UBIQUITIN AND UBIQUITIN-RELATIVE SUMO IN DNA DAMAGE RESPONSE

EDITED BY: Kristijan Ramadan and Ivan Dikic

PUBLISHED IN: Frontiers in Genetics

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88945-441-9
DOI 10.3389/978-2-88945-441-9

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

UBIQUITIN AND UBIQUITIN-RELATIVE SUMO IN DNA DAMAGE RESPONSE

Topic Editors:
Kristijan Ramadan, University of Oxford, United Kingdom
Ivan Dikic, Goethe University, Germany

DNA damage response (DDR) is a term that includes a variety of highly sophisticated mechanisms that cells have evolved in safeguarding the genome from the deleterious consequences of DNA damage. It is estimated that every single cell receives tens of thousands of DNA lesions per day. Failure of DDR to properly respond to DNA damage leads to stem cell dysfunction, accelerated ageing, various degenerative diseases or cancer. The sole function of DDR is to recognize diverse DNA lesions, signal their presence, activate cell cycle arrest and finally recruit specific DNA repair proteins to fix the DNA damage and thus prevent genomic instability. DDR is composed of hundreds of spatiotemporally regulated and interconnected proteins, which are able to promptly respond to various DNA lesions. So it is not surprising that mutations in genes encoding various DDR proteins cause embryonic lethality, malignancies, neurodegenerative diseases and premature ageing.

The importance of DDR for cell survival and genome stability is unquestionable, but how the sophisticated network of hundreds of different DDR proteins is spatiotemporally coordinated is far from being understood. In the last ten years ubiquitin (ubiquitination) and the ubiquitin-relative SUMO (sumoylation) have emerged as essential posttranslational modifications that regulate DDR. Beside a plethora of ubiqutin and sumo E1-activating enzymes, E2-conjugating enzymes, E3-ligases and ubiquitin/sumo proteases involved in ubiquitination and sumoylation, the complexity of ubiqutin and sumo systems is additionally increased by the fact that both ubiquitin and sumo can form a variety of different chains on substrates which govern the substrate fate, such as its interaction with other proteins, changing its enzymatic activity or promoting substrate degradation.

The importance of ubiquitin/SUMO systems in the orchestration of DDR is best illustrated in patients with mutations in E3-ubiquitin ligases BRCA1 or RNF168. BRCA1 is essential for proper function of DDR and its mutations lead to triple-negative breast and ovarian cancers. RNF168 is an E3 ubiquitin ligase, which creates the ubiquitin docking platform for recruitment of different DNA damage signalling and repair proteins at sites of DNA lesion, and its mutations cause RIDDLE syndrome characterized by radiosensitivity, immunodeficiency and learning disability. In addition, recently discovered the ubiquitin receptor protein SPRTN is part of the DNA replication machinery and its mutations cause early-onset hepatocellular carcinoma and premature ageing in humans.

Despite more than 700 different enzymes directly involved in ubiquitination and sumoylation processes only few of them are known to play a role in DDR. Therefore, we feel that the role of ubiquitin and the ubiquitin-related SUMO in DDR is far from being understood, and that this

is the emerging field that will hugely expand in the next decade due to the rapid development of a new generation of technologies, which will allow us a more robust and precise analyses of human genome, transcriptome and proteome.

In this Research Topic we provide a comprehensive overview of our current understanding of ubiquitin and SUMO pathways in all aspects of DDR, from DNA replication to different DNA repair pathways, and demonstrate how alterations in these pathways cause genomic instability that is linked to degenerative diseases, cancer and pathological ageing.

Citation: Ramadan, K., Dikic, I., eds. (2018). Ubiquitin and Ubiquitin-Relative SUMO in DNA damage response. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-441-9

Table of Contents

frontiers in Genetics

EDITORIAL
published: 27 November 2017
doi: 10.3389/fgene.2017.00188

Editorial: Ubiquitin and Ubiquitin-Relative SUMO in DNA Damage Response

Kristijan Ramadan[1]* *and Ivan Dikic*[2,3]*

[1] Department of Oncology, CRUK/MRC Oxford Institute for Radiation Oncology (MRC), Oxford University, Oxford, United Kingdom, [2] Institute of Biochemistry II, School of Medicine, Goethe University, Frankfurt, Germany, [3] Molecular Signaling Unit, Buchmann Institute for Molecular Life Sciences, Goethe University, Frankfurt, Germany

Keywords: DNA damage response, ubiquitin, ubiquitination, SUMO, sumoylation, cancer, genome stability

OPEN ACCESS

Edited by:
Paul S. Meltzer, National Cancer Institute (NIH), United States

Reviewed by:
Petra Beli, Institute of Molecular Biology, Germany

***Correspondence:**
Kristijan Ramadan
kristijan.ramadan@oncology.ox.ac.uk
Ivan Dikic
dikic@biochem2.uni-frankfurt.de

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *16 May 2017*
Accepted: *10 November 2017*
Published: *27 November 2017*

Citation:
Ramadan K and Dikic I (2017) Editorial: Ubiquitin and Ubiquitin-Relative SUMO in DNA Damage Response. Front. Genet. 8:188. doi: 10.3389/fgene.2017.00188

Editorial on the Research Topic

Ubiquitin and Ubiquitin-Relative SUMO in DNA Damage Response

Ubiquitin (UB) is a small inactive peptide which dramatically changes the fate of ubiquitinated proteins when enzymatically activated and covalently attached to proteins in the process known as ubiquitination (Ciechanover et al., 1984). UB was initially discovered as a signal for UB-dependent protein degradation by the proteasome system in the late 1970s and early 1980s (Ciechanover et al., 1980, 1984; Hershko et al., 1980). However, it is now clear that the cellular role of ubiqutination is much more complex than initially thought (Grabbe et al., 2011). Ubiquitination is the most complex posttranslational modification (PTM) that regulates virtually all cellular processes (Komander, 2009; Heride et al., 2014; Swatek and Komander, 2016). Avram Hershko, Aaron Ciechanover and Irwin A. Rose were awarded with the Nobel Prize in Chemistry for 2004 for the discovery of the UB-mediated protein degradation (proteolysis) (Kresge et al., 2006). This award tremendously boosted scientific curiosity towards UB and ubiqutination as can be demonstrated by there currently being more than 70,000 Pubmed research articles on ubiqutination, compared to less than 12,000 before 2004. There are several ubiquitin like modifiers (Welchman et al., 2005), but SUMO is the best investigated one in DNA damage response (Schwertman et al., 2016). Therefore, this issue is focusing on UB and its main relative SUMO.

The DNA damage response (DDR) has been defined as a multifaceted network of cellular pathways that are activated after DNA damage (Jeggo et al., 2016). Various DNA lesions activate the DDR, which first senses DNA damage and then transduce this signal to downstream effectors that consequently govern a robust cellular response visualized as cell cycle arrest, DNA repair and/or apoptosis (Jackson and Bartek, 2009). The discovery of the cellular toolbox for repairing damaged DNA was commemorated in 2015 when the Nobel Prize in Chemistry was awarded to Tomas Lindahl, Paul Modrich and Aziz Sancar (Lindahl et al., 2016). The DDR is composed of hundreds of different proteins, the function of which needs to be spatiotemporally orchestrated, and this occurs via various PTMs. In the last decade the PTMs, ubiquitination and SUMOylation,

have emerged as the essential and most critical PTMs in the regulation of the DDR (Jackson and Durocher, 2013; Schwertman et al., 2016). Defects in the components of UB system in DDR are associated with many human diseases, including cancer and accelerated ageing. Thus, we decided to systematically review advances in this relatively young field, and to cover its role in DNA replication, DNA repair and mitosis. Our intention is to invite the most prominent scientists in the field, together with a selection of young and promising scientists, and give them the opportunity to summarize our current knowledge of UB and SUMO in the regulation of the DDR. The main goal is to share current visions and directions that will shape the priorities in this field for the next 10 years. The majority of invited scientists gladly contributed to this special issue, either by writing review articles or reviewing submitted manuscripts. Thus, we would like to thank them all for their enormous and professional contribution to the issue.

Helle Ulrich (Institute of Molecular Biology - Mainz) and her group highlighted that ubiquitination and SUMOylation control all aspects of DNA replication, from its initiation, elongation and termination, and not only translesion DNA synthesis as was initially proposed (Garcia-Rodriguez et al.). The group of Simone Sabbioneda (National Research Centre - Pavia) discussed how UB and SUMO control DNA damage tolerance, the last line of defense that allows completion of DNA replication in the presence of an unrepaired template. They focused on post-replication repair, the mechanism cells use to bypass highly distorted templates caused by damaged bases (Cipolla et al.). Jacqueline Jacobs (Netherlands Cancer Institute) and her group demonstrated that UB and SUMO play an essential role in both telomere maintenance and protection, but are also key contributors for the cellular response to dysfunctional telomeres (Yalçin et al.). Besides the physiological role of the UB and SUMO pathways in DNA replication and telomere function, this issue also covers the majority of DNA repair pathways. Thus, Coleman and Huang (New York University School of Medicine) nicely summarized how SUMOylation plays a major role in fine-tuning of the Fanconi-Anemia Pathway, the main pathway for repairing DNA interstrand crosslinks. The group of Hanspeter Naegeli (University of Zurich) highlighted the essential importance of ubiquitination, SUMOylation but also Neddylation in the regulation of nucleotide excision repair, the main mechanism that protects us from UV-light (Rüthemann et al.). Smeenk and Mailand (University of Copenhagen) gave us comprehensive overview of UB and SUMO in the repair of DNA double strand break (DSB) repair, the most cytotoxic DNA lesion. Their work clearly demonstrates how ubiquitination and SUMOylation are highly sophisticated and complex PTMs in the DDR. Harding and Greenberg (University of Pennsylvania) presented an additional perspective on DSB repair, with a special focus on nuclear architecture, chromatin dynamics and chromatin organization in DSB repair and how UB and SUMO control and connect these processes. Himmels and Sartori (University of Zurich) went even deeper in the understanding of DSB repair and described how UB and SUMO regulate DNA-end resection, the initial step in DSB repair. Interestingly, they concluded that the UB pathway in DNA-end resection is mostly linked to protein degradation processes, where SUMO acts as an intermolecular "glue" in modulating protein-protein or protein-DNA interactions required for homologous recombination rather than specifically affecting the activity of individual proteins. Dantuma and Pfeiffer (Karolinska Institute, Stockholm) discussed how the E3-UB and E3-SUMO ligases are recruited to sites of DNA damage and the importance of the spatiotemporal relationship among different DNA repair proteins and PTMs. Pellegrino and Altmeyer (University of Zurich) nicely explained how the crosstalk between ubiqutination, SUMOylation and PARylation, another PTM that also forms a chain signal (PAR), regulate genome stability. Pinto-Fernandez and Kessler (University of Oxford) demonstrated the importance of inactivation of the ubiquitin signal in the DDR in their summary of how deubiquitinating enzymes counteract DDR-related ubiquitination. Beside the essential role of UB, SUMO and PAR in the spatiotemporal recruitment of different DNA replication and repair proteins at sites of DNA damage, the group of Thorsten Hoppe (University of Cologne) discussed how protein disassembly is equally as important as protein recruitment for genome stability (Franz et al.). The disassembly of proteins from chromatin is mostly orchestrated by the ubiquitin-dependent AAA+ATPase p97/Cdc48, also known as VCP in humans, that serves as the unfoldase and segregase to remove ubiquitinated proteins (Vaz et al., 2013; Bodnar and Rapoport, 2017). Ferrari and Gentili (University of Zurich) described the involvement of the DDR in the G2/M-checkpoint and mitosis and how these two processes are regulated by PTMs. In addition to molecular mechanisms of UB and SUMO in DDR and genome stability, this issue also contains one technical article, which helps us to better understand how to quantitatively investigate UB and SUMO pathways in DDR. Heidelberger et al. (Institute of Molecular Biology - Mainz and Goethe University, Frankfurt) described mass spectrometry-based approaches for quantitative analyses of site-specific protein ubiqutination in the context of the DDR.

CONCLUDING REMARKS

By reading these outstanding articles one can easily conclude that all authors strongly emphasize the promising therapeutic potential that targeting two PTMs- ubiquitination and SUMOylation- as well as other components of the DDR, has for cancer therapy (Hoeller and Dikic, 2009; Shen et al., 2013; Bassermann et al., 2014). As editors, we share the opinion of the authors. The best examples are the recently approved PARP inhibitor Olaparib for the treatment of BRCA-deficient cancers and the proteasome inhibitor Bortezomib for treating B-cell lymphomas. Indeed, many pharmaceutical companies have been intensively working on the inhibitors that target the components of the DDR and UB system. Many of these inhibitors are currently in pre-clinical or clinical trials (Deshaies, 2014). We would be extremely happy if this special issue helps researchers to better understand the involvement of the UB and SUMO systems in the DDR. We also believe that the knowledge gathered here will help scientists and pharmaceutical

companies to better understand how to utilize the enormous potential of the UB and SUMO system in DDR for cancer therapy. Last but not least, we would like to dedicate this special issue on UB and SUMO in the DDR to Prof Stefan Jentsch, who passed away recently. As a postdoc in Alexander Varshavsky laboratory, Stefan was the first to discover the link between the UB-system and DDR (Jentsch et al., 1987). During his independent scientific career Stefan's discoveries have shaped the field of UB, SUMO, and DDR (Hoppe and Branzei, 2017).

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

We thank to John Fielden for critical reading of this editorial. KR acknowledges the Medical Research Council programme grant (MC_PC_12001/1).

REFERENCES

Bassermann, F., Eichner, R., and Pagano, M. (2014). The ubiquitin proteasome system - implications for cell cycle control and the targeted treatment of cancer. *Biochim. Biophys. Acta* 1843, 150–162. doi: 10.1016/j.bbamcr.2013.02.028

Bodnar, N. O., and Rapoport, T. A. (2017). Molecular Mechanism of substrate processing by the Cdc48 ATPase Complex. *Cell* 169, 722–735.e9. doi: 10.1016/j.cell.2017.04.020

Ciechanover, A., Finley, D., and Varshavsky, A. (1984). The ubiquitin-mediated proteolytic pathway and mechanisms of energy-dependent intracellular protein degradation. *J. Cell. Biochem.* 24, 27–53. doi: 10.1002/jcb.240240104

Ciechanover, A., Heller, H., Elias, S., Haas, A. L., and Hershko, A. (1980). ATP-dependent conjugation of reticulocyte proteins with the polypeptide required for protein degradation. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1365–1368. doi: 10.1073/pnas.77.3.1365

Deshaies, R. J. (2014). Proteotoxic crisis, the ubiquitin-proteasome system, and cancer therapy. *BMC Biol.* 12:94. doi: 10.1186/s12915-014-0094-0

Grabbe, C., Husnjak, K., and Dikic, I. (2011). The spatial and temporal organization of ubiquitin networks. *Nat. Rev. Mol. Cell Biol.* 12, 295–307. doi: 10.1038/nrm3099

Heride, C., Urbé, S., and Clague, M. J. (2014). Ubiquitin code assembly and disassembly. *Curr. Biol.* 24, R215–R220. doi: 10.1016/j.cub.2014.02.002

Hershko, A., Ciechanover, A., Heller, H., Haas, A. L., and Rose, I. A. (1980). Proposed role of ATP in protein breakdown: conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1783–1786. doi: 10.1073/pnas.77.4.1783

Hoeller, D., and Dikic, I. (2009). Targeting the ubiquitin system in cancer therapy. *Nature* 458, 438–444. doi: 10.1038/nature07960

Hoppe, T., and Branzei, D. (2017). Stefan Jentsch (1955–2016)-Maestro of the ubiquitin family. *EMBO J.* 36, 1–2. doi: 10.15252/embj.201696176

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078. doi: 10.1038/nature08467

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jeggo, P. A., Pearl, L. H., and Carr, A. M. (2016). DNA repair, genome stability and cancer: a historical perspective. *Nat. Rev. Cancer* 16, 35–42. doi: 10.1038/nrc.2015.4

Jentsch, S., McGrath, J. P., and Varshavsky, A. (1987). The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* 329, 131–134. doi: 10.1038/329131a0

Komander, D. (2009). The emerging complexity of protein ubiquitination. *Biochem. Soc. Trans.* 37(Pt 5), 937–953. doi: 10.1042/BST0370937

Kresge, N., Simoni, R. D., and Hill, R. L. (2006). The Discovery of Ubiquitin-mediated Proteolysis by Aaron Ciechanover, Avram Hershko, and Irwin Rose. *J. Biol. Chem.* 281:e32.

Lindahl, T., Modrich, P., and Sancar, A. (2016). The 2015 Nobel prize in chemistry the discovery of essential mechanisms that repair DNA Damage. *J. Assoc. Genet. Technol.* 42, 37–41.

Schwertman, P., Bekker-Jensen, S., and Mailand, N. (2016). Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat. Rev. Mol. Cell Biol.* 17, 379–394. doi: 10.1038/nrm.2016.58

Shen, M., Schmitt, S., Buac, D., and Dou, Q. P. (2013). Targeting the ubiquitin-proteasome system for cancer therapy. *Expert Opin. Ther. Targets* 17, 1091–1108. doi: 10.1517/14728222.2013.815728

Swatek, K. N., and Komander, D. (2016). Ubiquitin modifications. *Cell Res.* 26, 399–422. doi: 10.1038/cr.2016.39

Vaz, B., Halder, S., and Ramadan, K. (2013). Role of p97/VCP (Cdc48) in genome stability. *Front. Genet.* 4:60. doi: 10.3389/fgene.2013.00060

Welchman, R. L., Gordon, C., and Mayer, R. J. (2005). Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat. Rev. Mol. Cell Biol.* 6, 599–609. doi: 10.1038/nrm1700

Conflict of Interest Statement: 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.

The reviewer PB declared a past co-authorship with one of the authors ID to the handling Editor.

frontiers in Genetics

REVIEW
published: 23 May 2017
doi: 10.3389/fgene.2017.00067

Ubiquitination and SUMOylation in Telomere Maintenance and Dysfunction

*Zeliha Yalçin, Carolin Selenz and Jacqueline J. L. Jacobs**

Department of Molecular Oncology, Netherlands Cancer Institute, Amsterdam, Netherlands

Telomeres are essential nucleoprotein structures at linear chromosomes that maintain genome integrity by protecting chromosome ends from being recognized and processed as damaged DNA. In addition, they limit the cell's proliferative capacity, as progressive loss of telomeric DNA during successive rounds of cell division eventually causes a state of telomere dysfunction that prevents further cell division. When telomeres become critically short, the cell elicits a DNA damage response resulting in senescence, apoptosis or genomic instability, thereby impacting on aging and tumorigenesis. Over the past years substantial progress has been made in understanding the role of post-translational modifications in telomere-related processes, including telomere maintenance, replication and dysfunction. This review will focus on recent findings that establish an essential role for ubiquitination and SUMOylation at telomeres.

Keywords: ubiquitin, SUMO, telomere maintenance, telomere dysfunction, DNA damage, DNA repair, shelterin, telomerase

OPEN ACCESS

Edited by:
Kristijan Ramadan,
University of Oxford, United Kingdom

Reviewed by:
Anabelle Decottignies,
Université catholique de Louvain, Belgium
Howard Donninger,
University of Louisville, United States

****Correspondence:***
Jacqueline J. L. Jacobs
j.jacobs@nki.nl

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *16 December 2016*
Accepted: *10 May 2017*
Published: *23 May 2017*

Citation:
Yalçin Z, Selenz C and Jacobs JJL (2017) Ubiquitination and SUMOylation in Telomere Maintenance and Dysfunction. Front. Genet. 8:67. doi: 10.3389/fgene.2017.00067

INTRODUCTION

Genome stability is essential for cells to function properly and ensure the survival of an organism. At the ends of chromosomes this stability is maintained by telomeres. In vertebrates telomeres consist of long double-stranded stretches of TTAGGG repeats, ending in a ~50–500 base pair overhang of the G-rich 3′-strand (Palm and de Lange, 2008). The protein complex shelterin, consisting of TRF1, TRF2, TIN2, POT1, TPP1 and RAP1, binds to telomeric repeats and mediates the formation of a telomeric loop (T-loop) in which the single-stranded 3′-overhang is concealed in a D-loop (Griffith et al., 1999; Doksani et al., 2013). This is necessary to prevent DNA damage response (DDR) and repair mechanisms from recognizing the single-stranded DNA (ssDNA) overhang. Due to incomplete replication of chromosome ends, each round of DNA replication progressively shortens linear chromosomes, risking loss of essential genes or important regulatory regions. To prevent this, telomeres act as a buffer region to maintain genome integrity (Harley et al., 1990). Replication of telomeres is initiated by the polymerase alpha-primase (PP) complex, which consists of subunits that have polymerase and primase activity (Pellegrini, 2012). During lagging-strand synthesis the ultimate RNA primer is removed, but cannot be replaced with DNA, resulting in an overhang. Additionally, leading-strand synthesis creates a transient blunt end that is processed by nucleases to generate a short 3′-overhang. Therefore, incomplete replication of the lagging strand and resection of the leading strand result in 3′-overhang generation, which contributes to telomere shortening and is known as the "end-replication problem" (Chow et al., 2012; Chen and Lingner, 2013; Martinez and Blasco, 2015). Besides the end-replication problem, replication at telomeres is extra challenging because of topological barriers, such as the T-loop and the presence of G-quadruplexes. Proper telomere replication requires G-quadruplex resolution and

suppression of G-quadruplex formation by the helicases BLM, DNA2, WRN and RTEL1, and also T-loop disassembly by WRN and RTEL1 (Uringa et al., 2012; Vannier et al., 2012, 2013; Crabbe et al., 2004; Edwards et al., 2014; Martinez and Blasco, 2015).

In many stem cells and in the majority of cancer cells telomere shortening is, respectively, partially or completely, compensated by telomerase. Telomerase consists of a telomerase reverse transcriptase (TERT) catalytic subunit and an RNA template (TERC) that add *de novo* TTAGGG repeats to chromosome ends. Telomerase is recruited to telomeres via TIN2-TPP1, whereby TPP1 promotes telomerase activity and telomere extension. First, the 3′-strand is extended by TERT using TERC as the complementary template to synthesize telomeric repeats. Subsequently, in humans, the CST complex binds to this newly generated 3′-strand and recruits the PP-complex to sequentially fill-in the 5′-strand (Greider and Blackburn, 1985; Reveal et al., 1997; Huang et al., 2012; Nandakumar and Cech, 2013). Alternatively, cancer cells that do not express telomerase can counteract telomere shortening by activating the alternative lengthening of telomeres (ALT) pathway. This pathway makes use of homologous recombination (HR)-dependent exchange/synthesis of telomeric DNA. Telomeric DNA can, for example, be copied from a nearby template (the same telomere or the sister telomere), but also from a more distant template such as a telomere from another chromosome (Pickett and Reddel, 2015). In addition, specialized types of promyelocytic leukemia (PML) bodies, so-called ALT-associated PML bodies (APBs), are essential for telomere maintenance in ALT-positive cells (Yeager et al., 1999). Telomeres cluster in APBs, which in addition to telomere-binding factors and telomeric DNA also contain proteins involved in HR to perform ALT (Pickett and Reddel, 2015). HR is a DNA repair pathway that outside of telomeres is used to correctly repair a DNA break by using the sister chromatid as template.

However, when cells proliferate in the absence of telomerase or ALT, telomeres become critically short and shelterin is not able to bind to chromosome ends in sufficient amounts (Nandakumar and Cech, 2013). This leads to initiation of DDR signaling and DNA repair activities that can impair cell proliferation and harm genome stability (d'Adda di Fagagna et al., 2003; Jacobs and de Lange, 2005; Davoli and de Lange, 2011; Jacobs, 2013). Also, when replication at telomeres stalls because of topological barriers that cannot be resolved by helicases, a DDR is activated to restart replication through HR (Badie et al., 2010; Tacconi and Tarsounas, 2015; Zimmer et al., 2016). The DDR and DNA repair mechanisms at dysfunctional telomeres are tightly regulated by post-translational modifications (PTMs). In addition, telomere maintenance and protection, which function to prevent DDR initiation at telomeres, are also affected by PTMs, including ubiquitination and SUMOylation (Peuscher and Jacobs, 2012).

In the process of ubiquitination, the 76 amino acid protein ubiquitin is covalently conjugated via its C-terminus to the ε-amino group of lysine residues or to the N-terminus of a target protein. Ubiquitination is implicated in many cellular pathways in almost all eukaryotic organisms and can target proteins for proteasomal degradation or affect their activity, localization and interaction with other molecules. The attachment of ubiquitin occurs via an enzymatic cascade consisting of E1 ubiquitin-activating, E2 ubiquitin-conjugating and E3 ubiquitin-ligating enzymes (Ciechanover et al., 1982; Hershko et al., 1983; Komander and Rape, 2012). Moreover, ubiquitin itself can also be ubiquitinated at its N-terminal M1 residue and at one of its seven internal lysine residues K6, K11, K27, K29, K33, K48 and K63. Therefore, ubiquitin-chains with many different linkages can be formed, significantly increasing their signaling potential and specificity. For example, K48-linked chains usually target proteins for proteasomal degradation (Komander and Rape, 2012). Ubiquitination is reversible through the action of deubiquitinating enzymes (DUBs), of which approximately 100 are known in humans. DUBs are able to cleave off an individual ubiquitin or break the bonds within the ubiquitin-chain, allowing for removal and editing at these sites (Komander et al., 2009).

Another PTM that is very similar to ubiquitination is SUMOylation. In this process, a small ubiquitin-related modifier (SUMO) protein is conjugated to target proteins. This also occurs via an enzymatic cascade, mediated by E1, E2 and E3 SUMO enzymes, which conjugate SUMO to the substrate protein in the same manner as ubiquitin (Johnson, 2004). Additionally, deSUMOylating enzymes can reverse this process (Mukhopadhyay and Dasso, 2007). In contrast to the ubiquitin system, for which over 600 E3 ligases are known to exist in humans, only a few SUMO ligases have been identified so far. In addition, multiple SUMO isoforms exist, with SUMO1 (101 amino acids), SUMO2 (95 amino acids) and SUMO3 (103 amino acids) being the ones that have been studied best (Cubenas-Potts and Matunis, 2013). In contrast to ubiquitin-chains, SUMO-chains do not directly target proteins for proteasomal degradation, but can prime the target for ubiquitin ligase-mediated degradation. Moreover, SUMOylation can influence protein activity, localization and interactions between proteins containing SUMO-interacting motifs (SIMs) (Geiss-Friedlander and Melchior, 2007; Kerscher, 2007). In the past years evidence increased for crucial roles of ubiquitination and SUMOylation in the cellular response to telomere dysfunction that potentially leads to genomic instability. Therefore, the aim of this review is to provide an overview of new findings obtained about ubiquitination and SUMOylation involved in telomere maintenance, replication and dysfunction.

TELOMERE MAINTENANCE: SHELTERIN IN CONTROL

Aberrant telomere function can have severe cellular consequences by leading to genomic instability, cellular senescence and early apoptosis. Therefore, tightly regulated telomere maintenance is required to ensure protection of chromosome ends. The most significant complex involved in telomere maintenance and protection is shelterin (**Figure 1**). Shelterin governs telomere maintenance and protection in essentially three main ways: (1) by preventing activation of the DDR and DNA repair mechanisms at telomeres, (2) by facilitating telomere replication and (3) by regulating

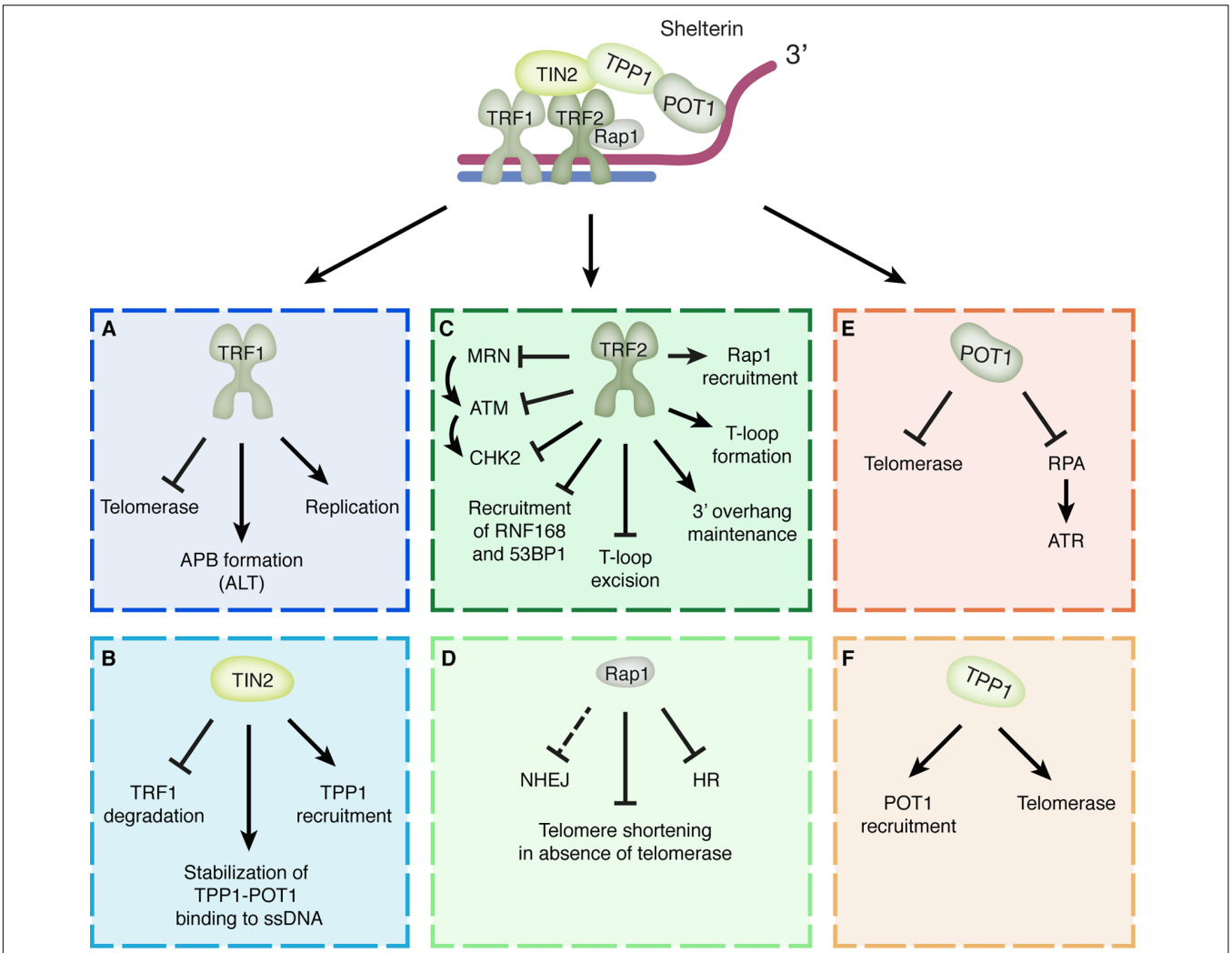

FIGURE 1 | Shelterin components and their functions in telomere protection. (A) TRF1 facilitates telomere replication, restricts telomerase access and promotes the formation of APBs associated with ALT. **(B)** TIN2 recruits TPP1 to telomeres, stabilizes TPP1-POT1 binding to the ssDNA and prevents proteasomal degradation of TRF1. **(C)** TRF2 is involved in T-loop formation and stabilization, prevents T-loop excision, promotes maintenance of the 3′-overhang, recruits RAP1 to telomeres and prevents the recruitment of RNF168 and 53BP1. Furthermore, TRF2 interferes with ATM signaling by (1) preventing binding of the MRN complex and thereby activation of ATM, (2) binding ATM and interfering with its activation directly and (3) interacting with CHK2 and interfering with its phosphorylation. **(D)** RAP1 inhibits HR at telomeres and prevents telomere shortening in the absence of telomerase. In addition, RAP1 appears able to provide a back-up mechanism for inhibition of NHEJ when TRF2 function is impaired. **(E)** POT1 inhibits RPA binding and access of telomerase to the telomere single-stranded 3′-overhang. **(F)** TPP1 recruits POT1 to telomeres and stimulates the recruitment and activity of telomerase.

telomerase-mediated telomere elongation. The shelterin components TRF1 and TRF2 directly interact with telomeric DNA and are structurally very similar. Although, both proteins have a TRF homology (TRFH) domain and a SANT/Myb DNA-binding domain, TRF1 and TRF2 do not physically interact and have separate functions (Stewart et al., 2012; Doksani and de Lange, 2014). TRF1 has been shown to be required for proper telomere replication, for example by recruiting the necessary helicases, such as BLM, and for restricting telomerase access to the telomeres (Sfeir et al., 2009). In contrast, TRF2 is involved in T-loop formation and stabilization, prevents T-loop excision and promotes maintenance of the 3′-overhang by recruiting the Apollo nuclease. It is also essential for inhibition of the ATM kinase to repress DNA damage signaling and inhibit classical non-homologous end-joining (c-NHEJ), an error-prone repair pathway that promotes ligation of broken DNA ends (Karlseder et al., 2004; Wang et al., 2004; Denchi and de Lange, 2007; Wu et al., 2010; Doksani et al., 2013; Okamoto et al., 2013). TRF2 interacts with the shelterin component RAP1 and recruits it to the telomeres. Unlike for TRF2, the contribution of RAP1 to protection of mammalian telomeres against NHEJ is less evident and only noticeable in experimental conditions where RAP1 is artificially recruited to TRF2-depleted telomeres or TRF2 function is partially compromised (Sarthy et al., 2009; Kabir et al., 2014; Benarroch-Popivker et al., 2016). A more obvious role for RAP1 appears

to be in protecting telomeres against HR. RAP1 deletion in a *Ku70*$^{-/-}$ background resulted in increased telomere-sister chromatid exchanges, indicating that RAP1 represses HR (Sfeir et al., 2010). Furthermore, telomeres devoid of RAP1 and the N-terminal basic domain of TRF2 are rapidly resected by HR factors, resulting in telomere loss and telomere-free fusions (Rai et al., 2016). Finally, mice lacking both RAP1 and telomerase show increased telomere shortening and progressively decreased survival compared to single telomerase knockout mice (Martinez et al., 2016). Thus RAP1 seems to aid both in protecting telomeres from DNA repair activities and in maintaining telomeres in absence of telomerase. Much of the mechanistic basis for these roles of RAP1 still remains to be discovered.

TRF1 and TRF2 are bridged by TIN2, which acts as a linker protein. This ensures the integrity of the whole shelterin complex by connecting not only the double-strand DNA-binding TRF1 and TRF2, but also by linking TRF1 and TRF2 to the ssDNA-binding TPP1-POT1 heterodimer. In addition, TIN2 also recruits TPP1 to the telomere and stabilizes TPP1-POT1 binding to the ssDNA (O'Connor et al., 2006; Palm and de Lange, 2008). POT1 is the only shelterin component that directly interacts with the single-stranded 3′-overhang through its two OB-fold domains (Zhong et al., 2012). It is recruited to the telomeres by interacting with TPP1 (Loayza and De Lange, 2003; Liu et al., 2004). Binding of the POT1-TPP1 complex to telomeres contributes to telomere maintenance by preventing other factors, such as replication protein A (RPA), from binding to the 3′-overhang and promoting DNA damage signaling. In addition, both POT1 and TPP1 are involved in the regulation of telomerase activity, showing opposing effects on telomerase (Stewart et al., 2012). Whereas POT1 negatively regulates telomerase binding by making the 3′-overhang inaccessible, TPP1 has been observed to promote recruitment of telomerase and stimulate its activity (Stewart et al., 2012). Although it is not yet exactly known how these opposing functions of POT1 and TPP1 are coordinated, recent work suggests that POT1 inhibits telomerase recruitment by suppressing phosphorylation of TPP1 at Ser255 by the M-phase kinase NEK6 (Hirai et al., 2016). According to the proposed model, POT1 might dissociate from the telomeres during replication, after the T-loop is dismantled, thereby relieving the inhibitory effect of POT1 on TPP1. This could then allow for phosphorylation of TPP1 and thereby promote recruitment of telomerase to telomeres to maintain the telomeric sequence (Hirai et al., 2016).

Regulation of TRF1 by Ubiquitin

In recent years, shelterin components have been shown to be regulated by PTMs. TRF1 levels are regulated by ubiquitin-mediated degradation that is facilitated by three E3 ligases: RLIM (RING H2 zinc finger or RNF12) and the F-box proteins FBX4 and β-TRCP1 (Lee et al., 2006; Her and Chung, 2009; Wang C. et al., 2013) (**Figure 2**). RLIM binds to a region between the dimerization and Myb domain of TRF1 and targets TRF1 for proteasomal degradation (Her and Chung, 2009). Similarly, FBX4 binds to the N-terminal region of the TRFH dimerization domain of free TRF1 (unbound to telomeric DNA) and also targets TRF1 for proteasomal degradation (Lee et al., 2006). When either RLIM or FBX4 is depleted TRF1 levels are stabilized, resulting in impaired cell growth and a decrease in telomere length, as TRF1 binding to telomeres is inhibitory toward telomerase. Accordingly, upon RLIM or FBX4 overexpression, levels of TRF1 decline, indicating a negative regulatory role for RLIM and FBX4 on TRF1 (Her and Chung, 2009). Moreover, a recent study has identified a novel TRF1-interacting protein that prevents FBX4 binding to TRF1. The splicing factor U2AF65 acts as a positive regulator of TRF1 by preventing FBX4-mediated ubiquitination and subsequent degradation of TRF1 (Kim and Chung, 2014). It has been proposed that U2AF65 only interacts with telomere unbound TRF1, as U2AF65 interacts with the Myb domain of TRF1 that is used by TRF1 to bind telomeric DNA. The Myb domain of TRF1 would therefore be inaccessible to U2AF65 when TRF1 is bound to DNA. Although U2AF65 interacts with a different domain of TRF1 than FBX4, which interacts with the TRFH domain of TRF1, TRF1 cannot interact with both proteins simultaneously (Kim and Chung, 2014). In addition, the shelterin component TIN2 also interferes with FBX4-mediated TRF1 turnover. TIN2 interacts with the TRFH dimerization domain of TRF1 (Ye and de Lange, 2004), preventing FBX4 association and thereby TRF1 ubiquitination and subsequent degradation (Zeng et al., 2010). TIN2 itself is also affected by ubiquitination. Its turnover is regulated by the E3 ligase SIAH2, which interacts with TIN2 to facilitate its proteasomal degradation (Bhanot and Smith, 2012). Finally, the F-box protein β-TRCP1 has also been shown to interact with TRF1 and promote its degradation. Similar to RLIM and FBX4, β-TRCP1 overexpression results in a reduced half-life of TRF1, while β-TRCP1 depletion leads to stabilization of TRF1 (Wang C. et al., 2013). Interestingly, β-TRCP1 overexpression also resulted in an increase in the percentage of APBs, which is surprising as TRF1 is known to be required for APB formation. Although an explanation could be that perhaps β-TRCP1 degrades not all but only a specific pool of TRF1, further studies are necessary to determine how TRF1 degradation by β-TRCP1 can be correlated with a function for β-TRCP1 in APB formation.

In contrast to the E3 ligases RLIM, FBX4 and β-TRCP1 promoting TRF1 turnover, the GCN5 and USP22 components of the chromatin modifying complex SAGA have been shown to oppose TRF1 ubiquitination (Atanassov et al., 2009). Depletion of either the histone acetyltransferase GCN5 or the DUB USP22 results in a decrease in TRF1 levels, which can be prevented by inhibition of the proteasome. GCN5 was found to be required for USP22 to properly associate with the SAGA complex and to be able to deubiquitinate TRF1 and prevent its turnover (Atanassov et al., 2009). In conclusion, the above-discussed studies indicate that TRF1 levels in cells are tightly regulated by numerous different proteins and on multiple levels.

Ubiquitination- and SUMOylation-Mediated Regulation of TPP1, TRF2 and RAP1

Another shelterin subunit that is subjected to ubiquitin-mediated proteolysis is TPP1, which is evidenced by stabilization of TPP1

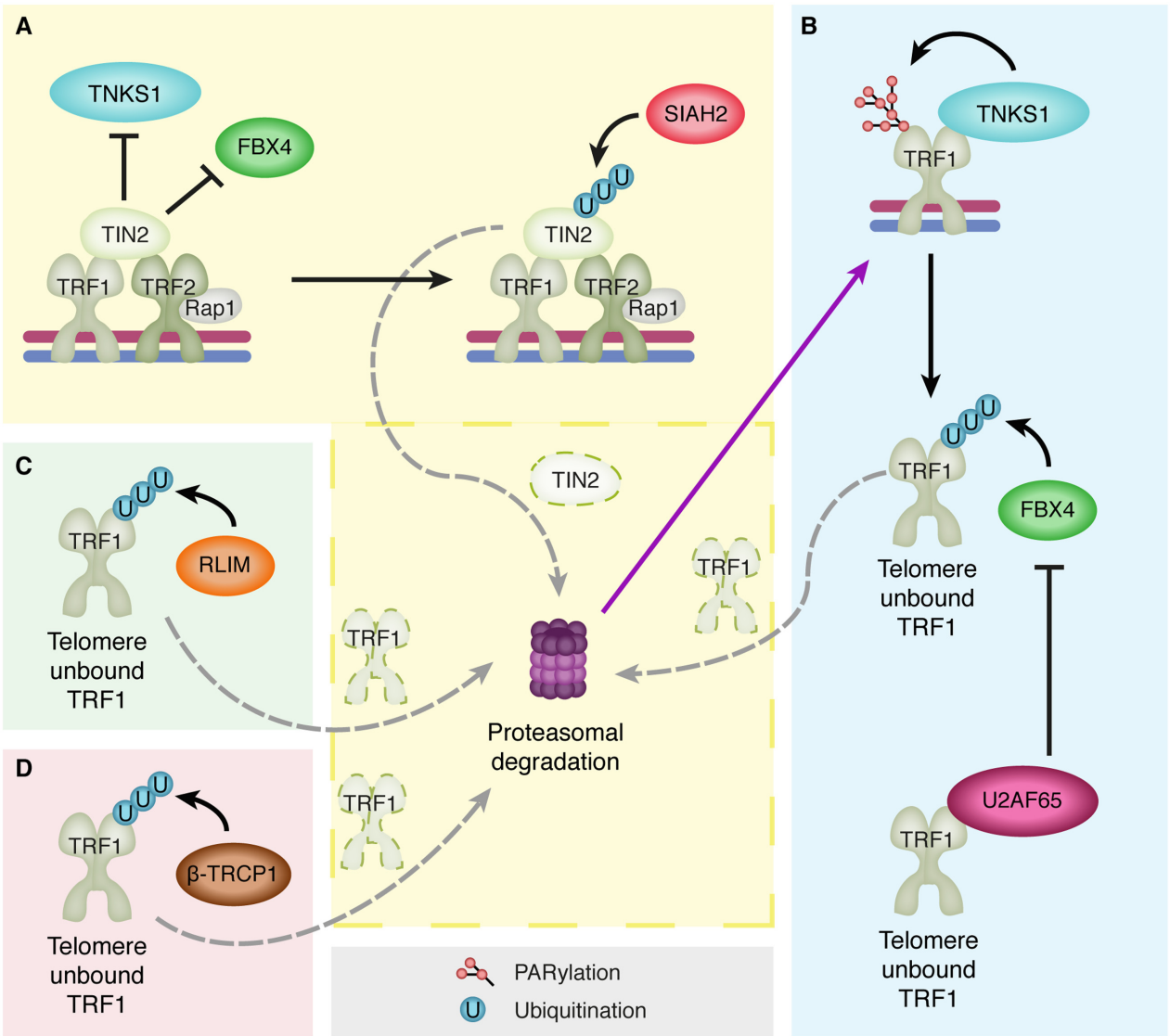

FIGURE 2 | The multilevel regulation of TRF1 degradation. (A) TIN2 protects TRF1 from proteasomal degradation by preventing binding of Tankyrase 1 (TNKS1) and the E3 ligase FBX4 to TRF1. However, TIN2 itself is also targeted for proteasomal degradation by ubiquitination through the E3 ligase SIAH2, which releases the inhibition on TNKS1 and FBX4. **(B)** Subsequently, TNKS1 can PARylate TRF1 resulting in its dissociation from the telomeric DNA. This allows for FBX4-mediated TRF1 ubiquitination and proteasomal degradation. However, U2AF65 also binds telomere-unbound TRF1, which inhibits FBX4 binding and subsequent TRF1 degradation. **(C,D)** The E3 ligases RLIM **(C)** and β-TRCP1 **(D)** also ubiquitinate telomere-unbound TRF1 and promote its proteolysis.

protein levels upon inhibition of the proteasome. Although the E3 ubiquitin ligases targeting TPP1 are still unknown, the DUB USP7 has been shown to interact with human TPP1 and to remove ubiquitin chains from its surface. While USP7 depletion did not affect proteasome-regulated TPP1 levels, USP7 might interact in a redundant manner with other DUBs to stabilize TPP1 (Zemp and Lingner, 2014). In mice, TPP1 ubiquitination by the E3 ligase RNF8 is also required for its stabilization at telomeres (Rai et al., 2011). However, in humans such a regulatory role of ubiquitination on TPP1, beyond regulation of its turnover, has not been observed. Changes in ubiquitination of TPP1 in humans have not resulted in aberrant TPP1 function nor have shown effects on TPP1 interaction with other proteins, such as TIN2, POT1 and telomerase (Zemp and Lingner, 2014). While this could be related to the use of overexpressed tagged TPP1 in human cells, it might also potentially indicate species differences in the extent of regulatory roles of ubiquitination on TPP1. Nevertheless, additional roles of human TPP1 ubiquitination may still await discovery.

Furthermore, the shelterin subunit TRF2 has also been shown to be ubiquitinated. TRF2 turnover is regulated by the E3 ligase SIAH1 as part of a positive feedback loop involving TRF2, ATM and p53 (Fujita et al., 2010). When telomere shortening causes loss of TRF2-mediated telomere protection, the ATM kinase is

activated, which induces p53 activity and results in replicative senescence. Subsequently, p53 induces transcription of SIAH1, which targets TRF2 for proteasomal degradation. This results in increased p53 activation, further decreasing TRF2 levels through SIAH1-mediated ubiquitination of TRF2 (Fujita et al., 2010). In addition, a crosstalk between ubiquitination and SUMOylation has recently been observed to contribute to regulation of TRF2 (Her et al., 2015). The E3 SUMO ligase PIAS1 was identified as a novel TRF2-interacting protein and shown to SUMOylate TRF2. SUMOylated TRF2 is subsequently recognized by the SUMO-targeted ubiquitin ligase (STUbL) RNF4 through its SIM. This results in ubiquitination of TRF2 and subsequent proteasomal degradation (Her et al., 2015). This probably affects only a fraction of the total pool of TRF2 in the cell, as TRF2 is essential for chromosome end protection and extensive turnover of TRF2 would result in telomere uncapping.

Finally, in *Saccharomyces cerevisiae* (budding yeast) Rap1 has been shown to be SUMOylated and subsequently targeted for proteasomal degradation by the STUbL Uls1 (Lescasse et al., 2013). Loss of Uls1 was shown to result in accumulation of poly-SUMOylated Rap1 and telomere fusions. These fusions could be prevented by introduction of *rap1* alleles lacking SUMOylation sites. This indicates that accumulation of poly-SUMOylated Rap1 promotes telomere fusion and suggests that poly-SUMOylated Rap1 is non-functional in telomere protection. The proposed model suggests that Uls1 promotes ubiquitination and subsequent degradation of poly-SUMOylated Rap1, thereby allowing for recruitment of non-SUMOylated Rap1 that is able to protect chromosome ends from fusing through NHEJ (Lescasse et al., 2013). To what extent these results can be translated to mammalian systems remains unclear, as in budding yeast Rap1 interacts directly with telomeres and protects against NHEJ, while in mammals no direct interaction between RAP1 and telomeres is detectable but RAP1 is recruited by TRF2 and seems to mainly protect against HR.

Altogether, it has become clear that telomere maintenance does not only depend on the binding-capability of shelterin itself to the telomeric DNA, but also on its regulation by PTMs. Both features are important in facilitating protection of genome stability by telomeres. Although ubiquitination has been shown to contribute to telomere maintenance in multiple ways, emerging data show that SUMOylation also plays an important role in this process. Further studies are likely to provide additional insight in how these modifications affect and regulate telomere function. Additionally, it would be beneficial to verify the extent to which findings from yeast studies are conserved in mammalian systems.

TELOMERE ELONGATION

The majority of cancer cells (± 90%), as well as many stem cells, express telomerase to elongate telomeres (Lazzerini-Denchi and Sfeir, 2016). Recently, it has been shown that in human embryonic stem cells (hESCs) telomere length is stabilized by a tight balance between telomere elongation through telomerase and telomere trimming by XRCC3 and NBS1 (Rivera et al., 2017). In tumors, telomere length is less stable, as telomere length between cancer cells is variable and telomerase extends most of the telomeres during every replication cycle (Martinez and Blasco, 2015). Although the majority of cancer cells maintains telomere length by activation of telomerase, a smaller number uses the ALT mechanism. During ALT, a HR-dependent mechanism copies telomeric DNA from a nearby template, resulting in telomere lengthening but also telomeres loss, which account for the heterogeneous telomere length typically observed in ALT cells (Pickett and Reddel, 2015).

Telomerase and ALT have been shown to be regulated by various PTMs, including ubiquitination and SUMOylation. Below we will discuss the roles of these two PTMs in regulation of telomerase activity and stability, and in ALT.

Telomerase in the Spotlight

Especially the TERT subunit of telomerase has been shown to be modified by multiple ubiquitin E3 ligases, most of them regulating its proteasomal degradation (**Figure 3**). The first E3 ubiquitin ligase that was identified to interact with and ubiquitinate human (h) TERT is MKRN1 (Makorin-1 or RNF61) (Kim et al., 2005). Overexpression of this E3 ligase was shown to decrease telomerase activity and telomere length through ubiquitination and subsequent degradation of hTERT. In addition, MKRN1 has also been specifically implicated in modulation of telomerase activity during cell differentiation (Salvatico et al., 2010). The cancer cell line HL-60 normally expresses the *MKRN1* gene at very low levels and MKRN1 protein levels cannot be detected. However, upon retinoic acid induced differentiation of HL-60 cells MKRN1 expression significantly increased, coinciding with a strong down regulation of telomerase activity. As hTERT has a long half-life, MKRN1-mediated degradation of hTERT could provide efficient degradation of hTERT when telomerase activity is no longer needed (Salvatico et al., 2010).

A second E3 ubiquitin ligase that directly acts on hTERT is MDM2 (also known as HDM2). MDM2 and hTERT can physically interact through multiple domains on both proteins, whereby hTERT is polyubiquitinated and degraded by the proteasome. In line with this, depletion of MDM2 resulted in increased hTERT protein levels and increased telomerase activity (Oh et al., 2010). In addition, the E2 ubiquitin-conjugating enzyme UBE2D3 (or UBCH5C) has also been shown to regulate hTERT ubiquitination. Similar to MDM2 depletion, UBE2D3 depletion results in hTERT accumulation and increased telomerase activity (Wang W. et al., 2013; Gao et al., 2016; Yang et al., 2016). As it is known that UBE2D3 and MDM2 function together in the ubiquitination of p53 (Saville et al., 2004) and both were shown to regulate hTERT ubiquitination, it is tempting to speculate that this E2–E3 couple might also act together in the ubiquitination of hTERT.

A third E3 ligase that interacts with hTERT is the co-chaperone protein CHIP (C-terminus of Hsc70-interacting protein). In contrast to the ligases mentioned above, CHIP binds to the premature form of hTERT in the cytoplasm to inhibit its transport into the nucleus and subsequent integration into the telomerase complex. This inhibitory function of CHIP on the

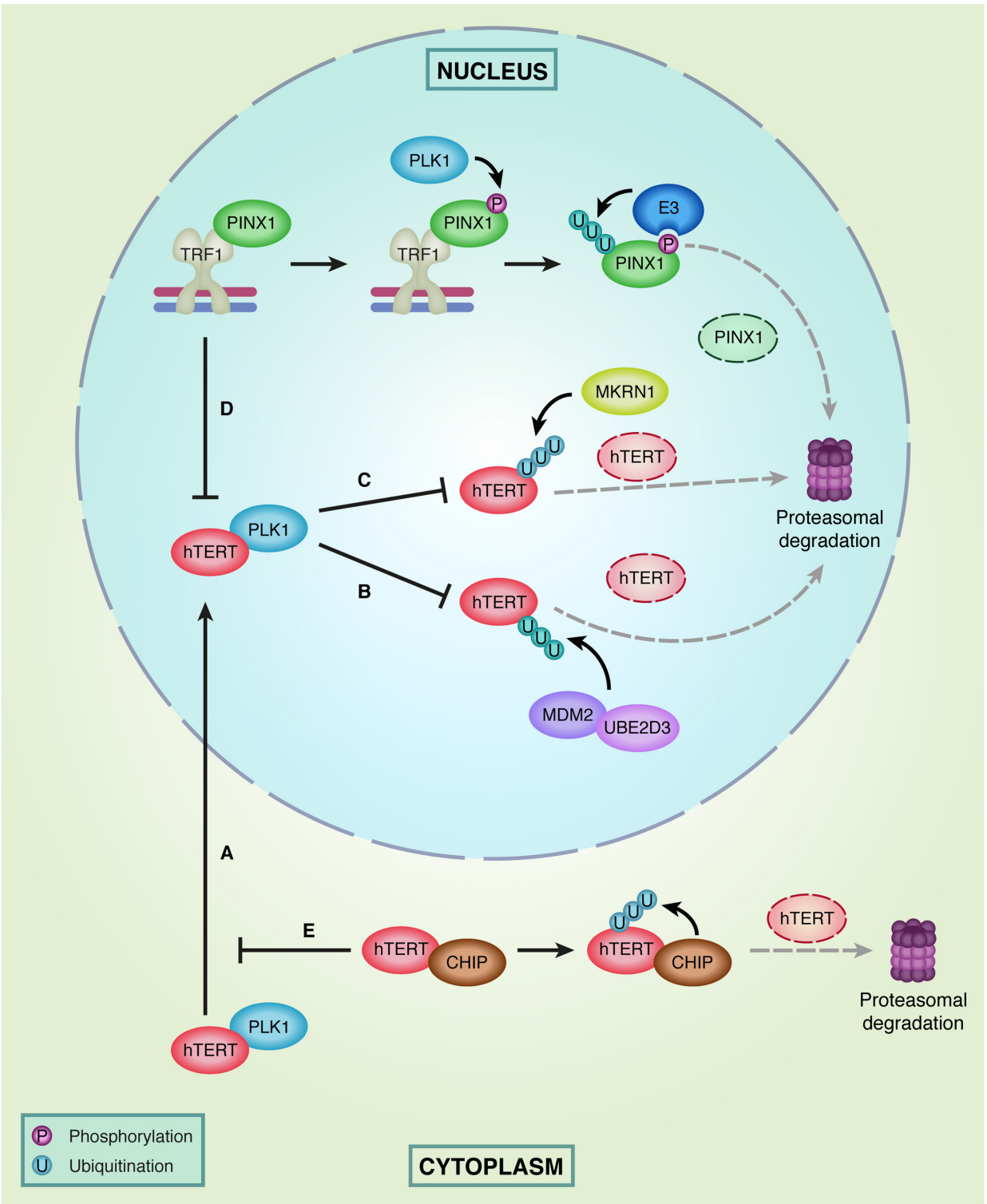

FIGURE 3 | Regulation of hTERT levels in the nucleus and cytoplasm. (A) PLK1 facilitates hTERT localization to the nucleus. **(B)** In the nucleus PLK1 interferes with hTERT ubiquitination by MDM2 and UBE2D3 and subsequent degradation of hTERT by the proteasome. **(C)** In addition, PLK1 prevents hTERT proteasomal degradation by interfering with ubiquitination of hTERT by MKRN1. **(D)** hTERT binding to telomeres is prevented by TRF1, which is stabilized at telomeres by PINX1. However, PINX1 is a target of phosphorylation by PLK1, resulting in recruitment of E3 ligases that recognize phosphorylated PINX1. This promotes ubiquitination and proteasomal degradation of PINX1. **(E)** In the cytoplasm, the E3 ligase CHIP inhibits PLK1-facilitated transport of hTERT to the nucleus by interacting with hTERT. Subsequently, CHIP can ubiquitinate hTERT and target it for proteasomal degradation.

nuclear import of hTERT has been shown to occur independently from the ubiquitin ligase activity of CHIP, which requires its U-box domain. However, for ubiquitination and subsequent degradation of hTERT, the U-box and E3 ligase activity of CHIP are necessary (Lee et al., 2010). Furthermore, the interaction between CHIP and hTERT was found to peak in G2/M phase and decrease during S-phase, suggesting that degradation of hTERT by CHIP is cell cycle regulated and exerted when telomerase does not act on telomeres.

In addition to MKRN1, MDM2 and CHIP, Polo-like kinase 1 (PLK1) was recently identified to directly interact with hTERT, but with positive effects on hTERT levels (Huang et al., 2015). PLK1 overexpression was shown to prevent ubiquitination and proteasomal degradation of hTERT and to result in increased total protein levels of hTERT, including increased levels of nuclear hTERT and chromatin-bound hTERT. Therefore, it was suggested that PLK1 facilitates localization of hTERT to the nucleus and might interfere with the function or binding of E3 ligases, such as MKRN1 and MDM2, to hTERT. This then prevents hTERT's proteasomal degradation and stabilizes telomerase activity (Huang et al., 2015). Interestingly, elevated PLK1 expression has been observed in several tumors. This might contribute to increased hTERT levels and excessive telomerase activity in cancer cells, thereby increasing the proliferative capacity of these cells (Holtrich et al., 1994).

Additionally, PLK1 has also been implicated in mediating the turnover of PINX1 (Wang et al., 2010). PINX1 is known as an hTERT inhibitor that directly interacts with TRF1 (Zhou and Lu, 2001). PINX1 binding to TRF1 has been shown to promote TRF1 association with telomeric DNA, thereby contributing to telomerase inhibition by decreasing accessibility of telomeric DNA to telomerase (Yoo et al., 2009). PLK1 can phosphorylate PINX1 and induce its ubiquitin-mediated proteasomal degradation. It has been proposed that phosphorylation of PINX1 by PLK1 stimulates the activity of E3 ubiquitin ligases, which subsequently ubiquitinate PINX1 and target it for degradation (Wang et al., 2010). When PINX1 is degraded, other factors can gain access to TRF1 and might affect the binding of TRF1 to telomeres, thereby increasing telomerase recruitment. One of these factors is the poly (ADP-ribose) polymerase Tankyrase 1 (TNKS1). TNKS1 has been shown to interact with TRF1 and to PARylate it, resulting in TRF1 dissociation from telomeres and subsequent degradation of TRF1 by the E3 ligase FBX4 (Smith et al., 1998; Ye and de Lange, 2004; Lee et al., 2006) (**Figure 2**). Consequently, telomerase gains access to telomeres, leading to telomere elongation. Interestingly, PLK1 can also phosphorylate TNKS1 directly, which stabilizes TNKS1 protein levels and thereby increases TRF1 degradation and telomerase activity (Ha et al., 2012). The shelterin protein TIN2 adds a final layer of complexity to this, as TIN2 can interact with TNKS1 and TRF1, thereby preventing TRF1 inactivation by TNKS1, resulting in TRF1 accumulation at telomeres and inhibition of telomerase loading (Ye and de Lange, 2004).

While ubiquitination evidently plays an important role in the regulation of telomerase activity in mammals, the contribution of SUMOylation to regulation of mammalian telomerase is not yet evident, despite the discovery of SUMOylation-dependent mechanisms of telomerase control in yeast. In *Schizosaccharomyces pombe* (fission yeast) SUMOylation of Tpz1, the homolog of TPP1, has been connected to negative regulation of telomere elongation (Hang et al., 2011; Garg et al., 2014; Miyagawa et al., 2014). SUMOylation of Tpz1 decreases telomerase binding to telomeres. This occurs through recruitment of the Stn1–Ten1 subunits of the CST complex, known to be involved in telomere length regulation and chromosome end capping by preventing telomerase accumulation at telomeres (Price et al., 2010). In contrast, Tpz1 has also been shown to interact with Ccq1-Est1 and thereby promote the recruitment and activity of telomerase, indicating a double role for Tpz1 in maintaining telomere length homeostasis (Miyoshi et al., 2008; Miyagawa et al., 2014). In budding yeast, the Stn1 protein has also been observed as a negative regulator of telomerase activity and telomere elongation. Stn1 interacts with the POT1 homolog Cdc13 and this interaction is increased when Cdc13 is SUMOylated, strengthening the inhibitory effect of Stn1 on telomerase. This increased interaction between Stn1 and Cdc13 has been associated with reduced telomerase levels, supporting a negative regulatory effect of SUMOylation on telomerase activity (Hang et al., 2011).

In conclusion, multiple different proteins and post-translational modifications, including ubiquitination, phosphorylation and PARylation, directly or indirectly affect the activity of telomerase. Whether there is also a role for SUMOylation in the control of telomerase regulation in mammalian cells still needs to be uncovered.

Alternative Lengthening of Telomeres: Surviving without Telomerase

Ubiquitination and SUMOylation are also important for promoting telomere elongation by ALT in cells without telomerase activity. The shelterin protein TRF1 has been shown to be an important factor for ALT by promoting APB formation and localization of telomeres to PML bodies. To be able to facilitate this, TRF1 needs to be SUMOylated by MMS21, the E3 SUMO ligase component of the SMC5/6 complex (Potts and Yu, 2007). If MMS21-mediated SUMOylation is prevented, TRF1 recruitment to PML bodies is inhibited and APB formation is impaired. In addition to TRF1, also TRF2, TIN2 and RAP1 are SUMOylated by MMS21. When MMS21 is depleted, SUMOylation of all these shelterin subunits is inhibited (Potts and Yu, 2007). SUMOylation of multiple components of a complex could affect its stability; therefore, MMS21-mediated SUMOylation of shelterin components was suggested to induce disassembly of the shelterin complex within APBs. This could result in telomere deprotection, thereby potentially facilitating telomere recombination in ALT cells. As mentioned before, TRF1 can also be ubiquitinated by the E3 ligase β-TRCP1, promoting degradation of TRF1. However, β-TRCP1 has also been found to be essential for APB formation, as inhibition of β-TRCP1 decreased the percentage of APBs (Wang C. et al., 2013). This seems contradictory for a negative regulator of TRF1, but it has been suggested that β-TRCP1 only degrades telomere-unbound TRF1. If SUMOylation of the shelterin subunits indeed results in

disassembly of the shelterin complex, β-TRCP1 might assist in the degradation of telomere-unbound TRF1.

Another factor involved in ALT is the scaffold protein SLX4, which recognizes DNA lesions and facilitates DNA repair by interacting with multiple endonucleases. Its structure includes both ubiquitin-binding zinc fingers (UBZs), involved in DNA interstrand crosslink repair, as well as SIMs that are important for localization of SLX4 to ALT telomeres (Ouyang et al., 2015). In addition, SLX4 contributes to telomere maintenance and protection by directly interacting with TRF2 via a motif resembling the TRF2-binding motif (TBM) present in other proteins known to interact with TRF2 (Wilson et al., 2013). The SUMO binding capacity of SLX4 was shown to enhance its interaction with several DNA damage sensors and telomere-binding proteins, including RPA, the MRE11-RAD50-NBS1 (MRN) complex and TRF2 (Ouyang et al., 2015). Therefore, increased SUMOylation of proteins at ALT telomeres may assist in the recruitment of SLX4 and other factors involved in ALT.

In budding yeast, telomere-bound proteins become increasingly SUMOylated when cells without telomerase activity obtain critically short telomeres that induce crisis. These SUMOylated proteins are recognized by the STUbL Slx5–Slx8, which mediates the relocalization of critically short telomeres to nuclear pore complexes (NPCs), where recombination events similar to the mammalian ALT pathway occur (Churikov et al., 2016). As Slx5–Slx8 can interact with SUMOylated telomere-bound proteins, as well as with the Nup84 complex of NPCs, it is believed to tether telomeres to the NPCs, thereby enabling recombination (Nagai et al., 2008; Churikov et al., 2016). Interestingly RNF4, the human homolog of Slx5–Slx8, has been shown to localize to PML bodies in human cells (Lallemand-Breitenbach et al., 2008; Tatham et al., 2008; Weisshaar et al., 2008). Although it is unknown whether RNF4 activity is necessary for ALT, it would be interesting to investigate if RNF4 has a similar function in humans as Slx5–Slx8 has in budding yeast. As discussed above, the MMS21 component of the SMC5/6 complex is necessary for SUMOylation at telomeres, which is needed for the recruitment of telomeres to PML bodies. RNF4 could potentially be involved in this by recognizing the MMS21-SUMOylated TRF1 at telomeres and promoting telomere recruitment to APBs.

Altogether, the above-discussed data show that ubiquitination and SUMOylation are crucial in the regulation of telomere elongation by telomerase and ALT. Multiple E3 ligases were shown to control the ubiquitination of hTERT to tightly regulate its levels and activity. In addition, various proteins and PTMs regulate the inhibitory function of TRF1 on telomerase. The complexity of telomerase regulation at telomeres suggests that this regulation is strictly controlled to prevent unscheduled access of telomerase to telomeres. However, further studies are necessary to understand how these mechanisms are coordinated and whether they are interrelated. In addition, ubiquitination and SUMOylation of shelterin components also seems to be crucial in promoting ALT, indicating that post-translational modification of shelterin components contributes to multiple processes involved in telomere maintenance and elongation.

TELOMERE DEPROTECTION

Successive rounds of cell division in the absence of telomerase or ALT ultimately lead to critically short telomeres and deprotected chromosome ends. These deprotected ends are recognized as damaged DNA by the DDR machinery. This results in activation of the p53 and Rb pathways and entry into senescence to limit telomere fusions and prevent subsequent genomic instability. However, when these pathways are impaired, senescence is bypassed and cells continue to divide and further lose telomere repeats until they reach crisis, a state of massive genome instability and cell death (d'Adda di Fagagna et al., 2003; Jacobs and de Lange, 2005; Palm and de Lange, 2008). At this stage, DNA repair pathways are activated and telomeres fuse in G1-phase either through Artemis and DNA ligase IV mediated c-NHEJ, or through alternative NHEJ (alt-NHEJ), a pathway mediated by PARP1 and DNA Ligase III. During subsequent cell divisions breakage-fusion-bridge cycles occur, inducing genomic instability and cell death. Nevertheless, a small portion of cells might escape crisis by reactivating telomerase or inducing ALT to maintain their telomeres, which results in expansion of cells with aberrant genomes, thereby promoting tumorigenesis (Arnoult and Karlseder, 2015; Lazzerini-Denchi and Sfeir, 2016).

Signaling through the RNF8–RNF168 Pathway at Uncapped Telomeres

The DDR activated by deprotected telomeres in many ways resembles the DDR at general DNA double-strand breaks (DSBs). The DDR at telomeres starts with the recognition of uncapped telomeres by the MRN complex and activation of the ATM kinase, resulting in phosphorylation of histone H2AX at serine 139, generating γH2AX. This serves as a binding platform for MDC1, which initiates a positive feedback loop by promoting further accumulation of MRN and ATM (Peuscher and Jacobs, 2012). This results in spreading of γH2AX along the chromatin and amplification of DDR signaling and repair factor recruitment (Cimprich and Cortez, 2008; Brown and Jackson, 2015). At DSBs MDC1 is known to recruit RNF8, which interacts with phosphorylated MDC1 via its FHA domain (Kolas et al., 2007; Mailand et al., 2007). Depletion of either MDC1 or RNF8 causes a similar defect in the accumulation of 53BP1 at dysfunctional telomeres and reduces telomere fusions upon TRF2 inhibition (Dimitrova and de Lange, 2006; Peuscher and Jacobs, 2011). Furthermore, RNF8 requires its FHA domain to accumulate and promote NHEJ at uncapped telomeres, suggesting that RNF8 also recognizes phosphorylated MDC1 in this setting. Together this indicates that MDC1 and RNF8 function in the same pathway at telomeres and in a way that is identical to the DDR at genome-wide DSBs (Peuscher and Jacobs, 2011).

Upon RNF8 recruitment to uncapped telomeres, the RNF8/RNF168 signaling cascade is activated, promoting ubiquitination of histone H2A and subsequent recruitment of 53BP1. In addition, the recruitment of 53BP1 is dependent on the recognition of H4K20me2 by the Tudor domains of 53BP1

(Dimitrova et al., 2008). 53BP1 recruitment to telomeres results in accrual of RIF1 and MAD2L2 to promote NHEJ. This has been shown to block 5′ end-resection and HR through inhibition of BRCA1 recruitment to uncapped telomeres (Dimitrova et al., 2008; Chapman et al., 2013; Zimmermann et al., 2013; Boersma et al., 2015). The same mechanism has also been described at DNA DSBs and in immunoglobulin class-switch recombination (CSR) (Manis et al., 2004; Bothmer et al., 2010; Di Virgilio et al., 2013; Escribano-Diaz et al., 2013; Feng et al., 2013; Boersma et al., 2015; Xu et al., 2015). In addition, 53BP1 also recruits PTIP, which was also reported to contribute to fusion of dysfunctional telomeres. Furthermore, in BRCA1 deficient cells PTIP was shown to inhibit DSB resection and thereby promote genomic instability (Callen et al., 2013).

Depletion of RNF8 in cells with inactivated TRF2 leads to decreased H2A ubiquitination and 53BP1 recruitment at telomeres and a reduction in telomere fusions (Peuscher and Jacobs, 2011). In addition, inhibition of RNF168 recruitment to telomeres by the iDDR domain of TRF2 (part of the hinge domain) also results in a decrease in 53BP1 accumulation at dysfunctional telomeres (Okamoto et al., 2013). It has been shown that the iDDR domain of TRF2 prevents RNF168 recruitment to telomeres by accrual of the E3 ubiquitin ligase UBR5 and the MRN complex, resulting in inhibition of the signaling cascade downstream of ATM and protecting against telomere fusions through inhibition of NHEJ. UBR5 has been shown to function together with the E3 ligase TRIP12 at DSBs to control the levels and recruitment of RNF168 by targeting it for proteasomal degradation (Gudjonsson et al., 2012). The interaction of TRF2 with UBR5 could therefore inhibit RNF168 recruitment to telomeres. Interestingly, the MRN complex was shown to recruit the DUB BRCC3, which is part of the BRCA1-A complex and has been suggested to counteract the action of RNF8-UBC13 at DSBs (Shao et al., 2009). In this way, BRCC3 could inhibit recruitment of RNF168 to telomeres. Although BRCA1 is usually implicated in facilitating end-resection and HR, the BRCA1-A complex has also been suggested to restrict end-resection at DNA breaks (Coleman and Greenberg, 2011). In addition, BRCA1 has been shown to contribute to chromosome end protection (Al-Wahiby and Slijepcevic, 2005). Altogether, these studies indicate that also at dysfunctional telomeres the RNF8/RNF168 pathway promotes H2A ubiquitination and recruitment of 53BP1 to activate NHEJ.

Recently, RNF8 has been shown to ubiquitinate histone H1 at DSBs, together with the E2 ubiquitin-conjugating enzyme UBC13 (Thorslund et al., 2015). Ubiquitinated histone H1 serves as a binding platform for RNF168, which subsequently ubiquitinates histone H2A on K13/K15 and allows for recruitment of several repair factors, such as 53BP1 and BRCA1 (Mattiroli et al., 2012; Fradet-Turcotte et al., 2013; Thorslund et al., 2015). Furthermore, the DUB USP51 was shown to reverse the ubiquitination of histone H2A at K13/K15. Depletion of USP51 induces an increase in H2A K13/K15 ubiquitination and a delay in DDR foci resolution (Wang et al., 2016). However, many other DUBs have also been shown to be able to deubiquitinate histone H2A, including USP3, USP16, USP26 and USP44 (Citterio, 2015). Whether RNF8 ubiquitinates histone H1 at telomeres and whether RNF168 modifies histone H2A at K13/K15 at telomeres remains to be elucidated.

Finally, the STUbL RNF4 has also been shown to promote NHEJ at uncapped telomeres (Groocock et al., 2014). It usually recognizes SUMO-modified targets and is only activated upon dimerization in the presence of SUMO-chains (Rojas-Fernandez et al., 2014). RNF4 was suggested to promote 53BP1 recruitment to uncapped telomeres and telomere fusions, depending on a nucleosome-targeting motif in its RING domain and its SIMs (Groocock et al., 2014). This suggests that RNF4 recognizes chromatin-bound SUMO conjugates via its SIM domains (interaction with SUMO proteins) and its RING domain (binding to chromatin) and can subsequently ubiquitinate nearby chromatin or target proteins.

Repression of the DDR and DNA Repair at Telomeres

In contrast to repair at genome-wide DSBs, which contributes to genome stability by fixing the break, repair at uncapped telomeres can be deleterious. When DNA repair factors gain access to chromosome ends and create end-to-end fusions in an attempt to 'heal the break', this can result in genomic instability. Therefore, telomeres need to be protected from unwanted actions of DNA repair factors. This protection is mainly achieved by the TRF2 and POT1 subunits of the shelterin complex (Doksani and de Lange, 2014). TRF2 has been shown to protect telomeres in at least five ways. First, TRF2 binding to telomeric DNA stimulates strand invasion and thereby T-loop formation, which hides telomere ends and prevents binding of the MRN complex and subsequent activation of ATM (Griffith et al., 1999; Stansel et al., 2001; Doksani et al., 2013). Secondly, TRF2 interferes directly with activation of the ATM kinase. TRF2 was found to be able to bind to ATM and prevent its phosphorylation at S1981, resulting in inhibition of the signaling cascade downstream of ATM (Karlseder et al., 2004). Thirdly, TRF2 has been shown to interact with the ATM target CHK2 at a position close to its Thr68 phosphorylation site, preventing activation of CHK2 (Buscemi et al., 2009). Fourthly, as discussed above, the iDDR domain of TRF2 prevents RNF168 and 53BP1 recruitment to telomeres, inhibiting the ATM signaling cascade downstream of ATM itself (Okamoto et al., 2013). Finally, TRF2 interacts with the α-helix 5 domain of Ku70, preventing Ku70–Ku80 heterotetramerization and activation of NHEJ (Ribes-Zamora et al., 2013). In addition to the protective function of TRF2, POT1 protects telomeres by repressing ATR activity. POT1 binds to the ssDNA of the 3′-overhang and is believed to prevent the recruitment of RPA, which is crucial for ATR activation (Gong and de Lange, 2010).

Although the shelterin complex protects telomeres throughout most of the cell cycle, telomeres are briefly deprotected during and after replication, before shelterin mediated protection has been re-established on newly replicated telomeres (Verdun and Karlseder, 2006). In addition, telomeres

appear to be in an underprotected state during mitosis when the mitotic kinase Aurora B promotes telomere deprotection (Hayashi et al., 2012). This makes mitotic telomeres vulnerable to form sister-telomere associations (Orthwein et al., 2014). Therefore, during mitosis DNA repair is actively suppressed to prevent genomic instability. This suppression is achieved by CDK1-mediated phosphorylation of RNF8 and CDK1- and PLK1-mediated phosphorylation of 53BP1 (Orthwein et al., 2014). RNF8 phosphorylation interferes with the binding of RNF8 to MDC1, thereby inhibiting its recruitment to DSBs and preventing subsequent DDR signaling. In addition, phosphorylation of 53BP1 interferes with its ability to recognize H4K20me2 and K15-ubiquitinated histone H2A, which are both critical for 53BP1 recruitment to DSBs. This results in suppression of DNA repair activity during mitosis. When RNF168 and 53BP1 were artificially recruited to DSBs and telomeres, DNA repair was restored, resulting in sister telomere fusions (Orthwein et al., 2014). Thus phosphorylation of RNF168 and 53BP1 in mitosis plays an important role in maintaining genome stability and represents a shelterin-independent way of preventing DNA repair activities from acting on telomeres.

In general, it is increasingly becoming clear that ubiquitination and SUMOylation are highly involved in the regulation of the DDR triggered by DSBs in the genome. Evidence that this is also the case in response to dysfunctional telomeres has started to emerge, although there is still relatively little known about telomere-specific mechanisms. So far, the initial observations have indicated similar signaling processes in the cell's response to DSBs and dysfunctional telomeres. However, also differences between these processes have been reported. For example at telomeres, in contrast to DSBs, the Ku70–Ku80 complex is not only recruited upon damage or uncapping, but is constitutively present to prevent deletion of telomeric repeats (Wang et al., 2009). However, it also promotes NHEJ at uncapped telomeres and is therefore restricted in its activity by TRF2. TRF2, as mentioned above, inhibits Ku70–Ku80 heterotetramerization, which interferes with Ku70–Ku80 activation and NHEJ induction, but does not deplete Ku from healthy telomeres (Ribes-Zamora et al., 2013). Furthermore, telomere deprotection has been shown to occur in different degrees, depending on the amount of TRF2 still bound to the telomeres (Cesare et al., 2013). Cells containing partially deprotected telomeres, with low amounts of TRF2 bound, will bypass the G2/M checkpoint, cycle to G1-phase and enter senescence, but will still be protected from telomeric NHEJ. However, when telomeres are completely uncapped, the DDR is fully activated and telomeres are fused through NHEJ (Cesare et al., 2013). Completely uncapped telomeres also avoid G2/M arrest, but the mechanism behind this is not yet known. These examples further emphasize that new findings regarding responses at DSBs, including ubiquitination and SUMOylation events, should also be studied at dysfunctional telomeres and vice versa, to understand whether the underlying mechanisms are identical or different between DSBs and dysfunctional telomeres.

PERSPECTIVES

A tight regulation of telomere maintenance, replication and protection is required to ensure safeguarding of genome integrity by telomeres. If factors in these processes are impaired or exhibit aberrant functions, genome stability is at risk, potentially promoting tumorigenesis. Therefore, it is crucial to further investigate the processes and factors that ensure proper telomere function. Over the last decade, the importance and functions of ubiquitination and SUMOylation at telomeres have started to become clear. These PTMs are not only essential for telomere maintenance and protection, but are also key contributors to the cell's response to dysfunctional telomeres. Although many studies have already explored ubiquitination and SUMOylation in different telomeric contexts and thereby identified various targets, the underlying mechanisms, as well as the precise contribution of PTMs are often still undetermined. Moreover, PTMs have also been shown to affect each other. So far, crosstalk between ubiquitination and SUMOylation has been shown to not only contribute to the general DDR, but also to DDR at telomeres, such as RNF4-mediated ubiquitination that was shown to require the presence of SUMO-chains (Groocock et al., 2014; Rojas-Fernandez et al., 2014). Therefore, it would be interesting to investigate whether additional crosstalk occurs at telomeres and if one aspect of telomere biology, for example DNA repair or maintenance, is more affected by the combination of ubiquitin and SUMO modifications than others. Further studies concerning telomere-specific ubiquitination and SUMOylation will be required to increase our understanding of the complex mechanisms that ensure proper telomere function or contribute to DNA repair at dysfunctional telomeres. It would be beneficial to distinguish which modifications are unique to telomeric DNA, as these might offer a tool to specifically target DNA repair at telomeres without interfering in an unwanted manner with genome-wide repair at DNA breaks.

AUTHOR CONTRIBUTIONS

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

FUNDING

Work in the laboratory of the authors is funded by grants from the Dutch Cancer Society (NKI2012-5305), the Netherlands Organization for Scientific Research (ALW822.02.004) and the European Research Council (ERCStG 311565).

ACKNOWLEDGMENTS

We apologize to authors whose work could not be cited due to space limitations. We thank Inge de Krijger, Vera Boersma and Aurora Cerutti for critical reading of our manuscript.

REFERENCES

Al-Wahiby, S., and Slijepcevic, P. (2005). Chromosomal aberrations involving telomeres in BRCA1 deficient human and mouse cell lines. *Cytogenet. Genome. Res.* 109, 491–496. doi: 10.1159/000084208

Arnoult, N., and Karlseder, J. (2015). Complex interactions between the DNA-damage response and mammalian telomeres. *Nat. Struct. Mol. Biol.* 22, 859–866. doi: 10.1038/nsmb.3092

Atanassov, B. S., Evrard, Y. A., Multani, A. S., Zhang, Z., Tora, L., Devys, D., et al. (2009). Gcn5 and SAGA regulate shelterin protein turnover and telomere maintenance. *Mol. Cell* 35, 352–364. doi: 10.1016/j.molcel.2009.06.015

Badie, S., Escandell, J. M., Bouwman, P., Carlos, A. R., Thanasoula, M., Gallardo, M. M., et al. (2010). BRCA2 acts as a RAD51 loader to facilitate telomere replication and capping. *Nat. Struct. Mol. Biol.* 17, 1461–1469. doi: 10.1038/nsmb.1943

Benarroch-Popivker, D., Pisano, S., Mendez-Bermudez, A., Lototska, L., Kaur, P., Bauwens, S., et al. (2016). TRF2-mediated control of telomere DNA topology as a mechanism for chromosome-end protection. *Mol. Cell* 61, 274–286. doi: 10.1016/j.molcel.2015.12.009

Bhanot, M., and Smith, S. (2012). TIN2 stability is regulated by the E3 ligase Siah2. *Mol. Cell. Biol.* 32, 376–384. doi: 10.1128/MCB.06227-11

Boersma, V., Moatti, N., Segura-Bayona, S., Peuscher, M. H., Van Der Torre, J., Wevers, B. A., et al. (2015). MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5' end resection. *Nature* 521, 537–540. doi: 10.1038/nature14216

Bothmer, A., Robbiani, D. F., Feldhahn, N., Gazumyan, A., Nussenzweig, A., and Nussenzweig, M. C. (2010). 53BP1 regulates DNA resection and the choice between classical and alternative end joining during class switch recombination. *J. Exp. Med.* 207, 855–865. doi: 10.1084/jem.20100244

Brown, J. S., and Jackson, S. P. (2015). Ubiquitylation, neddylation and the DNA damage response. *Open Biol.* 5:150018. doi: 10.1098/rsob.150018

Buscemi, G., Zannini, L., Fontanella, E., Lecis, D., Lisanti, S., and Delia, D. (2009). The shelterin protein TRF2 inhibits Chk2 activity at telomeres in the absence of DNA damage. *Curr. Biol.* 19, 874–879. doi: 10.1016/j.cub.2009.03.064

Callen, E., Di Virgilio, M., Kruhlak, M. J., Nieto-Soler, M., Wong, N., Chen, H. T., et al. (2013). 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell* 153, 1266–1280. doi: 10.1016/j.cell.2013.05.023

Cesare, A. J., Hayashi, M. T., Crabbe, L., and Karlseder, J. (2013). The telomere deprotection response is functionally distinct from the genomic DNA damage response. *Mol. Cell* 51, 141–155. doi: 10.1016/j.molcel.2013.06.006

Chapman, J. R., Barral, P., Vannier, J. B., Borel, V., Steger, M., Tomas-Loba, A., et al. (2013). RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol. Cell* 49, 858–871. doi: 10.1016/j.molcel.2013.01.002

Chen, L. Y., and Lingner, J. (2013). CST for the grand finale of telomere replication. *Nucleus* 4, 277–282. doi: 10.4161/nucl.25701

Chow, T. T., Zhao, Y., Mak, S. S., Shay, J. W., and Wright, W. E. (2012). Early and late steps in telomere overhang processing in normal human cells: the position of the final RNA primer drives telomere shortening. *Genes Dev.* 26, 1167–1178. doi: 10.1101/gad.187211.112

Churikov, D., Charifi, F., Eckert-Boulet, N., Silva, S., Simon, M. N., Lisby, M., et al. (2016). SUMO-dependent relocalization of eroded telomeres to nuclear pore complexes controls telomere recombination. *Cell Rep.* 15, 1242–1253. doi: 10.1016/j.celrep.2016.04.008

Ciechanover, A., Elias, S., Heller, H., and Hershko, A. (1982). "Covalent affinity" purification of ubiquitin-activating enzyme. *J. Biol. Chem.* 257, 2537–2542.

Cimprich, K. A., and Cortez, D. (2008). ATR: an essential regulator of genome integrity. *Nat. Rev. Mol. Cell Biol.* 9, 616–627. doi: 10.1038/nrm2450

Citterio, E. (2015). Fine-tuning the ubiquitin code at DNA double-strand breaks: deubiquitinating enzymes at work. *Front. Genet.* 6:282. doi: 10.3389/fgene.2015.00282

Coleman, K. A., and Greenberg, R. A. (2011). The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. *J. Biol. Chem.* 286, 13669–13680. doi: 10.1074/jbc.M110.213728

Crabbe, L., Verdun, R. E., Haggblom, C. I., and Karlseder, J. (2004). Defective telomere lagging strand synthesis in cells lacking WRN helicase activity. *Science* 306, 1951–1953. doi: 10.1126/science.1103619

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'Adda di Fagagna, F., Reaper, P. M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., et al. (2003). A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 426, 194–198. doi: 10.1038/nature02118

Davoli, T., and de Lange, T. (2011). The causes and consequences of polyploidy in normal development and cancer. *Annu. Rev. Cell Dev. Biol.* 27, 585–610. doi: 10.1146/annurev-cellbio-092910-154234

Denchi, E. L., and de Lange, T. (2007). Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature* 448, 1068–1071. doi: 10.1038/nature06065

Di Virgilio, M., Callen, E., Yamane, A., Zhang, W., Jankovic, M., Gitlin, A. D., et al. (2013). Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 339, 711–715. doi: 10.1126/science.1230624

Dimitrova, N., Chen, Y. C., Spector, D. L., and De Lange, T. (2008). 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 456, 524–528. doi: 10.1038/nature07433

Dimitrova, N., and de Lange, T. (2006). MDC1 accelerates nonhomologous end-joining of dysfunctional telomeres. *Genes Dev.* 20, 3238–3243. doi: 10.1101/gad.1496606

Doksani, Y., and de Lange, T. (2014). The role of double-strand break repair pathways at functional and dysfunctional telomeres. *Cold Spring Harb. Perspect. Biol.* 6:a016576. doi: 10.1101/cshperspect.a016576

Doksani, Y., Wu, J. Y., De Lange, T., and Zhuang, X. (2013). Super-resolution fluorescence imaging of telomeres reveals TRF2-dependent T-loop formation. *Cell* 155, 345–356. doi: 10.1016/j.cell.2013.09.048

Edwards, D. N., Orren, D. K., and Machwe, A. (2014). Strand exchange of telomeric DNA catalyzed by the Werner syndrome protein (WRN) is specifically stimulated by TRF2. *Nucleic Acids Res.* 42, 7748–7761. doi: 10.1093/nar/gku454

Escribano-Diaz, C., Orthwein, A., Fradet-Turcotte, A., Xing, M., Young, J. T., Tkac, J., et al. (2013). A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol. Cell* 49, 872–883. doi: 10.1016/j.molcel.2013.01.001

Feng, L., Fong, K. W., Wang, J., Wang, W., and Chen, J. (2013). RIF1 counteracts BRCA1-mediated end resection during DNA repair. *J. Biol. Chem.* 288, 11135–11143. doi: 10.1074/jbc.M113.457440

Fradet-Turcotte, A., Canny, M. D., Escribano-Diaz, C., Orthwein, A., Leung, C. C., Huang, H., et al. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 499, 50–54. doi: 10.1038/nature12318

Fujita, K., Horikawa, I., Mondal, A. M., Jenkins, L. M., Appella, E., Vojtesek, B., et al. (2010). Positive feedback between p53 and TRF2 during telomere-damage signalling and cellular senescence. *Nat. Cell Biol.* 12, 1205–1212. doi: 10.1038/ncb2123

Gao, X., Wang, W., Yang, H., Wu, L., He, Z., Zhou, S., et al. (2016). UBE2D3 gene overexpression increases radiosensitivity of EC109 esophageal cancer cells in vitro and in vivo. *Oncotarget* 7, 32543–32553. doi: 10.18632/oncotarget.8869

Garg, M., Gurung, R. L., Mansoubi, S., Ahmed, J. O., Dave, A., Watts, F. Z., et al. (2014). Tpz1TPP1 SUMOylation reveals evolutionary conservation of SUMO-dependent Stn1 telomere association. *EMBO Rep.* 15, 871–877. doi: 10.15252/embr.201438919

Geiss-Friedlander, R., and Melchior, F. (2007). Concepts in sumoylation: a decade on. *Nat. Rev. Mol. Cell Biol.* 8, 947–956. doi: 10.1038/nrm2293

Gong, Y., and de Lange, T. (2010). A Shld1-controlled POT1a provides support for repression of ATR signaling at telomeres through RPA exclusion. *Mol. Cell* 40, 377–387. doi: 10.1016/j.molcel.2010.10.016

Greider, C. W., and Blackburn, E. H. (1985). Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* 43, 405–413.

Griffith, J. D., Comeau, L., Rosenfield, S., Stansel, R. M., Bianchi, A., Moss, H., et al. (1999). Mammalian telomeres end in a large duplex loop. *Cell* 97, 503–514.

Groocock, L. M., Nie, M., Prudden, J., Moiani, D., Wang, T., Cheltsov, A., et al. (2014). RNF4 interacts with both SUMO and nucleosomes to promote the DNA damage response. *EMBO Rep.* 15, 601–608. doi: 10.1002/embr.201338369

Gudjonsson, T., Altmeyer, M., Savic, V., Toledo, L., Dinant, C., Grofte, M., et al. (2012). TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. *Cell* 150, 697–709. doi: 10.1016/j.cell.2012.06.039

Ha, G. H., Kim, H. S., Go, H., Lee, H., Seimiya, H., Chung, D. H., et al. (2012). Tankyrase-1 function at telomeres and during mitosis is regulated by Polo-like kinase-1-mediated phosphorylation. *Cell Death Differ.* 19, 321–332. doi: 10.1038/cdd.2011.101

Hang, L. E., Liu, X., Cheung, I., Yang, Y., and Zhao, X. (2011). SUMOylation regulates telomere length homeostasis by targeting Cdc13. *Nat. Struct. Mol. Biol.* 18, 920–926. doi: 10.1038/nsmb.2100

Harley, C. B., Futcher, A. B., and Greider, C. W. (1990). Telomeres shorten during ageing of human fibroblasts. *Nature* 345, 458–460. doi: 10.1038/345458a0

Hayashi, M. T., Cesare, A. J., Fitzpatrick, J. A., Lazzerini-Denchi, E., and Karlseder, J. (2012). A telomere-dependent DNA damage checkpoint induced by prolonged mitotic arrest. *Nat. Struct. Mol. Biol.* 19, 387–394. doi: 10.1038/nsmb.2245

Her, J., Jeong, Y. Y., and Chung, I. K. (2015). PIAS1-mediated sumoylation promotes STUbL-dependent proteasomal degradation of the human telomeric protein TRF2. *FEBS Lett.* 589, 3277–3286. doi: 10.1016/j.febslet.2015.09.030

Her, Y. R., and Chung, I. K. (2009). Ubiquitin Ligase RLIM modulates telomere length homeostasis through a Proteolysis of TRF1. *J. Biol. Chem.* 284, 8557–8566. doi: 10.1074/jbc.M806702200

Hershko, A., Heller, H., Elias, S., and Ciechanover, A. (1983). Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *J. Biol. Chem.* 258, 8206–8214.

Hirai, Y., Tamura, M., Otani, J., and Ishikawa, F. (2016). NEK6-mediated phosphorylation of human TPP1 regulates telomere length through telomerase recruitment. *Genes Cells* 21, 874–889. doi: 10.1111/gtc.12391

Holtrich, U., Wolf, G., Brauninger, A., Karn, T., Bohme, B., Rubsamen-Waigmann, H., et al. (1994). Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors. *Proc. Natl. Acad. Sci. U.S.A.* 91, 1736–1740.

Huang, C., Dai, X., and Chai, W. (2012). Human Stn1 protects telomere integrity by promoting efficient lagging-strand synthesis at telomeres and mediating C-strand fill-in. *Cell Res.* 22, 1681–1695. doi: 10.1038/cr.2012.132

Huang, Y., Sun, L., Liu, N., Wei, Q., Jiang, L., Tong, X., et al. (2015). Polo-like Kinase 1 (Plk1) Up-regulates telomerase activity by affecting human telomerase reverse transcriptase (hTERT) stability. *J. Biol. Chem.* 290, 18865–18873. doi: 10.1074/jbc.M114.635375

Jacobs, J. J. (2013). Loss of telomere protection: consequences and opportunities. *Front. Oncol.* 3:88. doi: 10.3389/fonc.2013.00088

Jacobs, J. J., and de Lange, T. (2005). p16INK4a as a second effector of the telomere damage pathway. *Cell Cycle* 4, 1364–1368. doi: 10.4161/cc.4.10.2104

Johnson, E. S. (2004). Protein modification by SUMO. *Annu. Rev. Biochem.* 73, 355–382. doi: 10.1146/annurev.biochem.73.011303.074118

Kabir, S., Hockemeyer, D., and De Lange, T. (2014). TALEN gene knockouts reveal no requirement for the conserved human shelterin protein Rap1 in telomere protection and length regulation. *Cell Rep.* 9, 1273–1280. doi: 10.1016/j.celrep.2014.10.014

Karlseder, J., Hoke, K., Mirzoeva, O. K., Bakkenist, C., Kastan, M. B., Petrini, J. H., et al. (2004). The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. *PLoS Biol.* 2:E240. doi: 10.1371/journal.pbio.0020240

Kerscher, O. (2007). SUMO junction-what's your function? New insights through SUMO-interacting motifs. *EMBO Rep.* 8, 550–555. doi: 10.1038/sj.embor.7400980

Kim, J., and Chung, I. K. (2014). The splicing factor U2AF65 stabilizes TRF1 protein by inhibiting its ubiquitin-dependent proteolysis. *Biochem. Biophys. Res. Commun.* 443, 1124–1130. doi: 10.1016/j.bbrc.2013.12.118

Kim, J. H., Park, S. M., Kang, M. R., Oh, S. Y., Lee, T. H., Muller, M. T., et al. (2005). Ubiquitin ligase MKRN1 modulates telomere length homeostasis through a proteolysis of hTERT. *Genes Dev.* 19, 776–781. doi: 10.1101/gad.1289405

Kolas, N. K., Chapman, J. R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F. D., et al. (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 318, 1637–1640. doi: 10.1126/science.1150034

Komander, D., Clague, M. J., and Urbe, S. (2009). Breaking the chains: structure and function of the deubiquitinases. *Nat. Rev. Mol. Cell Biol.* 10, 550–563. doi: 10.1038/nrm2731

Komander, D., and Rape, M. (2012). The ubiquitin code. *Annu. Rev. Biochem.* 81, 203–229. doi: 10.1146/annurev-biochem-060310-170328

Lallemand-Breitenbach, V., Jeanne, M., Benhenda, S., Nasr, R., Lei, M., Peres, L., et al. (2008). Arsenic degrades PML or PML-RARalpha through a SUMO-triggered RNF4/ubiquitin-mediated pathway. *Nat. Cell Biol.* 10, 547–555. doi: 10.1038/ncb1717

Lazzerini-Denchi, E., and Sfeir, A. (2016). Stop pulling my strings - what telomeres taught us about the DNA damage response. *Nat. Rev. Mol. Cell Biol.* 17, 364–378. doi: 10.1038/nrm.2016.43

Lee, J. H., Khadka, P., Baek, S. H., and Chung, I. K. (2010). CHIP promotes human telomerase reverse transcriptase degradation and negatively regulates telomerase activity. *J. Biol. Chem.* 285, 42033–42045. doi: 10.1074/jbc.M110.149831

Lee, T. H., Perrem, K., Harper, J. W., Lu, K. P., and Zhou, X. Z. (2006). The F-box protein FBX4 targets PIN2/TRF1 for ubiquitin-mediated degradation and regulates telomere maintenance. *J. Biol. Chem.* 281, 759–768. doi: 10.1074/jbc.M509855200

Lescasse, R., Pobiega, S., Callebaut, I., and Marcand, S. (2013). End-joining inhibition at telomeres requires the translocase and polySUMO-dependent ubiquitin ligase Uls1. *EMBO J.* 32, 805–815. doi: 10.1038/emboj.2013.24

Liu, D., Safari, A., O'connor, M. S., Chan, D. W., Laegeler, A., Qin, J., et al. (2004). PTOP interacts with POT1 and regulates its localization to telomeres. *Nat. Cell Biol.* 6, 673–680. doi: 10.1038/ncb1142

Loayza, D., and De Lange, T. (2003). POT1 as a terminal transducer of TRF1 telomere length control. *Nature* 423, 1013–1018. doi: 10.1038/nature01688

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., et al. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 131, 887–900. doi: 10.1016/j.cell.2007.09.040

Manis, J. P., Morales, J. C., Xia, Z., Kutok, J. L., Alt, F. W., and Carpenter, P. B. (2004). 53BP1 links DNA damage-response pathways to immunoglobulin heavy chain class-switch recombination. *Nat. Immunol.* 5, 481–487. doi: 10.1038/ni1067

Martinez, P., and Blasco, M. A. (2015). Replicating through telomeres: a means to an end. *Trends Biochem. Sci.* 40, 504–515. doi: 10.1016/j.tibs.2015.06.003

Martinez, P., Gomez-Lopez, G., Pisano, D. G., Flores, J. M., and Blasco, M. A. (2016). A genetic interaction between RAP1 and telomerase reveals an unanticipated role for RAP1 in telomere maintenance. *Aging Cell* doi: 10.1111/acel.12517 [Epub ahead of print].

