohen, S. M. (2014). miRNAs and Aging: A Genetic Perspective. *Ageing Res. Rev.* 17, 3–8. doi:10.1016/j.arr.2014.04.001

Greer, E. L., Dowlatshahi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., et al. (2007). An AMPK-FOXO Pathway Mediates Longevity Induced by a Novel Method of Dietary Restriction in *C. elegans*. *Curr. Biol.* 17 (19), 1646–1656. doi:10.1016/j.cub.2007.08.047

Grishok, A., Pasquinelli, A. E., Conte, D., Li, N., Parrish, S., Ha, I., et al. (2001). Genes and Mechanisms Related to RNA Interference Regulate Expression of the

Small Temporal RNAs that Control *C. elegans* Developmental Timing. *Cell* 106 (1), 23–34. doi:10.1016/S0092-8674(01)00431-7

Grosswendt, S., Filipchyk, A., Manzano, M., Klironomos, F., Schilling, M., Herzog, M., et al. (2014). Unambiguous Identification of miRNA:target Site Interactions by Different Types of Ligation Reactions. *Mol. Cel.* 54 (6), 1042–1054. doi:10.1016/j.molcel.2014.03.049

Hills-Muckey, K., Martinez, M. A. Q., Stec, N., Hebbar, S., Saldanha, J., Medwig-Kinney, T. N., et al. (2021). An Engineered, Orthogonal Auxin Analog/at TIR1(F79G) Pairing Improves Both Specificity and Efficacy of the Auxin Degradation System in Caenorhabditis Elegans. *Genetics*. doi:10.1093/genetics/iyab174

Honjoh, S., Yamamoto, T., Uno, M., and Nishida, E. (2009). Signalling through RHEB-1 Mediates Intermittent Fasting-Induced Longevity in *C. elegans*. *Nature* 457 (7230), 726–730. doi:10.1038/nature07583

Inukai, S., and Slack, F. (2013). A microRNAs and the Genetic Network in Aging. *J. Mol. Biol.* 425 (19), 3601–3608. doi:10.1016/j.jmb.2013.01.023

Inukai, S., Pincus, Z., De Lencastre, A., and Slack, F. J. (2018). A MicroRNA Feedback Loop Regulates Global MicroRNA Abundance during Aging. *RNA* 24 (2), 159–172. doi:10.1261/rna.062190.117

Johnston, R. J., and Hobert, O. (2003). A MicroRNA Controlling Left/Right Neuronal Asymmetry in *Caenorhabditis elegans*. *Nature* 426 (6968), 845–849. doi:10.1038/NATURE02255

Jung, H. J., and Suh, Y. (2012). MicroRNA in Aging: From Discovery to Biology. *Curr. Genomics* 13 (7), 548–557. doi:10.2174/138920212803251436

Kaeberlein, T. L., Smith, E. D., Tsuchiya, M., Welton, K. L., Thomas, J. H., Fields, S., et al. (2006). Lifespan Extension in *Caenorhabditis elegans* by Complete Removal of Food. *Aging Cell* 5 (6), 487–494. doi:10.1111/j.1474-9726.2006.00238.x

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

Kenyon, C. J. (2010). The Genetics of Ageing. *Nature* 464 (7288), 504–512. doi:10.1038/nature08980

Khan, A. H., Zou, Z., Yang, X., Chen, S., and Tian, X. L. (2019). Conserved Signaling Pathways Genetically Associated with Longevity across the Species. *Biochim. Biophys. Acta - Mol. Basis Dis.* 1865 (7), 1745–1755. doi:10.1016/j.bbadis.2018.09.001

Kim, S. S., and Lee, S. J. V. (2019). Non-Coding RNAs in *Caenorhabditis elegans* Aging. *Mol. Cell* 42 (5), 379. doi:10.14348/MOLCELLS.2019.0077

Kinser, H. E., and Pincus, Z. (2020). MicroRNAs as Modulators of Longevity and the Aging Process. *Hum. Genet.* 139 (3), 291–308. doi:10.1007/s00439-019-02046-0

Kogure, A., Uno, M., Ikeda, T., and Nishida, E. (2017). The MicroRNA Machinery Regulates Fasting-Induced Changes in Gene Expression and Longevity in *Caenorhabditis elegans*. *J. Biol. Chem.* 292 (27), 11300–11309. doi:10.1074/jbc.M116.765065

Kozomara, A., Birgaoanu, M., and Griffiths-Jones, S. (2019). MiRBase: From MicroRNA Sequences to Function. *Nucleic Acids Res.* 47 (D1), D155–D162. doi:10.1093/nar/gky1141

Kudlow, B. A., Zhang, L., and Han, M. (2012). Systematic Analysis of Tissue-Restricted MiRISCs Reveals a Broad Role for MicroRNAs in Suppressing Basal Activity of the *C. elegans* Pathogen Response. *Mol. Cel.* 46 (4), 530–541. doi:10.1016/j.molcel.2012.03.011

Lee, G. D., Wilson, M. A., Zhu, M., Wolkow, C. A., De Cabo, R., Ingram, D. K., et al. (2006). Dietary Deprivation Extends Lifespan in *Caenorhabditis elegans*. *Aging Cell* 5 (6), 515–524. doi:10.1111/j.1474-9726.2006.00241.x

Lee, R. C., Feinbaum, R. L., and Ambros, V. (1993). The *C. elegans* Heterochronic Gene Lin-4 Encodes Small RNAs with Antisense Complementarity to Lin-14. *Cell* 75 (5), 843–854. doi:10.1016/0092-8674(93)90529-Y

Lehrbach, N. J., Castro, C., Murfitt, K. J., Abreu-Goodger, C., Griffin, J. L., and Miska, E. A. (2012). Post-developmental MicroRNA Expression Is Required for Normal Physiology, and Regulates Aging in Parallel to Insulin/IGF-1 Signaling in *C. elegans*. *RNA* 18 (12), 2220–2235. doi:10.1261/rna.035402.112

Levine, B., and Kroemer, G. (2008). Autophagy in the Pathogenesis of Disease. *Cell* 132 (1), 27–42. doi:10.1016/j.cell.2007.12.018

Loose, J. A., and Ghazi, A. (2021). Auxin Treatment Increases Lifespan in *Caenorhabditis elegans*. *Biol. Open* 10 (5). doi:10.1242/bio.058703

Mack, H. I. D., Heimbucher, T., and Murphy, C. T. (2018). The Nematode *Caenorhabditis elegans* as a Model for Aging Research. *Drug Discov. Today Disea. Models* 27 (March), 3–13. doi:10.1016/j.ddmod.2018.11.001

Martinez, N. J., Ow, M. C., Reece-Hoyes, J. S., Barrasa, M. I., Ambros, V. R., and Walhout, A. J. M. (2008). Genome-Scale Spatiotemporal Analysis of *Caenorhabditis elegans* MicroRNA Promoter Activity. *Genome Res.* 18 (12), 2005–2015. doi:10.1101/gr.083055.108

Miller, H. A., Dean, E. S., Pletcher, S. D., and Leiser, S. F. (2020). Cell Non-autonomous Regulation of Health and Longevity. *ELife* 9, 1–28. doi:10.7554/ELIFE.62659

Mizushima, N. (2007). Autophagy: Process and Function. *Genes Develop.* 21 (22), 2861–2873. doi:10.1101/gad.1599207

Murphy, C. T., and Hu., P. J. (2013). Insulin/Insulin-like Growth Factor Signaling in *C. elegans*. *Wormbook: Online Rev. C. Elegans Biol. Dec* 26, 1–43. doi:10.1895/wormbook.1.164

Negishi, T., Kitagawa, S., Horii, N., Tanaka, Y., Haruta, N., Sugimoto, A., et al. (2021). The Auxin-Inducible Degron 2 (AID2) System Enables Controlled Protein Knockdown during Embryogenesis and Development in *Caenorhabditis elegans*. *Genetics* 220, iyab218. doi:10.1093/genetics/iyab218

Ng, L. F., Kaur, P., Bunnag, N., Suresh, J., Sung, I. C. H., Tan, Q. H., et al. (2019). WNT Signaling in Disease. *Cells* 8 (8). doi:10.3390/cells8080826

Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T., and Kanemaki, M. (2009). An Auxin-Based Degron System for the Rapid Depletion of Proteins in Nonplant Cells. *Nat. Methods* 6 (12), 917–922. doi:10.1038/nmeth.1401

Olsen, P. H., and Ambros., V. (1999). The *Lin-4* Regulatory RNA Controls Developmental Timing in *Caenorhabditis elegans* by Blocking LIN-14 Protein Synthesis after the Initiation of Translation. *Develop. Biol.* 216 (2), 671–680. doi:10.1006/dbio.1999.9523

Pagliuso, D. C., Bodas, D. M., and Pasquinelli, A. E. (2021). Recovery from Heat Shock Requires the MicroRNA Pathway in *Caenorhabditis elegans*. *PLoS Genet.* 17 (8). doi:10.1371/JOURNAL.PGEN.1009734

Panowski, S. H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa Mediates Diet-Restriction-Induced Longevity of *C. elegans*. *Nature* 447 (7144), 550–555. doi:10.1038/nature05837

Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., and Rougvie, A. E. (2000). The 21-nucleotide *Let-7* RNA Regulates Developmental Timing in *Caenorhabditis elegans*. *Nature* 403, 901–906. doi:10.1038/35002607

Rollins, J. A., Shaffer, D., Snow, S. S., Kapahi, P., and Rogers, A. N. (2019). Dietary Restriction Induces Posttranscriptional Regulation of Longevity Genes. *Life Sci. Alliance* 2 (4), e201800281. doi:10.26508/lsa.201800281

Sarkis, G. J., Ashcom, J. D., Hawdon, J. M., and Jacobson, L. A. (1988). Decline in Protease Activities with Age in the Nematode *Caenorhabditis elegans*. *Mech. Ageing Develop.* 45 (3), 191–201. doi:10.1016/0047-6374(88)90001-2

Schiksnis, E., Nicholson, A., Modena, M., Pule, M., Arribere, J., and Pasquinelli, A. (2020). Auxin-Independent Depletion of Degron-Tagged Proteins by TIR1. *MicroPublication Biol.* 2020, doi:10.17912/micropub.biology.000213

Sethi, J. K., and Vidal-Puig, A. (2010). Wnt Signalling and the Control of Cellular Metabolism. *Biochem. J.* 427 (1), 1–17. doi:10.1042/BJ20091866

Smith-Vikos, T., De Lencastre, A., Inukai, S., Shlomchik, M., Holtrup, B., Slack, F. J., et al. (2014). MicroRNAs Mediate Dietary-Restriction-Induced Longevity through PHA-4/FOXA and SKN-1/Nrf Transcription Factors. *Curr. Biol.* 24 (19), 2238–2246. doi:10.1016/j.cub.2014.08.013,

Smith-Vikos, T., and Slack, F. J. (2012). MicroRNAs and Their Roles in Aging. *J. Cel. Sci.* 125 (1), 7–17. doi:10.1242/jcs.099200

Son, H. G., Altintas, O., Kim, E. J. E., Kwon, S., and Lee, S. J. V. (2019). Age-Dependent Changes and Biomarkers of Aging in *Caenorhabditis elegans*. *Aging Cell* 18 (2), e12853. doi:10.1111/acel.12853

Taylor, R. C., and Dillin, A. (2011). Aging as an Event of Proteostasis Collapse. *Cold Spring Harbor Perspect. Biol.* 3 (5), 1–17. doi:10.1101/cshperspect.a004440

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. Develop. Biol.* 9 (August), 2268. doi:10.3389/fcell.2021.692020

Uno, M., Honjoh, S., Matsuda, M., Hoshikawa, H., Kishimoto, S., Yamamoto, T., et al. (2013). A Fasting-Responsive Signaling Pathway that Extends Life Span in *C. elegans*. *Cel. Rep.* 3 (1), 79–91. doi:10.1016/j.celrep.2012.12.018

Uno, M., and Nishida, E. (2016). Lifespan-Regulating Genes in *C. elegans*. *Npj Aging Mech. Dis.* 2 (1), 1–8. doi:10.1038/npjamd.2016.10

Vasquez-Rifo, A., Jannot, G., Armisen, J., Labouesse, M., Bukhari, S. I. A., Rondeau, E. L., et al. (2012). Developmental Characterization of the MicroRNA-specific *C. elegans* Argonautes Alg-1 and Alg-2. *PLoS ONE* 7 (3), e33750. doi:10.1371/journal.pone.0033750

Vella, M. C., Choi, E. Y., Lin, S. Y., Reinert, K., and Slack, F. J. (2004). The *C. elegans* MicroRNA *Let-7* Binds to Imperfect *Let-7* Complementary Sites from the *Lin-41* 3′UTR. *Genes Develop.* 18 (2), 132–137. doi:10.1101/gad.1165404

Vora, M., Shah, M., Ostafi, S., Onken, B., Xue, J., Ni, J. Z., et al. (2013). Deletion of *microRNA-80* Activates Dietary Restriction to Extend *C. elegans* Healthspan and Lifespan. *PLoS Genet.* 9 (8), 1003. doi:10.1371/journal.pgen.1003737

Wang, S., Tang, N. H., Lara-Gonzalez, P., Zhao, Z., Cheerambathur, D. K., Prevo, B., et al. (2017). A Toolkit for GFP-Mediated Tissue-specific Protein Degradation in *C. elegans*. *Development (Cambridge)* 144 (14), 2694–2701. doi:10.1242/dev.150094

Wightman, B., Ha, I., and Ruvkun, G. (1993). Posttranscriptional Regulation of the Heterochronic Gene *Lin-14* by *Lin-4* Mediates Temporal Pattern Formation in *C. elegans*. *Cell* 75 (5), 855–862. doi:10.1016/0092-8674(93)90530-4

Xu, Y., He, Z., Song, M., Zhou, Y., and Shen, Y. (2019). A Micro RNA Switch Controls Dietary Restriction-induced Longevity through Wnt Signaling. *EMBO Rep.* 20 (5), e46888. doi:10.15252/embr.201846888

Youngman, E. M., and Claycomb, J. M. (2014). From Early Lessons to New Frontiers: The Worm as a Treasure Trove of Small RNA Biology. *Front. Genet.* 5 (NOV), 416. doi:10.3389/fgene.2014.00416

Zhang, L., Ding, L., Cheung, T. H., Dong, M. Q., Chen, J., Sewell, A. K., et al. (2007). Systematic Identification of *C. elegans* MiRISC Proteins, MiRNAs, and MRNA Targets by Their Interactions with GW182 Proteins AIN-1 and AIN-2. *Mol. Cel.* 28 (4), 598–613. doi:10.1016/j.molcel.2007.09.014

Zhang, L., Ward, J. D., Cheng, Z., and Dernburg, A. F. (2015). The Auxin-Inducible Degradation (AID) System Enables Versatile Conditional Protein Depletion in *C. elegans*. *Development (Cambridge)* 142 (24), 4374–4384. doi:10.1242/dev.129635

Zhang, S., Li, F., Zhou, T., Wang, G., and Li, Z. (2020). *Caenorhabditis elegans* as a Useful Model for Studying Aging Mutations. *Front. Endocrinol.* 11, 731. doi:10.3389/fendo.2020.554994

Zhi, L., Yu, Y., Li, X., and Wang, D. (2017). Molecular Control of Innate Immune Response to Pseudomonas Aeruginosa Infection by Intestinal *Let-7* in *Caenorhabditis elegans*. *PLoS Pathog.* 13 (1), e1006152. doi:10.1371/JOURNAL.PPAT.1006152

Zhou, Y., Wang, X., Song, M., He, Z., Cui, G., Peng, G., et al. (2019). A Secreted MicroRNA Disrupts Autophagy in Distinct Tissues of *Caenorhabditis elegans* upon Ageing. *Nat. Commun.* 10 (1), 1–14. doi:10.1038/s41467-019-12821-2

Zisoulis, D. G., Lovci, M. T., Wilbert, M. L., Hutt, K. R., Liang, T. Y., Pasquinelli, A. E., et al. (2010). Comprehensive Discovery of Endogenous Argonaute Binding Sites in *Caenorhabditis elegans*. *Nat. Struct. Mol. Biol.* 17 (2), 173–179. doi:10.1038/nsmb.1745

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Geranylgeranylacetone Ameliorates Beta-Amyloid Toxicity and Extends Lifespan *via* the Heat Shock Response in *Caenorhabditis elegans*

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Activation of a cytoprotective cellular pathway known as the heat shock response (HSR) is a promising strategy for the treatment of Alzheimer's disease and other neurodegenerative diseases. Geranylgeranylacetone (GGA) is a commonly used anti-ulcer drug in Japan that has been shown to activate the HSR. Here, we establish *C. elegans* as a model system to investigate the effects of GGA. First, we show that GGA-mediated activation of the HSR is conserved in worms. Then, we show that GGA can ameliorate beta-amyloid toxicity in both muscle and neuronal worm Alzheimer's disease models. Finally, we find that exposure to GGA is sufficient to extend the lifespan of wild-type worms. Significantly, the beneficial effects of GGA on both beta-amyloid toxicity and lifespan are dependent on HSR activation. Taken together, this research supports further development of GGA as a therapeutic for Alzheimer's disease, provides evidence that HSR activation is a relevant therapeutic mechanism, and indicates that the beneficial effects of GGA are not limited to disease.

Keywords: geranylgeranylacetone, HSF1, heat shock response, Alzheimer's disease, aging, longevity, lifespan, drug repurposing

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INTRODUCTION

Translation of biomedical science discoveries into disease-modifying therapeutics is a slow and laborious process that requires enormous financial resources. The estimated cost of developing a new pharmaceutical is between 1 and 3 billion dollars (DiMasi et al., 2016; Nosengo, 2016). One analysis suggests that the returned value for investment in the pharmaceutical industry may be less than the value of the initial investments (Pushpakom et al., 2019).

Repurposing of current therapeutics is a strategy that can substantially reduce development costs (Pushpakom et al., 2019). Drug repurposing significantly reduces the attrition of candidate molecules during therapeutic development due to safety concerns, rapid metabolism, and lack of bioavailability. Therefore, drug repurposing has the potential to facilitate the development of new disease treatments. One successful example of this approach is Viagra, which was originally an antihypertensive drug that was repurposed for the treatment of erectile dysfunction.

Alzheimer's disease (AD) is a devastating neurodegenerative disease with an urgent and desperate need for therapeutic development (Sierksma et al., 2020; Walsh and Selkoe, 2020). Two of the key pathological features of AD, senile plaques and neurofibrillary tangles, are associated with protein misfolding and aggregation. In the brains of AD patients, beta-amyloid peptides accumulate in senile

plaques while Tau proteins accumulate in neurofibrillary tangles. Distinct protein aggregates also accumulate in a stunning variety of other neurodegenerative diseases (Hipp et al., 2019). While the specific connections between protein folding and pathology remain an active area of research, the consequences of protein misfolding accumulation include general loss of cellular protein folding homeostasis, synaptic loss, and neuronal death. Therefore, preventing or reversing protein misfolding is being pursued as a therapeutic strategy for AD.

The heat shock response (HSR) is a cytoprotective, cellular stress response that responds to protein misfolding induced by temperature and other stresses (Guisbert and Morimoto, 2012). The HSR is mediated by the highly conserved HSF1 transcription factor. HSF1 regulates a suite of beneficial heat shock genes, including molecular chaperones that directly facilitate the refolding and/or degradation of misfolded proteins.

Genetic activation of HSR genes has been shown to have beneficial effects in disease models for Alzheimer's disease and other neurodegenerative diseases. For example, overexpression of the HSP70 heat shock gene suppresses toxicity in a mouse AD model (Hoshino et al., 2011). Furthermore, HSR activation has been shown to extend lifespan even in the absence of disease (Hsu et al., 2003; Morley and Morimoto, 2004). These results have prompted efforts to identify and develop small molecule HSR inducers (Calamini et al., 2011; Neef et al., 2011; West et al., 2012).

Geranylgeranylacetone (GGA), also known as Teprenone, is a drug that has had widespread use in Japan as an anti-ulcer agent since the 1980s (Zeng et al., 2018). While the mechanism of action for this drug remains incompletely characterized, it has been shown to activate the HSR in cultured cells (Hirakawa et al., 1996). HSR activation by GGA occurs via disruption of the interaction between the HSP70 moleculer chaperone and HSF1, as HSP70 is a key negative regulator of HSF1 (Otaka et al., 2007).

Administration of GGA was found to have dramatic effects in a mouse Alzheimer's disease model. GGA treated APP23 mice exhibited decreased levels of beta-amyloid peptide, beta-amyloid aggregates, and synaptic loss (Sun et al., 2017). Importantly, these benefits are accompanied by improvements in cognitive function. Furthermore, these effects occurred during oral administration of GGA, indicating that GGA can cross the blood-brain barrier (Katsuno et al., 2005).

The promising results with GGA in the mouse Alzheimer's disease model and its established clinical use in humans makes GGA an ideal candidate for drug repurposing. Continued development of GGA would benefit from a model system that is experimentally feasible and genetically tractable. Therefore, we establish *Caenorhabditis elegans* as a model to study the mechanism of GGA and determine if the beneficial effects of GGA against beta-amyloid toxicity requires HSR activation.

MATERIALS AND METHODS

Nematode Strains and Cultures

Nematodes were maintained using standard laboratory techniques on NGM plates seeded with OP50 bacteria at 20°C unless otherwise indicated (Brenner, 1974). Worms were age-matched by bleaching with hypochlorite (NaOCl) and hatching overnight in M9 buffer or with synchronized egg-laying. The following strains were used: 1) N2 (wild-type) (Brenner, 1974); 2) CL 2006 [pCL12 (*unc-54*/human Aβ peptide 3-42 minigene) + *rol-6(su1006)*] (Link, 1995); 3) CL2355 [pCL45 (*snb-1*:Aβ 1-42:3′ UTR (long) + *mtl-2:GFP*]I (Wu et al., 2006); 4) CL2122 [(pPD30.38) *unc-54* (vector) + (pCL26) *mtl-2::GFP*] (Wu et al., 2006); 5) PS3551 *hsf-1(sy441)* (Hajdu-Cronin et al., 2004); 6) CF1038 *daf-16(mu86)* (Hsin and Kenyon, 1999).

Chemicals and Reagents

GGA was obtained from Santa Cruz Biotechnology. Diacetyl was obtained from TCI America. RNAi constructs were obtained from the Ahringer RNAi library and used for feeding RNAi as previously described (Kamath and Ahringer, 2003; Ma et al., 2017). The following oligos were used: *hsp-16.2* forward (ACT TTACCACTATTTCCGTCCAGC), *hsp-16.2* reverse (CCTTGA ACCGCTTCTTTCTTTG), *hsp-16.11* forward (GGCTCAGAT GGAACGTCAA), *hsp-16.11* reverse (GCTTGAACTGCGAGA CATTG), *F44E5.5* forward (CAACTGCTGGTGATACCCATC TC), *F44E5.5* reverse (CTTGAAAGTGTTCTCTTGGCACG), *hsp-70* forward (GTACTACGTACTCATGTGTCGGTATTT ATC), *hsp-70* reverse (ACGGGCTTTCCTTGTTTTCC), *cdc-42* forward (TTTGCTTCTCCGTGGTTGCTCC), and *cdc-42* reverse (TCCGTTGACACTGGTTTCTGCTTG).

RT-qPCR

N2 worms were grown at 20°C on NGM control or 10 μM GGA plates starting at the L1 larval stage until day one of adulthood. RNA was isolated using Trizol Reagent (Thermo Fisher) as previously described with the addition of a freeze-thaw in liquid nitrogen and use of a Direct-zol RNA kit (Zymo Research) (Golden et al., 2020). RNA was reverse transcribed with an iScript cDNA Synthesis Kit (Bio-Rad) and RT-qPCR was performed using an iTaq Universal SYBR Green Supermix (Bio-Rad) and the CFX Connect cycler (Bio-Rad). HSR gene expression was normalized to expression of *cdc-42*. All qPCR experiments were performed with biological triplicates and p-values were calculated using the Student's t-test.

Paralysis Assay

Transgenic CL 2006 (muscle beta-amyloid) worms were grown at 20°C on NGM control or 10 μM GGA plates seeded with empty vector RNAi (L4440) or *hsf-1* RNAi containing bacteria starting at the L1 larval stage. Worms were scored for paralysis starting on day 1 of adulthood. Individuals were considered paralyzed if the body of the worm did not move when gently prodded. Worms were transferred by picking onto new plates as needed to separate adults from eggs and larva. Data was analyzed using OASIS 2 (Han et al., 2016).

Associative Memory Assay

Transgenic CL2122 (control) and CL2355 (neuronal beta-amyloid) worms were grown at 16°C on NGM control or 10 μM GGA plates starting at the L1 larval stage until day one of adulthood. Then, worms were shifted to 25°C for 24 h to induce

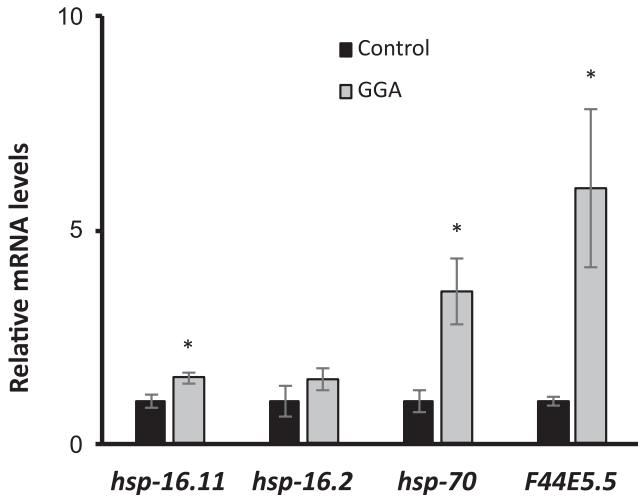

FIGURE 1 | GGA activates the HSR in *C. elegans*. Wild-type (N2) worms were synchronized and incubated on NGM control or 10 μM GGA plates until day 1 of adulthood. RT-qPCR analysis showed a significant increase in basal expression of heat shock gene mRNA in worms exposed to GGA. Averages shown are from 3 biological replicates. Error bars represent SEM; * indicates p-value < 0.05 (Student's t-test).

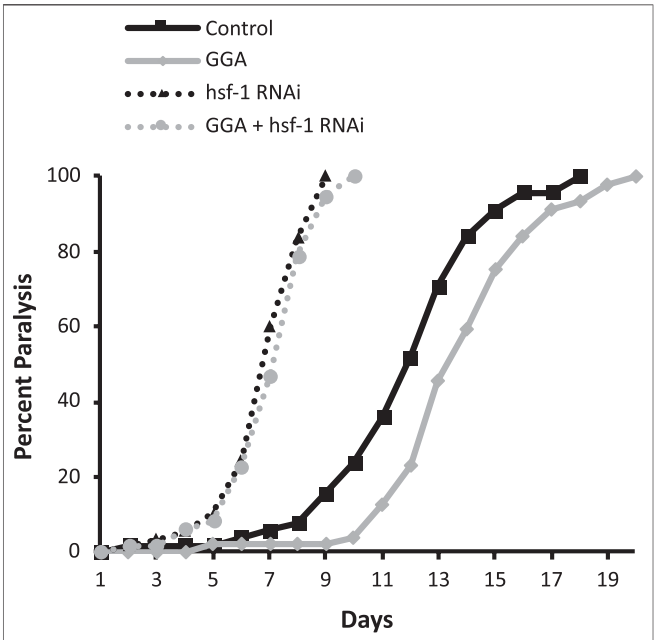

FIGURE 2 | GGA alleviates paralysis in a *C. elegans* Alzheimer's disease model. Transgenic CL2006 worms expressing beta-amyloid in muscle were synchronized and incubated on NGM control or 10 μM GGA plates seeded with empty vector (L4440) or *hsf-1* RNAi containing bacteria. Worms were scored for paralysis starting at day 1 of adulthood. Worms exposed to GGA had significantly delayed paralysis compared to control worms (p-value < 0.05, Mantel-Cox log-rank test). Each curve represents data from 3 independent trials of n ≥ 20 individuals.

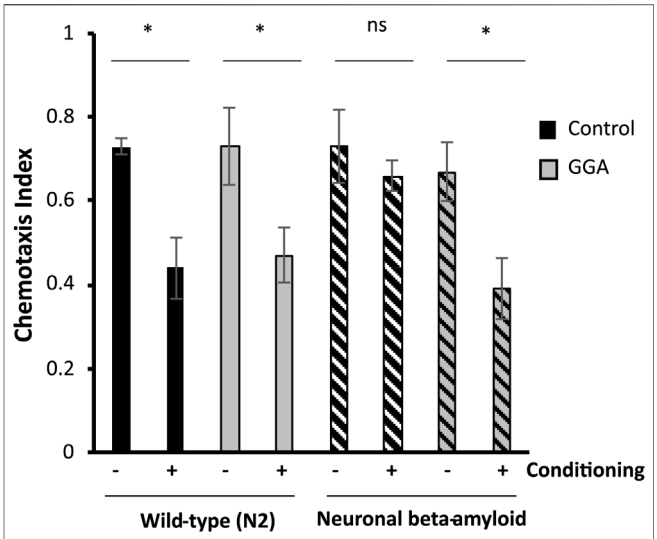

FIGURE 3 | GGA restores associative learning in a neuronal Alzheimer's disease model. Transgenic worms with or without neuronal beta-amyloid expression were synchronized and incubated on NGM control or 10 μM GGA plates at 16°C. On day one of adulthood, worms were shifted to 25°C for 24 h to induce beta-amyloid expression. Then, worms were starved in the presence or absence of the odorant diacetyl for 2 h and scored for chemotaxis. Worms expressing neuronal beta-amyloid exhibited a defect in conditioned learning that was restored upon exposure to GGA. Averages shown are from 3 biological replicates of $n \geq 30$ adults each. Error bars represent SEM; * indicates p-value < 0.05; ns = non-significant (Student's t-test).

neuronal beta-amyloid expression. Day 2 adults were conditioned by transferring to bacteria-free NGM plates with 6 μL of 0.04% diacetyl added to the plate lid (Dosanjh et al., 2010). Control worms were transferred to bacteria-free NGM plates but not exposed to diacetyl. Plates were inverted and incubated for 2 h at 25°C. The chemotaxis assay was conducted by spotting 2 μL of 0.04% diacetyl 1 inch from the center of the assay plate on one side and 95% ethanol on the other. Worms were quickly placed at the center of each plate, followed by an additional 2 μL of odorant and 2 μL of 1 M sodium azide in each spot to paralyze the worms. Plates were then incubated at 20°C for 1 h and immediately scored. The chemotaxis index was calculated using the following formula (number of worms at the diacetyl spot—number of worms at the ethanol spot)/total number of worms.

Lifespan

N2, PS3551 (*hsf-1* mutant), and CF1038 (*daf-16* mutant) worms were grown at 20°C on NGM control or 10 μM GGA plates starting at the L1 or L4 larval stages. Viability was scored starting on day 1 of adulthood. Individuals were considered dead if they did not have a motile response to gentle prodding and lacked pharyngeal pumping. Worms were transferred by picking onto new control or GGA plates as needed to separate adults from eggs and larva. Data was analyzed using OASIS 2 (Han et al., 2016).

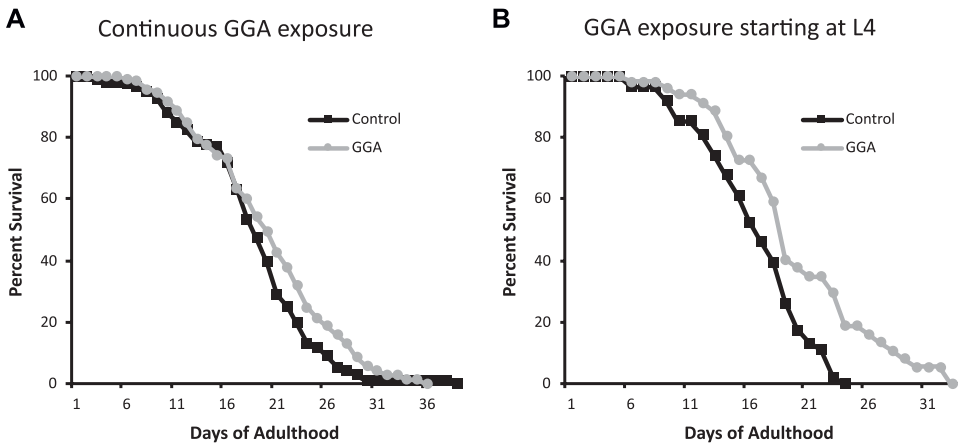

FIGURE 4 | GGA extends lifespan in *C. elegans*. Wild-type (N2) worms were synchronized and incubated on NGM control plates or plates containing 10 μM GGA starting at the L1 **(A)** or L4 **(B)** larval stages. Worms were scored for viability starting at day 1 of adulthood. Worms exposed to GGA in either case lived significantly longer than control worms (p-value < 0.05, Mantel-Cox log-rank test). Each curve represents ≥3 pooled trials of $n \geq 20$ individuals each.

TABLE 1 | Effects of GGA on lifespan.

Strain	Condition	Replicate	Number of Worms	Mean Lifespan	Standard Error
N2	Control	Combined	180	18.72	0.48
N2	GGA	Combined	190	20.2	0.69
daf-16(mu86)	Control	Combined	130	14.79	0.41
daf-16(mu86)	GGA	Combined	140	16.3	0.55
hsf-1(sy441)	Control	Combined	130	12.77	0.33
hsf-1(sy441)	GGA	Combined	130	13.08	0.33
N2	Control (L4)	Combined	60	16.45	0.65
N2	GGA (L4)	Combined	70	19.92	0.97
N2	Control	1	30	20.05	1.19
		2	30	17.98	1.57
		3	40	19.77	1.04
		4	80	18.25	0.51
N2	GGA	1	30	21.70	1.82
		2	40	19.15	1.21
		3	40	20.13	1.07
		4	80	20.07	1.18
daf-16(mu86)	Control	1	30	13.77	0.78
		2	30	14.67	1.15
		3	30	16.02	0.76
		4	40	14.78	0.50
daf-16(mu86)	GGA	1	30	14.50	1.02
		2	40	15.92	1.03
		3	30	15.57	0.85
		4	40	18.37	1.10
hsf-1(sy441)	Control	1	30	12.78	0.75
		2	30	13.01	0.69
		3	30	13.34	0.76
		4	40	12.17	0.45
hsf-1(sy441)	GGA	1	30	13.77	0.69
		2	30	12.66	0.62
		3	30	14.26	0.69
		4	40	12.09	0.61
N2	Control (L4)	5	20	17.29	1.00
		6	20	16.05	1.28
		7	20	15.91	1.03
N2	GGA (L4)	5	35	21.12	1.16
		6	20	19.57	1.48
		7	20	18.13	1.96

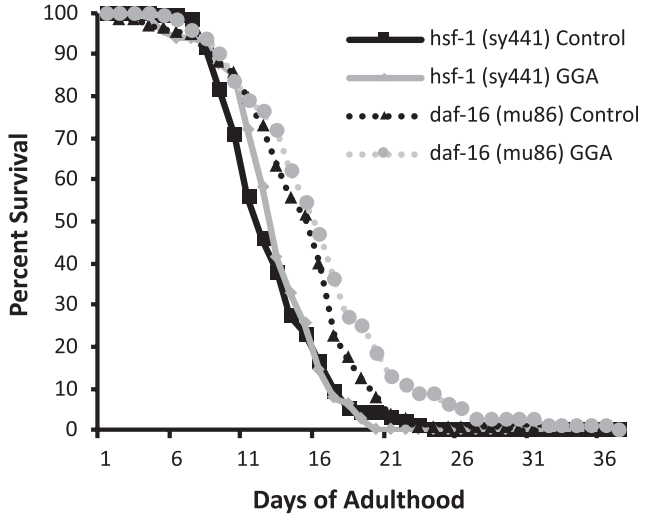

FIGURE 5 | The HSR is required for GGA-mediated lifespan extension. Worms containing mutations in *daf-16 (mu86)* and *hsf-1 (sy441)* were synchronized and incubated on control NGM plates or plates containing 10 μM GGA. Worms were scored for viability starting at day 1 of adulthood. Incubation with GGA caused significant lifespan extension in *daf-16* mutant worms (p-value < 0.05 Mantel-Cox log-rank test) but not worms containing a mutation in *hsf-1*. Each curve represents ≥4 pooled trials of $n \geq 30$ individuals each.

RESULTS

The ability of Geranylgeranylacetone (GGA) to ameliorate beta-amyloid toxicity in mice motivated further investigation of this drug using the genetically tractable model nematode *Caenorhabditis elegans*. Previously, GGA has been shown to activate the cytoprotective heat shock response (HSR) in cultured human cells and in mice (Zeng et al., 2018). Therefore, we first investigated the ability of GGA to activate the HSR in worms. Worms were exposed to GGA from the first larval stage (L1) to adulthood on standard NGM agar plates or NGM plates containing GGA. It was found that incubation of worms on plates containing 10 μM GGA was sufficient to increase the expression of the *hsp-16.11*, *hsp-70*, and *F44E5.5* HSR-dependent genes between 1.5-fold and 6-fold measured by RT-qPCR (**Figure 1**). GGA also induced a fourth HSR gene, *hsp-16.2*, 1.5-fold, but this effect was not statistically significant. The *hsp-70* and *F44E5.5* genes both encode HSP70 family proteins while *hsp-16.11* and *hsp-16.2* genes encode small HSP proteins. These results demonstrate that the effects of GGA on the HSR are conserved from humans and mice to worms and establish the basis for using *C. elegans* as model system for investigation of the effects of GGA on the HSR.

Previously, GGA was shown to have beneficial effects on a mouse model for Alzheimer's disease (AD) containing expression of the human beta-amyloid peptide (Sun et al., 2017). Therefore, we next tested whether the effects of GGA on beta-amyloid toxicity were conserved in worms. A well-established worm AD model was used that features transgenic expression of the human A-beta peptide (Link, 1995). In this model, beta-amyloid is expressed in muscle cells and the worms develop progressive paralysis as they age with 50% of the worms becoming paralyzed on day 11 of adulthood (**Figure 2**). We found that incubation with GGA led to a reduction in this age-dependent paralysis with the median paralysis occurring nearly 2 days later. These results indicate that GGA can ameliorate toxicity from beta-amyloid expression in a worm disease model.

Having established that the effects of GGA on HSR activation and beta-amyloid-induced paralysis were conserved in worms, we next tested whether the effects on beta-amyloid were dependent on the HSR. The HSR was inhibited by RNAi knockdown of the HSF1 transcription factor that is required for the response. Consistent with previous work, we found that inhibition of HSF1 alone enhanced the toxicity in this disease model, with median paralysis occurring around day 6 of adulthood. In contrast to its effects in wild-type worms, GGA did not have a significant effect on beta-amyloid toxicity when HSF-1 was inhibited. These results indicate that activation of the HSR is required for the beneficial effects of GGA.

Having shown that GGA can reverse the toxicity from beta-amyloid expression in muscle tissue, we next tested the effects of GGA in neurons, a more relevant tissue type for AD. In a second worm AD model, neuronal expression of human beta-amyloid has been shown to cause neuronal toxicity and results in a defect in associative learning (Dosanjh et al., 2010). In the associative learning assay, worms are simultaneously exposed to a chemoattractant and a lack of food. Upon subsequent exposures, worms display associative learning by reducing their chemotaxis towards the odorant. Naïve wild type worms exhibited chemotaxis towards diacetyl, measured by a chemotaxis index of 0.73 (**Figure 3**). When they were conditioned by exposure to diacetyl in the absence of food, they reduced their preference for this odorant to a chemotaxis index of 0.44. In the AD model, neuronal expression of beta-amyloid disrupted associative learning but did not affect chemotaxis of naïve worms (0.73 naïve *verses* 0.66 conditioned).

We tested the ability of GGA to reverse the associative learning defect in worms expressing neuronal beta-amyloid. GGA did not affect chemotaxis nor associative learning in wild-type worms (0.73 naïve *verses* 0.47 conditioned). Significantly, exposure to GGA restored associative learning in worms with neuronal beta-amyloid (0.68 naïve *verses* 0.39 conditioned). Taken together, these experiments demonstrate that the beneficial effects of incubation with GGA in worms extend to neurons.

Having established the effects of GGA on disease models, we next tested whether its beneficial effects could extend to longevity. A wide range of protein aggregates accumulate during the normal process of aging and disruption of protein folding homeostasis, or proteostasis, is considered a hallmark of aging (David et al., 2010; López-Otín et al., 2013). Overexpression of HSF1 has been shown to extend lifespan in worms while inhibition of HSF1 causes premature aging (Hsu et al., 2003; Morley and Morimoto, 2004). Therefore, we tested whether incubation with GGA was sufficient to extend lifespan in wild-type worms. Excitingly, incubation of wild-type worms with GGA extended the mean lifespan (18.7 ± 0.5 days for control *versus* 20.2 ± 0.7 days, p-value 0.03) (**Figure 4A**; **Table 1**). This lifespan extension did not require early developmental exposure as a lifespan extension

was still observed upon exposure of worms to GGA starting at the L4 stage (16.5 ± 0.7 days for control *versus* 19.9 ± 1.0 days, p-value 0.002) (**Figure 4B**). We tested whether this phenotype was dependent on the HSR using a worm strain containing a mutation in HSF1 that blocks HSR activation. We found that GGA was no longer was able to extend lifespan in the HSF1 mutant background (12.8 ± 0.3 days *versus* 13.1 ± 0.3 days, p-value 0.54) (**Figure 5**; **Table 1**). This dependency was specific for the HSR as the effects of GGA were not reduced in worms containing a mutation in DAF-16, a distinct longevity pathway known as insulin-like signaling (14.8 ± 0.4 *versus* 16.3 ± 0.6, p-value 0.01). Together, these data indicate that GGA can extend lifespan in worms via activation of the HSR and that its beneficial effects are not limited to neurodegenerative disease models.

DISCUSSION

This manuscript establishes *C. elegans* as a model system for investigation of the anti-ulcer drug geranylgeranylacetone (GGA). First, conservation of the effects of GGA in worms was shown with respect to both HSR activation and amelioration of beta-amyloid toxicity. Then, genetic manipulation of the system was used to demonstrate that activation of the HSR is required for GGA's beneficial effects. Finally, it was discovered that GGA can also extend lifespan in wild-type worms. Taken together, this research not only supports further development of GGA as a novel Alzheimer's disease (AD) therapeutic, but also provides a foundation that can be used for new insights into HSR regulation and lifespan.

Activation of the HSR by GGA in worms establishes a new tool in the *C. elegans* HSR field. This activation is not surprising given the high degree of conservation in the HSF1 transcription factor that mediates this response. However, worms are resistant to the effects of many small molecules due to the barrier function of their cuticle. The ability to activate the HSR independent of temperature increases and associated protein misfolding stress will enable new experimental approaches to explore HSR regulatory mechanisms. Furthermore, as HSR-inducible promoters have been one of the mainstays for ectopic expression in *C. elegans*, this tool may also be useful for other fields of biology that use worms as a model organism including developmental biology and neuroscience.

Amelioration of the detrimental effects of beta-amyloid expression in worm muscle and neuronal AD models by GGA is consistent with previous results showing that GGA has beneficial effects in a mouse AD model. However, the establishment of its effects in worms facilitated an explicit test of its dependence on the HSR. The powerful genetic tools and other advantages of this model system should set the stage for further exploration of the effects of GGA on beta-amyloid toxicity. Moreover, the use of worms can facilitate investigation of GGA in other neurodegenerative disease models such as Parkinson's disease, ALS, and Huntington's disease.

Excitingly, we discovered that GGA can extend the lifespan of wild-type worms. This lifespan extension is also dependent on HSF1 but not dependent on insulin-like signaling, one of the major cellular pathways that influences longevity. These results are consistent with disruption of proteostasis as a hallmark of aging, further supporting that interventions targeting this pathway can have beneficial effects. To our knowledge, our results for the first time provide experimental evidence that a small molecule HSR activator can positively affect longevity in worms. Importantly, GGA is already approved for human use to treat ulcers, indicating that it is both bioavailable and does not have substantial off-target toxicity. Therefore, these findings provide strong support for future work on GGA and other HSR inducers.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

IM, SP, TS, MF, and EG performed the experiments. EG and KSKG supervised the project and wrote the manuscript.

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REFERENCES

Brenner, S. (1974). The Genetics of *Caenorhabditis elegans*. *Genetics* 77 (1), 71–94. doi:10.1093/genetics/77.1.71

Calamini, B., Silva, M. C., Madoux, F., Hutt, D. M., Khanna, S., Chalfant, M. A., et al. (2011). Small-molecule Proteostasis Regulators for Protein Conformational Diseases. *Nat. Chem. Biol.* 8 (2), 185–196. doi:10.1038/nchembio.763

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 (8), e1000450. doi:10.1371/journal.pbio.1000450

DiMasi, J. A., Grabowski, H. G., and Hansen, R. W. (2016). Innovation in the Pharmaceutical Industry: New Estimates of R and D Costs. *J. Health Econ.* 47, 20–33. doi:10.1016/j.jhealeco.2016.01.012

Dosanjh, L. E., Brown, M. K., Rao, G., Link, C. D., and Luo, Y. (2010). Behavioral Phenotyping of a Transgenic Caenorhabditis Elegans

Expressing Neuronal Amyloid-β. *J. Alzheimer's Dis.* 19(2), 681–690. doi:10.3233/JAD-2010-1267

Golden, N. L., Plagens, R. N., Kim Guisbert, K. S., and Guisbert, E. (2020). Standardized Methods for Measuring Induction of the Heat Shock Response in *Caenorhabditis elegans*. *J. Visualized Experiments* 161. doi:10.3791/61030

Guisbert, E., and Morimoto, R. I. (2012). "The Regulation and Function of the Heat Shock Response," in *Protein Quality Control in Neurodegenerative Diseases* (Springer Berlin Heidelberg), 1–18. doi:10.1007/978-3-642-27928-7_1

Hajdu-Cronin, Y. M., Chen, W. J., and Sternberg, P. W. (2004). The L-type Cyclin CYL-1 and the Heat-Shock-Factor HSF-1 Are Required for Heat-Shock-Induced Protein Expression in *Caenorhabditis elegans*. *Genetics* 168 (4), 1937–1949. doi:10.1534/genetics.104.028423

Han, S. K., Lee, D., Lee, H., Kim, D., Son, H. G., Yang, J.-S., et al. (2016). OASIS 2: Online Application for Survival Analysis 2 with Features for the Analysis of Maximal Lifespan and Healthspan in Aging Research. *Oncotarget* 7 (35), 56147–56152. doi:10.18632/oncotarget.11269

Hipp, M. S., Kasturi, P., and Hartl, F. U. (2019). The Proteostasis Network and its Decline in Ageing. *Nat. Rev. Mol. Cel Biol* 20 (7), 421–435. doi:10.1038/s41580-019-0101-y

Hirakawa, T., Rokutan, K., Nikawa, T., and Kishi, K. (1996). Geranylgeranylacetone Induces Heat Shock Proteins in Cultured guinea Pig Gastric Mucosal Cells and Rat Gastric Mucosa. *Gastroenterology* 111 (2), 345–357. doi:10.1053/gast.1996.v111.pm8690199

Hoshino, T., Murao, N., Namba, T., Takehara, M., Adachi, H., Katsuno, M., et al. (2011). Suppression of Alzheimer's Disease-Related Phenotypes by Expression of Heat Shock Protein 70 in Mice. *J. Neurosci.* 31 (14), 5225–5234. doi:10.1523/JNEUROSCI.5478-10.2011

Hsin, H., and Kenyon, C. (1999). Signals from the Reproductive System Regulate the Lifespan of *C. elegans*. *Nature* 399 (6734), 362–366. doi:10.1038/20694

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 (5622), 1142–1145. doi:10.1126/science.1083701

Kamath, R., and Ahringer, J. (2003). Genome-wide RNAi Screening in *Caenorhabditis elegans*. *Methods* 30 (4), 313–321. doi:10.1016/s1046-2023(03)00050-1

Katsuno, M., Sang, C., Adachi, H., Minamiyama, M., Waza, M., Tanaka, F., et al. (2005). Pharmacological Induction of Heat-Shock Proteins Alleviates Polyglutamine-Mediated Motor Neuron Disease. *Proc. Natl. Acad. Sci. U.S.A.* 102 (46), 16801–16806. doi:10.1073/pnas.0506249102

Link, C. D. (1995). Expression of Human Beta-Amyloid Peptide in Transgenic *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 92 (20), 9368–9372. doi:10.1073/pnas.92.20.9368

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

Ma, J., Grant, C. E., Plagens, R. N., Barrett, L. N., Guisbert, K. S. K., and Guisbert, E. (2017). Cellular Proteomes Drive Tissue-specific Regulation of the Heat Shock Response. *G3 Bethesda, Md.* 7 (3), 1011–1018. doi:10.1534/g3.116.038232

Morley, J. F., and Morimoto, R. I. (2004). Regulation of Longevity inCaenorhabditis Elegansby Heat Shock Factor and Molecular Chaperones. *Mol. Biol. Cel* 15 (2), 657–664. doi:10.1091/mbc.e03-07-0532

Neef, D. W., Jaeger, A. M., and Thiele, D. J. (2011). Heat Shock Transcription Factor 1 as a Therapeutic Target in Neurodegenerative Diseases. *Nat. Rev. Drug Discov.* 10 (12), 930–944. doi:10.1038/nrd3453

Nosengo, N. (2016). Can You Teach Old Drugs New Tricks? *Nature* 534 (7607), 314–316. doi:10.1038/534314a

Otaka, M., Yamamoto, S., Ogasawara, K., Takaoka, Y., Noguchi, S., Miyazaki, T., et al. (2007). The Induction Mechanism of the Molecular Chaperone HSP70 in the Gastric Mucosa by Geranylgeranylacetone (HSP-Inducer). *Biochem. Biophysical Res. Commun.* 353 (2), 399–404. doi:10.1016/j.bbrc.2006.12.031

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., et al. (2019). Drug Repurposing: Progress, Challenges and Recommendations. *Nat. Rev. Drug Discov.* 18 (1), 41–58. doi:10.1038/nrd.2018.168

Sierksma, A., Escott-Price, V., and De Strooper, B. (2020). Translating Genetic Risk of Alzheimer's Disease into Mechanistic Insight and Drug Targets. *Science* 370 (6512), 61–66. doi:10.1126/science.abb8575

Sun, Y., Zhang, J. R., and Chen, S. (2017). Suppression of Alzheimer's Disease-related P-henotypes by the H-eat S-hock P-rotein 70 I-nducer, Geranylgeranylacetone, in APP/PS1 Transgenic Mice via the ERK/p38 MAPK Signaling Pathway. *Exp. Ther. Med.* 14 (6), 5267–5274. doi:10.3892/etm.2017.5253

Walsh, D. M., and Selkoe, D. J. (2020). Amyloid β-protein and beyond: the Path Forward in Alzheimer's Disease. *Curr. Opin. Neurobiol.* 61, 116–124. doi:10.1016/j.conb.2020.02.003

West, J. D., Wang, Y., and Morano, K. A. (2012). Small Molecule Activators of the Heat Shock Response: Chemical Properties, Molecular Targets, and Therapeutic Promise. *Chem. Res. Toxicol.* 25 (10), 2036–2053. doi:10.1021/tx300264x

Wu, Y., Wu, Z., Butko, P., Christen, Y., Lambert, M. P., Klein, W. L., et al. (2006). Amyloid- -Induced Pathological Behaviors Are Suppressed by Ginkgo Biloba Extract EGb 761 and Ginkgolides in Transgenic *Caenorhabditis elegans*. *J. Neurosci.* 26 (50), 13102–13113. doi:10.1523/JNEUROSCI.3448-06.2006

Zeng, S., Wang, H., Chen, Z., Cao, Q., Hu, L., and Wu, Y. (2018). Effects of Geranylgeranylacetone upon Cardiovascular Diseases. *Cardiovasc. Ther.* 36 (4), e12331. doi:10.1111/1755-5922.12331

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The CBP-1/p300 Lysine Acetyltransferase Regulates the Heat Shock Response in *C. elegans*

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The decline of proteostasis is a hallmark of aging that is, in part, affected by the dysregulation of the heat shock response (HSR), a highly conserved cellular response to proteotoxic stress in the cell. The heat shock transcription factor HSF-1 is well-studied as a key regulator of proteostasis, but mechanisms that could be used to modulate HSF-1 function to enhance proteostasis during aging are largely unknown. In this study, we examined lysine acetyltransferase regulation of the HSR and HSF-1 in *C. elegans*. We performed an RNA interference screen of lysine acetyltransferases and examined mRNA expression of the heat-shock inducible gene *hsp-16.2*, a widely used marker for HSR activation. From this screen, we identified one acetyltransferase, CBP-1, the *C. elegans* homolog of mammalian CREB-binding protein CBP/p300, as a negative regulator of the HSR. We found that while knockdown of CBP-1 decreases the overall lifespan of the worm, it also enhances heat shock protein production upon heat shock and increases thermotolerance of the worm in an HSF-1 dependent manner. Similarly, we examined a hallmark of HSF-1 activation, the formation of nuclear stress bodies (nSBs). In analyzing the recovery rate of nSBs, we found that knockdown of CBP-1 enhanced the recovery and resolution of nSBs after stress. Collectively, our studies demonstrate a role of CBP-1 as a negative regulator of HSF-1 activity and its physiological effects at the organismal level upon stress.

Keywords: HSF-1, CBP-1, heat shock response, aging, *C. elegans*

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INTRODUCTION

One of the hallmarks of aging is the dysregulation of protein homeostasis, or proteostasis, in the cell which is critical for cell survival and function (López-Otín et al., 2013). The heat shock response (HSR) is a key player in the proteostasis network, known to play a role in a variety of physiological processes, including development, reproduction, aging, and age-related diseases (Labbadia and Morimoto, 2015; Joutsen and Sistonen, 2019). The HSR is a highly conserved cellular response that maintains proteostasis in the cell through the activation of heat shock transcription factor 1 (HSF1). HSF1 is the master regulator of the HSR and when activated, drives the expression of heat shock proteins (HSPs) that act as molecular chaperones to restore proteostasis after stress (Hartl et al., 2011). HSF1 has been implicated in a wide array of age-related diseases, including neurodegenerative diseases, metabolic diseases, and cancer (Powers et al., 2009; Gomez-Pastor et al., 2017). Understanding HSF1 regulation with aging may be useful when developing therapeutic strategies for such diseases.

The transcriptional activity of HSF1 is stress-inducible and highly regulated (Westerheide et al., 2012). Human HSF1 is regulated in part through post-translational modifications. These modifications include acetylation, phosphorylation, and sumoylation, and they function in both the activation and attenuation of HSF1 (Gomez-Pastor et al., 2017). Studies have shown multiple site-specific, reversible acetylation modifications on HSF1 that affect its overall protein stability and attenuation (Westerheide et al., 2009; Raychaudhuri et al., 2014; Zelin and Freeman, 2015). Under non-stress conditions, HSF1 levels are stabilized by the acetylation of Lys208 and Lys298 by the lysine acetyltransferase (KAT) CBP/p300 (Raychaudhuri et al., 2014). During stress, CBP/p300 also mediates the acetylation of Lys80 of HSF1, which aids in inhibiting HSF1 binding to DNA to attenuate the HSR (Westerheide et al., 2009; Raychaudhuri et al., 2014). There are also multiple other known sites of HSF1 acetylation where the effect of acetylation has yet to be elucidated.

The activity of HSF-1, the *C. elegans* homolog of HSF1, is highly conserved and makes *C. elegans* a useful model organism to study the regulation of HSF-1 during stress and aging. HSF-1 has been shown to interact with CREB binding protein CBP-1, the *C. elegans* homolog of CBP/p300, in lifespan regulation (Zhang et al., 2009); however, the mechanism by which CBP-1 regulates HSF-1 has not been examined.

CBP-1 in *C. elegans* functions as a chromatin remodeler and lysine acetyltransferase that can regulate transcription (Victor et al., 2002). CBP-1 is an essential protein for embryonic development, cell differentiation, and aging, and knockdown of *cbp-1* significantly reduces lifespan (Shi and Mello 1998; Victor et al., 2002; Zhang et al., 2009; Cai et al., 2019). CBP-1 also acts in multiple stress response pathways, including the mitochondrial unfolded protein response and the oxidative stress response (Ganner et al., 2019; Li et al., 2021). Although CBP-1 interactions with HSF-1 have been described, its role, as well as the potential role of other lysine acetyltransferases, in regulating HSF-1 and the HSR are still unknown.

In this study, we utilized *C. elegans* to screen for lysine acetyltransferase modulators of the HSR. We identified a role of CBP-1 in negatively regulating the HSR in *C. elegans*, confirming the importance of this regulator in this stress response pathway. Collectively, our studies demonstrate the ability of CBP-1 to regulate the HSR in an HSF-1 dependent-manner and highlight its effects on stress resistance and longevity.

METHODS

C. elegans Strains and Maintenance

The following strains were used in this study: N2 (Bristol), PS3551–*hsf-1* (sy441), MH2430—*cbp-1* (ku258), SDW015–*hsf-1* (asd002 (*hsf-1:GFP* + *unc-119* (+)), and SDW173. The MH2430 strain was outcrossed three times to the N2 wildtype strain to generate the SDW173 strain. Strains were maintained at 20°C on standard NGM plates seeded with *Escherichia coli* OP50-1. The synchronous population of nematodes was obtained by bleach synchronization and plated for 19 h on NGM plates without food.

RNA Interference

Synchronous L1 nematodes were grown on standard NGM plates seeded with OP50-1 bacteria for 19 h to prevent potential RNAi-mediated effects on early development. Worms were transferred onto standard NGM plates supplemented with 25 μg/ml carbenicillin and 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) and seeded with either HT115 bacteria containing an empty vector (EV/L4440 control) or with sequence-verified gene-specific RNAi strains isolated from the Ahringer RNAi library (J. Ahringer, University of Cambridge, Cambridge, U.K.), as previously described (Kamath et al., 2003). To induce dsRNA production, HT115 bacteria were supplemented with 1 mM IPTG shaking at 37°C for 1 h before seeding.

RNA Isolation and Quantitative PCR

RNA was extracted with TRIzol® reagent (Ambion®, cat# 15,596–026) by standard protocol. RNA was reverse-transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat# 4,368,814). cDNA was diluted to 100 ng/μl to be used as a template for qRT–PCR performed with the StepOne Plus Real-time PCR system (Applied Biosystems, cat # 4,376,600) using iTaq™ Universal SYBR® Green Supermix (Bio-Rad, cat# 1,725,121) according to the manufacturer's instructions. Expression levels were analyzed *via* qPCR using the ΔΔCt method. The housekeeping gene *cdc-42* (R07G3.1) was used for normalization. Results show averages of independent biological triplicates performed in technical triplicates. Statistical analysis was performed with GraphPad (GraphPad Software, https://www.graphpad.com) using an unpaired Student's t-test.

Primers used: *hsp16.2* (Y46H3A.3) Fwd: ACGCCAATTTGC TCCAGTCT, Rvs: TGATGGCAAACTTTTGATCATTGT; *hsp-70* (C12C8.1) Fwd: TTCAATGGGAAGGACCTCAACT, Rvs: GGCTGCACCAAAGGCTACTG; *cdc-42* (R07G3.1) Fwd: CTT CTGAGTATGTGCCGACAGTCT, Rvs: GGCTCGCCACCGATCAT; *hsp16.48* (T27E4.3) Fwd: TTG GAGAAATGCTGATCACAACTC, Rvs: TTTTTAGTTCTCTTC CATCCAATTCA; F44E5.5 Fwd: CTTCATGCAAAGCTATTG GTATCG, Rvs: CTTCCGAGTTGGCGAGGAT; *cbp-1* (R10E11.1) Fwd: GCAGCGAAAACGGAGGAA, Rvs: GCATGG AACAAATGTGGAGTCTT; *hsf-1* (Y53C10A.12) Fwd: TGCAGC CAGGATTGTCGA, Rvs: GGCGGCGCAAAAGTCTATT; *hat-1* (M03C11.4) Fwd: ACGGACTTGCTGTCGTTAAA, Rvs: CCG AAGATTGTCTCCTCATCTC; *kat-1* (T02G5.8) Fwd: CCACAT CTGCTGCACTATCA, Rvs: GCAGTTACCGAAGAGAGAGAA G; *taf-1* (W04A8.7) Fwd: TACGAGGCCACAGCTTATTG, Rvs: CGCTTCTCCTCCTTATACTGTTC; *mys-1* (VC5.4) Fwd: CGA GCTGCAAATGGTTCAATTA, Rvs: GTAGCTCACACGACG CTAAA; *mys-2* (K03D10.3) Fwd: GGAGCGAAAGAGCTCATG TATT; Rvs: GCTCGACTACCACTTCGTTTAC; *mec-17* (F57H12.7) Fwd: GGTCATCAGAGCAAGGGAAA, Rvs: TTG GCCTGATGAGCTCTATTG; *atat-2* (W06B11.1) Fwd: GTT CAGCTGTGTCCAGTCAT, Rvs: TAACATATCCTGGCGCAT CAA; T02G5.4 Fwd: GCAGTTACCGAAGAGAGAGAAG, Rvs: CCACATCTGCTGCACTATCA; *hsp-1* (F26D10.3) Fwd: TCA AGAGAAACACCACCATCC, Rvs: GGCACGTTCTCCTTC GTAAA; *hsp-90* (C47E8.5) Fwd: AGTACTGCGTCCAACAAC TC, Rvs: TCTTCTCCTCCTCGGTTTCT.

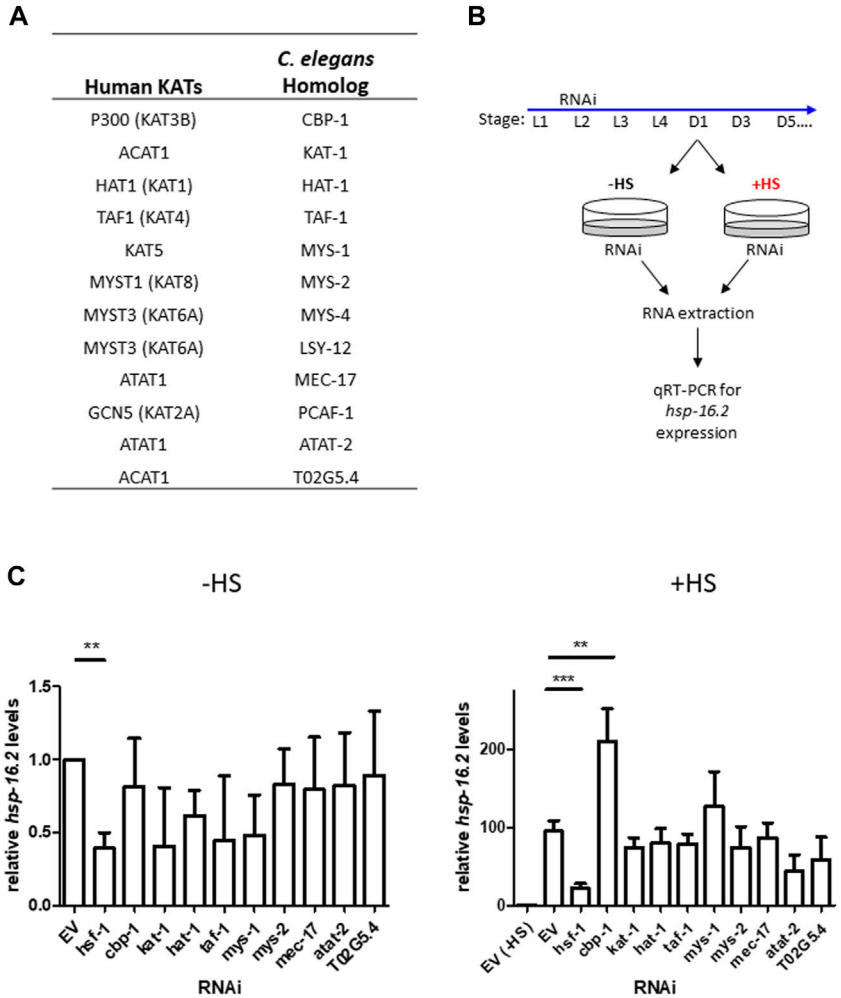

FIGURE 1 | Targeted-RNAi screen for lysine acetyltransferase modulators of the HSR identifies CBP-1. **(A)** Table of all putative lysine acetyltransferases (KATs) and their human homologs. **(B)** Schematic of the targeted RNAi screen for lysine acetyltransferase modulators of the HSR. ~ 75% of worm KATs were screened by examining endogenous *hsp-16.2 gene expression*, a heat-inducible heat shock protein gene, before and after heat shock using qRT-PCR. **(C)** N2 (wildtype) animals were fed empty vector (EV) control or KAT RNAi from 19 h after L1 larval stage to Day 1 of adulthood. RNA was extracted from Day 1 animals before and immediately after a 1-h heat shock at 33°C and *hsp-16.2* levels were quantified by qRT-PCR using the ΔΔCt method ($n = 3$ biologically independent samples). The housekeeping gene cdc-42 (R07G3.1) was used for normalization. Statistical analysis was determined by conducting a One-Way ANOVA using GraphPad Prism (GraphPad Software, www.graphpad.com) followed by a Tukey post hoc test comparison of all columns. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001.

Thermotolerance Analysis

Thermotolerance was tested by exposing Day 1 animals to 37°C heat shock for 3.5 h and then determining survival 48 h later by assessing response to a gentle touch. For each trial, ~ 100 randomly selected individual animals were assessed for the fraction alive (live/total). Thermotolerance assay data reflects three biologically independent trials. Data was plotted as the % survival using GraphPad Prism (GraphPad Software, https://www.graphpad.com) and was analyzed with a two-tailed *t* test.

Lifespan Analysis

All lifespan assays were performed at 20°C with approximately 100 worms per condition in biological triplicate. Animals were transferred to fresh plates daily for 5 days to avoid progeny contamination. Adult worms were scored approximately every day and counted as dead when no response was observed by poking with a platinum wire. The average survivability of three replicates was plotted using OASIS 2 (https://sbi.postech.ac.kr/oasis2/) (Han et al., 2016), and statistical analysis was done by Log-rank (Mantel-Cox) Test.

Fluorescent Microscopy and Nuclear Stress Body Assessment

Fluorescent images were obtained using a Keyence BZ-X fluorescent microscope. Animals were picked free of bacteria and anesthetized with 10 mM levamisole. Nuclear stress body formation was quantified by assessing for the presence of nuclear foci containing HSF-1:GFP in hypodermal cells. The heat shock conditions for nuclear stress body assessment were 15 min in a

33°C water bath on plates wrapped in parafilm. After anesthetizing and placing the cover slip on top of the worms, they were imaged within 10–15 min to avoid the formation of nuclear stress bodies which may be due to hypoxia or other cytotoxic stress (n = ~ 10 worms per condition). Quantification was performed in GraphPad Prism (GraphPad Software, www.graphpad.com) and significance was determined using 2-way ANOVA.

Statistical Analyses

Statistical analyses were carried out with GraphPad Software (GraphPad Software, La Jolla, CA, United States, https://www.graphpad.com) unless otherwise stated. All error bars are representative of standard deviation between independent biological replicates, as indicated.

RESULTS

Targeted-RNAi Screen for Lysine Acetyltransferase Modulators of the HSR Identifies CBP-1

To identify lysine acetyltransferases that may regulate the HSR, we performed an RNA interference (RNAi) screen by targeting ~ 75% of all putative lysine acetyltransferases (KATs) in *C. elegans* using RNAi knockdown (**Figure 1A**) *C. elegans* KATs were identified by searching the *C. elegans* protein database for proteins containing conserved acetyltransferase domains to those of known human KATs (Sheikh and Akhtar, 2019). We utilized quantitative RT-PCR to assess the heat shock inducibility of *hsp-16.2* mRNA expression, a widely used marker of HSR activity, after KAT RNAi knockdown (Golden et al., 2020). We induced RNAi 19 h after the L1 stage until Day 1 of adulthood, when we extracted RNA before and immediately after a 1-h heat shock at 33°C to assess *hsp-16.2* expression (**Figure 1B**). Our KAT and *hsf-1* RNAi conditions suppress expression of their corresponding genes by about 50% (**Supplementary Figure S1**). From this screen, we found no significant changes in *hsp-16.2* expression in non-heat shock conditions after KAT RNAi knockdown (**Figure 1C**). After heat shock, only RNAi for *cbp-1* (R10E11.1), the homolog of human CBP/p300 CREB binding protein, significantly altered the mRNA expression of *hsp16.2* expression, resulting in an increase in expression of approximately 2-fold (**Figure 1C**).

CBP-1 Regulates the Expression of Multiple HSF-1 Target Genes in an HSF-1-Dependent Manner

To further examine the role of CBP-1 on the HSR, we examined how knockdown of *cbp*-1 affects various heat shock inducible HSF-1 target genes using qRT-PCR. The target genes tested, including *hsp-16.2*, *hsp.16.48, hsp-70,* and F44E5.5 (an inducible *hsp-70* family gene), are highly inducible upon heat shock. We found that while *cbp*-1 RNAi didn't significantly change the expression of the *hsp* target genes tested under basal conditions (**Supplementary Figure S2A**), *cbp-1* RNAi did significantly increase the expression of these genes after heat shock as compared to EV control (**Figure 2A**). We also found that *cbp-1* knockdown does not affect *hsf-1* expression, or vice versa (**Supplementary Figure S1A**). HSF-1 also regulates the expression of a distinct subset of developmental genes independent of the HSR, including *hsp-1* and *hsp-90* (Li et al., 2016). We found that *cbp-1* knockdown does not affect expression of these HSF-1 target genes (**Supplementary Figure S2B**). Thus, CBP-1 is negatively regulating HSP but not HSF-1 expression after heat stress.

To determine whether this negative regulation of CBP-1 is mediated directly through HSF-1, we used the PS3551 worm strain containing a non-functional HSF-1 (Zhou et al., 2018). This mutant contains a point mutation in the HR-C domain of HSF-1 that causes the expression of a truncated non-functional HSF-1 mutant. We then measured the expression of *hsp-16.2* and *hsp-70* (C12C8.1) in response to *cbp-1* RNAi (beginning 19 h after L1) before and after heat shock in this mutant to determine dependence on HSF-1. While we found that knockdown of *cbp-1* increased *hsp-16.2* expression after heat shock with wildtype HSF-1 (**Figure 1A**), that increase is abolished with a nonfunctional HSF-1. Knockdown of *cbp-1* does not significantly increase *hsp-16.2* or *hsp*-70 (*C12C8*.1) expression after heat shock compared to control (**Figure 2B**). We conclude that CBP-1 depends on HSF-1 in order to regulate *hsp* gene expression.

Effect of *cbp-1* and *hsf-1* RNAi on Thermotolerance and Lifespan

We next wanted to test whether CBP-1 also regulates other physiological readouts of the HSR. To test for resistance to heat stress, we examined thermotolerance, a physiological effect known to be modulated by HSF-1. To test this, we fed the worms *cbp-1*, *hsf-1*, or control RNAi from 19 h after L1 until Day 1 of adulthood prior to subjecting the worms to a lethal heat shock of 37°C for 3.5 h. After a 48-h recovery, the worms were scored alive/dead after a gentle touch. Compared to empty vector control RNAi-treated worms, *hsf-1* RNAi-treated worms had significantly reduced survival after heat shock (~ 20% decrease), as expected (**Figure 3A**). However, knockdown of *cbp-1* increased the % survival of the worms by ~ 40% (**Figure 3A**). Thus, knockdown of *cbp-1* increases thermotolerance.

We then performed a complimentary experiment, assessing how an increase in CBP-1 activity would affect thermotolerance. We utilized the MH3420 strain that contains two point mutations in *cbp-1,* resulting in a gain-of-function allele with increased KAT activity (Eastburn and Han, 2005). This strain was outcrossed three times with our laboratory N2 strain to create the SDW173 strain. N2 and SDW173 worms were synchronized and grown to Day 1 of adulthood then subjected to a lethal heat shock of 37°C for 3.5 h. We found that SDW173 worms had an ~20% decrease

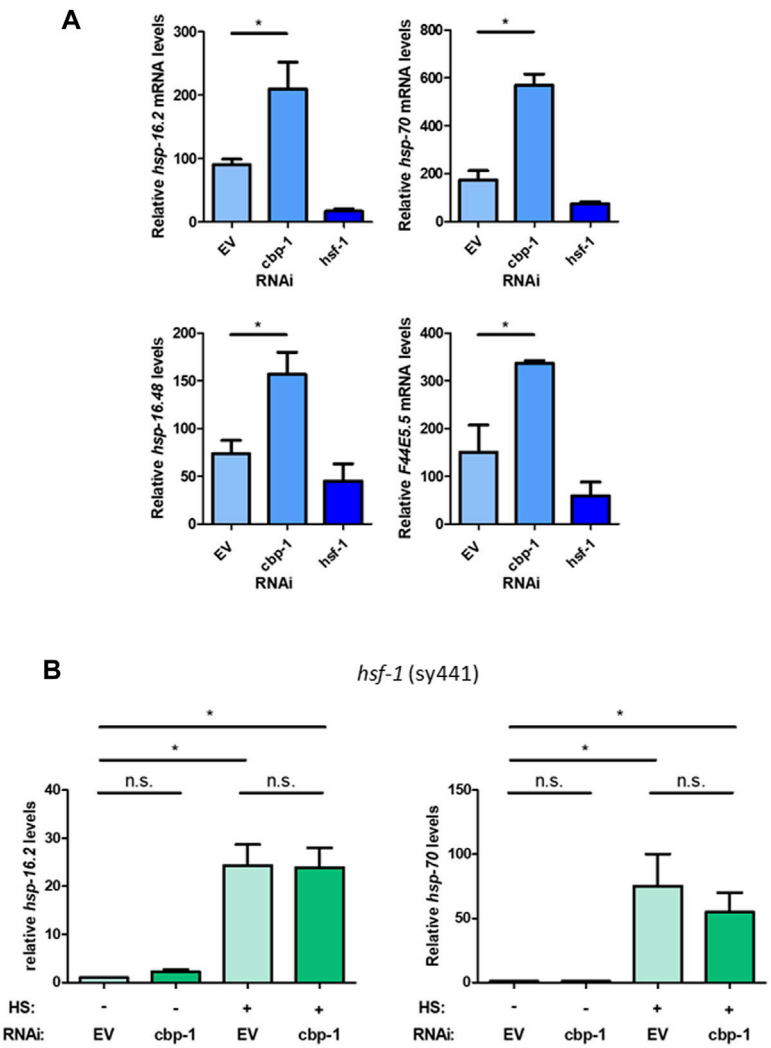

FIGURE 2 | CBP-1 regulates the expression of multiple HSF-1 target genes in an HSF-1-dependent manner. **(A)** RNA was extracted from N2 (wildtype) worms on Day 1 of adulthood with a 1-h heat shock at 33°C that were fed empty vector (EV), *cbp-1,* or *hsf-1* RNAi beginning 19 h after L1. Heat shock inducible heat shock protein gene expression levels for *hsp-16.2, hsp-16.48, F44E5.5,* and *hsp-70* were quantified by qRT-PCR. **(B)** RNA was extracted from an HSF-1 deletion worm strain (PS3551) on Day 1 of adulthood with and without a 1-h heat shock at 33°C with animals fed EV or *cbp-1* RNAi. PS3551 animals were fed empty vector (EV) control or *cbp-1* RNAi from 19 h after L1 larval stage to Day 1 of adulthood. Heat shock inducible *hsp-16.2* and *hsp-70* levels were quantified by qRT-PCR. Statistical analysis was determined by determined by conducting a One-Way ANOVA using GraphPad Prism (GraphPad Software, www.graphpad.com) followed by a Tukey post hoc test comparison of all columns. *p-value < 0.05.

in percent survival as compared to N2 wildtype worms (**Figure 3B**). This suggests that an increase in CBP-1 acetyltransferase activity decreases thermotolerance. In summary, *cbp-1* RNAi activates thermotolerance, while a *cbp-1* gain-of-function mutant inhibits thermotolerance.

Previous studies have found that *cbp-1* RNAi reduces the lifespan of the worm (Zhang et al., 2009; Cai et al., 2019). We wanted to test whether this effect depends on HSF-1. Worms were fed with EV control, *cbp-1*, *hsf-1*, or *cbp-1/hsf-1* double RNAi from 19 h after L1 throughout their lifespans. The worms were scored approximately every day starting at day 1 of adulthood for survival, and dead worms were scored when non-responsive to poking with a platinum wire. We found that *cbp-1* RNAi decreased lifespan to a significantly lesser extent than *hsf-1* RNAi (**Figure 3C**). However, the *cbp-1/hsf-1* double RNAi did not further reduce lifespan (**Figure 3C**).

cbp-1 RNAi Increases Recovery Rate of Nuclear Stress Bodies After Heat Shock

Upon activation of the HSR, HSF-1 undergoes localization changes and forms nuclear stress bodies (nSBs), a hallmark of HSF-1 activation (Deonarine et al., 2021). nSBs require HSF-1 binding and are a sign of activation of the HSR (Morton and Lamitina, 2013). After HSR activation, nSBs form within 5 min of stress detection and gradually dissolve until HSF-1 is diffuse in

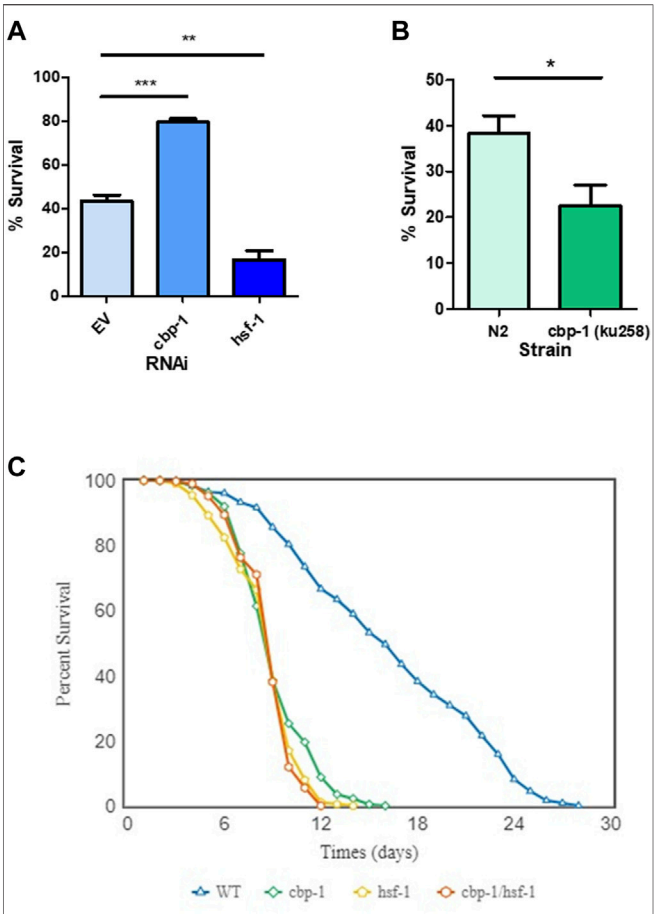

FIGURE 3 | Effect of CBP-1 on thermotolerance and lifespan. **(A)** In order to assess CBP-1 activity on resistance to heat stress, N2 worms were given a lethal heat shock of 3.5 h at 37°C on Day 1 of adulthood and then ~ 100 animals were randomly scored for survival after a 48-h recovery. Percent survival was determined for worms fed with empty vector (EV), *cbp-1*, and *hsf-1* RNAi. Data was plotted as % survival using GraphPad Prism (GraphPad Software, www.graphpad.com) and was analyzed with a two-tailed *t* test. **p*-value < 0.05, ***p*-value < 0.01, ****p*-value < 0.001. **(B)** Thermotolerance was assessed in N2 and SDW173, a strain containing a gain-of-function allele of *cbp-1* that increases acetyltransferase activity (MH2430 strain outcrossed 3x to N2). Data was plotted as % survival using GraphPad Prism (GraphPad Software, www.graphpad.com) and was analyzed with a two-tailed *t* test. **p*-value < 0.05. **(C)** Lifespan analysis was performed in wild type N2 animals fed with EV, *cbp-1* RNAi, *hsf-1* RNAi, or *cbp-1/hsf-1* double RNAi throughout the lifespan. Worms were scored approximately every day for survival. The average survivability of three replicates was plotted using OASIS 2 (https://sbi.postech.ac.kr/oasis2/) (Han et al., 2016), and statistical analysis was done by Log-rank (Mantel-Cox) Test (Shown in **Supplementary Table S1**).

the nucleus (**Figure 4A**) (Deonarine et al., 2021). We examined the effect of *cbp-1* RNAi on the rate of nSB recovery after stress. We used the SDW015 (HSF-1:GFP) strain to visualize nSB formation before and after a 15-min heat shock at 33°C on Day 2 of adulthood. We utilized fluorescence microscopy to image hypodermal cells of the worm in 30-min increments until the majority of cells containing nSBs had diffuse HSF-1: GFP expression in the nuclei (**Figure 4A**). After heat shock, control worms took an average of 1.7 h to recover and resolve their nSBs, whereas worms fed *cbp-1* RNAi took an average of 1.1 h to resolve nSBs (**Figure 4B**). Thus, knockdown of *cbp-1* significantly increased the rate of recovery of nSBs after heat shock.

DISCUSSION

Given the importance of post-translational modifications on HSF-1 regulation and function, we sought to elucidate the effect of lysine acetyltransferase regulation on the HSR. Here, we conducted a targeted RNAi screen for lysine acetyltransferase regulation of *hsp-16.2* expression levels and identified CBP-1 as a regulator of *hsp* expression. In addition, *cbp-1* knockdown also increased the expression of multiple other heat shock genes, increased thermotolerance, and improved the recovery rate of nuclear stress bodies after heat stress. Overall, these studies suggest a role for CBP-1 in negatively regulating HSF-1 activity during stress.

CBP-1 is widely known to be a transcriptional coactivator that aids in transcription (Victor et al., 2002). We further examined various other heat-shock inducible *hsp* expression and found that *cbp-1* knockdown increases the expression of multiple *hsp*s (*hsp-16.2, hsp.16.48, hsp-70,* and *F44E5.5*) after heat shock. This type of negative transcriptional regulation is a novel role for CBP-1 in *C. elegans*. However, human CBP/p300 has some characterized instances in which it negatively regulates transcription through effects on transcription factors. For example, acetylation of the elongation factor AFF1 by CBP/p300 inhibits transcription during genotoxic stress (Kumari et al., 2019).

CBP-1 is an essential protein that regulates multiple physiological processes including cell differentiation, embryonic development, metabolism, lifespan, and aging (Shi and Mello, 1998; Zhang et al., 2009; Vora et al., 2013; Cai et al., 2019). Specifically, CBP-1 interacts with DAF-16 and HSF-1 to regulate lifespan during caloric restriction (Zhang et al., 2009). Similarly, our results suggest that CBP-1 and HSF-1 work in the same pathway to regulate lifespan. While our work suggests that CBP-1 negatively regulates the HSR, our lifespan results suggest that it positively affects lifespan and aging. This was unexpected, as activating the HSR generally protects from aging (Hsu et al., 2003; Morley and Morimoto, 2004). Since CBP-1 plays a positive role in a variety of physiological pathways that control aging (Zhang et al., 2009; Cai et al., 2019; Ganner et al., 2019; Zhou et al., 2019; Li et al., 2021) it may be that the effects on these pathways are overriding the effects of CBP on HSF-1 and HSP expression in terms of life span regulation. It will be informative in future work to determine how the regulation of HSF-1 activity by CBP-1 can benefit aging and potentially lifespan in the worm.

While our data indicates that CBP-1 regulates HSF-1 activity, the mechanism of this regulation is still unknown. CBP-1 could directly acetylate HSF-1 as one potential mechanism. Mammalian CBP/p300 acetylates HSF-1 under both stress and non-stress conditions to both stabilize HSF-1 and attenuate HSF-1 from the DNA (Westerheide et al., 2009; Raychaudhuri et al., 2014). CBP/p300 acetylation of HSF1 could be conserved in *C. elegans* and function to attenuate HSF-1 activity. While human CBP/p300 also acts as a scaffolding protein to recruit the

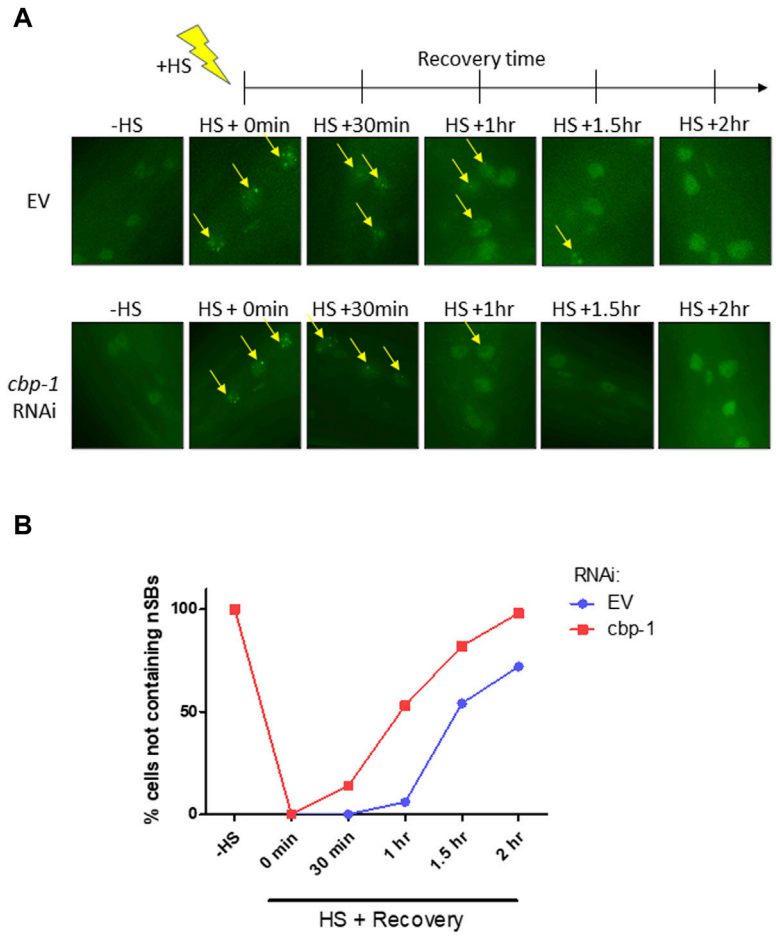

FIGURE 4 | *cbp-1* RNAi increases recovery rate of nuclear stress bodies (nSBs) after heat shock. **(A)** Fluorescent images of HSF-1:GFP in the SDW015 strain were examined before heat shock and in 30-min increments after a 15-min heat shock at 33°C for 2 h at 20°C until the majority of hypodermal cells containing nuclear stress bodies (nSBs) had been resolved. Images represent fluorescent nSB formation in hypodermal cells under control and *cbp-1* RNAi conditions at each time point in Day 2 adult worms. Yellow arrows indicate hypodermal cell nuclei containing nSB foci. **(B)** The percentage of hypodermal cells not containing nSBs were quantitated following fluorescent imaging of worms fed empty vector (EV) control and *cbp-1* RNAi (n = 10 worms). Significance was determined using 2-way ANOVA. Column *p-value = 0.0497; row **p-value = 0.0022.

transcriptional machinery (Holmqvist and Mannervik, 2013), it is likely that worm CBP-1 regulation of the HSR requires CBP-1 acetyltransferase activity since we found that the SDW173 mutant containing CBP-1 with hyperactive KAT activity decreased thermotolerance. There is also the possibility of CBP-1 regulating HSF-1 through an indirect regulator, as CBP-1 also binds to a multitude of other factors including the stress-responsive factors SKN-1 and DAF-16 (Nasrin et al., 2000; Ganner et al., 2019). Future work to uncover the mechanism behind HSF-1 regulation will allow for a more detailed analysis of the role of CBP-1 during stress and aging.

A hallmark of HSF1 activation in both mammalian cells and *C. elegans* is the formation and resolution of nuclear stress bodies (nSBs) containing HSF1 (Jolly et al., 1999; Jolly et al., 2004; Biamonti and Vourc'h, 2010; Morton and Lamitina, 2013; Deonarine et al., 2021). In mammalian cells, the resolution of nSBs correlates with HSF1 activity, transcription of *hsp* target genes, and cell survival at the single cell level (Gaglia et al., 2020). A recent mammalian study discovered the existence of both HSF1 nSBs at non-*hsp* gene loci and smaller HSF1 condensates at transcriptionally active *hsp* gene loci upon heat shock (Zhang et al., 2022). In this study, HSF1 in the condensates was found to colocalize with transcription apparatus factors, including RNA Pol II, BRD4, MED1, and CYCT1 (Zhang et al., 2022). It is thus possible that upon heat shock, HSF1 in nSBs sequesters transcription factors to halt general transcription, while HSF1 in the smaller condensates functions to induce the transcription of *hsp* target genes. HSF1 nSBs that are not able to resolve may go on to transition from liquid to gel phase condensates, which could then permanently hinder HSF1 activity and the HSR. Our study did not analyze small HSF-1 condensates, and it is not yet known if these are present in the worm. However, we found that *cbp-1* knockdown promotes the recovery of large HSF-1 nSBs after heat shock. Thus, CBP-1 may facilitate the stabilization of HSF-1 nSBs, which could promote the gel phase state upon prolonged stress, leading to ultimate HSF-1 inactivation. We hypothesize that decreasing CBP-1 levels through RNAi could thus enhance the HSR. We do not know how

CBP-1 may stabilize HSF-1 nSBs. It could be through the acetylation of HSF-1 or another protein, through providing a scaffolding, or through an alternative mechanism. It will be interesting to investigate these possibilities in future work.

CBP-1 also functions as a regulator of multiple other stress response pathways. Interestingly, CBP-1 positively regulates SKN-1/Nrf activation and the oxidative stress response, where it regulates SKN-1 activity and protein abundance (Ganner et al., 2019). CBP-1 is also an essential regulator of the mitochondrial unfolded protein response (Li et al., 2021). Collectively, our studies identify a new role of CBP-1 in HSR regulation in *C. elegans* and its physiological effects at the organismal level. CBP-1 negatively regulates HSP expression, nuclear stress body recovery, and thermotolerance ability of the worm. Future work is needed to determine the precise mechanism of action of CBP-1 in the HSR pathway and any potential acetylation changes of HSF-1 that are induced by CBP-1.

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.

AUTHOR CONTRIBUTIONS

LB and SW designed the study. LB performed the experiments and the data analyses. LB and SW contributed to figure design and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fragi.2022.861761/full#supplementary-material

Supplementary Figure S1 | Validation of KAT and *hsf-1* RNAi. **(A)** RNA was extracted from wildtype (N2) worms on Day 1 of adulthood that were fed empty vector (EV), *cbp-1,* or *hsf-1* RNAi beginning 19 hrs after L1. *cbp-1* and *hsf-1* levels were quantified by qRT-PCR (n = 3 biologically independent samples). **(B)** RNA was extracted from N2 worms on Day 1 of adulthood that were fed EV or KAT RNAi beginning 19 hrs after L1. Validation of KAT knockdown was examined by quantifying KAT levels by qRT-PCR (n = 3 biologically independent samples). Statistical analysis determined by determined by conducting a One-Way ANOVA using GraphPad Prism (GraphPad Software, www.graphpad.com) followed by a Tukey post hoc test comparison of all columns. *p-value < 0.05, **p-value < 0.01, ***p-value < 0.001.

Supplementary Figure S2 | *cbp-1* RNAi does not change the expression of HSF-1 target genes under basal conditions. **(A)** RNA was extracted from N2 (wildtype) worms on Day 1 of adulthood under basal conditions at 20°C that were fed empty vector (EV), *cbp-1,* or *hsf-1* RNAi beginning 19 hrs after L1. Heat shock inducible heat shock protein gene expression levels for *hsp-16.2, hsp-16.48, F44E5.5,* and *hsp-70* were quantified by qRT-PCR. **(B)** RNA was extracted from N2 worms on Day 1 of adulthood under basal conditions that were fed EV or *cbp-1* RNAi beginning 19 hrs after L1. Developmental HSF-1 target genes independent of the heat shock response, *hsp-1* and *hsp-90,* were quantified by qRT-PCR. Statistical analysis was determined by determined by conducting a One-Way ANOVA using GraphPad Prism (GraphPad Software, www.graphpad.com) followed by a Tukey post hoc test comparison of all columns. *p-value < 0.05.

Supplementary Table S1 | Lifespan analysis. Analysis of lifespan of EV, *cbp-1, hsf-1,* and *cbp-1/hsf-1* RNAi including mean lifespan and total/censored/scored number of worms per trial (done in biological triplicate). Survivability was plotted using OASIS 2 (https://sbi.postech.ac.kr/oasis2/) (Han *et al.* 2016), and statistical analysis was done by Log-rank (Mantel-Cox) Test.

REFERENCES

Biamonti, G., and Vourc'h, C. (2010). Nuclear Stress Bodies. *Cold Spring Harbor. Perspect. Biol.* 2 (6), a000695. doi:10.1101/cshperspect.a000695

Cai, H., Dhondt, I., Vandemeulebroucke, L., Vlaeminck, C., Rasulova, M., and Braeckman, B. P. (2019). CBP-1 Acts in GABAergic Neurons to Double Life Span in Axenically Cultured *Caenorhabditis elegans*. *Journals Gerontol. Ser. A Biol. Sci. Med. Sci.* 74 (8), 1198–1205. doi:10.1093/gerona/glx206

Deonarine, A., Walker, M. W. G., and Westerheide, S. D. (2021). HSF-1 Displays Nuclear Stress Body Formation in Multiple Tissues in *Caenorhabditis elegans* upon Stress and Following the Transition to Adulthood. *Cell Stress Chaperones* 26 (2), 417–431. doi:10.1007/s12192-020-01188-9

Eastburn, D. J., and Han, M. (2005). A Gain-Of-Function Allele of Cbp-1 , the *Caenorhabditis elegans* Ortholog of the Mammalian CBP/p300 Gene, Causes an Increase in Histone Acetyltransferase Activity and Antagonism of Activated Ras. *Mol. Cel Biol.* 25 (21), 9427–9434. doi:10.1128/mcb.25.21.9427-9434.2005

Gaglia, G., Rashid, R., Yapp, C., Joshi, G. N., Li, C. G., Lindquist, S. L., et al. (2020). HSF1 Phase Transition Mediates Stress Adaptation and Cell Fate Decisions. *Nat. Cel Biol.* 22 (2), 151–158. doi:10.1038/s41556-019-0458-3

Ganner, A., Gerber, J., Ziegler, A.-K., Li, Y., Kandzia, J., Matulenski, T., et al. (2019). CBP-1/p300 Acetyltransferase Regulates SKN-1/Nrf Cellular Levels, Nuclear Localization, and Activity in *C. elegans*. *Exp. Gerontol.* 126, 110690. doi:10.1016/j.exger.2019.110690

Golden, N. L., Plagens, R. N., Kim Guisbert, K. S., and Guisbert, E. (2020). Standardized Methods for Measuring Induction of the Heat Shock Response in *Caenorhabditis elegans*. *J. Vis. Exp.* 161. doi:10.3791/61030

Gomez-Pastor, R., Burchfiel, E. T., and Thiele, D. J. (2017). Regulation of Heat Shock Transcription Factors and Their Roles in Physiology and Disease. *Nat. Rev. Mol. Cel Biol.* 19, 4–19. doi:10.1038/nrm.2017.73

Han, S. K., Lee, D., Lee, H., Kim, D., Son, H. G., Yang, J. S., et al. (2016). OASIS 2: Online Application for Survival Analysis 2 with Features for the Analysis of Maximal Lifespan and Healthspan in Aging Research. *Oncotarget* 7, 56147–56152. doi:10.18632/oncotarget.11269

Hartl, F. U., Bracher, A., and Hayer-Hartl, M. (2011). Molecular Chaperones in Protein Folding and Proteostasis. *Nature* 475 (7356), 324–332. doi:10.1038/nature10317

Holmqvist, P.-H., and Mannervik, M. (2013). Genomic Occupancy of the Transcriptional Co-activators P300 and CBP. *Transcription* 4 (1), 18–23. doi:10.4161/trns.22601

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 (5622), 1142–1145. doi:10.1126/science.1083701

Jolly, C., Usson, Y., and Morimoto, R. I. (1999). Rapid and Reversible Relocalization of Heat Shock Factor 1 within Seconds to Nuclear Stress Granules. *Proc. Natl. Acad. Sci. U.S.A.* 96 (12), 6769–6774. doi:10.1073/pnas.96.12.6769

Jolly, C., Metz, A., Govin, J., Vigneron, M., Turner, B. M., Khochbin, S., et al. (2004). Stress-induced Transcription of Satellite III Repeats. *J. Cel. Biol.* 164 (1), 25–33. doi:10.1083/jcb.200306104

Joutsen, J., and Sistonen, L. (2019). Tailoring of Proteostasis Networks with Heat Shock Factors. *Cold Spring Harb. Perspect. Biol.* 11 (4), a034066. doi:10.1101/cshperspect.a034066

Kamath, R. S., Fraser, A. G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., et al. (2003). Systematic Functional Analysis of the *Caenorhabditis elegans* Genome Using RNAi. *Nature* 421 (6920), 231–237. doi:10.1038/nature01278

Kumari, N., Hassan, M. A., Lu, X., Roeder, R. G., and Biswas, D. (2019). AFF1 Acetylation by P300 Temporally Inhibits Transcription during Genotoxic Stress Response. *Proc. Natl. Acad. Sci. U.S.A.* 116 (44), 22140–22151. doi:10.1073/pnas.1907097116

Labbadia, J., and Morimoto, R. I. (2015). The Biology of Proteostasis in Aging and Disease. *Annu. Rev. Biochem.* 84, 435–464. doi:10.1146/annurev-biochem-060614-033955

Li, J., Chauve, L., Phelps, G., Brielmann, R. M., and Morimoto, R. I. (2016). E2F Coregulates an Essential HSF Developmental Program that Is Distinct from the Heat-Shock Response. *Genes Dev.* 30 (18), 2062–2075. doi:10.1101/gad.283317.116

Li, T. Y., Sleiman, M. B., Li, H., Gao, A. W., Mottis, A., Bachmann, A. M., et al. (2021). The Transcriptional Coactivator CBP/p300 Is an Evolutionarily Conserved Node that Promotes Longevity in Response to Mitochondrial Stress. *Nat. Aging* 1 (2), 165–178. doi:10.1038/s43587-020-00025-z

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

Morley, J. F., and Morimoto, R. I. (2004). Regulation of Longevity inCaenorhabditis Elegansby Heat Shock Factor and Molecular Chaperones. *MBoC* 15 (2), 657–664. doi:10.1091/mbc.e03-07-0532

Morton, E. A., and Lamitina, T. (2013). Caenorhabditis elegans HSF-1 is an Essential Nuclear Protein that Forms Stress Granule-like Structures Following Heat Shock. *Aging Cell* 12 (1), 112–120. doi:10.1111/acel.12024

Nasrin, N., Ogg, S., Cahill, C. M., Biggs, W., Nui, S., Dore, J., et al. (2000). DAF-16 Recruits the CREB-Binding Protein Coactivator Complex to the Insulin-like Growth Factor Binding Protein 1 Promoter in HepG2 Cells. *Proc. Natl. Acad. Sci. U.S.A.* 97 (19), 10412–10417. doi:10.1073/pnas.190326997

Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W., and Balch, W. E. (2009). Biological and Chemical Approaches to Diseases of Proteostasis Deficiency. *Annu. Rev. Biochem.* 78, 959–991. doi:10.1146/annurev.biochem.052308.114844

Raychaudhuri, S., Loew, C., Körner, R., Pinkert, S., Theis, M., Hayer-Hartl, M., et al. (2014). Interplay of Acetyltransferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. *Cell* 156 (5), 975–985. doi:10.1016/j.cell.2014.01.055

Sheikh, B. N., and Akhtar, A. (2019). The many Lives of KATs - Detectors, Integrators and Modulators of the Cellular Environment. *Nat. Rev. Genet.* 20 (1), 7–23. doi:10.1038/s41576-018-0072-4

Shi, Y., and Mello, C. (1998). A CBP/p300 Homolog Specifies Multiple Differentiation Pathways in *Caenorhabditis elegans*. *Genes Dev.* 12 (7), 943–955. doi:10.1101/gad.12.7.943

Victor, M., Bei, Y., Gay, F., Calvo, D., Mello, C., and Shi, Y. (2002). HAT Activity Is Essential for CBP-1-dependent Transcription and Differentiation inCaenorhabditis Elegans. *EMBO Rep.* 3 (1), 50–55. doi:10.1093/embo-reports/kvf006

Vora, M., Shah, M., Ostafi, S., Onken, B., Xue, J., Ni, J. Z., et al. (2013). Deletion of microRNA-80 Activates Dietary Restriction to Extend *C. elegans* Healthspan and Lifespan. *Plos Genet.* 9 (8), e1003737. doi:10.1371/journal.pgen.1003737

Westerheide, S. D., Anckar, J., Stevens, S. M., Sistonen, L., and Morimoto, R. I. (2009). Stress-inducible Regulation of Heat Shock Factor 1 by the Deacetylase SIRT1. *Science* 323 (5917), 1063–1066. doi:10.1126/science.1165946

Westerheide, S. D., Raynes, R., Powell, C., Xue, B., and Uversky, V. N. (2012). HSF Transcription Factor Family, Heat Shock Response, and Protein Intrinsic Disorder. *Curr. Protein Pept. Sci.* 13 (1), 86–103. doi:10.2174/138920312799277956

Zelin, E., and Freeman, B. C. (2015). Lysine Deacetylases Regulate the Heat Shock Response Including the Age-Associated Impairment of HSF1. *J. Mol. Biol.* 427 (7), 1644–1654. doi:10.1016/j.jmb.2015.02.010

Zhang, M., Poplawski, M., Yen, K., Cheng, H., Bloss, E., Zhu, X., et al. (2009). Role of CBP and SATB-1 in Aging, Dietary Restriction, and Insulin-like Signaling. *Plos Biol.* 7 (11), e1000245. doi:10.1371/journal.pbio.1000245

Zhang, H., Shao, S., Zeng, Y., Wang, X., Qin, Y., Ren, Q., et al. (2022). Reversible Phase Separation of HSF1 Is Required for an Acute Transcriptional Response during Heat Shock. *Nat. Cel Biol.* 24 (3), 340–352. doi:10.1038/s41556-022-00846-7

Zhou, J.-B., Zheng, Y.-L., Zeng, Y.-X., Wang, J.-W., Pei, Z., and Pang, J.-Y. (2018). Marine Derived Xyloketal Derivatives Exhibit Anti-stress and Anti-ageing Effects through HSF Pathway in *Caenorhabditis elegans*. *Eur. J. Med. Chem.* 148, 63–72. doi:10.1016/j.ejmech.2018.02.028

Zhou, L., He, B., Deng, J., Pang, S., and Tang, H. (2019). Histone Acetylation Promotes Long-Lasting Defense Responses and Longevity Following Early Life Heat Stress. *Plos Genet.* 15 (4), e1008122. doi:10.1371/journal.pgen.1008122

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The Replica Set Method is a Robust, Accurate, and High-Throughput Approach for Assessing and Comparing Lifespan in *C. elegans* Experiments

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The advent of feeding based RNAi in *Caenorhabditis elegans* led to an era of gene discovery in aging research. Hundreds of gerogenes were discovered, and many are evolutionarily conserved, raising the exciting possibility that the underlying genetic basis for healthy aging in higher vertebrates could be quickly deciphered. Yet, the majority of putative gerogenes have still only been cursorily characterized, highlighting the need for high-throughput, quantitative assessments of changes in aging. A widely used surrogate measure of aging is lifespan. The traditional way to measure mortality in *C. elegans* tracks the deaths of individual animals over time within a relatively small population. This traditional method provides straightforward, direct measurements of median and maximum lifespan for the sampled population. However, this method is time consuming, often underpowered, and involves repeated handling of a set of animals over time, which in turn can introduce contamination or possibly damage increasingly fragile, aged animals. We have previously developed an alternative "Replica Set" methodology, which minimizes handling and increases throughput by at least an order of magnitude. The Replica Set method allows changes in lifespan to be measured for over one hundred feeding-based RNAi clones by one investigator in a single experiment- facilitating the generation of large quantitative phenotypic datasets, a prerequisite for development of biological models at a systems level. Here, we demonstrate through analysis of lifespan experiments simulated *in silico* that the Replica Set method is at least as precise and accurate as the traditional method in evaluating and estimating lifespan, and requires many fewer total animal observations across the course of an experiment. Furthermore, we show that the traditional approach to lifespan experiments is more vulnerable than the Replica Set method to experimental and measurement error. We find no compromise in statistical power for Replica Set experiments, even for moderate effect sizes, or when simulated experimental errors are introduced. We compare and contrast the statistical analysis of data generated by the two approaches, and highlight pitfalls common with the traditional methodology. Collectively, our analysis provides a standard of measure for each method

across comparable parameters, which will be invaluable in both experimental design and evaluation of published data for lifespan studies.

Keywords: *Caenorhabditis elegans*, lifespan, survival modeling, biostatistics, high throughput

INTRODUCTION

Aging is a gradual and progressive decline in physiological function, most prominently reflected in the rising probability of death over time for a given a population. Concordantly, lifespan is often used as a surrogate measure of aging as it is a straightforward and unambiguous measurement: an animal is either alive or dead. The traditional longitudinal method (TLM) of measuring lifespan in model organisms, including *C. elegans*, entails following a relatively small population of animals and tracking when death events occur. The *population* is observed at specific time points, and at each time point dead animals are counted and consequently removed (**Figure 1A**). Thus, the increasing mortality observed within a population is dependent on each previous measure of mortality. For example, if 5 animals within a population of 35 animals die on day 13, then there will be only 30 remaining animals to assess whether they are alive or dead at the next time point. Mortality within a chronologically age-matched population has been found to rise exponentially- with some natural variation between individuals- even within an isogenic population in a common environment, which cumulatively provides a distribution of how death events occurred within the population (Finch et al., 1990; Brooks et al., 1994).

Traditional Assays to Follow *C. Elegans* Lifespan have a Number of Weaknesses

While using the TLM to measure lifespan in *C. elegans* is straightforward, it is relatively low-throughput, limiting the number of conditions that can simultaneously be measured. Comprehensive genome wide RNAi-based gene knockdown screens to date have identified in *C. elegans* 1147 putative gerogenes (broadly defined as genes whose function either extends or shortens longevity) (WormBase WS282). However, in many cases these gerogenes have been identified based on measuring viability at a single or a few time points (Lee et al., 2003; Hamilton et al., 2005; Hansen et al., 2005), which fails to provide a quantifiable measure of change in lifespan. Full longitudinal lifespan analysis is one prerequisite for identifying genetic interactions between gerogenes (*e.g.* epistatic, asynthetic interactions, etc.).

C. elegans viability is most often scored based on observable movement, which becomes less frequent and subtler as animals age. Young animals actively explore their environment and feed upon a lawn of *E. coli*, making scoring young animals straightforward. However, as an animal ages movement progressively declines, becoming increasingly uncoordinated and lethargic (Hosono et al., 1980; Bolanowski et al., 1981; Johnson, 1987; Herndon et al., 2002; Huang et al., 2004). Specifically, by day seven of adulthood, wild-type *C. elegans* may be observed that no longer display spontaneous active behavior in the absence of outside stimuli (Herndon et al., 2002; Huang et al., 2004). At more advanced age, increasing sarcopenia results in progressive degeneration of movement, ultimately resulting in paralyzed animals. Viability in an older animal is ascertained by observing subtle head movements at the very tip of the animal, increasing probability of scoring mistakes associated with progressing age (Duhon and Johnson, 1995; Herndon et al., 2002; Glenn et al., 2004; Gerstbrein et al., 2005). Additionally, scoring of the same population across multiple timepoints requires repeated handling, environmental exposure, and potential introduction of airborne contamination, which is not a trivial concern. For example, *C. elegans* lifespan can be altered by even subtle environmental changes: animals exposed to ambient light in a laboratory for as little as 20 min per day during scoring exhibit mean lifespan up to 12% shorter than animals scored in the dark (De Magalhaes Filho et al., 2018). Collectively, these drawbacks limit throughput, introduce variability, and increase the probability of experimental error with the TLM approach.

The Replica Set Method

To overcome the limitations of the TLM, we previously developed an alternative experimental design to measure *C. elegans* viability that relies on the use of replica sets (Samuelson et al., 2007b, 2007a; Johnson et al., 2014; Cornwell et al., 2018; Cornwell and Samuelson, 2020). To this end, a large population of age-synchronized, isogenic animals are divided into a number of smaller samples (which we term as "replicas") of approximately 15–20 animals each. Enough replicates are generated to cover each time point in the planned experiment. At each time point, one of the replicas is scored for the number of living, dead and censored animals, then discarded. Thus, over the period of time that covers the expected lifespan of the population as a whole, a series of independent subpopulations are sampled at each time point (**Figure 1B**). In using replica sets there is no repeated prodding of animals, and no repeated exposure to potential environmental contamination from opening and closing the same plates. The viability observed at one time-point is independent of every other observation, and hundreds of conditions can be tested in parallel– which increases throughput by at least an order of magnitude (for an example of increased throughput through application of the replica set method (hereafter RSM), see (Samuelson et al., 2007a)).

Systematically Assessing the Performance of Replica Set and Traditional Methods

Understanding the nature of what each longevity assay measures and how each is analyzed is crucial; to that end we undertook a comparison of the accuracy, precision, and resilience between the methodologies using an *in silico* approach to contrast lifespan estimates obtained by both methods against a known standard.

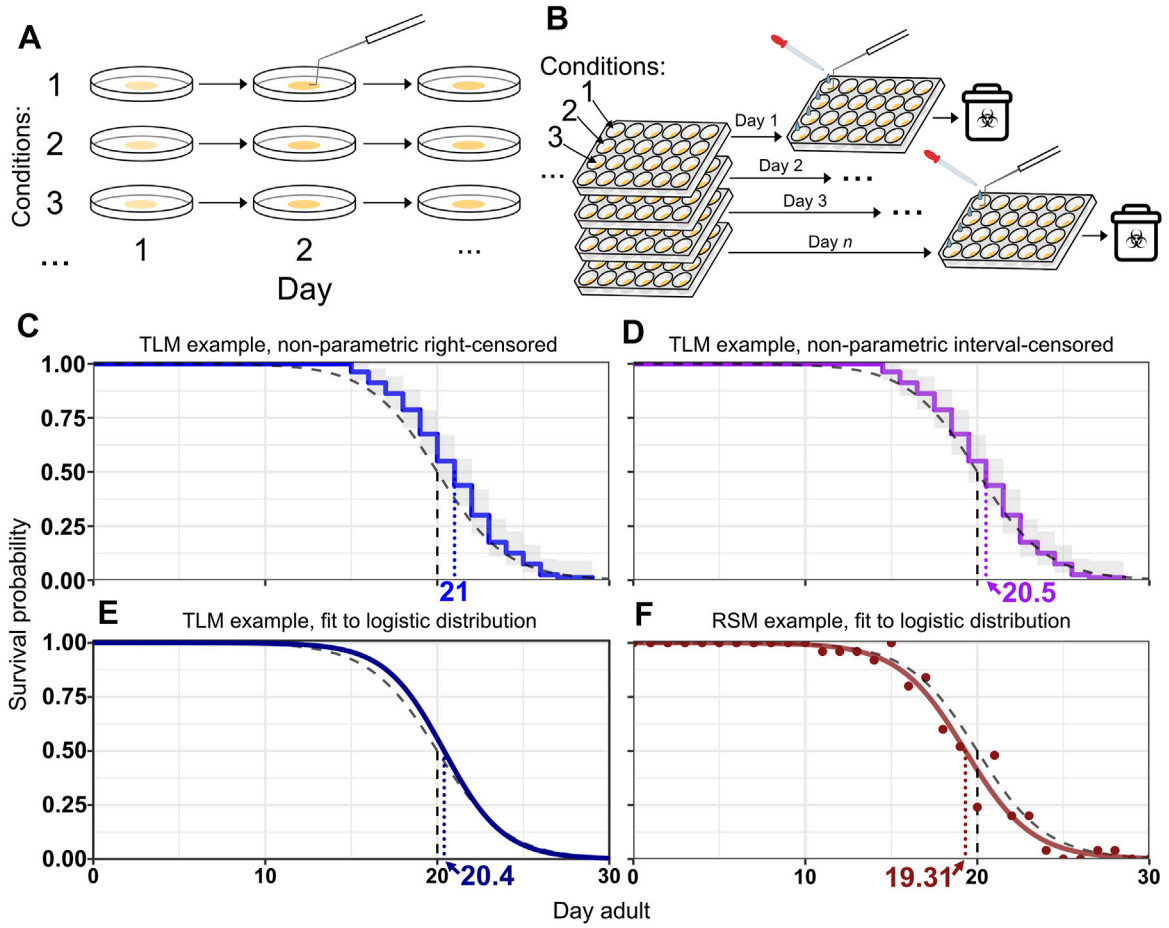

FIGURE 1 | RSM and TLM assays employ different strategies for population sampling. **(A)** Handling in traditional lifespan assays (TLM). A dish of animals is maintained for the duration of the experiment. Vital status observation may be facilitated by prodding animals to stimulate movement. When more than one experimental condition is included, such as different strains or RNAi treatments, a separate plate is maintained throughout the experiment for each condition. **(B)** Handling in replica set lifespan assays (RSM). Each observation uses a different plate derived from a synchronized population, with all the plates for expected observations of the experiment having been set up at the beginning of the experiment. M9 solution is typically added to wells to separate animals from the bacterial lawn and to help assess vital status, in conjunction with prodding as necessary. While replica set assays can be done with single-well dishes as well, the approach is particularly well-suited to use of multi-well plates, in which wells of the plate can be different RNAi conditions. Every plate in the replicate group is set up with an identical layout. **(C–F)** Example survival curves from simulated lifespan experiments, assuming daily scoring. The black dashed curve represents the generating logistic distribution with mean/median of 20 days and shape parameter $s = 2$. The vertical dashed lines indicate the median for the respective curve. **(C–E)** A simulated TLM experiment was run with 80 animals and fit with Kaplan-Meier (KM) using right-censoring (blue curve) **(C)**, interval-censoring (purple line) **(D)**, or with parametric fitting to a logistic distribution (fit logistic curve is dark blue) **(E)**. The grey shaded region is the confidence interval for KM curves. **(F)** shows an example simulated replica set experiment using 25 animals per observation with the proportions of live animals for each observation shown as points, and the fit logistic curve, both in dark red. The median survival for the simulated TLM experiment is 21 days with right censoring **(C)**, 20.5 days with interval censoring **(D)**, and 20.4 days with parametric fitting **(E)**. The median survival for the simulated replica set experiment **(F)** is 19.3 days.

We show that the TLM is susceptible to an analysis-induced intrinsic bias in estimated median and mean lifespan, which is an artifact arising from the assumption that death occurred at the time of observation. Most of the widely used software to plot and analyze lifespan data-using the non-parametric Kaplan-Meier approach by default- assume death occurred at the time of observation, known as right-censoring (**Figure 1C**), rather than at an unknown time in the interval between observations, the latter of which is known as interval-censoring (**Figure 1D**) (Kaplan and Meier, 1958; Finkelstein, 1986). When observation intervals are consistent within an experiment, this bias does not affect the power to detect statistical differences, but is important when considering mean or median estimates of lifespan across experiments, or when the observation interval is varied between conditions to be compared within one experiment. We found that the most appropriate way to analyze data from a TLM experiment having discrete observation times with day-long (or longer) periods is to use interval censoring. We demonstrate that both TLM and RSM approaches have similar accuracy and precision in estimating lifespan using sample sizes and scoring frequency reflective of values from many published studies, when non-parametric analysis with interval censoring (**Figure 1D**) or

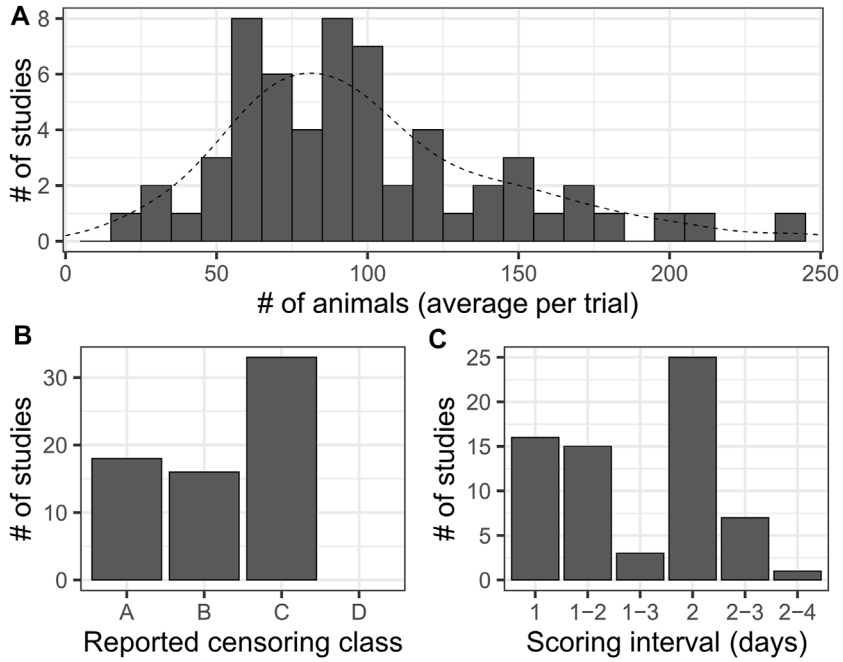

FIGURE 2 | A Literature survey for lifespan studies using *C. elegans* highlights dramatic variation in sample sizes and levels of detail reported. **(A)** Histogram of average number of animals per trial for each study considered in a survey of literature reporting lifespan experiments for N2 (wild-type). Histogram bar width represents 10 animals. The dashed line is the smoothed density curve for the same data. Median: 90, mean: 99.46. This excludes two outlying data points, each reported once, that were unlikely representative of single-trial results (360 and 721 animals). **(B)** A summary of the level of detail reported on censored observations in the surveyed publications. We divided results into categories as follows: [A]- censoring was not mentioned in the manuscript and no censor information was reported in the data, [B]- Censoring was mentioned in the manuscript (*e.g.* "animals that crawled off plates were censored"), but no censor information was reported in the accompanying data, [C]- Censoring was mentioned in the manuscript and a summary of censored animal information (*e.g.* "12 animals were censored for trial 1") was reported in the included data. [D]- Censoring was mentioned in the manuscript, and data for censored animals was provided for each time point of each trial. Note that we did not find an example of this last level among the literature surveyed. **(C)** Histogram of the reported scoring intervals from across the studies. Studies where a variable scoring interval was specified are indicated by a range of values. The most common scoring schedule was every-other-day scoring, indicated by an interval of two.

parametric analysis (i.e. distribution-based curve fitting, in this case to the logistic distribution) is applied for TLM (see **Figures 1E,F** for examples of parametric analysis of TLM and RSM data, respectively). However, when several forms of scoring error are simulated, we discovered the estimated median generated through the TLM significantly deviates from the true median. In contrast, the RSM is robust to various experimental and measurement errors that are likely to be common in examination of *C. elegans* lifespan. Thus, the replica set method represents a resilient and high-throughput approach to quantitatively assess changes in *C. elegans* lifespan.

MATERIALS AND METHODS

Lifespan Experiment Literature Survey and Assembly of a Gerogene Compendium

67 publications incorporating lifespan experiments with N2 animals were manually reviewed, spanning the years 2007–2020. For each paper, we noted the number of animals assayed per experimental trial (average across trials within a paper, when multiple unique values were provided), the interval between scoring occurrences, the lifespan and statistical analysis methods utilized, and the level of detail in reported censoring information. When scoring intervals were variable, the range was recorded (*e.g.* 2-3 for every 2 or 3 days). Reported censored observation information was classified into categorical bins A through D with increasing detail: A, no mention of censoring and no censored data reported; B, censoring mentioned but no censored data reported; C, censoring mentioned and censored observations were reported at a summary level (# of animals censored per condition or per trial); D, censoring mentioned and censored observations were reported per observation or timepoint including the reason for censoring (**Figure 2C**). In some cases, the complete details of the statistical analysis were not mentioned, but were able to be inferred, *e.g.* stating the log-rank test was utilized and showing stepped survival curves but not mentioning the Kaplan-Meier estimator. Papers for which it was not clear how the reported animal numbers were split into trials, which had a very high number of animals (1000+) were excluded from plots and summary calculations as such a large number of animals is unlikely to be derived from a single experimental trial of a single condition.

To establish the degree to which high-throughput lifespan studies have contributed to our knowledge of genetic interactions

with lifespan in *C. elegans,* a collection of gerogenes and associated publications was assembled based on annotation curated in WormBase (Harris et al., 2009) version WS282 using the WormBase API through R version 4.0.2 (R Core Team, 2015). Genes, both coding and non-coding, were identified that were associated with one of the following phenotypes through RNAi or mutation experiments: "life span phenotype" (WBPhenotype:0000039), "shortened life span" (WBPhenotype:0001171), or "extended life span" (WBPhenotype:0000061). The citation information for the publications providing the evidence for the relationships were also retrieved.

Parametric and Non-Parametric Fitting of Experimentally-Derived Lifespan Datasets

Four TLM experiments from two experimenters, six RSM experiments from two different experimenters, and five Lifespan Machine experiments from two different experimenters for N2 lifespan of animals kept at 20°C as adults were assembled from unpublished datasets. In order to obtain lifespan estimates, as well as determine the logistic curve shape parameter *s* from experimentally-derived datasets, TLM experiment data was fit with both parametric logistic and Kaplan-Meier approaches, RSM experiments were fit with the parametric logistic model, and non-parametric Lifespan Machine experiments were fit non-parametrically, all as described here under "Estimation of median, mean, and maximum lifespan".

Simulation of Lifespan Experiments

To simulate lifespan experiments, random virtual animals were generated from a probability distribution, either logistic or Gompertz, as indicated. Survival times were drawn from the distribution in batches, representing the population samples of cohabiting animals on plates (TLM) or wells (RSM) for lifespan experiments. For each batch of simulated animals, the corresponding number of survival times were independently drawn randomly. Each simulated trial was "scored" at time points corresponding to an interval of 1, 2, or 3 days which was fixed for a given trial. All simulation and evaluation work was performed in R version 4.0.2 (R Core Team, 2015).

For a TLM experiment, we simulated survival times on a single plate of N animals. This plate was then scored at each timepoint, with dead animals removed when they were "observed", continuing for each increment in observation time until no animals were left "alive".

For the RSM with N animals and T planned measurement timepoints, T plates of N animals each were drawn (N*T animals total). Analogous to real experiments, in which an estimate of expected maximum lifespan is obtained prior to setup of RSM trials, T was selected to include two observations past the expected maximum lifespan (99% survival of the generating distribution). For each time point, proportional survival was observed for a new "plate", and each "plate" was scored once only. Scoring continued until reaching either two consecutive observations with no "live" animals, or until the prepared replicate plates (T) were exhausted. Across conditions of both methods, each experiment was repeated for 10,000 trials. Note that for two-sample testing and power analysis, 10,000 trials were generated but only 100 were considered due to constraints on computation time.

Reproducibility of these simulated experiments was facilitated by using a consistent pseudorandom number generator seeding strategy across all experiments. To generate simulated animals, a number of values from 0 to 1 was randomly drawn at uniform probability corresponding to the starting population sample size N (TLM) or the size of the population sample for each replicate N*T (RSM); these values were in turn used as quantiles to determine simulated animal death times based on the generating distribution. The quantile function for logistic is $T(p) = s*\ln(\frac{1}{p} - 1) + \mu$ where $T(p)$ is the time of death at quantile p, s is a parameter controlling curve shape, and μ is the mean/median. To represent survival of a wild-type population, we chose μ = 20 and s = 2, with s derived from logistic curve fits to our own N2 lifespan data from both RSM and TLM assays, and mean/median of 20 days as a convenient approximation (**Supplementary Figure S1**).

To complete computation of the simulations in a reasonable time-frame, execution was parallelized to use multiple available cores on a desktop computer using the R package *future* or on a high-performance computing cluster (UR CIRC BlueHive) using the package *slurmR* (Yon and Marjoram, 2019; Bengtsson, 2021); animal populations were generated on the former, and permutation testing performed on the latter. To ensure reproducibility for generating animal population samples in parallel, the L'Ecuyer-CMRG random number generator was used for population generation, and the default R RNG was employed elsewhere for reduced computational overhead.

Estimation of Median, Mean, and Maximum Lifespan

Each time a simulated plate was scored with either method, the time (day adult) and number of live and dead animals were recorded. For non-parametric analysis of TLM experiments, data was formatted with one record per individual with a death event. For right-censoring, which assumes the event occurred at the time of observation, only the observation time t and the death-indicating event code 1 were necessary. For interval-censoring, which assumes the event occurred between the observation at time t and the previous observation at t-1, the two times specifying the interval (t—1, t) were provided for each event. In both non-parametric analyses, the data was fit using the R package *survival* (Therneau, 2021) and the mean, median, and 95% quantile lifespan estimates were recorded for each simulated trial.

For analysis of RSM experiments, or parametric treatment of TLM data, the experiment result tables were converted to interval format, similar to TLM with interval censoring. For RSM, as each animal is only observed once and only the current status is known, an observed death at time t yields an interval of $(0, t)$, while animals observed alive have an interval of (t, ∞) (*i.e.* we do not know when any given live

animal eventually died). Parameters were estimated using non-linear minimization as implemented in the R function *nlm* (R Core Team, 2015). Specifically, $P(t) = \frac{1}{e^{u+1}}$ where $u = \frac{t-\mu}{s}$ with parameters μ (mean/median) and s (curve shape) selected to maximize likelihood of the observed survival intervals, calculated as $P(right\ edge) - P(left\ edge)$ for each interval. The returned parameters were then used to find the 95% quantile lifespan estimate; mean and median lifespan are given by the parameter μ.

Determining Accuracy and Precision Across Simulated Experiment Trials

10,000 simulated trials were performed for sample sizes of 5–50 for RSM and 5—150 for TLM, both in increments of 5 animals, for scoring intervals of 1, 2, and 3 days. For each combination of assay, scoring interval, population size, and analysis type, standard error (SE), and mean-squared error (MSE) were computed across the median lifespan estimates from the 10,000 trials as metrics of precision and accuracy, respectively. For daily scoring, sample sizes were identified that resulted in similar accuracy and precision across assay types, and these sample sizes were the focus of further analysis.

Simulating Systemically Flawed Experiments and Fitting Data From a Different Generating Distribution

An experiment where two populations with differing lifespans are accidentally mixed was used as the motivating example for a case of a fundamentally flawed experiment which would not be able to produce the expected result with any lifespan assay methodology. To simulate this experiment, 67% of animals were generated with parameters $\mu = 20$ and $s = 2$, and the remaining 33% of animals were generated with $\mu = 16$ and $s = 1.6$, with the logistic distribution. Experiments for RSM and TLM were then run as described earlier.

To evaluate RSM performance when the generating distribution is different from the fitting distribution, simulations were performed where Gompertz was used as the generating distribution across the same set of assay types and analysis treatments as utilized elsewhere for single-sample experiments (*e.g.* experiment sample sizes, scoring interval). Experiment samples were generated as otherwise described for logistic, but with $T(p) = \frac{\ln(1-\frac{\beta}{\alpha}\ln(1-p))}{\beta}$ for obtaining death times, corresponding to a two-parameter formulation of the Gompertz function (Benjamin, 1825; Pollard and Valkovics, 1992; Wilson, 1994). Selected parameter values were $\alpha = 0.0003271342$ and $\beta = 0.3271342007$, such that median lifespan is 20 days and slope is comparable to logistic with $\mu = 20$ and $s = 2$ (see curves in **Figure 5A**).

Model for Experimenter Mis-Scoring and Scoring Hazard in Simulated Experiments

We simulated the effects of mis-scoring- either deeming a live animal as dead, or vice-versa- occurring at a probability, $P(t)$, which was either held constant, or modulated as a function of time. The probability of mis-scoring was modeled independently for each animal. In TLM experiments, mis-scoring affected which animals stayed on the plate or were removed: dead animals mis-scored as alive stayed on the plate to be scored again the following day, whereas live animals scored as dead were removed from the plate and never scored again. For the RSM, each animal was scored only once, and thus exposed to the possibility of being incorrectly scored only once.

We also simulated the possibility of formerly live animals being accidentally killed by the investigator and then recorded as dead through rough handling when making an observation- a scoring hazard. This type of error was simulated as a function of time only.

For constant-rate mis-scoring, a fixed error probability p was applied across the trial. When error rates were modeled as a function of time, the error rate started at zero, then rose to $maxP$ according to the equation: $P(t) = maxP*(1 - \frac{1}{e^{-(\frac{u-t}{s})}+1})$ where $u = \frac{(1.5t-\mu)}{s}$, typically with $\mu = 20$ and $s = 2$, or otherwise the same parameters as for the generating logistic distribution. Error probabilities p and $maxP$ were set to 2, 4, or 10%. A set of results is provided for the case where the rise in error rate is inversely proportional to the decrease in survival at time t ("late-life onset" error), as well as for the biologically motivated scenario where error rate starts to increase in advance of mortality concomitant with the loss of mobility and onset of paralysis ("mid-life onset" error); the latter is obtained by using $p(t*1.5)$ to shift the error rate curve (**Figure 6**).

Calculation of P-values and Power for Two-Sample Comparison Analyses

For analysis of statistical power, first 100 trials for RSM and TLM experiments were simulated across medians ranging from 16 to 24 days (+/- 20% from the reference of 20 days) in increments of 0.1 days all at population sample sizes for each trial from 5 to 50 animals for RSM and 5 to 150 animals for TLM. The generating distribution slope parameter was held at $s = 2$ for all cases. The result of a given trial was compared to the reference population (median of 20 days) with the same sample size and experiment type. p-values for comparison of parametric analysis for 100 generated experiment trials were computed using the R package *statmod* after 10,000 iterations of label permutation per trial as otherwise previously described (Phipson and Smyth, 2010; Cornwell et al., 2018). For non-parametric analysis, tests for differences in survival were performed with functions *survdiff* for right-censored data (Therneau, 2021), and *ictest* for interval-censored data (Fay and Shaw, 2010) for 100 generated trials for each relevant set of conditions. p-values were corrected for multiple testing across the $n = 100$ trial comparisons within a set of conditions using the Benjamini–Hochberg FDR adjustment (Benjamini and Hochberg, 1995). Power was then calculated as *power* =

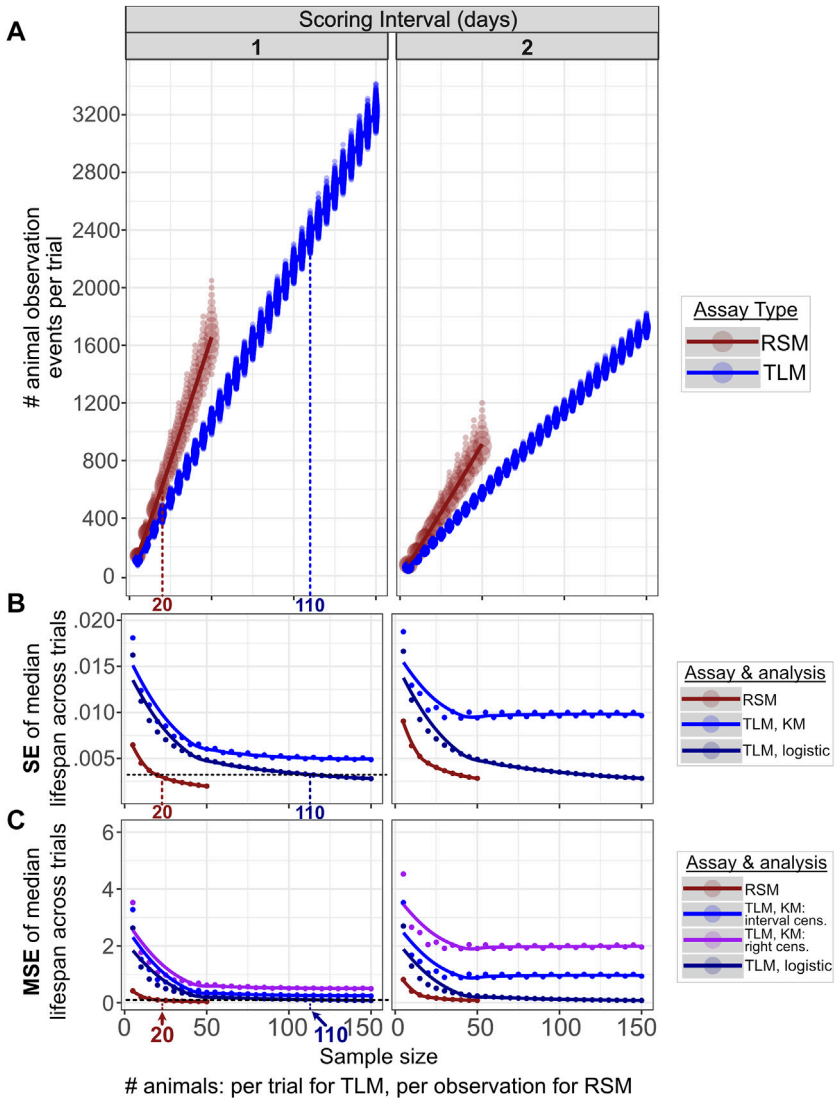

FIGURE 3 | Comparable accuracy and precision can be obtained for RSM and TLM, but with many fewer total animal observations for RSM. 10,000 simulated RSM and TLM experiment trials were run for each increment in sample sizes across a range of 5–50 for RSM and 5—150 for TLM, both in increments of 5. For RSM, "sample size" refers to the number of animals per timepoint scored, whereas for TLM this is the number of animals at the start of the trial. The columns correspond to scoring intervals from 1 day (every day) or 2 days (every other day). We find fewer total animal observations are necessary to obtain a given level of precision (standard error of median lifespans and accuracy) **(B)**, (mean-squared error of median lifespans) **(C)** for RSM (median of 620 observations) compared to TLM (median of 2366 observations), with corresponding population sizes of 20 and 110 animals respectively. Increasing the interval between scoring timepoints negatively impacts both metrics for both assay types, particularly at small sample sizes. The same set of simulated TLM experiment data was used for all TLM analysis treatments. **(A)** The total number of animal observations (i.e. the number of times any animal is scored during the course of a trial, including live and dead animals, and those that have been scored on previous observations for TLM) scales differently with sample size between RSM (dark red) and TLM (blue) assays. "Sample size" is the number of animals per observation for RSM, and the number of animals per trial for TLM. The size of the circle at a given position indicates the number of trials which had that result. **(B)** SE (standard error) across the median lifespans for the 10,000 simulated lifespan trials for each sample size, for the given assay type and analysis method, indicating how precision improves with increasing sample size between the assay types. Right-censoring *vs*. interval-censoring shifts the medians but does not alter the dispersion, so only one set of results is shown for Kaplan-Meier analyses (denoted "KM"). For the left-most column (daily scoring) the horizontal black dashed line indicates a sample size for which SE is similarly low (0.003) across RSM and TLM (with parametric fitting to a logistic distribution, denoted "logistic"), 20 animals per observation for RSM and 110 animals per trial for TLM. **(C)** MSE (mean-squared error) across the median lifespans for the 10,000 simulated lifespan trials for each sample size, for the given assay type and analysis method, indicating how accuracy improves with increasing sample size between the assay types. For the left-most column (daily scoring), the horizontal black dashed line indicates a sample size for which MSE is similarly low (0.1) across RSM and TLM (with parametric fitting to a logistic distribution, denoted "logistic"), 20 animals per observation for RSM and 110 animals per trial for TLM.

$\frac{\sum_{i=1}^{n} \begin{matrix} X_i < \propto & 1 \\ X_i \geq \propto & 0 \end{matrix}}{n}$ for $\propto = 0.01$ where i is the index in a vector of p-values X of length n.

RESULTS

Previously Published Lifespan Studies Using Traditional Methods Vary Widely in Sample Size and Scoring Frequency

Among researchers measuring *C. elegans* lifespan using the traditional longitudinal method, a wide range of sample sizes and scoring periods have been reported. For example, one laboratory might survey a population of 50 animals for death events once per day. In contrast, another laboratory might measure survival within a population of 100 animals every third day. Thus, we compared and contrasted the accuracy and resiliency of the TLM and RSM across a wide range of scoring conditions. We posited that the sample size and scoring period have substantial effects on the accuracy and precision of lifespan estimates, thus we first surveyed the literature to identify the ranges actually used in published studies, and then modeled these *in silico* in order to identify how they altered the accuracy and precision of each method in estimating lifespan.

To assess the sample sizes and scoring frequencies actually used in published research, we surveyed a representative sample of 67 manuscripts published between 2007 and 2020 incorporating *C. elegans* longevity estimates across a diverse array of high quality journals (**Supplementary Table S1**). We chose experiments using wild type animals, which have been reported to live between 16.6 and 20 days based on mean or median lifespan of N2 (wild-type strain) hermaphrodite animals maintained at 20°C (Kenyon et al., 1993; Gems and Riddle, 2000; Johnson et al., 2001, 2014). We assessed the number of animals included per experiment, the number of trials performed, the interval between scoring instances, and statistical aspects of the analysis (censoring, statistical software employed). Studies used a very broad range of values for the number of animals observed per experiment- from 10 to 1425 (mean = 115.2, median = 80) (**Figure 2A**). We also summarized the level of detail reported on censored observations- animals removed from an experiment due to alternative phenotypes or non-aging-associated death- on a scale from A (censoring not mentioned and no data reported) to D (censored animals reported for each time point) (**Figure 3B**) as high rates of censoring can impact the reported sample size of an experiment. Surprisingly, we did not find any examples of "class D" censoring among this set of publications, while nearly one quarter did not report any data on censored animals.

The interval between scoring was harder to assess, as some publications report variable scoring frequencies such as "every day to every other day". Scoring frequency ranged from 1 to 4 days, with every other day being the most common observation interval (**Figure 2C**). From this we noted that the average interval between scoring instances is more than 10% of the median lifespan of wild-type animals, and sample size can vary by more than an order of magnitude. Thus, actual *C. elegans* lifespan studies using traditional methods vary widely in sample size and scoring frequency, which likely influences reproducibility and relative comparability between results.

In Silico Modeling of Mortality and Accuracy of TLM and RSM Approaches

To evaluate the accuracy of the two methods in predicting median lifespan we first created a parametric model based on the logistic distribution from which to generate simulated populations of animals, with two parameters- one for mean/median and one for the shape/slope of the curve (denoted *s*). While the logistic function is symmetric, such that the mean and median are the same, for analysis in other contexts we focus on the median- the point at which 50% of the animals of a given population have died- as a summary value of lifespan in most cases. This "known standard" generating model represents an arbitrarily large theoretical population where the exact time of death for each animal is known. From this we then conducted an *in silico* analysis to determine how well each method captures the characteristics of our simulated standard. We set the slope parameter $s = 2$ for our generating model based on logistic curve fits to our own lifespan data across multiple experiments for wild-type (N2) *C. elegans* at 20°C with *E. coli* feeding on agar plates (**Supplementary Figure S1**), and assumed a mean/median of 20 days; this yields a generating distribution with mean, median, and 99% (maximum) survival of 20, 20 and 30 days, respectively.

To test how accurately and precisely the TLM and RSM approximate the median lifespan of the model population, we used a Monte-Carlo approach to generate 10,000 simulated experiment trials each for TLM and RSM (Dwass, 1957; Good, 2006), and calculated median as well as mean lifespan by both a parametric (i.e. fit to a distribution, in this case logistic) and non-parametric analysis (i.e. Kaplan-Meier) for TLM, and parametric analysis for RSM. Parametric analysis was performed for both assay types to ensure that the apparent benefits of RSM were not due to a difference in analysis methods; for both RSM and TLM this analysis was performed by finding parameters of a logistic curve which fit the data using non-linear minimization, as *C. elegans* lifespan data has been previously shown to fit a logistic distribution well (Vanfleteren et al., 1998). Concurrently, we assessed whether varying the sample size (5–50 animals per observation for RSM, 5—150 animals per trial for TLM) or scoring frequency (1–3 days) altered the accuracy or precision of either method.

The TLM and RSM have Similar Precision and Accuracy in Predicting Median Lifespan, With RSM Requiring Fewer Total Animal Observations During the Course of an Experiment

To assess the precision of the experimental and analytical approaches, we investigated the variability resulting from the parametric and non-parametric analysis methods for TLM, and parametric analysis methods for RSM. The median lifespan estimates across experiment sizes for simulated

daily scoring of TLM experiments had minimal differences in precision after both parametric and non-parametric analysis (as indicated by SEM) (**Figure 3B**). Increasing sample size (number of animals) substantially reduces variance in estimated average lifespan in both parametric treatments of the TLM, and, to a lesser extent, with RSM (**Figure 3B**). A similar trend is observed for the accuracy, as indicated by mean-squared error (MSE) of the median lifespan estimates (**Figure 3C**). It is worth noting that sample size is defined differently between the two methods- the TLM "sample size" reflects the total starting experiment sample size, whereas the RSM sample size represents the number of independent animals sampled at each time point- we sought to find experimental criteria for which the two methods were maximally comparable. We note that for daily scoring of TLM experiments starting with 110 animals per condition, the SEM and MSE of estimated lifespan is equivalent to an RSM experiment with 20 independent animals scored per condition (**Figures 3B,C**, respectively). Having demonstrated concordance between parametric and nonparametric models, we focus hereafter on simulation and comparison of TLM and RSM assuming 110 animals per condition for TLM, and 20 animals per observation for RSM, for daily scoring, with analyses based on the parametric approach. Finally, as these sample sizes are within the range of those commonly used in actual experiments, we conclude that the TLM and RSM methods have a similar level of precision and accuracy in estimating lifespan.

It must be noted that the number of observations necessary- *i.e.* the number of total animals observed during the course of an experiment- to obtain adequate power to detect a lifespan effect for each method is inherently different. As an example, in the TLM approach, for a given population, 110 individual animals may be observed in total, but in RSM 20 animals are measured per time point, with different individuals at each observation for the latter. The longitudinal nature of the TLM approach means that fewer measurements are made with progressing observations, until no animals are left alive. This is in contrast to an RSM experiment, where the number of measurements is the number of plates (*i.e.* time points) multiplied by number of animals per plate, with the number of animals remaining generally consistent. While the number of "unique animals" observed is much larger in the RSM experiment, the total number of animal observations- the number of times the researcher scores any animal per time point- is lower for RSM than TLM at a given level of precision and variance across simulated experiment trials (**Figures 3A–C**). As it takes time to determine if an animal is alive or dead, a reduction in the number of total animal observations indicates less time will be necessary to score the experiment. We find the nature of the assay for vital status in RSM, with the addition of liquid to a well to stimulate movement, further reduces the time needed to score an animal (Cornwell et al., 2018), and means that the net "hands-on" time is much shorter for a comparable RSM experiment.

Analysis of TLM Data With a Right-Censoring Approach, But Not With Interval Censoring or Parametric Treatment, Leads to Biased Summary Survival Estimates

To our surprise, an initial comparison of parametric (logistic) treatment to a non-parametric (Kaplan-Meier) analysis of TLM-style data based on the simulated dataset revealed a bias in the non-parametric average lifespan estimates. This intrinsic bias increases the predicted median (**Figure 4A**) and mean (**Figure 4B**) lifespan by approximately one half of the scoring period (*e.g.* a bias of 0.5 days for daily scoring, 1.5 days for scoring every three days). The nature of this bias is a statistical artifact from assuming that death occurred at the time of observation, which is the case when right-censoring is employed- often the default for lifespan analysis software. To correct for this anomaly while maintaining a non-parametric analysis approach, one can use interval censoring, which places the time of death as occurring within an interval between the two observations. Correspondingly we found that the application of interval censoring eliminated this bias, as does parametric analysis of TLM data (**Figures 4A,B**).

Among the 67 papers that were part of our literature survey, we found that a Kaplan-Meier estimator was applied in 60 manuscripts (90%), but the use of interval censoring was not explicitly reported in any cases. In a brief investigation of recent versions of statistical software implementing survival analysis methods, we found some level of support (built-in or *via* community-derived scripts/packages) for interval censoring in nine out of 21 programs (**Supplementary Table S2**). Out of the representative literature we surveyed, only ~25% utilized software that implemented interval censoring, regardless of whether it was utilized for the study (note that the older versions of the software used for the actual studies may not have supported interval censoring at the time); for about 23% of the studies considered we were not able to determine which statistical software, if any, was utilized. Thus, it is possible that right-edge bias, which is introduced by applying right-censoring instead of interval censoring, is likely to be prevalent in many reported *C. elegans* lifespan studies.

Analysis of Replica Set Experiment Data With Logistic Curve Fitting Produces Reasonable Lifespan Estimates Even With Animals Drawn From a Different Distribution

Although wild-type *C. elegans* lifespan has been previously shown to fit well to a logistic distribution (Vanfleteren et al., 1998), and a number of lifespan-modulating perturbations and treatments have been found to temporally scale lifespan in *C. elegans* rather than change the type of distribution (Stroustrup et al., 2016), we cannot exclude the possibility of encountering a condition that alters the shape of the survival curve. We posited that if we were to simulate survival experiments based on a different generating distribution, we would still obtain

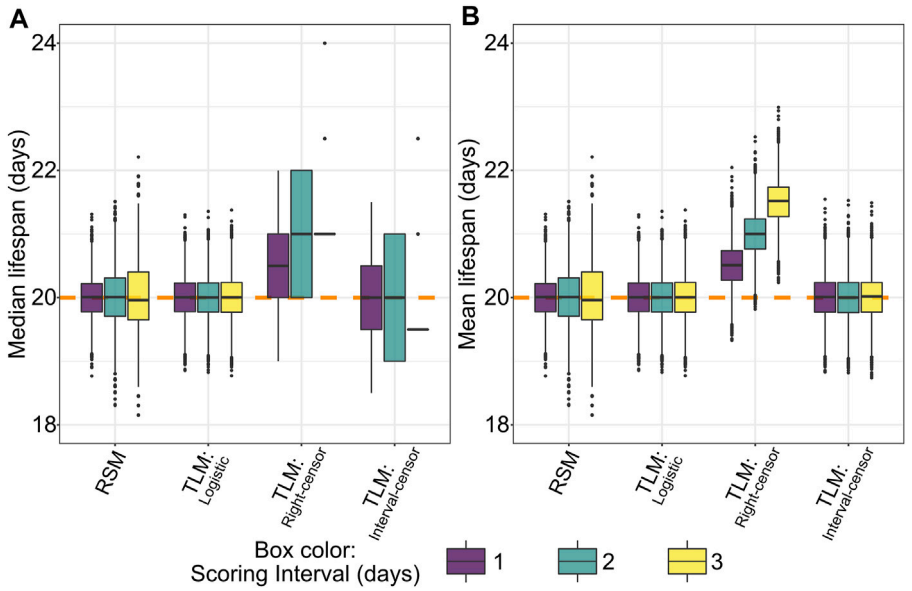

FIGURE 4 | Non-parametric right-censored treatment of TLM data leads to right-edge bias, which is not observed with interval censoring, nor with parametric (logistic) fitting of TLM or RSM data. Across 10,000 simulated experiments for a sample size of 20 for RSM (# animals per observation) and 110 for TLM (# animals per trial) we find that non-parametric analysis of TLM data with right-censoring (denoted "Right-censor") leads to a positive bias in estimated median **(A)** and mean **(B)** lifespan equivalent to approximately half the scoring interval. The dashed orange line indicates the mean/median of the generating logistic distribution (20 days). This bias is not observed in the TLM experiments analyzed with either a non-parametric treatment and interval censoring (denoted "Interval-censor"), or parametric treatment (logistic curve fit, denoted "Logistic"), nor with simulated RSM experiments and associated parametric analysis.

reasonable estimates of median survival from an RSM experiment with fitting the data to logisitic curves. To test this, we repeated our lifespan simulations, except with animal lifespan samples drawn from a Gompertz distribution, rather than a logistic distribution, while maintaining a median survival of 20 days (**Figure 5A**). We find that the change in generating distribution results in a small underestimate of lifespan for both the RSM and parametric treatment for TLM, a reduction in median lifespan of approximately 0.5 and 0.25 days, respectively (**Figures 5B,C** right side), while not changing the dispersion of the estimates across the simulated trials (**Figure 5C** left side). As expected, altering the shape of the generating distribution while maintaining the same median did not affect the estimates for non-parametric treatment of TLM experiments (**Figure 5B**). Thus, even when the experiment data does not match the assumptions of our logistic-based parametric analysis, we find that RSM still provides reasonable estimates of lifespan- with greater accuracy than the non-parametric right-censored analysis of TLM- without loss of precision.

Assessing Effects of Scoring and Experimental Error

In addition to biological variation due to intrinsic stochastic factors within a single population of animals (Herndon et al., 2002), a lifespan experiment may suffer from extrinsic error, such as: incorrect scoring of individual viability, strain contamination, incubator temperature fluctuation, mating, insufficient food availability, or extended exposure to blue spectrum light (Gems and Riddle, 1996; De Magalhaes Filho et al., 2018; Baugh and Hu, 2020). We systematically investigated some of these extrinsic sources of variability by adding error terms to our simulations of both RSM and TLM experiments.

Systemic Experimental Error Simulation

For an error that shifts the expected survival of every animal by an equal amount, independent of measurement parameters (*e.g.* observation frequency, sample size) and methods (TLM or RSM), we would expect equal effects on the outputs from both methods. This would occur in a real experiment if, for example, there was a contaminating strain leading to a mixed population, the wrong strain was used in one comparative trial, or RNAi was inefficient. To this end, we simulated a mixed population case such that a third of the animals were short-lived by 20% (median lifespan of 16 days) compared to our standard population at 20 days (**Figure 5D**). We posited that this would shift the median lifespan estimates similarly across assay and analysis types. Indeed, we found that for either TLM or RSM there was a congruent decrease in estimated lifespan (**Figure 5E**). Thus, we can conclude that systemic error has a consistent effect across experimental methodologies, and regardless of the data analysis strategies considered for TLM.

Mis-Scoring Error Simulation

We next modeled how scoring error- inaccurately determining whether an animal is alive or dead- affected the accuracy and precision of the output of each method. We posit that as animals age and lose the ability to respond to stimuli through body-wall

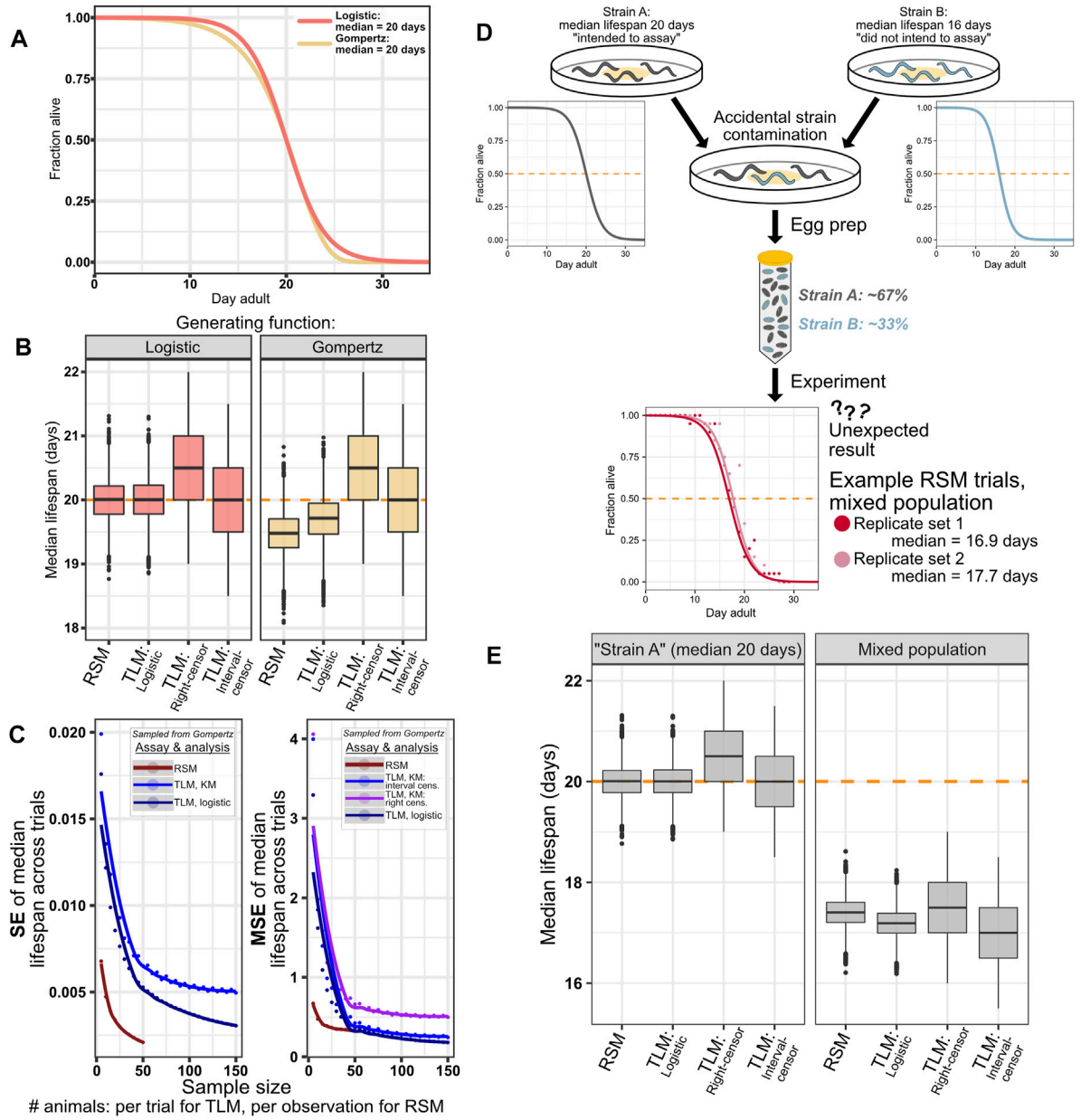

FIGURE 5 | RSM shows reasonable performance even with populations generated from a distribution other than logistic. As expected, simulation of systemic errors in an experiment similarly impacts all assay and analysis types. **(A–C)** While *C. elegans* survival data has been shown to fit better to a logistic distribution than to Gompertz (Vanfleteren et al., 1998; Stroustrup et al., 2016), the possibility of treatments or genetic perturbations that can alter the shape of the curve cannot be dismissed. As such, we performed an experiment to ascertain how such a change in the generating distribution would affect the performance of the two assay types, while maintaining fitting to the logistic distribution for parametric treatments. **(A)** Animals were drawn from a logistic (orange) or Gompertz (yellow) distribution, both having median lifespan of 20 days. Parameters were chosen for the Gompertz curve such that the slopes were similar (see Materials and Methods). Note that unlike with logistic, the median and mean are not equal for Gompertz. **(B)** Median lifespan across 10,000 simulated lifespan trials for the indicated assay type and analysis treatment, for simulated animals with lifespan drawn from a logistic distribution (left) or Gompertz distribution (right). 20 animals per observation were used for simulated RSM experiments, and 110 animals per trial were used for the simulated TLM experiments. The input TLM experiment data was the same across the different analysis types. The "true median" of the generating distribution is indicated by the dashed orange line. As expected, non-parametric analysis of TLM experiments is not affected by the change in generating distribution, while the mismatch between the generating and fitting distributions results in a slight underestimate of lifespan for the RSM and parametric treatments of TLM- 19.48 and 19.71 days, respectively (median of the median lifespan across the 10,000 simulated trials, **Supplementary Table S3**). Precision is minimally affected by the change in distribution, as indicated by standard error (SE) of the median lifespans (**(C)**, left side), while mean-squared error (MSE) (**(C)**, right side) reflects the shift observed in **(B)** when compared to the similar plots of SE and MSE for the case of a non-mismatched distribution in **Figure 3**. **(D,E)** Systemic experimental errors, exemplified by a simulated case of mixing two *C. elegans* strains with different lifespans, affect all assay and analysis treatments similarly. **(D)** Schematic representation of a simulation of an experiment in which two strains with different lifespans are mixed. "Strain A" (dark gray) has median lifespan of 20 days, and represents the strain for which the assay might have been intended, but it becomes contaminated with "Strain B" (blue) which has substantially shorter

(*Continued*)

FIGURE 5 | lifespan of 16 days. The corresponding logistic curves show the generating distributions for these two populations. The result is a mixed population with 67% of animals having median lifespan of 20 days, and 33% of animals having median lifespan of 16 days, which yields a lifespan experiment result that is not the 20 days our experimenter expected (two example RSM experiment results from the mixed simulation are shown in red and light red). **(E)** Both assay types, and all TLM analysis approaches similarly reflect the result of the mixed experiment, yielding median lifespan estimates in between 16 and 20 days. 20 animals per observation were used for simulated RSM experiments, and 110 animals per trial were used for the simulated TLM experiments.

muscle movement, there is an increased probability that an experimenter will make a mistake in scoring viability. As errors in scoring can propagate through the remaining time-course for TLM but not RSM, we hypothesized that such scoring errors would introduce both more variability and bias in TLM compared to RSM experiments.

We sought to test this hypothesis through three simulation conditions: a constant mis-scoring error rate throughout lifespan, mis-scoring that begins at mid-life, and mis-scoring that begins late in life. For each condition we simulated error-rates of 2, 4, and 10%. The first condition serves as a proof of principle positive control to demonstrate whether incorrect assessment of viability affects the perceived lifespan by either RSM or TLM (**Figure 6B**, dotted lines). As expected, a constant probability of incorrect scoring resulted in both a loss of precision (i.e. increased variability) and accuracy in estimating lifespan with the TLM. Specifically, introduction of a constant 2% mis-scoring error yielded a median lifespan estimate of 16.8 days; shorter than the actual median lifespan of 20 days by 15.9% (with parametric analysis) (**Figure 6D**, **Supplementary Table S3**). In contrast, the same error applied to the RSM resulted in no appreciable change in accuracy (19.85 days, a decrease of 0.75%) (**Figure 6D**, **Supplementary Table S3**). If we increase the mis-scoring rate to 10%, the accuracy of both assays suffers, but the difference in bias is stark: the TLM median lifespan is 6.36 days (with parametric analysis), while RSM is 19.46 days (**Figure 6D**). It may be observed that the TLM estimates from non-parametric analysis with right-censoring appear to be less compromised with respect to accuracy than the other TLM treatments, but this is incidental due the aforementioned right-hand bias which yields an overestimate of lifespan under non-error conditions (**Figures 6C,D**). Thus, we conclude that the TLM is more sensitive to scoring error than the RSM, and shows a downward-shifted bias when even a small scoring error is introduced.

We next sought to determine how incorrect scoring would affect accuracy in a more realistic context, taking into account the behavioral changes that occur during aging in *C. elegans*. Assessing whether an individual is alive or dead typically relies on observing animal movement for both experimental approaches (TLM and RSM). As *C. elegans* animals age, they undergo a progressive decline in movement, which ultimately results in paralysis of most of the body in the last 3rd of an animal's lifespan for WT (Hosono et al., 1980; Bolanowski et al., 1981; Johnson, 1987; Herndon et al., 2002; Huang et al., 2004) (**Figure 6A**). From the onset of paralysis, it becomes necessary to determine the status of animals from subtle head movement, often only in response to touch- making scoring much more difficult and time-consuming. Conversely, it is easy to determine whether an animal is alive or dead early in life, as movement is frequent and spontaneous (**Figure 6A**). To simulate the increase in scoring difficulty that follows this progressive decline in movement with age, we simulated scoring error- either false positive, or false negative- with probability rising from 0% for young animals up to 2, 4, or 10% later in life. The timing of the rise in the error rate was also modeled in two ways. The first simulates a "mid-life onset", which represents incorrect scoring of animals starting from the decline in movement that occurs in advance of the highest rate of mortality, but within the period of time when a sub-set of individual wild-type animals have been empirically observed to enter a decrepit state (Herndon et al., 2002) (**Figure 6B**, dashed lines). This condition simulates a population where the vast majority of animals will still be responsive to touch, and therefore scored correctly, but a small subset of paralyzed animals within the larger population will be incorrectly scored at a higher rate. Even if the maximal probability of making such a mistake is just one in 50 animals, the shift in accuracy for TLM is nearly ten times that of RSM, at 19.4 days compared to 20.07 days, respectively (**Figure 6E**, **Supplementary Table S3**). This difference is exacerbated further when assaying long-lived animals, as just a 2% mis-scoring rate increasing from mid-life reduced median survival results from TLM by approximately two days when populations were generated from a distribution based on *daf-2(e1370)* lifespan characteristics (median 42 days, maximum 60 days (Kenyon et al., 1993; Bansal et al., 2015; Hahm et al., 2015; Podshivalova et al., 2017; Zhao et al., 2021)) (**Supplementary Figure S3**). When the possibility of mis-scoring increases to a maximum of 10%, the TLM estimate dips to 17.34 days (**Figure 6E**). Interestingly, we find that the RSM lifespan estimate actually increases- to 20.58 days- this is attributable to a difference in when RSM experiments are ended. Whereas TLM experiments end when there are no animals left alive from the starting population, typically an RSM experiment is terminated when no live animals are observed on two consecutive observations; consequently, mis-scoring a dead animal as alive near the end of the experiment could "reset the clock" and extend the experiment if the termination condition is strictly enforced, as it was for these simulations.

We also simulated a "late-life onset" of mis-scoring which represents incorrect scoring of animals that are entirely paralyzed or dead (**Figure 6B**, solid colored lines). In this scenario, the difference in accuracy between the assays appears to be less stark, which may be due to the smaller population remaining in the TLM experiments by the time the rate of mis-scoring peaks; when the possibility of mis-scoring rises to 10% starting from mid-life, the median TLM estimate is 19.43 days (**Figure 6F**). Here again, we observe an apparent over-estimate in lifespan for the RSM, attributable to the termination condition in the RSM simulations being reset when "dead" animals are scored as "live" near the end of the experiment. Thus, RSM provides a more accurate estimate of lifespan when the possibility of mistaking if an animal is alive or dead starts to increase near the time when movement begins to decline, even when the rate of such errors is low.

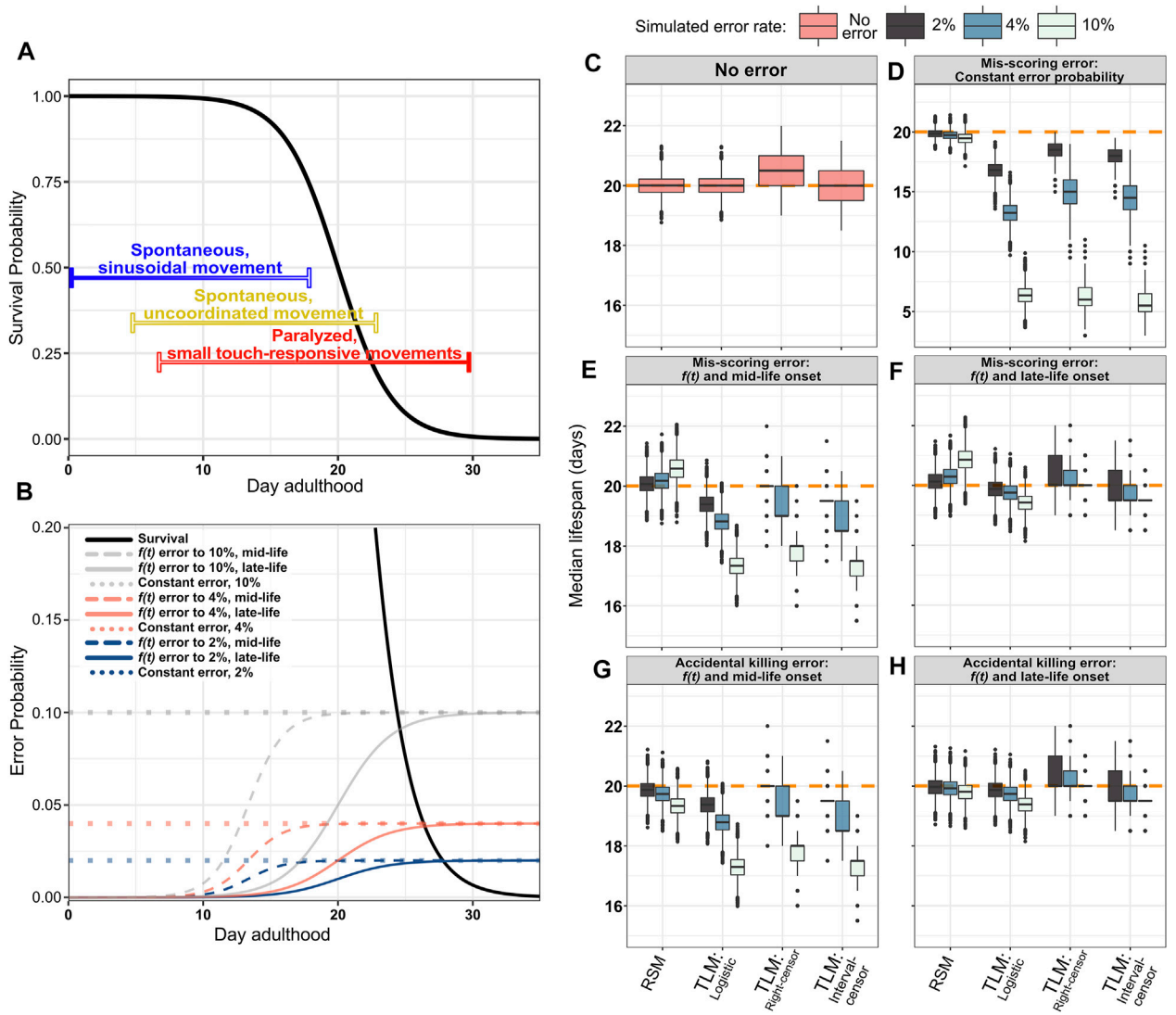

FIGURE 6 | The RSM is more resilient to incorrect scoring of survival, as well as premature animal death caused by accidentally rough handling. As animals age, they demonstrate locomotory decline and increased fragility, making them more difficult to score for viability, and increasing the possibility of scoring errors to which RSM is more robust. **(A)** An illustration of the phases of movement and the decline thereof during *C. elegans* lifespan (Huang, C., Xiong, C., and Kornfeld, K. (2004), Newell Stamper, Breanne L., et al. (2018)). Young animals move spontaneously and frequently, with a consistent sinusoidal motion (indicated by blue bar). For scoring lifespan assays, it is usually quickly determined if animals in this stage are alive, as any that have temporarily stopped moving can be stimulated by gently tapping the plate. In "middle aged" animals, movement becomes less coordinated and frequent, but they still may be moving without any external stimulus (indicated by yellow bar). A stronger plate tap, or occasional prod with a pick or fiber may occasionally be necessary to stimulate movement. In aged animals, spontaneous translational body movement has largely ceased, with only occasional posture shifts of the head or tail (indicated by red bar). In this stage, it is usually not immediately evident if an animal is alive or dead, and touch stimulation is usually necessary to determine their vital state. As this can be laborious, the possibility of making an error in determining the state of an animal may increase, as does the chance of accidentally killing animals that were actually alive in the process of poking them to stimulate movement. Even in isogenic *C. elegans* populations in identical conditions housed on a single plate, there may be substantial variability between the movement states of different animals, represented as the color gradients of the bars. **(B)** Simulated error probability curves shown in relation to the generating logistic distribution (black solid curve) for single-sample experiments (mean/median = 20 and $s = 2$). Error rates were simulated at a fixed probability or as a function of time. Constant error rates were set at either 2, 4, or 10% (dotted lines). Modeling error as a function of time more closely mimics the increased likelihood that an experimenter will make a mistake as animals become paralyzed or fragile with age. The hazard function from the logistic distribution is used to increase the error probability as animals age, starting from either mid-life (dashed lines) or late-life (solid colored lines) reaching the maximum (2, 4, or 10%) near the end of life. The constant error and 10% error scenarios are not intended as a simulation of likely error rates during actual experiments, but serve to demonstrate the degree to which RSM improves robustness to accidental scoring and handling errors under extreme conditions. **(C–H)** Median lifespan across 10,000 simulated RSM and TLM trials for the indicated error models and analysis treatments, with a sample size of 20 animals for RSM (per observation) and 110 for TLM (per trial), and assuming daily scoring. The orange dashed line indicates the mean/median of the generating logistic distribution (20 days). **(C)** No simulated error. **(D)** Constant probability of mis-scoring. **(E,F)** Mis-scoring with probability modeled as function of time, rising from 0% to the indicated maximum rate starting in mid-life **(E)** or late-life **(F)**. **(G,H)** Simulated accidental death, in which an animal which was actually alive is accidentally killed by rough handling and subsequently called as dead, with probability modeled as function of time, rising from 0% to the indicated maximum rate starting in mid-life **(G)** or late-life **(H)**.

Age-Associated Fragility Hazard Simulation

During aging, *C. elegans* become increasingly fragile, making them more susceptible to injury by physical handling. As such, an intrusive method of scoring vital status could damage or possibly kill older animals, affecting their survival. Typically, older animals are gently prodded with the end of a platinum wire to induce movement, which could damage or kill those animals if too much force is accidentally applied. Therefore, we introduced an increasing probability of "killing" the animal in the process of scoring, from 0% and increasing to a 2, 4, or 10% starting from either mid-life (**Figure 6B**, dashed lines) or late-life (**Figure 6B**, solid colored lines). We found that introducing even a small probability of investigator-induced accidental death of an animal late in life, such that only one in 25 animals is affected (4%), resulted in a decrease in estimated median lifespan by 1.3% when followed through the TLM (parametric analysis); in contrast, the RSM estimated lifespan decreased by only 0.4 and 0.97% for a late life scoring hazard of 4 and 10%, respectively (**Figure 6H**, **Supplementary Table S3**). As expected, if the onset of frailty- and the associated increase in hazard-is shifted to mid-life, the TLM again yields dramatically shorter lifespan estimates, from 19.38 days with 2% hazard rate to 17.29 days with 10% hazard rate, compared to 19.33 days for RSM even at the 10% rate (**Figure 6G**, **Supplementary Table S3**). Thus, the lifespan estimates of a population of *C. elegans* determined through the TLM is much more prone to error than the RSM if the process of scoring vital status of an animal impairs survival.

RSM Requires Fewer Total Animal Observations to Obtain Adequate Statistical Power in Comparing Lifespan Across Conditions

In aging research, comparative lifespan has been widely used between many types of conditions to ascertain if a statistically significant change has occurred between conditions (e.g. mutant versus wild-type animals, et. cetera). In order to determine how the TLM and RSM approaches influence the power of detection between two samples, we performed additional simulations, varying the median of the generating distributions (−20% to +20% in 0.1 day increments with respect to a reference population with median survival of 20 days) across a range of sample sizes (5–50 animals for RSM, and 5 to 150 animals for TLM) with daily scoring. We calculated power assuming an alpha of 0.01 after computing *p*-values for the comparison of 100 simulated trials against the reference population with all other conditions held constant, using the log-rank test and label permutation for non-parametric and parametric analyses, respectively. When we focus on the sample sizes previously determined to yield similar accuracy and precision between the assays for single-sample estimates of lifespan (**Figure 3**)- 110 animals per trial for TLM, and 20 animals per observation for RSM- we find that statistical power is also broadly comparable for the assays and analysis approaches across the range of effect sizes tested (**Figure 7A**). To see how the TLM and RSM differ in the amount of investigator effort necessary to achieve sufficient statistical power for a moderate difference in lifespan, we looked at the number of total animal observations per trial when the effect size was held at+/-10% (**Figure 7C**). The RSM experiments actually require fewer than half the number of total observations as the TLM to reach a power of 1, indicating that a corrected *p*-value < 0.01 was obtained for all 100 trial comparisons. We next repeated these comparisons, but with experiments for which mis-scoring error had been simulated, to determine how mistakes in scoring could end up influencing statistical power differently depending on the chosen type of lifespan assay. We see that even with a moderate rate of mis-scoring-up to a rate of 4% rising from late-life (**Figure 6B**, solid orange curve)- RSM power of detection remains robust, again with many fewer total observations necessary than TLM (**Supplementary Figure S2**). Overall, this corroborates our empirical experience with RSM enabling more conditions to be scored in the same amount of time as a given TLM experiment, without compromising the interpretability or utility of the results.

DISCUSSION

The Replica Set Method is Distinct From the Traditional Longitudinal Method

We demonstrate that the TLM approach for assessing lifespan is less accurate and precise, as well as less resilient to several forms of error compared to the RSM. Furthermore, the RSM yields comparable statistical power with many fewer necessary total observations. This arises from the fundamental difference in their physical setup, and the statistical implications of the structure of each method. The survival observed at any time point using the TLM is dependent on the survival observed at each previous point. In contrast, observations of animal survival in the RSM are completely independent. It is this independence that helps reduce both intrinsic and extrinsic variability in the RSM.

We find that the intrinsic variability, accuracy, and statistical power of an TLM experiment with a sample size of 110 is comparable that of the RSM with a sample size of 20. Taking these values as an example, if one were to sum the total number of times the vital status of any animal is determined over the course of an experiment by both methods, with daily scoring, the RSM would generate about 600 observations and the TLM would produce about 2300 observations (**Figure 3**). However, in the RSM viability of 600 distinct animals is measured, while the TLM would only have measured mortality of 110 unique animals. Consequently, the RSM measures more individual animals, while the TLM makes more total observations. These two parameters have different weights between the two methods: a single additional animal at the start of a TLM experiment adds more observations than a single animal added to an observation of an RSM experiment. In contrast, increasing

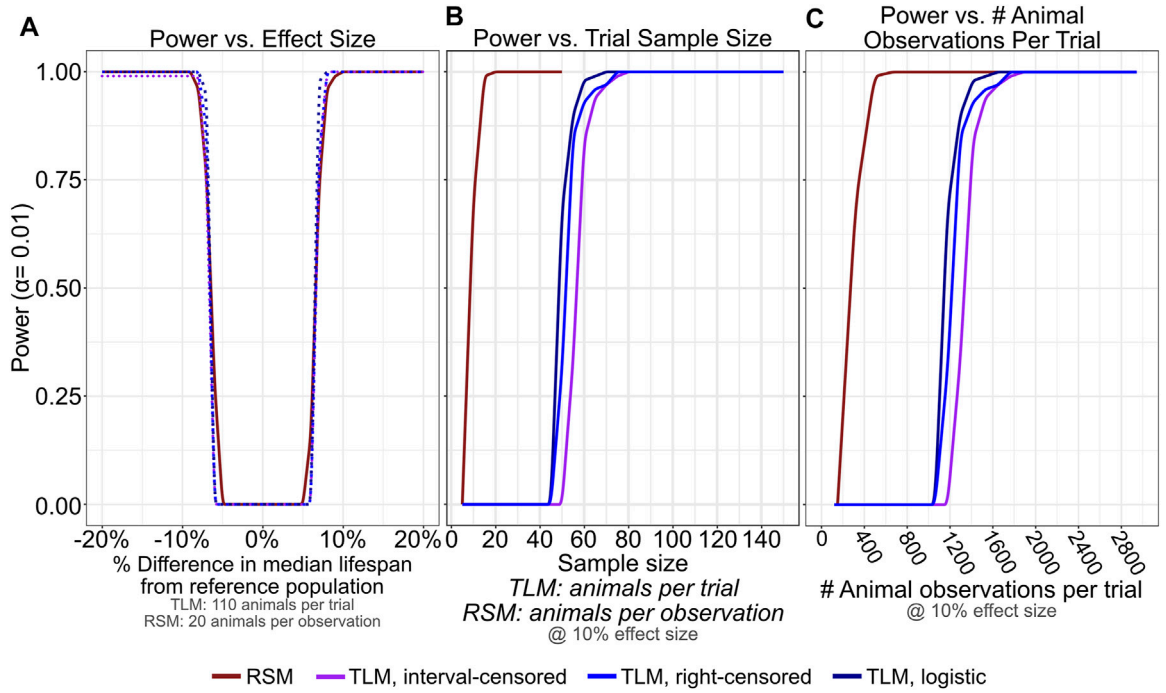

FIGURE 7 | RSM provides similar statistical detection power to TLM, with many fewer necessary animal observations. 100 lifespan experiment trials were simulated for RSM and TLM each for every combination of sample size (5–50 per observation for RSM, and 2–150 per trial for TLM, both in increments of 5) and generating distribution mean/medians from 16—24 days (in increments of 0.1 days), all assuming daily scoring. For a given sample size and assay/analysis type, each trial was compared using an appropriate testing strategy against a reference population generated from a distribution with mean/median of 20 days. *p*-values determined from these tests were corrected for multiple testing (FDR) within the set of likewise trials and used to calculate power at alpha level of 0.01. **(A)** Statistical detection power is comparable across RSM and all analysis treatments of TLM for a broad range of effect sizes when considering 20 animals per observation for RSM and 110 animals per trial for TLM. **(B,C)** For an effect size of 10% (+/- 2 days compared to the reference population) RSM saturates power at a number of animals per observation **(B)** that corresponds to a far smaller number of total animal observations per trial compared to TLM **(C)**. The analysis treatments of TLM experiments all perform similarly when the scoring interval is held constant.

sample size by one animal produces not only more observations in the RSM, but each of those added measures is more informative as each observation is independent. This asymmetry helps explain why increasing sample size reduces RSM variability- and increases statistical power- more quickly than it would with TLM. Thus, real TLM and RSM data with a sample size of 110 and 20 animals, respectively, have similar precision in estimating lifespan.

The Traditional Method Suffers Bias due to Right-Censoring

An artificial increase in estimated lifespan as a function of the scoring interval has been previously observed for *C. elegans* TLM experiments using simulation (Petrascheck and Miller, 2017). In concordance with their finding, we showed that the TLM has an intrinsic bias that increases estimated mean and median lifespan by approximately one half of the scoring period (e.g. bias of 0.5 days for once-daily scoring) (**Figures 4A,B**). This is due to scoring frequency: daily or alternate day scoring makes a discrete, periodic measurement of survival. However, survival is a continuous phenotype, thus the time between actual and observed death creates a bias, with magnitude directly proportional to the time between survival measurements. This is not evident when observation intervals are dramatically shortened- as in the case of automated experiment platforms such as the Lifespan Machine (Stroustrup et al., 2013)- or in studies involving longer-lived organisms where the scoring precision is very fine relative to the total lifespan.

Importantly, we find that analysis of the same experiment data non-parametrically but with interval censoring, or parametrically by logistic curve fitting, abrogates the right-censoring-induced bias. Rather than assuming an event occurred at the time of observation as with right-censoring, interval-censoring places the time of death in the middle of the interval between observations (Gomez et al., 2009; Zhang and Sun, 2010). Our simulations show very little mean or median bias when interval censoring is applied, up to a scoring interval of 3 days, which we find is on the upper end of the interval actually used across a range of *C. elegans* survival studies (**Figure 2B**). Fitting the same simulated TLM experiments to logistic curves- similar to the analysis approach taken with RSM data- also avoids inducing the bias we observe in the right-censored non-parametric analysis (**Figures 4A,B**). As the bias represents a shift in the mean and median survival estimates, the dispersion of the data is not affected, and there is minimal effect on statistical power between right-censoring and interval-censoring when

comparing groups where observations were made with the same observation schedule (**Figures 7A,B**). In contrast, if the groups compared were observed at variable intervals ("every 2–3 days"), or if scoring was performed at different intervals between the groups (*e.g.* every day for group 1, alternate days for group 2), this right-censoring bias would influence the results, possibly producing false positive or negative conclusions. Thus, it is critical to consider interval censoring or parametric analysis when designing experiments or reviewing disparate experimental approaches. In our literature survey of 67 papers including N2 lifespan experiments, we found no cases where interval censoring was utilized (**Supplementary Table S1**), despite support for such analysis being available in some common statistical software packages such as SPSS, STATA, JMP, and R (**Supplementary Table S2**). Similarly, in considering the information on analysis software collected from 824 publications including N2 lifespan experiments in a recent meta-analysis (Urban et al., 2021), more than 40% of publications used software that does not presently support interval censoring- much of which is accounted for by Graphpad Prism alone- an indication that there is not yet awareness of the benefits of interval censoring for *C. elegans* survival studies.

In contrast to the TLM, the RSM makes a discrete measure of a discrete phenotype: each day an independent sample of individuals is counted, and each animal is either alive or dead at this time. With RSM we are indifferent to the timing of death outside of this single time point; instead we measure proportional survival, a quantity unbiased by scoring time.

The Traditional Method is Less Resilient to Error

The TLM is less resilient then the RSM in accurately estimating mean and median lifespan of a population when even a small probability of incorrectly scoring viability later in life is introduced. The difference in response to scoring error stems entirely from the fact that dead animals are removed from the plate once observed in TLM. If a live animal is incorrectly scored as dead, its lifespan is artificially shortened at least one day, but potentially more as individual decrepit and frail animals can remain alive for an extended period of time (Johnson, 1987; Herndon et al., 2002). Conversely, if a dead animal is accidentally scored as live, it is likely to be properly scored dead at the next time point. Thus, TLM scoring error is heavily biased towards death. In contrast, the RSM measures an individual animal once, thus incorrectly scoring viability affects only a single observation, and is consequently more robust to such bias.

In addition to the increasing difficulty with age in determining whether an animal is alive or dead, aging *C. elegans* become increasingly fragile. Touching animals with a platinum wire and looking for movement is a common technique for applying a stimulus intended to cause a locomotory response in animals that are alive but not otherwise moving. Loss of touch responsiveness to light touch in aged animals makes scoring viability more difficult, and may promote the use of a more forceful touch stimuli that kills a decrepit animal. While it is difficult to know the actual hazard rate that occurs from poking animals without a non-damaging approach to ascertain true live/dead status, for simulation we chose maximal hazard rates of 1 in 50 (2%), 1 in 25 (4%), and 1 in 10 (10%) intuitively (**Figures 6G,H**). The actual rate of animal death from damage due to touch will be highly dependent on the individual conducting the experiment. Surprisingly, when we apply a fragility hazard rising with time from around mid-life, the median lifespan from the TLM is decreased by 3.1% compared to 0.7% for RSM at a maximum of 2% error, and up to 15% for TLM compared to just 3.3% for RSM at a maximum of 10% error, when both assays were treated parametrically (**Figure 6G**). An alternative touch response approach to using a typical platinum wire would be to use an eyelash or similar fiber, which is less damaging to aged animals (Sulston and Horvitz, 1977). If a live animal is killed by touching it in the process of stimulating movement, in the TLM the error propagates to affect multiple measurements. Importantly, like mis-scoring, the RSM is also resilient towards this sort of error. This makes sense intuitively, as the scoring hazard does not accumulate. Collectively, the independence of individual measurements in the RSM insulates it from errors introduced by incorrect scoring or repeated handling of increasingly fragile animals.

Limitations of Both Methods for Assessing Lifespan

The TLM and RSM for scoring lifespan have distinct limitations. For instance, throughput is a major limitation to the TLM; scoring a sample by the RSM can be done much faster, for several major reasons. First, addition of liquid to the wells in the RSM acts as a mild stimulus that causes animals to swim, which aids in discriminating live animals from dead ones. Furthermore, sarcopenia in older animals limits movement particularly in the viscous bacterial lawn, and the addition of liquid in the RSM scoring approach helps to liberate the animals. Second, as the RSM insulates the experimenter from propagating scoring errors, it allows one to more quickly make decisions on whether an individual animal is alive or dead without concern of how a mistake would influence the rest of the experiment. Third, each well of the multi-well plates used for RSM are small, such that the entire well can fit in a microscope field of view, which makes for faster tracking of the whole population. In contrast, the Petri-style plates commonly used for TLM are too large to view the entire population at one time, which requires scanning the whole plate. While RSM experiments can involve a large number of plates, the required initial setup time may be reduced by employing common laboratory aids such as multi-well electronic pipettes. If incubator space is found to be limiting, particularly relevant for experiments in long-lived mutant backgrounds or where an intervention is expected to extend lifespan, the number of plates may be reduced by planning to score less frequently until signs of aging- such as movement decline- are observed. It is worth noting that specific equipment and software to facilitate the use of the RSM can be found described in: (Cornwell et al., 2018; Cornwell and Samuelson, 2020). A limitation inherent to the RSM is that progeny production must be prevented: either through the use of fluorodeoxyuridine (FUdR), which in some cases produces lifespan phenotypes that can confound analysis (Aitlhadj and Stürzenbaum, 2010; Van Raamsdonk and Hekimi, 2011), or sterile genetic mutants. However, some genetic interactions that

influence lifespan are only revealed when progeny production is actively inhibited. For example, it was long thought that the TGFβ pathway did not regulate *C. elegans* aging (Kenyon et al., 1993; Larsen et al., 1995), however, the use of FUdR revealed that TGFβ signaling regulates longevity through insulin signaling (Shaw et al., 2007). The link between TGFβ signaling and longevity was missed without the use of FUdR, as TGFβ pathway mutations produce a slight egg laying defect (*egl*) and extend reproductive longevity, which causes internal hatching of progeny later in life that kills the parent prematurely. Of note, starved wild-type animals also manifest an *egl* phenotype, perhaps as an adaptive survival advantage to progeny under conditions of low food (Baugh and Hu, 2020); this has implications for studies on the genetics of dietary restriction (DR), which may be complicated by overlap between models of DR and starvation responses (Mair and Dillin, 2008). Thus, using either a chemical inhibitor or genetic mutant to limit progeny production versus manually transferring animals are both valid; each has advantages and disadvantages, and is one aspect to consider when choosing a methodology.

The type and amount of the bacterial food source is another important consideration in planning either type of lifespan experiment. Starvation must be avoided for accurate assessment of lifespan (Johnson et al., 1984), which is typically managed by adjusting the bacterial volume added to a well or plate, the number of animals added, and by concentrating bacterial cultures if necessary. While using FUdR to inhibit reproduction obviates the need to transfer parents away from progeny that will quickly deplete the available food, it is still possible to accidentally distribute too little bacterial culture or too many animals to a given plate or well, making for a higher probability of a single well "starving out". In TLM, this is avoided by keeping spare plates prepared at the same time as the others, but without animals added, onto which the animals are manually transferred from the at-risk plate- a process which is prone to error, particularly when animals are aged and fragile, and can introduce contamination. For RSM, this is avoided by preparing extra replicate plates at the outset of the experiment- which also have animals added like all other plates in the set- which can be scored as necessary to replace a plate with starved wells (Cornwell et al., 2018; Cornwell and Samuelson, 2020). Starved wells are easy to identify and are censored. Additionally, the common bacterial food sources used in *C. elegans* culture have been found to differ in nutritional composition and influence developmental rate and lifespan of *C. elegans* (Stuhr and Curran, 2020). Thus, with either lifespan method care must be taken to ensure consistency in food source (*e.g.* strain and concentration), and proactive preparation of spare plates or replicates will reduce the possibility of having to discard or restart a trial due to starvation.

TLM, RSM, and Lifespan Assay Automation

Improvements in affordable imaging hardware as well as machine learning have led to an increasing number of options for automating *C. elegans* longitudinal assays such as lifespan. Most such systems utilize cameras and record images at intervals or video, and use software to identify animals and determine the time at which movement ceases. Present approaches represent different positions across a balance between throughput and detail on the activity of individual animals: full-motion video is necessary to reconstruct movement tracks or record activity levels across the entire adult life of animals, but these systems are difficult to scale to thousands of animals or conditions (*e.g.* the Nemalife Infinity system, SiViS), while periodic imaging is suitable for constructing a time-lapse across life of many more animals or conditions, but is not able to track the movement path of individual animals early in life when used with multiple plates (*e.g.* the Lifespan Machine, WormBot) (Stroustrup et al., 2013; Pitt et al., 2019; Rahman et al., 2020; Puchalt et al., 2021). Others utilize alternative experiment or measurement paradigms in order to approach the middle of the balance, such as the Phylum Arena system (PhylumTech, Santa Fé, Argentina), which uses an array of infrared microbeams to detect movement of animals rather than traditional imaging, and WorMotel (Jushaj et al., 2020) which is based on singling animals in microplate-sized wells, thus removing the need for continuous video to build a life history of activity for individual animals. While all of these systems track the same population of animals across time, the scoring approaches are passive, nondestructive, and generally obviate the need to re-expose animals to the environment after the start of the experiment, thereby reducing exposure to airborne contaminants similar to RSM experiments. However, mechanically stimulating movement- as with adding liquid to a well in RSM or poking with a wire- may be advantageous when working with animals harboring mutations that increase the time spent in a behaviorally quiescent sleep-like state, such as *daf-2* mutants, which, if unstimulated, can cease movement for periods long enough to be called as dead by the default movement analysis models that are bundled with the Lifespan Machine software, in our experience (Gaglia and Kenyon, 2009; Stroustrup et al., 2013). These hardware platforms may also be unsuitable for chronic heat tolerance assays, as this could require elevating the temperature of the surrounding environment or enclosure and thus also the instruments themselves. The "set it and forget it" nature may also lead to mistakes that are not caught until the planned end of the experiment- starvation, progeny, contamination, *etc*- unless the researcher checks for anomalies periodically. Large-scale automation of lifespan experiments still involves substantial upfront costs, such that RSM remains an attractive option for applications such as RNAi library screening across large gene families or across the genome. Even when automated approaches are available, investigators might consider running a manually-scored TLM or RSM trial alongside the automated experiment, thereby acquiring two trials worth of data, with the hands-on time of just one.

Statistical Analysis of the Traditional Longitudinal and Replica Set Methods

At the completion of a lifespan experiment, it is important to determine the mean, median, and maximum lifespan within a sample, as well as to identify significant differences in lifespan

between conditions of interest. For TLM experiments, summary statistics of lifespan can be estimated using the Kaplan-Meier estimator. The Kaplan-Meier estimator is a non-parametric statistical estimator frequently applied to lifespan data because it provides a number of benefits for survival analysis applicable to many studies in research and clinical settings (Kaplan and Meier, 1958). First, the Kaplan-Meier estimator accounts for one-sided censoring of animals; censoring is important in situations where an animal must be removed from study (*i.e.* died for reasons other than aging). For instance, in the case of *C. elegans,* animals that crawl up the side of the plastic dish will desiccate and die. In these cases, it is known approximately how long the animal lived before the event that warranted censoring, but when the animal would eventually have died due to aging is unknown. The non-parametric nature of Kaplan-Meier removes the necessity of verifying that the data is appropriate for a given distribution- there are very few assumptions to satisfy. However, parametric survival functions- which require ensuring that the data is a good fit to the chosen distribution (often Gompertz, logistic, or Weibull)- are also available for longitudinal lifespan data, and may be more accurate for cases when the surviving fraction approaches zero at the termination of the study (Miller, 1983; Wilson, 1994; Mudholkar et al., 1996).

To determine whether there are significant differences in lifespan between two populations when using Kaplan-Meier analysis, the Mantel-Cox log-rank test is applied (Mantel, 1966). This statistical test has been a standard within the field for many years and allows one to identify significant differences in mean/maximum lifespan. The log-rank test is non-parametric, and takes censoring into account (Bland and Altman, 2004). A popular alternative to the log-rank test is the Wilcoxon test, which is also non-parametric, but whereas log-rank has been found to be biased toward differences near the end of the experiment timeline (*i.e.* termination of the study), Wilcoxon can be biased toward differences at the beginning (Tarone, 1981).

The RSM yields current status data, representing a form of interval censoring in which every data point is either left-censored (for animals that are dead at time of observation) or right-censored (for animals that are alive at time of observation). As the basic Kaplan-Meier estimator does not handle current status data, calculation of mean, median, and maximal lifespan of data generated through the replica set method must be determined by other methods. While non-parametric methods for handling current status data have been described and implemented, this remains an active area of development. In particular, the nonparametric maximum likelihood estimate (NPMLE) (Gomez et al., 2009) as implemented in the R packages "Interval" (Fay and Shaw, 2010) or "icenReg" (Anderson-Bergman, 2017) may provide a basis for nonparametric analysis of RSM data. For the present work, we chose to model this data parametrically using the logistic distribution, as *C. elegans* lifespan has previously been shown to fit this distribution well (Vanfleteren et al., 1998). Consistently, we have also previously found that large lifespan datasets derived from the RSM fit best to a logistic model, but can also fit a Gompertz distribution (Samuelson et al., 2007b; 2007a), the latter of which is frequently used for demographic analysis of mortality in mammals (Finch and Pike, 1996). Parametric and nonparametric methods of survival analysis have been shown to perform similarly when the data is a good fit for the chosen distribution (Efron, 1988).

Identifying significant differences in lifespan between two populations through the RSM is determined by first fitting the data from a given condition to a logistic model. The median lifespan values are calculated from these model fits; logistic curves are symmetrical about their inflection point, such that the median is equal to the mean (Winsor, 1932). The *p*-values for the desired comparison are then computed using a Monte Carlo resampling approach using 10,000 iterations to obtain precise *p*-values (Robinson, 2007; Phipson and Smyth, 2010). Confidence intervals for the median lifespan values can also be derived from the Monte Carlo simulation. Although these results can take some time to compute for a large number of comparisons, it is trivial to parallelize for significant speedup on most modern computers.

Our analysis of power to detect differences for traditional lifespan is concordant with previous findings using simulations and non-parametric analysis (Petrascheck and Miller, 2017). Additionally, both studies highlight the importance of adequate sample size for detection of a given magnitude difference in lifespan for traditional longitudinal experiments, and identify that accuracy is compromised with longer scoring intervals in traditional experiments. We further highlight that scoring frequency can influence accuracy and precision in determining mean and median lifespan, and that analysis of TLM experiments with interval censoring may produce estimates of longevity that are more comparable between studies. Importantly, we show that Replica Set experiments require relatively few animals per observation to obtain similar or better detection power compared to TLM experiments (**Figure 7**). Furthermore, our results demonstrate that scoring errors- simulating an incorrect assessment of animal vital status or accidentally damaging fragile aged animals during scoring- even at low probability and introduced late within an experiment, can drastically influence lifespan estimates for TLM assays.

We demonstrate that the RSM is more accurate, precise, and robust to error then the TLM for estimating lifespan in *C. elegans*. A lack of repeated handling reduces exposure to airborne contaminants, and removes possible error from prodding of increasingly fragile aging animals. Furthermore, the RSM is amenable to measuring lifespan at high throughput and at low cost. Lifespan of several hundred test conditions (e.g. feeding based RNAi) can be simultaneously followed, allowing one to hasten the discovery of genetic interactions and pathways (Samuelson et al., 2007a). Furthermore, when applying the RSM to *C. elegans*, liquid is added to the well at the time of scoring, which not only increases scoring accuracy, but also provides a stimulus for spontaneous movement, reducing the need for time-consuming manual touch stimulation (Cornwell et al., 2018; Cornwell and Samuelson, 2020). Through video capture of thrashing/swimming *C. elegans* it is possible to quantify activity, using pixel displacement (A.V.S. unpublished observations), to obtain an estimate of healthspan (i.e. the period of life that animals actively respond to stimuli, see (Samuelson et al., 2007a)). Thus the RSM can easily and simultaneously track both survival and health during aging. Collectively this study highlights the limitations and pitfalls of the TLM for measuring lifespan of *C. elegans* and provides, in the RSM, an accurate, precise, high-throughput alternative that is less susceptible to common types of experimental errors.

DATA AVAILABILITY STATEMENT

The R source code and simulated datasets used for this study will be made available at https://github.com/samuelsonlab-urmc or from the authors by reasonable request.

AUTHOR CONTRIBUTIONS

AC conceived and performed experiments, analyzed results, developed software, and wrote the manuscript. JL conceived and performed experiments, analyzed results, developed software, and wrote the manuscript. PS conceived experiments and analyzed results related to statistical analysis of simulations. NR gathered and analyzed the details on published *C. elegans* lifespan experiments. JT analyzed results and wrote the manuscript. AVS conceived the experiments, analyzed results, and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fragi.2022.861701/full#supplementary-material

Supplementary Figure S1 | N2 (WT) lifespan experiment data examples for RSM, TLM, and an automated lifespan platform (the "Lifespan Machine").

Supplementary Figure S2 | Even with moderate rates of mis-scoring, RSM provides statistical power of detection similar to TLM, with many fewer total animal observations necessary.

Supplementary Figure S3 | RSM is more robust than TLM to errors from incorrect scoring of survival in in simulated experiments based on long-lived animals.

Supplementary Table S1 | *C. elegans* lifespan assay literature survey.

Supplementary Table S2 | A survey of statistical software supporting lifespan analysis.

Supplementary Table S3 | Summary values (median and mean) across single-sample simulated lifespan experiments.

REFERENCES

Aitlhadj, L., and Stürzenbaum, S. R. (2010). The Use of FUdR Can Cause Prolonged Longevity in Mutant Nematodes. *Mech. Ageing Development* 131, 364–365. doi:10.1016/j.mad.2010.03.002

Anderson-Bergman, C. (2017). icenReg: Regression Models for Interval Censored Data in R. *J. Stat. Soft.* 81. doi:10.18637/jss.v081.i12

Bansal, A., Zhu, L. J., Yen, K., and Tissenbaum, H. A. (2015). Uncoupling Lifespan and Healthspan in *Caenorhabditis elegans* Longevity Mutants. *Proc. Natl. Acad. Sci. U.S.A.* 112, E277–E286. doi:10.1073/pnas.1412192112

Baugh, L. R., and Hu, P. J. (2020). Starvation Responses throughout the caenorhabditis Elegans Life Cycle. *Genetics* 216, 837–878. doi:10.1534/genetics.120.303565

Bengtsson, H. (2021). A Unifying Framework for Parallel and Distributed Processing in R Using Futures. *R. J.* 13, 208. doi:10.32614/rj-2021-048

Benjamin, G. (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.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B (Methodological)* 57, 289–300. doi:10.1111/j.2517-6161.1995.tb02031.x

Bland, J. M., and Altman, D. G. (2004). The Logrank Test. *BMJ* 328, 1073. doi:10.1136/bmj.328.7447.1073

Bolanowski, M. A., Russell, R. L., and Jacobson, L. A. (1981). Quantitative Measures of Aging in the Nematode *Caenorhabditis elegans*. I. Population and Longitudinal Studies of Two Behavioral Parameters. *Mech. Ageing Dev.* 15, 279–295. doi:10.1016/0047-6374(81)90136-6

Brooks, A., Lithgow, G. J., Johnson, T. E., and Lithgow, G. J. (1994). Mortality Rates in a Genetically Heterogeneous Population of *Caenorhabditis elegans*. *Science* 263, 668–671. doi:10.1126/science.8303273

Cornwell, A. B., Llop, J. R., Salzman, P., Thakar, J., and Samuelson, A. V (2018). The Replica Set Method: A High-Throughput Approach to Quantitatively Measure *Caenorhabditis elegans* Lifespan. *Jove J. Vis. Exp.* 29, e57819. doi:10.3791/57819

Cornwell, A. B., and Samuelson, A. V. (2020). "Analysis of Lifespan in *C. elegans*: Low- and High-Throughput Approaches. *Methods Mol. Biol.* 2144, 7–27. doi:10.1007/978-1-0716-0592-9_2

De Magalhaes Filho, C. D., Henriquez, B., Seah, N. E., Evans, R. M., Lapierre, L. R., and Dillin, A. (2018). Visible Light Reduces *C. elegans* Longevity. *Nat. Commun.* 9. doi:10.1038/s41467-018-02934-5

Duhon, S. A., and Johnson, T. E. (1995). Movement as an index of vitality: Comparing Wild Type and the Age-1 Mutant of *Caenorhabditis elegans*. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 50A, B254–B261. doi:10.1093/gerona/50A.5.B254

Dwass, M. (1957). Modified Randomization Tests for Nonparametric Hypotheses. *Ann. Math. Statist.* 28, 181–187. doi:10.1214/aoms/1177707045

Efron, B. (1988). Logistic Regression, Survival Analysis, and the Kaplan-Meier Curve. *J. Am. Stat. Assoc.* 83, 414–425. doi:10.1080/01621459.1988.10478612

Fay, M. P., and Shaw, P. A. (2010). Exact and Asymptotic Weighted Logrank Tests for Interval Censored Data: TheintervalRPackage. *J. Stat. Soft.* 36, 1–34. doi:10.18637/jss.v036.i02

Finch, C. E., and Pike, M. C. (1996). Maximum Life Span Predictions from the Gompertz Mortality Model. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 51A, B183–B194. doi:10.1093/gerona/51A.3.B183

Finch, C. E., Pike, M. C., and Witten, M. (1990). Slow Mortality Rate Accelerations during Aging in Some Animals Approximate that of Humans. *Science* 249, 902–905. doi:10.1126/science.2392680

Finkelstein, D. M. (1986). A Proportional Hazards Model for Interval-Censored Failure Time Data. *Biometrics* 42, 845–854. doi:10.2307/2530698

Gaglia, M. M., and Kenyon, C. (2009). Stimulation of Movement in a Quiescent, Hibernation-like Form of *Caenorhabditis elegans* by Dopamine Signaling. *J. Neurosci.* 29, 7302–7314. doi:10.1523/JNEUROSCI.3429-08.2009

Gems, D., and Riddle, D. L. (2000). Defining Wild-type Life Span in *Caenorhabditis elegans*. *J. Gerontol. A. Biol. Sci. Med. Sci.* 55, B215–B219. doi:10.1093/gerona/55.5.b215

Gems, D., and Riddle, D. L. (1996). Longevity in *Caenorhabditis elegans* Reduced by Mating but Not Gamete Production. *Nature* 379, 723–725. doi:10.1038/379723a0

Gems, D., Sutton, A. J., Sundermeyer, M. L., Albert, P. S., King, K. V., Edgley, M. L., 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

Gerstbrein, B., Stamatas, G., Kollias, N., and Driscoll, M. (2005). *In Vivo* spectrofluorimetry Reveals Endogenous Biomarkers that Report Healthspan and Dietary Restriction in *Caenorhabditis elegans*. *Aging Cell* 4, 127–137. doi:10.1111/j.1474-9726.2005.00153.x

Glenn, C. F., Chow, D. K., David, L., Cooke, C. A., Gami, M. S., Iser, W. B., et al. (2004). Behavioral Deficits during Early Stages of Aging in *Caenorhabditis elegans* Result from Locomotory Deficits Possibly Linked to Muscle Frailty. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 59, 1251–1260. doi:10.1093/gerona/59.12.1251

Gómez, G., Calle, M. L., Oller, R., and Langohr, K. (2009). Tutorial on Methods for Interval-Censored Data and Their Implementation in R. *Stat. Model.* 9, 259–297. doi:10.1177/1471082X0900900402

Good, P. I. (2006). *Resampling Methods: A Practical Guide to Data Analysis*. doi:10.1007/0-8176-4444-X

Hahm, J.-H., Kim, S., DiLoreto, R., Shi, C., Lee, S.-J. V., Murphy, C. T., et al. (2015). *C. elegans* Maximum Velocity Correlates with Healthspan and Is Maintained in Worms with an Insulin Receptor Mutation. *Nat. Commun.* 6, 8919. doi:10.1038/ncomms9919

Hamilton, B., Dong, Y., Shindo, M., Liu, W., Odell, I., Ruvkun, G., et al. (2005). A Systematic RNAi Screen for Longevity Genes in *C. elegans*. *Genes Dev.* 19, 1544–1555. doi:10.1101/gad.1308205

Hansen, M., Hsu, A.-L., Dillin, A., and Kenyon, C. (2005). New Genes Tied to Endocrine, Metabolic, and Dietary Regulation of Lifespan from a *Caenorhabditis elegans* Genomic RNAi Screen. *Plos Genet.* 1, e17–0128. doi:10.1371/journal.pgen.0010017

Harris, T. W., Antoshechkin, I., Bieri, T., Blasiar, D., Chan, J., Chen, W. J., et al. (2009). Wormbase: A Comprehensive Resource for Nematode Research. *Nucleic Acids Res.* 38, D463–D467. doi:10.1093/nar/gkp952

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

Hosono, R., Sato, Y., Aizawa, S. I., and Mitsui, Y. (1980). Age-dependent Changes in Mobility and Separation of the Nematode *Caenorhabditis elegans*. *Exp. Gerontol.* 15, 285–289. doi:10.1016/0531-5565(80)90032-7

Huang, C., Xiong, C., and Kornfeld, K. (2004). Measurements of Age-Related Changes of Physiological Processes that Predict Lifespan of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8084–8089. doi:10.1073/pnas.0400848101

Johnson, D. W., Llop, J. R., Farrell, S. F., Yuan, J., Stolzenburg, L. R., and Samuelson, A. V. (2014). The *Caenorhabditis elegans* Myc-Mondo/Mad Complexes Integrate Diverse Longevity Signals. *Plos Genet.* 10, e1004278. doi:10.1371/journal.pgen.1004278

Johnson, T. E., De Castro, E., Hegi de Castro, S., Cypser, J., Henderson, S., and Tedesco, P. (2001). Relationship between Increased Longevity and Stress Resistance as Assessed through Gerontogene Mutations in *Caenorhabditis elegans*. *Exp. Gerontol.* 36, 1609–1617. doi:10.1016/S0531-5565(01)00144-9

Johnson, T. E. (1987). Aging Can Be Genetically Dissected into Component Processes Using Long-Lived Lines of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 84, 3777–3781. doi:10.1073/pnas.84.11.3777

Johnson, T. E., Mitchell, D. H., Kline, S., Kemal, R., and Foy, J. (1984). Arresting Development Arrests Aging in the Nematode *Caenorhabditis elegans*. *Mech. Ageing Development* 28, 23–40. doi:10.1016/0047-6374(84)90150-7

Jushaj, A., Churgin, M., Yao, B., De La Torre, M., Fang-Yen, C., and Temmerman, L. (2020). Optimized Criteria for Locomotion-Based Healthspan Evaluation in *C. elegans* Using the WorMotel System. *PLoS One* 15, e0229583. doi:10.1371/journal.pone.0229583

Kaplan, E. L., and Meier, P. (1958). Nonparametric Estimation from Incomplete Observations. *J. Am. Stat. Assoc.* 53, 457–481. doi:10.2307/228186810.1080/01621459.1958.10501452

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

Larsen, P. L., Albert, P. S., and Riddle, D. L. (1995). Genes that Regulate Both Development and Longevity in *Caenorhabditis elegans*. *Genetics* 139, 1567–1583. doi:10.1093/genetics/139.4.1567

Lee, S. S., Lee, R. Y. N., Fraser, A. G., Kamath, R. S., Ahringer, J., and Ruvkun, G. (2003). A Systematic RNAi Screen Identifies a Critical Role for Mitochondria in *C. elegans* Longevity. *Nat. Genet.* 33, 40–48. doi:10.1038/ng1056

Mair, W., and Dillin, A. (2008). Aging and Survival: The Genetics of Life Span Extension by Dietary Restriction. *Annu. Rev. Biochem.* 77, 727–754. doi:10.1146/annurev.biochem.77.061206.171059

Mantel, N. (1966). Evaluation of Survival Data and Two New Rank Order Statistics Arising in its Consideration. *Cancer Chemother. Rep.* 50, 163–170.