Mattiroli, F., Vissers, J. H., Van Dijk, W. J., Ikpa, P., Citterio, E., Vermeulen, W., et al. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150, 1182–1195. doi: 10.1016/j.cell.2012.08.005

Miyagawa, K., Low, R. S., Santosa, V., Tsuji, H., Moser, B. A., Fujisawa, S., et al. (2014). SUMOylation regulates telomere length by targeting the shelterin subunit Tpz1(Tpp1) to modulate shelterin-Stn1 interaction in fission yeast. *Proc. Natl. Acad. Sci. U.S.A.* 111, 5950–5955. doi: 10.1073/pnas.1401359111

Miyoshi, T., Kanoh, J., Saito, M., and Ishikawa, F. (2008). Fission yeast Pot1-Tpp1 protects telomeres and regulates telomere length. *Science* 320, 1341–1344. doi: 10.1126/science.1154819

Mukhopadhyay, D., and Dasso, M. (2007). Modification in reverse: the SUMO proteases. *Trends Biochem. Sci.* 32, 286–295. doi: 10.1016/j.tibs.2007.05.002

Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M. B., Roberts, T. M., Brown, G. W., et al. (2008). Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 322, 597–602. doi: 10.1126/science.1162790

Nandakumar, J., and Cech, T. R. (2013). Finding the end: recruitment of telomerase to telomeres. *Nat. Rev. Mol. Cell Biol.* 14, 69–82. doi: 10.1038/nrm3505

O'Connor, M. S., Safari, A., Xin, H., Liu, D., and Songyang, Z. (2006). A critical role for TPP1 and TIN2 interaction in high-order telomeric complex assembly. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11874–11879. doi: 10.1073/pnas.0605303103

Oh, W., Lee, E. W., Lee, D., Yang, M. R., Ko, A., Yoon, C. H., et al. (2010). Hdm2 negatively regulates telomerase activity by functioning as an E3 ligase of hTERT. *Oncogene* 29, 4101–4112. doi: 10.1038/onc.2010.160

Okamoto, K., Bartocci, C., Ouzounov, I., Diedrich, J. K., Yates, J. R. III, and Denchi, E. L. (2013). A two-step mechanism for TRF2-mediated chromosome-end protection. *Nature* 494, 502–505. doi: 10.1038/nature11873

Orthwein, A., Fradet-Turcotte, A., Noordermeer, S. M., Canny, M. D., Brun, C. M., Strecker, J., et al. (2014). Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. *Science* 344, 189–193. doi: 10.1126/science.1248024

Ouyang, J., Garner, E., Hallet, A., Nguyen, H. D., Rickman, K. A., Gill, G., et al. (2015). Noncovalent interactions with SUMO and ubiquitin orchestrate distinct functions of the SLX4 complex in genome maintenance. *Mol. Cell* 57, 108–122. doi: 10.1016/j.molcel.2014.11.015

Palm, W., and de Lange, T. (2008). How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* 42, 301–334. doi: 10.1146/annurev.genet.41.110306.130350

Pellegrini, L. (2012). The Pol alpha-primase complex. *Subcell. Biochem.* 62, 157–169. doi: 10.1007/978-94-007-4572-8_9

Peuscher, M. H., and Jacobs, J. J. (2011). DNA-damage response and repair activities at uncapped telomeres depend on RNF8. *Nat. Cell Biol.* 13, 1139–1145. doi: 10.1038/ncb2326

Peuscher, M. H., and Jacobs, J. J. (2012). Posttranslational control of telomere maintenance and the telomere damage response. *Cell Cycle* 11, 1524–1534. doi: 10.4161/cc.19847

Pickett, H. A., and Reddel, R. R. (2015). Molecular mechanisms of activity and derepression of alternative lengthening of telomeres. *Nat. Struct. Mol. Biol.* 22, 875–880. doi: 10.1038/nsmb.3106

Potts, P. R., and Yu, H. (2007). The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. *Nat. Struct. Mol. Biol.* 14, 581–590. doi: 10.1038/nsmb1259

Price, C. M., Boltz, K. A., Chaiken, M. F., Stewart, J. A., Beilstein, M. A., and Shippen, D. E. (2010). Evolution of CST function in telomere maintenance. *Cell Cycle* 9, 3157–3165. doi: 10.4161/cc.9.16.12547

Rai, R., Chen, Y., Lei, M., and Chang, S. (2016). TRF2-RAP1 is required to protect telomeres from engaging in homologous recombination-mediated deletions and fusions. *Nat. Commun.* 7:10881. doi: 10.1038/ncomms10881

Rai, R., Li, J. M., Zheng, H., Lok, G. T., Deng, Y., Huen, M. S., et al. (2011). The E3 ubiquitin ligase Rnf8 stabilizes Tpp1 to promote telomere end protection. *Nat. Struct. Mol. Biol.* 18, 1400–1407. doi: 10.1038/nsmb.2172

Reveal, P. M., Henkels, K. M., and Turchi, J. J. (1997). Synthesis of the mammalian telomere lagging strand in vitro. *J. Biol. Chem.* 272, 11678–11681.

Ribes-Zamora, A., Indiviglio, S. M., Mihalek, I., Williams, C. L., and Bertuch, A. A. (2013). TRF2 interaction with Ku heterotetramerization interface gives insight into c-NHEJ prevention at human telomeres. *Cell Rep.* 5, 194–206. doi: 10.1016/j.celrep.2013.08.040

Rivera, T., Haggblom, C., Cosconati, S., and Karlseder, J. (2017). A balance between elongation and trimming regulates telomere stability in stem cells. *Nat. Struct. Mol. Biol.* 24, 30–39. doi: 10.1038/nsmb.3335

Rojas-Fernandez, A., Plechanovova, A., Hattersley, N., Jaffray, E., Tatham, M. H., and Hay, R. T. (2014). SUMO chain-induced dimerization activates RNF4. *Mol. Cell* 53, 880–892. doi: 10.1016/j.molcel.2014.02.031

Salvatico, J., Kim, J. H., Chung, I. K., and Muller, M. T. (2010). Differentiation linked regulation of telomerase activity by Makorin-1. *Mol. Cell. Biochem.* 342, 241–250. doi: 10.1007/s11010-010-0490-x

Sarthy, J., Bae, N. S., Scrafford, J., and Baumann, P. (2009). Human RAP1 inhibits non-homologous end joining at telomeres. *EMBO J.* 28, 3390–3399. doi: 10.1038/emboj.2009.275

Saville, M. K., Sparks, A., Xirodimas, D. P., Wardrop, J., Stevenson, L. F., Bourdon, J. C., et al. (2004). Regulation of p53 by the ubiquitin-conjugating enzymes UbcH5B/C in vivo. *J. Biol. Chem.* 279, 42169–42181. doi: 10.1074/jbc.M403362200

Sfeir, A., Kabir, S., Van Overbeek, M., Celli, G. B., and De Lange, T. (2010). Loss of Rap1 induces telomere recombination in the absence of NHEJ or a DNA damage signal. *Science* 327, 1657–1661. doi: 10.1126/science.1185100

Sfeir, A., Kosiyatrakul, S. T., Hockemeyer, D., Macrae, S. L., Karlseder, J., Schildkraut, C. L., et al. (2009). Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell* 138, 90–103. doi: 10.1016/j.cell.2009.06.021

Shao, G., Lilli, D. R., Patterson-Fortin, J., Coleman, K. A., Morrissey, D. E., and Greenberg, R. A. (2009). The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3166–3171. doi: 10.1073/pnas.0807485106

Smith, S., Giriat, I., Schmitt, A., and De Lange, T. (1998). Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science* 282, 1484–1487.

Stansel, R. M., De Lange, T., and Griffith, J. D. (2001). T-loop assembly in vitro involves binding of TRF2 near the 3' telomeric overhang. *EMBO J.* 20, 5532–5540. doi: 10.1093/emboj/20.19.5532

Stewart, J. A., Chaiken, M. F., Wang, F., and Price, C. M. (2012). Maintaining the end: roles of telomere proteins in end-protection, telomere replication and length regulation. *Mutat. Res.* 730, 12–19. doi: 10.1016/j.mrfmmm.2011.08.011

Tacconi, E. M., and Tarsounas, M. (2015). How homologous recombination maintains telomere integrity. *Chromosoma* 124, 119–130. doi: 10.1007/s00412-014-0497-2

Tatham, M. H., Geoffroy, M. C., Shen, L., Plechanovova, A., Hattersley, N., Jaffray, E. G., et al. (2008). RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. *Nat. Cell Biol.* 10, 538–546. doi: 10.1038/ncb1716

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., et al. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393. doi: 10.1038/nature15401

Uringa, E. J., Lisaingo, K., Pickett, H. A., Brind'amour, J., Rohde, J. H., Zelensky, A., et al. (2012). RTEL1 contributes to DNA replication and repair and telomere maintenance. *Mol. Biol. Cell* 23, 2782–2792. doi: 10.1091/mbc.E12-03-0179

Vannier, J. B., Pavicic-Kaltenbrunner, V., Petalcorin, M. I., Ding, H., and Boulton, S. J. (2012). RTEL1 dismantles T loops and counteracts telomeric G4-DNA to maintain telomere integrity. *Cell* 149, 795–806. doi: 10.1016/j.cell.2012.03.030

Vannier, J. B., Sandhu, S., Petalcorin, M. I., Wu, X., Nabi, Z., Ding, H., et al. (2013). RTEL1 is a replisome-associated helicase that promotes telomere and genome-wide replication. *Science* 342, 239–242. doi: 10.1126/science.1241779

Verdun, R. E., and Karlseder, J. (2006). The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. *Cell* 127, 709–720. doi: 10.1016/j.cell.2006.09.034

Wang, C., Xiao, H., Ma, J., Zhu, Y., Yu, J., Sun, L., et al. (2013). The F-box protein beta-TrCP promotes ubiquitination of TRF1 and regulates the ALT-associated PML bodies formation in U2OS cells. *Biochem. Biophys. Res. Commun.* 434, 728–734. doi: 10.1016/j.bbrc.2013.03.096

Wang, C., Yu, J., Yuan, K., Lan, J., Jin, C., and Huang, H. (2010). Plk1-mediated mitotic phosphorylation of PinX1 regulates its stability. *Eur. J. Cell Biol.* 89, 748–756. doi: 10.1016/j.ejcb.2010.05.005

Wang, R. C., Smogorzewska, A., and De Lange, T. (2004). Homologous recombination generates T-loop-sized deletions at human telomeres. *Cell* 119, 355–368. doi: 10.1016/j.cell.2004.10.011

Wang, W., Yang, L., Hu, L., Li, F., Ren, L., Yu, H., et al. (2013). Inhibition of UBE2D3 expression attenuates radiosensitivity of MCF-7 human breast cancer cells by increasing hTERT expression and activity. *PLoS ONE* 8:e64660. doi: 10.1371/journal.pone.0064660

Wang, Y., Ghosh, G., and Hendrickson, E. A. (2009). Ku86 represses lethal telomere deletion events in human somatic cells. *Proc. Natl. Acad. Sci. U.S.A.* 106, 12430–12435. doi: 10.1073/pnas.0903362106

Wang, Z., Zhang, H., Liu, J., Cheruiyot, A., Lee, J. H., Ordog, T., et al. (2016). USP51 deubiquitylates H2AK13,15ub and regulates DNA damage response. *Genes Dev.* 30, 946–959. doi: 10.1101/gad.271841.115

Weisshaar, S. R., Keusekotten, K., Krause, A., Horst, C., Springer, H. M., Gottsche, K., et al. (2008). Arsenic trioxide stimulates SUMO-2/3 modification leading to RNF4-dependent proteolytic targeting of PML. *FEBS Lett.* 582, 3174–3178. doi: 10.1016/j.febslet.2008.08.008

Wilson, J. S., Tejera, A. M., Castor, D., Toth, R., Blasco, M. A., and Rouse, J. (2013). Localization-dependent and -independent roles of SLX4 in regulating telomeres. *Cell Rep.* 4, 853–860. doi: 10.1016/j.celrep.2013.07.033

Wu, P., Van Overbeek, M., Rooney, S., and De Lange, T. (2010). Apollo contributes to G overhang maintenance and protects leading-end telomeres. *Mol. Cell* 39, 606–617. doi: 10.1016/j.molcel.2010.06.031

Xu, G., Chapman, J. R., Brandsma, I., Yuan, J., Mistrik, M., Bouwman, P., et al. (2015). REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 521, 541–544. doi: 10.1038/nature14328

Yang, H., Wu, L., Ke, S., Wang, W., Yang, L., Gao, X., et al. (2016). Downregulation of ubiquitin-conjugating enzyme UBE2D3 promotes telomere maintenance and radioresistance of Eca-109 human esophageal carcinoma cells. *J. Cancer* 7, 1152–1162. doi: 10.7150/jca.14745

Ye, J. Z., and de Lange, T. (2004). TIN2 is a tankyrase 1 PARP modulator in the TRF1 telomere length control complex. *Nat. Genet.* 36, 618–623. doi: 10.1038/ng1360

Yeager, T. R., Neumann, A. A., Englezou, A., Huschtscha, L. I., Noble, J. R., and Reddel, R. R. (1999). Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. *Cancer Res.* 59, 4175–4179.

Yoo, J. E., Oh, B. K., and Park, Y. N. (2009). Human PinX1 mediates TRF1 accumulation in nucleolus and enhances TRF1 binding to telomeres. *J. Mol. Biol.* 388, 928–940. doi: 10.1016/j.jmb.2009.02.051

Zemp, I., and Lingner, J. (2014). The shelterin component TPP1 is a binding partner and substrate for the deubiquitinating enzyme USP7. *J. Biol. Chem.* 289, 28595–28606. doi: 10.1074/jbc.M114.596056

Zeng, Z., Wang, W., Yang, Y., Chen, Y., Yang, X., Diehl, J. A., et al. (2010). Structural basis of selective ubiquitination of TRF1 by SCFFbx4. *Dev. Cell* 18, 214–225. doi: 10.1016/j.devcel.2010.01.007

Zhong, F. L., Batista, L. F., Freund, A., Pech, M. F., Venteicher, A. S., and Artandi, S. E. (2012). TPP1 OB-fold domain controls telomere maintenance by recruiting telomerase to chromosome ends. *Cell* 150, 481–494. doi: 10.1016/j.cell.2012.07.012

Zhou, X. Z., and Lu, K. P. (2001). The Pin2/TRF1-interacting protein PinX1 is a potent telomerase inhibitor. *Cell* 107, 347–359.

Zimmer, J., Tacconi, E. M., Folio, C., Badie, S., Porru, M., Klare, K., et al. (2016). Targeting BRCA1 and BRCA2 Deficiencies with G-Quadruplex-Interacting Compounds. *Mol. Cell* 61, 449–460. doi: 10.1016/j.molcel.2015.12.004

Zimmermann, M., Lottersberger, F., Buonomo, S. B., Sfeir, A., and De Lange, T. (2013). 53BP1 regulates DSB repair using Rif1 to control 5' end resection. *Science* 339, 700–704. doi: 10.1126/science.1231573

Conflict of Interest Statement: 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.

frontiers in Genetics

MINI REVIEW
published: 23 August 2016
doi: 10.3389/fgene.2016.00152

Controlling DNA-End Resection: An Emerging Task for Ubiquitin and SUMO

*Sarah-Felicitas Himmels and Alessandro A. Sartori**

Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland

DNA double-strand breaks (DSBs) are one of the most detrimental lesions, as their incorrect or incomplete repair can lead to genomic instability, a hallmark of cancer. Cells have evolved two major competing DSB repair mechanisms: Homologous recombination (HR) and non-homologous end joining (NHEJ). HR is initiated by DNA-end resection, an evolutionarily conserved process that generates stretches of single-stranded DNA tails that are no longer substrates for religation by the NHEJ machinery. Ubiquitylation and sumoylation, the covalent attachment of ubiquitin and SUMO moieties to target proteins, play multifaceted roles in DNA damage signaling and have been shown to regulate HR and NHEJ, thus ensuring appropriate DSB repair. Here, we give a comprehensive overview about the current knowledge of how ubiquitylation and sumoylation control DSB repair by modulating the DNA-end resection machinery.

Keywords: DNA double-strand break repair, DNA-end resection, homologous recombination, ubiquitylation, sumoylation, CtIP/Sae2

OPEN ACCESS

Edited by:
Kristijan Ramadan,
University of Oxford, UK

Reviewed by:
Nhan Le Tran,
Mayo Clinic, USA
Wojciech Niedzwiedz,
University of Oxford, UK

***Correspondence:**
Alessandro A. Sartori
sartori@imcr.uzh.ch

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *12 June 2016*
Accepted: *05 August 2016*
Published: *23 August 2016*

Citation:
Himmels S-F and Sartori AA (2016) Controlling DNA-End Resection: An Emerging Task for Ubiquitin and SUMO. Front. Genet. 7:152. doi: 10.3389/fgene.2016.00152

INTRODUCTION

The capacity of our cells to detect and repair damaged DNA is key to prevent genomic instability and consequently the development of cancer (Jackson and Bartek, 2009; Ciccia and Elledge, 2010). DNA double-strand breaks (DSBs) are particularly hazardous lesions as their inappropriate repair can result in chromosomal translocations, an important driving force of tumorigenesis (Hanahan and Weinberg, 2011; Forment et al., 2012; Bunting and Nussenzweig, 2013; Rodgers and McVey, 2016). To circumvent this threat, the balance between the two major DSB repair pathways – homologous recombination (HR) and classical non-homologous end-joining (C-NHEJ) – is governed by various factors (Chapman et al., 2012; Ceccaldi et al., 2016). C-NHEJ operates with fast kinetics throughout the entire cell cycle and directly ligates broken DNA ends without requiring extended sequence complementarities to guide repair (Chiruvella et al., 2013; Radhakrishnan et al., 2014; Graham et al., 2016). In contrast, HR is slower and restricted to the S and G2 phases of the cell cycle because it requires an intact sister chromatid as a template for homology-directed repair (Karanam et al., 2012; Jasin and Rothstein, 2013; Orthwein et al., 2015). HR is initiated by DNA-end resection, an evolutionarily conserved mechanism that generates long stretches of 3′ single-stranded DNA (ssDNA) overhangs by nucleolytic degradation of the 5′ terminated strand of the DSB (Symington, 2014; Cejka, 2015; Daley et al., 2015). Consequently, DNA-end resection is a prerequisite for the formation of the Rad51-ssDNA presynaptic filament to promote HR. At the same time, it precludes the assembly of the C-NHEJ machinery, most prominently the Ku70-Ku80 (Ku) heterodimer, to bridge and ligate the broken DNA ends (Symington and Gautier, 2011). Thus, being a critical determinant of DSB repair pathway choice, DNA-end resection is tightly

controlled through multiple mechanisms, including post-translational modifications (PTMs). For instance, core components of the DSB resection machinery as well as resection antagonists undergo phosphorylation by cyclin-dependent kinases to gradually shift DSB repair from NHEJ to HR in the postreplicative stages of the cell cycle (Ferretti et al., 2013; Tomimatsu et al., 2014; Tkáč et al., 2016). In addition to phosphorylation, recent evidence highlighted that ubiquitylation and sumoylation control almost every aspect of cellular responses to DNA damage, including the repair of DSBs (Jackson and Durocher, 2013; Schwertman et al., 2016). This was exemplified by high-throughput proteomics studies revealing that DSB repair is facilitated by waves of global DNA damage-induced ubiquitylation and sumoylation (Cremona et al., 2012; Psakhye and Jentsch, 2012; Elia et al., 2015).

Ubiquitin and small ubiquitin-related modifier (SUMO), the most prominent members of a conserved protein family of ubiquitin-like proteins, can be attached to lysine residues of target proteins *via* an isopeptide bond (Bergink and Jentsch, 2009). There is only one SUMO in yeast (encoded by the essential *smt3* gene), whereas vertebrates express three independent SUMO isoforms (SUMO-1,-2,-3), of which SUMO-2/3 share 97% sequence identity (Hay, 2013). Different from other PTMs, ubiquitin-like modifications are carried out in a three-step cascade mechanism requiring the consecutive action of activating enzymes (E1s), conjugating enzymes (E2s), and ligases (E3s), which confer substrate specificity. In humans, ubiquitylation is mediated by two E1s, ∼35 active E2s, and more than 600 E3s, while sumoylation is conducted by a single heterodimeric E1, one E2 (UBC9), and approximately 10 E3s (Komander and Rape, 2012; Flotho and Melchior, 2013; Berndsen and Wolberger, 2014; Brown and Jackson, 2015; Stewart et al., 2016). Both processes are reversible with the removal of ubiquitin and SUMO from substrate proteins performed by deubiquitinases (DUBs) and SUMO/sentrin-specific proteases (SENPs), respectively (Ronau et al., 2016). Ubiquitin can be attached to target proteins either as monoubiquitin or as different types of polyubiquitin chains, depending on which of the seven lysine residues of ubiquitin is used for chain assembly (Swatek and Komander, 2016; Yau and Rape, 2016). The diverse ubiquitin chain types having different structural properties can change a variety of attributes in the target proteins. For example, while K48-linked ubiquitin chains promote proteasomal degradation, K63-linked chains are generally considered to regulate protein-protein interactions. In contrast, poly-SUMO chains primarily form through a single consensus sumoylation motif in mammalian SUMO-2/3, which is missing in SUMO-1 (Hay, 2013).

In this review, we want to highlight the importance of ubiquitin and SUMO in DSB repair with a special focus on the regulation of DNA-end resection.

DNA-END RESECTION IN A NUTSHELL

DNA-end resection in eukaryotes is a bidirectional two-step process initiated by the MRX (Mre11-Rad50-Xrs2) nuclease complex in conjunction with Sae2 in yeast, and by the MRN (MRE11-RAD50-NBS1) complex in conjunction with CtIP in human cells (**Figure 1A**). Subsequently, extended resection is performed by two redundant mechanisms involving either the 5′ to 3′ exonuclease Exo1 or the endonuclease Dna2 in concert with the RecQ helicase Sgs1 in yeast, and either EXO1 or DNA2 in concert with BLM (or WRN) in human cells (**Figure 1A**) (Sturzenegger et al., 2014; Cejka, 2015; Symington, 2016). As a result of this process, stretches of ssDNA are rapidly coated by RPA, the heterotrimeric ssDNA-binding protein, which serves as a platform to activate cell cycle checkpoints. For the ssDNA to be used as a substrate for homology-directed repair, RPA needs to be replaced by Rad51 with the help of recombination mediators (e.g., BRCA2).

UBIQUITYLATION AND SUMOYLATION OF THE DNA-END RESECTION MACHINERY

MRN/MRX Nuclease Complex

Mass spectrometric analysis revealed several potential ubiquitylation sites in all three subunits of the MRN complex (Kim et al., 2011; Symington and Gautier, 2011; Wagner et al., 2011; Mertins et al., 2013; Elia et al., 2015). However, with the exception of NBS1, none of them have yet been experimentally validated. Skp2, an F-box protein and component of the SCF (Skp1-Cullin1-F-box) E3 ligase complex, was found to interact with NBS1 and conjugate K63-linked ubiquitin chains onto NBS1-K735 in response to DSBs (**Figure 1B**) (Wu et al., 2012). Although DNA-end resection was not investigated in this study, cells deficient for Skp2 exhibited defects in ATM activation and HR (Wu et al., 2012). Lu et al. (2012) reported that RNF8 ubiquitinates NBS1 at two lysine residues and this was further shown to promote NBS1 recruitment to laser-induced DSB sites. Interestingly, cells ectopically expressing the ubiquitylation-deficient mutant of NBS1 exhibited reduced RPA foci formation after IR treatment and decreased HR frequency (**Figure 1B**) (Lu et al., 2012).

It has been discovered through proteomics studies that DNA damage-induced multisite sumoylation of a subset of HR proteins in yeast, including MRX, accelerates DSB repair and that this global 'SUMO response' depends on both MRX and DNA-end resection (**Figure 1B**) (Cremona et al., 2012; Psakhye and Jentsch, 2012). Consistently, Psakhye and Jentsch (2012) reported that *S. cerevisiae* Mre11 is sumoylated and exhibits strong two-hybrid interactions with Ubc9 (E2) and Siz2 (E3). However, very recent findings suggested that sumoylation of Mre11 is unlikely to be required for MRX-dependent DNA-end resection but that SUMO-interacting motifs (SIMs) in Mre11 non-covalently recruit poly-SUMO chains to facilitate MRX complex assembly (Chen et al., 2016).

CtIP/Sae2

Several E3 ubiquitin ligases have been described to interact with and modify CtIP, thereby possibly affecting DNA-end resection and DSB repair pathway choice. An early study reported that the

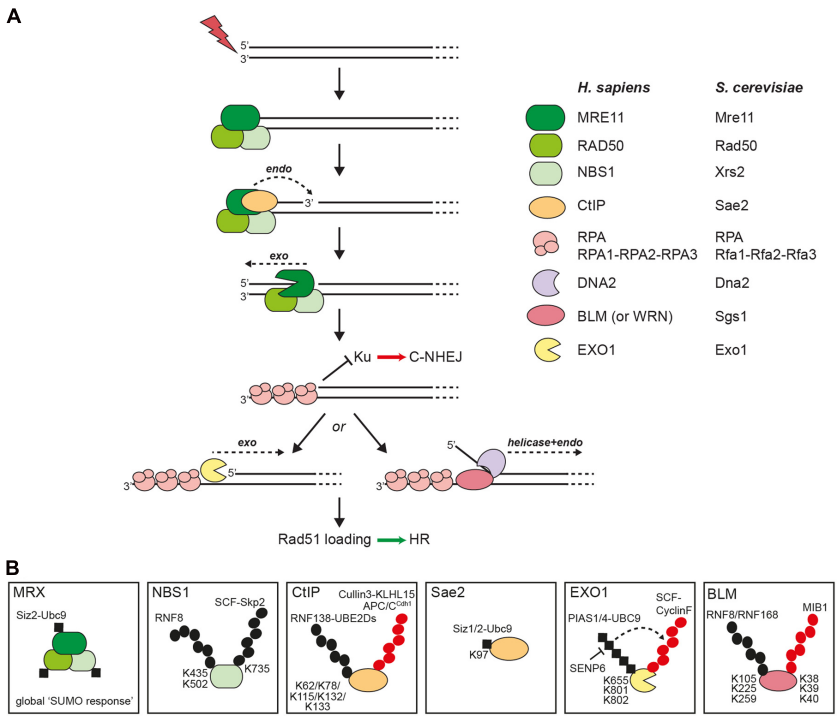

FIGURE 1 | DNA-end resection factors are modified by ubiquitin and SUMO. (A) Simplified scheme of the bidirectional DNA-end resection model. Upon DSB induction the MRX/N complex rapidly localizes to the damaged site. During S and G2 phases of the cell cycle, DNA-end resection is needed for the repair of DSBs via homologous recombination (HR). According to the newest biochemical evidence in yeast, MRX and Sae2 collaborate in the initiation of DNA-end resection through endonucleolytic cleavage of the 5′-terminated strand upstream from the DSB end. Starting from the nick, the exonuclease activity of Mre11 is then supposed to degrade DNA in a 3′ to 5′ direction back toward the DSB end. The resulting single-stranded DNA (ssDNA) overhang is immediately coated by RPA to protect the ssDNA from degradation. The 5′-recessed end now represents a preferred substrate for the 5′ to 3′ exonuclease Exo1 to carry out more processive resection. Alternatively, extended resection is catalyzed by the combined endonuclease and helicase activities of Dna2-Sgs1 in yeast or DNA2-BLM (or WRN) in human cells. Importantly, processed DSB ends are no longer a substrate for Ku binding, a prerequisite for DSB repair by classical non-homologous end joining (C-NHEJ). Ultimately, RPA is removed from ssDNA and replaced by the Rad51 recombinase to initiate strand invasion of the sister chromatid and further downstream steps in HR. **(B)** Schematic illustration of selected resection factors undergoing ubiquitylation and/or sumoylation. Please refer to the main text for details. Black dots, ubiquitin modifications involved in modulating protein function; red dots, ubiquitin modifications involved in protein degradation; black squares, SUMO modification; K, ubiquitin- or SUMO-modified lysine residues in substrate proteins.

heterodimeric RING-type E3 ligase BRCA1/BARD1 ubiquitylates CtIP to promote its stable retention at sites of DNA damage (Yu et al., 2006). However, more recent data indicated that BRCA1 specifically ubiquitylates histone H2A, thereby rendering the chromatin permissive for long-range resection after initial resection by CtIP-MRN has occurred (Kalb et al., 2014; Densham et al., 2016). Moreover, Schmidt et al. (2015) demonstrated that RNF138 in complex with the UBE2D family of E2 conjugating enzymes interacts with CtIP to foster its ubiquitylation and accumulation at DSBs (**Figure 1B**). The authors further observed that depletion of pivotal RING-type E3 ligases involved in the DDR including BRCA1, RNF8 and RNF168 does not compromise DNA damage accrual of CtIP (Schmidt et al., 2015). Therefore, the physiological role of BRCA1-dependent CtIP ubiquitylation in DNA repair still remains to be determined (Barber and Boulton, 2006). Mass spectrometry analysis of CtIP from irradiated cells revealed 13 potential ubiquitylation sites (Schmidt et al., 2015). Furthermore, the same authors found that CtIP polyubiquitylation and redistribution to DSBs was impaired in cells expressing a CtIP mutant in which five N-terminal lysine residues were simultaneously substituted with arginines (5KR) (Schmidt et al., 2015). Finally, as ectopic expression of CtIP-5KR did not restore DNA-end resection in CtIP-depleted cells, it was proposed that ubiquitylation of CtIP by RNF138-UBE2D is a key event in promoting HR (Schmidt et al., 2015). Further support for a pro-resection function of RNF138 emerged from another study showing that RNF138 ubiquitylates Ku80 to facilitate the removal of Ku from DSBs, thereby allowing access of the DNA-end resection machinery and subsequent HR (Ismail et al., 2015). Taken together, one could envision that RNF138-mediated CtIP recruitment to, and Ku displacement from DSBs act in parallel to promote DNA-end resection. Yet it may be possible that RNF138 targets additional proteins involved in DSB repair pathway choice (Bekker-Jensen and Mailand, 2015). Interestingly, two independent studies have reported that the deubiquitinase (DUB) activity of USP4 functions in DNA-end resection (Liu et al., 2015; Wijnhoven et al., 2015). They both demonstrated that USP4 interacts with CtIP and MRN and regulates the recruitment of CtIP to DSBs. However, they further observed that USP4 auto-deubiquitylation

rather than USP4-mediated deubiquitylation of CtIP is essential for HR.

The anaphase-promoting complex/cyclosome-Cdh1 (APC/C^{Cdh1}) E3 ubiquitin ligase was shown to control cell cycle-dependent repair of DSBs by specifically targeting CtIP for proteasomal degradation after mitotic exit as well as after DNA damage in G2 phase (**Figure 1B**) (Lafranchi et al., 2014). Conceivably, such a mechanism would counteract resection of DSBs and allow efficient C-NHEJ in G1 cells, where the intact sister chromatid is not available for HR. Consistently, it was demonstrated that expression of a CtIP mutant defective in Cdh1 interaction abolished CtIP ubiquitylation, leading to its accumulation and prolonged retention at DSBs, oversized DNA-end resection and impaired DSB repair (Lafranchi et al., 2014). Furthermore, a similar cell cycle-dependent mechanism resulting in ubiquitin-mediated proteolysis of CtIP was shown to involve the peptidyl-prolyl *cis*/*trans* isomerase PIN1 (Steger et al., 2013). Following DSB induction in G2, PIN1 was found to specifically interact with CtIP through two phosphorylated S/T-P motifs, leading to its ubiquitylation and subsequent proteasomal degradation (Steger et al., 2013). Consequently, the PIN1-CtIP axis was equally proposed to antagonize DNA-end resection, particularly in situations where NHEJ is the preferred pathway. Moreover, it has been suggested that PIN1-mediated CtIP isomerization triggers a conformational change which facilitates the binding of a E3 ubiquitin ligase (Sartori and Steger, 2013). Our most recent findings point toward a role for the Cullin3 (CUL3) E3 ubiquitin ligase in cooperating with PIN1 in the regulation of CtIP protein stability (**Figure 1B**) (Ferretti et al., 2016). In brief, we discovered that the CUL3 substrate adaptor Kelch-like protein 15 (KLHL15) interacts with CtIP to promote its degradation via the ubiquitin-proteasome pathway. Accordingly, we observed that DNA-end resection is strongly decreased in cells overexpressing KLHL15 but enhanced in cells lacking KLHL15, thus impacting the balance between HR and NHEJ.

Using reconstituted SUMO conjugating systems, both CtIP and Sae2 were found to be sumoylated (Sarangi et al., 2015). Moreover, Ubc9-Siz1/2-mediated Sae2 sumoylation at a single conserved lysine residue (K97) was induced by DNA damage and found to increase the levels of soluble Sae2 (**Figure 1B**). Further genetic analysis revealed that Sae2-K97R mutant cells are impaired in the processing and repair of DSBs, indicating that Sae2 sumoylation is critical for DNA-end resection.

In summary, ubiquitin and SUMO control CtIP/Sae2 resection function at various levels, including its redistribution at DSBs, protein-protein interactions and protein stability.

EXO1 5′ to 3′ Exonuclease

It has been known for quite some time that human EXO1 is targeted for degradation by the ubiquitin-proteasome pathway in response to treatment with agents that block DNA replication (El-Shemerly et al., 2005, 2008). Interestingly, work from the same group could recently demonstrate that EXO1 is constitutively sumoylated by PIAS1/4-UBC9 *in vitro* and *in vivo* and that this is a prerequisite for ubiquitin-mediated EXO1 degradation at stalled replication forks avoiding excessive resection of free DNA ends (**Figure 1B**) (Bologna et al., 2015). Moreover, they found that the SENP6 de-sumoylating enzyme interacts with EXO1 to antagonize this process. However, since mutating three major SUMO acceptor sites in EXO1 did not effectively rescue EXO1 degradation it remains to be determined how, mechanistically, EXO1 sumoylation controls its enzymatic activity (Bologna et al., 2015). Consistent with these findings, Elia et al. (2015) reported that EXO1 is ubiquitylated and degraded by the proteasome in response to replication stress induced by UV radiation and 4NQO. They further identified EXO1 as a new substrate of the SCF-Cyclin F E3 ubiquitin ligase, which possibly mediates EXO1 degradation to prevent unwanted resection of stalled forks (Elia et al., 2015). Finally, adding another layer of complexity to the regulation of EXO1 by ubiquitin and SUMO, Nishi et al. (2014) recently discovered that the proteasome-associated DUB UCHL5 contributes to DNA-end resection, at least in part, by regulating the recruitment of EXO1 (but not CtIP) to sites of DNA damage.

DNA2/Dna2 Structure-Specific Endonuclease

More than 20 potential ubiquitylation sites on human DNA2 have so far been identified in different mass spectrometry approaches, but their role in DNA damage/repair has not yet been experimentally addressed (Kim et al., 2011; Wagner et al., 2011; Mertins et al., 2013).

BLM/Sgs1 3′ to 5′ DNA Helicase

Besides promoting long-range resection of DSBs in conjunction with DNA2, BLM has important functions in other DNA metabolic pathways including DNA replication, telomere maintenance and transcription (Croteau et al., 2014). BLM

TABLE 1 | DNA-end resection proteins targeted by ubiquitin or SUMO E3 ligases.

DNA-end resection factor	E3 Ligase	Modification	Reference
Mre11/Rad50/Xrs2	*global sumoylation response*	SUMO	Cremona et al., 2012; Psakhye and Jentsch, 2012
NBS1	RNF8	Ubiquitin	Lu et al., 2012
	SCF^{Skp2}	Ubiquitin	Wu et al., 2012
CtIP	BRCA1/BARD1	Ubiquitin	Yu et al., 2006
	$CUL3^{KLHL15}$	Ubiquitin	Ferretti et al., 2016
	APC/C^{Cdh1}	Ubiquitin	Lafranchi et al., 2014
	RNF138	Ubiquitin	Schmidt et al., 2015
Sae2	Siz1/2	SUMO	Sarangi et al., 2015
BLM	RNF8 and RNF168	Ubiquitin	Tikoo et al., 2013
	MIB-1	Ubiquitin	Blackford et al., 2015; Wang et al., 2013
EXO1	PIAS1/4	SUMO	Bologna et al., 2015
	$SCF^{CyclinF}$	Ubiquitin	Elia et al., 2015

sumoylation and ubiquitylation has previously been proposed to control its spatiotemporal localization and to promote or suppress HR particularly in the context of stalled replication forks (Eladad et al., 2005; Ouyang et al., 2013; Tikoo et al., 2013). Akin to these observations, Sgs1 and BLM nuclear foci formation in response to hydroxyurea (HU) treatment was found to be negatively regulated by the SUMO-targeted ubiquitin ligase complexes Slx5-Slx8 and RNF4 in yeast and mammalian cells, respectively (Böhm et al., 2015). Moreover, Tikoo et al. (2013) reported that cells lacking either RNF8 or RNF168 E3 ligases failed to efficiently promote K63-linked ubiquitylation of BLM following HU exposure, which is otherwise required for BLM-RAP80 interaction and, thus, BLM recruitment to damaged chromatin (**Figure 1B**). A controversial issue relates to the question as to whether or not TOPBP1-BLM interaction, which is important for genome maintenance, protects BLM from MIB1-mediated ubiquitylation and subsequent proteasomal degradation when cells encounter DNA damage during S phase (**Figure 1B**) (Wang et al., 2013, 2015; Blackford et al., 2015).

Notably, following DNA-end resection, the RPA-ssDNA platform becomes extensively modified by ubiquitin and SUMO to promote checkpoint activation and HR in both yeast and human cells, as it has been recently reviewed elsewhere (Maréchal and Zou, 2015; Schwertman et al., 2016). Finally, emerging data from the Durocher lab demonstrates that ubiquitylation of PALB2, a major binding partner of BRCA2, by the E3 ligase $CUL3^{KEAP1}$ blocks its interaction with BRCA1 and, consequently, the recruitment of BRCA2 to DSBs, thereby suppressing HR in G1 cells (Orthwein et al., 2015).

CONCLUSION AND FUTURE PERSPECTIVES

The key discovery that E3 ubiquitin ligases RNF8 and RNF168 play an integral part in the crosstalk between chromatin state and DNA damage signaling has opened the door for scientists to investigate how ubiquitin and SUMO orchestrate DSB repair pathways. In the last few years, it became clear that RNF8-RNF168-mediated ubiquitylation of histones mainly serves to generate recruitment platforms for the coordinated assembly of various ubiquitin-binding domain (UBD)-containing proteins (e.g., 53BP1) to DSB sites (Schwertman et al., 2016). In contrast, ubiquitin-mediated recruitment seems to play a minor role in the regulation of DNA-end resection, which is further supported by the fact that resection factors are devoid of any canonical UBDs. Although Murina et al. (2014) reported that CtIP can interact with ubiquitin *in vitro*, further investigations are clearly needed to establish a role for CtIP-ubiquitin interaction in DNA-end resection.

Our survey revealed that ubiquitylation and sumoylation of DNA-end resection factors predominantly influences protein stability, thereby facilitating their timely removal to enable the completion of HR (**Table 1**). Another emerging theme is that ubiquitin-mediated proteolysis of resection proteins is dependent on the cell cycle stage and may therefore need to be primed by an upstream phosphorylation event. In other words, an important challenge for the future will be to investigate whether and how ubiquitin and SUMO are able to fine-tune nuclease and/or helicase activities of specific resection enzymes. Current evidence suggests that SUMO may preferentially function as an intermolecular 'glue' in modulating protein-protein or protein-DNA interactions required for HR rather than specifically affecting the activity of individual proteins (Sarangi and Zhao, 2015). Finally, there is only very limited data available yet regarding the role of deconjugating enzymes in DSB repair. As they belong to a family of cysteine proteases and are therefore considered more 'druggable' than E3 ligases, the identification of DUBs or SENPs promoting DNA-end resection and HR could provide a new basis for the development of inhibitors for targeted cancer therapy (Hühn et al., 2013; Carvalho and Kanaar, 2014; D'Arcy et al., 2015).

AUTHOR CONTRIBUTIONS

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

FUNDING

Our research is supported by the Swiss National Science Foundation (SNSF Project Grant 31003A_156023) and the Promedica Foundation.

ACKNOWLEDGMENTS

We apologize to authors whose work could not be cited due to space limitations. We thank Antonio Porro for critical discussion and helpful advice on the manuscript.

REFERENCES

Barber, L. J., and Boulton, S. J. (2006). BRCA1 ubiquitylation of CtIP: just the tIP of the iceberg? *DNA Repair (Amst.)* 5, 1499–1504. doi: 10.1016/j.dnarep.2006.08.009

Bekker-Jensen, S., and Mailand, N. (2015). RNF138 joins the HR team. *Nat. Cell Biol.* 17, 1375–1377. doi: 10.1038/ncb3262

Bergink, S., and Jentsch, S. (2009). Principles of ubiquitin and SUMO modifications in DNA repair. *Nature* 458, 461–467. doi: 10.1038/nature07963

Berndsen, C. E., and Wolberger, C. (2014). New insights into ubiquitin E3 ligase mechanism. *Nat. Struct. Mol. Biol.* 21, 301–307. doi: 10.1038/nsmb.2780

Blackford, A. N., Nieminuszczy, J., Schwab, R. A., Galanty, Y., Jackson, S. P., and Niedzwiedz, W. (2015). TopBP1 interacts with BLM to maintain genome stability but is dispensable for preventing BLM degradation. *Mol. Cell* 57, 1133–1141. doi: 10.1016/j.molcel.2015.02.012

Böhm, S., Mihalevic, M. J., Casal, M. A., and Bernstein, K. A. (2015). Disruption of SUMO-targeted ubiquitin ligases Slx5-Slx8/RNF4 alters RecQ-like helicase

Sgs1/BLM localization in yeast and human cells. *DNA Repair (Amst.)* 26, 1–14. doi: 10.1016/j.dnarep.2014.12.004

Bologna, S., Altmannova, V., Valtorta, E., Koenig, C., Liberali, P., Gentili, C., et al. (2015). Sumoylation regulates EXO1 stability and processing of DNA damage. *Cell Cycle* 14, 2439–2450. doi: 10.1080/15384101.2015.1060381

Brown, J. S., and Jackson, S. P. (2015). Ubiquitylation, neddylation and the DNA damage response. *Open Biol.* 5:150018. doi: 10.1098/rsob.150018

Bunting, S. F., and Nussenzweig, A. (2013). End-joining, translocations and cancer. *Nat. Rev. Cancer* 13, 443–454. doi: 10.1038/nrc3537

Carvalho, J. F. S., and Kanaar, R. (2014). Targeting homologous recombination-mediated DNA repair in cancer. *Expert Opin. Ther. Targets* 18, 427–458. doi: 10.1517/14728222.2014.882900

Ceccaldi, R., Rondinelli, B., and D'Andrea, A. D. (2016). Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol.* 26, 52–64. doi: 10.1016/j.tcb.2015.07.009

Cejka, P. (2015). DNA end resection: nucleases team up with the right partners to initiate homologous recombination. *J. Biol. Chem.* 290, 22931–22938. doi: 10.1074/jbc.R115.675942

Chapman, J. R., Taylor, M. R. G., and Boulton, S. J. (2012). Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* 47, 497–510. doi: 10.1016/j.molcel.2012.07.029

Chen, Y.-J., Chuang, Y.-C., Chuang, C.-N., Cheng, Y.-H., Chang, C.-R., Leng, C.-H., et al. (2016). *S. cerevisiae* Mre11 recruits conjugated SUMO moieties to facilitate the assembly and function of the Mre11-Rad50-Xrs2 complex. *Nucleic Acids Res.* 44, 2199–2213. doi: 10.1093/nar/gkv1523

Chiruvella, K. K., Liang, Z., and Wilson, T. E. (2013). Repair of double-strand breaks by end joining. *Cold Spring Harb. Perspect. Biol.* 5:a012757. doi: 10.1101/cshperspect.a012757

Ciccia, A., and Elledge, S. J. (2010). The DNA damage response: making it safe to play with knives. *Mol. Cell* 40, 179–204. doi: 10.1016/j.molcel.2010.09.019

Cremona, C. A., Sarangi, P., Yang, Y., Hang, L. E., Rahman, S., and Zhao, X. (2012). Extensive DNA damage-induced sumoylation contributes to replication and repair and acts in addition to the mec1 checkpoint. *Mol. Cell* 45, 422–432. doi: 10.1016/j.molcel.2011.11.028

Croteau, D. L., Popuri, V., Opresko, P. L., and Bohr, V. A. (2014). Human RecQ helicases in DNA repair, recombination, and replication. *Annu. Rev. Biochem.* 83, 519–552. doi: 10.1146/annurev-biochem-060713-035428

Daley, J. M., Niu, H., Miller, A. S., and Sung, P. (2015). Biochemical mechanism of DSB end resection and its regulation. *DNA Repair (Amst.)* 32, 66–74. doi: 10.1016/j.dnarep.2015.04.015

D'Arcy, P., Wang, X., and Linder, S. (2015). Deubiquitinase inhibition as a cancer therapeutic strategy. *Pharmacol. Ther.* 147, 32–54. doi: 10.1016/j.pharmthera.2014.11.002

Densham, R. M., Garvin, A. J., Stone, H. R., Strachan, J., Baldock, R. A., Daza-Martin, M., et al. (2016). Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection. *Nat. Struct. Mol. Biol.* 23, 647–655. doi: 10.1038/nsmb.3236

Eladad, S., Ye, T.-Z., Hu, P., Leversha, M., Beresten, S., Matunis, M. J., et al. (2005). Intra-nuclear trafficking of the BLM helicase to DNA damage-induced foci is regulated by SUMO modification. *Hum. Mol. Genet.* 14, 1351–1365. doi: 10.1093/hmg/ddi145

Elia, A. E. H., Boardman, A. P., Wang, D. C., Huttlin, E. L., Everley, R. A., Dephoure, N., et al. (2015). Quantitative proteomic atlas of ubiquitination and acetylation in the DNA damage response. *Mol. Cell* 59, 867–881. doi: 10.1016/j.molcel.2015.05.006

El-Shemerly, M., Hess, D., Pyakurel, A. K., Moselhy, S., and Ferrari, S. (2008). ATR-dependent pathways control hEXO1 stability in response to stalled forks. *Nucleic Acids Res.* 36, 511–519. doi: 10.1093/nar/gkm1052

El-Shemerly, M., Janscak, P., Hess, D., Jiricny, J., and Ferrari, S. (2005). Degradation of human exonuclease 1b upon DNA synthesis inhibition. *Cancer Res.* 65, 3604–3609. doi: 10.1158/0008-5472.CAN-04-4069

Ferretti, L. P., Himmels, S., Trenner, A., Walker, C., von Aesch, C., Eggenschwiler, A., et al. (2016). Cullin3-KLHL15 ubiquitin ligase mediates CtIP protein turnover to fine-tune DNA-end resection. *Nat. Commun.* (in press).

Ferretti, L. P., Lafranchi, L., and Sartori, A. A. (2013). Controlling DNA-end resection: a new task for CDKs. *Front. Genet.* 4:99. doi: 10.3389/fgene.2013.00099

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

Forment, J. V., Kaidi, A., and Jackson, S. P. (2012). Chromothripsis and cancer: causes and consequences of chromosome shattering. *Nat. Rev. Cancer* 12, 663–670. doi: 10.1038/nrc3352

Graham, T. G. W., Walter, J. C., and Loparo, J. J. (2016). Two-stage synapsis of DNA ends during non-homologous end joining. *Mol. Cell* 61, 850–858. doi: 10.1016/j.molcel.2016.02.010

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

Hay, R. T. (2013). Decoding the SUMO signal. *Biochem. Soc. Trans.* 41, 463–473. doi: 10.1042/BST20130015

Hühn, D., Bolck, H. A., and Sartori, A. A. (2013). Targeting DNA double-strand break signalling and repair: recent advances in cancer therapy. *Swiss Med. Wkly.* 143, w13837. doi: 10.4414/smw.2013.13837

Ismail, I. H., Gagné, J.-P., Genois, M.-M., Strickfaden, H., McDonald, D., Xu, Z., et al. (2015). The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. *Nat. Cell Biol.* 17, 1446–1457. doi: 10.1038/ncb3259

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078. doi: 10.1038/nature08467

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jasin, M., and Rothstein, R. (2013). Repair of strand breaks by homologous recombination. *Cold Spring Harb. Perspect. Biol.* 5:a012740. doi: 10.1101/cshperspect.a012740

Kalb, R., Mallery, D. L., Larkin, C., Huang, J. T. J., and Hiom, K. (2014). BRCA1 is a histone-H2A-specific ubiquitin ligase. *Cell Rep.* 8, 999–1005. doi: 10.1016/j.celrep.2014.07.025

Karanam, K., Kafri, R., Loewer, A., and Lahav, G. (2012). Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. *Mol. Cell* 47, 320–329. doi: 10.1016/j.molcel.2012.05.052

Kim, W., Bennett, E. J., Huttlin, E. L., Guo, A., Li, J., Possemato, A., et al. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* 44, 325–340. doi: 10.1016/j.molcel.2011.08.025

Komander, D., and Rape, M. (2012). The ubiquitin code. *Annu. Rev. Biochem.* 81, 203–229. doi: 10.1146/annurev-biochem-060310-170328

Lafranchi, L., de Boer, H. R., de Vries, E. G. E., Ong, S.-E., Sartori, A. A., and van Vugt, M. A. T. M. (2014). APC/C(Cdh1) controls CtIP stability during the cell cycle and in response to DNA damage. *EMBO J.* 33, 2860–2879. doi: 10.15252/embj.201489017

Liu, H., Zhang, H., Wang, X., Tian, Q., Hu, Z., Peng, C., et al. (2015). The deubiquitylating enzyme USP4 cooperates with CtIP in DNA double-strand break end resection. *Cell Rep.* 13, 93–107. doi: 10.1016/j.celrep.2015.08.056

Lu, C.-S., Truong, L. N., Aslanian, A., Shi, L. Z., Li, Y., Hwang, P. Y.-H., et al. (2012). The RING finger protein RNF8 ubiquitinates Nbs1 to promote DNA double-strand break repair by homologous recombination. *J. Biol. Chem.* 287, 43984–43994. doi: 10.1074/jbc.M112.421545

Maréchal, A., and Zou, L. (2015). RPA-coated single-stranded DNA as a platform for post-translational modifications in the DNA damage response. *Cell Res.* 25, 9–23. doi: 10.1038/cr.2014.147

Mertins, P., Qiao, J. W., Patel, J., Udeshi, N. D., Clauser, K. R., Mani, D. R., et al. (2013). Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat. Methods* 10, 634–637. doi: 10.1038/nmeth.2518

Murina, O., von Aesch, C., Karakus, U., Ferretti, L. P., Bolck, H. A., Hänggi, K., et al. (2014). FANCD2 and CtIP cooperate to repair DNA interstrand crosslinks. *Cell Rep.* 7, 1030–1038. doi: 10.1016/j.celrep.2014.03.069

Nishi, R., Wijnhoven, P., le Sage, C., Tjeertes, J., Galanty, Y., Forment, J. V., et al. (2014). Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. *Nat. Cell Biol.* 16, 1016–1026. doi: 10.1038/ncb3028

Orthwein, A., Noordermeer, S. M., Wilson, M. D., Landry, S., Enchev, R. I., Sherker, A., et al. (2015). A mechanism for the suppression of homologous recombination in G1 cells. *Nature* 528, 422–426. doi: 10.1038/nature16142

Ouyang, K. J., Yagle, M. K., Matunis, M. J., and Ellis, N. A. (2013). BLM SUMOylation regulates ssDNA accumulation at stalled replication forks. *Front. Genet.* 4:167. doi: 10.3389/fgene.2013.00167

Psakhye, I., and Jentsch, S. (2012). Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* 151, 807–820. doi: 10.1016/j.cell.2012.10.021

Radhakrishnan, S. K., Jette, N., and Lees-Miller, S. P. (2014). Non-homologous end joining: emerging themes and unanswered questions. *DNA Repair (Amst.)* 17, 2–8. doi: 10.1016/j.dnarep.2014.01.009

Rodgers, K., and McVey, M. (2016). Error-prone repair of DNA double-strand breaks. *J. Cell. Physiol.* 231, 15–24. doi: 10.1002/jcp.25053

Ronau, J. A., Beckmann, J. F., and Hochstrasser, M. (2016). Substrate specificity of the ubiquitin and Ubl proteases. *Cell Res.* 26, 441–456. doi: 10.1038/cr.2016.38

Sarangi, P., Steinacher, R., Altmannova, V., Fu, Q., Paull, T. T., Krejci, L., et al. (2015). Sumoylation influences DNA break repair partly by increasing the solubility of a conserved end resection protein. *PLoS Genet.* 11:e1004899. doi: 10.1371/journal.pgen.1004899

Sarangi, P., and Zhao, X. (2015). SUMO-mediated regulation of DNA damage repair and responses. *Trends Biochem. Sci.* 40, 233–242. doi: 10.1016/j.tibs.2015.02.006

Sartori, A. A., and Steger, M. (2013). Prolyl isomerization: a new PIN code for DSB repair. *Cell Cycle* 12, 2717–2718. doi: 10.4161/cc.26077

Schmidt, C. K., Galanty, Y., Sczaniecka-Clift, M., Coates, J., Jhujh, S., Demir, M., et al. (2015). Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. *Nat. Cell Biol.* 17, 1458–1470. doi: 10.1038/ncb3260

Schwertman, P., Bekker-Jensen, S., and Mailand, N. (2016). Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat. Rev. Mol. Cell Biol.* 17, 379–394. doi: 10.1038/nrm.2016.58

Steger, M., Murina, O., Hühn, D., Ferretti, L. P., Walser, R., Hänggi, K., et al. (2013). Prolyl isomerase PIN1 regulates DNA double-strand break repair by counteracting DNA end resection. *Mol. Cell* 50, 333–343. doi: 10.1016/j.molcel.2013.03.023

Stewart, M. D., Ritterhoff, T., Klevit, R. E., and Brzovic, P. S. (2016). E2 enzymes: more than just middle men. *Cell Res.* 26, 423–440. doi: 10.1038/cr.2016.35

Sturzenegger, A., Burdova, K., Kanagaraj, R., Levikova, M., Pinto, C., Cejka, P., et al. (2014). DNA2 cooperates with the WRN and BLM RecQ helicases to mediate long-range DNA end resection in human cells. *J. Biol. Chem.* 289, 27314–27326. doi: 10.1074/jbc.M114.578823

Swatek, K. N., and Komander, D. (2016). Ubiquitin modifications. *Cell Res.* 26, 399–422. doi: 10.1038/cr.2016.39

Symington, L. S. (2014). End resection at double-strand breaks: mechanism and regulation. *Cold Spring Harb. Perspect. Biol.* 6:a016436. doi: 10.1101/cshperspect.a016436

Symington, L. S. (2016). Mechanism and regulation of DNA end resection in eukaryotes. *Crit. Rev. Biochem. Mol. Biol.* 51, 195–212. doi: 10.3109/10409238.2016.1172552

Symington, L. S., and Gautier, J. (2011). Double-strand break end resection and repair pathway choice. *Annu. Rev. Genet.* 45, 247–271. doi: 10.1146/annurev-genet-110410-132435

Tikoo, S., Madhavan, V., Hussain, M., Miller, E. S., Arora, P., Zlatanou, A., et al. (2013). Ubiquitin-dependent recruitment of the Bloom syndrome helicase upon replication stress is required to suppress homologous recombination. *EMBO J.* 32, 1778–1792. doi: 10.1038/emboj.2013.117

Tkáč, J., Xu, G., Adhikary, H., Young, J. T. F., Gallo, D., Escribano-Díaz, C., et al. (2016). HELB is a feedback inhibitor of DNA end resection. *Mol. Cell* 61, 405–418. doi: 10.1016/j.molcel.2015.12.013

Tomimatsu, N., Mukherjee, B., Catherine Hardebeck, M., Ilcheva, M., Vanessa Camacho, C., Louise Harris, J., et al. (2014). Phosphorylation of EXO1 by CDKs 1 and 2 regulates DNA end resection and repair pathway choice. *Nat. Commun.* 5:3561. doi: 10.1038/ncomms4561

Wagner, S. A., Beli, P., Weinert, B. T., Nielsen, M. L., Cox, J., Mann, M., et al. (2011). A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol. Cell Proteomics* 10:M111.013284. doi: 10.1074/mcp.M111.013284

Wang, J., Chen, J., and Gong, Z. (2013). TopBP1 controls BLM protein level to maintain genome stability. *Mol. Cell* 52, 667–678. doi: 10.1016/j.molcel.2013.10.012

Wang, J., Chen, J., and Gong, Z. (2015). TopBP1 stabilizes BLM protein to suppress sister chromatid exchange. *Mol. Cell* 57, 955–956. doi: 10.1016/j.molcel.2015.02.011

Wijnhoven, P., Konietzny, R., Blackford, A. N., Travers, J., Kessler, B. M., Nishi, R., et al. (2015). USP4 auto-deubiquitylation promotes homologous recombination. *Mol. Cell* 60, 362–373. doi: 10.1016/j.molcel.2015.09.019

Wu, J., Zhang, X., Zhang, L., Wu, C.-Y., Rezaeian, A. H., Chan, C.-H., et al. (2012). Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. *Mol. Cell* 46, 351–361. doi: 10.1016/j.molcel.2012.02.018

Yau, R., and Rape, M. (2016). The increasing complexity of the ubiquitin code. *Nat. Cell Biol.* 18, 579–586. doi: 10.1038/ncb3358

Yu, X., Fu, S., Lai, M., Baer, R., and Chen, J. (2006). BRCA1 ubiquitinates its phosphorylation-dependent binding partner CtIP. *Genes Dev.* 20, 1721–1726. doi: 10.1101/gad.1431006

Conflict of Interest Statement: 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.

The reviewer WN and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

frontiers in Genetics

REVIEW
published: 28 July 2016
doi: 10.3389/fgene.2016.00133

DUBbing Cancer: Deubiquitylating Enzymes Involved in Epigenetics, DNA Damage and the Cell Cycle As Therapeutic Targets

Adan Pinto-Fernandez and Benedikt M. Kessler*

Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK

Controlling cell proliferation is one of the hallmarks of cancer. A number of critical checkpoints ascertain progression through the different stages of the cell cycle, which can be aborted when perturbed, for instance by errors in DNA replication and repair. These molecular checkpoints are regulated by a number of proteins that need to be present at the right time and quantity. The ubiquitin system has emerged as a central player controlling the fate and function of such molecules such as cyclins, oncogenes and components of the DNA repair machinery. In particular, proteases that cleave ubiquitin chains, referred to as deubiquitylating enzymes (DUBs), have attracted recent attention due to their accessibility to modulation by small molecules. In this review, we describe recent evidence of the critical role of DUBs in aspects of cell cycle checkpoint control, associated DNA repair mechanisms and regulation of transcription, representing pathways altered in cancer. Therefore, DUBs involved in these processes emerge as potentially critical targets for the treatment of not only hematological, but potentially also solid tumors.

Keywords: ubiquitin, deubiquitylating enzyme, transcription, epigenetics, DNA damage response, small molecule inhibitors, multiple myeloma, cell cycle checkpoints

OPEN ACCESS

Edited by:
Ivan Dikic,
Goethe Business School, Germany

Reviewed by:
Henning Walczak,
Imperial College London, UK
Ramin Massoumi,
Lund University, Sweden

****Correspondence:***
Adan Pinto-Fernandez
adan.pintofernandez@ndm.ox.ac.uk

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *31 March 2016*
Accepted: *12 July 2016*
Published: *28 July 2016*

Citation:
Pinto-Fernandez A and Kessler BM (2016) DUBbing Cancer: Deubiquitylating Enzymes Involved in Epigenetics, DNA Damage and the Cell Cycle As Therapeutic Targets. Front. Genet. 7:133. doi: 10.3389/fgene.2016.00133

INTRODUCTION

Posttranslational modifications dictate the fate and function of most proteins. Chemical modifications by phosphate groups and ubiquitin, a small 76 amino acid protein, are amongst the most common ones. Targeting enzymes that modulate protein phosphorylation, such as protein kinases, has been proven to be a suitable inroad to novel anti-cancer therapeutics. In the case of the ubiquitin system, drug development efforts have been lagging behind due to the complexity of the ubiquitin conjugating and deconjugating mechanisms, and because many aspects of the fundamental biology of this pathway, in particular the topology of poly ubiquitin chains and post-translational modifications present on ubiquitin itself, are not yet fully understood (Cohen and Tcherpakov, 2010; Swatek and Komander, 2016). Despite this, the clinical approval of the proteasome inhibitors Bortezomib, Carfilzomib, and Ixazomib has boosted new drug discovery programs targeting different components of the ubiquitin system (Adams, 2002; Cohen and Tcherpakov, 2010; Ernst et al., 2013; Herndon et al., 2013; Shirley, 2016).

The ubiquitin system is involved in the regulation of almost every cellular activity through proteolytic and non-proteolytic events, including protein degradation by the 26S proteasome or through the lysosomal pathway and autophagy, protein–protein interactions, protein activity and protein localization (Herndon et al., 2013; Swatek and Komander, 2016). The covalent attachment of ubiquitin to a target protein is catalyzed by the sequential action of three enzymes: E1 activating enzyme, E2 conjugating enzyme and E3 ligase. In the final ubiquitylation step, Ub is usually transferred to an ε-NH_2 of a lysine residue in the target protein. The addition of one or more ubiquitin monomers to another substrate-attached ubiquitin is possible and leads to formation of polymeric chains. There are different types of Ub polymers depending on the linkage and on the topology of the chain. The process is reversible and the removal of ubiquitin is catalyzed by a subclass of isopeptidases referred to as deubiquitylating enzymes or DUBs (Hershko and Ciechanover, 1998; Komander and Rape, 2012; Mevissen et al., 2013; Swatek and Komander, 2016).

DEUBIQUITYLATING ENZYMES

There are ∼90 DUBs encoded in the human genome, which are sub classified into seven different families: ubiquitin-specific proteases (USPs), ubiquitin carboxy-terminal hydrolases (UCHs), ovarian tumor domain con-taining proteases (OTUs), Machado-Joseph disease protein domain proteases (MJD), JAMM/MPN domain-associated metallopeptidases (JAMMs), the monocyte chemo-tactic protein-induced protein (MCPIP) and the motif interacting with Ub-containing novel DUB family (MINDY). Apart from the JAMMs family which has zinc metalloprotease activity, DUBs are cysteine proteases (Komander et al., 2009; Fraile et al., 2012; Kolattukudy and Niu, 2012; Abdul Rehman et al., 2016).

DUB hydrolase activity (predominantly IsoT/USP5) is required to generate free ubiquitin from its precursors because Ub is transcribed as a fusion of multiple Ub molecules or as a fusion with other proteins (Hadari et al., 1992). Keeping a steady-state level of free Ub is essential for cell viability (Wang C.H. et al., 2014). Therefore, a second process involving DUBs is the recycling of ubiquitin by preventing its degradation, which is mediated by proteasome associated DUBs USP14, UCH-L5/UCH37, and POH1, or receptor mediated endocytosis and lysosomal degradation associated DUBs USP8 and AMSH (Reyes-Turcu et al., 2009; Lee et al., 2011). More specifically, DUBs antagonize the action of ubiquitin E3 ligases that target protein substrates for degradation or by regulating E3 ligases activity and/or stability. Generally, the addition of Ub monomers or polymers to a protein can also generate non-proteolytic signals. Thus, DUBs can modulate the outcome of those signals by two main mechanisms: by removing ubiquitin polymers or monomers from proteins involved in these signaling events, but also by editing the linkage and topology of the ubiquitin chains present in the substrate (Komander et al., 2009; Bennett, 2010; Fraile et al., 2012; Komander and Rape, 2012).

Deubiquitylating enzymes activity can be tightly regulated by different means, including transcriptional changes in their gene expressions, microRNAs, post-translational modifications including phosphorylation and auto-ubiquitylation, protein interactions and by changing their subcellular localization (Komander et al., 2009; Fraile et al., 2012; Yang et al., 2012; Wijnhoven et al., 2015). Despite that the number of encoded DUBs is moderate, it is anticipated that most of them act on a discrete set of protein substrates due to restrictions in Ub chain linkage recognition as observed for OTUs (Mevissen et al., 2013) or metalloprotease DUBs (Komander et al., 2009), or the requirement of interactions with specific adaptors or scaffold proteins, as noted for JAMMs and a subset of USPs (Ventii and Wilkinson, 2008; Komander et al., 2009; Rahighi et al., 2009; Reyes-Turcu et al., 2009; Bremm et al., 2010; Mevissen et al., 2013).

As we will highlight in this review, DUBs emerge as regulators of many cellular signaling pathways critical for cell survival, proliferation, genome stability, and transcriptional control, all of which are important processes that when altered can contribute to the development of neoplasia and tumorigenesis. In particular, ubiquitylation events linked to chromatin-dependent processes appear to involve a large subset of E3 ligases and DUBs that are recognized to be prominent targets in cancer. The possibility of using small molecule inhibitors against DUBs as inroads for anti-cancer strategies are now receiving a prominent focus in pharma and academia (Nicholson et al., 2007; Sgorbissa et al., 2010; Shi and Grossman, 2010; Edelmann et al., 2011; Fraile et al., 2012; Lim and Baek, 2013; McClurg and Robson, 2015; Lim et al., 2016).

DUBs AFFECTING CHROMATIN FUNCTION

Histone and other chromatin-associated protein modifications, together with DNA methylation, provide the cell with long-term epigenetic gene transcription regulations without affecting its DNA sequence. Deregulation of these processes is a common event in cancer development and progression (Esteller, 2007; Gronbaek et al., 2007; Segal and Widom, 2009; Simo-Riudalbas and Esteller, 2015). Most histones are post-translationally modified by the addition of different molecules including acetylation, methylation, phosphorylation, SUMOylation, and ubiquitylation. All these histone PTMs (post-translational modifications) play an important role in gene transcription regulation and chromatin remodeling, but also in the DNA damage response (DDR; Weake and Workman, 2008; Zhou et al., 2009; Zhang T. et al., 2015). Consequences of histone ubiquitylation have been extensively reviewed (Weake and Workman, 2008; Belle and Nijnik, 2014). Up to 10% of cellular histone H2A is mono-ubiquitylated on lysine 119 (H2AK119Ub), and this modification is crucial for the regulation of transcription, cell cycle progression, and DDRs (Clague et al., 2015). Poly-ubiquitylation of the same histone H2A (and its variant H2AX) on K13/15 is important for the DDR. In the case of Histone H2B, H2BK120Ub has been identified as a marker of gene activation. Other histones are also ubiquitylated, such as histone

H3, which has an important role in nucleosome assembly. These histone modifications seem to be non-redundant, and whereas ubiquitylation of histone H2B is related to transcription activation and silencing, ubiquitylated H2A accumulates at repressed promoters. Not surprisingly, a number of DUBs have been identified as histone modifiers (listed in **Table 1**). Some of these DUBs present specificity for H2A, others are specific for H2B, but many of them present dual specificity toward these two histones (see **Table 1**). The high number of DUBs targeting histones suggests redundant or context-specific roles for these enzymes (Nakagawa et al., 2008; Feng et al., 2010; Cao and Yan, 2012; Lee et al., 2013; Mosbech et al., 2013; Zhang et al., 2013). The regulation of histones by DUBs has already been linked to cancer. Two good examples are USP22 and BAP1. USP22 deubiquitylates both, H2A and H2B (Zhang et al., 2008a,b; Atanassov et al., 2009; Wang and Dent, 2014). In a recent clinicopathological study in colon carcinoma samples, ubiquitylation of H2B (uH2B) was found to be decreased in colon cancers as compared to normal colon epithelium. Interestingly, high expression levels of USP22 in these tumor samples statistically correlated with reduced levels of uH2B (Wang Z. et al., 2015). USP22 has been linked to poor prognosis in cancer, making it a very attractive target in cancer research. Overexpression of this DUB has been found in colorectal cancer (Liu Y.L. et al., 2011), gastric cancer (Yang et al., 2011; He et al., 2015), liver cancer (Tang et al., 2015a,b), breast cancer (Zhang Y. et al., 2011), glioma (Liang J. et al., 2014), pancreatic cancer (He et al., 2015), non-small-cell lung cancer (Hu et al., 2012), salivary adenoid cystic carcinoma (Dai et al., 2014), human pharyngeal squamous cell carcinoma (Dou et al., 2014), and oral squamous cell carcinoma (Piao et al., 2012). BAP1 is a nuclear DUB that targets histone 2A mono-ubiquitylation on lysine 119 (H2AK119ub1; as part of the Polycomb repressor unit; Scheuermann et al., 2010) and regulates histone H3 lysine methylation and chromatin functions (Dey et al., 2012). The forkhead transcription factor FOXK2 acts a scaffold protein between BAP1 and DNA, promoting targeted epigenetic regulation by BAP1 (Ji et al., 2014; Okino et al., 2015). BAP1 has been identified as a tumor suppressor and as a potential prognostic marker for a number of cancer types. Both, germline and somatic mutations and nuclear expression loss of BAP1 have been linked to increased susceptibility and poor prognosis in malignant melanocytic proliferations (Wiesner et al., 2011; Piris et al., 2015), mesothelioma (Bott et al., 2011), basal cell carcinoma (Mochel et al., 2015), meningioma (Abdel-Rahman et al., 2011), lung cancer (Abdel-Rahman et al., 2011), bladder cancer (Nickerson et al., 2014), thymic carcinoma (Wang Y. et al., 2014), and clear cell renal cell carcinoma (Murali et al., 2013). Although the majority of the published studies suggest that the effects of BAP1 in cancer involve a disruption of the epigenetic homeostasis in these tumors (Wang Y. et al., 2014), for some researchers, it is not so clear that the antitumor effect of BAP1 is only dependent on H2A deubiquitylation (Pena-Llopis et al., 2012).

DUBs ROLE IN THE CROSSTALK BETWEEN THE DIFFERENT HISTONE PTMs

The crosstalk between different histone PTMs has been described (Zhang T. et al., 2015). JAMM/MPN, a member of the domain-associated metallopeptidases, plays an important role in gene expression regulation by coordinating acetylation of histones with deubiquitylation of histone H2A and regulating by this way the association of histone H1 with nucleosomes (Zhu et al., 2007). USP22 has been found to be associated with the Spt-Ada-Gcn5-acetyltransferase (SAGA) histone acetyltransferase (HAT) complex. In this context, USP22 deubiquitylates histone H2B and other components of the shelterin complex (Atanassov

TABLE 1 | Selection of deubiquitylating enzymes (DUBs) involved in modulating histone H2A/B ubiquitylation.

DUB	Histone (substrate)	Process	Selected reference
USP3	H2A, γH2AX and H2B	Cell cycle and DNA double-strand break response	Nicassio et al., 2007
USP7	H2A and H2B	Gene expression	van der Knaap et al., 2005; Luo et al., 2015
USP10	H2A (H2A.Z)	Transcriptional activation	Draker et al., 2011
USP12	H2A and H2B	*Xenopus* development	Joo et al., 2011
USP16	H2A	Cell cycle and gene expression	Joo et al., 2007; Gu et al., 2016
USP21	H2A	Transcriptional activation	Nakagawa et al., 2008
USP22	H2A and H2B	Embryonic development and telomere integrity	Zhang et al., 2008a,b; Atanassov et al., 2009; Wang and Dent, 2014
USP29	H2A and H2B	DNA double-strand break response	Mosbech et al., 2013
USP36	H2B	Unknown	Taillebourg et al., 2012
USP44	H2A and H2B	DNA double-strand break response (H2A) and stem cell differentiation (H2B)	Fuchs et al., 2012; Mosbech et al., 2013
USP46	H2A and H2B	*Xenopus* development	Joo et al., 2011
USP49	H2B	Co-transcriptional pre-mRNA processing	Zhang et al., 2013
BAP1	H2A	Gene expression	Scheuermann et al., 2010
OTUB1	Histones (unspecified)	DNA double-strand break response	Sato et al., 2012
BRCC36	H2A and γH2AX	DNA double-strand break response	Shao et al., 2009
MYSM1	H2A	Gene expression	Zhu et al., 2007; Jiang et al., 2015; Li et al., 2016

et al., 2009). USP17 regulates histone acetylation through deubiquitylation of K63-polyubiquitylated SDS3, inhibiting the histone deacetylase activity (HDAC) of SDS3 and subsequently the proliferation and anchorage-independent growth of tumor cells (Ramakrishna et al., 2011, 2012). A recent study describes that USP7 interacts with and deubiquitylates Tip60, an acetyltransferase targeting histones, resulting in its stabilization (Dar et al., 2013). As mentioned above, BAP1 forms together with ASXL1 the PR-DUB complex that removes ubiquitin from H2AK119Ub. In a recent study, LaFave et al. (2015) found that BAP1 deletion in mice increased the levels of tri-methylated histone H3 (H3K27me3) and reduced mono-methylation of the histone H4 (H4K20me1). A member of the OTU family of DUBs called TRABID (also ZRANB1) was found to be an innate immunological regulator of inflammatory T cell responses. TRABID regulates histone methylation (H3K9me2, H3K9me3, and H3K4me3) at the promoter of IL-12 by deubiquitylating and stabilizing the histone demethylase JMJD2D (Jin et al., 2016). In the same study, ectopic expression of TRABID reduced K29, and to a lesser extent K11 ubiquitylation of JMJD2D. This data is consistent with previous published studies describing specificity of this DUB toward K29-linked ubiquitin chains in *in vitro* assays (Virdee et al., 2010; Licchesi et al., 2012). The protein TIP5 is part of the nucleolar remodeling complex (NoRC) that modulates the silencing of a fraction of rDNA by recruiting histone and DNA methyltransferases. TIP5 is deubiquitylated and stabilized by USP21, resulting in an increase of H3K4me3 and rDNA promoter methylation (Khan et al., 2015). It had been previously described that H2A ubiquitylation controls the di- and tri-methylation of H3K4. In the same study, the authors describe the indirect effects of USP21 on H3K4m2, and H3K4m3 modifications through its H2A histone deubiquitylating activity (Nakagawa et al., 2008). An interesting study has recently linked the roles of the DUB USP7 on epigenetic regulation, cell cycle, and DNA repair (Wang Q. et al., 2016). Upon DNA damage, USP7 interacts, deubiquitylates and stabilizes the histone demethylase PHF8, inducing the specific expression of a subset of genes, including the cell cycle regulator cyclin A2. In the same article, USP7, PHF8, and cyclin A2 were found to be overexpressed in breast carcinomas, correlating with the histological grade of disease. USP24 was also found to target histones by controlling the levels of the histone-lysine *N*-methyltransferase Suv39h1, resulting in a modulation of the H3K9me levels. Single-nucleotide polymorphisms (SNPs) of USP24 were found in lung cancer. The variants 930C/T and 7656T/C were increased in tumor samples and were found to induce USP24 expression by stabilizing RNA. (Wang Y.C. et al., 2015). LSD1 (lysine-specific demethylase 1) removes methyl groups from H3K4 and also from H3K9. LSD1 has been found to be upregulated in many tumors and its protein levels regulated by the ubiquitin proteasome system. Using a library of siRNA against all human DUBs, USP28 was found as the DUB involved in the stabilization of LSD1. USP28 interacts and deubiquitylates LSD1, and the expression levels of the two proteins correlate well in tumor cell lines and tumor samples (Wu et al., 2013). In conclusion, all these examples are starting to point toward an emerging and important role of the DUBs in the regulation of gene expression by epigenetic events in cancer.

DUBs and DNA METHYLATION

Only one DUB, USP7, has been described to have a role in the DNA methylation process. USP7 regulates the inheritance of DNA methylation patterns through control of the abundance of Dnmt1, the DNA methyltransferase responsible for this epigenetic mark (Bronner, 2011; Qin et al., 2011). USP7 is part of a protein complex with Dnmt1, the histone acetyl transferase Tip60 and the ubiquitin ligase Uhrf1. USP7 and Urf1 tightly regulate the abundance of Dnmt1 in order to control DNA methylation inheritance and replication of methylation patterns. Supporting the role of ubiquitylation in the maintenance of DNA methylation, Nishiyama et al. (2013) have discovered that the ubiquitylation of histone H3 is necessary to keep the DNA methylation mark by Dnmt1.

DUBs and DNA DAMAGE

In order to achieve the mutational status that leads to malignancy, tumor cells often deregulate the DDR and the genome maintenance systems (Hanahan and Weinberg, 2011). Under normal physiological conditions, cells have sensor proteins that check for damage in their genome, and once these proteins detect the lesion, repair enzymes are recruited to the damage site to promote repair. Small lesions are repaired by base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and the Fanconi anemia (FA) pathways. More harmful damages such as double-stranded breaks (DSBs) can be repaired by non-homologous end joining (NHEJ) or through homologous recombination (HR). All the DDR pathways are tightly regulated by PTMs, which includes ubiquitylation/deubiquitylation events. Therefore, DUBs are involved in multiple DDR checkpoints in addition to their capability to modulate histone modifications (**Table 2** and **Figure 1**; Bennett and Harper, 2008; Jacq et al., 2013; Liu et al., 2016; Nie and Boddy, 2016). FA complementation group D2 protein (FANCD2) and proliferating cell nuclear (PCNA), when mono-ubiquitylated, induce the DDR. USP1 targets FANCD2, FANC1, and PCNA for deubiquitylation. FANCD2 and FANC1 are implicated in the FA pathway and PCNA in the translesion synthesis process (Nijman et al., 2005; Oestergaard et al., 2007; Hendel et al., 2011; Villamil et al., 2013; Kim and Kim, 2016). USP1 mutations and deregulated expression levels have been reported in different tumors (Garcia-Santisteban et al., 2013). The Chk2-p53-PUMA pathway is another regulator of the DDR generated by double-strand breaks. USP28 was found to stabilize two components of this pathway upon DNA damage, Chk2 and 53BP1 (Zhang et al., 2006). The proteasome-associated DUB Rpn11/POH1 has been described as an important regulator of ubiquitin conjugates generated after DNA damage, thereby representing an important component of the double-strand break response (Butler et al., 2012). BRIT1 is an early DDR factor that is recruited upon DSBs by phosphorylated H2AX histone (γ-H2AX), and it contributes to the final repair process by inducing chromatin relaxation. BRIT1 is deubiquitylated and stabilized by USP8 with the help of the scaffold protein BRUCE, tightly regulating the action

TABLE 2 | Deubiquitylating enzymes associated with DNA damage responses (DDR).

DUB	Non-histone substrate	DDR pathway	Selected reference
USP1	FANCD2, FANCI and PCNA	Fanconi anemia, post-replication repair (PRR) and translesion DNA synthesis (TLS)	Nijman et al., 2005; Oestergaard et al., 2007; Hendel et al., 2011; Villamil et al., 2013; Kim and Kim, 2016
USP2a	Mdm2	p53	Stevenson et al., 2007
USP4	Auto-deubiquitylation and ARF-BP1	DSB-response (HR), p53	Zhang X. et al., 2011; Wijnhoven et al., 2015
USP5	p53	p53	Dayal et al., 2009
USP7	Mdm2, p53, Claspin, Chk1, Ring1b, Bmi1 and RNF168	p53, ATR-Chk1 and γ-H2AX (DSBs and SSBs)	Brooks et al., 2007; Faustrup et al., 2009; Brooks and Gu, 2011; Alonso-de Vega et al., 2014; Qian et al., 2015; Zhu et al., 2015
USP8	BRIT1	BRIT1–SWI–SNF DSB-response	Ge et al., 2015
USP9X	Claspin	ATR-Chk1	McGarry et al., 2016
USP10	p53 and MSH2	ATM-p53 and mismatch repair (MMR)	Yuan et al., 2010; Zhang et al., 2016
USP11	p53	DDR to etoposide	Ke et al., 2014
USP20	Claspin	ATR-Chk1	Yuan et al., 2014; Zhu et al., 2014
USP24	p53 and DDB2	p53-PUMA	Zhang L. et al., 2012, 2015
USP28	Chk2 and 53BP1	Chk2-p53-PUMA	Zhang et al., 2006
USP29	p53 and Claspin	p53 and ATR-Chk1	Liu et al., 2011a; Martin et al., 2015
UCH-L5	NFRKB	DSB-response (HR)	Nishi et al., 2014
OTUB1	p53	p53	Sun et al., 2012
OTUD5	p53 and PDCD5	p53	Luo et al., 2013; Park et al., 2015
Rpn11	Ubiquitin conjugates generated by DNA damage	DNA double-strand break response	Butler et al., 2012

of BRIT1 at damaged sites (Ge et al., 2015). USP4 has been found to be important for DSB repair by promoting homologous recombination. USP4 is auto-deubiquitylated on lysine, but potentially also on cysteine residues, and its deubiquitylation is an important step to permit interaction with the DNA-end resection factor CtIP and MRE11-RAD50-NBS1 (MRN) complex, thereby recruiting CtIP to the damaged sites (Wijnhoven et al., 2015). USP10 was recently identified as a partner of MSH2 by mass spectrometry-based interactome studies. MSH2 is an important factor for the mismatch repair pathway and for the resistance to DNA-damaging agents. USP10 stabilizes MSH2, and knockdown of USP10 in lung cancer cells reduces the sensitivity of these cells to DNA damaging agents. A well-known mechanism of DDR involves histone H2AX, which is phosphorylated and accumulated at damaged sites. Then, the ubiquitin ligases RNF168 and RNF8 ubiquitylate γ-H2AX, thereby inducing the accumulation of repair factors. USP7 has been identified as a regulator of γ-H2AX and H2A ubiquitylation by modulating the stability of the E3 ubiquitin ligases RNF168, RINGB1, and BMI1 (Zhu et al., 2015). USP7 is a regulator of different pathways of the DDR, and it is also a modulator of the ATR-Chk1 pathway since it controls the levels of two main components of this response, Chk1 (an essential checkpoint kinase in the DDR) and Claspin (an important component of the ATR-Chk1 axis) (Faustrup et al., 2009; Alonso-de Vega et al., 2014). Other DUBs have also been identified to stabilize Claspin and, therefore, to modulate the ATR-Chk1 pathway, such as is the case for USP9X (McGarry et al., 2016), USP29 (Martin et al., 2015), and USP20 (Yuan et al., 2014; Zhu et al., 2014). In an interesting study, Nishi et al., identified UCH-L5 as the only DUB from a library of 90 DUBs that were able to promote changes in the DDR during three different assays: recruitment at the damaged sites, DDR signaling modulation and DSB repair. The authors found that UCH-L5 interacted with and stabilized NFRKB. NFRKB is a component of the INO80 complex that promotes HR and DNA-end resection (Nishi et al., 2014).

REGULATION OF p53, c-MYC AND OTHER ONCOGENES BY DUBs

The tumor suppressor p53 is a transcription factor able to control important cellular pathways including DDR, cell cycle, apoptosis, angiogenesis, and senescence. It is called "the guardian of the genome" because of its ability to prevent genome mutation and tumor onset and progression (Nag et al., 2013). p53 levels and subcellular localization are mainly regulated by ubiquitylation (Brooks and Gu, 2011). A number of DUBs can modulate p53 signals: USP7 deubiquitylates both p53 and MDM2, one of the ubiquitin ligases that ubiquitylates p53, thereby stabilizing both proteins (Brooks et al., 2007; Brooks and Gu, 2011). Upon DNA damage, USP10 deubiquitylates and activates p53. The same study shows that USP10 suppresses tumor cell growth in cells expressing wild-type p53 (Yuan et al., 2010). USP2a associates with and deubiquitylates MDM2, but not p53, and promotes MDM2-dependent p53 degradation (Stevenson et al., 2007). Overexpression of this DUB was found in different tumors such as glioma (Boustani et al., 2016), bladder cancer (Jeong et al., 2015), prostate cancer (Nelson et al., 2012), and oral squamous carcinoma (da Silva et al., 2009). USP4 interacts with and deubiquitylates another E3 ubiquitin ligase for p53, ARF-BP1/Mule/HUWE, leading to the stabilization of ARF-BP1 and subsequent reduction of p53 levels. The same authors found that

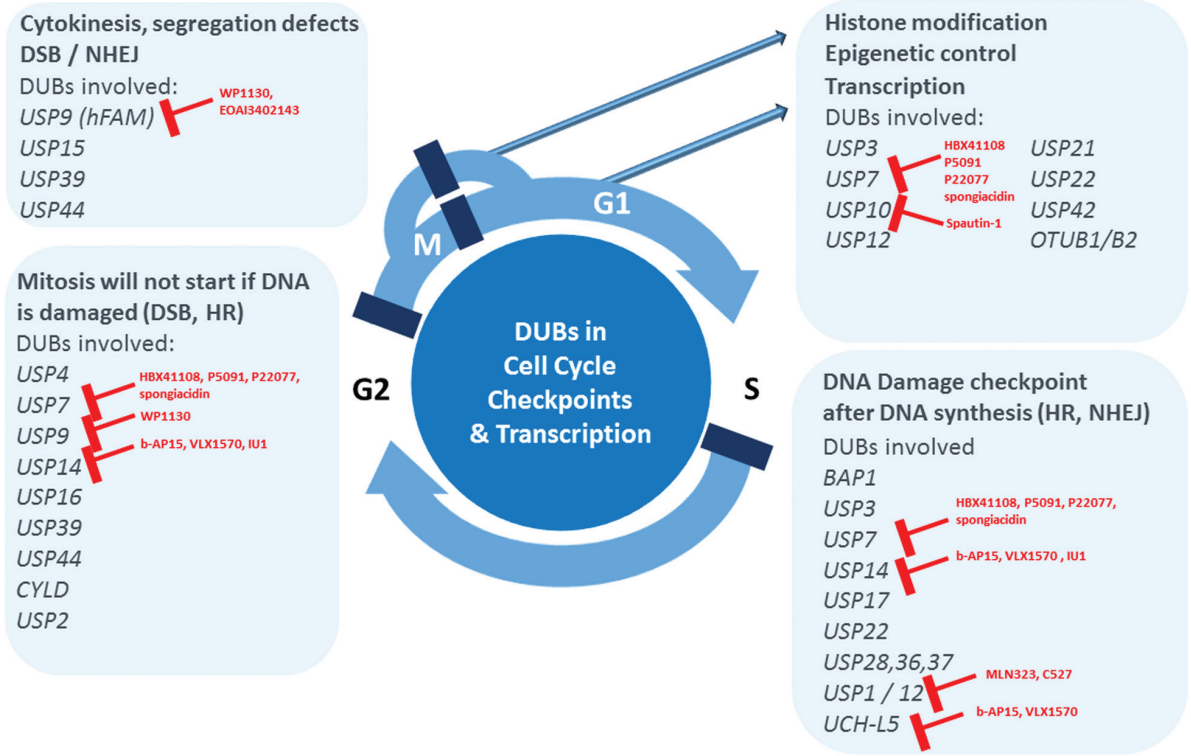

FIGURE 1 | Deubiquitylating enzymes (DUBs) emerge as major pharmacological targets. Uncontrolled cell proliferation is one of the hallmarks of cancer. Recent literature evidence establishes the critical role of DUBs in aspects of cell cycle checkpoint control, associated DNA repair mechanisms and regulation of transcription, representing pathways altered in cancer. Therefore, DUBs involved in these processes emerge as potentially critical targets for the treatment of not only hematological, but potentially also solid tumors. Small molecule inhibitors available for a subset of DUBs are indicated in red.

USP4 is overexpressed in several types of human cancer, and they suggest that USP4 could be a potential oncogene (Zhang X. et al., 2011; Xing et al., 2016). In response to oxidative stress, USP29 binds to and stabilizes p53. Accumulated p53 quickly induces apoptosis under these conditions (Liu et al., 2011a). OTUB1 is a DUB with preference for substrates with poly ubiquitin K48 linked chains (Mevissen et al., 2013; Altun et al., 2015) that was reported to suppress MDM2-mediated p53 ubiquitylation (Sun et al., 2012). Interestingly, the mechanism by which OTUB1 controls p53 ubiquitylation is independent of its DUB activity. OTUB1 blocks p53 ubiquitylation by MDM2 by interacting with and inhibiting UbcH5, and E2 conjugating enzyme for MDM2. Overexpression of OTUB1 in cells drastically stabilizes and activates p53, leading to apoptosis and to a marked inhibition of cell proliferation in a p53-dependent manner (Sun et al., 2012). OTUB1 is also involved in the inhibition of another E2 enzyme, UBC13 (Sato et al., 2012). Since UBC13 is the only E2 involved in the conjugation of K63 Ub chains, this makes OTUB1 an interesting regulator of both, K63 and K48 polyubiquitin chains signals. OTUB1 is also an activator of a very important oncogene in cancer, RAS. Remarkably, OTUB1 deubiquitylates mono- and di-ubiquitylated RAS, independently of its activation status, resulting in the translocation of the RAS protein to the plasma membrane where it is normally activated (Baietti et al., 2016). OTUB1 expression levels are related with poor prognosis and metastasis in colorectal cancer (Zhou et al., 2014), ovarian cancer (Wang Y. et al., 2016), non-small-cell lung carcinomas (Baietti et al., 2016), and it has been linked to resistance to chemotherapy in breast cancer bearing patients (Karunarathna et al., 2016) and prostate cancer cell invasion (Iglesias-Gato et al., 2015). Knock-down of USP5, the only DUB with specificity for unanchored poly ubiquitin, stabilizes p53 as well. The authors propose a model in which p53 is selectively stabilized because the unanchored poly ubiquitin that accumulates after USP5 knockdown is able to compete with ubiquitylated p53 for proteasomal proteolysis (Dayal et al., 2009). Another DUB, OTUD5, interacts and deubiquitylates p53 in response to DNA damage stress (Luo et al., 2013). PDCD5 is an additional factor accumulated upon DNA damage and regulates the p53 pathway. In a two yeast two-hybrid study to identify partners of PDCD5 in the presence of genotoxic stress, OTUD5 was found to interact with and stabilize PDCD, thereby unveiling a dual role for this enzyme in the regulation of the p53 signals (Park et al., 2015). In another two-hybrid study, USP24 was found to interact with and stabilize DDB2 (UV damage binding protein; Zhang L. et al., 2012). Posteriori, the same authors identified an upregulation of USP24 in a number of tumor cell lines. They found also that USP24 deubiquitylates p53, activating the PUMA pathway, a regulator of DNA-damage-induced apoptosis (Zhang L. et al., 2015). Similarly, it was found that

USP11 deubiquitylates p53 in response to genotoxicity induced by etoposide (Ke et al., 2014). Other DUBs are linked to the turn-over of important tumor suppressors and oncogenes. For instance, oncogenic transformation by the stabilization of the dual specificity (Tyr/Thr) phosphatase Cdc25A appears to be controlled by Dub3/USP17 (Pereg et al., 2010). USP17 knockdown does lead to a cell cycle arrest in G1/S and G2/M, and high levels of USP17 have been observed in lung, colon, esophagus, and cervix tumor biopsies, underpinning its role in cell cycle control (McFarlane et al., 2010). USP7 is abundantly expressed in many cell types and, as mentioned above, it was shown to deubiquitylate MDM2, thereby modulating p53 stability, but it also has other cellular substrates including FOXO4, Claspin and FOXO3 (Nicholson and Suresh Kumar, 2011). Knockdown of USP7 leads to cell cycle arrest in G1 or G2 (Khoronenkova et al., 2012), which is underpinning its role in controlling several aspects of cell division. The JAMM-domain containing DUB BRCC36 stimulates activity of BRCA1, leading to G2/M checkpoint arrest/control (Mallery et al., 2002). In addition to its role in the p53 pathway, USP28 was also shown to stabilize the oncogene c-MYC after DNA damage. The same authors and others found high expression levels of this DUB in colon, lung, glioma, bladder, and breast carcinomas (Popov et al., 2007a,b; Diefenbacher et al., 2014, 2015; Guo et al., 2014; Wang Z. et al., 2016). USP36 and USP37 also control the stability of c-Myc and thereby affect c-Myc oncogene driven cellular proliferation (Zhang et al., 2006; Pan et al., 2015; Sun et al., 2015).

DUBs AFFECTING CELL CYCLE REGULATORS

The accumulation and turnover of proteins that regulate the cell cycle such as cyclins, CDKs, and checkpoint signaling molecules is highly orchestrated and controlled to ensure the timely progression through the cell cycle. Inappropriate expression of one or more of these proteins is a common feature of virtually all human tumors. A large number of studies have underscored the importance of E3 ubiquitin ligases and the role they play in regulating cell cycle components [reviewed in Li and Jin (2012)]. However, DUBs that counterbalance E3 ligase activity may also be critical in cell cycle progression (Song and Rape, 2008; Fraile et al., 2012). Indeed, DUB function is frequently miss- regulated in cancer, and our knowledge concerning DUB expression and activity during the different phases of the cell cycle is expanding (for a specific review, see Lim et al., 2016). The regulation of chromatin structure and transcription is one of the key mechanisms by which DUBs exert cell cycle control. DUBs involved in DNA damage checkpoints are exerting effects on cell cycle progression, and these include USP1, USP3, USP7, USP10, USP11, USP16, USP21, USP22, USP28, BRCC36, MYSM1, and BAP1 (Jacq et al., 2013; see also section above). Another key cell cycle checkpoint is the one controlling the correct mitotic spindle assembly, and DUBs such as USP44, CYLD, and USP15 were reported to modulate this process. USP44 acts as a tumor suppressor by preventing chromosome segregation errors (Holland and Cleveland, 2012) via deubiquitylation of the anaphase promoting complex (APC) coactivator Cdc20 (Stegmeier et al., 2007a), and USP44 deletion leads to spontaneous tumor formation, preferentially in the lungs (Zhang Y. et al., 2012). USP15 stabilizes newly synthesized REST and rescues its expression at mitotic exit (Faronato et al., 2013). CYLD targets Plk1 and contributes to regulating mitotic entry (Stegmeier et al., 2007b). USP3 modifies chromatin and is required for S-phase progression (Nicassio et al., 2007). USP2a, as mentioned above, has been linked to different types of cancer. In bladder cancer cells, USP2a was found to deubiquitylate and to stabilize the cell cycle regulator, Cyclin A1, controlling proliferation of these cells (Kim et al., 2012). Taken together, ∼15 (out of ∼90) DUBs have been directly linked to molecular processes of the cell cycle (**Figure 1**).

WILL DUB INHIBITION WORK IN CANCER?

Generally, DUB function is linked to most cellular processes, but in particular appears to cluster around three major pathways that are commonly deregulated in tumorigenesis. These include transcriptional and epigenetic control of gene expression, DDR pathways and cell cycle checkpoint control (**Figure 1**). These processes are functionally interconnected, for instance DNA repair mechanisms that are part of cell cycle control checkpoints in the transitions from G2 to S and M to cytokinesis. The associated subset of DUBs are therefore attractive drug candidates, although so far small molecule inhibitors for only a few of them have been reported many of them are subject to intense screening activities (for a comprehensive review, see Kemp, 2016) including natural compounds (Tsukamoto, 2016). The most relevant DUBs related to these pathways with existing chemical matter are UCH-L1, USP1, USP7, USP9, USP14, and UCH-37. Inhibitors of the USP1/UAF complex have been reported, such as ML323, developed based on a N-Benzyl-2-phenylpyrimidin scaffold (Liang Q. et al., 2014), and C527 (SJB3-019A; Mistry et al., 2013). Interestingly, ML323 was found to sensitize the non-small lung cancer cell line H596 to cisplatin (Liang Q. et al., 2014). Most efforts so far have been focused on USP7 (HAUSP), because of its effect on the MDM2-p53 axis. Reported inhibitors include Hybrigenix HBX41108/HBX19818, all based on quinazoline core structures (Colland et al., 2009; Reverdy et al., 2012), Progenra P22077/P5091 developed from a phenyl-thio-2-thienyl building block (Altun et al., 2011; Chauhan et al., 2012), but also natural compounds such as spongiacidin (Yamaguchi et al., 2013). Most promisingly, the USP7 inhibitor P5091 was shown to be able to overcome Bortezomib resistance in Multiple Myeloma cells (Chauhan et al., 2012). However, this chemical scaffold has limited pharmacodynamics properties. A different small molecule, WP1130 and its improved derivative EOAI3402143 based on second-generation tyrphostin derivatives [initially identified as Janus-activated kinase (JAK)-signal transducer], appear to inhibit USP9X and USP24 and consequently increased Myeloma tumor cell apoptosis *in vitro* and *in vivo*

(Peterson et al., 2015). USP9X affects chromosome alignment and segregation via ubiquitylation of survivin (Vong et al., 2005), but has potentially other roles including cell sensitization by affecting the stability of MCL-1, BCR-ABL, and ITCH (Schwickart et al., 2010; Kushwaha et al., 2015). Spautin-1, a quinazolinamine derivative, was characterized as a USP10 inhibitor that to some extent also targets USP13 (Liu et al., 2011b). The resemblance of the quinazoline chemical core element to USP7 inhibitors may offer an opportunity to use this scaffold to further develop inhibitors specific for other USPs.