Miller, R. G. (1983). What Price Kaplan-Meier? *Biometrics* 39, 1077. doi:10.2307/2531341

Mudholkar, G. S., Srivastava, D. K., and Kollia, G. D. (1996). A Generalization of the Weibull Distribution with Application to the Analysis of Survival Data. *J. Am. Stat. Assoc.* 91, 1575–1583. doi:10.1080/01621459.1996.10476725

Petrascheck, M., and Miller, D. L. (2017). Computational Analysis of Lifespan Experiment Reproducibility. *Front. Genet.* 8. doi:10.3389/fgene.2017.00092

Phipson, B., and Smyth, G. K. (2010). Permutation P-Values Should Never Be Zero: Calculating Exact P-Values when Permutations Are Randomly Drawn. *Stat. Appl. Genet. Mol. Biol.* 9, 39. doi:10.2202/1544-6115.1585

Pitt, J. N., Strait, N. L., Vayndorf, E. M., Blue, B. W., Tran, C. H., Davis, B. E. M., et al. (2019). WormBot, an Open-Source Robotics Platform for Survival and Behavior Analysis in *C. elegans*. *GeroScience* 41, 961–973. doi:10.1007/s11357-019-00124-9

Podshivalova, K., Kerr, R. A., and Kenyon, C. (2017). How a Mutation that Slows Aging Can Also Disproportionately Extend End-Of-Life Decrepitude. *Cel Rep.* 19, 441–450. doi:10.1016/j.celrep.2017.03.062

Pollard, J. H., and Valkovics, E. J. (1992). The Gompertz Distribution and its Applications. *Genus* 48, 15–28.

Puchalt, J. C., Sánchez-Salmerón, A.-J., Ivorra, E., Llopis, S., Martínez, R., and Martorell, P. (2021). Small Flexible Automated System for Monitoring *Caenorhabditis elegans* Lifespan Based on Active Vision and Image Processing Techniques. *Sci. Rep.* 11. doi:10.1038/s41598-021-91898-6

R Core Team, R. (2015). A Language and Environment for Statistical Computing. Available at: https://www.r-project.org/.

Rahman, M., Edwards, H., Birze, N., Gabrilska, R., Rumbaugh, K. P., Blawzdziewicz, J., et al. (2020). NemaLife Chip: a Micropillar-Based Microfluidic Culture Device Optimized for Aging Studies in Crawling *C. elegans*. *Sci. Rep.* 10. doi:10.1038/s41598-020-73002-6

Robinson, A. (2007). Randomization, Bootstrap and Monte Carlo Methods in Biology. *J. R. Stat. Soc A* 170, 856. doi:10.1111/j.1467-985x.2007.00485_5.x

Samuelson, A. V., Carr, C. E., and Ruvkun, G. (2007a). Gene Activities that Mediate Increased Life Span of *C. elegans* Insulin-like Signaling Mutants. *Genes Dev.* 21, 2976–2994. doi:10.1101/gad.1588907

Samuelson, A. V., Klimczak, R. R., Thompson, D. B., Carr, C. E., and Ruvkun, G. (2007b). Identification ofCaenorhabditis elegansGenes Regulating Longevity Using Enhanced RNAi-Sensitive Strains. *Cold Spring Harbor Symposia Quantitative Biol.* 72, 489–497. doi:10.1101/sqb.2007.72.068

Shaw, W. M., Luo, S., Landis, J., Ashraf, J., and Murphy, C. T. (2007). The *C. elegans* TGF-β Dauer Pathway Regulates Longevity *via* Insulin Signaling. *Curr. Biol.* 17, 1635–1645. doi:10.1016/j.cub.2007.08.058

Stroustrup, N., Anthony, W. E., Nash, Z. M., Gowda, V., Gomez, A., López-Moyado, I. F., et al. (2016). The Temporal Scaling of *Caenorhabditis elegans* Ageing. *Nature* 530, 103–107. doi:10.1038/nature16550

Stroustrup, N., Ulmschneider, B. E., Nash, Z. M., López-Moyado, I. F., Apfeld, J., and Fontana, W. (2013). The caenorhabditis Elegans Lifespan Machine. *Nat. Methods* 10, 665–670. doi:10.1038/nmeth.2475

Stuhr, N. L., and Curran, S. P. (2020). Bacterial Diets Differentially Alter Lifespan and Healthspan Trajectories in *C. elegans*. *Commun. Biol.* 3, 653. doi:10.1038/s42003-020-01379-1

Sulston, J. E., and Horvitz, H. R. (1977). Post-embryonic Cell Lineages of the Nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56, 110–156. doi:10.1016/0012-1606(77)90158-0

Tarone, R. E. (1981). On the Distribution of the Maximum of the Logrank Statistic and the Modified Wilcoxon Statistic. *Biometrics* 37, 79. doi:10.2307/2530524

Therneau, T. M. (2021). *A Package for Survival Analysis in R. R Package*. version 3.2-12. Available at: https://cran.r-project.org/package=survival.

Urban, N. D., Cavataio, J. P., Berry, Y., Vang, B., Maddali, A., Sukpraphrute, R. J., et al. (2021). Explaining Inter-lab Variance in *C. elegans* N2 Lifespan: Making a Case for Standardized Reporting to Enhance Reproducibility. *Exp. Gerontol.* 156, 111622. doi:10.1016/j.exger.2021.111622

Van Raamsdonk, J. M., and Hekimi, S. (2011). FUdR Causes a Twofold Increase in the Lifespan of the Mitochondrial Mutant Gas-1. *Mech. Ageing Development* 132, 519–521. doi:10.1016/j.mad.2011.08.006

Vanfleteren, J. R., De Vreese, A., and Braeckman, B. P. (1998). Two-Parameter Logistic and Weibull Equations Provide Better Fits to Survival Data from Isogenic Populations of *Caenorhabditis elegans* in Axenic Culture Than Does the Gompertz Model. *Journals Gerontol. Ser. A: Biol. Sci. Med. Sci.* 53A, B393–B403. doi:10.1093/gerona/53A.6.B393

Wilson, D. L. (1994). The Analysis of Survival (Mortality) Data: Fitting Gompertz, Weibull, and Logistic Functions. *Mech. Ageing Dev.* 74, 15–33. doi:10.1016/0047-6374(94)90095-7

Winsor, C. P. (1932). The Gompertz Curve as a Growth Curve. *Proc. Natl. Acad. Sci. U.S.A.* 18, 1–8. doi:10.1073/pnas.18.1.1

Yon, G., and Marjoram, P. (2019). slurmR: A Lightweight Wrapper for HPC with Slurm. *Joss* 4, 1493. doi:10.21105/joss.01493

Zhao, Y., Zhang, B., Marcu, I., Athar, F., Wang, H., Galimov, E. R., et al. (2021). Mutation Ofdaf-2extends Lifespan *via* Tissue-specific Effectors that Suppress Distinct Life-limiting Pathologies. *Aging Cell* 20. doi:10.1111/acel.13324

Zhigang Zhang, Z., and Jianguo Sun, J. (2010). Interval Censoring. *Stat. Methods Med. Res.* 19, 53–70. doi:10.1177/0962280209105023

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BRIEF RESEARCH REPORT
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The FGFR4 Homolog KIN-9 Regulates Lifespan and Stress Responses in *Caenorhabditis elegans*

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Fibroblast growth factor receptors (FGFRs) regulate diverse biological processes in eukaryotes. The nematode *Caenorhabditis elegans* is a good animal model for studying the roles of FGFR signaling and its mechanism of regulation. In this study, we report that KIN-9 is an FGFR homolog in *C. elegans* that plays essential roles in aging and stress response maintenance. *kin-9* was discovered as a target of *miR-246*, a microRNA that is positively regulated by the Axin family member *pry-1*. We found that animals lacking *kin-9* function were long-lived and resistant to chemically induced stress. Furthermore, they showed a reduced expression of endoplasmic reticulum unfolded protein response (ER-UPR) pathway genes, suggesting that *kin-9* is required to maintain a normal ER-UPR. The analysis of GFP reporter-based expression in transgenic animals revealed that KIN-9 is localized in the intestine. Overall, our findings demonstrate that *kin-9* is regulated by *miR-246* and may function downstream of *pry-1*. This study prompts future investigations to understand the mechanism of miRNA-mediated FGFR function in maintaining aging and stress response processes.

Keywords: *kin-9*, FGFR4, *pry-1*, *miR-246*, aging, stress response, ER-UPR, Fgf signaling

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INTRODUCTION

Aging is the gradual deterioration of cellular and tissue functions that are regulated by both genetic and environmental factors (Kenyon, 2010; Lapierre and Hansen, 2012; Uno and Nishida, 2016). Genetic factors include components of conserved signaling pathways that regulate a host of cellular processes that are crucial for the development and proper functioning of healthy adults. Thus, it is essential to identify and understand the function of pathway components so that they can be targeted not only to promote normal growth, but also to develop potential treatments for associated diseases. To this end, our group is investigating the function of an Axin scaffolding protein homolog in the nematode *Caenorhabditis elegans*, PRY-1, which is necessary for the formation of many tissues and cell types, as well as for maintaining stress responses and aging (Mallick et al., 2019b, 2020, 2021a; Mallick and Gupta, 2022). To gain insights into the genetic network of *pry-1,* we performed mRNA and miRNA transcriptome profiling experiments (Ranawade et al., 2018; Mallick et al., 2019a). Together with biochemical and genetic studies, these data identified many factors that interact with *pry-1*, including *aak-2/AMPK*, *daf-16/FOXO*, and *crtc-1/CRTCs* (Mallick et al., 2020; 2021b). Our miRNA transcriptome analysis revealed six differentially expressed miRNAs in *pry-1* mutants, five of which (*lin-4*, *miR-237*, *miR-48*, *miR-84*, and *miR-241*) were upregulated, and one (*miR-246*) was downregulated (Mallick et al., 2019a). miRNAs are non-coding RNAs that regulate target gene expression by binding to their conserved 3′-untranslated region (UTR) or, less commonly, to the 5′-

UTR and coding sequences (Stefani and Slack, 2008; Ambros and Ruvkun, 2018; O'Brien et al., 2018). Studies have shown that miRNAs regulate diverse biological processes (Ambros and Ruvkun, 2018; O'Brien et al., 2018).

The present study focuses on *miR-246* and one of its targets in mediating aging and stress resistance. *miR-246* was reported to be the highest-fold upregulated gene during aging in both wild-type and long-lived *daf-2* (insulin/insulin-like growth factor-1 signaling (IIS) receptor homolog) mutant (De Lencastre et al., 2010; Pincus et al., 2011). However, the mechanism underlying the action of *miR-246* remains unclear. The results described here suggest that *miR-246* acts genetically downstream of *pry-1* to regulate the expression of a fibroblast growth factor receptor (FGFR) homolog to promote longevity and stress resistance in animals.

FGF signaling is conserved in eukaryotes and plays an important role in development and disease (DeVore et al., 1995; Ornitz and Itoh, 2015; Xie et al., 2020). Studies in mammalian models have revealed that the pathway is regulated by miRNAs (Yin et al., 2015; Copeland and Simoes-Costa, 2020); however, detailed mechanisms of interactions, specific processes, and pathway components are currently lacking. In this regard, the *C. elegans* system holds significant potential because it contains conserved miRNA families and FGF signaling that can be targeted by forward and reverse genetic approaches. While EGL-17 (FGF) and EGL-15 (FGFR) were previously shown to be necessary for sex specific muscle development (DeVore et al., 1995), they are not essential for the lifespan and stress responses in worms. Here, we provide evidence that KIN-9 is an FGFR4 family member that is repressed by *mir-246*. The receptor tyrosine kinase (RTK) domain of KIN-9 shows a high degree of sequence and structural similarity to those of FGFR4 mammalian family members. In agreement with this, we found that the KIN-9 overexpression phenotype resembled that of activated FGF signaling in *C. elegans*. A phenotypic analysis revealed that while *kin-9* mutants are long-lived and stress-resistant, *miR-246* mutants show the opposite phenotypes. We also found that *kin-9* RNAi fully suppressed the lifespan and stress sensitivity of the *miR-246* mutants. To further validate the regulatory relationship between *miR-246* and *kin-9*, a chimeric GFP-*kin-9*-3′ UTR reporter was used, which showed increased fluorescence in *miR-246* mutant animals. These data, together with the upregulation of KIN-9 in both the *miR-246* and *pry-1* mutants, support a model where *pry-1* positively regulates *miR-246*, which in turn inhibits *kin-9* expression. Analysis of *kin-9::GFP* transgenic animals revealed that the gene is expressed in the pharynx and intestine. Its presence in the intestine supports the role of *kin-9* in lifespan maintenance, similar to that described for many other long-lived mutant strains (An and Blackwell, 2003; Libina et al., 2003; Taylor and Dillin, 2013). As *kin-9* mutants are resistant to stress, we examined the expression of unfolded protein response (UPR) pathway components and chaperones. The results showed that the expression of the endoplasmic reticulum (ER) UPR components were downregulated, suggesting that lower *kin-9* activity is beneficial for protein homeostasis. Overall, the results described in this study demonstrate the essential role of *kin-9* in regulating *mir-246*-mediated lifespan and stress response in *C. elegans*.

MATERIALS AND METHODS

Parts of the methods section are in the **Supplementary Data S1**.

Strains and Culture Conditions

Worms were cultured on standard NGM plates using established protocols and *Escherichia coli* strain OP50 as a food source (Brenner, 1974). The strains were maintained at 20°C unless otherwise mentioned.

RNAi

RNAi-mediated gene silencing was performed using a protocol previously published by our laboratory (Mallick et al., 2021a). Plates were seeded with *Escherichia coli* HT115 expressing either dsRNA specific to candidate genes or empty vector (L4440). Synchronized gravid adults were bleached, and eggs were plated. After becoming young adults, animals were analyzed for stress sensitivity and lifespan (Mallick et al., 2021a).

Computational Analysis

To identify the targets of *miR-246*, the computational algorithms TargetScan (Jan et al., 2011), PicTar (Lall et al., 2006), PITA (Kertesz et al., 2007) and STarMirDB (Rennie et al., 2016) were used. These tools predict miRNA targets based on 3′ UTR seed matches of genes. The searches generated *miR-246* target candidates (**Supplementary Figure S1**; **Supplementary Table S2**).

Lifespan Analysis

Lifespan experiments were done following adult-specific RNAi treatment using a previously described protocol (Mallick et al., 2020). Animals were grown on NGM OP50 seeded plates till the late L4 stage after which they were transferred to RNAi plates. For lifespan analysis at different temperatures, animals were grown till the late L4 stage at 20°C following which they were shifted to either 15°C or 25°C. Plates were then screened daily for dead animals and surviving worms were transferred every other day till the progeny production ceased. Censoring was done for animals that either escaped, burrowed into the medium, showed a bursting of intestine from the vulva, or underwent bagging of worms (larvae hatch inside the worm and the mother dies).

U0126 Inhibitor Assay

NGM plates with U0126 inhibitor (U120-1 MG, Sigma-Aldrich) of 30 μM was adapted from described previously (Reiner et al., 2008; Sharanya et al., 2015). A stock concentration of 10 mM U0126 was prepared in DMSO and was added to plates right before pouring to achieve a plate concentration of 30 μM. Synchronized L1 animals were plated and allowed to grow till young adult stage following which stress sensitivity to 200 mM was examined.

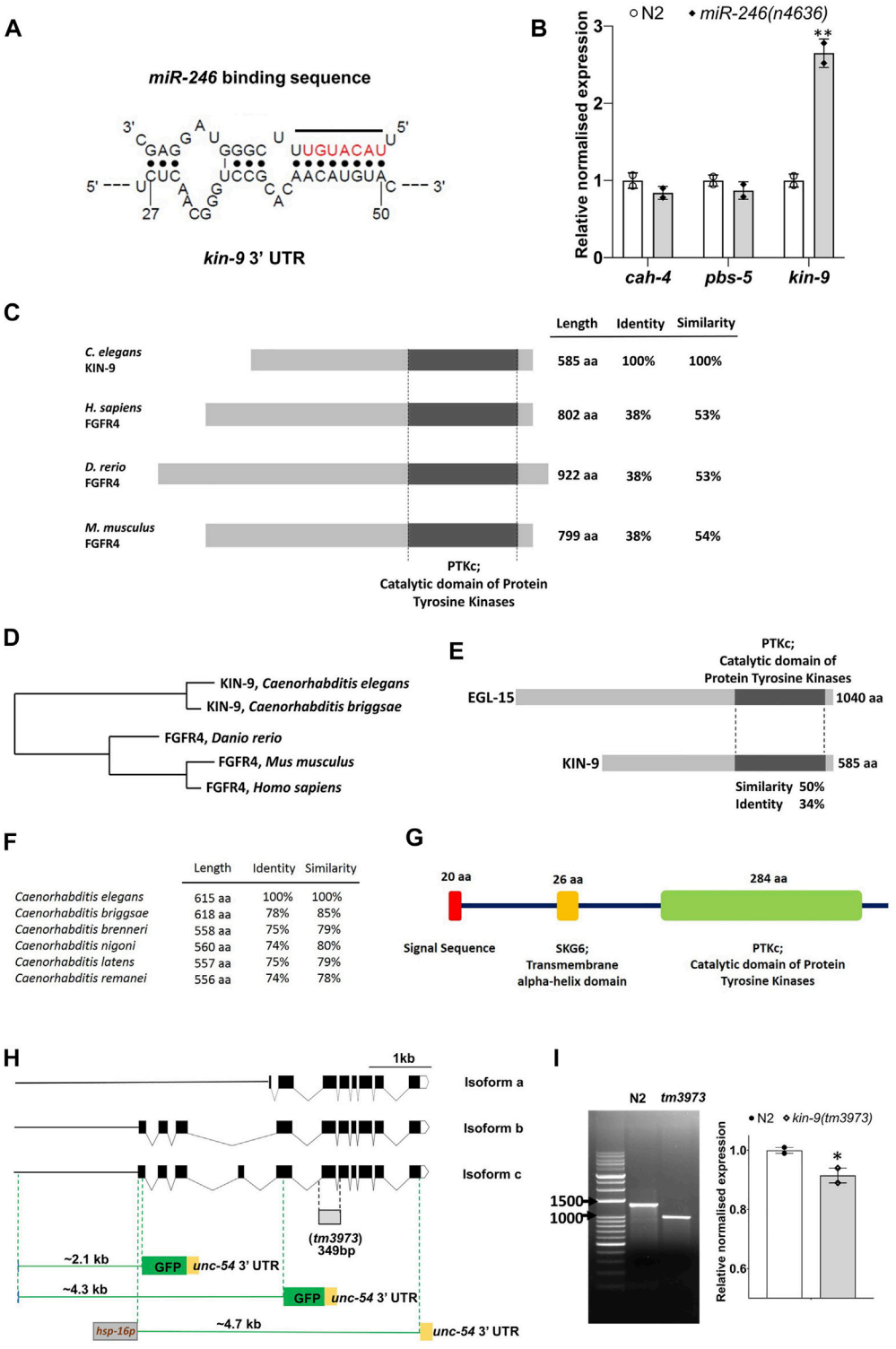

FIGURE 1 | *miR-246* mutants show overexpression of *kin-9*. **(A)** The predicted binding site of *miR-246* at the 3′ UTR of *kin-9* mRNA. **(B)** Expression analysis of three candidate genes *cah-4, pbs-5* and *kin-9* in the *miR-246* mutants. **(C)** Schematic representation of KIN-9 and FGFR4 proteins from *Caenorhabditis elegans*, *Homo sapiens*, *Danio rerio*, and *Mus musculus* with percent identity and similarity indicated relative to *C. elegans* KIN-9. Conserved domains are aligned and are depicted with sizes, all presented to scale. **(D)** Phylogenetic tree of proteins shown in panel (C). **(E)** Schematic representation of *C. elegans* KIN-9 and EGL-15 proteins with percent identity and similarity indicated. **(F)** Similarities and identities between the KIN-9 proteins in the *Caenorhabditis* genus. **(G)** Protein domains and structure of KIN-9 protein. **(H)** Schematic dendrograms showing all the isoforms and tm3973 deletion allele of *kin-9* with exons (black solid boxes), introns (bent lines) and upstream

(*Continued*)

FIGURE 1 | sequences (solid straight line). Regions used for creating transcriptional reporters and heat shock promoter-driven *kin-9* overexpression are also shown. **(I)** PCR and qPCR analyses of the *tm3973* allele. Gel image showing the shorter fragment of *kin-9* transcript and bar graph showing *kin-9* mRNA levels in the *tm3973* mutants. **(B, I)** Each data represents the mean of two replicates and error bars the standard error of means. Significance was calculated using Bio-Rad software (one-way ANOVA) and significant differences are indicated by stars (*): * ($p < 0.05$), ** ($p < 0.01$).

Oil Red O Staining

Neutral lipid staining was done on synchronized day-1 adult animals using Oil Red O dye (Thermo Fisher Scientific, United States) following the previously published protocol. Quantification was then done using ImageJ software as described previously (Mallick and Gupta, 2020).

RESULTS

We previously reported that PRY-1 regulates the expression of a set of heterochronic miRNAs, including the *lin-4* and *let-7* family members (Mallick et al., 2019a). *miR-246*, which affects the stress response and aging-related processes (De Lencastre et al., 2010), was also discovered in that study. It was found that *miR-246* is not involved in the *pry-1*-mediated heterochronic pathway. Furthermore, its expression was downregulated in *pry-1(mu38)* larvae and adults (Mallick et al., 2019a).

kin-9 Expression is Regulated by *miR-246* and its 3′ UTR Contains miRNA Consensus Binding Sites

To understand the biological role of *miR-246*, we focused on identifying its target genes. Because the phenotype caused by a miRNA deletion is expected to result from the increased activity of its target(s), we hypothesized that *miR-246* loss-induced short lifespan and enhanced stress sensitivity would be suppressed by depletion of its target gene activity. The predicted targets of *miR-246* were identified using computational algorithms (see Methods). We assessed the transcript levels of top three potential targets (*cah-4*, *kin-9*, and *pbs-5*) in an *miR-246* deletion mutant that lacks the entire transcript and 5′ upstream sequence (*n4636:* 518 bp length) (Miska et al., 2007) (**Figures 1A,B**). It was expected that the transcript level of the target gene would be higher than normal in the miRNA mutant background. Our results revealed that, while *kin-9* expression was significantly upregulated compared to the control, there was no change in *cah-4* and *pbs-5* expression (**Figure 1B**; **Supplementary Figure S2**).

Sequence analysis revealed that KIN-9 is a member of the FGFR4 family (http://www.wormbase.org). The RTK domain of KIN-9 is approximately 53% similar between the mouse and human FGFR4 proteins (**Figures 1C,D**). Outside this domain, no significant sequence similarity with FGFR4 was detected (**Figure 1C**). We also used the secondary structure prediction tool Jpred4 (http://www.compbio.dundee.ac.uk/jpred/) (Drozdetskiy et al., 2015), which confirmed KIN-9 homology with human FGFR4 (PDB ID: 6jpj, 6jpe, 5jkg, 4uxq, 4qrc, and 4qqt). Finally, to determine whether the KIN-9 RTK domain possesses conserved tyrosine kinase phosphorylation sites present in FGFR, the online program group-based prediction system (GPS 5) was utilized (http://gps.biocuckoo.cn/online.php) (Xue et al., 2011). The analysis revealed six such sites that, together with secondary structure prediction, established KIN-9 as a bona fide FGFR family member in *C. elegans* (**Supplementary Figure S3**). It is worth mentioning that a previously characterized FGFR in *C. elegans*, EGL-15, shares 50% sequence similarity with the RTK domain of KIN-9 (**Figure 1E**; **Supplementary Figure S4**) (Schutzman et al., 2001). KIN-9 orthologs were also found in other nematode species (http://www.wormbase.org) (**Figures 1F**). The *kin-9* gene is predicted to produce three isoforms of varied sizes (478 aa (a), 585 aa (b), and 615 aa (c), all of which have a conserved RTK domain. The longest isoforms (b and c) also possess an N-terminal signal sequence and a transmembrane alpha-helix domain (**Figures 1G,H**).

kin-9 mutants are Stress Resistant and Long-Lived Whereas *hsp::kin-9* Animals are Stress Sensitive and Die Prematurely

A deletion mutant of *kin-9*, *tm3973*, was used to investigate the gene function. The mutation was found to remove a portion of the RTK domain, leading to multiple in-frame stop codons (see **Supplementary Data S1**). These changes are expected to cause truncated proteins for all three isoforms (167 aa, 271 aa, and 304 aa) (**Figure 1H**). Interestingly, a cDNA analysis showed the presence of a roughly 990 bp transcript in *kin-9* deletion mutant (*tm3973*) worms, indicating a readthrough transcription (**Figure 1I**). While it is unclear whether the *tm3973* mutation allows translation, any products arising from this allele are expected to be non-functional.

kin-9 deletion mutant (*tm3973*) animals exhibited no obvious morphological defects but appeared to have a slight growth delay and lay significantly fewer eggs (**Figures 2A,B**); in addition, L1 larvae showed resistance to starvation (**Figure 2C**). Since brood size and L1 survival may be affected by lipid levels (Watts and Ristow, 2017; Ranawade et al., 2018), we performed Oil Red O staining and found no change in neutral lipid levels (**Figure 2D**). The *tm3973* animals also showed increased resistance to paraquat and tunicamycin but were insensitive to heat stress (**Figures 2E–G**). These data suggest that *kin-9* plays an important role in the stress response maintenance. Further support for this conclusion comes from the expression analysis of the endoplasmic reticulum unfolded protein response (ER-UPR) genes. We found that *kin-9* mutants affected the ER-UPR pathway, as evidenced by the reduced expression of *ire-1*, *pek-1*, *xbp-1*, and the chaperone *hsp-4* (**Figures 2H,I**). Consistent with these results, the mutants exhibited electrotaxis defects associated with chronic stress (**Figure 2J**) (Taylor et al., 2021). No change

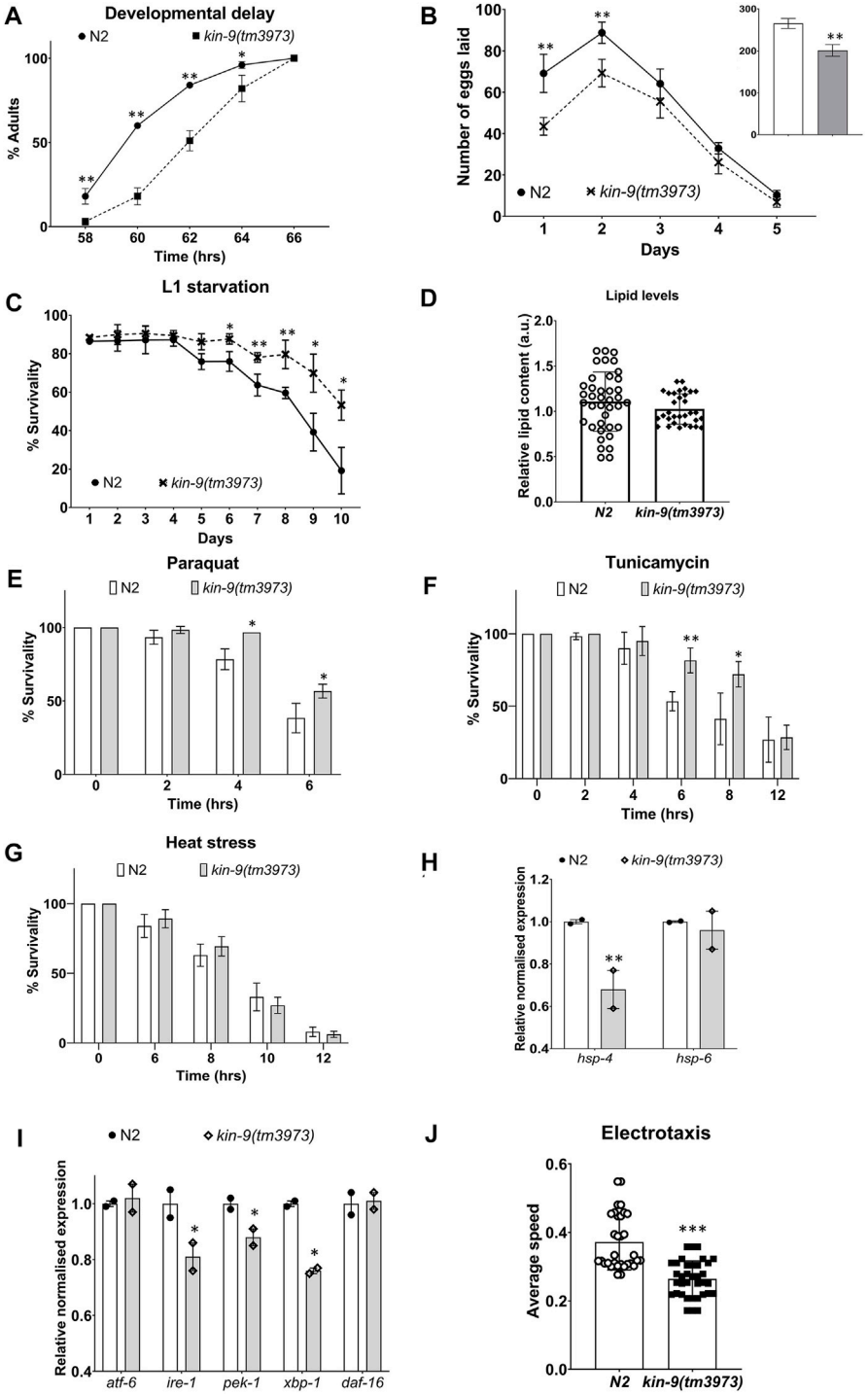

FIGURE 2 | *kin-9* mutants exhibit resistance to heat and chemical-induced stress. **(A)** Graph showing the time taken to reach adulthood. **(B)** Line graph showing the number of eggs laid by *kin-9* mutants compared to N2. Smaller bar graph showing the total number of eggs laid (N2- clear bar; *kin-9(tm3973)* grey bar). **(C)** Survival graph of *kin-9(tm3973)* L1 worms upon starvation compared to N2. **(D)** Quantification of neutral lipids using Oil Red O staining. **(E,F)** Bar graph showing the survivability of *kin-9* mutants compared to N2 following paraquat (200 mM) and tunicamycin (25 ng/μL) over a period of 6 and 12hrs respectively. **(G)** Survivability of *kin-9* mutants compared to N2 at 35°C over a period of 12hrs. **(H,I)** qPCR analysis of daf-16 genes in *kin-9(tm3973)* adults compared to N2. Data in **(H–I)** represent the mean of two replicates and error bars the standard error of means. Significance was calculated using Bio-Rad software (one-way ANOVA). **(J)** Electrotaxis analysis of day-1 *kin-9(tm3973)* adults. **(A–G,I)** Data represent the mean of two replicates (n > 40 animals in each replicate) and error bars represent the standard deviation. Statistical analyses were done using multiple unpaired *t*-tests and significant differences are indicated by stars (*): * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

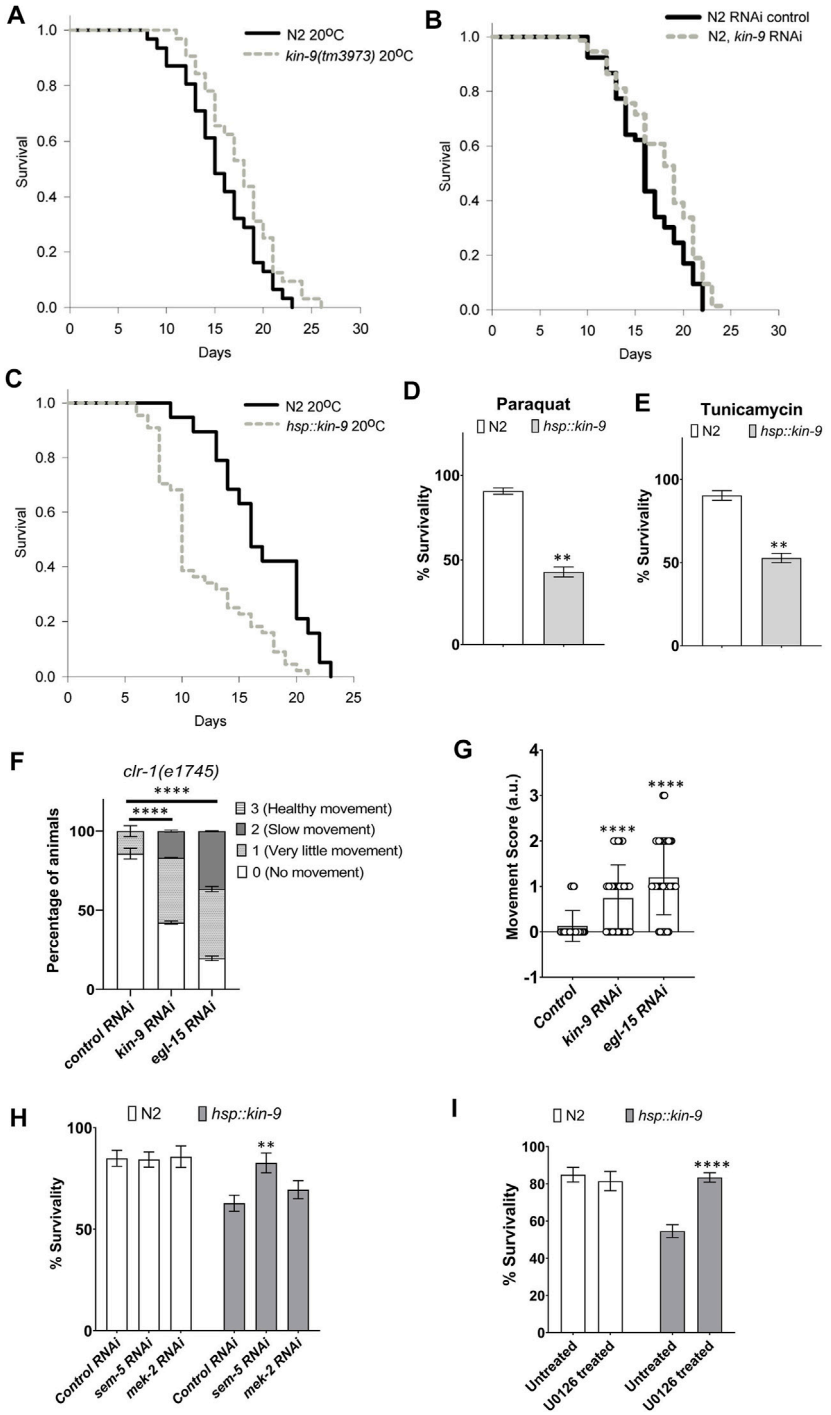

FIGURE 3 | Loss of *kin-9* function extends lifespan. **(A)** Lifespan graphs of *kin-9 (tm3973)* mutants compared to N2 **(B)** Lifespan analysis of animals following control and *kin-9* RNAi knockdown during adulthood. **(C)** Lifespan graphs of *hsp::kin-9* animals compared to N2. **(A–C)** See the Methods section and **Supplementary Table S3** for statistics performed. **(D,E)** Bar graph showing the survivability of *hsp::kin-9* adults compared to N2 following paraquat (200 mM) and tunicamycin (25 ng/µL) exposure for 2hrs. **(F,G)** Staggered bar graphs **(F)** and bar graphs **(G)** showing the analyses of movement defect in *clr-1* mutants following control, *kin-9* and *egl-15* RNAi. In panel F movement is quantified on a scale of 0–3 and in panel G average score for movement has been plotted. **(H)** Bar graphs showing the survivability of N2 and *hsp::kin-9* adults in 200 mM paraquat for 2hrs following control, *sem-5* and *mek-2* RNAi knockdown. **(I)** Bar graphs showing the survivability of N2 and *hsp::kin-9* adults in 200 mM paraquat for 2hrs following U0126 inhibitor treatment. **(D–I)** Data represent the mean of two to three replicates ($n > 50$ animals in each replicate) and error bars represent the standard deviation. Statistical analyses were done using multiple unpaired *t*-tests with Welch correction **(D,E,H,I)**, one-way ANOVA with multiple comparison test **(G)** and 2-way ANOVA with Sidak multiple comparison test **(F)**. Significant differences are indicated by stars (*): ** ($p < 0.01$), *** ($p < 0.001$) and **** ($p < 0.001$).

in the expression of mitochondrial UPR components was detected in the absence of *kin-9* function (**Figures 2H,I**).

Because *miR-246* mutants are short-lived (De Lencastre et al., 2010), we examined the lifespan phenotype of animals lacking *kin-9* function. Consistent with *kin-9* being a downstream target, mutant animals showed an extended lifespan (**Figure 3A**; **Supplementary Table S3**). Similar lifespan changes were observed in the RNAi-treated animals (**Figure 3B**; **Supplementary Table S3**). The mutants exhibited slightly increased body bending rates but no change in pharyngeal pumping (**Supplementary Figure S5**).

If the absence of *kin-9* results in a lifespan extension, then its increased levels should give rise to the opposite phenotype. To test this hypothesis, *hsp::kin-9* transgenic lines were generated that overexpressed *kin-9* following heat treatment (see **Supplementary Data S1**). High levels of *kin-9* expression in these animals were confirmed using qPCR (**Supplementary Figure S6**). Consistent with the longer lifespan of *kin-9* mutants, transgenic animals overexpressing *kin-9* were small, short-lived, and exhibited slower pharyngeal pumping and body bending (**Figure 3C**; **Supplementary Figures S5, S7, S8**). The aging phenotype was similar at other growth temperatures (15 and 25°C, **Supplementary Figure S7**; **Supplementary Table S3**). Moreover, *hsp::kin-9* animals exhibited an increased stress sensitivity to both paraquat and tunicamycin (**Figures 3D,E**). Altogether, these data demonstrate an important role for *kin-9* in regulating the normal lifespan and stress responses of animals.

Interestingly, we found that *hsp::kin-9* animals were unusually transparent when grown at 25°C (**Supplementary Figure S8**), an appearance that resembled 'Clear (Clr)' phenotype reported earlier in *C. elegans* that have activated FGF signaling (**Supplementary Figure S8**) (Borland et al., 2001). To investigate whether *kin-9* genetically interacts with *clr-1*, we performed RNAi experiments. The results showed that while *kin-9* RNAi had no effect on the Clr phenotype, it suppressed movement defects of animals (**Figures 3F,G**). In control experiments, *egl-15* knockdown suppressed both the Clr and movement defects of *clr-1* mutants (**Figures 3F,G**).

Additionally, we performed RNAi knockdowns of two of the FGF pathway components, MEK-2 (Erk Kinase) and SEM-5 (Grb2) (Borland et al., 2001). The results showed that *sem-5* RNAi suppressed stress sensitivity of *hsp::kin-9* animals (**Figure 3H**). No such response was not observed in the case of *mek-2* (**Figure 3H**). Since *C. elegans* contains two Erk kinases (*mek-1* and *mek-2*), we reasoned that there may be a genetic redundancy and, therefore, used a MEK inhibitor U0126 (Favata et al., 1998; Reiner et al., 2008; Sharanya et al., 2015). As expected, treating *hsp::kin-9* animals with U0126 fully rescued their stress sensitivity to PQ (**Figure 3I**). Altogether, these data support our conclusion that KIN-9 function is mediated by the ERK kinase signaling, consistent with its role in regulating FGF signaling.

kin-9 is Expressed in the Pharynx, Intestine, and Tail Region

Considering the *kin-9's* essential role in *C. elegans*, we wanted to identify the cells and tissues in which the gene is expressed. To this end, transgenic strains carrying *kin-9::GFP* reporters were generated. Two different constructs were utilized (see **Supplementary Data S1**), the longest of which (4.3 kb) contained a part of an exon that is common to all isoforms, whereas the shorter one (2.1 kb) is specific to isoforms b and c (**Figure 1H**). The analysis of transgenic lines showed GFP fluorescence throughout their lifespan, which agrees well with previously published transcriptomic data (Golden et al., 2008; Grün et al., 2014). We found that *kin-9p(2.1 kb)::GFP* adults exhibited fluorescence in the pharynx, intestine, and certain cells located in the tail (**Figure 4A**). While *kin-9p(4.3 kb)::GFP* animals exhibited a similar pattern, interestingly, no fluorescence was observed in the posterior region (**Supplementary Figure S8**). It is possible that the longer fragment has certain inhibitory sequences that contribute to differences in expression. More experiments involving the dissection of regulatory sequences are needed to investigate this possibility. The intestinal localization of KIN-9 and its persistence during adulthood agree well with the expression of other genes that promote lifespan and stress response maintenance (An and Blackwell, 2003; Libina et al., 2003; Taylor and Dillin, 2013). However, the specific role of KIN-9 in the pharynx remains to be determined.

kin-9 3′ UTR is Targeted by *miR-246* and *kin-9* RNAi Rescues *miR-246(n4636)* Defects

Since miRNAs function mainly by binding to the 3′ UTR of target genes (Ambros and Ruvkun, 2018; O'Brien et al., 2018), we examined whether *miR-246* affects the transcriptional regulation of *kin-9*. To this end, transgenic lines were generated containing a chimera of GFP and *kin-9* 3′ UTR under the control of the *kin-9* promoter (*kin-9p(2.1 kb)::GFP::kin-9 UTR*) (**Supplementary Data S1**; **Figure 4B**). As expected, GFP fluorescence increased approximately four-fold when the construct was introduced into *miR-246(n4636)* animals (**Figure 4C**). Since *miR-246* is positively regulated by *pry-1* (Mallick et al., 2019a), we also examined *kin-9p(2.1 kb)::GFP::kin-9UTR* fluorescence in *pry-1* mutants and found similar upregulation (**Figure 4C**). Consistent with this, qPCR analysis revealed that *kin-9* transcript levels were high in animals lacking the *pry-1* function (**Figure 4D**). Altogether, these data show that *pry-1* and *miR-246* negatively regulate *kin-9* expression.

As *kin-9* expression is upregulated in *miR-246* mutants and *miR-246* regulates *kin-9* transcript levels, we tested whether *miR-246(n4636)* phenotypes could be rescued by reducing *kin-9* function. The lifespan and stress sensitivity defects of *miR-246* mutants were significantly rescued by *kin-9* RNAi (**Figures 4E–H**; **Supplementary Figure S9**, **Supplementary Table S3**). Together with transcript analysis and data from previous sections, these results demonstrate that *kin-9* is involved in lifespan regulation and acts downstream of *miR-246*. Interestingly, no phenotypic rescue was observed by knocking down *kin-9* in the *pry-1* mutant animals (**Supplementary Figure S10**, **Supplementary Table S3**), suggesting that *kin-9* is not the sole effector of *pry-1* function in aging-related processes. These findings are consistent with the interaction of *pry-1* with multiple pathway components to regulate aging and the stress response in *C. elegans*.

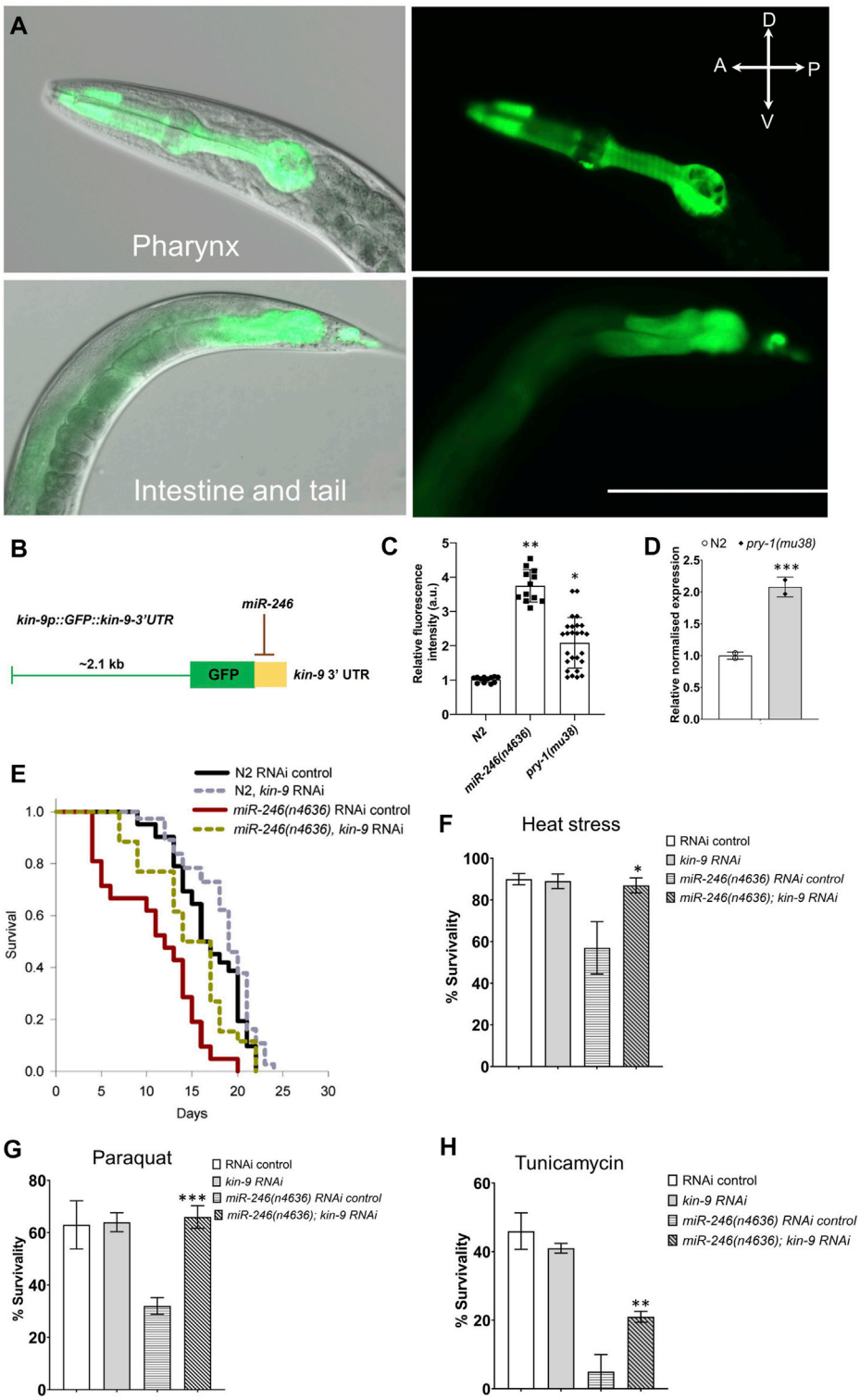

FIGURE 4 | Lowering *kin-9* activity suppresses *miR-246* mutant defects. **(A)** *kin-9p (2.1 kb):GFP* analysis shows expression in the pharynx, intestine, and tail neurons. **(B)** Schematic diagram of a *kin-9p::GFP::kin-9* 3′ UTR construct showing potential *miR-246* binding at the 3′ UTR of the kin-9 mRNA transcript. **(C)** Bar graph showing the GFP analysis using the array *kin-9p::GFP::kin-9* 3′ UTR in *miR-246 (n4636)* and *pry-1(mu38)* adults compared to control. Data represent the mean of two replicates ($n > 25$ animals in each replicate) and error bars represent the standard deviation. Statistical analyses were done using one-way ANOVA with Dunnett's post hoc test and significant differences are indicated by stars (*): * ($p < 0.05$), ** ($p < 0.01$). **(D)** qPCR analysis of *kin-9* gene in the *pry-1(mu38)* adults compared to control. Data represent the mean of two replicates and error bars the standard error of means. Significance was calculated using Bio-Rad software (one-way ANOVA)

(*Continued*)

FIGURE 4 | and significant differences are indicated by stars (*): *** ($p < 0.001$). **(E)** Lifespan analysis of N2 and *miR-246 (n4636)* animals following control and *kin-9* RNAi knockdown. See the **Supplementary Data S1** and **Supplementary Table S3** for statistics performed. **(F–H)** Bar graphs showing survivability of N2 and *miR-246 (n4636)* animals following control and *kin-9* RNAi when exposed to heat stress (35°C), paraquat (200 mM for 2 h) and tunicamycin (25 ng/μl for 2 h). Data represent the mean of two replicates ($n > 50$ animals in each replicate) and error bars represent the standard deviation. Statistical analyses were done using one-way ANOVA with Dunnett's post hoc test and significant differences are indicated by stars (*): * ($p < 0.05$), ** ($p < 0.01$).

DISCUSSION

We identified *kin-9* as a new target of the miRNA *miR-246* in *C. elegans* and demonstrated its essential function in regulating stress responses and the lifespan of animals. Sequence analysis of *kin-9* revealed that it is a member of the FGFR family, with the RTK domain being the most similar to FGFR4. Furthermore, its overexpression using a *kin-9* transgenic system resulted in a Clr phenotype similar to that observed in activated FGF signaling conditions (Borland et al., 2001; Schutzman et al., 2001). The Clr phenotype is characterized by the accumulation of clear fluid within the pseudocoelomic cavity. Such animals appear to have floating intestines with fluid-filled body cavities and are short, immobile, and sterile (Borland et al., 2001; Schutzman et al., 2001). A similar trait was also observed in other FGF pathway component mutants (Schutzman et al., 2001). Interestingly, *kin-9* RNAi did not affect the Clr phenotype of *clr-1* mutants. However, movement defect of animals was rescued similar to *egl-15* knockdown. Furthermore, we found that the MEK inhibitor U0126 fully suppressed the stress sensitivity of animals overexpressing *kin-9*. Overall, these data lead us to conclude that *kin-9* regulates FGF signaling in *C. elegans*.

Studies in other animal models have reported the miRNA-mediated regulation of FGFs and FGFRs. For example, *miR-140* regulates *FGF9* during lung development, and *miR-200a*, *miR-20a*, and *miR-217* regulate *FGF4*, *FGF13*, and *FGFR12*, respectively, during the establishment of the neural crest territory (Yin et al., 2015; Copeland and Simoes-Costa, 2020). The work described here represents the first such study in *C. elegans* and provides a unique opportunity to investigate the miRNA-mediated regulation of FGFR. It is worth mentioning that, while the mammalian systems contain twenty-three FGF family members (FGF1-23) and four FGFRs (FGFR1-4), *C. elegans* carries only two ligands (EGL-17 and LET-756) and two receptors (EGL-15 and KIN-9) (Borland et al., 2001; Ornitz and Itoh, 2015; Xie et al., 2020). The simplicity of the worm system, together with its short lifespan and powerful genetic approaches to manipulate the genes, make it possible to investigate the mechanism of *kin-9* function and identify other pathway components that act genetically downstream of *miR-246*.

Previously, *miR-246* was identified in a transcriptomic study in our lab as being positively regulated by *pry-1* (Mallick et al., 2019a). Considering that miRNAs are necessary for lifespan maintenance (De Lencastre et al., 2010), we aimed to identify their targets to further understand the role of the *pry-1-miR-246* genetic network. While our *in-silico* analysis revealed three genes with consensus *miR-246* binding sites in their 3′ UTR, *kin-9* was the only gene with increased expression in the *miR-246* mutant background. Consistent with this, a *GFP* transgene containing the *kin-9* 3′ UTR was responsive to *miR-246* activity, that is, in the absence of *miR-246*, fluorescence was significantly increased.

Analysis of *kin-9* mutants has revealed the essential role of this gene in regulating stress responses and aging-related processes. While animals lacking *kin-9* function showed resistance to stress treatments, reduced expression of heat shock chaperone and ER-UPR genes, and a longer lifespan, transgenic lines overexpressing *kin-9* showed the opposite phenotypes. These data show that perturbations in *kin-9* function have opposite effects on stress response and ER-UPR gene expression. Studies have shown that *hsp-4* expression levels do not always correlate with stress sensitivity of animals. Thus, for example, *hsp-4* is downregulated in both *atf-6* (stress resistant) and *xbp-1* (stress sensitive) mutants (Taylor and Dillin, 2013; Burkewitz et al., 2020). More work is needed to fully elucidate the cellular and molecular mechanisms of *kin-9* in ER-UPR and stress response maintenance. It may be that a reduction in *hsp-4* and other ER-UPR genes in *kin-9* mutants is associated with beneficial effects such as improved proteostasis, thereby contributing to increased stress resistance and better health of animals. In this regard, experiments examining *kin-9*'s involvement in protein aggregation, various forms of stress, and degradation and clearing of intracellular factors should provide valuable information.

Consistent with the lifespan phenotype of *kin-9* mutants, the gene is expressed in the intestine, a tissue known to be the primary player in nutrient uptake and metabolic activities (Libina et al., 2003; Rera et al., 2013). Studies on aging have shown that the intestine communicates with other parts of the body, such as neurons and muscles, leading to the activation of downstream effectors. For example, the IIS transcription factor DAF-16, which functions mainly in neurons and the intestine, affects muscle health and mitochondrial mass, suggesting cross-talk between these tissues (Libina et al., 2003; Uno and Nishida, 2016; Wang et al., 2019; Mallick et al., 2020; Mallick and Gupta 2022). Thus, it is conceivable that *kin-9* regulates stress responses and lifespan by maintaining a healthy gut, which in turn signals to other tissues to promote their health.

Our data support a model in which *pry-1* promotes *miR-246* expression which in turn inhibits *kin-9* expression. However, the precise mechanism underlying this genetic relationship remains unclear. Although *kin-9* expression was inhibited by *pry-1*, knockdown of the gene did not suppress the *pry-1* phenotype. It may be that *kin-9* regulates only a small set of *pry-1* downstream components and is unable to alter *pry-1* signaling significantly in an independent manner. Previous studies on *pry-1/Axin* have revealed its genetic network, which includes multiple signaling components (Mallick et al., 2019b). For example, PRY-1 interacts with AAK-2/AMPK in the muscle in a cell-non-autonomous manner to regulate DAF-16 expression in the

intestine to promote the lifespan and muscle health of animals (Mallick et al., 2020). PRY-1 also regulates the CABIN1 domain-containing protein, PICD-1, to affect calcineurin signaling and CRTC-1-dependent transcription (Mallick et al., 2021b). Recently, we identified several genes (*cpz-1/CTSZ*, *cdk-1/CDK1*, *rnr-1/RRM1*, *his-7/H2AX*, and *ard-1/HSD17B10*) that function downstream of *pry-1* to regulate the lifespan and stress response of animals (Mallick et al., 2021a). Taken together, these findings demonstrate that *pry-1* acts as a master regulator of aging-related processes and functions by coordinating the activities of diverse genes and pathways.

KIN-9 is the first identified FGFR family member in *C. elegans* that plays an essential role in aging and stress response maintenance. Previously, FGF signaling (EGL-15-EGL-17) was reported to be necessary to promote similar processes mediated by the homologs of the transmembrane proteins Klotho (KLO-1 and KLO-2), although EGL-15 and EGL-17 mutants independently do not have any such phenotypes (Château et al., 2010). Studies in higher eukaryotes have also shown that Klotho promotes lifespan and functions as a co-receptor for FGFRs (Kuro-o et al., 1999; Ornitz and Itoh, 2015). Moreover, there is evidence that mammalian FGFs cause age-related changes. For example, age-associated impairment of human mesenchyme-derived progenitor cells can be reversed by FGF2 treatment (Hurley et al., 2016). Additionally, the activated FGF2 pathway causes increased fat accumulation in aged human skeletal muscles (Mathes et al., 2021).

Our analysis of *kin-9* as a target of *miR-246* and its potential role genetically downstream of *pry-1* prompt future studies to investigate the mechanism of its regulation and conservation across eukaryotes. Whether *kin-9* utilizes the known components of FGF signaling remains to be determined. Interestingly, *kin-9* RNAi was shown earlier to delay the development of *let-60/Ras* mutant animals (Byrne et al., 2007). The identification of *kin-9* pathway components and their interactions with *pry-1* holds significant promise in advancing our understanding of Axin signaling in stress maintenance and aging.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

BPG and AM designed the study. AM, SKBT, LX, SM, and HH performed the experiments and analyzed data. A.M. produced the final figures and tables. BPG, AM, and SKBT wrote the manuscript.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Ambros, V., and Ruvkun, G. (2018). Recent Molecular Genetic Explorations of caenorhabditis Elegans microRNAs. *Genetics* 209, 651–673. doi:10.1534/genetics.118.300291

An, J. H., and Blackwell, T. K. (2003). SKN-1 linksC. Elegansmesendodermal Specification to a Conserved Oxidative Stress Response. *Genes Dev.* 17, 1882–1893. doi:10.1101/gad.1107803

Borland, C. Z., Schutzman, J. L., and Stern, M. J. (2001). Fibroblast Growth Factor Signaling inCaenorhabditis Elegans. *BioEssays* 23, 1120–1130. doi:10.1002/bies.10007

Brenner, S. (1974). THE GENETICS OF CAENORHABDITIS ELEGANS. *Genetics* 77, 71–94. doi:10.1002/cbic.20030062510.1093/genetics/77.1.71

Burkewitz, K., Feng, G., Dutta, S., Kelley, C. A., Steinbaugh, M., Cram, E. J., et al. (2020). Atf-6 Regulates Lifespan through ER-Mitochondrial Calcium Homeostasis. *Cell Rep.* 32, 108125. doi:10.1016/j.celrep.2020.108125

Byrne, A. B., Weirauch, M. T., Wong, V., Koeva, M., Dixon, S. J., Stuart, J. M., et al. (2007). A Global Analysis of Genetic Interactions in *Caenorhabditis elegans*. *J. Biol.* 6, 8. doi:10.1186/jbiol58

Château, M.-T., Araiz, C., Descamps, S., and Galas, S. (2010). Klotho Interferes with a Novel FGF-Signalling Pathway and insulin/Igf-like Signalling to Improve Longevity and Stress Resistance in *Caenorhabditis elegans*. *Aging* 2, 567–581. doi:10.18632/aging.100195

Copeland, J., and Simoes-Costa, M. (2020). Post-transcriptional Tuning of FGF Signaling Mediates Neural Crest Induction. *Proc. Natl. Acad. Sci. U.S.A.* 117, 33305–33316. doi:10.1073/PNAS.2009997117

De Lencastre, A., Pincus, Z., Zhou, K., Kato, M., Lee, S. S., and Slack, F. J. (2010). MicroRNAs Both Promote and Antagonize Longevity in *C. elegans*. *Curr. Biol.* 20, 2159–2168. doi:10.1016/j.cub.2010.11.015

DeVore, D. L., Horvitz, H. R., and J.Stern, M. M. (1995). An FGF Receptor Signaling Pathway Is Required for the Normal Cell Migrations of the Sex Myoblasts in *C. elegans* Hermaphrodites. *Cell* 83, 611–620. doi:10.1016/0092-8674(95)90101-9

Drozdetskiy, A., Cole, C., Procter, J., and Barton, G. J. (2015). JPred4: A Protein Secondary Structure Prediction Server. *Nucleic Acids Res.* 43, W389–W394. doi:10.1093/nar/gkv332

Favata, M. F., Horiuchi, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feeser, W. S., et al. (1998). Identification of a Novel Inhibitor of Mitogen-Activated Protein Kinase Kinase. *J. Biol. Chem.* 273, 18623–18632. doi:10.1074/jbc.273.29.18623

Golden, T. R., Hubbard, A., Dando, C., Herren, M. A., and Melov, S. (2008). Age-related Behaviors Have Distinct Transcriptional Profiles inCaenorhabditis Elegans. *Aging Cell* 7, 850–865. doi:10.1111/j.1474-9726.2008.00433.x

Grün, D., Kirchner, M., Thierfelder, N., Stoeckius, M., Selbach, M., and Rajewsky, N. (2014). Conservation of mRNA and Protein Expression during Development of *C. elegans*. *Cell Rep.* 6, 565–577. doi:10.1016/j.celrep.2014.01.001

Hurley, M. M., Gronowicz, G., Zhu, L., Kuhn, L. T., Rodner, C., and Xiao, L. (2016). Age-Related Changes in FGF-2, Fibroblast Growth Factor Receptors and β-Catenin Expression in Human Mesenchyme-Derived Progenitor Cells. *J. Cell. Biochem.* 117, 721–729. doi:10.1002/jcb.25357

Jan, C. H., Friedman, R. C., Ruby, J. G., and Bartel, D. P. (2011). Formation, Regulation and Evolution of *Caenorhabditis elegans* 3′UTRs. *Nature* 469, 97–101. doi:10.1038/nature09616

Kenyon, C. J. (2010). The Genetics of Ageing. *Nature* 464, 504–512. doi:10.1038/nature08980

Kertesz, M., Iovino, N., Unnerstall, U., Gaul, U., and Segal, E. (2007). The Role of Site Accessibility in microRNA Target Recognition. *Nat. Genet.* 39, 1278–1284. doi:10.1038/ng2135

Kuro-o, M., Matsumura, Y., Arawa, H., Kawaguchi, H., Suga, T., Utsugi, T., et al. (1999). Mutation of the Mouse Klotho Gene Leads to a Syndrome Resembling Ageing. *Chemtracts* 12, 703–707.

Lall, S., Grün, D., Krek, A., Chen, K., Wang, Y.-L., Dewey, C. N., et al. (2006). A Genome-wide Map of Conserved MicroRNA Targets in *C. elegans*. *Curr. Biol.* 16, 460–471. doi:10.1016/j.cub.2006.01.050

Lapierre, L. R., and Hansen, M. (2012). Lessons from *C. elegans*: Signaling Pathways for Longevity. *Trends Endocrinol. Metabolism* 23, 637–644. doi:10.1016/j.tem.2012.07.007

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. Available at: www.cell.com/cgi/content/full/115/4/489/DC1. doi:10.1016/s0092-8674(03)00889-4

Mallick, A., and Gupta, B. P. (2020). Vitellogenin-2 Acts Downstream of PRY-1/Axin to Regulate Lipids and Lifespan in *C. elegans*. *Micropubl. Biol.* 2020, 19–21. doi:10.17912/micropub.biology.000281

Mallick, A., and Gupta, B. P. (2022). AXIN-AMPK Signaling: Implications for Healthy Aging. *F1000Res* 10, 1259–1314. doi:10.12688/f1000research.74220.1

Mallick, A., Jhaveri, N., Jeon, J., Chang, Y., Shah, K., Hosein, H., et al. (2021a). Genetic Analysis of *Caenorhabditis elegans* pry-1/Axin Suppressors Identifies Genes Involved in Reproductive Structure Development, Stress Responses, and Aging. *G3 Genes* 12, jkab430. doi:10.1093/g3journal/jkab430

Mallick, A., Ranawade, A., and Gupta, B. P. (2019a). Role of PRY-1/Axin in Heterochronic miRNA-Mediated Seam Cell Development. *BMC Dev. Biol.* 19, 1–12. doi:10.1186/s12861-019-0197-5

Mallick, A., Ranawade, A., van den Berg, W., and Gupta, B. P. (2020). Axin-Mediated Regulation of Lifespan and Muscle Health in C. elegans Requires AMPK-FOXO Signaling. *iScience* 23, 101843. doi:10.1016/j.isci.2020.101843

Mallick, A., Taylor, S. K. B., Mehta, S., and Gupta, B. P. (2021b). picd-1, a Gene that Encodes CABIN1 Domain-Containing Protein, Interacts with pry-1/Axin to Regulate Multiple Processes in Caenorhabditis elegans. *bioRxiv* 1, 37. doi:10.1101/2021.09.27.462077

Mallick, A., Taylor, S. K. B., Ranawade, A., and Gupta, B. P. (2019b). Axin Family of Scaffolding Proteins in Development: Lessons from C. elegans. *Jdb* 7, 20–23. doi:10.3390/jdb7040020

Mathes, S., Fahrner, A., Ghoshdastider, U., Rüdiger, H. A., Leunig, M., Wolfrum, C., et al. (2021). FGF-2-dependent Signaling Activated in Aged Human Skeletal Muscle Promotes Intramuscular Adipogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 118, 1–12. doi:10.1073/pnas.2021013118

Miska, E. A., Alvarez-Saavedra, E., Abbott, A. L., Lau, N. C., Hellman, A. B., McGonagle, S. M., et al. (2007). Most *Caenorhabditis elegans* microRNAs Are Individually Not Essential for Development or Viability. *PLoS Genet.* 3, e215–2403. doi:10.1371/journal.pgen.0030215

O'Brien, J., Hayder, H., Zayed, Y., and Peng, C. (2018). Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front. Endocrinol.* 9, 1–12. doi:10.3389/fendo.2018.00402

Ornitz, D. M., and Itoh, N. (2015). The fibroblast growth factor signaling pathway. *WIREs Developmental Biology* 4, 215–266. doi:10.1002/wdev.176

Pincus, Z., Smith-Vikos, T., and Slack, F. J. (2011). MicroRNA predictors of longevity in caenorhabditis elegans. *PLoS Genet* 7, e1002306. doi:10.1371/journal.pgen.1002306

Ranawade, A., Mallick, A., and Gupta, B. P. (2018). PRY-1/Axin Signaling Regulates Lipid Metabolism in *Caenorhabditis elegans*. *PLoS One* 13, e0206540. doi:10.1371/journal.pone.0206540

Reiner, D. J., González-Pérez, V., Der, C. J., and Cox, A. D. (2008). Use of *Caenorhabditis elegans* to Evaluate Inhibitors of Ras Function *In Vivo*. *Methods Enzymol.* 439, 425–449. doi:10.1016/S0076-6879(07)00430-2

Rennie, W., Kanoria, S., Liu, C., Mallick, B., Long, D., Wolenc, A., et al. (2016). STarMirDB: A Database of microRNA Binding Sites. *RNA Biol.* 13, 554–560. doi:10.1080/15476286.2016.1182279

Rera, M., Azizi, M. J., and Walker, D. W. (2013). Organ-specific Mediation of Lifespan Extension: More Than a Gut Feeling? *Ageing Res. Rev.* 12, 436–444. doi:10.1016/j.arr.2012.05.003

Schutzman, J. L., Borland, C. Z., Newman, J. C., Robinson, M. K., Kokel, M., and Stern, M. J. (2001). The *Caenorhabditis elegans* EGL-15 Signaling Pathway Implicates a DOS-like Multisubstrate Adaptor Protein in Fibroblast Growth Factor Signal Transduction. *Mol. Cell. Biol.* 21, 8104–8116. doi:10.1128/mcb.21.23.8104-8116.2001

Sharanya, D., Fillis, C. J., Kim, J., Zitnik, E. M., Ward, K. A., Gallagher, M. E., et al. (2015). Mutations inCaenorhabditis Briggsaeidentify New Genes Important for Limiting the Response to EGF Signaling during Vulval Development. *Evol. Dev.* 17, 34–48. doi:10.1111/ede.12105

Stefani, G., and Slack, F. J. (2008). Small Non-coding RNAs in Animal Development. *Nat. Rev. Mol. Cell Biol.* 9, 219–230. doi:10.1038/nrm2347

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, S. K. B., Minhas, M. H., Tong, J., Selvaganapathy, P. R., Mishra, R. K., and Gupta, B. P. (2021). *C. elegans* Electrotaxis Behavior Is Modulated by Heat Shock Response and Unfolded Protein Response Signaling Pathways. *Sci. Rep.* 11, 1–17. doi:10.1038/s41598-021-82466-z

Uno, M., and Nishida, E. (2016). Lifespan-regulating Genes in c. Elegans. *npj Aging Mech. Dis.* 2, 16010. doi:10.1038/npjamd.2016.10

Wang, H., Webster, P., Chen, L., and Fisher, A. L. (2019). Cell-autonomous and Non-autonomous Roles of Daf-16 in Muscle Function and Mitochondrial Capacity in Aging *C. elegans*. *Aging* 11, 2295–2311. doi:10.18632/aging.101914

Watts, J. L., and Ristow, M. (2017). Lipid and Carbohydrate Metabolism in *Caenorhabditis elegans*. *Genetics* 207, 413–446. doi:10.1534/genetics.117.300106

Xie, Y., Su, N., Yang, J., Tan, Q., Huang, S., Jin, M., et al. (2020). FGF/FGFR Signaling in Health and Disease. *Sig Transduct. Target Ther.* 5, 181. doi:10.1038/s41392-020-00222-7

Xue, Y., Liu, Z., Cao, J., Ma, Q., Gao, X., Wang, Q., et al. (2011). GPS 2.1: Enhanced Prediction of Kinase-specific Phosphorylation Sites with an Algorithm of Motif Length Selection. *Protein Eng. Des. Sel.* 24, 255–260. doi:10.1093/protein/gzq094

Yin, Y., Castro, A. M., Hoekstra, M., Yan, T. J., Kanakamedala, A. C., Dehner, L. P., et al. (2015). Fibroblast Growth Factor 9 Regulation by MicroRNAs Controls Lung Development and Links DICER1 Loss to the Pathogenesis of Pleuropulmonary Blastoma. *PLoS Genet.* 11, e1005242–22. doi:10.1371/journal.pgen.1005242

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REVIEW
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The Intestine as a Lifespan- and Proteostasis-Promoting Signaling Tissue

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In multicellular organisms such as *Caenorhabditis elegans*, cellular stress stimuli and responses are communicated between tissues to promote organismal health- and lifespan. The nervous system is the predominant regulator of cell nonautonomous proteostasis that orchestrates systemic stress responses to integrate both internal and external stimuli. This review highlights the role of the intestine in mediating cell nonautonomous stress responses and explores recent findings that suggest a central role for the intestine to regulate organismal proteostasis. As a tissue that receives and further transduces signals from the nervous system in response to dietary restriction, heat- and oxidative stress, and hypoxia, we explore evidence suggesting the intestine is a key regulatory organ itself. From the perspective of naturally occurring stressors such as dietary restriction and pathogen infection we highlight how the intestine can function as a key regulator of organismal proteostasis by integrating insulin/IGF-like signaling, miRNA-, neuropeptide- and metabolic signaling to alter distal tissue functions in promoting survival, health- and lifespan.

Keywords: intercellular signaling, intestine, cell-nonautonomous, proteostasis, *C. elegans*, stress, organismal aging, neurons

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INTRODUCTION

Efficient protein folding is essential for the vitality of all cells within an organism throughout the different challenges that occur in a lifetime. Maintaining a functional proteome is therefore crucial for cellular health in all living organisms. This is achieved by the cellular proteostasis network, and predominantly enacted by molecular chaperones as well as degradation machineries such as the proteasome and autophagy [reviewed in Hipp et al. (2019)]. In order to combat protein misfolding and environmental fluctuations, multicellular organisms require a coordinated response mounted between tissues. Recognition of this led to a shift in focus towards investigations into the cell nonautonomous regulation of proteostasis and how it impacts both health- and lifespan in normal and pathological aging [reviewed in O'Brien and van Oosten-Hawle (2016); reviewed in Sala et al. (2017); reviewed in Hipp et al. (2019); reviewed in Morimoto (2020)]. Recent evidence suggests that cell nonautonomous regulation of proteostasis is a highly complex process with different outcomes based on the tissue experiencing the stress (Taylor and Dillin, 2013; van Oosten-Hawle et al., 2013; Shao et al., 2016).

A crucial component to regulate organismal proteostasis is the cell nonautonomous control of stress responses such as the unfolded protein response of the ER (UPR^{ER}) (Lee, 2021), the unfolded protein response of the mitochondria (UPR^{MIT}) [reviewed in Jovaisaite et al. (2014)], the heat shock

response (HSR) (Prahlad et al., 2008) and transcellular chaperone signaling (TCS) (van Oosten-Hawle et al., 2013). Throughout the aging process the competency of stress responses are known to decline in *C. elegans* [Ben-Zvi et al. (2009); reviewed in; Labbadia and Morimoto (2014); Kim et al. (2018); reviewed in; Muñoz-Carvajal and Sanhueza (2020)] which often correlates with the onset of age-related diseases such as Alzheimer's (AD) [reviewed in Campanella et al. (2018)] and Huntington's disease (HD), as shown in *C. elegans* models (Labbadia et al., 2011) and mouse models (Wang et al., 2008), as well as in the human pathology; for example, HSF1 is degraded in brain samples of HD patients (Gomez-Pastor et al., 2017; Koyuncu et al., 2018). Enhancing and "hijacking" cellular stress responses in animals modeling these disease states can be beneficial in delaying or even preventing age-associated pathologies [reviewed in Ben-Zvi et al. (2009); Vidal et al. (2012); Bakula and Scheibye-Knudsen (2020)] which is highlighted and described in detail elsewhere in this special issue.

Many of the cell nonautonomous stress responses are controlled by the nervous system, in both *C. elegans* as well as vertebrate models (Bishop and Guarente, 2007; Durieux et al., 2011; Taylor and Dillin, 2013; Williams et al., 2014; Shao et al., 2016), opening new questions on the exact neural circuits orchestrating organismal proteostasis. Beyond the nervous system the intestine has been shown to not only integrate stress signals received from the neurons but is yet another organ central for the regulation of proteostasis. This review will explore the role of the intestine as a proteostasis-regulating tissue and the consequences for organismal health- and lifespan with an emphasis on findings from the model organism *C. elegans*.

THE INTEGRATION OF NERVOUS SYSTEM SIGNALS TO THE INTESTINE TO PROMOTE ORGANISMAL PROTEOSTASIS AND LIFESPAN

Cell nonautonomous neuroendocrine signaling pathways, such as those which initiate trans-tissue communication from the olfactory neurons to the intestine, are important for *C. elegans* health and lifespan and have been shown to regulate proteotoxic stress and quality control [reviewed in Miller et al. (2020)]. For example, chemosensory neurons send neuroendocrine signals in response to food cues, such as low food quantity, also known as dietary restriction (DR), to regulate the activity of the FOXO transcription factor DAF-16/FOXO in the intestine, the main component of the insulin-like signaling (IlS) pathway, that regulates longevity (Fletcher and Kim, 2017). DR induces the expression of the neuroendocrine ligand DAF-7 in the ASI chemosensory neurons, which signal to the RIM and RIC interneurons to suppress the co-SMAD DAF-3. This enables the induction of the DR response and the activation of DAF-16 in the intestine to promote longevity (Fletcher and Kim, 2017). However, in aging nematodes, DAF-7 expression levels are decreased and thus are no longer inhibiting DAF-3, which reduces the capacity for DR-induced lifespan extension in older animals (Fletcher and Kim, 2017) (**Figure 1A**).

Furthermore, DR, as well as the induction of ER stress *via* neuronal overexpression of *xbp-1s*, can activate the neuronal IRE-1-XBP-1 branch of the UPR^{ER} (Matai et al., 2019; Özbey et al., 2020), a stress-response pathway counteracting unfolded protein stress in the endoplasmic reticulum, that is, involved in lifespan regulation [reviewed in Taylor and Hetz (2020); reviewed in Walter and Ron (2011)]. DR can induce the splicing of XBP-1 into XBP-1s in the RIM and RIC neurons which drives cell nonautonomous UPR^{ER} activation in the intestine *via* acetylcholine and tyramine signaling leading to metabolic and lysosomal changes, and lifespan- and proteostasis-enhancing effects on an organismal level (Imanikia et al., 2019a, 2019b; Özbey et al., 2020) (**Figure 1B**).

In addition, DR as well as reduced IlS signaling (as modelled *via* the use of *daf-2* mutants) can cause an increase in the autophagy protein ATG–18 activity in chemosensory neurons, and the intestine, resulting in lifespan extension in a cell nonautonomous manner (Minnerly et al., 2017). The exact cell nonautonomous mechanism of the ATG-18 mediated lifespan extension upon DR is not yet fully known, however in loss of function *daf-2* mutants an increased activity of ATG-18 in ADF, ADL, ASG, and AWA neurons sends a signal through neurotransmitters to an unknown neuron which in turn signals to the intestine *via* neuropeptides to activate DAF-16 and thus increase longevity (Minnerly et al., 2017) (**Figure 1C**).

Interestingly the perception of food odor itself can maintain organismal proteostasis and regulate lifespan through the microRNA *miR-71* mediated inhibition of *tir-1* mRNA stability in olfactory AWC neurons (Finger et al., 2019) (**Figure 1D**). Importantly, AWC neuron activity has a direct impact on ubiquitin-dependent protein degradation in the intestine, which is regulated *via* secretion of the neuropeptides NLP-9 and NLP-14, leading to increased longevity and proteostasis (Finger et al., 2019) (**Figure 1D**).

Efficient communication between the nervous system and the intestine is also required for effective survival upon exposure to cold and warm temperatures (Zhang et al., 2018). Under both circumstances, neurons require DAF-16 activity specifically within the intestine to integrate cues from the environment as well as the nervous system to impact longevity under different temperature conditions (Zhang et al., 2018). For example, low temperature sensing IL1 and NSM neurons send signals to the intestine to extend lifespan through glutamate and serotonin neurotransmitters, whereas the warm temperature sensing ASJ neurons send signals to the intestine through insulin-like neuropeptides to shorten lifespan (Zhang et al., 2018) (**Figure 1E**). These opposing effects on longevity are *via* differential regulation of the transcription factor DAF-16 where IL1 and NSM neurons send signals to activate DAF-16 expression (**Figure 1Ei**) whereas ASJ neurons send signals to inhibit DAF-16 expression in the intestine (Zhang et al., 2018) (**Figure 1Eii**). In addition, exposure to high temperatures activates the HSR, which has proteostasis and longevity promoting effects (Morley and Morimoto, 2004). In *C. elegans*, thermo-sensory AFD neurons respond to an increase in

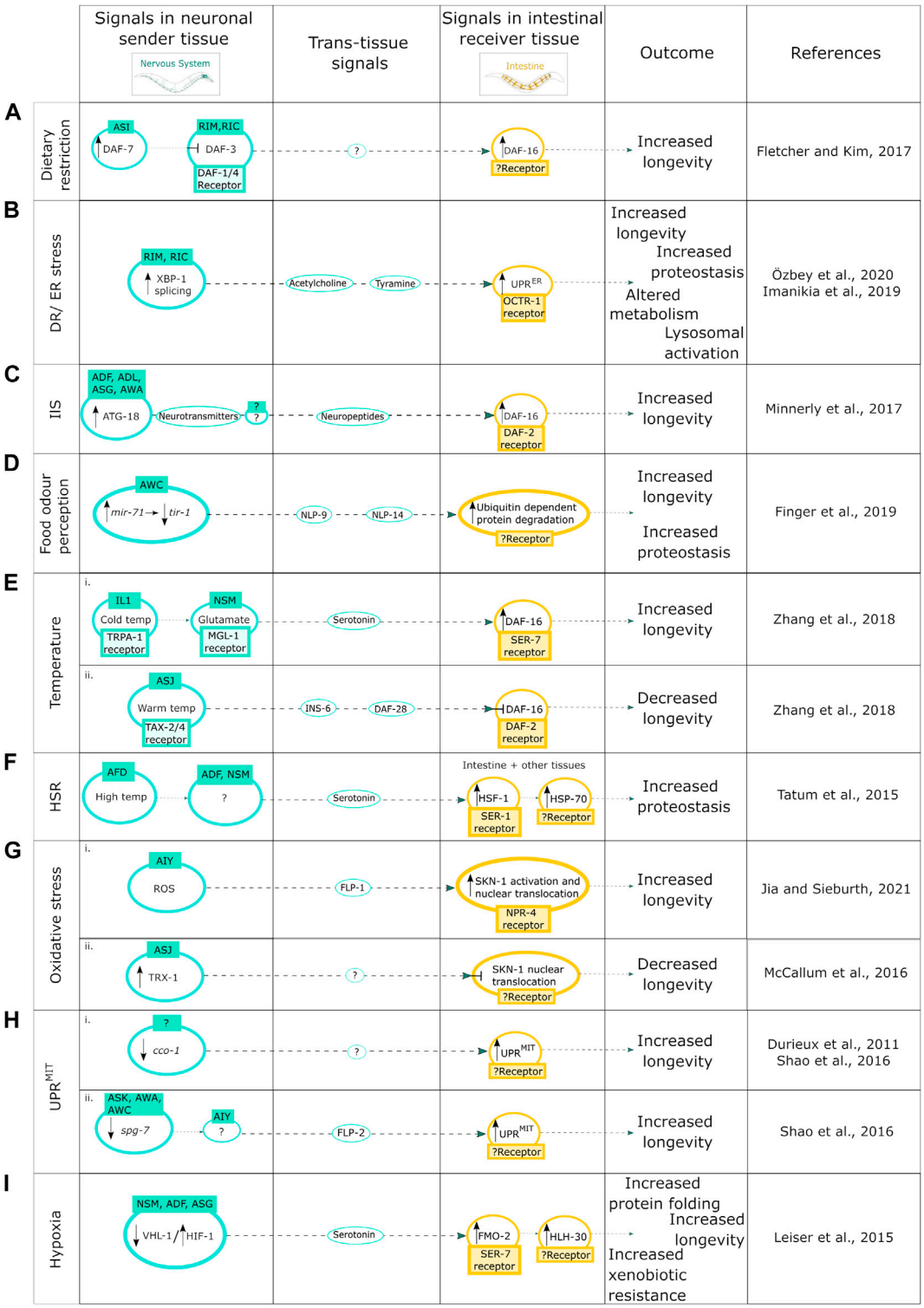

FIGURE 1 | Neuron-to-intestine communication in organismal proteostasis and aging. **(A)** Mechanism of DR induced neuronal cell nonautonomous signaling upon expression of DAF-7. **(B)** DR/ER stress-induced neuronal cell nonautonomous signaling upon activation of the IRE-1-XBP-1 branch of the UPR^{ER}. **(C)** Neuronal cell nonautonomous signaling upon increase of the autophagy protein ATG-18; **(D)** upon food odor perception through the miRNA pathway; **(E)** in response to (i) cold temperature and (ii) high temperature; **(F)** in response to the HSR; **(G)** in response to oxidative stress; induced by (i) ROS, (ii) TRX-1 activation; **(H)** upon induction of neuronal UPR^{MIT} by the KD of the ETC component (i) *cco-1* and (ii) *spg-7*; and **(I)** in response to hypoxia.

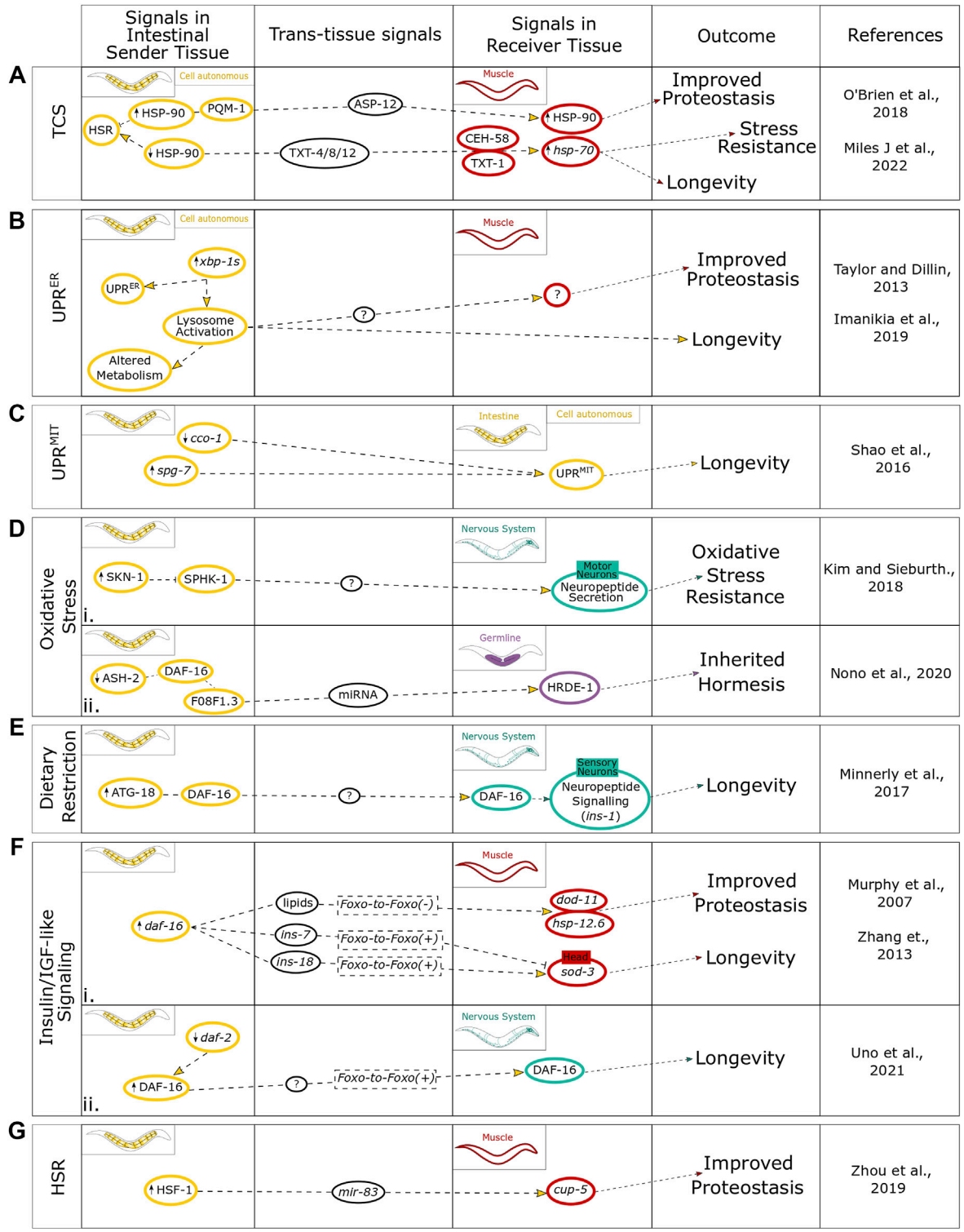

FIGURE 2 | Summary of intestinal regulation of cell nonautonomous stress signals in mediating organism physiology. The findings for intestine-induced stresses within the context of **(A)** TCS cell autonomously and communicated to the muscle, **(B)** the UPR^{ER} cell autonomously and communicated to the muscle, **(C)** the UPR^{MIT} contained to a cell autonomous response, **(D)** oxidative stress communicated to both (i) the nervous system and (ii) the germline, **(E)** dietary restriction communicated to the nervous system, **(F)** IIS communicated to both (i) the muscle and (ii) the nervous system, and **(G)** the age-associated changes in the competency of the HSR. This figure highlights the signals within the intestinal sender tissue, signals involved in the trans-tissue communication, and signals within the different receiver tissues and how they link to different physiological outcomes.

temperature by regulating the cell nonautonomous HSR (Prahlad et al., 2008) by activating the heat shock transcription factor heat shock factor-1 (HSF-1), in distal tissues including the intestine (Tatum et al., 2015). This cell nonautonomous HSF-1 activation is mediated *via* the release of serotonin from ADF and NSM neurons and activation of the SER-1 receptor and results in induction of heat shock proteins such as HSP-70 that facilitates the reduction of protein misfolding (Tatum et al., 2015) (**Figure 1F**).

Integration of neuronal signals by the intestine also plays an important role in the response to oxidative stress (Kim and Sieburth, 2018). Reactive oxygen species (ROS), which are the main cause of oxidative stress, can promote premature and pathological aging, however, they can also increase organismal stress resistance and longevity, thus having a dual effect [reviewed in Miranda-Vizuete and Veal (2017)]. The ROS-induced oxidative stress response can be activated by the neuroendocrine stress signal, FLP-1, which upon secretion from the AIY neurons activates the antioxidant response in the intestine, and positively regulates the oxidative stress response transcription factor SKN-1 to modulate lifespan (Jia and Sieburth, 2021) (**Figure 1Gi**). SKN-1, however, also has a redox-independent role through cell nonautonomous regulation *via* the thioredoxin, TRX-1, that is, expressed in ASJ neurons and suppresses the cell nonautonomous nuclear localization of SKN-1 in the intestine through the p38 MAPK pathway (McCallum et al., 2016) (**Figure 1Gii**). Neuronal ROS can also induce cell nonautonomous activation of intestinal UPR^{MIT}, however this cell nonautonomous activation can also be induced *via* the knockdown of mitochondrial components (Durieux et al., 2011; Shao et al., 2016). Both the reduction in activity of the complex IV subunit *cco-1* and the knockdown of the mt-AAA protease *spg-7* in the intestine induce cell nonautonomous activation of the UPR^{MIT} leading to an increased lifespan (**Figure 2C**) (Durieux et al., 2011; Shao et al., 2016). The signal/mediator of cell nonautonomous UPR^{MIT} induction *via cco-1* knockdown in either neurons or intestine is not yet known (Durieux et al., 2011; Shao et al., 2016) (**Figure 1Hi**). The cell nonautonomous induction of UPR^{MIT}, when induced by neuronal *spg-7* knockdown, is mediated *via* the secretion of the neuropeptide FLP-2 from AIA interneurons (Shao et al., 2016) (**Figure 1Hii**). It will be interesting to investigate whether similar or the same mediating components regulate the cell nonautonomous UPR^{MIT} upon *cco-1* knockdown.

Another cellular stress response pathway that requires cell nonautonomous neuron-to-intestine signaling is the hypoxic stress response. Chronic low levels of oxygen induce the hypoxic stress response, regulated *via* the hypoxia-inducible factor (HIF-1), which drives cytoprotective mechanisms in response to this stress and modulates neural circuit function and activity (Pender and Horvitz, 2018). Activation of HIF-1 has been shown to increase the expression of serotonin in NSM, ADF, and ASG sensory neurons (Pocock and Hobert, 2010). Increased pan-neuronal expression of HIF-1 in neurons activates increased production of the longevity gene FMO-2 in the intestine through serotonergic signaling and the SER-7 receptor, which in turn activates the induction of the transcription factor HLH-30 to increase longevity (Leiser et al., 2015) (**Figure 1I**). Interestingly, intestinal FMO-2 induction can also be activated *via* DR, however, this is potentially regulated through a different signaling pathway (Leiser et al., 2015).

Overall, neuron-to-intestine cell nonautonomous signaling has a crucial role in the pro-longevity actions through dietary restriction, ER stress, IlS, food odor perception and *via* the temperature-, heat shock-, oxidative-, and hypoxic stress responses.

THE INTESTINE AS A REGULATING AND INTEGRATING ORGAN FOR ORGANISMAL PROTEOSTASIS

While the intestine is an important organ for the integration of neuronal signals to benefit organismal proteostasis, it can potentially act independent of neuronal input. An example of this is that HSP-90 overexpression solely in the intestine is able to communicate *via* the transcription factor PQM-1 and the extracellular immune-peptide ASP-12 to upregulate HSP-90 in the neighboring muscle cells (O'Brien et al., 2018). The subsequent muscular HSP-90 upregulation is able to suppress the age-dependent aggregation of amyloid beta (Aβ) in the muscle (O'Brien et al., 2018). Conversely, intestinal *hsp-90* knockdown has been found to signal *via* secreted peptides TXT-4, TXT-8, and TXT-12 to induce the guanylate cyclase TXT-1 and the transcription factor CEH-58 in muscle cells leading to upregulation of *hsp-70* which results in an improved survival when exposed to elevated temperatures (Miles et al., 2022). These findings provide evidence that the intestine can be a major regulator of proteostasis and stress resistance through cell nonautonomous regulation of molecular chaperones *via* transcellular chaperone signaling (TCS) (**Figure 2A**) (O'Brien et al., 2018). Intestinal induction of the UPR^{ER} through expression of *xbp-1s* led to a modest increase in lifespan and reduced Aβ aggregation in muscle cells. However, the intestine by itself appears to be unable to induce an UPR^{ER} stress response in distal tissues (**Figure 2B**) (Taylor and Dillin, 2013). Intercellular activation of the UPR^{ER} from one organ to another specifically requires neuronal expression of *xbp-1s,* with just two interneurons triggering the activation of the UPR^{ER} to distal tissues by tyramine (Özbey et al., 2020). While intestinally induced UPR^{ER} remains cell autonomous, the nervous system requires transcellular activation of the UPR^{ER} in the intestine as an intermediate signaling tissue to produce the organismal benefits on longevity and proteotoxicity, potentially due to intestinal activation of lysosomal factors including *rde-1*, *lmp-1*, *vha-18*, and *asp-3* that exert these beneficial effects in distal tissues (**Figure 2B**) (Imanikia et al., 2019a). A comparable finding was put forward for intestinal induction of the mitochondrial UPR, with pan-neuronal induction leading to activation of the UPR^{MIT} in distal tissues, whereas intestinal induction of UPR^{MIT} remained cell autonomous but was still able to enhance longevity (**Figure 2C**) (Shao et al., 2016). Thus, even though the intestine is unable to transduce the activation of a stress response to distal tissues, as shown by the examples of the UPR^{ER} and UPR^{MIT}, it is

instrumental to mediate the consequences on proteostasis at an organismal level.

When observing naturally occurring stresses that impact the intestine, such as DR or pathogen infection, the intestine becomes an undeniably major tissue required for the tissue-specific as well as "transcellular" regulation of stress stimuli impacting *C. elegans* health- and lifespan. The importance of the intestine is highlighted through its role in the gut-brain axis in humans where the intestine plays a key role in the regulation of brain health that influences the development of neurodegenerative diseases [reviewed in Houser and Tansey (2017); reviewed in Peterson (2020)].

OXIDATIVE STRESS RESPONSES REGULATED BY THE INTESTINE

In response to oxidative stress, the intestine has been found to integrate neuronal and environmental cues *via* intestinal expression of the conserved transcription factor SKN-1 (Nrf2 in humans) [reviewed in Blackwell et al. (2015)]. The loss of function of this transcription factor significantly reduces oxidative stress tolerance leading to reduced survival (Bishop and Guarente, 2007). *C. elegans skn-1* null mutants that express wild type SKN-1 solely in the intestine are able to survive oxidative stress, whereas animals expressing SKN-1 solely in neurons remained unable to tolerate oxidative stress with extremely low survival rates (Bishop and Guarente, 2007). Interestingly, the intestine mediates the response to oxidative stress by utilizing the neuropeptide network *via* motor neurons (**Figure 2Di**) (Kim and Sieburth, 2018). Similarly, intestinal IlS regulates resistance to oxidative stress, with knockdown of *daf-2* in the intestine increasing oxidative stress resistance in a *daf-16* dependent manner (Uno et al., 2021). This demonstrates the importance of the intestine as a central orchestrating organ, with the capacity to control proteostasis, perhaps *via* feedback signaling to neural circuits. Oxidative stress is also an important factor in the immune response upon pathogen infection affecting the intestine. For example, *C. elegans* can produce ROS within the intestine upon infection with pathogenic bacteria, such as *Enterococcus faecalis*, as a means of immune defense (Chávez et al., 2007). While ROS can increase *C. elegans* survival rate infected by pathogenic bacteria, high levels of ROS production is cytotoxic and has the potential to also damage host tissues and organs. This can be counteracted by the upregulation of oxidative stress enzymes including *sod-3* and *clt-2* (Chávez et al., 2007). Beyond their role in pathogen defense, antioxidants also have effects on lifespan, behavior and proteostasis (Brown et al., 2006). For example, *C. elegans* exposed to the antioxidant epigallocatechin gallate (EGCG) *via* feeding reduces the age-associated decline of pharyngeal pumping but did not lead to a lifespan extension (Brown et al., 2006); whereas the antioxidant α-lipoic acid has the opposite effect by increasing lifespan, potentially through differences in specific gene expression with different molecular targets. Both antioxidants are able to improve age-associated decline in chemotaxis ability, indicating roles in influencing neuronal signaling and behavioral consequences (Brown et al., 2006).

In humans, oxidative stress has been linked with the progression of AD, as the gut-brain axis is crucial for brain health [reviewed in Dumitrescu et al. (2018); reviewed in Luca et al. (2019)]. For example, upon a 12-week oral probiotic supplementation program the oxidative stress biomarker 'malondialdehyde' was reduced which correlated with an increase in cognition in AD patients (Akbari et al., 2016). Therefore, further characterization of this link between the intestine and nervous system in the context of oxidative stress may provide potential targets for therapeutics in AD.

INTESTINAL REGULATION OF DIETARY RESTRICTION

DR is a common stress that *C. elegans* will encounter naturally when food abundance is low. The molecular process by which DR is sensed, interpreted and resolved is considered a complex and incompletely understood process. There is, however, a common consensus that the major tissues perceiving dietary restriction are the neurons and the intestine with information on environmental cues of food abundance interpreted by the nervous system and actual nutritional uptake by the intestine (Walker et al., 2005). A recent study investigated the role of autophagy in the regulation of lifespan through DR as well as the IlS pathway in *C. elegans* (Minnerly et al., 2017). Intestinal expression of autophagy factor ATG-18 is required to respond to DR, enabling DR-mediated longevity by targeting neuropeptide communication in the nervous system (**Figure 2E**). In parallel, and independent of DR, intestinal expression of ATG-18 also influences the expression of insulin like-peptide *ins-1* in neurons and requires neurotransmitter release to promote longevity *via* the IlS pathway (Minnerly et al., 2017). Importantly, the intestine plays a crucial role for DR-induced life- and health-span extension through increased autophagic flux by alleviating the age-related decline in motility and improving the intestinal barrier function (Gelino et al., 2016). This contribution of autophagy to healthspan extension is largely observed in the genetically dietary restricted *eat-2* mutant, where autophagic flux is increased, resulting in a reduced age-dependent loss of gut integrity (Gelino et al., 2016). Interestingly, in humans, gut dysbiosis also reduces intestinal integrity and can be a source of oxidative stress that contributes to the initiation of neurodegenerative diseases [reviewed in Dumitrescu et al. (2018)].

INTESTINAL REGULATION OF THE INSULIN/INSULIN-LIKE GROWTH FACTOR-LIKE SIGNALING PATHWAY AND FOXO-TO-FOXO SIGNALING

The IlS pathway is the predominant pathway integrating different nutritional cues resulting in system-wide impacts through several

aspects of healthspan including fertility (Klass, 1977; Tissenbaum and Ruvkun, 1998); reviewed in Neirijnck et al. (2019), stress resistance [reviewed in Yu and Chung (2001)], immunity (Singh and Aballay, 2006) and metabolism [reviewed in Anderson and Weindruch (2007)], as well as having direct impacts on lifespan (Kenyon et al., 1993). The IlS pathway is inherently a cell nonautonomous pathway with activation signals in the form of extracellular insulin-like peptides binding the cell-surface receptor DAF-2/IGFR. This in turn triggers a kinase cascade culminating in the phosphorylation of the main FOXO transcription factor DAF-16 (Ogg et al., 1997) which subsequently translocates to the nucleus leading to a specific transcriptional program promoting longevity and stress resistance (Henderson and Johnson, 2001). Intestinal DAF-16 has been found to control proteostasis and longevity through cell nonautonomous mechanisms which can be either dependent on DAF-16 in receiver tissues [*FOXO-to-FOXO* (+) signaling] (Murphy et al., 2007; Uno et al., 2021) or through an alternative mechanism independent of DAF-16 function in distal tissues [*FOXO-to-FOXO* (-) signaling] (Murphy et al., 2003; Zhang et al., 2013). In *FOXO-to-FOXO* (+) signaling, intestinal DAF-16 induces an upregulation of the DAF-16-dependent gene *sod-3* in the epidermis and muscle, correlating with an increased lifespan (**Figure 2Fi**). This intestinal *FOXO-to-FOXO* (+) signaling was found to be dependent on the positive and negative regulation of the insulin-like genes *ins-18* and *ins-7*, respectively. In particular, *ins-7* knockdown in the intestine induced *sod-3* expression in head muscles and enhanced lifespan whereas intestinal *ins-18* knockdown prevented *sod-3* expression in head muscles and caused a shortened lifespan creating a positive feedback loop that coordinates system-wide aging (**Figure 2Fi**) (Murphy et al., 2007). The intestinal-neuronal axis is vital in this cell non-autonomous regulation of lifespan as well as reproductive span, with knockdown of *daf-2* in the gut being sufficient for lifespan extension (Uno et al., 2021). Interestingly, *daf-2* knockdown in the intestine also induced nuclear localization and activation of DAF-16 in the neurons (**Figure 2Fii**), further suggesting IlS-mediated *FOXO-to-FOXO* (+) signaling can be employed from the intestine to the neurons to regulate lifespan.

The intestine has also been found to employ IlS in *FOXO-to-FOXO* (-) trans-tissue communication (**Figure 2Fi**). For example, intestine-specific overexpression of DAF-16 extended the lifespan of *daf-16* and *daf-2* null mutants by up to 70%, suggesting this longevity phenotype is not dependent on DAF-16 in distal tissues (Libina et al., 2003). The intestine was also the only tissue requiring DAF-16 activity to maintain germline-defective induced longevity, suggesting a potential cell nonautonomous connection between the intestine and germline within the context of fitness trade-off. Interestingly, intestinal DAF-16 overexpression was able to upregulate the expression of proteostasis and metabolic genes, such as *hsp-12.6* and *dod-11*, respectively, *via FOXO-to-FOXO* (-) signaling (**Figure 2Fi**), in tissues lacking DAF-16 (Zhang et al., 2013). These *FOXO-to-FOXO* (-) signals were found to be dependent on the lipid signal *mdt-15* a subunit of a mediator complex that regulates the expression of genes involved in lipid metabolism, suggesting lipids could be enactors of this pathway. In age-associated protein misfolding disease models, the intestine was able to utilize this *mdt-15*-dependent *FOXO-to-FOXO* (-) signaling to improve muscular proteostasis and alleviated the age-dependent paralysis caused by muscular Aβ expression (Zhang et al., 2013). When human insulin is introduced into the *C. elegans* intestine through feeding bacteria supplemented with a buffered suspension of insulin complexed with protamine sulfate, used to treat type II diabetes in humans, this inhibited α-synuclein aggregation in the muscle through antagonizing the DAF-2/IGFR receptor (Haque et al., 2020). This suggests there is some commonality in the functioning of this pathway between humans and *C. elegans* which may be beneficial in neurodegenerative diseases. Low levels of the Insulin-like growth factor 1 (IGF-1) is a risk factor for developing neurodegenerative disease (Westwood et al., 2014) and modulation of IGF-1 by estrogens is thought to cause slower progression of Parkinson's disease in women compared to men [reviewed in González et al. (2008); reviewed in Labandeira-Garcia et al. (2016); reviewed in Castilla-Cortázar et al. (2020)]. Therefore, deepening our understanding of how to modulate the function of the IlS pathway and what off-target effects this may produce could prove therapeutically beneficial.

INTESTINAL REGULATION OF THE MICRORNA PATHWAY

Another regulatory pathway which is becoming increasingly detailed in its role in trans-tissue communication of stress responses and aging is the microRNA (miRNA) pathway [reviewed in Leung and Sharp (2010); reviewed in Inukai and Slack (2013); reviewed in Son et al. (2019)]. MiRNAs are single-stranded RNAs approximately 22 nucleotides in length which play roles in regulating expression patterns with the ability to either stabilize or destabilize complementary target mRNAs to either increase or decrease translation [reviewed in Fabian et al. (2010)]. The intestine has been implicated in utilizing miRNAs to modulate distal tissue function in promoting health- and lifespan. For example, the age-associated increase of HSF-1 was found to regulate the miRNA *mir-83* specifically within the intestine which impaired intestinal autophagy through targeting a lysosomal calcium channel, *cup-5* (Zhou et al., 2019). This suppression of autophagy by intestine-specific expression of *mir-83* extended to the body wall muscle, by directly impacting the age-associated aggregation of PolyQ (**Figure 2G**). *mir-83* null mutants showed reduced PolyQ aggregation not only within the intestine but also in the muscle tissue and resulted in an increased lifespan (Zhou et al., 2019). Despite not being directly expressed in muscle, *mir-83* was found to directly regulate *cup-5* transcription in the muscle through interaction with its 3′UTR. Importantly, *mir-83* was detected in both extracellular vesicles and in coelomocytes, suggesting that *mir-83* was transported directly from the intestine to the muscle to enact the cell nonautonomous regulation of autophagy and lifespan.