CLINICAL PERSPECTIVE FOR DUB INHIBITORS

The USP14 inhibitor IU1 and dual inhibitors of USP14/UCHL5 (proteasomal DUBs) such as b-AP15 (D'Arcy et al., 2011), a *bis*[(4-nitrophenyl)methylene]-piperidinone derivative or its more recently developed analog VLX1570 (Wang X. et al., 2015), show potentially promising effects that could be potentially translated into the clinic. IU1 appears to accelerate degradation of protein aggregates (Lee et al., 2010), and b-AP15 also overcomes Bortezomib resistance in Multiple Myeloma cells (Tian et al., 2014). As a consequence, VLX1570 has now been cleared to enter Phase I/II for the treatment against Multiple Myeloma patients for whom every other drug combination failed (Taylor, 2016). This represents the first DUB inhibitor reaching the clinical phase, and it is expected that within the next one to two years, a number of other DUB inhibitor candidates will follow also for the potential treatment of solid tumors. For instance, p53 wildtype expressing tumors (e.g., certain colon cancers) may be suitable for USP7/10 inhibitor based treatment strategies. c-Myc-dependent tumors (adenocarcinomas and non-small lung, breast, and colon cancer) could potentially respond to USP28, USP36, USP37, all DUBs affecting c-Myc protein turnover. One third of human cancers present mutations in the oncogene RAS or in components of its effector pathways (Matallanas and Crespo, 2010). The recent discovery describing how OTUB1 control RAS activity (Baietti et al., 2016) may lead to the development of OTUB1 inhibitors targeting RAS mutation based cancers such as pancreatic cancer, colon and melanoma. Clearly, DUBs have now reached center stage as cancer targets, and novel inhibitors for this enzyme class will provide the framework for more effective single agent or combination therapies to better treat hematological and solid tumors.

AUTHOR CONTRIBUTIONS

BK derived the concept and structure of the manuscript and **Figure 1**, and he wrote parts of the review. AP-F wrote parts of the review, prepared the tables and contributed to proof-reading.

FUNDING

This work was supported by the John Fell Fund 133/075 and the Wellcome Trust grant 097813/Z/11/Z awarded to BK.

REFERENCES

Abdel-Rahman, M. H., Pilarski, R., Cebulla, C. M., Massengill, J. B., Christopher, B. N., Boru, G., et al. (2011). Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. *J. Med. Genet.* 48, 856–859. doi: 10.1136/jmedgenet-2011-100156

Abdul Rehman, S. A., Kristariyanto, Y. A., Choi, S.-Y., Nkosi, P. J., Weidlich, S., Labib, K., et al. (2016). MINDY-1 is a member of an evolutionarily conserved and structurally distinct new family of deubiquitinating enzymes. *Mol. Cell* 63, 146–155. doi: 10.1016/j.molcel.2016.05.009

Adams, J. (2002). Development of the proteasome inhibitor PS-341. *Oncologist* 7, 9–16. doi: 10.1634/theoncologist.7-1-9

Alonso-de Vega, I., Martin, Y., and Smits, V. A. (2014). USP7 controls Chk1 protein stability by direct deubiquitination. *Cell Cycle* 13, 3921–3926. doi: 10.4161/15384101.2014.973324

Altun, M., Kramer, H. B., Willems, L. I., McDermott, J. L., Leach, C. A., Goldenberg, S. J., et al. (2011). Activity-based chemical proteomics accelerates inhibitor development for deubiquitylating enzymes. *Chem. Biol.* 18, 1401–1412. doi: 10.1016/j.chembiol.2011.08.018

Altun, M., Walter, T. S., Kramer, H. B., Herr, P., Iphofer, A., Bostrom, J., et al. (2015). The human otubain2-ubiquitin structure provides insights into the cleavage specificity of poly-ubiquitin-linkages. *PLoS ONE* 10:e0115344. doi: 10.1371/journal.pone.0115344

Atanassov, B. S., Evrard, Y. A., Multani, A. S., Zhang, Z., Tora, L., Devys, D., et al. (2009). Gcn5 and SAGA regulate shelterin protein turnover and telomere maintenance. *Mol. Cell* 35, 352–364. doi: 10.1016/j.molcel.2009.06.015

Baietti, M. F., Simicek, M., Abbasi Asbagh, L., Radaelli, E., Lievens, S., Crowther, J., et al. (2016). OTUB1 triggers lung cancer development by inhibiting RAS monoubiquitination. *EMBO Mol. Med.* 8, 288–303. doi: 10.15252/emmm.201505972

Belle, J. I., and Nijnik, A. (2014). H2A-DUBbing the mammalian epigenome: expanding frontiers for histone H2A deubiquitinating enzymes in cell biology and physiology. *Int. J. Biochem. Cell Biol.* 50, 161–174. doi: 10.1016/j.biocel.2014.03.004

Bennett, E. J. (2010). Cellular regulation by deubiquitinating enzymes. *Nat. Rev. Mol. Cell Biol.* Available at: http://www.nature.com/nrm/posters/dubs/index.html

Bennett, E. J., and Harper, J. W. (2008). DNA damage: ubiquitin marks the spot. *Nat. Struct. Mol. Biol.* 15, 20–22. doi: 10.1038/nsmb0108-20

Bott, M., Brevet, M., Taylor, B. S., Shimizu, S., Ito, T., Wang, L., et al. (2011). The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat. Genet.* 43, 668–672. doi: 10.1038/ng.855

Boustani, M. R., Khoshnood, R. J., Nikpasand, F., Taleshi, Z., Ahmadi, K., Yahaghi, E., et al. (2016). Overexpression of ubiquitin-specific protease 2a (USP2a) and nuclear factor erythroid 2-related factor 2 (Nrf2) in human gliomas. *J. Neurol. Sci.* 363, 249–252. doi: 10.1016/j.jns.2016.03.003

Bremm, A., Freund, S. M., and Komander, D. (2010). Lys11-linked ubiquitin chains adopt compact conformations and are preferentially hydrolyzed by the deubiquitinase Cezanne. *Nat. Struct. Mol. Biol.* 17, 939–947. doi: 10.1038/nsmb.1873

Bronner, C. (2011). Control of DNMT1 abundance in epigenetic inheritance by acetylation, ubiquitylation, and the histone code. *Sci. Signal.* 4:e3. doi: 10.1126/scisignal.2001764

Brooks, C. L., and Gu, W. (2011). p53 regulation by ubiquitin. *FEBS Lett.* 585, 2803–2809. doi: 10.1016/j.febslet.2011.05.022

Brooks, C. L., Li, M., Hu, M., Shi, Y., and Gu, W. (2007). The p53–Mdm2–HAUSP complex is involved in p53 stabilization by HAUSP. *Oncogene* 26, 7262–7266. doi: 10.1038/sj.onc.1210531

Butler, L. R., Densham, R. M., Jia, J., Garvin, A. J., Stone, H. R., Shah, V., et al. (2012). The proteasomal de-ubiquitinating enzyme POH1 promotes

the double-strand DNA break response. *EMBO J.* 31, 3918–3934. doi: 10.1038/emboj.2012.232

Cao, J., and Yan, Q. (2012). Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Front. Oncol.* 2:26. doi: 10.3389/fonc.2012.00026

Chauhan, D., Tian, Z., Nicholson, B., Kumar, K. G., Zhou, B., Carrasco, R., et al. (2012). A small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. *Cancer Cell* 22, 345–358. doi: 10.1016/j.ccr.2012.08.007

Clague, M. J., Heride, C., and Urbe, S. (2015). The demographics of the ubiquitin system. *Trends Cell Biol.* 25, 417–426. doi: 10.1016/j.tcb.2015.03.002

Cohen, P., and Tcherpakov, M. (2010). Will the ubiquitin system furnish as many drug targets as protein kinases? *Cell* 143, 686–693. doi: 10.1016/j.cell.2010.11.016

Colland, F., Formstecher, E., Jacq, X., Reverdy, C., Planquette, C., Conrath, S., et al. (2009). Small-molecule inhibitor of USP7/HAUSP ubiquitin protease stabilizes and activates p53 in cells. *Mol. Cancer Ther.* 8, 2286–2295. doi: 10.1158/1535-7163.MCT-09-0097

D'Arcy, P., Brnjic, S., Olofsson, M. H., Fryknas, M., Lindsten, K., De Cesare, M., et al. (2011). Inhibition of proteasome deubiquitinating activity as a new cancer therapy. *Nat. Med.* 17, 1636–1640. doi: 10.1038/nm.2536

da Silva, S. D., Cunha, I. W., Nishimoto, I. N., Soares, F. A., Carraro, D. M., Kowalski, L. P., et al. (2009). Clinicopathological significance of ubiquitin-specific protease 2a (USP2a), fatty acid synthase (FASN), and ErbB2 expression in oral squamous cell carcinomas. *Oral Oncol.* 45, e134–e139. doi: 10.1016/j.oraloncology.2009.02.004

Dai, W., Yao, Y., Zhou, Q., and Sun, C. F. (2014). Ubiquitin-specific peptidase 22, a histone deubiquitinating enzyme, is a novel poor prognostic factor for salivary adenoid cystic carcinoma. *PLoS ONE* 9:e87148. doi: 10.1371/journal.pone.0087148

Dar, A., Shibata, E., and Dutta, A. (2013). Deubiquitination of Tip60 by USP7 determines the activity of the p53-dependent apoptotic pathway. *Mol. Cell. Biol.* 33, 3309–3320. doi: 10.1128/MCB.00358-13

Dayal, S., Sparks, A., Jacob, J., Allende-Vega, N., Lane, D. P., and Saville, M. K. (2009). Suppression of the deubiquitinating enzyme USP5 causes the accumulation of unanchored polyubiquitin and the activation of p53. *J. Biol. Chem.* 284, 5030–5041. doi: 10.1074/jbc.M805871200

Dey, A., Seshasayee, D., Noubade, R., French, D. M., Liu, J., Chaurushiya, M. S., et al. (2012). Loss of the tumor suppressor BAP1 causes myeloid transformation. *Science* 337, 1541–1546. doi: 10.1126/science.1221711

Diefenbacher, M. E., Chakraborty, A., Blake, S. M., Mitter, R., Popov, N., Eilers, M., et al. (2015). Usp28 counteracts Fbw7 in intestinal homeostasis and cancer. *Cancer Res.* 75, 1181–1186. doi: 10.1158/0008-5472.CAN-14-1726

Diefenbacher, M. E., Popov, N., Blake, S. M., Schulein-Volk, C., Nye, E., Spencer-Dene, B., et al. (2014). The deubiquitinase USP28 controls intestinal homeostasis and promotes colorectal cancer. *J. Clin. Invest.* 124, 3407–3418. doi: 10.1172/JCI73733

Dou, Y., Lin, J., Shu, H., and Jiang, N. (2014). Role of ubiquitin-specific peptidase 22 in carcinogenesis of human pharyngeal squamous cell carcinoma. *Mol. Med. Rep.* 10, 2973–2978. doi: 10.3892/mmr.2014.2589

Draker, R., Sarcinella, E., and Cheung, P. (2011). USP10 deubiquitylates the histone variant H2A. Z and both are required for androgen receptor-mediated gene activation. *Nucleic Acids Res.* 39, 3529–3542. doi: 10.1093/nar/gkq1352

Edelmann, M. J., Nicholson, B., and Kessler, B. M. (2011). Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases. *Expert Rev. Mol. Med.* 13:e35. doi: 10.1017/S1462399411002031

Ernst, A., Avvakumov, G., Tong, J., Fan, Y., Zhao, Y., Alberts, P., et al. (2013). A strategy for modulation of enzymes in the ubiquitin system. *Science* 339, 590–595. doi: 10.1126/science.1230161

Esteller, M. (2007). Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat. Rev. Genet.* 8, 286–298. doi: 10.1038/nrg2005

Faronato, M., Patel, V., Darling, S., Dearden, L., Clague, M. J., Urbe, S., et al. (2013). The deubiquitylase USP15 stabilizes newly synthesized REST and rescues its expression at mitotic exit. *Cell Cycle* 12, 1964–1977. doi: 10.4161/cc.25035

Faustrup, H., Bekker-Jensen, S., Bartek, J., Lukas, J., and Mailand, N. (2009). USP7 counteracts SCFbetaTrCP- but not APCCdh1-mediated proteolysis of Claspin. *J. Cell Biol.* 184, 13–19. doi: 10.1083/jcb.200807137

Feng, L., Wang, J., and Chen, J. (2010). The Lys63-specific deubiquitinating enzyme BRCC36 is regulated by two scaffold proteins localizing in different subcellular compartments. *J. Biol. Chem.* 285, 30982–30988. doi: 10.1074/jbc.M110.135392

Fraile, J. M., Quesada, V., Rodriguez, D., Freije, J. M., and Lopez-Otin, C. (2012). Deubiquitinases in cancer: new functions and therapeutic options. *Oncogene* 31, 2373–2388. doi: 10.1038/onc.2011.443

Fuchs, G., Shema, E., Vesterman, R., Kotler, E., Wolchinsky, Z., Wilder, S., et al. (2012). RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. *Mol. Cell* 46, 662–673. doi: 10.1016/j.molcel.2012.05.023

Garcia-Santisteban, I., Peters, G. J., Giovannetti, E., and Rodriguez, J. A. (2013). USP1 deubiquitinase: cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. *Mol Cancer* 12, 91. doi: 10.1186/1476-4598-12-91

Ge, C., Che, L., Ren, J., Pandita, R. K., Lu, J., Li, K., et al. (2015). BRUCE regulates DNA double-strand break response by promoting USP8 deubiquitination of BRIT1. *Proc. Natl. Acad. Sci. U.S.A.* 112, E1210–E1219. doi: 10.1073/pnas.1418335112

Gronbaek, K., Hother, C., and Jones, P. A. (2007). Epigenetic changes in cancer. *APMIS* 115, 1039–1059. doi: 10.1111/j.1600-0463.2007.apm_636.xml.x

Gu, Y., Jones, A. E., Yang, W., Liu, S., Dai, Q., Liu, Y., et al. (2016). The histone H2A deubiquitinase Usp16 regulates hematopoiesis and hematopoietic stem cell function. *Proc. Natl. Acad. Sci. U.S.A.* 113, E51–E60. doi: 10.1073/pnas.1517041113

Guo, G., Xu, Y., Gong, M., Cao, Y., and An, R. (2014). USP28 is a potential prognostic marker for bladder cancer. *Tumour Biol.* 35, 4017–4022. doi: 10.1007/s13277-013-1525-1

Hadari, T., Warms, J. V., Rose, I. A., and Hershko, A. (1992). A ubiquitin C-terminal isopeptidase that acts on polyubiquitin chains. Role in protein degradation. *J. Biol. Chem.* 267, 719–727.

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

He, Y., Jin, Y. J., Zhang, Y. H., Meng, H. X., Zhao, B. S., Jiang, Y., et al. (2015). Ubiquitin-specific peptidase 22 overexpression may promote cancer progression and poor prognosis in human gastric carcinoma. *Transl. Res.* 165, 407–416. doi: 10.1016/j.trsl.2014.09.005

Hendel, A., Krijger, P. H., Diamant, N., Goren, Z., Langerak, P., Kim, J., et al. (2011). PCNA ubiquitination is important, but not essential for translesion DNA synthesis in mammalian cells. *PLoS Genet.* 7:e1002262. doi: 10.1371/journal.pgen.1002262

Herndon, T. M., Deisseroth, A., Kaminskas, E., Kane, R. C., Koti, K. M., Rothmann, M. D., et al. (2013). U.s. Food and Drug Administration approval: carfilzomib for the treatment of multiple myeloma. *Clin. Cancer Res.* 19, 4559–4563. doi: 10.1158/1078-0432.CCR-13-0755

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

Holland, A. J., and Cleveland, D. W. (2012). The deubiquitinase USP44 is a tumor suppressor that protects against chromosome missegregation. *J. Clin. Invest.* 122, 4325–4328. doi: 10.1172/JCI66420

Hu, J., Liu, Y. L., Piao, S. L., Yang, D. D., Yang, Y. M., and Cai, L. (2012). Expression patterns of USP22 and potential targets BMI-1, PTEN, p-AKT in non-small-cell lung cancer. *Lung Cancer* 77, 593–599. doi: 10.1016/j.lungcan.2012.05.112

Iglesias-Gato, D., Chuan, Y. C., Jiang, N., Svensson, C., Bao, J., Paul, I., et al. (2015). OTUB1 de-ubiquitinating enzyme promotes prostate cancer cell invasion in vitro and tumorigenesis in vivo. *Mol. Cancer* 14, 8. doi: 10.1186/s12943-014-0280-2

Jacq, X., Kemp, M., Martin, N. M., and Jackson, S. P. (2013). Deubiquitylating enzymes and DNA damage response pathways. *Cell Biochem. Biophys.* 67, 25–43. doi: 10.1007/s12013-013-9635-3

Jeong, P., Ha, Y. S., Yun, S. J., Yoon, H. Y., Freeman, M. R., Kim, J., et al. (2015). Assess the expression of ubiquitin specific protease USP2a for bladder cancer diagnosis. *BMC Urol.* 15:80. doi: 10.1186/s12894-015-0074-x

Ji, Z., Mohammed, H., Webber, A., Ridsdale, J., Han, N., Carroll, J. S., et al. (2014). The forkhead transcription factor FOXK2 acts as a chromatin targeting factor

for the BAP1-containing histone deubiquitinase complex. *Nucleic Acids Res.* 42, 6232–6242. doi: 10.1093/nar/gku274

Jiang, X. X., Chou, Y., Jones, L., Wang, T., Sanchez, S., Huang, X. F., et al. (2015). Epigenetic regulation of antibody responses by the histone H2A deubiquitinase MYSM1. *Sci. Rep.* 5, 13755. doi: 10.1038/srep13755

Jin, J., Xie, X., Xiao, Y., Hu, H., Zou, Q., Cheng, X., et al. (2016). Epigenetic regulation of the expression of Il12 and Il23 and autoimmune inflammation by the deubiquitinase Trabid. *Nat. Immunol.* 17, 259–268. doi: 10.1038/ni.3347

Joo, H. Y., Jones, A., Yang, C., Zhai, L., Smith, A. D. I. V., Zhang, Z., et al. (2011). Regulation of histone H2A and H2B deubiquitination and *Xenopus* development by USP12 and USP46. *J. Biol. Chem.* 286, 7190–7201. doi: 10.1074/jbc.M110.158311

Joo, H. Y., Zhai, L., Yang, C., Nie, S., Erdjument-Bromage, H., Tempst, P., et al. (2007). Regulation of cell cycle progression and gene expression by H2A deubiquitination. *Nature* 449, 1068–1072. doi: 10.1038/nature06256

Karunarathna, U., Kongsema, M., Zona, S., Gong, C., Cabrera, E., Gomes, A. R., et al. (2016). OTUB1 inhibits the ubiquitination and degradation of FOXM1 in breast cancer and epirubicin resistance. *Oncogene* 35, 1433–1444. doi: 10.1038/onc.2015.208

Ke, J. Y., Dai, C. J., Wu, W. L., Gao, J. H., Xia, A. J., Liu, G. P., et al. (2014). USP11 regulates p53 stability by deubiquitinating p53. *J. Zhejiang Univ. Sci. B* 15, 1032–1038. doi: 10.1631/jzus.B1400180

Kemp, M. (2016). Recent advances in the discovery of deubiquitinating enzyme inhibitors. *Prog. Med. Chem.* 55, 149–192. doi: 10.1016/bs.pmch.2015.10.002

Khan, A., Giri, S., Wang, Y., Chakraborty, A., Ghosh, A. K., Anantharaman, A., et al. (2015). BEND3 represses rDNA transcription by stabilizing a NoRC component via USP21 deubiquitinase. *Proc. Natl. Acad. Sci. U.S.A.* 112, 8338–8343. doi: 10.1073/pnas.1424705112

Khoronenkova, S. V., Dianova, I. I., Ternette, N., Kessler, B. M., Parsons, J. L., and Dianov, G. L. (2012). ATM-dependent downregulation of USP7/HAUSP by PPM1G activates p53 response to DNA damage. *Mol. Cell* 45, 801–813. doi: 10.1016/j.molcel.2012.01.021

Kim, J., Kim, W. J., Liu, Z., Loda, M., and Freeman, M. R. (2012). The ubiquitin-specific protease USP2a enhances tumor progression by targeting cyclin A1 in bladder cancer. *Cell Cycle* 11, 1123–1130. doi: 10.4161/cc.11.6.19550

Kim, M., and Kim, J. M. (2016). The role of USP1 autocleavage in DNA interstrand crosslink repair. *FEBS Lett.* 590, 340–348. doi: 10.1002/1873-3468.12060

Kolattukudy, P. E., and Niu, J. (2012). Inflammation, endoplasmic reticulum stress, autophagy, and the monocyte chemoattractant protein-1/CCR2 pathway. *Circ. Res.* 110, 174–189. doi: 10.1161/CIRCRESAHA.111.243212

Komander, D., Clague, M. J., and Urbe, S. (2009). Breaking the chains: structure and function of the deubiquitinases. *Nat. Rev. Mol. Cell Biol.* 10, 550–563. doi: 10.1038/nrm2731

Komander, D., and Rape, M. (2012). The ubiquitin code. *Annu. Rev. Biochem.* 81, 203–229. doi: 10.1146/annurev-biochem-060310-170328

Kushwaha, D., O'Leary, C., Cron, K. R., Deraska, P., Zhu, K., D'Andrea, A. D., et al. (2015). USP9X inhibition promotes radiation-induced apoptosis in non-small cell lung cancer cells expressing mid-to-high MCL1. *Cancer Biol. Ther.* 16, 392–401. doi: 10.1080/15384047.2014.1002358

LaFave, L. M., Beguelin, W., Koche, R., Teater, M., Spitzer, B., Chramiec, A., et al. (2015). Loss of BAP1 function leads to EZH2-dependent transformation. *Nat. Med.* 21, 1344–1349. doi: 10.1038/nm.3947

Lee, B. H., Lee, M. J., Park, S., Oh, D. C., Elsasser, S., Chen, P. C., et al. (2010). Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* 467, 179–184. doi: 10.1038/nature09299

Lee, M. J., Lee, B. H., Hanna, J., King, R. W., and Finley, D. (2011). Trimming of ubiquitin chains by proteasome-associated deubiquitinating enzymes. *Mol. Cell. Proteomics* 10:R110003871. doi: 10.1074/mcp.R110.003871

Lee, S. W., Youn, H., Kim, E. J., and Um, S. J. (2013). Retracted: histone H2B ubquitination regulates retinoic acid signaling through the cooperation of ASXL1 and BAP1. *Mol. Cell* 51, 200–210. doi: 10.1016/j.molcel.2013.06.005

Li, J. M., and Jin, J. (2012). CRL ubiquitin ligases and DNA damage response. *Front. Oncol.* 2:29. doi: 10.3389/fonc.2012.00029

Li, P., Yang, Y. M., Sanchez, S., Cui, D. C., Dang, R. J., Wang, X. Y., et al. (2016). Deubiquitinase MYSM1 is essential for normal bone formation and mesenchymal stem cell differentiation. *Sci. Rep.* 6, 22211. doi: 10.1038/srep22211

Liang, J., Zhang, X., Xie, S., Zhou, X., Shi, Q., Hu, J., et al. (2014). Ubiquitin-specific protease 22: a novel molecular biomarker in glioma prognosis and therapeutics. *Med. Oncol.* 31, 899. doi: 10.1007/s12032-014-0899-2

Liang, Q., Dexheimer, T. S., Zhang, P., Rosenthal, A. S., Villamil, M. A., You, C., et al. (2014). A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. *Nat. Chem. Biol.* 10, 298–304. doi: 10.1038/nchembio.1455

Licchesi, J. D., Mieszczanek, J., Mevissen, T. E., Rutherford, T. J., Akutsu, M., Virdee, S., et al. (2012). An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. *Nat. Struct. Mol. Biol.* 19, 62–71. doi: 10.1038/nsmb.2169

Lim, K. H., and Baek, K. H. (2013). Deubiquitinating enzymes as therapeutic targets in cancer. *Curr. Pharm. Des.* 19, 4039–4052. doi: 10.2174/1381612811319220013

Lim, K. H., Song, M. H., and Baek, K. H. (2016). Decision for cell fate: deubiquitinating enzymes in cell cycle checkpoint. *Cell. Mol. Life Sci.* 73, 1439–1455. doi: 10.1007/s00018-015-2129-2

Liu, J., Chung, H. J., Vogt, M., Jin, Y., Malide, D., He, L., et al. (2011a). JTV1 co-activates FBP to induce USP29 transcription and stabilize p53 in response to oxidative stress. *EMBO J.* 30, 846–858. doi: 10.1038/emboj.2011.11

Liu, J., Xia, H., Kim, M., Xu, L., Li, Y., Zhang, L., et al. (2011b). Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. *Cell* 147, 223–234. doi: 10.1016/j.cell.2011.08.037

Liu, Y., Li, Y., and Lu, X. (2016). Regulators in the DNA damage response. *Arch. Biochem. Biophys.* 594, 18–25. doi: 10.1016/j.abb.2016.02.018

Liu, Y. L., Yang, Y. M., Xu, H., and Dong, X. S. (2011). Aberrant expression of USP22 is associated with liver metastasis and poor prognosis of colorectal cancer. *J. Surg. Oncol.* 103, 283–289. doi: 10.1002/jso.21802

Luo, J., Lu, Z., Lu, X., Chen, L., Cao, J., Zhang, S., et al. (2013). OTUD5 regulates p53 stability by deubiquitinating p53. *PLoS ONE* 8:e77682. doi: 10.1371/journal.pone.0077682

Luo, M., Zhou, J., Leu, N. A., Abreu, C. M., Wang, J., Anguera, M. C., et al. (2015). Polycomb protein SCML2 associates with USP7 and counteracts histone H2A ubiquitination in the XY chromatin during male meiosis. *PLoS Genet.* 11:e1004954. doi: 10.1371/journal.pgen.1004954

Mallery, D. L., Vandenberg, C. J., and Hiom, K. (2002). Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. *EMBO J.* 21, 6755–6762. doi: 10.1093/emboj/cdf691

Martin, Y., Cabrera, E., Amoedo, H., Hernandez-Perez, S., Dominguez-Kelly, R., and Freire, R. (2015). USP29 controls the stability of checkpoint adaptor Claspin by deubiquitination. *Oncogene* 34, 1058–1063. doi: 10.1038/onc.2014.38

Matallanas, D., and Crespo, P. (2010). New druggable targets in the Ras pathway? *Curr. Opin. Mol. Ther.* 12, 674–683.

McClurg, U. L., and Robson, C. N. (2015). Deubiquitinating enzymes as oncotargets. *Oncotarget* 6, 9657–9668. doi: 10.18632/oncotarget.3922

McFarlane, C., Kelvin, A. A., de la Vega, M., Govender, U., Scott, C. J., Burrows, J. F., et al. (2010). The deubiquitinating enzyme USP17 is highly expressed in tumor biopsies, is cell cycle regulated, and is required for G1-S progression. *Cancer Res.* 70, 3329–3339. doi: 10.1158/0008-5472.CAN-09-4152

McGarry, E., Gaboriau, D., Rainey, M., Restuccia, U., Bachi, A., and Santocanale, C. (2016). The deubiquitinase USP9X maintains DNA replication fork stability and DNA damage checkpoint responses by regulating CLASPIN during S-phase. *Cancer Res.* 76, 2384–2393. doi: 10.1158/0008-5472.CAN-15-2890

Mevissen, T. E., Hospenthal, M. K., Geurink, P. P., Elliott, P. R., Akutsu, M., Arnaudo, N., et al. (2013). OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell* 154, 169–184. doi: 10.1016/j.cell.2013.05.046

Mistry, H., Hsieh, G., Buhrlage, S. J., Huang, M., Park, E., Cuny, G. D., et al. (2013). Small-molecule inhibitors of USP1 target ID1 degradation in leukemic cells. *Mol. Cancer Ther.* 12, 2651–2662. doi: 10.1158/1535-7163.MCT-13-0103-T

Mochel, M. C., Piris, A., Nose, V., and Hoang, M. P. (2015). Loss of BAP1 expression in basal cell carcinomas in patients with germline BAP1 mutations. *Am. J. Clin. Pathol.* 143, 901–904. doi: 10.1309/AJCPG8LFJC0DHDQT

Mosbech, A., Lukas, C., Bekker-Jensen, S., and Mailand, N. (2013). The deubiquitylating enzyme USP44 counteracts the DNA double-strand break response mediated by the RNF8 and RNF168 ubiquitin ligases. *J. Biol. Chem.* 288, 16579–16587. doi: 10.1074/jbc.M113.459917

Murali, R., Wiesner, T., and Scolyer, R. A. (2013). Tumours associated with BAP1 mutations. *Pathology* 45, 116–126. doi: 10.1097/PAT.0b013e32835d0efb

Nag, S., Qin, J., Srivenugopal, K. S., Wang, M., and Zhang, R. (2013). The MDM2-p53 pathway revisited. *J. Biomed. Res.* 27, 254–271. doi: 10.7555/JBR.27.20130030

Nakagawa, T., Kajitani, T., Togo, S., Masuko, N., Ohdan, H., Hishikawa, Y., et al. (2008). Deubiquitylation of histone H2A activates transcriptional initiation via trans-histone cross-talk with H3K4 di- and trimethylation. *Genes Dev.* 22, 37–49. doi: 10.1101/gad.1609708

Nelson, W. G., De Marzo, M. A., and Yegnasubramanian, S. (2012). USP2a activation of MYC in prostate cancer. *Cancer Discov.* 2, 206–207. doi: 10.1158/2159-8290.CD-12-0027

Nicassio, F., Corrado, N., Vissers, J. H., Areces, L. B., Bergink, S., Marteijn, J. A., et al. (2007). Human USP3 is a chromatin modifier required for S phase progression and genome stability. *Curr. Biol.* 17, 1972–1977. doi: 10.1016/j.cub.2007.10.034

Nicholson, B., Marblestone, J. G., Butt, T. R., and Mattern, M. R. (2007). Deubiquitinating enzymes as novel anticancer targets. *Future Oncol.* 3, 191–199. doi: 10.2217/14796694.3.2.191

Nicholson, B., and Suresh Kumar, K. G. (2011). The multifaceted roles of USP7; new therapeutic opportunities. *Cell Biochem. Biophys.* 60, 61–68. doi: 10.1007/s12013-011-9185-5

Nickerson, M. L., Dancik, G. M., Im, K. M., Edwards, M. G., Turan, S., Brown, J., et al. (2014). Concurrent alterations in TERT, KDM6A, and the BRCA pathway in bladder cancer. *Clin. Cancer Res.* 20, 4935–4948. doi: 10.1158/1078-0432.CCR-14-0330

Nie, M., and Boddy, M. N. (2016). Cooperativity of the SUMO and ubiquitin pathways in genome stability. *Biomolecules* 6, 14. doi: 10.3390/biom6010014

Nijman, S. M., Huang, T. T., Dirac, A. M., Brummelkamp, T. R., Kerkhoven, R. M., D'Andrea, A. D., et al. (2005). The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol. Cell* 17, 331–339. doi: 10.1016/j.molcel.2005.01.008

Nishi, R., Wijnhoven, P., le Sage, C., Tjeertes, J., Galanty, Y., Forment, J. V., et al. (2014). Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. *Nat. Cell Biol.* 16, 1016–1026. doi: 10.1038/ncb3028

Nishiyama, A., Yamaguchi, L., Sharif, J., Johmura, Y., Kawamura, T., Nakanishi, K., et al. (2013). Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. *Nature* 502, 249–253. doi: 10.1038/nature12488

Oestergaard, V. H., Langevin, F., Kuiken, H. J., Pace, P., Niedzwiedz, W., Simpson, L. J., et al. (2007). Deubiquitination of FANCD2 is required for DNA crosslink repair. *Mol. Cell* 28, 798–809. doi: 10.1016/j.molcel.2007.09.020

Okino, Y., Machida, Y., Frankland-Searby, S., and Machida, Y. J. (2015). BRCA1-associated protein 1 (BAP1) deubiquitinase antagonizes the ubiquitin-mediated activation of FoxK2 target genes. *J. Biol. Chem.* 290, 1580–1591. doi: 10.1074/jbc.M114.609834

Pan, J., Deng, Q., Jiang, C., Wang, X., Niu, T., Li, H., et al. (2015). USP37 directly deubiquitinates and stabilizes c-Myc in lung cancer. *Oncogene* 34, 3957–3967. doi: 10.1038/onc.2014.327

Park, S. Y., Choi, H. K., Choi, Y., Kwak, S., Choi, K. C., and Yoon, H. G. (2015). Deubiquitinase OTUD5 mediates the sequential activation of PDCD5 and p53 in response to genotoxic stress. *Cancer Lett.* 357, 419–427. doi: 10.1016/j.canlet.2014.12.005

Pena-Llopis, S., Vega-Rubin-de-Celis, S., Liao, A., Leng, N., Pavia-Jimenez, A., Wang, S., et al. (2012). BAP1 loss defines a new class of renal cell carcinoma. *Nat. Genet.* 44, 751–759. doi: 10.1038/ng.2323

Pereg, Y., Liu, B. Y., O'Rourke, K. M., Sagolla, M., Dey, A., Komuves, L., et al. (2010). Ubiquitin hydrolase Dub3 promotes oncogenic transformation by stabilizing Cdc25A. *Nat. Cell Biol.* 12, 400–406. doi: 10.1038/ncb2041

Peterson, L. F., Sun, H., Liu, Y., Potu, H., Kandarpa, M., Ermann, M., et al. (2015). Targeting deubiquitinase activity with a novel small-molecule inhibitor as therapy for B-cell malignancies. *Blood* 125, 3588–3597. doi: 10.1182/blood-2014-10-605584

Piao, S., Liu, Y., Hu, J., Guo, F., Ma, J., Sun, Y., et al. (2012). USP22 is useful as a novel molecular marker for predicting disease progression and patient prognosis of oral squamous cell carcinoma. *PLoS ONE* 7:e42540. doi: 10.1371/journal.pone.0042540

Piris, A., Mihm, M. C. Jr., and Hoang, M. P. (2015). BAP1 and BRAFV600E expression in benign and malignant melanocytic proliferations. *Hum. Pathol.* 46, 239–245. doi: 10.1016/j.humpath.2014.10.015

Popov, N., Herold, S., Llamazares, M., Schulein, C., and Eilers, M. (2007a). Fbw7 and Usp28 regulate myc protein stability in response to DNA damage. *Cell Cycle* 6, 2327–2331. doi: 10.4161/cc.6.19.4804

Popov, N., Wanzel, M., Madiredjo, M., Zhang, D., Beijersbergen, R., Bernards, R., et al. (2007b). The ubiquitin-specific protease USP28 is required for MYC stability. *Nat. Cell Biol.* 9, 765–774. doi: 10.1038/ncb1601

Qian, J., Pentz, K., Zhu, Q., Wang, Q., He, J., Srivastava, A. K., et al. (2015). USP7 modulates UV-induced PCNA monoubiquitination by regulating DNA polymerase eta stability. *Oncogene* 34, 4791–4796. doi: 10.1038/onc.2014.394

Qin, W., Leonhardt, H., and Spada, F. (2011). Usp7 and Uhrf1 control ubiquitination and stability of the maintenance DNA methyltransferase Dnmt1. *J. Cell. Biochem.* 112, 439–444. doi: 10.1002/jcb.22998

Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R., et al. (2009). Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* 136, 1098–1109. doi: 10.1016/j.cell.2009.03.007

Ramakrishna, S., Suresh, B., Bae, S. M., Ahn, W. S., Lim, K. H., and Baek, K. H. (2012). Hyaluronan binding motifs of USP17 and SDS3 exhibit anti-tumor activity. *PLoS ONE* 7:e37772. doi: 10.1371/journal.pone.0037772

Ramakrishna, S., Suresh, B., Lee, E. J., Lee, H. J., Ahn, W. S., and Baek, K. H. (2011). Lys-63-specific deubiquitination of SDS3 by USP17 regulates HDAC activity. *J. Biol. Chem.* 286, 10505–10514. doi: 10.1074/jbc.M110.162321

Reverdy, C., Conrath, S., Lopez, R., Planquette, C., Atmanene, C., Collura, V., et al. (2012). Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. *Chem. Biol.* 19, 467–477. doi: 10.1016/j.chembiol.2012.02.007

Reyes-Turcu, F. E., Ventii, K. H., and Wilkinson, K. D. (2009). Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu. Rev. Biochem.* 78, 363–397. doi: 10.1146/annurev.biochem.78.082307.091526

Sato, Y., Yamagata, A., Goto-Ito, S., Kubota, K., Miyamoto, R., Nakada, S., et al. (2012). Molecular basis of Lys-63-linked polyubiquitination inhibition by the interaction between human deubiquitinating enzyme OTUB1 and ubiquitin-conjugating enzyme UBC13. *J. Biol. Chem.* 287, 25860–25868. doi: 10.1074/jbc.M112.364752

Scheuermann, J. C., de Ayala Alonso, A. G., Oktaba, K., Ly-Hartig, N., McGinty, R. K., Fraterman, S., et al. (2010). Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature* 465, 243–247. doi: 10.1038/nature08966

Schwickart, M., Huang, X., Lill, J. R., Liu, J., Ferrando, R., French, D. M., et al. (2010). Deubiquitinase USP9X stabilizes MCL1 and promotes tumour cell survival. *Nature* 463, 103–107. doi: 10.1038/nature08646

Segal, E., and Widom, J. (2009). What controls nucleosome positions? *Trends Genet.* 25, 335–343. doi: 10.1016/j.tig.2009.06.002

Sgorbissa, A., Potu, H., and Brancolini, C. (2010). Isopeptidases in anticancer therapy: looking for inhibitors. *Am. J. Transl. Res.* 2, 235–247.

Shao, G., Lilli, D. R., Patterson-Fortin, J., Coleman, K. A., Morrissey, D. E., and Greenberg, R. A. (2009). The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3166–3171. doi: 10.1073/pnas.0807485106

Shi, D., and Grossman, S. R. (2010). Ubiquitin becomes ubiquitous in cancer: emerging roles of ubiquitin ligases and deubiquitinases in tumorigenesis and as therapeutic targets. *Cancer Biol. Ther.* 10, 737–747. doi: 10.4161/cbt.10.8.13417

Shirley, M. (2016). Ixazomib: first global approval. *Drugs* 76, 405–411. doi: 10.1007/s40265-016-0548-5

Simo-Riudalbas, L., and Esteller, M. (2015). Targeting the histone orthography of cancer: drugs for writers, erasers and readers. *Br. J. Pharmacol.* 172, 2716–2732. doi: 10.1111/bph.12844

Song, L., and Rape, M. (2008). Reverse the curse–the role of deubiquitination in cell cycle control. *Curr. Opin. Cell Biol.* 20, 156–163. doi: 10.1016/j.ceb.2008.01.012

Stegmeier, F., Rape, M., Draviam, V. M., Nalepa, G., Sowa, M. E., Ang, X. L., et al. (2007a). Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* 446, 876–881. doi: 10.1038/nature05694

Stegmeier, F., Sowa, M. E., Nalepa, G., Gygi, S. P., Harper, J. W., and Elledge, S. J. (2007b). The tumor suppressor CYLD regulates entry into mitosis. *Proc. Natl. Acad. Sci. U.S.A.* 104, 8869–8874. doi: 10.1073/pnas.0703268104

Stevenson, L. F., Sparks, A., Allende-Vega, N., Xirodimas, D. P., Lane, D. P., and Saville, M. K. (2007). The deubiquitinating enzyme USP2a regulates the p53 pathway by targeting Mdm2. *EMBO J.* 26, 976–986. doi: 10.1038/sj.emboj.7601567

Sun, X. X., Challagundla, K. B., and Dai, M. S. (2012). Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. *EMBO J.* 31, 576–592. doi: 10.1038/emboj.2011.434

Sun, X. X., He, X., Yin, L., Komada, M., Sears, R. C., and Dai, M. S. (2015). The nucleolar ubiquitin-specific protease USP36 deubiquitinates and stabilizes c-Myc. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3734–3739. doi: 10.1073/pnas.1411713112

Swatek, K. N., and Komander, D. (2016). Ubiquitin modifications. *Cell Res.* 26, 399–422. doi: 10.1038/cr.2016.39

Taillebourg, E., Gregoire, I., Viargues, P., Jacomin, A. C., Thevenon, D., Faure, M., et al. (2012). The deubiquitinating enzyme USP36 controls selective autophagy activation by ubiquitinated proteins. *Autophagy* 8, 767–779. doi: 10.4161/auto.19381

Tang, B., Liang, X., Tang, F., Zhang, J., Zeng, S., Jin, S., et al. (2015a). Expression of USP22 and Survivin is an indicator of malignant behavior in hepatocellular carcinoma. *Int. J. Oncol.* 47, 2208–2216. doi: 10.3892/ijo.2015.3214

Tang, B., Tang, F., Li, B., Yuan, S., Xu, Q., Tomlinson, S., et al. (2015b). High USP22 expression indicates poor prognosis in hepatocellular carcinoma. *Oncotarget* 6, 12654–12667. doi: 10.18632/oncotarget.3705

Taylor, D. (2016). Multiple Myeloma Therapy in 2015: an extraordinary moment in oncology. *Am. Health Drug Benefits* 9, 1–12.

Tian, Z., D'Arcy, P., Wang, X., Ray, A., Tai, Y. T., Hu, Y., et al. (2014). A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in multiple myeloma and overcomes bortezomib resistance. *Blood* 123, 706–716. doi: 10.1182/blood-2013-05-500033

Tsukamoto, S. (2016). Search for inhibitors of the ubiquitin-proteasome system from natural sources for cancer therapy. *Chem. Pharm. Bull. (Tokyo)* 64, 112–118. doi: 10.1248/cpb.c15-00768

van der Knaap, J. A., Kumar, B. R., Moshkin, Y. M., Langenberg, K., Krijgsveld, J., Heck, A. J., et al. (2005). GMP synthetase stimulates histone H2B deubiquitylation by the epigenetic silencer USP7. *Mol. Cell* 17, 695–707. doi: 10.1016/j.molcel.2005.02.013

Ventii, K. H., and Wilkinson, K. D. (2008). Protein partners of deubiquitinating enzymes. *Biochem. J.* 414, 161–175. doi: 10.1042/BJ20080798

Villamil, M. A., Liang, Q., and Zhuang, Z. (2013). The WD40-repeat protein-containing deubiquitinase complex: catalysis, regulation, and potential for therapeutic intervention. *Cell Biochem. Biophys.* 67, 111–126. doi: 10.1007/s12013-013-9637-1

Virdee, S., Ye, Y., Nguyen, D. P., Komander, D., and Chin, J. W. (2010). Engineered diubiquitin synthesis reveals Lys29-isopeptide specificity of an OTU deubiquitinase. *Nat. Chem. Biol.* 6, 750–757. doi: 10.1038/nchembio.426

Vong, Q. P., Cao, K., Li, H. Y., Iglesias, P. A., and Zheng, Y. (2005). Chromosome alignment and segregation regulated by ubiquitination of survivin. *Science* 310, 1499–1504. doi: 10.1126/science.1120160

Wang, C. H., Chen, G. C., and Chien, C. T. (2014). The deubiquitinase Leon/USP5 regulates ubiquitin homeostasis during Drosophila development. *Biochem. Biophys. Res. Commun.* 452, 369–375. doi: 10.1016/j.bbrc.2014.08.069

Wang, L., and Dent, S. Y. (2014). Functions of SAGA in development and disease. *Epigenomics* 6, 329–339. doi: 10.2217/epi.14.22

Wang, Q., Ma, S., Song, N., Li, X., Liu, L., Yang, S., et al. (2016). Stabilization of histone demethylase PHF8 by USP7 promotes breast carcinogenesis. *J. Clin. Invest.* 126, 2205–2220. doi: 10.1172/JCI85747

Wang, X., D'Arcy, P., Caulfield, T. R., Paulus, A., Chitta, K., Mohanty, C., et al. (2015). Synthesis and evaluation of derivatives of the proteasome deubiquitinase inhibitor b-AP15. *Chem. Biol. Drug Des.* 86, 1036–1048. doi: 10.1111/cbdd.12571

Wang, Y., Thomas, A., Lau, C., Rajan, A., Zhu, Y., Killian, J. K., et al. (2014). Mutations of epigenetic regulatory genes are common in thymic carcinomas. *Sci. Rep.* 4:7336. doi: 10.1038/srep07336

Wang, Y., Zhou, X., Xu, M., Weng, W., Zhang, Q., Yang, Y., et al. (2016). OTUB1-catalyzed deubiquitination of FOXM1 facilitates tumor progression and predicts a poor prognosis in ovarian cancer. *Oncotarget* doi: 10.18632/oncotarget.9160 [Epub ahead of print].

Wang, Y. C., Wang, S. A., Chen, P. H., Hsu, T. I., Yang, W. B., Chuang, Y. P., et al. (2015). Variants of ubiquitin-specific peptidase 24 play a crucial role in lung cancer malignancy. *Oncogene* doi: 10.1038/onc.2015.432 [Epub ahead of print].

Wang, Z., Song, Q., Xue, J., Zhao, Y., and Qin, S. (2016). Ubiquitin-specific protease 28 is overexpressed in human glioblastomas and contributes to glioma tumorigenicity by regulating MYC expression. *Exp. Biol. Med. (Maywood)* 241, 255–264. doi: 10.1177/1535370215595468

Wang, Z., Zhu, L., Guo, T., Wang, Y., and Yang, J. (2015). Decreased H2B monoubiquitination and overexpression of ubiquitin-specific protease enzyme 22 in malignant colon carcinoma. *Hum. Pathol.* 46, 1006–1014. doi: 10.1016/j.humpath.2015.04.001

Weake, V. M., and Workman, J. L. (2008). Histone ubiquitination: triggering gene activity. *Mol. Cell* 29, 653–663. doi: 10.1016/j.molcel.2008.02.014

Wiesner, T., Obenauf, A. C., Murali, R., Fried, I., Griewank, K. G., Ulz, P., et al. (2011). Germline mutations in BAP1 predispose to melanocytic tumors. *Nat. Genet.* 43, 1018–1021. doi: 10.1038/ng.910

Wijnhoven, P., Konietzny, R., Blackford, A. N., Travers, J., Kessler, B. M., Nishi, R., et al. (2015). USP4 auto-deubiquitylation promotes homologous recombination. *Mol. Cell* 60, 362–373. doi: 10.1016/j.molcel.2015.09.019

Wu, Y., Wang, Y., Yang, X. H., Kang, T., Zhao, Y., Wang, C., et al. (2013). The deubiquitinase USP28 stabilizes LSD1 and confers stem-cell-like traits to breast cancer cells. *Cell Rep.* 5, 224–236. doi: 10.1016/j.celrep.2013.08.030

Xing, C., Lu, X. X., Guo, P. D., Shen, T., Zhang, S., He, X. S., et al. (2016). Ubiquitin-specific protease 4-mediated deubiquitination and stabilization of PRL-3 is required for potentiating colorectal oncogenesis. *Cancer Res.* 76, 83–95. doi: 10.1158/0008-5472.CAN-14-3595

Yamaguchi, M., Miyazaki, M., Kodrasov, M. P., Rotinsulu, H., Losung, F., Mangindaan, R. E., et al. (2013). Spongiacidin C, a pyrrole alkaloid from the marine sponge Stylissa massa, functions as a USP7 inhibitor. *Bioorg. Med. Chem. Lett.* 23, 3884–3886. doi: 10.1016/j.bmcl.2013.04.066

Yang, D. D., Cui, B. B., Sun, L. Y., Zheng, H. Q., Huang, Q., Tong, J. X., et al. (2011). The co-expression of USP22 and BMI-1 may promote cancer progression and predict therapy failure in gastric carcinoma. *Cell Biochem. Biophys.* 61, 703–710. doi: 10.1007/s12013-011-9229-x

Yang, S., Banerjee, S., Freitas, A., Cui, H., Xie, N., Abraham, E., et al. (2012). miR-21 regulates chronic hypoxia-induced pulmonary vascular remodeling. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 302, L521–L529. doi: 10.1152/ajplung.00316.2011

Yuan, J., Luo, K., Deng, M., Li, Y., Yin, P., Gao, B., et al. (2014). HERC2-USP20 axis regulates DNA damage checkpoint through Claspin. *Nucleic Acids Res.* 42, 13110–13121. doi: 10.1093/nar/gku1034

Yuan, J., Luo, K., Zhang, L., Cheville, J. C., and Lou, Z. (2010). USP10 regulates p53 localization and stability by deubiquitinating p53. *Cell* 140, 384–396. doi: 10.1016/j.cell.2009.12.032

Zhang, D., Zaugg, K., Mak, T. W., and Elledge, S. J. (2006). A role for the deubiquitinating enzyme USP28 in control of the DNA-damage response. *Cell* 126, 529–542. doi: 10.1016/j.cell.2006.06.039

Zhang, L., Lubin, A., Chen, H., Sun, Z., and Gong, F. (2012). The deubiquitinating protein USP24 interacts with DDB2 and regulates DDB2 stability. *Cell Cycle* 11, 4378–4384. doi: 10.4161/cc.22688

Zhang, L., Nemzow, L., Chen, H., Lubin, A., Rong, X., Sun, Z., et al. (2015). The deubiquitinating enzyme USP24 is a regulator of the UV damage response. *Cell Rep.* 10, 140–147. doi: 10.1016/j.celrep.2014.12.024

Zhang, M., Hu, C., Tong, D., Xiang, S., Williams, K., Bai, W., et al. (2016). Ubiquitin-specific peptidase 10 (USP10) deubiquitinates and stabilizes MutS homolog 2 (MSH2) to regulate cellular sensitivity to DNA damage. *J. Biol. Chem.* 291, 10783–10791. doi: 10.1074/jbc.M115.700047

Zhang, T., Cooper, S., and Brockdorff, N. (2015). The interplay of histone modifications – writers that read. *EMBO Rep.* 16, 1467–1481. doi: 10.15252/embr.201540945

Zhang, X., Berger, F. G., Yang, J., and Lu, X. (2011). USP4 inhibits p53 through deubiquitinating and stabilizing ARF-BP1. *EMBO J.* 30, 2177–2189. doi: 10.1038/emboj.2011.125

Zhang, X. Y., Pfeiffer, H. K., Thorne, A. W., and McMahon, S. B. (2008a). USP22, an hSAGA subunit and potential cancer stem cell marker, reverses the polycomb-catalyzed ubiquitylation of histone H2A. *Cell Cycle* 7, 1522–1524. doi: 10.4161/cc.7.11.5962

Zhang, X. Y., Varthi, M., Sykes, S. M., Phillips, C., Warzecha, C., Zhu, W., et al. (2008b). The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. *Mol. Cell* 29, 102–111. doi: 10.1016/j.molcel.2007.12.015

Zhang, Y., Foreman, O., Wigle, D. A., Kosari, F., Vasmatzis, G., Salisbury, J. L., et al. (2012). USP44 regulates centrosome positioning to prevent aneuploidy and suppress tumorigenesis. *J. Clin. Invest.* 122, 4362–4374. doi: 10.1172/JCI 63084

Zhang, Y., Yao, L., Zhang, X., Ji, H., Wang, L., Sun, S., et al. (2011). Elevated expression of USP22 in correlation with poor prognosis in patients with invasive breast cancer. *J. Cancer Res. Clin. Oncol.* 137, 1245–1253. doi: 10.1007/s00432-011-0998-9

Zhang, Z., Jones, A., Joo, H. Y., Zhou, D., Cao, Y., Chen, S., et al. (2013). USP49 deubiquitinates histone H2B and regulates cotranscriptional pre-mRNA splicing. *Genes Dev.* 27, 1581–1595. doi: 10.1101/gad.211037.112

Zhou, W., Wang, X., and Rosenfeld, M. G. (2009). Histone H2A ubiquitination in transcriptional regulation and DNA damage repair. *Int. J. Biochem. Cell Biol.* 41, 12–15. doi: 10.1016/j.biocel.2008.09.016

Zhou, Y., Wu, J., Fu, X., Du, W., Zhou, L., Meng, X., et al. (2014). OTUB1 promotes metastasis and serves as a marker of poor prognosis in colorectal cancer. *Mol. Cancer* 13, 258. doi: 10.1186/1476-4598-13-258

Zhu, M., Zhao, H., Liao, J., and Xu, X. (2014). HERC2/USP20 coordinates CHK1 activation by modulating CLASPIN stability. *Nucleic Acids Res.* 42, 13074–13081. doi: 10.1093/nar/gku978

Zhu, P., Zhou, W., Wang, J., Puc, J., Ohgi, K. A., Erdjument-Bromage, H., et al. (2007). A histone H2A deubiquitinase complex coordinating histone acetylation and H1 dissociation in transcriptional regulation. *Mol. Cell* 27, 609–621. doi: 10.1016/j.molcel.2007.07.024

Zhu, Q., Sharma, N., He, J., Wani, G., and Wani, A. A. (2015). USP7 deubiquitinase promotes ubiquitin-dependent DNA damage signaling by stabilizing RNF168. *Cell Cycle* 14, 1413–1425. doi: 10.1080/15384101.2015.1007785

Conflict of Interest Statement: AP and BK are both associated with Cancer Research Technologies and Forma Therapeutics.

frontiers in Genetics

REVIEW
published: 21 July 2016
doi: 10.3389/fgene.2016.00128

Maintaining Genome Stability in Defiance of Mitotic DNA Damage

*Stefano Ferrari * and Christian Gentili*

Institute of Molecular Cancer Research, University of Zurich, Zurich, Switzerland

The implementation of decisions affecting cell viability and proliferation is based on prompt detection of the issue to be addressed, formulation and transmission of a correct set of instructions and fidelity in the execution of orders. While the first and the last are purely mechanical processes relying on the faithful functioning of single proteins or macromolecular complexes (sensors and effectors), information is the real cue, with signal amplitude, duration, and frequency ultimately determining the type of response. The cellular response to DNA damage is no exception to the rule. In this review article we focus on DNA damage responses in G2 and Mitosis. First, we set the stage describing mitosis and the machineries in charge of assembling the apparatus responsible for chromosome alignment and segregation as well as the inputs that control its function (checkpoints). Next, we examine the type of issues that a cell approaching mitosis might face, presenting the impact of post-translational modifications (PTMs) on the correct and timely functioning of pathways correcting errors or damage before chromosome segregation. We conclude this essay with a perspective on the current status of mitotic signaling pathway inhibitors and their potential use in cancer therapy.

Keywords: cancer therapy, checkpoint, DNA damage, mitosis, phosphorylation, ubiquitylation

OPEN ACCESS

Edited by:
Kristijan Ramadan,
University of Oxford, UK

Reviewed by:
Howard Donninger,
University of Louisville, USA
Claude Prigent,
Centre National de la Recherche Scientifique, France

****Correspondence:***
Stefano Ferrari
sferrari@imcr.uzh.ch

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *24 May 2016*
Accepted: *06 July 2016*
Published: *21 July 2016*

Citation:
Ferrari S and Gentili C (2016) Maintaining Genome Stability in Defiance of Mitotic DNA Damage. Front. Genet. 7:128. doi: 10.3389/fgene.2016.00128

INTRODUCTION

Signaling pathways have been initially depicted as linear cascades, with elements organized in a hierarchical manner and unidirectional arrows connecting a stimulus to the final response through a defined number of intermediates (Rodbell, 1980). The advent of systems biology, following completion of animal and plant genome sequencing, has changed this view. The amount of information available today allows to more realistically depict signaling pathways as networks, where the arrangement of components (nodes) is such that some are more connected than others in a so-called scale-free topology, and where sets of components are organized in modular fashion, with a clear hierarchy among modules (Barabási and Oltvai, 2004). Such architecture has been shown to ensure fault tolerance (robustness) in response to challenges (Barabási and Oltvai, 2004; Zhu et al., 2007). Corollary to system-level approaches has been the development of mathematical models where the fluctuation of variables as it actually occurs in defined biological systems can be computed, hence realistically representing the dynamic flow of information in signaling networks (Samaga and Klamt, 2013; Gerard et al., 2015).

The descriptive power of systems biology and its ability to predict scenarios do not, however, dwarf the contribution of reductionism when it comes to identification of network components and to dissection of their molecular mechanism of action, including elucidation of the inputs that affect their sub-cellular localization, the interaction with partner proteins and biochemical properties such as stability and enzymatic activity. It is only thanks to the wealth of information

provided by reductionist approaches that rational design of small molecule inhibitors able to interfere with the correct functioning of networks could be successfully guided (Asghar et al., 2015). Since the constitutive elements of network modules hierarchically relate to each other, modification of structural or enzymatic traits of one or more elements in a network will necessary affect network properties and result in outputs that are directly observable. Protein post-translational modification (PTM), in form of covalent addition of chemical groups or entire peptidyl moieties to one or more amino acids of a protein target, is the means to rapidly and, in most cases, reversibly affect such traits. The hierarchical, synergistic or antagonistic combination of PTMs defines a code that translates into distinct outputs, hence contributing to shape the emergent properties of complex systems like living organisms (Lorenz et al., 2011).

In this review, we focus on mitosis and examine how DNA damage occurring during transition through mitosis is addressed to avoid genome instability. Special emphasis will be set on the impact of PTMs on mechanisms of genome surveillance. We conclude with an up-to-date perspective on drugs designed for therapeutic purposes and that entered clinical trials.

MITOSIS AND CHECKPOINTS

Transition through the cell cycle sets the conditions for cell division. This results in the generation of two daughter cells genetically identical to the mother, according to a principle originally formulated by Rudolf Virchow who first made such observation in 1858 and stated that every cell derives from a pre-exiting cell, "*omnis cellula e cellula*" (Mazzarello, 1999). The major events characterizing transition through the cell cycle are cell growth, by which means cells increase their size and the number of organelles, and duplication of genetic material in S-phase. If not perturbed, upon completion of DNA replication cells enter mitosis, a term that originally described nuclear division (Mazzarello, 1999). Perturbations of this program may be caused by external agents such as ionizing radiation or certain chemotherapeutic drugs as well as by endogenous metabolic processes, leading to the formation of double-strand breaks (DSBs). Inappropriate repair of DSBs may cause gross chromosomal aberrations, the activation of oncogenes or the inactivation of tumor suppressor genes resulting in carcinogenesis. Direct demonstration of the importance of surveillance pathways in the maintenance of genome stability (Hanahan and Weinberg, 2011) is provided by genetic conditions characterized by dysfunction of the machinery that signals DNA damage and/or addresses its repair, which are associated with a predisposition to the development of cancer (Curtin, 2012).

Mitosis

Mitosis is probably the most spectacular event a cell undergoes to during its lifetime and it is essentially the process by which the duplicated genetic information is equally distributed to the daughter cells. Morphological changes that are easily observable with a microscope allow distinguishing sub-phases of mitosis consisting of prophase, metaphase, anaphase and telophase. These are followed by cytokinesis, ultimately causing physical separation of the daughter cells. The use of suitable model organisms and the support provided by modern technology has led us to a deep understanding of mechanistic aspects and regulatory pathways controlling the onset, execution and completion of mitosis. Briefly, in S-phase newly synthesized DNA emerging behind replication complexes that processively move on template DNA is maintained catenated throughout its length by ring-shaped cohesins and sister chromatids are held together at the centromeric region where kinetochores have been assembled (Kenney and Heald, 2006; Walczak et al., 2010). As cells move to prophase, chromatin condensation takes place, leading to the formation of visible rod-shaped structures, with a reduction of the length of DNA to an extent compatible with the distance that chromatids cover when moving to the opposite poles of the mitotic spindle (Walczak et al., 2010). Chromatin condensation results from the action of a multi-subunit protein complex called condensin, whose recruitment and activity are positively controlled by phosphorylation through CDK1, Aurora-B and PLKs and opposed by phosphorylation through CK2 (Hirano, 2012). Topoisomerase II, which undergoes phosphorylation and sumoylation in mitosis (Dephoure et al., 2008; Hendriks et al., 2014), ensures decatenation of sister chromatids prior to condensation (Hirano, 2015). Segregation of compacted chromosomes is initially prevented by cohesins (Peters et al., 2008) that are controlled by a combination of PTMs at lysine residues involving acetylation and sumoylation (Rudra and Skibbens, 2013) and are first removed at chromosome arms during prophase through PLK1-mediated phosphorylation (Hauf et al., 2005). At this time centromeric regions are protected by the protein shugoshin that, through recruitment of the phosphatase PP2A, counteracts PLK1 activity (Kitajima et al., 2006; Liu et al., 2013b). Construction of the mitotic spindle is the necessary step for physical separation of chromatids, with different strategies employed in distinct organisms to promote microtubule-to-kinetochore contacts (Boettcher and Barral, 2013). Microtubules forming the cell's cytoskeleton are disassembled in late prophase and highly dynamic microtubules radiate at this point from mature centrosomes or self-organize around chromosomes (Heald et al., 1996, 1997; Karsenti and Vernos, 2001), driving migration of centrosomes to opposite poles of the cell (inter-polar microtubules), anchoring centrosomes to the plasma membrane and positioning the spindle (astral microtubules) and initiating the capture of chromosomes (kinetochore microtubules). All these events are controlled by mitotic kinases (Nigg, 2001; Walczak et al., 2010).

In prophase, more than 100 proteins assemble around each centromeric region forming the kinetochore, while in the cytoplasm pairs of centrioles that have duplicated during S phase remain linked together at the proximal ends by a proteinaceous link containing C-Nap1 and rootletin, which is removed at mitotic entry through NEK2-mediated phosphorylation (Bahe et al., 2005; Hardy et al., 2014). Microtubule-chromosome interactions are characterized by the dynamic process of capture and release of erroneous attachments, as for instance merotelic attachments, which defines the condition of a single kinetochore being attached to microtubules nucleated from opposite spindle poles. Such interactions are principally regulated by

Aurora-B-mediated phosphorylation of kinetochore components (Cheeseman, 2014), occur in prometaphase and metaphase, and largely affect the duration of these sub-phases (Pereira and Maiato, 2012). The subsequent chromosome congression to the spindle equator (metaphase plate) is coordinated by the action of motor proteins such as dynein and CENP-E, the latter being controlled by an Aurora-A/PP1-dependent phosphorylation switch (Kim et al., 2010), and is followed by a process called bi-orientation, where kinetochores of sister chromatids attach to microtubule bundles that have nucleated from opposite centrosomes (Tanaka et al., 2005). Upon congression of all sister pairs to the metaphase plate, licensing of a multimeric E3-ligase, the anaphase promoting complex/cyclosome (APC/C), ensues and leads to ubiquitylation and degradation of proteins such as Cyclin B, switching off CDK1 activity, and securin, freeing the enzyme separase that is now able to cleave and remove centromeric cohesins (Sivakumar and Gorbsky, 2015). This point marks the metaphase-to-anaphase transition where mechanical processes, consisting of inter-polar microtubule elongation and kinetochore microtubule shortening, as well as biochemical events mediated by the action of APC/C, determine the movement of chromatids to spindle poles (Castro et al., 2005; Goshima and Scholey, 2010). The process is completed by re-establishment of the nuclear membrane around decondensing chromosomes at telophase and is followed by physical separation of daughter cells, or cytokinesis (Pines and Rieder, 2001), assisted by the action of an acto-myosin contractile ring (D'Avino, 2009).

G2/M Checkpoint

Entry and transition through mitosis is highly controlled by molecular constrains (checkpoints) that have evolved to prevent genomic instability and consist of the G2/M and the spindle assembly checkpoints. The G2/M checkpoint prevents mitotic entry to cells that have suffered DNA damage during G2 or that have progressed into G2 with unrepaired DNA lesions from previous cell cycle phases. Final target of the G2/M DNA damage checkpoint is CDK1, the master regulator of mitosis. The cascade of phosphorylation events impinging on CDK1 is briefly sketched below. Signals from unfinished DNA replication (through ATR/CHK1), damaged DNA (through ATM/CHK2) or DNA resected at sites of damage (through ATR/CHK1), activate the kinases WEE1/MYT1 that, in turn, phosphorylate T_{14} and Y_{15} in the Gly-rich P-loop of CDK1, causing inhibition of enzymatic activity (Heald et al., 1993; **Figure 1**). Phosphorylation at these sites does not impair ATP binding, neither sterically nor by electrostatic repulsion (Gould and Nurse, 1989), but rather hampers catalysis (Atherton-Fessler et al., 1993). Additionally, WEE1 enforces the signal of "NO-entry" into mitosis by inactivating CDC25 (Donzelli and Draetta, 2003), the phosphatase responsible for CDK1 dephosphorylation. Specifically, CHK1-dependent phosphorylation of CDC25A at Ser_{124}/Thr_{507} and of CDC25C at Ser_{216} mediates interaction with 14-3-3 proteins that, in turn, displace the phosphatases from the nucleus, a mechanism that appears to be the primary way to inhibit the function of these two phosphatases during G_2 and mitosis (Uto et al., 2004). On the other hand, inhibition of CDC25B, the phosphatase mediating activation of CDK1/Cyclin B at centrosomes during prophase, has been extensively studied in relation to its mitotic role (Gabrielli et al., 1996) but is less characterized in the context of the DNA damage response. Factors upstream of CDC25 or Cyclin B/CDK1, such as the Polo-like kinases PLK1 and PLK3 (Nyberg et al., 2002; Bahassi el et al., 2006), Aurora-A (Ferrari et al., 2005; Krystyniak et al., 2006; Bhatia et al., 2010) and protein phosphatase PP2A (Yan et al., 2010) are also part of the G2/M checkpoint signaling network. Maintenance of the G2/M checkpoint activation partly relies on transcriptional regulation by p53 that induces transcription of the cell-cycle inhibitor $p21^{CIP1/WAF1}$, and on expressions of 14-3-3s (a scaffold and signaling protein), PUMA (BCL2 binding component 3), BAX (BCL2 partner and apoptotic activator) and GADD45 (growth arrest and DNA-damage-inducible gene) (Nyberg et al., 2002; Riley et al., 2008). Upon completion of DNA synthesis or repair of damage, signals from WEE1 cease, resulting in progressive dephosphorylation and reactivation of CDC25C. The latter, in turn, initiates selective dephosphorylation of the inhibiting sites in CDK1 (Izumi and Maller, 1993), creating an auto-catalytic loop in which CDK1- (Hoffmann et al., 1993; Strausfeld et al., 1994) and Polo-like kinase 1 (PLK1)-dependent CDC25C phosphorylation (Strausfeld et al., 1994; Toyoshima-Morimoto et al., 2002) increase phosphatase activity leading to full dephosphorylation and activation of CDK1. As a result, the checkpoint is silenced and cell cycle progression ensues.

Spindle Assembly Checkpoint

The spindle assembly checkpoint (SAC) ensures that chromosomes are properly bi-oriented, preventing missegregation that would otherwise result in aneuploidy (Musacchio and Salmon, 2007). Target of the SAC is the APC/C, an E3 ubiquitin ligase composed of approximately 15 subunits, which binds its substrates by recognizing so-called degron sequences (Pines, 2011). APC/C is activated in mitosis by its co-activators CDC20 and CDH1 in a Cyclin B/CDK1-dependent manner (Wieser and Pines, 2015) and works in tandem with two distinct E2 conjugating enzymes: UBCH5 or UBCH10 that add the first ubiquitin moiety to APC/C substrates, and UBE2S that extends the chain (Rodrigo-Brenni and Morgan, 2007; Garnett et al., 2009) mediating preferentially the formation of K_{11}-linked ubiquitin chains (Wu et al., 2010; Bremm and Komander, 2012). K_{11}-chains show a distinct fold with respect to K_{48}- or K_{63}-linked ubiquitin (Matsumoto et al., 2010). A phosphorylation-dependent switch controls timely activation of the E2 UBE2S by the APC/C complex, whereby phosphorylation of Ser_{92} in CDC20 prevents delivery of UBE2S to the APC/C, and its dephosphorylation by $PP2A^{B56}$ allows UBE2S to bind the APC/C, catalyzing ubiquitin chain elongation (Craney et al., 2016).

Major players of the SAC are Mad1, Mad2, Bub1, BubR1/Mad3, Bub3, and Mps1, proteins that essentially monitor kinetochore—microtubule attachments and convert this to signals that inhibit metaphase-to-anaphase transition (Cheeseman, 2014). The main trigger of signals from SAC is Mad2, a protein that can assume an "open" (inactive) or a "close" (active) conformation. Mechanistically, the closed conformation

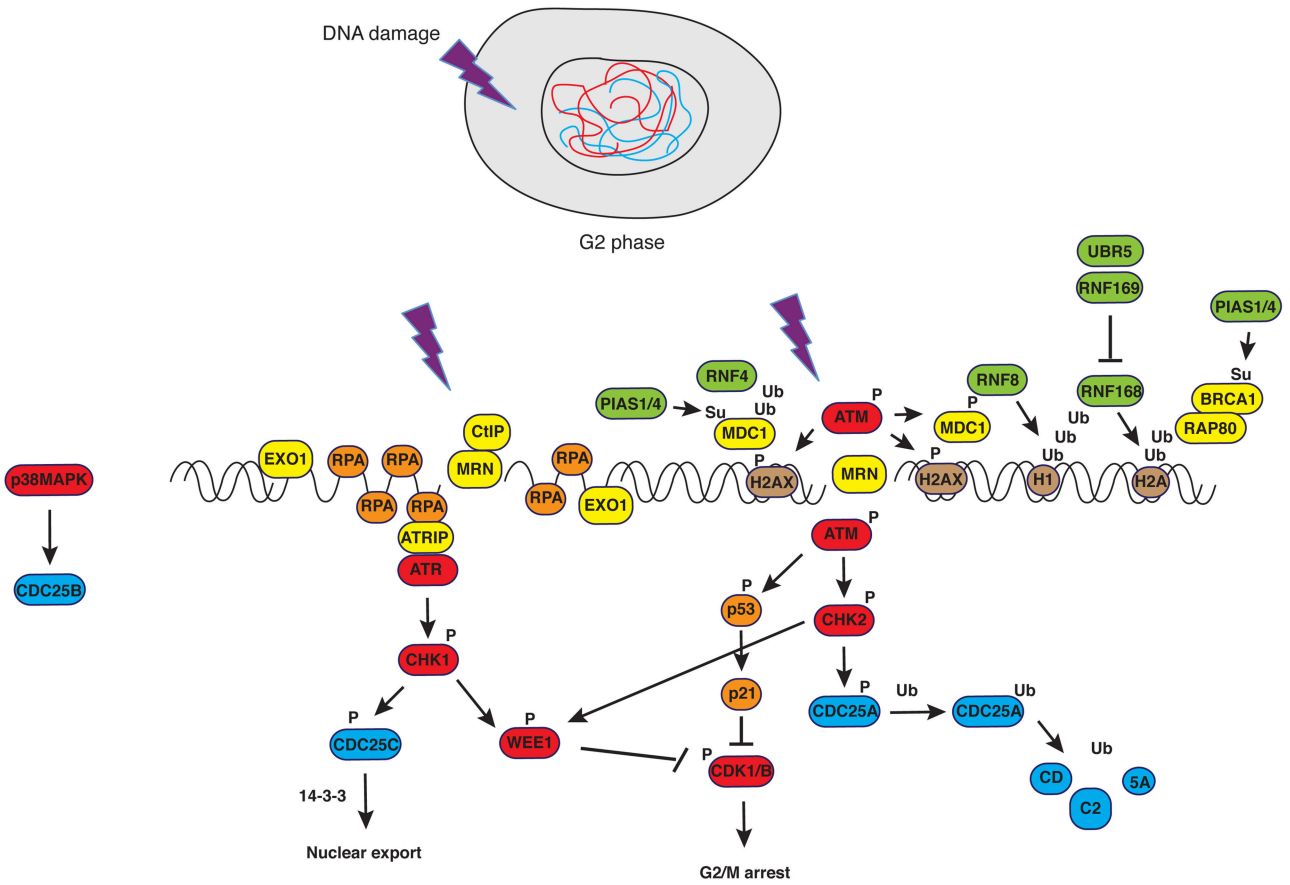

FIGURE 1 | DNA damage response in G2. Upon generation of double strand breaks (DSBs), ATM is recruited to DNA ends in a MRN-dependent manner. Phosphorylation of H2AX creates epitopes facilitating the recruitment of DNA damage signaling and repair factors in a manner that depends on PTMs such as ubiquitylation and sumoylation (see text for details and Bologna and Ferrari, 2013). Successful activation of ATM-dependent signals causes controlled resection of DNA ends that, in turn, trigger ATR-dependent pathways. The latter converge with the former on the master regulator of mitosis, CDK1, blocking its activity.

into which Mad2 folds once bound to kinetochores that are improperly attached to spindle microtubules is induced in further neighboring Mad2 molecules that diffuse away from kinetochores and associate with BubR1 and Bub3 forming the so-called mitotic checkpoint complex (MCC). The latter binds and sequesters the first co-activator of APC/C, Cdc20, in an MPS1-dependent manner (Wieser and Pines, 2015), blocking degradation of securin and effectively arresting cells in metaphase (Cheeseman, 2014; Sivakumar and Gorbsky, 2015). The kinases Aurora-B, CDK1 and PLK1 participate in regulating kinetochore function, with Aurora-B-dependent phosphorylation of Ndc80 N-terminus reducing the microtubule-binding affinity of the Ndc80 complex and eliminating incorrect kinetochore-microtubule attachments (Cheeseman et al., 2006). PLK1 associates and regulates several kinetochore proteins, including those localized in the inner centromere like CENP-U, phosphorylation of which facilitates PLK1 recruitment to the kinetochore (Kang et al., 2006), and PLK1-interacting checkpoint helicase (PICH) that binds the kinase through its Polo-box domain (Baumann et al., 2007). Once appropriate attachment is established (i.e., bi-orientation) such that sufficient tension is created and the kinase is spatially separated from its substrates (Liu et al., 2009), PP1 dephosphorylates Aurora-B targets (Cheeseman, 2014), with additional support from PP2A (Foley et al., 2011). Satisfaction of the checkpoint upon appropriate bi-orientation of chromosomes triggers the metaphase to anaphase transition.

CHALLENGES TO THE GENOME AND RESPONSES IN MITOSIS

In order to preserve the integrity of information contained in the genome, DNA is continuously monitored by proteins that recognize distinct types of damage. Such proteins or protein complexes—so called sensors—inform signal transducers that, in turn, prompt effectors to orchestrate repair of the damage (Bologna and Ferrari, 2013; Jackson and Durocher, 2013; Dantuma and van Attikum, 2016). In parallel, transducers trigger checkpoint pathways impinging on key cell cycle controllers (see above) that ultimately slow down or arrests transition through the cell cycle (Kastan and Bartek, 2004). Inappropriate detection or untimely repair of DNA damage before the onset

of mitosis may lead to chromosome breaks, rearrangements or fusions—comprehensively know as "structural abnormalities"—that facilitate the development of cancer (Branzei and Foiani, 2010; Curtin, 2012) and have been focus of intense research in the last decades (Aguilera and García-Muse, 2013).

DNA repair involves chromatin remodeling that, in turn, facilitates binding of repair factors to the region(s) where the lesion occurred (Aydin et al., 2014). This sequence of events has been observed during transition through the cell cycle, when the DNA repair machinery called to action faces simple or more challenging tasks, depending on whether damage is in euchromatin or in heterochromatin (Lemaître and Soutoglou, 2014). It appears, however, that DNA damage responses operative till completion of G2 and mediated through checkpoint kinases converging on WEE1 and CDK1 (Boddy et al., 1998; Smith et al., 2010; **Figure 1**), must be blocked at the time of chromosome condensation and segregation. In cells carrying a wild type complement of checkpoint genes, entry into prometaphase with ensuing chromosome condensation and nuclear envelope breakdown defines a point of non-return and puts an end to the checkpoint that was operative in G2.

Termination of activities on DNA in mitosis is exemplified by the repression of transcriptional activity (Martínez-Bálbas et al., 1995) that occurs through a passive process, consisting in limited access of transcription machinery to compacted chromatin, and an active mechanism, entailing CDK1-dependent phosphorylation of its components (Gottesfeld and Forbes, 1997). Similar mechanisms control DNA repair proteins, to avoid that active DSB repair during mitosis may result in telomere fusions, aneuploidy (Cesare, 2014; Orthwein et al., 2014) and whole chromosome missegregation through collateral stabilization of kinetochore-microtubules interactions (Bakhoum et al., 2014). Indeed, it was observed that in the absence of genotoxic stress, DNA repair proteins are phosphorylated in mitosis in a CDK- or PLK1-dependent manner to exclude them from chromatin (**Figure 2**). This is the case for BRCA2 (Lee et al., 2004), RAP80 (Cho et al., 2013), 53BP1 (Orthwein et al., 2014; Benada et al., 2015), RNF8 (Orthwein et al., 2014) and XRCC4 (Terasawa et al., 2014), to mention just few examples. RNF8, a well-characterized E3 ubiquitin ligase recruited to sites of damage through interaction of its N-terminal FHA domain with phosphorylated MDC1 and HERC2 (Bologna and Ferrari, 2013), the latter acting as coordinator of ubiquitin-dependent assembly of DNA repair factors (Bekker-Jensen et al., 2010), is phosphorylated by mitotic kinases to suppress its interaction with MDC1 (Orthwein et al., 2014). In the case of 53BP1, phosphorylation of two residues within the ubiquitylation-dependent recruitment (UDR) motif of 53BP1 in mitosis blocks binding to K_{15}-ubiquitylated histone H2A, thus impairing its recruitment to foci (Benada et al., 2015). On the other hand, PP4C/R3β-mediated dephosphorylation of these sites in G1 re-establishes 53BP1 binding to chromatin (Lee et al., 2014). In the case of BRCA2, PLK1-dependent phosphorylation at $S_{193,205,206}$ and $T_{203,207}$ causes dissociation from the histone acetyltransferase protein p300/CBP-associated factor (P/CAF) (Lin et al., 2003), and CDK-dependent S_{3291} phosphorylation at the onset of mitosis inhibits BRCA2-mediated stabilization of RAD51 nucleofilaments that are normally generated at sites of recombination (Esashi et al., 2005).

Shutting off repair in mitosis, however, does not imply that DNA damage is ignored if it occurs in this phase of the cell cycle. Evidence obtained in early studies conducted in vertebrate somatic cells showed that chromosome fragmentation caused by irradiation at the beginning of mitosis persisted till anaphase (Zirkle and Bloom, 1953), possibly indicating that repair pathways were not activated in this period of time. On the other hand, recent studies on the outcome of laser irradiation of mitotic chromosomes indicated that DNA damage response is triggered within 30 s from the treatment (Gomez-Godinez et al., 2010). Studies in the budding yeast *S. cerevisiae,* where activation of a dicentric chromosome was used to introduce a double strand DNA break into a chromosome at mitosis, showed that cells paused in mid-anaphase, triggering RAD9-dependent events that were reminiscent of a DNA damage response (Yang et al., 1997). Subsequent work conducted in yeast, where cells were irradiated in mitosis, showed that stabilization of Pds1, an anaphase inhibitor and APC/Cdc20 target, led to delay of anaphase and mitotic exit, facilitating repair of damage (Tinker-Kulberg and Morgan, 1999). Delay of the metaphase-to-anaphase transition was also reported for *Drosophila* embryos undergoing irradiation or being treated with methly metane sulfonate (MMS) and it was shown to depend on the stabilization of Cyclin A (Su and Jaklevic, 2001). Mitotic DNA damage in *X. laevis* and DT40 cells was shown to prevent spindle assembly in an ATM/ATR-dependent manner (Smith et al., 2009), and proposed to be an additional means to monitor chromosome breaks that have escaped the G2/M checkpoint.

It has been reported that eukaryotic cells are able to delay the execution of mitosis or, in some instances, reverse progression through mitosis, in response to DNA damage (Rieder and Cole, 1998) or microtubule poisons (Rieder and Cole, 2000) administered in antephase, a time when microscopic changes in the cell are not yet detectable and that physically spans from the conclusion of G2 to the initiation of chromosome condensation (Chin and Yeong, 2010). The same response was observed upon damage caused in early prophase (Rieder and Cole, 1998). In antephase, cells activate a checkpoint that is not mediated by PI-3K-like kinases such as ATM but rather depends on two proteins, the CHFR E3-ubiquitin ligase (Matsusaka and Pines, 2004; Shinde et al., 2013) that principally catalyzes poly-ubiquitylation of its substrates via K_{48} and K_{63} (Kang et al., 2002; Bothos et al., 2003; Oh et al., 2009) and is involved in the first wave of ubiquitylation at DNA damage sites (Liu et al., 2013a), and the Pro-directed p38 MAPK (Mikhailov et al., 2004; **Figure 2**). Cells containing a wild-type antephase checkpoint undergo chromosome decondensation and revert to a G2-like state (Rieder and Cole, 1998; Matsusaka and Pines, 2004), whereas cells lacking a functional CHFR progress into mitosis (Scolnick and Halazonetis, 2000). Extensive damage occurring upon completion of antephase does not normally cause reversion to an early stage of the cell cycle but rather triggers mitotic arrest through activation of SAC (Mikhailov et al., 2002, 2004; Choi and Lee, 2008), the only mechanism left in the arsenal of cells at this point of the cell cycle. SAC, however, does not orchestrate

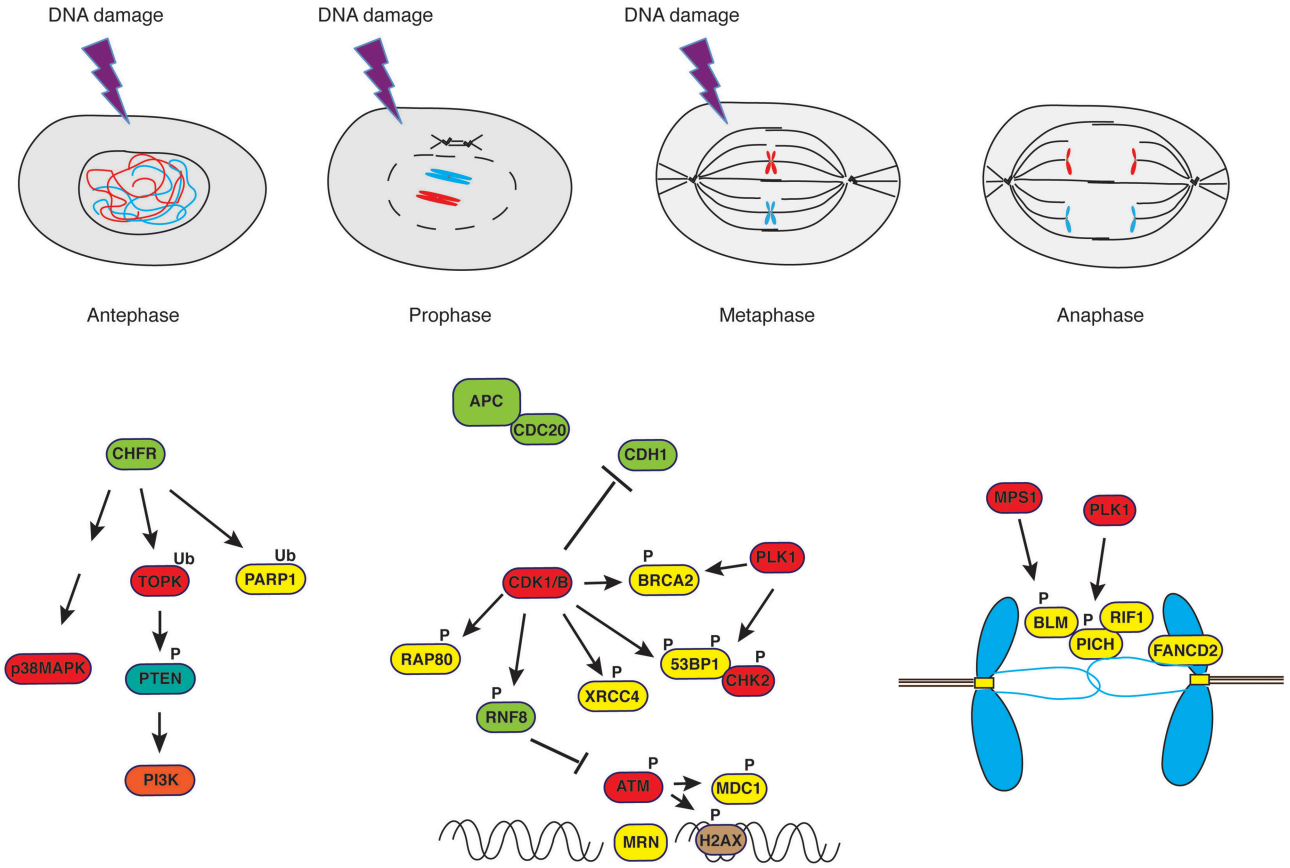

FIGURE 2 | DNA damage response in mitosis. Irradiation of cells in antephase or in early prophase triggers a response that is independent of PI-3K-like kinases such as ATM but rather depends on the E3-ubiquitin ligase CHFR and the stress-response kinase p38MAPK. On the other hand, irradiation of cells in late prophase or in metaphase leads to a curtailed DNA damage response. Ultrafine anaphase bridges, caused by improper resolution of replication or recombination intermediates, are addressed by the coordinated action of the helicases PICH and BLM supported by RIF1 (see text for details).

repair of damaged DNA but monitors that distribution of chromosomes to daughter cells occurs equally, hence avoiding aneuploidy. As mentioned above, SAC is active at kinetochores where the state of microtubule attachment is monitored, and signaling pathways preventing anaphase remain active as long as mono-oriented or incorrectly attached kinetochores are detected (Mikhailov et al., 2002; Cheeseman and Desai, 2008). Observations made in yeast (Pangilinan and Spencer, 1996) and in mammalian cells (Mikhailov et al., 2002) indicate that altering the topology of chromatin, particularly at regions that affect kinetochore structure, prevents satisfaction of SAC and delays transition through mitosis. These studies showed that ATM-dependent pathways (Mikhailov et al., 2002) or DDR genes (Pangilinan and Spencer, 1996) are not involved in the response to chromosome damage and that the metaphase block can be rapidly overridden by dominant-negative Mad2 (Mikhailov et al., 2002). However, a study addressing the effect of decatenation inhibitors (topoisomerase-II inhibitors) on nocodazole-arrested cells described a number of ATM-dependent events in response to these drugs, including H2AX phosphorylation, CDK1 inactivation, histone H3 dephosphorylation and chromosome decondensation, paralleled by stabilization of Cyclins A and B1, with cells apparently unable to exit mitosis (Chow et al., 2003). Studies conducted in our laboratory on cells that were synchronized in mitosis without disturbing microtubule dynamics, hence without "pre-sensitizing" cells by activation of stress responses that are known to increase levels of γH2AX (Giunta et al., 2010), and that we treated with ionizing radiation at metaphase, showed CHK2 activation, rapid inhibition of CDK1 and Aurora-A activities accompanied by reactivation of PP1, increased APC/CDH1 E3-ubiquitin ligase activity and chromosome decondensation (Bhatia et al., 2010). Our data clearly showed that metaphase-irradiated cells completed mitosis at the expenses of genome stability, displaying increased chromosome segregation defects and the formation of micronuclei (Bhatia et al., 2010).

A comprehensive study that assessed entity and amplitude of the DDR in mitosis by scoring formation of IR-induced foci (IRIF) and comparing mitotic to interphase cells concluded that only a subset of IRIF could form in mitosis, namely those comprising γ-H2AX, NBS1 and MDC1, but not RNF8, RNF168, BRCA1 or 53BP1 (Giunta et al., 2010) as also

confirmed by others (Nelson et al., 2009). Exclusion of RNF8 and 53BP1 from chromatin was shown to be the consequence of phosphorylation by mitotic kinases (see above) (Orthwein et al., 2014) and association of 53BP1 to IRIF was observed only upon nuclear envelope reformation around decompacting chromosomes in telophase (Giunta et al., 2010; **Figure 2**).

Specifically regarding ATM, its activation has been examined in mammalian cells both during undisturbed transition through mitosis or upon stress. In the absence of DNA damage, the kinase Aurora-B phosphorylates ATM on S_{1403} in mitosis, and abrogation of this event was shown to impair signaling through the spindle assembly checkpoint (Yang et al., 2011). Administration of taxol, a drug suppressing microtubule dynamics and causing mitotic stress, was reported to trigger ATM activity, though none of the known ATM targets in DDR such as SMC-1, NBS-1 or CHK-2 was phosphorylated under these conditions (Shen et al., 2006). DNA damage response-related roles for ATM in mitosis were inferred from early observations made in lymphoblastoid cells derived from A-T patients, which displayed a defective SAC upon treatment with radiation (Takagi et al., 1998; Shigeta et al., 1999). Another report described the activation of ATM in response to chromosomal breaks generated during mitotic catastrophe (Imreh et al., 2011). ATM activation was also examined upon irradiation of cells synchronized in mitosis with drugs that interfere with microtubule polymerization. Under these conditions, ionizing radiation triggered ATM activity, though CHK2 failed to fire and cells remained in mitosis with elevated phosphorylation at MPM-2 epitopes, indicative of high CDK1 activity. Mechanistically, the absence of a productive DDR signal following ATM activation was proposed to result from PLK1-dependent phosphorylation of CHK2, with 53BP1 acting as platform to bring PLK1 and CHK2 in close proximity (van Vugt et al., 2010; **Figure 2**).

As a whole, these studies confirm that ATM can fire when the minimal requirement for its activation is satisfied, namely the presence of exposed double-stranded ends (You et al., 2007), independently on the cell cycle position, though a productive DDR downstream of ATM seems not to be triggered in early mitosis.

In addition to DNA damage occurring during transition through mitosis, cells reaching mitosis are confronted with other problems: these are the structures resulting from incomplete DNA replication, improper resolution of replication intermediates or unresolved intermediates of homology-directed repair carried over from S-phase (Liu et al., 2014). Such structures become a threat at the time of chromosome segregation since they can cause sister chromatid entanglement and non-disjunction (Gelot et al., 2015). Incomplete DNA replication occurs at regions encompassing so-called "replication barriers." Predominant among those are common-fragile sites (CFSs) (Durkin and Glover, 2007), cytologically defined as segments in metaphase chromosomes displaying brakes at runs of flexible AT-rich repeats (Aguilera and García-Muse, 2013). CFSs constitute up to 80% of the breakpoints that lead to the gross chromosomal rearrangements (GCRs) observed in precancerous cells (Bartkova et al., 2006). Part of under-replicated CFSs observed in cells at anaphase remain connected through thin threads of DNA called ultrafine bridges (UFBs) (Liu et al., 2014). To avoid DNA breaks resulting from segregation of incompletely replicated chromosomes, these structures are addressed before cell division. It has been observed that BLM, along with topoisomerase IIIα, RMI1, RMI2 (BTRR complex) and PICH (PLK1-Interacting Checkpoint Helicase), coat anaphase UFBs (Baumann et al., 2007; Chan and Hickson, 2009; Chan et al., 2009; Naim and Rosselli, 2009; **Figure 2**). An earlier report on BLM phosphorylation by MPS1, facilitating accurate chromosome segregation (Leng et al., 2006), anticipated the important role played by this DNA helicase in mitosis. BLM recruitment to UBFs is facilitated by FANCD2, a key component of the Fanconi Anemia pathway, which was shown to form sister *foci* in mitosis (Naim and Rosselli, 2009; Harrigan et al., 2011; Lukas et al., 2011; **Figure 2**) and be necessary to prevent the generation of micronuclei (Naim and Rosselli, 2009). The SNF2 ATPase family member PICH plays an essential role at kinetochores and the inner centromere, as demonstrated by studies in which PICH depletion caused loss of Mad2 from kinetochores and abrogated the spindle checkpoint, events that were followed by chromosome missegregation (Baumann et al., 2007). Also PICH was proposed to help recruiting the BTRR complex at UFBs, cooperating to the resolution of DNA bridges by the end of anaphase (Liu et al., 2014; **Figure 2**). A recent addition to the pool of proteins present at UFBs is RIF1, ortholog of a yeast telomeric protein. RIF1 is recruited to UFBs in a PICH-dependent manner but independently of 53BP1, ATM or BLM, and phosphorylation by CDK1 restricts its ability to bind DNA at anaphase (Hengeveld et al., 2015). In addition to the BTRR complex, the Holliday Junction resolvases SLX1–SLX4–MUS81–EME1 (SLX–MUS complex) and GEN1 (Wyatt et al., 2013; Chan and West, 2014) contribute to process structures caused by under-replication at CFSs (Naim et al., 2013; Ying et al., 2013). The SLX–MUS complex cooperates with TopBP1, a scaffold protein composed of nine BRCT domains and recruited at sites of DNA damage in a 9-1-1-dependent manner (Delacroix et al., 2007; Lee et al., 2007; Wardlaw et al., 2014). TopBP1 is necessary for ATR activation (Kumagai et al., 2006), colocalizes with RPA and FANCD2 (Pedersen et al., 2015) forming *foci* on condensing chromatin through its BRCT5 domain, and recruits TOP2A to help resolving DNA entanglements between sister chromatids (Broderick et al., 2015). CDK1-dependent phosphorylation of EME1 in the MUS81-EME1 structure-specific endonuclease, promoting interaction with SLX1-SLX4, controls the resolution of DNA recombination intermediates in mitosis (Matos et al., 2011; Matos and West, 2014). Proteome-wide studies have identified a number of ubiquitylation sites in GEN1, MUS81, EME1, TopBP1 (Kim et al., 2011; Wagner et al., 2011; Mertins et al., 2013), though the biological function of such PTM and its eventual connection with mitotic functions of these proteins has not been addressed to date. Finally, human GEN1 acts as back up to the above-mentioned machinery at anaphase, moving in place and gaining access to DNA after nuclear envelope breakdown (NEB) (Wechsler et al., 2011; Chan and West, 2014; Sarbajna et al., 2014). For the yeast homolog of GEN1, Yen1, it was shown that activity and access to the nucleus depend on a reversible

CDK1/Cdc14 phosphorylation switch (Eissler et al., 2014; Matos and West, 2014).

In addition to the role of the above mentioned scaffold proteins in tethering nucleases to UFBs to the end of resolving DNA bridges in anaphase, unscheduled DNA synthesis at UFBs marked by TopBP1 (Pedersen et al., 2015) or SLX4 (Minocherhomji et al., 2015) has been reported and interpreted as an attempt to fill-in unreplicated regions, hence restoring genome integrity before cell division.

In case lesions generated by replication stress remain unrepaired, they are passed to daughter cells in a manner that shelters them from further damage through sequestration in 53BP1 nuclear bodies, thus allowing repair in the next cell cycle (Lukas et al., 2011). In the presence of extensive damage that remains unaddressed, cells experience sudden mitotic death also known as mitotic catastrophe (Morrison and Rieder, 2004; Vitale et al., 2011).

We have mentioned above that the DNA damage checkpoint is in place to facilitate DNA repair by blocking transition from G2 to M (**Figure 1**). A non-trivial consequence of prolonged arrest before mitosis is centrosome amplification, an event that is observed with high incidence in cancer cells carrying mutations of DNA repair genes. This event, which is alleviated upon bypass of the checkpoint in a manner that is only partially dependent on ATM, was postulated to be a mechanism ensuring death of cells that manage to evade the G2/M checkpoint or the SAC (Dodson et al., 2004). The metaphase-to-anaphase transition is a critical cell cycle stage during which chromosome missegregation may occur. Loss or gain of entire chromosomes—known as "numerical abnormalities"—resulting from chromosome missegregation during mitosis, is a characteristic of tumors known from more than a century and described as "aneuploidy" (Pellman, 2007). Mechanisms leading to aneuploidy have been amply reviewed elsewhere (Holland and Cleveland, 2009) and comprise (i) defective attachment of sister chromatids to spindle microtubules (merotelic attachment), often linked to centrosome amplification, (ii) malfunction of the spindle assembly checkpoint and (iii) defects in chromosome cohesion.

Key to a fully-fledged response to DNA damage is the network of signals that orchestrate assembly of DNA repair proteins at sites of damage and informs the cell cycle machinery. Ultimate target of G2/M checkpoint pathways is CDK1, the master regulator of mitosis (see "Mitosis and Checkpoints") that is maintained in an "OFF" status by direct negative inputs (WEE1) and inactivation of its positive regulators (CDC25), in conjunction with modulation of other enzymatic activities such as those of the kinases PLK1 (Smits et al., 2000), Aurora-A (Krystyniak et al., 2006) and protein phosphatase PP2A (Yan et al., 2010; **Figure 1**). The budding yeast *S. cerevisiae* represents a notable exception in this respect. Whereas in high eukaryotes CDKs have acquired specific functions throughout evolution, with CDK1 being the master controller of mitosis and undergoing immediate inhibition in an ATM/ATR-CHK1/CHK2-dependent manner upon DNA damage, *S. cerevisiae* possesses only one Cyclin-dependent kinase, Cdc28, controlling pathways and transitions in all phases of the cell cycle and whose activity depends on interaction with different Cyclins (Enserink and Kolodner, 2010). As opposed to CDK1, budding yeast Cdc28 is not inhibited by DNA damage response pathways, since the status of Tyr_{19} phosphorylation in the P-loop of Cdc28 is not a determinant for entry into mitosis (Amon et al., 1992). The key control of budding yeast mitosis is operative at the metaphase-to-anaphase transition, where degradation of the Esp1 (separase) inhibitor Pds1 (securin) allows cleavage of the Scc1 subunit in the cohesin complex and separation of the sisters (Ciosk et al., 1998; Sanchez et al., 1999). Hence, in yeast, mitotic arrest in response to DNA damage occurs in metaphase and depends on the abundance of Pds1 (Sanchez et al., 1999). This mechanism liberates Cdc28 of the control that CDK1 undergoes to in higher eukaryotes. Contrary to rapid inhibition upon DNA damage, Cdc28 is absolutely required in DDR and participates to the control of genome stability (Enserink et al., 2009). Cdc28 triggers homologous directed repair of DSBs through phosphorylation of Sae2 (Huertas et al., 2008), prompting initial resection of DNA ends (Ira et al., 2004), and other components of error-free repair pathways such as Dna2 (Ubersax et al., 2003) and Srs2 (Chiolo et al., 2005; Saponaro et al., 2010). Interestingly, Cdc28 targets such as Sae2 are also phosphorylated by classic DDR kinases, whereby mutation of phosphorylation sites for either set of kinases hampers repair and recombination functions of the protein (Baroni et al., 2004).

Hence, the rapid inactivation of vertebrate CDK1 in response to damage is difficult to reconcile with claims on its involvement in DNA damage responses at G2/M. Although it has been suggested that the gap between checkpoint triggering and CDK1 shutoff in vertebrate cells may be sufficient for CDK1 to orchestrate initial phases of repair, a much wiser interpretation of the experimental evidence is that repair of DNA damage in checkpoint-arrested cells depends on CDK2 (Wohlbold and Fisher, 2009) and other Proline-directed kinases involved in stress responses (Bulavin et al., 2001). As a matter of fact, high CDK1 activity, along with the activity of other mitotic kinases (Benada et al., 2015), is sufficient to suppress responses to DNA damage occurring during transition through mitosis in mammalian cells (Zhang et al., 2011). A further layer of regulation is imposed by phosphatases such as WIP1, a CDK1 target that undergoes ubiquitin-mediated degradation in mitosis, which sets the threshold for DDR signaling in mitosis by controlling the phosphorylation state of DDR proteins (Macurek et al., 2013).

MITOTIC PTMs AND CANCER THERAPY

Mitosis is the cell cycle phase that is most vulnerable to injury, regardless on whether damage is caused by radiation, heat-shock or chemicals (Westra and Dewey, 1971; Stobbe et al., 2002; Chan et al., 2012). Based on this indication, targeting mitotic cells has been largely exploited in the clinic as means to contain tumor growth (Doménech and Malumbres, 2013; Marzo and Naval, 2013). Molecular studies have highlighted the role of PTMs, and the enzymes that mediate them, in mechanisms controlling the

mitotic responses to stress (Pearce and Humphrey, 2001). This comes as no surprise, considering that essentially all mechanistic aspects of normal transition through mitosis are controlled by PTMs of mitotic machinery components, with reversible PTMs allowing a certain degree of flexibility in the decisions implemented and irreversible PTMs conferring directionality to the process (Nigg, 2001; Ma and Poon, 2011; Teixeira and Reed, 2013). Hence, mitotic protein kinases and E3-ubiquitin ligases with established role in cancer have become the focus of interest for chemists and pharmacologists designing and testing novel therapeutics that target cells in mitosis (Dominguez-Brauer et al., 2015). Such interest was also motivated by considerations on the side effects of classic anti-mitotic drugs like taxanes and vinca alkaloids that are currently deployed to the treatment of a variety of solid tumors such as breast, ovarian and lung cancer. Anti-mitotic drugs, due to their mode of action that alters microtubules' dynamic instability, result in neurotoxicity and neutropenia (Marzo and Naval, 2013). Furthermore, their lack of efficiency when used as single agents has evidenced another important limitation of these anti-mitotics (Doménech and Malumbres, 2013; Marzo and Naval, 2013). Shifting the focus to the discovery of drugs that target mitotic kinases or E3-ubiquitin ligases, however, did not solve the major caveat for cell cycle—and mitotic—inhibitors, namely the fact that the efficacy of a drug depends on the tumor proliferative rate: fast proliferation makes leukemia and myeloma relatively favorable conditions to treat, whereas a mitotic index (i.e., the percentage of mitotic cells in the whole populations) as little as 1% and doubling time of more than 1 year, as observed in some solid tumors, are negative factors to be taken into account when planning a treatment and its length.

Here below we provide a report on the current status of drug discovery and clinical trials for compounds targeting mitotic kinases and phosphatases as well as ubiquitin-proteasome system components (**Table 1** and **Figure 3**).

Inhibition of Mitotic Kinases

CDK1 Inhibitors

CDK1 is the master regulator of mitosis (Nigg, 2001). Flavopiridol is the first CDK1 inhibitor that underwent >60 clinical trials to date (www.clinicaltrials.gov). The poor efficacy of the compound, however, prevented its approval as anti-tumor drug (Shapiro, 2006; Stone et al., 2012; Galons et al., 2013). Other CDK1 inhibitors displaying high potency on cancer cell lines are currently in Phase I or II (with none of them being yet available for patients, see **Table 1** and references therein). So far, only the CDK1 inhibitor Dinaciclib was tested in a phase III study that was concluded in 2015 and aimed at treating refractory chronic lymphocytic leukemia patients (NCT01580228). In general, however, inhibition of CDK1 in healthy cells and the poor selectivity of CDK1 inhibitors, possibly due to the high degree of sequence conservation in the catalytic domain of CDK members, often result in major side effects when deployed in the clinic. For such reason, CDK1 inhibitors are currently used in combination therapies with other mitotic inhibitors (see below).

Aurora Kinases Inhibitors

Aurora kinase family members, Aurora-A, -B and -C exert different roles in the cell. Early studies showed that Aurora-A controls centrosomes maturation and separation, bipolar spindle formation and chromosomes segregation, while Aurora-B, as member of the Chromosome Passenger Complex, participates in the control of chromosome condensation and orientation on the mitotic spindle, ensuring correct kinetochore-microtubule attachments (Nigg, 2001). Both Aurora-A and -B were shown to stabilize midzone microtubules and regulate cytokinesis (Carmena et al., 2009). Aurora-C has a role in gametogenesis, it is expressed in testis, thyroid, and placenta and its contribution to cancer development was shown in mouse models (Khan et al., 2011). Several inhibitors against Aurora-A and Aurora-B have been developed during the last decade (Doménech and Malumbres, 2013; Bavetsias and Linardopoulos, 2015; D'Assoro et al., 2015; Falchook et al., 2015). As for CDKs, most Aurora kinases inhibitors target all family members and a major effort has been done to develop drugs that are more selective for individual Aurora kinases (**Table 1** and **Figure 3**). Among them, two reversible ATP competitive inhibitors, MLN8054 (Manfredi et al., 2007) and its derivative MLN8237 (Manfredi et al., 2011) have shown to be potent and selective Aurora-A inhibitors (Sells et al., 2015). Both were deployed in several studies and since MLN8237 has shown to be safer, it is currently under evaluation in Phase III clinical trials (NCT01482962).

Polo-Like Kinases Inhibitors

Polo-like family members constitute another class of Serine/Threonine (Ser/Thr) kinases with key roles in mitosis (Nigg, 2001). Five PLKs are expressed in human cells, PLK1-5, with PLK1 and PLK4 being the major representatives of this family (Zitouni et al., 2014). Distinguishing feature of PLKs is the polo-box domain that flanks the catalytic domain and allows docking to substrates primed by CDKs to carry on their phosphorylation (Zitouni et al., 2014).

Mechanistically, PLK1 is activated by Aurora-A (Ferrari et al., 2005; Macurek et al., 2008) at the onset of mitosis and functions to promote centrosome maturation and separation, assembly and elongation of the mitotic spindle as well as cytokinesis (Barr et al., 2004; Degenhardt and Lampkin, 2010). PLK1 is overexpressed in various malignancies (Holtrich et al., 1994; Eckerdt et al., 2005; Mito et al., 2005; Takai et al., 2005; Strebhardt and Ullrich, 2006; Renner et al., 2009; Weiß and Efferth, 2012). PLK1 inhibition in cancer patients has been pursued with some success using two ATP-competitive kinase inhibitors: BI-2536 and BI-6727 (see **Table 1**). The potency, pharmacokinetic and pharmacodynamic properties of BI-6727 as well as its antitumor activity in a number of cancer models (Rudolph et al., 2009) has promoted the drug to a phase III trial for acute myeloid leukemia patients where BI-6727 was tested in combination with the DNA-synthesis blocking agent cytarabine (NCT01721876).

PLK4 has a fundamental role in centriole duplication (Bettencourt-Dias et al., 2005; Habedanck et al., 2005). PLK4 overexpression leads to the formation of extra centrosomes

TABLE 1 | List of drugs, their mitotic targets and current clinical trial phase.

Drug	Target	Status	References
MITOTIC KINASE INHIBITORS			
Roscovitine (Cyclacel)	CDK2, CDK7, CDK9	Phase I-II	De Azevedo et al., 1997
AT7519 (Astex)	pan-CDKs	Phase I-II	Wyatt et al., 2008
Dinaciclib (Merck)	pan-CDKs	Phase I-II-III	Parry et al., 2010
Flavopiridol (Sanofi-Aventis)	pan-CDKs	Phase I-II	De Azevedo et al., 1996
P276-00 (Piramal)	pan-CDKs	Phase I-II	Joshi et al., 2007
RGB 286638 (Agennix)	pan-CDKs and others	Phase I-II	Cirstea et al., 2013
Terameprocol (Erimos)	CDK1 and Survivin	Phase I-II	Heller et al., 2001; Chang et al., 2004
TG02 (Tragara)	pan-CDKs, JAK2, FLT3	Phase I	Goh et al., 2012
MK-1775 (Merk)	Wee1	Phase I-II	Hirai et al., 2009
BI-2536 (Boehringer Ingelheim)	Plk1	Phase I-II	Lenart et al., 2007
Volasertib/BI-6727 (Boehringer Ingelheim)	Plk1	Phase I-II-III	Rudolph et al., 2009
CFI-400945 (Campbell Family Institute, CAN)	Plk4	Phase I	Mason et al., 2014
AMG-900 (Amgen)	Aurora-kinases	Phase I	Payton et al., 2010
AT-9283 (Astex)	Aurora-kinases	Phase I-II	Howard et al., 2009
CYC-116 (Cyclacel)	Aurora-kinases	Phase I	Wang et al., 2010
PHA-680632 (Pfizer/Nerviano MS)	Aurora-kinases	Phase II-III	Soncini et al., 2006
GSK1070916 (GlaxoSmithKline)	Aurora-kinases	Phase I	Hardwicke et al., 2009
PF-03814735 (Pfizer)	Aurora-kinases	Phase I	Jani et al., 2010
Danusertib/PHA-739358 (Pfizer/Nerviano MS)	Aurora-kinases	Phase II	Carpinelli et al., 2007
R763/AS703569 (Rigel)	Aurora-kinases	Pre-Clinical	McLaughlin et al., 2010
SNS-314 (Sunesis)	Aurora-kinases	Phase I	Oslob et al., 2008
MK-0457 (VX-680) (Vertex/Merck) Tozasertib	Aurora-kinases	Phase I-II	Harrington et al., 2004
ENMD-2076 (EntreMed)	Aurora-A	Phase I-II	Tentler et al., 2010
Alisertib/MLN8237 (Millennium)	Aurora-A	Phase I-II	Görgün et al., 2010
Barasertib/AZD1152 (AstraZeneca)	Aurora B	Phase I-II-III	Mortlock et al., 2007; Wilkinson et al., 2007
2OH-BNPP1	Bub1	Pre-Clinical	Kang et al., 2008; Nyati et al., 2015
BAY-320/BAY-524 (Bayer)	Bub1	Pre-Clinical	Baron et al., 2016
Cycloalkenepyrazoles	Bub1	Pre-Clinical	Brazeau and Rosse, 2014
BAY 1161909/BAY 1217389 (Bayer)	Mps1	Phase I	Wengner et al., 2016
CFI-402257 (Campbell Family Institute, CAN)	Mps1	Pre-Clinical	Dominguez-Brauer et al., 2015
S81694 (Nerviano MS)	Mps1	Pre-Clinical	Colombo et al., 2015
CRT0105446	LIMK1 and LIMK2	Pre-Clinical	Mardilovich et al., 2015
CRT0105950	LIMK1 and LIMK2	Pre-Clinical	Mardilovich et al., 2015
MITOTIC PHOSPHATASE INHIBITORS			
IRC 083864/Debio 0931 (Ipsen -DebioPharma)	CDC25	Phase II (*)	Lavecchia et al., 2010
LB100 (Lixte biotechnology)	PP2A	Pre-clinical/Phase I	Lu et al., 2009
UBIQUITIN-PROTEASOME SYSTEM INHIBITORS			
Bortezomib (Millennium)	Proteasome	Phase I-II	Hideshima et al., 2001
Carfilzomib (Onyx Pharmaceuticals)	Proteasome	Phase I-II	Kortuem and Stewart, 2013
MLN9708 (Millennium)	Proteasome	Phase I-II	Chauhan et al., 2011
CEP-18770 (Cephalon)	Proteasome	Phase I-II	Seavey et al., 2012
TAK-243 (MLN7243, Millennium - Takeda)	E1 (UBA1)	Pre-clinical/Phase I	Milhollen et al., 2015
Nutlins (Roche)	E3 (MDM2)	Pre-clinical	Vassilev, 2007
TAME	E3 (APC/C - Cdc20)	Pre-clinical	Zeng et al., 2010
Apcin (Harvard U - Boston Biochem)	E3 (APC/C - Cdc20)	Pre-clinical	Sackton et al., 2014
MLN4924 (Millennium)	NEDD8 activating enzyme (NAE)	Phase I-II	Soucy et al., 2009

**Since launch in Phase II, no additional information has been rendered available at ClinicalTrials.gov.*

resulting in aberrant mitotic spindles and aneuploid daughter cells (Basto et al., 2008; Ganem et al., 2009; Holland et al., 2010). Evidence on PLK4 overexpression in tumors (Macmillan et al., 2001; van de Vijver et al., 2002; Miller et al., 2005; Hu et al., 2006; Salvatore et al., 2007; Chng et al., 2008) raised the interest to develop small molecule inhibitors of this kinase. CFI-400945

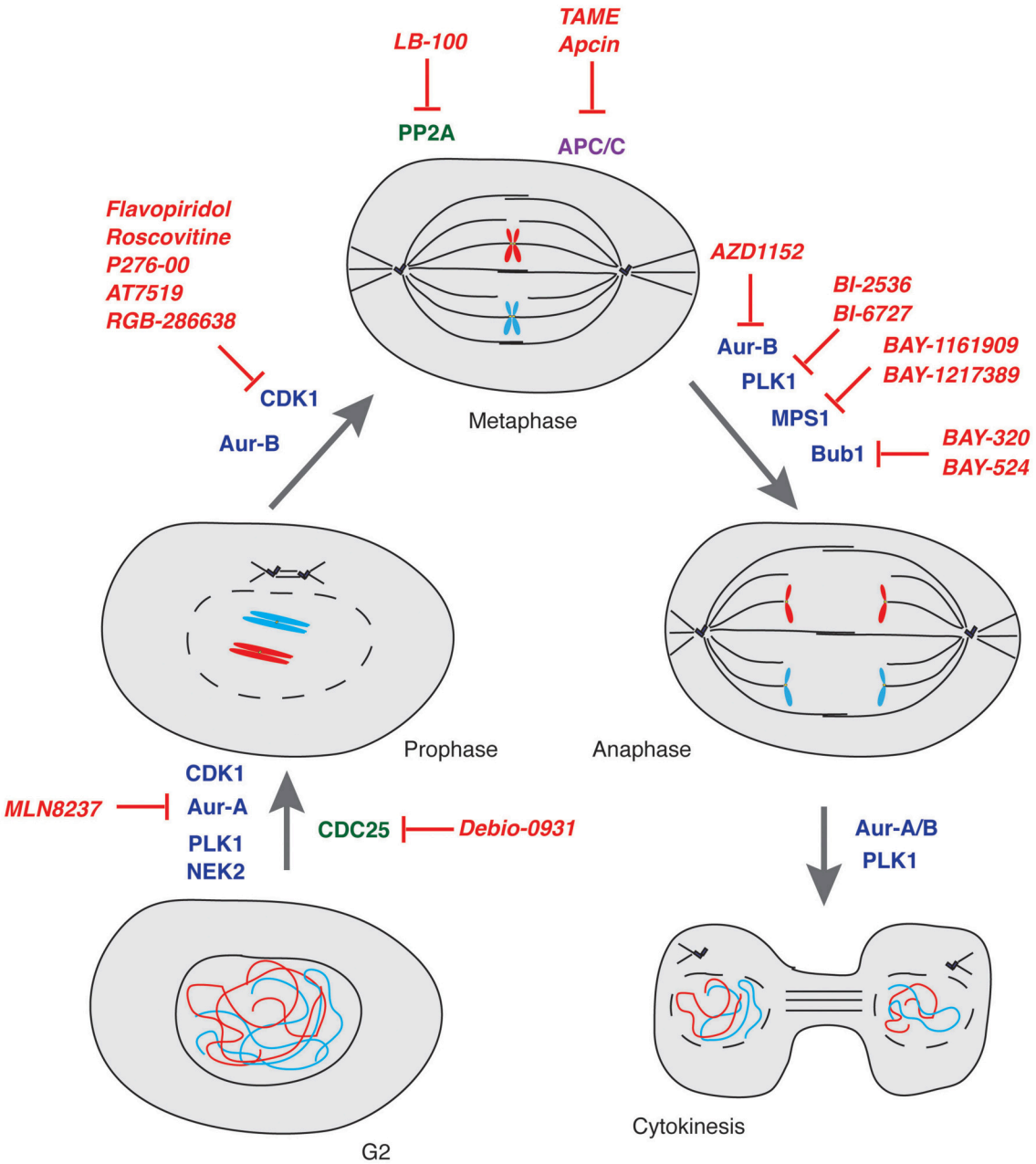

FIGURE 3 | Mitosis and its control by kinases, phosphatases and E3-ubiquitin ligases. Schematic representation of key controllers of the onset, transition and exit from mitosis with indication of the major drugs inhibiting their function. Kinases: blue; Phosphatases: green; E3-ubiquitin ligases: purple; Drugs: red.

was shown to be a potent and selective PLK4 inhibitor exerting a dose-dependent effect on centriole biogenesis (Mason et al., 2014). At high concentration, CFI-400945 inhibits centriole duplication, while at low concentration it causes the generation of supernumerary centrosomes. Interestingly, in both cases, cells arrest or die (Mason et al., 2014). In the same study, the anti-cancer potential of CFI-400945 was also shown in mice and the drug is currently under evaluation in advanced cancer patients (NCT01954316).

Supernumerary centrosomes occur at high frequency in cancer cells but not in non-transformed cells and were originally proposed by Theodor Boveri to be linked to cancer development (Brinkley and Goepfert, 1998; Brinkley, 2001). Supernumerary centrosomes tend to cluster at mitosis forming pseudo-bipolar spindles to avoid multipolar mitoses that would result in the generation of unviable progeny (Ganem et al., 2009). Formation of pseudo-bipolar spindles where merotelic chromosome attachments is frequent, is among the major

causes of aneuploidy (Ganem et al., 2009). The anti-fungal drug griseofulvin was shown to freeze the process of centrosome clustering (Raab et al., 2012) and since then a number of small molecule inhibitors of this process have been synthesized and examined (Kawamura et al., 2013; Ogden et al., 2014; Bhakta-Guha et al., 2015). Centrosome declustering drugs are, however, still in pre-clinical studies (Krämer et al., 2011; Pannu et al., 2014) given two main considerations: The first is that eliminating the subpopulation of cancer cells carrying centrosome amplifications in the heterogeneous collection of cells making up a tumor is yet to be proven beneficial in anticancer therapy. The second is that identification of individuals suitable to treatment with centrosome declustering drugs still awaits routine screening methods to define the genetic makeup of patients with centrosome amplification who would benefit of such treatment (Godinho and Pellman, 2014).

Mitotic Phosphatase Inhibitors

Members of the CDC25 family of protein phosphatases act as positive regulators of CDKs that are their unique targets (see above). The only report on CDC25 targeting drugs is for phase II clinical trials initiated in 2010 with IRC 083864 under the name Debio-0931 (Lavecchia et al., 2010), a drug that has previously shown activity against pancreatic and cervical cancer xenografts (Brezak et al., 2009). To date, LB-100 is the only know drug targeting the Ser/Thr phosphatase PP2A to have entered phase I trials in combination with cytotoxic drugs or irradiation for the indication "solid tumors" (NCT01837667) (Hong et al., 2015). Inhibition of enzymes with multiple functions such as PP2A, by many considered unfeasible due to the associated high toxicity of such treatments, was shown to be well-tolerated if the drug is administered intermittently (http://www.lixte.com/Product_Development.php). LB-100 has been granted licensing in Asia for treatment of Hepatocellular Carcinoma in December 2015 (http://adisinsight.springer.com/drugs/800037966).

Ubiquitin-Proteasome Inhibitors

The established role of ubiquitin-dependent pathways in the degradation of mitotic apparatus components has made them an ideal site of intervention in cancer therapy and possible applications of proteasome inhibitors to the treatment of cancer, their mode of action and mechanisms of resistance have been amply reviewed (Crawford et al., 2011; Zhang et al., 2013). Approval of Bortezomid over a decade ago for the indications multiple myeloma and multiple cell lymphoma paved the way to the discovery of candidates with reduced side effects and improved efficacy that are currently in clinical trial (Zhang et al., 2013). Specifically to mitosis, a new perspective was provided in a report describing the use of spindle poisons in conjunction with inhibition of the ATPase activity of components of the proteasome to increase apoptosis in cancer cells (Yamada and Gorbsky, 2006), offering further possibilities of intervention.

The majority of drugs that we discussed above halt cells before mitosis or in early mitosis. Prolonged treatment with drugs interfering with microtubules dynamics has been described to lead to mitotic exit—operationally defined mitotic slippage—(Brito and Rieder, 2006), a condition that leads to the acquisition of further aneuploidy and aggressiveness (Kuukasjarvi et al., 1997; McClelland et al., 2009). Hence, significant effort has been devoted in recent years to block mitotic exit. Inhibiting the interaction of CDC20 with APC/C by TAME (tosyl-L-arginine methyl ester) has shown to effectively halt cells in mitosis and channel them to death (Zeng et al., 2010; Zeng and King, 2012). The more recently developed APC/C inhibitor Apcin, showing the ability to bind CDC20 and to prevent ubiquitylation of D-box containing APC/C targets, has provided an additional means to block mitotic exit (Sackton et al., 2014). The combined use of Apcin and TAME was reported to synergistically halt mitotic exit, hence opening new therapeutic perspectives (Sackton et al., 2014).

In a similar fashion Nutlins were described to impair physical interaction between p53 and the E3 ubiquitin ligase MDM2, promoting p53 stabilization and enhancing its tumor suppressor activity (Vassilev et al., 2004; Vassilev, 2007). Enthusiasm for these drugs, however, was mitigated by two major drawbacks: first the observation that MDM2 interacts preferentially with wild-type p53 (Lukashchuk and Vousden, 2007) and, second, the report that Nutlins exert a cytostatic effects in p53-deficient cells, indicating that they do not solely inhibit the p53/MDM2 interaction (VanderBorght et al., 2006).

In conclusion, it is foreseeable that the development of novel and specific drugs targeting components of pathways that control mitosis and/or interfere with signals that fine-tune their function, in conjunction with stratification of patients based on their genetic background, will allow to better determine combination therapies for each individual patient, taking us a step closer to personalized medicine.

AUTHOR CONTRIBUTIONS

SF conceived the review topic and wrote the manuscript. CG contributed to write the manuscript.

FUNDING

This work was supported by grants of the Promedica-Stiftung, the Stiftung zur Krebsbekämpfung and the Stiftung für wissenschaftliche Forschung.

ACKNOWLEDGMENTS

We are grateful to Pavel Janscak and members of SF laboratory for critical reading of the manuscript as well as to Doriano Fabbro for helpful suggestions. We apologize to those colleagues whose work has not been cited due to space constraints.

REFERENCES

Aguilera, A., and García-Muse, T. (2013). Causes of genome instability. *Annu. Rev. Genet.* 47, 1–32. doi: 10.1146/annurev-genet-111212-133232

Amon, A., Surana, U., Muroff, I., and Nasmyth, K. (1992). Regulation of p34CDC28 tyrosine phosphorylation is not required for entry into mitosis in *S. cerevisiae*. *Nature* 355, 368–371. doi: 10.1038/355368a0

Asghar, U., Witkiewicz, A. K., Turner, N. C., and Knudsen, E. S. (2015). The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat. Rev. Drug Discov.* 14, 130–146. doi: 10.1038/nrd4504

Atherton-Fessler, S., Parker, L. L., Geahlen, R. L., and Piwnica-Worms, H. (1993). Mechanisms of p34cdc2 regulation. *Mol. Cell. Biol.* 13, 1675–1685. doi: 10.1128/MCB.13.3.1675

Aydin, Ö. Z., Vermeulen, W., and Lans, H. (2014). ISWI chromatin remodeling complexes in the DNA damage response. *Cell Cycle* 13, 3016–3025. doi: 10.4161/15384101.2014.956551

Bahassi el, M., Myer, D. L., McKenney, R. J., Hennigan, R. F., and Stambrook, P. J. (2006). Priming phosphorylation of Chk2 by polo-like kinase 3 (Plk3) mediates its full activation by ATM and a downstream checkpoint in response to DNA damage. *Mutat. Res.* 596, 166–176. doi: 10.1016/j.mrfmmm.2005.12.002

Bahe, S., Stierhof, Y. D., Wilkinson, C. J., Leiss, F., and Nigg, E. A. (2005). Rootletin forms centriole-associated filaments and functions in centrosome cohesion. *J. Cell Biol.* 171, 27–33. doi: 10.1083/jcb.200504107

Bakhoum, S. F., Kabeche, L., Murnane, J. P., Zaki, B. I., and Compton, D. A. (2014). DNA-damage response during mitosis induces whole-chromosome missegregation. *Cancer Discov.* 4, 1281–1289. doi: 10.1158/2159-8290.CD-14-0403

Barabási, A. L., and Oltvai, Z. N. (2004). Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* 5, 101–113. doi: 10.1038/nrg1272

Baron, A. P., von Schubert, C., Cubizolles, F., Siemeister, G., Hitchcock, M., Mengel, A., et al. (2016). Probing the catalytic functions of Bub1 kinase using the small molecule inhibitors BAY-320 and BAY-524. *Elife* 5:e12187. doi: 10.7554/eLife.12187

Baroni, E., Viscardi, V., Cartagena-Lirola, H., Lucchini, G., and Longhese, M. P. (2004). The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. *Mol. Cell. Biol.* 24, 4151–4165. doi: 10.1128/MCB.24.10.4151-4165.2004

Barr, F. A., Silljé, H. H., and Nigg, E. A. (2004). Polo-like kinases and the orchestration of cell division. *Nat. Rev. Mol. Cell Biol.* 5, 429–440. doi: 10.1038/nrm1401

Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., et al. (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. *Nature* 444, 633–637. doi: 10.1038/nature05268

Basto, R., Brunk, K., Vinadogrova, T., Peel, N., Franz, A., Khodjakov, A., et al. (2008). Centrosome amplification can initiate tumorigenesis in flies. *Cell* 133, 1032–1042. doi: 10.1016/j.cell.2008.05.039

Baumann, C., Körner, R., Hofmann, K., and Nigg, E. A. (2007). PICH, a centromere-associated SNF2 family ATPase, is regulated by Plk1 and required for the spindle checkpoint. *Cell* 128, 101–114. doi: 10.1016/j.cell.2006.11.041

Bavetsias, V., and Linardopoulos, S. (2015). Aurora kinase inhibitors: current status and outlook. *Front. Oncol.* 5:278. doi: 10.3389/fonc.2015.00278

Bekker-Jensen, S., Rendtlew Danielsen, J., Fugger, K., Gromova, I., Nerstedt, A., Lukas, C., et al. (2010). HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. *Nat. Cell Biol.* 12, 80–86; sup pp 1–12. doi: 10.1038/ncb2008

Benada, J., Burdová, K., Lidak, T., von Morgen, P., and Macurek, L. (2015). Polo-like kinase 1 inhibits DNA damage response during mitosis. *Cell Cycle* 14, 219–231. doi: 10.4161/15384101.2014.977067

Bettencourt-Dias, M., Rodrigues-Martins, A., Carpenter, L., Riparbelli, M., Lehmann, L., Gatt, M. K., et al. (2005). SAK/PLK4 is required for centriole duplication and flagella development. *Curr. Biol.* 15, 2199–2207. doi: 10.1016/j.cub.2005.11.042

Bhakta-Guha, D., Saeed, M. E., Greten, H. J., and Efferth, T. (2015). Dis-organizing centrosomal clusters: specific cancer therapy for a generic spread? *Curr. Med. Chem.* 22, 685–694. doi: 10.2174/0929867322666141212114529

Bhatia, P., Menigatti, M., Brocard, M., Morley, S. J., and Ferrari, S. (2010). Mitotic DNA damage targets the Aurora A/TPX2 complex. *Cell Cycle* 9, 4592–4599. doi: 10.4161/cc.9.22.13665

Boddy, M. N., Furnari, B., Mondesert, O., and Russell, P. (1998). Replication checkpoint enforced by kinases Cds1 and Chk1. *Science* 280, 909–912. doi: 10.1126/science.280.5365.909

Boettcher, B., and Barral, Y. (2013). The cell biology of open and closed mitosis. *Nucleus* 4, 160–165. doi: 10.4161/nucl.24676

Bologna, S., and Ferrari, S. (2013). It takes two to tango: Ubiquitin and SUMO in the DNA damage response. *Front. Genet.* 4:106. doi: 10.3389/fgene.2013.00106

Bothos, J., Summers, M. K., Venere, M., Scolnick, D. M., and Halazonetis, T. D. (2003). The Chfr mitotic checkpoint protein functions with Ubc13-Mms2 to form Lys63-linked polyubiquitin chains. *Oncogene* 22, 7101–7107. doi: 10.1038/sj.onc.1206831

Branzei, D., and Foiani, M. (2010). Maintaining genome stability at the replication fork. *Nat. Rev. Mol. Cell Biol.* 11, 208–219. doi: 10.1038/nrm2852

Brazeau, J. F., and Rosse, G. (2014). Novel cycloalkenepyrazoles as inhibitors of bub1 kinase. *ACS Med. Chem. Lett.* 5, 280–281. doi: 10.1021/ml5000492

Bremm, A., and Komander, D. (2012). Synthesis and analysis of K11-linked ubiquitin chains. *Methods Mol. Biol.* 832, 219–228. doi: 10.1007/978-1-61779-474-2_15

Brezak, M. C., Valette, A., Quaranta, M., Contour-Galcera, M. O., Jullien, D., Lavergne, O., et al. (2009). IRC-083864, a novel bis quinone inhibitor of CDC25 phosphatases active against human cancer cells. *Int. J. Cancer* 124, 1449–1456. doi: 10.1002/ijc.24080

Brinkley, B. R. (2001). Managing the centrosome numbers game: from chaos to stability in cancer cell division. *Trends Cell Biol.* 11, 18–21. doi: 10.1016/S0962-8924(00)01872-9

Brinkley, B. R., and Goepfert, T. M. (1998). Supernumerary centrosomes and cancer: Boveri's hypothesis resurrected. *Cell Motil. Cytoskeleton* 41, 281–288.

Brito, D. A., and Rieder, C. L. (2006). Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. *Curr. Biol.* 16, 1194–1200. doi: 10.1016/j.cub.2006.04.043

Broderick, R., Nieminuszczy, J., Blackford, A. N., Winczura, A., and Niedzwiedz, W. (2015). TOPBP1 recruits TOP2A to ultra-fine anaphase bridges to aid in their resolution. *Nat. Commun.* 6, 6572. doi: 10.1038/ncomms7572

Bulavin, D. V., Higashimoto, Y., Popoff, I. J., Gaarde, W. A., Basrur, V., Potapova, O., et al. (2001). Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature* 411, 102–107. doi: 10.1038/35075107

Carmena, M., Ruchaud, S., and Earnshaw, W. C. (2009). Making the Auroras glow: regulation of Aurora, A., and B kinase function by interacting proteins. *Curr. Opin. Cell Biol.* 21, 796–805. doi: 10.1016/j.ceb.2009.09.008

Carpinelli, P., Ceruti, R., Giorgini, M. L., Cappella, P., Gianellini, L., Croci, V., et al. (2007). PHA-739358, a potent inhibitor of Aurora kinases with a selective target inhibition profile relevant to cancer. *Mol. Cancer Ther.* 6, 3158–3168. doi: 10.1158/1535-7163.MCT-07-0444

Castro, A., Bernis, C., Vigneron, S., Labbé, J. C., and Lorca, T. (2005). The anaphase-promoting complex: a key factor in the regulation of cell cycle. *Oncogene* 24, 314–325. doi: 10.1038/sj.onc.1207973

Cesare, A. J. (2014). Mitosis, double strand break repair, and telomeres: a view from the end: how telomeres and the DNA damage response cooperate during mitosis to maintain genome stability. *Bioessays* 36, 1054–1061. doi: 10.1002/bies.201400104

Chan, K. L., and Hickson, I. D. (2009). On the origins of ultra-fine anaphase bridges. *Cell Cycle* 8, 3065–3066. doi: 10.4161/cc.8.19.9513

Chan, K. L., Palmai-Pallag, T., Ying, S., and Hickson, I. D. (2009). Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. *Nat. Cell Biol.* 11, 753–760. doi: 10.1038/ncb1882

Chan, K. S., Koh, C. G., and Li, H. Y. (2012). Mitosis-targeted anti-cancer therapies: where they stand. *Cell Death Dis.* 3, e411. doi: 10.1038/cddis.2012.148

Chan, Y. W., and West, S. C. (2014). Spatial control of the GEN1 Holliday junction resolvase ensures genome stability. *Nat. Commun.* 5, 4844. doi: 10.1038/ncomms5844

Chang, Y. C., Chou, F. P., Huang, H. P., Hsu, J. D., and Wang, C. J. (2004). Inhibition of cell cycle progression by penta-acetyl geniposide in rat C6 glioma cells. *Toxicol. Appl. Pharmacol.* 198, 11–20. doi: 10.1016/j.taap.2004.03.004

Chauhan, D., Tian, Z., Zhou, B., Kuhn, D., Orlowski, R., Raje, N., et al. (2011). *In vitro* and *in vivo* selective antitumor activity of a novel orally bioavailable

proteasome inhibitor MLN9708 against multiple myeloma cells. *Clin. Cancer Res.* 17, 5311–5321. doi: 10.1158/1078-0432.CCR-11-0476

Cheeseman, I. M. (2014). The kinetochore. *Cold Spring Harb. Perspect. Biol.* 6:a015826. doi: 10.1101/cshperspect.a015826

Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M., and Desai, A. (2006). The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell* 127, 983–997. doi: 10.1016/j.cell.2006.09.039

Cheeseman, I. M., and Desai, A. (2008). Molecular architecture of the kinetochore-microtubule interface. *Nat. Rev. Mol. Cell Biol.* 9, 33–46. doi: 10.1038/nrm2310

Chin, C. F., and Yeong, F. M. (2010). Safeguarding entry into mitosis: the antephase checkpoint. *Mol. Cell Biol.* 30, 22–32. doi: 10.1128/MCB.00687-09

Chiolo, I., Carotenuto, W., Maffioletti, G., Petrini, J. H., Foiani, M., and Liberi, G. (2005). Srs2 and Sgs1 DNA helicases associate with Mre11 in different subcomplexes following checkpoint activation and CDK1-mediated Srs2 phosphorylation. *Mol. Cell. Biol.* 25, 5738–5751. doi: 10.1128/MCB.25.13.5738-5751.2005

Chng, W. J., Braggio, E., Mulligan, G., Bryant, B., Remstein, E., Valdez, R., et al. (2008). The centrosome index is a powerful prognostic marker in myeloma and identifies a cohort of patients that might benefit from aurora kinase inhibition. *Blood* 111, 1603–1609. doi: 10.1182/blood-2007-06-097774

Cho, H. J., Oh, Y. J., Han, S. H., Chung, H. J., Kim, C. H., Lee, N. S., et al. (2013). Cdk1 protein-mediated phosphorylation of receptor-associated protein 80 (RAP80) serine 677 modulates DNA damage-induced G2/M checkpoint and cell survival. *J. Biol. Chem.* 288, 3768–3776. doi: 10.1074/jbc.M112.401299

Choi, E., and Lee, H. (2008). Chromosome damage in mitosis induces BubR1 activation and prometaphase arrest. *FEBS Lett.* 582, 1700–1706. doi: 10.1016/j.febslet.2008.04.028

Chow, J. P., Siu, W. Y., Fung, T. K., Chan, W. M., Lau, A., Arooz, T., et al. (2003). DNA damage during the spindle-assembly checkpoint degrades CDC25A, inhibits cyclin-CDC2 complexes, and reverses cells to interphase. *Mol. Biol. Cell* 14, 3989–4002. doi: 10.1091/mbc.E03-03-0168

Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M., and Nasmyth, K. (1998). An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell* 93, 1067–1076. doi: 10.1016/S0092-8674(00)81211-8

Cirstea, D., Hideshima, T., Santo, L., Eda, H., Mishima, Y., Nemani, N., et al. (2013). Small-molecule multi-targeted kinase inhibitor RGB-286638 triggers P53-dependent and -independent anti-multiple myeloma activity through inhibition of transcriptional CDKs. *Leukemia* 27, 2366–2375. doi: 10.1038/leu.2013.194

Colombo, R., Burbridge, M., Rodriguez, M., Cantero, F., Caldarelli, M., Giorgini, M. L., et al. (2015). Abstract 1638: Preclinical characterization of the novel TTK kinase inhibitor S81694 for the treatment of triple negative breast cancer. *Cancer Res.* 75:1638. doi: 10.1158/1538-7445.AM2015-1638

Craney, A., Kelly, A., Jia, L., Fedrigo, I., Yu, H., and Rape, M. (2016). Control of APC/C-dependent ubiquitin chain elongation by reversible phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* 113, 1540–1545. doi: 10.1073/pnas.1522423113

Crawford, L. J., Walker, B., and Irvine, A. E. (2011). Proteasome inhibitors in cancer therapy. *J. Cell Commun. Signal.* 5, 101–110. doi: 10.1007/s12079-011-0121-7

Curtin, N. J. (2012). DNA repair dysregulation from cancer driver to therapeutic target. *Nat. Rev. Cancer* 12, 801–817. doi: 10.1038/nrc3399

Dantuma, N. P., and van Attikum, H. (2016). Spatiotemporal regulation of posttranslational modifications in the DNA damage response. *EMBO J.* 35, 6–23. doi: 10.15252/embj.201592595

D'Assoro, A. B., Haddad, T., and Galanis, E. (2015). Aurora-A kinase as a promising therapeutic target in cancer. *Front. Oncol.* 5:295. doi: 10.3389/fonc.2015.00295

D'Avino, P. P. (2009). How to scaffold the contractile ring for a safe cytokinesis - lessons from Anillin-related proteins. *J. Cell Sci.* 122, 1071–1079. doi: 10.1242/jcs.034785

De Azevedo, W. F., Leclerc, S., Meijer, L., Havlicek, L., Strnad, M., and Kim, S. H. (1997). Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine. *Eur. J. Biochem.* 243, 518–526. doi: 10.1111/j.1432-1033.1997.0518a.x

De Azevedo, W. F. Jr., Mueller-Dieckmann, H. J., Schulze-Gahmen, U., Worland, P. J., Sausville, E., and Kim, S. H. (1996). Structural basis for specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2735–2740. doi: 10.1073/pnas.93.7.2735

Degenhardt, Y., and Lampkin, T. (2010). Targeting Polo-like kinase in cancer therapy. *Clin. Cancer Res.* 16, 384–389. doi: 10.1158/1078-0432.CCR-09-1380

Delacroix, S., Wagner, J. M., Kobayashi, M., Yamamoto, K., and Karnitz, L. M. (2007). The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes Dev.* 21, 1472–1477. doi: 10.1101/gad.1547007

Dephoure, N., Zhou, C., Villén, J., Beausoleil, S. A., Bakalarski, C. E., Elledge, S. J., et al. (2008). A quantitative atlas of mitotic phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10762–10767. doi: 10.1073/pnas.0805139105

Dodson, H., Bourke, E., Jeffers, L. J., Vagnarelli, P., Sonoda, E., Takeda, S., et al. (2004). Centrosome amplification induced by DNA damage occurs during a prolonged G2 phase and involves ATM. *EMBO J.* 23, 3864–3873. doi: 10.1038/sj.emboj.7600393

Doménech, E., and Malumbres, M. (2013). Mitosis-targeting therapies: a troubleshooting guide. *Curr. Opin. Pharmacol.* 13, 519–528. doi: 10.1016/j.coph.2013.03.011

Dominguez-Brauer, C., Thu, K. L., Mason, J. M., Blaser, H., Bray, M. R., and Mak, T. W. (2015). Targeting Mitosis in Cancer: Emerging Strategies. *Mol. Cell* 60, 524–536. doi: 10.1016/j.molcel.2015.11.006

Donzelli, M., and Draetta, G. F. (2003). Regulating mammalian checkpoints through Cdc25 inactivation. *EMBO Rep.* 4, 671–677. doi: 10.1038/sj.embor.embor887

Durkin, S. G., and Glover, T. W. (2007). Chromosome fragile sites. *Annu. Rev. Genet.* 41, 169–192. doi: 10.1146/annurev.genet.41.042007.165900

Eckerdt, F., Yuan, J., and Strebhardt, K. (2005). Polo-like kinases and oncogenesis. *Oncogene* 24, 267–276. doi: 10.1038/sj.onc.1208273

Eissler, C. L., Mazón, G., Powers, B. L., Savinov, S. N., Symington, L. S., and Hall, M. C. (2014). The Cdk/cDc14 module controls activation of the Yen1 holliday junction resolvase to promote genome stability. *Mol. Cell* 54, 80–93. doi: 10.1016/j.molcel.2014.02.012

Enserink, J. M., Hombauer, H., Huang, M. E., and Kolodner, R. D. (2009). Cdc28/Cdk1 positively and negatively affects genome stability in S. *cerevisiae. J Cell Biol* 185, 423–437. doi: 10.1083/jcb.200811083

Enserink, J. M., and Kolodner, R. D. (2010). An overview of Cdk1-controlled targets and processes. *Cell Div.* 5:11. doi: 10.1186/1747-1028-5-11

Esashi, F., Christ, N., Gannon, J., Liu, Y., Hunt, T., Jasin, M., et al. (2005). CDK-dependent phosphorylation of BRCA2 as a regulatory mechanism for recombinational repair. *Nature* 434, 598–604. doi: 10.1038/nature03404

Falchook, G. S., Bastida, C. C., and Kurzrock, R. (2015). Aurora kinase inhibitors in oncology clinical trials: current state of the progress. *Semin. Oncol.* 42, 832–848. doi: 10.1053/j.seminoncol.2015.09.022

Ferrari, S., Marin, O., Pagano, M. A., Meggio, F., Hess, D., El-Shemerly, M., et al. (2005). Aurora-A site specificity: a study with synthetic peptide substrates. *Biochem. J.* 390, 293–302. doi: 10.1042/BJ20050343

Foley, E. A., Maldonado, M., and Kapoor, T. M. (2011). Formation of stable attachments between kinetochores and microtubules depends on the B56-PP2A phosphatase. *Nat. Cell Biol.* 13, 1265–1271. doi: 10.1038/ncb2327

Gabrielli, B. G., De Souza, C. P., Tonks, I. D., Clark, J. M., Hayward, N. K., and Ellem, K. A. (1996). Cytoplasmic accumulation of cdc25B phosphatase in mitosis triggers centrosomal microtubule nucleation in HeLa cells. *J. Cell Sci.* 109 (Pt 5), 1081–1093.

Galons, H., Oumata, N., Gloulou, O., and Meijer, L. (2013). Cyclin-dependent kinase inhibitors closer to market launch? *Expert Opin. Ther. Pat.* 23, 945–963. doi: 10.1517/13543776.2013.789861

Ganem, N. J., Godinho, S. A., and Pellman, D. (2009). A mechanism linking extra centrosomes to chromosomal instability. *Nature* 460, 278–282. doi: 10.1038/nature08136

Garnett, M. J., Mansfeld, J., Godwin, C., Matsusaka, T., Wu, J., Russell, P., et al. (2009). UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. *Nat. Cell Biol.* 11, 1363–1369. doi: 10.1038/ncb1983

Gelot, C., Magdalou, I., and Lopez, B. S. (2015). Replication stress in Mammalian cells and its consequences for mitosis. *Genes (Basel).* 6, 267–298. doi: 10.3390/genes6020267

Gerard, C., Tyson, J. J., Coudreuse, D., and Novak, B. (2015). Cell cycle control by a minimal Cdk network. *PLoS Comput. Biol.* 11:e1004056. doi: 10.1371/journal.pcbi.1004056

Giunta, S., Belotserkovskaya, R., and Jackson, S. P. (2010). DNA damage signaling in response to double-strand breaks during mitosis. *J. Cell Biol.* 190, 197–207. doi: 10.1083/jcb.200911156

Godinho, S. A., and Pellman, D. (2014). Causes and consequences of centrosome abnormalities in cancer. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 369:20130467. doi: 10.1098/rstb.2013.0467

Goh, K. C., Novotny-Diermayr, V., Hart, S., Ong, L. C., Loh, Y. K., Cheong, A., et al. (2012). TG02, a novel oral multi-kinase inhibitor of CDKs, JAK2 and FLT3 with potent anti-leukemic properties. *Leukemia* 26, 236–243. doi: 10.1038/leu.2011.218

Gomez-Godinez, V., Wu, T., Sherman, A. J., Lee, C. S., Liaw, L. H., Zhongsheng, Y., et al. (2010). Analysis of DNA double-strand break response and chromatin structure in mitosis using laser microirradiation. *Nucleic Acids Res.* 38, e202. doi: 10.1093/nar/gkq836

Görgün, G., Calabrese, E., Hideshima, T., Ecsedy, J., Perrone, G., Mani, M., et al. (2010). A novel Aurora-A kinase inhibitor MLN8237 induces cytotoxicity and cell-cycle arrest in multiple myeloma. *Blood* 115, 5202–5213. doi: 10.1182/blood-2009-12-259523

Goshima, G., and Scholey, J. M. (2010). Control of mitotic spindle length. *Annu. Rev. Cell Dev. Biol.* 26, 21–57. doi: 10.1146/annurev-cellbio-100109-104006

Gottesfeld, J. M., and Forbes, D. J. (1997). Mitotic repression of the transcriptional machinery. *Trends Biochem. Sci.* 22, 197–202. doi: 10.1016/S0968-0004(97)01045-1

Gould, K. L., and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. *Nature* 342, 39–45. doi: 10.1038/342039a0

Habedanck, R., Stierhof, Y. D., Wilkinson, C. J., and Nigg, E. A. (2005). The Polo kinase Plk4 functions in centriole duplication. *Nat. Cell Biol.* 7, 1140–1146. doi: 10.1038/ncb1320

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

Hardwicke, M. A., Oleykowski, C. A., Plant, R., Wang, J., Liao, Q., Moss, K., et al. (2009). GSK1070916, a potent Aurora B/C kinase inhibitor with broad antitumor activity in tissue culture cells and human tumor xenograft models. *Mol. Cancer Ther.* 8, 1808–1817. doi: 10.1158/1535-7163.MCT-09-0041

Hardy, T., Lee, M., Hames, R. S., Prosser, S. L., Cheary, D. M., Samant, M. D., et al. (2014). Multisite phosphorylation of C-Nap1 releases it from Cep135 to trigger centrosome disjunction. *J. Cell Sci.* 127, 2493–2506. doi: 10.1242/jcs.142331

Harrigan, J. A., Belotserkovskaya, R., Coates, J., Dimitrova, D. S., Polo, S. E., Bradshaw, C. R., et al. (2011). Replication stress induces 53BP1-containing OPT domains in G1 cells. *J. Cell Biol.* 193, 97–108. doi: 10.1083/jcb.201011083

Harrington, E. A., Bebbington, D., Moore, J., Rasmussen, R. K., Ajose-Adeogun, A. O., Nakayama, T., et al. (2004). VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth *in vivo*. *Nat. Med.* 10, 262–267. doi: 10.1038/nm1003

Hauf, S., Roitinger, E., Koch, B., Dittrich, C. M., Mechtler, K., and Peters, J. M. (2005). Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. *PLoS Biol.* 3:e69. doi: 10.1371/journal.pbio.0030069

Heald, R., McLoughlin, M., and McKeon, F. (1993). Human wee1 maintains mitotic timing by protecting the nucleus from cytoplasmically activated Cdc2 kinase. *Cell* 74, 463–474. doi: 10.1016/0092-8674(93)80048-J

Heald, R., Tournebize, R., Blank, T., Sandaltzopoulos, R., Becker, P., Hyman, A., et al. (1996). Self-organization of microtubules into bipolar spindles around artificial chromosomes in Xenopus egg extracts. *Nature* 382, 420–425. doi: 10.1038/382420a0

Heald, R., Tournebize, R., Habermann, A., Karsenti, E., and Hyman, A. (1997). Spindle assembly in Xenopus egg extracts: respective roles of centrosomes and microtubule self-organization. *J. Cell Biol.* 138, 615–628. doi: 10.1083/jcb.138.3.615

Heller, J. D., Kuo, J., Wu, T. C., Kast, W. M., and Huang, R. C. (2001). Tetra-O-methyl nordihydroguaiaretic acid induces G2 arrest in mammalian cells and exhibits tumoricidal activity *in vivo*. *Cancer Res.* 61, 5499–5504.

Hendriks, I. A., D'Souza, R. C., Yang, B., Verlaan-de Vries, M., Mann, M., and Vertegaal, A. C. (2014). Uncovering global SUMOylation signaling networks in a site-specific manner. *Nat. Struct. Mol. Biol.* 21, 927–936. doi: 10.1038/nsmb.2890

Hengeveld, R. C., de Boer, H. R., Schoonen, P. M., de Vries, E. G., Lens, S. M., and van Vugt, M. A. (2015). Rif1 is required for resolution of ultrafine DNA bridges in anaphase to ensure genomic stability. *Dev. Cell* 34, 466–474. doi: 10.1016/j.devcel.2015.06.014

Hideshima, T., Richardson, P., Chauhan, D., Palombella, V. J., Elliott, P. J., Adams, J., et al. (2001). The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res.* 61, 3071–3076.

Hirai, H., Iwasawa, Y., Okada, M., Arai, T., Nishibata, T., Kobayashi, M., et al. (2009). Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. *Mol. Cancer Ther.* 8, 2992–3000. doi: 10.1158/1535-7163.MCT-09-0463

Hirano, T. (2012). Condensins: universal organizers of chromosomes with diverse functions. *Genes Dev.* 26, 1659–1678. doi: 10.1101/gad.194746.112

Hirano, T. (2015). Chromosome Dynamics during Mitosis. *Cold Spring Harb. Perspect. Biol.* 7:a015792. doi: 10.1101/cshperspect.a015792

Hoffmann, I., Clarke, P. R., Marcote, M. J., Karsenti, E., and Draetta, G. (1993). Phosphorylation and activation of human cdc25-C by cdc2–cyclin B and its involvement in the self-amplification of MPF at mitosis. *EMBO J.* 12, 53–63.

Holland, A. J., and Cleveland, D. W. (2009). Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat. Rev. Mol. Cell Biol.* 10, 478–487. doi: 10.1038/nrm2718

Holland, A. J., Lan, W., Niessen, S., Hoover, H., and Cleveland, D. W. (2010). Polo-like kinase 4 kinase activity limits centrosome overduplication by autoregulating its own stability. *J. Cell Biol.* 188, 191–198. doi: 10.1083/jcb.200911102

Holtrich, U., Wolf, G., Bräuninger, A., Karn, T., Böhme, B., Rübsamen-Waigmann, H., et al. (1994). Induction and down-regulation of PLK, a human serine/threonine kinase expressed in proliferating cells and tumors. *Proc. Natl. Acad. Sci. U.S.A.* 91, 1736–1740. doi: 10.1073/pnas.91.5.1736

Hong, C. S., Ho, W., Zhang, C., Yang, C., Elder, J. B., and Zhuang, Z. (2015). LB100, a small molecule inhibitor of PP2A with potent chemo- and radio-sensitizing potential. *Cancer Biol. Ther.* 16, 821–833. doi: 10.1080/15384047.2015.1040961

Howard, S., Berdini, V., Boulstridge, J. A., Carr, M. G., Cross, D. M., Curry, J., et al. (2009). Fragment-based discovery of the pyrazol-4-yl urea (AT9283), a multitargeted kinase inhibitor with potent aurora kinase activity. *J. Med. Chem.* 52, 379–388. doi: 10.1021/jm800984v

Hu, Z., Fan, C., Oh, D. S., Marron, J. S., He, X., Qaqish, B. F., et al. (2006). The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics* 7:96. doi: 10.1186/1471-2164-7-96

Huertas, P., Cortés-Ledesma, F., Sartori, A. A., Aguilera, A., and Jackson, S. P. (2008). CDK targets Sae2 to control DNA-end resection and homologous recombination. *Nature* 455, 689–692. doi: 10.1038/nature07215

Imreh, G., Norberg, H. V., Imreh, S., and Zhivotovsky, B. (2011). Chromosomal breaks during mitotic catastrophe trigger gammaH2AX-ATM-p53-mediated apoptosis. *J. Cell Sci.* 124, 2951–2963. doi: 10.1242/jcs.081612

Ira, G., Pellicioli, A., Balijja, A., Wang, X., Fiorani, S., Carotenuto, W., et al. (2004). DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. *Nature* 431, 1011–1017. doi: 10.1038/nature02964

Izumi, T., and Maller, J. L. (1993). Elimination of cdc2 phosphorylation sites in the cdc25 phosphatase blocks initiation of M-phase. *Mol. Biol. Cell* 4, 1337–1350. doi: 10.1091/mbc.4.12.1337

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jani, J. P., Arcari, J., Bernardo, V., Bhattacharya, S. K., Briere, D., Cohen, B. D., et al. (2010). PF-03814735, an orally bioavailable small molecule aurora kinase inhibitor for cancer therapy. *Mol. Cancer Ther.* 9, 883–894. doi: 10.1158/1535-7163.MCT-09-0915

Joshi, K. S., Rathos, M. J., Joshi, R. D., Sivakumar, M., Mascarenhas, M., Kamble, S., et al. (2007). *In vitro* antitumor properties of a novel cyclin-dependent kinase inhibitor, P276-00. *Mol. Cancer Ther.* 6, 918–925. doi: 10.1158/1535-7163.MCT-06-0613

Kang, D., Chen, J., Wong, J., and Fang, G. (2002). The checkpoint protein Chfr is a ligase that ubiquitinates Plk1 and inhibits Cdc2 at the G2 to M transition. *J. Cell Biol.* 156, 249–259. doi: 10.1083/jcb.200108016

Kang, J., Yang, M., Li, B., Qi, W., Zhang, C., Shokat, K. M., et al. (2008). Structure and substrate recruitment of the human spindle checkpoint kinase Bub1. *Mol. Cell* 32, 394–405. doi: 10.1016/j.molcel.2008.09.017

Kang, Y. H., Park, J. E., Yu, L. R., Soung, N. K., Yun, S. M., Bang, J. K., et al. (2006). Self-regulated Plk1 recruitment to kinetochores by the Plk1-PBIP1 interaction is critical for proper chromosome segregation. *Mol. Cell* 24, 409–422. doi: 10.1016/j.molcel.2006.10.016

Karsenti, E., and Vernos, I. (2001). The mitotic spindle: a self-made machine. *Science* 294, 543–547. doi: 10.1126/science.1063488

Kastan, M. B., and Bartek, J. (2004). Cell-cycle checkpoints and cancer. *Nature* 432, 316–323. doi: 10.1038/nature03097

Kawamura, E., Fielding, A. B., Kannan, N., Balgi, A., Eaves, C. J., Roberge, M., et al. (2013). Identification of novel small molecule inhibitors of centrosome clustering in cancer cells. *Oncotarget* 4, 1763–1776. doi: 10.18632/oncotarget.1198

Kenney, R. D., and Heald, R. (2006). Essential roles for cohesin in kinetochore and spindle function in Xenopus egg extracts. *J. Cell Sci.* 119, 5057–5066. doi: 10.1242/jcs.03277

Khan, J., Ezan, F., Crémet, J. Y., Fautrel, A., Gilot, D., Lambert, M., et al. (2011). Overexpression of active Aurora-C kinase results in cell transformation and tumour formation. *PLoS ONE* 6:e26512. doi: 10.1371/journal.pone.0026512

Kim, W., Bennett, E. J., Huttlin, E. L., Guo, A., Li, J., Possemato, A., et al. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* 44, 325–340. doi: 10.1016/j.molcel.2011.08.025

Kim, Y., Holland, A. J., Lan, W., and Cleveland, D. W. (2010). Aurora kinases and protein phosphatase 1 mediate chromosome congression through regulation of CENP-E. *Cell* 142, 444–455. doi: 10.1016/j.cell.2010.06.039

Kitajima, T. S., Sakuno, T., Ishiguro, K., Iemura, S., Natsume, T., Kawashima, S. A., et al. (2006). Shugoshin collaborates with protein phosphatase 2A to protect cohesin. *Nature* 441, 46–52. doi: 10.1038/nature04663

Kortuem, K. M., and Stewart, A. K. (2013). Carfilzomib. *Blood* 121, 893–897. doi: 10.1182/blood-2012-10-459883

Krämer, A., Maier, B., and Bartek, J. (2011). Centrosome clustering and chromosomal (in)stability: a matter of life and death. *Mol. Oncol.* 5, 324–335. doi: 10.1016/j.molonc.2011.05.003

Krystyniak, A., Garcia-Echeverria, C., Prigent, C., and Ferrari, S. (2006). Inhibition of Aurora A in response to DNA damage. *Oncogene* 25, 338–348.

Kumagai, A., Lee, J., Yoo, H. Y., and Dunphy, W. G. (2006). TopBP1 activates the ATR-ATRIP complex. *Cell* 124, 943–955. doi: 10.1016/j.cell.2005.12.041

Kuukasjärvi, T., Karhu, R., Tanner, M., Kähkönen, M., Schäffer, A., Nupponen, N., et al. (1997). Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer. *Cancer Res.* 57, 1597–1604.

Lavecchia, A., Di Giovanni, C., and Novellino, E. (2010). Inhibitors of Cdc25 phosphatases as anticancer agents: a patent review. *Expert Opin. Ther. Pat.* 20, 405–425. doi: 10.1517/13543771003623232

Lee, D. H., Acharya, S. S., Kwon, M., Drane, P., Guan, Y., Adelmant, G., et al. (2014). Dephosphorylation enables the recruitment of 53BP1 to double-strand DNA breaks. *Mol. Cell* 54, 512–525. doi: 10.1016/j.molcel.2014.03.020

Lee, J., Kumagai, A., and Dunphy, W. G. (2007). The Rad9-Hus1-Rad1 checkpoint clamp regulates interaction of TopBP1 with ATR. *J. Biol. Chem.* 282, 28036–28044. doi: 10.1074/jbc.M704635200

Lee, M., Daniels, M. J., and Venkitaraman, A. R. (2004). Phosphorylation of BRCA2 by the Polo-like kinase Plk1 is regulated by DNA damage and mitotic progression. *Oncogene* 23, 865–872. doi: 10.1038/sj.onc.1207223

Lemaître, C., and Soutoglou, E. (2014). Double strand break (DSB) repair in heterochromatin and heterochromatin proteins in DSB repair. *DNA Repair (Amst).* 19, 163–168. doi: 10.1016/j.dnarep.2014.03.015

Lénárt, P., Petronczki, M., Steegmaier, M., Di Fiore, B., Lipp, J. J., Hoffmann, M., et al. (2007). The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. *Curr. Biol.* 17, 304–315. doi: 10.1016/j.cub.2006.12.046

Leng, M., Chan, D. W., Luo, H., Zhu, C., Qin, J., and Wang, Y. (2006). MPS1-dependent mitotic BLM phosphorylation is important for chromosome stability. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11485–11490. doi: 10.1073/pnas.0601828103

Lin, H. R., Ting, N. S., Qin, J., and Lee, W. H. (2003). M phase-specific phosphorylation of BRCA2 by Polo-like kinase 1 correlates with the dissociation of the BRCA2-P/CAF complex. *J. Biol. Chem.* 278, 35979–35987. doi: 10.1074/jbc.M210659200

Liu, C., Wu, J., Paudyal, S. C., You, Z., and Yu, X. (2013a). CHFR is important for the first wave of ubiquitination at DNA damage sites. *Nucleic Acids Res.* 41, 1698–1710. doi: 10.1093/nar/gks1278

Liu, D., Vader, G., Vromans, M. J., Lampson, M. A., and Lens, S. M. (2009). Sensing chromosome bi-orientation by spatial separation of aurora B kinase from kinetochore substrates. *Science* 323, 1350–1353. doi: 10.1126/science.1167000

Liu, H., Rankin, S., and Yu, H. (2013b). Phosphorylation-enabled binding of SGO1-PP2A to cohesin protects sororin and centromeric cohesion during mitosis. *Nat. Cell Biol.* 15, 40–49. doi: 10.1038/ncb2637

Liu, Y., Nielsen, C. F., Yao, Q., and Hickson, I. D. (2014). The origins and processing of ultra fine anaphase DNA bridges. *Curr. Opin. Genet. Dev.* 26, 1–5. doi: 10.1016/j.gde.2014.03.003

Lorenz, D. M., Jeng, A., and Deem, M. W. (2011). The emergence of modularity in biological systems. *Phys. Life Rev.* 8, 129–160. doi: 10.1016/j.plrev.2011.02.003

Lu, J., Kovach, J. S., Johnson, F., Chiang, J., Hodes, R., Lonser, R., et al. (2009). Inhibition of serine/threonine phosphatase PP2A enhances cancer chemotherapy by blocking DNA damage induced defense mechanisms. *Proc. Natl. Acad. Sci. U.S.A.* 106, 11697–11702. doi: 10.1073/pnas.0905930106

Lukas, C., Savic, V., Bekker-Jensen, S., Doil, C., Neumann, B., Pedersen, R. S., et al. (2011). 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. *Nat. Cell Biol.* 13, 243–253. doi: 10.1038/ncb2201

Lukashchuk, N., and Vousden, K. H. (2007). Ubiquitination and degradation of mutant p53. *Mol. Cell Biol.* 27, 8284–8295. doi: 10.1128/MCB.00050-07

Ma, H. T., and Poon, R. Y. (2011). How protein kinases co-ordinate mitosis in animal cells. *Biochem. J.* 435, 17–31. doi: 10.1042/BJ20100284

Macmillan, J. C., Hudson, J. W., Bull, S., Dennis, J. W., and Swallow, C. J. (2001). Comparative expression of the mitotic regulators SAK and PLK in colorectal cancer. *Ann. Surg. Oncol.* 8, 729–740. doi: 10.1007/s10434-001-0729-6

Macurek, L., Benada, J., Müllers, E., Halim, V. A., Krejcíková, K., Burdová, K., et al. (2013). Downregulation of Wip1 phosphatase modulates the cellular threshold of DNA damage signaling in mitosis. *Cell Cycle* 12, 251–262. doi: 10.4161/cc.23057

Macurek, L., Lindqvist, A., Lim, D., Lampson, M. A., Klompmaker, R., Freire, R., et al. (2008). Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. *Nature* 455, 119–123. doi: 10.1038/nature07185

Manfredi, M. G., Ecsedy, J. A., Chakravarty, A., Silverman, L., Zhang, M., Hoar, K. M., et al. (2011). Characterization of Alisertib (MLN8237), an investigational small-molecule inhibitor of aurora A kinase using novel *in vivo* pharmacodynamic assays. *Clin. Cancer Res.* 17, 7614–7624. doi: 10.1158/1078-0432.CCR-11-1536

Manfredi, M. G., Ecsedy, J. A., Meetze, K. A., Balani, S. K., Burenkova, O., Chen, W., et al. (2007). Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4106–4111. doi: 10.1073/pnas.0608798104

Mardilovich, K., Baugh, M., Crighton, D., Kowalczyk, D., Gabrielsen, M., Munro, J., et al. (2015). LIM kinase inhibitors disrupt mitotic microtubule organization and impair tumor cell proliferation. *Oncotarget* 6, 38469–38486. doi: 10.18632/oncotarget.6288

Martínez-Bálbas, M. A., Dey, A., Rabindran, S. K., Ozato, K., and Wu, C. (1995). Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* 83, 29–38. doi: 10.1016/0092-8674(95)90231-7

Marzo, I., and Naval, J. (2013). Antimitotic drugs in cancer chemotherapy: promises and pitfalls. *Biochem. Pharmacol.* 86, 703–710. doi: 10.1016/j.bcp.2013.07.010

Mason, J. M., Lin, D. C., Wei, X., Che, Y., Yao, Y., Kiarash, R., et al. (2014). Functional characterization of CFI-400945, a Polo-like kinase 4 inhibitor, as a potential anticancer agent. *Cancer Cell* 26, 163–176. doi: 10.1016/j.ccr.2014.05.006

Matos, J., Blanco, M. G., Maslen, S., Skehel, J. M., and West, S. C. (2011). Regulatory control of the resolution of DNA recombination intermediates during meiosis and mitosis. *Cell* 147, 158–172. doi: 10.1016/j.cell.2011.08.032

Matos, J., and West, S. C. (2014). Holliday junction resolution: regulation in space and time. *DNA Repair (Amst).* 19, 176–181. doi: 10.1016/j.dnarep.2014.03.013

Matsumoto, M. L., Wickliffe, K. E., Dong, K. C., Yu, C., Bosanac, I., Bustos, D., et al. (2010). K11-linked polyubiquitination in cell cycle control

revealed by a K11 linkage-specific antibody. *Mol. Cell* 39, 477–484. doi: 10.1016/j.molcel.2010.07.001

Matsusaka, T., and Pines, J. (2004). Chfr acts with the p38 stress kinases to block entry to mitosis in mammalian cells. *J. Cell Biol.* 166, 507–516. doi: 10.1083/jcb.200401139

Mazzarello, P. (1999). A unifying concept: the history of cell theory. *Nat. Cell Biol.* 1, E13–E15. doi: 10.1038/8964

McClelland, S. E., Burrell, R. A., and Swanton, C. (2009). Chromosomal instability: a composite phenotype that influences sensitivity to chemotherapy. *Cell Cycle* 8, 3262–3266. doi: 10.4161/cc.8.20.9690

McLaughlin, J., Markovtsov, V., Li, H., Wong, S., Gelman, M., Zhu, Y., et al. (2010). Preclinical characterization of Aurora kinase inhibitor R763/AS703569 identified through an image-based phenotypic screen. *J. Cancer Res. Clin. Oncol.* 136, 99–113. doi: 10.1007/s00432-009-0641-1

Mertins, P., Qiao, J. W., Patel, J., Udeshi, N. D., Clauser, K. R., Mani, D. R., et al. (2013). Integrated proteomic analysis of post-translational modifications by serial enrichment. *Nat. Methods* 10, 634–637. doi: 10.1038/nmeth.2518

Mikhailov, A., Cole, R. W., and Rieder, C. L. (2002). DNA damage during mitosis in human cells delays the metaphase/anaphase transition via the spindle-assembly checkpoint. *Curr. Biol.* 12, 1797–1806. doi: 10.1016/S0960-9822(02)01226-5

Mikhailov, A., Shinohara, M., and Rieder, C. L. (2004). Topoisomerase, I. I., and histone deacetylase inhibitors delay the G2/M transition by triggering the p38 MAPK checkpoint pathway. *J. Cell Biol.* 166, 517–526. doi: 10.1083/jcb.200405167

Milhollen, M. A., Shi, J., Traore, T., Huck, J., Sappal, D., Duffy, J., et al. (2015). Abstract A164: The small molecule UAE inhibitor TAK-243 (MLN7243) prevents DNA damage repair and reduces cell viability/tumor growth when combined with radiation, carboplatin and docetaxel. *Mol. Cancer Ther.* 14:A164. doi: 10.1158/1535-7163.TARG-15-A164

Miller, L. D., Smeds, J., George, J., Vega, V. B., Vergara, L., Ploner, A., et al. (2005). An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc. Natl. Acad. Sci. U.S.A.* 102, 13550–13555. doi: 10.1073/pnas.0506230102

Minocherhomji, S., Ying, S., Bjerregaard, V. A., Bursomanno, S., Aleliunaite, A., Wu, W., et al. (2015). Replication stress activates DNA repair synthesis in mitosis. *Nature* 528, 286–290. doi: 10.1038/nature16139

Mito, K., Kashima, K., Kikuchi, H., Daa, T., Nakayama, I., and Yokoyama, S. (2005). Expression of Polo-Like Kinase (PLK1) in non-Hodgkin's lymphomas. *Leuk. Lymphoma* 46, 225–231. doi: 10.1080/10428190400015709

Morrison, C., and Rieder, C. L. (2004). Chromosome damage and progression into and through mitosis in vertebrates. *DNA Repair (Amst).* 3, 1133–1139. doi: 10.1016/j.dnarep.2004.03.005

Mortlock, A. A., Foote, K. M., Heron, N. M., Jung, F. H., Pasquet, G., Lohmann, J. J., et al. (2007). Discovery, synthesis, and *in vivo* activity of a new class of pyrazoloquinazolines as selective inhibitors of aurora B kinase. *J. Med. Chem.* 50, 2213–2224. doi: 10.1021/jm061335f

Musacchio, A., and Salmon, E. D. (2007). The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* 8, 379–393. doi: 10.1038/nrm2163

Naim, V., and Rosselli, F. (2009). The FANC pathway and BLM collaborate during mitosis to prevent micro-nucleation and chromosome abnormalities. *Nat. Cell Biol.* 11, 761–768. doi: 10.1038/ncb1883

Naim, V., Wilhelm, T., Debatisse, M., and Rosselli, F. (2013). ERCC1 and MUS81-EME1 promote sister chromatid separation by processing late replication intermediates at common fragile sites during mitosis. *Nat. Cell Biol.* 15, 1008–1015. doi: 10.1038/ncb2793

Nelson, G., Buhmann, M., and von Zglinicki, T. (2009). DNA damage foci in mitosis are devoid of 53BP1. *Cell Cycle* 8, 3379–3383. doi: 10.4161/cc.8.20.9857

Nigg, E. A. (2001). Mitotic kinases as regulators of cell division and its checkpoints. *Nat. Rev. Mol. Cell Biol.* 2, 21–32. doi: 10.1038/35048096

Nyati, S., Schinske-Sebolt, K., Pitchiaya, S., Chekhovskiy, K., Chator, A., Chaudhry, N., et al. (2015). The kinase activity of the Ser/Thr kinase BUB1 promotes TGF-beta signaling. *Sci. Signal.* 8, ra1. doi: 10.1126/scisignal.2005379

Nyberg, K. A., Michelson, R. J., Putnam, C. W., and Weinert, T. A. (2002). Toward maintaining the genome: DNA damage and replication checkpoints. *Annu. Rev. Genet.* 36, 617–656. doi: 10.1146/annurev.genet.36.060402.113540

Ogden, A., Cheng, A., Rida, P. C., Pannu, V., Osan, R., Clewley, R., et al. (2014). Quantitative multi-parametric evaluation of centrosome declustering drugs: centrosome amplification, mitotic phenotype, cell cycle and death. *Cell Death Dis.* 5, e1204. doi: 10.1038/cddis.2014.164

Oh, Y. M., Kwon, Y. E., Kim, J. M., Bae, S. J., Lee, B. K., Yoo, S. J., et al. (2009). Chfr is linked to tumour metastasis through the downregulation of HDAC1. *Nat. Cell Biol.* 11, 295–302. doi: 10.1038/ncb1837

Orthwein, A., Fradet-Turcotte, A., Noordermeer, S. M., Canny, M. D., Brun, C. M., Strecker, J., et al. (2014). Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. *Science* 344, 189–193. doi: 10.1126/science.1248024

Oslob, J. D., Romanowski, M. J., Allen, D. A., Baskaran, S., Bui, M., Elling, R. A., et al. (2008). Discovery of a potent and selective aurora kinase inhibitor. *Bioorg. Med. Chem. Lett.* 18, 4880–4884. doi: 10.1016/j.bmcl.2008.07.073

Pangilinan, F., and Spencer, F. (1996). Abnormal kinetochore structure activates the spindle assembly checkpoint in budding yeast. *Mol. Biol. Cell* 7, 1195–1208. doi: 10.1091/mbc.7.8.1195

Pannu, V., Rida, P. C., Celik, B., Turaga, R. C., Ogden, A., Cantuaria, G., et al. (2014). Centrosome-declustering drugs mediate a two-pronged attack on interphase and mitosis in supercentrosomal cancer cells. *Cell Death Dis.* 5, e1538. doi: 10.1038/cddis.2014.505

Parry, D., Guzi, T., Shanahan, F., Davis, N., Prabhavalkar, D., Wiswell, D., et al. (2010). Dinaciclib (SCH 727965), a novel and potent cyclin-dependent kinase inhibitor. *Mol. Cancer Ther.* 9, 2344–2353. doi: 10.1158/1535-7163.MCT-10-0324

Payton, M., Bush, T. L., Chung, G., Ziegler, B., Eden, P., McElroy, P., et al. (2010). Preclinical evaluation of AMG 900, a novel potent and highly selective pan-aurora kinase inhibitor with activity in taxane-resistant tumor cell lines. *Cancer Res.* 70, 9846–9854. doi: 10.1158/0008-5472.CAN-10-3001

Pearce, A. K., and Humphrey, T. C. (2001). Integrating stress-response and cell-cycle checkpoint pathways. *Trends Cell Biol.* 11, 426–433. doi: 10.1016/S0962-8924(01)02119-5

Pedersen, R. T., Kruse, T., Nilsson, J., Oestergaard, V. H., and Lisby, M. (2015). TopBP1 is required at mitosis to reduce transmission of DNA damage to G1 daughter cells. *J. Cell Biol.* 210, 565–582. doi: 10.1083/jcb.201502107

Pellman, D. (2007). Cell biology: aneuploidy and cancer. *Nature* 446, 38–39. doi: 10.1038/446038a

Pereira, A. J., and Maiato, H. (2012). Maturation of the kinetochore-microtubule interface and the meaning of metaphase. *Chromosome Res.* 20, 563–577. doi: 10.1007/s10577-012-9298-8

Peters, J. M., Tedeschi, A., and Schmitz, J. (2008). The cohesin complex and its roles in chromosome biology. *Genes Dev.* 22, 3089–3114. doi: 10.1101/gad.1724308

Pines, J. (2011). Cubism and the cell cycle: the many faces of the APC/C. *Nat. Rev. Mol. Cell Biol.* 12, 427–438. doi: 10.1038/nrm3132

Pines, J., and Rieder, C. L. (2001). Re-staging mitosis: a contemporary view of mitotic progression. *Nat. Cell Biol.* 3, E3–E6. doi: 10.1038/35050676

Raab, M. S., Breitkreutz, I., Anderhub, S., Rønnest, M. H., Leber, B., Larsen, T. O., et al. (2012). GF-15, a novel inhibitor of centrosomal clustering, suppresses tumor cell growth *in vitro* and *in vivo*. *Cancer Res.* 72, 5374–5385. doi: 10.1158/0008-5472.CAN-12-2026

Renner, A. G., Dos Santos, C., Recher, C., Bailly, C., Créancier, L., Kruczynski, A., et al. (2009). Polo-like kinase 1 is overexpressed in acute myeloid leukemia and its inhibition preferentially targets the proliferation of leukemic cells. *Blood* 114, 659–662. doi: 10.1182/blood-2008-12-195867

Rieder, C. L., and Cole, R. (2000). Microtubule disassembly delays the G2-M transition in vertebrates. *Curr. Biol.* 10, 1067–1070. doi: 10.1016/S0960-9822(00)00678-3

Rieder, C. L., and Cole, R. W. (1998). Entry into mitosis in vertebrate somatic cells is guarded by a chromosome damage checkpoint that reverses the cell cycle when triggered during early but not late prophase. *J. Cell Biol.* 142, 1013–1022. doi: 10.1083/jcb.142.4.1013

Riley, T., Sontag, E., Chen, P., and Levine, A. (2008). Transcriptional control of human p53-regulated genes. *Nat. Rev. Mol. Cell Biol.* 9, 402–412. doi: 10.1038/nrm2395

Rodbell, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature* 284, 17–22. doi: 10.1038/284017a0

Rodrigo-Brenni, M. C., and Morgan, D. O. (2007). Sequential E2s drive polyubiquitin chain assembly on APC targets. *Cell* 130, 127–139. doi: 10.1016/j.cell.2007.05.027

Rudolph, D., Steegmaier, M., Hoffmann, M., Grauert, M., Baum, A., Quant, J., et al. (2009). BI 6727, a Polo-like kinase inhibitor with improved pharmacokinetic profile and broad antitumor activity. *Clin. Cancer Res.* 15, 3094–3102. doi: 10.1158/1078-0432.CCR-08-2445

Rudra, S., and Skibbens, R. V. (2013). Cohesin codes - interpreting chromatin architecture and the many facets of cohesin function. *J. Cell Sci.* 126, 31–41. doi: 10.1242/jcs.116566

Sackton, K. L., Dimova, N., Zeng, X., Tian, W., Zhang, M., Sackton, T. B., et al. (2014). Synergistic blockade of mitotic exit by two chemical inhibitors of the APC/C. *Nature* 514, 646–649. doi: 10.1038/nature13660

Salvatore, G., Nappi, T. C., Salerno, P., Jiang, Y., Garbi, C., Ugolini, C., et al. (2007). A cell proliferation and chromosomal instability signature in anaplastic thyroid carcinoma. *Cancer Res.* 67, 10148–10158. doi: 10.1158/0008-5472.CAN-07-1887

Samaga, R., and Klamt, S. (2013). Modeling approaches for qualitative and semi-quantitative analysis of cellular signaling networks. *Cell Commun. Signal.* 11:43. doi: 10.1186/1478-811X-11-43

Sanchez, Y., Bachant, J., Wang, H., Hu, F., Liu, D., Tetzlaff, M., et al. (1999). Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science* 286, 1166–1171. doi: 10.1126/science.286.5442.1166

Saponaro, M., Callahan, D., Zheng, X., Krejci, L., Haber, J. E., Klein, H. L., et al. (2010). Cdk1 targets Srs2 to complete synthesis-dependent strand annealing and to promote recombinational repair. *PLoS Genet.* 6:e1000858. doi: 10.1371/journal.pgen.1000858

Sarbajna, S., Davies, D., and West, S. C. (2014). Roles of SLX1-SLX4, MUS81-EME1, and GEN1 in avoiding genome instability and mitotic catastrophe. *Genes Dev.* 28, 1124–1136. doi: 10.1101/gad.238303.114

Scolnick, D. M., and Halazonetis, T. D. (2000). Chfr defines a mitotic stress checkpoint that delays entry into metaphase. *Nature* 406, 430–435. doi: 10.1038/35019108

Seavey, M. M., Lu, L. D., Stump, K. L., Wallace, N. H., and Ruggeri, B. A. (2012). Novel, orally active, proteasome inhibitor, delanzomib (CEP-18770), ameliorates disease symptoms and glomerulonephritis in two preclinical mouse models of SLE. *Int. Immunopharmacol.* 12, 257–270. doi: 10.1016/j.intimp.2011.11.019

Sells, T. B., Chau, R., Ecsedy, J. A., Gershman, R. E., Hoar, K., Huck, J., et al. (2015). MLN8054 and Alisertib (MLN8237): discovery of selective oral Aurora A inhibitors. *ACS Med. Chem. Lett.* 6, 630–634. doi: 10.1021/ml500409n

Shapiro, G. I. (2006). Cyclin-dependent kinase pathways as targets for cancer treatment. *J. Clin. Oncol.* 24, 1770–1783. doi: 10.1200/JCO.2005.03.7689

Shen, K., Wang, Y., Brooks, S. C., Raz, A., and Wang, Y. A. (2006). ATM is activated by mitotic stress and suppresses centrosome amplification in primary but not in tumor cells. *J. Cell. Biochem.* 99, 1267–1274. doi: 10.1002/jcb.20848

Shigeta, T., Takagi, M., Delia, D., Chessa, L., Iwata, S., Kanke, Y., et al. (1999). Defective control of apoptosis and mitotic spindle checkpoint in heterozygous carriers of ATM mutations. *Cancer Res.* 59, 2602–2607.

Shinde, S. R., Gangula, N. R., Kavela, S., Pandey, V., and Maddika, S. (2013). TOPK and PTEN participate in CHFR mediated mitotic checkpoint. *Cell Signal.* 25, 2511–2517. doi: 10.1016/j.cellsig.2013.08.013

Sivakumar, S., and Gorbsky, G. J. (2015). Spatiotemporal regulation of the anaphase-promoting complex in mitosis. *Nat. Rev. Mol. Cell Biol.* 16, 82–94. doi: 10.1038/nrm3934

Smith, E., Dejsuphong, D., Balestrini, A., Hampel, M., Lenz, C., Takeda, S., et al. (2009). An ATM- and ATR-dependent checkpoint inactivates spindle assembly by targeting CEP63. *Nat. Cell Biol.* 11, 278–285. doi: 10.1038/ncb1835

Smith, J., Tho, L. M., Xu, N., and Gillespie, D. A. (2010). The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Adv. Cancer Res.* 108, 73–112. doi: 10.1016/B978-0-12-380888-2.00003-0

Smits, V. A., Klompmaker, R., Arnaud, L., Rijksen, G., Nigg, E. A., and Medema, R. H. (2000). Polo-like kinase-1 is a target of the DNA damage checkpoint. *Nat. Cell Biol.* 2, 672–676. doi: 10.1038/35023629

Soncini, C., Carpinelli, P., Gianellini, L., Fancelli, D., Vianello, P., Rusconi, L., et al. (2006). PHA-680632, a novel Aurora kinase inhibitor with potent antitumoral activity. *Clin. Cancer Res.* 12, 4080–4089. doi: 10.1158/1078-0432.CCR-05-1964

Soucy, T. A., Smith, P. G., and Rolfe, M. (2009). Targeting NEDD8-activated cullin-RING ligases for the treatment of cancer. *Clin. Cancer Res.* 15, 3912–3916. doi: 10.1158/1078-0432.CCR-09-0343

Stobbe, C. C., Park, S. J., and Chapman, J. D. (2002). The radiation hypersensitivity of cells at mitosis. *Int. J. Radiat. Biol.* 78, 1149–1157. doi: 10.1080/09553000210166570

Stone, A., Sutherland, R. L., and Musgrove, E. A. (2012). Inhibitors of cell cycle kinases: recent advances and future prospects as cancer therapeutics. *Crit. Rev. Oncog.* 17, 175–198. doi: 10.1615/CritRevOncog.v17.i2.40

Strausfeld, U., Fernandez, A., Capony, J. P., Girard, F., Lautredou, N., Derancourt, J., et al. (1994). Activation of p34cdc2 protein kinase by microinjection of human cdc25C into mammalian cells. Requirement for prior phosphorylation of cdc25C by p34cdc2 on sites phosphorylated at mitosis. *J. Biol. Chem.* 269, 5989–6000.

Strebhardt, K., and Ullrich, A. (2006). Targeting polo-like kinase 1 for cancer therapy. *Nat. Rev. Cancer* 6, 321–330. doi: 10.1038/nrc1841

Su, T. T., and Jaklevic, B. (2001). DNA damage leads to a Cyclin A-dependent delay in metaphase-anaphase transition in the Drosophila gastrula. *Curr. Biol.* 11, 8–17. doi: 10.1016/S0960-9822(00)00042-7

Takagi, M., Delia, D., Chessa, L., Iwata, S., Shigeta, T., Kanke, Y., et al. (1998). Defective control of apoptosis, radiosensitivity, and spindle checkpoint in ataxia telangiectasia. *Cancer Res.* 58, 4923–4929.

Takai, N., Hamanaka, R., Yoshimatsu, J., and Miyakawa, I. (2005). Polo-like kinases (Plks) and cancer. *Oncogene* 24, 287–291. doi: 10.1038/sj.onc.1208272

Tanaka, T. U., Stark, M. J., and Tanaka, K. (2005). Kinetochore capture and bi-orientation on the mitotic spindle. *Nat. Rev. Mol. Cell Biol.* 6, 929–942. doi: 10.1038/nrm1764

Teixeira, L. K., and Reed, S. I. (2013). Ubiquitin ligases and cell cycle control. *Annu. Rev. Biochem.* 82, 387–414. doi: 10.1146/annurev-biochem-060410-105307

Tentler, J. J., Bradshaw-Pierce, E. L., Serkova, N. J., Hasebroock, K. M., Pitts, T. M., Diamond, J. R., et al. (2010). Assessment of the *in vivo* antitumor effects of ENMD-2076, a novel multitargeted kinase inhibitor, against primary and cell line-derived human colorectal cancer xenograft models. *Clin. Cancer Res.* 16, 2989–2998. doi: 10.1158/1078-0432.CCR-10-0325

Terasawa, M., Shinohara, A., and Shinohara, M. (2014). Canonical non-homologous end joining in mitosis induces genome instability and is suppressed by M-phase-specific phosphorylation of XRCC4. *PLoS Genet.* 10:e1004563. doi: 10.1371/journal.pgen.1004563

Tinker-Kulberg, R. L., and Morgan, D. O. (1999). Pds1 and Esp1 control both anaphase and mitotic exit in normal cells and after DNA damage. *Genes Dev.* 13:1936–1949. doi: 10.1101/gad.13.15.1936

Toyoshima-Morimoto, F., Taniguchi, E., and Nishida, E. (2002). Plk1 promotes nuclear translocation of human Cdc25C during prophase. *EMBO Rep.* 3, 341–348. doi: 10.1093/embo-reports/kvf069

Ubersax, J. A., Woodbury, E. L., Quang, P. N., Paraz, M., Blethrow, J. D., Shah, K., et al. (2003). Targets of the cyclin-dependent kinase Cdk1. *Nature* 425, 859–864. doi: 10.1038/nature02062

Uto, K., Inoue, D., Shimuta, K., Nakajo, N., and Sagata, N. (2004). Chk1, but not Chk2, inhibits Cdc25 phosphatases by a novel common mechanism. *EMBO J.* 23, 3386–3396. doi: 10.1038/sj.emboj.7600328

VanderBorght, A., Valckx, A., Van Dun, J., Grand-Perret, T., De Schepper, S., Vialard, J., et al. (2006). Effect of an hdm-2 antagonist peptide inhibitor on cell cycle progression in p53-deficient H1299 human lung carcinoma cells. *Oncogene* 25, 6672–6677. doi: 10.1038/sj.onc.1209667

van de Vijver, M. J., He, Y. D., van't Veer, L. J., Dai, H., Hart, A. A., Voskuil, D. W., et al. (2002). A gene-expression signature as a predictor of survival in breast cancer. *N. Engl. J. Med.* 347, 1999–2009. doi: 10.1056/NEJMoa021967

van Vugt, M. A., Gardino, A. K., Linding, R., Ostheimer, G. J., Reinhardt, H. C., Ong, S. E., et al. (2010). A mitotic phosphorylation feedback network connects Cdk1, Plk1, 53BP1, and Chk2 to inactivate the G(2)/M DNA damage checkpoint. *PLoS Biol.* 8:e1000287. doi: 10.1371/journal.pbio.1000287

Vassilev, L. T. (2007). MDM2 inhibitors for cancer therapy. *Trends Mol. Med.* 13, 23–31. doi: 10.1016/j.molmed.2006.11.002

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., et al. (2004). *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303, 844–848. doi: 10.1126/science.1092472

Vitale, I., Galluzzi, L., Castedo, M., and Kroemer, G. (2011). Mitotic catastrophe: a mechanism for avoiding genomic instability. *Nat. Rev. Mol. Cell Biol.* 12, 385–392. doi: 10.1038/nrm3115

Wagner, S. A., Beli, P., Weinert, B. T., Nielsen, M. L., Cox, J., Mann, M., et al. (2011). A proteome-wide, quantitative survey of *in vivo* ubiquitylation sites

reveals widespread regulatory roles. *Mol. Cell. Proteomics* 10:M111 013284. doi: 10.1074/mcp.M111.013284

Walczak, C. E., Cai, S., and Khodjakov, A. (2010). Mechanisms of chromosome behaviour during mitosis. *Nat. Rev. Mol. Cell Biol.* 11, 91–102. doi: 10.1038/nrm2832

Wang, S., Midgley, C. A., Scaërou, F., Grabarek, J. B., Griffiths, G., Jackson, W., et al. (2010). Discovery of N-phenyl-4-(thiazol-5-yl)pyrimidin-2-amine aurora kinase inhibitors. *J. Med. Chem.* 53, 4367–4378. doi: 10.1021/jm90 1913s

Wardlaw, C. P., Carr, A. M., and Oliver, A. W. (2014). TopBP1: A BRCT-scaffold protein functioning in multiple cellular pathways. *DNA Repair (Amst).* 22, 165–174. doi: 10.1016/j.dnarep.2014.06.004

Wechsler, T., Newman, S., and West, S. C. (2011). Aberrant chromosome morphology in human cells defective for Holliday junction resolution. *Nature* 471, 642–646. doi: 10.1038/nature09790

Weiß, L., and Efferth, T. (2012). Polo-like kinase 1 as target for cancer therapy. *Exp. Hematol. Oncol.* 1:38. doi: 10.1186/2162-3619-1-38

Wengner, A. M., Siemeister, G., Koppitz, M., Schulze, V., Kosemund, D., Klar, U., et al. (2016). Novel Mps1 Kinase Inhibitors with Potent Antitumor Activity. *Mol. Cancer Ther.* 15, 583–592. doi: 10.1158/1535-7163.MCT-15-0500

Westra, A., and Dewey, W. C. (1971). Variation in sensitivity to heat shock during the cell-cycle of Chinese hamster cells *in vitro*. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* 19, 467–477. doi: 10.1080/095530071145 50601

Wieser, S., and Pines, J. (2015). The biochemistry of mitosis. *Cold Spring Harb. Perspect. Biol.* 7:a015776. doi: 10.1101/cshperspect.a015776

Wilkinson, R. W., Odedra, R., Heaton, S. P., Wedge, S. R., Keen, N. J., Crafter, C., et al. (2007). AZD1152, a selective inhibitor of Aurora B kinase, inhibits human tumor xenograft growth by inducing apoptosis. *Clin. Cancer Res.* 13, 3682–3688. doi: 10.1158/1078-0432.CCR-06-2979

Wohlbold, L., and Fisher, R. P. (2009). Behind the wheel and under the hood: functions of cyclin-dependent kinases in response to DNA damage. *DNA Repair (Amst).* 8, 1018–1024. doi: 10.1016/j.dnarep.2009.04.009

Wu, T., Merbl, Y., Huo, Y., Gallop, J. L., Tzur, A., and Kirschner, M. W. (2010). UBE2S drives elongation of K11-linked ubiquitin chains by the anaphase-promoting complex. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1355–1360. doi: 10.1073/pnas.0912802107

Wyatt, H. D., Sarbajna, S., Matos, J., and West, S. C. (2013). Coordinated actions of SLX1-SLX4 and MUS81-EME1 for Holliday junction resolution in human cells. *Mol. Cell* 52, 234–247. doi: 10.1016/j.molcel.2013.08.035

Wyatt, P. G., Woodhead, A. J., Berdini, V., Boulstridge, J. A., Carr, M. G., Cross, D. M., et al. (2008). Identification of N-(4-piperidinyl)-4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxamide (AT7519), a novel cyclin dependent kinase inhibitor using fragment-based X-ray crystallography and structure based drug design. *J. Med. Chem.* 51, 4986–4999. doi: 10.1021/jm800382h

Yamada, H. Y., and Gorbsky, G. J. (2006). Inhibition of TRIP1/S8/hSug1, a component of the human 19S proteasome, enhances mitotic apoptosis induced by spindle poisons. *Mol. Cancer Ther.* 5, 29–38. doi: 10.1158/1535-7163.MCT-05-0126

Yan, Y., Cao, P. T., Greer, P. M., Nagengast, E. S., Kolb, R. H., Mumby, M. C., et al. (2010). Protein phosphatase 2A has an essential role in the activation of gamma-irradiation-induced G2/M checkpoint response. *Oncogene* 29, 4317–4329. doi: 10.1038/onc.2010.187

Yang, C., Tang, X., Guo, X., Niikura, Y., Kitagawa, K., Cui, K., et al. (2011). Aurora-B mediated ATM serine 1403 phosphorylation is required for mitotic ATM activation and the spindle checkpoint. *Mol. Cell* 44, 597–608. doi: 10.1016/j.molcel.2011.09.016

Yang, S. S., Yeh, E., Salmon, E. D., and Bloom, K. (1997). Identification of a mid-anaphase checkpoint in budding yeast. *J. Cell Biol.* 136, 345–354. doi: 10.1083/jcb.136.2.345

Ying, S., Minocherhomji, S., Chan, K. L., Palmai-Pallag, T., Chu, W. K., Wass, T., et al. (2013). MUS81 promotes common fragile site expression. *Nat. Cell Biol.* 15, 1001–1007. doi: 10.1038/ncb2773

You, Z., Bailis, J. M., Johnson, S. A., Dilworth, S. M., and Hunter, T. (2007). Rapid activation of ATM on DNA flanking double-strand breaks. *Nat. Cell Biol.* 9, 1311–1318. doi: 10.1038/ncb1651

Zeng, X., and King, R. W. (2012). An APC/C inhibitor stabilizes cyclin B1 by prematurely terminating ubiquitination. *Nat. Chem. Biol.* 8, 383–392. doi: 10.1038/nchembio.801

Zeng, X., Sigoillot, F., Gaur, S., Choi, S., Pfaff, K. L., Oh, D. C., et al. (2010). Pharmacologic inhibition of the anaphase-promoting complex induces a spindle checkpoint-dependent mitotic arrest in the absence of spindle damage. *Cancer Cell* 18, 382–395. doi: 10.1016/j.ccr.2010.08.010

Zhang, J., Wu, P., and Hu, Y. (2013). Clinical and marketed proteasome inhibitors for cancer treatment. *Curr. Med. Chem.* 20, 2537–2551. doi: 10.2174/09298673113209990122

Zhang, W., Peng, G., Lin, S. Y., and Zhang, P. (2011). DNA damage response is suppressed by the high cyclin-dependent kinase 1 activity in mitotic mammalian cells. *J. Biol. Chem.* 286, 35899–35905. doi: 10.1074/jbc.M111.267690

Zhu, X., Gerstein, M., and Snyder, M. (2007). Getting connected: analysis and principles of biological networks. *Genes Dev.* 21, 1010–1024. doi: 10.1101/gad.1528707

Zirkle, R. E., and Bloom, W. (1953). Irradiation of parts of individual cells. *Science* 117, 487–493. doi: 10.1126/science.117.3045.487

Zitouni, S., Nabais, C., Jana, S. C., Guerrero, A., and Bettencourt-Dias, M. (2014). Polo-like kinases: structural variations lead to multiple functions. *Nat. Rev. Mol. Cell Biol.* 15, 433–452. doi: 10.1038/nrm3819

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 28 June 2016
doi: 10.3389/fgene.2016.00122

Writers, Readers, and Erasers of Histone Ubiquitylation in DNA Double-Strand Break Repair

Godelieve Smeenk and Niels Mailand *

Ubiquitin Signaling Group, Protein Signaling Program, Faculty of Health and Medical Sciences, The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Copenhagen, Denmark

DNA double-strand breaks (DSBs) are highly cytotoxic DNA lesions, whose faulty repair may alter the content and organization of cellular genomes. To counteract this threat, numerous signaling and repair proteins are recruited hierarchically to the chromatin areas surrounding DSBs to facilitate accurate lesion repair and restoration of genome integrity. In vertebrate cells, ubiquitin-dependent modifications of histones adjacent to DSBs by RNF8, RNF168, and other ubiquitin ligases have a key role in promoting the assembly of repair protein complexes, serving as direct recruitment platforms for a range of genome caretaker proteins and their associated factors. These DNA damage-induced chromatin ubiquitylation marks provide an essential component of a histone code for DSB repair that is controlled by multifaceted regulatory circuits, underscoring its importance for genome stability maintenance. In this review, we provide a comprehensive account of how DSB-induced histone ubiquitylation is sensed, decoded and modulated by an elaborate array of repair factors and regulators. We discuss how these mechanisms impact DSB repair pathway choice and functionality for optimal protection of genome integrity, as well as cell and organismal fitness.