Aside from autophagy, the intestine has also been found to modulate oxidative stress *via* the miRNA *mir-60* by maintaining cellular homeostasis to promote survival and lifespan (Kato et al., 2016). Intestinal miRNAs are also required for inherited hormesis in response to oxidative stress. When *C. elegans* are exposed to

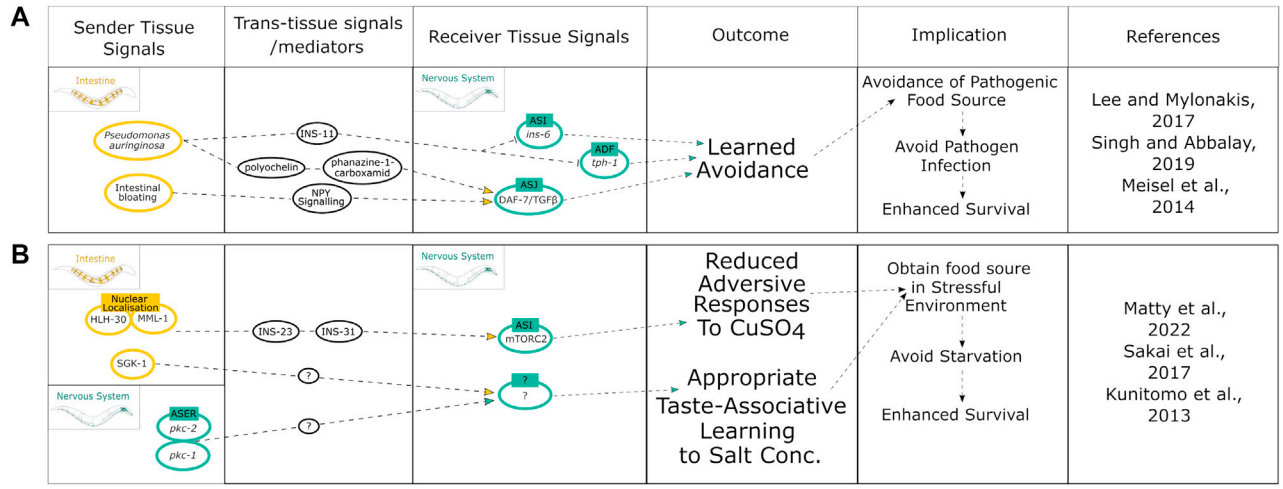

FIGURE 3 | Summary of intestinal modulation of behavioral responses linked to improved stress-associated survival. **(A)** Mechanisms initiated by the intestine that modulate behavior upon pathogen infection to promote survival. **(B)** Integration of both neuronal and intestinal cues to pursue food cues by downregulating avoidance of risk stimuli to promote survival.

different stresses during developmental stages including osmotic stress, heavy metal stress, and DR this results in increased resistance to oxidative stress which can be passed on to subsequent generations (Kishimoto et al., 2017). Disrupting miRNA transport from the intestine partially suppressed the increased oxidative stress resistance of animals experiencing this as early life stress, but the oxidative stress resistance was completely abolished in their first-generation progeny (Okabe et al., 2021). This suggested that transmission of miRNA from the intestine to the germline may regulate the inheritance of oxidative stress resistance. Further support that the intestine regulates the intergenerational transmission of oxidative stress resistance through epigenetic mechanisms, as well as miRNAs showed that this was dependent on the IlS intestinal transcription factor DAF-16 (Nono et al., 2020). Intestinal knockdown of the histone trimethylation (H3K4) demethylase modifier ASH-2 resulted in increased oxidative stress resistance in two subsequent generations (**Figure 2Dii**). This effect was dependent on DAF-16 activity in the intestine through regulation of a downstream target, F08F1.3, which targeted several histone modifiers in the germline as well as being required the function of HRDE-1, a germline argonaut, which regulates miRNA activity (Nono et al., 2020). This suggests that there may be a cell nonautonomous regulation of inherited stress resistance integrating both the miRNA and IlS pathways between the intestine and germline in promoting the survival of offspring.

THE INTESTINE AS AN INTEGRATOR OF BEHAVIORAL AND DEFENSE MECHANISMS

All examples discussed thus far have focused on how the intestine affects health- and lifespan directly. The intestine also influences neuronal cues that modulate behaviors to promote survival in response to stress stimuli and enhance proteostasis. This intestine-to-neuron crosstalk is important to drive behavioral change that can be beneficial for organismal proteostasis. Behavioral modulation is vital as a mechanism to evade pathogens and promote survival and longevity. Certain pathogenic bacteria such as *Pseudomonas aeruginosa* induce initial attraction in *C. elegans* as a food source but then lead to a subsequently learned avoidance once it has been recognized as a pathogen (Singh and Aballay, 2019). This switch to avoidance has been linked to intestinal signals. One study from 2017 points towards an intestine-derived neuropeptide, INS-11, to mediate this learned avoidance behavior through modulating IlS (*ins-6*) and serotonin signaling (*tph-1*) in ASI and ADF neurons, respectively (**Figure 3A**) (Lee and Mylonakis, 2017). The neuropeptide Y (NPY)-related signaling neuroendocrine pathway is activated as a result of intestinal bloating and directly targets DAF-7/TGF-β signaling in ASJ neurons creating a learned avoidance for the low-oxygen environment associated with *P. aeruginosa* (**Figure 3A**) (Singh and Aballay, 2019). Another study suggested that it is the release of the secondary metabolites pyochelin and phenazine-1-carboxamide, produced by *P. aeruginosa* in the intestine, which targets DAF-7/TGF-β signaling in ASJ neurons to initiate this learned avoidance behavior (**Figure 3A**) (Meisel et al., 2014).

Interestingly, the *C. elegans* intestine may even dictate behavioral decisions based on the level of damage sustained from a particular sensory cue (Hajdú et al., 2021). Upon exposure to toxic concentrations of benzaldehyde or diacetyl there was a cytoprotective response initiated in the intestine. This response effectively restored proteostasis in the intestine damaged by benzaldehyde but not diacetyl. The intestinal damage was suggested to be signaled from the intestine to the nervous system to confer either flexible or robust avoidance responses upon re-exposure to each chemical (Hajdú et al., 2021). Although

this mechanism needs further characterization, it presents a potential role of the intestine in controlling memory formation in the nervous system to dictate future behaviors based on damage levels as a form of decision making between defensive avoidance and food searching. This balances the risk of damage with the risk of starvation to promote survival and therefore longevity. Similar findings have also implicated the intestine to integrate cues involved in "risk-versus-reward" behavioral decision making (Matty et al., 2022): *C. elegans* that had experienced food deprivation will cross a toxic barrier quicker than well-fed animals to reach a food source. The mechanism behind this elevated risk-taking was shown to involve the translocation of the transcription factors MML-1 and HLH-30 from the intestinal nuclei to the cytoplasm, as well as insulin-like peptides INS-23 and INS-31, that target the DAF-2 receptors on the ASI neurons, thereby modulating TORC2 signaling to reduce aversive chemotaxis responses (**Figure 3B**) (Matty et al., 2022). In addition, *C. elegans* can associate being well-fed or starved with the specific concentration of salt that either nutritional state was experienced in a manner that depends on the protein kinase PKC-1 (Gq/DAG/PKC pathway) (Kunitomo et al., 2013) and the TORC2 substrate PKC-2 in ASE neurons (Sakai et al., 2017) (**Figure 3B**). Moreover, the TORC2 substrate SGK-1 is required specifically in the intestine to associate low salt with starvation leading to an avoidance of low salt concentrations and attraction to high concentrations of salt, albeit the exact neuronal targets remain to be determined (**Figure 3B**) (Sakai et al., 2017). Thus, the intestine is required for feedback signaling to the nervous system to initiate protective behaviors that in turn benefit organismal proteostasis.

Similarly, behavioral cues initiated in the intestine can influence the responses to thermal stress by migrating away from dangerously high and low temperatures. For example, intestinal activation of the TORC2 substrate PKC-2, promotes cold-directed migration (Land and Rubin, 2017).

Together these findings indicate the intestine is able to modulate risk-taking behaviors as well as pathogen avoidance to promote organismal proteostasis.

DISCUSSION AND OUTLOOK

The nervous system has been the focus of trans-tissue regulation in pro survival, healthspan, and longevity signals. However, increasing knowledge points to the intestine as a key organ that feeds information towards the nervous system and other tissues in response to a variety of stressors including nutrition availability, pathogen infection, oxidative- and heat stress. The intestine has been proven to affect not only stress survival but is implicated in passing on epigenetic information to promote survival in proceeding generations. This suggests the intestine is not only a hub for health- and lifespan regulation but also a potential modulator of evolutionary adaptation to stressful environments. However, there remains much to be understood about how the intestine safeguards proteostasis across tissues and how this role can be harnessed to delay the onset of neurodegenerative diseases in patients, such as, for example, through the gut microbiome. Rats exposed to curli-producing bacteria in their gut, showed increased neuronal alpha-synuclein deposition in both gut and brain, potentially directly *via* cross-seeding of amyloid species and priming certain responses of innate immune cells in the brain, such as glial cells (Chen et al., 2016).

In mammals, the gut-brain axis plays an important role in the development of multiple age-dependent protein folding diseases, including AD, PD and amyotrophic lateral sclerosis (ALS), that is, often preceded by changes in gut microbiota. For example, a lack or decrease of certain bacterial species in the gut, can have a negative impact on PD or ALS pathogenesis in patients at an early disease stage (Jin et al., 2019; Hertzberg et al., 2022).

Interestingly, the mammalian brain senses gut stimuli *via* the passive release of hormones and other macromolecules from the gut. Epithelial sensor cells in the gut, the enteroendocrine cell or neuropod cells, can also directly connect with vagal neurons to transduce mechanical, chemical, or bacterially derived sensory signals from the gut lumen to the brain, using glutamate as a neurotransmitter [reviewed in Furness et al. (2013); reviewed in Psichas et al. (2015); Kaelberer et al. (2018)]. Thus, this allows the intestine a direct impact on neuroplasticity, brain health and proteostasis-promoting behaviors that influence overall health which is likely conserved throughout evolution.

As highlighted in this review, the intestine differentially affects different tissues, therefore further understanding of intercellular signaling events occurring between the gut and distal tissues, and in particular gut-to-neuron communication, could open exciting possibilities for future therapeutic interventions to improve brain homeostasis during aging.

AUTHOR CONTRIBUTIONS

VB prepared **Figure 1**, FH prepared **Figures 2**, **3**. FH, VB and PVO-H prepared, reviewed, and edited all sections of the manuscript.

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REFERENCES

Akbari, E., Asemi, Z., Daneshvar Kakhaki, R., Bahmani, F., Kouchaki, E., Tamtaji, O. R., et al. (2016). Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial. *Front. Aging Neurosci.* 8, 256. doi:10.3389/fnagi.2016.00256

Anderson, R. M., and Weindruch, R. (2007). Metabolic Reprogramming in Dietary Restriction. *Interdiscip. Top. Gerontol.* 35, 18–38. doi:10.1159/000096554

Bakula, D., and Scheibye-Knudsen, M. (2020). MitophAging: Mitophagy in Aging and Disease. *Front. Cell Dev. Biol.* 8, 239. doi:10.3389/fcell.2020.00239

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

Bishop, N. A., and Guarente, L. (2007). Two Neurons Mediate Diet-Restriction-Induced Longevity in *C. elegans*. *Nature* 447, 545–549. doi:10.1038/nature05904

Blackwell, T. K., Steinbaugh, M. J., Hourihan, J. M., Ewald, C. Y., and Isik, M. (2015). SKN-1/Nrf, Stress Responses, and Aging in *Caenorhabditis elegans*. *Free Radic. Biol. Med.* 88, 290–301. doi:10.1016/j.freeradbiomed.2015.06.008

Brown, M., Evans, J., and Luo, Y. (2006). Beneficial Effects of Natural Antioxidants EGCG and α-lipoic Acid on Life Span and Age-dependent Behavioral Declines in *Caenorhabditis elegans*. *Pharmacol. Biochem. Behav.* 85, 620–628. doi:10.1016/j.pbb.2006.10.017

Campanella, C., Pace, A., Caruso Bavisotto, C., Marzullo, P., Marino Gammazza, A., Buscemi, S., et al. (2018). Heat Shock Proteins in Alzheimer's Disease: Role and Targeting. *Ijms* 19, 2603. doi:10.3390/ijms19092603

Castilla-Cortázar, I., Aguirre, G. A., Femat-Roldán, G., Martín-Estal, I., and Espinosa, L. (2020). Is Insulin-like Growth Factor-1 Involved in Parkinson's Disease Development? *J. Transl. Med.* 18, 70. doi:10.1186/s12967-020-02223-0

Cha´vez, V., Mohri-Shiomi, A., Maadani, A., Vega, L. A., and Garsin, D. A. (2007). Oxidative Stress Enzymes Are Required for DAF-16-Mediated Immunity Due to Generation of Reactive Oxygen Species by *Caenorhabditis elegans*. *Genetics* 176, 1567–1577. doi:10.1534/genetics.107.072587

Chen, S. G., Stribinskis, V., Rane, M. J., Demuth, D. R., Gozal, E., Roberts, A. M., et al. (2016). Exposure to the Functional Bacterial Amyloid Protein Curli Enhances Alpha-Synuclein Aggregation in Aged Fischer 344 Rats and *Caenorhabditis elegans*. *Sci. Rep.* 6, 34477. doi:10.1038/srep34477

Dumitrescu, L., Popescu-Olaru, I., Cozma, L., Tulbă, D., Hinescu, M. E., Ceafalan, L. C., et al. (2018). Oxidative Stress and the Microbiota-Gut-Brain Axis. *Oxid. Med. Cell. Longev.* 2018, 1–12. doi:10.1155/2018/2406594

Durieux, J., Wolff, S., and Dillin, A. (2011). The Cell-Non-Autonomous Nature of Electron Transport Chain-Mediated Longevity. *Cell* 144, 79–91. doi:10.1016/j.cell.2010.12.016

Fabian, M. R., Sonenberg, N., and Filipowicz, W. (2010). Regulation of mRNA Translation and Stability by microRNAs. *Annu. Rev. Biochem.* 79, 351–379. doi:10.1146/annurev-biochem-060308-103103

Finger, F., Ottens, F., Springhorn, A., Drexel, T., Proksch, L., Metz, S., et al. (2019). Olfaction Regulates Organismal Proteostasis and Longevity *via* microRNA-dependent Signalling. *Nat. Metab.* 1, 350–359. doi:10.1038/s42255-019-0033-z

Fletcher, M., and Kim, D. H. (2017). Age-Dependent Neuroendocrine Signaling from Sensory Neurons Modulates the Effect of Dietary Restriction on Longevity of *Caenorhabditis elegans*. *PLOS Genet.* 13, e1006544. doi:10.1371/journal.pgen.1006544

Furness, J. B., Rivera, L. R., Cho, H.-J., Bravo, D. M., and Callaghan, B. (2013). The Gut as a Sensory Organ. *Nat. Rev. Gastroenterol. Hepatol.* 10, 729–740. doi:10.1038/nrgastro.2013.180

Gelino, S., Chang, J. T., Kumsta, C., She, X., Davis, A., Nguyen, C., et al. (2016). Intestinal Autophagy Improves Healthspan and Longevity in *C. elegans* during Dietary Restriction. *PLOS Genet.* 12, e1006135. doi:10.1371/journal.pgen.1006135

Gomez-Pastor, R., Burchfiel, E. T., Neef, D. W., Jaeger, A. M., Cabiscol, E., McKinstry, S. U., et al. (2017). Abnormal Degradation of the Neuronal Stress-Protective Transcription Factor HSF1 in Huntington's Disease. *Nat. Commun.* 8, 14405. doi:10.1038/ncomms14405

González, C., Diaz, F., and Alonso, A. (2008). Neuroprotective Effects of Estrogens: Cross-Talk between Estrogen and Intracellular Insulin Signalling. *Iddt* 8, 65–67. doi:10.2174/187152608784139659

Hajdú, G., Gecse, E., Taisz, I., Móra, I., and Sőti, C. (2021). Toxic Stress-specific Cytoprotective Responses Regulate Learned Behavioral Decisions in *C. elegans*. *BMC Biol.* 19, 26. doi:10.1186/s12915-021-00956-y

Haque, R., Shamsuzzama, Kumar, L., Sharma, T., Fatima, S., Jadiya, P., et al. (2020). Human Insulin Modulates α-synuclein Aggregation *via* DAF-2/DAF-16 Signalling Pathway by Antagonising DAF-2 Receptor in *C. elegans* Model of Parkinson's Disease. *Oncotarget* 11, 634–649. doi:10.18632/oncotarget.27366

Henderson, S. T., and Johnson, T. E. (2001). daf-16 Integrates Developmental and Environmental Inputs to Mediate Aging in the Nematode *Caenorhabditis elegans*. *Curr. Biol.* 11, 1975–1980. doi:10.1016/S0960-9822(01)00594-2

Hertzberg, V. S., Singh, H., Fournier, C. N., Moustafa, A., Polak, M., Kuelbs, C. A., et al. (2022). Gut Microbiome Differences between Amyotrophic Lateral Sclerosis Patients and Spouse Controls. *Amyotroph. Lateral Scler. Front. Degener.* 23, 91–99. doi:10.1080/21678421.2021.1904994

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

Houser, M. C., and Tansey, M. G. (2017). The Gut-Brain axis: Is Intestinal Inflammation a Silent Driver of Parkinson's Disease Pathogenesis? *Npj Park. Dis.* 3, 1–9. doi:10.1038/s41531-016-0002-0

Imanikia, S., Özbey, N. P., Krueger, C., Casanueva, M. O., and Taylor, R. C. (2019a). Neuronal XBP-1 Activates Intestinal Lysosomes to Improve Proteostasis in *C. elegans*. *Curr. Biol.* 29, 2322–2338.e7. doi:10.1016/j.cub.2019.06.031

Imanikia, S., Sheng, M., Castro, C., Griffin, J. L., and Taylor, R. C. (2019b). XBP-1 Remodels Lipid Metabolism to Extend Longevity. *Cell Rep.* 28, 581–589.e4. doi:10.1016/j.celrep.2019.06.057

Inukai, S., and Slack, F. (2013). MicroRNAs and the Genetic Network in Aging. *J. Mol. Biol.* 425, 3601–3608. doi:10.1016/j.jmb.2013.01.023

Jia, Q., and Sieburth, D. (2021). Mitochondrial Hydrogen Peroxide Positively Regulates Neuropeptide Secretion during Diet-Induced Activation of the Oxidative Stress Response. *Nat. Commun.* 12, 2304. doi:10.1038/s41467-021-22561-x

Jin, M., Li, J., Liu, F., Lyu, N., Wang, K., Wang, L., et al. (2019). Analysis of the Gut Microflora in Patients with Parkinson's Disease. *Front. Neurosci.* 13, 1184. doi:10.3389/fnins.2019.01184

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

Kaelberer, M. M., Buchanan, K. L., Klein, M. E., Barth, B. B., Montoya, M. M., Shen, X., et al. (2018). A Gut-Brain Neural Circuit for Nutrient Sensory Transduction. *Science* 361, eaat5236. doi:10.1126/science.aat5236

Kato, M., Kashem, M. A., and Cheng, C. (2016). An Intestinal microRNA Modulates the Homeostatic Adaptation to Chronic Oxidative Stress in *C. elegans*. *Aging* 8, 1979–1996. doi:10.18632/aging.101029

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, S., and Sieburth, D. (2018). Sphingosine Kinase Regulates Neuropeptide Secretion during the Oxidative Stress-Response through Intertissue Signaling. *J. Neurosci.* 38, 8160–8176. doi:10.1523/JNEUROSCI.0536-18.2018

Kim, Y., Zheng, X., Ansari, Z., Bunnell, M. C., Herdy, J. R., Traxler, L., et al. (2018). Mitochondrial Aging Defects Emerge in Directly Reprogrammed Human Neurons Due to Their Metabolic Profile. *Cell Rep.* 23, 2550–2558. doi:10.1016/j.celrep.2018.04.105

Kishimoto, S., Uno, M., Okabe, E., Nono, M., and Nishida, E. (2017). Environmental Stresses Induce Transgenerationally Inheritable Survival Advantages *via* Germline-To-Soma Communication in *Caenorhabditis elegans*. *Nat. Commun.* 8, 14031. doi:10.1038/ncomms14031

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

Koyuncu, S., Saez, I., Lee, H. J., Gutierrez-Garcia, R., Pokrzywa, W., Fatima, A., et al. (2018). The Ubiquitin Ligase UBR5 Suppresses Proteostasis Collapse in

Pluripotent Stem Cells from Huntington's Disease Patients. *Nat. Commun.* 9, 2886. doi:10.1038/s41467-018-05320-3

Kunitomo, H., Sato, H., Iwata, R., Satoh, Y., Ohno, H., Yamada, K., et al. (2013). Concentration Memory-dependent Synaptic Plasticity of a Taste Circuit Regulates Salt Concentration Chemotaxis in *Caenorhabditis elegans*. *Nat. Commun.* 4, 2210. doi:10.1038/ncomms3210

Labandeira-Garcia, J. L., Rodriguez-Perez, A. I., Valenzuela, R., Costa-Besada, M. A., and Guerra, M. J. (2016). Menopause and Parkinson's Disease. Interaction between Estrogens and Brain Renin-Angiotensin System in Dopaminergic Degeneration. *Front. Neuroendocrinol.* 43, 44–59. doi:10.1016/j.yfrne.2016.09.003

Labbadia, J., and Morimoto, R. I. (2014). Proteostasis and Longevity: when Does Aging Really Begin? *F1000Prime Rep.* 6, 7. doi:10.12703/P6-7

Labbadia, J., Cunliffe, H., Weiss, A., Katsyuba, E., Sathasivam, K., Seredenina, T., et al. (2011). Altered Chromatin Architecture Underlies Progressive Impairment of the Heat Shock Response in Mouse Models of Huntington Disease. *J. Clin. Invest.* 121, 3306–3319. doi:10.1172/JCI57413

Land, M., and Rubin, C. S. (2017). A Calcium- and Diacylglycerol-Stimulated Protein Kinase C (PKC), *Caenorhabditis elegans* PKC-2, Links Thermal Signals to Learned Behavior by Acting in Sensory Neurons and Intestinal Cells. *Mol. Cell. Biol.* 37, e00192–17. doi:10.1128/MCB.00192-17

Lee, K., and Mylonakis, E. (2017). An Intestine-Derived Neuropeptide Controls Avoidance Behavior in *Caenorhabditis elegans*. *Cell Rep.* 20, 2501–2512. doi:10.1016/j.celrep.2017.08.053

Lee, S.-K. (2021). "Endoplasmic Reticulum Homeostasis and Stress Responses in *Caenorhabditis elegans*," in *Cellular Biology of the Endoplasmic Reticulum, Progress in Molecular and Subcellular Biology*. Editors L. B. Agellon and M. Michalak (Cham: Springer International Publishing), 279–303. doi:10.1007/978-3-030-67696-4_13

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

Leung, A. K. L., and Sharp, P. A. (2010). MicroRNA Functions in Stress Responses. *Mol. Cell* 40, 205–215. doi:10.1016/j.molcel.2010.09.027

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

Luca, M., Di Mauro, M., Di Mauro, M., and Luca, A. (2019). Gut Microbiota in Alzheimer's Disease, Depression, and Type 2 Diabetes Mellitus: The Role of Oxidative Stress. *Oxid. Med. Cell. Longev.* 2019, 4730539. doi:10.1155/2019/4730539

Matai, L., Sarkar, G. C., Chamoli, M., Malik, Y., Kumar, S. S., Rautela, U., et al. (2019). Dietary Restriction Improves Proteostasis and Increases Life Span through Endoplasmic Reticulum Hormesis. *Proc. Natl. Acad. Sci.* 116, 17383–17392. doi:10.1073/pnas.1900055116

Matty, M. A., Lau, H. E., Haley, J. A., Singh, A., Chakraborty, A., Kono, K., et al. (2022). Intestine-to-neuronal Signaling Alters Risk-Taking Behaviors in Food-Deprived *Caenorhabditis elegans PLOS Genetics* 18, e1010178. doi:10.1371/journal.pgen.1010178

McCallum, K. C., Liu, B., Fierro-González, J. C., Swoboda, P., Arur, S., Miranda-Vizuete, A., et al. (2016). TRX-1 Regulates SKN-1 Nuclear Localization Cell Non-autonomously in *Caenorhabditis elegans*. *Genetics* 203, 387–402. doi:10.1534/genetics.115.185272

Meisel, J. D., Panda, O., Mahanti, P., Schroeder, F. C., and Kim, D. H. (2014). Chemosensation of Bacterial Secondary Metabolites Modulates Neuroendocrine Signaling and Behavior of *C. elegans*. *Cell* 159, 267–280. doi:10.1016/j.cell.2014.09.011

Miles, J., Townend, S., Smith, W., Westhead, D. R., and Oosten-Hawle, P. van, (2022). Transcellular Chaperone Signaling Is an Intercellular Stress-Response Distinct from the HSF-1 Mediated HSR. *bioRxiv*. doi:10.1101/2022.03.17.484707

Miller, H. A., Dean, E. S., Pletcher, S. D., and Leiser, S. F. (2020). Cell Non-Autonomous Regulation of Health and Longevity. *eLife* 9, e62659. doi:10.7554/eLife.62659

Minnerly, J., Zhang, J., Parker, T., Kaul, T., and Jia, K. (2017). The Cell Non-Autonomous Function of ATG-18 Is Essential for Neuroendocrine Regulation of *Caenorhabditis elegans* Lifespan. *PLoS Genet.* 13, e1006764. doi:10.1371/journal.pgen.1006764

Miranda-Vizuete, A., and Veal, E. A. (2017). *Caenorhabditis elegans* as a Model for Understanding ROS Function in Physiology and Disease. *Redox Biol.* 11, 708–714. doi:10.1016/j.redox.2016.12.020

Morimoto, R. I. (2020). Cell-Nonautonomous Regulation of Proteostasis in Aging and Disease. *Cold Spring Harb. Perspect. Biol.* 12, a034074. doi:10.1101/cshperspect.a034074

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

Muñoz-Carvajal, F., and Sanhueza, M. (2020). The Mitochondrial Unfolded Protein Response: A Hinge between Healthy and Pathological Aging. *Front. Aging Neurosci.* 12. doi:10.3389/fnagi.2020.581849

Murphy, C. T., McCarroll, S. A., Bargmann, C. I., Fraser, A., Kamath, R. S., Ahringer, J., et al. (2003). Genes that Act Downstream of DAF-16 to Influence the Lifespan of *Caenorhabditis elegans*. *Nature* 424, 277–283. doi:10.1038/nature01789

Murphy, C. T., Lee, S.-J., and Kenyon, C. (2007). Tissue Entrainment by Feedback Regulation of Insulin Gene Expression in the Endoderm of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci.* 104, 19046–19050. doi:10.1073/pnas.0709613104

Neirijnck, Y., Papaioannou, M. D., and Nef, S. (2019). The Insulin/IGF System in Mammalian Sexual Development and Reproduction. *Int. J. Mol. Sci.* 20, 4440. doi:10.3390/ijms20184440

Nono, M., Kishimoto, S., Sato-Carlton, A., Carlton, P. M., Nishida, E., and Uno, M. (2020). Intestine-to-Germline Transmission of Epigenetic Information Intergenerationally Ensures Systemic Stress Resistance in *C. elegans*. *Cell Rep.* 30, 3207–3217.e4. doi:10.1016/j.celrep.2020.02.050

O'Brien, D., and van Oosten-Hawle, P. (2016). Regulation of Cell-Non-Autonomous Proteostasis in Metazoans. *Essays Biochem.* 60, 133–142. doi:10.1042/EBC20160006

O'Brien, D., Jones, L. M., Good, S., Miles, J., Vijayabaskar, M. S., Aston, R., et al. (2018). A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis. *Cell Rep.* 23, 3905–3919. doi:10.1016/j.celrep.2018.05.093

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A., et al. (1997). The Fork Head Transcription Factor DAF-16 Transduces Insulin-like Metabolic and Longevity Signals in *C. elegans*. *Nature* 389, 994–999. doi:10.1038/40194

Okabe, E., Uno, M., Kishimoto, S., and Nishida, E. (2021). Intertissue Small RNA Communication Mediates the Acquisition and Inheritance of Hormesis in *Caenorhabditis elegans. Commun. Biol.* 4, 1–10. doi:10.1038/s42003-021-01692-3

Özbey, N. P., Imanikia, S., Krueger, C., Hardege, I., Morud, J., Sheng, M., et al. (2020). Tyramine Acts Downstream of Neuronal XBP-1s to Coordinate Inter-tissue UPRER Activation and Behavior in *C. elegans*. *Dev. Cell* 55, 754–770.e6. doi:10.1016/j.devcel.2020.10.024

Pender, C. L., and Horvitz, H. R. (2018). Hypoxia-inducible Factor Cell Non-autonomously Regulates *C. elegans* Stress Responses and Behavior *via* a Nuclear Receptor. *eLife* 7, e36828. doi:10.7554/eLife.36828

Peterson, C. T. (2020). Dysfunction of the Microbiota-Gut-Brain Axis in Neurodegenerative Disease: The Promise of Therapeutic Modulation with Prebiotics, Medicinal Herbs, Probiotics, and Synbiotics. *J. Evid.-Based Integr. Med.* 25, 2515690X20957225. doi:10.1177/2515690X20957225

Pocock, R., and Hobert, O. (2010). Hypoxia Activates a Latent Circuit for Processing Gustatory Information in *C. elegans*. *Nat. Neurosci.* 13, 610–614. doi:10.1038/nn.2537

Prahlad, V., Cornelius, T., and Morimoto, R. I. (2008). Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons. *Science* 320, 811–814. doi:10.1126/science.1156093

Psichas, A., Reimann, F., and Gribble, F. M. (2015). Gut Chemosensing Mechanisms. *J. Clin. Invest.* 125, 908–917. doi:10.1172/JCI76309

Sakai, N., Ohno, H., Tomioka, M., and Iino, Y. (2017). The Intestinal TORC2 Signaling Pathway Contributes to Associative Learning in *Caenorhabditis elegans*. *PLoS ONE* 12, e0177900. doi:10.1371/journal.pone.0177900

Sala, A. J., Bott, L. C., and Morimoto, R. I. (2017). Shaping Proteostasis at the Cellular, Tissue, and Organismal Level. *J. Cell Biol.* 216, 1231–1241. doi:10.1083/jcb.201612111

Shao, L.-W., Niu, R., and Liu, Y. (2016). Neuropeptide Signals Cell Non-autonomous Mitochondrial Unfolded Protein Response. *Cell Res.* 26, 1182–1196. doi:10.1038/cr.2016.118

Singh, V., and Aballay, A. (2006). Heat-shock Transcription Factor (HSF)-1 Pathway Required for *Caenorhabditis elegans* Immunity. *Proc. Natl. Acad. Sci. U. S. A.* 103, 13092–13097. doi:10.1073/pnas.0604050103

Singh, J., and Aballay, A. (2019). Intestinal Infection Regulates Behavior and Learning *via* Neuroendocrine Signaling. *eLife* 8, e50033. doi:10.7554/eLife.50033

Son, H. G., Altintas, O., Kim, E. J. E., Kwon, S., and Lee, S.-J. V. (2019). Age-dependent Changes and Biomarkers of Aging in *Caenorhabditis elegans*. *Aging Cell* 18, e12853. doi:10.1111/acel.12853

Tatum, M. C., Ooi, F. K., Chikka, M. R., Chauve, L., Martinez-Velazquez, L. A., Steinbusch, H. W. M., et al. (2015). Neuronal Serotonin Release Triggers the Heat Shock Response in *C. elegans* in the Absence of Temperature Increase. *Curr. Biol. CB* 25, 163–174. doi:10.1016/j.cub.2014.11.040

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

Tissenbaum, H. A., and Ruvkun, G. (1998). An Insulin-like Signaling Pathway Affects Both Longevity and Reproduction in *Caenorhabditis elegans*. *Genetics* 148, 703–717. doi:10.1093/genetics/148.2.703

Uno, M., Tani, Y., Nono, M., Okabe, E., Kishimoto, S., Takahashi, C., et al. (2021). Neuronal DAF-16-To-Intestinal DAF-16 Communication Underlies Organismal Lifespan Extension in *C. elegans*. *iScience* 24, 102706. doi:10.1016/j.isci.2021.102706

van Oosten-Hawle, P., Porter, R. S., and Morimoto, R. I. (2013). Regulation of Organismal Proteostasis by Transcellular Chaperone Signaling. *Cell* 153, 1366–1378. doi:10.1016/j.cell.2013.05.015

Vidal, R. L., Figueroa, A., Court, F. A., Thielen, P., Molina, C., Wirth, C., et al. (2012). Targeting the UPR Transcription Factor XBP1 Protects against Huntington's Disease through the Regulation of FoxO1 and Autophagy. *Hum. Mol. Genet.* 21, 2245–2262. doi:10.1093/hmg/dds040

Walker, G., Houthoofd, K., Vanfleteren, J. R., and Gems, D. (2005). Dietary Restriction in *C. elegans*: From Rate-Of-Living Effects to Nutrient Sensing Pathways. *Mech. Ageing Dev.* 126, 929–937. doi:10.1016/j.mad.2005.03.014

Walter, P., and Ron, D. (2011). The Unfolded Protein Response: From Stress Pathway to Homeostatic Regulation. *Science* 334, 1081–1086. doi:10.1126/science.1209038

Wang, J., Wang, C.-E., Orr, A., Tydlacka, S., Li, S.-H., and Li, X.-J. (2008). Impaired Ubiquitin–Proteasome System Activity in the Synapses of Huntington's Disease Mice. *J. Cell Biol.* 180, 1177–1189. doi:10.1083/jcb.200709080

Westwood, A. J., Beiser, A., DeCarli, C., Harris, T. B., Chen, T. C., He, X., et al. (2014). Insulin-like Growth Factor-1 and Risk of Alzheimer Dementia and Brain Atrophy. *Neurology* 82, 1613–1619. doi:10.1212/WNL.0000000000000382

Williams, K. W., Liu, T., Kong, X., Fukuda, M., Deng, Y., Berglund, E. D., et al. (2014). Xbp1s in Pomc Neurons Connects ER Stress with Energy Balance and Glucose Homeostasis. *Cell Metab.* 20, 471–482. doi:10.1016/j.cmet.2014.06.002

Yu, B. P., and Chung, H. Y. (2001). Stress Resistance by Caloric Restriction for Longevity. *Ann. N. Y. Acad. Sci.* 928, 39–47. doi:10.1111/j.1749-6632.2001.tb05633.x

Zhang, P., Judy, M., Lee, S.-J., and Kenyon, C. (2013). Direct and Indirect Gene Regulation by a Life-Extending FOXO Protein in *C. elegans*: Roles for GATA Factors and Lipid Gene Regulators. *Cell Metab.* 17, 85–100. doi:10.1016/j.cmet.2012.12.013

Zhang, B., Gong, J., Zhang, W., Xiao, R., Liu, J., and Xu, X. Z. S. (2018). Brain–gut Communications *via* Distinct Neuroendocrine Signals Bidirectionally Regulate Longevity in *C. elegans*. *Genes. Dev.* 32, 258–270. doi:10.1101/gad.309625.117

Zhou, Y., Wang, X., Song, M., He, Z., Cui, G., Peng, G., et al. (2019). A Secreted microRNA Disrupts Autophagy in Distinct Tissues of *Caenorhabditis elegans* upon Ageing. *Nat. Commun.* 10, 4827. doi:10.1038/s41467-019-12821-2

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C. elegans as an Animal Model to Study the Intersection of DNA Repair, Aging and Neurodegeneration

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Since its introduction as a genetic model organism, *Caenorhabditis elegans* has yielded insights into the causes of aging. In addition, it has provided a molecular understanding of mechanisms of neurodegeneration, one of the devastating effects of aging. However, *C. elegans* has been less popular as an animal model to investigate DNA repair and genomic instability, which is a major hallmark of aging and also a cause of many rare neurological disorders. This article provides an overview of DNA repair pathways in *C. elegans* and the impact of DNA repair on aging hallmarks, such as mitochondrial dysfunction, telomere maintenance, and autophagy. In addition, we discuss how the combination of biological characteristics, new technical tools, and the potential of following precise phenotypic assays through a natural life-course make *C. elegans* an ideal model organism to study how DNA repair impact neurodegeneration in models of common age-related neurodegenerative diseases.

Keywords: aging, neurodegenerative diseases, DNA repair, Parkinson's disease, *Caenorhabditis elegans*, Alzheimer's disease

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INTRODUCTION

Neurodegenerative diseases like Parkinson's disease (PD) and Alzheimer's disease (AD) present a significant healthcare challenge. Aging is the major risk factor for these neurodegenerative diseases (Fang et al., 2017; Hou et al., 2019). Many cellular processes contribute to aging (**Figure 1**). These processes are often referred to as the "hallmarks of aging" and include genomic instability, epigenetic alteration, mitochondrial dysfunction, loss of proteostasis, senescence, telomere shortening, altered metabolism and cell-cell communication, stem cell exhaustion and, as recently proposed, compromised autophagy (López-Otín et al., 2013; Hansen et al., 2018; Aman et al., 2021). A major challenge for future research is to understand how these complex processes interact to influence aging. Simple model systems, such as *C. elegans*, remain important tools because they allow us to study the interaction between different mechanisms of aging, and how the aging process leads to development of age-related diseases.

The Nobel Laureate Sydney Brenner established the nematode *C. elegans* as a model system (Brenner, 1974). *C. elegans* is sexually dimorphic, with the majority of the population consisting of self-fertilizing hermaphrodites. Males constitute a small portion of the population (0.1%) (Brenner, 1974). The full genome sequence of this small nematode was completed in 1998 (Consortium*, 1998) and almost 70% of the 19,000 genes are conserved between *C. elegans* and humans (Consortium*, 1998). *C. elegans* develops through a program that takes it through the embryonic stage, four larval stages (L1 through L4) into a reproductive adult in 3 days (at 20°C). In case the environment is not

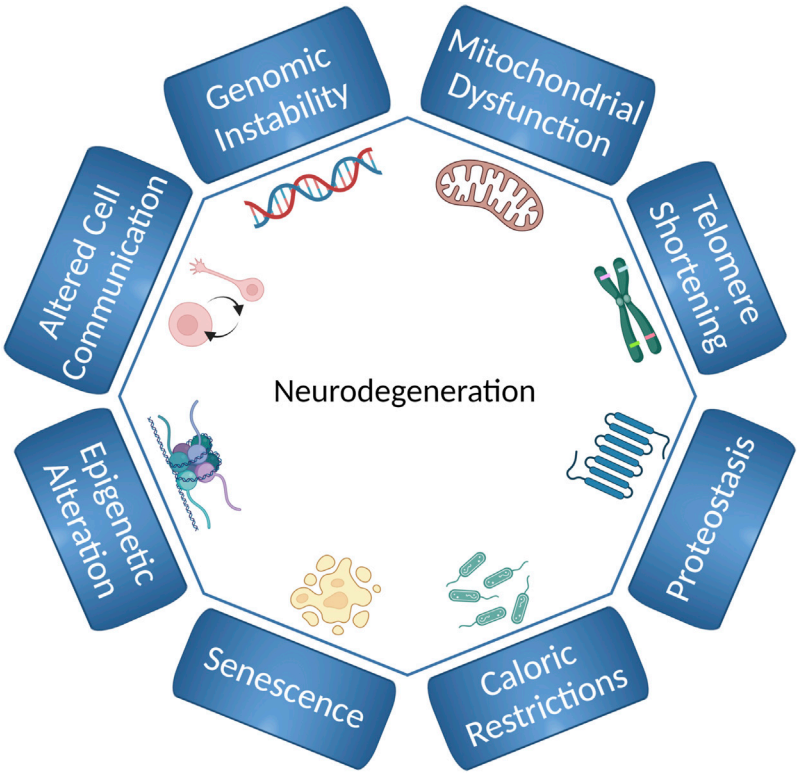

FIGURE 1 | Hallmarks of aging and neurodegeneration in *C. elegans*. The hallmarks of aging and neurodegeneration in *C. elegans* include genomic instability, telomere shortening, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, and altered cell communication. The figure has been generated using Biorender.com by the author.

favorable, e.g., due to overpopulation or lack of food, larvae may go into an alternative developmental stage, referred to as the dauer stage, where they become stress-resistant and may survive for several months until they, upon encountering food, go back to the developmental cycle (Riddle et al., 1981).

A large proportion of the 959 post-mitotic somatic cells in *C. elegans* (Sulston and Horvitz, 1977) belong to the nervous system. This includes 302 neurons and 56 glia-like cells, and 7600 synapses (White et al., 1986). Most classical neurotransmitters such as glutamate (Glu), gamma-Aminobutyric acid (GABA), dopamine (DA), serotonin (5-hydroxytryptamine; 5-HT), and acetylcholine (ACh) are present in the worms (Brownlee and Fairweather, 1999). The interactions of *C. elegans* neurons, synapses, and neurotransmitters are similar to those of mammals (Bargmann, 1998). Here, we will discuss how *C. elegans* is used as model system to study various hallmarks of aging and neurodegeneration with a special focus on genomic instability and mitochondrial dysfunction. Additionally, we also discuss limits, recent advancements, and new techniques that can be implemented to study the role of DNA damage as a driver of aging and neurodegeneration.

C. elegans in Aging Research

Aging is the process of gradual functional decline that an organism experiences over time. Because aging is the main risk factor for neurodegenerative diseases (NDs) (Hou et al., 2019), an understanding of the aging processes is highly relevant in a perspective of translational research.

Nematodes were regarded as a preferred model for aging research (Gershon, 1970) due to characteristics like their morphological simplicity and the possibility to follow large populations through a natural short life course. This, combined with Sydney Brenner's influential article in 1974 highlighting the possibilities of *C. elegans* linking mutations and their phenotypic effects (Brenner, 1974), established the possibility to use *C. elegans* to find modifiers of aging and lifespan (Gershon, 1970). Researchers started describing the aging process in *C. elegans* (Croll et al., 1977; Klass, 1977) and the impact of factors like temperature and food (Klass, 1977) while developing methods for the isolation of mutants with altered longevity (Klass, 1983). Progressively, it was established that aging modified measurable parameters like behavior, chemotaxis or locomotion (Hosono, 1978; Hosono et al., 1980; Johnson, 1987), highlighting how the study of aging implicates more variables than the lifespan itself. The first mutants associated with lifespan extension were subsequently characterized and linked to variants in the *age-1* gene (Friedman and Johnson, 1988). As the field progressed, a new gene was identified as a modifier of lifespan, *daf-2* (Kenyon et al., 1993). Mutants in *daf-2* had

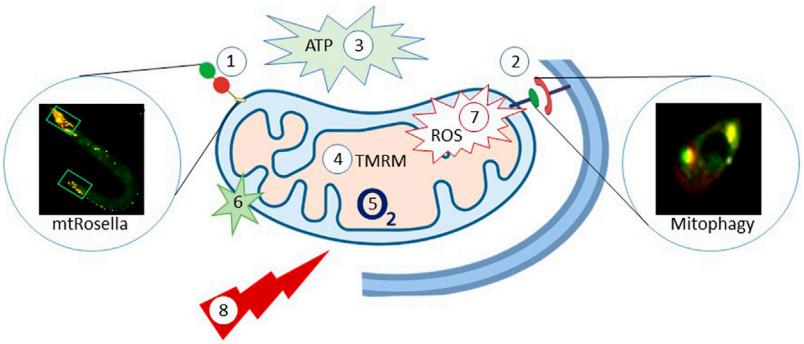

FIGURE 2 | *C. elegans* as model organism to study mitochondrial homeostasis and function. The *C. elegans* provide a way to monitor mitophagy; 1: Mitochondria-target Rosella (mtRosella), using the Rosella biosensor combining a pH-insensitive DsRed and pH-insensitive GFP. 2: Co-localization between DCT-1 (outer mitochondrial membrane protein) and LGG-1 (autophagosomal membrane protein, homolog of the mammalian LC3). 3: Luciferase-based methods to evaluate how different conditions affects the production of ATP in C. elegans; The Roche ATP bioluminescent assay kit HSII is another way to determine ATP content. 4: TMRM: the TMRM staining-tetramethylrhodamine, ethyl ester, perchlorate, a dye that accumulates in intact, respiring mitochondria. 5: Oxygen consumption rate (OCR) is another parameter to reflect mitochondria function. 6: Mito Tracker Green FM is a green-fluorescent dye that stains mitochondria in live cells and its accumulation is dependent on membrane potential. 7: mitochondrial ROS and cellular ROS can be quantification via mtROS (MitoTracker Red CM-H2X ROS); DHE (dihydroethidium), respectively. 8: Stress resistance-survival can also be detected via a heat stress assay, CCCP stress assay, ultraviolet light stress assay, paraquat-induced oxidative stress assay and starvation assays. The figure has been generated using power point image building tool by the author.

lifespan double that of the wild type, a phenotype suppressed by mutations in *daf-16*, that would later be shown to suppress also the long-lived phenotype of *age-1* (Murakami and Johnson, 1996).

Interestingly, these genes had been linked previously with another process that modified lifespan, lifecycle and aging: dauer formation. The screens for genes that modified dauer formation had been done previously (Albert et al., 1981; Riddle et al., 1981), identifying *daf-2* and *age-1* mutants (in previous publications denominated *daf-23*) as dauer constitutive which means that even in favorable growth conditions, a percentage of those populations would still go into the dauer stage; with mutations in *daf-16* suppressing this dauer constitutive phenotype (Vowels and Thomas, 1992). This interplay between AGE-1 and DAF-2, with DAF-16 opposing their functions in lifespan and dauer formation, would culminate in a series of publications identifying these genes as the components of the insulin/IGF-1 signaling (IIS) pathway in the nematode (Morris et al., 1996; Morris et al., 1996; Kimura et al., 1997; Lin et al., 1997; Ogg et al., 1997; Tissenbaum and Ruvkun, 1998), a pathway conserved throughout evolution and that regulates lifespan across different organisms (Tissenbaum and Ruvkun, 1998; Tatar et al., 2001; Barbieri et al., 2003; Blüher et al., 2003; Holzenberger et al., 2003; Hwangbo et al., 2004), including humans (Hwangbo et al., 2004; Suh et al., 2008; Willcox et al., 2008).

Later, many different factors have been identified to impact aging using *C. elegans* as a model, including oxidative stress (Larsen, 1993; Vanfleteren, 1993; Park et al., 2009), DNA repair (Hyun et al., 2008; Arczewska et al., 2013; Lans et al., 2013; Fang et al., 2016; SenGupta et al., 2021) and epigenetics (Maures et al., 2011; Li and Casanueva, 2016; Martin-Herranz et al., 2019). Thus, *C. elegans* is established as a model organism for aging. In the following chapters, we highlight some of these processes and how they affect the development of NDs.

Hallmarks of Aging

Among the known hallmarks of aging (**Figure 1**), we briefly address some hallmarks that are affected by genomic instability and discuss how in *C. elegans* can be used to study the contribution of these processes to neurodegeneration and aging.

Mitochondrial Dysfunction

Mitochondria support neurons by generating ATP (Mattson et al., 2008) that provides the energy for cellular activities that maintain neuronal function and structure. Mitochondria regulate Ca^{2+} -and redox signaling which impact on synaptic plasticity (Jung et al., 2020). Mitochondrial dysfunction, caused by different reasons, such as mutations in mitochondrial genes and as well as intracellular and extracellular stresses to mitochondria, contributes to aging (Sun et al., 2016) and neurological disorders (Mattson et al., 2008) (**Figure 1** and **Figure 2**). For instance, in animal/cellular models of AD bearing Aβ pathology, higher mitochondria-mediated oxidative stress, impaired Ca^{2+} homeostasis, derailed energy metabolism, and apoptosis were evident (Mattson et al., 2008). Indeed, Aβ directly cause mitochondrial damage, including increased mitochondrial ROS, higher mitochondrial Ca^{2+} uptake, and decreased ATP production (Hashimoto et al., 2003). In PD, mitochondrial complex I activity is decreased resulting in ATP depletion, ROS production, and excitotoxic Ca^{2+} overload. In AD, dysfunction of proteins like α-synuclein, Parkin, DJ-1, PINK1, UCHL1, and LRRK2 result in mitochondrial dysfunction (Lautrup et al., 2019). In a *C. elegans* PD model, mitochondrial fusion and fission defect increases sensitivity of dopaminergic neurons to UVC but makes them resistant to the neurotoxin 6-OHDA (Hartman et al., 2019). In Huntington's disease (HD), adverse effects on mitochondria have been reported including mitochondrial electron transport impairment (Brouillet et al., 2005), mitochondrial trafficking impairment (Chang et al., 2006), ATP reduction in synaptic terminals (Orr

et al., 2008), and mitochondrial depolarization (Panov et al., 2002). In a *C. elegans* model of HD, disruption of the mitochondrial fission gene *drp-1* exacerbates the phenotype, whereas decreasing mitochondrial fragmentation improved protection (Machiela et al., 2021).

Mitochondrial function in *C. elegans* can be measured using several strategies: for example, luciferase-based reporters are available to evaluate oxidative phosphorylation (OXPHOS), glycolysis, and fatty acid oxidation (Luz et al., 2016). The oxygen consumption rate is widely used to study mitochondrial function. ATP levels can also be measured in *C. elegans* (Palikaras and Tavernarakis, 2016). ROS sensitive dyes, like DHE (Dihydroethidium) or Mitotracker Red can be used to detect cellular ROS. Some stress resistance/survival parameters can also reflect mitochondrial function, including assays to stress induced by heat (Zevian and Yanowitz, 2014), mitochondrial uncoupling (Palikaras et al., 2015), ultraviolet light (Park et al., 2017), paraquat and juglone (Senchuk et al., 2017), and starvation (Palikaras et al., 2015).

Autophagy

Autophagy is a dynamic process dedicated to maintain the cellular homeostasis, normal growth and development (Meléndez et al., 2003). Ample studies have shown that loss of autophagy genes either extend or shorten lifespan in *C. elegans* (Hars et al., 2007; Hashimoto et al., 2009). However, neuronal selective autophagy may have a particular role in the development of neurodegenerative disorders (Conway et al., 2020; Konstantinidis and Tavernarakis, 2021). Selective autophagy of mitochondria, mitophagy, seems to be critical for neuronal health (Aman et al., 2021). Mitophagy functions as a machinery that selectively degrades damaged mitochondria in response to numerous stresses such as starvation and oxidative stress (Palikaras et al., 2018; Pickles et al., 2018). Mitophagy deficiency reduces lifespan and healthspan. In humans and mice, mitophagy levels were shown to be lower in the hippocampus dentate gyrus, hearts, and skeletal muscle satellite cells (Hoshino et al., 2013; Sun et al., 2015; Bravo-San Pedro et al., 2017). Transgenic reporter strains (**Figure 2**) of mitophagy (Palikaras et al., 2015), has been used to reveal improvements in healthspan and lifespan when mitophagy-related genes, such as mitochondrial fission protein dynamin-related protein 1 (DRP1), Parkin, and PTEN-induced kinase-1 (PINK-1) are up-regulated (Todd and Staveley, 2012; Palikaras et al., 2015; Schiavi et al., 2015).

Deregulation of mitophagy has been linked to several age-related neurodegenerative diseases, including AD (Batlevi and La Spada, 2011), PD (Schapira, 2011), and HD (Batlevi and La Spada, 2011). In AD, for example, mitophagy stimulation restores memory loss via PINK-1, PDR-1 (Parkinson's disease-related-1; parkin), or DCT-1 (DAF-16/FOXO-controlled germline-tumor-tumor-affecting-1)-dependent pathways (Fang et al., 2019a). Loss of function mutations in mitophagy genes (PINK-1 and PARK2) has been linked to PD (Narendra et al., 2008; Narendra et al., 2010; Hartman et al., 2019).

Loss of Proteostasis

Protein homeostasis (proteostasis) is well conserved in eukaryotes and functions to maintain the proteome and prevent misfolding and protein aggregation (Santra et al., 2019). Chaperones, the organelle-specific UPR pathway, the ubiquitin-proteasome system (UPS), ERAD and autophagy machinery all contribute to proteostasis (Labbadia and Morimoto, 2015) (**Figure 1**). In *C. elegans* loss of proteostasis has been linked to aging and cell death as a result of exhaustion, or failure, in chaperone activity (Li and Casanueva, 2016). The mitochondrial UPR (UPRmt) has been shown to be activated when mitochondrial protein import is inhibited, reducing mitochondrial load and subsequently improving longevity in *C. elegans* (Lionaki et al., 2022). The endoplasmic reticulum UPR (UPR^{ER}) transcription factor XBP-1 in the neurons may activate intestinal UPR^{ER}in *C. elegans* via neuronal signal, leading to improved proteostasis and subsequently improving the lifespan (Imanikia et al., 2019). Nematodes has been widely used to investigate age-related organelle-specific proteostasis failure and its implications for lifespan regulation (Hsu et al., 2003; Meléndez et al., 2003; Morley and Morimoto, 2004; Palikaras et al., 2015). *C. elegans* AD, PD, HD and ALS models have been used to show that proteostasis collapse are associated to a failure in amyloid, tau, α-synuclein, and extended polyQ clearance (Voisine et al., 2010; Lehrbach and Ruvkun, 2019; Ruz et al., 2020) (see below).

Telomere Shortening and Stem Cell Exhaustion

Telomere length and senescence impact longevity in humans and higher species. Likewise, in *C. elegans*, telomere length is a determinant of longevity and lifespan (Lim et al., 2001; Joeng et al., 2004) (**Figure 1**). Although the senescence-related secretory phenotype in *C. elegans* is still poorly understood, senescence-like atrophy is responsible for the age-dependent loss of gonad cells in the distal tip of the germline (de la Guardia et al., 2016), as well as insulin/IGF-1 signaling-dependent self-destruction of intestinal biomass (Ezcurra et al., 2018; Kern et al., 2021). This indicates that this model organism might experience age-related senescence, but stem cell exhaustion has not been directly linked to aging in *C. elegans*. However, a growing body of evidence suggests that germline stem cells may have a role in neuronal fate maintenance (Kimble and Seidel, 2008; Tursun et al., 2011; Devanapally et al., 2015; Wu et al., 2015; Marchal and Tursun, 2021), and a new study further substantiates that thermosensory neurons regulate longevity and germline stem cell exhaustion (Lee et al., 2019).

Genomic Instability

Genomic instability is a key hallmark of aging (**Figure 1**) (López-Otín et al., 2013). Failure to maintain genome stability, e.g. by defects in DNA repair, is associated with a range of phenotypes, from severe developmental defects to very modest disorders. Some DNA repair diseases display some, but not all, signs of aging—a characteristic referred to as 'segmental progeria' (Rieckher et al., 2021). These syndromes highlight that inefficient DNA repair contributes to aging and neurodegeneration (Keijzers et al., 2017). Importantly, genomic instability may also exacerbate, or even drive, other

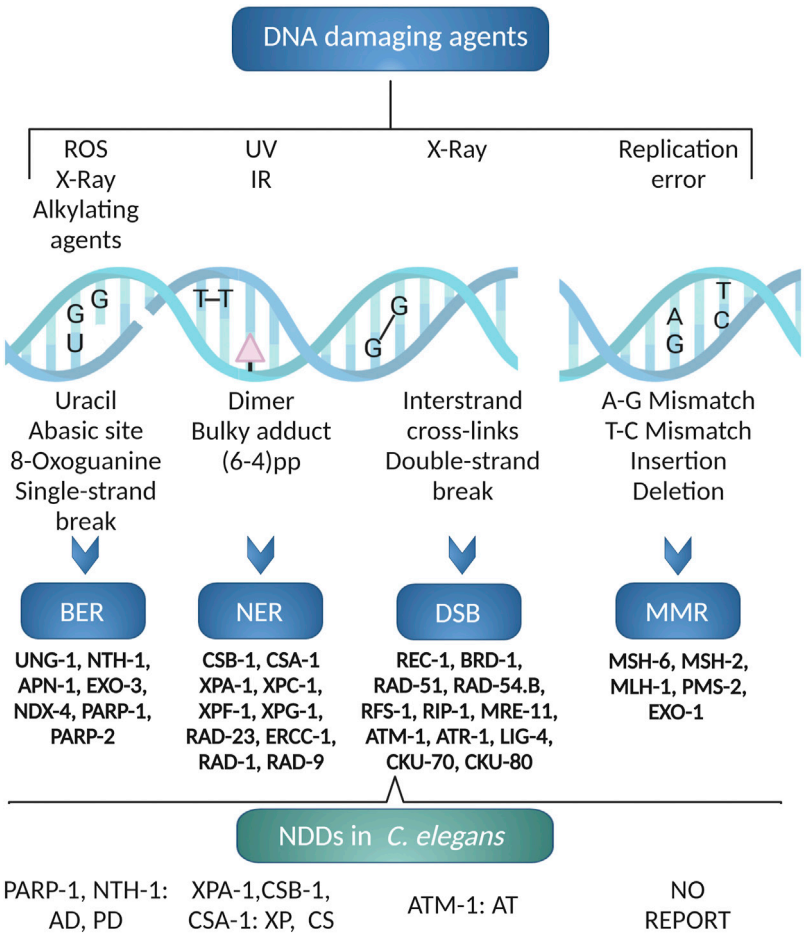

FIGURE 3 | *C. elegans* DNA repair pathways. The excision repair pathways BER, NER, DSB and MMR are operative in *C. elegans*. Using *C. elegans* various neurodegenerative diseases like AD, PD, XP, CS and AT associated to DNA repair defects has been modeled. The figure has been generated using Biorender.com by the author.

hallmarks, such as telomere erosion and mitochondrial dysfunction (van der Rijt et al., 2020).

The major DNA repair pathways are represented in *C. elegans* and most are well conserved (Rieckher et al., 2018) (**Figure 3**). Most of our knowledge on DNA repair in *C. elegans* comes from studies of germline genome stability which is the only organ containing proliferating cells in the adult worm (Vermezovic et al., 2012). Germline DNA repair can be followed through phenotypic end-points such as survival assays, brood size, and male frequency as a marker of germline meiotic crossing-over defects (initiated by programmed introduction of double-stranded breaks by SPO-11) (Craig et al., 2012). Immunohistochemistry and fluorescence-based reporters are standardized tools to monitor apoptosis and activation of classical DNA damage response signaling of germline cells (and embryos) (Vermezovic et al., 2012). Direct measurements of nuclear and mitochondrial DNA damage are also possible from whole worm extracts (Arczewska et al., 2013; SenGupta et al., 2021), but as the number of germline nuclei exceeds the somatic cells, such assays will be dominated by germline effects. Similarly, the comet assay can be used to measure DNA single-or double strand breaks in germline nuclei or embryos (Park et al., 2016). Methods to visualize DNA damage response markers in somatic cells are still lacking although, immunohistochemical analyses has been used to detect single-stranded breaks (SenGupta et al., 2021).

Mature neurons are post mitotic. The DNA repair pathways that are not primarily coupled to DNA replication, that is base excision repair (BER), nucleotide excision repair (NER) and non-homologous end joining (NHEJ) (McKinnon, 2009), are therefore likely to be more important for their maintenance. NER is a versatile pathway needed for repair of covalent helix-distorting adducts that has been extensively studied in *C. elegans*. Both transcription-coupled (TC-NER) and global genome (GG-NER) NER pathways are active (**Figure 3**). In response to UV-induced DNA damage, germline cells rely primarily on GG-NER; TC-NER serves as a backup pathway when GG-NER is not present (Lans and Vermeulen, 2011). TC-NER is, however,

required for maintenance of genetic integrity in post-mitotic somatic cells (Lans et al., 2010). We refer the readers to excellent reviews on this pathway (Marteijn et al., 2014).

The Base Excision Repair (BER) pathway is the main pathway for repair of helix-non distorting chemically modified DNA bases. BER is less studied in *C. elegans* (Elsakrmy et al., 2020). The BER pathway is initiated by a DNA glycosylase that identify and excise certain modified bases (**Figure 3**). Whereas mammals have 11 DNA glycosylases there are only two DNA-glycosylases in *C. elegans*, the monofunctional uracil-DNA glycosylase (UNG-1) (Nakamura et al., 2008; Skjeldam et al., 2010) and bifunctional endonuclease III homolog NTH-1 with AP lyase activity (NTH-1) (Morinaga et al., 2009). UNG-1 repairs uracil, one of the most common types of DNA damage that arises through deamination of cytosine or misincorporation of dUMP from the nucleotide pool (Skjeldam et al., 2010). As it´s human homolog, NTH-1 repairs oxidised pyrimidines (Hazra et al., 2007). The substrate specificities of the *C. elegans* DNA glycosylases are verified only for a few characteristic substrates, and we do not know whether loss of redundancy is compensated by broader substrate specificities. UNG-1 is active on the classical UNG substrates, such as uracil and 5-hydroxymethyluracil (Papaluca et al., 2018). NTH-1 activity has been demonstrated on thymine glycol (Tg), 5-formyluracil (5-foU), and 5- hydroxymethyluracil (5-hmU) containing DNA (Morinaga et al., 2009). The lack of a DNA glycosylase dealing with oxidized purines in *C. elegans* in puzzling, but it has been suggested that RPS-3 might contribute 8-oxoG DNA glycosylase activity (Skjeldam et al., 2010). It was also reported that NTH-1 shows weak activity towards 8-oxodG paired with G, although the physiological relevance of this activity is unclear (Morinaga et al., 2009). It is also possible that oxidised pyriminines are repaired by the NER pathway in *C. elegans* (Arczewska et al., 2013). *ung-1* and *nth-1* mutant are viable, fertile with normal life span albeit with a weak mutator phenotype (Fensgård et al., 2010; Skjeldam et al., 2010; Kassahun et al., 2018). Excision of damaged bases by DNA glycosylases generates an abasic site (AP-site) that must be processed in order to generate a substrate for a DNA polymerase. The AP-site are mainly processed by AP endonucleases. EXO-3 and APN-1 are the two AP endonucleases in *C. elegans*. APN-1 harbours both 3′-diesterase and 3′-5′ exonuclease activity making it distinct from EXO-3, which lacks 3′-5′ exonuclease activity (Yang et al., 2012; Elsakrmy et al., 2020). The *exo-3* mutant animals exhibit reduced brood size and reduced lifespan (Kato et al., 2015). In contrast, the *apn-1* mutants, have normal lifespan despite having a high mutation frequency (Zakaria et al., 2010). There is no ortholog of the classical BER polymerase, DNA polymerase β. In *C. elegans* gap filling and removal of the 5'dRp end is performed by the low-fidelity DNA Polymerase θ (Asagoshi et al., 2012). Finally, BER is completed by DNA ligase I.

In *C. elegans* (**Figure 3**), highly deleterious double strand breaks (DSBs) are mostly repaired by error-free homologous recombination (HR) or error-prone non-homologous end joining (NHEJ) or single-strand annealing (SSA) (Lemmens and Tijsterman, 2011). The source of damage, the cell cycle phase, and the animal's developmental stage all influence which pathway is engaged. HR is cell cycle dependent and NHEJ is cell-cycle independent (Clejan et al., 2006). The Canonical HR pathway is primarily responsible for the repair of DNA DSBs in the germline. In somatic cells, however, DSBs are mostly repaired through NHEJ. Intriguingly, loss of HR and NHEJ genes, which are lethal in mammals, are well tolerated in *C. elegans* (Lemmens and Tijsterman, 2011; Belan et al., 2021).

DNA replication errors are repaired by Mismatch Repair (MMR) (**Figure 3**). Orthologues of the core MMR genes; MSH-2, MSH-6, MLH-1 and PMS-2, are present in *C. elegans* (**Figure 3**) but *C. elegans* lacks an ortholog of MSH3 and PMS1. Thus, mismatch surveillance is mainly carried out by the MSH-2/MSH-6 heterodimer (Denver et al., 2006). RNAi-mediated depletion (Tijsterman et al., 2002) or loss of function (Meier et al., 2018) of the core MMR genes, lead to strong mutator phenotypes. As in mammals, *C. elegans msh-2* mutant showed microsatellite instability (Tijsterman et al., 2002) and reduced DNA-damage induced germline apoptosis in response to genotoxic stress (Degtyareva et al., 2002; SenGupta et al., 2013).

C. elegans as a Model to Study Mechanisms of Neurodegeneration and Aging

The advantages of *C. elegans* as a model system of aging (**Figure 4**) are also highly relevant for studies of NDs. In addition, the transparent body makes it possible to use fluorescent reporters (Chalfie et al., 1994) for *in vivo* visualization of the neuron(s) of interest or the whole neuronal network. The CEP neurons for example, are mechanosensory neurons responding to the neurotransmitter dopamine. *C. elegans* strains expressing Green Fluorescent Protein (GFP) in dopaminergic neurons are frequently used to study dopaminergic neuron function and integrity by live imaging. In this way, it is possible to track the integrity of neurons, but also how its state translates to a functional challenge (SenGupta et al., 2021; Palikaras et al., 2022).

The generation of RNAi libraries (Kamath et al., 2003; Rual et al., 2004) that target more than 95% of *C. elegans* genes, allows for genome-wide screens for modulators of aging and neurodegeneration (Hamilton et al., 2005; Hamamichi et al., 2008; Kuwahara et al., 2008). Although RNAi was initially found to be inefficient is neurons (Timmons et al., 2001), strains are constructed to make neurons less refractory to RNAi *e.g.* by expressing SID-1, an essential protein in the RNAi machinery, that enables intracellular transport of RNAi (Winston et al., 2002), exclusively in neurons (Calixto et al., 2010). In the same way, compound screenings can be performed in a high-throughput manner by adding the screened drugs in the media at the desired concentration/s where the worms are cultured, and analyzed. In recent years, the adaptation of gene editing methods, like the CRISPR/Cas9 system (Jinek et al., 2012), into *C. elegans* have proven again how accessible it is to adapt new techniques to the organism for easy creation and screening of tailor-made strains (Dickinson et al., 2013; Paix et al., 2015; Ward, 2015). Other available tools and methods, like the use of

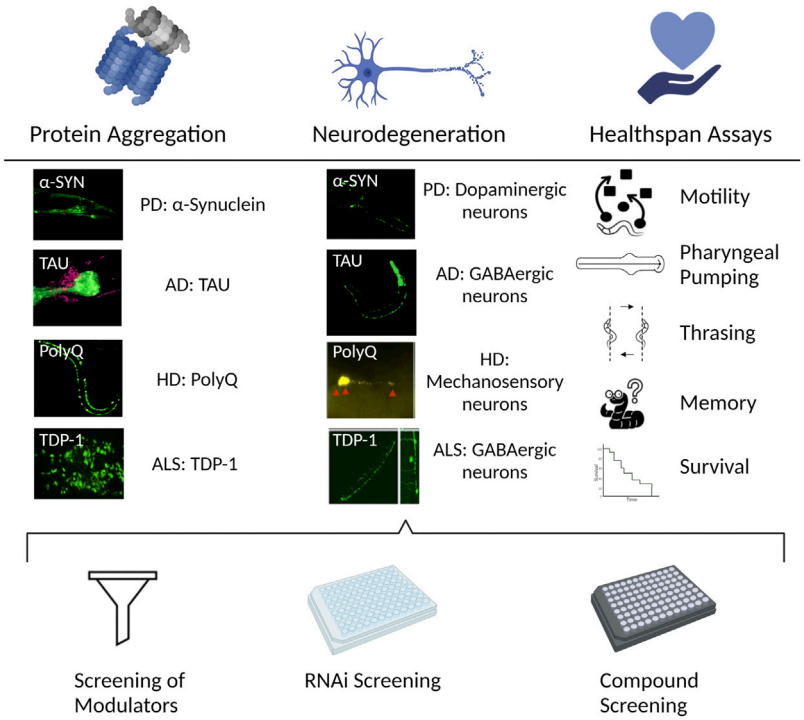

FIGURE 4 | *C. elegans* to study NDs. The nematode allows for different perspectives in the study and understanding of NDs. **Left**. Protein misfolding and aggregation is one of the common features of different NDs. The transparency of *C. elegans* in combination with the use of fluorescent tags allows for the in vivo visualization of the protein aggregation process in the NDs of interest. **Center**. A consequence of the progression of NDs is the degeneration of the affected neurons. Strains can be engineered to express the aggregating proteins in the neuronal circuit of interest in the nematode, allowing to study the neurodegeneration process. **Right.** The morphological changes, featured in the different ND models like aggregation or neurodegeneration, also translate into functional challenges. The performance in the different available assays corresponds to the health status of the neuron/s of interest. **Bottom.** The above-mentioned assessments of an ND using *C. elegans* can be combined with available mutant strains for the gene/s of interest, or with screenings, either with the available existing RNAi libraries, to find genetic modulators; or compound screenings where chemical regulators of the ND progression can be discovered. The figure has been generated using Biorender.com by the author.

conditional gene expression systems or single copy transgene insertions can be found in existing reviews (Nance and Frøkjær-Jensen, 2019; Driesschaert et al., 2021).

An important strength of *C. elegans* as a model for NDs is that different subsets of neurons have been paired with assays that assess the functional status of the desired group of cells. There are methods to assess general neuronal function, like the thrashing assay, where the animals are placed in liquid media and the number of body bends while the animals "swim" are quantified (Buckingham and Sattelle, 2009). Other experiments test the locomotion or the chemotaxis of the nematode (Hosono et al., 1980; Sawin et al., 2000). On the other hand, there are assays designed to explore the health status of a specific subset of neurons. In that manner, the study of neurodegeneration is not just limited to the morphological loss of the neuron, but that process is coupled with experiments that give us an indication on the fitness status of the neurons of interest, like the basal slowing response assay, or the capacity to slow down and alter direction of movement when it reaches food, to assess dopaminergic function (SenGupta et al., 2021; Palikaras et al., 2022); or response to be touched in the head or the tail, to evaluate glutamatergic neuron status (Hart et al., 1995).

Genomic Instability as a Driver of Aging

Many rare DNA repair diseases are modeled in *C. elegans*. *C. elegans* NER mutants phenocopy many aspects of mouse models of NER syndromes; *C. elegans* NER endonuclease ERCC-1 and XPF-1 mutants show growth arrest, developmental failure, shorter lifespan (Lans et al., 2013) and neurons are hypersensitive to UV irradiation (Sabatella et al., 2021). Loss of CSB-1 and XPA-1 in *C. elegans* result in progressive mitochondrial dysfunction (Scheibye-Knudsen et al., 2014), neurodegeneration of ALM and PLM mechanosensory neurons in response to UV irradiation (Lopes et al., 2020), and premature aging (Fang et al., 2014).

Werner syndrome (WS) is a DNA repair syndrome recognized to be a very good representation of accelerated aging. WS is caused by mutations in the *WRN* gene, encoding the WRN RecQ helicase. WS patients show early signs of aging followed by the emergence of age-related disorders such as type 2 diabetes, atherosclerosis, and osteoporosis (Lautrup et al., 2019). Malignancy and myocardial infarction are the major causes of death in these patients, as is the case in the general population, but in WS patients, the average life expectancy is only 54 years (Oshima et al., 2017). The *C. elegans* WRN-1 contains the RecQ helicase function of human WRN, but lacks the exonuclease which is encoded by a separate gene, *mut-7*. Yet,

C. elegans wrn-1 mutants domain recapitulates many phenotypes seen in the human disease; *wrn-1* mutants show shorter lifespan and impaired mitochondrial function (Fang et al., 2019b). *wrn-1* mutants also show loss of gonad cells in the distal tip of the germline but it is possible that this reflects a primary replication defect rather than a senescence - like phenotype. In an elegant recent study, plasticity of olfactory neurons was shown to depend on recruitment of epigenetic regulators through the combined activities of the MUT-7 exonuclease and the WRN-1 helicase (Hsu et al., 2021). This study is an example that shows the power of *C. elegans* to reveal novel functions and pathogenic mechanisms.

Ataxia Telangiectasia (AT) is another disease at the intersection of genetic instability, neurodegeneration, and accelerated aging. AT is caused by mutations in the *Ataxia-Telangiectasia Mutated* (*ATM*) gene, which codes for the ATM kinase. ATM was initially defined as a protein essential for DNA double-stranded break repair, but now it is recognized as a master regulator and coordinator of cellular responses to DNA damage (Shiloh and Ziv, 2013). Human AT is a pleiotropic disease with symptoms originating in a variety of physiological systems. The disease is characterized by mild immunodeficiency due to defects in cellular immunity, but also progressive cerebellar neurodegeneration (Amirifar et al., 2019). The progressive neurological manifestations are, at least in part, understood as reflecting accelerated brain aging. Other aging signs in AT children arise as they approach puberty, such as metabolic syndrome, type 2 diabetes, and osteoporosis (Shiloh and Lederman, 2017). In *C. elegans,* loss of ATM-1 is accompanied by a modest shortening of lifespan. More importantly, the *atm-1* mutants recapitulate the progressive neurodegeneration of the human disease and studies in *C. elegans* helped to pin down mitochondrial dysfunction and reduced mitophagy as a driver of this phenotype (Fang et al., 2016).

In mammals, direct induction of apoptosis signaling via the classical DDR signaling pathways appears to be a dominant mechanism driving neurodegeneration in many DNA repair diseases (Hoch et al., 2017). We recently described another mechanism driving neurodegeneration and aging using *C. elegans* as a model; We demonstrated that the loss-of-function mutation in NER (CSA-1, CSB-1 and XPA-1), ATM-1 and WRN-1 result in progressive mitochondrial dysfunction because failure to resolve constitutive DNA damage leads to depletion of the cellular NAD^+ pool (Fang et al., 2014; Scheibye-Knudsen et al., 2014; Fang et al., 2016; Fang et al., 2019a). The activation of the DNA damage sensor poly-ADP-ribose polymerase 1 (PARP-1) is a critical event in this pathway: PARP-1 uses NAD^+ to create poly-ADP-ribose (PAR) polymers that help to recruit the DNA damage response machinery. Constitutive activation of PARP-1 depletes the NAD^+ pool over time. In our animal models, NAD^+ became scarce, and enzymes that rely on NAD^+ as a cofactor cease to function. SIR-2.1 (ortholog to mammalian SIRT1) for example, affects mitochondrial biogenesis, function, and the clearance of defective mitochondria via mitophagy. As a result, NAD^+ deficiency causes an accumulation of damaged and malfunctioning mitochondria. Thus, in these animal models, the neurological abnormalities were indirect results of a dysmetabolic impact generated by chronic DNA damage response signaling rather than direct consequences of conventional DNA damage response signaling. Preventing NAD^+ depletion or increasing mitochondrial biogenesis might significantly delay the development and progression of neurological symptoms using both genetic and pharmacological techniques (Fang et al., 2014; Scheibye-Knudsen et al., 2014; Fang et al., 2016; Fang et al., 2019b). To understand the effect of restoring NAD^+ levels, multiple species (*C. elegans*, *Drosophila*, and mice) have been used to investigate the effects on the lifespan and health span of different NAD^+ precursors (Fang et al., 2016; Schöndorf et al., 2018). In *C. elegans*, growth in the presence of 500 μM nicotinamide riboside, an NAD^+ precursor, extended lifespan via a SIR-2.1-dependent pathway. At the same time, improvements in healthspan have also been reported including mitochondrial health, muscle strength, and motor function in different models (Belenky et al., 2007; Fang et al., 2016) (**Figure 4**).

Genomic Instability as a Driver of Common Age-Related Diseases

Since *C. elegans* was introduced as a genetic model in the neurobiology field half a century ago it has provided insights into the mechanisms involving aggregation, neurodegeneration and several strains have been created to model specific human diseases (**Figure 4**) (**Table 1**).

Parkinson's Disease

Parkinson's disease (PD) is the second most common ND and aging is the most important risk factor (Poewe et al., 2017; Armstrong and Okun, 2020). Loss of dopaminergic neurons in the substantia nigra region in the early stages of the disease plus formation of α-synuclein aggregates are the cardinal features of the disease (Poewe et al., 2017). Motor symptoms like trembling or coordination difficulties are characteristic but also problems in cognition, sleep or emotional stability.

In *C. elegans* dopaminergic neurons were identified already in 1975 (Sulston et al., 1975). Later, it was proposed as a PD model given its approachability, conservation of genes and pathways and available techniques and methods (Wintle and Van Tol, 2001), for example the possibility of using fluorescent proteins in the neuronal circuit of choice (Chalfie et al., 1994). The health state of dopaminergic neurons was initially addressed in neurotoxicity studies using 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenylpyridinium (MPP+) (Nass et al., 2002; Braungart et al., 2004). As worms do not have the *SNCA* gene, several groups generated strains expressing human α-synuclein in selected neuron classes (the dopaminergic circuit, in motor neurons or pan-neuronally) (Lakso et al., 2003; Cao et al., 2005; Cooper et al., 2006; Kuwahara et al., 2006) (**Figure 4**) (**Table 1**). Furthermore, variants of the *SNCA* gene that increases the misfolding tendency and assembly of α-synuclein fibrils in humans (Polymeropoulos et al., 1997; Krüger et al., 1998), like the A53T or the A30P mutant isoforms (Lakso et al., 2003; Kuwahara et al., 2006), were used to better mimic the human disease. Strains were generated

TABLE 1 | *C. elegans* strains used to model neurodegenerative disease.

NDD	Strain	Genotype	Tissue	Use	Reference
PD	BY273	*vtIs [Pdat-1::GFP; Pdat-1::WTα-synuclein]*	DA neurons	Expression of α-synuclein in DA neurons to assess neurodegeneration and its functional effect	Nass et al. (2002) Proc Natl Acad Sci USA 99: 3264–9
	Panneuronal-WTα-syn	*(Paex-3::α-syn; Pdat-1::GFP)*	Pan-neuronal	Panneuronal expression of WT α-syn to assess neurodegeneration and its functional effect	Lakso et al. (2003) J Neurochem 2003 July; 86 (1):165–72
	Dopaminergic-A53Tα-syn	*(Pdat-1::α-synA53T; Pdat-1::GFP)*	DA neurons	Dopaminergic expression of mutant α-syn (A53T) to assess neurodegeneration and its functional effect	Lakso et al. (2003) J Neurochem 2003 July; 86 (1):165–73
	Panneuronal-A53Tα-syn	*(Paex-3::α-synA53T; Paex-3::GFP)*	Pan-nenuronal	Panneuronal expression of α-syn (A53T) to assess neurodegeneration and its functional effect	Lakso et al. (2003) J Neurochem 2003 July; 86 (1):165–74
	UA44	*baIn11 [Pdat-1:: α-syn, Pdat-1::GFP]*	DA neurons	Expression of α-synuclein in DA neurons to assess neurodegeneration and its functional effect	Cooper et al. (2006) Science 313: 324–8
	UA49	*baIn2 [Punc-54::α-syn::GFP, rol-6 (su1006)]*	Muscle	Expression of α-synuclein in muscle to assess the aggregation process	Hamamichi et al. (2008) Proc Natl Acad Sci USA 105: 728–33
	UA50	*baInl13 [Punc-54::α-syn::GFP, Punc-54::tor-2], rol-6 (su1006)]*	Muscle	Expression of α-synuclein in muscle to assess the aggregation process but at a reduced rate due to the expresion of TOR2	Hamamichi et al. (2008) Proc Natl Acad Sci USA 105: 728–33
	NL5901	*pkIs2386 [Punc-54::alphasynuclein:: YFP + unc-119 (+)]*	Muscle	Expression of α-synuclein in muscle to assess the aggregation process	van Ham et al. (2008) PLoS Genet 4: e1000027
	JVR406	*jerEx30 [ddr-2p::BiFC1 (EGFH1-LINK-SYN) + tph-1p::BIFC2 (SYN-EGFH2) + rol-6 (su1006)]*	Serotonergic neurons (tph-1p) and head, tail, ventral and dorsal nerve cords (ddr-2p)	Expression of α-synuclein in two different neural circuits expressing BiFC when there is a transfer of α-synuclein between the two neuronal populations	Tyson et al. (2017) Sci Rep. 2017 August 8; 7 (1):7506
AD	CL2355	*dvIs50 [pCL45 (snb-1::Abeta 1–42:: 3′ UTR (long) + mtl-2::GFP]*	Pan-neuronal	Pan-neuronal expression of hAβ3-42	Wu et al. J Neurosci. 2006 December 13; 26 (50):13102–13
	JKM2	*Is [rgef-1p::Signalpeptide-Abeta (1–42)::hsp-3(IRES)::wrmScarlet-Abeta (1–42)::unc-54 (3′UTR) +rps-0p::HygroR]*	Pan-neuronal	Pan-neuronal expression of hAβ1-42	Gallrein et al. Progress in Neurobiology 198 (2021): 101907
	UA198	*baIn34 [Peat-4::Abeta42; Pmyo-2:: mCherry]; adIs1240 [Peat-4::GFP]*	Glutamatergic neuron	Glutamatergic neuronal expression of the hAβ1-42	Griffin et al. (2019) Dis Mod Mech 12.2 (2019): dmm037218
	BR5270	*byIs161 [rab-3p::F3 (delta)K280 + myo-2p::mCherry]*	Pan-neuronal	Pan-neuronal expression of the human tau mutant hTau [F3Δ280]	Fatuouros et al. Hum Mol Genet. 2012 August 15; 21 (16):3587–603
	CK12	*Is [aex-3::tau4R1N(P301L) + myo-2p::gfp]*	Pan-neuronal	Pan-neuronal expression of the human tau mutant hTau [P301L]	Kraemer et al. Proc Natl Acad Sci USA 100.17 (2003): 9980–9985
	FX11962	*tmIs389(Punc-119::WT hTau0N4R + Pges-1::egfp)*	Pan-neuronal	Pan-neuronal expression of the human WT tau0N4R at low level (no memory impairment)	Miyasaka et al. Front Neurosci 12 (2018): 415
	FX11974	*tmIs390(Punc-119::WT hTau0N4R + Pges-1::egfp)*	Pan-neuronal	Pan-neuronal expression of the human WT tau0N4R at high level with memory impairment	Miyasaka et al. Front Neurosci 12 (2018): 415
	UM0001	*dvIs50 [PCL45 (snb-1::Abeta 1–42:: 3′ UTR (long) + mtl-2::GFP]; byIs161 [Prab-3::F3 (delta)K280 + Pmyo-2:: mCherry]*	Pan-neuronal	Pan-neuronal expression of hAβ1-42; hTau [F3Δ280]	Yang et al. Aging (Albany NY) 12.17 (2020): 16852
HD	ID1	*igIs1 [Pmec-3::htt57-128Q::cfp; lin-15 (+); Pmec-7::yfp]*	Mechanosensory neurons	Expression of polyQ repeats (128Q) in touch receptor neurons, resulting in extended aggregation in the mechanosensory neuronal circuit and mec phenotype	Parker et al. (2001) Proc Natl Acad Sci U S A 98: 13318–23

(Continued on following page)

TABLE 1 | (*Continued*) *C. elegans* strains used to model neurodegenerative disease.

NDD	Strain	Genotype	Tissue	Use	Reference
	ID245	*igIs245 [Pmec-3::htt57-19Q::cfp; lin-15 (+); Pmec-7::yfp]*	Mechanosensory neurons	Expression of polyQ repeats (19Q) in touch receptor neurons resulting in very low/rare aggregation in the mechanosensory neuronal circuit	Parker et al. (2001) Proc Natl Acad Sci U S A 98: 13318–23
	AM140	*rmIs132 [Punc-54::Q35::YFP]*	Muscle	Expression of polyQ repeats (35Q) in muscle. Soluble in early stages transitioning to aggregation in adult stage	Morley et al. (2002) Proc Natl Acad Sci USA 99: 10417–22
	AM141	*rmIs133 [Punc-54::Q40::YFP]*	Muscle	Expression of polyQ repeats (40Q) in muscle. Rapid aggregation since early stages showing a full aggregation phenotype in adulthood	Morley et al. (2002) Proc Natl Acad Sci USA 99: 10417–22
	AM101	*rmIs110 [Prgef-1::Q40::YFP]*	Pan-neuronal	Pan-neuronal expression of polyQ repeats (40Q) resulting in neuronal dysfunction	Gidalevitz et al. (2006), Science. March 10; 311 (5766):1471–4
	Prion-like Q25	*Ex [(myo-2p::V1Q25) + (flp-21p:: Q25V2-ICR-DsRed) + pRF4 (rol-6 (su1006)]*	Pharyngeal muscle (myo-2p) and pharyngeal neurons (flp-21p)	Expression of 25Q in two different tissues expressing BiFC when there is a transfer of polyQ between the different tissues	Kim et al. (2017), Exp Neurobiol. 2017 December; 26 (6): 321–328
	Prion-like Q97	*Ex [(myo-2p::V1Q97) + (flp-21p:: Q97V2-ICR-DsRed) + pRF4 (rol-6 (su1006)]*	Pharyngeal muscle (myo-2p) and pharyngeal neurons (flp-21p)	Expression of 97Q in two different tissues expressing BiFC when there is a transfer of polyQ between the different tissues	Kim et al. (2017), Exp Neurobiol. 2017 December; 26 (6): 321–328
ALS	Panneuronal-Human WT SOD1	*Is(Psnb-1::SOD1WT; Pmyo-2::GFP)*	Pan-neuronal	Expression of panneuronal WT human SOD1	Wang et al. (2009) PLoS Genet January; 5 (1): e1000350
	Panneuronal-Human-G85R-SOD1	*Is(Psnb-1::SOD1 (G85R); Pmyo-2::GFP)*	Pan-neuronal	Expression of panneuronal mutant G85R human SOD1 resulting in locomotor defects and aggregation	Wang et al. (2009) PLoS Genet January; 5 (1): e1000350
	Panneuronal-Human WT SOD1	*Is(Psnb-1::SOD1WT::YFP)*	Pan-neuronal	Expression of panneuronal WT human SOD1 tagged with YFP	Wang et al. (2009) PLoS Genet January; 5 (1): e1000350
	Panneuronal-Human-G85R-SOD1	*Is(Psnb-1::SOD1 (G85R)::YFP)*	Pan-neuronal	Expression of panneuronal mutant G85R human SOD1 resulting in locomotor defects and aggregation	Wang et al. (2009) PLoS Genet January; 5 (1): e1000350
	CL6049	*dvIs62 [snb-1p::hTDP-43/3′ long UTR + mtl-2p::GFP] X*	Pan-neuronal	Expression of pan-neuronal WT human TDP43	Ash et al. (2010) Hum Mol Genet. 2010 August 15; 19 (16):3206–18
	alfa-1 mutant (5x outcrossed) + GABA tag	*alfa-1 (ok3062); oxIs12 [Punc-47:: GFP + lin-15 (+)]*	GABAergic neurons	Deletion mutant of alfa-1 (orthologue of human C9ORF72) with GABAergic neurons tagged. Observable degeneration of that neuronal circuit, locomotion defects and sensitive to osmotic stress	Therrien et al. (2013) PLoS One December 12; 8 (12):e83450
	GABAergic-DPR (GR)50	*Is [Punc-47::(GR)50::GFP; Pmyo-3:: HIS-58::mCherry]*	GABAergic neurons	Expression of GABAergic DPR (GR)50 resulting in locomotion defects and blebbing in the GABAergic circuit	Rudich et al. (2017) Hum Mol Genet December 15; 26 (24): 4916–4928
	GABAergic-DPR (PR)50	*Is [Punc-47::(PR)50::GFP; Pmyo-3:: HIS-58::mCherry]*	GABAergic neurons	Expression of GABAergic DPR (PR)50 resulting in locomotion defects and blebbing in the GABAergic circuit	Rudich et al. (2017) Hum Mol Genet December 15; 26 (24): 4916–4928
	Muscle-DPR (GR)50	*Is [Pmyo-3::(GR)50::GFP; Pmyo-3:: HIS-58::mCherry]*	Muscle	Expression of muscle DPR (GR)50 resulting in locomotion defects and brood size decrease	Rudich et al. (2017) Hum Mol Genet December 15; 26 (24): 4916–4928
	Muscle-DPR (PR)50	*Is [Pmyo-3::(PR)50::GFP; Pmyo-3:: HIS-58::mCherry]*	Muscle	Expression of muscle DPR (PR)50 resulting in locomotion defects and brood size decrease	Rudich et al. (2017) Hum Mol Genet December 15; 26 (24): 4916–4928

expressing α-synuclein in the body wall muscles (Hamamichi et al., 2008; van Ham et al., 2008). The bigger size of the cells in this tissue, made it easier to observe aggregation *in vivo*, facilitating identification of genetic modifiers of the aggregation phenotype through RNAi based screens (Hamamichi et al., 2008; van Ham et al., 2008). Newer models created to understand dynamics and specifics of PD and α-synuclein aggregation, points to misfolded α-synuclein being transferred from neuron to neuron by seeding (Desplats et al., 2009; Hansen et al., 2011). A model was created some years ago to

track this α-synuclein transfer by using bimolecular fluorescence complementation (BiFC) with synaptic transmission influencing the propagation of α-synuclein (Cooper et al., 2018) (**Table 1**).

Alzheimer's Disease

Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disease globally, accounting for 60–70% of dementia cases. AD is marked by increasing cognitive impairment, decreased decision-making ability, behavioral abnormalities, and gradual memory loss that leads to dementia. The neuropathological features of AD are extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) due to hyper-phosphorylation of Tau (pTau) (Armstrong, 2006; Canter et al., 2016).

In 1998, the genome sequence of *C. elegans* revealed homologues of the AD-related proteins APP (*apl-1*) and *T*au (*ptl-1*). *C. elegans* lacks the specific features of the human APP amino acid sequence which is essential for the generation of aggregation prone Aβ peptides. *C. elegans* also lacks beta-secretase (BACE1) which generates Aβ peptides (Canter et al., 2016). To enable the use of *C. elegans* as a model for AD, Christopher Link's group created a transgenic worm that expresses human beta-amyloid peptides, specifically Aβ3-42, in body wall muscle cells (Dosanjh et al., 2010). In this model, muscle-associated deposits accumulate. Immunostaining with the anti-Aβ antibody verified these deposits as extracellular Aβ plaques. Later a strain expressing full-length Aβ1-42 in muscle cells was made (McColl et al., 2012). In addition, a strain with pan-neuronal expression of Aβ for AD research was generated (Link, 2006). In this model, neuronal impairment was observed, such as impairments in odor-associated learning behavior, serotonin-controlled behavior, and experience-dependent learning (Dosanjh et al., 2010). NFTs formed by tau are another characteristic of AD (Strang et al., 2019) and pan-neuronal expression of Tau (normal or mutated) has been generated (**Figure 4**) (**Table 1**). Human apolipoprotein E (ApoE) is a characterized genetic predisposition marker in AD (Corder et al., 1994). Models with co-expression of distinct human ApoE alleles and an Aβ peptide was created to provide an *in vivo* platform to explore Aβ-associated neurotoxic effects in distinct glutamatergic neurons (Griffin et al., 2019) and serotonergic hermaphrodite specific neurons (HSNs) (Sae-Lee et al., 2020). Combined with machine learning and other laboratory techniques, the *C. elegans* AD model has been used as a model system in drug development (Xie et al., 2022).

Huntington's Disease

Huntington's disease (HD) is a neurodegenerative disorder caused by dominantly inherited glutamine repeats (polyQ) in the huntingtin gene (*HTT*). The product of *HTT* is the protein huntingtin (HTT). These glutamine repeats, become pathogenic when the number of repeats reach 36 (Tabrizi et al., 2020). Then, HTT folds abnormally, forming aggregates that disrupt cellular functions and result in cell death. The progression of the disease causes loss of control of voluntary and involuntary movements, and dysfunction in cognition and behavior.

Early efforts to use *C. elegans* as a model for HD included the assessment of sensory neurons by dye filling assays and the impact of endogenous expression of diverse lengths of polyQ repeats in aggregation, neurodegeneration and response to stimulus (Faber et al., 1999). Several models were established expressing different lengths of polyQ repeats in different tissues, from body wall muscle (Satyal et al., 2000; Morley et al., 2002) to mechanosensory neurons (Parker et al., 2001). When expressed in body wall muscle, tracking the process of polyQ aggregation was easy. Thus, it was possible to study how the different tracts of polyQ assembled at different rates, and it became evident that the motility of the nematode was affected in a directly proportional way as the organism aged. When expressed in touch receptor neurons, the observable aggregation was accompanied with defects in the response to touch, especially in the tail, accompanied by abnormalities in the neuronal processes. It is also important to remark that the effect of polyQ aggregation did not impact only the expressed tissue of its function, but altered and deregulated other processes, like the heat shock response (Satyal et al., 2000). The latter illustrates how the small animal system can reveal layers of insight in a way that cellular model system cannot (**Figure 4**) (**Table 1**).

These studies provided the tools necessary for carrying out screenings for modifiers of polyQ aggregation (Nollen et al., 2004; Voisine et al., 2007) and show how the presence of misfolded proteins affect the cellular environment (Holmberg et al., 2004; Gidalevitz et al., 2006). An advantage of this system is the difference in rate of aggregation between the strains with different number of repeats, which bypasses the problem of the lack of expansion of the polyQ tracts in *C. elegans*. In that way, one could use a strain with a low rate of aggregation and try to find a modifier, genetic or drug that increases aggregation. On the other hand, it is possible to use strains that have a higher level of aggregation in order to find modifiers that would decrease it. The possibility of using strains that produce misfolded proteins also allows to study regulation and processes like the unfolded protein response (UPR) or ER-associated degradation (ERAD), not just being useful for HD research but also to the understanding of proteostasis in general (Silva et al., 2011; Muñoz-Lobato et al., 2014). After studies suggesting the transmission of polyglutamine aggregates between cells as the mechanism for the progress of the disease (Ren et al., 2009; Pecho-Vrieseling et al., 2014; Pearce et al., 2015), a strain was engineered to assess the transmission of polyQ between different group of cells by BiFC, with neurodegenerative phenotypes increasing with the length of the polyQ tract (Kim et al., 2017).

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a common degenerative motor neuron disease, impacting the motor cortex, brain stem, and spinal cord (Ragagnin et al., 2019). Mutations in four genes, *SOD1, C9ORF72, TARDBP*, and *FUS/TLS* cause the majority of cases. The disease is manifested by progressive locomotor impairment and weakness, speech disturbance and pulmonary complications that lead to respiratory failure and death (Zarei et al., 2015).

C. elegans has been used to model pathology emanating from the specific mutations; for example, expression of mutant isoforms of human SOD1 systemically induced by heat shock or in different tissues muscle (Oeda et al., 2001), motor neurons (Witan et al., 2008) or pan-neuronally (Wang et al., 2009), result in proteotoxicity and/or locomotion defects. *C. elegans* has an ortholog of *C9ORF72*, *alfa-1,* and the null mutant evidenced locomotion problems, suffered neurodegeneration in GABAergic neurons and was sensitive to osmotic stress (Therrien et al., 2013). An intronic hexanucleotide repeat (GGGGCC) in *C9ORF72* was identified as the most common genetic variant in familial ALS (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Translation of this transgene resulted in the production of toxic dipeptide repeats proteins (DPRs) (Rudich et al., 2017), as seen in the human disease. When expressed in motor neurons, this resulted in neurodegeneration and motility defects. When expressed in muscle, the motility defects were accompanied by reduction in brood size and developmental defects.

Other efforts have focused on *TARDBP* and *FUS*. The products of both genes, TDP-43 and FUS, respectively, are DNA/RNA binding proteins involved in transcription and splicing regulation (Mackenzie et al., 2010). Mutants of the *C. elegans* homologues of these genes, *tdp-1* and *fust-1*, respectively, suggest functional conservation. Lack of TDP-1 induces accumulation of double stranded RNA (dsRNA), genomic instability and changes in the chromatin organization (Saldi et al., 2014; Saldi et al., 2018; Mitra et al., 2019), accompanied by locomotion defects (Zhang et al., 2012) and sensitization to osmotic and oxidative stress (Vaccaro et al., 2012b). Loss of FUST-1 suppressed miRNA-mediated gene silencing (Zhang et al., 2018) and dysregulated circRNA formation (Cao, 2021). When expressing human TDP-43 in a pan-neuronal manner, motility defects were observed (Ash et al., 2010). Interestingly, pan-neuronal expression of human FUS did not cause any observable defect (Murakami et al., 2012). However, expression of disease-causing mutations of TDP-43 and FUS both caused neurodegeneration and locomotion defects when expressed pan-neuronally (Murakami et al., 2012; Liachko et al., 2013) or in the GABAergic circuit (Vaccaro et al., 2012a) (**Figure 4**) (**Table 1**). Defective DNA damage response has been linked to many subtypes of ALS (Guerrero et al., 2016). Recently, in an *C. elegans* ALS model, it was shown that TDP-43 is recruited in DSB site and plays a vital role in TDP-43 driven pathology (Mitra et al., 2019). Overexpression of another DNA damage response modulator, FUS, in *C. elegans* leads to defective neuro muscular signalling and motor defects (Markert et al., 2020). Loss of RAD-23, a homolog of the human HRAD23A and HR23B proteins that serve as accessory proteins in the damage recognition step of NER and in coupling of NER-mediated DNA repair to the ubiquitin/proteasomal system (UPS), protects motor neurons and improves movement disorders by enhancing the clearance of TDP-43 and SOD-1 aggregation in a *C. elegans* ALS model (Jablonski et al., 2015).

Perspectives

Evolutionarily *C. elegans* is distant from humans. It lacks organ systems, like the brain, liver, kidney, blood, and a blood-brain barrier. Hence, there are some relevant constraints for the use of this model research into the intersection of genome maintenance, aging and neurodegeneration. To begin with, it feeds on bacteria, making biochemistry inherently difficult. Secondly, despite possessing 959 somatic cells, investigating somatic stem cell biology is difficult (Sulston and Horvitz, 1977). Thirdly, *C. elegans* lacks adaptive immunity. Fourthly, *C. elegans* lacks DNA methylation, which is critical in aging and neurodegeneration (Corsi et al., 2015). Finally, achieving proper sample sizes for biochemical assays such as immunohistochemistry, immunoprecipitation, chromatin immunoprecipitation, microarray, RNA and DNA sequencing requires whole animal extract, which may mask effects in certain cell types.

Despite these obvious limitations, *C. elegans* has been used as a model to study NDs for decades (**Figure 5**). But what at a glance could be taken as a disadvantage, is actually one of its main strengths, there are numerous possibilities for adaptation to different studies, techniques and scopes. This potential remains to this day, with many new methods that allow for more specific studies of the neuronal milieu of the nematode. Most remarkably, in the past few years, the common effort of different *C. elegans* labs led to the creation of the CeNGEN project (Hammarlund et al., 2018), a consortium formed with the goal of identifying the neuronal gene expression of the nematode with single cell resolution (Taylor et al., 2021) and that was made available online (https://cengen.shinyapps.io/CengenApp/). This work built upon previous work consisting of the creation of strains with the neuron/s of interest tagged with a fluorescent reporter, culturing big quantities of the desired strain and the posterior digestion and processing of the animals in order to obtain a suspension of cells. Lastly, the fluorescent cells would be sorted using FACS and, in this case, the pipeline continued with RNA-seq in order to analyze their gene expression (Spencer et al., 2014). These methods permit to limit a study to the desired neuronal group. Nonetheless, an adult *C. elegans* hermaphrodite has 959 somatic cells of which 302 are neurons; which means that subtle changes in neuronal gene expression could be masked, plus it enables to spot differences between the different groups of neurons. Furthermore, although the downstream step in the mentioned publications was gene expression studies, when the desired cell population is sorted, the possibilities for techniques and methods are almost infinite. The study and identification of neuropeptides (Van Bael et al., 2018) or the measurement of oxygen consumption rate to assess mitochondrial function (Koopman et al., 2016), would become specific to the population of neurons of interest; and it is obvious how this can impact and be revolutionary in the study of NDs on *C. elegans.*

Different approaches have been used for bypassing the possible masking of changes in the neuronal expression by analyzing whole animals. Other labs generated methods to tag the transcripts of the desired tissue. In (Ma et al., 2016), the muscle transcriptome was analyzed by tagging the spliced leader (SL) RNA gene using a muscle specific promoter, allowing to process whole animals collecting total RNA, but then focusing their study in the transcripts carrying the SL tag. Of course, this

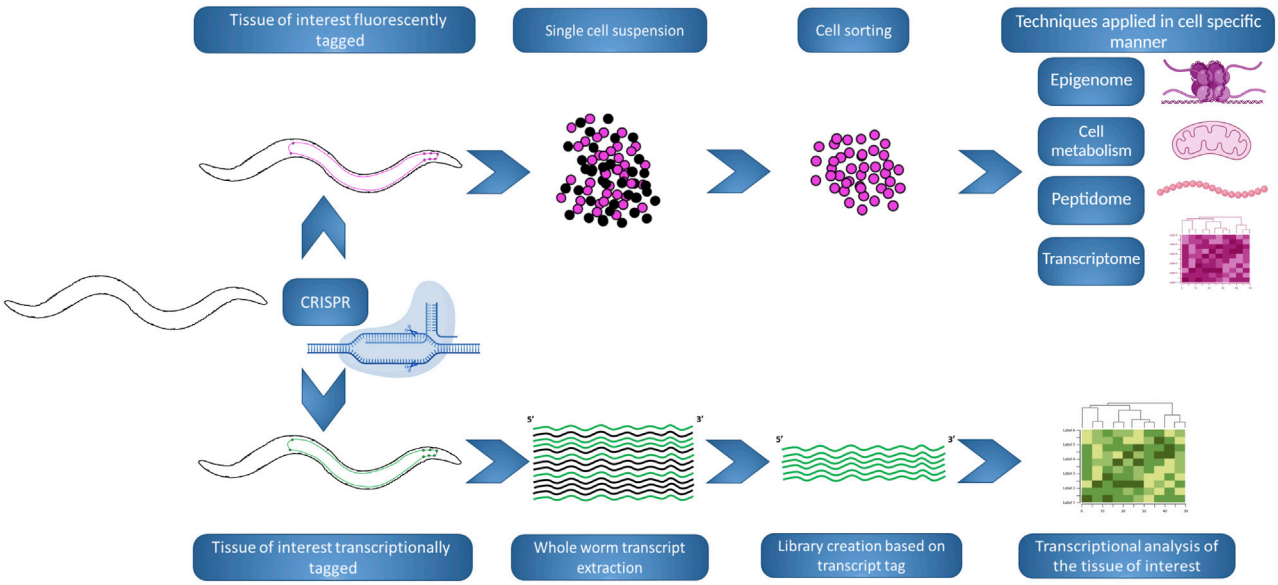

FIGURE 5 | Innovative workflows using *C. elegans* to study NDs. The development of new workflows and or techniques in the nematode allows for news scopes in the study of NDs, allowing to switch between whole-organism to single cell perspectives, at one's convenience. **(Top).** A strain can be generated by CRISPR in order to tag with a fluorescence marker (pink) the tissue of interest. Posteriorly, large synchronized populations of the strain can be raised and chemically digested, in order to obtain a cell suspension that be FACS sorted based on the fluorescence tag. The resulting sorted population can be used in different analyses and techniques, where small variations could be masked if analysing whole worms. **(Bottom).** A strain can be generated by CRISPR in order to tag biochemically (green) the transcripts produced in the tissue of interest. Posteriorly, large synchronized populations of the strain can be raised and whole organism extraction is performed, but the creation of the transcript library will be biased by the presence of the biochemical tag. Following transcriptional analysis will inform specifically about changes in the tissue of interest. The figure has been generated using Biorender.com by the author.

method could be adapted to other tissues, being possible to exploit in neuronal subsets. In (Alberti et al., 2018), cell type specific microRNAs are methylated by expressing a plant specific methyltransferase in the desired subset of neurons, and biasing the cloning and creation of the library for RNA-seq in the existence of methylation, permitting cell specific resolution. This work does not highlight just the importance of neuron specific expression, but also the study of other RNA families, that although not generating protein products, are very important in the regulation and therefore the metabolism and functionality of a cell. With that in mind, the study of circular RNAs, a fraction of RNAs that lack 5′ and 3′ ends, may become of importance in the NDs research on *C. elegans*. Circular RNAs are the products of pre-mRNA transcripts that undergo backsplicing events. Although their functions are not fully understood, they are involved in regulation of gene expression, therefore, being potential modulators of numerous processes in the cell. They are exceptionally stable and thought to accumulate during the aging process (Cortés-López et al., 2018). They could become a major factor to understand and assess the fitness of a neuron. Additionally, small RNAs resulting from transfer RNAs (tRNA), tRNA-derived fragments (tRFs), are also accumulated as the organism ages, being potential candidates as aging biomarkers (Shin et al., 2021).