Keywords: DNA damage response, ubiquitin, DNA double-strand breaks, chromatin, DNA repair, histones

OPEN ACCESS

Edited by:
Kristijan Ramadan, University of Oxford, UK

Reviewed by:
Massimo Broggini, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Italy
Simone Sabbioneda, Istituto di Genetica Molecolare, Italy

***Correspondence:**
Niels Mailand
niels.mailand@cpr.ku.dk

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *10 May 2016*
Accepted: *15 June 2016*
Published: *28 June 2016*

Citation:
Smeenk G and Mailand N (2016) Writers, Readers, and Erasers of Histone Ubiquitylation in DNA Double-Strand Break Repair. Front. Genet. 7:122. doi: 10.3389/fgene.2016.00122

INTRODUCTION

Conserving the integrity of DNA and the information stored within its sequence is critical for the viability and fitness of any living cell and organism. DNA is continuously subjected to genotoxic insults inflicted by endogenous as well as exogenous sources (Barnes and Lindahl, 2004; Jackson and Bartek, 2009). Among the resulting spectrum of DNA lesions, one of the most cytotoxic types is the DNA double-strand break (DSB; Wyman and Kanaar, 2006). If left unrepaired or repaired incorrectly, such breaks can give rise to mutations or chromosomal rearrangements, which may lead to cell death or cancer development. In parallel with these stochastic DSBs, programmed DSBs play a central role in various biological processes in both uni- and multicellular organisms. These intentional DSBs are generated to facilitate the exchange of genetic information between homologous chromosomes during meiosis in diploid and polyploid organisms, as well as in processes such as mating type switching in haploid yeast (De Massy, 2013). In addition, programmed DSBs are instrumental for the genetic exchanges required for T-cell receptor rearrangement and V(D)J- and class-switch recombination (CSR) during lymphocyte maturation (Alt et al., 2013). The potentially malignant consequences of improperly processed DSBs on human physiology are illustrated by the fact that many leukemias result from chromosomal translocations

caused by faulty rejoining of programmed breaks at the immunoglobulin locus in B cells (Alt et al., 2013).

Two major pathways are employed by eukaryotic cells for the repair of DSBs. Non-homologous end-joining (NHEJ) is active throughout interphase and promotes rapid religation of broken DNA ends that do not require extensive end-processing, but is an error-prone process (Lieber, 2010). Homologous recombination (HR) mainly functions during S and G2 phases, when a newly replicated sister chromatid is available as a template for error-free repair (Heyer et al., 2010). This pathway is used for the repair of more complex DSBs and occurs with slower kinetics than NHEJ. HR is initiated by resection of the broken DNA ends by the Mre11-Nbs1-Rad50 (MRN) complex, CtIP, and EXO1 and BLM-DNA2 exonucleases. The choice of pathway for repair of individual DSBs is influenced by several parameters, including cell cycle status, the complexity of the break and whether it resides in euchromatic or heterochromatic regions of the genome (Shibata et al., 2011; Chapman et al., 2012b; Ceccaldi et al., 2016).

To counteract the threat posed by potentially deleterious DNA lesions, cells have evolved a complex network of interwoven, protective pathways that are collectively referred to as the DNA damage response (DDR; Jackson and Bartek, 2009; Ciccia and Elledge, 2010). DSBs are particularly potent triggers of the DDR, stimulating DNA repair pathways, transient arrest of the cell cycle, and transcriptional reprogramming. A striking feature of the cellular response to DSBs is the massive accumulation of DDR factors directly at the sites of DNA damage. The resulting structures, often referred to as Ionizing Radiation Induced Foci (IRIF), can be readily visualized by immunofluorescence microscopy (Bekker-Jensen and Mailand, 2010; Lukas et al., 2011). To a large extent, this rapid accumulation of proteins at DSBs relies on DNA damage-induced posttranslational modifications (PTMs) of histones and other chromatin-associated proteins that are in turn recognized by specific effector proteins (Lukas et al., 2011; Polo and Jackson, 2011). The functional consequences of this DSB recruitment programme range from chromatin relaxation to protection of the broken ends and assembly of repair protein complexes (Bekker-Jensen and Mailand, 2010; Lukas et al., 2011). Inherited mutations in genes encoding DSB signaling factors are associated with cancer and other genetic instability syndromes, illustrating their pivotal importance for DDR functionality and genome maintenance. For example, biallelic mutations in the *RNF168* gene, which encodes a ubiquitin ligase that catalyzes histone H2A ubiquitylation near DSBs to attract downstream repair factors, is the underlying cause of the ataxia-telangiectasia-like RIDDLE syndrome (Stewart et al., 2009). Patients with this rare disease present with symptoms typical of genomic instability syndromes, including radiosensitivity, immunodeficiency, and neurodegeneration (Stewart et al., 2007; Devgan et al., 2011).

A large body of work has given rise to a model in which DSB formation is accompanied by the propagation of a DNA damage-induced histone code that is written, read and ultimately erased by an elaborate network of effector proteins and regulators. Central to this process is the ubiquitylation of histones in the vicinity of DSBs by the two E3 ubiquitin ligases RNF8 and RNF168, coupling DSB detection to efficient repair of the lesions. In this review, we summarize and discuss how RNF8- and RNF168-mediated chromatin ubiquitylation orchestrates DSB signaling and repair mechanisms in mammalian cells, and how the DSB-associated histone ubiquitylation marks generated by these E3s are subsequently interpreted and turned over during the course of DNA repair to protect genome stability.

WRITERS OF DSB-ASSOCIATED HISTONE UBIQUITYLATION

The formation of DSBs sets in motion a cascade of signaling events that collectively facilitates faithful repair of the lesions. DSBs trigger rapid activation of the ATM kinase in a process that involves its acetylation by TIP60 (KAT5), induced by chromatin alterations (Sun et al., 2007, 2009; Kaidi and Jackson, 2013). A key target of activated ATM is the histone H2A variant H2AX, which contains a unique ATM phosphorylation site in its C-terminal tail (Rogakou et al., 1998). The product of this phosphorylation event, known as γ-H2AX, provides a binding site for the MDC1 protein via its tandem BRCT domain, a phosphopeptide-binding module found in a range of DDR proteins (Stucki et al., 2005; Mermershtain and Glover, 2013). MDC1 is a scaffold protein that recruits a number of factors to DNA damage sites. Among these is the E3 ubiquitin ligase RNF8, which initiates a dynamic ubiquitin-dependent DSB signaling response that culminates in the generation of specific ubiquitin marks on H2A-type histones near the breaks, laid down by another E3 ligase, RNF168 (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Doil et al., 2009; Pinato et al., 2009; Stewart et al., 2009; Thorslund et al., 2015). These ubiquitin modifications at damaged chromatin serve as recruitment platforms for a range of important DSB repair factors. The DSB signaling response thus undergoes a switch from being extensively driven by phosphorylation, targeting H2AX and associated factors, to relying also on a wave of ubiquitylation events mediated by RNF8, RNF168 and other ubiquitin ligases.

RNF8 is recruited to sites of DNA damage via its FHA domain, which recognizes ATM phosphorylation sites in MDC1 (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; **Figure 1**). While it has long been clear that RNF8 collaborates with the E2 ubiquitin-conjugating enzyme Ubc13 to deposit K63-linked ubiquitin chains at DSB sites (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007), the identity of its chromatin-bound substrate(s) has been more puzzling. Initially, RNF8 and RNF168 were thought to share H2A-type histones as substrates. Recently, however, it was shown that RNF8 is inert toward ubiquitylation of nucleosomal H2A and mainly promotes K63-linked polyubiquitylation of H1 linker histones but not core histones at DSB sites (Mattiroli et al., 2012; Thorslund et al., 2015). This ubiquitylation event serves as a recruitment signal for RNF168, which in turn ubiquitylates H2A-type histones at K13/K15 (Gatti et al., 2012; Mattiroli et al., 2012; Fradet-Turcotte et al., 2013). RNF168 is recruited to DSBs by recognizing ubiquitylated histone H1 via its UDM1 region, composed of two ubiquitin-binding motifs (MIU1 and UMI) and a flanking target recognition motif (LRM1; Panier

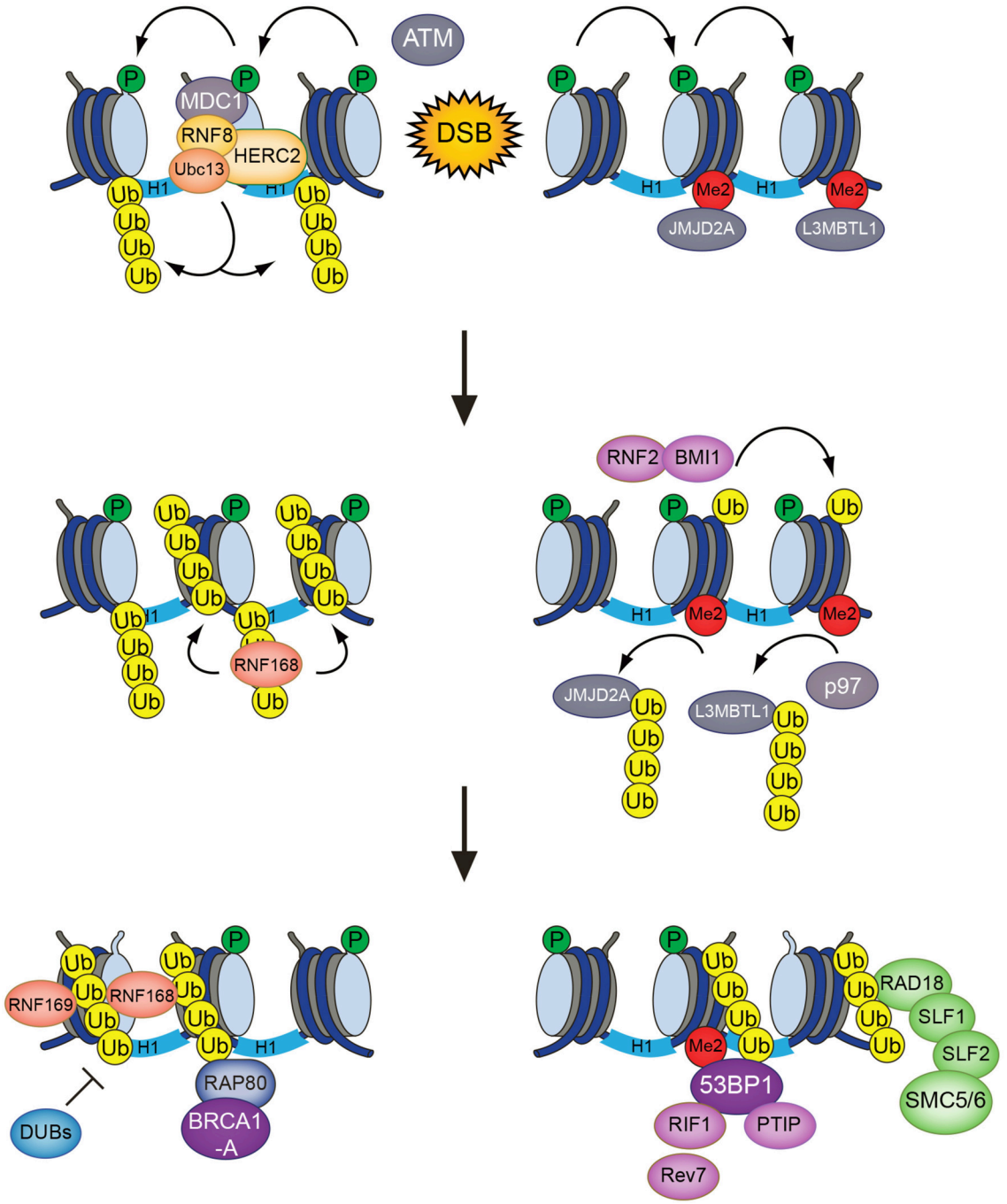

FIGURE 1 | The RNF8/RNF168-mediated histone ubiquitylation pathway. DSB-associated histone ubiquitylation requires the sequential action of the RNF8 and RNF168 ubiquitin ligases. RNF8 is targeted to DSB sites through interaction with phosphorylated MDC1, promoting Ubc13-dependent K63-linked polyubiquitylation of H1-type linker histones. This serves as a recruitment signal for RNF168, which catalyzes and propagates ubiquitylation of H2A-type histones at K13/K15 to an expanded chromatin area surrounding the break sites. These modifications provide loading platforms for a range of important components of DSB repair pathways, including 53BP1, BRCA1 (via RAP80), and the SMC5/6 complex (via RAD18-SLF1-SLF2), which have key roles in promoting DSB repair efficiency, fidelity, and pathway choice.

et al., 2012; Thorslund et al., 2015). At the break sites, RNF168 potently catalyzes ubiquitylation of H2A-type histones at the N-terminal K13/15 residues. The ability of RNF168 to also bind ubiquitylated H2A via its C-terminal UDM2 motif, composed of MIU2 and LRM2, enables it to efficiently propagate this modification to the surrounding chromatin areas once recruited

(Gatti et al., 2012; Mattiroli et al., 2012; Panier et al., 2012). The acidic patch on the nucleosomal surface, composed of residues in H2A and H2B, plays an important role in promoting N-terminal H2A ubiquitylation by RNF168. Changing essential residues in this patch impairs the binding of RNF168 and subsequent H2A-K13/K15 ubiquitylation (Leung et al., 2014; Mattiroli et al., 2014). As it is becoming apparent that RNF8 and RNF168 target different histone substrates at DSB sites, an important question concerns the nature of RNF168-generated ubiquitylations on H2A-K13/K15. Early studies showed that RNF168 deposits K63 linked ubiquitin chains on H2A-type histones, in part because it was found to interact with Ubc13, which exclusively catalyzes K63-linked polyubiquitylation in conjunction with its partner proteins Uev1 or Mms2 (Hofmann and Pickart, 1999; Doil et al., 2009; Stewart et al., 2009). However, a recent *in vitro* study suggested that, at least when overexpressed, RNF168 promotes K27-linked ubiquitylation of H2A(X), and that these atypical ubiquitin chains have an important role in promoting DSB signaling (Gatti et al., 2015). The nature of the cognate E2 enzyme(s) that catalyze K27-linked ubiquitylation in conjunction with RNF168 remains to be established. Another study showed that RNF168 forms ubiquitin chains together with UbcH5c, but not Ubc13, suggesting that RNF168 may not primarily assemble K63-linked polyubiquitin chains at damaged chromatin (Mattiroli et al., 2012). Indeed, RNF168 is capable of promoting recruitment of repair factors to DSB sites even in cells lacking Ubc13 (Thorslund et al., 2015). Moreover, it was shown that 53BP1, a direct reader of RNF168-generated ubiquitylation, binds to monoubiquitylated H2A-K15 (Fradet-Turcotte et al., 2013), suggesting that at least in some cases, RNF168 can drive protein recruitment to DSB sites without assembling polyubiquitin chains. Thus, it is possible that RNF168 has several substrates at damaged chromatin that are differentially ubiquitylated, or that RNF168 catalyzes different types of ubiquitin modifications on H2A. It is also conceivable that RNF168 may, at least in part, amplify K63-linked ubiquitylation at DSB sites indirectly by promoting the robust accumulation of other E3 ubiquitin ligases.

Although RNF8 and RNF168 are pivotal ubiquitin ligases in DSB signaling, additional layers of histone ubiquitylation and E3s are also involved, highlighting the complexity of chromatin ubiquitylation at DNA damage sites. RNF8 interacts with an auxiliary E3 ligase, HERC2, via its FHA domain that recognizes a specific C-terminal ATM phosphorylation site in this protein (Bekker-Jensen et al., 2010). The association with HERC2 appears to facilitate preferential binding of RNF8 to Ubc13 among its cognate E2 ubiquitin-conjugating enzyme partners. Due to its sheer size, however, the precise function(s) of the 530-kDa HERC2 protein and its intrinsic ubiquitin ligase activity in the DSB response has not yet been conclusively established.

Canonical monoubiquitylation of H2A on K119 is a highly abundant chromatin modification, generated by the E3 ligase heterodimer BMI1 (RING1a) and RNF2 (RING1b), which forms the stable core of the Polycomb Repressive Complex 1 (PRC1; Wang et al., 2004). Monoubiquitylation of H2AK119 plays a fundamental role in transcriptional silencing of genes, but BMI1 and RNF2 also accumulate at DSB sites, suggesting that gene silencing takes place at DSBs (Chou et al., 2010; Facchino et al., 2010; Ismail et al., 2010; Ginjala et al., 2011; Pan et al., 2011; Wu et al., 2011). Indeed, a reporter system visualizing transcription at DSB sites showed that transcription is silenced locally when DSBs are induced (Shanbhag et al., 2010). Interestingly, both inhibition of RNA polymerase I and II activity at DSB sites is dependent on ATM activation and RNF2/BMI-dependent ubiquitylation of H2AK119 (Kruhlak et al., 2007; Shanbhag et al., 2010; Kakarougkas et al., 2014; Larsen et al., 2014; Harding et al., 2015; Ui et al., 2015). Whether ubiquitylation of H2AK119 and H2AK13/K15 at DSB sites initiate separate pathways that facilitate transcriptional silencing and DSB signaling, respectively, or whether the PRC1 complex also has a more direct role in the latter process remains an important outstanding question.

Similar to H2AK119, H2B is monoubiquitylated at K120 in vertebrates (Thorne et al., 1987). However, whereas H2A K119 ubiquitylation induces transcriptional repression, ubiquitylation of H2B is associated with active transcription. The monoubiquitylation of H2B is catalyzed by the E2 enzyme RAD6 in conjunction with the E3 heterodimer RNF20-RNF40 (Kim et al., 2009). The mechanism of H2B ubiquitylation in transcriptional elongation has been studied extensively in yeast (Henry et al., 2003; Wood et al., 2003), and a similar mode of action has been found in human cells. When RNA Polymerase II encounters a nucleosome, the PAF1C transcription complex is recruited, which in turn binds RNF20-RNF40-RAD6 to ubiquitylate H2B (Zhu et al., 2005; Kim et al., 2009). The histone chaperone FACT, which also binds to RAD6 and ubiquitylated H2B, in turn shuttles away the other H2A-H2B dimer to facilitate nucleosome reorganization and chromatin relaxation (Pavri et al., 2006; Hondele et al., 2013). H2B ubiquitylation is a prerequisite for methylation of H3K4 and H3K79, which are associated with active transcription, potentially explaining, at least in part, the role of H2B ubiquitylation in this process (Briggs et al., 2002; Ng et al., 2002; Sun and Allis, 2002). Interestingly, RNF20 and RNF40 are also recruited to DSB sites, suggesting that local monoubiquitylation of H2BK120 takes place near DSBs (Moyal et al., 2011; Nakamura et al., 2011). In this context, H2B ubiquitylation might trigger conformational changes leading to H3K4 methylation or the *de novo* formation of this mark, generating a recruitment platform for the chromatin remodeler SNF2h (Nakamura et al., 2011). This in turn promotes chromatin relaxation and facilitates efficient recruitment of factors involved in HR (Moyal et al., 2011; Nakamura et al., 2011; Oliveira et al., 2014). The FACT complex was also found to be required for SNF2h accumulation at DSB sites, suggesting a mode of action similar to that at transcriptional sites (Oliveira et al., 2014). The opposing effects of H2AK119 and H2BK120 monoubiquitylation on transcription could be explained by the finding that H2BK120 ubiquitylation sterically disrupts chromatin compaction, while H2AK119 ubiquitylation, which lies at the other side of the nucleosomal surface, does not have the same effect (Jason et al., 2001; Fierz et al., 2011). Instead, H2AK119 ubiquitylation could act as a binding platform for proteins that facilitate transcriptional repression or DSB signaling, similar to H2AK13/K15 ubiquitylation.

Finally, a role of the E3 ligase BBAP in conjunction with its binding partner BAL1 (PARP9) in catalyzing monoubiquitylation of H4K91 has been reported to be a PARP-dependent mechanism acting in parallel with the RNF8/RNF168 pathway to enhance the accrual of DSB repair factors including 53BP1 and BRCA1 (Yan et al., 2009, 2013). However, although BBAP and BAL1 accumulate at DSB sites, it is not yet known whether H4K91 ubiquitylation has a direct role in promoting DSB repair events.

DECODING DSB-INDUCED HISTONE UBIQUITYLATION

The ubiquitylation products of RNF8 and RNF168 provide affinity platforms at DNA damage sites for a range of factors that can be classified as "readers" of DSB-associated histone ubiquitylation marks (**Figure 2**). These reader proteins, several of whose functions are now relatively well understood, recognize DSB-induced chromatin ubiquitylation marks via intrinsic ubiquitin-binding domains (UBD) of the UIM-, MIU,- and UBZ-types (Dikic et al., 2009). Using the DSB signaling response as a model system for studying the specificity of ubiquitin recognition, it has been shown that target-specific binding to ubiquitylated ligands often relies on a dual interaction mode, in which the combination of low-affinity ubiquitin recognition by a UBD and target-binding specificity imparted by an adjacent module together enables specific, high-affinity interaction with damaged chromatin areas. Thus, several readers of RNF8/RNF168-dependent chromatin ubiquitylation in the DSB response were shown to contain so-called LR motifs that interact weakly with H2A or other factors, juxtaposed to their UBDs (Panier et al., 2012). In spatio-temporal order of recruitment, the range of chromatin ubiquitylation readers containing LR motifs in combination with UBDs is headed by RNF168, which binds K63-linked ubiquitin chains on histone H1 via its UDM1 domain (Thorslund et al., 2015). The subsequent RNF168-generated ubiquitylation products at DSB sites are then recognized by several factors, including RNF168 itself, RAP80, 53BP1, RAD18, and RNF169 (Panier et al., 2012; Fradet-Turcotte et al., 2013; **Figure 2**). The ability of RNF168 to both catalyze and recognize ubiquitin-modified H2A, the latter via its C-terminal UDM2 motif, may allow robust and efficient propagation of the DSB-induced H2A-ubiquitin signal along the chromatin fiber.

Several readers of RNF168-catalyzed ubiquitylation function as scaffolds for the assembly of DNA repair factor complexes at DSB sites. RAP80 recruits the key HR factor BRCA1 and other components of the multimeric BRCA1-A complex to damaged chromatin (Kim et al., 2007; Sobhian et al., 2007; Wang et al., 2007; Yan et al., 2007). RAP80 contains two tandem UIMs with a characteristic spacing that allows for specific binding to K63-linked ubiquitin chains *in vitro* (Kim et al., 2007; Sobhian et al., 2007; Sato et al., 2009; Sims and Cohen, 2009). Whether such linkage-specific chain recognition by RAP80 occurs *in vivo* is not fully clear, but this feature of RAP80 underscores the need to dissect the precise nature of the RNF168-catalyzed ubiquitylations on H2A and perhaps additional chromatin-bound proteins. Given that other readers of these modifications only rely on single UBDs, it seems unlikely that binding to K63-linked chains on H2A is a prerequisite for their recruitment to DSB sites. Indeed, it was shown that both RNF168, RAP80, and RAD18 can bind to RNF168-generated K27-linked ubiquitin chains on H2AK13/15 (Gatti et al., 2015).

53BP1, a key factor in DSB repair, does not contain a classical UBD, and its specific recruitment to RNF8- and RNF168-generated ubiquitin products at DSBs therefore long remained enigmatic. 53BP1 uses its tandem Tudor domain to bind mono- and di-methylated H4K20 (Botuyan et al., 2006), generated by SET8 (PR-SET7) and SUV4-20 h1/h2, respectively (Nishioka et al., 2002; Schotta et al., 2004; Yang et al., 2008). Since more than 80% of histone H4K20 is constitutively dimethylated in human cells (Yang et al., 2008), a range of potential mechanisms that would make H4K20me2 available for 53PB1 binding at damaged chromatin have been proposed. On the one hand, it has been suggested that SET8 and MMSET, another histone methyltransferase, are recruited to DSB sites where they methylate H4K20 *de novo* (Oda et al., 2010; Pei et al., 2011; Dulev et al., 2014; Tuzon et al., 2014). On the other hand, SET8, SUV4-20 h1/h2, and MMSET were all found to be dispensable for the DSB recruitment of 53BP1 in MEFs (Hartlerode et al., 2012). Other studies found that H4K20me2 is exposed at DSB sites through deacetylation of H4K16 (Hsiao and Mizzen, 2013) or by the displacement or degradation of the H4K20me2-binding factors L3MBTL1 and JMJD2A/B (Acs et al., 2011; Mallette et al., 2012). While none of these models are mutually exclusive, the precise scope of regulatory mechanisms impinging on H4K20 methylation at DSB sites and their relative importance for controlling 53BP1 recruitment were unclear. However, more recent findings established that 53BP1 not only relies on interaction with methylated H4K20, but also has an adjacent, cryptic ubiquitin recognition module (termed the UDR motif) that is required for its recruitment to sites of DNA damage (Fradet-Turcotte et al., 2013). The UDR preferentially recognizes H2AK15 ubiquitylation, which is specifically generated by RNF168 (Fradet-Turcotte et al., 2013). Several additional mechanisms contributing to 53BP1 accrual have been suggested, including direct SUMOylation and ubiquitylation of 53BP1 by PIAS4 and RNF168, respectively (Galanty et al., 2009; Bohgaki et al., 2013).

Other readers of histone ubiquitylation in the DSB response include the E3 ubiquitin ligases RAD18 and RNF169, both of which modulate the DSB response through mechanisms that do not appear to involve their catalytic activities. RAD18 promotes HR-mediated DSB repair through interaction with the RAD51 paralog RAD51C (Huang et al., 2009). More recently, RAD18 has been shown to be instrumental for recruiting the SMC5/6 cohesion complex to DNA damage sites downstream of RNF8/RNF168-generated histone ubiquitylation. This function requires two adaptor proteins, SLF1 and SLF2, which form a complex and physically bridge RAD18 and the SMC5/6 complex (Raschle et al., 2015). This recruitment pathway may promote faithful DSB repair and genome stability maintenance by suppressing illegitimate recombination events. Importantly,

Writers	Function	References
RNF8	Ubiquitylation of H1	(Thorslund et al., 2015)
RNF168	Ubiquitylation of H2A at K13/K15	(Mattiroli et al., 2012; Gatti et al., 2012)
RNF2-BMI1	Ubiquitylation of H2A at K119	(Ismail et al., 2011; Ginjala et al., 2011; Pan et al., 2011)
BRCA1-BARD1	Ubiquitylation of H2A at K127/K129	(Kalb et al., 2014)
RNF20-RNF40	Ubiquitylation of H2B at K120	(Moyal et al., 2011; Nakamura et al., 2011)

Readers	Effector(s)	Function	References
RNF168		Propagation of H2A-K13/K15 ubiquitylation	(Doil et al., 2009; Stewart et al., 2009)
RAP80	**BRCA1**	Regulation of DSB end resection and HR	(Sobhian et al., 2007; Kim et al., 2007; Wang et al., 2007; Hu et al., 2011; Coleman et al., 2011)
53BP1	**RIF1, REV7, PTIP, Artemis**	Stimulation of NHEJ and inhibition of DSB end resection	(Bunting et al., 2010; Bouwman et al., 2010; Chapman et al., 2013; Di Virgilio et al., 2013; Escribano-Diaz et al., 2013; Zimmermann et al., 2013; Boersma et al., 2015; Xu et al., 2015; Callen et al., 2013; Wang et al., 2014)
RAD18	**RAD51C, SLF1, SLF2**	Recruitment of HR factors (RAD51 and the SMC5/6 complex)	(Huang et al., 2009; Räschle et al., 2015)
RNF169		Suppression of reader binding to ubiquitylated H2A	(Panier et al., 2012; Poulsen et al., 2012; Chen et al., 2012)

Erasers	Function	References
USP3, USP16, USP26, USP37, USP44	Reversal of H2A ubiquitylation	(Nicassio et al., 2007; Shanbhag et al., 2010; Typas et al., 2015; Mosbech et al., 2013)
OTUB1, OTUB2, BRCC36, POH1	Antagonism of RNF8/RNF168-mediated chromatin ubiquitylation	(Nakada et al., 2010; Kato et al., 2014; Shao et al., 2009; Butler et al., 2012)

FIGURE 2 | Major writers, readers, and erasers of DSB-associated histone ubiquitylation.

the involvement of RAD18 in DSB repair is distinct from its role in DNA damage bypass during DNA replication, where it facilitates exchanges of replicative DNA polymerases with UBD-containing damage-tolerant translesion DNA synthesis (TLS) polymerases such as DNA polymerase η (polη) at the replication fork by catalyzing PCNA monoubiquitylation in conjunction with RAD6 (Mailand et al., 2013). RNF169, a paralog of RNF168, accumulates at DSB sites by directly recognizing RNF168-generated ubiquitylation products, yet it does not cooperate with RNF168 in propagation of the H2A-ubiquitin mark (Chen et al., 2012; Panier et al., 2012; Poulsen et al., 2012). Instead, RNF169 appears to act as a negative regulator of downstream protein recruitment to DSBs by competing with other readers for binding to RNF168-generated ubiquitylation products, possibly helping to restrain the magnitude and propagation of the RNF8/RNF168-dependent DSB response.

ERASERS AND REGULATORS OF DSB-INDUCED HISTONE UBIQUITYLATION

Similar to the remarkable complexity by which RNF8 and RNF168 ubiquitylation products are laid down and interpreted, the regulation, maintenance, and removal of these modifications are governed by a range of different mechanisms. Several PTMs, including phosphorylation, SUMOylation, acetylation, and methylation are employed in the regulatory control of factors that affect the kinetics of DSB-associated chromatin ubiquitylation (Lukas et al., 2011; Polo and Jackson, 2011). For example, inactivating phosphorylations on RNF8 and 53BP1 by mitotic kinases suppress DSB-induced signaling and repair during mitosis. This has a critical role in protecting the genome from the formation of toxic repair products such as sister telomere fusions that can be otherwise generated during this window of the cell cycle (Orthwein et al., 2014).

The unique ability of RNF168 to both generate and interact with ubiquitylated H2A (Panier et al., 2012) allows for the dynamic and efficient spreading of DSB-induced chromatin marks along the chromatin fiber. At the same time, however, this begs the existence of regulatory mechanisms for keeping this potentially deleterious activity in check. One means of limiting the magnitude of the RNF8/RNF168-mediated chromatin ubiquitylation response involves the ubiquitin ligases UBR5 and TRIP12, which curb RNF168 expression by promoting its proteasomal degradation. In the absence of these factors, the DSB-specific histone ubiquitylation marks can undergo excessive spreading to entire damaged chromosomes as well as to adjacent ones (Gudjonsson et al., 2012). Interestingly, the destabilization of RNF168 by knockdown of HERC2 (Bekker-Jensen et al., 2010) is abrogated by co-depletion of TRIP12, suggesting that TRIP12 may target RNF168 molecules that are not in complex with HERC2 (Gudjonsson et al., 2012). In contrast, the deubiquitylating enzyme (DUB) USP34 has been speculated to stabilize RNF168 at DSB sites by counteracting proteasomal degradation of RNF168 through removal of K48-linked ubiquitin chains (Sy et al., 2013).

Several DUBs, including USP3, USP16, USP26, USP37, and USP44, target H2A for deubiquitylation in the context of the DDR, and when overexpressed, each of these DUBs efficiently reverse ubiquitin-dependent protein assembly at DSB sites (**Figure 2**). USP3, the first among these DUBs to be implicated in the DSB response, deubiquitylates H2A on a genome-wide scale (Nicassio et al., 2007; Doil et al., 2009; Lancini et al., 2014). Its ablation leads to increased DNA breakage, but to which extent this is due to hyperactivation of the DDR by ubiquitylated H2A or enhanced replication stress remains to be resolved (Nicassio et al., 2007; Lancini et al., 2014). USP16, which is predominantly cytoplasmic during interphase, has been implicated in deubiquitylation of H2A during mitosis, but has also been shown to counteract transcriptional silencing associated with DSB-induced histone ubiquitylation (Cai et al., 1999; Joo et al., 2007; Shanbhag et al., 2010). In addition, it was suggested that USP16 interacts with and is regulated by HERC2 and acts downstream of RNF8 and RNF168 at DSB sites (Zhang et al., 2014). USP44 was identified in a screen for DUBs whose overexpression prevent 53BP1 recruitment to DSB sites and has been shown to reverse ubiquitylation of both H2A and H2B (Fuchs et al., 2012; Mosbech et al., 2013). The OTU family DUB OTUB1 is highly active against K48-linked ubiquitin chains but negatively impacts the DSB response in a non-canonical manner independent of its catalytic activity. OTUB1 associates with and inhibits a range of E2s, including Ubc13 and UbcH5, thereby preventing RNF168 recruitment to, and H2A ubiquitylation at, DSBs (Nakada et al., 2010; Juang et al., 2012; Sato et al., 2012; Wiener et al., 2012). OTUB2 and BRCC36, a component of the BRCA1-A complex, both preferentially cleave K63-linked ubiquitin chains and have been proposed to antagonize RNF8-Ubc13-dependent ubiquitylation at DSB sites (Shao et al., 2009; Kato et al., 2014). Thus, an elaborate array of DUBs are engaged in regulating and fine-tuning the RNF8/RNF168-mediated chromatin ubiquitylation response to DSBs, emphasizing its biological importance and plasticity. The collective antagonistic actions of these DUBs may both allow cells to set a basal threshold for triggering the full-blown ubiquitin-dependent response to DSBs, and to regulate and fine-tune its magnitude during the course of lesion repair.

HISTONE UBIQUITYLATION AND DSB REPAIR PATHWAY CHOICE

By mediating the recruitment of a range of key DSB repair factors, including BRCA1, 53BP1 and associated proteins, chromatin ubiquitylation at DNA damage sites by RNF8 and RNF168 plays an important role in promoting DSB repair pathway efficiency and utilization. The choice between NHEJ and HR for the repair of a DSB is primarily made at the level of DSB end protection, which favors NHEJ, and end resection, which commits the break to repair by HR (Chapman et al., 2012b). 53BP1, whose relocalization to DSB sites is strongly dependent on RNF8/RNF168-mediated chromatin ubiquitylation, functions at the crossroads of DSB pathway choice. Accordingly, 53BP1 actively promotes NHEJ in several contexts while inhibiting

BRCA1-mediated HR through mechanisms that are still being elucidated. 53BP1 limits HR by posing a barrier for the DSB end resection machinery to approach the break sites, a function that is specifically antagonized by BRCA1 under HR-permissive conditions during the S and G2 phases, causing the relocalization of 53BP1 to the periphery of IRIF (Cao et al., 2009; Bouwman et al., 2010; Bunting et al., 2010; Chapman et al., 2012a; Karanam et al., 2012). Several effector proteins collaborate with 53BP1 in these NHEJ-promoting processes. The proteins RIF1 and PTIP bind 53BP1 via non-overlapping, phosphorylation-dependent interactions involving a large cluster of ATM phosphorylation sites in its N-terminal half (Munoz et al., 2007; Callen et al., 2013; Chapman et al., 2013; Di Virgilio et al., 2013; Escribano-Díaz et al., 2013; Feng et al., 2013). While RIF1 appears to act as an HR inhibitor that antagonizes BRCA1, PTIP mediates pro-NHEJ functions of 53BP1 involving its binding to the nuclease Artemis, a known NHEJ component (Wang et al., 2014). PTIP is responsible for mutagenic DNA repair processes such as the fusion of unprotected telomeres during mitosis (Callen et al., 2013). On the other hand, the requirement of 53BP1 for productive CSR in developing B cells, a process that impinges on the repair of a programmed DSB by NHEJ, appears to be channeled mainly through RIF1 (Callen et al., 2013; Chapman et al., 2013; Di Virgilio et al., 2013; Escribano-Díaz et al., 2013). Recently, REV7 (also known as MAD2L2) was found to act downstream of 53BP1 and RIF1 in inhibiting end resection and promoting CSR, independent of its role in TLS in conjunction with REV3L (Boersma et al., 2015; Xu et al., 2015). However, because REV7 does not appear to form complexes with 53BP1 and RIF1, the precise mechanism by which it promotes these processes remains to be established. The emerging linear RNF8-RNF168-53BP1-RIF1-REV7 cascade also mediates the DDR at uncapped telomeres, promoting illegitimate NHEJ-dependent fusions of deprotected chromosome ends that lead to massive genome instability (Peuscher and Jacobs, 2011; Okamoto et al., 2013; Zimmermann et al., 2013; Boersma et al., 2015).

BRCA1 binds to resected DNA independently of the RAP80-containing BRCA1-A complex, promoting HR in complex with either CtIP or BACH1 (Huen et al., 2010). RAP80 prevents hyper-recombination by limiting the access of BRCA1 to end-resected DSBs (Coleman and Greenberg, 2011; Hu et al., 2011). When bound to its partner protein BARD1, BRCA1 is active as an E3 ubiquitin ligase (Wu et al., 1996), and cancer-predisposing germline mutations in the *BRCA1* gene cluster in its N-terminal RING domain that underlies this activity (Maxwell and Domchek, 2012). However, ES cells expressing an E3 ligase-deficient BRCA1 allele containing an I26A mutation in the RING domain, which preserves the binding to BARD1, are viable, unlike *BRCA1* null cells (Reid et al., 2008). Mice expressing this BRCA1 mutant display no increase in tumor formation, indicating that the ubiquitin ligase activity of BRCA1 may not be absolutely essential for its tumor suppressor function (Shakya et al., 2011). In addition, the nature of the physiologically relevant BRCA1 E3 ligase substrate(s) remains unclear. Recently, BRCA1 was found to ubiquitylate H2A *in vitro* at the C-terminal residues K127 and K129 (Kalb et al., 2014), sites that are not known to be targeted by other H2A ubiquitin ligases. In line with this finding, it has been known for some time that expression of a chimeric protein in which ubiquitin is fused in-frame to the C-terminus of H2A rescues the defect in heterochromatin silencing observed in cells lacking BRCA1 (Zhu et al., 2011). Whether BRCA1-mediated H2A K127/K129 ubiquitylation has any functional role in DSB repair is therefore an important question that awaits clarification.

As RNF168 is required for the recruitment of both 53BP1 and the BRCA1-A complex to DSBs, it is feasible that this protein also plays a role in the regulation of DSB repair pathway choice. Interestingly, RNF168 depletion in BRCA1-deficient cells rescued HR and RAD51 IRIF formation, similar to 53BP1 loss, but was not able to restore HR in cells lacking CtIP, RAD50, RAD51, or BRCA2 (Muñoz et al., 2012). A truncated form of RNF168 unable to form IRIF still promoted HR, suggesting that the function of RNF168 in HR does not require its localization to DSB sites (Muñoz et al., 2014). Moreover, ectopic expression of RNF168 or 53BP1 in BRCA1-deficient cells exacerbates their hypersensitivity to PARP inhibitors by promoting 53BP1 accrual and suppression of resection (Zong et al., 2015). Although some of the DUBs that antagonize H2A ubiquitylation and/or RNF168 recruitment to DSB sites likely play a role in regulating the balance between 53BP1 and BRCA1 and DSB repair pathway choice, only a few have been directly implicated in this process. POH1 (also known as PSMD14) is a proteasome-associated DUB that accumulates at DSB sites, and whose knockdown gives rise to enlarged 53BP1 foci, suggesting that the proteasome is involved in the turnover of ubiquitin conjugates at sites of DNA damage (Butler et al., 2012; Galanty et al., 2012). POH1 collaborates with BRCA1 to overcome the 53BP1-mediated barrier to DNA end resection, leading to DSB repair by HR (Kakarougkas et al., 2013). USP26 and USP37 also accumulate at DSBs and deubiquitylate H2A. These DUBs have been suggested to counteract RNF168-dependent BRCA1 repression by RAP80, thereby promoting HR via BRCA1-dependent loading of PALB2-BRCA2 and RAD51 onto the resected DNA (Typas et al., 2015). While tremendous progress has thus been made toward understanding the mechanisms underlying DSB repair pathway choice that are critically dependent on the tug of war between BRCA1 and 53BP1, many questions remain about how they exert their functions in this process. In particular, despite its clinical importance as a tumor suppressor that has been recognized for decades, the reason why BRCA1 is so important for aborting tumorigenic events remains enigmatic. Further efforts to decipher the mechanisms underlying the antagonistic relationship between 53BP1 and BRCA1 in DSB signaling should help to clarify the role of BRCA1 in protecting against breast and ovarian cancers, and may lead to improved cancer treatment strategies.

In addition to histone ubiquitylation mediated by RNF8, RNF168, and other E3 ubiquitin ligases, the choice between NHEJ and HR for repair of individual DSBs is also controlled and regulated by a range of other ubiquitin-dependent processes (reviewed in detail in Schwertman et al., 2016). For example, ubiquitin has a direct key role in suppressing HR during G1 phase. This is mediated by KEAP1, a Cullin 3-RING ubiquitin ligase (CRL3) complex substrate adaptor (Cullinan et al., 2004;

Kobayashi et al., 2004; Zhang et al., 2004), which ubiquitylates PALB2 to inhibit the interaction between BRCA1 and PALB2-BRCA2 (Orthwein et al., 2015). USP11, a DUB that has previously been implicated in HR and interacts with BRCA2 (Schoenfeld et al., 2004; Wiltshire et al., 2010), deubiquitylates PALB2 but is degraded in G1 upon DSB formation, thereby effectively suppressing HR specifically during this window of the cell cycle (Orthwein et al., 2015). Likewise, the E3 ligase RNF138 was recently shown to play an important role in promoting DSB end resection, through its dual ability to ubiquitylate Ku80, promoting displacement of the Ku70-Ku80 complex from DSB ends, and the resection factor CtIP, enabling its recruitment to DSB sites (Ismail et al., 2015; Schmidt et al., 2015). The integration of multiple ubiquitin-dependent signaling processes targeting chromatin and DNA repair factors thus provides a critical regulatory framework for controlling DSB repair pathway utilization and efficiency during the cell cycle.

FUTURE PERSPECTIVES

Histone ubiquitylation by the RNF8/RNF168 cascade forms the nexus of a multipronged signaling pathway that entails the recruitment of many important repair factors to DNA damage sites, playing a central role in regulating the choice and efficiency of DSB repair mechanisms. A decade of studies of the RNF8/RNF168 pathway and its role in shaping the dynamic chromatin ubiquitylation landscape in the vicinity of DSBs has revealed many important insights into its complex spatio-temporal organization and regulation, as well as its impact on genome stability maintenance. However, many questions about the workings of this signaling response remain. Although it is becoming increasingly clear that RNF8 and RNF168 target different histones at DSB-surrounding chromatin areas, it is possible that they may have additional substrates relevant to their DSB signaling and repair functions. In addition, while RNF168 specifically ubiquitylates N-terminal lysines in H2A-type histones, the residue(s) in linker histones targeted by RNF8 are not yet known. For H2A, however, the nature of the physiologically relevant ubiquitin modifications assembled by RNF168 needs to be further addressed, with both H2A monoubiquitylation and polyubiquitylation via K63- and K27-linkages potentially playing important roles in the context of DSB responses. Whether other DSB-responsive ubiquitin ligases, including RAD18, HERC2, and RNF169, are actively engaged in ubiquitylating histones or other chromatin-bound factors at DSB sites also awaits clarification. Finally, it remains to be conclusively established whether some readers of RNF168-catalyzed ubiquitylation products at DSB sites, including RAP80 and RAD18, mainly recognize ubiquitylated H2A or perhaps other RNF168-modified proteins on chromatin. It is also possible that there are as-yet unknown readers of RNF8- and RNF168-dependent ubiquitylation marks at damaged chromatin. Structural studies will be of key value for a better understanding of the relationships between ubiquitylated histones and their writers, readers and erasers in DSB repair. The recent breakthroughs in cryo-EM technology hold promise that it will be feasible to obtain structures of ubiquitin-modified histones and nucleosomes in complex with such proteins and complexes, thus paving the way for atomic-level resolution of ubiquitin-dependent DSB signaling responses.

The identification of H1-type linker histones as targets of RNF8-dependent K63 polyubiquitylation adds a new and dynamic dimension to the histone code for DSB repair. While the ubiquitylated forms of H1 histones can serve as an anchor for initial RNF168 recruitment to the break sites, it is conceivable that DSB-induced linker histone modifications may also play a role in remodeling chromatin structure to render it more permissive for efficient repair. To this end, it has been shown that murine cells lacking three of the six linker histone isoforms are more resistant to DNA damage, and that the K63-ubiquitylated forms of H1 are more loosely associated with chromatin than their unmodified counterparts (Murga et al., 2007; Thorslund et al., 2015). Thus, dynamic DNA damage-induced modifications of linker histones by ubiquitin and perhaps other PTMs, numerous of which have been mapped by proteomic studies (Harshman et al., 2013), might promote restructuring of chromatin composition and compaction to facilitate the accessibility of the DSB repair machinery. How linker histones and their modifications impact chromatin structure dynamics during the course of DNA repair is an underexplored issue that merits further attention in the coming years.

Downstream of RNF8/RNF168-dependent chromatin ubiquitylation, the molecular transactions governing DSB repair pathway choice as a function of the well-established tug of war between 53BP1 and BRCA1 and their associated proteins are an intensely studied and clinically relevant topic, with many important questions still to be addressed. In particular, the mechanism(s) by which BRCA1 promotes DSB end resection, HR and tumor suppression remain incompletely understood. From the perspective of chromatin ubiquitylation, an especially pertinent issue that has so far eluded clear-cut answers concerns the role and substrate(s) of BRCA1-BARD1 E3 ligase activity in DSB repair. BRCA1 has been shown to contribute to chromatin ubiquitylation at DSB sites and modifies C-terminal lysine residues in H2A (Morris and Solomon, 2004; Polanowska et al., 2006; Kalb et al., 2014). Resolving if and how such BRCA1-mediated ubiquitylation at damaged chromatin contributes to its role in DSB repair may provide much-needed insights into the molecular mechanisms underlying its pro-HR function.

As many key players involved in the ubiquitin-dependent chromatin response to DSBs have now been identified and characterized, a major goal for the coming years will be to further delineate the mechanisms underlying these regulatory signaling processes and their integration into biologically meaningful cellular responses and circuitries that enable genome stability protection after DSBs in a ubiquitin-driven manner.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

We apologize to researchers whose important work could not be included due to space constraints. Work in the laboratory of the authors is funded by the Novo Nordisk Foundation (Grants no. NNF14CC0001 and NNF15OC0016926), European Research Council (ERC), Danish Council for Independent Research, Danish Cancer Society, and the Lundbeck Foundation.

REFERENCES

Acs, K., Luijsterburg, M. S., Ackermann, L., Salomons, F. A., Hoppe, T., and Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 18, 1345–1350. doi: 10.1038/nsmb.2188

Alt, F. W., Zhang, Y., Meng, F. L., Guo, C., and Schwer, B. (2013). Mechanisms of programmed DNA lesions and genomic instability in the immune system. *Cell* 152, 417–429. doi: 10.1016/j.cell.2013.01.007

Barnes, D. E., and Lindahl, T. (2004). Repair and genetic consequences of endogenous DNA base damage in mammalian cells. *Annu. Rev. Genet.* 38, 445–476. doi: 10.1146/annurev.genet.38.072902.092448

Bekker-Jensen, S., and Mailand, N. (2010). Assembly and function of DNA double-strand break repair foci in mammalian cells. *DNA Repair (Amst).* 9, 1219–1228. doi: 10.1016/j.dnarep.2010.09.010

Bekker-Jensen, S., Rendtlew Danielsen, J., Fugger, K., Gromova, I., Nerstedt, A., Lukas, C., et al. (2010). HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. *Nat. Cell Biol.* 12, 80–86. doi: 10.1038/ncb2008

Boersma, V., Moatti, N., Segura-Bayona, S., Peuscher, M. H., van der Torre, J., Wevers, B. A., et al. (2015). MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5′ end resection. *Nature* 521, 537–540. doi: 10.1038/nature14216

Bohgaki, M., Bohgaki, T., El Ghamrasni, S., Srikumar, T., Maire, G., Panier, S., et al. (2013). RNF168 ubiquitylates 53BP1 and controls its response to DNA double-strand breaks. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20982–20987. doi: 10.1073/pnas.1320302111

Botuyan, M. V., Lee, J., Ward, I. M., Kim, J. E., Thompson, J. R., Chen, J., et al. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* 127, 1361–1373. doi: 10.1016/j.cell.2006.10.043

Bouwman, P., Aly, A., Escandell, J. M., Pieterse, M., Bartkova, J., van der Gulden, H., et al. (2010). 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. *Nat. Struct. Mol. Biol.* 17, 688–695. doi: 10.1038/nsmb.1831

Briggs, S. D., Xiao, T., Sun, Z. W., Caldwell, J. A., Shabanowitz, J., Hunt, D. F., et al. (2002). Gene silencing: trans-histone regulatory pathway in chromatin. *Nature* 418, 498. doi: 10.1038/nature00970

Bunting, S. F., Callén, E., Wong, N., Chen, H. T., Polato, F., Gunn, A., et al. (2010). 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell* 141, 243–254. doi: 10.1016/j.cell.2010.03.012

Butler, L. R., Densham, R. M., Jia, J., Garvin, A. J., Stone, H. R., Shah, V., et al. (2012). The proteasomal de-ubiquitinating enzyme POH1 promotes the double-strand DNA break response. *EMBO J.* 31, 3918–3934. doi: 10.1038/emboj.2012.232

Cai, S. Y., Babbitt, R. W., and Marchesi, V. T. (1999). A mutant deubiquitinating enzyme (Ubp-M) associates with mitotic chromosomes and blocks cell division. *Proc. Natl. Acad. Sci. U.S.A.* 96, 2828–2833. doi: 10.1073/pnas.96.6.2828

Callen, E., Di Virgilio, M., Kruhlak, M. J., Nieto-Soler, M., Wong, N., Chen, H. T., et al. (2013). 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell* 153, 1266–1280. doi: 10.1016/j.cell.2013.05.023

Cao, L., Xu, X., Bunting, S. F., Liu, J., Wang, R. H., Cao, L. L., et al. (2009). A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. *Mol. Cell* 35, 534–541. doi: 10.1016/j.molcel.2009.06.037

Ceccaldi, R., Rondinelli, B., and D'andrea, A. D. (2016). Repair pathway choices and consequences at the double-strand break. *Trends Cell Biol.* 26, 52–64. doi: 10.1016/j.tcb.2015.07.009

Chapman, J. R., Barral, P., Vannier, J. B., Borel, V., Steger, M., Tomas-Loba, A., et al. (2013). RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol. Cell* 49, 858–871. doi: 10.1016/j.molcel.2013.01.002

Chapman, J. R., Sossick, A. J., Boulton, S. J., and Jackson, S. P. (2012a). BRCA1-associated exclusion of 53BP1 from DNA damage sites underlies temporal control of DNA repair. *J. Cell Sci.* 125, 3529–3534. doi: 10.1242/jcs.105353

Chapman, J. R., Taylor, M. R., and Boulton, S. J. (2012b). Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* 47, 497–510. doi: 10.1016/j.molcel.2012.07.029

Chen, J., Feng, W., Jiang, J., Deng, Y., and Huen, M. S. (2012). Ring finger protein RNF169 antagonizes the ubiquitin-dependent signaling cascade at sites of DNA damage. *J. Biol. Chem.* 287, 27715–27722. doi: 10.1074/jbc.M112.373530

Chou, D. M., Adamson, B., Dephoure, N. E., Tan, X., Nottke, A. C., Hurov, K. E., et al. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18475–18480. doi: 10.1073/pnas.1012946107

Ciccia, A., and Elledge, S. J. (2010). The DNA damage response: making it safe to play with knives. *Mol. Cell* 40, 179–204. doi: 10.1016/j.molcel.2010.09.019

Coleman, K. A., and Greenberg, R. A. (2011). The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. *J. Biol. Chem.* 286, 13669–13680. doi: 10.1074/jbc.M110.213728

Cullinan, S. B., Gordan, J. D., Jin, J., Harper, J. W., and Diehl, J. A. (2004). The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. *Mol. Cell. Biol.* 24, 8477–8486. doi: 10.1128/MCB.24.19.8477-8486.2004

De Massy, B. (2013). Initiation of meiotic recombination: how and where? Conservation and specificities among eukaryotes. *Annu. Rev. Genet.* 47, 563–599. doi: 10.1146/annurev-genet-110711-155423

Devgan, S. S., Sanal, O., Doil, C., Nakamura, K., Nahas, S. A., Pettijohn, K., et al. (2011). Homozygous deficiency of ubiquitin-ligase ring-finger protein RNF168 mimics the radiosensitivity syndrome of ataxia-telangiectasia. *Cell Death Differ.* 18, 1500–1506. doi: 10.1038/cdd.2011.18

Dikic, I., Wakatsuki, S., and Walters, K. J. (2009). Ubiquitin-binding domains - from structures to functions. *Nat. Rev. Mol. Cell Biol.* 10, 659–671. doi: 10.1038/nrm2767

Di Virgilio, M., Callen, E., Yamane, A., Zhang, W., Jankovic, M., Gitlin, A. D., et al. (2013). Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 339, 711–715. doi: 10.1126/science.1230624

Doil, C., Mailand, N., Bekker-Jensen, S., Menard, P., Larsen, D. H., Pepperkok, R., et al. (2009). RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 136, 435–446. doi: 10.1016/j.cell.2008.12.041

Dulev, S., Tkach, J., Lin, S., and Batada, N. N. (2014). SET8 methyltransferase activity during the DNA double-strand break response is required for recruitment of 53BP1. *EMBO Rep.* 15, 1163–1174. doi: 10.15252/embr.201439434

Escribano-Díaz, C., Orthwein, A., Fradet-Turcotte, A., Xing, M., Young, J. T., Tkac, J., et al. (2013). A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol. Cell* 49, 872–883. doi: 10.1016/j.molcel.2013.01.001

Facchino, S., Abdouh, M., Chatoo, W., and Bernier, G. (2010). BMI1 confers radioresistance to normal and cancerous neural stem cells through recruitment of the DNA damage response machinery. *J. Neurosci.* 30, 10096–10111. doi: 10.1523/JNEUROSCI.1634-10.2010

Feng, L., Fong, K. W., Wang, J., Wang, W., and Chen, J. (2013). RIF1 counteracts BRCA1-mediated end resection during DNA repair. *J. Biol. Chem.* 288, 11135–11143. doi: 10.1074/jbc.M113.457440

Fierz, B., Chatterjee, C., McGinty, R. K., Bar-Dagan, M., Raleigh, D. P., and Muir, T. W. (2011). Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. *Nat. Chem. Biol.* 7, 113–119. doi: 10.1038/nchembio.501

Fradet-Turcotte, A., Canny, M. D., Escribano-Díaz, C., Orthwein, A., Leung, C. C., Huang, H., et al. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 499, 50–54. doi: 10.1038/nature12318

Fuchs, G., Shema, E., Vesterman, R., Kotler, E., Wolchinsky, Z., Wilder, S., et al. (2012). RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. *Mol. Cell* 46, 662–673. doi: 10.1016/j.molcel.2012.05.023

Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012). RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* 26, 1179–1195. doi: 10.1101/gad.188284.112

Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009). Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* 462, 935–939. doi: 10.1038/nature08657

Gatti, M., Pinato, S., Maiolica, A., Rocchio, F., Prato, M. G., Aebersold, R., et al. (2015). RNF168 promotes noncanonical K27 ubiquitination to signal DNA damage. *Cell Rep.* 10, 226–238. doi: 10.1016/j.celrep.2014.12.021

Gatti, M., Pinato, S., Maspero, E., Soffientini, P., Polo, S., and Penengo, L. (2012). A novel ubiquitin mark at the N-terminal tail of histone H2As targeted by RNF168 ubiquitin ligase. *Cell Cycle* 11, 2538–2544. doi: 10.4161/cc.20919

Ginjala, V., Nacerddine, K., Kulkarni, A., Oza, J., Hill, S. J., Yao, M., et al. (2011). BMI1 is recruited to DNA breaks and contributes to DNA damage-induced H2A ubiquitination and repair. *Mol. Cell. Biol.* 31, 1972–1982. doi: 10.1128/MCB.00981-10

Gudjonsson, T., Altmeyer, M., Savic, V., Toledo, L., Dinant, C., Grofte, M., et al. (2012). TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. *Cell* 150, 697–709. doi: 10.1016/j.cell.2012.06.039

Harding, S. M., Boiarsky, J. A., and Greenberg, R. A. (2015). ATM dependent silencing links nucleolar chromatin reorganization to DNA damage recognition. *Cell Rep.* 13, 251–259. doi: 10.1016/j.celrep.2015.08.085

Harshman, S. W., Young, N. L., Parthun, M. R., and Freitas, M. A. (2013). H1 histones: current perspectives and challenges. *Nucleic Acids Res.* 41, 9593–9609. doi: 10.1093/nar/gkt700

Hartlerode, A. J., Guan, Y., Rajendran, A., Ura, K., Schotta, G., Xie, A., et al. (2012). Impact of histone H4 lysine 20 methylation on 53BP1 responses to chromosomal double strand breaks. *PLoS ONE* 7:e49211. doi: 10.1371/journal.pone.0049211

Henry, K. W., Wyce, A., Lo, W. S., Duggan, L. J., Emre, N. C., Kao, C. F., et al. (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes Dev.* 17, 2648–2663. doi: 10.1101/gad.1144003

Heyer, W. D., Ehmsen, K. T., and Liu, J. (2010). Regulation of homologous recombination in eukaryotes. *Annu. Rev. Genet.* 44, 113–139. doi: 10.1146/annurev-genet-051710-150955

Hofmann, R. M., and Pickart, C. M. (1999). Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* 96, 645–653. doi: 10.1016/S0092-8674(00)80575-9

Hondele, M., Stuwe, T., Hassler, M., Halbach, F., Bowman, A., Zhang, E. T., et al. (2013). Structural basis of histone H2A-H2B recognition by the essential chaperone FACT. *Nature* 499, 111–114. doi: 10.1038/nature12242

Hsiao, K. Y., and Mizzen, C. A. (2013). Histone H4 deacetylation facilitates 53BP1 DNA damage signaling and double-strand break repair. *J. Mol. Cell Biol.* 5, 157–165. doi: 10.1093/jmcb/mjs066

Hu, Y., Scully, R., Sobhian, B., Xie, A., Shestakova, E., and Livingston, D. M. (2011). RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. *Genes Dev.* 25, 685–700. doi: 10.1101/gad.2011011

Huang, J., Huen, M. S., Kim, H., Leung, C. C., Glover, J. N., Yu, X., et al. (2009). RAD18 transmits DNA damage signalling to elicit homologous recombination repair. *Nat. Cell Biol.* 11, 592–603. doi: 10.1038/ncb1865

Huen, M. S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., et al. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 131, 901–914. doi: 10.1016/j.cell.2007.09.041

Huen, M. S., Sy, S. M., and Chen, J. (2010). BRCA1 and its toolbox for the maintenance of genome integrity. *Nat. Rev. Mol. Cell Biol.* 11, 138–148. doi: 10.1038/nrm2831

Ismail, I. H., Andrin, C., McDonald, D., and Hendzel, M. J. (2010). BMI1-mediated histone ubiquitylation promotes DNA double-strand break repair. *J. Cell Biol.* 191, 45–60. doi: 10.1083/jcb.201003034

Ismail, I. H., Gagné, J. P., Genois, M. M., Strickfaden, H., McDonald, D., Xu, Z., et al. (2015). The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. *Nat. Cell Biol.* 17, 1446–1457. doi: 10.1038/ncb3259

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078. doi: 10.1038/nature08467

Jason, L. J., Moore, S. C., Ausio, J., and Lindsey, G. (2001). Magnesium-dependent association and folding of oligonucleosomes reconstituted with ubiquitinated H2A. *J. Biol. Chem.* 276, 14597–14601. doi: 10.1074/jbc.M011153200

Joo, H. Y., Zhai, L., Yang, C., Nie, S., Erdjument-Bromage, H., Tempst, P., et al. (2007). Regulation of cell cycle progression and gene expression by H2A deubiquitination. *Nature* 449, 1068–1072. doi: 10.1038/nature06256

Juang, Y. C., Landry, M. C., Sanches, M., Vittal, V., Leung, C. C., Ceccarelli, D. F., et al. (2012). OTUB1 co-opts Lys48-linked ubiquitin recognition to suppress E2 enzyme function. *Mol. Cell* 45, 384–397. doi: 10.1016/j.molcel.2012.01.011

Kaidi, A., and Jackson, S. P. (2013). KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling. *Nature* 498, 70–74. doi: 10.1038/nature12201

Kakarougkas, A., Ismail, A., Chambers, A. L., Riballo, E., Herbert, A. D., Kunzel, J., et al. (2014). Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. *Mol. Cell* 55, 723–732. doi: 10.1016/j.molcel.2014.06.028

Kakarougkas, A., Ismail, A., Katsuki, Y., Freire, R., Shibata, A., and Jeggo, P. A. (2013). Co-operation of BRCA1 and POH1 relieves the barriers posed by 53BP1 and RAP80 to resection. *Nucleic Acids Res.* 41, 10298–10311. doi: 10.1093/nar/gkt802

Kalb, R., Mallery, D. L., Larkin, C., Huang, J. T., and Hiom, K. (2014). BRCA1 is a histone-H2A-specific ubiquitin ligase. *Cell Rep.* 8, 999–1005. doi: 10.1016/j.celrep.2014.07.025

Karanam, K., Kafri, R., Loewer, A., and Lahav, G. (2012). Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. *Mol. Cell* 47, 320–329. doi: 10.1016/j.molcel.2012.05.052

Kato, K., Nakajima, K., Ui, A., Muto-Terao, Y., Ogiwara, H., and Nakada, S. (2014). Fine-tuning of DNA damage-dependent ubiquitination by OTUB2 supports the DNA repair pathway choice. *Mol. Cell* 53, 617–630. doi: 10.1016/j.molcel.2014.01.030

Kim, H., Chen, J., and Yu, X. (2007). Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* 316, 1202–1205. doi: 10.1126/science.1139621

Kim, J., Guermah, M., McGinty, R. K., Lee, J. S., Tang, Z., Milne, T. A., et al. (2009). RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. *Cell* 137, 459–471. doi: 10.1016/j.cell.2009.02.027

Kobayashi, A., Kang, M. I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T., et al. (2004). Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell. Biol.* 24, 7130–7139. doi: 10.1128/MCB.24.16.7130-7139.2004

Kolas, N. K., Chapman, J. R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F. D., et al. (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 318, 1637–1640. doi: 10.1126/science.1150034

Kruhlak, M., Crouch, E. E., Orlov, M., Montaño, C., Gorski, S. A., Nussenzweig, A., et al. (2007). The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. *Nature* 447, 730–734. doi: 10.1038/nature05842

Lancini, C., van den Berk, P. C., Vissers, J. H., Gargiulo, G., Song, J. Y., Hulsman, D., et al. (2014). Tight regulation of ubiquitin-mediated DNA damage response by USP3 preserves the functional integrity of hematopoietic stem cells. *J. Exp. Med.* 211, 1759–1777. doi: 10.1084/jem.20131436

Larsen, D. H., Hari, F., Clapperton, J. A., Gwerder, M., Gutsche, K., Altmeyer, M., et al. (2014). The NBS1-Treacle complex controls ribosomal RNA transcription in response to DNA damage. *Nat. Cell Biol.* 16, 792–803. doi: 10.1038/ncb3007

Leung, J. W., Agarwal, P., Canny, M. D., Gong, F., Robison, A. D., Finkelstein, I. J., et al. (2014). Nucleosome acidic patch promotes RNF168- and RING1B/BMI1-dependent H2AX and H2A ubiquitination and DNA damage signaling. *PLoS Genet.* 10:e1004178. doi: 10.1371/journal.pgen.1004178

Lieber, M. R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* 79, 181–211. doi: 10.1146/annurev.biochem.052308.093131

Lukas, J., Lukas, C., and Bartek, J. (2011). More than just a focus: the chromatin response to DNA damage and its role in genome integrity maintenance. *Nat. Cell Biol.* 13, 1161–1169. doi: 10.1038/ncb2344

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., et al. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 131, 887–900. doi: 10.1016/j.cell.2007.09.040

Mailand, N., Gibbs-Seymour, I., and Bekker-Jensen, S. (2013). Regulation of PCNA-protein interactions for genome stability. *Nat. Rev. Mol. Cell Biol.* 14, 269–282. doi: 10.1038/nrm3562

Mallette, F. A., Mattiroli, F., Cui, G., Young, L. C., Hendzel, M. J., Mer, G., et al. (2012). RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J.* 31, 1865–1878. doi: 10.1038/emboj.2012.47

Mattiroli, F., Uckelmann, M., Sahtoe, D. D., Van Dijk, W. J., and Sixma, T. K. (2014). The nucleosome acidic patch plays a critical role in RNF168-dependent ubiquitination of histone H2A. *Nat. Commun.* 5, 3291. doi: 10.1038/ncomms4291

Mattiroli, F., Vissers, J. H., van Dijk, W. J., Ikpa, P., Citterio, E., Vermeulen, W., et al. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150, 1182–1195. doi: 10.1016/j.cell.2012.08.005

Maxwell, K. N., and Domchek, S. M. (2012). Cancer treatment according to BRCA1 and BRCA2 mutations. *Nat. Rev. Clin. Oncol.* 9, 520–528. doi: 10.1038/nrclinonc.2012.123

Mermershtain, I., and Glover, J. N. (2013). Structural mechanisms underlying signaling in the cellular response to DNA double strand breaks. *Mutat. Res.* 750, 15–22. doi: 10.1016/j.mrfmmm.2013.07.004

Morris, J. R., and Solomon, E. (2004). BRCA1: BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum. Mol. Genet.* 13, 807–817. doi: 10.1093/hmg/ddh095

Mosbech, A., Lukas, C., Bekker-Jensen, S., and Mailand, N. (2013). The deubiquitylating enzyme USP44 counteracts the DNA double-strand break response mediated by the RNF8 and RNF168 ubiquitin ligases. *J. Biol. Chem.* 288, 16579–16587. doi: 10.1074/jbc.M113.459917

Moyal, L., Lerenthal, Y., Gana-Weisz, M., Mass, G., So, S., Wang, S. Y., et al. (2011). Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. *Mol. Cell* 41, 529–542. doi: 10.1016/j.molcel.2011.02.015

Munoz, I. M., Jowsey, P. A., Toth, R., and Rouse, J. (2007). Phospho-epitope binding by the BRCT domains of hPTIP controls multiple aspects of the cellular response to DNA damage. *Nucleic Acids Res.* 35, 5312–5322. doi: 10.1093/nar/gkm493

Muñoz, M. C., Laulier, C., Gunn, A., Cheng, A., Robbiani, D. F., Nussenzweig, A., et al. (2012). RING finger nuclear factor RNF168 is important for defects in homologous recombination caused by loss of the breast cancer susceptibility factor BRCA1. *J. Biol. Chem.* 287, 40618–40628. doi: 10.1074/jbc.M112.410951

Muñoz, M. C., Yanez, D. A., and Stark, J. M. (2014). An RNF168 fragment defective for focal accumulation at DNA damage is proficient for inhibition of homologous recombination in BRCA1 deficient cells. *Nucleic Acids Res.* 42, 7720–7733. doi: 10.1093/nar/gku421

Murga, M., Jaco, I., Fan, Y., Soria, R., Martinez-Pastor, B., Cuadrado, M., et al. (2007). Global chromatin compaction limits the strength of the DNA damage response. *J. Cell Biol.* 178, 1101–1108. doi: 10.1083/jcb.200704140

Nakada, S., Tai, I., Panier, S., Al-Hakim, A., Iemura, S., Juang, Y. C., et al. (2010). Non-canonical inhibition of DNA damage-dependent ubiquitination by OTUB1. *Nature* 466, 941–946. doi: 10.1038/nature09297

Nakamura, K., Kato, A., Kobayashi, J., Yanagihara, H., Sakamoto, S., Oliveira, D. V., et al. (2011). Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. *Mol. Cell* 41, 515–528. doi: 10.1016/j.molcel.2011.02.002

Ng, H. H., Xu, R. M., Zhang, Y., and Struhl, K. (2002). Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J. Biol. Chem.* 277, 34655–34657. doi: 10.1074/jbc.C200433200

Nicassio, F., Corrado, N., Vissers, J. H., Areces, L. B., Bergink, S., Marteijn, J. A., et al. (2007). Human USP3 is a chromatin modifier required for S phase progression and genome stability. *Curr. Biol.* 17, 1972–1977. doi: 10.1016/j.cub.2007.10.034

Nishioka, K., Rice, J. C., Sarma, K., Erdjument-Bromage, H., Werner, J., Wang, Y., et al. (2002). PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol. Cell* 9, 1201–1213. doi: 10.1016/S1097-2765(02)00548-8

Oda, H., Hübner, M. R., Beck, D. B., Vermeulen, M., Hurwitz, J., Spector, D. L., et al. (2010). Regulation of the histone H4 monomethylase PR-Set7 by CRL4(Cdt2)-mediated PCNA-dependent degradation during DNA damage. *Mol. Cell* 40, 364–376. doi: 10.1016/j.molcel.2010.10.011

Okamoto, K., Bartocci, C., Ouzounov, I., Diedrich, J. K., Yates, J. R. III., and Denchi, E. L. (2013). A two-step mechanism for TRF2-mediated chromosome-end protection. *Nature* 494, 502–505. doi: 10.1038/nature11873

Oliveira, D. V., Kato, A., Nakamura, K., Ikura, T., Okada, M., Kobayashi, J., et al. (2014). Histone chaperone FACT regulates homologous recombination by chromatin remodeling through interaction with RNF20. *J. Cell Sci.* 127, 763–772. doi: 10.1242/jcs.135855

Orthwein, A., Fradet-Turcotte, A., Noordermeer, S. M., Canny, M. D., Brun, C. M., Strecker, J., et al. (2014). Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. *Science* 344, 189–193. doi: 10.1126/science.1248024

Orthwein, A., Noordermeer, S. M., Wilson, M. D., Landry, S., Enchev, R. I., Sherker, A., et al. (2015). A mechanism for the suppression of homologous recombination in G1 cells. *Nature* 528, 422–426. doi: 10.1038/nature16142

Pan, M. R., Peng, G., Hung, W. C., and Lin, S. Y. (2011). Monoubiquitination of H2AX protein regulates DNA damage response signaling. *J. Biol. Chem.* 286, 28599–28607. doi: 10.1074/jbc.M111.256297

Panier, S., Ichijima, Y., Fradet-Turcotte, A., Leung, C. C., Kaustov, L., Arrowsmith, C. H., et al. (2012). Tandem protein interaction modules organize the ubiquitin-dependent response to DNA double-strand breaks. *Mol. Cell* 47, 383–395. doi: 10.1016/j.molcel.2012.05.045

Pavri, R., Zhu, B., Li, G., Trojer, P., Mandal, S., Shilatifard, A., et al. (2006). Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. *Cell* 125, 703–717. doi: 10.1016/j.cell.2006.04.029

Pei, H., Zhang, L., Luo, K., Qin, Y., Chesi, M., Fei, F., et al. (2011). MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. *Nature* 470, 124–128. doi: 10.1038/nature09658

Peuscher, M. H., and Jacobs, J. J. (2011). DNA-damage response and repair activities at uncapped telomeres depend on RNF8. *Nat. Cell Biol.* 13, 1139–1145. doi: 10.1038/ncb2326

Pinato, S., Scandiuzzi, C., Arnaudo, N., Citterio, E., Gaudino, G., and Penengo, L. (2009). RNF168, a new RING finger, MIU-containing protein that modifies chromatin by ubiquitination of histones H2A and H2AX. *BMC Mol. Biol.* 10:55. doi: 10.1186/1471-2199-10-55

Polanowska, J., Martin, J. S., Garcia-Muse, T., Petalcorin, M. I., and Boulton, S. J. (2006). A conserved pathway to activate BRCA1-dependent ubiquitylation at DNA damage sites. *EMBO J.* 25, 2178–2188. doi: 10.1038/sj.emboj.7601102

Polo, S. E., and Jackson, S. P. (2011). Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes Dev.* 25, 409–433. doi: 10.1101/gad.2021311

Poulsen, M., Lukas, C., Lukas, J., Bekker-Jensen, S., and Mailand, N. (2012). Human RNF169 is a negative regulator of the ubiquitin-dependent response to DNA double-strand breaks. *J. Cell Biol.* 197, 189–199. doi: 10.1083/jcb.201109100

Raschle, M., Smeenk, G., Hansen, R. K., Temu, T., Oka, Y., Hein, M. Y., et al. (2015). Proteomics reveals dynamic assembly of repair complexes during bypass of DNA cross-links. *Science* 348:1253671. doi: 10.1126/science.1253671

Reid, L. J., Shakya, R., Modi, A. P., Lokshin, M., Cheng, J. T., Jasin, M., et al. (2008). E3 ligase activity of BRCA1 is not essential for mammalian cell viability or homology-directed repair of double-strand DNA breaks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20876–20881. doi: 10.1073/pnas.0811203106

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* 273, 5858–5868. doi: 10.1074/jbc.273.10.5858

Sato, Y., Yamagata, A., Goto-Ito, S., Kubota, K., Miyamoto, R., Nakada, S., et al. (2012). Molecular basis of Lys-63-linked polyubiquitination inhibition by the interaction between human deubiquitinating enzyme OTUB1 and

ubiquitin-conjugating enzyme UBC13. *J. Biol. Chem.* 287, 25860–25868. doi: 10.1074/jbc.M112.364752

Sato, Y., Yoshikawa, A., Mimura, H., Yamashita, M., Yamagata, A., and Fukai, S. (2009). Structural basis for specific recognition of Lys 63-linked polyubiquitin chains by tandem UIMs of RAP80. *EMBO J.* 28, 2461–2468. doi: 10.1038/emboj.2009.160

Schmidt, C. K., Galanty, Y., Sczaniecka-Clift, M., Coates, J., Jhujh, S., Demir, M., et al. (2015). Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. *Nat. Cell Biol.* 17, 1458–1470. doi: 10.1038/ncb3260

Schoenfeld, A. R., Apgar, S., Dolios, G., Wang, R., and Aaronson, S. A. (2004). BRCA2 is ubiquitinated *in vivo* and interacts with USP11, a deubiquitinating enzyme that exhibits prosurvival function in the cellular response to DNA damage. *Mol. Cell. Biol.* 24, 7444–7455. doi: 10.1128/MCB.24.17.7444-7455.2004

Schotta, G., Lachner, M., Sarma, K., Ebert, A., Sengupta, R., Reuter, G., et al. (2004). A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev.* 18, 1251–1262. doi: 10.1101/gad.300704

Schwertman, P., Bekker-Jensen, S., and Mailand, N. (2016). Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. *Nat. Rev. Mol. Cell Biol.* 17, 379–394. doi: 10.1038/nrm.2016.58

Shakya, R., Reid, L. J., Reczek, C. R., Cole, F., Egli, D., Lin, C. S., et al. (2011). BRCA1 tumor suppression depends on BRCT phosphoprotein binding, but not its E3 ligase activity. *Science* 334, 525–528. doi: 10.1126/science.1209909

Shanbhag, N. M., Rafalska-Metcalf, I. U., Balane-Bolivar, C., Janicki, S. M., and Greenberg, R. A. (2010). ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141, 970–981. doi: 10.1016/j.cell.2010.04.038

Shao, G., Lilli, D. R., Patterson-Fortin, J., Coleman, K. A., Morrissey, D. E., and Greenberg, R. A. (2009). The Rap80-BRCC36 de-ubiquitinating enzyme complex antagonizes RNF8-Ubc13-dependent ubiquitination events at DNA double strand breaks. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3166–3171. doi: 10.1073/pnas.0807485106

Shibata, A., Conrad, S., Birraux, J., Geuting, V., Barton, O., Ismail, A., et al. (2011). Factors determining DNA double-strand break repair pathway choice in G2 phase. *EMBO J.* 30, 1079–1092. doi: 10.1038/emboj.2011.27

Sims, J. J., and Cohen, R. E. (2009). Linkage-specific avidity defines the lysine 63-linked polyubiquitin-binding preference of rap80. *Mol. Cell* 33, 775–783. doi: 10.1016/j.molcel.2009.02.011

Sobhian, B., Shao, G., Lilli, D. R., Culhane, A. C., Moreau, L. A., Xia, B., et al. (2007). RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* 316, 1198–1202. doi: 10.1126/science.1139516

Stewart, G. S., Panier, S., Townsend, K., Al-Hakim, A. K., Kolas, N. K., Miller, E. S., et al. (2009). The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* 136, 420–434. doi: 10.1016/j.cell.2008.12.042

Stewart, G. S., Stankovic, T., Byrd, P. J., Wechsler, T., Miller, E. S., Huissoon, A., et al. (2007). RIDDLE immunodeficiency syndrome is linked to defects in 53BP1-mediated DNA damage signaling. *Proc. Natl. Acad. Sci. U.S.A.* 104, 16910–16915. doi: 10.1073/pnas.0708408104

Stucki, M., Clapperton, J. A., Mohammad, D., Yaffe, M. B., Smerdon, S. J., and Jackson, S. P. (2005). MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* 123, 1213–1226. doi: 10.1016/j.cell.2005.09.038

Sun, Y., Jiang, X., Xu, Y., Ayrapetov, M. K., Moreau, L. A., Whetstine, J. R., et al. (2009). Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nat. Cell Biol.* 11, 1376–1382. doi: 10.1038/ncb1982

Sun, Y., Xu, Y., Roy, K., and Price, B. D. (2007). DNA damage-induced acetylation of lysine 3016 of ATM activates ATM kinase activity. *Mol. Cell. Biol.* 27, 8502–8509. doi: 10.1128/MCB.01382-07

Sun, Z. W., and Allis, C. D. (2002). Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* 418, 104–108. doi: 10.1038/nature00883

Sy, S. M., Jiang, J., O, W. S., Deng, Y., and Huen, M. S. (2013). The ubiquitin specific protease USP34 promotes ubiquitin signaling at DNA double-strand breaks. *Nucleic Acids Res.* 41, 8572–8580. doi: 10.1093/nar/gkt622

Thorne, A. W., Sautiere, P., Briand, G., and Crane-Robinson, C. (1987). The structure of ubiquitinated histone H2B. *EMBO J.* 6, 1005–1010.

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., et al. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393. doi: 10.1038/nature15401

Tuzon, C. T., Spektor, T., Kong, X., Congdon, L. M., Wu, S., Schotta, G., et al. (2014). Concerted activities of distinct H4K20 methyltransferases at DNA double-strand breaks regulate 53BP1 nucleation and NHEJ-directed repair. *Cell Rep.* 8, 430–438. doi: 10.1016/j.celrep.2014.06.013

Typas, D., Luijsterburg, M. S., Wiegant, W. W., Diakatou, M., Helfricht, A., Thijssen, P. E., et al. (2015). The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. *Nucleic Acids Res.* 43, 6919–6933. doi: 10.1093/nar/gkv613

Ui, A., Nagaura, Y., and Yasui, A. (2015). Transcriptional elongation factor ENL phosphorylated by ATM recruits polycomb and switches off transcription for DSB repair. *Mol. Cell* 58, 468–482. doi: 10.1016/j.molcel.2015.03.023

Wang, B., Matsuoka, S., Ballif, B. A., Zhang, D., Smogorzewska, A., Gygi, S. P., et al. (2007). Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* 316, 1194–1198. doi: 10.1126/science.1139476

Wang, H., Wang, L., Erdjument-Bromage, H., Vidal, M., Tempst, P., Jones, R. S., et al. (2004). Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 431, 873–878. doi: 10.1038/nature02985

Wang, J., Aroumougame, A., Lobrich, M., Li, Y., Chen, D., Chen, J., et al. (2014). PTIP associates with Artemis to dictate DNA repair pathway choice. *Genes Dev.* 28, 2693–2698. doi: 10.1101/gad.252478.114

Wiener, R., Zhang, X., Wang, T., and Wolberger, C. (2012). The mechanism of OTUB1-mediated inhibition of ubiquitination. *Nature* 483, 618–622. doi: 10.1038/nature10911

Wiltshire, T. D., Lovejoy, C. A., Wang, T., Xia, F., O'connor, M. J., and Cortez, D. (2010). Sensitivity to poly(ADP-ribose) polymerase (PARP) inhibition identifies ubiquitin-specific peptidase 11 (USP11) as a regulator of DNA double-strand break repair. *J. Biol. Chem.* 285, 14565–14571. doi: 10.1074/jbc.M110.104745

Wood, A., Krogan, N. J., Dover, J., Schneider, J., Heidt, J., Boateng, M. A., et al. (2003). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell* 11, 267–274. doi: 10.1016/S1097-2765(02)00802-X

Wu, C. Y., Kang, H. Y., Yang, W. L., Wu, J., Jeong, Y. S., Wang, J., et al. (2011). Critical role of monoubiquitination of histone H2AX protein in histone H2AX phosphorylation and DNA damage response. *J. Biol. Chem.* 286, 30806–30815. doi: 10.1074/jbc.M111.257469

Wu, L. C., Wang, Z. W., Tsan, J. T., Spillman, M. A., Phung, A., Xu, X. L., et al. (1996). Identification of a RING protein that can interact *in vivo* with the BRCA1 gene product. *Nat. Genet.* 14, 430–440. doi: 10.1038/ng1296-430

Wyman, C., and Kanaar, R. (2006). DNA double-strand break repair: all's well that ends well. *Annu. Rev. Genet.* 40, 363–383. doi: 10.1146/annurev.genet.40.110405.090451

Xu, G., Chapman, J. R., Brandsma, I., Yuan, J., Mistrik, M., Bouwman, P., et al. (2015). REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 521, 541–544. doi: 10.1038/nature14328

Yan, J., Kim, Y. S., Yang, X. P., Li, L. P., Liao, G., Xia, F., et al. (2007). The ubiquitin-interacting motif containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response. *Cancer Res.* 67, 6647–6656. doi: 10.1158/0008-5472.CAN-07-0924

Yan, Q., Dutt, S., Xu, R., Graves, K., Juszczynski, P., Manis, J. P., et al. (2009). BBAP monoubiquitylates histone H4 at lysine 91 and selectively modulates the DNA damage response. *Mol. Cell* 36, 110–120. doi: 10.1016/j.molcel.2009.08.019

Yan, Q., Xu, R., Zhu, L., Cheng, X., Wang, Z., Manis, J., et al. (2013). BAL1 and its partner E3 ligase, BBAP, link Poly(ADP-ribose) activation, ubiquitylation, and double-strand DNA repair independent of ATM, MDC1, and RNF8. *Mol. Cell. Biol.* 33, 845–857. doi: 10.1128/MCB.00990-12

Yang, H., Pesavento, J. J., Starnes, T. W., Cryderman, D. E., Wallrath, L. L., Kelleher, N. L., et al. (2008). Preferential dimethylation of histone H4 lysine 20 by Suv4-20. *J. Biol. Chem.* 283, 12085–12092. doi: 10.1074/jbc.M707974200

Zhang, D. D., Lo, S. C., Cross, J. V., Templeton, D. J., and Hannink, M. (2004). Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell. Biol.* 24, 10941–10953. doi: 10.1128/MCB.24.24.10941-10953.2004

Zhang, Z., Yang, H., and Wang, H. (2014). The histone H2A deubiquitinase USP16 interacts with HERC2 and fine-tunes cellular response to DNA damage. *J. Biol. Chem.* 289, 32883–32894. doi: 10.1074/jbc.M114.599605

Zhu, B., Zheng, Y., Pham, A. D., Mandal, S. S., Erdjument-Bromage, H., Tempst, P., et al. (2005). Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol. Cell* 20, 601–611. doi: 10.1016/j.molcel.2005.09.025

Zhu, Q., Pao, G. M., Huynh, A. M., Suh, H., Tonnu, N., Nederlof, P. M., et al. (2011). BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. *Nature* 477, 179–184. doi: 10.1038/nature10371

Zimmermann, M., Lottersberger, F., Buonomo, S. B., Sfeir, A., and de Lange, T. (2013). 53BP1 regulates DSB repair using Rif1 to control 5′ end resection. *Science* 339, 700–704. doi: 10.1126/science.1231573

Zong, D., Callen, E., Pegoraro, G., Lukas, C., Lukas, J., and Nussenzweig, A. (2015). Ectopic expression of RNF168 and 53BP1 increases mutagenic but not physiological non-homologous end joining. *Nucleic Acids Res.* 43, 4950–4961. doi: 10.1093/nar/gkv336

Conflict of Interest Statement: 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.

frontiers in Genetics

MINI REVIEW
published: 14 June 2016
doi: 10.3389/fgene.2016.00109

Mass Spectrometry-Based Proteomics for Investigating DNA Damage-Associated Protein Ubiquitylation

Jan B. Heidelberger[1], Sebastian A. Wagner[2] and Petra Beli[1]**

[1] Institute of Molecular Biology, Mainz, Germany, [2] Department of Medicine, Hematology/Oncology, Goethe University, Frankfurt, Germany

Modification of proteins with the 76 amino acid protein ubiquitin plays essential roles in cellular signaling. Development of methods for specific enrichment of ubiquitin remnant peptides and advances in high-resolution mass spectrometry have enabled proteome-wide identification of endogenous ubiquitylation sites. Moreover, ubiquitin remnant profiling has emerged as a powerful approach for investigating changes in protein ubiquitylation in response to cellular perturbations, such as DNA damage, as well as for identification of substrates of ubiquitin-modifying enzymes. Despite these advances, interrogation of ubiquitin chain topologies on substrate proteins remains a challenging task. Here, we describe mass spectrometry-based approaches for quantitative analyses of site-specific protein ubiquitylation and highlight recent studies that employed these methods for investigation of ubiquitylation in the context of the cellular DNA damage response. Furthermore, we provide an overview of experimental strategies for probing ubiquitin chain topologies on proteins and discuss how these methods can be applied to analyze functions of ubiquitylation in the DNA damage response.

Keywords: mass spectrometry-based proteomics, ubiquitin, ubiquitin-modifying enzymes, ubiquitin remnant profiling, DNA damage response

OPEN ACCESS

Edited by:
Kristijan Ramadan, University of Oxford, UK

Reviewed by:
Ivan Matic, Max Planck Institute for Biology of Ageing, Germany
Benedikt M. Kessler, University of Oxford, UK

****Correspondence:***
Petra Beli
p.beli@imb-mainz.de;
Sebastian A. Wagner
swagner@med.uni-frankfurt.de

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *06 March 2016*
Accepted: *30 May 2016*
Published: *14 June 2016*

Citation:
Heidelberger JB, Wagner SA and Beli P (2016) Mass Spectrometry-Based Proteomics for Investigating DNA Damage-Associated Protein Ubiquitylation. Front. Genet. 7:109. doi: 10.3389/fgene.2016.00109

MASS SPECTROMETRY-BASED PROTEOMICS FOR INVESTIGATING POSTTRANSLATIONAL MODIFICATIONS

Mass spectrometry (MS)-based proteomics has become a powerful tool for investigating posttranslational modifications (PTMs) of proteins in the context of cellular signaling (Larance and Lamond, 2015). In shotgun or bottom up proteomics, cellular proteins are subjected to proteolysis and the resulting peptides are separated according to hydrophobicity using high-pressure liquid chromatography (LC) and identified by tandem mass spectrometry (LC–MS/MS; Aebersold and Mann, 2003). The mass difference introduced by the presence of PTMs can be exploited for identification and localization of modifications. A major obstacle for proteome-wide analysis of posttranslational modification sites by LC–MS/MS is the sub-stoichiometric cellular occurrence of modified protein species and the inability of current mass spectrometers to identify all peptides resulting from digestion of the cellular proteome. Consequently, specific enrichment methods for modified peptide species are essential for the proteome-wide identification of PTMs by LC-MS/MS.

Identification of endogenous ubiquitylation sites by MS-based proteomics has long been hampered by a lack of specific enrichment methods for ubiquitylated peptides. Earlier studies have most commonly relied on the ectopic expression of polyhistidine tagged ubiquitin and enrichment of ubiquitylated proteins by nickel-nitrilotriacetic acid (Ni-NTA) chromatography under denaturing conditions. Alternatively, purified tagged ubiquitin-binding domains have been used to enrich ubiquitylated proteins from cells expressing endogenous ubiquitin (Hjerpe et al., 2009). These approaches have been successfully employed for the identification of putative ubiquitylated proteins and a limited number of ubiquitylation sites (Peng et al., 2003; Matsumoto et al., 2005; Tagwerker et al., 2006; Meierhofer et al., 2008; Danielsen et al., 2011). However, enrichment at the protein level does not sufficiently reduce sample complexity and thus does not permit efficient proteome-wide identification of endogenous ubiquitylation sites.

UBIQUITIN REMNANT PROFILING FOR PROTEOME-WIDE IDENTIFICATION OF UBIQUITYLATION SITES

Digestion of ubiquitylated proteins with trypsin leaves a di-glycine remnant from the C-terminus of ubiquitin covalently attached to the previously ubiquitylated lysine. The di-glycine remnant leads to a peptide mass shift (∼114 Da) and can be exploited to pinpoint the localization of the ubiquitin attachment site in the protein (Peng et al., 2003) (**Figure 1**). Xu et al. (2010) used di-glycine-modified histones as an antigen to produce the monoclonal antibody GX41 that specifically recognizes di-glycine adducts on the ε-amine of lysine. To demonstrate the applicability of the antibody for enrichment of di-glycine modified peptides, the authors purified ubiquitylated proteins from HEK293T cells ectopically expressing His_6-tagged ubiquitin. After proteolysis, di-glycine-modified peptides were enriched using di-glycine lysine specific antibodies and identified by LC–MS/MS.

The generation of di-glycine lysine specific antibodies and advances in high-resolution mass spectrometry have enabled first proteome-wide studies of endogenous ubiquitylation sites in human cells (Kim et al., 2011; Wagner et al., 2011) (**Figure 1**). Wagner et al. (2012) extended ubiquitin remnant profiling to murine tissues and demonstrated that different tissues display specific ubiquitylation patterns. In addition, they identified a number of core ubiquitylation sites that are present in all examined tissues.

One drawback of ubiquitin remnant profiling is that tryptic digestion of proteins modified with the ubiquitin-like modifiers NEDD8 and ISG15 also results in a di-glycine remnant attached to the previously modified lysine. Consequently, ubiquitin remnant profiling does not allow distinguishing NEDD8- and ISG15-modification sites from ubiquitylation sites. However, the expression of ISG15 in cells that are not stimulated with interferon (IFN)-α/β is very low (Skaug and Chen, 2010) and NEDD8 is considered to primarily modify Cullin-RING ligases (CRLs; Lydeard et al., 2013). Kim et al. (2011) used different experimental approaches to demonstrate that >94% of the di-glycine lysine containing peptides are indeed ubiquitin remnant peptides.

QUANTITATIVE ANALYSES OF PROTEIN UBIQUITYLATION BY UBIQUITIN REMNANT PROFILING

Ubiquitin remnant profiling can be combined with quantitative proteomics approaches based on metabolic (e.g., stable isotope labeling with amino acids in cell culture, SILAC) or chemical labeling (e.g., tandem mass tags or isobaric tags for relative and absolute quantitation) to quantify the relative abundance of ubiquitylation sites in different experimental conditions. In the last 5 years this strategy has been successfully implemented to investigate site-specific alterations of the ubiquitin-modified proteome after cellular stress or growth factor stimulation as well as to identify substrates of ubiquitin-modifying enzymes.

Initial studies employed ubiquitin remnant profiling and SILAC-based quantitative proteomics to analyze site-specific changes in ubiquitylation in different human cell lines after inhibition of the proteasome (Kim et al., 2011; Wagner et al., 2011; Udeshi et al., 2012). These studies have shown that proteasome inhibition globally perturbs cellular ubiquitylation patterns and leads to increased ubiquitylation of >40% of the quantified sites. Interestingly, proteasome inhibition also results in decreased abundance of a fraction of ubiquitylation sites, including sites on histones and DNA repair factors PCNA, FANCI, and FANCD2 that confer non-degradative, regulatory functions. These observations indicate that proteasome inhibition leads to depletion of the cellular ubiquitin pool and results in a shift from mono- to poly-ubiquitylation, and can therefore be used to distinguish degradative from non-degradative, regulatory ubiquitylation (Kim et al., 2011; Wagner et al., 2011; Udeshi et al., 2012; Higgins et al., 2015).

Ubiquitin remnant profiling and SILAC-based quantitative proteomics has been used to perform first proteome-wide, site-specific analyses of ubiquitylation after DNA damage. Povlsen et al. (2012) induced DNA damage in U2OS cells by ultraviolet light (UV) irradiation and identified up-regulated and down-regulated ubiquitylation sites on known components of DNA damage repair and signaling as well as on proteins that had not been previously implicated in this process, demonstrating that ubiquitin-modifying enzymes play an integral role in the regulation of the cellular response to DNA damage. This study also demonstrated that UV light affects the ubiquitylation status of the PCNA-associated factor PAF15 and that PAF15 ubiquitylation regulates the interaction between translesion synthesis polymerases and PCNA during the DNA damage bypass. Another study investigated site-specific changes in ubiquitylation after DNA damage induced by irradiation of U2OS cells with UV light or ionizing radiation (IR) and could demonstrate that centromere proteins are de-ubiquitylated in response to UV light- and IR-induced DNA damage (Elia et al., 2015a). Notably, in this study profiling of ubiquitylation sites has been performed with and without pre-treatment of cells with

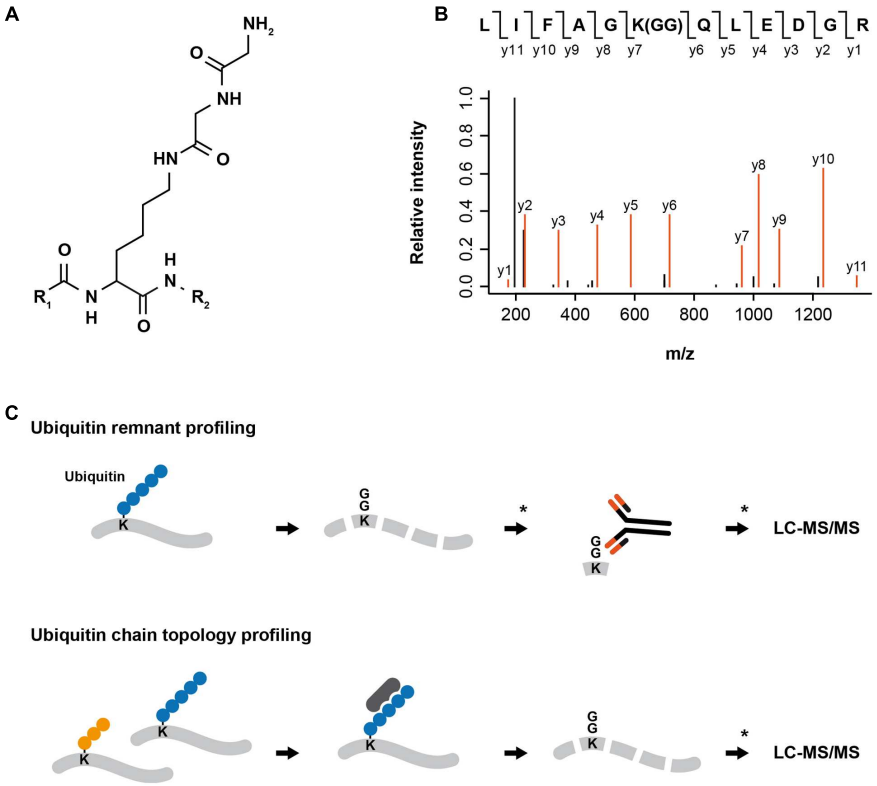

FIGURE 1 | MS-based proteomics approaches for analyzing protein ubiquitylation. (A) Digestion of ubiquitylated proteins with trypsin leaves a di-glycine remnant from the C-terminus of ubiquitin covalently attached to the previously modified lysine. **(B)** Exemplary fragment spectrum of a di-glycine modified peptide. The di-glycine remnant leads to a shift of ~114 Da in the peptide mass and can be exploited to pinpoint the localization of the ubiquitin attachment. **(C)** For ubiquitin remnant profiling, proteins extracted from cells are digested into peptides using trypsin, di-glycine modified peptides are enriched using di-glycine lysine specific antibodies and identified by LC-MS/MS. *Pre- and post-enrichment fractionation can be introduced to decrease sample complexity and increase the depth of the analysis. For ubiquitin chain topology profiling, proteins extracted from cells or tissues are incubated with an ubiquitin linkage-specific binder (e.g., antibody, affimer, TUBE). Enriched proteins modified by a specific type of ubiquitin chain are digested in-gel into peptides and peptide samples are analyzed by LC-MS/MS. *Post-enrichment fractionation can be introduced to decrease sample complexity and increase the depth of the analysis.

the proteasome inhibitor MG132 to facilitate the identification of both degradative and non-degradative, regulatory ubiquitylation sites, respectively. In another study, the same authors found that the single-stranded DNA binding protein RPA is ubiquitylated on multiple lysines after replication fork stalling by the ubiquitin ligase RFWD3 (Elia et al., 2015b). Ubiquitylation of RPA occurs on chromatin and does not mediate its degradation, but is important for the replication fork restart and homologous recombination at stalled replication forks.

Ubiquitin remnant profiling also enables global analyses of ubiquitin-linkage abundance and has been employed to reveal that UV light irradiation increases the cellular abundance of K6-linked ubiquitin chains (Povlsen et al., 2012; Elia et al., 2015a). Modification of proteins with K6-linked ubiquitylation by the ubiquitin ligase BRCA1 has been previously suggested to play a role in the cellular response to DNA damage (Wu-Baer et al., 2003, 2010; Morris and Solomon, 2004; Nishikawa et al., 2004).

Ubiquitin remnant profiling is a powerful approach for identification and quantification of ubiquitylation sites, however, several limitations should be considered when designing experiments: shotgun proteomics is biased towards more abundant peptide species and it is likely that ubiquitylation sites on low abundant, chromatin-associated proteins that often play an essential role in the regulation of DNA damage repair and signaling are missed. Also, the high amount of protein that is needed in order to achieve a satisfactory depth of the analysis hampers the application of this method for samples with limited quantity (e.g., primary cells). We envision that further improvements of MS instrumentation will facilitate the identification and quantification of low abundant ubiquitylation sites. Alternatively, targeted proteomics approaches can be employed to reproducibly quantify selected ubiquitylation sites across different experiments.

MAPPING SUBSTRATES OF UBIQUITIN-MODIFYING ENZYMES

In addition to analyzing ubiquitin-dependent processes after DNA damage, an increasing number of studies have successfully employed ubiquitin remnant profiling and quantitative proteomics to identify substrates of ubiquitin ligases and

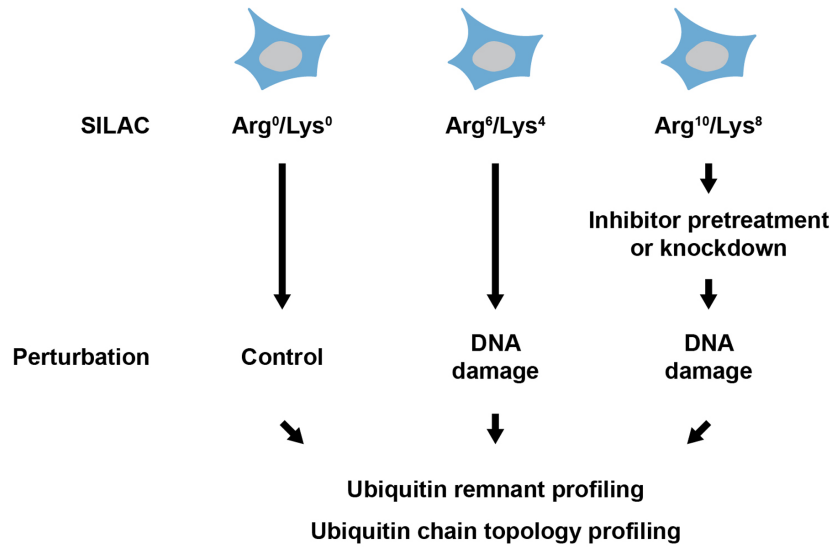

FIGURE 2 | Experimental strategy for analyzing DNA damage-associated ubiquitylation. Cells are metabolically labeled using heavy isotope containing lysine and arginine (SILAC) and subsequently treated with a DNA damage-inducing agent. Pretreatment with an inhibitor or knockdown of ubiquitin-modifying enzyme can be performed before treatment with the DNA damage-inducing agent. Proteins are extracted from cells and ubiquitylation is quantitatively investigated using ubiquitin remnant profiling and/or ubiquitin chain topology profiling.

deubiquitylating (DUBs) enzymes. In most studies the activity of the ubiquitin-modifying enzyme is inhibited using small molecules or the expression of the enzyme is down-regulated using knockdown or knockout approaches. Emanuele et al. (2011) employed ubiquitin remnant profiling and SILAC-based quantitative proteomics to quantify ubiquitylation sites in cells in which CRLs activity had been chemically inhibited by the NEDD8-activating enzyme inhibitor MLN4924. CRLs frequently target substrate proteins for degradation through the proteasome and therefore additional pre-treatment of cells with the proteasome inhibitor had been used to stabilize the proteins that are normally targeted for degradation by CRLs. The authors identified hundreds of ubiquitylation sites that decreased in abundance after CRL inhibition and demonstrated that NUSAP1, a protein involved in mitosis and DNA damage response, is a novel substrate of the Skp1, Cullin, F-box (SCF) Cyclin F (Emanuele et al., 2011). Elia et al. (2015a) employed a similar approach to demonstrate that 10% of UV light-induced ubiquitylation is dependent on CRLs and to show that SCF–Cyclin F ubiquitylates the double-stranded DNA exonuclease EXO1 after irradiation of cells with UV light.

Mutations in the substrate recognition domain of the CRL CUL3 adaptor speckle-type POZ protein (SPOP) are frequently found in primary prostate cancer (Barbieri et al., 2012). SPOP functions in DNA double strand break repair and SPOP mutations in prostate cancer are associated with genomic instability (Boysen et al., 2015). However, the identity of its substrates and whether or not cancer-associated mutations of SPOP affect the substrate landscape remained unclear. Theurillat et al. (2014) compared the abundance of ubiquitylation sites in immortalized prostate epithelial cells stably overexpressing wild type or mutant forms of SPOP. They were able to show that SPOP mutants lead to decreased ubiquitylation and impaired degradation of the chromatin remodeler DEK and thereby contribute to the oncogenic phenotype of prostate cancer cells.

Ubiquitin remnant profiling has also been employed to identify the substrates of the CRL adaptor cereblon (CRBN). Krönke et al. (2014) employed a chemical proteomics approach to show that lenalidomide, a thalidomide derivative used in treatment of multiple myeloma, binds to CRBN. Following up on this observation, they employed SILAC-based quantitative proteomics and ubiquitin remnant profiling to analyze alterations in ubiquitylation site abundance after treatment of MM1S multiple-myeloma cell line with lenalidomide. The authors demonstrated that lenalidomide regulates ubiquitylation and abundance of the transcription factors IKZF1 and IKZF3, and could further show that depletion of these proteins inhibited the growth of lenalidomide-sensitive multiple myeloma cell lines, while not having an effect on lenalidomide-insensitive cells (Krönke et al., 2014).

Furthermore, ubiquitin remnant profiling and inducible knockdown has been employed to identify DNA damage-inducible transcript 4 (DDIT4) as a novel substrate of the ubiquitin ligase HUWE1 that has been implicated in cancer development and DNA damage response (Thompson et al., 2014).

The above-mentioned studies demonstrate that ubiquitin remnant profiling is a powerful approach for identifying substrates of ubiquitin-modifying enzymes in the context of DNA damage signaling and disease. Development of novel specific small molecule inhibitors of ubiquitin-modifying enzymes as well as generation of knockdown/knockout cell lines will help to further decipher the complex relations between ubiquitin-modifying enzymes and their substrates.

INVESTIGATING UBIQUITIN CHAIN TOPOLOGY

The role of K48-linked ubiquitylation in the degradation of proteins through the proteasome and the function of K63-linked ubiquitylation in cellular signaling are well established (Hochstrasser, 1995; Haglund and Dikic, 2005). The cellular functions of atypical ubiquitin chains formed through K6, K11, K27, K29, and K33 are largely unknown and tools for detection and enrichment of proteins modified with these atypical ubiquitin chains are missing.

The development of linkage-specific antibodies for M1-, K48-, and K63-linked ubiquitin chains facilitated the identification of substrates and functions of these ubiquitin chains (Newton et al., 2008; Matsumoto et al., 2010, 2012). Recently, high affinity binders (affimers) for K6, K33, and K48 ubiquitin chains have been generated and are commercially available; however, the application of these ubiquitin linkage-specific affimers for enrichment of proteins modified by specific ubiquitin chains still remains to be demonstrated. Efficient enrichment of proteins modified with a particular ubiquitin chain would enable their identification by LC–MS/MS and greatly help to deepen the understanding of the cellular functions of atypical ubiquitin chains (**Figure 1**).

In addition to antibodies and affimers, the identification of ubiquitin-binding domains (UBDs) that recognize specific type of ubiquitin chains allowed generation of engineered tandem ubiquitin-binding entities (TUBEs) that can be used as affinity matrix for enrichment of proteins modified with specific types of ubiquitin chains (Hjerpe et al., 2009; Sims et al., 2012). Recently, a K63-specific TUBE and SILAC-based quantitative proteomics have been used to compare protein ubiquitylation in wild type and UBC13 knockout HCT116 cells. Using this strategy, Thorslund et al. (2015) identified 371 proteins, several of which had been previously reported to be modified by K63-linked ubiquitylation. To complement these results, the same authors employed a similar approach for identification of proteins with increased K63-linked ubiquitylation after DNA damage induced by IR, thus identifying histone H1 as substrate of UBC13/RNF8 at DNA double strand breaks (Thorslund et al., 2015). Another study conducted in *Saccharomyces cerevisiae* employed a K63-specific TUBE for enrichment of K63-linked ubiquitylated proteins from wild type and ubiquitin K63R strain after oxidative stress induced by H_2O_2. The authors identified >100 proteins modified with K63-linked ubiquitin chains after treatment of cells with H_2O_2 and demonstrated that ribosomal proteins are dynamically modified by K63-linked ubiquitylation during the cellular response to H_2O_2 (Silva et al., 2015). Besides above mentioned TUBEs for K63-linked ubiquitin chains, TUBEs specifically binding to M1- and K48-linked ubiquitin chains have been generated (Trempe et al., 2005; Rahighi et al., 2009).

Another approach for analyzing ubiquitin chain topology on substrate proteins has been developed in the Komander lab: In Ubiquitin Chain Restriction Enzyme Analysis (UbiCRest), the relative SDS–PAGE mobility of investigated proteins before and after treatment with different linkage-specific DUBs is monitored to identify the type of ubiquitin chains on the protein (Hospenthal et al., 2015). Multiple DUBs from the human ovarian tumor (OTU) DUB family that display various degrees of specificities towards different ubiquitin linkage types have been identified and can be used for UbiCRest: For instance, OTUB1 specifically cleaves K48-, OTUD1 K63-, Cezanne K11-, and OTULIN M1-linked ubiquitylation, whereas OTUD3 displays specificity towards K6- and K11-linked ubiquitylation (Mevissen et al., 2013). A current limitation of this method is that DUBs might display various specificities towards ubiquitin chains linkages depending on the set-up of the assay and the concentration of the enzyme used, and the fact that specific DUBs for all types of ubiquitin chains have not been unambiguously identified. To date, UbiCRest was only employed to study the ubiquitin chain topology on single proteins; however, it might be possible to combine this method with MS to identify ubiquitin chain topologies on a proteome-wide scale.

CONCLUSION

Development of methods for specific enrichment of ubiquitin remnant peptides and advances in high-resolution MS have enabled proteome-wide identification of ubiquitylation sites in cell lines and tissues. Furthermore, ubiquitin remnant profiling has been used for quantitative analysis of site-specific protein ubiquitylation after cellular perturbations, thereby providing a better understanding of the regulatory scope of ubiquitylation in different cellular processes, including the DNA damage response. Ubiquitin remnant profiling has also been successfully employed to identify substrates of ubiquitin-modifying enzymes, some of which have been implicated in the cellular response to DNA damage.

However, our understanding of the roles of ubiquitylation in the cellular DNA damage response is far from complete: little is known about the function of many of the dynamically modified ubiquitylation sites identified in ubiquitin remnant profiling studies. In addition, numerous ubiquitin-modifying enzymes have been implicated in the DNA damage response and for most of these enzymes the cellular substrate spectrum remains to be uncovered. Future studies employing ubiquitin remnant profiling and novel small molecule inhibitors or genetic knockdown/knockout approaches are likely to deepen the knowledge about the substrates and functions of these DNA damage-associated ubiquitin-modifying enzymes (**Figure 2**). Another major challenge lies in the investigation of the ubiquitin chain topology on proteins. In the last years, specific binders for M1-, K48- and K63-linked ubiquitin chains have been developed. Further development of tools for detection and enrichment of proteins modified with K6-, K11-, K27-, K29-, and K33-linked ubiquitin chains is essential to understand the cellular functions of atypical ubiquitylation. Probing the ubiquitin chain topology on proteins with DNA damage-regulated ubiquitylation sites will also help to understand the functions of ubiquitylation in the DNA damage response (**Figure 2**).

AUTHOR CONTRIBUTIONS

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

FUNDING

The research in PB's group is supported by the German Research Foundation (Emmy Noether Program, BE 5342/1-1 and SFB 1177 on Selective Autophagy) and the Marie Curie Career Integration Grant from the European Commission (grant agreement number: 630763) The research in SAW's group is supported by the LOEWE program Ubiquitin Networks (Ub-Net) of the State of Hesse (Germany) and the Else Kröner-Fresenius-Stiftung. The Institute of Molecular Biology (IMB) is funded by the Boehringer Ingelheim Foundation.

ACKNOWLEDGMENT

We thank the members of the Beli and Wagner group for helpful discussions.

REFERENCES

Aebersold, R., and Mann, M. (2003). Mass spectrometry-based proteomics. *Nature* 422, 198–207. doi: 10.1038/nature01511

Barbieri, C. E., Baca, S. C., Lawrence, M. S., Demichelis, F., Blattner, M., Theurillat, J.-P., et al. (2012). Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat. Genet.* 44, 685–689. doi: 10.1038/ng.2279

Boysen, G., Barbieri, C. E., Prandi, D., Blattner, M., Chae, S.-S., Dahija, A., et al. (2015). SPOP mutation leads to genomic instability in prostate cancer. *Elife* 4, e09207. doi: 10.7554/eLife.09207

Danielsen, J. M. R., Sylvestersen, K. B., Bekker-Jensen, S., Szklarczyk, D., Poulsen, J. W., Horn, H., et al. (2011). Mass spectrometric analysis of lysine ubiquitylation reveals promiscuity at site level. *Mol. Cell. Proteomics* 10, M110.003590. doi: 10.1074/mcp.M110.003590

Elia, A. E. H., Boardman, A. P., Wang, D. C., Huttlin, E. L., Everley, R. A., Dephoure, N., et al. (2015a). Quantitative proteomic atlas of ubiquitination and acetylation in the DNA damage response. *Mol. Cell* 59, 867–881. doi: 10.1016/j.molcel.2015.05.006

Elia, A. E. H., Wang, D. C., Willis, N. A., Boardman, A. P., Hajdu, I., Adeyemi, R. O., et al. (2015b). RFWD3-dependent ubiquitination of RPA regulates repair at stalled replication forks. *Mol. Cell* 60, 280–293. doi: 10.1016/j.molcel.2015.09.011

Emanuele, M. J., Elia, A. E. H., Xu, Q., Thoma, C. R., Izhar, L., Leng, Y., et al. (2011). Global identification of modular cullin-RING ligase substrates. *Cell* 147, 459–474. doi: 10.1016/j.cell.2011.09.019

Haglund, K., and Dikic, I. (2005). Ubiquitylation and cell signaling. *EMBO J.* 24, 3353–3359. doi: 10.1038/sj.emboj.7600808

Higgins, R., Gendron, J. M., Rising, L., Mak, R., Webb, K., Kaiser, S. E., et al. (2015). The unfolded protein response triggers site-specific regulatory ubiquitylation of 40S ribosomal proteins. *Mol. Cell* 59, 35–49. doi: 10.1016/j.molcel.2015.04.026

Hjerpe, R., Aillet, F., Lopitz-Otsoa, F., Lang, V., England, P., and Rodriguez, M. S. (2009). Efficient protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities. *EMBO Rep.* 10, 1250–1258. doi: 10.1038/embor.2009.192

Hochstrasser, M. (1995). Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* 7, 215–223. doi: 10.1016/0955-0674(95)80031-X

Hospenthal, M. K., Mevissen, T. E. T., and Komander, D. (2015). Deubiquitinase-based analysis of ubiquitin chain architecture using ubiquitin chain restriction (UbiCRest). *Nat. Protoc.* 10, 349–361. doi: 10.1038/nprot.2015.018

Kim, W., Bennett, E. J., Huttlin, E. L., Guo, A., Li, J., Possemato, A., et al. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* 44, 325–340. doi: 10.1016/j.molcel.2011.08.025

Krönke, J., Udeshi, N. D., Narla, A., Grauman, P., Hurst, S. N., McConkey, M., et al. (2014). Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science* 343, 301–305. doi: 10.1126/science.1244851

Larance, M., and Lamond, A. I. (2015). Multidimensional proteomics for cell biology. *Nat. Rev. Mol. Cell Biol.* 16, 269–280. doi: 10.1038/nrm3970

Lydeard, J. R., Schulman, B. A., and Harper, J. W. (2013). Building and remodelling Cullin-RING E3 ubiquitin ligases. *EMBO Rep.* 14, 1050–1061. doi: 10.1038/embor.2013.173

Matsumoto, M., Hatakeyama, S., Oyamada, K., Oda, Y., Nishimura, T., and Nakayama, K. I. (2005). Large-scale analysis of the human ubiquitin-related proteome. *Proteomics* 5, 4145–4151. doi: 10.1002/pmic.200401280

Matsumoto, M. L., Dong, K. C., Yu, C., Phu, L., Gao, X., Hannoush, R. N., et al. (2012). Engineering and structural characterization of a linear polyubiquitin-specific antibody. *J. Mol. Biol.* 418, 134–144. doi: 10.1016/j.jmb.2011.12.053

Matsumoto, M. L., Wickliffe, K. E., Dong, K. C., Yu, C., Bosanac, I., Bustos, D., et al. (2010). K11-linked polyubiquitination in cell cycle control revealed by a K11 linkage-specific antibody. *Mol. Cell* 39, 477–484. doi: 10.1016/j.molcel.2010.07.001

Meierhofer, D., Wang, X., Huang, L., and Kaiser, P. (2008). Quantitative analysis of global ubiquitination in HeLa cells by mass spectrometry. *J. Proteome Res.* 7, 4566–4576. doi: 10.1021/pr800468j

Mevissen, T. E. T., Hospenthal, M. K., Geurink, P. P., Elliott, P. R., Akutsu, M., Arnaudo, N., et al. (2013). OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell* 154, 169–184. doi: 10.1016/j.cell.2013.05.046

Morris, J. R., and Solomon, E. (2004). BRCA1: BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum. Mol. Genet.* 13, 807–817. doi: 10.1093/hmg/ddh095

Newton, K., Matsumoto, M. L., Wertz, I. E., Kirkpatrick, D. S., Lill, J. R., Tan, J., et al. (2008). Ubiquitin chain editing revealed by polyubiquitin linkage-specific antibodies. *Cell* 134, 668–678. doi: 10.1016/j.cell.2008.07.039

Nishikawa, H., Ooka, S., Sato, K., Arima, K., Okamoto, J., Klevit, R. E., et al. (2004). Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. *J. Biol. Chem.* 279, 3916–3924. doi: 10.1074/jbc.M308540200

Peng, J., Schwartz, D., Elias, J. E., Thoreen, C. C., Cheng, D., Marsischky, G., et al. (2003). A proteomics approach to understanding protein ubiquitination. *Nat. Biotechnol.* 21, 921–926. doi: 10.1038/nbt849

Povlsen, L. K., Beli, P., Wagner, S. A., Poulsen, S. L., Sylvestersen, K. B., Poulsen, J. W., et al. (2012). Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitylation in DNA-damage bypass. *Nat. Cell Biol.* 14, 1089–1098. doi: 10.1038/ncb2579

Rahighi, S., Ikeda, F., Kawasaki, M., Akutsu, M., Suzuki, N., Kato, R., et al. (2009). Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. *Cell* 136, 1098–1109. doi: 10.1016/j.cell.2009.03.007

Silva, G. M., Finley, D., and Vogel, C. (2015). K63 polyubiquitination is a new modulator of the oxidative stress response. *Nat. Struct. Mol. Biol.* 22, 116–123. doi: 10.1038/nsmb.2955

Sims, J. J., Scavone, F., Cooper, E. M., Kane, L. A., Youle, R. J., Boeke, J. D., et al. (2012). Polyubiquitin-sensor proteins reveal localization and linkage-type dependence of cellular ubiquitin signaling. *Nat. Methods* 9, 303–309. doi: 10.1038/nmeth.1888

Skaug, B., and Chen, Z. J. (2010). Emerging role of ISG15 in antiviral immunity. *Cell* 143, 187–190. doi: 10.1016/j.cell.2010.09.033

Tagwerker, C., Flick, K., Cui, M., Guerrero, C., Dou, Y., Auer, B., et al. (2006). A tandem affinity tag for two-step purification under fully denaturing conditions: application in ubiquitin profiling and protein complex identification combined with in vivocross-linking. *Mol. Cell. Proteomics* 5, 737–748. doi: 10.1074/mcp.M500368-MCP200

Theurillat, J.-P. P., Udeshi, N. D., Errington, W. J., Svinkina, T., Baca, S. C., Pop, M., et al. (2014). Prostate cancer. Ubiquitylome analysis identifies dysregulation of effector substrates in SPOP-mutant prostate cancer. *Science* 346, 85–89. doi: 10.1126/science.1250255

Thompson, J. W., Nagel, J., Hoving, S., Gerrits, B., Bauer, A., Thomas, J. R., et al. (2014). Quantitative Lys-ϵ-Gly-Gly (diGly) proteomics coupled with inducible RNAi reveals ubiquitin-mediated proteolysis of DNA damage-inducible transcript 4 (DDIT4) by the E3 ligase HUWE1. *J. Biol. Chem.* 289, 28942–28955. doi: 10.1074/jbc.M114.573352

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., et al. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393. doi: 10.1038/nature15401

Trempe, J.-F., Brown, N. R., Lowe, E. D., Gordon, C., Campbell, I. D., Noble, M. E. M., et al. (2005). Mechanism of Lys48-linked polyubiquitin chain recognition by the Mud1 UBA domain. *EMBO J.* 24, 3178–3189. doi: 10.1038/sj.emboj.7600797

Udeshi, N. D., Mani, D. R., Eisenhaure, T., Mertins, P., Jaffe, J. D., Clauser, K. R., et al. (2012). Methods for quantification of in vivo changes in protein ubiquitination following proteasome and deubiquitinase inhibition. *Mol. Cell. Proteomics* 11, 148–159. doi: 10.1074/mcp.M111.016857

Wagner, S. A., Beli, P., Weinert, B. T., Nielsen, M. L., Cox, J., Mann, M., et al. (2011). A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol. Cell. Proteomics* 10, M111013284. doi: 10.1074/mcp.M111.013284

Wagner, S. A., Beli, P., Weinert, B. T., Schölz, C., Kelstrup, C. D., Young, C., et al. (2012). Proteomic analyses reveal divergent ubiquitylation site patterns in murine tissues. *Mol. Cell. Proteomics* 11, 1578–1585. doi: 10.1074/mcp.M112.017905

Wu-Baer, F., Lagrazon, K., Yuan, W., and Baer, R. (2003). The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. *J. Biol. Chem.* 278, 34743–34746. doi: 10.1074/jbc.C300249200

Wu-Baer, F., Ludwig, T., and Baer, R. (2010). The UBXN1 protein associates with autoubiquitinated forms of the BRCA1 tumor suppressor and inhibits its enzymatic function. *Mol. Cell. Biol.* 30, 2787–2798. doi: 10.1128/MCB.01056-09

Xu, G., Paige, J. S., and Jaffrey, S. R. (2010). Global analysis of lysine ubiquitination by ubiquitin remnant immunoaffinity profiling. *Nat. Biotechnol.* 28, 868–873. doi: 10.1038/nbt.1654

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 13 June 2016
doi: 10.3389/fgene.2016.00105

The Regulation of DNA Damage Tolerance by Ubiquitin and Ubiquitin-Like Modifiers

*Lina Cipolla, Antonio Maffia, Federica Bertoletti and Simone Sabbioneda**

Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche, Pavia, Italia

DNA replication is an extremely complex process that needs to be executed in a highly accurate manner in order to propagate the genome. This task requires the coordination of a number of enzymatic activities and it is fragile and prone to arrest after DNA damage. DNA damage tolerance provides a last line of defense that allows completion of DNA replication in the presence of an unrepaired template. One of such mechanisms is called post-replication repair (PRR) and it is used by the cells to bypass highly distorted templates caused by damaged bases. PRR is extremely important for the cellular life and performs the bypass of the damage both in an error-free and in an error-prone manner. In light of these two possible outcomes, PRR needs to be tightly controlled in order to prevent the accumulation of mutations leading ultimately to genome instability. Post-translational modifications of PRR proteins provide the framework for this regulation with ubiquitylation and SUMOylation playing a pivotal role in choosing which pathway to activate, thus controlling the different outcomes of damage bypass. The proliferating cell nuclear antigen (PCNA), the DNA clamp for replicative polymerases, plays a central role in the regulation of damage tolerance and its modification by ubiquitin, and SUMO controls both the error-free and error-prone branches of PRR. Furthermore, a significant number of polymerases are involved in the bypass of DNA damage possess domains that can bind post-translational modifications and they are themselves target for ubiquitylation. In this review, we will focus on how ubiquitin and ubiquitin-like modifications can regulate the DNA damage tolerance systems and how they control the recruitment of different proteins to the replication fork.

Keywords: DNA damage tolerance, translesion synthesis, ubiquitylation, SUMOylation, ISGylation, PCNA

OPEN ACCESS

Edited by:
Kristijan Ramadan, University of Oxford, UK

Reviewed by:
Danilo Maddalo, Novartis Oncology, Switzerland
Barbara Van Loon, Norwegian University for Science and Technology, Norway

****Correspondence:***
Simone Sabbioneda
simone.sabbioneda@igm.cnr.it

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *01 March 2016*
Accepted: *25 May 2016*
Published: *13 June 2016*

Citation:
Cipolla L, Maffia A, Bertoletti F and Sabbioneda S (2016) The Regulation of DNA Damage Tolerance by Ubiquitin and Ubiquitin-Like Modifiers. Front. Genet. 7:105. doi: 10.3389/fgene.2016.00105

INTRODUCTION

DNA damage poses a constant threat to the genetic material. It can arise from products either of the cellular metabolism or by exposure to exogenous sources (physical or chemical). Regardless of its origin, DNA damage is addressed swiftly by the multitude of repair mechanisms that protect the integrity of the genome (Hoeijmakers, 2001). The DNA damage response provides an overall control network for the repair mechanisms and it allows the coordination of the complex biochemical reactions that lead to the elimination of DNA damage (Ciccia and Elledge, 2011). Unfortunately, in certain conditions, the cells are exposed to an amount of damage that the repair systems cannot handle completely. This could be caused either by an extreme insult, able to saturate one or multiple repair systems, or by damage that is repaired slowly. The result of both conditions

is the permanence of lesions in the template DNA. Nevertheless, the damaged template then must be replicated during S phase. Replicative DNA polymerases are extremely efficient and processive but are unable to cope with a distorted template caused by DNA damage. To solve this impasse, cells possess damage tolerance pathways that are tasked with the bypass of the damage, which eventually will be repaired at a later stage (Sale et al., 2012). Failure to bypass the damage is believed to be one of the main causes of replication fork blocks, cell cycle arrest and eventually cell death.

During S phase, the damaged template can be replicated by either a special class of DNA polymerases, in a process called DNA translesion synthesis (TLS), or by a damage avoidance pathway that uses the sister chromatid as a template, in a mechanism called template switch. TLS utilizes specialized low-fidelity DNA polymerases (η, ι, κ, ζ, and Rev1), mostly belonging to the Y-family, to bypass the damaged template, while template switch is proposed to use a recombination-like mechanism. A crucial difference between the two pathways is that the former is potentially error-prone, while the latter is thought to be error-free (Branzei and Foiani, 2007; Sale et al., 2012). Given this background, the choice of pathway is extremely important in order to bypass the damage with the lowest possible chance of introducing mutations. Post-translational modifications play a central role in controlling damage tolerance and, in the last few years, emerging evidence has shown that ubiquitylation and SUMOylation sit at a crucial crossroad that influences its outcomes (Huang and D'Andrea, 2006; Bergink and Jentsch, 2009; Bekker-Jensen and Mailand, 2011; Mailand et al., 2013; Pinder et al., 2013).

Ubiquitylation is a process that involves the addition of ubiquitin to a target protein. This process is conserved in all eukaryotes and it controls a variety of cellular functions, ranging from protein degradation to cell cycle progression. Ubiquitylation is reversible and utilizes three classes of enzymes to target ubiquitin to a desired protein (Hershko and Ciechanover, 1998). In the initial step, an ubiquitin activating enzyme (E1) forms a thioester bond with ubiquitin. Afterward, ubiquitin conjugating enzymes (E2) transfer the ubiquitin from the E1 to the target protein, either directly or with the help of an E3 ubiquitin ligase that confers specificity to its E2 partner. Ubiquitin is normally attached via its C-terminus to lysines on the target proteins. Once ubiquitin has been linked to its target, it can be further modified by the addition of additional ubiquitin moieties on one of the lysines that can be found on ubiquitin itself: K6, K11, K27, K29, K33, K48, and K63 (Ikeda and Dikic, 2008; Kulathu and Komander, 2012). The linkage to the different lysines confers diverse structural properties to the polyubiquitin chains, creating a different binding platform for a variety of processes. For example, K48-linked chains have a compact structure (closed chain) and they direct proteins to degradation by the proteasome (Varadan et al., 2002). On the other hand, K63 chains are linear and flexible and they seem to have a more prominent role in mediating protein–protein interactions (Varadan et al., 2004). SUMOylation shares a similar activating pathway with ubiquitin but uses SUMO (Small Ubiquitin MOdifier) as a substrate (Muller et al., 2001; Hay, 2005). In most organisms, a single SUMO is present but human cells express 4 different variants (SUMO1–4, Hay, 2005). Remarkably, while in the human genome we can find between 10 to 35 ubiquitin E2s and hundreds of putativeE3 ubiquitin ligases have been predicted, this number is greatly reduced in the case of SUMO, up to the point where UBC9 encodes the only known SUMO E2 (Hay, 2005). The aim of this review is to highlight the crucial role of both ubiquitylation and SUMOylation in the regulation of the DNA damage tolerance pathways.

UBIQUITYLATION OF PCNA

A number of E2 and E3 enzymes has been known for a long time to be involved in the replication of damaged DNA, among these the proteins encoded by Rad6, Rad18, Ubc13, Mms2, and Rad5 in the yeast Saccharomyces cerevisiae (Jentsch et al., 1987; Bailly et al., 1994, 1997; Xiao et al., 2000). All of these proteins have been shown to ubiquitylate, in different ways, the PCNA, assigning to PCNA a central role in the regulation of damage bypass during replication (Hoege et al., 2002; Mailand et al., 2013).

Proliferating cell nuclear antigen is a homotrimeric protein that acts as the processivity factor for DNA polymerases, in a role similar to *E. coli* β-clamp (Kuriyan and O'Donnell, 1993; Krishna et al., 1994a,b). Each subunit consists of two different domains connected by an interdomain connecting loop (IDCL). The IDCL makes contacts and tethers the DNA polymerases to the DNA. The binding to the IDCL of PCNA is mediated by a PCNA interacting peptide (PIP) motif present in the interacting partner. PCNA plays also crucial roles as a loading platform for a variety of proteins involved in different repair systems (Freudenthal et al., 2010; Dieckman et al., 2012). In yeast, PCNA was originally discovered to be ubiquitylated after the treatment with methyl methanesulfonate (MMS) by the complex formed by the ubiquitin ligase Rad18 and the ubiquitin conjugating enzyme Rad6 (Hoege et al., 2002) (**Figure 1**). Ubiquitylation was shown to be attached to lysine 164 that is located on the back side of the trimer, on the opposite side where the replicating polymerases make contact (front side, Freudenthal et al., 2010).

Once monoubiquitylated, PCNA (Ubi-PCNA) can be further modified resulting in the formation of K63-linked polyubiquitin chains (Hoege et al., 2002). The two modifications were proposed to channel the bypass toward different branches of damage tolerance, with monoubiquitylation leading to TLS and polyubiquitylation of PCNA steering the system toward template switch (Branzei, 2011; Giannattasio et al., 2014).

Orthologs of all the proteins involved in the process originally described in *S. cerevisiae* have been identified in both invertebrates and vertebrates and, overall, the system appears to be conserved across different organisms, although subtle differences are present. For example, in *Xenopus laevis*, PCNA is monoubiquitylated during an unperturbed S phase and this modification is required for the efficient progression of the replication fork in egg extracts, while polyubiquitylation of the trimer appears specifically only after DNA damage (Leach and Michael, 2005).

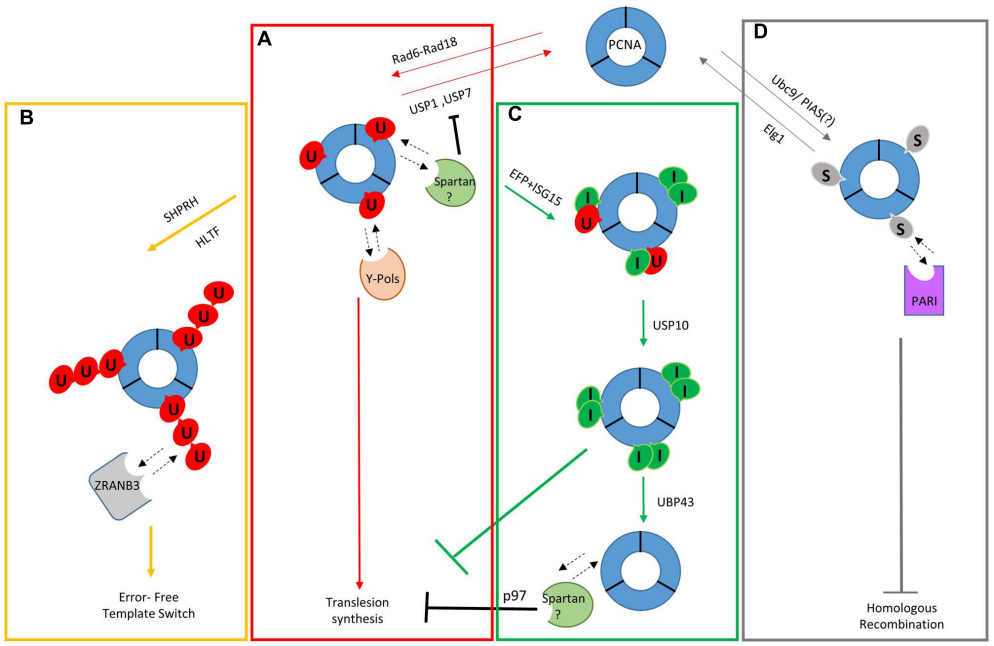

FIGURE 1 | Schematic model of ubiquitin and ubiquitin-like modifications in the DNA damage tolerance pathway. (A) Monoubiquitylation of PCNA leading to TLS. **(B)** Polyubiquitylation of PCNA leading to template switch. **(C)** ISGylation of PCNA and recovery from TLS. **(D)** SUMOylation of PCNA during unperturbed S phase and inhibition of Homologous Recombination. Dotted lines indicate interactions between regulators of the DDT and modified/unmodified PCNA.

In vertebrates, the main modification of PCNA is monoubiquitylation. It is observed after treatments that block the progression of the replication fork (Kannouche and Lehmann, 2004; Kannouche et al., 2004; Watanabe et al., 2004). In such conditions, it is possible to detect an accumulation of single-stranded DNA (ssDNA), likely caused by the uncoupling of the activities of the blocked replication fork and the DNA helicase. At this point, RPA readily binds the free ssDNA creating the substrate for the recruitment of Rad18 and Rad6 that ubiquitylate PCNA on lysine 164 (Davies et al., 2008). Rad18 and replication protein A (RPA) interact directly and the recruitment of Rad6/Rad18 to RPA-coated ssDNA has been observed *in vitro* (Huttner and Ulrich, 2008). Monoubiquitylated PCNA has increased affinity for TLS polymerases, whose interactions are mediated by their PIP-boxes (PCNA-interacting peptide) and ubiquitin-binding motifs (Kannouche et al., 2004; Bienko et al., 2005; Dikic et al., 2009). Upon fork stalling, replicative polymerases slow down and dissociate from the replisome followed by the recruitment of TLS polymerases (polymerase switching; **Figure 1A**). In the last few years, there has been a progressive discovery of new factors that help Rad18 in promoting the efficient ubiquitylation of PCNA. One of these factors is a TLS polymerase itself. It is interesting to point out that originally the recruitment of TLS polymerases was proposed to be an event that followed the monoubiquitylation of PCNA. New experimental data seem to suggest that TLS polymerases can influence themselves the state of PCNA, and an increase in PCNA ubiquitylation has been observed, in some cell types, after polη overexpression (Durando et al., 2013; Masuda et al., 2015). In these conditions, polη is believed to enhance and stabilize Rad18 in the proximity of PCNA. Rad18 and polη have been purified as a stable complex and their interaction has been proposed to be dependent on the phosphorylation of Rad18. Rad18 is phosphorylated, at a basal level even in unperturbed conditions but this modification is enhanced after DNA damage by DDK (Dbf4/Drf1-dependent Cdc7 kinase) and JNK (c-Jun N-terminal kinase; Day et al., 2010; Barkley et al., 2012). This hyper-phosphorylation is believed to increase the affinity of Rad18 for polη and promote their mutual recruitment to the chromatin, leading to the ubiquitylation of PCNA. However, this model of action is still controversial since it would make the accumulation of Ubi-PCNA an event dependent on ATR and Chk1, in contrast with previous established experimental evidence that demonstrated that ubiquitylation of PCNA is independent from both ATM and ATR kinases and their respective DNA damage checkpoints (Chang et al., 2006; Davies et al., 2008; Gohler et al., 2008; Niimi et al., 2008; Yang et al., 2008). A cohort of new factors that have been found to interact with Rad18 to promote efficient PCNA ubiquitylation include NBS1 (Yanagihara et al., 2011), Claspin and Chk1 (Yang et al., 2008), RPA (Davies et al., 2008), Spartan (see later in this review, Centore et al., 2012; Davis et al., 2012; Juhasz et al., 2012; Mosbech et al., 2012) and SIVA1 (Han et al., 2014).

In human cells, Rad18 is the principal E3 ligase that monoubiquitylates PCNA, but avian DT40 cells lacking Rad18 ($Rad18^{-/-}$) still show detectable levels of Ubi-PCNA, indicating the existence of another E3 ligase (Arakawa et al., 2006; Simpson et al., 2006). In fact, other minor pathways leading to the

ubiquitylation of PCNA have been proposed also in *S. cerevisiae* and in human cells under specific conditions. In human cells, RNF8 and $CRL4^{Cdt2}$ were identified as ubiquitin E3 ligases of PCNA, although their contribution is rather minor when compared to Rad18 (Zhang et al., 2008; Terai et al., 2010).

Rad18 is itself ubiquitylated and its modification is believed to control its availability and cellular localization. Rad18 has been reported to form a homodimer where the ubiquitin moiety on each Rad18 interacts with the UBZ (ubiquitin-binding zinc finger domain) of the other subunit (Miyase et al., 2005; Notenboom et al., 2007). Once Rad18 is de-ubiquitylated, it becomes active. The Rad18 dimer, which is considered inactive, is believed to localize mainly in the cytoplasm, while the active Rad18 monomer is distributed in the nucleoplasm. Recently, Rev1 has been shown to bind ubiquitylated Rad18 causing the release of non-modified Rad18 from the dimer, that is then free to ubiquitylate PCNA on the chromatin (Wang et al., 2016). This is another example of the extensive crosstalk between TLS polymerases, Rad18 and PCNA, further strengthening the idea that the regulation of DNA damage tolerance is far from a simple linear pathway.

Once ubiquitylated, PCNA can be further modified via K63-linked polyubiquitylation. In yeast, the complex formed by Ubc13-Mms2 (E2) and Rad5 (E3) is responsible for this modification (Hoege et al., 2002; Parker and Ulrich, 2009). In human cells, polyUbi-PCNA is hardly observed in comparison to yeast (Chiu et al., 2006) although all the proteins involved are believed to be conserved. Two Rad5 orthologs have been identified: helicase-like transcription factor (HLTF) and SNF2 histone linker PHD RING helicase (SHPRH; **Figure 1B**). HLTF is characterized by ATPase and HIRAN domains that promote fork regression *in vitro*, a crucial step in the stabilization of the replication fork in the presence of DNA damage (MacKay et al., 2009; Blastyak et al., 2010; Achar et al., 2015). Both HLTF and SHRPH can catalyze the addition of ubiquitin chains to Ubi-PCNA *in vitro* and their silencing, mediated by siRNA, results in a decrease in polyUbi-PCNA in living cells (Motegi et al., 2006, 2008; Unk et al., 2008, 2010). Recent evidence suggests that the loss of HLTF and SHPRH increases mutagenesis induced by UV and MMS treatment, respectively (Lin et al., 2011). HLTF has been shown to have also a role in the mono-ubiquitylation of PCNA and in the recruitment of polη (Lin et al., 2011). Surprisingly, mouse embyonic fibroblast (MEF) cells lacking both SHPRH and HLTF are still competent for PCNA polyubiquitylation and the double mutant is not hypersensitive to DNA-damaging agents (Krijger et al., 2011a). This seems to suggest the existence of yet another E3 ligase involved in PCNA ubiquitylation, at least in mouse. In light of all of this evidence, it is clear that further investigation will be required in order to understand the role of the Rad5 orthologs in higher eukaryotes.

GOING BACK: THE DE-UBIQUITYLATING ENZYMES

Ubi-PCNA plays a central role in the bypass of damaged DNA by facilitating the access of TLS polymerases to the replication fork. However, unscheduled recruitment of low-fidelity TLS polymerases would result in replication errors and mutagenesis on undamaged DNA, thus the level of Ubi-PCNA must be strictly controlled. Ubi-PCNA in human cells is negatively regulated by the ubiquitin-specific protease 1 (USP1; Huang et al., 2006) (**Figure 1A**). USP1 interacts with the activating protein partner UAF1 (USP1-associated factor 1) and de-ubiquitylate Ubi-PCNA in the absence of DNA damage (Cohn et al., 2007). USP1 is subjected to an auto-cleavage reaction, which regulates its cellular concentration (Cohn et al., 2007). Furthermore, high doses of UV-C light result in the down-regulation of the USP1 transcript, thus ensuring its down-regulation when the ubiquitylation of PCNA needs to be promoted (Huang et al., 2006). Indeed, Ubi-PCNA levels correlate nicely with the reduced expression levels of USP1 after UV treatment (Niimi et al., 2008). Differently from UV, USP1 is still present after hydroxyurea or MMS treatment, two genotoxic agents that induce a strong ubiquitylation of PCNA (Niimi et al., 2008). This observation suggests the possible presence of other negative regulators.

USP1 has been shown to protect the cells from genomic instability, as monitored by the formation of micronuclei, caused by the erroneous recruitment of polκ and the following decrease in fork progression (Jones et al., 2012). USP1 was the first and most prominent DUB involved in the negative regulation of PCNA ubiquitylation; however, recent data seem to suggest the involvement of more DUBs in the control of PCNA. Some of these DUBs either act directly on PCNA or can regulate other proteins that control its ubiquitylation. Among these, USP7, also called HAUSP, is the DUB that controls the stability of p53 by counteracting the activity of Mdm2, the E3 ligase responsible for its degradation (Li et al., 2002; Cummins and Vogelstein, 2004; Sheng et al., 2006). Recently USP7 has been shown to regulate indirectly the ubiquitylation of PCNA via the stabilization of either Rad18 or polη (Qian et al., 2015; Zlatanou et al., 2016). Other work has shown that USP7 can de-ubiquitylate Ubi-PCNA *in vitro* and it suppresses UV- and oxidative-stress-induced PCNA monoubiquitylation *in vivo* (Kashiwaba et al., 2015). PCNA ubiquitylation after DNA damage is normally very stable and can be detected days after the original genotoxic treatment (Niimi et al., 2008). Another DUB involved in the de-ubiquitylation of PCNA is USP10. USP10 can interact directly with PCNA via its PIP box and its silencing results in increased Ubi-PCNA 24 h after UV irradiation (Park et al., 2014). The activity of USP10 is remarkably deferred compared with USP1 as no difference could be appreciated in the levels Ubi-PCNA at 0 and 12 h after UV irradiation (Park et al., 2014), whereas silencing of USP1 results in the accumulation of Ubi-PCNA even in the absence of DNA damage (Huang et al., 2006). This seems to suggest that USP10 may control the de-ubiquitylation of Ubi-PCNA during the recovery from UV irradiation (see ISGylation, later on). An USP1 ortholog has not been identified in yeast. Recently, ubiquitin protease 10 (UBP10) was reported to de-ubiquitylate Ubi-PCNA in *S. cerevisiae* (Gallego-Sanchez et al., 2012). Cells lacking UBP10 accumulate Ubi-PCNA in response to DNA damage resulting in an increased interaction between PCNA and Rev1. UBP10 appears to de-ubiquitylate Ubi-PCNA during S phase and its protein levels remain constant

after UV treatment suggesting that UBP10 in yeast and USP1 in human regulate the de-ubiquitylation of PCNA by different mechanisms (Gallego-Sanchez et al., 2012).

NEW READERS OF UBIQUITYLATED PCNA

Once PCNA is ubiquitylated, it provides a loading platform for a variety of proteins involved in the replication of damaged DNA. As already mentioned, Ubi-PCNA can recruit a plethora of TLS polymerases allowing damage bypass and the restart of a stalled replication fork (Sale et al., 2012). Recently at least two new proteins have been described to be able to read the state of ubiquitylated PCNA and to help in maintaining the stability of the fork: Spartan, also called DVC1, and ZRANB3 (Centore et al., 2012; Davis et al., 2012; Mosbech et al., 2012) (**Figures 1A–C**).

Spartan is a substrate of the anaphase promoting complex and localizes to replication factories in a manner dependent on both its PIP and UBZ domains (Davis et al., 2012; Mosbech et al., 2012). In its absence, cells become hypersensitive to DNA damage agents and they are deficient in the DNA damage tolerance (DDT) response. Spartan can bind to p97 via its SHP domain (Davis et al., 2012; Mosbech et al., 2012). p97 encodes for a chaperone protein that can remodel ubiquitylated proteins in an ATP-dependent manner (Meyer et al., 2012).

As mentioned, Spartan PIP box and UBZ domain are needed for its accrual in replication factories and DNA damage foci. While all the data in the literature consistently report that PCNA is required for Spartan recruitment, the role of Ubi-PCNA as the target of Spartan's UBZ is still controversial. Spartan can bind Ubi-PCNA *in vitro* (Centore et al., 2012) but there are discording evidences that this may occur *in vivo*. Two groups reported that Spartan could relocalize to replication factories when Rad18 is depleted by siRNA, a condition that results in the absence of Ubi-PCNA (Davis et al., 2012; Mosbech et al., 2012). Spartan itself is ubiquitylated and this modification prevents further binding to ubiquitin targets and decreases its accumulation in focal structures (Centore et al., 2012).

Given all the conflicting evidence, the role of Spartan is still under scrutiny, with at least two proposed models of actions. In the first Spartan is thought to bind to Ubi-PCNA and to promote both Rad18 and polη recruitment to the chromatin. Its binding would shield Ubi-PCNA from being de-ubiquitylated by USP1 or by another DUB, and in its absence PCNA ubiquitylation appears to be reduced (Centore et al., 2012) (**Figure 1A**). At the opposite side of the spectrum, an alternative mechanism proposes Spartan acting as a negative regulator of TLS. In this scenario, Spartan is thought to recruit p97, which in turn will remove polη from the replication fork in order to resume processive replication (**Figure 1C**). This model is substantiated by increased focal retention of polη and increased mutagenesis when Spartan is silenced (Davis et al., 2012; Mosbech et al., 2012). Recently, three patients showing early onset hepatocellular carcinomas and progeroid syndrome have been found to carry a mutation in SPRTN (Lessel et al., 2014). When Spartan was mutated or depleted, the cells showed signs of genomic instability, defects in replication fork progression and cell proliferation. Interestingly, depletion of polη in a background mutated in SPRTN did not rescue the replication phenotypes, indicating that polη is potentially not the main target of Spartan activity (Lessel et al., 2014). The discovery of this new progeroid syndrome further stresses the importance of SPRTN, but additional investigation is needed to clarify the mechanism of action of this protein essential for the DDT.

Proliferating cell nuclear antigen polyubiquitylation is proposed to channel the DDT to an error-free damage avoidance branch named template switch (Hoege et al., 2002; Branzei and Foiani, 2007; Branzei, 2011). The molecular mechanism of this pathway is still not completely understood and, until recently, we did not know the role of K63-linked chains attached to PCNA. In the last couple of years the protein ZRANB3/AH2, has been proposed to be able to recognize specifically polyubiquitylated PCNA and to promote template switch by stimulating fork regression (Ciccia et al., 2012; Weston et al., 2012; Yuan et al., 2012). ZRANB3 encodes for an annealing helicase/translocase and it can interact with polyUbi-PCNA via multiple domains. A canonical PIP motif and an APIM (C-terminal AlkB2 PCNA-interaction motif) domain mediate the direct interaction with the PCNA trimer while an NPL4 zinc finger (NZF), a variant of ubiquitin-binding domain, recognizes K63-linked ubiquitin chains specifically (Ciccia et al., 2012). This domain is able to bind to polyUbi-PCNA *in vitro* and it is needed for the localization of ZRANB3 to damage sites. All these structural motifs are required for restarting the fork after DNA damage (**Figure 1B**).

Experimental observations suggest that ZRANB3 may play three different roles at the stalled replication fork: (1) it can stimulate fork regression in order to stabilize the fork and minimize the amount of ssDNA that is generated (Ciccia et al., 2012). (2) ZRANB3 can disrupt D-loop formation *in vitro* and this in turn could result in the prevention of inappropriate homologous recombination (HR) (Ciccia et al., 2012); (3) it can act as a strand-specific endonuclease pointing to a role not only in damage bypass but also in damage repair (Weston et al., 2012).

ZRANB3 may act in parallel or in conjunction with HLFT that also has a helicase activity and can stimulate fork regression *in vitro* (Blastyak et al., 2010; Achar et al., 2015). Further work will be needed in the future to completely elucidate ZRANB3 role in damage tolerance and repair.

PCNA SUMOylation AND ISGylation

Another prominent post-translational modification of PCNA is its SUMOylation. It was originally identified in yeast and only recently it was observed in human cells.

In yeast, PCNA is SUMOylated (S-PCNA) on Lys164 (major) and Lys127 (minor) by the combined action of Ubc9 (E2) and Siz2 (E3) or by Ubc9 alone, respectively (Hoege et al., 2002) (**Figure 1D**). SUMOylation occurs during normal S phase and/or in response to high doses of DNA damage (Juhasz et al., 2012). SUMOylated PCNA interacts with Srs2 helicase, which has been shown to prevent HR by disrupting Rad51 filaments (Papouli

et al., 2005; Pfander et al., 2005). Srs2 has a non-canonical PIP-box with limited affinity for PCNA and it binds stably only when the clamp is SUMOylated. A SUMO interacting motif that is located in tandem after the PIP in the protein carboxyl terminus of Srs2 mediates this interaction (Kim S.O. et al., 2012).

Given the catalytic activity of Srs2 and the timing of this modification, it is believed that SUMOylation of PCNA acts as a negative regulator of unscheduled HR during S phase, where this kind of pathway could be detrimental to the cell. In yeast, one of the replication factor C (RFC)-like complexes, Elg1-RFC also has a role in regulating S-PCNA. RFC is a complex consisting of Rfc1-5 and it works as clamp loader/unloader. All eukaryotic cells contain a series of three alternative RFCs, containing Elg1, Ctf18, or Rad24 in place of Rfc1(Kim and MacNeill, 2003). Elg1-RFC is required for the efficient unloading of SUMOylated PCNA from the chromatin during S phase. In cells lacking Elg1, PCNA accumulates on the chromatin and it is possible to detect an increase in SUMOylated PCNA (Parnas et al., 2010; Kubota et al., 2013b).

In *X. laevis* S-PCNA is present during unperturbed replication in cell extracts, but it is not required for the replication of either ssDNA or sperm chromatin (Leach and Michael, 2005).

In human cells, S-PCNA had eluded detection for a number of years and it has been detected only recently after overexpression of SUMO1, although to a much less extent than the levels detected in yeast (Moldovan et al., 2012). PCNA was found to be SUMOylated on both Lys164 and Lys254 under specific conditions (Gali et al., 2012). As in yeast, mammalian UBC9 acts as the E2 enzyme but surprisingly, at least *in vitro*, the SUMOylation of PCNA does not require the Siz1 orthologs (PIAS1-4) in either lysine residues (Gali et al., 2012).

A PCNA-SUMO fusion protein not only prevents HR, but also DNA double-strand break formation, as monitored by a marked reduction of γH2AX foci (Gali et al., 2012). Two putative functional homologs of Srs2 have been identified in human cells: PCNA-associated recombination inhibitor (PARI; Moldovan et al., 2012) and F-box DNA helicase (FBH1; Fugger et al., 2009; Bacquin et al., 2013). Both PARI and FBH1 have been reported to interact with PCNA and to have PCNA-dependent anti-recombinogenic activity, but only PARI seems to specifically interact with SUMOylated PCNA, at least *in vitro* (Moldovan et al., 2012). On the other hand, FBH1 needs to be degraded, via $CRL4^{Cdt2}$ pathway in order to allow efficient recruitment of polη to replication factories (Bacquin et al., 2013).

In human cells, ATAD5, the ortholog of yeast Elg1 appears to have a somehow different role from its yeast counterpart as it interacts, at stalled replication forks, with the USP1/UAF1 complex and facilitates USP1-mediated PCNA de-ubiquitylation (Lee et al., 2010; Kubota et al., 2013a).

Last year ISGyaltion, another ubiquitin-like modification, was discovered to affect PCNA.

ISG15 (interferon-stimulated gene 15) was the first identified ubiquitin-like protein and it is strongly stimulated by type I interferon (Haas et al., 1987; Loeb and Haas, 1992). As ubiquitin and SUMO this post-translational modification relies on a chain of three classes of enzymes to be linked to its substrates: UBE1L is the activating E1 enzyme, followed by UBCH8 (E2) and finally by EFP and HERC5 (E3s; Yuan and Krug, 2001; Kim et al., 2004; Zhao et al., 2004; Dastur et al., 2006; Zou and Zhang, 2006). PCNA was reported to be bi-ISGylated 24 h after UV irradiation by EFP on both K164 and K168 (Park et al., 2014). Mutations of either residues resulted in the complete disappearance of ISGylated PCNA indicating that ISGylation at one site influences the state of the other. The late response to UV irradiation suggested that ISG15 had a role in the recovery from DNA damage and post-replication repair (PRR). The E3 ligase EFP interacts with Ubi-PCNA and this interaction is propaedeutic to PCNA ISGylation (Park et al., 2014). This modification in turn recruits USP10 that de-ubiquitylates PCNA in order to block TLS and resume normal replication. Eventually, UBP43 removes ISG15 from PCNA (**Figure 1C**). ISGylation-deficient mutants of PCNA show increased recruitment of polη to the chromatin many hours after UV irradiation (Park et al., 2014).

UBIQUITYLATION OF TLS POLYMERASES

As mentioned before, PCNA is not the only player that is modified in order to control PRR. All the members of the Y-family of DNA polymerase (η, ι, κ, and Rev1) involved in DNA TLS have been identified to be modified by ubiquitin or ubiquitin-like modifiers (Sale et al., 2012). Furthermore, all four of them contain ubiquitin-binding domains (UBM or UBZ; (Bienko et al., 2005; Guo et al., 2006, 2008; Plosky et al., 2006).

Probably, the best characterized of the group is polη, the major TLS polymerase involved in the error-free bypass of cyclobutane pyrimidine dimers (CPDs), the main adduct created by UV irradiation. CPDs are repaired slowly by the nucleotide excision repair (NER) and have a higher probability to persist in the genome until DNA replication. The importance of the bypass performed by polη is exemplified by the fact that individuals carrying an inactivating mutation are affected by *Xeroderma pigmentosum* Variant (XPV; Masutani et al., 1999). Regardless of the importance of its function, polη shares a common characteristic with other Y-family polymerases, a wide catalytic site. This structural feature, while beneficial for damage bypass, makes the polymerase intrinsically error-prone compared to replicating polymerases when using undamaged DNA as a template. For this reason, its recruitment to the replication fork needs to be tightly regulated. Polη is recruited to replication factories in a manner dependent on its PIP-box and UBZ, a specialized ubiquitin-binding zinc finger (Kannouche et al., 2001, 2002; Bienko et al., 2005, 2010; Sabbioneda et al., 2009). The presence of both domains stabilizes the interaction between the polymerase and Ubi-PCNA after DNA damage (Kannouche et al., 2004; Bienko et al., 2010). Mutants in either the PIP-box or the UBZ are required for focal accumulation of the polymerase but they retain a partial bypass activity, indicating that they work in parallel to ensure efficient binding with PCNA (Bienko et al., 2010). Ubiquitylation of PCNA provides a positive regulation by increasing the affinity between polη and the clamp when the replication fork is blocked (Kannouche et al., 2004).

Conversely, ubiquitylation of the polymerase works as a negative regulator by preventing its recruitment on the chromatin (Bienko et al., 2010). *In vivo*, a small amount of polη is monoubiquitylated, in the absence of damage, in its nuclear localization signal directly adjacent the PIP-box. The modification occurs primarily on K682 but in its absence, also K686, K694 and K709 have been found to be ubiquitylated (Bienko et al., 2010; Jung et al., 2011). Ubiquitylation is strictly dependent on the UBZ of polη. Recently, PirH2 was discovered to be the E3 ligase responsible for this monoubiquitylation (Jung et al., 2011). Ubiquitylation of polη is believed to cause a conformational change in its C-terminus with the attached ubiquitin binding intra-molecularly to polη's UBZ. In this closed confirmation, neither the UBZ, blocked by the binding to the ubiquitin attached to polη, nor the PIP-box, that is located between the UBZ and K682, are available to stabilize its interaction with PCNA (Bienko et al., 2010). Ubi-polη is indeed excluded from the chromatin and replication foci. After DNA damage, ubiquitylated polη gradually disappears. The polymerase can be then recruited to the chromatin and it becomes proficient for TLS. The de-ubiquitylation of the polymerase is believed to be carried out by the DUB USP7 (Qian et al., 2015). It is important to note that only 10% of polη is ubiquitylated in the absence of damage at any given time, indicating that some other forms of regulation are keeping polη under negative control. In some cellular background, polη gradually disappears in the hours following UV irradiation. This process is believed to be mediated by Mdm2 that polyubiquitylate the polymerase and marks it for proteasomal degradation (Jung et al., 2012). A similar system, mediated by $CRL4^{Cdt2}$ has also been observed in *Caenorhabditis elegans*. Interestingly in this system, the degradation of polη is prevented by its SUMOylation by the SUMO E3 ligase GEI-17 (Kim and Michael, 2008). It is still unclear whether polη is SUMOylated in human cells.

Similarly, to polη also its paralog polι is ubiquitylated (Bienko et al., 2005; McIntyre et al., 2015). This polymerase is thought to bypass lesions when polη is not present (Wang et al., 2007; Vidal and Woodgate, 2009). *In vitro*, polι can bypass different typologies of DNA adducts with different degrees of fidelity (Washington et al., 2004a,b; Frank and Woodgate, 2007).

Polι is characterized by two UBMs that are needed for its modification and correct localization in replication foci (Bienko et al., 2005; Bomar et al., 2010). It is speculated that the ubiquitylation of polι might be important for its interaction with polη (McIntyre et al., 2013).

The deoxycytidyl transferase Rev1 possesses two UBMs (Bomar et al., 2010) and gets ubiquitylated *in vivo* (Guo et al., 2006; Kim H. et al., 2012). The UBMs are needed for the efficient interaction with Ubi-PCNA (Guo et al., 2006; Wood et al., 2007). In yeast, deletion of UBM2 severely affects UV-induced mutagenesis, a pathway that is strictly dependent on TLS (Wood et al., 2007; Terai et al., 2010). Mutations in Rev1's UBMs make the cells hypersensitive to UV in the DT40 system (Guo et al., 2006). In chicken cells, Rev1 and its UBMs have been shown to have a role in replication fork progression in the presence of UV in a process that is independent from Ubi-PCNA (Edmunds et al., 2008). Finally, Rev1 appears to be able to bind to the Fanconi core complex via FAAP20 and this interaction is believed to promote Rev1 recruitment to replication foci and ultimately Rev1-dependent mutagenesis (Mirchandani et al., 2008; Kim H. et al., 2012).

The last TLS polymerase that has been reported to be ubiquitylated is polκ (Guo et al., 2008). Polκ is characterized by two UBZ domains in its c-terminus (Bienko et al., 2005) that have been reported to be important for the interaction with PCNA and the localization in foci after UV irradiation (Guo et al., 2008). Polκ has also been shown to be important for NER, and its repair function depends on its UBZs (Ogi et al., 2010).

The role of polκ ubiquitylation is currently not clear but it is likely to promote protein–protein interaction similarly to the other members of the Y-family of DNA polymerases.

DNA DAMAGE TOLERANCE AND CANCER

Post-replication repair and the damage tolerance systems provide an essential safety mechanism that allows the completion of DNA replication and it is an important pathway to preserve genome stability. At the same time, it can act as a double-edged sword since a number of its components, such as TLS polymerases, are intrinsically error prone and can be a source of mutations if they are not correctly regulated. Mutations are one of the major driving forces that lead to cell transformation and tumorigenesis, therefore it is important to define the contribution of PRR in the context of cancer. The dichotomy of protection versus increased risk is emblematic in the case of polη. As already mentioned in this review, a deficiency in polη is the cause of XPV (Broughton et al., 2002). Like other XP groups that are mutated in NER, XPV patients are sensitive to sun light and are extremely prone to both melanoma and non-melanoma skin cancers (Fassihi et al., 2016). Polη is the main polymerase that is able to bypass CPDs in an error-free manner and it possible to envisage that when missing, its role is carried out by other TLS polymerases with different degrees of fidelity.

In these cases, the ultimate and less than desirable outcome would be the introduction of mutations that are responsible for the transformation of the skin cells. It is important to note that polη-deficient patients are the most prone to skin cancers among the *X. pigmentosum* groups (Fassihi et al., 2016). XPV patients tend to have milder skin phenotypes and are normally diagnosed much later in their life, when they have already accumulated a number of UV-induced mutations. This higher mutation load correlates with the possibility of developing more skin tumors in their adult life (Fassihi et al., 2016). In this context, it is clear that polη protects the cells from cancer. On the other hand, the survival capability conferred by this polymerase can be hijacked to make tumors more resilient. *In vitro*, cells lacking polη are more sensitive to cisplatin, one of the most used first line drug in chemotherapy (Albertella et al., 2005a). Increased expression of polη associates with worse prognosis and survival in a cohort of patients suffering from non-small cell lung cancer patients previously treated with platinum (Ceppi et al., 2009). Polη seems also to be involved in the cellular response after treatment with

nucleoside analogs, which are commonly used in the clinic as cancer drugs (Chen et al., 2006). Interestingly, mutations in polη are hardly found in patients with sporadic skin carcinomas (Glick et al., 2006; Flanagan et al., 2007; Lange et al., 2011) but its overexpression has been reported (Albertella et al., 2005b). Polη ortholog, polι, has been found to be elevated in breast cancer cells and in these cell lines a reduced mutation frequency was recorded when the polymerase was depleted *in vitro* (Yang et al., 2004). Furthermore, mutation in polι have been linked to an increased predisposition of developing lung cancer in both human (Sakiyama et al., 2005) and mouse (Wang et al., 2004; Lee and Matsushita, 2005).

Two of TLS polymerases extensively characterized for their role in mutagenesis and cancer are polζ and Rev1. Polζ is thought to be the major player involved in error-prone replication of damaged templates *in vivo*. In mice, conditional Rev3 knockout results in increased genome instability and tumorigenesis in a p53-null background (Wittschieben et al., 2006, 2010; Lange et al., 2013). Similarly to polη, there is experimental evidence indicating that the presence of both Rev1 and polζ can confer drug resistance both *in vitro* and *in vivo* (Xie et al., 2010). Conversely, Rev3 inhibition makes lymphoma and lung cancer cells more sensitive to platinum-derived drugs (Doles et al., 2010), once again underlying the dichotomy of TLS regarding cancer and genome protection. All of these evidences point to the idea that transient inhibition of TLS could be synthetically lethal to tumor cells that rely on the TLS mutator activity for survival. TLS polymerases are not the only proteins involved in damage tolerance that have been linked to cancer development. The expression of the E3 ligase HLTF has been found to be altered in transformed cells and in numerous tumors. A reduced expression of HLTF, due to hyper-methylation of its promoter, has been found in colon and colorectal cancer, esophageal squamous cell and gastric carcinomas (Debauve et al., 2008). Interestingly HLTF is overexpressed in transformed cells, indicating that a differential modulation of its expression could be needed at different stages of tumorigenesis (Debauve et al., 2008). Given the role of HLTF in the control of the error-free branch of damage tolerance, it is tempting to speculate that it could be beneficial for tumor cells to inactivate HLTF in order to channel the PRR pathway toward the more mutagenic TLS bypass, thus allowing the malignant cells to accumulate more mutations. As mentioned before a SPRTN deficiency has been linked with a new progeroid syndrome with propensity to develop early onset hepatocellular carcinomas, but it is still not clear whether this phenotype is directly linked with its proposed control of polη (Lessel et al., 2014). In conclusion, a tight regulation of TLS and the DNA damage tolerance pathway in general is required to preserve the delicate balance between protecting the genome stability and inducing cellular transformation.

THE UNANSWERED QUESTIONS

In the last decade, mounting evidence has pointed out the crucial role of ubiquitin, and other ubiquitin-like modifications, in the control of PCNA and TLS. Nevertheless, we still do not know whether PCNA ubiquitylation is strictly required for TLS. A series of experimental hints suggest that there is more to the story and we still have only a partial picture of the regulation of the damage tolerance pathway. For instance, MEF cells carrying the PCNA K164R mutation can be further sensitized by the deletion of other TLS genes, indicating that some steps of the pathway could be independent from Ubi-PCNA (Hendel et al., 2011). Furthermore, PCNA ubiquitylation is not required for polη-mediated somatic hyper-mutation in mouse B cells (Krijger et al., 2011b).

In human cells the phosphorylation of polη, that occurs on the chromatin, is dependent on its UBZ, indicating that the binding to ubiquitin is needed for this regulatory modification (Gohler et al., 2011). However, this phosphorylation does not require Ubi-PCNA and can occur in its absence (Gohler et al., 2011). Dynamic studies on polη show that Ubi-PCNA helps in stabilizing the polymerase in replication foci but do not exclude the possibility that other ubiquitylated proteins may play a role in its initial recruitment (Sabbioneda et al., 2008). Consistent with this hypothesis polη is still recruited to replication factories after chemical depletion of Ubi-PCNA caused by prolonged treatment with the proteasome inhibitors MG132 or epoxomicin (Sabbioneda et al., 2008). It must be noted that mouse cells carrying a homozygous K164R mutation appear to be deficient for polη recruitment (Krijger et al., 2011b), and so far no explanation has been found for these conflicting evidences.

CONCLUSION

We are now starting to grasp the complexities of the regulation of PRR and TLS, the continuous dance between protein partners and the intricacies that lie behind such an important tolerance pathway. Meanwhile, behind the scenes, the hunt for the next big ubiquitylated/SUMOylated target still rages on.

AUTHOR CONTRIBUTIONS

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

FUNDING

The work in the authors' laboratory is supported by the Associazione Italiana per la Ricerca sul Cancro Start-up Grant 12710 and by the European Commission Grant PCIG10-GA-2011-303806.

ACKNOWLEDGMENT

The authors apologize to all their colleagues for not being able to cite all the relevant literature due to space limitations.

REFERENCES

Achar, Y. J., Balogh, D., Neculai, D., Juhasz, S., Morocz, M., Gali, H., et al. (2015). Human HLTF mediates postreplication repair by its HIRAN domain-dependent replication fork remodelling. *Nucleic Acids Res.* 43, 10277–10291. doi: 10.1093/nar/gkv896

Albertella, M. R., Green, C. M., Lehmann, A. R., and O'Connor, M. J. (2005a). A role for polymerase eta in the cellular tolerance to cisplatin-induced damage. *Cancer Res.* 65, 9799–9806. doi: 10.1158/0008-5472.CAN-05-1095

Albertella, M. R., Lau, A., and O'Connor, M. J. (2005b). The overexpression of specialized DNA polymerases in cancer. *DNA Repair (Amst.)* 4, 583–593. doi: 10.1016/j.dnarep.2005.01.005

Arakawa, H., Moldovan, G. L., Saribasak, H., Saribasak, N. N., Jentsch, S., and Buerstedde, J. M. (2006). A role for PCNA ubiquitination in immunoglobulin hypermutation. *PLoS Biol.* 4:e366. doi: 10.1371/journal.pbio.0040366

Bacquin, A., Pouvelle, C., Siaud, N., Perderiset, M., Salome-Desnoulez, S., Tellier-Lebegue, C., et al. (2013). The helicase FBH1 is tightly regulated by PCNA via CRL4(Cdt2)-mediated proteolysis in human cells. *Nucleic Acids Res.* 41, 6501–6513. doi: 10.1093/nar/gkt397

Bailly, V., Lamb, J., Sung, P., Prakash, S., and Prakash, L. (1994). Specific complex formation between yeast RAD6 and RAD18 proteins: a potential mechanism for targeting RAD6 ubiquitin-conjugating activity to DNA damage sites. *Genes Dev.* 8, 811–820. doi: 10.1101/gad.8.7.811

Bailly, V., Lauder, S., Prakash, S., and Prakash, L. (1997). Yeast DNA repair proteins Rad6 and Rad18 form a heterodimer that has ubiquitin conjugating, DNA binding, and ATP hydrolytic activities. *J. Biol. Chem.* 272, 23360–23365. doi: 10.1074/jbc.272.37.23360

Barkley, L. R., Palle, K., Durando, M., Day, T. A., Gurkar, A., Kakusho, N., et al. (2012). c-Jun N-terminal kinase-mediated Rad18 phosphorylation facilitates Poleta recruitment to stalled replication forks. *Mol. Biol. Cell* 23, 1943–1954. doi: 10.1091/mbc.E11-10-0829

Bekker-Jensen, S., and Mailand, N. (2011). The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. *FEBS Lett.* 585, 2914–2919. doi: 10.1016/j.febslet.2011.05.056

Bergink, S., and Jentsch, S. (2009). Principles of ubiquitin and SUMO modifications in DNA repair. *Nature* 458, 461–467. doi: 10.1038/nature07963

Bienko, M., Green, C. M., Crosetto, N., Rudolf, F., Zapart, G., Coull, B., et al. (2005). Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* 310, 1821–1824. doi: 10.1126/science.1120615

Bienko, M., Green, C. M., Sabbioneda, S., Crosetto, N., Matic, I., Hibbert, R. G., et al. (2010). Regulation of translesion synthesis DNA polymerase eta by monoubiquitination. *Mol. Cell* 37, 396–407. doi: 10.1016/j.molcel.2009.12.039

Blastyak, A., Hajdu, I., Unk, I., and Haracska, L. (2010). Role of double-stranded DNA translocase activity of human HLTF in replication of damaged DNA. *Mol. Cell Biol.* 30, 684–693. doi: 10.1128/MCB.00863-09

Bomar, M. G., D'Souza, S., Bienko, M., Dikic, I., Walker, G. C., and Zhou, P. (2010). Unconventional ubiquitin recognition by the ubiquitin-binding motif within the Y family DNA polymerases iota and Rev1. *Mol. Cell* 37, 408–417. doi: 10.1016/j.molcel.2009.12.038

Branzei, D. (2011). Ubiquitin family modifications and template switching. *FEBS Lett.* 585, 2810–2817. doi: 10.1016/j.febslet.2011.04.053

Branzei, D., and Foiani, M. (2007). Template switching: from replication fork repair to genome rearrangements. *Cell* 131, 1228–1230. doi: 10.1016/j.cell.2007.12.007

Broughton, B. C., Cordonnier, A., Kleijer, W. J., Jaspers, N. G., Fawcett, H., Raams, A., et al. (2002). Molecular analysis of mutations in DNA polymerase eta in xeroderma pigmentosum-variant patients. *Proc. Natl. Acad. Sci. U.S.A.* 99, 815–820. doi: 10.1073/pnas.022473899

Centore, R. C., Yazinski, S. A., Tse, A., and Zou, L. (2012). Spartan/C1orf124, a reader of PCNA ubiquitylation and a regulator of UV-induced DNA damage response. *Mol. Cell* 46, 625–635. doi: 10.1016/j.molcel.2012.05.020

Ceppi, P., Novello, S., Cambieri, A., Longo, M., Monica, V., Lo Iacono, M., et al. (2009). Polymerase eta mRNA expression predicts survival of non-small cell lung cancer patients treated with platinum-based chemotherapy. *Clin. Cancer Res.* 15, 1039–1045. doi: 10.1158/1078-0432.CCR-08-1227

Chang, D. J., Lupardus, P. J., and Cimprich, K. A. (2006). Monoubiquitination of proliferating cell nuclear antigen induced by stalled replication requires uncoupling of DNA polymerase and mini-chromosome maintenance helicase activities. *J. Biol. Chem.* 281, 32081–32088. doi: 10.1074/jbc.M606799200

Chen, Y. W., Cleaver, J. E., Hanaoka, F., Chang, C. F., and Chou, K. M. (2006). A novel role of DNA polymerase eta in modulating cellular sensitivity to chemotherapeutic agents. *Mol. Cancer Res.* 4, 257–265. doi: 10.1158/1541-7786.MCR-05-0118

Chiu, R. K., Brun, J., Ramaekers, C., Theys, J., Weng, L., Lambin, P., et al. (2006). Lysine 63-polyubiquitination guards against translesion synthesis-induced mutations. *PLoS Genet*. 2:e116. doi: 10.1371/journal.pgen.0020116

Ciccia, A., and Elledge, S. J. (2011). The DNA damage response: making it safe to play with knives. *Mol. Cell* 40, 179–204. doi: 10.1016/j.molcel.2010.09.019

Ciccia, A., Nimonkar, A. V., Hu, Y., Hajdu, I., Achar, Y. J., Izhar, L., et al. (2012). Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress. *Mol. Cell* 47, 396–409. doi: 10.1016/j.molcel.2012.05.024

Cohn, M. A., Kowal, P., Yang, K., Haas, W., Huang, T. T., Gygi, S. P., et al. (2007). A UAF1-containing multisubunit protein complex regulates the *Fanconi anemia* pathway. *Mol. Cell* 28, 786–797. doi: 10.1016/j.molcel.2007.09.031

Cummins, J. M., and Vogelstein, B. (2004). HAUSP is required for p53 destabilization. *Cell Cycle* 3, 689–692. doi: 10.4161/cc.3.6.924

Dastur, A., Beaudenon, S., Kelley, M., Krug, R. M., and Huibregtse, J. M. (2006). Herc5, an interferon-induced HECT E3 enzyme, is required for conjugation of ISG15 in human cells. *J. Biol. Chem.* 281, 4334–4338. doi: 10.1074/jbc.M512830200

Davies, A. A., Huttner, D., Daigaku, Y., Chen, S., and Ulrich, H. D. (2008). Activation of ubiquitin-dependent DNA damage bypass is mediated by replication protein a. *Mol. Cell* 29, 625–636. doi: 10.1016/j.molcel.2007.12.016

Davis, E. J., Lachaud, C., Appleton, P., Macartney, T. J., Nathke, I., and Rouse, J. (2012). DVC1 (C1orf124) recruits the p97 protein segregase to sites of DNA damage. *Nat. Struct. Mol. Biol.* 19, 1093–1100. doi: 10.1038/nsmb.2394

Day, T. A., Palle, K., Barkley, L. R., Kakusho, N., Zou, Y., Tateishi, S., et al. (2010). Phosphorylated Rad18 directs DNA polymerase eta to sites of stalled replication. *J. Cell Biol.* 191, 953–966. doi: 10.1083/jcb.201006043

Debauve, G., Capouillez, A., Belayew, A., and Saussez, S. (2008). The helicase-like transcription factor and its implication in cancer progression. *Cell Mol. Life Sci.* 65, 591–604. doi: 10.1007/s00018-007-7392-4

Dieckman, L. M., Freudenthal, B. D., and Washington, M. T. (2012). PCNA structure and function: insights from structures of PCNA complexes and post-translationally modified PCNA. *Subcell. Biochem.* 62, 281–299. doi: 10.1007/978-94-007-4572-8_15

Dikic, I., Wakatsuki, S., and Walters, K. J. (2009). Ubiquitin-binding domains - from structures to functions. *Nat. Rev. Mol. Cell Biol.* 10, 659–671. doi: 10.1038/nrm2767

Doles, J., Oliver, T. G., Cameron, E. R., Hsu, G., Jacks, T., Walker, G. C., et al. (2010). Suppression of Rev3, the catalytic subunit of Pol{zeta}, sensitizes drug-resistant lung tumors to chemotherapy. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20786–20791. doi: 10.1073/pnas.1011409107

Durando, M., Tateishi, S., and Vaziri, C. (2013). A non-catalytic role of DNA polymerase eta in recruiting Rad18 and promoting PCNA monoubiquitination at stalled replication forks. *Nucleic Acids Res.* 41, 3079–3093. doi: 10.1093/nar/gkt016

Edmunds, C. E., Simpson, L. J., and Sale, J. E. (2008). PCNA ubiquitination and REV1 define temporally distinct mechanisms for controlling translesion synthesis in the avian cell line DT40. *Mol. Cell* 30, 519–529. doi: 10.1016/j.molcel.2008.03.024

Fassihi, H., Sethi, M., Fawcett, H., Wing, J., Chandler, N., Mohammed, S., et al. (2016). Deep phenotyping of 89 xeroderma pigmentosum patients reveals unexpected heterogeneity dependent on the precise molecular defect. *Proc. Natl. Acad. Sci. U.S.A.* 113, E1236–E1245. doi: 10.1073/pnas.1519444113

Flanagan, A. M., Rafferty, G., O'Neill, A., Rynne, L., Kelly, J., Mccann, J., et al. (2007). The human POLH gene is not mutated, and is expressed in a cohort of patients with basal or squamous cell carcinoma of the skin. *Int. J. Mol. Med.* 19, 589–596. doi: 10.3892/ijmm.19.4.589

Frank, E. G., and Woodgate, R. (2007). Increased catalytic activity and altered fidelity of human DNA polymerase iota in the presence of manganese. *J. Biol. Chem.* 282, 24689–24696. doi: 10.1074/jbc.M702159200

Freudenthal, B. D., Gakhar, L., Ramaswamy, S., and Washington, M. T. (2010). Structure of monoubiquitinated PCNA and implications for translesion synthesis and DNA polymerase exchange. *Nat. Struct. Mol. Biol.* 17, 479–484. doi: 10.1038/nsmb.1776

Fugger, K., Mistrik, M., Danielsen, J. R., Dinant, C., Falck, J., Bartek, J., et al. (2009). Human Fbh1 helicase contributes to genome maintenance via pro- and anti-recombinase activities. *J. Cell Biol.* 186, 655–663. doi: 10.1083/jcb.200812138

Gali, H., Juhasz, S., Morocz, M., Hajdu, I., Fatyol, K., Szukacsov, V., et al. (2012). Role of SUMO modification of human PCNA at stalled replication fork. *Nucleic Acids Res.* 40, 6049–6059. doi: 10.1093/nar/gks256

Gallego-Sanchez, A., Andres, S., Conde, F., San-Segundo, P. A., and Bueno, A. (2012). Reversal of PCNA ubiquitylation by Ubp10 in Saccharomyces cerevisiae. *PLoS Genet.* 8:e1002826. doi: 10.1371/journal.pgen.1002826

Giannattasio, M., Zwicky, K., Follonier, C., Foiani, M., Lopes, M., and Branzei, D. (2014). Visualization of recombination-mediated damage bypass by template switching. *Nat. Struct. Mol. Biol.* 21, 884–892. doi: 10.1038/nsmb.2888

Glick, E., White, L. M., Elliott, N. A., Berg, D., Kiviat, N. B., and Loeb, L. A. (2006). Mutations in DNA polymerase eta are not detected in squamous cell carcinoma of the skin. *Int. J. Cancer* 119, 2225–2227. doi: 10.1002/ijc.22099

Gohler, T., Munoz, I. M., Rouse, J., and Blow, J. J. (2008). PTIP/Swift is required for efficient PCNA ubiquitination in response to DNA damage. *DNA Repair (Amst.)* 7, 775–787. doi: 10.1016/j.dnarep.2008.02.001

Gohler, T., Sabbioneda, S., Green, C. M., and Lehmann, A. R. (2011). ATR-mediated phosphorylation of DNA polymerase eta is needed for efficient recovery from UV damage. *J. Cell Biol.* 192, 219–227. doi: 10.1083/jcb.201008076

Guo, C., Tang, T. S., Bienko, M., Dikic, I., and Friedberg, E. C. (2008). Requirements for the interaction of mouse Polkappa with ubiquitin and its biological significance. *J. Biol. Chem.* 283, 4658–4664. doi: 10.1074/jbc.M709275200

Guo, C., Tang, T. S., Bienko, M., Parker, J. L., Bielen, A. B., Sonoda, E., et al. (2006). Ubiquitin-binding motifs in REV1 protein are required for its role in the tolerance of DNA damage. *Mol. Cell Biol.* 26, 8892–8900. doi: 10.1128/MCB.01118-06

Haas, A. L., Ahrens, P., Bright, P. M., and Ankel, H. (1987). Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. *J. Biol. Chem.* 262, 11315–11323.

Han, J., Liu, T., Huen, M. S., Hu, L., Chen, Z., and Huang, J. (2014). SIVA1 directs the E3 ubiquitin ligase RAD18 for PCNA monoubiquitination. *J. Cell Biol.* 205, 811–827. doi: 10.1083/jcb.201311007

Hay, R. T. (2005). SUMO: a history of modification. *Mol. Cell* 18, 1–12. doi: 10.1016/j.molcel.2005.03.012

Hendel, A., Krijger, P. H., Diamant, N., Goren, Z., Langerak, P., Kim, J., et al. (2011). PCNA ubiquitination is important, but not essential for translesion DNA synthesis in mammalian cells. *PLoS Genet.* 7:e1002262. doi: 10.1371/journal.pgen.1002262

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

Hoege, C., Pfander, B., Moldovan, G. L., Pyrowolakis, G., and Jentsch, S. (2002). RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419, 135–141. doi: 10.1038/nature00991

Hoeijmakers, J. H. (2001). Genome maintenance mechanisms for preventing cancer. *Nature* 411, 366–374. doi: 10.1038/35077232

Huang, T. T., and D'Andrea, A. D. (2006). Regulation of DNA repair by ubiquitylation. *Nat. Rev. Mol. Cell Biol.* 7, 323–334. doi: 10.1038/nrm1908

Huang, T. T., Nijman, S. M., Mirchandani, K. D., Galardy, P. J., Cohn, M. A., Haas, W., et al. (2006). Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat. Cell Biol.* 8, 339–347. doi: 10.1038/ncb1378

Huttner, D., and Ulrich, H. D. (2008). Cooperation of replication protein A with the ubiquitin ligase Rad18 in DNA damage bypass. *Cell Cycle* 7, 3629–3633. doi: 10.4161/cc.7.23.7166

Ikeda, F., and Dikic, I. (2008). Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: beyond the Usual Suspects' review series. *EMBO Rep.* 9, 536–542. doi: 10.1038/embor.2008.93

Jentsch, S., Mcgrath, J. P., and Varshavsky, A. (1987). The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* 329, 131–134. doi: 10.1038/329131a0

Jones, M. J., Colnaghi, L., and Huang, T. T. (2012). Dysregulation of DNA polymerase kappa recruitment to replication forks results in genomic instability. *EMBO J.* 31, 908–918. doi: 10.1038/emboj.2011.457

Juhasz, S., Balogh, D., Hajdu, I., Burkovics, P., Villamil, M. A., Zhuang, Z., et al. (2012). Characterization of human Spartan/C1orf124, an ubiquitin-PCNA interacting regulator of DNA damage tolerance. *Nucleic Acids Res.* 40, 10795–10808. doi: 10.1093/nar/gks850

Jung, Y. S., Hakem, A., Hakem, R., and Chen, X. (2011). Pirh2 E3 ubiquitin ligase monoubiquitinates DNA polymerase eta to suppress translesion DNA synthesis. *Mol. Cell Biol.* 31, 3997–4006. doi: 10.1128/MCB.05808-11

Jung, Y. S., Qian, Y., and Chen, X. (2012). DNA polymerase eta is targeted by Mdm2 for polyubiquitination and proteasomal degradation in response to ultraviolet irradiation. *DNA Repair (Amst.)* 11, 177–184. doi: 10.1016/j.dnarep.2011.10.017

Kannouche, P., Broughton, B. C., Volker, M., Hanaoka, F., Mullenders, L. H., and Lehmann, A. R. (2001). Domain structure, localization, and function of DNA polymerase eta, defective in xeroderma pigmentosum variant cells. *Genes Dev.* 15, 158–172. doi: 10.1101/gad.187501

Kannouche, P., Fernandez de Henestrosa, A. R., Coull, B., Vidal, A. E., Gray, C., Zicha, D., et al. (2002). Localization of DNA polymerases eta and iota to the replication machinery is tightly co-ordinated in human cells. *EMBO J.* 21, 6246–6256. doi: 10.1093/emboj/cdf618

Kannouche, P. L., and Lehmann, A. R. (2004). Ubiquitination of PCNA and the polymerase switch in human cells. *Cell Cycle* 3, 1011–1013. doi: 10.4161/cc.3.8.1074

Kannouche, P. L., Wing, J., and Lehmann, A. R. (2004). Interaction of human DNA polymerase eta with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. *Mol. Cell* 14, 491–500. doi: 10.1016/S1097-2765(04)00259-X

Kashiwaba, S., Kanao, R., Masuda, Y., Kusumoto-Matsuo, R., Hanaoka, F., and Masutani, C. (2015). USP7 is a suppressor of PCNA ubiquitination and oxidative-stress-induced mutagenesis in human cells. *Cell Rep.* 13, 2072–2080. doi: 10.1016/j.celrep.2015.11.014

Kim, H., Yang, K., Dejsuphong, D., and D'Andrea, A. D. (2012). Regulation of Rev1 by the Fanconi anemia core complex. *Nat. Struct. Mol. Biol.* 19, 164–170. doi: 10.1038/nsmb.2222

Kim, J., and MacNeill, S. A. (2003). Genome stability: a new member of the RFC family. *Curr. Biol.* 13, R873–R875. doi: 10.1016/j.cub.2003.10.048

Kim, K. I., Giannakopoulos, N. V., Virgin, H. W., and Zhang, D. E. (2004). Interferon-inducible ubiquitin E2, Ubc8, is a conjugating enzyme for protein ISGylation. *Mol. Cell Biol.* 24, 9592–9600. doi: 10.1128/MCB.24.21.9592-9600.2004

Kim, S. H., and Michael, W. M. (2008). Regulated proteolysis of DNA polymerase eta during the DNA-damage response in *C. elegans*. *Mol. Cell* 32, 757–766. doi: 10.1016/j.molcel.2008.11.016

Kim, S. O., Yoon, H., Park, S. O., Lee, M., Shin, J. S., Ryu, K. S., et al. (2012). Srs2 possesses a non-canonical PIP box in front of its SBM for precise recognition of SUMOylated PCNA. *J. Mol. Cell Biol.* 4, 258–261. doi: 10.1093/jmcb/mjs026

Krijger, P. H., Lee, K. Y., Wit, N., Van Den Berk, P. C., Wu, X., Roest, H. P., et al. (2011a). HLTF and SHPRH are not essential for PCNA polyubiquitination, survival and somatic hypermutation: existence of an alternative E3 ligase. *DNA Repair (Amst.)* 10, 438–444. doi: 10.1016/j.dnarep.2010.12.008

Krijger, P. H., Van Den Berk, P. C., Wit, N., Langerak, P., Jansen, J. G., Reynaud, C. A., et al. (2011b). PCNA ubiquitination-independent activation of polymerase eta during somatic hypermutation and DNA damage tolerance. *DNA Repair (Amst.)* 10, 1051–1059. doi: 10.1016/j.dnarep.2011.08.005

Krishna, T. S., Fenyo, D., Kong, X. P., Gary, S., Chait, B. T., Burgers, P., et al. (1994a). Crystallization of proliferating cell nuclear antigen (PCNA) from *Saccharomyces cerevisiae*. *J. Mol. Biol.* 241, 265–268. doi: 10.1006/jmbi.1994.1495

Krishna, T. S., Kong, X. P., Gary, S., Burgers, P. M., and Kuriyan, J. (1994b). Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell* 79, 1233–1243. doi: 10.1016/0092-8674(94)90014-0

Kubota, T., Myung, K., and Donaldson, A. D. (2013a). Is PCNA unloading the central function of the Elg1/ATAD5 replication factor C-like complex? *Cell Cycle* 12, 2570–2579. doi: 10.4161/cc.25626

Kubota, T., Nishimura, K., Kanemaki, M. T., and Donaldson, A. D. (2013b). The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Mol. Cell* 50, 273–280. doi: 10.1016/j.molcel.2013.02.012

Kulathu, Y., and Komander, D. (2012). Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat. Rev. Mol. Cell Biol.* 13, 508–523. doi: 10.1038/nrm3394

Kuriyan, J., and O'Donnell, M. (1993). Sliding clamps of DNA polymerases. *J. Mol. Biol.* 234, 915–925. doi: 10.1006/jmbi.1993.1644

Lange, S. S., Bedford, E., Reh, S., Wittschieben, J. P., Carbajal, S., Kusewitt, D. F., et al. (2013). Dual role for mammalian DNA polymerase zeta in maintaining genome stability and proliferative responses. *Proc. Natl. Acad. Sci. U.S.A.* 110, E687–E696. doi: 10.1073/pnas.1217425110

Lange, S. S., Takata, K., and Wood, R. D. (2011). DNA polymerases and cancer. *Nat. Rev. Cancer* 11, 96–110. doi: 10.1038/nrc2998

Leach, C. A., and Michael, W. M. (2005). Ubiquitin/SUMO modification of PCNA promotes replication fork progression in *Xenopus laevis* egg extracts. *J. Cell Biol.* 171, 947–954. doi: 10.1083/jcb.200508100

Lee, G. H., and Matsushita, H. (2005). Genetic linkage between *Pol iota* deficiency and increased susceptibility to lung tumors in mice. *Cancer Sci.* 96, 256–259. doi: 10.1111/j.1349-7006.2005.00042.x

Lee, K. Y., Yang, K., Cohn, M. A., Sikdar, N., D'Andrea, A. D., and Myung, K. (2010). Human ELG1 regulates the level of ubiquitinated proliferating cell nuclear antigen (PCNA) through Its interactions with PCNA and USP1. *J. Biol. Chem.* 285, 10362–10369. doi: 10.1074/jbc.M109.092544

Lessel, D., Vaz, B., Halder, S., Lockhart, P. J., Marinovic-Terzic, I., Lopez-Mosqueda, J., et al. (2014). Mutations in SPRTN cause early onset hepatocellular carcinoma, genomic instability and progeroid features. *Nat. Genet.* 46, 1239–1244. doi: 10.1038/ng.3103

Li, M., Chen, D., Shiloh, A., Luo, J., Nikolaev, A. Y., Qin, J., et al. (2002). Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature* 416, 648–653. doi: 10.1038/nature737

Lin, J. R., Zeman, M. K., Chen, J. Y., Yee, M. C., and Cimprich, K. A. (2011). SHPRH and HLTF act in a damage-specific manner to coordinate different forms of postreplication repair and prevent mutagenesis. *Mol. Cell* 42, 237–249. doi: 10.1016/j.molcel.2011.02.026

Loeb, K. R., and Haas, A. L. (1992). The interferon-inducible 15-kDa ubiquitin homolog conjugates to intracellular proteins. *J. Biol. Chem.* 267, 7806–7813.