These approaches, which provide a more subtle understanding of the transcriptional context of the neurons, can be complemented with a series of new fluorophores with enhanced brightness. This increase in brightness makes it possible that lowly expressed genes can still be visualized *in vivo* in the worm. Examples like mNeonGreen or mScarlet (Shaner et al., 2013; Bindels et al., 2017) have appeared as alternatives to GFP or mCherry to label proteins that are not highly expressed, as in (Hostettler et al., 2017), where mNeonGreen consistently outperforms the levels of brightness of GFP.

C. elegans became the first organism with a described global description of its neural circuit or connectome (Cook et al., 2019; Alicea, 2020). Understanding the neuronal circuit as a dynamic network, that adapts to challenges as the case of a NDs model, may help us understand the adaptations the neuronal circuit goes through as a whole when subsets of neurons start the process of neurodegeneration. This could be combined with approaches like NeuroPAL, a *C. elegans* strain created where every single neuron can be identified based on its fluorophore colour and position in the neuronal circuit (Yemini et al., 2021), allowing to track whole-brain neuronal dynamics and activity. Furthermore, the NeuroPAL strain can be used for identifying neuronal gene expression or the effect of mutations in neuronal fate and development.

Other efforts focus on facilitating the way of carrying out assays in the field. The previously mentioned assays, although accessible and affordable, may require time consuming tedious tasks such as counting aggregates, body bends or paralyzed animals. Trying to ease these procedures by automating them, would make the assessment of NDs models on *C. elegans* an easier task and would permit to assess more strains or conditions in the

same assay, potentiating one of the advantages of the organism, the possibilities for high-throughput applications. The creation of systems like the WorMotel (Churgin and Fang-Yen, 2015), where assayed worms are cultured in multiwell plates and images of them are taken in the desired interval of times, allowing to discern their movement. Systems like this allow us to determine parameters like locomotion, lifespan and behavior (SenGupta et al., 2021). Other groups have developed methods to automatize the quantification of aggregate formation (Molenkamp et al., 2021; Vaziriyan-Sani et al., 2021) or the number of head thrashes (Zhang et al., 2022); both methods that have been described previously as traditional ways to analyze ND models (**Figure 5**). The continuous development and improvement of the existing methods and the automatization of traditionally laborious assays will allow for more precise and wide analysis; doing possible the inclusion of new variables or scopes in this kind of assays.

CONCLUSION

DNA damage and its repair are generally linked to the aging process. Effective studies of the interactions between these processes depends on a model system that allows physiological aging to be taken into account. Although some studies have emerged where *C. elegans*, with its simplified DNA repair mechanisms have been studied in light of the described aging pathways, there is still much that remains to be explored and we are probably only scratching the surface on the information that can be gained. Genetic interactions of different DNA repair pathways have been demonstrated, *e.g.* between the BER and NER pathway (Arczewska et al., 2013) but systematic studies to elucidate the extent of the collaboration between different DNA repair pathways are warranted. The segmental progeroid and other DNA repair deficiency syndromes have a plethora of phenotypes from different organs and organ systems and *C. elegans* serves as powerful model to understand the response and integration to different kinds of DNA repair pathways in different organ systems. Moreover, *C. elegans* has the potential to reveal how DNA repair impact on the induction of senescence, telomere attritions and possibly other, yet to be defined, mechanisms of aging and how different processes might operate simultaneously and are integrated. The recent identification of involvement of MUT-7/WRN-1 regulating neuronal plasticity (Hsu et al., 2021) is one example where *C. elegans* may have revealed a new mechanism. At present is not known, for example, whether non-canonical MMR, where MMR is uncoupled from DNA replication, may play a role in *C. elegans* neurons.

Another observation that has been made in *C. elegans* is that although DNA repair in general is protective, loss of function in individual DNA repair enzymes may be associated with beneficial phenotypes: A hypomorphic mutation of ATR in humans causes accelerated aging disease known as Seckel syndrome. The *C. elegans* ATL-1 is orthologous to human ATR. Interestingly, *atl-1* mutants exhibit extended life span, perhaps as a consequence of mild oxidative stress and mitochondrial dysfunction (Suetomi et al., 2013). We recently showed that removing the BER DNA glycosylase NTH-1 protected against age dependent loss of dopaminergic neurons in a *C. elegans* PD model. These animals showed significantly less accumulation of oxidative lesions and lethal SSBs in nuclear and mitochondrial DNA. The lack of NTH-1 prevented the accumulation of BER intermediates, which activated mitochondrial genotoxic stress and mitohormesis, altogether orchestrating a response that protects DA neurons from α-synuclein-induced neurotoxicity (SenGupta et al., 2021). Future work will show whether similar mechanisms compensating for loss of DNA repair activity are operating also in mammals.

Thus, the combination of approachability, versatility and decades of accumulated knowledge, from the very early days of genetic studies screening for phenotypes and measuring maximum lifespan; have led us to an exciting moment in the field. The new genetic tools and novel technologies, like single neuron resolution transcriptional studies or assay automatization, expand the existing traditional methods and allow for wider and newer scopes where we will be able to gain a deeper understanding of the role of DNA repair in NDs, maybe permitting us 1 day to expand our healthspan much closer to our lifespan.

AUTHOR CONTRIBUTIONS

Conceptualization HN and TS; Writing-original draft, validation, and editing FJNG, RA, TS, EF and HN; software and visual graphics, FJNG, TS and RA. All listed authors have read, made intellectual contribution and approved the published version of the manuscript.

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REFERENCES

Albert, P. S., Brown, S. J., and Riddle, D. L. (1981). Sensory Control of Dauer Larva Formation inCaenorhabditis Elegans. *J. Comp. Neurol.* 198, 435–451. doi:10.1002/cne.901980305

Alberti, C., Manzenreither, R. A., Sowemimo, I., Burkard, T. R., Wang, J., Mahofsky, K., et al. (2018). Cell-type Specific Sequencing of microRNAs from Complex Animal Tissues. *Nat. Methods* 15, 283–289. doi:10.1038/nmeth.4610

Alicea, B. (2020). Raising the Connectome: The Emergence of Neuronal Activity and Behavior in *Caenorhabditis elegans*. *Front. Cell. Neurosci.* 14, 524791. doi:10.3389/fncel.2020.524791

Aman, Y., Schmauck-Medina, T., Hansen, M., Morimoto, R. I., Simon, A. K., Bjedov, I., et al. (2021). Autophagy in Healthy Aging and Disease. *Nat. Aging* 1, 634–650. doi:10.1038/s43587-021-00098-4

Amirifar, P., Ranjouri, M. R., Yazdani, R., Abolhassani, H., and Aghamohammadi, A. (2019). Ataxia-telangiectasia: A Review of Clinical Features and Molecular Pathology. *Pediatr. Allergy Immunol.* 30, 277–288. doi:10.1111/pai.13020

Arczewska, K. D., Tomazella, G. G., Lindvall, J. M., Kassahun, H., Maglioni, S., Torgovnick, A., et al. (2013). Active Transcriptomic and Proteomic Reprogramming in the *C. elegans* Nucleotide Excision Repair Mutant Xpa-1. *Nucleic Acids Res.* 41, 5368–5381. doi:10.1093/nar/gkt225

Armstrong, M. J., and Okun, M. S. (2020). Diagnosis and Treatment of Parkinson Disease. *Jama* 323, 548–560. doi:10.1001/jama.2019.22360

Armstrong, R. A. (2006). Plaques and Tangles and the Pathogenesis of Alzheimer's Disease. *Folia Neuropathol.* 44, 1–11.

Asagoshi, K., Lehmann, W., Braithwaite, E. K., Santana-Santos, L., Prasad, R., Freedman, J. H., et al. (2012). Single-nucleotide Base Excision Repair DNA Polymerase Activity in *C. elegans* in the Absence of DNA Polymerase. *Nucleic Acids Res.* 40, 670–681. doi:10.1093/nar/gkr727

Ash, P. E. A., Zhang, Y.-J., Roberts, C. M., Saldi, T., Hutter, H., Buratti, E., et al. (2010). Neurotoxic Effects of TDP-43 Overexpression in *C. elegans*. *Hum. Mol. Genet.* 19, 3206–3218. doi:10.1093/hmg/ddq230

Barbieri, M., Bonafè, M., Franceschi, C., and Paolisso, G. (2003). Insulin/IGF-I-signaling Pathway: an Evolutionarily Conserved Mechanism of Longevity from Yeast to Humans. *Am. J. Physiology-Endocrinology Metabolism* 285, E1064–E1071. doi:10.1152/ajpendo.00296.2003

Bargmann, C. I. (1998). Neurobiology of the *Caenorhabditis elegans* Genome. *Science* 282, 2028–2033. doi:10.1126/science.282.5396.2028

Batlevi, Y., and La Spada, A. R. (2011). Mitochondrial Autophagy in Neural Function, Neurodegenerative Disease, Neuron Cell Death, and Aging. *Neurobiol. Dis.* 43, 46–51. doi:10.1016/j.nbd.2010.09.009

Belan, O., Anand, R., and Boulton, S. J. (2021). Mechanism of Mitotic Recombination: Insights from *C. elegans*. *Curr. Opin. Genet. Dev.* 71, 10–18. doi:10.1016/j.gde.2021.06.005

Belenky, P., Racette, F. G., Bogan, K. L., Mcclure, J. M., Smith, J. S., and Brenner, C. (2007). Nicotinamide Riboside Promotes Sir2 Silencing and Extends Lifespan via Nrk and Urh1/Pnp1/Meu1 Pathways to NAD+. *Cell.* 129, 473–484. doi:10.1016/j.cell.2007.03.024

Bindels, D. S., Haarbosch, L., van Weeren, L., Postma, M., Wiese, K. E., Mastop, M., et al. (2017). mScarlet: a Bright Monomeric Red Fluorescent Protein for Cellular Imaging. *Nat. Methods* 14, 53–56. doi:10.1038/nmeth.4074

Blüher, M., Kahn, B. B., and Kahn, C. R. (2003). Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue. *Science* 299, 572–574.

Braungart, E., Gerlach, M., Riederer, P., Baumeister, R., and Hoener, M. C. (2004). *Caenorhabditis elegans* MPP+ Model of Parkinson's Disease for High-Throughput Drug Screenings. *Neurodegener. Dis.* 1, 175–183. doi:10.1159/000080983

Bravo-San Pedro, J. M., Kroemer, G., and Galluzzi, L. (2017). Autophagy and Mitophagy in Cardiovascular Disease. *Circ. Res.* 120, 1812–1824. doi:10.1161/circresaha.117.311082

Brenner, S. (1974). The Genetics of *Caenorhabditis elegans*. *Genetics* 77, 71–94. doi:10.1093/genetics/77.1.71

Brouillet, E., Jacquard, C., Bizat, N., and Blum, D. (2005). 3-Nitropropionic Acid: a Mitochondrial Toxin to Uncover Physiopathological Mechanisms Underlying Striatal Degeneration in Huntington's Disease. *J. Neurochem.* 95, 1521–1540. doi:10.1111/j.1471-4159.2005.03515.x

Brownlee, D. J. A., and Fairweather, I. (1999). Exploring the Neurotransmitter Labyrinth in Nematodes. *Trends Neurosci.* 22, 16–24. doi:10.1016/s0166-2236(98)01281-8

Buckingham, S. D., and Sattelle, D. B. (2009). Fast, Automated Measurement of Nematode Swimming (Thrashing) without Morphometry. *BMC Neurosci.* 10, 84. doi:10.1186/1471-2202-10-84

Calixto, A., Chelur, D., Topalidou, I., Chen, X., and Chalfie, M. (2010). Enhanced Neuronal RNAi in *C. elegans* Using SID-1. *Nat. Methods* 7, 554–559. doi:10.1038/nmeth.1463

Canter, R. G., Penney, J., and Tsai, L.-H. (2016). The Road to Restoring Neural Circuits for the Treatment of Alzheimer's Disease. *Nature* 539, 187–196. doi:10.1038/nature20412

Cao, D. (2021). An Autoregulation Loop in Fust-1 for Circular RNA Regulation in *Caenorhabditis elegans*. *Genetics* 219. doi:10.1093/genetics/iyab145

Cao, S., Gelwix, C. C., Caldwell, K. A., and Caldwell, G. A. (2005). Torsin-mediated Protection from Cellular Stress in the Dopaminergic Neurons of *Caenorhabditis elegans*. *J. Neurosci.* 25, 3801–3812. doi:10.1523/jneurosci.5157-04.2005

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W., and Prasher, D. C. (1994). Green Fluorescent Protein as a Marker for Gene Expression. *Science* 263, 802–805. doi:10.1126/science.8303295

Chang, D. T. W., Rintoul, G. L., Pandipati, S., and Reynolds, I. J. (2006). Mutant Huntingtin Aggregates Impair Mitochondrial Movement and Trafficking in Cortical Neurons. *Neurobiol. Dis.* 22, 388–400. doi:10.1016/j.nbd.2005.12.007

Churgin, M. A., and Fang-Yen, C. (2015). An Imaging System for *C. elegans* Behavior. *Methods Mol. Biol.* 1327, 199–207. doi:10.1007/978-1-4939-2842-2_14

Clejan, I., Boerckel, J., and Ahmed, S. (2006). Developmental Modulation of Nonhomologous End Joining in *Caenorhabditis elegans*. *Genetics* 173, 1301–1317. doi:10.1534/genetics.106.058628

Consortium*, C. E. S. (1998). Genome Sequence of the Nematode *C. elegans*: a Platform for Investigating Biology. *Science* 282, 2012–2018. doi:10.1126/science.282.5396.2012

Conway, O., Akpinar, H. A., Rogov, V. V., and Kirkin, V. (2020). Selective Autophagy Receptors in Neuronal Health and Disease. *J. Mol. Biol.* 432, 2483–2509. doi:10.1016/j.jmb.2019.10.013

Cook, S. J., Jarrell, T. A., Brittin, C. A., Wang, Y., Bloniarz, A. E., Yakovlev, M. A., et al. (2019). Whole-animal Connectomes of Both *Caenorhabditis elegans* Sexes. *Nature* 571, 63–71. doi:10.1038/s41586-019-1352-7

Cooper, A. A., Gitler, A. D., Cashikar, A., Haynes, C. M., Hill, K. J., Bhullar, B., et al. (2006). α-Synuclein Blocks ER-Golgi Traffic and Rab1 Rescues Neuron Loss in Parkinson's Models. *Science* 313, 324–328. doi:10.1126/science.1129462

Cooper, J. F., Spielbauer, K. K., Senchuk, M. M., Nadarajan, S., Colaiácovo, M. P., and van Raamsdonk, J. M. (2018). α-Synuclein Expression from a Single Copy Transgene Increases Sensitivity to Stress and Accelerates Neuronal Loss in Genetic Models of Parkinson's Disease. *Exp. Neurol.* 310, 58–69. doi:10.1016/j.expneurol.2018.09.001

Corder, E. H., Saunders, A. M., Risch, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., JR., et al. (1994). Protective Effect of Apolipoprotein E Type 2 Allele for Late Onset Alzheimer Disease. *Nat. Genet.* 7, 180–184. doi:10.1038/ng0694-180

Corsi, A. K., Wightman, B., and Chalfie, M. (2015). A Transparent Window into Biology: A Primer on *Caenorhabditis elegans*. *Genetics* 200, 387–407. doi:10.1534/genetics.115.176099

Cortés-López, M., Gruner, M. R., Cooper, D. A., Gruner, H. N., Voda, A. I., van der Linden, A. M., et al. (2018). Global Accumulation of circRNAs during Aging in *Caenorhabditis elegans*. *BMC Genomics* 19, 8. doi:10.1186/s12864-017-4386-y

Craig, A. L., Moser, S. C., Bailly, A. P., and Gartner, A. (2012). Methods for Studying the DNA Damage Response in the Caenorhabdatis Elegans Germ Line. *Methods Cell. Biol.* 107, 321–352. doi:10.1016/b978-0-12-394620-1.00011-4

Croll, N. A., Smith, J. M., and Zuckerman, B. M. (1977). The Aging Process of the nematodeCaenorhabditis Elegansin Bacterial and Axenic Culture. *Exp. Aging Res.* 3, 175–189. doi:10.1080/03610737708257101

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

Degtyareva, N. P., Greenwell, P., Hofmann, E. R., Hengartner, M. O., Zhang, L., Culotti, J. G., et al. (2002). *Caenorhabditis elegans* DNA Mismatch Repair Gene

Msh-2 Is Required for Microsatellite Stability and Maintenance of Genome Integrity. *Proc. Natl. Acad. Sci. U.S.A.* 99, 2158–2163. doi:10.1073/pnas.032671599

DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., et al. (2011). Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. *Neuron* 72, 245–256. doi:10.1016/j.neuron.2011.09.011

Denver, D. R., Feinberg, S., Steding, C., Durbin, M., and Lynch, M. (2006). The Relative Roles of Three DNA Repair Pathways in Preventing *Caenorhabditis elegans* Mutation Accumulation. *Genetics* 174, 57–65. doi:10.1534/genetics.106.059840

Desplats, P., Lee, H.-J., Bae, E.-J., Patrick, C., Rockenstein, E., Crews, L., et al. (2009). Inclusion Formation and Neuronal Cell Death through Neuron-To-Neuron Transmission of α-synuclein. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13010–13015. doi:10.1073/pnas.0903691106

Devanapally, S., Ravikumar, S., and Jose, A. M. (2015). Double-stranded RNA Made in *C. elegans* Neurons Can Enter the Germline and Cause Transgenerational Gene Silencing. *Proc. Natl. Acad. Sci. U.S.A.* 112, 2133–2138. doi:10.1073/pnas.1423333112

Dickinson, D. J., Ward, J. D., Reiner, D. J., and Goldstein, B. (2013). Engineering the *Caenorhabditis elegans* Genome Using Cas9-Triggered Homologous Recombination. *Nat. Methods* 10, 1028–1034. doi:10.1038/nmeth.2641

Dosanjh, L. E., Brown, M. K., Rao, G., Link, C. D., and Luo, Y. (2010). Behavioral Phenotyping of a Transgenic Caenorhabditis Elegans Expressing Neuronal Amyloid-β. *Jad* 19, 681–690. doi:10.3233/jad-2010-1267

Driesschaert, B., Mergan, L., and Temmerman, L. (2021). Conditional Gene Expression in Invertebrate Animal Models. *J. Genet. Genomics* 48, 14–31. doi:10.1016/j.jgg.2021.01.005

Elsakrmy, N., Zhang-Akiyama, Q.-M., and Ramotar, D. (2020). The Base Excision Repair Pathway in the Nematode *Caenorhabditis elegans*. *Front. Cell. Dev. Biol.* 8, 598860. doi:10.3389/fcell.2020.598860

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. e5. doi:10.1016/j.cub.2018.06.035

Faber, P. W., Alter, J. R., Macdonald, M. E., and Hart, A. C. (1999). Polyglutamine-mediated Dysfunction and Apoptotic Death of a *Caenorhabditis elegans* Sensory Neuron. *Proc. Natl. Acad. Sci. U.S.A.* 96, 179–184. doi:10.1073/pnas.96.1.179

Fang, E. F., Hou, Y., Lautrup, S., Jensen, M. B., Yang, B., Sengupta, T., et al. (2019a). NAD+ Augmentation Restores Mitophagy and Limits Accelerated Aging in Werner Syndrome. *Nat. Commun.* 10, 5284. doi:10.1038/s41467-019-13172-8

Fang, E. F., Hou, Y., Palikaras, K., Adriaanse, B. A., Kerr, J. S., Yang, B., et al. (2019b). Mitophagy Inhibits Amyloid-β and Tau Pathology and Reverses Cognitive Deficits in Models of Alzheimer's Disease. *Nat. Neurosci.* 22, 401–412. doi:10.1038/s41593-018-0332-9

Fang, E. F., Kassahun, H., Croteau, D. L., Scheibye-Knudsen, M., Marosi, K., Lu, H., et al. (2016). NAD + Replenishment Improves Lifespan and Healthspan in Ataxia Telangiectasia Models via Mitophagy and DNA Repair. *Cell. Metab.* 24, 566–581. doi:10.1016/j.cmet.2016.09.004

Fang, E. F., Lautrup, S., Hou, Y., Demarest, T. G., Croteau, D. L., Mattson, M. P., et al. (2017). NAD + in Aging: Molecular Mechanisms and Translational Implications. *Trends Mol. Med.* 23, 899–916. doi:10.1016/j.molmed.2017.08.001

Fang, E. F., Scheibye-Knudsen, M., Brace, L. E., Kassahun, H., Sengupta, T., Nilsen, H., et al. (2014). Defective Mitophagy in XPA via PARP-1 Hyperactivation and NAD+/SIRT1 Reduction. *Cell.* 157, 882–896. doi:10.1016/j.cell.2014.03.026

Fensgård, Ø., Kassahun, H., Bombik, I., Rognes, T., Lindvall, J. M., and Nilsen, H. (2010). A Two-Tiered Compensatory Response to Loss of DNA Repair Modulates Aging and Stress Response Pathways. *Aging (Albany NY)* 2, 133–159. doi:10.18632/aging.100127

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., and Mello, C. C. (1998). Potent and Specific Genetic Interference by Double-Stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806–811. doi:10.1038/35888

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

Gershon, D. (1970). Studies on Aging in Nematodes. *Exp. Gerontol.* 5, 7–12. doi:10.1016/0531-5565(70)90023-9

Gidalevitz, T., Ben-Zvi, A., Ho, K. H., Brignull, H. R., and Morimoto, R. I. (2006). Progressive Disruption of Cellular Protein Folding in Models of Polyglutamine Diseases. *Science* 311, 1471–1474. doi:10.1126/science.1124514

Griffin, E. F., Scopel, S. E., Stephen, C. A., Holzhauer, A. C., Vaji, M. A., Tuckey, R. A., et al. (2019). ApoE-associated Modulation of Neuroprotection from Aβ-Mediated Neurodegeneration in Transgenic *Caenorhabditis elegans*. *Dis. Model. Mech.* 12. doi:10.1242/dmm.037218

Guerrero, E. N., Wang, H., Mitra, J., Hegde, P. M., Stowell, S. E., Liachko, N. F., et al. (2016). TDP-43/FUS in Motor Neuron Disease: Complexity and Challenges. *Prog. Neurobiol.* 145-146, 78–97. doi:10.1016/j.pneurobio.2016.09.004

Hamamichi, S., Rivas, R. N., Knight, A. L., Cao, S., Caldwell, K. A., and Caldwell, G. A. (2008). Hypothesis-based RNAi Screening Identifies Neuroprotective Genes in a Parkinson's Disease Model. *Proc. Natl. Acad. Sci. U.S.A.* 105, 728–733. doi:10.1073/pnas.0711018105

Hamilton, B., Dong, Y., Shindo, M., Liu, W., Odell, I., Ruvkun, G., et al. (2005). A Systematic RNAi Screen for Longevity Genes in *C. elegans*. *Genes. Dev.* 19, 1544–1555. doi:10.1101/gad.1308205

Hammarlund, M., Hobert, O., Miller, D. M., and Sestan, N. (2018). The CeNGEN Project: The Complete Gene Expression Map of an Entire Nervous System. *Neuron* 99, 430–433. doi:10.1016/j.neuron.2018.07.042

Han, I., You, Y., Kordower, J. H., Brady, S. T., and Morfini, G. A. (2010). Differential Vulnerability of Neurons in Huntington's Disease: the Role of Cell Type-specific Features. *J. Neurochem.* 113, 1073–1091. doi:10.1111/j.1471-4159.2010.06672.x

Hansen, C., Angot, E., Bergström, A.-L., Steiner, J. A., Pieri, L., Paul, G., et al. (2011). α-Synuclein Propagates from Mouse Brain to Grafted Dopaminergic Neurons and Seeds Aggregation in Cultured Human Cells. *J. Clin. Investig.* 121, 715–725. doi:10.1172/jci43366

Hansen, M., Rubinsztein, D. C., and Walker, D. W. (2018). Autophagy as a Promoter of Longevity: Insights from Model Organisms. *Nat. Rev. Mol. Cell. Biol.* 19, 579–593. doi:10.1038/s41580-018-0033-y

Hars, E. S., Qi, H., Jin, S. V., Cai, L., Hu, C., Liu, L. F., et al. (2007). Autophagy Regulates Ageing inC. Elegans. *Autophagy* 3, 93–95. doi:10.4161/auto.3636

Hart, A. C., Sims, S., and Kaplan, J. M. (1995). Synaptic Code for Sensory Modalities Revealed by *C. elegans* GLR-1 Glutamate Receptor. *Nature* 378, 82–85. doi:10.1038/378082a0

Hartman, J. H., Gonzalez-Hunt, C., Hall, S. M., Ryde, I. T., Caldwell, K. A., Caldwell, G. A., et al. (2019). Genetic Defects in Mitochondrial Dynamics in *Caenorhabditis elegans* Impact Ultraviolet C Radiation- and 6-Hydroxydopamine-Induced Neurodegeneration. *Int. J. Mol. Sci.* 20. doi:10.3390/ijms20133202

Hashimoto, M., Rockenstein, E., Crews, L., and Masliah, E. (2003). Role of Protein Aggregation in Mitochondrial Dysfunction and Neurodegeneration in Alzheimer's and Parkinson's Diseases. *Nmm* 4, 21–36. doi:10.1385/nmm:4:1-2:21

Hashimoto, Y., Ookuma, S., and Nishida, E. (2009). Lifespan Extension by Suppression of Autophagy Genes inCaenorhabditis Elegans. *Genes. cells.* 14, 717–726. doi:10.1111/j.1365-2443.2009.01306.x

Hazra, T., Das, A., Das, S., Choudhury, S., Kow, Y., and Roy, R. (2007). Oxidative DNA Damage Repair in Mammalian Cells: a New Perspective. *DNA Repair* 6, 470–480. doi:10.1016/j.dnarep.2006.10.011

Hoch, N. C., Hanzlikova, H., Hanzlikova, H., Rulten, S. L., Tétreault, M., Komulainen, E., et al. (2017). XRCC1 Mutation Is Associated with PARP1 Hyperactivation and Cerebellar Ataxia. *Nature* 541, 87–91. doi:10.1038/nature20790

Holmberg, C. I., Staniszewski, K. E., Mensah, K. N., Matouschek, A., and Morimoto, R. I. (2004). Inefficient Degradation of Truncated Polyglutamine Proteins by the Proteasome. *Embo J.* 23, 4307–4318. doi:10.1038/sj.emboj.7600426

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Géloën, A., Even, P. C., et al. (2003). IGF-1 Receptor Regulates Lifespan and Resistance to Oxidative Stress in Mice. *Nature* 421, 182–187. doi:10.1038/nature01298

Hoshino, A., Mita, Y., Okawa, Y., Ariyoshi, M., Iwai-Kanai, E., Ueyama, T., et al. (2013). Cytosolic P53 Inhibits Parkin-Mediated Mitophagy and Promotes Mitochondrial Dysfunction in the Mouse Heart. *Nat. Commun.* 4, 2308. doi:10.1038/ncomms3308

Hosono, R. (1978). Age Dependent Changes in the Behavior of *Caenorhabditis elegans* on Attraction to *Escherichia coli*. *Exp. Gerontol.* 13, 31–36. doi:10.1016/0531-5565(78)90027-x

Hosono, R., Sato, Y., Aizawa, S.-I., and Mitsui, Y. (1980). Age-dependent Changes in Mobility and Separation of the Nematode *Caenorhabditis elegans*. *Exp. Gerontol.* 15, 285–289. doi:10.1016/0531-5565(80)90032-7

Hostettler, L., Grundy, L., Käser-Pébernard, S., Wicky, C., Schafer, W. R., and Glauser, D. A. (2017). The Bright Fluorescent Protein mNeonGreen Facilitates Protein Expression Analysis *In Vivo*. *G3 (Bethesda)* 7, 607–615. doi:10.1534/g3.116.038133

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., et al. (2019). Ageing as a Risk Factor for Neurodegenerative Disease. *Nat. Rev. Neurol.* 15, 565–581. doi:10.1038/s41582-019-0244-7

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

Hsu, T. Y., Zhang, B., L'Etoile, N. D., and Juang, B. T. (2021). *C. elegans* Orthologs MUT-7/CeWRN-1 of Werner Syndrome Protein Regulate Neuronal Plasticity. *Elife* 10. doi:10.7554/eLife.62449

Hwangbo, D. S., Gersham, B., Tu, M.-P., Palmer, M., and Tatar, M. (2004). *Drosophila* dFOXO Controls Lifespan and Regulates Insulin Signalling in Brain and Fat Body. *Nature* 429, 562–566. doi:10.1038/nature02549

Hyun, M., Lee, J., Lee, K., May, A., Bohr, V. A., and Ahn, B. (2008). Longevity and Resistance to Stress Correlate with DNA Repair Capacity in *Caenorhabditis elegans*. *Nucleic Acids Res.* 36, 1380–1389. doi:10.1093/nar/gkm1161

Imanikia, S., Özbey, N. P., Krueger, C., Casanueva, M. O., and Taylor, R. C. (2019). Neuronal XBP-1 Activates Intestinal Lysosomes to Improve Proteostasis in *C. elegans*. *Curr. Biol.* 29, 2322–2338. e7. doi:10.1016/j.cub.2019.06.031

Jablonski, A. M., Lamitina, T., Liachko, N. F., Sabatella, M., Lu, J., Zhang, L., et al. (2015). Loss of RAD-23 Protects against Models of Motor Neuron Disease by Enhancing Mutant Protein Clearance. *J. Neurosci.* 35, 14286–14306. doi:10.1523/jneurosci.0642-15.2015

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., and Charpentier, E. (2012). A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science* 337, 816–821. doi:10.1126/science.1225829

Joeng, K. S., Song, E. J., Lee, K.-J., and Lee, J. (2004). Long Lifespan in Worms with Long Telomeric DNA. *Nat. Genet.* 36, 607–611. doi:10.1038/ng1356

Johnson, T. E. (1987). Aging Can Be Genetically Dissected into Component Processes Using Long-Lived Lines of *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 84, 3777–3781. doi:10.1073/pnas.84.11.3777

Jung, H., Kim, S. Y., Canbakis Cecen, F. S., Cho, Y., and Kwon, S.-K. (2020). Dysfunction of Mitochondrial Ca2+ Regulatory Machineries in Brain Aging and Neurodegenerative Diseases. *Front. Cell. Dev. Biol.* 8, 599792. doi:10.3389/fcell.2020.599792

Kamath, R. S., Fraser, A. G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., et al. (2003). Systematic Functional Analysis of the *Caenorhabditis elegans* Genome Using RNAi. *Nature* 421, 231–237. doi:10.1038/nature01278

Kassahun, H., Sengupta, T., Schiavi, A., Maglioni, S., Skjeldam, H. K., Arczewska, K., et al. (2018). Constitutive MAP-Kinase Activation Suppresses Germline Apoptosis in NTH-1 DNA Glycosylase Deficient *C. elegans*. *DNA Repair* 61, 46–55. doi:10.1016/j.dnarep.2017.11.009

Kato, Y., Moriwaki, T., Funakoshi, M., and Zhang-Akiyama, Q.-M. (2015). *Caenorhabditis elegans* EXO-3 Contributes to Longevity and Reproduction: Differential Roles in Somatic Cells and Germ Cells. *Mutat. Research/Fundamental Mol. Mech. Mutagen.* 772, 46–54. doi:10.1016/j.mrfmmm.2015.01.001

Keijzers, G., Bakula, D., and Scheibye-Knudsen, M. (2017). Monogenic Diseases of DNA Repair. *N. Engl. J. Med.* 377, 1868–1876. doi:10.1056/nejmra1703366

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

Kern, C. C., Townsend, S., Salzmann, A., Rendell, N. B., Taylor, G. W., Comisel, R. M., et al. (2021). *C. elegans* Feed Yolk to Their Young in a Form of Primitive Lactation. *Nat. Commun.* 12, 5801. doi:10.1038/s41467-021-25821-y

Kim, D.-K., Cho, K.-W., Ahn, W. J., Acuña, D., Jeong, H., Lee, H.-J., et al. (2017). Cell-to-cell Transmission of Polyglutamine Aggregates in *C. elegans*. *Exp. Neurobiol.* 26, 321–328. doi:10.5607/en.2017.26.6.321

Kimble, J., and Seidel, H. (2008). "*C. elegans* Germline Stem Cells and Their Niche." in *StemBook*. Cambridge (MA): Harvard Stem Cell Institute. Copyright: © 2013 Judith Kimble and Hannah Seidel.

Kimura, K. D., Tissenbaum, H. A., Liu, Y., and Ruvkun, G. (1997). daf-2 , an Insulin Receptor-like Gene that Regulates Longevity and Diapause in *Caenorhabditis elegans*. *Science* 277, 942–946. doi:10.1126/science.277.5328.942

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. (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

Konstantinidis, G., and Tavernarakis, N. (2021). Molecular Basis of Neuronal Autophagy in Ageing: Insights from *Caenorhabditis elegans*. *Cells* 10. doi:10.3390/cells10030694

Koopman, M., Michels, H., Dancy, B. M., Kamble, R., Mouchiroud, L., Auwerx, J., et al. (2016). A Screening-Based Platform for the Assessment of Cellular Respiration in *Caenorhabditis elegans*. *Nat. Protoc.* 11, 1798–1816. doi:10.1038/nprot.2016.106

Kremer, B., Goldberg, P., Andrew, S. E., Theilmann, J., Telenius, H., Zeisler, J., et al. (1994). A Worldwide Study of the Huntington's Disease Mutation: The Sensitivity and Specificity of Measuring CAG Repeats. *N. Engl. J. Med.* 330, 1401–1406. doi:10.1056/nejm199405193302001

Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., et al. (1998). Ala30Pro Mutation in the Gene Encoding Alpha-Synuclein in Parkinson's Disease. *Nat. Genet.* 18, 106–108.

Kuwahara, T., Koyama, A., Gengyo-Ando, K., Masuda, M., Kowa, H., Tsunoda, M., et al. (2006). Familial Parkinson Mutant α-Synuclein Causes Dopamine Neuron Dysfunction in Transgenic *Caenorhabditis elegans*. *J. Biol. Chem.* 281, 334–340. doi:10.1074/jbc.m504860200

Kuwahara, T., Koyama, A., Koyama, S., Yoshina, S., Ren, C.-H., Kato, T., et al. (2008). A Systematic RNAi Screen Reveals Involvement of Endocytic Pathway in Neuronal Dysfunction in α-synuclein Transgenic C . Elegans. C. elegans. *Hum. Mol. Genet.* 17, 2997–3009. doi:10.1093/hmg/ddn198

Labbadia, J., and Morimoto, R. I. (2015). The Biology of Proteostasis in Aging and Disease. *Annu. Rev. Biochem.* 84, 435–464. doi:10.1146/annurev-biochem-060614-033955

Lakso, M., Vartiainen, S., Moilanen, A. M., Sirviö, J., Thomas, J. H., Nass, R., et al. (2003). Dopaminergic Neuronal Loss and Motor Deficits in *Caenorhabditis elegans* Overexpressing Human Alpha-Synuclein. *J. Neurochem.* 86, 165–172. doi:10.1046/j.1471-4159.2003.01809.x

Lans, H., and Vermeulen, W. (2011). Nucleotide Excision Repair in *Caenorhabditis elegans*. *Mol. Biol. Int.* 2011, 542795. doi:10.4061/2011/542795

Lans, H., Lindvall, J. M., Thijssen, K., Karambelas, A. E., Cupac, D., Fensgård, Ø., et al. (2013). DNA Damage Leads to Progressive Replicative Decline but Extends the Life Span of Long-Lived Mutant Animals. *Cell. Death Differ.* 20, 1709–1718. doi:10.1038/cdd.2013.126

Lans, H., Marteijn, J. A., Schumacher, B., Hoeijmakers, J. H. J., Jansen, G., and Vermeulen, W. (2010). Involvement of Global Genome Repair, Transcription Coupled Repair, and Chromatin Remodeling in UV DNA Damage Response Changes during Development. *PLoS Genet.* 6, e1000941. doi:10.1371/journal.pgen.1000941

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

Lautrup, S., Sinclair, D. A., Mattson, M. P., and Fang, E. F. (2019). NAD+ in Brain Aging and Neurodegenerative Disorders. *Cell. Metab.* 30, 630–655. doi:10.1016/j.cmet.2019.09.001

Lee, H. J., Noormohammadi, A., Koyuncu, S., Calculli, G., Simic, M. S., Herholz, M., et al. (2019). Prostaglandin Signals from Adult Germline Stem Cells Delay Somatic Ageing of *Caenorhabditis elegans*. *Nat. Metab.* 1, 790–810. doi:10.1038/s42255-019-0097-9

Lehrbach, N. J., and Ruvkun, G. (2019). Endoplasmic Reticulum-Associated SKN-1A/Nrf1 Mediates a Cytoplasmic Unfolded Protein Response and Promotes Longevity. *Elife* 8. doi:10.7554/eLife.44425

Lemmens, B. B. L. G., and Tijsterman, M. (2011). DNA Double-Strand Break Repair in *Caenorhabditis elegans*. *Chromosoma* 120, 1–21. doi:10.1007/s00412-010-0296-3

Li, C., and Casanueva, O. (2016). Epigenetic Inheritance of Proteostasis and Ageing. *Essays Biochem.* 60, 191–202. doi:10.1042/ebc20160025

Liachko, N. F., Mcmillan, P. J., Guthrie, C. R., Bird, T. D., Leverenz, J. B., and Kraemer, B. C. (2013). CDC7 Inhibition Blocks Pathological TDP-43 Phosphorylation and Neurodegeneration. *Ann. Neurol.* 74, 39–52. doi:10.1002/ana.23870

Lim, C.-S., Mian, I. S., Dernburg, A. F., and Campisi, J. (2001). *C. elegans* Clk-2, a Gene that Limits Life Span, Encodes a Telomere Length Regulator Similar to Yeast Telomere Binding Protein Tel2p. *Curr. Biol.* 11, 1706–1710. doi:10.1016/s0960-9822(01)00526-7

Lin, K., Dorman, J. B., Rodan, A., and Kenyon, C. (1997). daf-16 : An HNF-3/forkhead Family Member that Can Function to Double the Life-Span of *Caenorhabditis elegans*. *Science* 278, 1319–1322. doi:10.1126/science.278.5341.1319

Link, C. D. (2006). *C. elegans* Models of Age-Associated Neurodegenerative Diseases: Lessons from Transgenic Worm Models of Alzheimer's Disease. *Exp. Gerontol.* 41, 1007–1013. doi:10.1016/j.exger.2006.06.059

Link, C. D. (1995). Expression of Human Beta-Amyloid Peptide in Transgenic *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 92, 9368–9372. doi:10.1073/pnas.92.20.9368

Lionaki, E., Gkikas, I., Daskalaki, I., Ioannidi, M.-K., Klapa, M. I., and Tavernarakis, N. (2022). Mitochondrial Protein Import Determines Lifespan through Metabolic Reprogramming and De Novo Serine Biosynthesis. *Nat. Commun.* 13, 651. doi:10.1038/s41467-022-28272-1

Lopes, A. F. C., Bozek, K., Herholz, M., Trifunovic, A., Rieckher, M., and Schumacher, B. (2020). A *C. elegans* Model for Neurodegeneration in Cockayne Syndrome. *Nucleic Acids Res.* 48, 10973–10985. doi:10.1093/nar/gkaa795

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

Luz, A. L., Lagido, C., Hirschey, M. D., and Meyer, J. N. (2016). *In Vivo* Determination of Mitochondrial Function Using Luciferase-Expressing *Caenorhabditis elegans*: Contribution of Oxidative Phosphorylation, Glycolysis, and Fatty Acid Oxidation to Toxicant-Induced Dysfunction. *Curr. Protoc. Toxicol.* 69, 25–22. doi:10.1002/cptx.10

Ma, X., Zhan, G., Sleumer, M. C., Chen, S., Liu, W., Zhang, M. Q., et al. (2016). Analysis of *C. elegans* Muscle Transcriptome Using Trans-splicing-based RNA Tagging (SRT). *Nucleic Acids Res.* 44, e156. doi:10.1093/nar/gkw734

Machiela, E., Rudich, P. D., Traa, A., Anglas, U., Soo, S. K., Senchuk, M. M., et al. (2021). Targeting Mitochondrial Network Disorganization Is Protective in *C. elegans* Models of Huntington's Disease. *Aging Dis.* 12, 1753–1772. doi:10.14336/ad.2021.0404

Mackenzie, I. R., Rademakers, R., and Neumann, M. (2010). TDP-43 and FUS in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Lancet Neurology* 9, 995–1007. doi:10.1016/s1474-4422(10)70195-2

Marchal, I., and Tursun, B. (2021). Induced Neurons from Germ Cells in *Caenorhabditis elegans*. *Front. Neurosci.* 15, 771687. doi:10.3389/fnins.2021.771687

Markert, S. M., Skoruppa, M., Yu, B., Mulcahy, B., Zhen, M., Gao, S., et al. (2020). Overexpression of an ALS-Associated FUS Mutation in *C. elegans* Disrupts NMJ Morphology and Leads to Defective Neuromuscular Transmission. *Biol. Open* 9. doi:10.1242/bio.055129

Marteijn, J. A., Lans, H., Vermeulen, W., and Hoeijmakers, J. H. J. (2014). Understanding Nucleotide Excision Repair and its Roles in Cancer and Ageing. *Nat. Rev. Mol. Cell. Biol.* 15, 465–481. doi:10.1038/nrm3822

Martin-Herranz, D. E., Aref-Eshghi, E., Bonder, M. J., Stubbs, T. M., Choufani, S., Weksberg, R., et al. (2019). Screening for Genes that Accelerate the Epigenetic Aging Clock in Humans Reveals a Role for the H3K36 Methyltransferase NSD1. *Genome Biol.* 20, 146. doi:10.1186/s13059-019-1753-9

Mattson, M. P., Gleichmann, M., and Cheng, A. (2008). Mitochondria in Neuroplasticity and Neurological Disorders. *Neuron* 60, 748–766. doi:10.1016/j.neuron.2008.10.010

Maures, T. J., Greer, E. L., Hauswirth, A. G., and Brunet, A. (2011). The H3K27 Demethylase UTX-1 Regulates *C. elegans* Lifespan in a Germline-independent, Insulin-dependent Manner. *Aging Cell.* 10, 980–990. doi:10.1111/j.1474-9726.2011.00738.x

McColl, G., Roberts, B. R., Pukala, T. L., Kenche, V. B., Roberts, C. M., Link, C. D., et al. (2012). Utility of an Improved Model of Amyloid-Beta (Aβ1-42) Toxicity in *Caenorhabditis elegans* for Drug Screening for Alzheimer's Disease. *Mol. Neurodegener.* 7, 57. doi:10.1186/1750-1326-7-57

McKinnon, P. J. (2009). DNA Repair Deficiency and Neurological Disease. *Nat. Rev. Neurosci.* 10, 100–112. doi:10.1038/nrn2559

McKinnon, P. J. (2013). Maintaining Genome Stability in the Nervous System. *Nat. Neurosci.* 16, 1523–1529. doi:10.1038/nn.3537

Meier, B., Volkova, N. V., Hong, Y., Schofield, P., Campbell, P. J., Gerstung, M., et al. (2018). Mutational Signatures of DNA Mismatch Repair Deficiency in *C. elegans* and Human Cancers. *Genome Res.* 28, 666–675. doi:10.1101/gr.226845.117

Meléndez, A., Tallóczy, Z., Seaman, M., Eskelinen, E. L., Hall, D. H., and Levine, B. (2003). Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in *C. elegans*. *Science* 301, 1387–1391.

Mitra, J., Guerrero, E. N., Hegde, P. M., Liachko, N. F., Wang, H., Vasquez, V., et al. (2019). Motor Neuron Disease-Associated Loss of Nuclear TDP-43 Is Linked to DNA Double-Strand Break Repair Defects. *Proc. Natl. Acad. Sci. U.S.A.* 116, 4696–4705. doi:10.1073/pnas.1818415116

Molenkamp, J., Den Outer, A., van Schijndel, V., and Sinnige, T. (2021). Monitoring Protein Aggregation Kinetics *In Vivo* Using Automated Inclusion Counting in *Caenorhabditis elegans*. *J. Vis. Exp.*

Morinaga, H., Yonekura, S.-I., Nakamura, N., Sugiyama, H., Yonei, S., and Zhang-Akiyama, Q.-M. (2009). Purification and Characterization of *Caenorhabditis elegans* NTH, a Homolog of Human Endonuclease III: Essential Role of N-Terminal Region. *DNA Repair* 8, 844–851. doi:10.1016/j.dnarep.2009.04.020

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 inCaenorhabditis Elegansby Heat Shock Factor and Molecular Chaperones. *MBoC* 15, 657–664. doi:10.1091/mbc.e03-07-0532

Morris, J. Z., Tissenbaum, H. A., and Ruvkun, G. (1996). A Phosphatidylinositol-3-OH Kinase Family Member Regulating Longevity and Diapause in *Caenorhabditis elegans*. *Nature* 382, 536–539. doi:10.1038/382536a0

Muñoz-Lobato, F., Rodríguez-Palero, M. J., Naranjo-Galindo, F. J., Shephard, F., Gaffney, C. J., Szewczyk, N. J., et al. (2014). Protective Role of DNJ-27/ERdj5 in *Caenorhabditis elegans* Models of Human Neurodegenerative Diseases. *Antioxid. Redox Signal* 20, 217–235.

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, T., Yang, S.-P., Xie, L., Kawano, T., Fu, D., Mukai, A., et al. (2012). ALS Mutations in FUS Cause Neuronal Dysfunction and Death in *Caenorhabditis elegans* by a Dominant Gain-Of-Function Mechanism. *Hum. Mol. Genet.* 21, 1–9. doi:10.1093/hmg/ddr417

Nakamura, N., Morinaga, H., Kikuchi, M., Yonekura, S.-I., Ishii, N., Yamamoto, K., et al. (2008). Cloning and Characterization of Uracil-DNA Glycosylase and the Biological Consequences of the Loss of its Function in the Nematode *Caenorhabditis elegans*. *Mutagenesis* 23, 407–413. doi:10.1093/mutage/gen030

Nance, J., and Frøkjær-Jensen, C. (2019). The *Caenorhabditis elegans* Transgenic Toolbox. *Genetics* 212, 959–990. doi:10.1534/genetics.119.301506

Narendra, D. P., Jin, S. M., Tanaka, A., Suen, D.-F., Gautier, C. A., Shen, J., et al. (2010). PINK1 Is Selectively Stabilized on Impaired Mitochondria to Activate Parkin. *PLoS Biol.* 8, e1000298. doi:10.1371/journal.pbio.1000298

Narendra, D., Tanaka, A., Suen, D.-F., and Youle, R. J. (2008). Parkin Is Recruited Selectively to Impaired Mitochondria and Promotes Their Autophagy. *J. Cell. Biol.* 183, 795–803. doi:10.1083/jcb.200809125

Nass, R., Hall, D. H., Miller, D. M., 3Rd, and Blakely, R. D. (2002). Neurotoxin-induced Degeneration of Dopamine Neurons in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 99, 3264–3269. doi:10.1073/pnas.042497999

Nollen, E. A. A., Garcia, S. M., van Haaften, G., Kim, S., Chavez, A., Morimoto, R. I., et al. (2004). Genome-wide RNA Interference Screen Identifies Previously Undescribed Regulators of Polyglutamine Aggregation. *Proc. Natl. Acad. Sci. U.S.A.* 101, 6403–6408. doi:10.1073/pnas.0307697101

Oeda, T., Shimohama, S., Kitagawa, N., Kohno, R., Imura, T., Shibasaki, H., et al. (2001). Oxidative Stress Causes Abnormal Accumulation of Familial Amyotrophic Lateral Sclerosis-Related Mutant SOD1 in Transgenic *Caenorhabditis elegans*. *Hum. Mol. Genet.* 10, 2013–2023. doi:10.1093/hmg/10.19.2013

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A., et al. (1997). The Fork Head Transcription Factor DAF-16 Transduces Insulin-like Metabolic and Longevity Signals in *C. elegans*. *Nature* 389, 994–999. doi:10.1038/40194

Orr, A. L., Li, S., Wang, C.-E., Li, H., Wang, J., Rong, J., et al. (2008). N-terminal Mutant Huntingtin Associates with Mitochondria and Impairs Mitochondrial Trafficking. *J. Neurosci.* 28, 2783–2792. doi:10.1523/jneurosci.0106-08.2008

Oshima, J., Sidorova, J. M., and Monnat, R. J., JR. (2017). Werner Syndrome: Clinical Features, Pathogenesis and Potential Therapeutic Interventions. *Ageing Res. Rev.* 33, 105–114. doi:10.1016/j.arr.2016.03.002

Paix, A., Folkmann, A., Rasoloson, D., and Seydoux, G. (2015). High Efficiency, Homology-Directed Genome Editing in *Caenorhabditis elegans* Using CRISPR-Cas9 Ribonucleoprotein Complexes. *Genetics* 201, 47–54. doi:10.1534/genetics.115.179382

Palikaras, K., and Tavernarakis, N. (2016). Intracellular Assessment of ATP Levels in *Caenorhabditis elegans*. *Bio Protoc.* 6. doi:10.21769/BioProtoc.2048

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

Palikaras, K., Lionaki, E., and Tavernarakis, N. (2018). Mechanisms of Mitophagy in Cellular Homeostasis, Physiology and Pathology. *Nat. Cell. Biol.* 20, 1013–1022. doi:10.1038/s41556-018-0176-2

Palikaras, K., Sengupta, T., Nilsen, H., and Tavernarakis, N. (2022). Assessment of Dopaminergic Neuron Degeneration in a *C. elegans* Model of Parkinson's Disease. *Star. Protoc.* 3, 101264. doi:10.1016/j.xpro.2022.101264

Panov, A. V., Gutekunst, C.-A., Leavitt, B. R., Hayden, M. R., Burke, J. R., Strittmatter, W. J., et al. (2002). Early Mitochondrial Calcium Defects in Huntington's Disease Are a Direct Effect of Polyglutamines. *Nat. Neurosci.* 5, 731–736. doi:10.1038/nn884

Papaluca, A., Wagner, J. R., Saragovi, H. U., and Ramotar, D. (2018). UNG-1 and APN-1 Are the Major Enzymes to Efficiently Repair 5-hydroxymethyluracil DNA Lesions in *C. elegans*. *Sci. Rep.* 8, 6860. doi:10.1038/s41598-018-25124-1

Park, H.-E. H., Jung, Y., and Lee, S.-J. V. (2017). Survival Assays Using *Caenorhabditis elegans*. *Mol. Cells* 40, 90–99. doi:10.14348/molcells.2017.0017

Park, S., Choi, S., and Ahn, B. (2016). DNA Strand Breaks in Mitotic Germ Cells of *Caenorhabditis elegans* Evaluated by Comet Assay. *Mol. Cells* 39, 204–210. doi:10.14348/molcells.2016.2206

Park, S.-K., Tedesco, P. M., and Johnson, T. E. (2009). Oxidative Stress and Longevity in *Caenorhabditis elegans* as Mediated by SKN-1. *Aging Cell.* 8, 258–269. doi:10.1111/j.1474-9726.2009.00473.x

Parker, J. A., Connolly, J. B., Wellington, C., Hayden, M., Dausset, J., and Neri, C. (2001). Expanded Polyglutamines in *Caenorhabditis elegans* Cause Axonal Abnormalities and Severe Dysfunction of PLM Mechanosensory Neurons without Cell Death. *Proc. Natl. Acad. Sci. U.S.A.* 98, 13318–13323. doi:10.1073/pnas.231476398

Pearce, M. M. P., Spartz, E. J., Hong, W., Luo, L., and Kopito, R. R. (2015). Prion-like Transmission of Neuronal Huntingtin Aggregates to Phagocytic Glia in the Drosophila Brain. *Nat. Commun.* 6, 6768. doi:10.1038/ncomms7768

Pecho-Vrieseling, E., Rieker, C., Fuchs, S., Bleckmann, D., Esposito, M. S., Botta, P., et al. (2014). Transneuronal Propagation of Mutant Huntingtin Contributes to Non-cell Autonomous Pathology in Neurons. *Nat. Neurosci.* 17, 1064–1072. doi:10.1038/nn.3761

Peters, O. M., Ghasemi, M., and Brown, R. H., JR. (2015). Emerging Mechanisms of Molecular Pathology in ALS. *J. Clin. Investig.* 125, 1767–1779. doi:10.1172/jci71601

Pickles, S., Vigié, P., and Youle, R. J. (2018). Mitophagy and Quality Control Mechanisms in Mitochondrial Maintenance. *Curr. Biol.* 28, R170–r185. doi:10.1016/j.cub.2018.01.004

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., et al. (2017). Parkinson Disease. *Nat. Rev. Dis. Prim.* 3, 17013. doi:10.1038/nrdp.2017.13

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., et al. (1997). Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease. *Science* 276, 2045–2047. doi:10.1126/science.276.5321.2045

Ragagnin, A. M. G., Shadfar, S., Vidal, M., Jamali, M. S., and Atkin, J. D. (2019). Motor Neuron Susceptibility in ALS/FTD. *Front. Neurosci.* 13, 532. doi:10.3389/fnins.2019.00532

Ren, P.-H., Lauckner, J. E., Kachirskaia, I., Heuser, J. E., Melki, R., and Kopito, R. R. (2009). Cytoplasmic Penetration and Persistent Infection of Mammalian Cells by Polyglutamine Aggregates. *Nat. Cell. Biol.* 11, 219–225. doi:10.1038/ncb1830

Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., et al. (2011). A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. *Neuron* 72, 257–268. doi:10.1016/j.neuron.2011.09.010

Riddle, D. L., Swanson, M. M., and Albert, P. S. (1981). Interacting Genes in Nematode Dauer Larva Formation. *Nature* 290, 668–671. doi:10.1038/290668a0

Rieckher, M., Bujarrabal, A., Doll, M. A., Soltanmohammadi, N., and Schumacher, B. (2018). A Simple Answer to Complex Questions: *Caenorhabditis elegans* as an Experimental Model for Examining the DNA Damage Response and Disease Genes. *J. Cell. Physiol.* 233, 2781–2790. doi:10.1002/jcp.25979

Rieckher, M., Garinis, G. A., and Schumacher, B. (2021). Molecular Pathology of Rare Progeroid Diseases. *Trends Mol. Med.* 27, 907–922. doi:10.1016/j.molmed.2021.06.011

Rual, J.-F., Ceron, J., Koreth, J., Hao, T., Nicot, A.-S., Hirozane-Kishikawa, T., et al. (2004). Toward Improving *Caenorhabditis elegans* Phenome Mapping with an ORFeome-Based RNAi Library. *Genome Res.* 14, 2162–2168. doi:10.1101/gr.2505604

Rudich, P., Snoznik, C., Watkins, S. C., Monaghan, J., Pandey, U. B., and Lamitina, S. T. (2017). Nuclear Localized C9orf72-Associated Arginine-Containing Dipeptides Exhibit Age-dependent Toxicity in *C. elegans*. *Hum. Mol. Genet.* 26, 4916–4928. doi:10.1093/hmg/ddx372

Ruz, C., Alcantud, J. L., Vives Montero, F., Duran, R., and Bandres-Ciga, S. (2020). Proteotoxicity and Neurodegenerative Diseases. *Int. J. Mol. Sci.* 21. doi:10.3390/ijms21165646

Sabatella, M., Thijssen, K. L., Davó-Martínez, C., Vermeulen, W., and Lans, H. (2021). Tissue-Specific DNA Repair Activity of ERCC-1/XPF-1. *Cell. Rep.* 34, 108608. doi:10.1016/j.celrep.2020.108608

Sae-Lee, W., Scott, L. L., Brose, L., Encarnacion, A. J., Shi, T., Kore, P., et al. (2020). APP-induced Patterned Neurodegeneration Is Exacerbated by APOE4 in *Caenorhabditis elegans*. *G3 (Bethesda)* 10, 2851–2861. doi:10.1534/g3.120.401486

Saldi, T. K., Gonzales, P., Garrido-Lecca, A., Dostal, V., Roberts, C. M., Petrucelli, L., et al. (2018). The *Caenorhabditis elegans* Ortholog of TDP-43 Regulates the Chromatin Localization of the Heterochromatin Protein 1 Homolog HPL-2. *Mol. Cell. Biol.* 38. doi:10.1128/MCB.00668-17

Saldi, T. K., Ash, P. E., Wilson, G., Gonzales, P., Garrido-Lecca, A., Roberts, C. M., et al. (2014). TDP -1, the C Aenorhabditis Elegans Ortholog of TDP -43, Limits the Accumulation of Double-stranded RNA. *Embo J.* 33, 2947–2966. doi:10.15252/embj.201488740

Santra, M., Dill, K. A., and de Graff, A. M. R. (2019). Proteostasis Collapse Is a Driver of Cell Aging and Death. *Proc. Natl. Acad. Sci. U.S.A.* 116, 22173–22178. doi:10.1073/pnas.1906592116

Satyal, S. H., Schmidt, E., Kitagawa, K., Sondheimer, N., Lindquist, S., Kramer, J. M., et al. (2000). Polyglutamine Aggregates Alter Protein Folding Homeostasis in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 97, 5750–5755. doi:10.1073/pnas.100107297

Saudou, F., and Humbert, S. (2016). The Biology of Huntingtin. *Neuron* 89, 910–926. doi:10.1016/j.neuron.2016.02.003

Sawin, E. R., Ranganathan, R., and Horvitz, H. R. (2000). *C. elegans* Locomotory Rate Is Modulated by the Environment through a Dopaminergic Pathway and by Experience through a Serotonergic Pathway. *Neuron* 26, 619–631. doi:10.1016/s0896-6273(00)81199-x

Schapira, A. H. V. (2011). Mitochondrial Pathology in Parkinson's Disease. *Mt. Sinai J. Med.* 78, 872–881. doi:10.1002/msj.20303

Scheibye-Knudsen, M., Mitchell, S. J., Fang, E. F., Iyama, T., Ward, T., Wang, J., et al. (2014). A High-Fat Diet and NAD + Activate Sirt1 to Rescue Premature Aging in Cockayne Syndrome. *Cell. Metab.* 20, 840–855. doi:10.1016/j.cmet.2014.10.005

Schiavi, A., Maglioni, S., Palikaras, K., Shaik, A., Strappazzon, F., Brinkmann, V., et al. (2015). Iron-Starvation-Induced Mitophagy Mediates Lifespan Extension upon Mitochondrial Stress in *C. elegans*. *Curr. Biol.* 25, 1810–1822. doi:10.1016/j.cub.2015.05.059

Schöndorf, D. C., Ivanyuk, D., Baden, P., Sanchez-Martinez, A., de Cicco, S., Yu, C., et al. (2018). The NAD+ Precursor Nicotinamide Riboside Rescues

Mitochondrial Defects and Neuronal Loss in iPSC and Fly Models of Parkinson's Disease. *Cell. Rep.* 23, 2976–2988. doi:10.1016/j.celrep.2018.05.009

Senchuk, M. M., Dues, D. J., and van Raamsdonk, J. M. (2017). Measuring Oxidative Stress in *Caenorhabditis elegans*: Paraquat and Juglone Sensitivity Assays. *Bio Protoc.* 7, 2086. doi:10.21769/BioProtoc.2086

SenGupta, T., Palikaras, K., Esbensen, Y. Q., Konstantinidis, G., Galindo, F. J. N., Achanta, K., et al. (2021). Base Excision Repair Causes Age-dependent Accumulation of Single-Stranded DNA Breaks that Contribute to Parkinson Disease Pathology. *Cell. Rep.* 36, 109668. doi:10.1016/j.celrep.2021.109668

SenGupta, T., Torgersen, M. L., Kassahun, H., Vellai, T., Simonsen, A., and Nilsen, H. (2013). Base Excision Repair AP Endonucleases and Mismatch Repair Act Together to Induce Checkpoint-Mediated Autophagy. *Nat. Commun.* 4, 2674. doi:10.1038/ncomms3674

Shaner, N. C., Lambert, G. G., Chammas, A., Ni, Y., Cranfill, P. J., Baird, M. A., et al. (2013). A Bright Monomeric Green Fluorescent Protein Derived from *Branchiostoma lanceolatum*. *Nat. Methods* 10, 407–409. doi:10.1038/nmeth.2413

Shiloh, Y., and Lederman, H. M. (2017). Ataxia-telangiectasia (A-T): An Emerging Dimension of Premature Ageing. *Ageing Res. Rev.* 33, 76–88. doi:10.1016/j.arr.2016.05.002

Shiloh, Y., and Ziv, Y. (2013). The ATM Protein Kinase: Regulating the Cellular Response to Genotoxic Stress, and More. *Nat. Rev. Mol. Cell. Biol.* 14, 197–210. doi:10.1038/nrm3546

Shin, G., Koo, H. J., Seo, M., Lee, S.-J. V., Nam, H. G., and Jung, G. Y. (2021). Transfer RNA-Derived Fragments in Aging *Caenorhabditis elegans* Originate from Abundant Homologous Gene Copies. *Sci. Rep.* 11, 12304. doi:10.1038/s41598-021-91724-z

Silva, M. C., Fox, S., Beam, M., Thakkar, H., Amaral, M. D., and Morimoto, R. I. (2011). A Genetic Screening Strategy Identifies Novel Regulators of the Proteostasis Network. *PLoS Genet.* 7, e1002438. doi:10.1371/journal.pgen.1002438

Skjeldam, H. K., Kassahun, H., Fensgård, Ø., Sengupta, T., Babaie, E., Lindvall, J. M., et al. (2010). Loss of *Caenorhabditis elegans* UNG-1 Uracil-DNA Glycosylase Affects Apoptosis in Response to DNA Damaging Agents. *DNA Repair* 9, 861–870. doi:10.1016/j.dnarep.2010.04.009

Spencer, W. C., Mcwhirter, R., Miller, T., Strasbourger, P., Thompson, O., Hillier, L. W., et al. (2014). Isolation of Specific Neurons from *C. elegans* Larvae for Gene Expression Profiling. *PLoS One* 9, e112102. doi:10.1371/journal.pone.0112102

Strang, K. H., Golde, T. E., and Giasson, B. I. (2019). MAPT Mutations, Tauopathy, and Mechanisms of Neurodegeneration. *Lab. Investig.* 99, 912–928. doi:10.1038/s41374-019-0197-x

Suetomi, K., Mereiter, S., Mori, C., Takanami, T., and Higashitani, A. (2013). *Caenorhabditis elegans* ATR Checkpoint Kinase ATL-1 Influences Life Span through Mitochondrial Maintenance. *Mitochondrion* 13, 729–735. doi:10.1016/j.mito.2013.02.004

Suh, Y., Atzmon, G., Cho, M.-O., Hwang, D., Liu, B., Leahy, D. J., et al. (2008). Functionally Significant Insulin-like Growth Factor I Receptor Mutations in Centenarians. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3438–3442. doi:10.1073/pnas.0705467105

Sulston, J., Dew, M., and Brenner, S. (1975). Dopaminergic Neurons in the nematodeCaenorhabditis Elegans. *J. Comp. Neurol.* 163, 215–226. doi:10.1002/cne.901630207

Sulston, J. E., and Horvitz, H. R. (1977). Post-embryonic Cell Lineages of the Nematode, *Caenorhabditis elegans*. *Dev. Biol.* 56, 110–156. doi:10.1016/0012-1606(77)90158-0

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

Sun, N., Yun, J., Liu, J., Malide, D., Liu, C., Rovira, I. I., et al. (2015). Measuring *In Vivo* Mitophagy. *Mol. Cell.* 60, 685–696. doi:10.1016/j.molcel.2015.10.009

Tabrizi, S. J., Flower, M. D., Ross, C. A., and Wild, E. J. (2020). Huntington Disease: New Insights into Molecular Pathogenesis and Therapeutic Opportunities. *Nat. Rev. Neurol.* 16, 529–546. doi:10.1038/s41582-020-0389-4

Tatar, M., Kopelman, A., Epstein, D., Tu, M.-P., Yin, C.-M., and Garofalo, R. S. (2001). A Mutant *Drosophila* Insulin Receptor Homolog that Extends Life-Span and Impairs Neuroendocrine Function. *Science* 292, 107–110. doi:10.1126/science.1057987

Taylor, S. R., Santpere, G., Weinreb, A., Barrett, A., Reilly, M. B., Xu, C., et al. (2021). Molecular Topography of an Entire Nervous System. *Cell.* 184, 4329–4347. e23. doi:10.1016/j.cell.2021.06.023

Therrien, M., Rouleau, G. A., Dion, P. A., and Parker, J. A. (2013). Deletion of C9ORF72 Results in Motor Neuron Degeneration and Stress Sensitivity in *C. elegans*. *PLoS One* 8, e83450. doi:10.1371/journal.pone.0083450

Tijsterman, M., Pothof, J., and Plasterk, R. H. A. (2002). Frequent Germline Mutations and Somatic Repeat Instability in DNA Mismatch-Repair-Deficient *Caenorhabditis elegans*. *Genetics* 161, 651–660. doi:10.1093/genetics/161.2.651

Timmons, L., Court, D. L., and Fire, A. (2001). Ingestion of Bacterially Expressed dsRNAs Can Produce Specific and Potent Genetic Interference in *Caenorhabditis elegans*. *Gene* 263, 103–112. doi:10.1016/s0378-1119(00)00579-5

Tissenbaum, H. A., and Ruvkun, G. (1998). An Insulin-like Signaling Pathway Affects Both Longevity and Reproduction in *Caenorhabditis elegans*. *Genetics* 148, 703–717. doi:10.1093/genetics/148.2.703

Tissenbaum, H. A. (2015). UsingC. Elegansfor Aging Research. *Invertebr. Reproduction Dev.* 59, 59–63. doi:10.1080/07924259.2014.940470

Todd, A. M., and Staveley, B. E. (2012). Expression of Pink1 with α-synuclein in the Dopaminergic Neurons of Drosophila Leads to Increases in Both Lifespan and Healthspan. *Genet. Mol. Res.* 11, 1497–1502. doi:10.4238/2012.may.21.6

Tursun, B., Patel, T., Kratsios, P., and Hobert, O. (2011). Direct Conversion of *C. elegans* Germ Cells into Specific Neuron Types. *Science* 331, 304–308. doi:10.1126/science.1199082

Vaccaro, A., Tauffenberger, A., Aggad, D., Rouleau, G., Drapeau, P., and Parker, J. A. (2012a). Mutant TDP-43 and FUS Cause Age-dependent Paralysis and Neurodegeneration in *C. elegans*. *PLoS One* 7, e31321. doi:10.1371/journal.pone.0031321

Vaccaro, A., Tauffenberger, A., Ash, P. E. A., Carlomagno, Y., Petrucelli, L., and Parker, J. A. (2012b). TDP-1/TDP-43 Regulates Stress Signaling and Age-dependent Proteotoxicity in *Caenorhabditis elegans*. *PLoS Genet.* 8, e1002806. doi:10.1371/journal.pgen.1002806

Van Bael, S., Zels, S., Boonen, K., Beets, I., Schoofs, L., and Temmerman, L. (2018). A *Caenorhabditis elegans* Mass Spectrometric Resource for Neuropeptidomics. *J. Am. Soc. Mass Spectrom.* 29, 879–889. doi:10.1007/s13361-017-1856-z

van der Rijt, S., Molenaars, M., Mcintyre, R. L., Janssens, G. E., and Houtkooper, R. H. (2020). Integrating the Hallmarks of Aging throughout the Tree of Life: A Focus on Mitochondrial Dysfunction. *Front. Cell. Dev. Biol.* 8, 594416. doi:10.3389/fcell.2020.594416

van Ham, T. J., Thijssen, K. L., Breitling, R., Hofstra, R. M. W., Plasterk, R. H. A., and Nollen, E. A. A. (2008). *C. elegans* Model Identifies Genetic Modifiers of α-Synuclein Inclusion Formation during Aging. *PLoS Genet.* 4, e1000027. doi:10.1371/journal.pgen.1000027

Van Zundert, B., Izaurieta, P., Fritz, E., and Alvarez, F. J. (2012). Early Pathogenesis in the Adult-onset Neurodegenerative Disease Amyotrophic Lateral Sclerosis. *J. Cell. Biochem.* 113, 3301–3312. doi:10.1002/jcb.24234

Vanfleteren, J. R. (1993). Oxidative Stress and Ageing in *Caenorhabditis elegans*. *Biochem. J.* 292 (Pt 2), 605–608. doi:10.1042/bj2920605

Vaziriyan-Sani, A. S., Handy, R. D., Walker, A. C., Pagolu, C. N., Enslow, S. M., and Czyż, D. M. (2021). Automating Aggregate Quantification in *Caenorhabditis elegans*. *J. Vis. Exp.*

Vekrellis, K., Xilouri, M., Emmanouilidou, E., Rideout, H. J., and Stefanis, L. (2011). Pathological Roles of α-synuclein in Neurological Disorders. *Lancet Neurology* 10, 1015–1025. doi:10.1016/s1474-4422(11)70213-7

Vermezovic, J., Stergiou, L., Hengartner, M. O., and d'Adda di Fagagna, F. (2012). Differential Regulation of DNA Damage Response Activation between Somatic and Germline Cells in *Caenorhabditis elegans*. *Cell. Death Differ.* 19, 1847–1855. doi:10.1038/cdd.2012.69

Voisine, C., Pedersen, J. S., and Morimoto, R. I. (2010). Chaperone Networks: Tipping the Balance in Protein Folding Diseases. *Neurobiol. Dis.* 40, 12–20. doi:10.1016/j.nbd.2010.05.007

Voisine, C., Varma, H., Walker, N., Bates, E. A., Stockwell, B. R., and Hart, A. C. (2007). Identification of Potential Therapeutic Drugs for Huntington's Disease Using *Caenorhabditis elegans*. *PLoS One* 2, e504. doi:10.1371/journal.pone.0000504

Vowels, J. J., and Thomas, J. H. (1992). Genetic Analysis of Chemosensory Control of Dauer Formation in *Caenorhabditis elegans*. *Genetics* 130, 105–123. doi:10.1093/genetics/130.1.105

Wang, J., Farr, G. W., Hall, D. H., Li, F., Furtak, K., Dreier, L., et al. (2009). An ALS-Linked Mutant SOD1 Produces a Locomotor Defect Associated with Aggregation and Synaptic Dysfunction when Expressed in Neurons of *Caenorhabditis elegans*. *PLoS Genet.* 5, e1000350. doi:10.1371/journal.pgen.1000350

Ward, J. D. (2015). Rapid and Precise Engineering of the *Caenorhabditis elegans* Genome with Lethal Mutation Co-conversion and Inactivation of NHEJ Repair. *Genetics* 199, 363–377. doi:10.1534/genetics.114.172361

White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. (1986). The Structure of the Nervous System of the Nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 314, 1–340. doi:10.1098/rstb.1986.0056

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

Winston, W. M., Molodowitch, C., and Hunter, C. P. (2002). Systemic RNAi in *C. elegans* Requires the Putative Transmembrane Protein SID-1. *Science* 295, 2456–2459. doi:10.1126/science.1068836

Wintle, R. F., and Van Tol, H. H. M. (2001). Dopamine Signaling in *Caenorhabditis Elegans*-Potential for Parkinsonism Research. *Park. Relat. Disord.* 7, 177–183. doi:10.1016/s1353-8020(00)00055-9

Witan, H., Kern, A., Koziollek-Drechsler, I., Wade, R., Behl, C., and Clement, A. M. (2008). Heterodimer Formation of Wild-type and Amyotrophic Lateral Sclerosis-Causing Mutant Cu/Zn-Superoxide Dismutase Induces Toxicity Independent of Protein Aggregation. *Hum. Mol. Genet.* 17, 1373–1385. doi:10.1093/hmg/ddn025

Wu, Q., Cao, X., Yan, D., Wang, D., and Aballay, A. (2015). Genetic Screen Reveals Link between the Maternal Effect Sterile Gene Mes-1 and Pseudomonas Aeruginosa-Induced Neurodegeneration in *Caenorhabditis elegans*. *J. Biol. Chem.* 290, 29231–29239. doi:10.1074/jbc.m115.674259

Xie, C., Zhuang, X.-X., Niu, Z., Ai, R., Lautrup, S., Zheng, S., et al. (2022). Amelioration of Alzheimer's Disease Pathology by Mitophagy Inducers Identified via Machine Learning and a Cross-Species Workflow. *Nat. Biomed. Eng.* 6, 76–93. doi:10.1038/s41551-021-00819-5

Yang, X., Fan, J., Ishchenko, A. A., Patel, D., Saparbaev, M. K., and Ramotar, D. (2012). Functional Characterization of the *Caenorhabditis elegans* DNA Repair Enzyme APN-1. *DNA Repair* 11, 811–822. doi:10.1016/j.dnarep.2012.06.009

Yemini, E., Lin, A., Nejatbakhsh, A., Varol, E., Sun, R., Mena, G. E., et al. (2021). NeuroPAL: A Multicolor Atlas for Whole-Brain Neuronal Identification in *C. elegans*. *Cell.* 184, 272–288. e11. doi:10.1016/j.cell.2020.12.012

Zakaria, C., Kassahun, H., Yang, X., Labbé, J. C., Nilsen, H., and Ramotar, D. (2010). *Caenorhabditis elegans* APN-1 Plays a Vital Role in Maintaining Genome Stability. *DNA Repair* 9, 169–176. doi:10.1016/j.dnarep.2009.11.007

Zarei, S., Carr, K., Reiley, L., Diaz, K., Guerra, O., Altamirano, P., et al. (2015). A Comprehensive Review of Amyotrophic Lateral Sclerosis. *Surg. Neurol. Int.* 6, 171. doi:10.4103/2152-7806.169561

Zevian, S. C., and Yanowitz, J. L. (2014). Methodological Considerations for Heat Shock of the Nematode *Caenorhabditis elegans*. *Methods* 68, 450–457. doi:10.1016/j.ymeth.2014.04.015

Zhang, H., Gao, S., and Chen, W. (2022). Automated Recognition and Analysis of Head Thrashes Behavior in *C. elegans*. *BMC Bioinforma.* 23, 87. doi:10.1186/s12859-022-04622-0

Zhang, T., Hwang, H.-Y., Hao, H., Talbot, C., JR., and Wang, J. (2012). *Caenorhabditis elegans* RNA-Processing Protein TDP-1 Regulates Protein Homeostasis and Life Span. *J. Biol. Chem.* 287, 8371–8382. doi:10.1074/jbc.m111.311977

Zhang, T., Wu, Y.-C., Mullane, P., Ji, Y. J., Liu, H., He, L., et al. (2018). FUS Regulates Activity of MicroRNA-Mediated Gene Silencing. *Mol. Cell.* 69, 787–801. e8. doi:10.1016/j.molcel.2018.02.001

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REVIEW
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HSF-1: Guardian of the Proteome Through Integration of Longevity Signals to the Proteostatic Network

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Discoveries made in the nematode *Caenorhabditis elegans* revealed that aging is under genetic control. Since these transformative initial studies, *C. elegans* has become a premier model system for aging research. Critically, the genes, pathways, and processes that have fundamental roles in organismal aging are deeply conserved throughout evolution. This conservation has led to a wealth of knowledge regarding both the processes that influence aging and the identification of molecular and cellular hallmarks that play a causative role in the physiological decline of organisms. One key feature of age-associated decline is the failure of mechanisms that maintain proper function of the proteome (proteostasis). Here we highlight components of the proteostatic network that act to maintain the proteome and how this network integrates into major longevity signaling pathways. We focus in depth on the heat shock transcription factor 1 (HSF1), the central regulator of gene expression for proteins that maintain the cytosolic and nuclear proteomes, and a key effector of longevity signals.

Keywords: aging, proteostasis, cell stress and aging, HSF-1 = heat-shock factor-1, longevity, *Caenorhabditis elegans* (*C. elegans*), genetics

OVERVIEW OF THE GENETICS OF LONGEVITY IN *C. ELEGANS*

Regulation of Insulin-Like Signaling Determines Metazoan Aging

Perhaps the most profound discovery in the last 30 years of aging research was the discovery in *Caenorhabditis elegans* that aging is under genetic control. Single mutations found in *age-1* and *daf-2* were the first genetic evidence that longevity is influenced by regulated processes (Friedman and Johnson 1988; Johnson 1990; Kenyon et al., 1993). Shortly after this discovery, genetic and molecular analysis revealed that *age*-1 *and daf*-2 function as a part of a conserved insulin-like signaling (ILS) pathway (Dorman et al., 1995). A seminal discovery in *C. elegans* was that the ILS promotes aging by repressing the FOXO transcription factor DAF-16 in wild-type animals (Lin et al., 1997; Ogg et al., 1997). This had major implications for the understanding of diabetes and cancer as it linked a specific transcription factor to mammalian insulin and insulin growth factor (IGF-1) for the first time [for a synopsis of this period of discovery see (Kenyon 2011)]. A large body of work has since demonstrated that this core signaling pathway influences aging across metazoan animals (Holzenberger et al., 2003; Kenyon 2005; Suh et al., 2008).

Early discoveries in the genetics of longevity using *C. elegans* were founded on prior genetic insight into dauer formation (Riddle et al., 1981). Developing *C. elegans* proceed through for larval stages (L1–L4), marked by molts before becoming reproductive adults (Cassada and Russell 1975; Felix and Duveau 2012; Frezal and Felix 2015; Lazetic and Fay 2017). At the L2 to L3 transition, *C. elegans* can enter an alternative L3 developmental stage called dauer, which is a genetic program of developmental diapause, allowing animals to survive food scarcity, overcrowding, or a number of harsh environmental conditions and survive for up to 4 months (Golden and Riddle 1984a; Butcher et al., 2007; Fielenbach and Antebi 2008; Felix and Duveau 2012). Genetic epistasis between mutations that favored constitutive dauer formation under normal conditions, with mutations that rendered animals unable to enter dauer (dauer defective), delineated not only the pathways that regulated dauer development but also informed early subsequent discoveries into the genetics of longevity, which occurred prior to knowing the molecular identity of pathway components (Vowels and Thomas 1992; Kenyon et al., 1993; Gottlieb and Ruvkun 1994; Dorman et al., 1995; Larsen et al., 1995; Gems et al., 1998; Fielenbach and Antebi 2008). It is worth noting that the mechanisms regulating dauer entry and longevity are separable; for example, *C. elegans* with temperature sensitive *daf-2* mutations grown at the permissive temperature bypass the dauer checkpoint and develop into normal adulthood, shifting to higher non-permissive temperatures reduces ILS and extends longevity in these adult animals (Golden and Riddle 1984b; Ailion and Thomas 2000; Ailion and Thomas 2003). This genetic analysis revealed two parallel genetic pathways that converge to regulate dauer development, which we now know are the ILS and TGF-β signaling pathways (Ren et al., 1996; Kimura et al., 1997).

Early studies to identify the Daf genes revealed that organismal longevity was determined via canonical ILS signaling. DAF-2 (insulin/IGF1 receptor) activates AGE-1 (PI3K) to generate phosphatidylinositol-3,4,5-triphosphate (PIP_3) (Morris et al., 1996; Kimura et al., 1997), which is opposed by DAF-18 (PTEN, Phosphatase And Tensin Homolog) (Ogg and Ruvkun 1998). Accumulation of PIP_3 activates PDK-1 (PDPK1, 3-phosphoinositide-dependent protein kinase-1), which activates AKT-1/2 (AKT Serine/Threonine Kinase, protein kinase B) via phosphorylation (Paradis et al., 1999). In turn activated AKT-1/2 directly inhibits DAF-16 (FOXO) through phosphorylation and sequestration in the cytosol (Paradis and Ruvkun 1998; Lee et al., 2001; Lin et al., 2001). Mutations within this pathway that decreased ILS lead to activation of DAF-16 and increased longevity (Kenyon et al., 1993; Lin et al., 1997; Ogg et al., 1997). Conversely, mutations that promote ILS, such as loss of *daf-18*, decrease lifespan (Larsen et al., 1995; Ogg and Ruvkun 1998).

Early genetic evidence suggested that TGF-β signaling regulated dauer formation but not lifespan (Larsen et al., 1995). However, it was later discovered that the longevity-regulating activity of the TGF-β pathway was masked by an egg-laying (Egl) phenotype that caused mortality from internal hatching of progeny; suppressing the latter by preventing production of progeny revealed that mutations within the TGF-β pathway doubled lifespan and induced transcriptional changes that overlapped with many DAF-16 regulated genes (Shaw et al., 2007). Molecular genetic analysis of Daf genes revealed an endocrine network that converges within steroidogenic tissues to promote production of a cholesterol derived ligand (dafachronic acid) of DAF-12, which encodes a nuclear hormone receptor orthologous to vertebrate farnesoid-X, liver-X and vitamin D-receptors [reviewed in (Antebi 2013)]. Significantly, many of the endocrine pathways that regulate dauer and longevity are evolutionarily conserved (Mooijaart et al., 2007; Keisala et al., 2009; Kenyon 2010; Tennessen and Thummel 2011).

One unresolved question is the *insulin paradox* in humans. Defects in insulin receptor signaling causes insulin resistance and diabetes. Deficiencies in IGF-1 or upstream growth hormone (GH) are associated with increased incidence of cardiovascular disease and atherosclerosis. Yet, polymorphisms in many components of insulin or IGF-1 pathways that decrease signaling is associated with improved longevity and found in centenarian populations (IGF-1R, PI3K, INSR, FOXO3). Furthermore, centenarian populations are associated with improved insulin sensitivity, low-serum IGF-1, and a mutation in the insulin receptor has been found in semi-supercentenarians (>105 years) [reviewed in (Arai et al., 2009; Calvo-Ochoa and Arias 2015)]. Early characterization of *C. elegans* with difference alleles of *daf-2,* all of which increase lifespan, noted different degrees of an effect on motility, stress resistance, morphology, development, reproductive lifespan and brood size (Gems et al., 1998). Mutations within the ligand binding domain tended to have less pleiotropies in contrast to mutations within the kinase domain of DAF-2, which suggested DAF-2 kinase activity has at least separable outputs on organismal physiology (i.e. longevity and other pleotropic effects) (Gems et al., 1998). Whether different alleles of *daf-2* differentially impact the molecular and cellular hallmarks of aging (discussed below) remain unexplored.

One possible explanation of the insulin paradox is that there is an optimal reduction in ILS to increase longevity, and reduction below this rate results in metabolic syndromes and premature aging (Cohen and Dillin 2008). Consistent with that possibility, null-mutations of *daf-2* and *age-1* in *C. elegans* result in lethal constitutive dauer formation (Malone and Thomas 1994; Dorman et al., 1995; Larsen et al., 1995; Gems et al., 1998) and several *daf-2* mutations are temperature sensitive loss of function: small increases in lifespan are observed at lower permissive temperatures and greater increases when temperature is increased (Gems et al., 1998). However, *age-1* null mutant animals raised at lower temperature can bypass dauer and eventually develop into adults that have near normal feeding rates, motility, and remarkably live up to 145–190 days, which is 10-times longer than wild-type animals (Ayyadevara et al., 2008). This suggests that the insulin paradox cannot be solved based simply on levels of ILS. Possible explanations to the insulin paradox that are not mutually exclusive include: differences between insulin and IGF-1 signaling, tissue- or cell-type specific effects, background mutations, the nature of mutation, and timing of alterations in ILS.

An Emerging Theme: Genes and Pathways Linked to Metabolic Control Determine Aging

Since the discovery that ILS regulates longevity, a common theme has emerged: the evolutionarily conserved genes and pathways that have the largest impact on lifespan often act in nutrient and energy sensing. For instance, a key controller of nutrient sensing is the target of Rapamycin (TOR) response to decreased levels of amino acids and carbohydrates (Kapahi et al., 2010). Under nutrient-rich conditions, TOR promotes cellular growth by simultaneously activating protein translation (*e.g.,* transcription of translation components) while inhibiting protein turnover (*e.g.,* transcription of chaperone and autophagy genes (McCormick et al., 2011; Seo et al., 2013; Lapierre et al., 2015)), and by inhibiting the initiation of autophagy. TOR inhibition, or activation of targets of TOR inhibition, results in extension of longevity (Jia et al., 2004; Hansen et al., 2008). Similarly, AMP-activated protein kinase (AMPK) acts as a conserved energy sensor of increased levels of AMP and ADP. Energy-stress activation of AMPK induces autophagy, the oxidative stress response (OSR), and extends longevity (Apfeld et al., 2004; Greer et al., 2007; Greer et al., 2009; Salminen and Kaarniranta 2012). Sirtuins (SIRT1-7 in mammals, *sir-2.1* and *sir-2.4* in *C. elegans*) also play a key role in nutrient sensing and extension of longevity (Jedrusik-Bode et al., 2013; Jedrusik-Bode 2014). Sirtuins are (NAD+)-dependent deacetylases, which sense levels of NAD+, an important metabolite linked to longevity (Verdin 2015). Sirtuin-mediated extension of longevity has been linked to ILS, AMPK, and TOR signaling, and sirtuins are essential for both dietary restriction (DR) and exercise to increase lifespan (Dai et al., 2018). From these and many additional studies two conclusions become self-evident: aging is under genetic control and these mechanisms have been deeply conserved throughout evolution.

Why would nutrient sensing, the abundance of key metabolites and energy currency, be causally linked to genetic programs that determine organismal longevity? It is tempting to speculate that very early in evolution organisms able to couple physiology to energy resources had a survival advantage. Under conditions of plentiful resources, organisms able to develop and reproduce quickly could dominate an ecological niche. However, when food is scarce, organisms able to conserve or recycle resources and delay energetically-costly physiological processes. such as development and reproduction, in favor of mechanisms that protect the Soma, would have a survival advantage. Delaying the production of offspring has the added benefit of limiting competition for limited resources. Consistent with this hypothesis, in *C. elegans* many long-lived mutant animals have one or more of the following characteristics: slower development, links to dauer formation (a form of developmental diapause), reduced numbers of overall progeny, and/or an extended period of progeny production (i.e., reproductive span) (Szewczyk et al., 2006; Mukhopadhyay and Tissenbaum 2007). In fact, loss of the *C. elegans* germline through mutation of *glp-1*, which encodes an ortholog to the Notch receptor, increases lifespan by preventing germ cell development in early adulthood, which also requires DAF-16, implying a connection to ILS (Arantes-Oliveira et al., 2002).

Refined genetic analysis has revealed that many of the phenotypes associated with reproduction or development are separable from longevity. For example, early discoveries in *C. elegans* aging research using temperature-sensitive alleles in the ILS pathway revealed that dauer formation and extended longevity were genetically separable (Kenyon et al., 1993), implying that strategies to improve healthy aging based on the genetics of longevity may not require a cost in developmental or reproductive fitness.

The aforementioned longevity signals converge on a limited number of transcription factors, which also respond to numerous additional stress signals. For example, *skn-1* encodes the *C. elegans* ortholog of the nuclear factor erythroid 2-related factor 2 (Nrf2), a member of the "Cap'n'Collar" basic leucine zipper family of transcription factors, which is best known for regulating the expression of the OSR (An and Blackwell 2003). However, specific splice isoforms of *skn-1* play key roles in the endoplasmic reticulum (ER) unfolded protein response (ER-UPR), maintaining proteostasis in the cytosol, and the response to starvation (Glover-Cutter et al., 2013; Lehrbach and Ruvkun 2016; Denzel et al., 2019; Lehrbach and Ruvkun 2019). DR activates SKN-1 within two head neurons (ASI) and is essential for increased longevity and cell non-autonomous changes in metabolic activity within peripheral tissues (Bishop and Guarente 2007). Additionally, amino acid and carbohydrate starvation activate *skn-1* through TOR signaling (Robida-Stubbs et al., 2012), and reduced ILS activates SKN-1 in conjunction with DAF-16 (Tullet et al., 2008). Additional evidence of signal convergence is AMPK phosphorylation and activation of DAF-16, after a distinct method of DR (Greer et al., 2007; Greer et al., 2009). Furthermore *pha-4,* which encodes the FOXA forkhead transcription factor is critical for lifespan extension phenotypes related to germline inhibition and DR, but not reduced ILS, through regulation of autophagy (Panowski et al., 2007; Lapierre et al., 2011). One of the key transcriptional effectors of longevity signaling is the heat shock transcription factor (HSF-1 in *C. elegans*, HSF1 in more complex metazoans), which we discuss in detail in the latter part of this review. Collectively, a growing number of *C. elegans* studies have begun to unravel the complex integrated networks that maintain organismal homeostasis from an extensive array of diverse extrinsic and intrinsic signals that converge on distinct but overlapping adaptive transcriptional responses (Greer and Brunet 2009; Denzel et al., 2019).

Longevity is Determined *via* Cell Non-Autonomous Signals

A strength of *C. elegans* as a model is the relative ease in achieving tissue- and cell-type specific genetic perturbation. Overexpression or rescue is easily achieved through the use of either tissue specific promoters (in wild-type or mutant backgrounds) or through mosaic analysis (Yochem et al., 2000; Yochem and Herman 2003; Meister et al., 2010; Prelich 2012). Spatial and temporal gene inactivation can be achieved classically through the use of

tissue specific RNAi (e.g., tissue specific expression of *rde-1* in RNAi-deficient *rde-1* mutant animals) (Qadota et al., 2007; Miles and van Oosten-Hawle 2020; Watts et al., 2020) or more recently with the development of the Tir1-auxin system, which provides spatial and temporally controlled protein degradation (Zhang et al., 2015). Collectively, these approaches allowed early efforts in the emerging field of aging research identify the tissues where a longevity gene or pathway functioned. These studies raised two possibilities: 1) longevity functions restricted to a specific tissue are regions with a metazoan that decline cell-intrinsically during aging and ultimately result in death, or 2) the longevity functions within a specific tissue generate cell non-autonomous paracrine or endocrine signals that orchestrate cellular aging across tissues. The latter view has become widely accepted and is based on numerous studies ranging from early observations to ongoing discoveries. Distinct tissues which influence aging throughout the Soma through endocrine signals include neurons, the somatic gonad, the germ line/gonadal stem cells, and intestinal cells (Kleemann and Murphy 2009). Examples of some of the longevity signaling pathways that act cell non-autonomously through endocrine signaling include ILS, bile acid signaling, TGF-β signaling, serotonin signaling, pregnenolone signaling, TORC1 signaling, and AMPK signaling (Kleemann and Murphy 2009; Ulgherait et al., 2014; Zhang et al., 2019). Signaling is likely to occur through lipophilic hormones (Hansen et al., 2005). These signals are distinct, for example: alterations in gonadal stem cell signaling communicates cell non-autonomously with somatic intestinal cells through lipophilic hormone signaling and *kri*-1 (ortholog of human KRIT1) to activate DAF-16. This cell non-autonomous mechanism is specific to germline to Soma signaling, as these functions are not required for ILS-mediated longevity or DAF-16 activation (Berman and Kenyon 2006). Similarly, endocrine germline signals and DR differentially regulate protein quality control mechanisms (Shpigel et al., 2019). For a recent review on cell non-autonomous signaling in longevity see (Miller et al., 2020).

Hallmarks of Molecular and Cellular Aging

Many molecular and cellular hallmarks of aging have been discovered, which have been broadly classified as: altered intercellular communication, genomic instability, telomere attrition, epigenomic alterations, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and dysfunction, and decline of protein homeostasis (proteostasis) (Lopez-Otin et al., 2013). While many of the mechanisms that alter aging impact multiple hallmarks, and similarly these hallmarks impact one another; in this review we focus primarily on proteostasis through an in depth discussion of the mechanisms that preserve proper function and folding of the cytosolic and nuclear proteomes, how these mechanisms intersect with the aforementioned longevity signals, and give special emphasis to the key transcription factor that acts as the guardian of the nuclear and cytosolic proteome: HSF1. We provide a detailed analysis of discoveries in mammals and *C. elegans,* and highlight areas of future investigation where iterative analysis between systems would provide deeper mechanistic insight to how proteostasis preserves the functional integrity of complex metazoans.

INHERENT CHALLENGES TO MAINTAINING PROTEOSTASIS

A challenge inherent to proteostasis is ensuring all proteins are properly folded *de novo* into a native conformation and maintained in a soluble state, despite overall protein concentrations approaching levels found in crystals (Powers et al., 2009). This is further amplified by the varied nature of protein size, amino acid composition, structural conformation, stability, turnover, and expression (Wolff et al., 2014). Thus, maintaining proteostasis is a generalized problem, not unique to a subset of proteins. Layered within this complexity is the need for proteins to be localized within the proper cellular compartment, to be maintained in correct stoichiometric ratios relative to other components of larger protein complexes, to undergo the correct modifications in response to a diverse array of internal and extrinsic cues, and to be maintained within a proteome with a composition unique to each cell type or tissue. To put it simply, the proper function and folding of the proteome is a complex and dynamic process, vital for maintaining cellular function.

OVERVIEW OF THE PROTEOSTATIC NETWORK

Proteostasis is maintained through the coordinated action of a large proteostatic network (PN), consisting of approximately 2,000 unique proteins, which regulate the synthesis, folding, trafficking, and degradation within the proteome [reviewed in (Morimoto 2008; Wolff et al., 2014; Hipp et al., 2019; Morimoto 2019)]. This network regulates *de novo* protein folding from the emergence of the nascent polypeptide from the ribosome to the final subcellular localization of the mature protein. The PN responds to acute stress on the proteome, and fine-tunes the rates of translation and degradation in response to a myriad of cell intrinsic and extrinsic cues. The major components are several large families of molecular chaperones, co-chaperones, and protein clearance mechanisms, predominately degradation through the ubiquitin-proteasome system (UPS) and autophagy at the lysosome (**Figure 1**).

DECLINING PROTEOSTASIS DURING AGING

Decline of Cellular Proteostasis in *C. elegans*

Key discoveries in *C. elegans* first revealed that declining proteostasis is a hallmark of aging, and provided a generalized cellular explanation for the manifestation and progression of neurodegenerative disease. The decline of cellular proteostasis is a hallmark of aging across species [reviewed in (Rubinsztein et al., 2011; Taylor and Dillin 2011; Labbadia and Morimoto 2015a; Klaips et al., 2018; Hipp et al., 2019)]. For instance, even in the absence of overt disease, there is a growing body of evidence demonstrating that the loss of autophagy and declining levels of molecular chaperones are conserved hallmarks of aging (Ben-Zvi et al., 2009; Morimoto and Cuervo 2009; Vellai et al.,

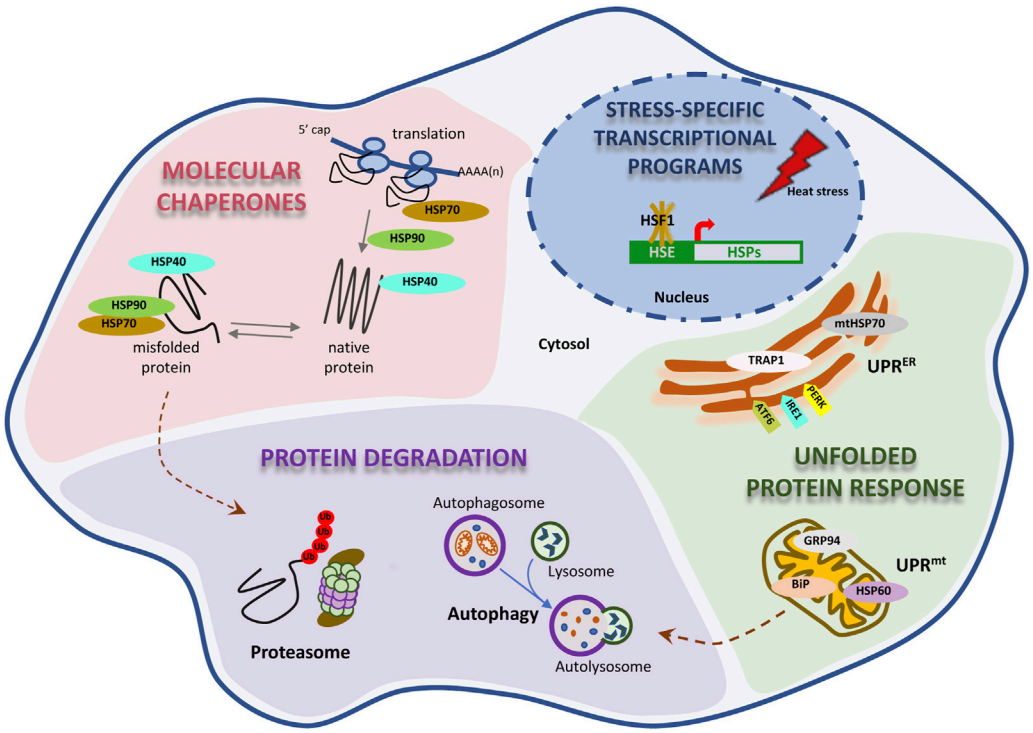

FIGURE 1 | Proteostasis is maintain by a proteostasis network. Proteostasis has multiple levels of regulation. A great number of molecular chaperones are present in the cytosol, assisting on the appropriate folding and quality of the proteome. The proteosome and autophagy protein degradation pathways are essential for the clearance of unneeded proteins. Two distinct, but analogous, unfolded protein responses in the mitochondria and endoplasmic reticulum (mitoUPR and ER-UPR, respectively) are necessary to activate the expression of chaperones and to protect the function of these organelles from unfolding stress. Ultimately, the PN network is equipped with the adaptive activation of different transcriptional programs that get induced by different types of stress.

2009). The use of transgenic *C. elegans* expressing polyglutamine fused to a fluorescent reporter remains a powerful system to identify age-associated declines in proteostasis *in vivo* (Satyal et al., 2000; Brignull et al., 2006; Morimoto 2006; Morimoto 2008). Early studies demonstrated that co-expression of both a proteotoxic polyQ reporter and endogenous metastable proteins further exacerbates protein misfolding and degenerative phenotypes, consistent with the notion that prolonged proteotoxic stress overloads the limited buffering capacity of the PN (Gidalevitz et al., 2006). Widespread failures in protein folding have since been shown to occur in early adulthood across tissues and coincides with reduced activation of HSF-1 and chaperone protein expression (Ben-Zvi et al., 2009). In *C. elegans,* the transcriptional inducibility of multiple forms of stress response rapidly decline within a few hours after the onset of reproduction due to the formation of repressive chromatin marks at stress loci (Shemesh et al., 2013; Labbadia and Morimoto 2015a; Labbadia and Morimoto 2015b). During normal aging, solubility of the *C. elegans* proteome declines, resulting in an accumulation of aggregates (David et al., 2010; Reis-Rodrigues et al., 2012; Walther et al., 2015). Additionally, during aging, there is an increase in misfolded and oxidatively-damaged proteins, especially in neurons (Powers et al., 2009; Kundra et al., 2017; Sala et al., 2017).

The age-associated collapse of proteostasis is also intrinsic to human senescence. During aging of higher metazoans, cells can enter a permanent form of G1 arrest commonly referred to as cellular senescence [reviewed in (McHugh and Gil 2018; Prata et al., 2018; Song et al., 2020)]. Cellular senescence is a genetic program (Serrano et al., 1997), and a key hallmark of aging, which can be induced by various age-associated damage drivers including: telomere damage, epigenetic dysregulation, DNA damage, and mitochondrial dysfunction. Accumulating senescence cells play a key role in age-associated diseases by promoting stem cell exhaustion, chronic inflammation, disruption of nutrient signaling, and proteostatic dysfunction (McHugh and Gil 2018; Prata et al., 2018; Borghesan et al., 2020; Song et al., 2020). The age-associated collapse of proteostatic networks observed in *C. elegans* also occurs in human senescent cells. For example, within senescent cells the transcriptional activation of the heat shock response deteriorates, activation of HSF1 is impaired, UPR-related transcriptional responses are impaired, and the proteosome is dysfunctional (Sabath et al., 2020).

COMPONENTS OF THE PROTEOSTATIC NETWORK AND LINKS TO LONGEVITY

Molecular Chaperones

The "workhorse" components of the PN are the molecular chaperones, which assist in all aspects of proteostasis both under normal conditions and in response to challenge from intrinsic and extrinsic factors. Chaperones are tightly

regulated; their relative abundance is closely linked to the rest of the proteome, with a limited buffering capacity (Gidalevitz et al., 2011). Thus, the consequence of proteotoxic stress is overload upon the chaperone system, resulting in protein misfolding. In the context of proteostatic disease, aggregate formation and toxic gain-of-function disruption of normal cellular physiology occurs and ultimately results in cell death if unresolved (Balch et al., 2008).

As key components of the PN, there are numerous molecular chaperones with diverse regulatory roles in maintaining proper function and folding of the proteome [for excellent reviews see (Richter et al., 2010; Balchin et al., 2016; Hipp et al., 2019)]. An informatic analysis identified 332 human genes encoding chaperones and co-chaperones (Brehme et al., 2014), which fall into nine families: HSP90, HSP70, HSP60, HSP40, prefoldin, small heat shock protein (sHSP), TPR-domain containing (Hartl and Hayer-Hartl 2002), and organellar-specific chaperones of the endoplasmic reticulum (Kleizen and Braakman 2004) and mitochondria (Tatsuta et al., 2005). The number of paralogous genes within a family can be extensive: members may be essential or dispensable for viability, act in specific contexts (e.g., *de novo* protein folding or refolding misfolded proteins), be expressed constitutively or regulated *via* specific signals, be expressed in specific cell types, or be localized in specific subcellular regions (Richter et al., 2010; Balchin et al., 2016). For example, cytosolic HSP70 paralogs differ in that heat shock cognate protein *HSC70* is constitutively expressed, while in contrast expression *HSP70* expression is induced after heat shock. The HSP90s and HSP70s are highly abundant chaperones in the cytosol and nucleus (Taipale et al., 2010; Labbadia and Morimoto 2015a). Paralogs, such as BiP and GRP94 are essential for ER function, while the paralogs mortalin and TRAP1 function in the mitochondria. HSP90s, HSP70s, HSP60s, and sHSPs mediate *de novo* protein folding of nascent polypeptides, a process directly coupled to translation, acting alone or in conjunction with co-chaperones. In response to stress, other HSP70 and HSP90 family members are induced to resolve protein misfolding. Small molecular chaperones are somewhat distinct, as they interact reversibly with a broad range of unfolded substrates independent of ATP. sHSPs do not primarily refold proteins, rather they prevent the formation of highly stable, proteotoxic aggregates, acting as a storage depot for unfolded proteins until they can be refolded or degraded [for detailed reviews on the chaperone system see (Bar-Lavan et al., 2016; Biebl and Buchner 2019; Rosenzweig et al., 2019; Jayaraj et al., 2020; Reinle et al., 2022)].