MacKay, C., Toth, R., and Rouse, J. (2009). Biochemical characterisation of the SWI/SNF family member HLTF. *Biochem. Biophys. Res. Commun.* 390, 187–191. doi: 10.1016/j.bbrc.2009.08.151

Mailand, N., Gibbs-Seymour, I., and Bekker-Jensen, S. (2013). Regulation of PCNA-protein interactions for genome stability. *Nat. Rev. Mol. Cell Biol.* 14, 269–282. doi: 10.1038/nrm3562

Masuda, Y., Kanao, R., Kaji, K., Ohmori, H., Hanaoka, F., and Masutani, C. (2015). Different types of interaction between PCNA and PIP boxes contribute to distinct cellular functions of Y-family DNA polymerases. *Nucleic Acids Res.* 43, 7898–7910. doi: 10.1093/nar/gkv712

Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., et al. (1999). The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* 399, 700–704. doi: 10.1038/21447

McIntyre, J., Mclenigan, M. P., Frank, E. G., Dai, X., Yang, W., Wang, Y., et al. (2015). Posttranslational regulation of Human DNA Polymerase iota. *J. Biol. Chem.* 290, 27332–27344. doi: 10.1074/jbc.M115.675769

McIntyre, J., Vidal, A. E., Mclenigan, M. P., Bomar, M. G., Curti, E., Mcdonald, J. P., et al. (2013). Ubiquitin mediates the physical and functional interaction between human DNA polymerases eta and iota. *Nucleic Acids Res.* 41, 1649–1660. doi: 10.1093/nar/gks1277

Meyer, H., Bug, M., and Bremer, S. (2012). Emerging functions of the VCP/p97 AAA-ATPase in the ubiquitin system. *Nat. Cell Biol.* 14, 117–123. doi: 10.1038/ncb2407

Mirchandani, K. D., Mccaffrey, R. M., and D'Andrea, A. D. (2008). The Fanconi anemia core complex is required for efficient point mutagenesis and Rev1 foci assembly. *DNA Repair (Amst.)* 7, 902–911. doi: 10.1016/j.dnarep.2008.03.001

Miyase, S., Tateishi, S., Watanabe, K., Tomita, K., Suzuki, K., Inoue, H., et al. (2005). Differential regulation of Rad18 through Rad6-dependent mono- and polyubiquitination. *J. Biol. Chem.* 280, 515–524. doi: 10.1074/jbc.M409219200

Moldovan, G. L., Dejsuphong, D., Petalcorin, M. I., Hofmann, K., Takeda, S., Boulton, S. J., et al. (2012). Inhibition of homologous recombination by the PCNA-interacting protein PARI. *Mol. Cell* 45, 75–86. doi: 10.1016/j.molcel.2011.11.010

Mosbech, A., Gibbs-Seymour, I., Kagias, K., Thorslund, T., Beli, P., Povlsen, L., et al. (2012). DVC1 (C1orf124) is a DNA damage-targeting p97 adaptor that promotes ubiquitin-dependent responses to replication blocks. *Nat. Struct. Mol. Biol.* 19, 1084–1092. doi: 10.1038/nsmb.2395

Motegi, A., Liaw, H. J., Lee, K. Y., Roest, H. P., Maas, A., Wu, X., et al. (2008). Polyubiquitination of proliferating cell nuclear antigen by HLTF and SHPRH prevents genomic instability from stalled replication forks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12411–12416. doi: 10.1073/pnas.0805685105

Motegi, A., Sood, R., Moinova, H., Markowitz, S. D., Liu, P. P., and Myung, K. (2006). Human SHPRH suppresses genomic instability through proliferating cell nuclear antigen polyubiquitination. *J. Cell Biol.* 175, 703–708. doi: 10.1083/jcb.200606145

Muller, S., Hoege, C., Pyrowolakis, G., and Jentsch, S. (2001). SUMO, ubiquitin's mysterious cousin. *Nat. Rev. Mol. Cell Biol.* 2, 202–210. doi: 10.1038/35056591

Niimi, A., Brown, S., Sabbioneda, S., Kannouche, P. L., Scott, A., Yasui, A., et al. (2008). Regulation of proliferating cell nuclear antigen ubiquitination in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16125–16130. doi: 10.1073/pnas.0802727105

Notenboom, V., Hibbert, R. G., Van Rossum-Fikkert, S. E., Olsen, J. V., Mann, M., and Sixma, T. K. (2007). Functional characterization of Rad18 domains for Rad6, ubiquitin, DNA binding and PCNA modification. *Nucleic Acids Res.* 35, 5819–5830. doi: 10.1093/nar/gkm615

Ogi, T., Limsirichaikul, S., Overmeer, R. M., Volker, M., Takenaka, K., Cloney, R., et al. (2010). Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. *Mol. Cell* 37, 714–727. doi: 10.1016/j.molcel.2010.02.009

Papouli, E., Chen, S., Davies, A. A., Huttner, D., Krejci, L., Sung, P., et al. (2005). Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell* 19, 123–133. doi: 10.1016/j.molcel.2005.06.001

Park, J. M., Yang, S. W., Yu, K. R., Ka, S. H., Lee, S. W., Seol, J. H., et al. (2014). Modification of PCNA by ISG15 plays a crucial role in termination of error-prone translesion DNA synthesis. *Mol. Cell* 54, 626–638. doi: 10.1016/j.molcel.2014.03.031

Parker, J. L., and Ulrich, H. D. (2009). Mechanistic analysis of PCNA poly-ubiquitylation by the ubiquitin protein ligases Rad18 and Rad5. *EMBO J.* 28, 3657–3666. doi: 10.1038/emboj.2009.303

Parnas, O., Zipin-Roitman, A., Pfander, B., Liefshitz, B., Mazor, Y., Ben-Aroya, S., et al. (2010). Elg1, an alternative subunit of the RFC clamp loader, preferentially interacts with SUMOylated PCNA. *EMBO J.* 29, 2611–2622. doi: 10.1038/emboj.2010.128

Pfander, B., Moldovan, G. L., Sacher, M., Hoege, C., and Jentsch, S. (2005). SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436, 428–433. doi: 10.1038/nature03665

Pinder, J. B., Attwood, K. M., and Dellaire, G. (2013). Reading, writing, and repair: the role of ubiquitin and the ubiquitin-like proteins in DNA damage signaling and repair. *Front. Genet.* 4:45. doi: 10.3389/fgene.2013.00045

Plosky, B. S., Vidal, A. E., Fernandez de Henestrosa, A. R., Mclenigan, M. P., Mcdonald, J. P., Mead, S., et al. (2006). Controlling the subcellular localization of DNA polymerases iota and eta via interactions with ubiquitin. *EMBO J.* 25, 2847–2855. doi: 10.1038/sj.emboj.7601178

Qian, J., Pentz, K., Zhu, Q., Wang, Q., He, J., Srivastava, A. K., et al. (2015). USP7 modulates UV-induced PCNA monoubiquitination by regulating DNA polymerase eta stability. *Oncogene* 34, 4791–4796. doi: 10.1038/onc.2014.394

Sabbioneda, S., Gourdin, A. M., Green, C. M., Zotter, A., Giglia-Mari, G., Houtsmuller, A., et al. (2008). Effect of proliferating cell nuclear antigen ubiquitination and chromatin structure on the dynamic properties of the Y-family DNA polymerases. *Mol. Biol. Cell* 19, 5193–5202. doi: 10.1091/mbc.E08-07-0724

Sabbioneda, S., Green, C. M., Bienko, M., Kannouche, P., Dikic, I., and Lehmann, A. R. (2009). Ubiquitin-binding motif of human DNA polymerase eta is required for correct localization. *Proc. Natl. Acad. Sci. U.S.A.* 106:E20. doi: 10.1073/pnas.0812744106

Sakiyama, T., Kohno, T., Mimaki, S., Ohta, T., Yanagitani, N., Sobue, T., et al. (2005). Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk. *Int. J. Cancer* 114, 730–737. doi: 10.1002/ijc.20790

Sale, J. E., Lehmann, A. R., and Woodgate, R. (2012). Y-family DNA polymerases and their role in tolerance of cellular DNA damage. *Nat. Rev. Mol. Cell Biol.* 13, 141–152. doi: 10.1038/nrm3289

Sheng, Y., Saridakis, V., Sarkari, F., Duan, S., Wu, T., Arrowsmith, C. H., et al. (2006). Molecular recognition of p53 and MDM2 by USP7/HAUSP. *Nat. Struct. Mol. Biol.* 13, 285–291. doi: 10.1038/nsmb1067

Simpson, L. J., Ross, A. L., Szuts, D., Alviani, C. A., Oestergaard, V. H., Patel, K. J., et al. (2006). RAD18-independent ubiquitination of proliferating-cell

nuclear antigen in the avian cell line DT40. *EMBO Rep.* 7, 927–932. doi: 10.1038/sj.embor.7400777

Terai, K., Abbas, T., Jazaeri, A. A., and Dutta, A. (2010). CRL4(Cdt2) E3 ubiquitin ligase monoubiquitinates PCNA to promote translesion DNA synthesis. *Mol. Cell* 37, 143–149. doi: 10.1016/j.molcel.2009.12.018

Unk, I., Hajdu, I., Blastyak, A., and Haracska, L. (2010). Role of yeast Rad5 and its human orthologs, HLTF and SHPRH in DNA damage tolerance. *DNA Repair (Amst.)* 9, 257–267. doi: 10.1016/j.dnarep.2009.12.013

Unk, I., Hajdu, I., Fatyol, K., Hurwitz, J., Yoon, J. H., Prakash, L., et al. (2008). Human HLTF functions as a ubiquitin ligase for proliferating cell nuclear antigen polyubiquitination. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3768–3773. doi: 10.1073/pnas.0800563105

Varadan, R., Assfalg, M., Haririnia, A., Raasi, S., Pickart, C., and Fushman, D. (2004). Solution conformation of Lys63-linked di-ubiquitin chain provides clues to functional diversity of polyubiquitin signaling. *J. Biol. Chem.* 279, 7055–7063. doi: 10.1074/jbc.M309184200

Varadan, R., Walker, O., Pickart, C., and Fushman, D. (2002). Structural properties of polyubiquitin chains in solution. *J. Mol. Biol.* 324, 637–647. doi: 10.1016/S0022-2836(02)01198-1

Vidal, A. E., and Woodgate, R. (2009). Insights into the cellular role of enigmatic DNA polymerase iota. *DNA Repair (Amst.)* 8, 420–423. doi: 10.1016/j.dnarep.2008.12.007

Wang, M., Devereux, T. R., Vikis, H. G., Mcculloch, S. D., Holliday, W., Anna, C., et al. (2004). Pol iota is a candidate for the mouse pulmonary adenoma resistance 2 locus, a major modifier of chemically induced lung neoplasia. *Cancer Res.* 64, 1924–1931. doi: 10.1158/0008-5472.CAN-03-3080

Wang, Y., Woodgate, R., Mcmanus, T. P., Mead, S., Mccormick, J. J., and Maher, V. M. (2007). Evidence that in xeroderma pigmentosum variant cells, which lack DNA polymerase eta, DNA polymerase iota causes the very high frequency and unique spectrum of UV-induced mutations. *Cancer Res.* 67, 3018–3026. doi: 10.1158/0008-5472.CAN-06-3073

Wang, Z., Huang, M., Ma, X., Li, H., Tang, T., and Guo, C. (2016). REV1 promotes PCNA monoubiquitination through interacting with ubiquitinated RAD18. *J. Cell Sci.* doi: 10.1242/jcs.179408 [Epub ahead of print].

Washington, M. T., Johnson, R. E., Prakash, L., and Prakash, S. (2004a). Human DNA polymerase iota utilizes different nucleotide incorporation mechanisms dependent upon the template base. *Mol. Cell Biol.* 24, 936–943. doi: 10.1128/MCB.24.2.936-943.2004

Washington, M. T., Minko, I. G., Johnson, R. E., Wolfle, W. T., Harris, T. M., Lloyd, R. S., et al. (2004b). Efficient and error-free replication past a minor-groove DNA adduct by the sequential action of human DNA polymerases iota and kappa. *Mol. Cell Biol.* 24, 5687–5693. doi: 10.1128/MCB.24.13.5687-5693.2004

Watanabe, K., Tateishi, S., Kawasuji, M., Tsurimoto, T., Inoue, H., and Yamaizumi, M. (2004). Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. *EMBO J.* 23, 3886–3896. doi: 10.1038/sj.emboj.7600383

Weston, R., Peeters, H., and Ahel, D. (2012). ZRANB3 is a structure-specific ATP-dependent endonuclease involved in replication stress response. *Genes Dev.* 26, 1558–1572. doi: 10.1101/gad.193516.112

Wittschieben, J. P., Patil, V., Glushets, V., Robinson, L. J., Kusewitt, D. F., and Wood, R. D. (2010). Loss of DNA polymerase zeta enhances spontaneous tumorigenesis. *Cancer Res.* 70, 2770–2778. doi: 10.1158/0008-5472.CAN-09-4267

Wittschieben, J. P., Reshmi, S. C., Gollin, S. M., and Wood, R. D. (2006). Loss of DNA polymerase zeta causes chromosomal instability in mammalian cells. *Cancer Res.* 66, 134–142. doi: 10.1158/0008-5472.CAN-05-2982

Wood, A., Garg, P., and Burgers, P. M. (2007). A ubiquitin-binding motif in the translesion DNA polymerase Rev1 mediates its essential functional interaction with ubiquitinated proliferating cell nuclear antigen in response to DNA damage. *J. Biol. Chem.* 282, 20256–20263. doi: 10.1074/jbc.M702366200

Xiao, W., Chow, B. L., Broomfield, S., and Hanna, M. (2000). The *Saccharomyces cerevisiae* RAD6 group is composed of an error-prone and two error-free postreplication repair pathways. *Genetics* 155, 1633–1641.

Xie, K., Doles, J., Hemann, M. T., and Walker, G. C. (2010). Error-prone translesion synthesis mediates acquired chemoresistance. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20792–20797. doi: 10.1073/pnas.1011412107

Yanagihara, H., Kobayashi, J., Tateishi, S., Kato, A., Matsuura, S., Tauchi, H., et al. (2011). NBS1 recruits RAD18 via a RAD6-like domain and regulates Pol eta-dependent translesion DNA synthesis. *Mol. Cell* 43, 788–797. doi: 10.1016/j.molcel.2011.07.026

Yang, J., Chen, Z., Liu, Y., Hickey, R. J., and Malkas, L. H. (2004). Altered DNA polymerase iota expression in breast cancer cells leads to a reduction in DNA replication fidelity and a higher rate of mutagenesis. *Cancer Res.* 64, 5597–5607. doi: 10.1158/0008-5472.CAN-04-0603

Yang, X. H., Shiotani, B., Classon, M., and Zou, L. (2008). Chk1 and *Claspin potentiate* PCNA ubiquitination. *Genes Dev.* 22, 1147–1152. doi: 10.1101/gad.1632808

Yuan, J., Ghosal, G., and Chen, J. (2012). The HARP-like domain-containing protein AH2/ZRANB3 binds to PCNA and participates in cellular response to replication stress. *Mol. Cell* 47, 410–421. doi: 10.1016/j.molcel.2012.05.025

Yuan, W., and Krug, R. M. (2001). Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like ISG15 protein. *EMBO J.* 20, 362–371. doi: 10.1093/emboj/20.3.362

Zhang, S., Chea, J., Meng, X., Zhou, Y., Lee, E. Y., and Lee, M. Y. (2008). PCNA is ubiquitinated by RNF8. *Cell Cycle* 7, 3399–3404. doi: 10.4161/cc.7.21.6949

Zhao, C., Beaudenon, S. L., Kelley, M. L., Waddell, M. B., Yuan, W., Schulman, B. A., et al. (2004). The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. *Proc. Natl. Acad. Sci. U.S.A.* 101, 7578–7582. doi: 10.1073/pnas.0402528101

Zlatanou, A., Sabbioneda, S., Miller, E. S., Greenwalt, A., Aggathanggelou, A., Maurice, M. M., et al. (2016). USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. *Oncogene* 35, 965–976. doi: 10.1038/onc.2015.149

Zou, W., and Zhang, D. E. (2006). The interferon-inducible ubiquitin-protein isopeptide ligase (E3) EFP also functions as an ISG15 E3 ligase. *J. Biol. Chem.* 281, 3989–3994. doi: 10.1074/jbc.M510787200

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 07 June 2016
doi: 10.3389/fgene.2016.00103

Choreographing the Double Strand Break Response: Ubiquitin and SUMO Control of Nuclear Architecture

*Shane M. Harding and Roger A. Greenberg**

Departments of Cancer Biology and Pathology, Abramson Family Cancer Research Institute, Basser Research Center for BRCA, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

The cellular response to DNA double strand breaks (DSBs) is a multifaceted signaling program that centers on post-translational modifications including phosphorylation, ubiquitylation and SUMOylation. In this review we discuss how ubiquitin and SUMO orchestrate the recognition of DSBs and explore how this influences chromatin organization. We discuss functional outcomes of this response including transcriptional silencing and how pre-existing chromatin states may control the DSB response and the maintenance of genomic stability.

Keywords: double-strand break repair, Ubiquitin, SUMO, RAP80, Telomere

OPEN ACCESS

Edited by:
Ivan Dikic,
Goethe University Frankfurt, Germany

Reviewed by:
Karen Hubbard,
The City College of New York, USA
Parvin Mehdipour,
Tehran University of Medical Sciences, Iran

***Correspondence:**
Roger A. Greenberg
rogergr@mail.med.upenn.edu

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *26 February 2016*
Accepted: *24 May 2016*
Published: *07 June 2016*

Citation:
Harding SM and Greenberg RA (2016) Choreographing the Double Strand Break Response: Ubiquitin and SUMO Control of Nuclear Architecture. Front. Genet. 7:103. doi: 10.3389/fgene.2016.00103

Every organism experiences challenges to the integrity of their DNA sequence from endogenous (i.e., replication errors) and exogenous (i.e., radiation) sources. Such challenges can take the form of base mismatches and base damages or single and DSBs in the DNA backbone. Discrete molecular pathways driven by posttranslational modifications have evolved to correct each of these DNA damage types and are crucial for cellular survival and for the maintenance of genomic integrity. Perhaps the most deleterious of these lesions is the DSB as even a single unrepaired DSB can cause cell death and inaccurate repair can lead to mutations that cause cancer and other genetic diseases (Jackson and Bartek, 2009).

The DSB response encompasses multiple post-translational modifications, including ubiquitylation (ub) and SUMOylation that primarily occur within the immediate vicinity of the DSB on chromatin and chromatin-associated proteins. Locally, this promotes DSB repair mechanisms and systemically activates cellular responses, including cell cycle checkpoints that collectively suppress genomic instability. Recent technological advances and conceptual insights have highlighted how the DSB response influences the dynamic structural organization of the nucleus. In this review we will first outline how the ubiquitin and SUMOylation systems contribute to the sensing of DSBs and then examine how these pathways affect higher order chromatin structure to maintain genetic stability.

UBIQUITIN AND SUMO IN THE DSB RESPONSE

At the apex of the molecular cascade that signals double strand breaks are three phosphatidylinositol 3-kinase related kinases (PIKKs): DNA-dependent protein kinase catalytic subunit (DNA-PKcs), Ataxia telangiectasia mutated (ATM), and ATM- and Rad3-related (ATR). DNA-PKcs forms an active holoenzyme, DNA-PK, with the heterodimer Ku70/80 at DNA ends and mainly contributes to DSB repair by non-homologous end-joining (NHEJ; Radhakrishnan et al., 2014). ATR, in cooperation with its binding partner ATRIP, binds to RPA

protein-bound single stranded DNA (ssDNA) and therefore mainly senses DSBs incurred during replication where long tracks of ssDNA may be generated (Cimprich and Cortez, 2008). The most extensively characterized of this PIKK triad is ATM for which thousands of phosphorylation targets have been identified, some of which overlap with ATR and DNA-PKcs (Matsuoka et al., 2007). All three kinases are able to phosphorylate the histone variant H2A.X at serine-139 forming "γH2AX" in megabase domains surrounding DSBs. The γH2AX-laced chromatin is the platform on which the remainder of the DSB response assembles (Bonner et al., 2008).

Ubiquitin is a 76 amino acid protein that covalently modifies protein substrates through linkages between lysine residues. Ubiquitin modification is catalyzed in a pathway whereby an E1 activating enzyme passes the ubiquitin molecule to an E2 conjugating enzyme that in turn passes the ubiquitin to a substrate molecule with the help of an E3 ubiquitin ligase (Ciechanover et al., 1980; Hershko et al., 1980, 1983; Bergink and Jentsch, 2009; Popovic et al., 2014). Ubiquitin itself contains seven lysine residues that can serve as locations for chain assembly, in addition to linkages through its *N*-terminus to form linear chains (Rajalingam and Dikic, 2016). The first indications of a role for ubiquitin in the response to DNA damage came when Jentsch et al. (1987) identified a ubiquitin conjugating activity of the DNA repair gene RAD6 in *Saccharomyces cerevisiae*. Subsequently, specific ubiquitin linkages were found to be functionally relevant when a K63R mutation in ubiquitin caused sensitivity to UV and base damages of the DNA in yeast (Spence et al., 1995) and that *Y*-family DNA polymerases are recruited to UV damage through interaction with ubiquitinated PCNA (Bienko et al., 2005). A link between K63-ub chains and the DSB response in mammalian cells remained elusive until several groups identified RAP80 as a binding partner of breast cancer 1, early onset (BRCA1; Kim et al., 2007; Sobhian et al., 2007; Wang et al., 2007). RAP80 contains a tandem ubiquitin-interacting motif (UIM) that binds with high affinity to K63 linkages *in vitro* and associates with K63-linkages *in vivo* following DNA damage (Sobhian et al., 2007). Interestingly, the BRCA1-RAP80 complex is comprised of several other proteins, including MERIT40 (Feng et al., 2009; Shao et al., 2009; Wang et al., 2009) and BRCC36, a deubiquitinating enzyme with K63-ub specificity (Sobhian et al., 2007; Cooper et al., 2010; Feng et al., 2010; Patterson-Fortin et al., 2010). The structural basis for BRCC36 DUB activity has recently been solved (Zeqiraj et al., 2015) and it was also recently shown that MERIT40 deficiency is synthetic lethal in the context of BRCA2 mutation (Jiang et al., 2015). Importantly, mutations in the RAP80 UIM and in the BRCA1-RAP80 associated protein Abraxas (Nikkilä et al., 2009; Solyom et al., 2012) have been described in familial breast cancer cases where the *BRCA1* and *BRCA2* genes are not affected. These disease associated mutations highlight the importance of this specific ubiquitin interaction for genome stability.

Using siRNA-screening approaches several groups identified RING finger protein 8 (RNF8) as the first E3 to catalyse K63 linkages at DSBs in mammals (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Wang and Elledge, 2007). Together these papers established that once γH2AX is generated, the mediator of DNA damage checkpoint 1 (MDC1) protein is rapidly recruited and phosphorylated by ATM at an N-terminal AQXF cluster. This phosphorylation event drives localization of RNF8 to the DSB site. Recent evidence supports a model where this rapid RNF8 recruitment drives histone H1 ubiquitylation via the UBC13 E2 ligase and this serves to recruit a second E3, RNF168, to ubiquitinate histone H2A at postions K13 and K15 (Mattiroli et al., 2012; Thorslund et al., 2015). Collectively these ubiquitylations establish chromatin changes that facilitate the recruitment of other DSB response factors including 53BP1 and BRCA1. The current models posit that in addition to providing direct docking sites for protein substrates these ubiquitin chains also drive large scale chromatin changes. One such proposition is that 53BP1 binds to pre-existing H4-K20 dimethylated residues that are exposed locally by these DSB-specific modifications (Huyen et al., 2004; Botuyan et al., 2006; Acs et al., 2011; Meerang et al., 2011; Mallette et al., 2012; Kocyłowski et al., 2015). More recent evidence demonstrates that 53BP1 is a specific reader of combinatorial histone modifications. 53BP1 DSB foci formation required H4K20 methylation by the Tudor domain as well as H2AK15-Ub recognition by a short conserved region C-terminal to the Tudor repeats called the UDR (Ubiquitin dependent recruitment) motif (Fradet-Turcotte et al., 2013). Importantly the degree of ubiquitylation surrounding DSBs is constrained in part by limited RNF168 protein levels. Deficiency in Ubr5 and TRIP12 increased RNF168 protein levels, resulting in excessive spreading of DSB ubiquitin and exhaustion of 53BP1 pools (Gudjonsson et al., 2012). It is now well established that ubiquitylation is a cornerstone of the DSB response and its precise control is essential for genome stability and tumor suppression.

In addition to ubiquitin, the small ubiquitin-like modifier (SUMO) proteins have been found to impact essentially every facet of the DNA damage response by modulating protein-protein interaction and enzymatic activity (Bergink and Jentsch, 2009). Discovered in 1996, SUMO is a small peptide that is covalently attached to proteins by E1, E2, and E3 SUMO ligases in a pathway analogous to ubiquitin conjugation (Matunis et al., 1996; Cubeñas-Potts and Matunis, 2013). The immunofluorescent and biochemical observation of SUMO1 and 2/3 isoforms at DSBs led to the identification of the PIAS1 and PIAS4 E3 SUMO ligases that drive SUMOylation of BRCA1 and 53BP1 (Galanty et al., 2009; Morris et al., 2009). Here, loss of either PIAS1 or PIAS4 severely impairs K63-ub at damage sites, reduces recruitment BRCA1 and 53BP1 and causes impaired DSB repair. Thus, in addition to ubiquitin, SUMO modifications occur at DSBs and modulate the DSB response.

Although conceptually it is easier to separate ubiquitin and SUMOylation, it is important to recognize that they can act in a combinatorial fashion. RNF4, a SUMO-targeted E3 ubiquitin ligase (STUbL), localizes to SUMO-modified MDC1 at DSB sites where its ubiquitin ligase activity is required for effective RAP80-BRCA1 recruitment as well as DSB repair and effective responses to replication stress (Galanty et al., 2012; Guzzo et al., 2012; Yin et al., 2012; Ragland et al., 2013; Gibbs-Seymour et al., 2015; Sarangi and Zhao, 2015). RAP80 itself contains a SIM domain adjacent to its UIM domains and each of these domains cooperates in

the productive association of RAP80-BRCA1 to damage sites (Guzzo et al., 2012; Hu et al., 2012). Importantly, BRCA1 itself is an E3 ubiquitin ligase that can catalyze K6 linkages of ubiquitin *in vitro* and this activity is stimulated by SUMOylation of BRCA1 (Wu-Baer et al., 2003; Morris and Solomon, 2004; Polanowska et al., 2006; Morris et al., 2009). Although the function of this particular BRCA1 activity is not well understood this serves as a clear example of the interconnection of ubiquitin and SUMOylation in the DSB response (Messick and Greenberg, 2009; Jackson and Durocher, 2013).

Recent proteomic studies have identified hundreds of damage-induced targets of both ubiquitylation and SUMOylation (Psakhye and Jentsch, 2012; Elia et al., 2015). The array of targets and the potential for combinatorial effects of these moieties poses a challenge to understanding how a particular modification on a particular protein impacts the DSB response. This problem may be particularly true for SUMOylation as one of these reports suggests that it is the bulk SUMOylation of a group of proteins rather than any one specific target that stimulates DSB repair (Psakhye and Jentsch, 2012). Despite these challenges it is immediately apparent that phosphorylation, ubiquitylation, and SUMOylation make the chromatin permissive to recruit the various effectors of the DSB response that collectively activate repair mechanisms and cell cycle checkpoints. More recently several groups have begun to explore how the DSB response impacts higher order chromatin structure and nuclear architecture that are strongly influenced by these and other post-translational modifications.

DSB RESPONSE-DRIVEN NUCLEAR REORGANIZATION

For the purposes of this review, we will separate chromatin reorganization into two interconnected categories. The first category includes large-scale chromatin redistributions in which the damaged locus changes its physical location within the 3D nuclear compartment (**Figure 1**). For example, moving from the nuclear interior to the periphery. We will discuss how these particular movements may influence the mechanism and fidelity of double strand break repair. These movements have been summarized in **Table 1**. The second category includes more localized epigenetic changes that cause transition between heterochromatic and euchromatic states. In this instance we will focus our discussion on recent insights into how these changes influence transcription near the DSBs.

Chromatin Movement and the DSB Response: Gross Chromatin Movements

Textbook descriptions of chromatin are, by necessity, static depictions of linear DNA bound by histones and other factors. Even in undamaged DNA this static arrangement is inaccurate and several mathematical models based on cellular data have described active chromatin movement as a non-directional random walk over relatively short distances (Dion and Gasser, 2013). These short-range movements are constrained by multiple cellular and physical properties and lead to occupancy of chromosomes within non-randomly defined nuclear volumes called "chromatin territories" (Cremer and Cremer, 2010). Less frequent longer-range movements have also been detected in various contexts. For example, targeting of the VP16 transcriptional activator to the nuclear periphery resulted in movement to the nuclear interior and inhibition of RNA Polymerase I (Pol I) transcription causes relocalization of chromatin to the nucleolar periphery (Tumbar and Belmont, 2001; Floutsakou et al., 2013).

In yeast, there is clear evidence that DSBs induce chromatin mobility. When DSBs were induced in the rDNA of *S. cerevisiae* these breaks relocalized to the exterior of the rDNA-containing nucleolus (Torres-Rosell et al., 2007). As even undamaged rDNA repeats transiently moved outside of the nucleolus the authors proposed that it was this underlying dynamic motion rather than a specific DSB-driven process led to the translocation. Importantly, this movement was dependent on a specific SUMOylation event in RAD52 that is also required for homology-directed repair (HR) of these rDNA loci. At other genomic loci in yeast SUMOylation also targets DSBs at defined genomic sites to the nuclear periphery (Nagai et al., 2008; Kalocsay et al., 2009). Breaks elsewhere in the yeast genome also led to a greater mobility of chromatin that was dependent on RAD51, RAD54, MEC1, RAD9 (similar to human MDC1, 53BP1 and BRCA1), and INO80 (Dion et al., 2012; Neumann et al., 2012). A recent report also found that the INO80-driven movement of DSBs within subtelomeres depends on actin polymerization (Spichal et al., 2016). These movements at least in part contributes to homology searches during HR (Miné-Hattab and Rothstein, 2012). A recent study found that DSB-induced MEC1-driven phosphorylation of the kinetochore component Cep1 causes release of the centromere from the spindle pole body and facilitates chromatin movement (Strecker et al., 2016). Additionally, the authors found that tethering of telomeres to the nuclear periphery constrains chromatin movement and the physical breakage of the chromatin from this linkage facilitates further chromatin mobility. Interestingly, in this instance the authors found no evidence for an HR defect but rather propose that the increased mobility facilitates cell cycle checkpoint activation. Therefore, a preponderance of evidence exists that DSBs in yeast are mobile and that SUMOylation and the DSB response drive this mobility. Even if the precise functional outcome of this movement is unclear, the consensus is that the movement has a positive impact on the ability of the yeast cell to survive DSBs and therefore sets precedent for study in mammalian cells.

There are now numerous reports of chromatin mobility in response to DSBs in mammalian cells, albeit the determinants for this mobility are incompletely defined, as many breaks appear to be stable in their nuclear position. One of the first examples of subnuclear DSBs induced by soft *X*-rays suggested that breaks are positionally stable during the initial phases of the damage response although the temporal and spatial resolution with this method is limited (Nelms et al., 1998). Using α-radiation to create DSBs along a confined linear track of the nucleus, Aten et al. (2004) found that breaks redistributed into clusters giving rise to

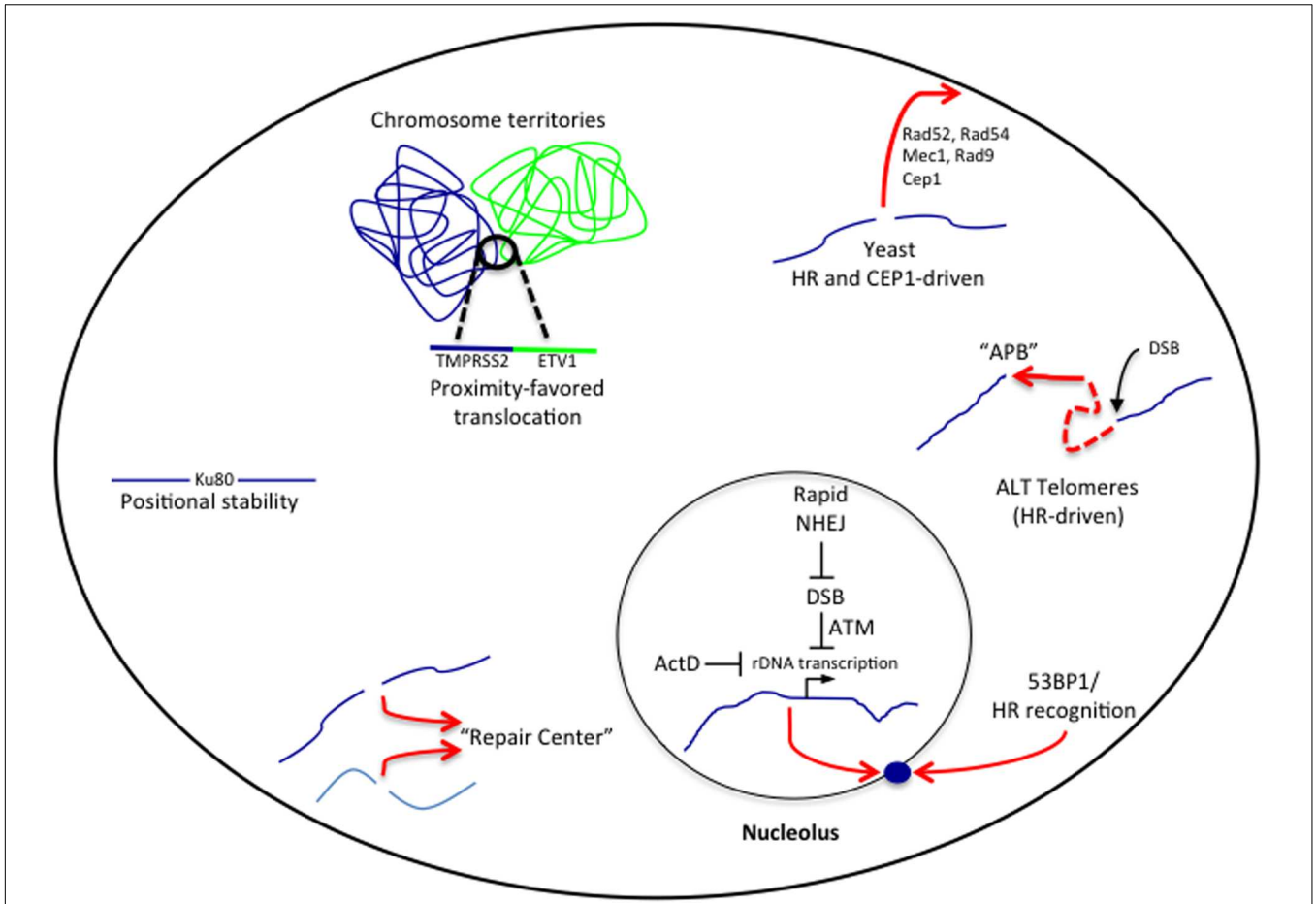

FIGURE 1 | Chromatin reorganization during double strand break (DSB) responses. In several contexts, DSBs induce chromatin reorganization. Errors in DSB repair can result in translocations that occur most frequently between chromosomes that are already in close spatial proximity through pre-existing organization into chromosome territories. Moving clockwise, persistent breaks in yeast are relocalized to the nuclear periphery in a SUMO dependent process for repair by homologous recombination (HR). Homology directed repair driven movement of DSBs induced at telomeres promotes clustering into ALT Promyelocytic Bodies (APBs). In nucleoli persistent breaks silence transcription, which leads to relocalization of the rDNA to the nucleolar periphery. In some instances multiple breaks appear to localize to "repair centers" while in other contexts breaks remain positionally stable.

MRE11 dependent "repair centers" that were most predominant in G1-phase (Stap et al., 2008). Similarly, DSBs created by γ-rays or etoposide induced movement of damaged chromatin >2 fold over that of undamaged loci (Krawczyk et al., 2012). Evidence for repair centers have also been reported in which IR induced GFP-53BP1 foci between 1 and 2 μm apart can rapidly gather into larger clusters (Neumaier et al., 2012). Loss of ATM reduced movements at both γ-rays and by charged nuclei (Becker et al., 2014) and at nuclease induced breaks (Caron et al., 2015). Conversely, DSBs induced by UV-microbeam or γ-rays were found to have limited mobility but led to a localized decondensation of chromatin (Kruhlak et al., 2006; Falk et al., 2007). Induction of multiply damaged sites (containing DSBs, single strand breaks and base damages) by charged nuclei was not found to cause significant movements nor did DSBs induced by a nuclease at an engineered multicopy transgene locus of likely >100 repeats that is heterochromatic (Soutoglou et al., 2007; Jakob et al., 2009). The reason for such discrepancy is not clear but may be related to cell types, modes of damaged induction employed, imaging methods used or the method to visualize the DSBs themselves. One plausible explanation is that the loci broken in the nuclease experiments were repetitive transgenes that did not share homology with sequences on different chromosomes. In this scenario, homology directed mobility and clustering would not be possible. Interestingly, the constraint on mobility was dependent on Ku80, a component of NHEJ repair of DSBs (Soutoglou et al., 2007). This suggests that the NHEJ machinery tethers or rapidly rejoins DSB ends to limit mobility; this may also underlie movements when breaks are induced in the nucleolus as discussed below. However, an additional report using such transgenes described long distance MRE11 dependent mobility that was associated with chromosome translocations (Roukos et al., 2013), perhaps related to the original reports from Aten et al. (2004). Interestingly, I-PpoI nuclease induced breaks were found to cause pairing of homologous genetic loci in an ATM and transcription dependent manner in G1 phase

TABLE 1 | Large-scale movement following double strand breaks (DSBs).

DSB location/break method	Movement	References
Yeast		
rDNA	To nucleolar periphery	Torres-Rosell et al., 2007
MAT Locus	To nuclear periphery	Kalocsay et al., 2009
MAT Locus	To nuclear periphery	Nagai et al., 2008
Single I-SceI site	Increased local mobility	Dion et al., 2012; Neumann et al., 2012
MAT Locus	Increased local mobility	Strecker et al., 2016
Mammalian		
α-radiation	Break clustering	Aten et al., 2004; Stap et al., 2008
γ-radiation	Break clustering	Neumaier et al., 2012
γ-radiation, etoposide	Local mobility	Krawczyk et al., 2012
Charged nuclei, nuclease	Local mobility	Becker et al., 2014; Caron et al., 2015
UV-microbeam, γ-radiation	Chromatin decondensation	Kruhlak et al., 2006; Falk et al., 2007
Charged nuclei	Minimal	Jakob et al., 2009
Ultrasoft *X*-rays	Minimal	Nelms et al., 1998
Single multicopy locus	Minimal	Soutoglou et al., 2007
I-SceI Chr1, 7, 10	Loci pairing	Roukos et al., 2013
I-PpoI	Homolog pairing	Gandhi et al., 2012
I-PpoI rDNA	To nucleolar periphery	Harding et al., 2015; van Sluis and McStay, 2015; Warmerdam et al., 2016
Telomere deprotection	Telomere fusion	Dimitrova et al., 2008; Lottersberger et al., 2015
TRF-FokI ALT Telomeres	Telomere clustering	Cho et al., 2014

of the cell cycle (Gandhi et al., 2012). Although canonical HR dependency was not examined, a possible explanation is the occurrence of homology directed clustering in G1 due to the absence of a sister chromatid. It is also important to note that the intercomparison of studies is difficult due to differences in measurement methods and the lack of standard comparators for movement in undamaged chromatin. The differences observed between yeast and mammalian cells may, in part result from the balance in repair pathways used. Yeast preferentially use HR, the less error prone mechanism of DSB repair that uses a sister chromatid as a template to resolve the break. NHEJ, the more error prone pathway that relies on the direct rejoining of broken ends, is more predominant in mammalian cells (Shrivastav et al., 2008). These differences in repair pathway between species may have important implications for the outcome of DSB responses in mammalian cells, as described below.

In light of these issues, one clear context where DSB movement occurs is at deprotected and damaged telomeres. To prevent their recognition as DSBs telomeres are protected in a complex called shelterin that blocks access to the ends by the DSB machinery (Palm and de Lange, 2008). When shelterin is depleted the DSB response is activated and telomeres are joined by NHEJ to cause striking telomere fusions (Doksani and de Lange, 2014). Loss of 53BP1 reduced the mobility of these telomere ends and resulted in almost complete loss of telomeric fusions (Dimitrova et al., 2008). These movements are driven at least in part by the LINC complex which connects dynamic microtubules to the inside of the nucleus; similar movements were also described at non-telomeric DSBs generated in BRCA1 deficient cells using an inhibitor of Poly (ADP-ribose) polymerase (PARPi) and this was proposed to contribute to 53BP1-dependent interchromosomal NHEJ (Lottersberger et al., 2015).

A second striking example of DSB dependent chromatin mobility also occurs at telomeres, but in a 53BP1 independent manner. Approximately 10–15% of cancer cells employ "alternative lengthening of telomeres (ALT)" to maintain their telomere length. Rather than activating telomerase, ALT cells utilize a homology driven mechanism to promote lengthening of telomeres (O'Sullivan and Almouzni, 2014; Dilley and Greenberg, 2015; Pickett and Reddel, 2015). Our laboratory recently developed a method whereby DSBs are generated specifically in telomeres to elicit a DSB response (Tang et al., 2013). In ALT, but not telomerase positive cells, such DSBs induced directed movement of telomere ends into clusters called ALT-associated PML bodies (APBs), a hallmark of ALT cells (Cho et al., 2014). Unlike other DSB movements described to date the movement in this case was biphasic. During the first phase damaged telomeres showed a significant increase in mobility as compared to undamaged telomeres; this mobility is similar to those movements described above. In the second phase the "incoming" telomere makes a long-range directed movement toward a relatively immobile "recipient" telomere. Both phases of this movement were dependent in part on the HR machinery (e.g., RAD51) and also on Mnd1-Hop2, a complex generally involved in meiotic interhomolog recombination. In agreement with these findings, ALT telomere replication stress due to SMARCAL1 deficiency also resulted in Rad51 telomere–telomere clustering and dramatic telomere enlargement (Cox et al., 2016). These results highlight the first example of directed DSB movement in mammalian cells mediated by HR, and collectively, reveal that dynamic chromatin movements contribute to genomic stability and cellular immortality through telomere maintenance.

In several contexts, it is clear that DSBs can induce chromatin movement. How the DSB response itself impacts these movements is slowly beginning to be elucidated. Unlike SUMOylation, a direct role for ubiquitin in DSB movement has not been described. The involvement of MEC1 and RAD9 in yeast implies that this may be the case. Although RAD52 is a clear SUMOylation target in yeast, it is also possible that movements are controlled by other SUMOylation events or simply by bulk SUMOylation of multiple factors (Psakhye and Jentsch, 2012). In *Drosophila melanogaster,* heterochromatic breaks are mobilized from heterochromatin to the nuclear periphery in a mechanism mediated by the STUbLs Dgrn and Rad60 (Chiolo et al., 2011; Ryu et al., 2015). This is reminiscent of the SUMO-dependent relocalization of yeast DSBs to the periphery of the

nucleus or nucleolus suggesting there is conservation of the mechanism across species. As in yeast we expect that in addition to recruiting DSB response factors for cell signaling and DSB repair, ubiquitylation and SUMOylation events serve to modulate chromatin movement. Indeed, at ALT telomeres the MMS21 subunit of the SMC5/6 complex SUMOylates multiple telomere binding proteins (e.g., TRF1) and contributes to HR of ALT telomeres and localization to APBs (Potts and Yu, 2007). It is also possible that the control of DSB movement is a fundamental aspect of the DSB response that controls the homology search during HR (as in telomeres described above) and may also limit illegitimate NHEJ as described below.

Chromatin Movement and the DSB Response: Localized Responses

The development of chromosome conformation capture (3C) and related high-throughput "C" technologies (e.g., Hi-C) has allowed the refinement of the chromosome territory models described above. These methods allow the interrogation of chromosome contacts that occur both within and between chromosomes. The most obvious of these contacts occur within topological associated domains (TADs) that are intrachromosomal regions of hundreds of kilobases in mammals that contain within them genes with similar expression dynamics (Dekker and Misteli, 2015). These TADs appear to arrange chromatin into regions whereby long-range interactions, such as between enhancers and promoters, can occur. Although markedly less frequent than TADs, interchromosomal contacts occur most often between chromosomes that are within the same chromosome territories as defined by FISH chromosome painting (Lieberman-Aiden et al., 2009). These concepts paint a picture of the nucleus whereby hierarchical levels of organization arrange chromatin in a dynamic non-random fashion. More recently indications that this organization can influence the DSB response itself and the outcome of DSB repair have arisen with implications for genetic stability and the generation of genetic abnormalities associated with cancer.

Studies of local chromatin dynamics at breaks are in their infancy. In yeast, 3C studies suggest that DSBs reduce the overall frequency of local (<100kb) interactions (Oza et al., 2009). This decrease appears to be correlated with the HR-dependent movement of the breaks to the nuclear periphery, as in G1-arrested cells where HR is inactive the interaction frequencies were less dynamic. This led to the proposal that damaged DNA is sequestered from the local chromatin environment to facilitate accurate DSB repair. This model is consistent with recent findings in mouse B-cells. By arresting cells in G1 to eliminate confounding HR-driven repair mechanisms, DSBs within a given chromosome most frequently led to translocation with genomic loci present *in cis* to the breaks (Hakim et al., 2012; Zhang et al., 2012).

A practical example of how differential localization can influence genome stability occurs in prostate cancer cells. When stimulated with dihydrotestosterone (DHT) TMPRSS2 gene expression is strongly induced in a manner dependent on topoisomerase II (TOP2) catalyzed DSBs that relieve torsional stresses that block transcription (Gómez-Herreros et al., 2014). These TOP2 dependent breaks have recently been mapped and frequently occur at breakpoints that are present in clinical fusions of TMPRSS2 with ETS transcription factors (e.g., ERG; Haffner et al., 2010). Linking back to nuclear organization, the TMPRSS2 and ETS transcription factor loci are frequently associated within the nuclear space (Lin et al., 2009; Mani et al., 2009). Consistent with the proximity model, fusions of TMPRSS2 to ERG (both localized on chromosome 21) occur in ∼90% of fusion cases whereas fusion to ETV1 (located on Chromosome 7) occurs at a much lower frequency (Tomlins et al., 2007). Thus chromosomal proximity can underlie translocations that are characteristic of cancer-associated genomic instability. Interestingly, mutations in TDP2, the enzyme that removes TOP2 that becomes trapped on DNA ends, results in persistent breaks and a human syndrome (Gómez-Herreros et al., 2014). Etoposide (a drug that traps TOP2 on broken DNA ends) causes DSBs that require TDP2 for break repair and resumption of transcription at TOP2 dependent loci, such as TMPRSS2. Indeed, TDP2 deficient mice also showed defective recovery of transcription in the developing mouse brain that was correlated with a reduction in the density of interneurons of the cerebellum. Given the clear relationship between transcription, a known modulator of local chromatin structure, and the DSB response it will be of prime interest to understand how these two interrelated cellular events impact on the higher order chromatin structure in combination and how this influences carcinogenesis and neurodevelopment.

It is becoming clear that the dynamic organization in 3D space of the nucleus has a direct influence on genomic stability and the DSB response. As technologies advance and methods for localized induction of DSBs in mammalian cells mature it will become possible to examine how these local chromatin interactions influence, and are influenced by, the DSB response.

COMMUNICATION BETWEEN THE DSB RESPONSE AND TRANSCRIPTION

Post-translational modifications on histones are a cornerstone of the DSB response. This has stimulated considerable interest in epigenetic marks on chromatin in the vicinity of the break site and the functional outcomes that this can entail. Recent studies from several labs have identified specific histone modifications driven by the DSB response that modulate transcription near the DSB site. We will outline some of these histone modifications in different physiological contexts and briefly discuss the functional outcomes of these events.

During meiosis SPO11, a TOP2-like enzyme, creates multiple DSBs to drive pairing between homologous chromosomes that initiates HR to induce crossovers and genetic variation (Lu and Yu, 2015). In males the *X*- and *Y*-chromosomes lack partners and remain largely unpaired during meiosis but remain replete with DSBs that activate a DSB response in an isolated structure called the *XY*-body (Turner, 2007). These DSBs are resected to initiate recombination events and are substrates for ATR activation and γH2AX formation throughout the sex chromosomes (Turner et al., 2004). As in mitotic cells this γH2AX laced chromatin

recruits MDC1 and RNF8, although whether RNF8 localization is strictly dependent on MDC1 in this context is unclear (Ichijima et al., 2011). In the *XY*-body MDC1 contributes to amplification of γH2AX signals and also induces SUMOylation but the target(s) remain undefined (Ichijima et al., 2011). As in somatic cells RNF8 drives H2A-ub formation in the *XY*-body and 53BP1 recruitment, however, BRCA1 does not spread throughout the *XY*-body but rather amplifies upstream signaling of ATR to γH2AX (Turner et al., 2004; Sin et al., 2012).

A striking outcome of this meiotic DSB signaling is the transcriptional silencing of genes on the *X*- and *Y*-chromosomes, a processed called meiotic sex chromosome inactivation (MSCI). MSCI occurs at least in part due to histone modifications including H2A-ub, H3 and H4 deacetylation, and H3K9 dimethylation; these marks persist during silencing throughout meiosis even after γH2AX has been resolved (Turner, 2007). MSCI fails in MDC1, H2AX, and BRCA1 null mice which correlates with male infertility, highlighting the role of transcriptional silencing in this context (Fernandez-Capetillo et al., 2003; Turner et al., 2004; Ichijima et al., 2011). Interestingly, RNF8 mice appear to maintain MSCI, however, males display reduced fertility possibly owing to other aspects of RNF8 dependent signaling in spermatogenesis (Li et al., 2010; Lu et al., 2010). These data highlight the importance of the DSB response in silencing transcription during meiosis to facilitate productive spermatogenesis.

The first study to suggest crosstalk between DSBs and transcription in somatic mammalian cells observed decreased RNA Pol I transcription in nucleoli of irradiated cells (Kruhlak et al., 2007). This silencing was dependent on ATM, MDC1, and NBS1 and prolonged in repair-deficient cells. Silencing in this context was independent of H2AX but the reasons for this uncoupling of the H2AX-MDC1 axis are unclear.

Persistent DSBs also silence transcription from RNA PolII-dependent promoters. Our group developed a system to simultaneously visualize DSB responses and nascent transcription in U2OS cells. Multiple breaks are induced within a LacO cassette 4 kb upstream of an inducible transcriptional unit in which the 3′-UTR (untranslated region) harbors 24 repeats of a stem loop that is recognized by the phage coat protein MS2 (Janicki et al., 2004; Shanbhag et al., 2010). This enables real time visualization of the DSB site and nascent transcription through the expression of mCherry-LacIFokI and YPF-MS2 fusion proteins, respectively. Introduction of a LacI molecule fused to the FOKI endonuclease creates a robust DSB response upstream of the transcriptional start site that effectively silences RNA PolII dependent transcription in an ATM and ubiquitin dependent manner. PolII was maintained at the locus, however, showed reduced levels of phosphorylation at the Serine 2 position of its carboxy terminal domain repeats, indicating impaired transitioning to elongating forms. This effect was strongly dependent on ATM and associated with H2A ubiquitylation. Transcription was rapidly restored upon nuclease termination and DSB repair, but persisted in the absence of the H2A-ub specific DUB USP16. Interestingly, deficiency in either RNF8 or RNF168 did not impact DSB silencing, albeit a modest reduction in silencing occurred upon combined knockdown. This implied that although these specific ubiquitylation events contribute to DSB silencing, other ATM-dependent events likely cooperate in suppressing transcription. This suggestion was recently supported by the finding that ATM-dependent phosphorylation of BAF180, a component of the chromatin remodeling PBAF complex, was required for H2AK119-ub and transcriptional silencing (Kakarougkas et al., 2014). Furthermore, depletion of BMI1 and EZH2, components of polycomb repressive complexes (PRC) 1 and 2, respectively, also contributed to DSB silencing (Ui et al., 2015). Importantly, ATM-dependent phosphorylation of ENL enhanced its interaction with BMI1 (i.e., PRC1) and led to transcriptional silencing. Together these data produce a model whereby multiple ATM-dependent signaling events lead to chromatin modifications that silence transcription *in cis* to DSBs. Interestingly, recent reports showed that transcriptional silencing of rDNA can occur *in trans* when DSBs are induced by UV-microbeam or IR (Ciccia et al., 2014; Larsen et al., 2014). Other studies have identified site-specific small RNAs generated by DICER-DROSHA in mammalian cells and in *Arabidopsis thaliana* that facilitates recognition of DSBs or repair by HR (Francia et al., 2012; Wei et al., 2012; Gao et al., 2014). It is important to note that this production of small RNAs at DSBs is distinct from silencing of RNA-PolI and PolII driven genes, as the ncRNAs do not appear to be promoter driven. Future studies exploring how specific DSBs influence transcriptional silencing both *in cis* and *in trans* on a global scale will be required to fully understand the extent of DSB silencing of promoter-driven transcription and in the production of small RNAs derived from the local chromatin.

During studies with our transcriptional reporter system we found that ATM-dependent silencing suppressed transcriptionally induced chromatin decondensation (Shanbhag et al., 2010). Despite observing overall positional stability of DSBs, Kruhlak et al. (2006) observed local expansion of chromatin following both IR and UV-microbeam damage. This correlated with decreased DNA density in electron microscopy but was independent of ATM and H2AX. In another example *D. melanogaster* IR induced local decondensation of HP1a-associated heterochromatin and this was proposed to facilitate DSB repair (Chiolo et al., 2011). At first, the finding that the DSB response can suppress transcription associated chromatin decompaction and that DSBs themselves induce decompaction appear at odds. However, it is highly likely that the pre-existing state of chromatin at the time of DSB induction influences the nature of the DSB response and the outcome of ATM signaling. To reconcile these issues it will be important to develop systems whereby DSBs can be induced within different chromatin states in the same biological system to determine how this influences chromatin dynamics.

CHROMATIN REORGANIZATION AS A REQUIREMENT FOR DSB REPAIR

DSBs are primarily repaired by one of two pathways in mammalian cells. In late S- and G2-phases after replication has

taken place, there is competition between rapid NHEJ and slower but more accurate HR. Understanding this balance has long been a goal of studies in the DSB repair field.

Tightly packed heterochromatin structures have been thought to be barriers to DSB repair and radioresistance (Chapman et al., 1999; Schaue and McBride, 2015). One possible reason for this is the limited accessibility of repair factors to highly compact chromatin structures. Indeed, it has often been observed that γH2AX preferentially forms in less dense euchromatin (Cowell et al., 2007). As mentioned above, correlative light and electron microscopy have demonstrated chromatin decompaction and that nucleosomes are disrupted in the vicinity of DSBs (Goldstein et al., 2013). Recently a pathway dependent on ATM that mediates DSB repair in heterochromatin regions has been described. It had long been known that ATM null cells repair the majority of DSBs (∼85%) with normal kinetics but that the remaining ∼15% of breaks remain unrepaired for long times after damage (Riballo et al., 2004). When analyzed by immunofluorescence of γH2AX in mouse cells these residual DSBs localize adjacent to heterochromatic "chromocenters" and required ATM-dependent phosphorylation of the KAP1 protein for resolution (Goodarzi et al., 2008). KAP1 is a component of heterochromatin and its phosphorylation by ATM drives relaxation of heterochromatin (Ziv et al., 2006). Phosphorylated KAP1 is maintained by the RNF8-RNF168-MDC1-53BP1 pathway and thus links ubiquitylation to DSB repair in heterochromatin.

As mentioned, translocations driven by NHEJ occur largely between chromosomes in close spatial proximity (Hakim et al., 2012; Zhang et al., 2012; Roukos et al., 2013). To prevent this happening at high frequency one may predict that the localization of chromatin on multiple levels must be controlled. In yeast, where HR is the dominant mechanism of repair, movement is viewed as a priority for DSB repair (Miné-Hattab and Rothstein, 2013). In mammals, where NHEJ predominates, limitations on movement may be necessary to prevent unwanted rejoining, but movement is observed in certain circumstances. As an example, breaks induced at the nuclear membrane were found to be positionally stable and did not relocalize to environments that were more permissive for HR rather they were repaired by alternative end-joining in place (Lemaître et al., 2014). Recently, studies from two laboratories generated DSBs within nucleoli of mammalian cells using endonucleases (Harding et al., 2015; van Sluis and McStay, 2015). In each case these DSBs and the rDNA chromatin itself were detected at the periphery of nucleoli indicating movement had occurred. This movement was associated with transcriptional silencing and when this silencing was blocked by inhibition of ATM the reorganization of nucleoli and the rDNA was prevented (**Figure 1**). We found that when NHEJ was blocked nucleolar reorganization and transcriptional silencing was enhanced; this was not observed when HR was inhibited. This suggested that NHEJ was the predominant mode of DSB repair in nucleoli, which was borne out by direct repair assays at the rDNA loci. Interestingly van Sluis and McStay (2015) observed HR-associated replication at the nucleolar periphery suggesting a role for HR in rDNA repair. Inefficient repair of rDNA by HR was also found to generate a loss of rDNA repeats; this effect was exacerbated by loss of NHEJ (Warmerdam et al., 2016). These complementary studies suggest that NHEJ occurs rapidly within nucleoli to maintain rDNA transcription. However, when these breaks remain unrepaired by NHEJ they are transcriptionally silencing and relocalize to the nucleolar periphery where they can be recognized by the HR machinery in a deleterious repair mechanism. Thus, DSBs in the rDNA recapitulated to some extent breaks in yeast where redistribution facilitates HR. This serves to highlight the role of nuclear organization in regulation of DSB repair pathway choice and may be a useful model system in which to study how ubiquitin and SUMO contribute to repair by NHEJ within the nucleolus and HR in the nucleolar periphery.

PERSPECTIVES

Over the last 30 years the mechanisms of the DSB response have been intensively studied and have provided an intricate model for the recognition and subsequent repair of DSBs dependent on post-translational modifications including phosphorylation, ubiquitylation, and SUMOylation. Of particular interest in the coming years will be how each of these modifications act in combination to drive accurate recognition of the breaks and repair pathway choice. SUMOylation and ubiquitylation offer a prime example of such concerted actions that are just beginning to be understood. Although technically challenging understanding how these multifaceted interactions are orchestrated is key to fully elucidating the DSB response. Recent evidence from many groups has begun to unravel these issues, and invariably they require multiple modifications rather than a single chromatin mark highlighting the importance of viewing the DSB response holistically rather than as singular distinct pathways.

Technological advances in the last decade have provided the tools necessary to interrogate how the organization of the nucleus both at the global (i.e., chromosome interaction) level and at the level of the epigenome. Key consideration in this regard include how the DSB response modulates chromatin interactions during the acute phase of the DNA damage response, and if persistent DNA damage signaling alters the epigenome. Equally important will be understanding how dynamic movements in the mammalian nucleus are controlled following DNA damage. Given recent evidence that such movements are important for the generation of chromosomal translocations a molecular understanding, such as that emerging in yeast, will be a fruitful area of future study.

It has also become increasingly apparent that the context (nuclear location, chromatin states, etc.) in which a DSB is induced has a significant effect on the nature of the response and outcome of repair. Several experimental approaches are

now available to induce breaks within defined chromatin environments and physical locations. These systems will undoubtedly facilitate a broader understanding of the contextual aspects of the DSB response and will lead to a more unified model of nuclear organization, cell signaling, and DSB repair.

AUTHOR CONTRIBUTIONS

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

FUNDING

This work was supported by NIH grants GM101149, CA17494, and CA138835 (to RAG), who is also supported by funds from the Abramson Family Cancer Research Institute and Basser Research Center for BRCA.

ACKNOWLEDGMENT

We apologize to colleagues whose work could not be cited due to space limitations.

REFERENCES

Acs, K., Luijsterburg, M. S., Ackermann, L., Salomons, F. A., Hoppe, T., and Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 18, 1345–1350. doi: 10.1038/nsmb.2188

Aten, J. A., Stap, J., Krawczyk, P. M., Van Oven, C. H., Hoebe, R. A., Essers, J., et al. (2004). Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* 303, 92–95. doi: 10.1126/science.1088845

Becker, A., Durante, M., Taucher-Scholz, G., and Jakob, B. (2014). ATM alters the otherwise robust chromatin mobility at sites of DNA double-strand breaks (DSBs) in human cells. *PLoS ONE* 9:e92640. doi: 10.1371/journal.pone.0092640

Bergink, S., and Jentsch, S. (2009). Principles of ubiquitin and SUMO modifications in DNA repair. *Nature* 458, 461–467. doi: 10.1038/nature07963

Bienko, M., Green, C. M., Crosetto, N., Rudolf, F., Zapart, G., Coull, B., et al. (2005). Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* 310, 1821–1824. doi: 10.1126/science.1120615

Bonner, W. M., Redon, C. E., Dickey, J. S., Nakamura, A. J., Sedelnikova, O. A., Solier, S., et al. (2008). GammaH2AX and cancer. *Nat. Rev. Cancer* 8, 957–967. doi: 10.1038/nrc2523

Botuyan, M. V., Lee, J., Ward, I. M., Kim, J.-E., Thompson, J. R., Chen, J., et al. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* 127, 1361–1373. doi: 10.1016/j.cell.2006.10.043

Caron, P., Choudjaye, J., Clouaire, T., Bugler, B., Daburon, V., Aguirrebengoa, M., et al. (2015). Non-redundant functions of ATM and DNA-PKcs in Response to DNA double-strand breaks. *Cell Rep.* 13, 1598–1609. doi: 10.1016/j.celrep.2015.10.024

Chapman, J. D., Stobbe, C. C., Gales, T., Das, I. J., Zellmer, D. L., Biade, S., et al. (1999). Condensed chromatin and cell inactivation by single-hit kinetics. *Radiat. Res.* 151, 433–441. doi: 10.2307/3579830

Chiolo, I., Minoda, A., Colmenares, S. U., Polyzos, A., Costes, S. V., and Karpen, G. H. (2011). Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* 144, 732–744. doi: 10.1016/j.cell.2011.02.012

Cho, N. W., Dilley, R. L., Lampson, M. A., and Greenberg, R. A. (2014). Interchromosomal homology searches drive directional ALT telomere movement and synapsis. *Cell* 159, 108–121. doi: 10.1016/j.cell.2014.08.030

Ciccia, A., Huang, J.-W., Izhar, L., Sowa, M. E., Harper, J. W., and Elledge, S. J. (2014). Treacher Collins syndrome TCOF1 protein cooperates with NBS1 in the DNA damage response. *Proc. Natl. Acad. Sci. U.S.A.* 111, 18631–18636. doi: 10.1073/pnas.1422488112

Ciechanover, A., Heller, H., Elias, S., Haas, A. L., and Hershko, A. (1980). ATP-dependent conjugation of reticulocyte proteins with the polypeptide required for protein degradation. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1365–1368. doi: 10.1073/pnas.77.3.1365

Cimprich, K. A., and Cortez, D. (2008). ATR: an essential regulator of genome integrity. *Nat. Rev. Mol. Cell Biol.* 9, 616–627. doi: 10.1038/nrm2450

Cooper, E. M., Boeke, J. D., and Cohen, R. E. (2010). Specificity of the BRISC deubiquitinating enzyme is not due to selective binding to Lys63-linked polyubiquitin. *J. Biol. Chem.* 285, 10344–10352. doi: 10.1074/jbc.M109.059667

Cowell, I. G., Sunter, N. J., Singh, P. B., Austin, C. A., Durkacz, B. W., and Tilby, M. J. (2007). γH2AX Foci form preferentially in euchromatin after ionising-radiation. *PLoS ONE* 2:e1057. doi: 10.1371/journal.pone.0001057

Cox, K. E., Maréchal, A., and Flynn, R. L. (2016). SMARCAL1 resolves replication stress at ALT telomeres. *Cell Rep.* 14, 1032–1040. doi: 10.1016/j.celrep.2016.01.011

Cremer, T., and Cremer, M. (2010). Chromosome territories. *Cold Spring Harb. Perspect. Biol.* 2, a003889. doi: 10.1101/cshperspect.a003889

Cubeñas-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

Dekker, J., and Misteli, T. (2015). Long-range chromatin interactions. *Cold Spring Harb. Perspect. Biol.* 7:a019356. doi: 10.1101/cshperspect.a019356

Dilley, R. L., and Greenberg, R. A. (2015). ALTernative telomere maintenance and cancer. *Trends Cancer* 1, 145–156. doi: 10.1016/j.trecan.2015.07.007

Dimitrova, N., Chen, Y.-C. M., Spector, D. L., and de Lange, T. (2008). 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. *Nature* 456, 524–528. doi: 10.1038/nature07433

Dion, V., and Gasser, S. M. (2013). Chromatin movement in the maintenance of genome stability. *Cell* 152, 1355–1364. doi: 10.1016/j.cell.2013.02.010

Dion, V., Kalck, V., Horigome, C., Towbin, B. D., and Gasser, S. M. (2012). Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat. Cell Biol.* 14, 502–509. doi: 10.1038/ncb2465

Doksani, Y., and de Lange, T. (2014). The role of double-strand break repair pathways at functional and dysfunctional telomeres. *Cold Spring Harb. Perspect. Biol.* 6, a016576. doi: 10.1101/cshperspect.a016576

Elia, A. E. H., Boardman, A. P., Wang, D. C., Huttlin, E. L., Everley, R. A., Dephoure, N., et al. (2015). Quantitative proteomic atlas of ubiquitination and acetylation in the DNA damage response. *Mol. Cell* 59, 867–881. doi: 10.1016/j.molcel.2015.05.006

Falk, M., Lukasova, E., Gabrielova, B., Ondrej, V., and Kozubek, S. (2007). Chromatin dynamics during DSB repair. *Biochim. Biophys. Acta* 1773, 1534–1545. doi: 10.1016/j.bbamcr.2007.07.002

Feng, L., Huang, J., and Chen, J. (2009). MERIT40 facilitates BRCA1 localization and DNA damage repair. *Genes Dev.* 23, 719–728. doi: 10.1101/gad.1770609

Feng, L., Wang, J., and Chen, J. (2010). The Lys63-specific deubiquitinating enzyme BRCC36 is regulated by two scaffold proteins localizing in different subcellular compartments. *J. Biol. Chem.* 285, 30982–30988. doi: 10.1074/jbc.M110.135392

Fernandez-Capetillo, O., Mahadevaiah, S. K., Celeste, A., Romanienko, P. J., Camerini-Otero, R. D., Bonner, W. M., et al. (2003). H2AX is required for chromatin remodeling and inactivation of sex chromosomes in male mouse meiosis. *Dev. Cell* 4, 497–508. doi: 10.1016/S1534-5807(03)00093-5

Floutsakou, I., Agrawal, S., Nguyen, T. T., Seoighe, C., Ganley, A. R. D., and McStay, B. (2013). The shared genomic architecture of human nucleolar organizer regions. *Genome Res.* 23, 2003–2012. doi: 10.1101/gr.157941.113

Fradet-Turcotte, A., Canny, M. D., Escribano-Díaz, C., Orthwein, A., Leung, C. C. Y., Huang, H., et al. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 499, 50–54. doi: 10.1038/nature12318

Francia, S., Michelini, F., Saxena, A., Tang, D., de Hoon, M., Anelli, V., et al. (2012). Site-specific DICER and DROSHA RNA products control the DNA-damage response. *Nat. Cell Biol.* 488, 1–8. doi: 10.1038/nature11179

Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012). RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* 26, 1179–1195. doi: 10.1101/gad.188284.112

Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009). Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* 462, 935–939. doi: 10.1038/nature08657

Gandhi, M., Evdokimova, V. N., T Cuenco, K., Nikiforova, M. N., Kelly, L. M., Stringer, J. R., et al. (2012). Homologous chromosomes make contact at the sites of double-strand breaks in genes in somatic G0/G1-phase human cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 9454–9459. doi: 10.1073/pnas.1205759109

Gao, M., Wei, W., Li, M.-M., Wu, Y.-S., Ba, Z., Jin, K.-X., et al. (2014). Ago2 facilitates Rad51 recruitment and DNA double-strand break repair by homologous recombination. *Cell Res.* 24, 532–541. doi: 10.1038/cr.2014.36

Gibbs-Seymour, I., Oka, Y., Rajendra, E., Weinert, B. T., Passmore, L. A., Patel, K. J., et al. (2015). Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. *Mol. Cell* 57, 150–164. doi: 10.1016/j.molcel.2014.12.001

Goldstein, M., Derheimer, F. A., Tait-Mulder, J., and Kastan, M. B. (2013). Nucleolin mediates nucleosome disruption critical for DNA double-strand break repair. *Proc. Natl. Acad. Sci. U.S.A.* 110, 16874–16879. doi: 10.1073/pnas.1306160110

Gómez-Herreros, F., Schuurs-Hoeijmakers, J. H. M., McCormack, M., Greally, M. T., Rulten, S., Romero-Granados, R., et al. (2014). TDP2 protects transcription from abortive topoisomerase activity and is required for normal neural function. *Nat. Genet.* 46, 516–521. doi: 10.1038/ng.2929

Goodarzi, A. A., Noon, A. T., Deckbar, D., Ziv, Y., Shiloh, Y., Löbrich, M., et al. (2008). ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol. Cell* 31, 167–177. doi: 10.1016/j.molcel.2008.05.017

Gudjonsson, T., Altmeyer, M., Savic, V., Toledo, L., Dinant, C., Grøfte, M., et al. (2012). TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. *Cell* 150, 697–709. doi: 10.1016/j.cell.2012.06.039

Guzzo, C. M., Berndsen, C. E., Zhu, J., Gupta, V., Datta, A., Greenberg, R. A., et al. (2012). RNF4-dependent hybrid SUMO-ubiquitin chains are signals for RAP80 and thereby mediate the recruitment of BRCA1 to sites of DNA damage. *Sci. Signal.* 5:ra88. doi: 10.1126/scisignal.2003485

Haffner, M. C., Aryee, M. J., Toubaji, A., Esopi, D. M., Albadine, R., Gurel, B., et al. (2010). Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat. Genet.* 42, 668–675. doi: 10.1038/ng.613

Hakim, O., Resch, W., Yamane, A., Klein, I., Kieffer-Kwon, K.-R., Jankovic, M., et al. (2012). DNA damage defines sites of recurrent chromosomal translocations in B lymphocytes. *Nature* 484, 69–74. doi: 10.1038/nature10909

Harding, S. M., Boiarsky, J. A., and Greenberg, R. A. (2015). ATM dependent silencing links nucleolar chromatin reorganization to DNA damage recognition. *Cell Rep.* 13, 251–259. doi: 10.1016/j.celrep.2015.08.085

Hershko, A., Ciechanover, A., Heller, H., Haas, A. L., and Rose, I. A. (1980). Proposed role of ATP in protein breakdown: conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc. Natl. Acad. Sci. U.S.A.* 77, 1783–1786. doi: 10.1073/pnas.77.4.1783

Hershko, A., Heller, H., Elias, S., and Ciechanover, A. (1983). Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *J. Biol. Chem.* 258, 8206–8214.

Hu, X., Paul, A., and Wang, B. (2012). Rap80 protein recruitment to DNA double-strand breaks requires binding to both small ubiquitin-like modifier (SUMO) and ubiquitin conjugates. *J. Biol. Chem.* 287, 25510–25519. doi: 10.1074/jbc.M112.374116

Huen, M. S. Y., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., et al. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 131, 901–914. doi: 10.1016/j.cell.2007.09.041

Huyen, Y., Zgheib, O., Ditullio, R. A., Gorgoulis, V. G., Zacharatos, P., Petty, T. J., et al. (2004). Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. *Nature* 432, 406–411. doi: 10.1038/nature03114

Ichijima, Y., Ichijima, M., Lou, Z., Nussenzweig, A., Camerini-Otero, R. D., Chen, J., et al. (2011). MDC1 directs chromosome-wide silencing of the sex chromosomes in male germ cells. *Genes Dev.* 25, 959–971. doi: 10.1101/gad.2030811

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078. doi: 10.1038/nature08467

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jakob, B., Splinter, J., Durante, M., and Taucher-Scholz, G. (2009). Live cell microscopy analysis of radiation-induced DNA double-strand break motion. *Proc. Natl. Acad. Sci. U.S.A.* 106, 3172–3177. doi: 10.1073/pnas.0810987106

Janicki, S. M., Tsukamoto, T., Salghetti, S. E., Tansey, W. P., Sachidanandam, R., Prasanth, K. V., et al. (2004). From silencing to gene expression: real-time analysis in single cells. *Cell* 116, 683–698. doi: 10.1016/S0092-8674(04)00171-0

Jentsch, S., McGrath, J. P., and Varshavsky, A. (1987). The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* 329, 131–134. doi: 10.1038/329131a0

Jiang, Q., Paramasivam, M., Aressy, B., Wu, J., Bellani, M., Tong, W., et al. (2015). MERIT40 cooperates with BRCA2 to resolve DNA interstrand cross-links. *Genes Dev.* 29, 1955–1968. doi: 10.1101/gad.264192.115

Kakarougkas, A., Ismail, A., Chambers, A. L., Riballo, E., Herbert, A. D., Künzel, J., et al. (2014). Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. *Mol. Cell* 55, 723–732. doi: 10.1016/j.molcel.2014.06.028

Kalocsay, M., Hiller, N. J., and Jentsch, S. (2009). Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol. Cell* 33, 335–343. doi: 10.1016/j.molcel.2009.01.016

Kim, H., Chen, J., and Yu, X. (2007). Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. *Science* 316, 1202–1205. doi: 10.1126/science.1139621

Kocyłowski, M. K., Rey, A. J., Stewart, G. S., and Halazonetis, T. D. (2015). Ubiquitin-H2AX fusions render 53BP1 recruitment to DNA damage sites independent of RNF8 or RNF168. *Cell Cycle* 14, 1748–1758. doi: 10.1080/15384101.2015.1010918

Kolas, N. K., Chapman, J. R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F. D., et al. (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 318, 1637–1640. doi: 10.1126/science.1150034

Krawczyk, P. M., Borovski, T., Stap, J., Cijsouw, T., ten Cate, R., Medema, J. P., et al. (2012). Chromatin mobility is increased at sites of DNA double-strand breaks. *J. Cell Sci.* 125, 1–7. doi: 10.1242/jcs.089847

Kruhlak, M., Crouch, E. E., Orlov, M., Montaño, C., Gorski, S. A., Nussenzweig, A., et al. (2007). The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. *Nature* 447, 730–734. doi: 10.1038/nature05842

Kruhlak, M. J., Celeste, A., Dellaire, G., Fernandez-Capetillo, O., Müller, W. G., McNally, J. G., et al. (2006). Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol.* 172, 823–834. doi: 10.1083/jcb.200510015

Larsen, D. H., Hari, F., Clapperton, J. A., Gwerder, M., Gutsche, K., Altmeyer, M., et al. (2014). The NBS1-Treacle complex controls ribosomal RNA transcription in response to DNA damage. *Nat. Cell Biol.* 16, 792–803. doi: 10.1038/ncb3007

Lemaître, C., Grabarz, A., Tsouroula, K., Andronov, L., Furst, A., Pankotai, T., et al. (2014). Nuclear position dictates DNA repair pathway choice. *Genes Dev.* 28, 2450–2463. doi: 10.1101/gad.248369.114

Li, L., Halaby, M.-J., Hakem, A., Cardoso, R., El Ghamrasni, S., Harding, S., et al. (2010). Rnf8 deficiency impairs class switch recombination, spermatogenesis, and genomic integrity and predisposes for cancer. *J. Exp. Med.* 207, 983–997. doi: 10.1084/jem.20092437

Lieberman-Aiden, E., van Berkum, N. L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326, 289–293. doi: 10.1126/science.1181369

Lin, C., Yang, L., Tanasa, B., Hutt, K., Ju, B.-G., Ohgi, K., et al. (2009). Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. *Cell* 139, 1069–1083. doi: 10.1016/j.cell.2009.11.030

Lottersberger, F., Karssemeijer, R. A., Dimitrova, N., and de Lange, T. (2015). 53BP1 and the LINC complex promote microtubule-dependent DSB mobility and DNA repair. *Cell* 163, 880–893. doi: 10.1016/j.cell.2015.09.057

Lu, L.-Y., Wu, J., Ye, L., Gavrilina, G. B., Saunders, T. L., and Yu, X. (2010). RNF8-dependent histone modifications regulate nucleosome removal during spermatogenesis. *Dev. Cell* 18, 371–384. doi: 10.1016/j.devcel.2010.01.010

Lu, L.-Y., and Yu, X. (2015). Double-strand break repair on sex chromosomes: challenges during male meiotic prophase. *Cell Cycle* 14, 516–525. doi: 10.1080/15384101.2014.998070

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., et al. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 131, 887–900. doi: 10.1016/j.cell.2007.09.040

Mallette, F. A., Mattiroli, F., Cui, G., Young, L. C., Hendzel, M. J., Mer, G., et al. (2012). RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J.* 31, 1865–1878. doi: 10.1038/emboj.2012.47

Mani, R.-S., Tomlins, S. A., Callahan, K., Ghosh, A., Nyati, M. K., Varambally, S., et al. (2009). Induced chromosomal proximity and gene fusions in prostate cancer. *Science* 326, 1230. doi: 10.1126/science.1178124

Matsuoka, S., Ballif, B. A., Smogorzewska, A., McDonald, E. R., Hurov, K. E., Luo, J., et al. (2007). ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* 316, 1160–1166. doi: 10.1126/science.1140321

Mattiroli, F., Vissers, J. H. A., van Dijk, W. J., Ikpa, P., Citterio, E., Vermeulen, W., et al. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150, 1182–1195. doi: 10.1016/j.cell.2012.08.005

Matunis, M. J., Coutavas, E., and Blobel, G. (1996). A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J. Cell Biol.* 135, 1457–1470. doi: 10.1083/jcb.135.6.1457

Meerang, M., Ritz, D., Paliwal, S., Garajova, Z., Bosshard, M., Mailand, N., et al. (2011). The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks. *Nat. Cell Biol.* 13, 1376–1382. doi: 10.1038/ncb2367

Messick, T. E., and Greenberg, R. A. (2009). The ubiquitin landscape at DNA double-strand breaks. *J. Cell Biol.* 187, 319–326. doi: 10.1083/jcb.200908074

Miné-Hattab, J., and Rothstein, R. (2012). Increased chromosome mobility facilitates homology search during recombination. *Nat. Cell Biol.* 14, 510–517. doi: 10.1038/ncb2472

Miné-Hattab, J., and Rothstein, R. (2013). DNA in motion during double-strand break repair. *Trends Cell Biol.* 23, 529–536. doi: 10.1016/j.tcb.2013.05.006

Morris, J. R., Boutell, C., Keppler, M., Densham, R., Weekes, D., Alamshah, A., et al. (2009). The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. *Nature* 462, 1–6. doi: 10.1038/nature 08593

Morris, J. R., and Solomon, E. (2004). BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum. Mol. Genet.* 13, 807–817. doi: 10.1093/hmg/ddh095

Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M. B., Roberts, T. M., Brown, G. W., et al. (2008). Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 322, 597–602. doi: 10.1126/science.1162790

Nelms, B. E., Maser, R. S., MacKay, J. F., Lagally, M. G., and Petrini, J. H. J. (1998). In situ visualization of DNA double-strand break repair in human fibroblasts. *Science* 280, 590–592. doi: 10.1126/science.280.5363.590

Neumaier, T., Swenson, J., Pham, C., Polyzos, A., Lo, A. T., Yang, P., et al. (2012). Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. *Proc. Natl. Acad. Sci. U.S.A.* 109, 443–448. doi: 10.1073/pnas.1117849108

Neumann, F. R., Dion, V., Gehlen, L. R., Tsai-Pflugfelder, M., Schmid, R., Taddei, A., et al. (2012). Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. *Genes Dev.* 26, 369–383. doi: 10.1101/gad.176156.111

Nikkilä, J., Coleman, K. A., Morrissey, D., Pylkäs, K., Erkko, H., Messick, T. E., et al. (2009). Familial breast cancer screening reveals an alteration in the RAP80 UIM domain that impairs DNA damage response function. *Oncogene* 28, 1843–1852. doi: 10.1038/onc.2009.33

O'Sullivan, R. J., and Almouzni, G. (2014). Assembly of telomeric chromatin to create ALTernative endings. *Trends Cell Biol.* 24, 675–685. doi: 10.1016/j.tcb.2014.07.007

Oza, P., Jaspersen, S. L., Miele, A., Dekker, J., Peterson, C. L., Oza, P., et al. (2009). Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. *Genes Dev.* 23, 912–927. doi: 10.1101/gad.1782209

Palm, W., and de Lange, T. (2008). How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* 42, 301–334. doi: 10.1146/annurev.genet.41.110306.130350

Patterson-Fortin, J., Shao, G., Bretscher, H., Messick, T. E., and Greenberg, R. A. (2010). Differential regulation of JAMM domain deubiquitinating enzyme activity within the RAP80 complex. *J. Biol. Chem.* 285, 30971–30981. doi: 10.1074/jbc.M110.135319

Pickett, H. A., and Reddel, R. R. (2015). Molecular mechanisms of activity and derepression of alternative lengthening of telomeres. *Nat. Struct. Mol. Biol.* 22, 875–880. doi: 10.1038/nsmb.3106

Polanowska, J., Martin, J. S., García-Muse, T., Petalcorin, M. I. R., and Boulton, S. J. (2006). A conserved pathway to activate BRCA1-dependent ubiquitylation at DNA damage sites. *EMBO J.* 25, 2178–2188. doi: 10.1038/sj.emboj.7601102

Popovic, D., Vucic, D., and Dikic, I. (2014). Ubiquitination in disease pathogenesis and treatment. *Nat. Med.* 20, 1242–1253. doi: 10.1038/nm.3739

Potts, P. R., and Yu, H. (2007). The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. *Nat. Struct. Mol. Biol.* 14, 581–590. doi: 10.1038/nsmb1259

Psakhye, I., and Jentsch, S. (2012). Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* 151, 807–820. doi: 10.1016/j.cell.2012.10.021

Radhakrishnan, S. K., Jette, N., and Lees-Miller, S. P. (2014). Non-homologous end joining: emerging themes and unanswered questions. *DNA Repair (Amst)* 17, 2–8. doi: 10.1016/j.dnarep.2014.01.009

Ragland, R. L., Patel, S., Rivard, R. S., Smith, K., Peters, A. A., Bielinsky, A.-K., et al. (2013). RNF4 and PLK1 are required for replication fork collapse in ATR-deficient cells. *Genes Dev.* 27, 2259–2273. doi: 10.1101/gad.223180.113

Rajalingam, K., and Dikic, I. (2016). SnapShot: expanding the ubiquitin code. *Cell* 164, 1074–1074.e1. doi: 10.1016/j.cell.2016.02.019

Riballo, E., Kühne, M., Rief, N., Doherty, A., Smith, G. C. M., Recio, M.-J., et al. (2004). A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. *Mol. Cell* 16, 715–724. doi: 10.1016/j.molcel.2004.10.029

Roukos, V., Voss, T. C., Schmidt, C. K., Lee, S., Wangsa, D., and Misteli, T. (2013). Spatial dynamics of chromosome translocations in living cells. *Science* 341, 660–664. doi: 10.1126/science.1237150

Ryu, T., Spatola, B., Delabaere, L., Bowlin, K., Hopp, H., Kunitake, R., et al. (2015). Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. *Nat. Cell Biol.* 17, 1401–1411. doi: 10.1038/ncb3258

Sarangi, P., and Zhao, X. (2015). SUMO-mediated regulation of DNA damage repair and responses. *Trends Biochem. Sci.* 40, 233–242. doi: 10.1016/j.tibs.2015.02.006

Schaue, D., and McBride, W. H. (2015). Opportunities and challenges of radiotherapy for treating cancer. *Nat. Rev. Clin. Oncol.* 12, 527–540. doi: 10.1038/nrclinonc.2015.120

Shanbhag, N. M., Rafalska-Metcalf, I. U., Balane-Bolivar, C., Janicki, S. M., and Greenberg, R. A. (2010). ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141, 970–981. doi: 10.1016/j.cell.2010.04.038

Shao, G., Patterson-Fortin, J., Messick, T. E., Feng, D., Shanbhag, N., Wang, Y., et al. (2009). MERIT40 controls BRCA1-Rap80 complex integrity and recruitment to DNA double-strand breaks. *Genes Dev.* 23, 740–754. doi: 10.1101/gad.1739609

Shrivastav, M., De Haro, L. P., and Nickoloff, J. A. (2008). Regulation of DNA double-strand break repair pathway choice. *Cell Res.* 18, 134–147. doi: 10.1038/cr.2007.111

Sin, H.-S., Barski, A., Zhang, F., Kartashov, A. V., Nussenzweig, A., Chen, J., et al. (2012). RNF8 regulates active epigenetic modifications and escape gene activation from inactive sex chromosomes in post-meiotic spermatids. *Genes Dev.* 26, 2737–2748. doi: 10.1101/gad.202713.112

Sobhian, B., Shao, G., Lilli, D. R., Culhane, A. C., Moreau, L. A., Xia, B., et al. (2007). RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* 316, 1198–1202. doi: 10.1126/science.11 39516

Solyom, S., Aressy, B., Pylkäs, K., Patterson-Fortin, J., Hartikainen, J. M., Kallioniemi, A., et al. (2012). Breast cancer-associated Abraxas mutation disrupts nuclear localization and DNA damage response functions. *Sci. Transl. Med.* 4:122ra23. doi: 10.1126/scitranslmed.3003223

Soutoglou, E., Dorn, J. F., Sengupta, K., Jasin, M., Nussenzweig, A., Ried, T., et al. (2007). Positional stability of single double-strand breaks in mammalian cells. *Nat. Cell Biol.* 9, 675–682. doi: 10.1038/ncb1591

Spence, J., Sadis, S., Haas, A. L., and Finley, D. (1995). A ubiquitin mutant with specific defects in DNA repair and multiubiquitination. *Mol. Cell. Biol.* 15, 1265–1273. doi: 10.1128/MCB.15.3.1265

Spichal, M., Brion, A., Herbert, S., Cournac, A., Marbouty, M., Zimmer, C., et al. (2016). Evidence for a dual role of actin in regulating chromosome organization and dynamics in yeast. *J. Cell Sci.* 129, 681–692. doi: 10.1242/jcs.175745

Stap, J., Krawczyk, P. M., Van Oven, C. H., Barendsen, G. W., Essers, J., Kanaar, R., et al. (2008). Induction of linear tracks of DNA double-strand breaks by alpha-particle irradiation of cells. *Nat. Methods* 5, 261–266. doi: 10.1038/nmeth.f.206

Strecker, J., Gupta, G. D., Zhang, W., Bashkurov, M., Landry, M.-C., Pelletier, L., et al. (2016). DNA damage signalling targets the kinetochore to promote chromatin mobility. *Nat. Cell Biol.* 18, 281–290. doi: 10.1038/ncb3308

Tang, J., Cho, N. W., Cui, G., Manion, E. M., Shanbhag, N. M., Botuyan, M. V., et al. (2013). Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. *Nat. Struct. Mol. Biol.* 20, 317–325. doi: 10.1038/nsmb.2499

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., et al. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393. doi: 10.1038/nature15401

Tomlins, S. A., Laxman, B., Dhanasekaran, S. M., Helgeson, B. E., Cao, X., Morris, D. S., et al. (2007). Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* 448, 595–599. doi: 10.1038/nature06024

Torres-Rosell, J., Sunjevaric, I., De Piccoli, G., Sacher, M., Eckert-Boulet, N., Reid, R., et al. (2007). The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat. Cell Biol.* 9, 923–931. doi: 10.1038/ncb1619

Tumbar, T., and Belmont, A. S. (2001). Interphase movements of a DNA chromosome region modulated by VP16 transcriptional activator. *Nat. Cell Biol.* 3, 134–139. doi: 10.1038/35055033

Turner, J. M. A. (2007). Meiotic sex chromosome inactivation. *Development* 134, 1823–1831. doi: 10.1242/dev.000018

Turner, J. M. A., Aprelikova, O., Xu, X., Wang, R., Kim, S., Chandramouli, G. V. R., et al. (2004). BRCA1, histone H2AX phosphorylation, and male meiotic sex chromosome inactivation. *Curr. Biol.* 14, 2135–2142. doi: 10.1016/j.cub.2004.11.032

Ui, A., Nagaura, Y., and Yasui, A. (2015). Transcriptional elongation factor ENL phosphorylated by ATM recruits polycomb and switches off transcription for DSB repair. *Mol. Cell* 58, 468–482. doi: 10.1016/j.molcel.2015.03.023

van Sluis, M., and McStay, B. (2015). A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. *Genes Dev.* 29, 1151–1163. doi: 10.1101/gad.260703.115

Wang, B., and Elledge, S. J. (2007). Ubc13/Rnf8 ubiquitin ligases control foci formation of the Rap80/Abraxas/Brca1/Brcc36 complex in response to DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 104, 20759–20763. doi: 10.1073/pnas.0710061104

Wang, B., Hurov, K., Hofmann, K., and Elledge, S. J. (2009). NBA1, a new player in the Brca1 A complex, is required for DNA damage resistance and checkpoint control. *Genes Dev.* 23, 729–739. doi: 10.1101/gad.1770309

Wang, B., Matsuoka, S., Ballif, B. A., Zhang, D., Smogorzewska, A., Gygi, S. P., et al. (2007). Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* 316, 1194–1198. doi: 10.1126/science.1139476

Warmerdam, D. O., van den Berg, J., and Medema, R. H. (2016). Breaks in the 45S rDNA lead to recombination-mediated loss of repeats. *Cell Rep.* 14, 2519–2527. doi: 10.1016/j.celrep.2016.02.048

Wei, W., Ba, Z., Gao, M., Wu, Y., Ma, Y., Amiard, S., et al. (2012). A role for small RNAs in DNA double-strand break repair. *Cell* 149, 101–112. doi: 10.1016/j.cell.2012.03.002

Wu-Baer, F., Lagrazon, K., Yuan, W., and Baer, R. (2003). The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. *J. Biol. Chem.* 278, 34743–34746. doi: 10.1074/jbc.C300249200

Yin, Y., Seifert, A., Chua, J. S., Maure, J.-F., Golebiowski, F., and Hay, R. T. (2012). SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. *Genes Dev.* 26, 1196–1208. doi: 10.1101/gad.189274.112

Zeqiraj, E., Tian, L., Piggott, C. A., Pillon, M. C., Duffy, N. M., Ceccarelli, D. F., et al. (2015). Higher-order assembly of BRCC36-KIAA0157 is required for DUB activity and biological function. *Mol. Cell* 59, 970–983. doi: 10.1016/j.molcel.2015.07.028

Zhang, Y., McCord, R. P., Ho, Y.-J., Lajoie, B. R., Hildebrand, D. G., Simon, A. C., et al. (2012). Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. *Cell* 148, 908–921. doi: 10.1016/j.cell.2012.02.002

Ziv, Y., Bielopolski, D., Galanty, Y., Lukas, C., Taya, Y., and Schultz, D. C. (2006). Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat. Cell Biol.* 8, 870–876. doi: 10.1038/ncb1446

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 13 May 2016
doi: 10.3389/fgene.2016.00087

Functions of Ubiquitin and SUMO in DNA Replication and Replication Stress

*Néstor García-Rodríguez†, Ronald P. Wong† and Helle D. Ulrich**

Institute of Molecular Biology, Mainz, Germany

Complete and faithful duplication of its entire genetic material is one of the essential prerequisites for a proliferating cell to maintain genome stability. Yet, during replication DNA is particularly vulnerable to insults. On the one hand, lesions in replicating DNA frequently cause a stalling of the replication machinery, as most DNA polymerases cannot cope with defective templates. This situation is aggravated by the fact that strand separation in preparation for DNA synthesis prevents common repair mechanisms relying on strand complementarity, such as base and nucleotide excision repair, from working properly. On the other hand, the replication process itself subjects the DNA to a series of hazardous transformations, ranging from the exposure of single-stranded DNA to topological contortions and the generation of nicks and fragments, which all bear the risk of inducing genomic instability. Dealing with these problems requires rapid and flexible responses, for which posttranslational protein modifications that act independently of protein synthesis are particularly well suited. Hence, it is not surprising that members of the ubiquitin family, particularly ubiquitin itself and SUMO, feature prominently in controlling many of the defensive and restorative measures involved in the protection of DNA during replication. In this review we will discuss the contributions of ubiquitin and SUMO to genome maintenance specifically as they relate to DNA replication. We will consider cases where the modifiers act during regular, i.e., unperturbed stages of replication, such as initiation, fork progression, and termination, but also give an account of their functions in dealing with lesions, replication stalling and fork collapse.

Keywords: ubiquitin, SUMO, DNA replication, DNA replication stress, DNA damage, DNA repair, genome stability

OPEN ACCESS

Edited by:
Kristijan Ramadan,
University of Oxford, UK

Reviewed by:
Dana Branzei,
IFOM, The FIRC Institute of Molecular Oncology, Italy
Aga Gambus,
University of Birmingham, UK

****Correspondence:***
Helle D. Ulrich
h.ulrich@imb-mainz.de

† These authors have contributed equally to this work.

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *21 March 2016*
Accepted: *27 April 2016*
Published: *13 May 2016*

Citation:
García-Rodríguez N, Wong RP and Ulrich HD (2016) Functions of Ubiquitin and SUMO in DNA Replication and Replication Stress. Front. Genet. 7:87. doi: 10.3389/fgene.2016.00087

INTRODUCTION

DNA replication in eukaryotes is a multi-step process that is tightly coupled to both cell cycle progression and the DNA damage response (Leman and Noguchi, 2013; Siddiqui et al., 2013; Berti and Vindigni, 2016). After completion of mitosis during the G1 stage of the cell cycle, replication origins are prepared for activation in a process called origin licensing (Siddiqui et al., 2013). This reaction results in the formation of pre-replicative complexes (pre-RCs) at replication origins, which include key components of the replicative helicase, albeit in an inactive form. Licensing restricts origin firing to once per cell cycle, thus preventing genome instability induced by re-replication. At the entry into S phase, DNA replication is initiated by the action of cell cycle-regulated kinases, resulting in the activation of the replicative helicase and the separation of strands to form the first replication forks. This is helicase and several DNA polymerases, but also a large number of accessory factors responsible accompanied by the assembly of replisomes, multi-protein complexes that comprise not only the for monitoring replication fork

progression, generating checkpoint and damage signals, and coordination of DNA synthesis with chromatin assembly (Leman and Noguchi, 2013). In eukaryotes, origin firing follows a temporally regulated program throughout S phase, giving rise to distinct early- and late-replicating regions of the genome (Renard-Guillet et al., 2014). The pattern of origin firing is flexible and reacts to situations such as the stalling of individual forks or the perception of a global damage signal by the cell. DNA synthesis proceeds bi-directionally, initiated by the deposition of short RNA primers that are subsequently extended by DNA polymerase α. Leading and lagging strand replication by the main replicative DNA polymerases ε and δ, respectively, is closely coordinated with the unwinding of the template DNA. As a consequence, accumulation of extended regions of single-stranded (ss)DNA is perceived as a sign of fork stalling and triggers a checkpoint response that suppresses the firing of late replication origins and prevents entry into mitosis (Leman and Noguchi, 2013). The nicks in the emerging lagging strand, arising from its discontinuous synthesis, are successively sealed by DNA ligase. As replication units (replicons) from neighboring origins meet, replication forks merge and replication is terminated by the disassembly of the replisomes. Since DNA replication takes place in the context of chromatin, removal of nucleosomes in front of the helicase and their renewed deposition after passage of the replication fork need to be synchronized with DNA synthesis (Groth, 2009). This coordination, actively mediated by components of the replisome, also protects against the loss of epigenetic marks during replication.

Accurate control over all stages of DNA replication is of vital importance for the maintenance of genome integrity in proliferating cells. Both incomplete replication and over-replication interfere with proper chromosome segregation, and defects in replication fidelity pose a serious threat to genome stability due to an increased mutation load. Hence, the mechanisms ensuring complete and accurate replication need to be considered as part of a cell's repertoire to defend itself against insults to its genome. By reversibly altering the properties of their target proteins, various different posttranslational protein modifications contribute significantly to these processes. Over the past decade, we have witnessed the emergence of ubiquitin and SUMO as key regulators of genome maintenance pathways (Ulrich and Walden, 2010; Jackson and Durocher, 2013). Although best known for mediating protein degradation, ubiquitin can convey a variety of non-proteolytic signals. This can partly be ascribed to the effects of mono-ubiquitylation, but also to ubiquitin's ability to form polymeric chains of different geometries, recognized by highly chain-selective effector proteins (Komander and Rape, 2012). More recently, it has been realized that SUMO can also trigger degradation of its targets by forming polymeric SUMO chains interacting with a class of enzymes known as SUMO-targeted ubiquitin ligases (STUbLs; Prudden et al., 2007; Sriramachandran and Dohmen, 2014). Hence, both proteolytic and non-proteolytic contributions need to be considered in discussing the effects of ubiquitin and SUMO on DNA replication.

This review will cover the functions of ubiquitin and SUMO during unperturbed replication, i.e., during origin licensing, replication elongation and termination, and with regard to chromatin assembly and nuclear structure. Another important aspect will be the response to replication stress. As much of the recent progress in the field can be ascribed to large-scale siRNA screens and proteomic approaches, mechanistic information is often lagging behind the identification of novel modification targets and conjugation factors. We will therefore refrain from giving a comprehensive account of all the enzymes and substrates involved in DNA replication and rather focus on representative examples where a relevant functional context is available.

REPLICATION OF INTACT DNA

The function of ubiquitylation in unperturbed DNA replication has been the subject of an excellent recent review (Moreno and Gambus, 2015), to which the reader is referred for details, particularly with respect to proteolytic functions of ubiquitylation. Here we complement this with information on the roles of protein SUMOylation, and we discuss the recurring problem of distinguishing modifications that are inherently part of the replication process from those occurring in response to spontaneous problems based on difficult-to-replicate sequences or chromatin regions.

Contributions of Ubiquitin and SUMO to Origin Licensing and Replication Initiation

At the entry into S phase, ubiquitin functions predominantly as an inducer of proteasomal degradation, owing to its prominent role in cell cycle regulation (Teixeira and Reed, 2013). Preparation for DNA replication requires loading of the hexameric ring-shaped Mcm2-7 complex onto origins of replication, mediated by the origin recognition complex (ORC) and two auxiliary factors, Cdt1 and Cdc6 (Siddiqui et al., 2013). Establishment of the pre-RC can only proceed late in mitosis and during G1 phase, when cyclin-dependent kinase (CDK) levels are low. This is achieved by a large, multi-subunit ubiquitin ligase, the anaphase promoting complex (APC/C), which induces degradation of mitotic cyclins and of the CDK-activating phosphatase Cdc25 (King et al., 1995; Donzelli et al., 2002; Teixeira and Reed, 2013). In vertebrates, the APC/C also targets the Cdt1 inhibitor geminin for degradation (McGarry and Kirschner, 1998).

In order to initiate S phase, two helicase coactivators – Cdc45 and the GINS complex – are recruited to pre-RCs, assembling the active replicative helicase, the CMG complex (Cdc45-Mcm2-7-GINS). Once the initial unwinding occurs, DNA polymerases and the sliding clamp, PCNA, are recruited to assemble the replisome and establish the replication fork (Leman and Noguchi, 2013). Origin firing requires a rise in CDK activity. Accordingly, APC/C activity is downregulated, mainly by inhibition of its regulatory subunit Cdh1 (Eldridge et al., 2006; Fukushima et al., 2013; Lau et al., 2013), but also by autoubiquitylation and degradation of its cognate ubiquitin conjugating enzyme (E2), UbcH10, a process induced in the absence of APC/C substrates (Rape and Kirschner, 2004). This allows an accumulation of G1-specific

cyclins. Additionally, CDK inhibitors, such as p27 and p21, are degraded (Starostina and Kipreos, 2012). Three different E3s, KPC, Pirh2 and the Skp1-Cullin-F-box complex SCF^{Skp2}, are known to act on p27 in a temporally and spatially ordered fashion during G1 and early S phase. The p21 protein is also a substrate of SCF^{Skp2}, but in addition, this factor is targeted by an intriguing mechanism that directly couples ubiquitylation to S phase entry (Abbas et al., 2008; Kim et al., 2008). The cognate E3, Cullin-RING ligase $CRL4^{Cdt2}$, recognizes its substrate only in conjunction with the replication clamp, PCNA, and only when PCNA is encircling DNA. The relevant degradation signal, which includes a PCNA-interacting peptide (PIP), is also found in other factors whose removal is associated with S phase, such as the fission yeast inhibitor of ribonucleotide reductase, Spd1, and the G1-specific transcription factor E2F1 from *Drosophila melanogaster*. (Havens and Walter, 2011; Ulrich, 2014). Thus, by coupling substrate recognition to binding of loaded PCNA, $CRL4^{Cdt2}$ is able to read the state of the replication machinery as an activating signal.

Firing of origins needs to be strictly limited to once per cell cycle to avoid problems of re-replication. This is achieved through a process known as origin licensing that restricts pre-RC assembly to G1 (Moreno and Gambus, 2015). In order to render the process irreversible, essential loading factors, such as Cdt1 and Cdc6, are eliminated when cells enter S phase. In many organisms, this is again mediated by ubiquitin-mediated proteolysis. Human Cdt1 is ubiquitylated by at least two different E3s of the CRL family, SCF^{Skp2} and – as described above – $CRL4^{Cdt2}$ (Li et al., 2003; Zhong et al., 2003). Cdc6 is deactivated either by export from the nucleus or by degradation following its ubiquitylation by $CRL4^{Cdt2}$ (Saha et al., 1998; Clijsters and Wolthuis, 2014). In budding yeast, SCF^{Cdc4} mediates ubiquitylation of Cdc6 (Drury et al., 1997).