Molecular chaperones are central to the proper regulation of protein homeostasis in aging cells (Labbadia and Morimoto 2015a; Margulis et al., 2020). Induction of molecular chaperones in response to stress is essential for normal development, and is significantly reduced during aging (Lopez-Otin et al., 2013; Dues et al., 2016; Sabath et al., 2020). In addition to regulating proteostasis, molecular chaperones and co-chaperones have been implicated in *C. elegans* longevity (Hsu et al., 2003; Morley and Morimoto 2004); direct manipulation of chaperone levels can alter *C. elegans* lifespan. For example, loss of HSP90 shortens lifespan (Somogyvari et al., 2018), while overexpression of HSP16 or HSP70 increases lifespan (Lithgow et al., 1995; Tatar et al., 1997; Yokoyama et al., 2002; Walker and Lithgow 2003). Molecular chaperone levels of expression are also regulated by longevity pathways. For instance, ILS regulates molecular chaperone expression, as the long-lived *daf-2* and *age-1* mutant animals have increased expression levels (Hsu et al., 2003; McElwee et al., 2003; Murphy et al., 2003; Walker and Lithgow 2003; Morley and Morimoto 2004; Halaschek-Wiener et al., 2005; Lamitina and Strange 2005; Wentz et al., 2018). Similarly, inactivation of TORC1 (e.g., *daf-15* encodes the *C. elegans* ortholog of Raptor) or downstream S6 kinase (*rsks*-1) is sufficient to induce chaperone expression and interestingly sHSPs, but *hsp70*s are not essential for the increased lifespan of *rsks-1* mutant animals (Seo et al., 2013). Chaperone functions also feedback to longevity signaling pathways. For example, the HSP90 family member DAF-21 directly regulates nuclear localization and transcriptional activity of the DAF-16A isoform (Somogyvari et al., 2018). Chaperones also coordinate adaptive transcriptional responses to changes in longevity signals. For instance, prefoldin 6 (*pfd-6*) encodes a chaperone, which under conditions of decreased ILS integrates HSF-1 and DAF-16 transcriptional activity (Son et al., 2018). These studies highlight a few examples of increasing evidence that molecular chaperones are not merely effectors of mechanisms to maintain the proteome through changes in protein folding, degradation and aggregation. Rather, molecular chaperones play key roles in connecting the PN with longevity signals and pathways.

Ubiquitin Proteasome System and Autophagy

Protein degradation is a fundamental mechanism for maintaining proteostasis. Turnover of unfolded polypeptides *via* proteasome- and autophagy-mediated degradation pathways are additional components of the PN. These effector mechanisms not only safeguard *de novo* protein quality control but are continually adjusted in response to stress from both internal cues and endocrine signals through transcriptional regulators of the PN.

The UPS is the main protein degradation system within the cell and is an integral part of the PN, assisting by clearing misfolded or toxic proteins. The proteasome is composed by a 19S regulatory cap and a 20S proteolytic core (Finley 2009). The ubiquitinated substrate attaches to the 19S regulatory cap via ubiquitin receptors to be translocated to the 20S core where it is hydrolyzed, effectively degrading it. Substrates targeted to the proteasome are tagged by polyubiquitin chains by a series of steps of E1, E2, and E3 ligases. Targets for degradation are redirected to the proteasome through interaction with co-chaperones, including the C-terminus of HSC70-interacting protein (CHIP) and Bcl2-associated athanogene 1 (BAG1), in conjunction with HSP70 and HSP90 complexes (Connell et al., 2001). When proteome stability cannot be maintained by protein degradation through the UPS, such as after heat shock, the accumulation of misfolded proteins is alleviated through increased autophagy. Thus, the major degradation mechanisms also function as a part of an integrated system.

A second major degradation system is mediated *via* the lysosome. The process of macroautophagy (hereafter referred to as autophagy), chaperone-mediated autophagy, and microautophagy are clearance mechanisms for a growing number of substrates, including proteins, aggregates, damaged organelles, nucleic acids, and pathogens (Rabinowitz and White 2010; Tooze and Yoshimori 2010; Workman and van Montfort 2010; Mizushima 2011; Benbrook and Long 2012; Pyo et al., 2012; Khaminets et al., 2015; Khaminets et al., 2016). Like the ubiquitin-dependent proteasome pathway, autophagy is tightly regulated (He and Klionsky 2009; Kroemer et al., 2010). Autophagosomes, which are double-membrane vesicles, form during autophagy to sequester substrates for degradation. These loaded autophagosomes then fuse to lysosomes to form autolysosomes, and substrates are degraded by lysosomal hydrolytic activity. The activation of autophagy is important to protect cells against multiple stressors such as heat shock and nutrient deprivation (Kroemer et al., 2010), protecting the organism from diseases associated with degeneration, infections, and inflammation, among others (Levine and Kroemer 2008; Mizushima et al., 2008; Aman et al., 2021; Kaushik et al., 2021; Nieto-Torres and Hansen 2021).

The UPS is essential for normal aging in *C. elegans* (Papaevgeniou and Chondrogianni 2016; Margulis et al., 2020; Ottens et al., 2021). The ubiquitin E3 ligase CHIP promotes longevity through ILS by regulating insulin receptor turnover (Tawo et al., 2017). Additionally, the CUL-1 E3 ligase complex regulates DAF-16 transcriptional activity (Ghazi et al., 2007). Another ubiquitin E3 ligase, RLE-1, targets DAF-16 for polyubiquitination-mediated degradation (Li et al., 2007). The proteasome itself also plays a key role in aging. Multiple studies have shown that loss of proteasome subunits lead to premature aging (Yun et al., 2008; Chondrogianni et al., 2015). Upregulated proteasomal activity is observed in the long-lived *glp-1* mutant animals and in DR models (Vilchez et al., 2012; Depuydt et al., 2013). The role of deubiquitination enzymes (DUBs) has also been implied in proteasome activity and aging in *C. elegans* (Papaevgeniou and Chondrogianni 2016). Repression of *ubh-4* (*C. elegans* DUB gene) by DAF-16 induces proteasome activity (Matilainen et al., 2013).

Lysosomal proteolytic activity deteriorates with aging (Sarkis et al., 1988; Kaushik et al., 2021). In *C. elegans*, many studies have reported the direct link between autophagy and longevity [e.g. (Melendez et al., 2003; Hansen et al., 2008; Chang et al., 2017), and many more]. Mutational inactivation of autophagy genes (*unc-51, bec-1, atg-18, atg-9, lgg-1*) shortens *C. elegans* lifespan (Toth et al., 2008). Many autophagy genes contribute to longevity paradigms; *Bec-1, unc-51, lgg-1,* and *atg-18* are crucial for lifespan extension through the ILS, TOR signaling, or under conditions of DR (Melendez et al., 2003; Jia and Levine 2007; Hansen et al., 2008; Kenyon 2010). Activation of the AMPK pathway to promote longevity is also autophagy-dependent (Egan et al., 2011; Mihaylova and Shaw 2011). Furthermore, some autophagy receptors promote autophagy-dependent proteostasis and longevity in a tissue specific manner (Kumsta et al., 2017).

The transcriptional regulation of autophagy genes affects longevity and some transcription factors extend lifespan or delay aging in an autophagy-dependent manner in *C. elegans* (Lapierre et al., 2015; Minnerly et al., 2017; Liu et al., 2020; Kaushik et al., 2021). For example, HLH-30/TFEB regulates multiple autophagy genes (*atg-18, vha-16, lmp-1, lipl-1, lipl-3*) and is required for lifespan extension in *glp-1, eat-2, daf-2, clk-1, rsks-1,* and TOR mutants, indicating roles in multiple longevity paradigms (Lapierre et al., 2013). HLH-30 is also implicated in lipid metabolism to promote longevity (Lapierre et al., 2011). PHA-4 regulates autophagy gene expression and is required to extend lifespan (Panowski et al., 2007; Sheaffer et al., 2008; Zhong et al., 2010; Lapierre et al., 2011). DAF-16 also regulates autophagy gene expression to extend longevity (Wang et al., 2008; McColl et al., 2010; Lapierre et al., 2015; Kaushik et al., 2021). The Myc-Mondo:Mlx transcriptional activation complex and the Mad:Max transcriptional repression complex links autophagy to longevity through ILS, DR, and germline signaling (Johnson et al., 2014; Nakamura et al., 2016). The homeodomain interacting protein kinase (*hpk-1*) is a transcriptional co-factor and nuclear kinase that regulates longevity and preserves proteostasis, at least in part, through an essential role in the induction of autophagy in response to inhibition of TOR or under conditions of DR (Das et al., 2017). How these transcription factors regulate autophagy under stress conditions (i.e., diverse metabolic or non-metabolic stressors), across cell types (either cell-intrinsically or cell non-autonomously), or compensate and coordinate specific types of autophagy, are all areas for future investigation.

Transcriptional Regulation of the Proteostatic Network

Vital components of the PN are adaptive transcriptional responses activated in response to acute or chronic damage to the proteome, as well as in response to metabolic and mitogenic signals. The most well-characterized adaptive transcriptional responses to proteotoxic stress are the HSR, OSR, mitochondrial unfolded protein response (mitoUPR), and ER-UPR. The HSR, ER-UPR, and mitoUPR are induced in response to proteotoxic stress within the cytosol/nucleus, ER, and mitochondria, respectively. Regulation of these adaptive transcriptional programs are critical aspects of the larger PN that act in concordance with each other and additional protein quality control components. There is growing evidence for crosstalk and compensatory mechanisms among these adaptive responses. Furthermore, each has cell-intrinsic and non-autonomous components [reviewed in (Dillin et al., 2014; Taylor et al., 2014; van Oosten-Hawle and Morimoto 2014; Morimoto 2019)]. Below, we focus on HSF1, which maintains proper function of the cytoplasmic and nuclear proteomes. Breakdown of the OSR, mitoUPR, and ER-UPR components of the PN have direct ties to cancer, neurodegenerative disease, and aging, but are beyond the scope of this review [see (Ryan and Hoogenraad 2007; Sykiotis et al., 2011; Wolff and Dillin 2013; Dufey et al., 2014; Jovaisaite et al., 2014; Mottis et al., 2014; Wolff et al., 2014; Karagoz et al., 2019a; Karagoz et al., 2019b; Preissler

and Ron 2019) for detailed reviews on the OSR, mitoUPR and ER-UPR]. For a comprehensive review of transcriptional and epigenetic regulation of stress response in *C. elegans* longevity, see (Denzel et al., 2019).

Tissue-Specific Utilization of the PN

While core components of the chaperone system are uniformly expressed across tissues, each cell type preferentially utilizes specific subsets of molecular chaperones, presumably in alignment with the demands of a proteome that is unique to each particular cell type [(Shemesh et al., 2021), and reviewed in (Labbadia and Morimoto 2015a; Sala et al., 2017)]. For example, the proteomes of cells within the pancreas and muscle significantly differ; the former have elevated expression of secreted proteins, while the latter are enriched in mitochondrial localized proteins (compared to the mean expression in all tissues) (Sala et al., 2017). Thus, it is not surprising that ER-specific chaperones are the major class of chaperones expressed in the secretory tissues of the pancreas, small intestine, and liver. In contrast, sHSPs are overrepresented in skeletal and cardiac muscle, consistent with their role in maintaining the folding of filament components. In accordance with their key role in proteome maintenance, the proportion of HSP70, HSP40, and HSP90 chaperones are relatively constant across tissues (Taipale et al., 2010; Guisbert et al., 2013; Sala et al., 2017; Nisaa and Ben-Zvi 2021). Nevertheless, specific members within these families, as well as other PN components, can be enriched to support specialized functions within a given tissue (Hageman and Kampinga 2009). Consistently, the regulatory pathways that control the heat shock response (HSR) comprise a heat shock regulatory network with tissue-selective effects: in total 59 regulators of the HSR were identified through a genome-wide functional genomic screen and include both molecular chaperones and additional components of the PN (Guisbert et al., 2013). Recent analysis of the transcriptional landscape of molecular chaperones have delineated core chaperones expressed across human tissues from variable chaperones differentially expressed to match tissue specific requirements, which collectively form conserved tissue-specific functional networks (Brehme et al., 2014; Shemesh et al., 2021). Interestingly, these networks are formed during development and differentiation to rewire the cell chaperoning capacities and alter usage of mechanisms to maintain protein quality control (Nisaa and Ben-Zvi 2021; Shemesh et al., 2021). Thus, when considering targeting the PN for the effective treatment of disease, one must account for the tissue of origin, the unique nature of the proteome within that tissue, cell fate/differentiation status, and specialized components of the PN acting within a tissue or cell type.

Cell Non-Autonomous Regulation of Proteostasis and Longevity are Linked

In addition to cell-intrinsic mechanisms, organisms maintain proteostasis through cell non-autonomous mechanisms. To date, the majority of these discoveries have come from studies in *C. elegans*. For instance, a regulatory component of the HSR has a cell non-autonomous component; the maintenance of proteostasis throughout the organism is controlled by thermosensory neurons in a serotonin-dependent manner (Prahlad et al., 2008; Tatum et al., 2015). In contrast, a second regulatory component from the GABAergic and cholinergic system normally limits muscle cell proteostasis (Garcia et al., 2007; Silva et al., 2013). Furthermore, a moderate increase in cholinergic signaling in the neuromuscular junction triggers calcium influx to the cytosol of muscle cells, activating a downstream signaling cascade leading to the transcriptional activation of HSF-1, and thereby the expression of molecular chaperones (Silva et al., 2013). These findings suggest that upstream neuronal signals regulate proteostatic mechanisms in distal tissues. The GATA transcription factor: PQM-1, functions as a mediator of transcellular chaperone signaling, acting in either a neuron or intestinal-specific route to trigger *hsp-90* in remote tissues to preserve proteostasis and metastable proteins in muscle cells induces systemic stress response across multiple tissues through transcellular chaperone signaling (van Oosten-Hawle and Morimoto 2014; O'Brien et al., 2018; Morimoto 2019). Moreover, the expression of HSP90 within intestinal or neuronal cells is sufficient to suppress protein misfolding in muscle cells (van Oosten-Hawle et al., 2013). Proteostasis and stress resilience in reproductive adult *C. elegans* are also regulated by communication from internally fertilized embryos (Sala et al., 2020).

Cell non-autonomous signals regulates components of the PN and extends longevity in *C. elegans*. For example, specific downregulation of electron transport chain components in the nervous system causes an increase in the mitoUPR in non-neuronal tissues (Durieux et al., 2011). Surprisingly, neuronal downregulation of the respiratory chain complex IV promotes longevity to a similar level as observed when it is downregulated throughout the Soma, and induces mitochondrial chaperones in the intestine. Furthermore, neuronal proteotoxic stress targeting mitochondria elicits a global induction of the mitoUPR through serotonin signaling (Berendzen et al., 2016). Interestingly, cell non-autonomous regulation of mitochondrial stress has also been observed in mammals, where the fibroblast growth factor 21 (FGF21) mediates a signaling event from muscle to peripheral tissue triggered by mitochondrial dysfunction in mice (Kim et al., 2013). Two lines of research indicate that the ER-UPR is cell non-autonomously regulated in *C. elegans*. First, neuronal expression of the spliced and activated form of the ER-UPR transcription factor X box-binding protein 1 (XBP-1) is sufficient to induce the activation of ER-UPR in intestinal cells, which in turn increases stress resistance and extends longevity (Taylor and Dillin 2013). Second, expression of octopamine receptor 1 (OCTR-1) in ASH, ASI, AIY or ADE chemosensory neurons is necessary to inhibit the activation of XBP-1 and the expression of non-canonical ER-UPR genes in distal cells (Urano et al., 2002; Sun et al., 2011). Interestingly, activation of the ER-UPR within distinct neuronal cell types activate unique responses in peripheral tissues (Higuchi-Sanabria et al., 2020). In mammals, ER-UPR interaction between tissues has been proposed as the mechanism of induction of ER stress in

TABLE 1 | HSF1 post-translational modifications.

Modification	Effect	Signal	Enzyme	References
Inhibitory Signals				
Ac-K80	loss of DNA binding		p300/SIRT1	Raychaudhuri et al. (2014) Westerheide et al. (2009)
Ph-S121	nuclear export	Metabolic	AMPK	Dai et al. (2015)
	Hsp90 interaction	Inflammation	MAPKAPK2	Wang et al. (2006)
Ph-S303	promotes SUMO-K298	Heat		Hietakangas et al. (2003)
	basal transcriptional repression			Kline and Morimoto, (1997)
			GSK3β	Chu et al. (1996) Chu et al. (1998) He et al. (1998)
	inhibits granules			He et al. (1998)
	cytoplasmic sequestration by 14-3-3		GSK3β	Wang et al. (2003)
	degradation *via* UPS			Kourtis et al. (2015)
Ph-S307	basal repression			Chu et al. (1996) Chu et al. (1998) He et al. (1998) Kline and Morimoto, (1997) Knauf et al. (1996) Xia and Voellmy, (1997)
	promote P-S303 by GSK3β		MAPK/ERK	Chu et al. (1996) Chu et al. (1998) Huang et al. (2018) Knauf et al. (1996) Wang et al. (2003)
	degradation via UPS			Kourtis et al. (2015)
Ph-S363	basal repression		PKC	Chu et al. (1998)
	loss of DNA binding, inhibits granules		JNK	Dai et al. (2000)
Ph-S216	degradation via UPS	Early Mitosis	PLK1	Lee et al. (2008)
Su-K298	limits activation	Heat	UBC9	Anckar et al. (2006) Hietakangas et al. (2003) Hietakangas et al. (2006) Hong et al. (2001)
Stimulatory Signals				
Ph-T142	DNA binding, increased transcription	Heat	CK2	Soncin et al. (2003)
Ph-S230	increased transcription	Stress	CaMKII	Holmberg et al. (2001)
Ph-S419	nuclear import	Heat	PLK1	Kim et al. (2005)
Ph-S320	nuclear localization	Heat	PKA	Murshid et al. (2010) Zhang et al. (2011)
Ph-S326	increased transcription	Heat		Guettouche et al. (2005)
			MEK	Tang et al. (2015)
			mTOR	Chou et al. (2012)
	increased stability, nuclear import		MEK	Tang et al. (2015)
Additional modifications				
Ac-K116, 118, 126, 148, 157, 208, 224, 298		Heat	p 300/SIRT1	Raychaudhuri et al. (2014) Westerheide et al. (2009)
Ph-S292, 314, 319, 344, 368, 444				Guettouche et al. (2005) Olsen et al. (2006) Olsen et al. (2010)
Ph-T323,367,369				Mayya et al. (2009) Olsen et al. (2006) Olsen et al. (2010)
O-glycosylation				Hamiel et al. (2009)

tumor cell lines that promotes the activation of the ER-UPR in macrophages, resulting in the production of pro-inflammatory cytokines leading to tumor growth (Mahadevan et al., 2011). Lastly, gonadal stem cell non-autonomous signaling also links proteotoxic stress resistance and longevity: loss of *C. elegans* gonadal stem cells results in increased somatic maintenance through increased proteosomal activity: overexpression of the 19S proteasome subunit *rpn-6* is

sufficient to fortify proteostasis and increase lifespan (Vilchez et al., 2012).

HSF1: GUARDIAN OF THE CYTOSOLIC AND NUCLEAR PROTEOME

Heat shock transcription factors are conserved throughout eukaryotes. The *Drosophila melanogaster, C. elegans,* and *Saccharomyces cerevisiae* genomes each encode one heat shock transcription factor, whereas HSF has expanded in vertebrates to encode four paralogs (HSF 1 through 4). Here we focus primarily focus on HSF1 [for more on HSF family members and their discovery, see (Sorger and Pelham 1988; Wiederrecht et al., 1988; Clos et al., 1990; Rabindran et al., 1991; Liu et al., 1997; Nakai 1999; Gomez-Pastor et al., 2018; Roos-Mattjus and Sistonen 2021)].

Regulation of HSF1

As a transcription factor, HSF1 is regulated extensively by post-translational modifications including acetylation, sumoylation, and phosphorylation (summarized in **Table 1**). These modifications regulate HSF1 activity at multiple stages, including release from inhibitors, nuclear translocation, homotrimerization, promoter binding, and recruitment of RNA Polymerase II [reviewed in (Gomez-Pastor et al., 2018; Roos-Mattjus and Sistonen 2021)].

Under normal conditions, HSF1 exists as an inactive monomer, stabilized by hydrophobic interactions between the heptad repeats in the N- and C-terminal regions (Sorger 1990; Sarge et al., 1993; Orosz et al., 1996; Farkas et al., 1998). One model for HSF1 activation is intrinsic activation; HSF1 effectively acts as a sensor responsive to changing thermodynamic conditions and thereby converts from an inactive monomer to an active trimer. This idea that HSF1 functions as an intrinsic "thermosensor" is consistent with *in vitro* data demonstrating that HSF1 can be activated in response to increasing temperature alone (Hentze et al., 2016). A second "chaperone titration model" posits that under basal conditions HSF1 is sequestered within the cytoplasm by molecular chaperones including HSP70, HSP90, and the chaperonin tailless complex polypeptide 1 (TCP1) ring complex (TRiC) (Abravaya et al., 1992; Baler et al., 1992; Baler et al., 1996; Duina et al., 1998; Shi et al., 1998; Zou et al., 1998; Guo et al., 2001; Akerfelt et al., 2010; Neef et al., 2010; Neef et al., 2011; Neef et al., 2014; Zheng et al., 2016). Upon acute proteotoxic stress, such as heat shock, protein misfolding titrates away chaperones that normally sequester HSF1 as a monomer. Released HSF1 monomers trimerize, translocate to the nucleus, bind to DNA promoters, and upregulate transcription of multiple genes. HSF1 activation induces expression of molecular chaperones, which initiates a negative feedback loop to inactivate HSF1 once stress upon the proteome is resolved (Zheng et al., 2016; Krakowiak et al., 2018). HSF1 has been shown to be regulated by additional means, including intrinsic refolding mechanisms (Mosser et al., 1990; Goodson and Sarge 1995; Xia and Voellmy 1997; Farkas et al., 1998; Zhong et al., 1998; Ahn and Thiele 2003), non-coding RNA (Shamovsky et al., 2006) and *in vivo* through cell non-autonomous signals from thermosensory neurons in *C. elegans* (Clark et al., 2007; Prahlad et al., 2008).

Conserved HSF-1 Regulation in *C. elegans*

There is a general conservation of mechanisms of HSF-1 regulation between *C. elegans* and mammals, yet the precise molecular mechanisms of control and how, for instance, post-translational modifications and direct interactors of mammalian HSF1, intersect with the wealth of genetic information linking *C. elegans* HSF-1 to longevity and stress signaling remains poorly understood. In both mammalian cell culture and *C. elegans,* under basal conditions HSF-1 is transcriptionally inactive due to cytoplasmic sequestration. Upon stress in either system, HSF-1 nuclear localization is governed by phosphorylation (Chiang et al., 2012; Dai et al., 2015; Tang et al., 2015). Similarly, nuclear HSF-1 forms stress granules upon heat stress in both *C. elegans* and mammals (Alastalo et al., 2003; Morton and Lamitina 2013). As in mammals, *C. elegans* HSF-1 transcriptional activation is dependent on the regulation of trimerization (Chiang et al., 2012). At least one direct regulator of HSF1 is conserved between mammals and *C. elegans*: the heat shock binding factor 1 (HSB-1) negatively regulates HSF-1 transcriptional activity (Satyal et al., 1998). Other direct regulators in mammals, such as TOR and AMPK, also show genetic interactions in *C. elegans* (discussed below), but whether direct interactions occur in *C. elegans* has yet to be determined. Knockdown of *hsp-70* also activates HSF-1, implying that the chaperone titration model regulation of HSF1 is conserved (Guisbert et al., 2013).

Secondary Structure of HSF1

Structurally, HSF1 is highly conserved across metazoan animals (**Figure 2**). The N-terminal region of approximately the first 100 amino acids is the most well-conserved region of the HSF protein family and encodes a helix-turn-helix loop DNA-binding domain (DBD), which recognizes DNA heat shock elements (HSE) (identified as nGAAn DNA repeats) (Wu 1995). Two regions of leucine zippers (HR-A/B and HR-C) allow oligomerization of HSF1 monomers (Bjork and Sistonen 2010; Anckar and Sistonen 2011; Dayalan Naidu and Dinkova-Kostova 2017). HR-A/B immediately follows the DBD and flanks a regulatory domain (RD) on one side, while HR-C is downstream of the RD. Intrinsic interactions of HR-A/B with HR-C prevents spontaneous HSF1 trimerization and activation under basal conditions. Approximately the last 100 amino acids of HSF1 contains the trans-activation domain (TAD), which is the region through which HSP70 interacts with HSF1.

HSF1 Regulation Through Phosphorylation

Phosphorylation of mammalian HSF1 is one of the most well studied mechanisms of HSF1 regulation; mass spectrometry and site-directed mutagenesis have identified phosphorylation on multiple serine or threonine residues (S121, S127, T142, S195, S216, S230, S292, S303, S307, S314, S319, S320, T323, S326, S338, S344, S363, T367, S368, T369, S419, and S444) [e.g. (Guettouche

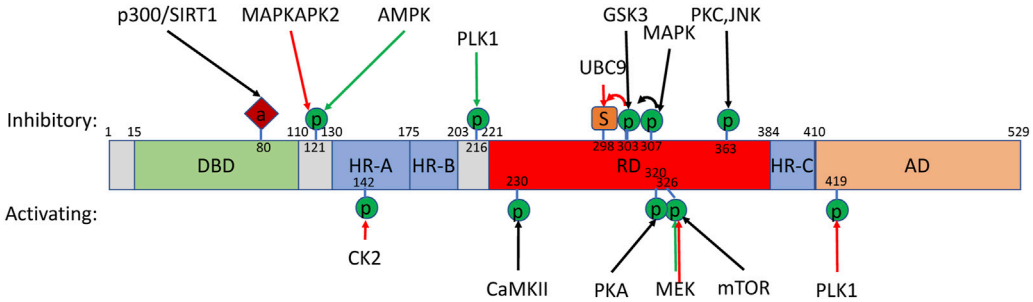

FIGURE 2 | HSF1 post-translational modifications. Schematic of mammalian HSF1 secondary structure with post-translational modifications with known regulators and overall effect on activity. Additional modifications without known regulators are listed in **Table 1**. Additional regulators where a specific post-translational modification has not been identified have been omitted. "DBD" indicates the helix-turn-helix loop DNA-binding domain. "HR-A/B" and "HR-C″ identify two regions of leucine zippers. "RD" indicates the regulatory domain. "TAD" indicates the transcriptional transactivation domain. Modifications in response to heat (red arrows) or metabolic/mitogenic signals (green) are shown. Amino acid numbers are indicated. "P" indicates phosphorylation. "S" indicates sumoylation. "A" indicates acetylation.

et al., 2005; Anckar and Sistonen 2011; Xu et al., 2012; Gomez-Pastor et al., 2018; Roos-Mattjus and Sistonen 2021), summary in **Table 1**]. Some residues appear to be phosphorylated under basal conditions (Chu et al., 1996; Knauf et al., 1996; Kline and Morimoto 1997; Chu et al., 1998; He et al., 1998) whereas other sites undergo inducible phosphorylation (Holmberg et al., 2002). Stress induces phosphorylation at multiple residues (i.e., hyperphosphorylation) and is concurrent with transactivation (Cotto et al., 1996; Xia and Voellmy 1997; Holmberg et al., 2001; Holmberg et al., 2002; Guettouche et al., 2005).

Most phosphorylation events repress transcriptional activity. For example, sequential phosphorylation of S307 by mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and S303 by glycogen synthase kinase 3 (GSK3) (Chu et al., 1996; Chu et al., 1998; He et al., 1998) recruits 14-3-3 proteins to export HSF1 from the nucleus and sequester it in the cytoplasm (Wang et al., 2003). Several serine residues are targeted by multiple kinases to repress HSF1 activity: acute inflammatory signals activate mitogen-activated protein kinase 2 (MAPK2) to phosphorylate HSF1 at S121, which promotes direct interaction with HSP90, subsequently reducing binding to DNA at heat shock elements and therefore lessening the activation of corresponding gene expression (Wang et al., 2006). This same residue is also phosphorylated by AMPK under conditions of metabolic stress to impair HSF1 activity by impeding nuclear translocation, thereby rendering cells sensitive to proteotoxic stress (Dai et al., 2015).

HSF1 (S363) phosphorylation also inhibits activity and is the target of both protein kinase C (PKC) and c-Jun N-terminal kinase (JNK). PKC was shown to inhibit HSF1 without affecting trimerization or binding to heat shock elements, and it may limit HSF1 activity during increases in PKC activity, such as after activation by growth receptors (Chu et al., 1998). JNK, a stress-responsive member of the MAPK pathway, also inactivates HSF1 through S363 phosphorylation, which rapidly clears HSF1 from the sites of transcription (Dai et al., 2000). Thus, the outcome of HSF1 (S363) phosphorylation on DNA binding is context-dependent. JNK has also been shown to phosphorylate the TAD of HSF1 after severe heat shock, and JNK inhibitors prevent the activation of HSF1 target genes, but whether this is occurring at S363 is unclear (Park and Liu 2001). Collectively, these findings indicate that diverse negative regulatory signals can converge on a common HSF1 residues to limit activity.

HSF1 phosphorylation can also activate transcription, which has been best-studied in the context of hyperphosphorylation due to acute heat stress. By mass spectrometry, twelve serine residues were identified that are phosphorylated after heat stress (Guettouche et al., 2005). During heat stress, Polo-like kinase (PLK1), one of the major protein kinases involved in cell division, and specifically in APC/C regulation, directly phosphorylates HSF1 on S419, and a S419A mutation inhibits HSF1 translocation to the nucleus, suggesting this is an early step in HSF1 activation (Kim et al., 2005). However, PLK1 also phosphorylates HSF1 (S216) in early mitosis, facilitating degradation, which is essential for proper mitotic progression (Lee et al., 2008). Thus, PLK1 can regulate HSF1 in opposing manners through phosphorylation of different residues in response to distinct signals. Similar to PLK1 action at HSF1 (S419), protein kinase A (PKA) phosphorylates HSF1 (S320) in response to heat shock, and this modification is required for translocation to the nucleus, DNA binding at HSE, and to activate expression of molecular chaperones (Murshid et al., 2010; Zhang et al., 2011). Casein kinase 2 phosphorylates HSF1 on T142 and mutation to alanine (T142A) inhibits trans-activation of *HSP70* by HSF1 and blocks binding to HSE, without affecting translocation to the nucleus (Soncin et al., 2003).

Both S230 and S326 have also been shown to be essential for the increased HSF1 transcriptional activity in response to acute heat shock (Holmberg et al., 2001; Guettouche et al., 2005; Tang et al., 2015). S230 lies within a consensus site for calcium/calmodulin-dependent protein kinase II (CaMKII), and CaMKII overexpression enhances both the level of *in vivo* S230 phosphorylation and transactivation of HSF1 (Holmberg et al., 2001). Furthermore, S230 is not needed for either the heat-induced DNA-binding activity or granule formation but is essential for the transcriptional activity of HSF1 (Holmberg et al., 2001). HSF1 (S326) has been shown to be directly

phosphorylated by mTOR, and inhibition of mTOR limits induction of molecular chaperones (Chou et al., 2012), yet it is unclear whether mTOR is essential for HSF1 (S326) phosphorylation after heat stress. mTOR is a major regulator of cellular growth and translation, which suggests that mTOR regulation of HSF1 activity may directly balance the total protein abundance within the proteome and molecular chaperone abundance in response to cell size. Interestingly, MAPK/ERK Kinase, or mitogen-activated protein kinase (MEK), phosphorylates S326 both *in vitro* and *in vivo* after heat stress to activate and stabilize HSF1 to preserve proteostasis (Tang et al., 2015). Furthermore, under heat stress, ERK, MEK, and HSF1 assemble into a ternary protein complex wherein ERK suppresses HSF1 (S326) phosphorylation through inhibitory phosphorylation of MEK.

The emerging picture of regulation of mammalian HSF1 through phosphorylation is one of an integrated, combinatorial process. Indeed, in contrast to the aforementioned studies where HSF1 function was compromised after mutating a single phosphorylation site, an HSF1 isoform with 15 phosphorylation sites mutated, including residues targeted by both inhibitory and stimulatory signals, was still able to translocate to the nucleus, bind DNA, and activate transcription (Budzynski et al., 2015). Thus, HSF1 activity is carefully balanced between positive and negative regulators, which is likely dependent on cell type and context. While in *C. elegans* HSF-1 has been shown to undergo phosphorylation (Chiang et al., 2012), only a fraction of modified amino acids in mammalian HSF1 are directly conserved (**Supplementary File S1**). Furthermore, the molecular and cellular details of regulation, and subsequent consequences on stress response, proteostasis, and longevity in an intact multicellular organism remains underexplored.

Ubiquitination of HSF1 and Degradation *via* the UPS

HSF1 protein levels are also regulated via degradation by the UPS. HSF1 can be ubiquitinated and degraded by the Skp1–Cul1–F box (SCF) ubiquitin ligase complex (Skaar et al., 2013). Ubiquitination of HSF1 by the SCF complex occurs during mitosis upon phosphorylation of HSF1 (S216) by PLK1, which releases HSF1 from the Cdc20 complex (Lee et al., 2008). In another study, the SCF complex was shown to target HSF1 for degradation *via* F-box and WD repeat domain containing protein 7 alpha (FBXW7α), a substrate-targeting subunit of the SCF complex. Interestingly, interaction occurs through a conserved degron motif phosphorylated by GSK3β and ERK1; FBXW7α ubiquitylates HSF1, and loss of FBXW7α results in impaired degradation of nuclear HSF1 and defective HSR attenuation (Kourtis et al., 2015). This suggests the possibility that distinct stimuli converge through phospho-regulation of HSF1 to recruit the SCF complex and target HSF1 for degradation. HSF1 may be transported to the proteasome through the Filamin A interacting protein 1-like (FILIP-1L) protein, which has been found in a complex with HSF1, Hsp72, and the ubiquitin-binding domain of hHR23A, a receptor that transports polyubiquitinated proteins to the proteasome for degradation; cells co-expressing HSF1 and FILIP-1L exhibit reduction in the HSF1 protein levels and inhibition of stress granule formation following exposure to heat shock (Hu and Mivechi 2011).

Sumoylation of HSF1

HSF1 is negatively regulated through sumoylation of HSF1 in both *C. elegans* and mammalian cell culture (Hong et al., 2001; Hietakangas et al., 2003; Anckar et al., 2006; Hietakangas et al., 2006; Das et al., 2017). SUMO protein catalyzes a small ubiquitin like modification, which frequently targets transcription factors to limit activity (Gill 2005; Cubenas-Potts and Matunis 2013; Deyrieux and Wilson 2017; Wotton et al., 2017). Mammals have four genes encoding SUMO, while *C. elegans* possess a single gene, *smo-1* (Kamitani et al., 1998; Jones et al., 2002). In mammals, mild heat stress results in HSF1 (S303) phosphorylation, which promotes subsequent SUMO-1 addition to HSF1 (K298) and limits HSF1 transcriptional activation. More severe and prolonged heat stress results in desumoylation and increased expression of heat shock genes (Hietakangas et al., 2003; Anckar et al., 2006). Thus, sumoylation has been proposed to act as a mechanism to fine-tune HSF1 activity to levels of acute proteotoxic stress (i.e., protein misfolding). Mapping of the human SUMO proteome has identified many additional lysine residues of HSF1 that putatively undergo sumoylation, but the biological significance of these modifications remains unknown (Hendriks et al., 2017; Gomez-Pastor et al., 2018; Roos-Mattjus and Sistonen 2021). In *C. elegans,* sumoylation also limits the inducibility of the HSR (Das et al., 2017), indicating that this mechanism regulates HSF-1 activity *in vivo*, as well as in mammalian cell culture. Consistently, loss of the SUMO isopeptidase *ulp-1* shortens lifespan under conditions of mild heat stress (Samuelson et al., 2007). Additionally, the transcriptional cofactor HPK-1 prevents HSF1 sumoylation under basal conditions (Das et al., 2017). Interestingly the yeast ortholog of HPK-1, Yak1, which directly phosphorylates HSF1 in response to altered metabolic conditions, is induced by heat stress and is required for thermal stress survival (Garrett et al., 1991; Hartley et al., 1994), suggesting that a regulatory role of HPK-1 upon HSF1 is evolutionarily conserved.

Acetylation of HSF1

HSF1 is also regulated through acetylation. At least nine lysine residues of HSF1 have been shown to be acetylated (**Table 1**). Perhaps the most well-studied is K80 acetylation of HSF1; the transcriptional co-activator p300/CBP (CREB-binding protein) acetylates HSF1 (K80) to attenuate the HSR by inhibiting DNA binding. This activity is opposed by the deacetylase activity of SIRT1: downregulation of *SIRT1* resulting in the weaker induction of molecular chaperones due to greater acetylation of HSF1, which prevents binding to HSE (Westerheide et al., 2009; Raychaudhuri et al., 2014). Interestingly, acetylation of HSF1 has been found to both increase and decrease stability (Westerheide et al., 2009; Kim et al., 2016); acetylation at different positions alters the HSF1 stability. Specifically, acetylation of HSF1 at position K118 and K80 lead to attenuation of the HSR due to HSF1 degradation by the ubiquitin-proteasome. In

contrast, acetylation at position K208 and K298 stabilizes HSF1 (Raychaudhuri et al., 2014). As SIRT1 is a critical deacetylase of HSF1, it is tempting to speculate that SIRT1 may promote HSF1 stability or degradation depending on the position of acetylation.

Additional Direct Regulators of Mammalian HSF1

A number of additional proteins have been identified that regulate HSF1 activity through direct interactions. For example, heat shock factor binding protein 1 (Hsbp1) is a negative regulator of HSF1 activity (Satyal et al., 1998). CHIP, Ral-binding protein 1 (RalBP1), and Death-associated protein 6 (Daxx) function in the activation of HSF1 (Dai et al., 2003; Hu and Mivechi 2003; Boellmann et al., 2004). Metastasis-associated protein 1 (MTA1) and Cdc20 are additional HSF1 regulators (Khaleque et al., 2008; Lee et al., 2008).

CONTROL OF GENE EXPRESSION BY HSF1

HSF1 preserves proteome vitality by commanding a transcriptional program whose physiological purpose is to maintain proper folding and function of the proteome in the face of both global and localized forms of protein misfolding stress. This transcriptional program marshals multiple chaperone systems when protein homeostasis is compromised, either in response to intrinsic or extrinsic cues. In addition to regulating the HSR, HSF1 activity is also responsive to metabolic and mitogenic signals and plays an important role in development and organismal longevity [reviewed in (Li et al., 2017)]. Additionally, HSF1 participates in physiological and pathological processes including: differentiation, immune response, multidrug resistance, longevity, neurodegeneration, and cancer. Interestingly, recent study comprehensively cataloged all known HSF1 target genes and preformed an enrichment analysis of HSF1 targets across tissues, cell types, and organisms (hsf1base.org) (Kovacs et al., 2019) and found that HSF1 targets, expressed in all tissues and cell types, are generally related to maintaining proteostasis. Furthermore, HSF1 targets that are conserved across various animal taxa operate mostly in cellular stress pathways (e.g., autophagy), chromatin remodeling, ribosome biogenesis, and aging; highlighting the diverse roles for HSF1 in regulating gene expression.

The Heat Shock Response (Unfolded Protein Response in the Cytosol and Nucleus)

The "heat shock response," defined as the rapid induction of heat shock proteins, was initially described in *Drosophila* 60 years ago (Ritossa 1962). We now know that the HSR is an ancient genetic program shared across all organisms and constitutes one key component of a larger network that responses to stress on the proteome. The HSR could more accurately be described as the Unfolded Protein Response to proteotoxic stress within the cytosol and nucleus, analogous to the mitoUPR and ER-UPR.

Protein stability, and therefore normal cellular function, are highly sensitive to changes in temperature. Acute heat stress not only denatures and aggregates proteins, but also damages the cytoskeleton, breaks down organelles such as Golgi apparatus and the ER, diminished the numbers of functional mitochondria and lysosomes, and produces cytoplasmic stress granules. The cellular consequences of this damage are: a collapse of actin and microtubule networks, disruption of intracellular transport, decreased availability of ATP, a global decrease in translation, a drop in cytosolic pH, and cell cycle arrest [reviewed in (Richter et al., 2010)]. Due to the inherent danger heat has on cellular function, it is not surprising that transcriptional programs evolved early in evolution to respond to heat. Life exists in a wide range of temperatures, for example *Pyrodictium abyssi* grows in hot vents over 100°C, and species of the *Thermoproteus* genus live in boiling mud. Yet, shifting *Pyrodictium occultum* from 102°C to 108°C induces transcriptional changes in response to heat stress (Stetter 2006). Thus, organisms thrive in only a narrow temperature range, and shifts of only a few degrees induces a universal and ancient transcriptional response to heat: the "heat shock response" (Brown and Lupas 1998; Takai et al., 1998; D'Amico et al., 2006; Richter et al., 2010).

Previous transcriptional and proteomic studies have identified a vast number of heat-inducible genes, which are involved in diverse cellular processes. The HSR not only induces the expression of molecular chaperones, but also: increases protein degradation *via* autophagy and expression of proteasome subunits, promotes stabilization of cellular energetics *via* altered expression of metabolic enzymes, inhibits unnecessary processes through the activation of additional regulatory proteins, induces repair of DNA/RNA and changes in gene expression to sustain cellular structures, and repairs membranes to restore transport and detoxification within the cell [reviewed in (Richter et al., 2010)].

HSF1 also restores proteostasis after stress by increased expression of genes involved in autophagy. As previously mentioned, autophagy is a crucial protagonist of the proteostasis network that functions to recycle cytosolic components after stress, including: toxic protein aggregates, nutrient deprivation, hypoxia, and damaged organelles, among others (Kroemer et al., 2010). In *C. elegans*, heat shock and *hsf-1* overexpression induce autophagy in multiple tissues (Kumsta et al., 2017). In mammals, HSF1 regulates the phosphorylation and activity of the SQSTM1/p62 autophagy receptor, suggesting that the HSF1 stress response pathway is involved in autophagic clearance of protein aggregates (Watanabe et al., 2017). Moreover, HSF1 controls autophagy activity induced by chemotherapeutic agents by regulating the transcription of autophagy-related protein 7 (ATG7) (Desai et al., 2013).

The Role of HSF1 in Development is Distinct From the HSR

Periods of rapid growth during development require proteome expansion, in turn demanding an expansion of

TABLE 2 | HSF1 loss and organismal development.

Mutation/allele	Outcome	References
Drosophila melanogaster		
Q78TAA	lethal at 1st or 2nd instar stage of development; homozygous lethal	Jedlicka et al. (1997)
Q373TAG	lethal at 1st or 2nd instar stage of development; decreased viability of adults	
S99N (DNA binding domain)	lethal at 1st or 2nd instar stage of development; homozygous lethal	
V57M (DNA binding domain)	lethal at 1st or 2nd instar stage of development; temperature sensitive	
Caenorhabditis elegans		
sy441 (truncation, lacks transactivation domain)	egg laying defect, arrested at the L2–L3 stage above 15°C	Hajdu-Cronin et al. (2004)
ok600 (frameshift deletion, putative null)	L2–L3 stage arrest	Li et al. (2016)
rsks-1(0); hsf-1(sy441)	rescued developmental phenotype	Chisnell et al. (2018)

the PN to regulate developmental processes. Certain windows during development demand an excess amount of energy and nutritional resources. Organisms consequently experience stress during stages where meeting these needs requires divergence from optimal developmental trajectory (Puscheck et al., 2015). In response to stress, HSF1, along with mammalian paralogs HSF2 and HSF3, acts to compensate and ensure survival of the developing organism (Akerfelt et al., 2010; Puscheck et al., 2015). The role of HSF1 in development with and without canonical heat shock stressors is still under ongoing investigation in other model systems.

In *Drosophila,* mutations in a single base of the HSF coding sequence causes arrest at the first or second larval instar stage of development. However, HSF mutations induced past these larval stages do not affect cell growth or viability under normal conditions, suggesting that HSF is only required in the early stages of development in *Drosophila* (**Table 2**). Additionally, the expression of canonical heat shock genes did not change in these mutants, suggesting that the developmental role of HSF1 could be distinct from the HSR (Jedlicka et al., 1997).

The developmental transcriptional program of HSF-1 in *C. elegans* is distinct from the canonical HSR (Li et al., 2016; Li et al., 2017). *hsf-1* null mutants arrest at the L2-L3 larval stage of development (**Table 2**). Activation of HSF-1 during development depends on a GC-rich E2F/DP transcription factor binding to a motif that allows HSF-1 to bind to a heat shock element distinct from the classical HSR. Through this, E2F and HSF-1 facilitate regulation of biogenesis and anabolic metabolism during development. However, loss of *hsf-1* also results in lower basal levels of molecular chaperones (Chiang et al., 2012). Additionally, knockdown of components of the mTOR pathway can rescue these defective developmental phenotypes. Specifically, rescue utilizing either knockdown of the TORC1 component *daf-15* (Raptor), a positive regulator of mTORC1 *ragc-1* (orthologous to RAG GTPase), or loss of the downstream effector that regulates rates of translation; *rsks-1* (ortholog of S6 Kinase), prevented developmental arrest (Chisnell et al., 2018). This implies that decreased rates of protein synthesis resulting from mTORC1 inactivation mitigates damage to the proteome associated with loss of HSF function. Alternatively, decreased mTORC1 activity may rescue *hsf-1* developmental defects resulting from diminished basal chaperone expression, at least in part by reducing either the total concentration or specific components of the cellular proteome.

HSF-1 INTEGRATES DIVERSE METABOLIC AND STRESS SIGNALS TO PRESERVE PROTEOSTASIS AND LONGEVITY

HSF-1 Preserves Longevity

HSF-1 has emerged as a key regulator of organismal longevity through the integration of signals of cellular energy metabolism and diverse forms of stress. This has been well studied in *C. elegans;* loss of *hsf-1* shortens lifespan, impairs survival to a diverse array of cellular stresses, and compromises proteostasis. Conversely, *hsf-1* overexpression increases lifespan, stress resistance, and delays age-associated proteostatic decline (Garigan et al., 2002; Hsu et al., 2003; Morley and Morimoto 2004; Kourtis et al., 2012; Das et al., 2017). Recently, it has been shown that HSF-1 requires the transcriptional cofactor *hpk-1* to extend longevity, to induce molecular chaperones after thermal stress, to enhance hormetic extension of longevity, and is required in conjunction with HSF-1 for maintenance of proteostasis (Das et al., 2017). HPK-1 antagonizes sumoylation of HSF-1 and inhibiting sumoylation increases the induction of molecular chaperones after heat shock (Das et al., 2017). While persistent heat stress is detrimental to nematode survival, either intermittent heat shock or mild hormetic heat shock also extends longevity *via* HSF-1 activation (Das et al., 2017; Kumsta et al., 2017). It is generally believed that hormesis extends longevity by bolstering organismal and cellular stress response pathways, which subsequently offsets aging-related decline in these pathways (Epel and Lithgow 2014).

How HSF-1 extends longevity remains an active area of investigation. Early work suggested that HSF-1 delays aging through expression of molecular chaperones, as overexpression of molecular chaperones can suppress polyglutamine aggregation in body wall muscle and increase *C. elegans* lifespan (Satyal et al., 2000; Hsu et al., 2003; Walker and Lithgow 2003). However, emerging evidence suggesting a more complex picture. First,

there are conflicting reports as to whether a hypomorphic *hsf-1* allele (premature stop codon removing the transactivation domain) sensitizes *C. elegans* to heat stress, despite having an impaired HSR (Prahlad et al., 2008; McColl et al., 2010). Interestingly, overexpression of a mutant HSF-1 lacking the transactivation domain is able to increase thermotolerance and lifespan through maintaining cytoskeletal integrity, despite being impaired in the ability to induce molecular chaperones after heat shock (Baird et al., 2014), but it is possible that the truncated *hsf-1* isoform is a neomorph. Another possibility is that HSF-1 may extend longevity through links to other components of the PN, such as the regulation of autophagy (Kumsta et al., 2017). As previously mentioned, efforts to directly link the genetic interactions and cell biological activity of HSF-1 in *C. elegans* to specific HSF1 post-translational modifications that occur in mammalian cells is still lacking, yet it is widely postulated that HSF-1 functions that extend longevity will be the same as those that preserve proteostasis, and by extension possibly delay aging and prevent the manifestation of neurodegenerative age-associated proteotoxic diseases in humans.

HSF-1 Functions Cell Non-Autonomously to Regulate Proteostasis, Stress Response and Longevity

One transformative discovery in *C. elegans* was the finding that HSF-1 functions cell non-autonomously within neurons to increase longevity and maintain proteostasis in distal tissues (Morley and Morimoto 2004; Prahlad et al., 2008; Prahlad and Morimoto 2011; Douglas et al., 2015), and that this occurs through serotonin signaling (Tatum et al., 2015). Increased neuronal expression of HSF-1 is sufficient to extend longevity and improve stress resistance. Interestingly, signals that increase the HSR in peripheral tissues through thermosensory neuronal circuits are separable from those that increase longevity (Douglas et al., 2015). In accordance to these findings, another study identifies that integrin-linked kinase (ILK) inhibition activates HSF-1 cell non-autonomous effect on stress resistance and lifespan in a thermosensory-dependent manner (Kumsta et al., 2014). Of note, HSF-1 acts in multiple tissues to regulate longevity (Morley and Morimoto 2004) and also acts cell non-autonomously outside of the nervous system. For example, intestinal HSF-1 activity upregulates the *mir*-83/miR-29 secreted microRNA to disrupt macroautophagy both within intestinal and body wall muscle (Zhou et al., 2019). However somewhat paradoxically, hormetic heat shock to activate HSF-1 or HSF-1 overexpression induces autophagy in multiple tissues (Kumsta et al., 2017), yet whether this occurs cell intrinsically or non-autonomously was not explored. Altogether, this implies that strategies to target HSF-1 in the treatment of disease should consider both cell intrinsic changes and effects in distal tissues. It will be interesting to learn whether pro-longevity functions of HSF-1 that are independent of molecular chaperone induction, can improve neuronal proteostasis but not protect cancer cells from chronic proteotoxic stress.

HSF-1 Integrates Metabolic Signals to Extend *C. elegans* Longevity

The most potent influencers of *C. elegans* longevity sense changes in metabolic status, which in turn leads to the activation of cytoprotective stress response and adaptive transcriptional programs, including the PN. HSF-1 is essential for many of these metabolic pathways or signals to extend longevity: including decreased ILS (Hsu et al., 2003; Morley and Morimoto 2004), germline deficiency (Hansen et al., 2005), reduced TORC1 signaling or inhibition of *rsks-1* (Seo et al., 2013), and dietary deprivation (a distinct method of *C. elegans* DR) (Steinkraus et al., 2008). Furthermore, HSF-1 is subjected to complex regulation at times of simultaneously applying thermal stress and DR, through the integrin-linked kinase PAT-4 (human integrin linked kinase) and the deacetylase SIR-2.1 (Raynes et al., 2012; Kumsta et al., 2014).

Not all longevity signals are dependent on HSF-1, or at least the interrelationship between longevity signaling and HSF-1 are complex. For instance, early work on disruption of the electron transport chain showed increased lifespan independently of *hsf-1* (Hsu et al., 2003). However, more recent work showed lifespan extension of HSB-1/HSF-1 signaling could be in part through modulation of mitochondrial function *via* mediating histone H4-dependent regulation of mtDNA gene expression (Sural et al., 2020), indicating that the complex signaling interactions between the mitochondrial and nuclear genomes is not fully understood. Another example is AMPK and HSF1. In mammals, AMPK activation directly phosphorylates HSF1 to suppress proteotoxic stress response and conversely, either proteotoxic stress or HSF1 itself inactivates AMPK (Dai et al., 2015; Su et al., 2019). In *C. elegans* as AMPK lifespan extension appears to be independent of HSF-1 (Greer and Brunet 2009; Hwang et al., 2014; Kuo et al., 2020). It is unclear whether this regulatory mechanism is simply not conserved, restricted to specific cell types, or compensated for when examined in a multicellular organism.

As discussed above, HSF-1 is required for a specific type of DR (referred to as dietary deprivation) to increase lifespan in *C. elegans* (Hsu et al., 2003; Greer and Brunet 2009). HSF-1 is integral in the extension of longevity under this specific form of DR, but surprisingly not the other methods of DR, indicating that HSF-1 plays a specific role. For example, *eat-2* mutant animals are a genetic model of DR: animals have reduced acetylcholine channel activity, which results in decreased pharyngeal pumping, increased lifespan, and stress resistance. The increased lifespan of *eat-2* animals is *hsf-1* independent (Hsu et al., 2003). In contrast, the increased stress resistance of *eat-2* mutant animals requires *hsf-1* (Shpigel et al., 2019). However, "dietary restriction" is a vague term that attempts to capture all forms of nutritional stress, and multiple methods for inducing DR have been described. Since empirical findings indicate that distinct types of DR have many different genetic requirements, it suggests one of the possible explanations: 1) different feeding regiments or nutrient uptake either titrate a similar signal, and differing

genetic requirements are observed when threshold effects trigger a specific response, 2) different types of DR trigger distinct forms of nutrient stress, or 3) some types of DR may trigger multiple forms of nutrient stress, which enact combinatorial responses.

HSF-1 is an essential transcriptional effector of the increased longevity in ILS mutant animals (Hsu et al., 2003; Morley and Morimoto 2004). In wild type animals, DAF-2 inhibits HSF-1 activity through DDL-1 and DDL-2 (Chiang et al., 2012). DDL-1 is homologous to the human coiled-coil domain-containing protein 53 (CCDC53), and DDL-2 is the homolog of human Wiskott-Aldrich syndrome protein and SCAR homolog (WASH2) protein. WASH2 and CCDC53 are both components of the Arp2/3 complex involved in actin polymerization, and intracellular motility of endosomes (Derivery et al., 2009). CCDC53 has also been reported to potentially interact with heat shock factor binding protein 1 (HSBP1) (Rual et al., 2005). Consistently, the Arp2/3 complex as well as multiple components involved in endocytic trafficking to the lysosome are essential for decreased ILS to extend longevity (Samuelson et al., 2007). As noted above, HSF-1 cooperates with DAF-16 through PFD-6, a component of the molecular chaperone prefoldin-like complex, which relays longevity signals from HSF-1 to FOXO under conditions of reduced ILS (Son et al., 2018).

As a key nutrient sensor for NAD + levels, SIRT1 deacetylates HSF1 during nutrient stress promoting stress survival, thus making the acetylation of HSF1 an integral part of sirtuin signaling (Westerheide et al., 2009; Raynes et al., 2013). Deacetylation of mammalian HSF1 frees up its DNA binding domain, allowing it to bind to a HSE, activate the HSR; and inhibition of SIRT1 accelerates the release from the HSE, decreases HSF1 protein expression, and activation of the HSR (Westerheide et al., 2009; Kim et al., 2016). In mammals SIRT1 is also required for maintenance of the proteome as SIRT1 deficiency results in defective protein quality control (Tomita et al., 2015), reinforcing the notion that SIRT1 regulation of HSF1 connects longevity signaling to the maintenance of proteostasis. HSF1 regulation by Sirtuins is conserved in *Saccharomyces cerevisiae,* and *C. elegans* (Anderson et al., 2003; Brunquell et al., 2018). In mammals, AROS (a positive regulator of SIRT1) increases deacetylation of HSF1, while CCAR2 and DBC1 negatively regulate SIRT1 dependent HSF1 deacetylation (Zhao et al., 2008; Raynes et al., 2013). In *C. elegans,* as CCAR-1 negatively regulates SIR-2.1, increasing HSF-1 acetylation and decreasing HSF-1 ability to regulate the HSR (Brunquell et al., 2018), demonstrating a conserved regulatory mechanism for HSF-1 activation. Furthermore, DR induces heat shock gene expression in a *sir-2.1* dependent manner (Raynes et al., 2012). This suggests that signals that modulate Sirtuin function can affect HSF-1. However, increasing NAD + levels promotes longevity in *C. elegans via sir-2.1,* DAF-16 activation, and the mito-UPR, without changing *hsf-1* expression (Mouchiroud et al., 2013). We could not find direct experimental evidence in *C. elegans* to demonstrate that the extended longevity conferred by *sir-2.1* overexpression is *hsf-1* dependent.

Mammalian HSF1 is directly activated by TOR [see above and reviewed in (Antikainen et al., 2017; Saxton and Sabatini 2017; Blackwell et al., 2019)]. Furthermore, experiments in yeast suggest that activated HSF1 might inhibit rapamycin resistance and TOR signaling (Bandhakavi et al., 2008). While a bulk of HSF1 and TOR interactions have been studied in cell culture and yeast, studies in *C. elegans* reveal that inactivation of TOR signaling increases lifespan in a manner dependent on *hsf-1*. Consistently, HSF-1 can also be activated with rapamycin treatment (Seo et al., 2013).

Loss of *C. elegans* germ cells cause a metabolic shift fat mobilization and lipolysis (Wang et al., 2008). This shift in results in aged animals assuming a younger metabolic transcriptional state (Wan et al., 2017). Gonadal stem cells are depleted in *glp-1* mutant animals, which encodes the *C. elegans* ortholog of the NOTCH receptor, and *glp-1* mutations increase lifespan dependent on *hsf-1* (Hansen et al., 2005). Interestingly, shortly after the onset of reproduction the inducibility of the HRS drops dramatically (Labbadia and Morimoto 2015b), and HSF-1 forms nuclear stress bodies at the initiation of reproduction throughout the germline and upon transition to adulthood HSF-1 stress bodies form in most somatic cells (Deonarine et al., 2021). However, this repression of the HSR does not occur in *glp-1* mutant animals (Shemesh et al., 2013) and genetic loss of the germline suppressed nuclear stress body formation with age (Deonarine et al., 2021), which suggests alterations in gonadal stem cell signaling cell non-autonomously reprograms somatic maintenance, at least in part through HSF-1 activity. Interestingly, genetic perturbation of the extracellular vitelline layer of an internally fertilized embryo initiates a transcellular signal to improve proteostasis and stress resistance in an HSF-1 dependent manner (Sala et al., 2020). Thus, HSF-1 links metabolic reprograming and stress signals from the germline to improved proteostasis in the Soma and extend longevity.

FUTURE PERSPECTIVES

Science is an iterative process, where discoveries in one model system informs and generates new hypotheses best tested in others. Emerging technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR) and single-cell RNA sequencing, along with the development of large datasets (e.g., human sequence data), and the field of systems biology hold the promise of translating discoveries made in model organisms to ultimately improve human healthspan. Conversely, model organisms provide a powerful platform to segregate causative mutations from correlations identified within human sequence variation; and can quickly elucidate gene function within an intact metazoan. The proteostasis field is currently undergoing a transformation thanks to the development of AlphaFold, an artificial intelligence program that can accurately predict protein structure (Jumper et al., 2021), which will ultimately provide a predictive iterative framework to merge findings in genetics, structural biology,

and biochemistry. These are just a few examples of how advancing technical innovation in the biological sciences will be applied to further unify our understanding of how aging negatively impacts biological systems.

Work in *C. elegans* has been instrumental in understanding the basis of aging, and is well positioned to remain a premiere system of discovery for years to come. For example, in the specific area of transcriptional control of proteostasis, HSF1 regulation through multiple post-translational modifications and direct interactors, reveals the underlying complexity required to maintain proteome function, yet surprisingly little is known about the specific HSF-1 residues that modified within an intact multicellular organism, such as *C. elegans*. Conversely, whether the longevity signals that converge on HSF-1 to delay *C. elegans* aging have analogous levels of regulation in mammals remains an area of exploration. Discovering how HSF-1 is regulated in a cell-type specific manner within an intact multicellular organism would provide insight into how extrinsic and intrinsic signals are integrated *via* HSF-1 to regulate the PN, and how this system breaks down during normal aging. As an integration point for diverse signals of metabolic, mitogenic, and proteotoxic stress; distinguishing between modifications acting as molecular switches, versus providing rheostat regulation to fine-tune gene expression levels, those responsible for selective gene expression and tissue specific regulation, could be paramount in guiding the development of strategies to extend human healthspan.

The past 30 years of aging research has led to a wealth of discovery into the major regulatory mechanisms that have a deterministic role on organismal aging, the molecular and cellular hallmarks of aging, and how organisms respond to myriad forms of stress. Major challenges will be to elucidate how organisms, as biological systems, integrate signals and coordinate adaptive responses to maintain homeostasis, how these processes breakdown during aging, and whether greater understanding will facilitate the development of strategies to maximize human health span and minimize the onset and progression of age-associated diseases.

AUTHOR CONTRIBUTIONS

AVS conceived and wrote the manuscript. MIP, ZCW, SY, and AS made significant intellectual contributions and major contributions to writing specific sections of the manuscript. AM and CS made significant intellectual contributions and minor contributions to writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fragi.2022.861686/full#supplementary-material

REFERENCES

Abravaya, K., Myers, M. P., Murphy, S. P., and Morimoto, R. I. (1992). The Human Heat Shock Protein Hsp70 Interacts with HSF, the Transcription Factor that Regulates Heat Shock Gene Expression. *Genes Dev.* 6, 1153–1164. doi:10.1101/gad.6.7.1153

Ahn, S.-G., and Thiele, D. J. (2003). Redox Regulation of Mammalian Heat Shock Factor 1 Is Essential for Hsp Gene Activation and Protection from Stress. *Genes Dev.* 17, 516–528. doi:10.1101/gad.1044503

Ailion, M., and Thomas, J. H. (2000). Dauer Formation Induced by High Temperatures in *Caenorhabditis elegans*. *Genetics* 156, 1047–1067. doi:10.1093/genetics/156.3.1047

Ailion, M., and Thomas, J. H. (2003). Isolation and Characterization of High-Temperature-Induced Dauer Formation Mutants in *Caenorhabditis elegans*. *Genetics* 165, 127–144. doi:10.1093/genetics/165.1.127

Åkerfelt, M., Morimoto, R. I., and Sistonen, L. (2010). Heat Shock Factors: Integrators of Cell Stress, Development and Lifespan. *Nat. Rev. Mol. Cell Biol.* 11, 545–555. doi:10.1038/nrm2938

Alastalo, T.-P., Hellesuo, M., Sandqvist, A., Hietakangas, V., Kallio, M., and Sistonen, L. (2003). Formation of Nuclear Stress Granules Involves HSF2 and Coincides with the Nucleolar Localization of Hsp70. *J. Cell Sci.* 116, 3557–3570. doi:10.1242/jcs.00671

Aman, Y., Schmauck-Medina, T., Hansen, M., Morimoto, R. I., Simon, A. K., Bjedov, I., et al. (2021). Autophagy in Healthy Aging and Disease. *Nat. Aging* 1, 634–650. doi:10.1038/s43587-021-00098-4

An, J. H., and Blackwell, T. K. (2003). SKN-1 Links *C. elegans* Mesendodermal Specification to a Conserved Oxidative Stress Response. *Genes Dev.* 17, 1882–1893. doi:10.1101/gad.1107803

Anckar, J., Hietakangas, V., Denessiouk, K., Thiele, D. J., Johnson, M. S., and Sistonen, L. (2006). Inhibition of DNA Binding by Differential Sumoylation of Heat Shock Factors. *Mol. Cell Biol.* 26, 955–964. doi:10.1128/mcb.26.3.955-964.2006

Anckar, J., and Sistonen, L. (2011). Regulation of HSF1 Function in the Heat Stress Response: Implications in Aging and Disease. *Annu. Rev. Biochem.* 80, 1089–1115. doi:10.1146/annurev-biochem-060809-095203

Anderson, R. M., Bitterman, K. J., Wood, J. G., Medvedik, O., and Sinclair, D. A. (2003). Nicotinamide and PNC1 Govern Lifespan Extension by Calorie Restriction in *Saccharomyces cerevisiae*. *Nature* 423, 181–185. doi:10.1038/nature01578

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

Antikainen, H., Driscoll, M., Haspel, G., and Dobrowolski, R. (2017). TOR-mediated Regulation of Metabolism in Aging. *Aging Cell* 16, 1219–1233. doi:10.1111/acel.12689

Apfeld, J., O'Connor, G., McDonagh, T., DiStefano, P. S., and Curtis, R. (2004). The AMP-Activated Protein Kinase AAK-2 Links Energy Levels and Insulin-like Signals to Lifespan in *C. elegans*. *Genes Dev.* 18, 3004–3009. doi:10.1101/gad.1255404

Arai, Y., Kojima, T., Takayama, M., and Hirose, N. (2009). The Metabolic Syndrome, IGF-1, and Insulin Action. *Mol. Cell Endocrinol.* 299, 124–128. doi:10.1016/j.mce.2008.07.002

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

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

Baird, N. A., Douglas, P. M., Simic, M. S., Grant, A. R., Moresco, J. J., Wolff, S. C., et al. (2014). HSF-1-mediated Cytoskeletal Integrity Determines Thermotolerance and Life Span. *Science* 346, 360–363. doi:10.1126/science.1253168

Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008). Adapting Proteostasis for Disease Intervention. *Science* 319, 916–919. doi:10.1126/science.1141448

Balchin, D., Hayer-Hartl, M., and Hartl, F. U. (2016). *In Vivo* aspects of Protein Folding and Quality Control. *Science* 353, aac4354. doi:10.1126/science.aac4354

Baler, R., Welch, W. J., and Voellmy, R. (1992). Heat Shock Gene Regulation by Nascent Polypeptides and Denatured Proteins: Hsp70 as a Potential Autoregulatory Factor. *J. Cell Biol.* 117, 1151–1159. doi:10.1083/jcb.117.6.1151

Baler, R., Zou, J., and Voellmy, R. (1996). Evidence for a Role of Hsp70 in the Regulation of the Heat Shock Response in Mammalian Cells. *Cell stress chaperones* 1, 33–39. doi:10.1379/1466-1268(1996)001<0033:efaroh>2.3.co;2

Bandhakavi, S., Xie, H., O'Callaghan, B., Sakurai, H., Kim, D. H., and Griffin, T. J. (2008). Hsf1 Activation Inhibits Rapamycin Resistance and TOR Signaling in Yeast Revealed by Combined Proteomic and Genetic Analysis. *PLoS One* 3, e1598. doi:10.1371/journal.pone.0001598

Bar-Lavan, Y., Shemesh, N., and Ben-Zvi, A. (2016). Chaperone Families and Interactions in Metazoa. *Essays Biochem.* 60, 237–253. doi:10.1042/ebc20160004

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

Benbrook, D. M., and Long, A. (2012). Integration of Autophagy, Proteasomal Degradation, Unfolded Protein Response and Apoptosis. *Exp. Oncol.* 34, 286–297.

Berendzen, K. M., Durieux, J., Shao, L. W., Tian, Y., Kim, H. E., Wolff, S., et al. (2016). Neuroendocrine Coordination of Mitochondrial Stress Signaling and Proteostasis. *Cell* 166, 1553–1563 e1510. doi:10.1016/j.cell.2016.08.042

Berman, J. R., and Kenyon, C. (2006). Germ-cell Loss Extends *C. elegans* Life Span through Regulation of DAF-16 by Kri-1 and Lipophilic-Hormone Signaling. *Cell* 124, 1055–1068. doi:10.1016/j.cell.2006.01.039

Biebl, M. M., and Buchner, J. (2019). Structure, Function, and Regulation of the Hsp90 Machinery. *Cold Spring Harb. Perspect. Biol.* 11. doi:10.1101/cshperspect.a034017

Bishop, N. A., and Guarente, L. (2007). Two Neurons Mediate Diet-Restriction-Induced Longevity in *C. elegans*. *Nature* 447, 545–549. doi:10.1038/nature05904

Bjork, J. K., and Sistonen, L. (2010). Regulation of the Members of the Mammalian Heat Shock Factor Family. *FEBS J.* 277, 4126–4139. doi:10.1111/j.1742-4658.2010.07828.x

Blackwell, T. K., Sewell, A. K., Wu, Z., and Han, M. (2019). TOR Signaling in *Caenorhabditis elegans* Development, Metabolism, and Aging. *Genetics* 213, 329–360. doi:10.1534/genetics.119.302504

Boellmann, F., Guettouche, T., Guo, Y., Fenna, M., Mnayer, L., and Voellmy, R. (2004). DAXX Interacts with Heat Shock Factor 1 during Stress Activation and Enhances its Transcriptional Activity. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4100–4105. doi:10.1073/pnas.0304768101

Borghesan, M., Hoogaars, W. M. H., Varela-Eirin, M., Talma, N., and Demaria, M. (2020). A Senescence-Centric View of Aging: Implications for Longevity and Disease. *Trends Cell Biol.* 30, 777–791. doi:10.1016/j.tcb.2020.07.002

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

Brignull, H. R., Moore, F. E., Tang, S. J., and Morimoto, R. I. (2006). Polyglutamine Proteins at the Pathogenic Threshold Display Neuron-specific Aggregation in a Pan-Neuronal *Caenorhabditis elegans* Model. *J. Neurosci.* 26, 7597–7606. doi:10.1523/jneurosci.0990-06.2006

Brown, J. R., and Lupas, A. N. (1998). What Makes a Thermophile? *Trends Microbiol.* 6, 349–350. doi:10.1016/s0966-842x(98)01351-1

Brunquell, J., Raynes, R., Bowers, P., Morris, S., Snyder, A., Lugano, D., et al. (2018). CCAR-1 Is a Negative Regulator of the Heat-Shock Response in *Caenorhabditis elegans*. *Aging Cell* 17, e12813. doi:10.1111/acel.12813

Budzynski, M. A., Puustinen, M. C., Joutsen, J., and Sistonen, L. (2015). Uncoupling Stress-Inducible Phosphorylation of Heat Shock Factor 1 from its Activation. *Mol. Cell Biol.* 35, 2530–2540. doi:10.1128/MCB.00816-14

Butcher, R. A., Fujita, M., Schroeder, F. C., and Clardy, J. (2007). Small-molecule Pheromones that Control Dauer Development in *Caenorhabditis elegans*. *Nat. Chem. Biol.* 3, 420–422. doi:10.1038/nchembio.2007.3

Calvo-Ochoa, E., and Arias, C. (2015). Cellular and Metabolic Alterations in the hippocampus Caused by Insulin Signalling Dysfunction and its Association with Cognitive Impairment during Aging and Alzheimer's Disease: Studies in Animal Models. *Diabetes Metab. Res. Rev.* 31, 1–13. doi:10.1002/dmrr.2531

Cassada, R. C., and Russell, R. L. (1975). The Dauerlarva, a Post-embryonic Developmental Variant of the Nematode *Caenorhabditis elegans*. *Dev. Biol.* 46, 326–342. doi:10.1016/0012-1606(75)90109-8

Chang, J. T., Kumsta, C., Hellman, A. B., Adams, L. M., and Hansen, M. (2017). Spatiotemporal Regulation of Autophagy during *Caenorhabditis elegans* Aging. *Elife* 6. doi:10.7554/eLife.18459

Chiang, W. C., Ching, T. T., Lee, H. C., Mousigian, C., and Hsu, A. L. (2012). HSF-1 Regulators DDL-1/2 Link Insulin-like Signaling to Heat-Shock Responses and Modulation of Longevity. *Cell* 148, 322–334. doi:10.1016/j.cell.2011.12.019

Chisnell, P., Parenteau, T. R., Tank, E., Ashrafi, K., and Kenyon, C. (2018). The mTOR Target S6 Kinase Arrests Development in *Caenorhabditis elegans* when the Heat-Shock Transcription Factor Is Impaired. *Genetics* 210, 999–1009. doi:10.1534/genetics.118.301533

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

Chou, S. D., Prince, T., Gong, J., and Calderwood, S. K. (2012). mTOR Is Essential for the Proteotoxic Stress Response, HSF1 Activation and Heat Shock Protein Synthesis. *PLoS One* 7, e39679. doi:10.1371/journal.pone.0039679

Chu, B., Soncin, F., Price, B. D., Stevenson, M. A., and Calderwood, S. K. (1996). Sequential Phosphorylation by Mitogen-Activated Protein Kinase and Glycogen Synthase Kinase 3 Represses Transcriptional Activation by Heat Shock Factor-1. *J. Biol. Chem.* 271, 30847–30857. doi:10.1074/jbc.271.48.30847

Chu, B., Zhong, R., Soncin, F., Stevenson, M. A., and Calderwood, S. K. (1998). Transcriptional Activity of Heat Shock Factor 1 at 37 Degrees C Is Repressed through Phosphorylation on Two Distinct Serine Residues by Glycogen Synthase Kinase 3 and Protein Kinases Calpha and Czeta. *J. Biol. Chem.* 273, 18640–18646. doi:10.1074/jbc.273.29.18640

Clark, D. A., Gabel, C. V., Gabel, H., and Samuel, A. D. (2007). Temporal Activity Patterns in Thermosensory Neurons of Freely Moving *Caenorhabditis elegans* Encode Spatial Thermal Gradients. *J. Neurosci.* 27, 6083–6090. doi:10.1523/jneurosci.1032-07.2007

Clos, J., Westwood, J. T., Becker, P. B., Wilson, S., Lambert, K., and Wu, C. (1990). Molecular Cloning and Expression of a Hexameric Drosophila Heat Shock Factor Subject to Negative Regulation. *Cell* 63, 1085–1097. doi:10.1016/0092-8674(90)90511-c

Cohen, E., and Dillin, A. (2008). The Insulin Paradox: Aging, Proteotoxicity and Neurodegeneration. *Nat. Rev.* 9, 759–767. doi:10.1038/nrn2474

Connell, P., Ballinger, C. A., Jiang, J., Wu, Y., Thompson, L. J., Hohfeld, J., et al. (2001). The Co-chaperone CHIP Regulates Protein Triage Decisions

Mediated by Heat-Shock Proteins. *Nat. Cell Biol.* 3, 93–96. doi:10.1038/35050618

Cotto, J. J., Kline, M., and Morimoto, R. I. (1996). Activation of Heat Shock Factor 1 DNA Binding Precedes Stress-Induced Serine Phosphorylation. Evidence for a Multistep Pathway of Regulation. *J. Biol. Chem.* 271, 3355–3358. doi:10.1074/jbc.271.7.3355

Cubenas-Potts, C., and Matunis, M. J. (2013). SUMO: a Multifaceted Modifier of Chromatin Structure and Function. *Dev. Cell* 24, 1–12. doi:10.1016/j.devcel.2012.11.020

D'Amico, S., Collins, T., Marx, J. C., Feller, G., and Gerday, C. (2006). Psychrophilic Microorganisms: Challenges for Life. *EMBO Rep.* 7, 385–389. doi:10.1038/sj.embor.7400662

Dai, H., Sinclair, D. A., Ellis, J. L., and Steegborn, C. (2018). Sirtuin Activators and Inhibitors: Promises, Achievements, and Challenges. *Pharmacol. Ther.* 188, 140–154. doi:10.1016/j.pharmthera.2018.03.004

Dai, Q., Zhang, C., Wu, Y., McDonough, H., Whaley, R. A., Godfrey, V., et al. (2003). CHIP Activates HSF1 and Confers Protection against Apoptosis and Cellular Stress. *EMBO J.* 22, 5446–5458. doi:10.1093/emboj/cdg529

Dai, R., Frejtag, W., He, B., Zhang, Y., and Mivechi, N. F. (2000). c-Jun NH2-terminal Kinase Targeting and Phosphorylation of Heat Shock Factor-1 Suppress its Transcriptional Activity. *J. Biol. Chem.* 275, 18210–18218. doi:10.1074/jbc.m000958200

Dai, S., Tang, Z., Cao, J., Zhou, W., Li, H., Sampson, S., et al. (2015). Suppression of the HSF1-Mediated Proteotoxic Stress Response by the Metabolic Stress Sensor AMPK. *EMBO J.* 34, 275–293. doi:10.15252/embj.201489062

Das, R., Melo, J. A., Thondamal, M., Morton, E. A., Cornwell, A. B., Crick, B., et al. (2017). The Homeodomain-Interacting Protein Kinase HPK-1 Preserves Protein Homeostasis and Longevity through Master Regulatory Control of the HSF-1 Chaperone Network and TORC1-Restricted Autophagy in *Caenorhabditis elegans*. *PLoS Genet.* 13, e1007038. doi:10.1371/journal.pgen.1007038

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

Dayalan Naidu, S., and Dinkova-Kostova, A. T. (2017). Regulation of the Mammalian Heat Shock Factor 1. *FEBS J.* 284, 1606–1627. doi:10.1111/febs.13999

Denzel, M. S., Lapierre, L. R., and Mack, H. I. D. (2019). Emerging Topics in *C. elegans* Aging Research: Transcriptional Regulation, Stress Response and Epigenetics. *Mech. Ageing Dev.* 177, 4–21. doi:10.1016/j.mad.2018.08.001

Deonarine, A., Walker, M. W. G., and Westerheide, S. D. (2021). HSF-1 Displays Nuclear Stress Body Formation in Multiple Tissues in *Caenorhabditis elegans* upon Stress and Following the Transition to Adulthood. *Cell stress chaperones* 26, 417–431. doi:10.1007/s12192-020-01188-9

Depuydt, G., Xie, F., Petyuk, V. A., Shanmugam, N., Smolders, A., Dhondt, I., et al. (2013). Reduced Insulin/insulin-like Growth Factor-1 Signaling and Dietary Restriction Inhibit Translation but Preserve Muscle Mass in *Caenorhabditis elegans*. *Mol. Cell Proteomics* 12, 3624–3639. doi:10.1074/mcp.m113.027383

Derivery, E., Sousa, C., Gautier, J. J., Lombard, B., Loew, D., and Gautreau, A. (2009). The Arp2/3 Activator WASH Controls the Fission of Endosomes through a Large Multiprotein Complex. *Dev. Cell* 17, 712–723. doi:10.1016/j.devcel.2009.09.010

Desai, S., Liu, Z., Yao, J., Patel, N., Chen, J., Wu, Y., et al. (2013). Heat Shock Factor 1 (HSF1) Controls Chemoresistance and Autophagy through Transcriptional Regulation of Autophagy-Related Protein 7 (ATG7). *J. Biol. Chem.* 288, 9165–9176. doi:10.1074/jbc.m112.422071

Deyrieux, A. F., and Wilson, V. G. (2017). Sumoylation in Development and Differentiation. *Adv. Exp. Med. Biol.* 963, 197–214. doi:10.1007/978-3-319-50044-7_12

Dillin, A., Gottschling, D. E., and Nystrom, T. (2014). The Good and the Bad of Being Connected: the Integrons of Aging. *Curr. Opin. Cell Biol.* 26, 107–112. doi:10.1016/j.ceb.2013.12.003

Dorman, J. B., Albinder, B., Shroyer, T., and Kenyon, C. (1995). The Age-1 and Daf-2 Genes Function in a Common Pathway to Control the Lifespan of *Caenorhabditis elegans*. *Genetics* 141, 1399–1406. doi:10.1093/genetics/141.4.1399

Douglas, P. M., Baird, N. A., Simic, M. S., Uhlein, S., McCormick, M. A., Wolff, S. C., et al. (2015). Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Longevity. *Cell Rep.* 12, 1196–1204. doi:10.1016/j.celrep.2015.07.026

Dues, D. J., Andrews, E. K., Schaar, C. E., Bergsma, A. L., Senchuk, M. M., and Van Raamsdonk, J. M. (2016). Aging Causes Decreased Resistance to Multiple Stresses and a Failure to Activate Specific Stress Response Pathways. *Aging (Albany NY)* 8, 777–795. doi:10.18632/aging.100939

Dufey, E., Sepulveda, D., Rojas-Rivera, D., and Hetz, C. (2014). Cellular Mechanisms of Endoplasmic Reticulum Stress Signaling in Health and Disease. 1. An Overview. *Am. J. Physiol. Cell Physiol.* 307, C582–C594. doi:10.1152/ajpcell.00258.2014

Duina, A. A., Kalton, H. M., and Gaber, R. F. (1998). Requirement for Hsp90 and a CyP-40-type Cyclophilin in Negative Regulation of the Heat Shock Response. *J. Biol. Chem.* 273, 18974–18978. doi:10.1074/jbc.273.30.18974

Durieux, J., Wolff, S., and Dillin, A. (2011). The Cell-Non-Autonomous Nature of Electron Transport Chain-Mediated Longevity. *Cell* 144, 79–91. doi:10.1016/j.cell.2010.12.016

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

Epel, E. S., and Lithgow, G. J. (2014). Stress Biology and Aging Mechanisms: toward Understanding the Deep Connection between Adaptation to Stress and Longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* 69 (Suppl. 1), S10–S16. doi:10.1093/gerona/glu055

Farkas, T., Kutskova, Y. A., and Zimarino, V. (1998). Intramolecular Repression of Mouse Heat Shock Factor 1. *Mol. Cell Biol.* 18, 906–918. doi:10.1128/mcb.18.2.906

Felix, M. A., and Duveau, F. (2012). Population Dynamics and Habitat Sharing of Natural Populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol.* 10, 59. doi:10.1186/1741-7007-10-59

Fielenbach, N., and Antebi, A. (2008). *C. elegans* Dauer Formation and the Molecular Basis of Plasticity. *Genes Dev.* 22, 2149–2165. doi:10.1101/gad.1701508

Finley, D. (2009). Recognition and Processing of Ubiquitin-Protein Conjugates by the Proteasome. *Annu. Rev. Biochem.* 78, 477–513. doi:10.1146/annurev.biochem.78.081507.101607

Frezal, L., and Felix, M. A. (2015). *C. elegans* outside the Petri Dish. *Elife* 4. doi:10.7554/eLife.05849

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

Garcia, S. M., Casanueva, M. O., Silva, M. C., Amaral, M. D., and Morimoto, R. I. (2007). Neuronal Signaling Modulates Protein Homeostasis in *Caenorhabditis elegans* Post-synaptic Muscle Cells. *Genes Dev.* 21, 3006–3016. doi:10.1101/gad.1575307

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

Garrett, S., Menold, M. M., and Broach, J. R. (1991). The *Saccharomyces cerevisiae* YAK1 Gene Encodes a Protein Kinase that Is Induced by Arrest Early in the Cell Cycle. *Mol. Cell Biol.* 11, 4045–4052. doi:10.1128/mcb.11.8.4045-4052.1991

Gems, D., Sutton, A. J., Sundermeyer, M. L., Albert, P. S., King, K. V., Edgley, M. L., 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

Ghazi, A., Henis-Korenblit, S., and Kenyon, C. (2007). Regulation of *Caenorhabditis elegans* Lifespan by a Proteasomal E3 Ligase Complex. *Proc. Natl. Acad. Sci. U. S. A.* 104, 5947–5952. doi:10.1073/pnas.0700638104

Gidalevitz, T., Ben-Zvi, A., Ho, K. H., Brignull, H. R., and Morimoto, R. I. (2006). Progressive Disruption of Cellular Protein Folding in Models of Polyglutamine Diseases. *Science* 311, 1471–1474. doi:10.1126/science.1124514

Gidalevitz, T., Prahlad, V., and Morimoto, R. I. (2011). The Stress of Protein Misfolding: from Single Cells to Multicellular Organisms. *Cold Spring Harb. Perspect. Biol.* 3. doi:10.1101/cshperspect.a009704

Gill, G. (2005). Something about SUMO Inhibits Transcription. *Curr. Opin. Genet. Dev.* 15, 536–541. doi:10.1016/j.gde.2005.07.004

Glover-Cutter, K. M., Lin, S., and Blackwell, T. K. (2013). Integration of the Unfolded Protein and Oxidative Stress Responses through SKN-1/Nrf. *PLoS Genet.* 9, e1003701. doi:10.1371/journal.pgen.1003701

Golden, J. W., and Riddle, D. L. (1984b). A Pheromone-Induced Developmental Switch in *Caenorhabditis elegans*: Temperature-Sensitive Mutants Reveal a Wild-type Temperature-dependent Process. *Proc. Natl. Acad. Sci. U. S. A.* 81, 819–823. doi:10.1073/pnas.81.3.819

Golden, J. W., and Riddle, D. L. (1984a). The *Caenorhabditis elegans* Dauer Larva: Developmental Effects of Pheromone, Food, and Temperature. *Dev. Biol.* 102, 368–378. doi:10.1016/0012-1606(84)90201-x

Gomez-Pastor, R., Burchfiel, E. T., and Thiele, D. J. (2018). Regulation of Heat Shock Transcription Factors and Their Roles in Physiology and Disease. *Nat. Rev. Mol. Cell Biol.* 19, 4–19. doi:10.1038/nrm.2017.73

Goodson, M. L., and Sarge, K. D. (1995). Heat-inducible DNA Binding of Purified Heat Shock Transcription Factor 1. *J. Biol. Chem.* 270, 2447–2450. doi:10.1074/jbc.270.6.2447

Gottlieb, S., and Ruvkun, G. (1994). daf-2, Daf-16 and Daf-23: Genetically Interacting Genes Controlling Dauer Formation in *Caenorhabditis elegans*. *Genetics* 137, 107–120. doi:10.1093/genetics/137.1.107

Greer, E. L., Banko, M. R., and Brunet, A. (2009). AMP-activated Protein Kinase and FoxO Transcription Factors in Dietary Restriction-Induced Longevity. *Ann. N. Y. Acad. Sci.* 1170, 688–692. doi:10.1111/j.1749-6632.2009.04019.x

Greer, E. L., and Brunet, A. (2009). Different Dietary Restriction Regimens Extend Lifespan by Both Independent and Overlapping Genetic Pathways in *C. elegans*. *Aging Cell* 8, 113–127. doi:10.1111/j.1474-9726.2009.00459.x

Greer, E. L., Dowlatshahi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., et al. (2007). An AMPK-FOXO Pathway Mediates Longevity Induced by a Novel Method of Dietary Restriction in *C. elegans*. *Curr. Biol.* 17, 1646–1656. doi:10.1016/j.cub.2007.08.047

Guettouche, T., Boellmann, F., Lane, W. S., and Voellmy, R. (2005). Analysis of Phosphorylation of Human Heat Shock Factor 1 in Cells Experiencing a Stress. *BMC Biochem.* 6, 4. doi:10.1186/1471-2091-6-4

Guisbert, E., Czyz, D. M., Richter, K., McMullen, P. D., and Morimoto, R. I. (2013). Identification of a Tissue-Selective Heat Shock Response Regulatory Network. *PLoS Genet.* 9, e1003466. doi:10.1371/journal.pgen.1003466

Guo, Y., Guettouche, T., Fenna, M., Boellmann, F., Pratt, W. B., Toft, D. O., et al. (2001). Evidence for a Mechanism of Repression of Heat Shock Factor 1 Transcriptional Activity by a Multichaperone Complex. *J. Biol. Chem.* 276, 45791–45799. doi:10.1074/jbc.m105931200

Hageman, J., and Kampinga, H. H. (2009). Computational Analysis of the Human HSPH/HSPA/DNAJ Family and Cloning of a Human HSPH/HSPA/DNAJ Expression Library. *Cell stress chaperones* 14, 1–21. doi:10.1007/s12192-008-0060-2

Hajdu-Cronin, Y. M., Chen, W. J., and Sternberg, P. W. (2004). The L-Type Cyclin CYL-1 And The Heat-Shock-Factor HSF-1 Are Required For Heat-Shockinduced Protein Expression In Caenorhabditis Elegans. *Genetics* 168, 1937–1949. doi:10.1534/genetics.104.028423

Halaschek-Wiener, J., Khattra, J. S., McKay, S., Pouzyrev, A., Stott, J. M., Yang, G. S., et al. (2005). Analysis of Long-Lived *C. elegans* Daf-2 Mutants Using Serial Analysis of Gene Expression. *Genome Res.* 15, 603–615. Epub 2005 Apr 2018. doi:10.1101/gr.3274805

Hamiel, C. R., Pinto, S., Hau, A., and Wischmeyer, P. E. (2009). Glutamine Enhances Heat Shock Protein 70 Expression Via Increased Hexosamine Biosynthetic Pathway Activity. *Am. J. Physiol. Cell Physiol.* 297, C1509–C1519. doi:10.1152/ajpcell.00240.2009

Hansen, M., Chandra, A., Mitic, L. L., Onken, B., Driscoll, M., and Kenyon, C. (2008). A Role for Autophagy in the Extension of Lifespan by Dietary Restriction in *C. elegans*. *PLoS Genet.* 4, e24. doi:10.1371/journal.pgen.0040024

Hansen, M., Hsu, A. L., Dillin, A., and Kenyon, C. (2005). New Genes Tied to Endocrine, Metabolic, and Dietary Regulation of Lifespan from a *Caenorhabditis elegans* Genomic RNAi Screen. *PLoS Genet.* 1, 119–128. doi:10.1371/journal.pgen.0010017

Hartl, F. U., and Hayer-Hartl, M. (2002). Molecular Chaperones in the Cytosol: from Nascent Chain to Folded Protein. *Science* 295, 1852–1858. doi:10.1126/science.1068408

Hartley, A. D., Ward, M. P., and Garrett, S. (1994). The Yak1 Protein Kinase of *Saccharomyces cerevisiae* Moderates Thermotolerance and Inhibits Growth by an Sch9 Protein Kinase-independent Mechanism. *Genetics* 136, 465–474. doi:10.1093/genetics/136.2.465

He, B., Meng, Y. H., and Mivechi, N. F. (1998). Glycogen Synthase Kinase 3beta and Extracellular Signal-Regulated Kinase Inactivate Heat Shock Transcription Factor 1 by Facilitating the Disappearance of Transcriptionally Active Granules after Heat Shock. *Mol. Cell Biol.* 18, 6624–6633. doi:10.1128/mcb.18.11.6624

He, C., and Klionsky, D. J. (2009). Regulation Mechanisms and Signaling Pathways of Autophagy. *Annu. Rev. Genet.* 43, 67–93. doi:10.1146/annurev-genet-102808-114910

Hendriks, I. A., Lyon, D., Young, C., Jensen, L. J., Vertegaal, A. C., and Nielsen, M. L. (2017). Site-specific Mapping of the Human SUMO Proteome Reveals Co-modification with Phosphorylation. *Nat. Struct. Mol. Biol.* 24, 325–336. doi:10.1038/nsmb.3366

Hentze, N., Le Breton, L., Wiesner, J., Kempf, G., and Mayer, M. P. (2016). Molecular Mechanism of Thermosensory Function of Human Heat Shock Transcription Factor Hsf1. *Elife* 5. doi:10.7554/eLife.11576

Hietakangas, V., Ahlskog, J. K., Jakobsson, A. M., Hellesuo, M., Sahlberg, N. M., Holmberg, C. I., et al. (2003). Phosphorylation of Serine 303 Is a Prerequisite for the Stress-Inducible SUMO Modification of Heat Shock Factor 1. *Mol. Cell Biol.* 23, 2953–2968. doi:10.1128/mcb.23.8.2953-2968.2003

Hietakangas, V., Anckar, J., Blomster, H. A., Fujimoto, M., Palvimo, J. J., Nakai, A., et al. (2006). PDSM, a Motif for Phosphorylation-dependent SUMO Modification. *Proc. Natl. Acad. Sci. U. S. A.* 103, 45–50. doi:10.1073/pnas.0503698102

Higuchi-Sanabria, R., Durieux, J., Kelet, N., Homentcovschi, S., de Los Rios Rogers, M., Monshietehadi, S., et al. (2020). Divergent Nodes of Non-autonomous UPR(ER) Signaling through Serotonergic and Dopaminergic Neurons. *Cell Rep.* 33, 108489. doi:10.1016/j.celrep.2020.108489

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

Holmberg, C. I., Hietakangas, V., Mikhailov, A., Rantanen, J. O., Kallio, M., Meinander, A., et al. (2001). Phosphorylation of Serine 230 Promotes Inducible Transcriptional Activity of Heat Shock Factor 1. *EMBO J.* 20, 3800–3810. doi:10.1093/emboj/20.14.3800

Holmberg, C. I., Tran, S. E., Eriksson, J. E., and Sistonen, L. (2002). Multisite Phosphorylation Provides Sophisticated Regulation of Transcription Factors. *Trends Biochem. Sci.* 27, 619–627. doi:10.1016/s0968-0004(02)02207-7

Holzenberger, M., Dupont, J., Ducos, B., Leneuve, P., Geloen, A., Even, P. C., et al. (2003). IGF-1 Receptor Regulates Lifespan and Resistance to Oxidative Stress in Mice. *Nature* 421, 182–187. doi:10.1038/nature01298

Hong, Y., Rogers, R., Matunis, M. J., Mayhew, C. N., Goodson, M. L., Park-Sarge, O. K., et al. (2001). Regulation of Heat Shock Transcription Factor 1 by Stress-Induced SUMO-1 Modification. *J. Biol. Chem.* 276, 40263–40267. doi:10.1074/jbc.m104714200

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

Hu, Y., and Mivechi, N. F. (2003). HSF-1 Interacts with Ral-Binding Protein 1 in a Stress-Responsive, Multiprotein Complex with HSP90 *In Vivo*. *J. Biol. Chem.* 278, 17299–17306. doi:10.1074/jbc.m300788200

Hu, Y., and Mivechi, N. F. (2011). Promotion of Heat Shock Factor Hsf1 Degradation via Adaptor Protein Filamin A-Interacting Protein 1-like (FILIP-1L). *J. Biol. Chem.* 286, 31397–31408. doi:10.1074/jbc.m111.255851

Huang, C.-Y., Lee, F.-L., Peng, S.-F., Lin, K.-H., Chen, R.-J., Ho, T.-J., et al. (2018). HSF1 Phosphorylation By ERK/GSK3 Suppressesrnf126 To Sustain IGF-IIR Expression For Hypertension-Inducedcardiomyocyte Hypertrophy. *J. Cell Physiol.* 233, 979–989. doi:10.1002/jcp.25945

Hwang, A. B., Ryu, E. A., Artan, M., Chang, H. W., Kabir, M. H., Nam, H. J., et al. (2014). Feedback Regulation via AMPK and HIF-1 Mediates ROS-dependent Longevity in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 111, E4458–E4467. doi:10.1073/pnas.1411199111

Jayaraj, G. G., Hipp, M. S., and Hartl, F. U. (2020). Functional Modules of the Proteostasis Network. *Cold Spring Harb. Perspect. Biol.* 12. doi:10.1101/cshperspect.a033951

Jedlicka, P., Mortin, M. A., and Wu, C. (1997). Multiple Functions of Drosophila Heat Shock Transcription Factor *In Vivo*. *EMBO J.* 16, 2452–2462. doi:10.1093/emboj/16.9.2452

Jedrusik-Bode, M. (2014). *C. elegans* Sirtuin SIR-2.4 and its Mammalian Homolog SIRT6 in Stress Response. *Worm* 3, e29102. doi:10.4161/worm.29102

Jedrusik-Bode, M., Studencka, M., Smolka, C., Baumann, T., Schmidt, H., Kampf, J., et al. (2013). The Sirtuin SIRT6 Regulates Stress Granule Formation in *C. elegans* and Mammals. *J. Cell Sci.* 126, 5166–5177. doi:10.1242/jcs.130708

Jia, K., Chen, D., and Riddle, D. L. (2004). The TOR Pathway Interacts with the Insulin Signaling Pathway to Regulate *C. elegans* Larval Development, Metabolism and Life Span. *Development* 131, 3897–3906. doi:10.1242/dev.01255

Jia, K., and Levine, B. (2007). Autophagy Is Required for Dietary Restriction-Mediated Life Span Extension in *C. elegans*. *Autophagy* 3, 597–599. doi:10.4161/auto.4989

Johnson, D. W., Llop, J. R., Farrell, S. F., Yuan, J., Stolzenburg, L. R., and Samuelson, A. V. (2014). The *Caenorhabditis elegans* Myc-Mondo/Mad Complexes Integrate Diverse Longevity Signals. *PLoS Genet.* 10, e1004278. doi:10.1371/journal.pgen.1004278

Johnson, T. E. (1990). Increased Life-Span of Age-1 Mutants in *Caenorhabditis elegans* and Lower Gompertz Rate of Aging. *Science* 249, 908–912. doi:10.1126/science.2392681

Jones, D., Crowe, E., Stevens, T. A., and Candido, E. P. (2002). Functional and Phylogenetic Analysis of the Ubiquitylation System in *Caenorhabditis elegans*: Ubiquitin-Conjugating Enzymes, Ubiquitin-Activating Enzymes, and Ubiquitin-like Proteins. *Genome Biol.* 3, RESEARCH0002. doi:10.1186/gb-2001-3-1-research0002

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

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., et al. (2021). Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* 596, 583–589. doi:10.1038/s41586-021-03819-2

Kamitani, T., Kito, K., Nguyen, H. P., Fukuda-Kamitani, T., and Yeh, E. T. (1998). Characterization of a Second Member of the Sentrin Family of Ubiquitin-like Proteins. *J. Biol. Chem.* 273, 11349–11353. doi:10.1074/jbc.273.18.11349

Kapahi, P., Chen, D., Rogers, A. N., Katewa, S. D., Li, P. W., Thomas, E. L., et al. (2010). With TOR, Less Is More: a Key Role for the Conserved Nutrient-Sensing TOR Pathway in Aging. *Cell metab.* 11, 453–465. doi:10.1016/j.cmet.2010.05.001

Karagoz, G. E., Acosta-Alvear, D., and Walter, P. (2019a). The Unfolded Protein Response: Detecting and Responding to Fluctuations in the Protein-Folding Capacity of the Endoplasmic Reticulum. *Cold Spring Harb. Perspect. Biol.* 11. doi:10.1101/cshperspect.a033886

Karagoz, G. E., Aragon, T., and Acosta-Alvear, D. (2019b). Recent Advances in Signal Integration Mechanisms in the Unfolded Protein Response. *F1000Res* 8. doi:10.12688/f1000research.19848.1

Kaushik, S., Tasset, I., Arias, E., Pampliega, O., Wong, E., Martinez-Vicente, M., et al. (2021). Autophagy and the Hallmarks of Aging. *Ageing Res. Rev.* 72, 101468. doi:10.1016/j.arr.2021.101468

Keisala, T., Minasyan, A., Lou, Y. R., Zou, J., Kalueff, A. V., Pyykko, I., et al. (2009). Premature Aging in Vitamin D Receptor Mutant Mice. *J. Steroid Biochem. Mol. Biol.* 115, 91–97. doi:10.1016/j.jsbmb.2009.03.007

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

Kenyon, C. (2011). The First Long-Lived Mutants: Discovery of the insulin/IGF-1 Pathway for Ageing. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 366, 9–16. doi:10.1098/rstb.2010.0276

Kenyon, C. (2005). The Plasticity of Aging: Insights from Long-Lived Mutants. *Cell* 120, 449–460. doi:10.1016/j.cell.2005.02.002

Kenyon, C. J. (2010). The Genetics of Ageing. *Nature* 464, 504–512. doi:10.1038/nature08980

Khaleque, M. A., Bharti, A., Gong, J., Gray, P. J., Sachdev, V., Ciocca, D. R., et al. (2008). Heat Shock Factor 1 Represses Estrogen-dependent Transcription through Association with MTA1. *Oncogene* 27, 1886–1893. doi:10.1038/sj.onc.1210834

Khaminets, A., Behl, C., and Dikic, I. (2016). Ubiquitin-Dependent and Independent Signals in Selective Autophagy. *Trends Cell Biol.* 26, 6–16. doi:10.1016/j.tcb.2015.08.010

Khaminets, A., Heinrich, T., Mari, M., Grumati, P., Huebner, A. K., Akutsu, M., et al. (2015). Regulation of Endoplasmic Reticulum Turnover by Selective Autophagy. *Nature* 522, 354–358. doi:10.1038/nature14498

Kim, H. W., Kim, S. A., and Ahn, S. G. (2016). Sirtuin Inhibitors, EX527 and AGK2, Suppress Cell Migration by Inhibiting HSF1 Protein Stability. *Oncol. Rep.* 35, 235–242. doi:10.3892/or.2015.4381

Kim, K. H., Jeong, Y. T., Oh, H., Kim, S. H., Cho, J. M., Kim, Y. N., et al. (2013). Autophagy Deficiency Leads to Protection from Obesity and Insulin Resistance by Inducing Fgf21 as a Mitokine. *Nat. Med.* 19, 83–92. doi:10.1038/nm.3014

Kim, S. A., Yoon, J. H., Lee, S. H., and Ahn, S. G. (2005). Polo-like Kinase 1 Phosphorylates Heat Shock Transcription Factor 1 and Mediates its Nuclear Translocation during Heat Stress. *J. Biol. Chem.* 280, 12653–12657. doi:10.1074/jbc.m411908200

Kimura, K. D., Tissenbaum, H. A., Liu, Y., and Ruvkun, G. (1997). daf-2, an Insulin Receptor-like Gene that Regulates Longevity and Diapause in *Caenorhabditis elegans*. *Science* 277, 942–946. doi:10.1126/science.277.5328.942

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

Kleemann, G. A., and Murphy, C. T. (2009). The Endocrine Regulation of Aging in *Caenorhabditis elegans*. *Mol. Cell Endocrinol.* 299, 51–57. doi:10.1016/j.mce.2008.10.048

Kleizen, B., and Braakman, I. (2004). Protein Folding and Quality Control in the Endoplasmic Reticulum. *Curr. Opin. Cell Biol.* 16, 343–349. doi:10.1016/j.ceb.2004.06.012

Kline, M. P., and Morimoto, R. I. (1997). Repression of the Heat Shock Factor 1 Transcriptional Activation Domain Is Modulated by Constitutive Phosphorylation. *Mol. Cell Biol.* 17, 2107–2115. doi:10.1128/mcb.17.4.2107

Knauf, U., Newton, E. M., Kyriakis, J., and Kingston, R. E. (1996). Repression of Human Heat Shock Factor 1 Activity at Control Temperature by Phosphorylation. *Genes Dev.* 10, 2782–2793. doi:10.1101/gad.10.21.2782

Kourtis, N., Moubarak, R. S., Aranda-Orgilles, B., Lui, K., Aydin, I. T., Trimarchi, T., et al. (2015). FBXW7 Modulates Cellular Stress Response and Metastatic Potential through HSF1 Post-translational Modification. *Nat. Cell Biol.* 17, 322–332. doi:10.1038/ncb3121

Kourtis, N., Nikoletopoulou, V., and Tavernarakis, N. (2012). Small Heat-Shock Proteins Protect from Heat-Stroke-Associated Neurodegeneration. *Nature* 490, 213–218. doi:10.1038/nature11417

Kovacs, D., Sigmond, T., Hotzi, B., Bohar, B., Fazekas, D., Deak, V., et al. (2019). HSF1Base: A Comprehensive Database of HSF1 (Heat Shock Factor 1) Target Genes. *Int. J. Mol. Sci.* 20. doi:10.3390/ijms20225815

Krakowiak, J., Zheng, X., Patel, N., Feder, Z. A., Anandhakumar, J., Valerius, K., et al. (2018). Hsf1 and Hsp70 Constitute a Two-Component Feedback Loop that Regulates the Yeast Heat Shock Response. *Elife* 7. doi:10.7554/eLife.31668

Kroemer, G., Marino, G., and Levine, B. (2010). Autophagy and the Integrated Stress Response. *Mol. Cell* 40, 280–293. doi:10.1016/j.molcel.2010.09.023

Kumsta, C., Chang, J. T., Schmalz, J., and Hansen, M. (2017). Hormetic Heat Stress and HSF-1 Induce Autophagy to Improve Survival and Proteostasis in *C. elegans*. *Nat. Commun.* 8, 14337. doi:10.1038/ncomms14337

Kumsta, C., Ching, T. T., Nishimura, M., Davis, A. E., Gelino, S., Catan, H. H., et al. (2014). Integrin-linked Kinase Modulates Longevity and Thermotolerance in *C. elegans* through Neuronal Control of HSF-1. *Aging Cell* 13, 419–430. doi:10.1111/acel.12189

Kundra, R., Ciryam, P., Morimoto, R. I., Dobson, C. M., and Vendruscolo, M. (2017). Protein Homeostasis of a Metastable Subproteome Associated with Alzheimer's Disease. *Proc. Natl. Acad. Sci. U. S. A.* 114, E5703–E5711. doi:10.1073/pnas.1618417114

Kuo, C. T., You, G. T., Jian, Y. J., Chen, T. S., Siao, Y. C., Hsu, A. L., et al. (2020). AMPK-mediated Formation of Stress Granules Is Required for Dietary Restriction-Induced Longevity in *Caenorhabditis elegans*. *Aging Cell* 19, e13157. doi:10.1111/acel.13157

Labbadia, J., and Morimoto, R. I. (2015b). 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

Labbadia, J., and Morimoto, R. I. (2015a). The Biology of Proteostasis in Aging and Disease. *Annu. Rev. Biochem.* 84, 435–464. doi:10.1146/annurev-biochem-060614-033955

Lamitina, S. T., and Strange, K. (2005). Transcriptional Targets of DAF-16 Insulin Signaling Pathway Protect *C. elegans* from Extreme Hypertonic Stress. *Am. J. Physiol. Cell Physiol.* 288, C467–C474. Epub 2004 Oct 2020. doi:10.1152/ajpcell.00451.2004

Lapierre, L. R., De Magalhaes Filho, C. D., McQuary, P. R., Chu, C. C., Visvikis, O., Chang, J. T., et al. (2013). The TFEB Orthologue HLH-30 Regulates Autophagy and Modulates Longevity in *Caenorhabditis elegans*. *Nat. Commun.* 4, 2267. doi:10.1038/ncomms3267

Lapierre, L. R., Gelino, S., Melendez, A., and Hansen, M. (2011). Autophagy and Lipid Metabolism Coordinately Modulate Life Span in Germline-Less *C. elegans*. *Curr. Biol.* 21, 1507–1514. doi:10.1016/j.cub.2011.07.042

Lapierre, L. R., Kumsta, C., Sandri, M., Ballabio, A., and Hansen, M. (2015). Transcriptional and Epigenetic Regulation of Autophagy in Aging. *Autophagy* 11, 867–880. doi:10.1080/15548627.2015.1034410

Larsen, P. L., Albert, P. S., and Riddle, D. L. (1995). Genes that Regulate Both Development and Longevity in *Caenorhabditis elegans*. *Genetics* 139, 1567–1583. doi:10.1093/genetics/139.4.1567

Lazetic, V., and Fay, D. S. (2017). Molting in *C. elegans*. *Worm* 6, e1330246.

Lee, R. Y., Hench, J., and Ruvkun, G. (2001). Regulation of *C. elegans* DAF-16 and its Human Ortholog FKHRL1 by the Daf-2 Insulin-like Signaling Pathway. *Curr. Biol.* 11, 1950–1957. doi:10.1016/s0960-9822(01)00595-4

Lee, Y. J., Kim, E. H., Lee, J. S., Jeoung, D., Bae, S., Kwon, S. H., et al. (2008). HSF1 as a Mitotic Regulator: Phosphorylation of HSF1 by Plk1 Is Essential for Mitotic Progression. *Cancer Res.* 68, 7550–7560. doi:10.1158/0008-5472.can-08-0129

Lehrbach, N. J., and Ruvkun, G. (2019). Endoplasmic Reticulum-Associated SKN-1A/Nrf1 Mediates a Cytoplasmic Unfolded Protein Response and Promotes Longevity. *Elife* 8. doi:10.7554/eLife.44425

Lehrbach, N. J., and Ruvkun, G. (2016). Proteasome Dysfunction Triggers Activation of SKN-1A/Nrf1 by the Aspartic Protease DDI-1. *Elife* 5. doi:10.7554/eLife.17721

Levine, B., and Kroemer, G. (2008). Autophagy in the Pathogenesis of Disease. *Cell* 132, 27–42. doi:10.1016/j.cell.2007.12.018

Li, J., Chauve, L., Phelps, G., Brielmann, R. M., and Morimoto, R. I. (2016). E2F Coregulates an Essential HSF Developmental Program that Is Distinct from the Heat-Shock Response. *Genes Dev.* 30, 2062–2075. doi:10.1101/gad.283317.116

Li, J., Labbadia, J., and Morimoto, R. I. (2017). Rethinking HSF1 in Stress, Development, and Organismal Health. *Trends Cell Biol.* 27, 895–905. doi:10.1016/j.tcb.2017.08.002

Li, W., Gao, B., Lee, S. M., Bennett, K., and Fang, D. (2007). RLE-1, an E3 Ubiquitin Ligase, Regulates *C. elegans* Aging by Catalyzing DAF-16 Polyubiquitination. *Dev. Cell* 12, 235–246. doi:10.1016/j.devcel.2006.12.002

Lin, K., Dorman, J. B., Rodan, A., and Kenyon, C. (1997). daf-16: An HNF-3/forkhead Family Member that Can Function to Double the Life-Span of *Caenorhabditis elegans*. *Science* 278, 1319–1322. doi:10.1126/science.278.5341.1319

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

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

Liu, W., Lin, H., Mao, Z., Zhang, L., Bao, K., Jiang, B., et al. (2020). Verapamil Extends Lifespan in *Caenorhabditis elegans* by Inhibiting Calcineurin Activity and Promoting Autophagy. *Aging (Albany NY)* 12, 5300–5317. doi:10.18632/aging.102951

Liu, X. D., Liu, P. C., Santoro, N., and Thiele, D. J. (1997). Conservation of a Stress Response: Human Heat Shock Transcription Factors Functionally Substitute for Yeast HSF. *EMBO J.* 16, 6466–6477. doi:10.1093/emboj/16.21.6466

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.2013.05.039

Mahadevan, N. R., Rodvold, J., Sepulveda, H., Rossi, S., Drew, A. F., and Zanetti, M. (2011). Transmission of Endoplasmic Reticulum Stress and Pro-inflammation from Tumor Cells to Myeloid Cells. *Proc. Natl. Acad. Sci. U. S. A.* 108, 6561–6566. doi:10.1073/pnas.1008942108

Malone, E. A., and Thomas, J. H. (1994). A Screen for Nonconditional Dauer-Constitutive Mutations in *Caenorhabditis elegans*. *Genetics* 136, 879–886. doi:10.1093/genetics/136.3.879

Margulis, B., Tsimokha, A., Zubova, S., and Guzhova, I. (2020). Molecular Chaperones and Proteolytic Machineries Regulate Protein Homeostasis in Aging Cells. *Cells* 9. doi:10.3390/cells9051308

Matilainen, O., Arpalahti, L., Rantanen, V., Hautaniemi, S., and Holmberg, C. I. (2013). Insulin/IGF-1 Signaling Regulates Proteasome Activity through the Deubiquitinating Enzyme UBH-4. *Cell Rep.* 3, 1980–1995. doi:10.1016/j.celrep.2013.05.012

Mayya, V., Lundgren, D. H., Hwang, S. I., Rezaul, K., Wu, L., Eng, J. K., et al. (2009). Quantitative Phosphoproteomic Analysis of T Cell Receptor Signaling Reveals System-Wide Modulation of Protein-Protein Interactions. *Sci. Signal.* 2 (84), ra46. doi:10.1126/scisignal.2000007

McColl, G., Rogers, A. N., Alavez, S., Hubbard, A. E., Melov, S., Link, C. D., et al. (2010). Insulin-like Signaling Determines Survival during Stress via Posttranscriptional Mechanisms in *C. elegans*. *Cell metab.* 12, 260–272. doi:10.1016/j.cmet.2010.08.004

McCormick, M. A., Tsai, S. Y., and Kennedy, B. K. (2011). TOR and Ageing: a Complex Pathway for a Complex Process. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 366, 17–27. doi:10.1098/rstb.2010.0198

McElwee, J., Bubb, K., and Thomas, J. H. (2003). Transcriptional Outputs of the *Caenorhabditis elegans* Forkhead Protein DAF-16. *Aging Cell* 2, 111–121. doi:10.1046/j.1474-9728.2003.00043.x

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

Meister, P., Towbin, B. D., Pike, B. L., Ponti, A., and Gasser, S. M. (2010). The Spatial Dynamics of Tissue-specific Promoters during *C. elegans* Development. *Genes Dev.* 24, 766–782. doi:10.1101/gad.559610

Melendez, A., Talloczy, Z., Seaman, M., Eskelinen, E. L., Hall, D. H., and Levine, B. (2003). Autophagy Genes Are Essential for Dauer Development and Life-Span Extension in *C. elegans*. *Science* 301, 1387–1391. doi:10.1126/science.1087782

Mihaylova, M. M., and Shaw, R. J. (2011). The AMPK Signalling Pathway Coordinates Cell Growth, Autophagy and Metabolism. *Nat. Cell Biol.* 13, 1016–1023. doi:10.1038/ncb2329

Miles, J., and van Oosten-Hawle, P. (2020). Tissue-Specific RNAi Tools to Identify Components for Systemic Stress Signaling. *J. Vis. Exp.* 159, 1. doi:10.3791/61357

Miller, H. A., Dean, E. S., Pletcher, S. D., and Leiser, S. F. (2020). Cell Non-autonomous Regulation of Health and Longevity. *Elife* 9. doi:10.7554/eLife.62659

Minnerly, J., Zhang, J., Parker, T., Kaul, T., and Jia, K. (2017). The Cell Non-autonomous Function of ATG-18 Is Essential for Neuroendocrine Regulation of *Caenorhabditis elegans* Lifespan. *PLoS Genet.* 13, e1006764. doi:10.1371/journal.pgen.1006764

Mizushima, N., Levine, B., Cuervo, A. M., and Klionsky, D. J. (2008). Autophagy Fights Disease through Cellular Self-Digestion. *Nature* 451, 1069–1075. doi:10.1038/nature06639

Mizushima, N. (2011). Autophagy in Protein and Organelle Turnover. *Cold Spring Harb. Symp. Quant. Biol.* 76, 397–402. doi:10.1101/sqb.2011.76.011023

Mooijaart, S. P., Kuningas, M., Westendorp, R. G., Houwing-Duistermaat, J. J., Slagboom, P. E., Rensen, P. C., et al. (2007). Liver X Receptor Alpha Associates with Human Life Span. *J. Gerontol. A Biol. Sci. Med. Sci.* 62, 343–349. doi:10.1093/gerona/62.4.343

Morimoto, R. I. (2019). Cell-Nonautonomous Regulation of Proteostasis in Aging and Disease. *Cold Spring Harb. Perspect. Biol.* 12 (4), a034074. doi:10.1101/cshperspect.a034074

Morimoto, R. I., and Cuervo, A. M. (2009). Protein Homeostasis and Aging: Taking Care of Proteins from the Cradle to the Grave. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 167–170. doi:10.1093/gerona/gln071

Morimoto, R. I. (2008). Proteotoxic Stress and Inducible Chaperone Networks in Neurodegenerative Disease and Aging. *Genes Dev.* 22, 1427–1438. doi:10.1101/gad.1657108

Morimoto, R. I. (2006). Stress, Aging, and Neurodegenerative Disease. *N. Engl. J. Med.* 355, 2254–2255. doi:10.1056/nejmcibr065573

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

Morris, J. Z., Tissenbaum, H. A., and Ruvkun, G. (1996). A Phosphatidylinositol-3-OH Kinase Family Member Regulating Longevity and Diapause in *Caenorhabditis elegans*. *Nature* 382, 536–539. doi:10.1038/382536a0

Morton, E. A., and Lamitina, T. (2013). *Caenorhabditis elegans* HSF-1 Is an Essential Nuclear Protein that Forms Stress Granule-like Structures Following Heat Shock. *Aging Cell* 12, 112–120. doi:10.1111/acel.12024

Mosser, D. D., Kotzbauer, P. T., Sarge, K. D., and Morimoto, R. I. (1990). *In Vitro* activation of Heat Shock Transcription Factor DNA-Binding by Calcium and Biochemical Conditions that Affect Protein Conformation. *Proc. Natl. Acad. Sci. U. S. A.* 87, 3748–3752. doi:10.1073/pnas.87.10.3748

Mottis, A., Jovaisaite, V., and Auwerx, J. (2014). The Mitochondrial Unfolded Protein Response in Mammalian Physiology. *Mamm. Genome* 25, 424–433. doi:10.1007/s00335-014-9525-z

Mouchiroud, L., Houtkooper, R. H., Moullan, N., Katsyuba, E., Ryu, D., Canto, 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

Mukhopadhyay, A., and Tissenbaum, H. A. (2007). Reproduction and Longevity: Secrets Revealed by *C. elegans*. *Trends Cell Biol.* 17, 65–71. doi:10.1016/j.tcb.2006.12.004

Murphy, C. T., McCarroll, S. A., Bargmann, C. I., Fraser, A., Kamath, R. S., Ahringer, J., et al. (2003). Genes that Act Downstream of DAF-16 to Influence the Lifespan of *Caenorhabditis elegans*. *Nature* 424, 277–283. doi:10.1038/nature01789

Murshid, A., Chou, S. D., Prince, T., Zhang, Y., Bharti, A., and Calderwood, S. K. (2010). Protein Kinase A Binds and Activates Heat Shock Factor 1. *PLoS One* 5, e13830. doi:10.1371/journal.pone.0013830

Nakai, A. (1999). New Aspects in the Vertebrate Heat Shock Factor System: Hsf3 and Hsf4. *Cell stress chaperones* 4, 86–93. doi:10.1379/1466-1268(1999)004<0086:naitvh>2.3.co;2

Nakamura, S., Karalay, O., Jager, P. S., Horikawa, M., Klein, C., Nakamura, K., et al. (2016). Mondo Complexes Regulate TFEB via TOR Inhibition to Promote Longevity in Response to Gonadal Signals. *Nat. Commun.* 7, 10944. doi:10.1038/ncomms10944

Neef, D. W., Jaeger, A. M., Gomez-Pastor, R., Willmund, F., Frydman, J., and Thiele, D. J. (2014). A Direct Regulatory Interaction between Chaperonin TRiC and Stress-Responsive Transcription Factor HSF1. *Cell Rep.* 9, 955–966. doi:10.1016/j.celrep.2014.09.056

Neef, D. W., Jaeger, A. M., and Thiele, D. J. (2011). Heat Shock Transcription Factor 1 as a Therapeutic Target in Neurodegenerative Diseases. *Nat. Rev. Drug Discov.* 10, 930–944. doi:10.1038/nrd3453

Neef, D. W., Turski, M. L., and Thiele, D. J. (2010). Modulation of Heat Shock Transcription Factor 1 as a Therapeutic Target for Small Molecule Intervention in Neurodegenerative Disease. *PLoS Biol.* 8, e1000291. doi:10.1371/journal.pbio.1000291

Nieto-Torres, J. L., and Hansen, M. (2021). Macroautophagy and Aging: The Impact of Cellular Recycling on Health and Longevity. *Mol. Asp. Med.* 82, 101020. doi:10.1016/j.mam.2021.101020

Nisaa, K., and Ben-Zvi, A. (2021). Chaperone Networks Are Shaped by Cellular Differentiation and Identity. *Trends Cell Biol.* 32 (6), 470–474. doi:10.1016/j.tcb.2021.11.001

O'Brien, D., Jones, L. M., Good, S., Miles, J., Vijayabaskar, M. S., Aston, R., et al. (2018). A PQM-1-Mediated Response Triggers Transcellular Chaperone Signaling and Regulates Organismal Proteostasis. *Cell Rep.* 23, 3905–3919. doi:10.1016/j.celrep.2018.05.093

Ogg, S., Paradis, S., Gottlieb, S., Patterson, G. I., Lee, L., Tissenbaum, H. A., et al. (1997). The Fork Head Transcription Factor DAF-16 Transduces Insulin-like Metabolic and Longevity Signals in *C. elegans*. *Nature* 389, 994–999. doi:10.1038/40194

Ogg, S., and Ruvkun, G. (1998). The *C. elegans* PTEN Homolog, DAF-18, Acts in the Insulin Receptor-like Metabolic Signaling Pathway. *Mol. Cell* 2, 887–893. doi:10.1016/s1097-2765(00)80303-2

Olsen, J. V., Blagoev, B., Gnad, F., Macek, B., Kumar, C., Mortensen, P., et al. (2006). Global, In Vivo, And Site-Specific Phosphorylation Dynamics in Signaling Networks. *Cell* 127, 635–648. doi:10.1016/j.cell.2006.09.026

Olsen, J. V., Vermeulen, M., Santamaria, A., Kumar, C., Miller, M. L., Jensen, L. J., et al. (2010). Quantitative Phosphoproteomics Reveals Widespread Full Phosphorylation Site Occupancy During Mitosis. *Science signaling* 3, ra3. doi:10.1126/scisignal.2000475

Orosz, A., Wisniewski, J., and Wu, C. (1996). Regulation of Drosophila Heat Shock Factor Trimerization: Global Sequence Requirements and Independence of Nuclear Localization. *Mol. Cell Biol.* 16, 7018–7030. doi:10.1128/mcb.16.12.7018

Ottens, F., Franz, A., and Hoppe, T. (2021). Build-UPS and Break-Downs: Metabolism Impacts on Proteostasis and Aging. *Cell Death Differ.* 28, 505–521. doi:10.1038/s41418-020-00682-y

Panowski, S. H., Wolff, S., Aguilaniu, H., Durieux, J., and Dillin, A. (2007). PHA-4/Foxa Mediates Diet-Restriction-Induced Longevity of *C. elegans*. *Nature* 447, 550–555. doi:10.1038/nature05837

Papaevgeniou, N., and Chondrogianni, N. (2016). UPS Activation in the Battle against Aging and Aggregation-Related Diseases: An Extended Review. *Methods Mol. Biol. Clift. N. J.* 1449, 1–70. doi:10.1007/978-1-4939-3756-1_1

Paradis, S., Ailion, M., Toker, A., Thomas, J. H., and Ruvkun, G. (1999). A PDK1 Homolog Is Necessary and Sufficient to Transduce AGE-1 PI3 Kinase Signals that Regulate Diapause in *Caenorhabditis elegans*. *Genes Dev.* 13, 1438–1452. doi:10.1101/gad.13.11.1438

Paradis, S., and Ruvkun, G. (1998). *Caenorhabditis elegans* Akt/PKB Transduces Insulin Receptor-like Signals from AGE-1 PI3 Kinase to the DAF-16 Transcription Factor. *Genes Dev.* 12, 2488–2498. doi:10.1101/gad.12.16.2488

Park, J., and Liu, A. Y. (2001). JNK Phosphorylates the HSF1 Transcriptional Activation Domain: Role of JNK in the Regulation of the Heat Shock Response. *J. Cell Biochem.* 82, 326–338. doi:10.1002/jcb.1163

Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W., and Balch, W. E. (2009). Biological and Chemical Approaches to Diseases of Proteostasis Deficiency. *Annu. Rev. Biochem.* 78, 959–991. doi:10.1146/annurev.biochem.052308.114844

Prahlad, V., Cornelius, T., and Morimoto, R. I. (2008). Regulation of the Cellular Heat Shock Response in *Caenorhabditis elegans* by Thermosensory Neurons. *Science* 320, 811–814. doi:10.1126/science.1156093

Prahlad, V., and Morimoto, R. I. (2011). Neuronal Circuitry Regulates the Response of *Caenorhabditis elegans* to Misfolded Proteins. *Proc. Natl. Acad. Sci. U. S. A.* 108, 14204–14209. doi:10.1073/pnas.1106557108

Prata, L., Ovsyannikova, I. G., Tchkonia, T., and Kirkland, J. L. (2018). Senescent Cell Clearance by the Immune System: Emerging Therapeutic Opportunities. *Semin. Immunol.* 40, 101275. doi:10.1016/j.smim.2019.04.003

Preissler, S., and Ron, D. (2019). Early Events in the Endoplasmic Reticulum Unfolded Protein Response. *Cold Spring Harb. Perspect. Biol.* 11. doi:10.1101/cshperspect.a033894

Prelich, G. (2012). Gene Overexpression: Uses, Mechanisms, and Interpretation. *Genetics* 190, 841–854. doi:10.1534/genetics.111.136911

Puscheck, E. E., Awonuga, A. O., Yang, Y., Jiang, Z., and Rappolee, D. A. (2015). Molecular Biology of the Stress Response in the Early Embryo and its Stem Cells. *Adv. Exp. Med. Biol.* 843, 77–128. doi:10.1007/978-1-4939-2480-6_4

Pyo, J. O., Nah, J., and Jung, Y. K. (2012). Molecules and Their Functions in Autophagy. *Exp. Mol. Med.* 44, 73–80. doi:10.3858/emm.2012.44.2.029

Qadota, H., Inoue, M., Hikita, T., Koppen, M., Hardin, J. D., Amano, M., et al. (2007). Establishment of a Tissue-specific RNAi System in *C. elegans*. *Gene* 400, 166–173. doi:10.1016/j.gene.2007.06.020

Rabindran, S. K., Giorgi, G., Clos, J., and Wu, C. (1991). Molecular Cloning and Expression of a Human Heat Shock Factor, HSF1. *Proc. Natl. Acad. Sci. U. S. A.* 88, 6906–6910. doi:10.1073/pnas.88.16.6906

Rabinowitz, J. D., and White, E. (2010). Autophagy and Metabolism. *Science* 330, 1344–1348. doi:10.1126/science.1193497

Raychaudhuri, S., Loew, C., Korner, R., Pinkert, S., Theis, M., Hayer-Hartl, M., et al. (2014). Interplay of Acetyltransferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. *Cell* 156, 975–985. doi:10.1016/j.cell.2014.01.055

Raynes, R., Leckey, B. D., Jr., Nguyen, K., and Westerheide, S. D. (2012). Heat Shock and Caloric Restriction Have a Synergistic Effect on the Heat Shock Response in a sir2.1-dependent Manner in *Caenorhabditis elegans*. *J. Biol. Chem.* 287, 29045–29053. doi:10.1074/jbc.m112.353714

Raynes, R., Pombier, K. M., Nguyen, K., Brunquell, J., Mendez, J. E., and Westerheide, S. D. (2013). The SIRT1 Modulators AROS and DBC1 Regulate HSF1 Activity and the Heat Shock Response. *PLoS One* 8, e54364. doi:10.1371/journal.pone.0054364

Reinle, K., Mogk, A., and Bukau, B. (2022). The Diverse Functions of Small Heat Shock Proteins in the Proteostasis Network. *J. Mol. Biol.* 434, 167157. doi:10.1016/j.jmb.2021.167157

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

Ren, P., Lim, C. S., Johnsen, R., Albert, P. S., Pilgrim, D., and Riddle, D. L. (1996). Control of *C. elegans* Larval Development by Neuronal Expression of a TGF-Beta Homolog. *Science* 274, 1389–1391. doi:10.1126/science.274.5291.1389

Richter, K., Haslbeck, M., and Buchner, J. (2010). The Heat Shock Response: Life on the Verge of Death. *Mol. Cell* 40, 253–266. doi:10.1016/j.molcel.2010.10.006

Riddle, D. L., Swanson, M. M., and Albert, P. S. (1981). Interacting Genes in Nematode Dauer Larva Formation. *Nature* 290, 668–671. doi:10.1038/290668a0

Ritossa, F. (1962). A New Puffing Pattern Induced by Temperature Shock and DNP in drosophila. *Experientia* 18, 571–573. doi:10.1007/bf02172188

Robida-Stubbs, S., Glover-Cutter, K., Lamming, D. W., Mizunuma, M., Narasimhan, S. D., Neumann-Haefelin, E., et al. (2012). TOR Signaling and Rapamycin Influence Longevity by Regulating SKN-1/Nrf and DAF-16/FoxO. *Cell metab.* 15, 713–724. doi:10.1016/j.cmet.2012.04.007

Roos-Mattjus, P., and Sistonen, L. (2021). Interplay between Mammalian Heat Shock Factors 1 and 2 in Physiology and Pathology. *FEBS J.* 2021, 1. doi:10.1111/febs.16178

Rosenzweig, R., Nillegoda, N. B., Mayer, M. P., and Bukau, B. (2019). The Hsp70 Chaperone Network. *Nat. Rev. Mol. Cell Biol.* 20, 665–680. doi:10.1038/s41580-019-0133-3

Rual, J. F., Venkatesan, K., Hao, T., Hirozane-Kishikawa, T., Dricot, A., Li, N., et al. (2005). Towards a Proteome-Scale Map of the Human Protein-Protein Interaction Network. *Nature* 437, 1173–1178. doi:10.1038/nature04209

Rubinsztein, D. C., Marino, G., and Kroemer, G. (2011). Autophagy and Aging. *Cell* 146, 682–695. doi:10.1016/j.cell.2011.07.030

Ryan, M. T., and Hoogenraad, N. J. (2007). Mitochondrial-nuclear Communications. *Annu. Rev. Biochem.* 76, 701–722. doi:10.1146/annurev.biochem.76.052305.091720

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, 31902–31913. doi:10.1073/pnas.2018138117

Sala, A. J., Bott, L. C., Brielmann, R. M., and Morimoto, R. I. (2020). Embryo Integrity Regulates Maternal Proteostasis and Stress Resilience. *Genes Dev.* 34, 678–687. doi:10.1101/gad.335422.119

Sala, A. J., Bott, L. C., and Morimoto, R. I. (2017). Shaping Proteostasis at the Cellular, Tissue, and Organismal Level. *J. Cell Biol.* 216, 1231–1241. doi:10.1083/jcb.201612111

Salminen, A., and Kaarniranta, K. (2012). AMP-activated Protein Kinase (AMPK) Controls the Aging Process via an Integrated Signaling Network. *Ageing Res. Rev.* 11, 230–241. doi:10.1016/j.arr.2011.12.005

Samuelson, A. V., Carr, C. E., and Ruvkun, G. (2007). Gene Activities that Mediate Increased Life Span of *C. elegans* Insulin-like Signaling Mutants. *Genes Dev.* 21, 2976–2994. doi:10.1101/gad.1588907

Sarge, K. D., Murphy, S. P., and Morimoto, R. I. (1993). Activation of Heat Shock Gene Transcription by Heat Shock Factor 1 Involves Oligomerization, Acquisition of DNA-Binding Activity, and Nuclear Localization and Can Occur in the Absence of Stress. *Mol. Cell Biol.* 13, 1392–1407. doi:10.1128/mcb.13.3.1392-1407.1993

Sarkis, G. J., Ashcom, J. D., Hawdon, J. M., and Jacobson, L. A. (1988). Decline in Protease Activities with Age in the Nematode *Caenorhabditis elegans*. *Mech. Ageing Dev.* 45, 191–201. doi:10.1016/0047-6374(88)90001-2

Satyal, S. H., Chen, D., Fox, S. G., Kramer, J. M., and Morimoto, R. I. (1998). Negative Regulation of the Heat Shock Transcriptional Response by HSBP1. *Genes Dev.* 12, 1962–1974. doi:10.1101/gad.12.13.1962

Satyal, S. H., Schmidt, E., Kitagawa, K., Sondheimer, N., Lindquist, S., Kramer, J. M., et al. (2000). Polyglutamine Aggregates Alter Protein Folding Homeostasis in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5750–5755. doi:10.1073/pnas.100107297

Saxton, R. A., and Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168, 960–976. doi:10.1016/j.cell.2017.02.004

Seo, K., Choi, E., Lee, D., Jeong, D. E., Jang, S. K., and Lee, S. J. (2013). Heat Shock Factor 1 Mediates the Longevity Conferred by Inhibition of TOR and insulin/IGF-1 Signaling Pathways in *C. elegans*. *Aging Cell* 12, 1073–1081. doi:10.1111/acel.12140

Serrano, M., Lin, A. W., McCurrach, M. E., Beach, D., and Lowe, S. W. (1997). Oncogenic Ras Provokes Premature Cell Senescence Associated with Accumulation of P53 and p16INK4a. *Cell* 88, 593–602. doi:10.1016/s0092-8674(00)81902-9

Shamovsky, I., Ivannikov, M., Kandel, E. S., Gershon, D., and Nudler, E. (2006). RNA-mediated Response to Heat Shock in Mammalian Cells. *Nature* 440, 556–560. doi:10.1038/nature04518

Shaw, W. M., Luo, S., Landis, J., Ashraf, J., and Murphy, C. T. (2007). The *C. elegans* TGF-Beta Dauer Pathway Regulates Longevity via Insulin Signaling. *Curr. Biol.* 17, 1635–1645. doi:10.1016/j.cub.2007.08.058

Sheaffer, K. L., Updike, D. L., and Mango, S. E. (2008). The Target of Rapamycin Pathway Antagonizes pha-4/FoxA to Control Development and Aging. *Curr. Biol.* 18, 1355–1364. doi:10.1016/j.cub.2008.07.097

Shemesh, N., Jubran, J., Dror, S., Simonovsky, E., Basha, O., Argov, C., et al. (2021). The Landscape of Molecular Chaperones across Human Tissues Reveals a Layered Architecture of Core and Variable Chaperones. *Nat. Commun.* 12, 2180. doi:10.1038/s41467-021-22369-9

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

Shi, Y., Mosser, D. D., and Morimoto, R. I. (1998). Molecular Chaperones as HSF1-specific Transcriptional Repressors. *Genes Dev.* 12, 654–666. doi:10.1101/gad.12.5.654

Shpigel, N., Shemesh, N., Kishner, M., and Ben-Zvi, A. (2019). Dietary Restriction and Gonadal Signaling Differentially Regulate Post-development Quality Control Functions in *Caenorhabditis elegans*. *Aging Cell* 18, e12891. doi:10.1111/acel.12891

Silva, M. C., Amaral, M. D., and Morimoto, R. I. (2013). Neuronal Reprograming of Protein Homeostasis by Calcium-dependent Regulation of the Heat Shock Response. *PLoS Genet.* 9, e1003711. doi:10.1371/journal.pgen.1003711

Skaar, J. R., Pagan, J. K., and Pagano, M. (2013). Mechanisms and Function of Substrate Recruitment by F-Box Proteins. *Nat. Rev. Mol. Cell Biol.* 14, 369–381. doi:10.1038/nrm3582

Somogyvari, M., Gecse, E., and Soti, C. (2018). DAF-21/Hsp90 Is Required for *C. elegans* Longevity by Ensuring DAF-16/FOXO Isoform A Function. *Sci. Rep.* 8, 12048. doi:10.1038/s41598-018-30592-6

Son, H. G., Seo, K., Seo, M., Park, S., Ham, S., An, S. W. A., et al. (2018). Prefoldin 6 Mediates Longevity Response from Heat Shock Factor 1 to FOXO in *C. elegans*. *Genes Dev.* 32, 1562–1575. doi:10.1101/gad.317362.118

Soncin, F., Zhang, X., Chu, B., Wang, X., Asea, A., Ann Stevenson, M., et al. (2003). Transcriptional Activity and DNA Binding of Heat Shock Factor-1 Involve Phosphorylation on Threonine 142 by CK2. *Biochem. Biophys. Res. Commun.* 303, 700–706. doi:10.1016/s0006-291x(03)00398-x

Song, S., Lam, E. W., Tchkonia, T., Kirkland, J. L., and Sun, Y. (2020). Senescent Cells: Emerging Targets for Human Aging and Age-Related Diseases. *Trends Biochem. Sci.* 45, 578–592. doi:10.1016/j.tibs.2020.03.008

Sorger, P. K., and Pelham, H. R. (1988). Yeast Heat Shock Factor Is an Essential DNA-Binding Protein that Exhibits Temperature-dependent Phosphorylation. *Cell* 54, 855–864. doi:10.1016/s0092-8674(88)91219-6

Sorger, P. K. (1990). Yeast Heat Shock Factor Contains Separable Transient and Sustained Response Transcriptional Activators. *Cell* 62, 793–805. doi:10.1016/0092-8674(90)90123-v

Steinkraus, K. A., Smith, E. D., Davis, C., Carr, D., Pendergrass, W. R., Sutphin, G. L., et al. (2008). Dietary Restriction Suppresses Proteotoxicity and Enhances Longevity by an Hsf-1-dependent Mechanism in *Caenorhabditis elegans*. *Aging Cell* 7, 394–404. doi:10.1111/j.1474-9726.2008.00385.x

Stetter, K. O. (2006). Hyperthermophiles in the History of Life. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 361, 1837–1842. doi:10.1098/rstb.2006.1907

Su, K. H., Dai, S., Tang, Z., Xu, M., and Dai, C. (2019). Heat Shock Factor 1 Is a Direct Antagonist of AMP-Activated Protein Kinase. *Mol. Cell* 76, 546–561. doi:10.1016/j.molcel.2019.08.021

Suh, Y., Atzmon, G., Cho, M. O., Hwang, D., Liu, B., Leahy, D. J., et al. (2008). Functionally Significant Insulin-like Growth Factor I Receptor Mutations in Centenarians. *Proc. Natl. Acad. Sci. U. S. A.* 105, 3438–3442. doi:10.1073/pnas.0705467105

Sun, J., Singh, V., Kajino-Sakamoto, R., and Aballay, A. (2011). Neuronal GPCR Controls Innate Immunity by Regulating Noncanonical Unfolded Protein Response Genes. *Science* 332, 729–732. doi:10.1126/science.1203411

Sural, S., Liang, C. Y., Wang, F. Y., Ching, T. T., and Hsu, A. L. (2020). HSB-1/HSF-1 Pathway Modulates Histone H4 in Mitochondria to Control mtDNA Transcription and Longevity. *Sci. Adv.* 6. doi:10.1126/sciadv.aaz4452

Sykiotis, G. P., Habeos, I. G., Samuelson, A. V., and Bohmann, D. (2011). The Role of the Antioxidant and Longevity-Promoting Nrf2 Pathway in Metabolic Regulation. *Curr. Opin. Clin. Nutr. Metab. Care* 14, 41–48. doi:10.1097/mco.0b013e32834136f2

Szewczyk, N. J., Udranszky, I. A., Kozak, E., Sunga, J., Kim, S. K., Jacobson, L. A., et al. (2006). Delayed Development and Lifespan Extension as Features of Metabolic Lifestyle Alteration in *C. elegans* under Dietary Restriction. *J. Exp. Biol.* 209, 4129–4139. doi:10.1242/jeb.02492

Taipale, M., Jarosz, D. F., and Lindquist, S. (2010). HSP90 at the Hub of Protein Homeostasis: Emerging Mechanistic Insights. *Nat. Rev. Mol. Cell Biol.* 11, 515–528. doi:10.1038/nrm2918

Takai, K., Nunoura, T., Sako, Y., and Uchida, A. (1998). Acquired Thermotolerance and Temperature-Induced Protein Accumulation in the Extremely Thermophilic Bacterium Rhodothermus Obamensis. *J. Bacteriol.* 180, 2770–2774. doi:10.1128/jb.180.10.2770-2774.1998

Tang, Z., Dai, S., He, Y., Doty, R. A., Shultz, L. D., Sampson, S. B., et al. (2015). MEK Guards Proteome Stability and Inhibits Tumor-Suppressive Amyloidogenesis via HSF1. *Cell* 160, 729–744. doi:10.1016/j.cell.2015.01.028

Tatar, M., Khazaeli, A. A., and Curtsinger, J. W. (1997). Chaperoning Extended Life. *Nature* 390, 30. doi:10.1038/36237

Tatsuta, T., Model, K., and Langer, T. (2005). Formation of Membrane-Bound Ring Complexes by Prohibitins in Mitochondria. *Mol. Biol. Cell* 16, 248–259. doi:10.1091/mbc.e04-09-0807

Tatum, M. C., Ooi, F. K., Chikka, M. R., Chauve, L., Martinez-Velazquez, L. A., Steinbusch, H. W., et al. (2015). Neuronal Serotonin Release Triggers the Heat Shock Response in *C. elegans* in the Absence of Temperature Increase. *Curr. Biol.* 25, 163–174. doi:10.1016/j.cub.2014.11.040

Tawo, R., Pokrzywa, W., Kevei, E., Akyuz, M. E., Balaji, V., Adrian, S., et al. (2017). The Ubiquitin Ligase CHIP Integrates Proteostasis and Aging by Regulation of Insulin Receptor Turnover. *Cell* 169, 470–482. e413. doi:10.1016/j.cell.2017.04.003

Taylor, R. C., Berendzen, K. M., and Dillin, A. (2014). Systemic Stress Signalling: Understanding the Cell Non-autonomous Control of Proteostasis. *Nat. Rev. Mol. Cell Biol.* 15, 211–217. doi:10.1038/nrm3752

Taylor, R. C., and Dillin, A. (2011). Aging as an Event of Proteostasis Collapse. *Cold Spring Harb. Perspect. Biol.* 3. doi:10.1101/cshperspect.a004440

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

Tennessen, J. M., and Thummel, C. S. (2011). Coordinating Growth and Maturation - Insights from Drosophila. *Curr. Biol.* 21, R750–R757. doi:10.1016/j.cub.2011.06.033

Tomita, T., Hamazaki, J., Hirayama, S., McBurney, M. W., Yashiroda, H., and Murata, S. (2015). Sirt1-deficiency Causes Defective Protein Quality Control. *Sci. Rep.* 5, 12613. doi:10.1038/srep12613

Tooze, S. A., and Yoshimori, T. (2010). The Origin of the Autophagosomal Membrane. *Nat. Cell Biol.* 12, 831–835. doi:10.1038/ncb0910-831

Toth, M. L., Sigmond, T., Borsos, E., Barna, J., Erdelyi, P., Takacs-Vellai, K., et al. (2008). Longevity Pathways Converge on Autophagy Genes to Regulate Life Span in *Caenorhabditis elegans*. *Autophagy* 4, 330–338. doi:10.4161/auto.5618

Tullet, J. M., Hertweck, M., An, J. H., Baker, J., Hwang, J. Y., Liu, S., et al. (2008). Direct Inhibition of the Longevity-Promoting Factor SKN-1 by Insulin-like Signaling in *C. elegans*. *Cell* 132, 1025–1038. doi:10.1016/j.cell.2008.01.030

Ulgherait, M., Rana, A., Rera, M., Graniel, J., and Walker, D. W. (2014). AMPK Modulates Tissue and Organismal Aging in a Non-cell-autonomous Manner. *Cell Rep.* 8, 1767–1780. doi:10.1016/j.celrep.2014.08.006

Urano, F., Calfon, M., Yoneda, T., Yun, C., Kiraly, M., Clark, S. G., et al. (2002). A Survival Pathway for *Caenorhabditis elegans* with a Blocked Unfolded Protein Response. *J. Cell Biol.* 158, 639–646. doi:10.1083/jcb.200203086

van Oosten-Hawle, P., and Morimoto, R. I. (2014). Organismal Proteostasis: Role of Cell-Nonautonomous Regulation and Transcellular Chaperone Signaling. *Genes Dev.* 28, 1533–1543. doi:10.1101/gad.241125.114

van Oosten-Hawle, P., Porter, R. S., and Morimoto, R. I. (2013). Regulation of Organismal Proteostasis by Transcellular Chaperone Signaling. *Cell* 153, 1366–1378. doi:10.1016/j.cell.2013.05.015

Vellai, T., Takacs-Vellai, K., Sass, M., and Klionsky, D. J. (2009). The Regulation of Aging: Does Autophagy Underlie Longevity? *Trends Cell Biol.* 19, 487–494. doi:10.1016/j.tcb.2009.07.007

Verdin, E. (2015). NAD(+) in Aging, Metabolism, and Neurodegeneration. *Science* 350, 1208–1213. doi:10.1126/science.aac4854

Vilchez, D., Morantte, I., Liu, Z., Douglas, P. M., Merkwirth, C., Rodrigues, A. P., et al. (2012). RPN-6 Determines *C. elegans* Longevity under Proteotoxic Stress Conditions. *Nature* 489, 263–268. doi:10.1038/nature11315

Vowels, J. J., and Thomas, J. H. (1992). Genetic Analysis of Chemosensory Control of Dauer Formation in *Caenorhabditis elegans*. *Genetics* 130, 105–123. doi:10.1093/genetics/130.1.105

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

Wan, Q. L., Shi, X., Liu, J., Ding, A. J., Pu, Y. Z., Li, Z., et al. (2017). Metabolomic Signature Associated with Reproduction-Regulated Aging in *Caenorhabditis elegans*. *Aging (Albany NY)* 9, 447–474. doi:10.18632/aging.101170

Wang, M. C., O'Rourke, E. J., and Ruvkun, G. (2008). Fat Metabolism Links Germline Stem Cells and Longevity in *C. elegans*. *Science* 322, 957–960. doi:10.1126/science.1162011

Wang, X., Grammatikakis, N., Siganou, A., and Calderwood, S. K. (2003). Regulation of Molecular Chaperone Gene Transcription Involves the Serine Phosphorylation, 14-3-3 Epsilon Binding, and Cytoplasmic Sequestration of Heat Shock Factor 1. *Mol. Cell Biol.* 23, 6013–6026. doi:10.1128/mcb.23.17.6013-6026.2003

Wang, X., Khaleque, M. A., Zhao, M. J., Zhong, R., Gaestel, M., and Calderwood, S. K. (2006). Phosphorylation of HSF1 by MAPK-Activated Protein Kinase 2 on Serine 121, Inhibits Transcriptional Activity and Promotes HSP90 Binding. *J. Biol. Chem.* 281, 782–791. doi:10.1074/jbc.m505822200

Watanabe, Y., Tsujimura, A., Taguchi, K., and Tanaka, M. (2017). HSF1 Stress Response Pathway Regulates Autophagy Receptor SQSTM1/p62-Associated Proteostasis. *Autophagy* 13, 133–148. doi:10.1080/15548627.2016.1248018

Watts, J. S., Harrison, H. F., Omi, S., Guenthers, Q., Dalelio, J., Pujol, N., et al. (2020). New Strains for Tissue-specific RNAi Studies in *Caenorhabditis elegans*. *G3* 10, 4167–4176. doi:10.1534/g3.120.401749

Wentz, J. M., Mendenhall, A. R., and Bortz, D. M. (2018). Pattern Formation in the Longevity-Related Expression of Heat Shock Protein-16.2 in *Caenorhabditis elegans*. *Bull. Math. Biol.* 80, 2669–2697. doi:10.1007/s11538-018-0482-7

Westerheide, S. D., Anckar, J., Stevens, S. M., Jr., Sistonen, L., and Morimoto, R. I. (2009). Stress-inducible Regulation of Heat Shock Factor 1 by the Deacetylase SIRT1. *Science* 323, 1063–1066. doi:10.1126/science.1165946

Wiederrecht, G., Seto, D., and Parker, C. S. (1988). Isolation of the Gene Encoding the *S. cerevisiae* Heat Shock Transcription Factor. *Cell* 54, 841–853. doi:10.1016/s0092-8674(88)91197-x

Wolff, S., and Dillin, A. (2013). Ageing: Beneficial Miscommunication. *Nature* 497, 442–443. doi:10.1038/497442a

Wolff, S., Weissman, J. S., and Dillin, A. (2014). Differential Scales of Protein Quality Control. *Cell* 157, 52–64. doi:10.1016/j.cell.2014.03.007

Workman, P., and van Montfort, R. L. (2010). Unveiling the Secrets of the Ancestral PI3 Kinase Vps34. *Cancer Cell* 17, 421–423. doi:10.1016/j.ccr.2010.04.016

Wotton, D., Pemberton, L. F., and Merrill-Schools, J. (2017). SUMO and Chromatin Remodeling. *Adv. Exp. Med. Biol.* 963, 35–50. doi:10.1007/978-3-319-50044-7_3

Wu, C. (1995). Heat Shock Transcription Factors: Structure and Regulation. *Annu. Rev. Cell Dev. Biol.* 11, 441–469. doi:10.1146/annurev.cb.11.110195.002301

Xia, W., and Voellmy, R. (1997). Hyperphosphorylation of Heat Shock Transcription Factor 1 Is Correlated with Transcriptional Competence and Slow Dissociation of Active Factor Trimers. *J. Biol. Chem.* 272, 4094–4102. doi:10.1074/jbc.272.7.4094

Xu, Y. M., Huang, D. Y., Chiu, J. F., and Lau, A. T. (2012). Post-translational Modification of Human Heat Shock Factors and Their Functions: a Recent Update by Proteomic Approach. *J. Proteome Res.* 11, 2625–2634. doi:10.1021/pr201151a

Yochem, J., and Herman, R. K. (2003). Investigating *C. elegans* Development through Mosaic Analysis. *Development* 130, 4761–4768. doi:10.1242/dev.00701

Yochem, J., Sundaram, M., and Bucher, E. A. (2000). Mosaic Analysis in *Caenorhabditis elegans*. *Methods Mol. Biol. Clift. N. J.* 135, 447–462. doi:10.1385/1-59259-685-1:447

Yokoyama, K., Fukumoto, K., Murakami, T., Harada, S., Hosono, R., Wadhwa, R., et al. (2002). Extended Longevity of *Caenorhabditis elegans* by Knocking in Extra Copies of hsp70F, a Homolog of Mot-2 (mortalin)/mthsp70/Grp75. *FEBS Lett.* 516, 53–57. doi:10.1016/s0014-5793(02)02470-5

Yun, C., Stanhill, A., Yang, Y., Zhang, Y., Haynes, C. M., Xu, C. F., et al. (2008). Proteasomal Adaptation to Environmental Stress Links Resistance to Proteotoxicity with Longevity in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7094–7099. doi:10.1073/pnas.0707025105

Zhang, L., Ward, J. D., Cheng, Z., and Dernburg, A. F. (2015). The Auxin-Inducible Degradation (AID) System Enables Versatile Conditional Protein Depletion in *C. elegans*. *Development* 142, 4374–4384. doi:10.1242/dev.129635

Zhang, Y., Lanjuin, A., Chowdhury, S. R., Mistry, M., Silva-Garcia, C. G., Weir, H. J., et al. (2019). Neuronal TORC1 Modulates Longevity via AMPK and Cell Nonautonomous Regulation of Mitochondrial Dynamics in *C. elegans*. *Elife* 8. doi:10.7554/eLife.49158

Zhang, Y., Murshid, A., Prince, T., and Calderwood, S. K. (2011). Protein Kinase A Regulates Molecular Chaperone Transcription and Protein Aggregation. *PLoS One* 6, e28950. doi:10.1371/journal.pone.0028950

Zhao, W., Kruse, J. P., Tang, Y., Jung, S. Y., Qin, J., and Gu, W. (2008). Negative Regulation of the Deacetylase SIRT1 by DBC1. *Nature* 451, 587–590. doi:10.1038/nature06515

Zheng, X., Krakowiak, J., Patel, N., Beyzavi, A., Ezike, J., Khalil, A. S., et al. (2016). Dynamic Control of Hsf1 during Heat Shock by a Chaperone Switch and Phosphorylation. *Elife* 5. doi:10.7554/eLife.18638

Zhong, M., Niu, W., Lu, Z. J., Sarov, M., Murray, J. I., Janette, J., et al. (2010). Genome-wide Identification of Binding Sites Defines Distinct Functions for *Caenorhabditis elegans* PHA-4/FOXA in Development and Environmental Response. *PLoS Genet.* 6, e1000848. doi:10.1371/journal.pgen.1000848

Zhong, M., Orosz, A., and Wu, C. (1998). Direct Sensing of Heat and Oxidation by Drosophila Heat Shock Transcription Factor. *Mol. Cell* 2, 101–108. doi:10.1016/s1097-2765(00)80118-5

Zhou, Y., Wang, X., Song, M., He, Z., Cui, G., Peng, G., et al. (2019). A Secreted microRNA Disrupts Autophagy in Distinct Tissues of *Caenorhabditis elegans* upon Ageing. *Nat. Commun.* 10, 4827. doi:10.1038/s41467-019-12821-2

Zou, J., Guo, Y., Guettouche, T., Smith, D. F., and Voellmy, R. (1998). Repression of Heat Shock Transcription Factor HSF1 Activation by HSP90 (HSP90 Complex) that Forms a Stress-Sensitive Complex with HSF1. *Cell* 94, 471–480. doi:10.1016/s0092-8674(00)81588-3

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Auxin-Inducible Degron System Reveals Temporal-Spatial Roles of HSF-1 and Its Transcriptional Program in Lifespan Assurance

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HSF-1 is a key regulator of cellular proteotoxic stress response and is required for animal lifespan. In *C. elegans*, HSF-1 mediated heat shock response (HSR) declines sharply on the first day of adulthood, and HSF-1 was proposed to function primarily during larval stages for lifespan assurance based on studies using RNAi. The tissue requirement for HSF-1 in lifespan, however, is not well understood. Using the auxin-inducible degron (AID) system, we manage to uncouple the roles of HSF-1 in development and longevity. In wild-type animals, we find HSF-1 is required during the whole self-reproductive period for lifespan. This period is extended in long-lived animals that have arrested germline stem cells (GSC) or reduced insulin/IGF-1 signaling (IIS). While depletion of HSF-1 from any major somatic tissues during development results in severe defects, HSF-1 primarily functions in the intestine and likely neural system of adults to support lifespan. Finally, by combining AID and genome-wide transcriptional analyses, we find HSF-1 directly activates the transcription of constitutively-expressed chaperone and co-chaperone genes among others in early adulthood, which underlies its roles in longevity assurance.

Keywords: aging, proteostasis, heat shock factor, auxin-inducible degron, temporal-spatial function, core chaperome, lifespan

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INTRODUCTION

HSF1 is best known as a key transcriptional activator of cellular heat shock response (HSR). Upon proteotoxic stress such as heat shock, HSF1 induces the expression of genes encoding molecular chaperones, detoxification enzymes, and protein degradation machinery to cope with stress-associated protein damage and misfolding (Vihervaara and Sistonen, 2014; Gomez-Pastor et al., 2018). It is well established that in *Drosophila* and mammalian cells, HSF1 activates the HSR by releasing paused RNA Polymerase II (Pol II) at promoter-proximal regions into productive elongation (Duarte et al., 2016; Mahat et al., 2016). Accumulating evidence suggests that HSF1 also has important roles in animal development, reproduction, and lifespan in both vertebrates and invertebrates (Li et al., 2017). These physiological functions of HSF1 are at least in part through promoting proteostasis, but HSF1's transcriptional programs in these conditions are not identical to the HSR (Li et al., 2016).

Proteostasis decline is a primary hallmark of aging as accumulated protein misfolding and aggregation are observed in aged animals and underlie age-related diseases such as neurodegenerative disorders (Balch et al., 2008; López-Otín et al., 2013). Consistent with its role in maintaining proteostasis, HSF1 is a prominent lifespan and healthspan promoting factor in *C. elegans*, *Drosophila,* and mammals (Hsu et al., 2003; Morley and Morimoto, 2004; Pierce et al., 2010;

Pierce et al., 2013; Merkling et al., 2015). Conversely, reduced HSF1 activities are observed in mouse models of Huntington's and Parkinson's diseases (Kim et al., 2016; Gomez-Pastor et al., 2017).

HSF-1, the *C. elegans* orthologue of HSF1, has well-established roles in lifespan assurance. RNAi-mediated knock-down or reduction-of-function mutant of HSF-1 significantly reduces lifespan and causes early onset of protein aggregation and physical declines (Hsu et al., 2003; Morley and Morimoto, 2004). On the contrary, over-expression of HSF-1 promotes longevity (Morley and Morimoto, 2004; Baird et al., 2014). In addition, longevity-promoting pathways including arrested germline stem cells (GSC) and reduced insulin/IGF-1 signaling (IIS) suppress proteotoxicity and extend lifespan in an HSF-1 dependent manner (Hansen et al., 2005; Cohen et al., 2006; Volovik et al., 2012; Shemesh et al., 2013). The contributions of HSF-1 in longevity have been attributed to its activities in stress response, as GSC arrest and low IIS enhance the HSR (Labbadia and Morimoto, 2015). However, HSF-1 is suggested to promote longevity beyond activating the HSR since over-expression of a C-terminally truncated HSF-1 that fails to robustly induce the HSR still extends *C. elegans* lifespan (Baird et al., 2014). However, the transcriptional program of endogenous HSF-1 and how HSF-1 regulates gene expression in the absence of external stress in adult somatic tissues are poorly understood.

To obtain deeper understanding of HSF-1's functions in longevity, it is also important to precisely determine the temporal-spatial requirement for HSF-1 in lifespan. Previous work using RNAi shows that HSF1 activity is primarily required during larval stages for longevity (Volovik et al., 2012). This temporal profile is consistent with the traditional view that HSF-1 functions through the HSR since the HSR is under programmed repression at the onset of reproductive maturity on Day 1 of adulthood (Labbadia and Morimoto, 2015). However, as HSF-1 is essential for *C. elegans* larval development, it is difficult to uncouple the lifespan shortening effects with developmental defects. On the other hand, several studies have shown that over-expression of HSF-1 in specific somatic tissues such as neural cells is sufficient to extend lifespan (Morley and Morimoto, 2004; Douglas et al., 2015). However, the tissue-specific contributions of endogenous HSF-1 in longevity are not well characterized.

To better understand the temporal-spatial requirement for HSF-1 in lifespan assurance, in this study, we applied an auxin-inducible degron (AID) system to enable rapid depletion of HSF-1 post-larval development in a tissue-specific manner. We also combined HSF-1 depletion by AID with RNA-seq and ChIP-seq analyses to determine the transcriptional program of HSF-1 that underlies its roles in longevity.

RESULTS

HSF-1 Predominantly Functions in Early Adulthood to Support Normal Lifespan

HSF-1 is required for larval development as the null mutant exhibits larval arrest and lethality (Li et al., 2016; Morton and Lamitina, 2013). To examine HSF-1's contribution to lifespan and uncouple it from its impacts on development, we took advantage of the AID system to deplete HSF-1 from the somatic tissues post-larval development. Our recent work has shown that expression of the TIR1 E3 ligase and degron tagging at the endogenous HSF-1 do not alter larval development while enabling efficient depletion of HSF-1 within 2 h of auxin treatment in adult animals (Edwards et al., 2021). Depletion of HSF-1 from Day 1 in young adults shortened the median lifespan by more than one third (**Figure 1A** and **Table 1**). Time-course analyses revealed that HSF-1 functions throughout the self-reproductive period in hermaphrodites to support lifespan (**Table 1**). On Day 5 of adulthood, when >98% of progenies were already produced (**Supplementary Figure S1A**), depletion of HSF-1 still resulted in a small yet significant lifespan shortening. It has been reported that auxin treatment itself extends lifespan in a concentration-dependent manner (Loose and Ghazi, 2021), and in one of our lifespan trials, we observed a modest lifespan extension when applying the standard 1 mM auxin to the control strain that expresses TIR1 but has no degron insertion at HSF-1 (**Table 1**, Trial #2). We, therefore, repeated the lifespan analyses using 0.5 mM auxin (**Figure 1B**). This experiment confirmed the results as using 1 mM auxin while it had much smaller effects on the control strain (**Table 1**, Trial #2). It is known that signals from the reproductive system could impact *C. elegans* lifespan (Hsin and Kenyon, 1999). Though depletion of HSF-1 from the adult germline dramatically reduced fecundity, depletion of HSF-1 from the soma had a modest impact on the brood size (**Supplementary Figure S1B**). Thus, our results suggest that HSF-1 functions directly through somatic maintenance to support lifespan. Collectively, our data determined the temporal requirement for HSF-1 in a normal lifespan, which overlaps with the self-reproductive period in early adulthood.

Long-Lived glp-1 and daf-2 Mutants Extend the Functional Period of HSF-1 in Lifespan Assurance

HSF-1 is required for lifespan extension in multiple longevity pathways including those mediated by arrested germline stem cells (GSC) and reduced insulin/IGF-1 signaling (IIS). To understand the roles of HSF-1 in longevity pathways post-larval development, we performed lifespan analyses with HSF-1 depleted at different time points of adulthood in the long-lived *glp-1(e2141)* <arrested GSC> and *daf-2(e1370)* <reduced IIS > mutants. As a control for *glp-1(e2141),* we included another temperature-sensitive mutant *fem-3(q20)* that is sterile as *glp-1(e2141)* at the restricted temperature of 25°C < only producing sperm but not oocytes > but has a normal lifespan in the analyses. Similar to wild-type, HSF-1 is required in early adulthood (to at least Day 4 at 25°C) for the lifespan of *fem-3(q20),* and pan-somatic depletion of HSF-1 since Day 1 of adulthood shortened lifespan by about one third (**Figure 2A**; **Table 2**). HSF-1 makes a bigger contribution to the *glp-1(e2141)* lifespan, and its functional period is extended in *glp-1(e2141)* (**Figure 2B**; **Table 2** Trial #1). These effects are more obvious when the median lifespan was

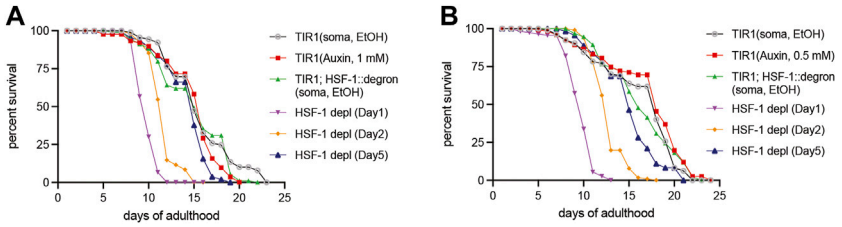

FIGURE 1 | Temporal requirement for HSF-1 in lifespan. Lifespan analysis at 20°C upon pan-somatic depletion of HSF-1 by AID using 1 mM of auxin **(A)** or 0.5 mM of auxin **(B)**. The control strain, CA1200 (*eft-3p:tir1*) was mock treated with ethanol (EtOH) or treated with auxin from Day 1 of adulthood. The HSF-1 AID model, JTL611 (*eft-3p:tir1; hsf-1: degron*) were mock treated with ethanol (EtOH) from Day 1 of adulthood or transferred from EtOH to auxin plates at indicated time to initiate HSF-1 depletion.

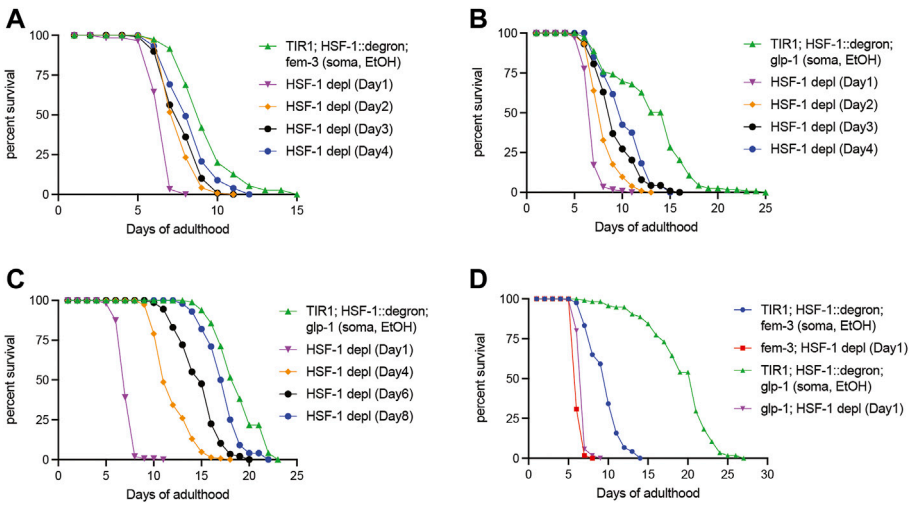

FIGURE 2 | Temporal requirement for HSF-1 in lifespan extension by GSC arrest. **(A,B)** Lifespan analysis at 25°C upon pan-somatic depletion of HSF-1 by AID in the control *fem-3(q20)* **(A)** or the long-lived *glp-1(e2141)* background **(B)**. Animals were mock treated with ethanol (EtOH) from Day 1 of adulthood or treated with auxin starting at the indicated time. **(C,D)** Lifespan analysis at 25°C upon pan-somatic depletion of HSF-1 by AID when fed with carbenicillin-treated OP50 bacteria. Animals were mock treated with ethanol (EtOH) since Day 1 of adulthood as controls. HSF-1 depletion was initiated in the long-lived *glp-1(e2141)* background at the indicated time **(C)**, or a comparison was made between the *fem-3(q20)* and *glp-1(e2141)* when both had HSF-1 depleted since Day 1 of adulthood **(D)**.

TABLE 1 | The lifespan data and statistical test upon pan-somatic HSF-1 depletion in the wild-type background, related to **Figure 1**.

Trial	Strain, treatment	Median Lifespan (Days of adulthood)	S.E.	Observed/Total	% Lifespan change	*p*-value (Log rank)
#1, 20°C	CA1200 (eft-3p::tir1), control	15.69	0.38	89/101		
	CA1200 (eft-3p::tir1), 1mM auxin	14.92	0.35	83/99	−4.91	0.098
#2, 20°C	CA1200 (eft-3p::tir1), control	15.71	0.41	117/130		
	CA1200 (eft-3p::tir1), 1mM auxin	17.74	0.47	110/130	12.92	0.0004
	CA1200 (eft-3p::tir1), control	16.25	0.43	98/130		
	CA1200 (eft-3p::tir1), 0.5mM auxin	17.02	0.42	117/130	4.74	0.0453
#1, 20°C	JTL611 (eft-3p::tir1; hsf-1::degron), control	14.94	0.37	95/108		
	JTL611, 1mM auxin (Day 1)	9.87	0.11	90/120	−33.94	<1e-8
	JTL611, 1mM auxin (Day 2)	11.65	0.15	95/120	−22.02	<1e-8
	JTL611, 1mM auxin (Day 3)	12.84	0.17	102/120	−14.06	<1e-8
	JTL611, 1mM auxin (Day 4)	13.43	0.2	119/120	−10.11	4.4e-8
	JTL611, 1mM auxin (Day 5)	14.3	0.25	109/120	−4.28	0.0005
#2, 20°C	JTL611 (eft-3p::tir1; hsf-1::degron), control	16.39	0.39	94/100		
	JTL611, 0.5mM auxin (Day 1)	9.65	0.16	110/120	−41.12	<1e-8
	JTL611, 0.5mM auxin (Day 2)	12.77	0.16	116/120	−22.09	<1e-8
	JTL611, 0.5mM auxin (Day 3)	13.46	0.2	113/120	−17.88	<1e-8
	JTL611, 0.5mM auxin (Day 4)	14.56	0.22	117/120	−11.17	2.4e-7
	JTL611, 0.5mM auxin (Day 5)	15.05	0.29	112/120	−8.18	0.0006

TABLE 2 | The lifespan data and statistical test upon pan-somatic HSF-1 depletion in long-lived animals, related to **Figure 2** and **Supplementary Figure S2**.

Trial without antibiotic	Strain, treatment	Median lifespan (Days of adulthood)	S.E.	Observed/ Total	% Lifespan change	*p*-value (Log rank)
#1, 25°C	JTL623 (glp-1; eft-3p::tir1), control	13.42	0.56	115/120		
	JTL623 (glp-1; eft-3p::tir1), 1mM auxin (Day 1)	13.97	0.46	116/120	4.10	3.20E-01
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), control	13.12	0.38	114/120		
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	6.98	0.08	116/120	−46.80	<1e-8
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 2)	8.21	0.14	116/120	−37.42	<1e-8
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 3)	9.38	0.2	117/120	−28.51	<1e-8
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 4)	10.28	0.2	120/120	−21.65	<1e-8
#1, 25°C	JTL624 (fem-3; eft-3p::tir1), control	9.23	0.15	114/120		
	JTL624 (fem-3; eft-3p::tir1), 1mM auxin (Day 1)	9.15	0.17	115/120	−0.87	8.48E-01
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), control	9.44	0.16	118/120		
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	6.61	0.07	120/121	−29.98	<1e-8
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 2)	7.77	0.09	120/120	−17.69	<1e-8
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 3)	7.92	0.11	119/120	−16.10	<1e-8
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 4)	8.45	0.13	116/120	−10.49	1.30E-05
Trial (with Carbenicillin)	**Strain, treatment**	**Median lifespan (Days of adulthood)**	**S.E.**	**Observed/ Total**	**% Lifespan change**	***p*-value (Log rank)**
#2, 25°C	JTL623 (glp-1; eft-3p::tir1), control	20.34	0.30	118/120		
	JTL623 (glp-1; eft-3p::tir1), 1mM auxin (Day 1)	20.72	0.53	61/61	1.87	8.92E-02
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), control	19.33	0.36	115/119		
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	6.87	0.05	120/120	−64.46	<1e-8
#3, 25°C	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), control	18.87	0.23	97/100		
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	7.28	0.06	151/151	−61.42	<1e-8
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 4)	12.05	0.16	147/150	−36.14	<1e-8
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 6)	14.89	0.18	147/150	−21.09	<1e-8
	JTL667 (glp-1; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 8)	17.36	0.19	100/100	−8.00	4.90E-07
#2, 25°C	JTL624 (fem-3; eft-3p::tir1), control	9.16	0.17	121/121		
	JTL624 (fem-3; eft-3p::tir1), 1mM auxin (Day 1)	9.75	0.13	120/120	6.44	3.82E-02
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), control	9.66	0.18	120/120		
	JTL670 (fem-3; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	6.33	0.05	119/120	−34.47	<1e-8
Trial (without antibiotic)	**Strain, treatment**	**Median lifespan (Days of adulthood)**	**S.E.**	**Observed/ Total**	**% Lifespan change**	***p*-value (Log rank)**
#4, 20°C	JTL618 (daf-2; eft-3p::tir1), control	32.92	1.57	84/99		
	JTL618 (daf-2; eft-3p::tir1), 1mM auxin (Day 1)	33.69	1.48	87/100	2.34	6.39E-01
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), control	31.93	1.65	82/101		
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 5)	20.85	0.79	94/100	−34.70	<1e-8
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 7)	25.47	0.79	98/100	−20.23	3.60E-08

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TABLE 2 | (*Continued*) The lifespan data and statistical test upon pan-somatic HSF-1 depletion in long-lived animals, related to Figure 2 and Supplementary Figure S2.

Trial with Carbenicillin	Strain, treatment	Median lifespan (Days of adulthood)	S.E.	Observed/ Total	% Lifespan change	*p*-value (Log rank)
#5, 20°C	JTL618 (daf-2; eft-3p::tir1), control	42.18	1.31	112/150		
	JTL618 (daf-2; eft-3p::tir1), 1mM auxin (Day 1)	43.41	1.25	100/150	2.92	8.10E-01
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), control	40.88	1.14	98/150		
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 7)	27.07	0.67	93/150	–33.78	<1e-8
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 11)	29.85	0.48	142/151	–26.98	<1e-8
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 15)	32.59	0.55	121/126	–20.28	<1e-8
	JTL641 (daf-2; eft-3p::tir1; hsf-1::degron), 1mM auxin (Day 19)	39.22	0.52	116/119	–4.06	1.10E-07

further increased as animals were fed with antibiotic-treated bacteria (**Figure 2C**; **Table 2** Trial #2 and #3). The additional lifespan extension was likely due to reduced bacterial infection since *glp-1(e2141)* delays later deaths with an atrophied pharynx but not earlier deaths from pharyngeal pathology by a bacterial infection (Zhao et al., 2017). In this growth condition, HSF-1 supported lifespan till at least Day 8, and depletion of HSF-1 in Day 1 adults resulted in >60% of lifespan reduction. It is noteworthy that depletion of HSF-1 was still effective in both the *fem-3 (q20)* and *glp-1(e2141)* animals on Day 4 (**Supplementary Figures S2A, B**), therefore, the relatively modest lifespan shortening effects in *fem-3(q20)* are not simply an artifact of defective AID during aging but rather suggest that HSF-1 is less active or its function is minimally required for the lifespan of *fem-3(q20)* beyond Day 4 at 25°C. While the signal of GSC arrest in *glp-1(e2141)* more than doubled the lifespan in the presence of HSF-1 (when fed with antibiotic-treated bacteria) (**Figure 2D**; **Table 2**, Trial #2), it had marginal effects if HSF-1 was depleted since Day 1 of young adults. These results indicate lifespan extension by arrested GSC is completely dependent on HSF-1 in adulthood, and provide an example that HSF-1's roles in larval development and longevity are temporally uncoupled.

We also tested the roles of HSF-1 in longevity by reducing IIS using the *daf-2(e1370)* mutant. Due to the high incidence of internal hatching upon HSF-1 depletion on Day 1 (54%, 189 out of 350) and Day 3 (39%, 136 out of 350) of *daf-2(e1370),* it is difficult to estimate the total contribution of HSF-1 to lifespan throughout adulthood. However, similar to the *glp-1(e2141)* mutant, *daf-2(e1370)* also seems to extend the functional period of HSF-1 as depletion of HSF-1 since Day 5 and Day 7 resulted in ~35% and ~20% of lifespan reduction in *daf-2(e1370)* (**Supplementary Figure S2C**, **Table 2** Trial #4) while depletion of HSF-1 since Day 5 in the wild-type only led to modest (4–8%) lifespan reduction (**Table 1**). This becomes apparent when *daf-2(e1370)* animals were fed with antibiotic-treated bacteria, in which HSF-1 contributes to longevity up to Day 19 of adulthood (**Supplementary Figure S2D**, **Table 2** Trial #5). Collectively, we found longevity by GSC arrest and reduced IIS correlates with extended functional period of HSF-1.

HSF-1 Directly Activates Transcription From its Associated Promoters in Somatic Cells of Young Adults in the Absence of Heat Stress

The prevailing view is that HSF-1 promotes longevity through its ability to activate the HSR. HSF-1 also drives a transcriptional program that is different from the HSR in *C. elegans* larval development (Li et al., 2016). In addition, enhancing HSF-1 activities by transgenic over-expression of HSF-1, mild mitochondrial perturbation, or ablation of its negative regulator HSB-1 extends lifespan through functions beyond inducing the canonical HSR (Baird et al., 2014; Kumsta et al., 2017; Higuchi-Sanabria et al., 2018; Egge et al., 2019; Williams et al., 2020; Sural et al., 2020). To better understand the molecular mechanism underlying the physiological roles of HSF-1 in lifespan, we set out to determine the transcriptional program of endogenous HSF-1 in somatic cells on Day 1 of young adults. Recently, we have determined HSF-1 binding sites specifically in the soma or the germline and binding sites used in both tissue types through whole animal ChIP-seq analyses following an acute depletion of HSF-1 in the soma or germline (Edwards et al., 2021). Among those sites, 79 promoter-associated HSF-1 binding peaks are either enriched in the soma (e.g., *Y94H6A.10*) or shared by the soma and germline (e.g. *hsp-1/hsc-70*) (**Figure 3A**). This result suggests that albeit the number of binding peaks is smaller compared to that in larval development (Li et al., 2016), HSF-1 can bind to promoters without thermal stress in adult somatic cells that are postmitotic.

We then examined the transcriptional impact of HSF-1 binding *via* RNA-seq analyses following HSF-1 depletion from somatic cells by AID. We performed RNA-seq in the control strains that express TIR1 but have no degron insertion in a time course of auxin treatment (**Supplementary Figures S3A–D**). This set of experiments, when compared to those using the experimental strains with degron tagging at HSF-1, identified the small groups of differentially expressed (DE) genes caused by auxin treatment (**Supplementary Figure S3A**, mock *vs*. auxin treatment in the control strain) or by degron insertion at HSF-1 (**Supplementary Figure S3B**, compare the control and experimental strains with mock treatment). As

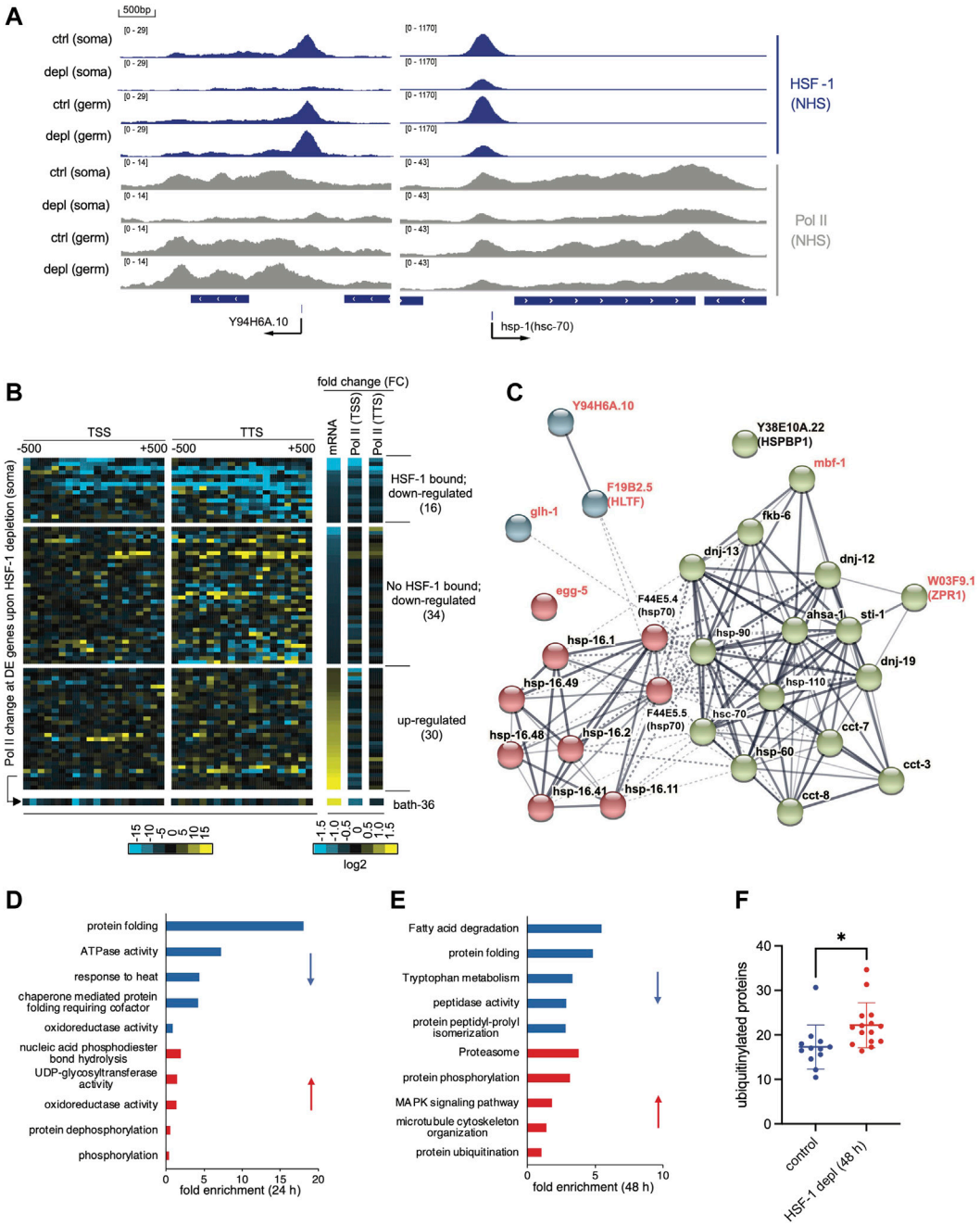

FIGURE 3 | Transcriptional program of HSF-1 in the soma of young adults. **(A)** G browser views of HSF-1 and RNA Polymerase II (Pol II) occupancy at the *Y94H6A.10* and *hsp-1 (hsc-70)* gene loci in young adults grown at the ambient temperature of 20°C with either the mock treatment (ctrl) or an acute HSF-1 depletion (depl) from the soma (soma) or the germline (germ) (auxin treatment for 2 h). **(B)** Heatmaps of Pol II occupancy changes upon HSF-1 depletion in young adults from the soma for 2 h and mRNA changes by HSF-1 depletion for 24 h at differentially expressed (DE) genes. The data shown are based on ChIP-seq and RNA-seq experiments in JTL611 (wild-type background). The DE genes are those that significantly altered mRNA levels (FDR: 0.05) upon pan-somatic depletion of HSF-1 for 24 h (**Supplementary Table S2**). Pol II occupancy change was calculated as the difference of normalized ChIP-seq reads (HSF-1 depletion vs. the control) mapped in 50 bp bins, ±500 bp from the transcription start sites (TSS) and transcription termination site (TTS). The fold change (FC) of Pol II occupancy (2 h of HSF-1 depletion) and mRNA (24 h of HSF-1 depletion) are shown in the log2 scale. The DE genes were first ranked by fold change of mRNA and then by whether bound by HSF-1 at the promoters (1 kb from TSS). The number of DE genes in each group is shown in parentheses. **(C)** The gene network is directly activated by HSF-1 in the somatic cells from *glp-1(e2141)*. Genes included are those with HSF-1 binding peaks at the promoters and significantly decreased expression upon HSF-1 depletion from the soma for 8 h or 24 h on Day 1 of adulthood. The protein-protein interaction network was retrieved from the STRING database and grouped by kmeans clustering ($n = 3$). The node color represents the cluster to which the gene belongs. The color saturation of edges represents the confidence score of functional interaction. Genes with names in black encode chaperones or co-chaperones, and genes with names in red are those with other functions. **(D,E)** Gene Ontology (GO) analyses of DE genes by HSF-1 depletion

(*Continued*)

FIGURE 3 | from the soma in *glp-1(e2141)* for 24 h **(D)** and 48 h **(E)**. The top5 GO terms based on enrichment score are shown for down-regulated genes (blue bars) and up-regulated genes (red bars) respectively. **(F)** Quantification of immunofluorescence of ubiquitinylated proteins in the control group or upon HSF-1 depletion from the soma since Day 1 of adulthood for 48 h in *glp-1(e2141)*. Data are shown as mean ± standard deviation (*n*>=12). Only animals with clear staining by the control antibody against REC-8 were included in analyses. Statistical significance was calculated by unpaired, two-tailed Student's t test. *$p < 0.05$.

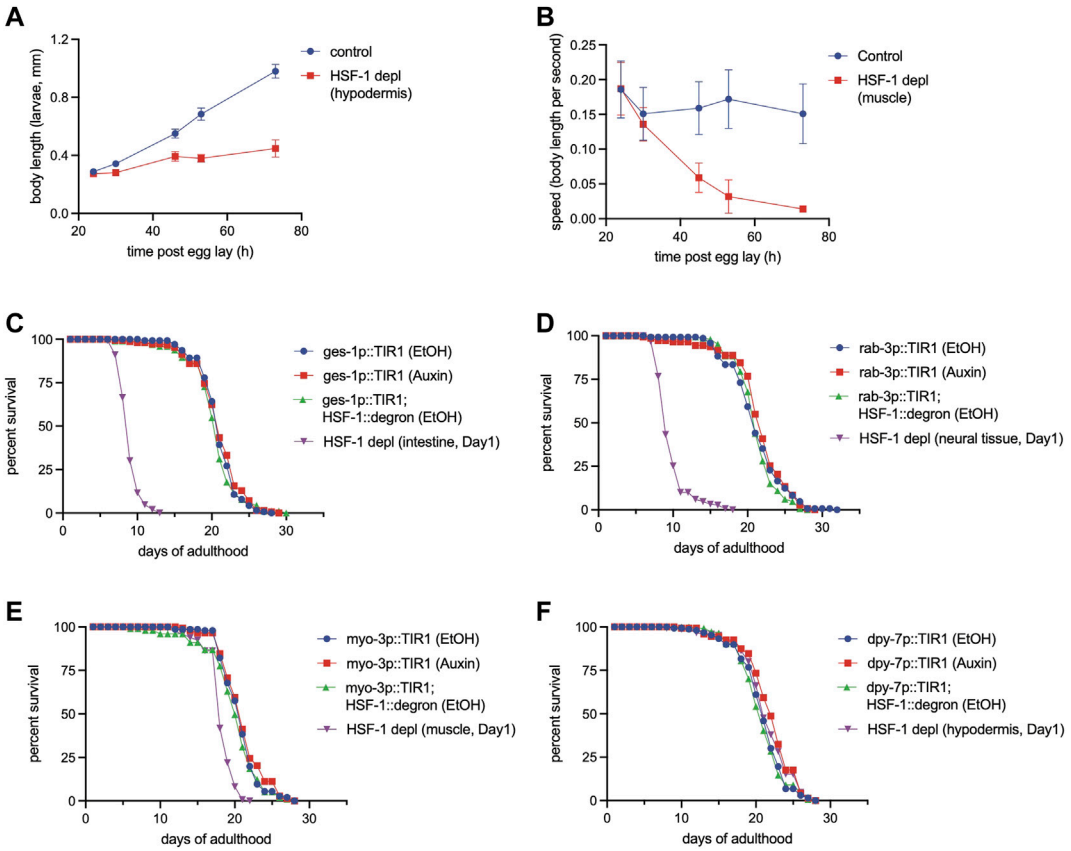

FIGURE 4 | Different tissue requirements for HSF-1 in larval development and lifespan assurance. **(A)** Size tracking of developing larvae with continuous HSF-1 depletion in the hypodermis initiated at egg lay. Experiments were done in the wild-type background at 20°C. Data are represented as mean ± standard deviation (*n*>=12). $p < 0.0001$ (control vs. HSF-1 depletion, two-way ANOVA). **(B)** Mobility of developing larvae measured as body length per second with continuous HSF-1 depletion in the muscle initiated at egg lay. Experiments were done in the wild-type background at 20°C. Data are represented as mean ± standard deviation (*n*>=12). $p < 0.0001$ (control vs. HSF-1 depletion, two-way ANOVA). **(C–F)** Lifespan analysis at 25°C upon depletion of HSF-1 by AID in the intestine **(C)**, neural cells **(D)**, muscle **(E)**, and hypodermis **(F)** since Day 1 of adulthood. Experiments were done in the control strains (expressing TIR1 only) and HSF1 AID models (expressing TIR1 and having degron insertion at endogenous *hsf-1*) in the long-lived *glp-1(e2141)* background. Animals were fed with carbenicillin treated OP50 bacteria and were mock treated with ethanol (EtOH) or treated with auxin since Day 1 of adulthood.

auxin treatment resulted in almost identical transcriptional changes in the two control strains that express TIR1 in the soma and germline, respectively (**Supplementary Figure S3C**), we conclude that these changes were due to auxin rather than off-target effects of TIR1. By filtering out HSF-1-independent changes (**Supplementary Figures S3A, B**, **Supplementary Table S1**), our methods specifically determined DE genes caused by HSF-1 depletion (**Supplementary Figure S3D**). Among the 80 DE genes resulting from 24 h of HSF-1 depletion in the soma, 16 out of 50 down-regulated genes have HSF-1 binding at the promoter (**Supplementary Figure S3E**, **Supplementary Table S2**) while only 1 out of 34 up-regulated genes are bound by HSF-1, suggesting that HSF-1 functions as a transcriptional activator in somatic cells of young adults. The group of 16 HSF-1-bound down-regulated genes exhibit a decrease of RNA Polymerase II (Pol II) occupancy both at the promoter and at the end of genes (**Figure 3B**) upon 2 h depletion of HSF-1 suggesting that they are likely the direct targets of HSF-1 and that HSF-1 functions at the step of Pol II recruitment. For examples, a decrease of Pol II occurs across the genes of *Y94H6A.10* and *hsp-1/hsc-70* upon HSF-1 depletion in the soma (**Figure 3A**). On the contrary, down-regulated genes without HSF-1 binding and up-regulated genes (including the only HSF-1 bound gene, *bath-36* in this group) lack a correlation between mRNA (24 h post HSF-1 depletion) and Pol II occupancy changes (2 h of

TABLE 3 | The lifespan data and statistical test upon tissue-specific HSF-1 depletion, related to **Figure 4**.

Targeted tissue (with Carbenicillin)	Strain, treatment	Median Lifespan (Days of adulthood)	S.E.	Observed/ Total	% Lifespan change	*p*-value (Log rank)
Intestine, 25°C	JTL658 (glp-1; ges-1p::tir1), control	21	0.22	140/150		
	JTL658 (glp-1; ges-1p::tir1), 1mM auxin (Day 1)	20.96	0.27	147/155	–0.19	3.53E-01
	JTL700 (glp-1; ges-1p::tir1; hsf-1::degron), control	20.47	0.34	93/100		
	JTL700 (glp-1; ges-1p::tir1; hsf-1::degron), 1mM auxin (Day 1)	9.05	0.11	146/150	–55.79	<1e-8
Neural cells, 25°C	JTL659 (glp-1; rab-3p::tir1), control	21.24	0.3	145/150		
	JTL659 (glp-1; rab-3p::tir1), 1mM auxin (Day 1)	21.62	0.33	142/149	1.79	2.78E-01
	JTL701 (glp-1; rab-3p::tir1; hsf-1::degron), control	21.21	0.24	146/150		
	JTL701 (glp-1; rab-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	9.79	0.17	150/150	–53.84	<1e-8
Muscle, 25°C	JTL660 (glp-1; myo-3p::tir1), control	20.82	0.21	146/150		
	JTL660 (glp-1; myo-3p::tir1), 1mM auxin (Day 1)	21.14	0.23	143/150	1.54	2.20E-01
	JTL702 (glp-1; myo-3p::tir1; hsf-1::degron), control	19.99	0.36	97/100		
	JTL702 (glp-1; myo-3p::tir1; hsf-1::degron), 1mM auxin (Day 1)	18.22	0.16	145/150	–8.85	<1e-8
Hypodermis, 25°C	JTL661 (glp-1; dpy-7p::tir1), control	20.94	0.27	140/150		
	JTL661 (glp-1; dpy-7p::tir1), 1mM auxin (Day 1)	21.95	0.28	142/150	4.82	3.10E-03
	JTL703 (glp-1; dpy-7p::tir1; hsf-1::degron), control	20.84	0.25	144/150		
	JTL703 (glp-1; dpy-7p::tir1; hsf-1::degron), 1mM auxin (Day 1)	21.46	0.29	145/150	2.98	2.72E-02

HSF-1 depletion), suggesting they are indirectly impacted by HSF-1 (**Figure 3B**).

HSF-1 Drives Expression of an Important Sub-Chaperome in Fully-Developed Somatic Cells

Among the direct targets of HSF-1 in somatic tissues of young adults (**Supplementary Figure S3F**), most have substantial HSF-1 binding in the germline as well (*Y94H6A.10* is the only exception). To control for the potential interference by mRNA from the germline and increase the specificity and sensitivity of RNA-seq analyses, we also performed the experiments in the germline deficient *glp-1(e2141)* background. Indeed, we have uncovered a bigger group of HSF-1 direct targets (28 genes), each of which is associated with one of the 79 HSF-1 binding peaks at the promoter in the soma and decreases mRNA upon 8 h and/or 24 h of HSF-1 depletion (**Figure 3C**, **Supplementary Table S2**). Similar to the list of genes identified in the wild-type background, the majority of these genes (22 out of 28) (**Figure 3C**, gene names in black) encode either molecular chaperones or co-chaperones, which function together in protein folding and protein conformation maintenance. These chaperone/co-chaperone genes are grouped into two clusters with one including canonical HSR genes encoding members of HSP70 and small heat shock proteins (HSP-16) whose expression is highly inducible by stress (**Figure 3C**, cluster #1: red nodes), and the other cluster enriched with constitutively expressed chaperones and co-chaperones (**Figure 3C**, cluster #2: green nodes). The latter group contains the essential HSP-90 and HSC-70 chaperones as well as their co-chaperones (DNJs, FKB-6, STI-1, AHSA-1, HSP-110, and the HSPBP1 orthologue Y38E10A.22). It also contains subunits of the chaperonin complexes in the cytosol (CCT-3, CCT-7, and CCT-8) and mitochondria (HSP-60). These HSF-1-dependent, constitutively expressed chaperone and co-chaperone genes span most the ATP-dependent chaperone systems in metazoan (Clare and Saibil, 2013) and dictate protein folding capacity in the cytosol, nuclei, and mitochondria. It is noteworthy that the majority of genes in cluster #2 (14 out of 16) belong to the developmental HSF-1 transcriptional program (Li et al., 2016), which are regulated by HSF-1 differently from the HSR. As genetic perturbation of the HSP-90, HSC-70 and cytosolic chaperonin systems accelerates age-dependent proteostatic and physiological declines (Brehme et al., 2014), our data suggest a role of HSF-1 in longevity by activating the expression of these chaperone systems in fully-developed somatic cells.

A decrease of this selective 'sub-chaperome' precedes massive transcriptomic changes upon HSF-1 depletion in *glp-1(e2141),* which also supports that they are the primary targets of HSF-1 in adult somatic cells. 19 out of 22 HSF-1 directly regulated chaperone/co-chaperone genes already decrease expression at 8 h of HSF-1 depletion (**Supplementary Table S2**). They remain the most prominent functional group ('protein folding') among ~400 DE genes at 24 h of HSF-1 depletion as shown by Gene Ontology (GO) analyses (note the enrichment of genes with 'ATPase activity' results from ATP-dependent chaperones, **Figure 3D**). 48 h of HSF-1 depletion, however, led to much bigger changes in the transcriptome (>1200 DE genes, **Supplementary Table S2**). Genes in the ubiquitin–proteasome system (UPS) are up-regulated (**Figure 3E**), which is a typical response to protein misfolding, implicating imbalanced proteome over time caused by loss of HSF-1 and consequent decline in protein folding. This idea is supported by the increased levels of ubiquitylated proteins in somatic tissues upon 48 h of HSF-1 depletion (**Figure 3F**, **Supplementary Figures S3G, H**), and is consistent with published results that HSF-1 RNAi leads to increased protein

misfolding and aggregation (Nollen et al., 2004; Ben-Zvi et al., 2009).

We also identified a few nonchaperone genes as HSF-1 direct targets (**Figure 3C**, gene names in red), although their roles in lifespan are not clear. Two of the encoded proteins, MBF-1 (Multiprotein Bridging Factor 1) and W03F9.1/ZPR1 in cluster #2 are proposed to be transcriptional coactivators and interact with chaperones/co-chaperones based on the studies of their orthologues in other model systems (Arce et al., 2006; Kannan et al., 2020). Especially, the *mbf-1* gene is a direct target of HSF-1 in *C. elegans* larval development (Li et al., 2016), and expression of its orthologues in yeast and *Drosophila* is also controlled by HSF1 (Birch-Machin et al., 2005; Pincus et al., 2018). Similar to *mbf-1*, expression of *Y94H6A.10* (a gene with unknown function) and *F19B2.5/HLTF* (encodes an SWI/SNF chromatin remodeler family protein) is activated by HSF-1 in larval development, suggesting that at least part of HSF-1 developmental transcription program sustains in adulthood.

To test whether the transcriptional program of HSF-1 identified in *glp-1(e2141)* is unique to this longevity model and how much it is linked to the higher growth temperature (25 *vs.* 20°C for wild-type), we performed RNA-seq analyses in the *fem-3(q20)* control at 25°C. The *fem-3(q20)* shows a similar decrease of HSF-1 direct targets including constitutively expressed chaperones, stress-inducible chaperones as well as nonchaperone genes upon 24-h HSF-1 depletion (**Supplementary Figures S3I, J**). Although there is a trend of a bigger difference in *glp-1(e2141)* compared to that in *fem-3(q20)* (**Supplementary Figure S3I**), not all HSF-1 direct targets are more highly expressed in *glp-1(e2141)* as one may expect if HSF-1 is hyperactivated by GSC-mediated longevity signal (**Supplementary Figure S3J**). The mRNA of stress-inducible *hsp70* and *hsp-16.2* are at higher levels in *glp-1(e2141)* compared to *fem-3(q20)*, suggesting expression levels of these canonical HSR genes are not solely dictated by the growth temperature. On the contrary, higher mRNA levels of constitutively expressed chaperones are observed in *fem-3(q20)*. This expression pattern is likely linked to the different cell compositions in these two types of animals. Though *fem-3(q20)* is sterile at 25°C, it still has a germline that makes sperm. The smaller changes upon depletion of HSF-1 from the soma in *fem-3(q20)* could be well explained by the 'masking effect' from unchanged mRNAs in the germline. Consistent with this idea, our recent paper shows that *hsc-70* and *hsp-90* mRNAs are enriched in the germline while HSR is more robust in the soma (Edwards et al., 2021), therefore *fem-3(q20)* with more germ cells shows higher levels of constitutive chaperones but lower levels of inducible chaperones in whole animal analyses. Collectively, the HSF-1 transcriptional program that we identified in the germline deficient *glp-1(e2141)* likely applies to somatic cells of animals with normal lifespan as seen in *fem-3(q20)*. Future studies are needed to determine whether HSF-1 activities are enhanced by longevity signals in the absence of external stress.

Tissue Requirements for HSF-1 in Larval Development and Lifespan Assurance are Different

While most of the HSF-1-dependent, constitutively-expressed chaperones and co-chaperones have roles in all cell types, over-expression of HSF-1 in specific tissues (e.g., neural cells) is sufficient to extend lifespan (Morley and Morimoto, 2004; Douglas et al., 2015). To understand the tissue requirement for endogenous HSF-1 in lifespan, we have made transgenic models that express TIR1 E3 ligase specifically in one of the major somatic tissue types (neural system, intestine, muscle, and hypodermis) to enable tissue-specific depletion of HSF-1 by AID. All of our AID models successfully depleted HSF-1 from the nuclei in the target tissues within 2 h of auxin treatment (**Supplementary Figures S4A–D**). We also checked HSF-1: degron:GFP in the neighboring tissues and were able to confirm the specificity of AID except for our neural model, in which the nuclear HSF-1 seemed also depleted in intestinal cells near the head but not in the center or toward the tail (**Supplementary Figure S4D**). The same neural TIR1 transgene did not deplete degron:GFP (not fused with HSF-1) in intestinal cells near the head as it did for HSF-1:degron:GFP upon auxin treatment (**Supplementary Figure S4E**). This result implies depletion of HSF-1 from a subset of intestinal nuclei in our neural AID model is unlikely due to leaky expression of TIR1 but instead dependent on neural HSF-1. However, due to high levels of auto-florescence in the intestine, future studies with more sensitive and quantitative methods to measure HSF-1 protein levels and its localization, and using alternative neural AID models are needed to confirm the results.

We then tested the tissue requirements of HSF-1 for larval development. Loss of HSF-1 in any of these tissues since egg lay led to larval developmental arrest or delay (**Figure 4A**, **Supplementary Figures S5A–C**). HSF-1 depletion from hypodermis caused larval arrest at L3-L4, and the animals were associated with molting defects (**Supplementary Figure S5D**). Animals with HSF-1 depleted in the intestine, neural cells or muscle managed to develop into adults. Intestinal depletion of HSF-1 led to a huge larval delay, more than doubling the time needed for developing into adults (**Supplementary Figure S5A**). Loss of HSF-1 in neural cells and muscle had a relatively milder larval delay (**Supplementary Figures S5B, C**) but greatly reduced mobility eventually making the animals paralyzed at the young adult stage (**Figure 4B**, **Supplementary Figure S5E**). Depletion of HSF-1 in the muscle also led to egg-laying defects, resulting in 100% internal hatching (20/20 animals, **Supplementary Figure S5F**). It is noteworthy that none of the tissue-specific HSF-1 depletion phenocopied the L1-L2 arrest by pan-somatic depletion of HSF-1 (Edwards et al., 2021), suggesting that HSF-1 functions cooperatively in all the tissue types tested to support larval development.

We then performed lifespan analysis with tissue-specific depletion of HSF-1 on Day 1 of adulthood. We chose *glp-1(e2141)* as the model since pan-somatic depletion of HSF-1 in this background exhibited the biggest lifespan shortening effect, therefore, providing sufficient dynamic range to examine potentially smaller effects from HSF-1 depletion in a single tissue type. Depletion of HSF-1 in the intestine and neural cells both resulted in ~55% lifespan shortening (**Figure 4C, D**, **Table 3**) which is only slightly smaller than that upon pan-somatic depletion (**Figure 2C**; **Table 2**). Depletion of HSF-1 from muscle led to a fairly small but still significant decrease (~9%) in median lifespan (**Figure 4E**; **Table 3**). Despite the essentiality of HSF-1 in hypodermis during larval development, loss of HSF-1

from hypodermis post-larval development did not significantly alter lifespan (**Figure 4F**; **Table 3**). Collectively, our results indicate the different tissue requirements for HSF-1 in development and lifespan, suggesting that the roles of HSF-1 in these two processes are uncoupled.

DISCUSSION

HSF-1 is known as a prominent lifespan promoting factor in *C. elegans* and has been proposed to contribute to lifespan largely from its activities at the larval stages based on RNAi experiments (Volovik et al., 2012) and through its ability to activate the HSR to cope with proteotoxic stress and maintain proteostasis (Hsu et al., 2003; Morley and Morimoto, 2004; Hansen et al., 2005; Cohen et al., 2006; Shemesh et al., 2013; Labbadia and Morimoto, 2015). HSF-1 is also required for larval development through a transcriptional program different from the HSR (Li et al., 2016). Therefore, it is important to distinguish the role of HSF-1 in the maintenance of adult somatic cells, which are all postmitotic, with that in development, since defects in both processes could shorten lifespan.

In this study, we used the auxin-inducible degron (AID) system to enable rapid and efficient depletion of HSF-1 and determined the spatiotemporal requirement for HSF-1 in lifespan post-larval development. We found that HSF-1 is predominantly required during the early adulthood to support lifespan, which overlaps with the self-reproductive period in wild-type animals (**Figure 1**; **Table 1**). This temporal correlation is interesting, implicating that somatic maintenance by HSF-1 may be coupled with reproductive activities to ensure a favorable environment for internal embryonic development and successful egg-laying. Supporting this idea, we have observed egg retention and increased internal hatching upon HSF-1 depletion from pan-soma or specifically from the muscle. It is not clear though whether HSF-1 has lost most of its activities after the reproductive period or the molecular decline has reached a threshold then so that the protective mechanism by HSF-1 makes no consequence. The functional period of HSF-1 in lifespan is extended in long-lived animals with GSC arrest or reduced IIS (**Figure 2**; **Table 2**). Importantly, GSC-arrest mediated lifespan extension is completely dependent on HSF-1 activity in adulthood (**Figure 2D**). This result suggests the functional impacts of HSF-1 in larval development and longevity can be uncoupled temporally, and also implies that germline signals in adults may regulate HSF-1's activities in the soma. Our RNA-seq analysis on long-lived *glp-1(2141)* and the control *fem-3(q20)* to compare HSF-1 activities is inconclusive due to the different cell compositions of these animals. Future work with tissue-specific transcription measurement is needed to test whether longevity signaling (e.g., GSC and IIS) hyperactivates HSF-1 in physiological condition (not upon stress) and/or sustains HSF-1 activities longer during aging. Consistent with the idea that functions of HSF-1 in development and longevity can be uncoupled, we found the tissue requirements for HSF-1 in larval development and lifespan are different (**Figure 4**). Loss of HSF-1 in the intestine or neural system showed >80% of lifespan shortening effects as the pan-somatic depletion of HSF-1. As neural cells and intestine are the endocrine centers in *C. elegans*, it is likely that functions of HSF-1 in neural cells and intestine impact neighboring tissues nonautonomously as well. One complication of our results is that despite using the classic *rab-3* promoter to express TIR1 in the neural system, auxin treatment depleted HSF-1 from a subset of intestinal nuclei as well. Given the very different phenotypes in larval development, we do not believe that neural depletion of HSF-1 in our AID models affected physiology solely through intestinal HSF-1. However, it calls for future studies to further confirm the specificity of our neural model, and understand the interaction of HSF-1 in the intestine and nervous system.

In this study, we also examined the transcriptional program of HSF-1 and its regulation in somatic tissues of young adults by RNA-seq and ChIP-seq analyses following HSF-1 depletion. With careful control experiments, we have determined the differentially expressed (DE) genes induced by auxin. This gene list (**Supplementary Table S1**) not only helped us identify transcriptomic changes specific to HSF-1 depletion but also provides a useful reference for any transcriptomic studies using AID. We have found that HSF-1 activates transcription at its associated promoters, and functions at the step of Pol II recruitment (**Figures 3A, B**). This is different from the HSR in *Drosophila* and mammalian cells, where HSF-1 functions at releasing promoter-proximally paused Pol II into productive elongation (Duarte et al., 2016; Mahat et al., 2016). It is also different from the HSR in *C. elegans,* in which HSF-1 promotes both Pol II recruitment and elongation as depletion of HSF-1 either decreases Pol II across the gene (e.g., inducible *hsp70s*) or causes Pol II accumulation at the promoters (e.g. *hsp-110*) during heat shock (Edwards et al., 2021). The different roles of HSF-1 in transcription regulation in physiology and the HSR may be due to the fact that Pol II pausing or stalling is not prevalent in physiological conditions in *C. elegans* as it lacks the pausing factor NELF (Kruesi et al., 2013; Maxwell et al., 2014).

Diverse mechanisms have been proposed for HSF-1's roles in longevity (Baird et al., 2014; Kumsta et al., 2017; Higuchi-Sanabria et al., 2018; Egge et al., 2019; Williams et al., 2020; Sural et al., 2020), and the HSF-1 direct target genes that we identified in adult soma provide a molecular basis for understanding functions of endogenous HSF-1 in comparison to gain-of-function phenotypes or pleotropic effects. One example is that expression of the troponin protein PAT-10 is activated and responsible for longevity by HSF-1 overexpression (16). We did not find that HSF-1 binds to the *pat-10* promoter or depletion of HSF-1 alters its expression, implicating regulation of PAT-10 is likely unique to HSF-1 overexpression. HSF-1 directly activates a compact transcriptional program including classical stress-inducible chaperones, constitutively-expressed chaperones, and co-chaperones as well as a few nonchaperone genes (**Figure 3C**). We conclude that protein folding is the primary function of this HSF-1 transcriptional program because 1 > chaperone and co-chaperone genes take more than three quarters of this group (22 out of 28), 2 > their expressions change early upon HSF-1 depletion, and 3 > transcriptomic signature at a later time (48 h after HSF-1 depletion) suggests proteotoxic stress response as a

consequence of losing folding capacity (**Figures 3D, E**). Despite the higher fold changes in expression of certain inducible chaperones upon HSF-1 depletion (**Supplementary Figure S3G**), the mRNA levels of constitutively-expressed chaperones are much higher (**Supplementary Figure S3H**). For example, the mRNA of *hsc-70* is ~50 fold as the inducible hsp70, *F44E5.5* in the presence of HSF-1. Thus, despite some differences in biochemical property, it is reasonable to think the constitutively expressed HSC-70 may have a bigger contribution to the overall folding capacity than the inducible HSP70 in the absence of stress. It is important to note that this group of constitutively-expressed chaperones and co-chaperones (cluster #2 in **Figure 3C**) overlap largely with the evolutionarily conserved 'core chaperome' defined by a previous study (in all the 5 functional groups and 50% of individual proteins) (Brehme et al., 2014). Their expression decline was proposed to underlie human brain aging, and genetic perturbation of this 'core chaperome' led to the early onset of proteome imbalance and healthspan shortening in *C. elegans* (Brehme et al., 2014). Collectively, we propose that HSF-1's role in lifespan assurance is primarily through activating the expression of a selective group of chaperone and co-chaperone genes and enhancing protein folding capacity.

HSF-1 also directly activates a few nonchaperone genes. Of those, MBF-1, W03F9.1/ZPR1, and F19B2.5/HLTF are proposed to have roles in transcription regulation and might be involved in the secondary transcriptional response to HSF-1 depletion. Future studies will examine whether they also affect animal lifespan, and understand the biological significance of their regulation by HSF-1.

MATERIALS AND METHODS

Worm Strains and Maintenance

Unless stated, *C. elegans* strains were maintained at 20°C on NGM plates seeded with OP50 bacteria and were handled using standard techniques (Brenner, 1974). The temperature-sensitive *glp-1 (e2141) and fem-3(q20)* animals were maintained at 15°C and grown at 25°C (since L1) for experiments.

The HSF-1 AID models were made by CRISPR knock-in of aid:gfp to the C-terminus of endogenous *hsf-1* gene as detailed in our previous publication (Edwards et al., 2021). New tissue-specific TIR1 models were made by modification of CA1200 (*eft-3p:tir1:mRuby*) and swapping the *eft-3* promoter with tissue-specific promoters. We first removed the *eft-3* promoter through microinjection of two chemically modified synthetic sgRNA (Synthego) in CA1200, which are against the upstream and downstream regions of the *eft-3* promoter, along with Cas9 Nuclease (Integrated DNA Technologies, IDT) following the previously published protocol (Prior et al., 2017). The DNA sequences targeted by the sgRNAs are GCT CTGGTACCCTCTAGTCA (upstream) and AGTTACGGT CCTTGTCGAGT (downstream) respectively. The resulting promoter-less allele contains a short insertion ('GGCATCCA') between the two cutting sites. We then inserted the tissue-specific promoters by microinjection of an sgRNA against that short insertion in the promoter-less allele (corresponding DNA sequence: GGTCCTTGTTGGATGCCT CA) with Cas9 Nuclease and PCR fragments of tissue-specific promoters as the repair templates. The *rab-3b* (1.2 kb), *dpy-7* (350 bp), and *myo-3* (2.5 kb) promoters were used for the expression of TIR1 in neural cells, hypodermis, and muscle. The published allele in CA1209 (*ges-1p:tir1:mRuby*) was used for intestinal expression of TIR1. All the transgenic models made by CRISPR were outcrossed 6 times before use.

Auxin Treatment

Auxin treatment was performed by transferring worms to bacteria-seeded NGM plates containing 1 mM (if not specified) or 0.5 mM auxin (Auxin: indole-3-acetic acid, Sigma). The preparation of auxin stock solution (400 mM in ethanol) and auxin-containing NGM plates was performed as previously described (Zhang et al., 2015). In all experiments, worms were also transferred to NGM plates containing 0.25% or 0.125% of ethanol (EtOH) to serve as the mock-treated control for 1 and 0.5 mM auxin respectively.

Measurements of Body Length, Mobility, and Brood Size

The HSF-1 AID animals and the corresponding control animals that only express TIR1 were age-synchronized by egg lay for 1 h on EtOH or Auxin plates. Larvae were grown for the indicated time (**Figure 4** and **Supplementary Figure S4**) and crawling animals were recorded using a Leica M205 FA microscope. Videos were imported into ImageJ and analyzed for the size of animals (body length, mm) and mobility (body length per second, BLPS) using the wrMTrck plugin.

For brood size analyses (**Supplementary Figure S1**), animals were synchronized by egg lay and singled at L4/young adult stage onto plates containing ethanol (control) or auxin (HSF-1 depl) to lay eggs for 24 h. Worms were then transferred to new plates every day and eggs were allowed to hatch and grow to the L3 stage, at which point the number of progeny was counted.

Lifespan Assays and Antibiotic Treatment of OP50

Age-synchronized worms were scored as dead and removed in the absence of touch response or pharyngeal pumping 6 days per week. For fertile animals, worms were transferred to fresh plates every day through the reproductive period to remove progeny. Bagged, desiccated, or missing animals were censored from analysis.

In a subset of lifespan experiments, carbenicillin treatment of OP50 was performed as previously described to prevent bacterial growth (Lenaerts et al., 2008). Briefly, freshly grown *E. coli* OP50 cells were spun at 3000 × g for 20 min and resuspended in the same volume of M9 buffer supplemented with 0.5 mM of carbenicillin. The bacteria were then incubated in the shaker at 37°C for 3 h. The bacteria were concentrated by 5 fold (pelleting the cells, removing 80% M9 buffer, and resuspending) and seeded onto 10 cm NGM plates using 400 ul of concentrated bacteria per plate.

Brightfield and Fluorescence Imaging

Imaging of live animals was done by immobilizing age-synchronized young adult animals in a drop of M9 buffer containing 6 mM levamisole on a 2% agarose pad. Fluorescent images (**Supplementary Figure S2**) were acquired immediately using a Zeiss LSM880 Confocal Microscope with a ×40 water objective. Zen software was used to obtain z-stacks and subsequent processing. Brightfield images (**Supplementary Figure S4**) were taken on a Zeiss axio observer inverted microscope with a ×20 air objective.

RNA Extraction and RNA-Seq

The HSF-1 AID animals and the corresponding control animals that only express TIR1 were synchronized by treatment of alkaline hypochlorite solution (bleach). The experiments in JTL611, JTL621 (HSF-1 AID models), and CA1200, CA1199 (control) were done as described in our recent paper (Edwards et al., 2021). For the experiments using the set of HSF-1 AID strains in *glp-1 (e2141)* and *fem-3(q20)* backgrounds, synchronized L1 larvae were grown for 48 h at 25°C on 10 cm normal NGM plates (~500 worms per plate) to develop into young adults. Approximately 120 young adult worms were picked onto 10 cm NGM plates containing either EtOH or auxin and kept for an indicated time before collection (8, 24, and 48 h). For each condition, RNA was extracted using a 300 µL Trizol reagent. Worms were vortexed continuously for 20 min at 4°C and then went through one cycle of freeze-thaw to help release RNA. Following this, RNA was purified using the Direct-zol RNA MiniPrep kit (Zymo Research) as per the manufacturer's instructions using on column DNase I digestion to remove genomic DNA.

Total RNAs were polyA enriched, and directional RNA-seq libraries were prepared using the NEBNext Ultra II RNA library prep Kit. Paired-end sequencing was done at a NovaSeq 6,000 sequencer at OMRF clinical genomics core. The majority of samples were sequenced by 50 bp. A subset of samples that were sequenced with longer reads was trimmed to 50 bp to make all downstream mapping and analyses consistent.

RNA-Seq and ChIP-Seq Data Analyses

RNA-seq analyses were conducted as previously described (Edwards et al., 2021). Briefly, RNA-seq reads were mapped to the Ensembl WBcel235 genome using RNA STAR (Dobin et al., 2013). The mapped reads were then subject to FeatureCounts in Rsubread (Liao et al., 2019) for quantification. Differential expression (DE) analyses were then done using edgeR (Robinson et al., 2010) with default settings except for using Likelihood Ratio Test and filtering out those lowly expressed genes with CPM (counts per million) value less than 1 in more than 75% samples. Gene ontology analysis (GO) of DE genes was conducted using the program DAVID (http://david.abcc.ncifcrf.gov/) with functional annotation clustering to collapse redundant GO terms. The enrichment score for each cluster was shown.

ChIP-seq reads mapping, peak calling, and generation of genomic occupancy files (bedgraph files) were detailed in our recent publication (Edwards et al., 2021). To assign HSF-1 ChIP-seq peaks to promoters, transcription start sites (TSSs) determined by GRO-cap (Kruesi et al., 2013) were used where available. To visualize and compare the ChIP-seq data in genome browser views, the bedgraph files were normalized to reads per million using MACS2 call peak -B –SPMR and visualized using Integrative Genomics Viewer (IGV) (Robinson et al., 2011) with WS235 genome. Quantification of genomic occupancy was done by mapping the center of ChIP fragments to a reference point (e.g. HSF-1 peak summits) using windowBed in bedtools (Quinlan and Hall, 2010) and Matrix in R. For quantitative comparison of Pol II occupancy between conditions, all Pol II ChIP-seq data was normalized to 8 million reads, corresponding to the lowest coverage after duplicate filtering among all conditions. Heatmaps were generated with the Java TreeView package (Saldanha, 2004).

Immunofluorescence

Worms were prepared by freeze-cracking and fixed in 4% paraformaldehyde, as described (Charlie et al., 2006) with the following changes. JTL667 (*glp-1; eft-3p:tir1; hsf-1:degron*) were synchronized by bleach synchronization, and L1 larvae were grown on NGM plates containing ethanol at 25°C for 48 h. Young adult worms were rinsed off in M9 buffer and plated on either ethanol or auxin plates for 48 h before rinsing for freeze-cracking. A methanol incubation for 15 min at 4°C after fixation was added to eliminate the TIR1:RFP signal in the worms. The samples were co-stained with Anti-Ubiquitinylated proteins (clone FK2, mouse, Sigma Cat# 04-263) at 1:200 and anti-REC-8 (rabbit, Novus Cat# 49230002) at 1:100 at 15°C overnight. We then labeled FK2 with anti-mouse-Alexa 647 (Invitrogen Cat# A32728) and anti-REC-8 with goat anti-rabbit-Alexa 488 (Invitrogen Cat# A32731) by incubating for 2 h at room temperature.

STATISTICAL ANALYSIS

Statistical significance was calculated by unpaired, two-tailed Student's t test in Microsoft excel or two-way ANOVA comparison in GraphPad Prism. Lifespan statistics were calculated by Log-rank test using OASIS2 online lifespan analysis software (https://sbi.postech.ac.kr/oasis2/). RNA-seq and ChIP-seq analyses are described in Materials And Methods.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GEO - GSE162067.

AUTHOR CONTRIBUTIONS

AM, SE, PE, LK, and JL performed experiments and analyzed data. JL conceived the project and wrote the manuscript. AM and SE edited the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fragi.2022.899744/full#supplementary-material

REFERENCES

Arce, D. P., Tonon, C., Zanetti, M. E., Godoy, A. V., Hirose, S., and Casalongue, C. A. (2006). The Potato Transcriptional Co-Activator StMBF1 Is Up-Regulated in Response to Oxidative Stress and Interacts with the TATA-Box Binding Protein. *BMB Rep.* 39 (4), 355–360. doi:10.5483/bmbrep.2006.39.4.355

Baird, N. A., Douglas, P. M., Simic, M. S., Grant, A. R., Moresco, J. J., Wolff, S. C., et al. (2014). HSF-1-Mediated Cytoskeletal Integrity Determines Thermotolerance and Life Span. *Science* 346 (6207), 360–363. doi:10.1126/science.1253168

Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008). Adapting Proteostasis for Disease Intervention. *Science* 319 (5865), 916–919. doi:10.1126/science.1141448

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 (35), 14914–14919. doi:10.1073/pnas.0902882106

Birch-Machin, I., Gao, S., Huen, D., McGirr, R., White, R. A., and Russell, S. (2005). Genomic Analysis of Heat-Shock Factor Targets in Drosophila. *Genome Biol.* 6 (7), R63. doi:10.1186/gb-2005-6-7-r63

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 (3), 1135–1150. doi:10.1016/j.celrep.2014.09.042

Brenner, S. (1974). The Genetics of *Caenorhabditis elegans*. *Genetics* 77 (1), 71–94. doi:10.1093/genetics/77.1.71

Charlie, N. K., Schade, M. A., Thomure, A. M., and Miller, K. G. (2006). Presynaptic UNC-31 (CAPS) Is Required to Activate the Gαs Pathway of the *Caenorhabditis elegans* Synaptic Signaling Network. *Genetics* 172 (2), 943–961. doi:10.1534/genetics.105.049577

Clare, D. K., and Saibil, H. R. (2013). ATP-Driven Molecular Chaperone Machines. *Biopolymers* 99 (11), 846–859. doi:10.1002/bip.22361

Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W., and Dillin, A. (2006). Opposing Activities Protect Against Age-Onset Proteotoxicity. *Science* 313 (5793), 1604–1610. doi:10.1126/science.1124646

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: Ultrafast Universal RNA-Seq Aligner. *Bioinformatics* 29 (1), 15–21. doi:10.1093/bioinformatics/bts635

Douglas, P. M., Baird, N. A., Simic, M. S., Uhlein, S., McCormick, M. A., Wolff, S. C., et al. (2015). Heterotypic Signals from Neural HSF-1 Separate Thermotolerance from Longevity. *Cell Rep.* 12 (7), 1196–1204. doi:10.1016/j.celrep.2015.07.026

Duarte, F. M., Fuda, N. J., Mahat, D. B., Core, L. J., Guertin, M. J., and Lis, J. T. (2016). Transcription Factors GAF and HSF Act at Distinct Regulatory Steps to Modulate Stress-Induced Gene Activation. *Genes Dev.* 30 (15), 1731–1746. doi:10.1101/gad.284430.116

Edwards, S. L., Erdenebat, P., Morphis, A. C., Kumar, L., Wang, L., Chamera, T., et al. (2021). Insulin/IGF-1 Signaling and Heat Stress Differentially Regulate HSF1 Activities in Germline Development. *Cell Rep.* 36 (9), 109623. doi:10.1016/j.celrep.2021.109623

Egge, N., Arneaud, S. L. B., Wales, P., Mihelakis, M., McClendon, J., Fonseca, R. S., et al. (2019). Age-Onset Phosphorylation of a Minor Actin Variant Promotes Intestinal Barrier Dysfunction. *Dev. Cell* 51 (5), 587–601. doi:10.1016/j.devcel.2019.11.001

Gomez-Pastor, R., Burchfiel, E. T., Neef, D. W., Jaeger, A. M., Cabiscol, E., McKinstry, S. U., et al. (2017). Abnormal Degradation of the Neuronal Stress-Protective Transcription Factor HSF1 in Huntington's Disease. *Nat. Commun.* 8, 14405. doi:10.1038/ncomms14405

Gomez-Pastor, R., Burchfiel, E. T., and Thiele, D. J. (2018). Regulation of Heat Shock Transcription Factors and Their Roles in Physiology and Disease. *Nat. Rev. Mol. Cell Biol.* 19 (1), 4–19. doi:10.1038/nrm.2017.73

Hansen, M., Hsu, A. L., Dillin, A., and Kenyon, C. (2005). New Genes Tied to Endocrine, Metabolic, and Dietary Regulation of Lifespan from a *Caenorhabditis elegans* Genomic RNAi Screen. *PLoS Genet.* 1 (1), 119–128. doi:10.1371/journal.pgen.0010017

Higuchi-Sanabria, R., Paul, J. W., 3rd, Durieux, J., Benitez, C., Frankino, P. A., Tronnes, S. U., et al. (2018). Spatial Regulation of the Actin Cytoskeleton by HSF-1 During Aging. *MBoC* 29 (21), 2522–2527. doi:10.1091/mbc.e18-06-0362

Hsin, H., and Kenyon, C. (1999). Signals from the Reproductive System Regulate the Lifespan of *C. elegans*. *Nature* 399 (6734), 362–366. doi:10.1038/20694

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 (5622), 1142–1145. doi:10.1126/science.1083701

Kannan, A., Jiang, X., He, L., Ahmad, S., and Gangwani, L. (2020). ZPR1 Prevents R-Loop Accumulation, Upregulates SMN2 Expression and Rescues Spinal Muscular Atrophy. *Brain* 143 (1), 69–93. doi:10.1093/brain/awz373

Kim, E., Wang, B., Sastry, N., Masliah, E., Nelson, P. T., Cai, H., et al. (2016). NEDD4-Mediated HSF1 Degradation Underlies α-Synucleinopathy. *Hum. Mol. Genet.* 25 (2), 211–222. doi:10.1093/hmg/ddv445

Kruesi, W. S., Core, L. J., Waters, C. T., Lis, J. T., and Meyer, B. J. (2013). Condensin Controls Recruitment of RNA Polymerase II to Achieve Nematode X-Chromosome Dosage Compensation. *Elife* 2, e00808. doi:10.7554/eLife.00808

Kumsta, C., Chang, J. T., Schmalz, J., and Hansen, M. (2017). Hormetic Heat Stress and HSF-1 Induce Autophagy to Improve Survival and Proteostasis in *C. elegans*. *Nat. Commun.* 8, 14337. doi:10.1038/ncomms14337

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 (4), 639–650. doi:10.1016/j.molcel.2015.06.027

Lenaerts, I., Walker, G. A., Van Hoorebeke, L., Gems, D., and Vanfleteren, J. R. (2008). Dietary Restriction of *Caenorhabditis elegans* by Axenic Culture Reflects Nutritional Requirement for Constituents Provided by Metabolically Active Microbes. *Journals Gerontology Ser. A Biol. Sci. Med. Sci.* 63 (3), 242–252. doi:10.1093/gerona/63.3.242

Li, J., Chauve, L., Phelps, G., Brielmann, R. M., and Morimoto, R. I. (2016). E2F Coregulates an Essential HSF Developmental Program that Is Distinct from the Heat-Shock Response. *Genes Dev.* 30 (18), 2062–2075. doi:10.1101/gad.283317.116

Li, J., Labbadia, J., and Morimoto, R. I. (2017). Rethinking HSF1 in Stress, Development, and Organismal Health. *Trends Cell Biol.* 27 (12), 895–905. doi:10.1016/j.tcb.2017.08.002

Liao, Y., Smyth, G. K., and Shi, W. (2019). The R Package Rsubread Is Easier, Faster, Cheaper and Better for Alignment and Quantification of RNA Sequencing Reads. *Nucleic Acids Res.* 47 (8), e47. doi:10.1093/nar/gkz114

Loose, J. A., and Ghazi, A. (2021). Auxin Treatment Increases Lifespan in *Caenorhabditis elegans*. *Biol. Open* 10 (5). doi:10.1242/bio.058703

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

Mahat, D. B., Salamanca, H. H., Duarte, F. M., Danko, C. G., and Lis, J. T. (2016). Mammalian Heat Shock Response and Mechanisms Underlying its Genome-Wide Transcriptional Regulation. *Mol. Cell* 62 (1), 63–78. doi:10.1016/j.molcel.2016.02.025

Maxwell, C. S., Kruesi, W. S., Core, L. J., Kurhanewicz, N., Waters, C. T., Lewarch, C. L., et al. (2014). Pol II Docking and Pausing at Growth and Stress Genes in *C. elegans*. *Cell Rep.* 6 (3), 455–466. doi:10.1016/j.celrep.2014.01.008

Merkling, S. H., Overheul, G. J., van Mierlo, J. T., Arends, D., Gilissen, C., and van Rij, R. P. (2015). The Heat Shock Response Restricts Virus Infection in Drosophila. *Sci. Rep.* 5, 12758. doi:10.1038/srep12758

Morley, J. F., and Morimoto, R. I. (2004). Regulation of Longevity in *Caenorhabditis elegans* by Heat Shock Factor and Molecular Chaperones. *MBoC* 15 (2), 657–664. doi:10.1091/mbc.e03-07-0532

Morton, E. A., and Lamitina, T. (2013). *Caenorhabditis elegans* HSF-1 Is an Essential Nuclear Protein that Forms Stress Granule-Like Structures Following Heat Shock. *Aging Cell* 12 (1), 112–120. doi:10.1111/acel.12024

Nollen, E. A. A., Garcia, S. M., van Haaften, G., Kim, S., Chavez, A., Morimoto, R. I., et al. (2004). Genome-Wide RNA Interference Screen Identifies Previously Undescribed Regulators of Polyglutamine Aggregation. *Proc. Natl. Acad. Sci. U.S.A.* 101 (17), 6403–6408. doi:10.1073/pnas.0307697101

Pierce, A., Podlutskaya, N., Halloran, J. J., Hussong, S. A., Lin, P.-Y., Burbank, R., et al. (2013). Over-Expression of Heat Shock Factor 1 Phenocopies the Effect of Chronic Inhibition of TOR by Rapamycin and Is Sufficient to Ameliorate Alzheimer's-Like Deficits in Mice Modeling the Disease. *J. Neurochem.* 124 (6), 880–893. doi:10.1111/jnc.12080

Pierce, A., Wei, R., Halade, D., Yoo, S.-E., Ran, Q., and Richardson, A. (2010). A Novel Mouse Model of Enhanced Proteostasis: Full-Length Human Heat Shock Factor 1 Transgenic Mice. *Biochem. Biophysical Res. Commun.* 402 (1), 59–65. doi:10.1016/j.bbrc.2010.09.111

Pincus, D., Anandhakumar, J., Thiru, P., Guertin, M. J., Erkine, A. M., and Gross, D. S. (2018). Genetic and Epigenetic Determinants Establish a Continuum of Hsf1 Occupancy and Activity across the Yeast Genome. *MBoC* 29 (26), 3168–3182. doi:10.1091/mbc.e18-06-0353

Prior, H., Jawad, A. K., MacConnachie, L., and Beg, A. A. (2017). Highly Efficient, Rapid and Co-CRISPR-independent Genome Editing in *Caenorhabditis elegans*. *G3 (Bethesda)* 7 (11), 3693–3698. doi:10.1534/g3.117.300216

Quinlan, A. R., and Hall, I. M. (2010). BEDTools: A Flexible Suite of Utilities for Comparing Genomic Features. *Bioinformatics* 26 (6), 841–842. doi:10.1093/bioinformatics/btq033

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., et al. (2011). Integrative Genomics Viewer. *Nat. Biotechnol.* 29 (1), 24–26. doi:10.1038/nbt.1754

Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data. *Bioinformatics* 26 (1), 139–140. doi:10.1093/bioinformatics/btp616

Saldanha, A. J. (2004). Java Treeview-Extensible Visualization of Microarray Data. *Bioinformatics* 20 (17), 3246–3248. doi:10.1093/bioinformatics/bth349

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 (5), 814–822. doi:10.1111/acel.12110

Sural, S., Liang, C. Y., Wang, F. Y., Ching, T. T., and Hsu, A. L. (2020). HSB-1/HSF-1 Pathway Modulates Histone H4 in Mitochondria to Control mtDNA Transcription and Longevity. *Sci. Adv.* 6 (43). doi:10.1126/sciadv.aaz4452

Vihervaara, A., and Sistonen, L. (2014). HSF1 at a Glance. *J. Cell Sci.* 127 (Pt 2), 261–266. doi:10.1242/jcs.132605

Volovik, Y., Maman, M., Dubnikov, T., Bejerano-Sagie, M., Joyce, D., Kapernick, E. A., et al. (2012). Temporal Requirements of Heat Shock Factor-1 for Longevity Assurance. *Aging Cell* 11 (3), 491–499. doi:10.1111/j.1474-9726.2012.00811.x

Williams, R., Laskovs, M., Williams, R. I., Mahadevan, A., and Labbadia, J. (2020). A Mitochondrial Stress-Specific Form of HSF1 Protects Against Age-Related Proteostasis Collapse. *Dev. Cell* 54 (6), 758–772. doi:10.1016/j.devcel.2020.06.038

Zhang, L., Ward, J. D., Cheng, Z., and Dernburg, A. F. (2015). The Auxin-Inducible Degradation (AID) System Enables Versatile Conditional Protein Depletion in *C. elegans*. *Development* 142 (24), 4374–4384. doi:10.1242/dev.129635

Zhao, Y., Gilliat, A. F., Ziehm, M., Turmaine, M., Wang, H., Ezcurra, M., et al. (2017). Two Forms of Death in Ageing *Caenorhabditis elegans*. *Nat. Commun.* 8, 15458. doi:10.1038/ncomms15458

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Age-dependent accumulation of tau aggregation in *Caenorhabditis elegans*

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Aging is the primary risk factor for Alzheimer's disease (AD) and related disorders (ADRDs). Tau aggregation is a hallmark of AD and other tauopathies. Even in normal aging, tau aggregation is found in brains, but in disease states, significantly more aggregated tau is present in brain regions demonstrating synaptic degeneration and neuronal loss. It is unclear how tau aggregation and aging interact to give rise to the phenotypes observed in disease states. Most AD/ADRD animal models have focused on late stages, after significant tau aggregation has occurred. There are fewer where we can observe the early aggregation events and progression during aging. In an attempt to address this gap, we created *C. elegans* models expressing a GFP-tagged version of the human tau protein. Here we examined how tau-gfp behaved during aging, comparing wild-type tau (hTau40), a disease-associated mutation (P301S), and an aggregation-prone variant (3PO). We measured age-dependent changes in GFP intensity and correlated those changes to normal aging in the nematode. We found differences in tau stability and accumulation depending on the tau variant expressed. hTau40GFP and P301SGFP were localized to axons and cell bodies, while 3POGFP was more concentrated within cell bodies. Expression of 3POGFP resulted in decreased lifespan and variations in locomotor rate, consistent with a pathological effect. Finally, we found that the human tau interacted genetically with the *C. elegans* ortholog of human tau, *ptl-1,* where the loss of *ptl-1* significantly accelerated the time to death in animals expressing 3PO.

KEYWORDS

***C. elegans*, tau, tauopathy, microtubule associated proteins, neuronal aging**

Introduction

Aging is the main risk factor for Alzheimer's disease (AD) and other AD-related disorders (ADRD). AD is the most common form of senile dementia and affects over 6.5 million people (Rajan et al., 2021). The population of people 65 years and older is projected to double by 2050 (Nations, 2019). These statistics demonstrate the urgency to understand how aging increases the chances of developing neurodegenerative disorders

(e.g., AD and ADRDs) and how the presence of aggregated proteins accelerates aging and increases the mortality rate.

Normal aging is characterized by a decline in protein homeostasis (Taylor and Dillin, 2011; López-Otín et al., 2013). The decline in cellular processes that maintain proteostasis can lead to protein aggregation (Ben-Zvi et al., 2009; Huang et al., 2019). Post-mortem brains of individuals diagnosed with AD and ADRDs have a high concentration of intracellular neurofibrillary tangles (NFTs) composed of the protein tau. Most people will spontaneously develop oligomeric complexes distinct from aggregated tau in addition to NFTs as they age (Huang et al., 2019), and at least one study has shown that NFTs correlate with normal, age-related cognitive decline (Guillozet et al., 2003). However, individuals with AD or ADRDs will develop a higher concentration of NFTs, especially in regions of the brain where synaptic loss and neuronal death can be observed (Chiu et al., 2017; Ziontz et al., 2019; Chen et al., 2021). Therefore, questions remain about the differences in tau aggregation during normal aging versus disease, and how age-related declines might be accelerated in the diseased state.

There are several animal models for AD and other ADRDs, with many that focus primarily on the aggregation of amyloid-ß (Edelstein-keshet and Spiros, 2002; Proctor and Gray, 2010; Puri and Li, 2010). Recent research, however, has highlighted the importance and complexity of the protein tau in these disorders. Age-related changes in tau have been shown to impair bioenergetics (reviewed in (Grimm, 2021)), autophagy (Chatterjee et al., 2021), calcium consumption (Datta et al., 2021), and other cellular events. In addition, structural studies have shown that tau adopts different conformations in disease that might have different disease causation capabilities (Oakley et al., 2020; Scheres et al., 2020; Shi et al., 2021). There is still a need for new *in vivo* models capable of visualizing tau protein dynamics, to measure the effect of aging, genetics, cell types or other environmental variables on aggregation.

Here we describe a model to visualize tau during aging in a *C. elegans* model. The nematode *C. elegans* has been used as a model organism to identify mechanisms that regulate lifespan (Kenyon, 2010; López-Otín et al., 2013). Several studies have highlighted the relationship between proteome remodeling, protein aggregation, and normal aging in the nematode (David et al., 2010; Ciryam et al., 2013; Walther et al., 2015; Huang et al., 2019). One recent study showed long-lived nematode mutants had a delayed onset of declining health, motivating a better understanding of how the aging process induces age-associated declines (Statzer et al., 2022). Animal models of tau aggregation have consistently reported a decrease in lifespan attributable to the overexpression of human tau (Wittmann et al., 2001; Sealey et al., 2017; Higham et al., 2019; Macdonald et al., 2019). Similarly, nematodes expressing human tau also showed a reduced lifespan and verified the presence of aggregated tau (Kraemer et al., 2003; Pir et al., 2016). Here we found that expression of gfp-tagged human tau reduced nematode lifespan, with a greater reduction associated with an aggressively aggregating version of tau (3PO). This decrease in lifespan was enhanced by a mutation that deleted the gene, encoding the endogenous microtubule associated protein, PTL-1, suggesting it was protective in this context.

Materials and methods

Strains and genetics

N2 (var. Bristol) was used as the wild-type reference strain in all experiment. Strains were maintained at 18–22°C, using standard maintenance techniques as described (Brenner, 1974). Strains used in this report include: RB809 [*ptl-1(ok621)*], NW1229 *evIs111* [P*rgef-1*:GFP] (Altun-Gultekin et al., 2001), EVL1654 *lhIs94* [P*rgef-1*:hTau40GFP], EVL1644 *lhIs92* [*Prgef-1*:hTau40-P301SGFP], and EVL1372 *lhIs84* [P*rgef-1*:3POTauGFP]. To create tau transgenes DNA was injected at the following concentrations: *lhEx274* (P*rgef-1*::hTau40GFP 5 ng/µl; *Pstr*-1:GFP 5 ng/µl); *lhEx646* (P*rgef-1*::hTau40(P301S)GFP 5 ng/µl; P*myo-2*:RFP 5 ng/µl; *Pstr*-1:GFP 5 ng/µl); *lhEx327* (P*rgef-1*::3POTauGFP 5 ng/µl; P*str-1::gfp* 5 ng/µl). Transgenes were integrated using Trimethylpsoralen and UV as described (Najarro et al., 2012). Animals were synchronized by starvation hatching and then placed on *E. coli* (OP50) to initiate development.

Whole-mount immunostaining

Anti-TAU staining was done using Bouin's fixation as described (Nonet et al., 1997). The primary antibodies used in this study were: rabbit anti-TAU (1:1000, A00024-A Agilent), mouse anti-TAU (1:500, TNT1) (Kanaan et al., 2011; Combs et al., 2016). The secondary antibodies used were Alexa 488-labeled goat anti-rabbit and Cy3 goat anti-mouse at 1: 500 dilution.

Fluorescence microscopy and image analysis

Images were obtained using an Olympus FV1000 laser-scanning confocal microscope equipped with the Fluoview software. Z-stacked images were taken and exported to ImageJ (Schneider et al., 2012). Images were z-projected using max intensity, and ROI was drawn around the relevant areas of the tail (for GFP images) or the ventral nerve cord (for the immunostaining). The following measurement options were selected: area, mean, minimum, maximum, integrated pixel intensity, and raw integrated pixel intensity. A region of the image adjacent to the animal was used to assess the background,

which was subtracted from the raw pixel density and then that value was divided by the area to arrive at a normalized value for the reported GFP fluorescence.

Biochemical characterization

Nematodes were grown on standard NGM plates and maintained at 20°C. Gravid nematodes were harvested and washed off the plate using M9 buffer and collected in a 15 ml conical tube. Embryos were extracted using bleach solution and incubated for 5 min while rotating. Embryos were washed four times and resuspended in M9 buffer, where they were allowed to hatch overnight, in the absence of food. Starvation-arrested L1 worms were grown in liquid culture containing *E. coli* (HB101), cholesterol, and 1x HyClone Antibiotic-Antimycotic Solution (Thermo Scientific). Worms were maintained with constant shaking at 20°C and collected and washed in M9 buffer on days one and three of adulthood. A final wash with ice-cold RAB reassembly buffer supplemented with protease/phosphatase inhibitor cocktail (Thermo Scientific, PI78440) and 5 mM EDTA. 100mg of compact worms were then aliquoted into 1.5 ml tubes and stored at −80°C.

Sequential protein extraction was performed as in (Kraemer et al., 2003) with minor modifications. Briefly, tau fractions were obtained by resuspending 100 mg worm pellet in two times (wt/vol) high salt RAB reassembly buffer (G-Biosciences, PI53113) supplemented with a 1x final concentration protease/phosphatase inhibitor cocktail (Thermo Scientific, PI78440). The same volume (compact worm/beads) of ice-cold 0.5 mm glass Beads (BioSpec Products) was added to the tube. Worms were lysed using a BeadBug Microtube Homogenizer (Benchmark Scientific) at max speed for 5 s, four times with 20-s rest on ice in between each session. The solution was incubated on ice for 30 min then centrifuged at 4°C (20817xg) for 30 min. The supernatant was considered the soluble fraction. The remaining pellet was washed with ice-cold RAB buffer and extracted with RIPA buffer with EDTA (G-Biosciences, AAJ61529AK), by incubation on ice for 20 min and centrifugation at 4°C (20817xg) for 15 min. The resulting supernatant was considered the detergent-soluble fraction. The pellet was washed with ice-cold RIPA buffer and extracted with urea (30 mM Tris, 7M urea, 2M thiourea, 4% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), pH 8.5] (Pir et al., 2016), incubated for 15 min at room temperature and centrifuged at room temperature (RT) (20817xg) for 10 min. The resulting supernatant was considered the urea soluble fraction.

Protein fractions were resolved in a 10% polyacrylamide gel and transferred to a PVDF membrane (Immobilon, IPFL00005). The following antibodies were used: Monoclonal anti-tau Tau-5 (1:2000; Thermo Scientific), Tau-7 (1:2000; Millipore Sigma), Tau-12 (1:2000; Millipore Sigma), Anti-Alpha-Tubulin (1:10,000; Thermo Scientific), anti-mouse HRP-labeled secondary antibody (1:5000; Thermo Scientific). PVDF membranes were visualized using Licor Odyssey Fc Imager. Western blot images were transferred to PowerPoint for cropping, alignment and annotation; no post-acquisition adjustments were made to brightness or contrast.

Survival analysis

50-late stage L4 were transferred to an OP50 seeded NGM plate and maintained at 20°C for the duration of the assay. Live animals were counted and moved to a fresh plate each day for the first 7 days of the assay and then every 2–3 days after the worms had stopped laying eggs. The number of dead worms was recorded. 2–5 independent experimental replicates were done per strain.

Locomotion analysis

Nematode locomotion was analyzed using WormLab Tracking Software (MBF Bioscience). L4 nematodes were selected and grown on standard NGM plates spotted with *E. coli* (OP50). Animals were transferred daily onto new plates and maintained at 20°C. On the day of the assay, nematodes were transferred to a fresh NGM plate (without food) to eliminate the excess OP50 from the nematode's cuticle. Individual worms were selected and placed in a new NGM plate (no food) and allowed to acclimate for 1 min before acquiring the video. A 1-min recording (11.1 frames per second) was captured using a CCD camera (Leica DFC 3000G) attached to a fluorescent stereo microscope (Leica M165 FC). Speed was measured on days 1, 3, 5, and 7 of adulthood.

Statistics

Analyses were performed using GraphPad (9.0.0) or using R in RStudio (Team, 2019; Team, 2020). For GFP analysis an Interaction ANOVA model (day*tau variant) was calculated, and significantly different interactions were assessed using a Tukey's HSD test, and a P-adjusted value of <0.01 was used to determine significance. A Log-rank (Mantel-Cox) analysis was used for the comparison of survival curves. A threshold for significance was established at $p < 0.05$ and is defined in the figure legend and noted with asterisks.

Cloning and molecular biology

Tau variants were amplified by PCR from bacterial expression clones. The original hTau40 expression vector (pT7C) was described in (Carmel et al., 1996). P301S was generated from hTau40 in the pT7C vector using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) following manufacturer's protocols. 3PO tau was generated from hTau40 by making the following modifications: I278N + N279I + ΔK280 + V309Y + Y310V + ΔP312 + S341I + ΔE342, using the QuikChange Site-Directed Mutagenesis Kit

(Stratagene), based on the previously described strategy for optimizing the spontaneous aggregation of tau (Iliev et al., 2006). PCR products were inserted into pCRII-TOPO (Invitrogen) using the manufacturer's protocol. The entry clones were recombined into a P*rgef-1*:GW:*gfp*::*unc-54* 3′UTR destination vector (pCZ#599, a generous gift of the Jin lab) using LR recombinase (Invitrogen) using manufacturer's protocol to generate pEVL415 (P*rgef-1*:*htau40*::*gfp*::*unc-54* 3′UTR), pEVL538 (P*rgef-1*:*3POtau*::*gfp*::*unc-54* 3′UTR), pEVL539 (P*rgef-1*:*htau40(P301S)*::*gfp*::*unc-54* 3′UTR).

Protein alignments

The microtubule binding repeats from the 2N4R human MAPT protein (NP_001116538.2) were aligned to the microtubule binding repeats from PTL-1 (NP_001367354.1) using Clustal Omega (Sievers and Higgins, 2021). The sequences are annotated with known human variants with the potential pathogenicity as described by the ClinVar database (Landrum et al., 2020).

Results

Wild-type tau expression in neurons is well tolerated

Our understanding of tau biology has increased dramatically since it was discovered to be a part of the neurofibrillary tangles in AD (Grundke-Iqbal et al., 1986; Wood et al., 1986; Goedert et al., 1988; Wischik et al., 1988). We now know that mutations along the MAPT gene, which codes for tau protein, can cause various disorders called tauopathies (Rossi and Tagliavini, 2015). Genetic mutations, or external factors, can lead to heterogenous tau aggregates that give rise to different phenotypic spectrums (Oakley et al., 2020; Scheres et al., 2020; Shi et al., 2021). However, understanding the spectrum of initiating events that cause tau to transition from monomeric to oligomeric to larger aggregates, and the different mechanisms of how tau polymers cause disease has proven challenging (Orr et al., 2017).

Animal models that mimic aspects of Alzheimer's disease (AD) and other Alzheimer' related disorders (ADRDs) are key to understanding the etiology of these diseases. Therefore, we set out to build an *in vivo* model to study tau using the nematode *C. elegans*. We created integrated transgenic animals expressing a GFP-tagged version of the longest human tau isoform (hTau40), pan-neuronally using the *rgef-1* promoter (Chen et al., 2011) (Figures 1A,B), because this promoter has been shown to have relatively stable expression throughout aging (Adamla and Ignatova, 2015). Additionally, we generated similar animals using tau variants, either P301S, which is a mutation associated with frontotemporal dementia (FTD), often used in research (Yoshiyama et al., 2007; Takeuchi et al., 2011), or 3PO, which is a lab construct that was optimized to spontaneously aggregate (Iliev et al., 2006) (Figure 1A). We found that, when DNA was injected at a relatively low concentration (5 ng/μl) expression of wild-type tau was well tolerated. That is, we did not observe any obvious defects in organismal morphology or impacts on survival during development, compared to animals expressing GFP alone under the same promoter as a negative control (*evIs111*) (data not shown). Thus, we used this concentration for all remaining experiments.

We observed tauGFP to be expressed throughout the entire nervous system of the nematode (Figure 1C'). In animals expressing wild-type tau, hTau40GFP appeared to be largely distributed through axons and neuronal cell bodies (Figures 1D,E). The P301S variant was like the wild-type protein (Figure 1D", E"). In contrast, the 3PO variant was concentrated in cell bodies, with less material present in processes (Figure 1D', E'). The appearance and localization of 3POtau is consistent with cell culture studies (Iliev et al., 2006). Based on these results, we concluded that we could observe tau localization inside *C. elegans* neurons.

TauGFP distribution and appearance change in aging with aggregation

Aging is the main risk factor for AD and ADRDs, so we monitored changes in tauGFP during aging. We imaged neurons located in the tail of the animals on days one, three, five, and seven of adulthood (Figure 2). This region provided a consistent and well-defined location and avoided potential background effects of the intestinal autofluorescence, which naturally increases with age and stress. Quantifying fluorescence via image analysis, we found discrete age-dependent changes in tauGFP localization and quantity that was different, depending on the tau variant expressed (Figure 3). Qualitatively, the pattern of hTau40GFP was largely the same over the first week of adulthood exhibiting localization to cell bodies and axons alike. There were no significant differences in GFP intensity in hTau40-expressing animals as a function of age during the first week of adulthood (Figure 3).

Next, when we examined the changes in the disease-associated variant, P301SGFP, we observed a different pattern. While the distribution of GFP on day one appeared to be roughly equal between cell bodies and processes, as the animals aged, we noticed a qualitative enrichment in the processes and a relative depletion in cell bodies, a pattern not observed for hTau40GFP (Figure 2). When we quantified the total fluorescence, there was a significant increase in the fluorescence observed by day three (Figure 3). Thus, we conclude that the P301S variant preferentially localized to neuronal processes, compared to cell bodies. The increased intensity over time, suggests it may be more stable *in vivo* compared to hTau40 (Figure 3). For the 3POGFP animals we also observed an increase in fluorescence intensity during aging, with protein largely concentrated in the cell bodies. From these studies we concluded that, compared to wild-type tau, both P301S and 3PO can accumulate significantly *in vivo* during aging, although the variants appear to be differentially distributed within the cells.

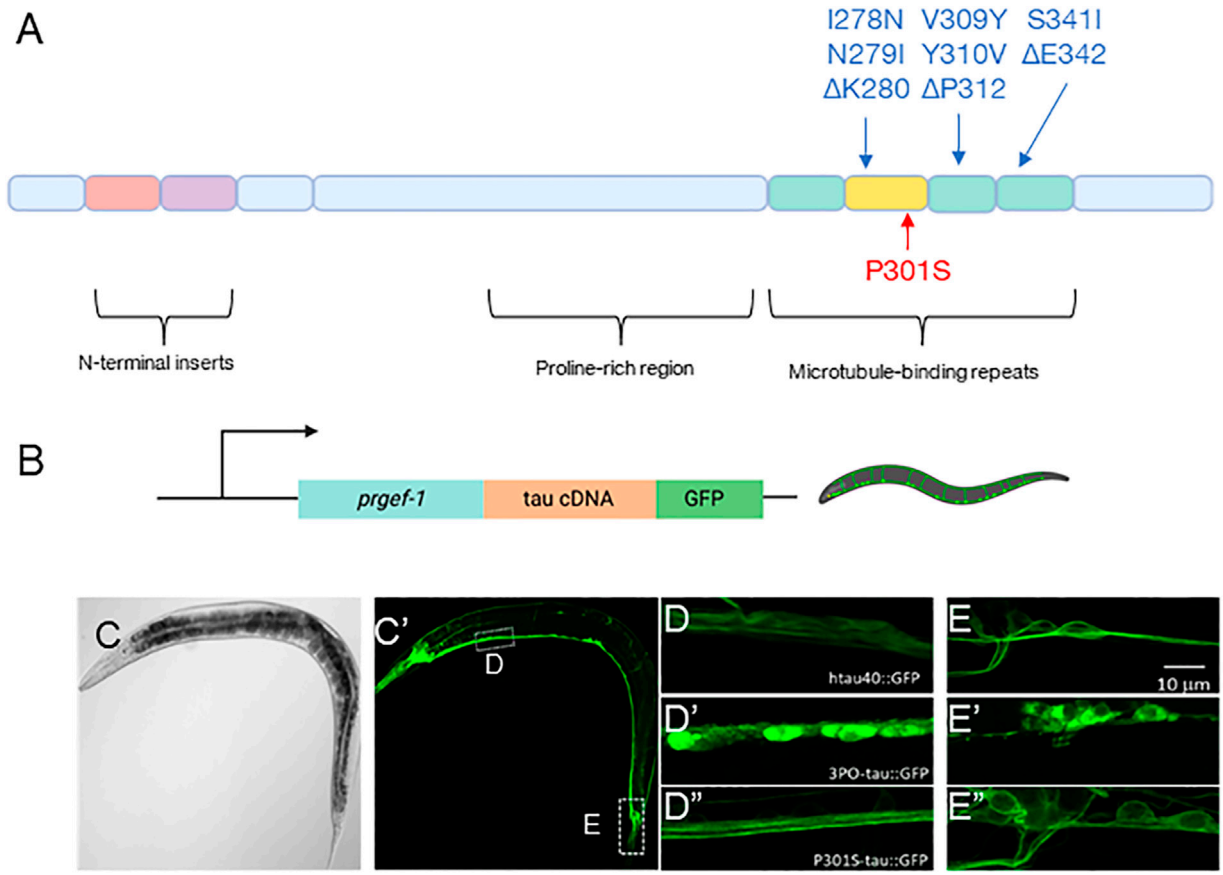

FIGURE 1
Pan-neuronal model of tau aggregation. **(A)** Schematic representation of the 2N4R tau isoform (hTau40) and the mutations used in this report. **(B)** A schematic representation of the DNA constructs injected (created with BioRender.com). **(C)** A transmitted light and **(C')** fluorescent image of a day one adult animal expressing hTau40GFP. Magnified confocal images of the **(D)** ventral nerve cord and **(E)** tail regions of the animals in the wild-type hTau40, 3PO **(D',E')** and P301S **(D", E")** expressing animals. Note, the images are taken from regions indicated by the hashed boxes in **(C)**. **(D)** We found that in the ventral nerve hTau40 was evenly concentrated throughout axons, in contrast to **(D')** 3POtau which was concentrated in the cell bodies. **(D")** P301S was similar to the wild-type tau. **(E,E',E")** In the tail we observed similar results, with tau present through the cell bodies.

P301S and 3PO tau form detergent insoluble aggregates in *C. elegans* neurons

Previous work has shown that tau aggregates in *C. elegans* can be biochemically characterized via serial extraction (Kraemer et al., 2003). We isolated protein from our tau transgenic animals, or a non-tau control (*evIs111*), on days one and three of adulthood and used serial extraction (Materials and methods) to characterize the state of aggregation (Figure 4). Briefly, we separated proteins into the soluble (RAB buffer), detergent soluble (RIPA buffer) or detergent insoluble (Urea extracted) phases, and then performed SDS-PAGE and western blots.

We found that, as expected protein isolated from our non-tau animals had no immunoreactivity to tau antibodies on Western blot. The wild-type protein, hTau40, was present in either the soluble or detergent soluble phase, with no protein detectable in the Urea-extracted fraction. In contrast, the 3PO protein was detected in all three phases, as early as day one of adulthood. Interestingly, for the P301S line, we found that on day one protein was found only in the soluble and detergent soluble fractions. However, we detected P301S tau in the detergent-insoluble fraction from day three adults. Based on this pattern, we concluded that the P301S tau was forming detergent-insoluble aggregates in *C. elegans,* albeit more slowly than 3PO. We also were able to conclude that the 3PO protein was more aggressively aggregating, as would be expected from previous biochemical analyses (Iliev et al., 2006).

Expression of aggregation-prone human tau reduces lifespan

To determine whether our tau expression lines were impacting the organisms we measured organismal lifespan. Several other tauopathy nematode models have shown that expression of tau can be detrimental using lifespan as a readout (Kraemer et al., 2003; Pir et al., 2016). This suggests

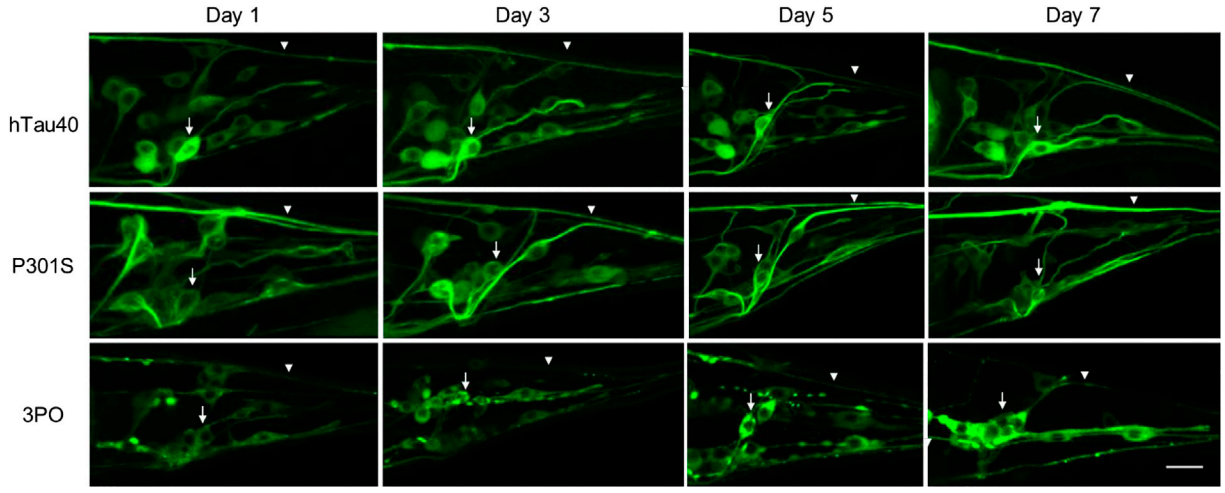

FIGURE 2
Age-dependent accumulation of tauGFP. We monitored TauGFP during aging in the animals. Across the first 7 days of adulthood, hTau40GFP was observed in cell bodies (arrows) and in axonal processes (arrowheads) consistently across aging. Compared to the wild-type we noticed that P301SGFP appears to be enriched in the axons (arrowheads), with a decrease in cell body fluorescence (arrows) during aging. 3POGFP was preferentially accumulated in cell bodies (arrows), with less fluorescence present in nerve processes (arrowheads). In addition, tau in the cell bodies in animals expressing 3POGFP appeared to be more clustered than those in animals expressing wild-type or P301S tau. Scale bar equals 10 μm.

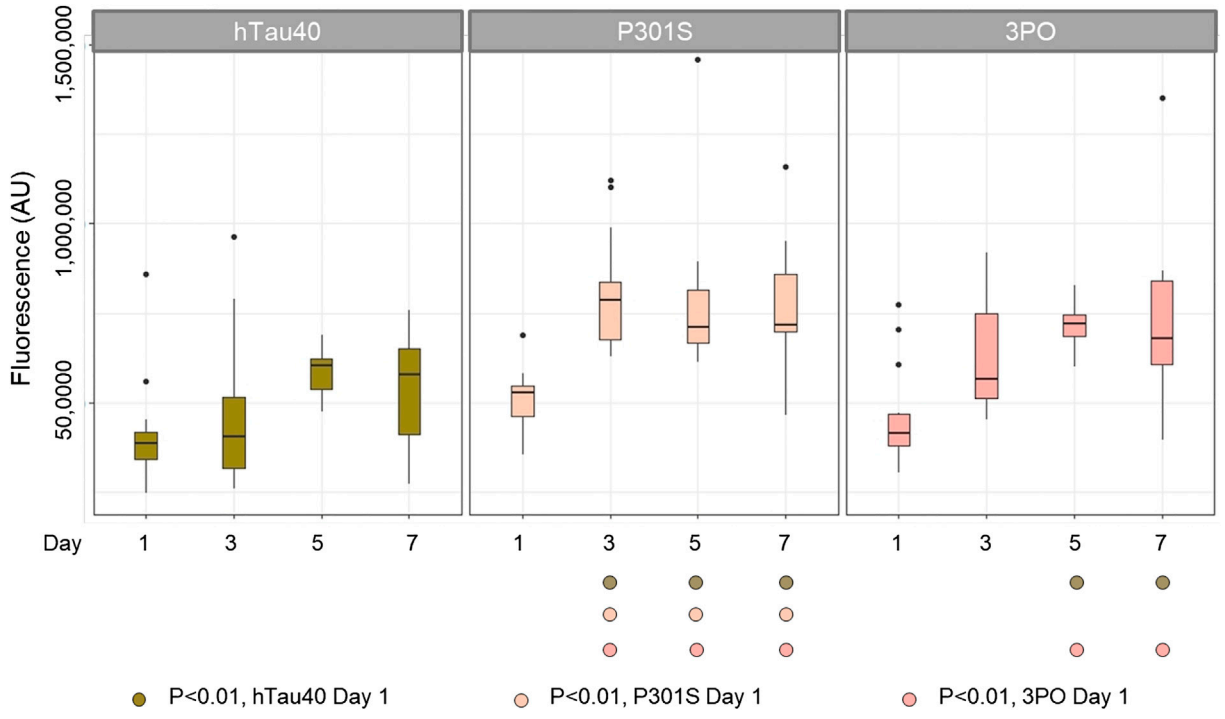

FIGURE 3
TauGFP is stable and accumulates in an age-dependent manner in neurons and axons. Day 1 nematodes strains have similar GFP intensity suggesting that the tau integrants have equivalent levels of expression. By day 3 (P301S) and day 5 (3PO) fluorescence was significantly increased, respectively, suggesting a significant buildup of tau while hTau40 remained statistically constant during the selected time points. ANOVA (Day*Variant) with Tukey post-hoc comparison. *N* = 12–15.

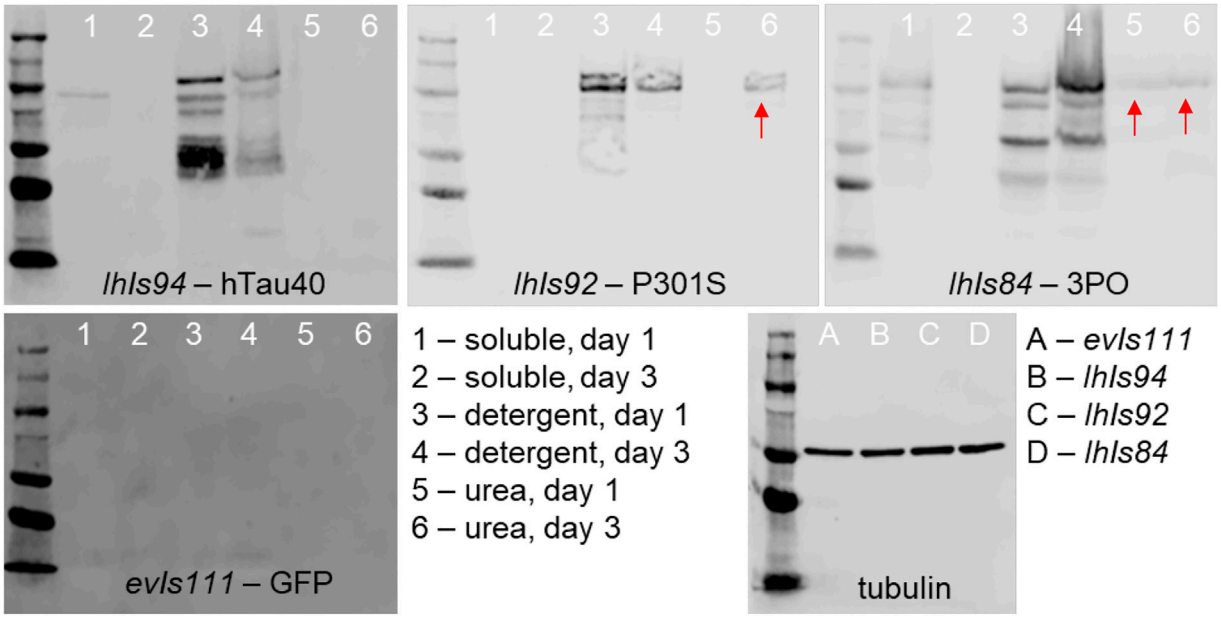

FIGURE 4
Serial biochemical extraction of aggregated tau. Protein was extracted from transgenic animals of the indicated genotype and day, then subjected to a series of extraction steps to collect the soluble (lanes 1 and 2), detergent soluble (lanes 3 and 4) and urea soluble (lanes 5 and 6) fractions. Fractions were separated by SDS-PAGE and detected using tau antibodies and chemiluminescence. The presence of tau in the urea fractions (red arrows) is indicative of aggregates that have formed. We found that tau is present in this fraction by day 1 of adulthood in 3PO expressing lines and by day 3 in P301S expressing lines. To the bottom right is a blot of extracts from day 1 animals of the indicated genotype of a control protein, tubulin, to demonstrate equal loading.

that lifespan assays can provide information that helps us assess healthspan deterioration in the nematode.

We age-synchronized nematodes and plated a cohort of 50 (per replicate) L4 staged animals (day 0). We then proceeded to count the number of living animals every day (see materials and methods) until all had died (Figure 5). We found that 50% of hTau40 and P301S animals died by day 14, neither of which were significantly different from the no tau control. In contrast, 50% of the 3PO animals had died by day 10, demonstrating a reduced lifespan (Figure 5). We observed a sharp drop in the number of living animals around day seven, which correlated with the significant increase of 3POGFP levels on day five (Figure 3). Interestingly, the P301S, a disease-associated variant, did not appreciably cause a reduced lifespan, when compared to hTau40 or GFP alone, despite the increased accumulation observed. Together these argue that accumulation, by itself is not sufficient to impact lifespan, but that a pro-aggregation variant of tau can negatively affect organismal survival.

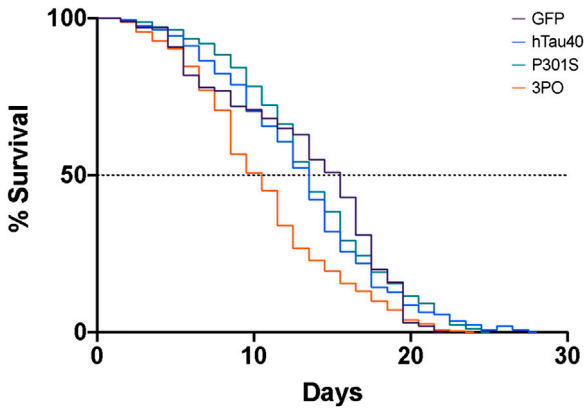

FIGURE 5
Expression of mutant tau in the nervous system induces premature lethality. A cohort of animals expressing GFP, hTau40GFP, P301SGFP or 3POGFP were counted daily for survival. We determined the day of 50% population survival (LD50). 3PO animals exhibit an LD50 of ~8 days, P301S ~11 days and WT tau of ~12 days (3PO is significantly different from GFP, $p < 0.001$, log-Rank test). Each line represents 2–5 independent experiments.

Aggregation-prone tau induces changes in locomotion

The *C. elegans* locomotor system relies on both excitatory and inhibitory motorneurons to alternately induce muscle contraction or relaxation, resulting in sinusoidal crawling behaviour (Zhen and Samuel, 2015). Forward and backward locomotion are controlled by different classes of motorneurons, while turning behaviours can be initiated by sensory events. Thus, evaluating locomotion is a way to

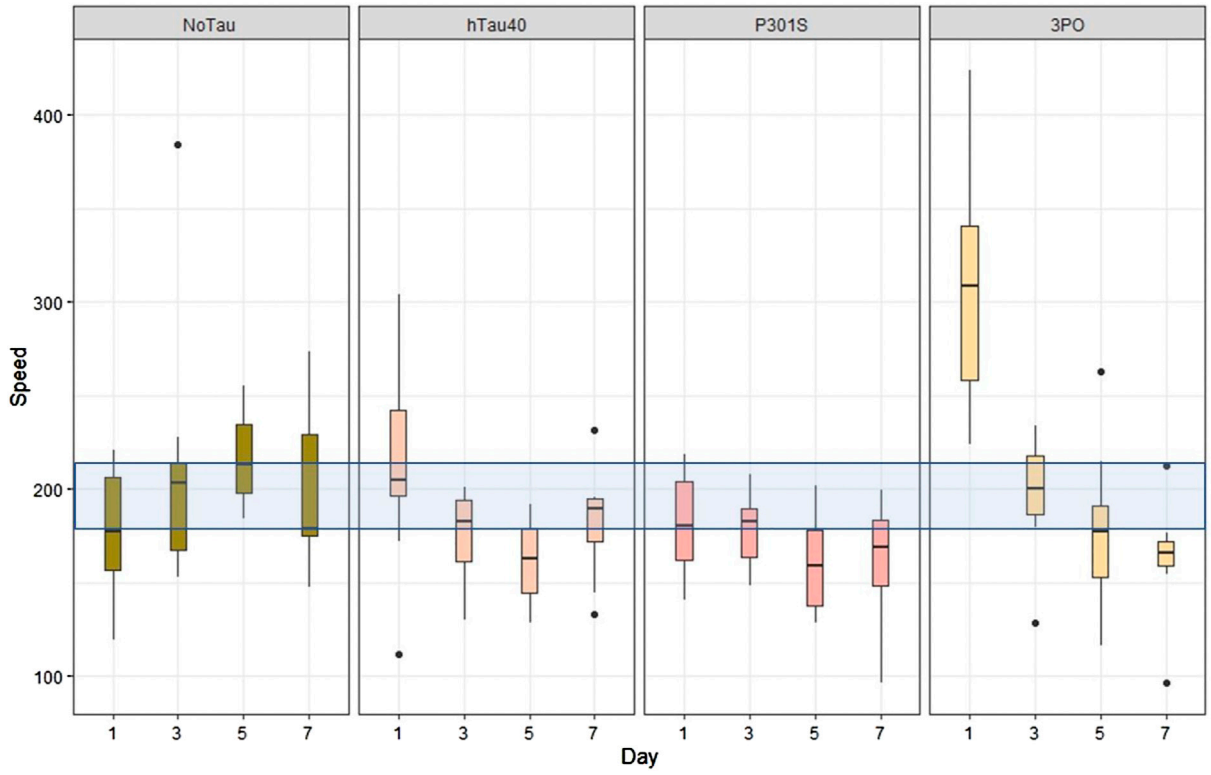

FIGURE 6
Expression of mutant tau affects locomotion. Animal motility was acquired by video microscopy and analyzed using tracking software (WormLab). When the speed of movement was quantified, the 3PO animals exhibited a hyperactive behavior on day one, but movement progressively slowed as the animals aged. Movement speed is plotted by day, with the blue transparent bar indicating the range of the mean speed of the control animals (*evIs111*) from all the days measured. The day one 3PO animals were significantly different from all the other lines on each day ($p < 0.001$). $N = 10$ for each transgene on each day.

examine the ensemble function of the *C. elegans* nervous system. We acquired movies of transgenic animals crawling on NGM plates (see Materials and methods) and analysed these using tracker software (WormLab). The most obvious pattern to emerge was that the 3PO animals were hyperactive, compared to the no tau control, hTau40 or P301S animals, on day one of adulthood, but that their movement decreased progressively, on each day tested (Figure 6). From these analyses we concluded that the expression of the 3PO version of tau was impacting neuronal function in the early stages of adulthood.

Human tau variants behave differently in the *C. elegans* ortholog of tau, ptl-1, mutant background

Tau is a microtubule-associated protein that binds tubulin and provides microtubule stability (Kadavath et al., 2015; Barbier et al., 2019). Thus, we asked whether there is an interaction between our expressed human tau and the *C. elegans* tau ortholog *ptl-1*. Previous reports suggest that human tau can rescue some, but not all, of the phenotypes observed in *ptl-1* mutants (Chew et al., 2013).

We crossed our tauGFP strains into the *ptl-1 (ok621)* background. The *ok621* mutation is a deletion that removes most of the *ptl-1* coding region, and the mutation has been reported to accelerate the onset of neuronal aging (Gordon et al., 2008; Chew et al., 2013).

We again measured the total pixel intensity of tauGFP in the tail (Figure 7). Like the wild-type animals, the levels of hTau40 remained relatively consistent over the time observed. Interestingly, in animals lacking *ptl-1* the P301S variant did not display increased accumulation with aging. Rather, the levels stayed relatively constant over time. Qualitatively, the hTau40GFP and P301SGFP remained localized in neurons and axons in the *ptl-1* mutant animals. Thus, we hypothesize that the axonal accumulation of the P301S was dependent on the endogenous *ptl-1*, or that in the absence of *ptl-1* there are compensatory mechanisms that facilitate the clearance of the P301S protein. We continued to observe significant accumulation of 3POtau when *ptl-1* was absent, (Day 1 vs. Day 3 $p < 0.0077$, Day 1 vs. Day 5 $p < 0.00001$ and Day 1 vs. Day 7 $p < 0.0004$; Two-way ANOVA with Tukey's correction). Overall,

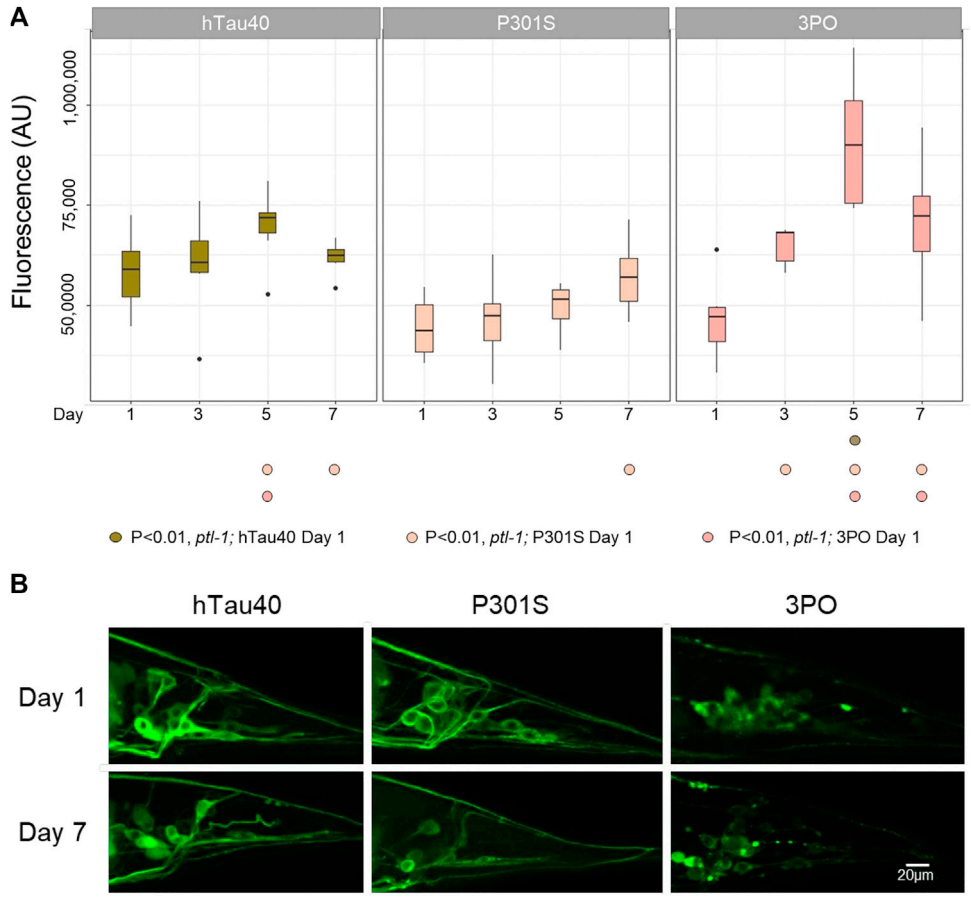

FIGURE 7
TauGFP variant accumulation in ptl-1 mutants. **(A)**. The *ok621* mutation reduced the accumulation of P301SGFP that occurred in wild-type background, but not the increased stability of 3POGFP. ANOVA (Day*Variant) with Tukey post-hoc comparison. *N* = 10 for each sample. **(B)**. Representative images of GFP accumulation in *ptl-1* mutants on days one and seven.

this provides further evidence for the differences in the accumulation of the protein variants, with the P301S affected by the loss of *ptl-1*, but the 3PO less so.

ptl-1 protects the animal from tau3PO effects

We repeated the lifespan assay in the *ptl-1* mutant background. There were no differences in the lifespans of wild-type or *ptl-1(ok621)* animals expressing GFP alone as a control ($p = 0.18$) (Figure 8). In the *ptl-1* background we observed a reduced lifespan due to hTau40GFP ($p < 0.001$) and 3POGFP animals ($p < 0.001$), while the P301SGFP lifespans were not different from *ptl-1* alone (Figure 8). It is not clear why the expression of wild-type human tau in the *ptl-1* mutant background was detrimental, while the P301S was not. However, the observation that the *ptl-1;*3POtau animals died more quickly than the expression of 3PO in the wild-type background suggested that the presence of the tau ortholog was beneficial to organismal survival.

Tau adopts an aggregation-prone conformation detected by TNT1

Finally, to determine if the tau accumulation observed was relevant to the human disease we stained our transgenic lines using an antibody that preferentially binds to conformational changes in tau associated with tau aggregation (TNT1) (Combs et al., 2017) and an antibody that recognizes tau in general (A0021-A). We quantified the ratio of TNT1 intensity to total tau intensity in the ventral nerve cord of the animals during aging (Figure 9). We saw no staining in our negative controls (*evIs111*, GFP alone), indicating that the staining was specific to the tau-expression lines. We found that the ratio of TNT1/total tau was not significantly different between animals expressing the hTau40 or P301S on day 1, but there was a significantly higher ratio of TNT1 staining in the animals expressing the 3PO variant. As aging progressed the ratio of aggregated to total tau was stable in the hTau40 lines, but was

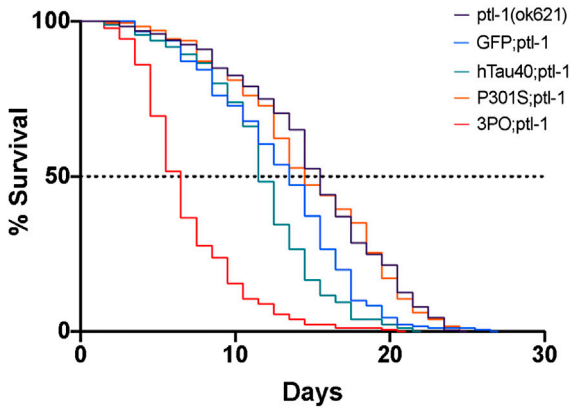

FIGURE 8
Lifespan of ptl-1 mutants expressing human tau. Animals were monitored daily for survival. All the lines expressing mutant tau died prematurely, compared to the *ptl-1(ok621)* single mutants (log-Rank test). We determined the LD50 as follows: 3POGFP; *ptl-1* ~5 days, P301SGFP; *ptl-1* ~15 days, hTau40GF; *ptl-1* ~12 days, and *ptl-1(ok621)* ~ 13 days. Curves represent 4 independently conducted experiments.

consistently higher in the 3PO tau. By day seven we found a significant increase in the TNT1 immunoreactivity in P301S expressing animals. Thus, we concluded that the conformation of some of the tau species formed in the nematodes was like that formed in human disease early in the aging process and the ratio of aggregated tau increases in animals expressing 3PO or P301S.

Discussion

Expression of a gfp-tagged wild-type tau permits visualization of age-related changes

In vivo animal models are extremely valuable for understanding neurodegenerative conditions like Alzheimer's disease and related disorders. While vertebrate models have significant utility in terms of their neuronal complexity and genetic relationship to humans, there are some drawbacks that can be better addressed using invertebrate models. For example, it is much easier to create new transgenic animals and, in general, phenotypes develop much more quickly. It is also simpler to cross these transgenes into animals with mutations in genes of interest that have been isolated in a common genetic background, thereby reducing potential confounds of genetic variation on the phenotypes observed.

To address some of the novel potential for invertebrate models we relied on the fact that *C. elegans* are optically transparent throughout the entire lifecycle, enabling us to visualize tau in living animals. Importantly, we observed that wild-type tau (hTau40), was well-tolerated by the animals at the DNA concentration we injected to create the transgenes. This contrasts with other *C. elegans* tauopathy models where over-expression of wild-type tau had a negative impact on survival (Kraemer et al., 2003). One difference could be the use of the 1N4R isoform in that study, while here we used the 2N4R isoform. However, the identification of expression levels in which the wild-type tau is non-pathological allowed us to observe the "normal" behaviour of tau inside an organismal nervous system during aging.

We did notice some differences between the accumulation in neurons of the ventral nerve cord and in the tail, where more of the tauGFP was observed in cell bodies. There are two potential explanations for this difference. First, the cells in the ventral nerve cord are primarily motor neurons, while the neurons observed in the tail are either sensory or interneurons, and thus, it is possible that tau accumulates differently in the various classes of neurons. Second, the morphology of the neurons is distinct, with the neurons in the tail having the cell body "between" the axonal and dendritic compartments, whereas the motor neurons have cell bodies that are somewhat lateral to the processes in the nerve cord (White et al., 1976, 1986). Thus, the observed differences may be more of a consequence of the morphology. Future experiments limiting tau to a subset of neurons should enable us to better distinguish which of these is more likely.

Interestingly, over the first 7 days of adulthood (approximately one-third to one-half of the animal's lifespan) we did observe some differences in the intensity of GFP for wild-type tau across different cells. While this could simply be a product of differences in expression, it might also suggest that some neurons are more susceptible to tauGFP accumulation, as has been previously reported. In two different studies using mice, it appears that GABAergic neurons are more likely to accumulate neurofibrillary tangles (NFTs) and display deficits in activity (Andrews-Zwilling et al., 2010; Levenga et al., 2013; Najm et al., 2020).

Finally, it is important to note that we do observe immunoreactivity with an antibody that preferentially detects a disease-associated conformation of tau in post-mortem tissue from humans (TNT1). Thus, even in the short time frame of a few days, we find that in the *C. elegans* nervous system, tau adopts conformations similar to that of human disease. We followed this up by extracting tau from our transgenic animals using increasingly stringent buffers. We found evidence of detergent-insoluble tau only in 3PO and older P301S animals, but not hTau40. Thus, at least some of the TNT1 staining in these animals is indicative of a soluble form of tau. There is growing evidence in the field that larger aggregates may be protective and smaller oligomeric species more pathogenic, and thus it is interesting that we observe tau species detected by TNT1 that appear to be soluble.

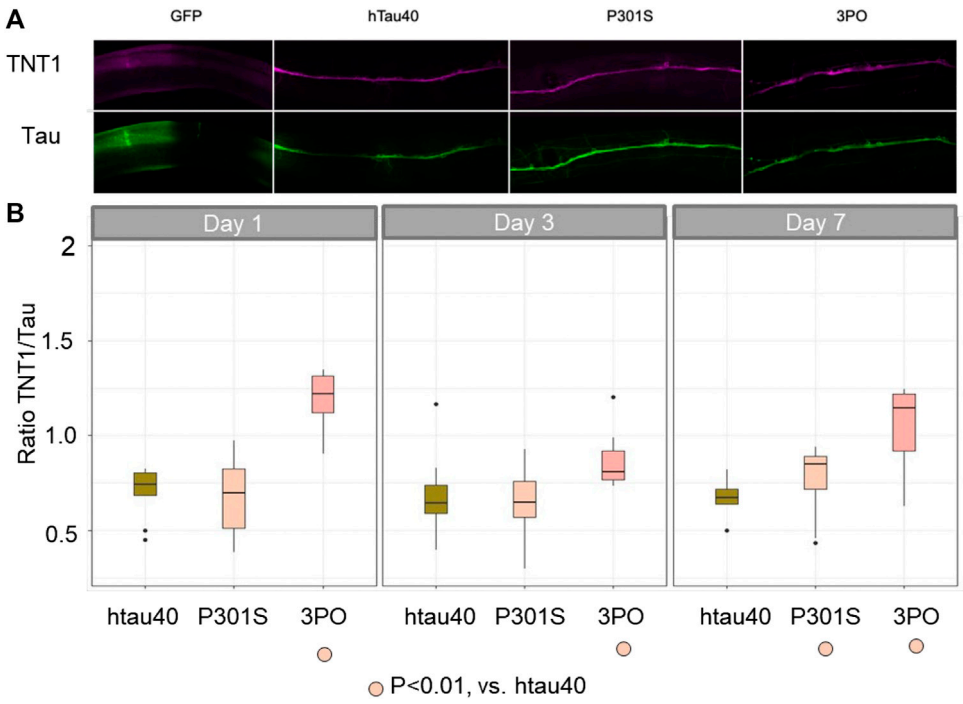

FIGURE 9
TNT1 staining indicates tau can adopt disease-relevant conformations in ***C. elegans*** We used the TNT1 antibody and A002401 antibody to stain animals for the early disease conformation aggregates compared to total tau. **(A)** Day one adult animals stained with TNT1 (magenta) and total Tau (green) antibodies. **(B)** We quantified the staining on days one, three and seven of adulthood ($N = 10$ per day per genotype), deriving the ratio of TNT1/Total tau. The 3PO lines had a higher ratio of pathogenic tau than hTau40 or P301S, which were not significantly different from each other on day one. By day seven the TNT1 immunoreactivity was significantly increased in the P301S animals compared to hTau40 (ANOVA, Tukey post-hoc comparison, $p < 0.05$).

Tau variants can act differently from wild type

We evaluated two different tau variants, one, the P301S mutation, is a disease associated variant that has been linked to multiple tauopathies in humans, including frontotemporal dementia (FTD), corticobasal degeneration (CBD), and progressive supranuclear palsy (PSP) (Bugiani et al., 1999; Kametani et al., 2020). The second, 3PO, has been used both *in vitro* and *in vivo* to model a rapidly polymerizing version of tau that does not require an inducer molecule to initiate aggregation.

One of the results we observed was that, for the most part, the P301S variant behaved like wild-type tau in our models, with a few notable differences. Like wild-type tau, it was well-tolerated in its effects on lifespan and organismal motility.

Unlike the wild-type tau protein, P301SGFP appeared to be more stable, increasing in intensity over the time frame we observed, preferentially accumulating in nerve processes, and could aggregate into detergent-insoluble species by day three of adulthood. Some tau mutants, including the P301L variants can be resistant to cellular degradation, independent of their ability to aggregate (Caballero et al., 2018). Interestingly, the observed increased stability of P301S was dependent on the endogenous *C. elegans* MAP, PTL-1. Whether this indicates a physical interaction between the P301S and PTL-1, or that there are other compensatory changes in the *ptl-1* mutants that reduced P301S from accumulating needs to be further studied.

As would be expected, we observed significantly different consequences using the rapidly polymerizing variant. First, we confirmed biochemically that 3PO protein was forming aggregates, and that TNT1 immunoreactivity was increased in the 3PO-expressing animals. The 3POtau was more concentrated in cell bodies, and even in nerve processes appeared different from P301S or wildtype. These are consistent with previous work *in vitro* documenting the kinds of aggregates formed by 3PO compared to P301 mutants (Combs and Gamblin, 2012; Mutreja et al., 2019). Animals expressing 3POtau died sooner than expected, and the decline in organismal lifespan was around the time we begin to see an increase in overall tau protein levels, which is consistent with other nematode tauopathy models where increased expression of tau or tau variants was increasingly detrimental to the animals. We observed an interaction between 3PO and the loss of *ptl-1,* and others have previously reported an interaction between another rapidly polymerizing variant (ΔK280) and *ptl-1* (Ko et al., 2020).

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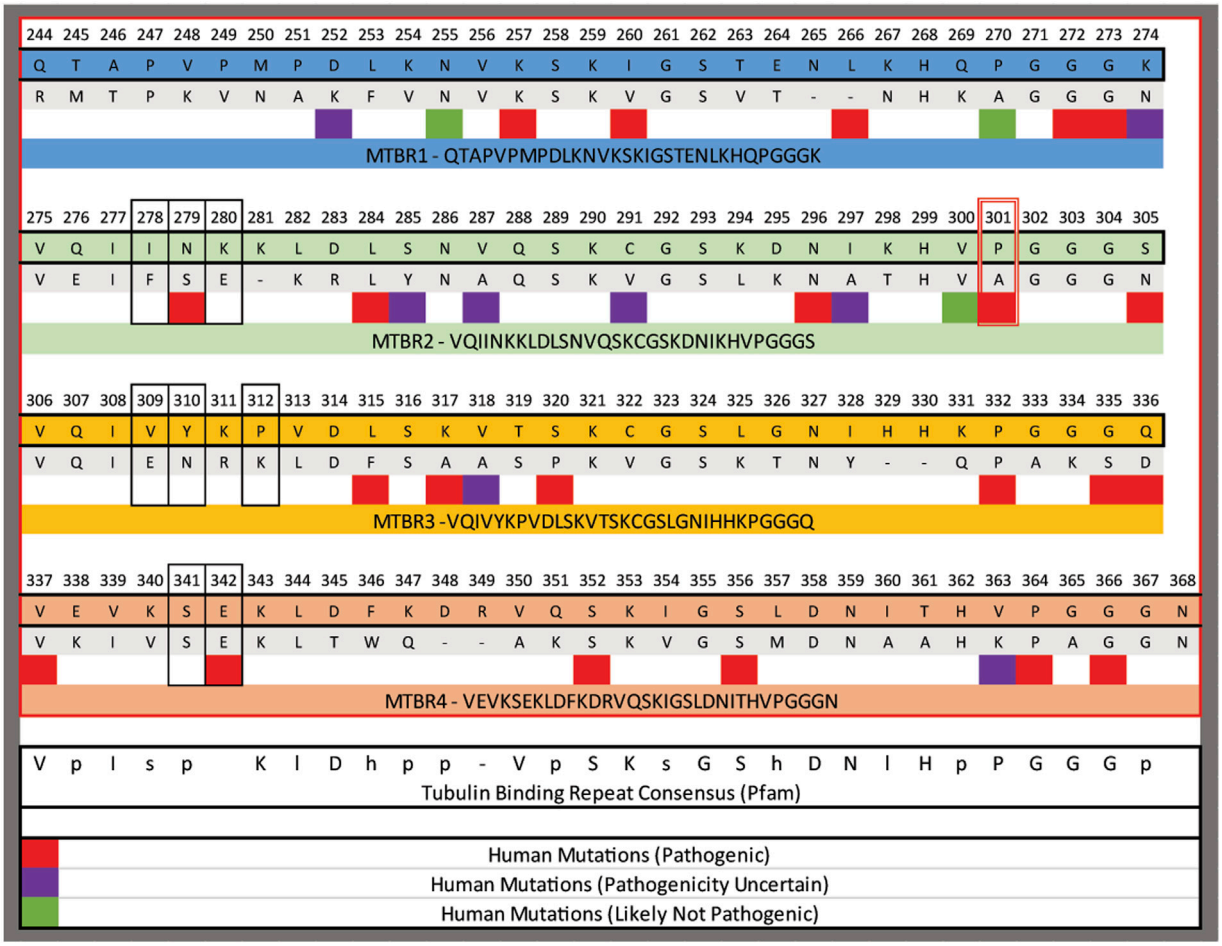

FIGURE 10
Alignment of the microtubule binding repeats (MTBR) from human tau and the C. ***elegans*** PTL-1. The domains were aligned to each other using Clustal omega, numbering is from hTau40. The red box outlines the position of P301 mutated in the P301S lines and the black boxes outline residues modified in the 3PO variant. Below specific amino acids are regions that have been identified to in human populations, and the pathogenicity indicated by the colour code as indicated.

Why was the P301S variant not particularly pathological in our model, given that other tau variants, including the oft-studied P301L have been shown to induce phenotypes? It is possible that the P301S takes longer to accumulate in a pathological form, and that we cannot observe that in a *C. elegans* lifetime (2–3 weeks). There may also be isoform differences in the aggregation properties (Mutreja et al., 2019). Thus, it will be interesting to study this in our system, asking whether 0N or 1N isoforms behave differently when the P301 residue is mutated.

Another possibility is that the serine substitution is better tolerated by *C. elegans* neurons. The mutation is in the second microtubule binding repeat. In those repeats there is a highly conserved P-G-G-G-X motif that is predicted to enable folding of the MTBRs. And, in most mammalian tau proteins, the P is invariant. In contrast, when the MTBRs from the PTL-1 protein and human tau are aligned that proline residue is either an alanine or a proline (Figure 10). Thus, it is possible that that position has more flexibility inside the environment of *C. elegans* neurons. Finally, it is possible that the location within the neurons that we observe protein accumulation (cell bodies vs. processes) could be relevant.

There are differences in our results from previous work studying tau aggregation in *C. elegans*. Primarily, we do not observe any obvious cell death in the animals, at least in the time frame in which we focused. This contrasts with a recent study where the authors used a single-copy knock-in approach to study the effects of tau post-translational modification effects *in vivo* (Guha et al., 2020). Other tauopathy models using over-expression of tau have noted cases of neuronal degeneration as well (Miyasaka et al., 2005; Kraemer and Schellenberg, 2007). There are differences in the isoforms being used in those studies, which motivates a more thorough investigation of isoform differences in tau-induced phenotypes.

Amount of aggregated to non-aggregated tau impacts survival

Our quantification of the GFP fluorescence suggests that tau levels are being maintained. Since the activity of the *rgef-1* promoter is constant during the phase of adulthood we are analysing, the simplest interpretation for the stable levels of fluorescence is that tau is being produced and turned over at a constant rate. For the P301S and 3PO we observe an increase in fluorescence in an age-dependent manner, consistent with a reduced capacity to remove those proteins. However, only the 3POtau results in an impact on lifespan. There was significantly more TNT1 staining in animals expressing 3PO, suggesting more of the total protein was in a pathological conformation, consistent its generation as an aggressively pro-aggregating form of tau (Iliev et al., 2006). From this, we hypothesize that there is a threshold at which tau aggregates begin to impact organismal health. Together these results suggest that, like many other studies have suggested, tau-aggregates concentration must be maintained intracellularly to be toxic, and higher the concentration of tau deposition results in a worsening of the phenotype (Simón et al., 2012; Shigemoto et al., 2018). Whether that threshold is dependent just on the amount of aggregation, or the localization of the aggregation, or even other cellular factors remains to be determined.

MAP proteins can protect against the effects of aggregated tau

PTL-1 is the *C. elegans* ortholog of human tau and other MAPs; it binds to tubulin and provides cytoskeleton support to the neurons and axons (Gordon et al., 2008; Chew et al., 2013). We found that a mutation ablating *ptl-1* function had multiple interactions with our tau expression lines. The most critical of these seemed to be that the loss of *ptl-1* resulted in a significant decrease in organismal lifespan in animals expressing 3POtau, which suggested that PTL-1 was protecting the animals from the consequences of the aggregated tau. Consistent with that effect, we found a reduction in the ability of the P301S variant to accumulate in axons when *ptl-1* was removed.

Overall, we have demonstrated that adding the GFP moiety to tau can help visualize tau in a living organism. Our current results suggest that aggregation, when confined to nerve processes may be better tolerated than when it is primarily in the somas. Using this system, we can begin to test genetic or environmental risk factors, trying to correlate the location and speed of aggregation to the changes in organismal lifespan. Ultimately, because of the simplicity of this system and rapidity with which phenotypes emerge, this model can be used to address many of the open questions in the field of tauopathies.

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Data availability statement

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

Author contributions

Study conception: BA and TG, Experimental Procedures: WA, BC, and BA; Data Analysis: WN and BA; Manuscript Preparation and Editing: WN, BC, TG, and BA.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Adamla, F., and Ignatova, Z. (2015). Somatic expression of unc-54 and vha-6 mRNAs declines but not pan-neuronal rgef-1 and unc-119 expression in aging *Caenorhabditis elegans*. *Sci. Rep.* 5, 10692. doi:10.1038/srep10692

Altun-Gultekin, Z., Andachi, Y., Tsalik, E. L., Pilgrim, D., Kohara, Y., and Hobert, O. (2001). A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in *C. elegans*. *Development* 128 (11), 1951–1969. doi:10.1242/dev.128.11.1951

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S. Y., et al. (2010). Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. *J. Neurosci.* 30 (41), 13707–13717. doi:10.1523/jneurosci.4040-10.2010

Barbier, P., Zejneli, O., Martinho, M., Lasorsa, A., Belle, V., Smet-Nocca, C., et al. (2019). Role of tau as a microtubule-associated protein: Structural and functional aspects. *Front. Aging Neurosci.* 11, 204. doi:10.3389/fnagi.2019.00204

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 (35), 14914–14919. doi:10.1073/pnas.0902882106

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77 (1), 71–94. doi:10.1093/genetics/77.1.71

Bugiani, O., Murrell, J. R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M., et al. (1999). Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J. Neuropathol. Exp. Neurol.* 58 (6), 667–677. doi:10.1097/00005072-199906000-00011

Caballero, B., Wang, Y., Diaz, A., Tasset, I., Juste, Y. R., Stiller, B., et al. (2018). Interplay of pathogenic forms of human tau with different autophagic pathways. *Aging Cell* 17 (1), e12692. doi:10.1111/acel.12692

Carmel, G., Mager, E. M., Binder, L. I., and Kuret, J. (1996). The structural basis of monoclonal antibody Alz50's selectivity for Alzheimer's disease pathology. *J. Biol. Chem.* 271 (51), 32789–32795. doi:10.1074/jbc.271.51.32789

Chatterjee, S., Sealey, M., Ruiz, E., Pegasiou, C. M., Green, S., Crisford, A., et al. (2021). Age-related changes in Tau and Autophagy in human brain in the absence of neurodegeneration. *bioRxiv* 2021 (2008), 456385. doi:10.1101/2021.08.21.456385

Chen, L., Fu, Y., Ren, M., Xiao, B., and Rubin, C. S. (2011). A RasGRP, *C. elegans* RGEF-1b, couples external stimuli to behavior by activating LET-60 (Ras) in sensory neurons. *Neuron* 70 (1), 51–65. doi:10.1016/j.neuron.2011.02.039

Chen, X., Cassady, K. E., Adams, J. N., Harrison, T. M., Baker, S. L., and Jagust, W. J. (2021). Regional tau effects on prospective cognitive change in cognitively normal older adults. *J. Neurosci.* 41 (2), 366–375. doi:10.1523/jneurosci.2111-20.2020

Chew, Y. L., Fan, X., Gotz, J., and Nicholas, H. R. (2013). PTL-1 regulates neuronal integrity and lifespan in *C. elegans*. *J. Cell Sci.* 126 (9), 2079–2091. doi:10.1242/jcs.jcs124404

Chiu, M. J., Fan, L. Y., Chen, T. F., Chen, Y. F., Chieh, J. J., and Horng, H. E. (2017). Plasma tau levels in cognitively normal middle-aged and older adults. *Front. Aging Neurosci.* 9, 51. doi:10.3389/fnagi.2017.00051

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 (3), 781–790. doi:10.1016/j.celrep.2013.09.043

Combs, B., and Gamblin, T. C. (2012). FTDP-17 tau mutations induce distinct effects on aggregation and microtubule interactions. *Biochemistry* 51 (43), 8597–8607. doi:10.1021/bi3010818

Combs, B., Hamel, C., and Kanaan, N. M. (2016). Pathological conformations involving the amino terminus of tau occur early in Alzheimer's disease and are differentially detected by monoclonal antibodies. *Neurobiol. Dis.* 94, 18–31. doi:10.1016/j.nbd.2016.05.016

Combs, B., Tiernan, C. T., Hamel, C., and Kanaan, N. M. (2017). Production of recombinant tau oligomers *in vitro*. *Methods Cell Biol.* 141, 45–64. doi:10.1016/bs.mcb.2017.06.005

Datta, D., Leslie, S. N., Wang, M., Morozov, Y. M., Yang, S., Mentone, S., et al. (2021). Age-related calcium dysregulation linked with tau pathology and impaired cognition in non-human primates. *Alzheimers Dement* 17 (6), 920–932. doi:10.1002/alz.12325

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 (8), e1000450. doi:10.1371/journal.pbio.1000450

Edelstein-keshet, L., and Spiros, A. (2002). Exploring the formation of Alzheimer's disease senile plaques *in silico*. *J. Theor. Biol.* 216 (3), 301–326. doi:10.1006/jtbi.2002.2540

Goedert, M., Wischik, C. M., Crowther, R. A., Walker, J. E., and Klug, A. (1988). Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of alzheimer disease: Identification as the microtubule-associated protein tau. *Proc. Natl. Acad. Sci. U. S. A.* 85 (11), 4051–4055. doi:10.1073/pnas.85.11.4051

Gordon, P., Hingula, L., Krasny, M. L., Swienckowski, J. L., Pokrywka, N. J., and Raley-Susman, K. M. (2008). The invertebrate microtubule-associated protein PTL-1 functions in mechanosensation and development in *Caenorhabditis elegans*. *Dev. Genes Evol.* 218 (10), 541–551. doi:10.1007/s00427-008-0250-z

Grimm, A. (2021). Impairments in brain bioenergetics in aging and tau pathology: A chicken and egg situation? *Cells* 10 (10), 2531. doi:10.3390/cells10102531

Grundke-Iqbal, I., Iqbal, K., Tung, Y. C., Quinlan, M., Wisniewski, H. M., and Binder, L. I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U. S. A.* 83 (13), 4913–4917. doi:10.1073/pnas.83.13.4913

Guha, S., Fischer, S., Johnson, G. V. W., and Nehrke, K. (2020). Tauopathy-associated tau modifications selectively impact neurodegeneration and mitophagy in a novel *C. elegans* single-copy transgenic model. *Mol. Neurodegener.* 15 (1), 65. doi:10.1186/s13024-020-00410-7

Guillozet, A. L., Weintraub, S., Mash, D. C., and Mesulam, M. M. (2003). Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch. Neurol.* 60 (5), 729–736. doi:10.1001/archneur.60.5.729

Higham, J. P., Malik, B. R., Buhl, E., Dawson, J. M., Ogier, A. S., Lunnon, K., et al. (2019). Alzheimer's disease associated genes ankyrin and tau cause shortened lifespan and memory loss in Drosophila. *Front. Cell. Neurosci.* 13, 260. doi:10.3389/fncel.2019.00260

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

Iliev, A. I., Ganesan, S., Bunt, G., and Wouters, F. S. (2006). Removal of pattern-breaking sequences in microtubule binding repeats produces instantaneous tau aggregation and toxicity. *J. Biol. Chem.* 281 (48), 37195–37204. doi:10.1074/jbc.M604863200

Kadavath, H., Hofele, R. V., Biernat, J., Kumar, S., Tepper, K., Urlaub, H., et al. (2015). Tau stabilizes microtubules by binding at the interface between tubulin heterodimers. *Proc. Natl. Acad. Sci. U. S. A.* 112 (24), 7501–7506. doi:10.1073/pnas.1504081112

Kametani, F., Yoshida, M., Matsubara, T., Murayama, S., Saito, Y., Kawakami, I., et al. (2020). Comparison of common and disease-specific post-translational modifications of pathological tau associated with a wide range of tauopathies. *Front. Neurosci.* 14, 581936. doi:10.3389/fnins.2020.581936

Kanaan, N. M., Morfini, G. A., LaPointe, N. E., Pigino, G. F., Patterson, K. R., Song, Y., et al. (2011). Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases. *J. Neurosci.* 31 (27), 9858–9868. doi:10.1523/JNEUROSCI.0560-11.2011

Kenyon, C. J. (2010). The genetics of ageing. *Nature* 464 (7288), 504–512. doi:10.1038/nature08980

Ko, S. H., Gonzalez, G., Liu, Z., and Chen, L. (2020). Axon injury-induced autophagy activation is impaired in a *C. elegans* model of tauopathy. *Int. J. Mol. Sci.* 21 (22), E8559. doi:10.3390/ijms21228559

Kraemer, B. C., and Schellenberg, G. D. (2007). SUT-1 enables tau-induced neurotoxicity in *C. elegans*. *Hum. Mol. Genet.* 16 (16), 1959–1971. doi:10.1093/hmg/ddm143

Kraemer, B. C., Zhang, B., Leverenz, J. B., Thomas, J. H., Trojanowski, J. Q., and Schellenberg, G. D. (2003). Neurodegeneration and defective neurotransmission in a *Caenorhabditis elegans* model of tauopathy. *Proc. Natl. Acad. Sci. U. S. A.* 100 (17), 9980–9985. pii. doi:10.1073/pnas.1533448100

Landrum, M. J., Chitipiralla, S., Brown, G. R., Chen, C., Gu, B., Hart, J., et al. (2020). ClinVar: Improvements to accessing data. *Nucleic Acids Res.* 48 (D1), D835–D844. doi:10.1093/nar/gkz972

Levenga, J., Krishnamurthy, P., Rajamohamedsait, H., Wong, H., Franke, T. F., Cain, P., et al. (2013). Tau pathology induces loss of GABAergic interneurons leading to altered synaptic plasticity and behavioral impairments. *Acta Neuropathol. Commun.* 1, 34. doi:10.1186/2051-5960-1-34

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

Macdonald, J. A., Bronner, I. F., Drynan, L., Fan, J., Curry, A., Fraser, G., et al. (2019). Assembly of transgenic human P301S Tau is necessary for neurodegeneration in murine spinal cord. *Acta Neuropathol. Commun.* 7 (1), 44. doi:10.1186/s40478-019-0695-5

Miyasaka, T., Ding, Z., Gengyo-Ando, K., Oue, M., Yamaguchi, H., Mitani, S., et al. (2005). Progressive neurodegeneration in *C. elegans* model of tauopathy. *Neurobiol. Dis.* 20 (2), 372–383. doi:10.1016/j.nbd.2005.03.017

Mutreja, Y., Combs, B., and Gamblin, T. C. (2019). FTDP-17 mutations alter the aggregation and microtubule stabilization propensity of tau in an isoform-specific fashion. *Biochemistry* 58 (6), 742–754. doi:10.1021/acs.biochem.8b01039

Najarro, E. H., Wong, L., Zhen, M., Carpio, E. P., Goncharov, A., Garriga, G., et al. (2012). *Caenorhabditis elegans* flamingo cadherin fmi-1 regulates GABAergic neuronal development. *J. Neurosci.* 32 (12), 4196–4211. doi:10.1523/JNEUROSCI.3094-11.2012

Najm, R., Rao, A., and Huang, Y. (2020). Too much tau in interneurons impairs adult hippocampal neurogenesis in Alzheimer's disease. *Cell Stem Cell* 26 (3), 297–299. doi:10.1016/j.stem.2020.02.004

Nations, U. (2019). "World Population Prospects 2019: Highlights (ST/ESA/SER.A/423)," in *Department of Economic and Social Affairs*. Available at: https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html

Nonet, M. L., Staunton, J. E., Kilgard, M. P., Fergestad, T., Hartwieg, E., Horvitz, H. R., et al. (1997). *Caenorhabditis elegans* rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. *J. Neurosci.* 17 (21), 8061–8073. doi:10.1523/jneurosci.17-21-08061.1997

Oakley, S. S., Maina, M. B., Marshall, K. E., Al-Hilaly, Y. K., Harrington, C. R., Wischik, C. M., et al. (2020). Tau filament self-assembly and structure: Tau as a therapeutic target. *Front. Neurol.* 11, 590754. doi:10.3389/fneur.2020.590754

Orr, M. E., Sullivan, A. C., and Frost, B. (2017). A brief overview of tauopathy: Causes, consequences, and therapeutic strategies. *Trends Pharmacol. Sci.* 38 (7), 637–648. doi:10.1016/j.tips.2017.03.011

Pir, G. J., Choudhary, B., Mandelkow, E., and Mandelkow, E. M. (2016). Tau mutant A152T, a risk factor for FTD/PSP, induces neuronal dysfunction and reduced lifespan independently of aggregation in a *C. elegans* Tauopathy model. *Mol. Neurodegener.* 11, 33. doi:10.1186/s13024-016-0096-1

Proctor, C. J., and Gray, D. A. (2010). GSK3 and p53 - is there a link in Alzheimer's disease? *Mol. Neurodegener.* 5, 7. doi:10.1186/1750-1326-5-7

Puri, I. K., and Li, L. (2010). Mathematical modeling for the pathogenesis of Alzheimer's disease. *PLoS One* 5 (12), e15176. doi:10.1371/journal.pone.0015176

Rajan, K. B., Weuve, J., Barnes, L. L., McAninch, E. A., Wilson, R. S., and Evans, D. A. (2021). Population estimate of people with clinical Alzheimer's disease and mild cognitive impairment in the United States (2020-2060). *Alzheimers Dement.* 17 (12), 1966–1975. doi:10.1002/alz.12362

Rossi, G., and Tagliavini, F. (2015). Frontotemporal lobar degeneration: Old knowledge and new insight into the pathogenetic mechanisms of tau mutations. *Front. Aging Neurosci.* 7, 192. doi:10.3389/fnagi.2015.00192

Scheres, S. H., Zhang, W., Falcon, B., and Goedert, M. (2020). Cryo-EM structures of tau filaments. *Curr. Opin. Struct. Biol.* 64, 17–25. doi:10.1016/j.sbi.2020.05.011

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nat. Methods* 9 (7), 671–675. doi:10.1038/nmeth.2089

Sealey, M. A., Vourkou, E., Cowan, C. M., Bossing, T., Quraishe, S., Grammenoudi, S., et al. (2017). Distinct phenotypes of three-repeat and four-repeat human tau in a transgenic model of tauopathy. *Neurobiol. Dis.* 105, 74–83. doi:10.1016/j.nbd.2017.05.003

Shi, Y., Zhang, W., Yang, Y., Murzin, A. G., Falcon, B., Kotecha, A., et al. (2021). Structure-based classification of tauopathies. *Nature* 598 (7880), 359–363. doi:10.1038/s41586-021-03911-7

Shigemoto, Y., Sone, D., Imabayashi, E., Maikusa, N., Okamura, N., Furumoto, S., et al. (2018). Dissociation of tau deposits and brain atrophy in early Alzheimer's disease: A combined positron emission tomography/magnetic resonance imaging study. *Front. Aging Neurosci.* 10, 223. doi:10.3389/fnagi.2018.00223

Sievers, F., and Higgins, D. G. (2021). The clustal omega multiple alignment package. *Methods Mol. Biol.* 2231, 3–16. doi:10.1007/978-1-0716-1036-7_1

Simón, D., García-García, E., Gómez-Ramos, A., Falcón-Pérez, J. M., Díaz-Hernández, M., Hernández, F., et al. (2012). Tau overexpression results in its secretion via membrane vesicles. *Neurodegener. Dis.* 10 (1-4), 73–75. doi:10.1159/000334915

Statzer, C., Reichert, P., Dual, J., and Ewald, C. Y. (2022). Longevity interventions temporally scale healthspan in *Caenorhabditis elegans*. *iScience* 25 (3), 103983. doi:10.1016/j.isci.2022.103983

Takeuchi, H., Iba, M., Inoue, H., Higuchi, M., Takao, K., Tsukita, K., et al. (2011). P301S mutant human tau transgenic mice manifest early symptoms of human tauopathies with dementia and altered sensorimotor gating. *PLoS One* 6 (6), e21050. doi:10.1371/journal.pone.0021050

Taylor, R. C., and Dillin, A. (2011). Aging as an event of proteostasis collapse. *Cold Spring Harb. Perspect. Biol.* 3 (5), a004440. doi:10.1101/cshperspect.a004440

Team, R. C. (2020). *R: A language and environment for statistical computing*. (Vienna, Austria: R foundation for statistical computing).

Team, R. (2019). *RStudio: Integrated development for R*. Boston, MA: RStudio, Inc.

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 (4), 919–932. doi:10.1016/j.cell.2015.03.032

White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 314 (1165), 1–340. doi:10.1098/rstb.1986.0056

White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. (1976). The structure of the ventral nerve cord of *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 275 (938), 327–348. doi:10.1098/rstb.1976.0086

Wischik, C. M., Novak, M., Thogersen, H. C., Edwards, P. C., Runswick, M. J., Jakes, R., et al. (1988). Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* 85 (12), 4506–4510. doi:10.1073/pnas.85.12.4506

Wittmann, C. W., Wszolek, M. F., Shulman, J. M., Salvaterra, P. M., Lewis, J., Hutton, M., et al. (2001). Tauopathy in Drosophila: Neurodegeneration without neurofibrillary tangles. *Science* 293 (5530), 711–714. doi:10.1126/science.1062382

Wood, J. G., Mirra, S. S., Pollock, N. J., and Binder, L. I. (1986). Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau). *Proc. Natl. Acad. Sci. U. S. A.* 83 (11), 4040–4043. doi:10.1073/pnas.83.11.4040

Yoshiyama, Y., Higuchi, M., Zhang, B., Huang, S. M., Iwata, N., Saido, T. C., et al. (2007). Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* 53 (3), 337–351. doi:10.1016/j.neuron.2007.01.010

Zhen, M., and Samuel, A. D. (2015). *C. elegans* locomotion: Small circuits, complex functions. *Curr. Opin. Neurobiol.* 33, 117–126. doi:10.1016/j.conb.2015.03.009

Ziontz, J., Bilgel, M., Shafer, A. T., Moghekar, A., Elkins, W., Helphrey, J., et al. (2019). Tau pathology in cognitively normal older adults. *Alzheimers Dement.* 11, 637–645. doi:10.1016/j.dadm.2019.07.007

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