In contrast to the pervasive influence of ubiquitin, SUMO appears to exert more subtle regulatory effects on replication initiation. In a cell-free system based on *Xenopus laevis* egg extracts, inhibition of SUMOylation was found to increase replication rates by allowing a larger number of origins to fire (Bonne-Andrea et al., 2013). The negative effect of SUMO on origin firing was attributable to the modification of cyclin E following recruitment of the cyclin E-CDK complex to pre-RCs. The notion that most cells only use a sub-set of their potential origins in each S phase suggests that SUMO may in this context contribute to limiting excessive origin firing. In the budding yeast, Wei and Zhao (2016) recently reported an apparently unrelated phenomenon that likewise suggests a negative impact of SUMO on origin firing. They observed a cell-cycle regulated SUMOylation of Mcm2-7, peaking at the pre-RC stage when the complex is loaded onto origins, but declining upon origin firing at the G1-to-S transition. Artificial enhancement of local SUMOylation inhibited CMG assembly and origin firing, most likely by means of recruiting a phosphatase that reversed essential phosphorylation events required for CMG activation. Intriguingly, both SUMOylation and deSUMOylation of Mcm proteins are accomplished by multiple E3s and isopeptidases in a subunit-specific manner, and significant differences were noted in the cell cycle regulation of individual Mcm subunits (de Albuquerque et al., 2016). Moreover, it is important to note that other components of pre-RCs have also been identified as SUMOylation targets, among them the subunits of ORC (Golebiowski et al., 2009). Hence, it remains to be established whether the negative effect of SUMO on origin firing observed in this study is due to the modification of an individual Mcm subunit, the Mcm2-7 complex in its entirety, or a general accumulation of SUMO around the pre-RC.

Proteomic Analyses of Replicating Chromatin

A wealth of information has emerged from the isolation of chromatin-associated proteins from proliferating cells, followed by mass spectrometry. Proteome-wide analyses identified numerous replication factors as ubiquitylation targets in human cells, including integral components of the replisome such as GINS and the Mcm2-7 helicase complex, the Replication Factor C (RFC) clamp loader complex, as well as all the replicative DNA polymerases and many associated factors (Wagner et al., 2011). Comparison of substrate spectra in the absence and presence of the proteasome inhibitor MG132 revealed both proteolytic and non-proteolytic roles of ubiquitylation. Similarly, a systematic screen in the budding yeast *Saccharomyces cerevisiae* identified a significant number of replisome components targeted by SUMO, including components of the Mcm2-7 complex, subunits of DNA polymerases and the RFC complex, the Rad27 flap endonuclease and topoisomerases Top1 and Top2 (Cremona et al., 2012). A recent study, using a procedure to isolate proteins on nascent DNA (iPOND) followed by mass spectrometry, characterized proteins enriched in the proximity of replisomes in an unprecedented spatial resolution. Interestingly, SUMOylation was predominant on factors near the replisome, while ubiquitylated proteins prevailed on mature chromatin (Lopez-Contreras et al., 2013). Although the implications of this distribution are not well understood, an appropriate balance appears to be important for replication and genome stability, as the ubiquitin isopeptidase USP7 was found to be responsible for maintaining SUMOylated proteins at replication forks by means of protecting them from ubiquitylation (Lecona et al., 2016). USP7 activity was found essential for origin firing as well as replisome progression, and intriguingly, one of its functions appears to be the deubiquitylation of SUMO itself.

Despite these observations, the functions of most replisome-associated modifications remain to be explored, and the notion that many of the SUMOylation events were found to be enriched after exposure of the cells to DNA damage (Cremona et al., 2012) raises the question of whether these modifications are inherent in the replication process or represent a response to spontaneous replication problems or low-level DNA damage.

PCNA Modifications during Unperturbed DNA Replication

Posttranslational modifications heavily modulate the function of the eukaryotic sliding clamp. PCNA is a homotrimeric,

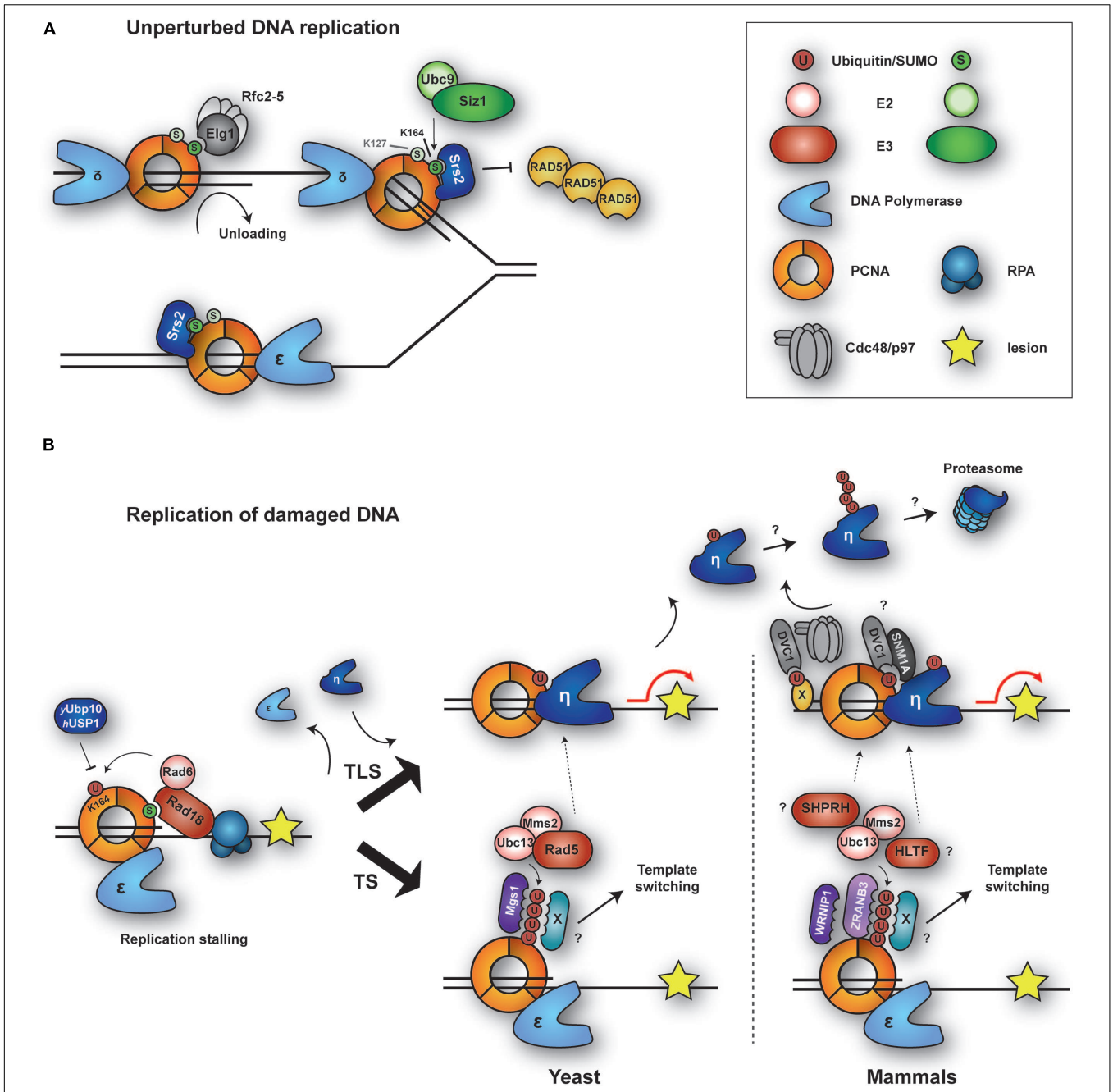

FIGURE 1 | Modifications of PCNA by ubiquitin and SUMO during replication of intact and damaged DNA. (A) During unperturbed replication, budding yeast PCNA is SUMOylated at K164 and to a minor extent at K127. Modification at K164 is mediated by SUMO E2-E3 Ubc9-Siz1. $PCNA^{SUMO}$ recruits anti-recombinogenic helicase Srs2 to counteract Rad51 filament formation. RFC-like complex RLC-Elg1 interacts with $PCNA^{SUMO}$ and unloads PCNA from DNA. **(B)** Upon replication stalling and exposure of ssDNA, E2-E3 complex Rad6-Rad18 is recruited by interaction with the RPA complex and (in yeast) SUMO and monoubiquitylates PCNA at K164. This modification is removed by Ubp10 (yeast) or USP1 (humans). Monoubiquitylated PCNA recruits damage-tolerant DNA polymerases for translesion synthesis (TLS), while polyubiquitylated PCNA initiates template switching (TS) by a poorly defined mechanism. Auxiliary factors DVC1 and SNM1A modulate TLS. DVC1 cooperates with the AAA ATPase p97 to extract polymerase η from chromatin. Mgs1/WRNIP1 and ZRANB3 bind to polyubiquitylated PCNA and might contribute to TS. Ubiquitylation of TLS polymerases prevents association with $PCNA^{Ub}$ and may induce their degradation.

ring-shaped complex that encircles DNA and functions as a processivity factor for DNA polymerases. In addition, PCNA serves as an interaction platform for numerous factors involved in DNA replication, repair, chromatin dynamics, cohesion and cell cycle regulation (Moldovan et al., 2007; Ulrich and Takahashi, 2013).

During unperturbed replication, budding yeast PCNA is modified by SUMO at a highly conserved lysine, K164, and

to a minor extent at K127 (Hoege et al., 2002; **Figure 1A**). Modification at K164 is mediated by the SUMO E2 Ubc9 in combination with the SUMO E3 Siz1 and is triggered by loading of the clamp onto DNA (Parker et al., 2008). SUMOylation at K127 *in vivo* requires Siz2 (Parker et al., 2008). The modification enhances interaction with an antirecombinogenic helicase, Srs2, at replication forks. Srs2 interacts with $PCNA^{SUMO}$ via its carboxy-terminal tail containing a PIP-like PCNA interaction motif adjacent to a canonical SUMO interacting motif (SIM; Pfander et al., 2005; Armstrong et al., 2012). Recruitment of Srs2 prevents unwanted homologous recombination (HR) by disrupting Rad51 filaments (Krejci et al., 2003; Veaute et al., 2003; Papouli et al., 2005; Pfander et al., 2005). In addition, the presence of SUMO on PCNA boosts the damage-induced activity of the ubiquitin ligase Rad18 toward PCNA, again through a SIM in the E3 sequence (Parker and Ulrich, 2012). As a consequence, upon encounter of replication-stalling DNA lesions, damage processing is channeled into a bypass pathway that depends on PCNA ubiquitylation (**Figure 1B**, and see below). Hence, PCNA SUMOylation appears to function as a pre-emptive defense measure to influence pathway choice in response to replication stress. The modification also appears to enhance interaction with an alternative clamp loader complex, RLC-Elg1, which has been proposed to mediate PCNA unloading during replication (Parnas et al., 2010; Kubota et al., 2013). However, SUMOylation is not essential for Elg1 action on PCNA.

SUMOylation at K164 has been observed not only in budding yeast, but also in *X. laevis* egg extracts, chicken DT40 cells and, more recently, in mammalian cells (Leach and Michael, 2005; Arakawa et al., 2006; Gali et al., 2012; Moldovan et al., 2012). In human cells, expression of a PCNA-SUMO fusion

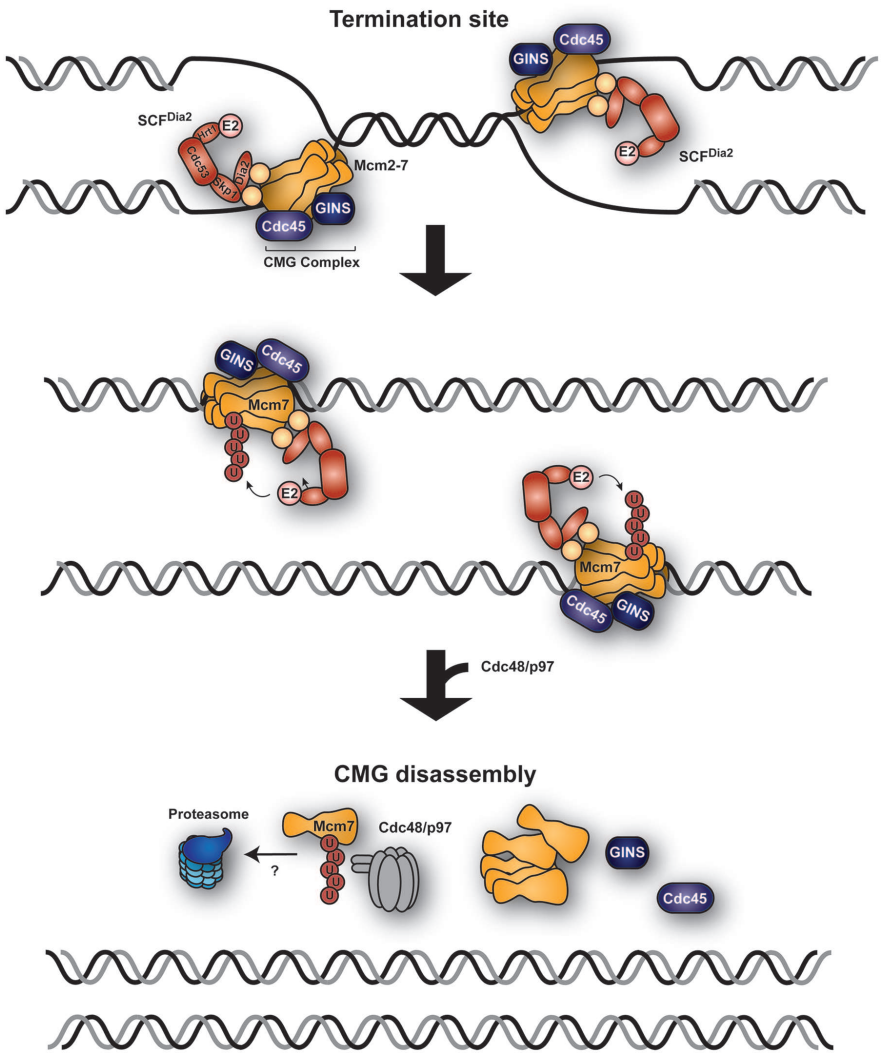

FIGURE 2 | Replication termination via ubiquitin-mediated CMG helicase extraction. A model, derived from observations in budding yeast and *Xenopus laevis* egg extracts, proposes ubiquitylation of Mcm7 by the replisome-associated E3 SCF^{Dia2} at the sites of replication termination where two forks converge. The CMG helicase (Mcm2-7, Cdc45, and GINS) is subsequently extracted from the chromatin by Cdc48/p97 in a ubiquitin-dependent manner.

protein inhibits spontaneous as well as damage-induced HR (Gali et al., 2012). Furthermore, a novel PCNA-interacting factor, the helicase PARI, has been suggested to function analogously to Srs2 in humans: it contains PIP and SIM motifs for interaction with $PCNA^{SUMO}$ and suppresses HR by removing Rad51 from DNA (Moldovan et al., 2012). However, SUMOylated PCNA is present at very low levels in mammalian when compared to yeast cells, and its detection requires overexpression of epitope-tagged SUMO alleles (Gali et al., 2012). Whether this reflects the need for a tighter regulation of the process in the yeast system with its naturally higher rate of recombination remains to be explored.

In response to DNA damage, PCNA is mono- and polyubiquitylated at K164 (Hoege et al., 2002), which facilitates the bypass of replication-blocking lesions (see below). In fission yeast, however, these modifications are observed during S phase even in the absence of exogenous DNA-damaging agents (Frampton et al., 2006). Similarly, PCNA monoubiquitylation has been detected during replication of undamaged DNA in *X. laevis* egg extracts and was found to be required for efficient chromosomal replication (Leach and Michael, 2005). It is currently unclear, however, whether PCNA ubiquitylation contributes to the normal replication process itself or rather reflects higher levels of endogenous damage or fork problems in these systems.

Modification of DNA Polymerases

All replicative DNA polymerases have been identified as ubiquitin and/or SUMO targets in budding yeast and mammalian cells (Wagner et al., 2011; Cremona et al., 2012). Mammalian DNA polymerase δ, responsible mainly for lagging strand synthesis, consists of four subunits (Hubscher et al., 2002), two of which, p12 and p66, are ubiquitylated during a normal S phase without leading to proteasomal degradation (Liu and Warbrick, 2006). Additionally, p66 is modified by SUMO at two different residues, K258 and K433 (Liu and Warbrick, 2006). Although the biological significance of these modifications remains unclear, it has been proposed that they might regulate protein–protein interactions within the polymerase complex or with other replication factors (Liu and Warbrick, 2006). A study in *Schizosaccharomyces pombe* showed that the catalytic subunit of the leading strand polymerase ε, Pol2, is polyubiquitylated and undergoes significant proteasome-dependent degradation during unperturbed S phase, involving the ubiquitin ligase SCF^{Pof3} (the homolog of budding yeast SCF^{Dia2}; Roseaulin et al., 2013). In contrast, Pol3, the catalytic subunit of polymerase δ, remained stable despite being ubiquitylated. The authors propose that the high rate of Pol2 turnover might ensure a continuous supply of "fresh" polymerase at the leading strand, while the discontinuous nature of lagging strand synthesis would not require an active exchange mechanism (Roseaulin et al., 2013). It will be interesting to address whether polymerase ε degradation serves a regulatory or a quality control purpose, and whether the phenomenon is conserved in other organisms.

Modification of Mcm10

The essential, conserved minichromosome maintenance protein 10 (Mcm10) facilitates initiation of DNA replication. The protein is loaded onto replication origins at the G1/S transition, where it promotes strand separation either by activating the helicase or by stabilizing the formation of ssDNA, but it is dispensable for assembly of the helicase itself (Kanke et al., 2012; van Deursen et al., 2012; Thu and Bielinsky, 2013). A contribution of Mcm10 to the elongation step of DNA replication remains controversial (Thu and Bielinsky, 2013). Mcm10 has been shown to interact with the catalytic subunit of DNA polymerase α (Pol1) and regulate its stability, suggesting a role of Mcm10 in lagging strand synthesis (Ricke and Bielinsky, 2004). Ricke and Bielinsky (2004) reported that a small fraction of Mcm10 is monoubiquitylated at two distinct lysine residues during G1 and S phase of the cell cycle (Das-Bradoo et al., 2006). The modification promotes interaction with PCNA, but inhibits binding of Mcm10 to polymerase α. Moreover, mutations within Mcm10's PIP box render cells inviable, suggesting that the interaction between Mcm10 and PCNA is essential (Das-Bradoo et al., 2006). Based on these findings, it was speculated that ubiquitylation of Mcm10 might induce a conformational change to expose its PIP box, thus allowing interaction with PCNA and release of polymerase α after the priming event. This might in turn facilitate the recruitment of polymerase δ and thereby Okazaki fragment extension (Das-Bradoo et al., 2006; Thu and Bielinsky, 2013).

Replication Termination

Convergence of two replication forks leads to replication termination via disassembly of the replicative helicase. This process must be tightly controlled, as the CMG complex cannot be reloaded after initiation and must remain associated with the replication fork until completion of the replication unit, the replicon. However, in contrast to replication initiation, the mechanism of replisome disassembly is not well understood.

Two recent reports have helped to shed light on this reaction in budding yeast and *X. laevis* egg extracts, uncovering a key role for the ubiquitin system (**Figure 2**; Maric et al., 2014; Moreno et al., 2014). Helicase disassembly is triggered by K48-polyubiquitylation of the helicase subunit Mcm7 by a member of the SCF family of ubiquitin ligases. In budding yeast, the relevant F-box protein is Dia2 (Maric et al., 2014). Interestingly, SCF^{Dia2} had previously been identified as a component of the replication progression complex (RPC), tethered to the Ctf4 and Mrc1 subunits via a TPR domain within Dia2 (Mimura et al., 2009; Morohashi et al., 2009). SCF^{Dia2} was also proposed to mediate degradation of Ctf4 and Mrc1 (Mimura et al., 2009); however, a recent study has challenged this model and reported instead that SCF^{Dia2} tethering to the RPC is important for the efficient ubiquitylation of Mcm7 (Maculins et al., 2015). Inhibition of replication fork progression prevents Mcm7 ubiquitylation, suggesting that Mcm7 ubiquitylation is restricted to terminating replisomes (Maric et al., 2014; Moreno et al., 2014). How cells distinguish these from elongating complexes to avoid premature ubiquitylation and disassembly

of the helicase is currently unknown. One possible scenario is that ubiquitylation is triggered by a DNA-mediated signal: while during replication the CMG helicase encircles ssDNA, it must enclose dsDNA upon termination (Bell, 2014). The mechanism of CMG helicase disassembly is likely to be conserved in higher eukaryotes. Homologs of Dia2 have yet to be identified, but other ubiquitin ligases might be involved in the process. Notably, disassembly of the CMG helicase requires the ubiquitin-dependent segregase p97, also called VCP (in yeast: Cdc48; Maric et al., 2014; Moreno et al., 2014), an AAA ATPase that remodels and thus extracts ubiquitylated proteins from protein complexes, membranes or chromatin, in many cases presenting them for proteasomal degradation (Vaz et al., 2013; Franz et al., 2016). Inactivation of p97 led to the accumulation of ubiquitylated forms of CMG on the chromatin, while inhibition of the proteasome did not block CMG disassembly (Maric et al., 2014; Moreno et al., 2014). Thus, whether $Mcm7^{Ub}$ is degraded after extraction remains to be seen, although the K48-linkage of the polyubiquitin chain on Mcm7 would imply proteasomal action.

UBIQUITIN AND SUMO IN DNA REPLICATION STRESS

Replication stress is broadly defined as a condition that interferes with replication fork progression (Zeman and Cimprich, 2014). It is caused by a range of intrinsic or exogenous factors, including polymerase inhibition or nucleotide depletion, imbalances in the levels of replication proteins, interference from ongoing transcription, incorporation of ribonucleotides, or physical barriers to the DNA polymerases, such as sequences inherently prone to form secondary structures, tightly bound proteins, or DNA lesions arising from chemical alterations or strand breaks. Conditions that impair the replicative DNA polymerases without impeding strand unwinding by the helicase result in an accumulation of ssDNA. This in turn initiates a replication-specific checkpoint response via the protein kinase ATR in order to stabilize stalled replication intermediates, suppress the firing of late origins and prevent entry into mitosis (Jossen and Bermejo, 2013). Depending on the nature of the blockage, ATR signaling promotes replication fork rescue or restart in one of several ways, for example by means of re-priming downstream of the problematic region, fork reversal, translesion synthesis or strand exchange between the sister chromatids (Jossen and Bermejo, 2013; Leman and Noguchi, 2013). Prolonged replication fork stalling or lack of an appropriate checkpoint response can cause replication fork collapse. This poorly defined event may include a dissociation of the replisome and/or the formation of strand breaks, caused either passively or by the action of nucleases. Importantly, fork collapse triggers the transition to a genuine DNA damage response, mediated by the checkpoint kinase ATM, as is generally observed in response to DSBs. Over the past decade it has become clear that ubiquitin and SUMO are key regulators of both the replication- and the damage-associated branches of the checkpoint response (Ulrich, 2012; Jackson and Durocher, 2013).

Proteomic Analyses of the DNA Replication Stress Response

A number of large-scale proteomic studies and systematic analyses of chromatin-associated factors have illustrated the dynamics of ubiquitylation and SUMOylation specifically in response to replication stress (Povlsen et al., 2012; Bursomanno et al., 2015; Xiao et al., 2015). According to those studies, when replication forks encounter DNA lesions, a plethora of SUMO and ubiquitin modifications on multiple factors is upregulated to either protect replication forks or initiate DNA repair mechanisms. In many cases, their consequences are mechanistically and functionally not well characterized, and it is clear today that modification of entire protein groups is sometimes more important than ubiquitylation or SUMOylation of individual factors (Psakhye and Jentsch, 2012). Moreover, a clear distinction between replication stress triggered by fork stalling and a full-blown damage response that might result from subsequent fork collapse has not always been attempted.

Control of Homologous Recombination during DNA Replication

Homologous recombination serves as a means to repair DNA DSBs, to promote exchange of genetic material and proper chromosome segregation during meiotic cell divisions, and to rescue stalled or collapsed replication forks (Krejci et al., 2012). The process is initiated by strand breaks or – in particular at stalled replication forks – regions of ssDNA, tightly bound by the Replication Protein A (RPA) complex. RPA is exchanged for the recombination factor RAD51. In yeast, this step is promoted by the RAD52 protein. In human cells, the exchange is mainly mediated by BRCA2 (Jensen et al., 2010; Liu J. et al., 2010; Thorslund et al., 2010). The RAD51-ssDNA filament invades dsDNA, forming a so-called D-loop, and exchange of genetic material proceeds via a combination of DNA synthesis, branch migration and resolution or dissolution of recombination intermediates by the action of nucleases. Numerous auxiliary factors, among them DNA helicases and DNA-dependent ATPases, modulate HR activity either positively or negatively at every step (Krejci et al., 2012), and many of them are modulated in their activities by ubiquitin and/or SUMO.

Replication Protein A (RPA)

Replication protein A is a ssDNA-binding protein complex with a central role as a scaffold in virtually all DNA transactions. In eukaryotes, RPA consists of three subunits: RPA1, RPA2, and RPA3 (Zou et al., 2006). In mammals, the largest subunit, RPA1, is stably associated with the Sentrin/SUMO-specific protease SENP6 during S phase, which keeps RPA1 in a hypoSUMOylated state (Dou et al., 2010; **Figure 3A**). In response to replication-mediated or radiation-induced DSBs, SENP6 dissociates, resulting in modification of RPA1 with SUMO through the action of unknown SUMO ligases. Two lysine residues were identified as SUMO acceptor sites: K449 was modified by a poly-SUMO chain, whereas K577 was mono-SUMOylated. Importantly, treatment with hydroxyurea (HU) or

UV irradiation, which stalls replication forks without causing DSBs, did not alter the association between SENP6 and RPA1. SUMOylation of RPA enhanced its interaction with RAD51 *in vitro* and promoted HR *in vivo* (**Figure 3A**). Taken together, $RPA1^{SUMO}$ seems to facilitate recruitment of RAD51 to collapsed forks and DSBs, thereby initiating HR (Dou et al., 2010). Interestingly, RAD51 contains a SIM motif that is necessary for its accumulation at damage sites (Shima et al., 2013). However, whether $RPA1^{SUMO}$ is indeed the *in vivo* target of this SIM relevant for recruitment of RAD51 to damage sites needs to be demonstrated, considering that SUMOylation of other proteins might act synergistically or redundantly in the assembly of repair complexes (Psakhye and Jentsch, 2012). The yeast homolog of RPA1, Rfa1, is also modified by SUMO upon treatment with the alkylating agent methyl-methanesulfonate (MMS; Burgess et al., 2007; Cremona et al., 2012), although the functional significance of this modification remains unclear.

More recently, Galanty et al. (2012) posited a plausible mechanism for the transition from RPA to RAD51on ssDNA, relying on the SUMO-targeted ubiquitin ligase (STUbL) RNF4: in RNF4-depleted cells RAD51 fails to accumulate and RPA persists at lesions. A SUMOylation-defective RPA1 mutant exhibited a similar behavior. Based on these findings, RNF4 was proposed to target $RPA1^{SUMO}$ for proteasomal degradation (**Figure 3A**). Consistent with this model, RNF4 and RPA1 coimmunoprecipitated in a manner dependent on the SIM region of RNF4, suggesting that RNF4 directly recognizes $RPA1^{SUMO}$ as a ubiquitylation target. As a consequence, RPA1 accumulates in RNF4-depleted cells after exposure to DNA damage, and several proteasome subunits become detectable at damage sites in an RNF4-dependent manner. Thus, RNF4-mediated RPA1 turnover might promote the exchange of RPA1 for RAD51 on ssDNA, stimulating HR (Galanty et al., 2012). This mechanism and the recruitment of RAD51 through RPA^{SUMO} are not mutually exclusive, as both could cooperate in promoting RAD51 filament formation (**Figure 3A**). However, the direct ubiquitylation of SUMO-modified RPA1 by RNF4 has yet to be demonstrated.

On the other hand, RPA is also ubiquitylated under conditions complementary to those that trigger its SUMOylation (**Figure 3B**). Ubiquitylation on multiple sites of all three RPA subunits was observed in response to replication fork stalling upon UV irradiation or treatment with other fork-stalling agents such as 4-nitroquinoline oxide or HU, but not after exposure to ionizing radiation (Elia et al., 2015). Thus, apparently cells respond to different types of damage in distinct ways, either ubiquitylating or SUMOylating RPA. Ubiquitylation of RPA, mediated by the E3 RFWD3, does not lead to proteasomal degradation. Inhibition of the modification by RFWD3 depletion or by means of a ubiquitylation-deficient RPA mutant caused defects in fork restart and persistence of γ-H2AX foci after release from prolonged HU treatment, as well as a reduction in HR in response to both fork stalling and direct induction of DSBs. These findings imply that RFWD3-dependent ubiquitylation of RPA promotes fork stability and HR-mediated restart of collapsed forks upon exposure to replication stress (Elia et al., 2015). The exact mechanism by which RPA^{Ub} stimulates these effects remains elusive, even though it has been suggested that the modification may promote release of the RPA complex from DNA and/or facilitate the recruitment of HR factors, similar to RPA^{SUMO} (Elia et al., 2015). Whether ubiquitin and SUMO can coexist on the same RPA complex remains to be explored. An independent study identified another ubiquitin ligase, PRP19, as the E3 responsible for RPA ubiquitylation (Marechal et al., 2014).

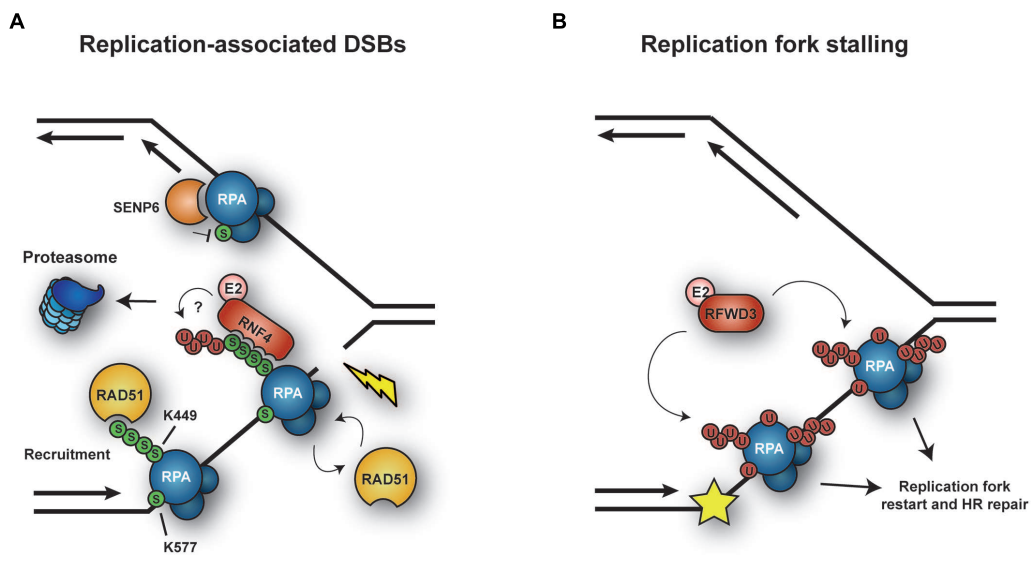

FIGURE 3 | Ubiquitylation and SUMOylation of RPA under conditions of replicative stress. (A) SUMOylation of RPA1 is counteracted by the protease SENP6 during unperturbed S phase. In response to replication-mediated DSBs, SENP6 dissociates, allowing SUMOylation of RPA1 in order to facilitate HR via recruitment of RAD51. In addition, RPA^{SUMO} is recognized by the STUbL RNF4, which mediates proteasomal turnover of RPA1, thereby promoting exchange of RPA1 for RAD51. **(B)** Upon replication fork stalling, the ubiquitin E3 RFWD3 ubiquitylates RPA at multiple sites, thereby promoting replication fork restart and HR repair.

In this study, depletion of PRP19 was found to reduce damage-induced RPA ubiquitylation and compromise the accumulation of the ATR-ATRIP checkpoint complex at sites of damage. In addition, ATRIP was reported to exhibit an affinity for K63-linked ubiquitin chains, suggesting that this modification on RPA might contribute to the recruitment of ATR-ATRIP (Marechal et al., 2014). However, these findings have been called into question by the observation of an unintentional side effect of the siRNAs used for the depletion of PRP19, on exogenously expressed ubiquitin (Elia et al., 2015). Hence, an involvement of PRP19 in RPA ubiquitylation needs to be reconfirmed.

BLM

The RecQ DNA helicase BLM plays an important role in genome maintenance by facilitating HR-mediated DNA repair in various ways (Bohm and Bernstein, 2014). BLM protein levels are regulated during the cell cycle, being lowest in G1 and peaking in late S phase (Dutertre et al., 2000). BLM normally resides in promyelocytic leukaemia (PML) nuclear bodies but re-localizes to stalled replication forks in response to DNA damage (Sengupta et al., 2003). At replication forks, BLM can exert both pro- and anti-recombinogenic functions (**Figure 4**): it protects replication forks by suppressing the formation of aberrant recombination events or, upon fork collapse, it promotes repair by HR. Posttranslational modifications of BLM by ubiquitin and/or SUMO make key contributions to the regulation of these processes (Bohm and Bernstein, 2014). Monoubiquitylation of BLM in the absence of DNA damage appears to be important for its normal localization in PML nuclear bodies (**Figure 4A**). Following HU treatment, BLM is further polyubiquitylated with K63-linked chains at K105, K225, and K259 by the E3s RNF8 and RNF168. Polyubiquitylation of BLM was found to be required for its recruitment to stalled replication forks, mediated via interaction with the ubiquitin-interacting motifs of the adaptor protein RAP80 (Tikoo et al., 2013). Once at stalled replication forks, BLM suppresses excessive HR (Tikoo et al., 2013) by dismantling RAD51-ssDNA filaments and disrupting D-loops (Bugreev et al., 2007). Polyubiquitylation of BLM might also potentiate the protein's anti-recombinogenic effect. However, constitutive association of BLM with chromatin, achieved by fusion with histone H2AX or the FHA domain of MDC1, was sufficient to suppress the elevated levels of HR caused by depletion of either RNF8 or RNF168, indicating that polyubiquitylation of BLM might function more as a means to recruit rather than to activate the protein (Tikoo et al., 2013).

In addition to being ubiquitylated, BLM is modified by SUMO at multiple sites, preferentially at K317 and K331 (Eladad et al., 2005). Expression of a SUMOylation-defective BLM mutant induces an excess of γ-H2AX foci, DSBs and cell death under conditions of replication stress, such as prolonged HU treatment, uncovering a role of BLM SUMOylation in protecting and/or restarting replication forks (**Figure 4B**). Interestingly, cells unable to SUMOylate BLM also fail to recruit RAD51 and to induce HR at stalled replication forks (Ouyang et al., 2009). In fact, as described for RPA (Dou et al., 2010), SUMOylation of the helicase enhances binding to RAD51 *in vitro* (Ouyang et al., 2009). However, in contrast to its ubiquitylation, its SUMOylation was not required for the trafficking of BLM itself to stalled forks (Ouyang et al., 2009). Thus, BLM SUMOylation might function as a molecular switch to regulate its activity: unSUMOylated, polyubiquitylated BLM is recruited to stalled replication forks, protecting them from deleterious HR, while BLM^{SUMO} facilitates HR by promoting RAD51 recruitment to collapsed forks (**Figure 3**; Ouyang et al., 2009). Future studies will certainly provide insight into the molecular mechanism by which these modifications regulate BLM function.

Little is known about posttranslational modifications of the BLM ortholog in budding yeast, Sgs1. In the absence of Sgs1, cells accumulate Rad51-dependent cruciform structures at damaged replication forks (Liberi et al., 2005). The same is observed in mutants of the SUMO-conjugating enzyme, *ubc9* (Branzei et al., 2006), and interestingly, Sgs1 is indeed a target of SUMOylation, suggesting the possibility that the modification might be important to prevent the accumulation of aberrant recombinogenic structures during replication of damaged templates (Branzei et al., 2006). However, in contrast to BLM modification, SUMOylation of Sgs1 does not seem to influence recombination frequencies (Lu et al., 2010).

Sgs1 modifications also appear to impinge on the protein's subcellular localization (Bohm et al., 2015): During S phase, Sgs1 forms nuclear foci that likely indicate spontaneous recombination events, as they increase with ionizing radiation treatment. Upon replication fork stalling by nucleotide depletion, the number of these foci is strongly reduced in a manner depending on the STUbL Slx5/8 – suggesting that STUbL-mediated ubiquitylation contributes to removing Sgs1 from stalled forks, thus possibly preventing unwanted recombination. However, as overall Sgs1 levels do not decrease, the process does not appear to involve degradation of the helicase, but rather its re-localization. The mechanism is likely conserved, since BLM^{SUMO} is also targeted by the mammalian STUbL RNF4 (Galanty et al., 2012). Thus, SUMOylation of BLM/Sgs1 seems essential for the fine-tuning of the protein's function: it facilitates HR repair at collapsed forks, but it also induces removal of the protein from stalled forks, adding an additional level of regulation.

SRS2

A recent study by Urulangodi et al. (2015) has uncovered a new mechanism promoting local recombination at sites of compromised replication in budding yeast. As described above, $PCNA^{SUMO}$ recruits the helicase Srs2 to prevent unwanted recombination during unperturbed S phase. Hence, removal of Srs2 should be critical in order to engage HR after fork stalling. Urulangodi et al. (2015) identified Esc2, a protein containing two SUMO-like domains (SLDs), as a new factor associated with stalled replication forks and controlling Srs2 levels. Via its SLDs, Esc2 interacts with the SIM of Srs2, thereby promoting interaction of Srs2 with the STUbL complex Slx5/8 and subsequent degradation by the proteasome. Consistent with these findings, Srs2 SUMOylation is induced by DNA damage (Saponaro et al., 2010). Thus, local down-regulation of Srs2 appears to enable recruitment of Rad51 and thereby HR-mediated rescue of stalled forks (Urulangodi

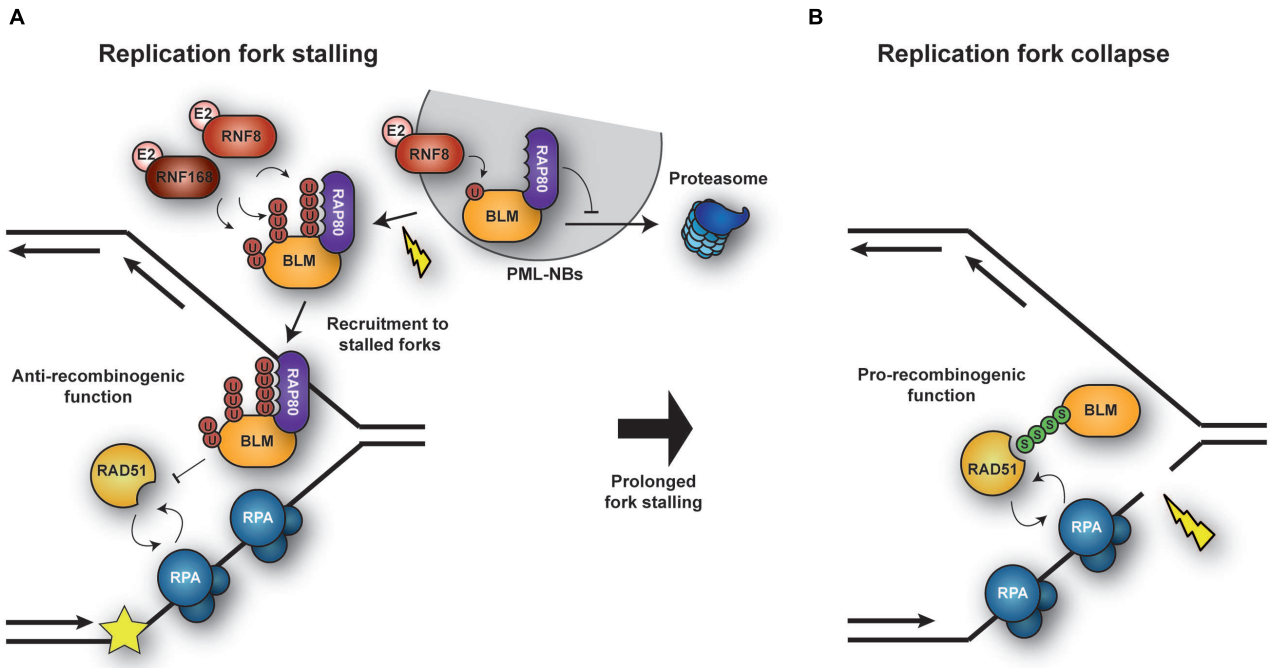

FIGURE 4 | Regulation of BLM activity under conditions of replicative stress. (A) Upon replication fork stalling, BLM is polyubiquitylated by the ubiquitn E3s RNF8 and RNF168. Polyubiquitylated BLM is recognized by RAP80, which mediates relocation of BLM from PML nuclear bodies and recruitment to stalled forks. At the fork BLM suppresses unwanted HR events. **(B)** Upon collapse of a stalled fork, BLM is SUMOylated, thereby facilitating the recruitment of RAD51 and repair by HR.

et al., 2015). In addition, it has been shown *in vitro* that Srs2 SUMOylation and interaction with $PCNA^{SUMO}$ are mutually inhibitory (Kolesar et al., 2012), suggesting that Esc2 might help to dismantle the association between $PCNA^{SUMO}$ and Srs2. Upon dissociation from $PCNA^{SUMO}$, Srs2 would be free to undergo SUMOylation, which would disfavor re-association with $PCNA^{SUMO}$. Alternatively, Esc2 might bypass the need for Srs2 SUMOylation by acting as a platform to recruit Slx5/8 to its substrate via physical interaction of Esc2 with Slx5 (Urulangodi et al., 2015).

SLX4

The binding of a multitasking protein to either SUMO or ubiquitin can modulate its function by conveying different contextual specificities. An example is provided by the scaffold protein SLX4, which coordinates multiple DNA repair pathways through its ability to bind several nucleases. Human SLX4 contains two ubiquitin-binding zinc finger (UBZ) domains that are essential for its role in the FA pathway, facilitating repair of DNA interstrand crosslinks (ICLs; see below and Coleman and Huang, 2016). In addition, SLX4 contains a cluster of SIMs, which recognizes SUMO chains. How much this cluster contributes to the repair of ICLs is not entirely clear, as expression of a SIM-defective mutant of SLX4 was able to effectively rescue the mitomycin C (MMC) sensitivity of SLX4-deficient human cells (Guervilly et al., 2015; Ouyang et al., 2015), whereas rescue was only partial in mouse cells (Gonzalez-Prieto et al., 2015). However, the SIM cluster is exclusively required for efficient recruitment and retention of SLX4 to laser-induced DNA damage sites, where it might enhance the association of SLX4 with multiple SUMOylated targets, including RPA and MRN (Gonzalez-Prieto et al., 2015; Ouyang et al., 2015). Once at stalled forks, SLX4 might, for instance, promote replication fork restart when associated with the endonuclease MUS81 (Hanada et al., 2007).

Interestingly, the SIMs perform yet another, unexpected function: by interacting with SUMO-charged Ubc9, they promote the SUMOylation of SLX4 itself and its binding partner, XPF. This presumed SUMO ligase activity appears to be toxic under some conditions, as mild overexpression of SLX4, but not mutation of the SIM or BTB domain, sensitizes cells to replication fork stalling upon HU treatment and promotes DSBs. In contrast, E3 activity was found to be required to prevent mitotic catastrophe at chromosome fragile sites, suggesting that promotion of DSB formation might be beneficial in difficult-to-replicate regions of the genome (Guervilly et al., 2015). Additional work will be needed to understand how the SUMO ligase activity of SLX4 contributes to genome stability and whether it can target other substrates besides SLX4 and XPF.

Structural Maintenance of Chromosomes 5 and 6 (Smc5/6)

The structural maintenance of chromosomes 5 and 6 (Smc5/6) complex (**Figure 5**) belongs to a family of multisubunit ATPases that also includes cohesion and condensin. The complex consists of eight subunits, Smc5, Smc6 and six non-Smc element (Nse)

subunits, Nse1– 6 (Murray and Carr, 2008). Smc5 and Smc6 adopt extended coiled-coil structures with globular heads at the C- and N-termini that form an ATPase domain. It is believed that Smc5/6, like the related cohesin and condensin complexes, is able to embrace DNA double-strands and thereby influence higher chromatin organization. Consistent with this idea, Smc5/6 has been shown to sequester sister chromatid intertwinings and assist replication fork rotation to relieve super-helical tension generated as DNA unwinds ahead of the fork (Kegel et al., 2011).

In most organisms, all subunits of the Smc5/6 complex are essential for cell survival. Hypomorphic mutants of Smc5/6 show mark sensitivity to perturbation of replication such as reduced dNTP levels and DNA damage (Murray and Carr, 2008; Stephan et al., 2011). Moreover, Smc5/6 localizes to natural replication pausing sites such as rDNA, centromeres and telomeres, and to collapsed forks (Ampatzidou et al., 2006; Lindroos et al., 2006; Menolfi et al., 2015). It has been shown that HR intermediates such as X-shaped molecules accumulate in Smc5/6 mutants in the course of replication in yeast. This leads to lethality in mitosis due to failure of these mutants to properly segregate their chromosomes (Ampatzidou et al., 2006; Branzei et al., 2006; Chen et al., 2009; Irmisch et al., 2009; Bermudez-Lopez et al., 2010; Choi et al., 2010). Interestingly, restricting Smc5/6-activity to G2, i.e., after completion of genome replication, is compatible with survival (Bermudez-Lopez et al., 2010; Menolfi et al., 2015). These data suggest that Smc5/6 is important for resolving recombination structures formed during DNA replication.

One of the Smc5/6 subunits, Nse2, also known as Mms21, is known to be a SUMO ligase (Andrews et al., 2005; Potts and Yu, 2005; Zhao and Blobel, 2005), whereas Nse1 was proposed and subsequently shown to harbor ubiquitin ligase activity (Pebernard et al., 2008; Doyle et al., 2010).

Nse2/Mms21, a SUMO ligase associated with the Smc5/6 complex

Nse2 associates with the coiled-coil domain of Smc5 via an essential N-terminal domain. In contrast, its C-terminal SUMO E3 domain is dispensable for survival, but important for resistance to DNA damage (McDonald et al., 2003; Andrews et al., 2005; Potts and Yu, 2005; Zhao and Blobel, 2005; Duan et al., 2009). Cells lacking the Nse2 SUMO E3 activity accumulate recombination intermediates following DNA replication stress, similar to *smc5/6* hypomorphic mutants (Branzei et al., 2006; Chavez et al., 2010). This suggests that the Smc5/6 complex responds to DNA damage primarily through its associated SUMOylation activity.

A recent study has provided new insight into the activation mechanism of Nse2's SUMO E3 activity toward its mostly chromatin-bound targets. Bermudez-Lopez et al. (2015) reported that ATP binding by the globular head of Smc5 induces a conformational change in the coiled-coil region, which was found to enhance E3 activity of Nse2. This mechanism appears to couple the loading of Smc5/6 onto chromatin to the activation of its enzymatic activity and suggests that the Smc5/6 complex as a whole behaves like a giant SUMO E3. In contrast to the

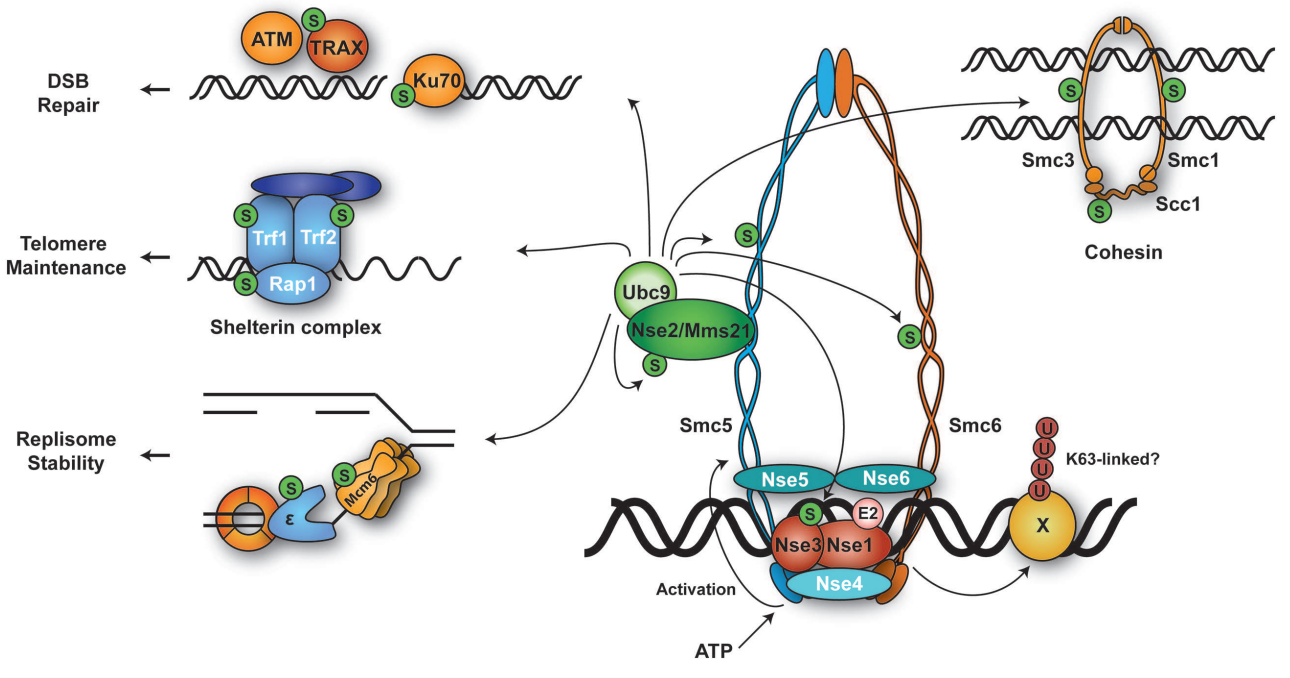

FIGURE 5 | Ubiquitylation and SUMOylation targets of Smc5/6 and their associated processes. The Smc5/6 complex harbors SUMO ligase (Nse2) and ubiquitin ligase (Nse1-Nse3) subunits. DNA binding stimulates the ATPase domains at the globular heads of Smc5/6, inducing a conformational change in the coiled-coil region of Smc5 that activates the SUMO E3 activity of Nse2. Smc5/6 likely selects its ubiquitylation and/or SUMOylation targets at relevant loci where the complex is recruited.

SUMO ligases of the PIAS family, Nse2 lacks a DNA-binding domain (Jackson, 2001; Ulrich, 2014). Therefore, loading of the entire Smc5/6 complex is likely required for selecting chromatin-associated substrates. In fact, many of Nse2's targets have been found to co-localize with Smc5/6 or with its associated repair sites. Not surprisingly, Nse2 SUMOylates several subunits within the Smc5/6 complex, such as Smc5, Smc6, Nse3, and Nse2 itself (Andrews et al., 2005; Potts and Yu, 2005; Zhao and Blobel, 2005). Interestingly, SUMOylation by the Smc5/6 complex impinges on the structurally related cohesin complex: in response to DNA damage Nse2 SUMOylates all cohesin subunits, Smc1, Smc3, and Scc1. The modification is required for proper loading of the cohesin complex under these conditions. Abolishing SUMOylation of cohesin by point mutations or by tethering a SUMO-specific isopeptidase to the complex caused defects in the establishment of sister chromatid cohesion and impaired cellular survival (Almedawar et al., 2012; Wu et al., 2012). Other substrates include DNA repair factors such as Ku70 and TRAX (Potts and Yu, 2005; Zhao and Blobel, 2005). In human cells, the complex modifies telomere-binding proteins like RAP1, TRF1, and TRF2 (Potts and Yu, 2007). In budding yeast, rDNA-associated proteins such as RNA polymerase I, Fob1, and Tof2, and the replication factors Pol2 and Mcm6 have been identified as substrates (Albuquerque et al., 2013; Hang et al., 2015). The functional consequences of these SUMOylation events are yet to be clarified.

Nse1, an Smc5/6 subunit with ubiquitin ligase activity

The Nse1 subunit of the Smc5/6 complex, a RING finger protein, exhibits weak ubiquitin ligase activity on its own (Pebernard et al., 2008). This activity is significantly enhanced in the presence of its direct interaction partner, Nse3. In collaboration with the E2 Ubc13/Mms2, Nse1/3 is capable of assembling K63-linked ubiquitin chains (Doyle et al., 2010). In *S. pombe*, the RING-like motif of Nse1 is not essential, but inactivation of the domain leads to hypersensitivity toward genotoxic stress (Pebernard et al., 2008). Recently, Nse3 was found to harbor DNA binding activity, and mutations in the relevant domain caused damage sensitivity and chromosome aberrations (Zabrady et al., 2015). These data indicate that Nse1/3 contribute to the activity of the Smc5/6 complex in chromosome maintenance upon genotoxic stress. However, the targets of such ubiquitin ligase activity have not been identified.

DNA Damage Bypass

DNA damage bypass, also called DNA damage tolerance, is important in situations where fork stalling has been triggered by lesions in the replication template that cannot be copied by the replicative DNA polymerases (Saugar et al., 2014). Such lesions mostly represent damage that is subject to base or nucleotide excision repair, i.e., small or bulky adducts, oxidative lesions, abasic sites and UV-induced pyrimidine dimers. In order to prevent a permanent replication arrest, damage bypass ensures complete duplication of the affected region without actually removing the lesion, and excision-based repair can act subsequently when the DNA has regained its double-stranded form. Two major pathways of damage bypass can be distinguished, which differ significantly in their overall accuracy: on the one hand, specialized damage-tolerant DNA polymerases can copy damaged DNA in a process named translesion synthesis (TLS). Due to the low fidelity of the enzymes involved, this pathway is a major cause of damage-induced mutagenesis. On the other hand, error-free damage bypass can be accomplished by means of a so-called template switching (TS) pathway, which altogether avoids the use of the damaged DNA as a replication template and instead relies on the (undamaged) sister chromatid to provide accurate sequence information. This process involves recombination factors and joint molecules as intermediates (Giannattasio et al., 2014), but appears to be distinct from the classical HR mechanism used for DSB repair. Both branches of damage bypass can act in a postreplicative manner; thus, they are not necessarily coupled to replication fork progression (Daigaku et al., 2010; Karras and Jentsch, 2010).

Mono- and Polyubiquitylation of PCNA

The profound impact of damage bypass on replication efficiency and fidelity is reflected by an intricate regulation of the pathway in cells (Ulrich, 2009; McIntyre and Woodgate, 2015). Central to its activation is the ubiquitylation of PCNA on a conserved lysine residue, K164 (**Figure 1B**). Whereas monoubiquitylation by the E2-E3 pair Rad6-Rad18 promotes TLS, extension of the modification to a K63-linked polyubiquitin chain by the heterodimeric E2 Ubc13-Mms2 triggers error-free TS (Hoege et al., 2002; Stelter and Ulrich, 2003). The cognate E3 in budding yeast is the RING finger protein Rad5; its human homologs are HLTF and SHPRH (Motegi et al., 2008). Rad18, which is rate-limiting for both TLS and TS, is recruited by RPA-covered ssDNA through physical interactions with the RPA complex (Davies et al., 2008; Niimi et al., 2008). In budding yeast, damage-independent SUMOylation of PCNA (see above) provides a second signal that strongly stimulates Rad18's activity toward PCNA (Parker and Ulrich, 2012). Additional E3s have been reported to operate on mammalian PCNA, such as RNF8 and $CRL4^{Cdt2}$ (Simpson et al., 2006; Zhang et al., 2008; Terai et al., 2010; Krijger et al., 2011). Moreover, large-scale mass spectrometry studies have identified multiple other ubiquitylation sites (McIntyre and Woodgate, 2015). However, the relevance of these conjugation factors and modifications for damage bypass is still a matter of debate, and links to proteasomal degradation may not be excluded (Yu et al., 2009; Cazzalini et al., 2014).

Activation of TLS by monoubiquitylated PCNA can largely be explained by the presence of ubiquitin-binding domains within the major family of damage-tolerant polymerases, which convey an enhanced affinity for the modified form of PCNA (Watanabe et al., 2004; Bienko et al., 2005; Bi et al., 2006; Plosky et al., 2006). In mammals, direct interactions with Rad18 also contribute to the recruitment of TLS polymerases (Watanabe et al., 2004). Whereas in yeast TLS-mediated damage-induced mutagenesis nearly completely depends on PCNA ubiquitylation, the process appears to be less dependent on this modification in vertebrate cells (Stelter and Ulrich, 2003; Edmunds et al., 2008; Hendel et al., 2011). In addition to the damage-tolerant polymerases, a number of auxiliary factors have been proposed

to modulate TLS via recognition of monoubiquitylated PCNA in mammals. These include the UBZ domain-containing proteins SNM1A, a nuclease that might provide a link between TLS and the repair of ICLs, and DVC1 (also called Spartan), an adaptor for the ubiquitin-dependent chaperone p97 (Yang et al., 2010; Centore et al., 2012). The selectivity of DVC1's UBZ domain for $PCNA^{Ub}$ has been contested, however, and it has been proposed that the protein binds to other ubiquitylated proteins at sites of replication stalling, where it would mediate extraction of polymerase η in order to limit TLS activity (Davis et al., 2012; Mosbech et al., 2012). Downregulation of TLS appears to be important for preventing excessive mutagenesis during replication. In human cells, this is accomplished mainly by PCNA deubiquitylation via the isopeptidase USP1 (Huang et al., 2006). In addition, the ubiquitin-like modifier ISG15 was recently found to contribute to the termination of TLS by modification of PCNA, which in turn mediated the recruitment of USP10 for PCNA deubiquitylation and dissociation of polymerase η (Park et al., 2014). Intriguingly, a viral isopeptidase, BPLF1, was also shown to deubiquitylate human PCNA during replication of the Epstein–Barr genome, thus inhibiting polymerase η recruitment during the lytic phase of infection (Whitehurst et al., 2012). How an inhibition of TLS may promote viral replication is not yet understood. In yeast, PCNA deubiquitylation is mediated by Ubp10; however, despite an accumulation of $PCNA^{Ub}$, inactivation of the enzyme does not cause a noticeable increase in mutation rates, indicating that reversal of the modification may be less critical for damage bypass in this organism (Gallego-Sanchez et al., 2012).

How polyubiquitylation of PCNA triggers TS is still an unresolved question. From experiments using linear head-to-tail fusions of ubiquitin moieties as mimics of polyubiquitin chains it was inferred that the K63-linkage itself is important for TS activity (Zhao and Ulrich, 2010). Although putative effectors that preferentially interact with polyubiquitylated PCNA have been identified, they are unlikely to be directly responsible for activating TS: the human ATPase WRNIP1 and its yeast homolog Mgs1 accumulate at stalled replication intermediates in a manner that depends on their UBZ domain as well as PCNA ubiquitylation (Crosetto et al., 2008; Saugar et al., 2012). However, even though a subset of the phenotypes of *mgs1* mutants is consistent with a function downstream of $PCNA^{Ub}$ (Hishida et al., 2006; Saugar et al., 2012), no obvious TS defects are observed in such mutants. In human cells, WRNIP1 appears to contribute to checkpoint activation as a bridging factor that promotes interaction of $PCNA^{Ub}$ with the ATM-associated ATMIN protein (Kanu et al., 2015). Yet, this function is unlikely to be related to polyubiquitylation, as a single ubiquitin moiety is sufficient to stimulate the interaction between WRNIP1/Mgs1 with PCNA (Saugar et al., 2012). A second ATPase, ZRANB3, has also been implicated in PCNA-dependent damage bypass, based on its localization to laser-induced DNA damage, its preferential interaction with polyubiquitylated PCNA, and a general sensitivity to replication stress upon depletion of the protein (Ciccia et al., 2012; Weston et al., 2012). However, a function in the TS pathway has yet to be properly established by means of genetic analysis. Moreover, a convincing yeast homolog has not been identified, which argues for an auxiliary function of ZRANB3 rather than a key role in activating TS.

Analysis of the TS pathway is further complicated by the multi-functionality of Rad5 and its two human homologs, whose catalytic RING domains are embedded in SWI/SNF-like domains with helicase and DNA-dependent ATPase activity. Although this helicase function can be genetically separated from Rad5's role in ubiquitin-dependent TS, it does contribute to survival of replication stress (Choi et al., 2015). Interestingly, Rad5 and its homologs have been implicated not only in TS, but also in TLS in budding and fission yeast as well as humans (Minesinger and Jinks-Robertson, 2005; Gangavarapu et al., 2006; Pages et al., 2008; Coulon et al., 2010; Lin et al., 2011; Kuang et al., 2013; Xu et al., 2016). Mechanistically, this activity remains controversial, as some studies have invoked PCNA polyubiquitylation in the process, whereas others have reported a RING- and ATPase-independent function or a dependence on a physical interaction with the TLS polymerase Rev1. Moreover, although both HLTF and SHPRH are capable of polyubiquitylating PCNA *in vitro*, they have been postulated to fulfill non-redundant functions in cooperation with TLS polymerases η and κ, respectively, depending on the nature of the damaging agent (Lin et al., 2011). Based on these observations, Lin et al. (2011) have put forth a model where a damage-tolerant polymerase harboring multiple UBDs, such as polymerase κ, might preferentially recognize polyubiquitylated PCNA, while monoubiquitylation might stimulate those polymerases with only one UBD, such as polymerase η. Along similar lines, Fuchs and coworkers proposed that polyubiquitylated PCNA might serve to simultaneously attract several different TLS polymerases for cooperation in damage bypass (Coulon et al., 2010). In contrast, observations by Yang et al. (2014) in an *in vitro* set-up have led to the opposite conclusion: rather than promoting TLS, polyubiquitylation of PCNA was found to inhibit the activity of polymerase η in the bypass of an abasic site, suggesting that the K63-chains trap the polymerase in a non-productive mode. In budding yeast, genetic analysis supports a positive effect of Rad5 on TLS in some situations. At the same time, however, PCNA polyubiquitylation promotes damage resistance even in the absence of any damage-tolerant polymerase, thus clearly implying a TLS-independent function in TS (Zhao and Ulrich, 2010). In summary, the consequences of PCNA polyubiquitylation remain to be elucidated in molecular terms, and future studies will be needed in order to gain insight into how the balance between mutagenic TLS and error-free TS is controlled *in vivo*.

Ubiquitylation of Other Damage Bypass Factors

Besides PCNA, numerous other factors involved in DNA damage bypass have been identified as ubiquitylation and/or SUMOylation targets (McIntyre and Woodgate, 2015). As many of the modifications were detected in the context of large-scale proteomics screens, their relevance for damage bypass has not always been confirmed. Nevertheless, some common patterns indicate potential regulatory impacts. For example, all

human TLS polymerases of the Y-family are ubiquitylated, and in many cases this depends on their own ubiquitin-binding domains. Although this is reminiscent of the E3-independent phenomenon of coupled ubiquitylation (Hoeller et al., 2007), relevant ubiquitin ligases have actually been identified, such as Pirh2, Mdm2, and TRIP in the case of polymerase η (Jung et al., 2010, 2011, 2012; Wallace et al., 2014). While Pirh2 attaches monoubiquitin, which apparently inhibits TLS by preventing interaction of the polymerase with $PCNA^{Ub}$ (Bienko et al., 2010), Mdm2 achieves the same effect by targeting polymerase η for polyubiquitylation and proteasomal degradation. In contrast, polyubiquitylation by the TRAF-interacting protein TRIP was reported to promote polymerase η localization to nuclear foci. The *D. melanogaster* homolog of TRIP, NOPO, is known to assemble K63-linked chains, possibly indicating a regulatory function of TRIP-mediated polymerase η modification as well, and interactions of both TRIP and NOPO with several Y-family polymerases suggest a conservation of the process (Wallace et al., 2014). In budding yeast, polymerase η has been found to be ubiquitylated as well; however, the effects of this modification on protein stability remain controversial (Parker et al., 2007; Skoneczna et al., 2007; Pabla et al., 2008; Plachta et al., 2015). Ubiquitin-mediated proteolysis also controls the levels of budding yeast TLS polymerase Rev1 along the cell cycle, thus limiting the bulk of its mutagenic activity to the G2/M phase (Waters and Walker, 2006). Finally, McIntyre et al. (2013) showed that ubiquitylation of human Y-family polymerases appears to promote mutual interactions via their ubiquitin-binding domains and – as a consequence – facilitate their cooperation in TLS.

Another recurring theme in the regulation of damage bypass is the protection of critical factors from proteolysis. This may be achieved by the SUMOylation of a protein, as is observed in the nematode *Caenorhabditis elegans*, where SUMOylation of polymerase η and potentially κ prevents their ubiquitin-mediated degradation during early embryonic development (Kim and Michael, 2008; Roerink et al., 2012). Alternatively, deubiquitylation can effectively stabilize a chromatin-associated protein by preventing its extraction or proteasomal degradation. In human cells, the isopeptidase USP7 appears to play a major role in this manner. As described above, its activity is important even during unperturbed replication (Lecona et al., 2016). In response to replication stress, USP7 deubiquitylates a number of proteins, among them polymerase η, Mdm2, Rad18, and HLTF. By acting on polymerase η and Mdm2, USP7 directly and indirectly stabilizes the polymerase and thereby facilitates TLS (Qian et al., 2015). Deubiquitylation of Rad18 and HLTF was also observed to stabilize the E3s and thus contribute positively to PCNA mono- and polyubiquitylation, respectively (Qing et al., 2011; Zlatanou et al., 2016). In addition, Zeman et al. (2014) reported that a failure to deubiquitylate Rad18 prevented its efficient recruitment and interaction with SHPRH, thus promoting mutagenic TLS at the expense of error-free TS. Surprisingly, USP7 also deubiquitylates PCNA (Kashiwaba et al., 2015). However, unlike the S phase-associated activity of USP1, USP7 activity toward PCNA was not found to be coupled to replication and was therefore proposed to prevent damage-induced mutagenesis during cell cycle-independent processes such as other DNA repair events. In summary, USP7 appears to be an important modulator of replication efficiency and fidelity not only during unperturbed replication, but also during DNA damage bypass.

The Fanconi Anemia Pathway

DNA interstrand cross-links (ICLs) are strongly replication fork-stalling lesions that are not only refractory to copying by replicative DNA polymerases, but also prevent strand separation and passage of the helicase. Accordingly, their processing in replicating cells requires an intricate operation involving components of several repair pathways, namely TLS polymerases, HR proteins and structure-specific nucleases. In vertebrate cells, cooperation between these factors is mediated by the FA pathway, named after a rare hereditary disease associated with bone-marrow failure, congenital abnormalities, cancer predisposition and a marked sensitivity to ICL-causing agents (Kottemann and Smogorzewska, 2013; Walden and Deans, 2014). Nineteen genes have been assigned to this pathway by means of epistasis analysis, and eight of these encode subunits of a multimeric ubiquitin ligase, the FA core complex (Coleman and Huang, 2016). This E3 is recruited to chromatin upon stalling of the replisome upstream of an ICL, where its catalytic subunit, FANCL, monoubiquitylates a heterodimer of two other FA proteins, FANCD2 and FANCI (Alpi et al., 2008; Longerich et al., 2009; Sato et al., 2012). The ubiquitylated form of this "ID complex" initiates ICL processing, which involves the generation of a collapsed fork as a step toward the unhooking of the cross-link by dual incisions on either side of the lesion. It is followed by TLS-mediated repair synthesis and HR-mediated reactivation of the replication fork. How these downstream events are accomplished has only recently been elucidated. Central to the unhooking step is the scaffold protein, SLX4/FANCP, which recognizes the ubiquitylated ID complex by means of two ubiquitin-binding UBZ domains and interacts with a number of structure-specific nucleases that mediate the actual incisions (Zhang and Walter, 2014). Ubiquitin binding by SLX4 is required for cellular resistance specifically toward DNA cross-linking agents (Kim et al., 2013), and mutations in SLX4 confer a FA phenotype in humans, highlighting the importance of SLX4 for ICL repair (Kim et al., 2011; Stoepker et al., 2011).

Another structure-specific endonuclease, FANCD2/FANCI-associated nuclease 1 (FAN1), was identified to act downstream of the ID complex (Kratz et al., 2010; Liu T. et al., 2010; MacKay et al., 2010; Smogorzewska et al., 2010). Like SLX4, FAN1 carries a UBZ domain that was reported to mediate the recruitment to damage sites via binding to monoubiquitylated FANCD2. However, FAN1 was found to be dispensable for ICL incision in a cell-free system (Klein Douwel et al., 2014). Moreover, patients carrying a *FAN1* homozygous microdeletion do not suffer from typical FA conditions (Trujillo et al., 2012), thus arguing against a contribution of the nuclease to the FA pathway. Insight into this conundrum has very recently come from the observation that FAN1 instead prevents

genomic instability induced by replication fork stalling events unrelated to ICLs (Lachaud et al., 2016). Thus, ubiquitylation of the ID complex by the FA core complex appears to serve a twofold purpose in response to replication stress, depending on the downstream effectors: a highly specialized ICL repair pathway triggered by SLX4 recruitment, and an independent, more general fork protection mechanism by means of FAN1.

Interestingly, the FA pathway appears to be intimately connected with another system for replication fork protection, the Rad18- and PCNA-dependent damage bypass mechanism described above. Not only does ICL processing require the activity of TLS polymerases, but the central initiating event of the FA pathway, the activation of the ID complex, was actually found to depend on Rad18, the E3 responsible for PCNA monoubiquitylation. The exact relationship between the two pathways is still a matter of controversy, as one study observed an interaction between FANCL and $PCNA^{Ub}$ that was required for efficient recruitment of FANCL to chromatin (Geng et al., 2010), whereas another report postulated a direct role of Rad18 in binding and recruitment of FANCD2 in a manner independent of PCNA modification (Williams et al., 2011). Another piece of evidence for a tight coordination between the two pathways is the notion that the isopeptidase USP1 mediates deubiquitylation of both PCNA and the ID complex (Nijman et al., 2005; Huang et al., 2006). Controlling the ubiquitylation of these two key players appears to be essential for proper replication fork maintenance, as loss of USP1 causes high levels of genome instability and mutagenesis (Huang et al., 2006).

A recent study discovered a regulatory circuit of polyubiquitin and SUMO that also appears to contribute to controlling FA pathway activity at sites of replication problems (Gibbs-Seymour et al., 2015): upon treatment with replication fork-stalling agents, FANCD2 and FANCI are SUMOylated by two SUMO E3 ligases, PIAS1 and PIAS4, in a manner dependent on prior activation of the ID complex by monoubiquitylation. The modification targets the proteins for RNF4-mediated polyubiquitylation and subsequent extraction from the chromatin by the p97 segregase in complex with DVC1. Hence, this mechanism may limit ID complex dosage at the sites of replication stress in order to terminate the response or avoid excessive activity of the FA pathway.

REPLICATION OF CHROMATIN

Genome replication occurs in the context of chromatin. Hence, for efficient copying of genomic DNA, nucleosomes must be disrupted ahead of an advancing replication fork. Upon passage of the fork, chromatin structure must rapidly be restored, and loss of epigenetic information in the process needs to be avoided. It is therefore not surprising that many chromatin components are targets of the ubiquitin and/or SUMO system for regulatory purposes, and these modifications are known to be important for the replication process itself.

Ubiquitylation of Histones H2A and H2B

Histone H2A was the first protein discovered to be modified by ubiquitin (Goldknopf et al., 1975). In fact, H2A and H2B are two of the most abundant ubiquitylation targets in the nucleus (Cao and Yan, 2012). Both H2A and H2B are predominantly modified by monoubiquitin. H2B was found to be monoubiquitylated at K123 in *S. cerevisiae* or K123 and 120 in human cells, which plays an important role in transcriptional regulation (Henry et al., 2003; Wood et al., 2003; Kao et al., 2004; Nakanishi et al., 2009; Song and Ahn, 2010). In yeast, H2B monoubiquitylation is mediated by the E2 Rad6 and the E3 Bre1 (Robzyk et al., 2000; Wood et al., 2003). The mammalian homologs of Bre1, RNF20, and RNF40 (Kim et al., 2005), cooperate with the E2s hRad6 and UbcH6 (Koken et al., 1991). $H2B^{Ub}$ promotes di- and tri-methylation of H3 at K4, which controls various aspects of transcription (Dover et al., 2002; Sun and Allis, 2002; Krogan et al., 2003), among them a stabilization of the histone chaperone complex FACT (Fleming et al., 2008).

Beyond its role in transcriptional regulation, $H2B^{Ub}$ has been implicated in DNA replication (**Figure 6A**). This connection was established by the observation that Bre1 is enriched around replication origins, where it contributes to maintaining $H2B^{Ub}$ levels on newly replicated DNA (Trujillo and Osley, 2012). Whereas a ubiquitylation-deficient mutant of H2B, K123R, is highly sensitive to replication fork-stalling agents (Trujillo and Osley, 2012; Lin et al., 2014), H3 mutants that abolish methylation are significantly less sensitive (Trujillo and Osley, 2012). This argues that the contribution of $H2B^{Ub}$ to replication is independent of its regulatory role in transcription, mediated through histone methylation. In cells lacking $H2B^{Ub}$, despite efficient formation of the pre-RC, association of replisome components such as polymerases ε and α and RPA with origins is impaired (Trujillo and Osley, 2012), as is replication progression after HU treatment (Lin et al., 2014). Also, PCNA associates normally at origins, but its levels are reduced at more distal sites, suggesting that the H2B-K123R mutant does not affect origin firing, but rather fork progression under conditions of replication stress (Trujillo and Osley, 2012). In addition, lack of $H2B^{Ub}$ leads to a defect in the binding of the FACT complex and reduced nucleosome occupancy in newly replicated DNA under the same stress condition (Trujillo and Osley, 2012; Lin et al., 2014). $H2B^{Ub}$'s effect on FACT in this context is reminiscent of its role during transcription. Since FACT is known to stimulate the activity of the Mcm helicase (Tan et al., 2006), it was speculated that $H2B^{Ub}$ could play a role in facilitating the unwinding of DNA ahead of the fork to promote replication progression. However, this view was challenged by a recent report from Lin et al. (2014), who postulated that $H2B^{Ub}$ may instead function to limit uncontrolled fork progression. They observed significant elongation of replication tracts in the absence of $H2B^{Ub}$ after HU treatment, together with increased levels of H2A phosphorylation, a sign of fork damage (Lin et al., 2014). In support of this model, fork progression under conditions of replication stress is also strongly enhanced in *rad6*Δ cells (Yu et al., 2014).

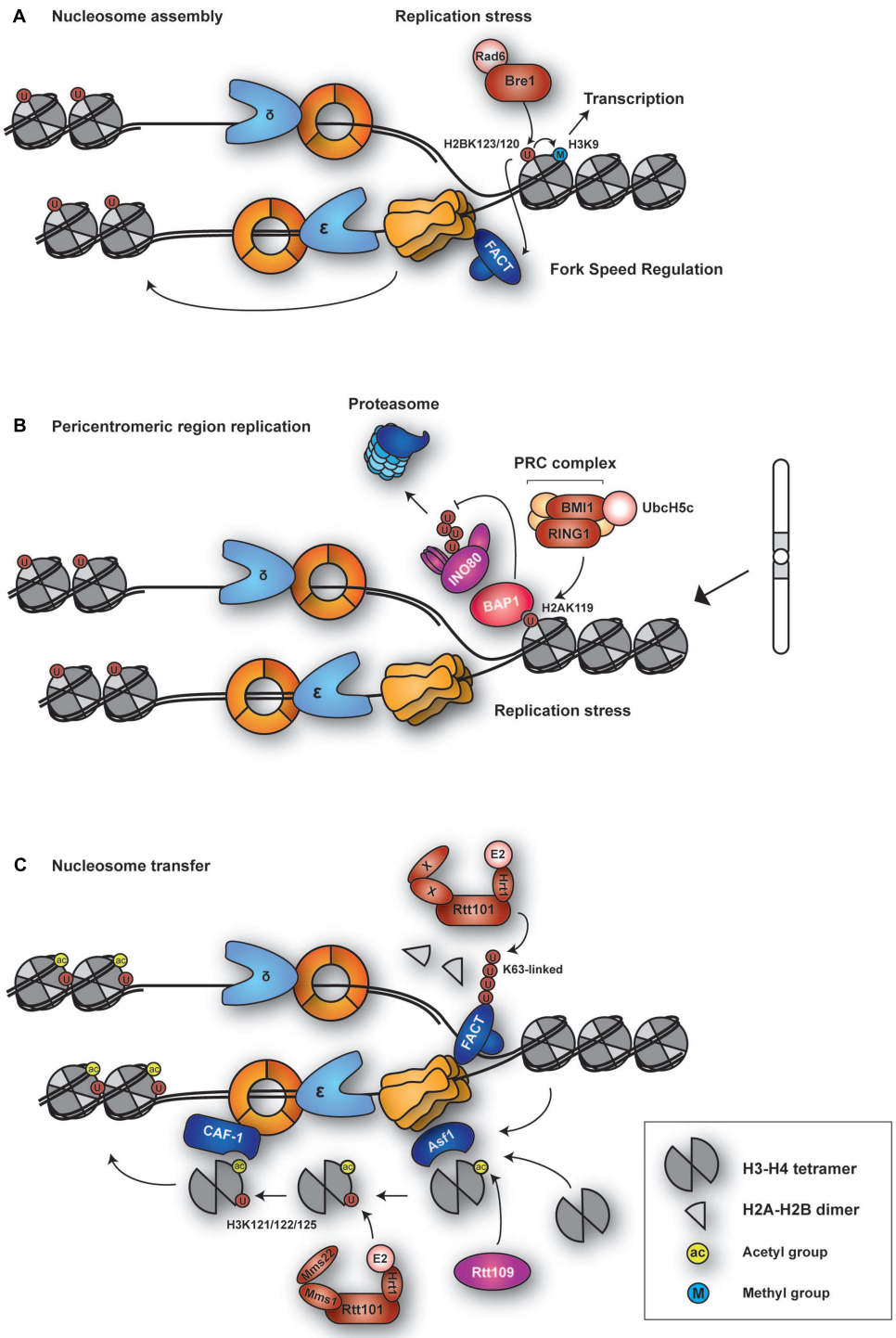

FIGURE 6 | Functions of histone ubiquitylation in DNA replication. (A) Budding yeast E2–E3 complex Rad6-Bre1 is recruited to sites of replication stress for H2B ubiquitylation at K123. $H2B^{Ub}$ regulates fork speed and nucleosome assembly behind the fork. Via H3K9 methylation, it independently contributes to transcriptional regulation. **(B)** The Polycomb Repressive Complex (PRC) is recruited to sites of replication stress or at problematic sequences to ubiquitylate H2A at K119. $H2A^{Ub}$ recruits BAP1, which maintains fork stability by protecting chromatin-remodeling INO80 complex from proteasomal degradation. **(C)** The E3 SCF^{Rtt101} ubiquitylates H3 at K121, 122, and 125. The reaction is stimulated by acetylation of H3 at K56 by histone acetyltransferase Rtt109. This facilitates transfer of the H3-H4 tetramer to CAF-1 for nucleosome deposition behind the fork. Rtt101 also ubiquitylates the chromatin-reorganizing FACT complex.

Taken together, ubiquitylation of H2B appears to coordinate nucleosome assembly with fork progression, an activity that becomes particularly important when the replisome is challenged by replication stress such as nucleotide depletion or DNA damage. However, the precise mechanism and the effectors of the modification are yet to be defined.

H2A is well known to be ubiquitylated at the conserved residue K119 by the polycomb repressive complex 1 (PRC1), which comprises the RING-E3 subunits RING1A or RING1B and BMI1 together with the E2 UbcH5c (Gao et al., 2012; McGinty et al., 2014). The mark is essential for establishing repressive chromatin during development (Lanzuolo and Orlando, 2012; Di Croce and Helin, 2013). However, $H2A^{Ub}$ may also play a role in the replication of intact and damaged DNA (**Figure 6B**). RING1B localizes to sites of replication (Lee et al., 2014; Piunti et al., 2014), and several Polycomb proteins were also found to be recruited to sites of DNA damage (Bergink et al., 2006; Chou et al., 2010; Ginjala et al., 2011), suggesting that $H2A^{Ub}$ may contribute to damage signaling at replication forks. In fact, loss of PRC function causes an increase in asymmetric forks, indicating perturbed replication dynamics (Piunti et al., 2014; Bravo et al., 2015). Conversely, enhancement of H2A ubiquitylation by depletion of the ubiquitin-specific protease USP3 in mammalian cells causes delays in S phase progression and increased formation of ssDNA and DNA breaks (Nicassio et al., 2007). These observations are consistent with $H2A^{Ub}$ acting as a damage signal that – when present in excess – leads to a hyperactivation of the damage response that would generate abnormal replication or repair structures causing genomic instability.

A recent study suggests a special role of $H2A^{Ub}$ in the replication of pericentromeric heterochromatic domains, which are duplicated late in S phase (Bravo et al., 2015). Cells deficient in all RING1 activities were found to accumulate high levels of ssDNA in these regions, along with increased spontaneous levels of γH2AX and a delayed transition from middle to late S phase. Consistent with these findings, $H2A^{Ub}$ colocalizes with PCNA in late S phase (Vassilev et al., 1995). Interestingly, selective restoration of $H2A^{Ub}$ within the pericentromeric heterocromatic domains by means of a fusion construct of RING1B, BMI1 and methyl-CpG binding domain protein 1 (MBD1) rescued the defect in S phase progression in RING1-deficient cells (Bravo et al., 2015). Given the enrichment of major satellite repeats in pericentric heterochromatin and their propensity to form secondary structures, the strong effect of $H2A^{Ub}$ in these regions may well reflect a general contribution of the modification to the replication of problematic sequences.

The mechanism by which $H2A^{Ub}$ influences DNA replication is still unknown, but some insight comes from the observation that BRCA1-associated protein-1 (BAP1) recognizes $H2A^{Ub}$ at replication forks and recruits the ATP-dependent chromatin remodeler Ino80. BAP1 deubiquitylates INO80 and thereby protects the protein from ubiquitin-mediated proteolysis (Lee et al., 2014). Hence, via BAP1 recruitment $H2A^{Ub}$ might allow the INO80 complex to exert its well-known role in stabilizing stalled replication forks and assisting fork restart (Papamichos-Chronakis and Peterson, 2008; Shimada et al., 2008; Vincent et al., 2008; Falbo et al., 2009; Vassileva et al., 2014).

Ubiquitylation of Histone H3 in Replication-Coupled Nucleosome Assembly

In order to ensure proper restoration of chromatin structure upon genome replication, the nucleosome assembly machinery is tightly coupled to replication fork progression. In budding yeast, this is achieved by means of a pathway involving acetylation of histone H3, a marker of newly synthesized histones (**Figure 6C**). In front of a replication fork, nucleosomes are disassembled by the action of the Mcm2-7 complex and histone chaperone Asf1 (Groth et al., 2007; Huang et al., 2015). Behind the fork, both parental and newly synthesized histones contribute to the restoration of chromatin structure. In *S. cerevisiae*, preferential binding of Asf1 to the H3-H4 dimer stimulates acetylation of newly synthesized H3 at K56 by the histone acetyltransferase Rtt109 (Masumoto et al., 2005; Driscoll et al., 2007; Han et al., 2007a). $H3K56^{ac}$ enhances binding of H3-H4 to histone chaperones Rtt106 and CAF-1 (Li et al., 2008). CAF-1 in turn interacts with PCNA and assists in histone deposition behind the fork (Shibahara and Stillman, 1999; Moggs et al., 2000; Zhang et al., 2000). H3 acetylation peaks in S phase and is removed upon completion of genome replication (Masumoto et al., 2005). $H3K56^{ac}$ is also detectable in mammalian cells, although in much lower abundance compared to yeast (Garcia et al., 2007; Das et al., 2009; Tjeertes et al., 2009; Yuan et al., 2009; Jasencakova et al., 2010), suggesting that either the modification is much more transient, or other acetylation sites can substitute for H3K56. Defects in the Asf1-Rtt109-$H3K56^{ac}$ pathway result in various aspects of genome instability, including reduced replisome function under conditions of replication stress (Franco et al., 2005; Schulz and Tyler, 2006; Han et al., 2007b; Clemente-Ruiz et al., 2011), sensitivity to DNA-damaging agents (Driscoll et al., 2007; Li et al., 2008), loss of sister chromatid cohesion, excessive recombination and high rates of gross chromosomal rearrangements (Myung et al., 2003; Prado et al., 2004; Ramey et al., 2004; Thaminy et al., 2007; Kadyrova et al., 2013; Munoz-Galvan et al., 2013).

Intriguingly, a large-scale genetic screen in budding yeast identified the CRL ubiquitin ligase complex, $Rtt101^{Mms1/Mms22}$, as a downstream effector of the pathway (Collins et al., 2007b). $Rtt101^{Mms1}$ is believed to be the budding yeast ortholog of human $CRL4^{DDB1}$ and assembles with the substrate adaptor Mms22 in a DNA damage-induced manner (Zaidi et al., 2008; Han et al., 2010, 2013). Inactivation of the complex causes damage sensitivity and defects in fork progression through damaged DNA and natural replication pause sites such as ribosomal DNA loci, and these defects are epistatic with the lack of Rtt109 (Luke et al., 2006; Duro et al., 2008; Zaidi et al., 2008; Wurtele et al., 2012). Moreover, Rtt109 was indeed found to recruit Rtt101 to chromatin (Roberts et al., 2008).

Despite the strong genetic link between the Asf1-Rtt109-$H3K56^{ac}$ nucleosome assembly pathway and $Rtt101^{Mms1/Mms22}$

in replisome functions and genome maintenance, their cooperation is not well understood in mechanistic terms. A recent report might provide insight into the process. Han et al. (2013) found that Rtt101$^{Mms1/Mms22}$ preferentially binds H3K56^{ac}-H4 over unmodified H3-H4 and can directly ubiquitylate H3 at lysine residues 121, 122, and 125. This in turn weakens H3 interaction with Asf1 and instead facilitates association with CAF-1 for subsequent deposition behind the replication fork. This function appears to be conserved in human cells, as depletion of CRL4^{DDB1} results in enhanced interaction of H3-H4 with Asf1 and reduced deposition of new H3 (Han et al., 2013). Hence, Rtt101$^{Mms1/Mms22}$-mediated ubiquitylation of H3 appears to assist in a hand-off mechanism that ensures the transfer of H3–H4 from Asf1 ahead of an advancing fork to other chaperones such as CAF-1 and Rtt106 behind the fork.

How does nucleosome assembly influence replisome stability? There is growing evidence indicating that coupling of nucleosome assembly and replication progression is essential for maintenance of intact replisomes. This view is supported by the observation that deregulation of histone supply causes replication forks to collapse, followed by recombination-mediated rescue (Groth et al., 2007; Clemente-Ruiz and Prado, 2009; Takayama and Toda, 2010; Clemente-Ruiz et al., 2011; Mejlvang et al., 2014). This effect is reminiscent of the situation where lack of the Asf1-Rtt109-H3K56^{ac} or Rtt101$^{Mms1/Mms22}$ pathway causes a decoupling of nucleosome assembly and replication progression.

Nevertheless, many mechanistic questions remain. For example, the majority of Cullin-based ubiquitin ligases are known to produce K48-linked polyubiquitin chains that target their substrates for proteasomal degradation (Komander and Rape, 2012; Mattiroli and Sixma, 2014), and the E2 Cdc34 that associates with Rtt101 is also known to assemble K48-chains (Ye and Rape, 2009). Yet, H3 itself is unlikely to be a substrate of the proteasome. This has led to the idea that Rtt101$^{Mms1/Mms22}$ may target other components at stalled forks for degradation in order to facilitate repair or restart. Indeed, one such substrate could be the FACT complex (Han et al., 2010), which requires Rtt101 specifically for localization to sites of replication, but not to transcription sites. Intriguingly, however, in this case a K63-linked ubiquitin chain was detected on FACT (Han et al., 2010). Hence, it is still an open question whether Rtt101$^{Mms1/Mms22}$ plays any role in proteasomal degradation mediated via K48-chains.

Ubiquitylation of Histone H3 in Replication-Coupled Epigenetic Inheritance

In order to maintain its identity and gene expression patterns, it is crucial for a cell to restore its epigenetic information after every round of replication. Due to the semiconservative nature of DNA replication, DNA is hemi-methylated after every replication cycle, and full DNA methylation has to be restored in order to reestablish gene silencing. It is known that a RING-type ubiquitin ligase, UHRF1 (ubiquitin-like with PHD and ring finger domains 1, also known as NP95 in mouse and ICBP90 in humans), is recruited to nascent DNA after replication (Lopez-Contreras et al., 2013). UHRF1 binds to hemi-methylated DNA through its SET and RING finger-associated (SRA) domain (Arita et al., 2008; Avvakumov et al., 2008). Recently, Nishiyama et al. (2013) found that UHRF1 ubiquitylates histone H3 at K23 in *X. laevis* egg extracts. Methylation is then restored by DNMT1, which recognizes H3K23^{Ub} through its replication foci targeting sequence. A similar mechanism is observed in mammalian cells, where UHRF1 ubiquitylates H3 at K18. Here, DNMT1 binds to H3K18^{Ub} via a ubiquitin-interacting UIM motif (Qin et al., 2015). This is an interesting example of how cells can use the ubiquitin system to establish other epigenetic marks following DNA replication.

SPATIAL REGULATION OF UBIQUITYLATION AND SUMOYLATION DURING DNA REPLICATION

The ubiquitin and SUMO systems are organized within the cell in a spatially controlled manner. One important hub for the coordination of nuclear ubiquitylation and SUMOylation activities appears to be the nuclear pore complex (NPC). The NPC is responsible for the transport of macromolecules between the nucleus and the cytoplasm, but genetic data from budding yeast suggest that it has additional functions in coordinating DNA damage signaling and repair (**Figure 7**). For example, it has been observed that cells deficient in components of the Nup84 nuclear pore subcomplex are hypersensitive to DNA-damaging agents (Bennett et al., 2001; Loeillet et al., 2005; Therizols et al., 2006) and accumulate spontaneous recombination foci in S and G2 phase (Loeillet et al., 2005; Palancade et al., 2007; Nagai et al., 2008). Mutations in both Nup84 and the HR pathway are synthetically lethal (Loeillet et al., 2005). These findings suggest that the NPC plays a role in replication during both unperturbed and stress conditions, and HR-based mechanisms to resolve fork problems become essential when NPC function is compromised.

A number of ubiquitin- and SUMO-related enzymes are found at the nuclear pore. For instance, SUMO protease Ulp1 is anchored to the nucleoporin Nup60 through myosin-like proteins (MLPs) Mlp1 and Mlp2 (Zhao et al., 2004). Mutation of *ULP1* or loss of MLPs shows synthetic effects when combined with mutations in HR (Zhao et al., 2004; Collins et al., 2007a; Palancade et al., 2007), and deleting MLPs leads to mislocalization of Ulp1, DNA damage sensitivity and clonal lethality (Zhao et al., 2004). Moreover, cells with impaired Ulp1function accumulate ssDNA during replication (Soustelle et al., 2004). It is therefore conceivable that the presence of deSUMOylating activity at the nuclear pore either prevents the accumulation of toxic recombination intermediates during replication or is required to resolve these structures. Proper localization of the SUMO conjugation system thus impinges on the process of DNA replication itself.

Intriguingly, the nuclear pore is also the site of accumulation of STUbLs in yeast (Nagai et al., 2011). Deletion of the STUbL complex Slx5/8 renders yeast hypersensitive to DNA-damaging

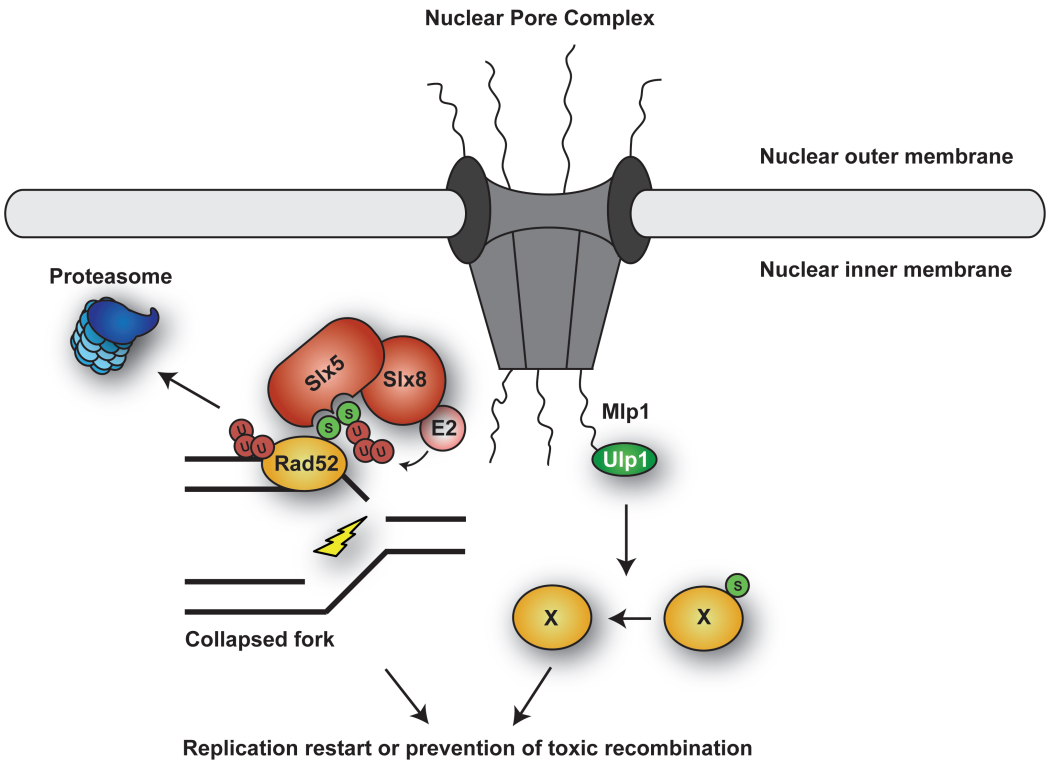

FIGURE 7 | Spatial organization of ubiquitin and SUMO metabolism in the nucleus. Both ubiquitin and SUMO conjugating and deconjugating enzymes are enriched at the Nuclear Pore Complex (NPC). Collapsed forks relocalize to NPCs, which modulates local ubiquitylation and/or SUMOylation of relevant components in order to facilitate fork restart or prevent toxic recombination events.

agents and replication stress, and the mutants show higher rates of spontaneous gross chromosomal rearrangements (Zhang et al., 2006; Prudden et al., 2007; Nagai et al., 2008). Consistent with these findings, collapsed replication forks – like DSBs – are redirected to the nuclear pore (Nagai et al., 2008; Horigome et al., 2014). These observations prompted the hypothesis that relocalization to the nuclear pore facilitates HR-mediated fork restart by means of STUbL activity, possibly via degradation of SUMOylated proteins such as Srs2 (Urulangodi et al., 2015). In support of this idea, Su et al. (2015) recently observed that sites of replication blockage created by expanded CAG repeats are relocated to nuclear pores particularly in late S phase. The authors suggested that such relocalization may target $Rad52^{SUMO}$ for degradation, which would then alter the outcome of HR pathways in the context of replication restart. In humans, it has been proposed that the PML nuclear bodies functionally resemble the yeast nuclear pores as a site where the mammalian STUbL RNF4 accumulates (Nagai et al., 2011). However, it remains to be tested whether perturbed replication forks are redirected to PML bodies in human cells.

Considering the large number of repair factors and replisome components that are SUMOylated during replication (Cremona et al., 2012; Psakhye and Jentsch, 2012), directing a collapsed fork to the nuclear pore may provide a window of opportunity for cells to fine-tune repair events by altering the fate of various repair and replication factors via posttranslational modification. However, it is still not fully understood how these activities are coordinated at the pore, for instance whether a certain factor is deSUMOylated by Ulp1 or directed to proteasomal degradation through STUbL activity. How such events would impact on the outcome of repair and the consequences for genome integrity awaits further investigation.

CONCLUDING REMARKS

The extensive range of mechanisms by which ubiquitin and SUMO impinge on eukaryotic DNA replication is a very good reflection of the diversity of these two posttranslational modification systems in general. Several recurring concepts, including proteasomal targeting, either by ubiquitin alone or in a SUMO-dependent manner as mediated by the SUMO-targeted ubiquitin ligases, but also SUMO-mediated protection from ubiquitylation and proteolysis, can be observed to operate on replicating chromatin. Non-proteolytic functions, such as the enhancement of protein–protein interactions via SUMOylation, monoubiquitylation or linkage-specific polyubiquitylation, play an even more prominent role in the recruitment of various regulatory factors to active or stalled replisomes. Importantly, when compared to replication initiation, which is largely coupled to cell cycle regulatory events, replication fork progression appears to be an extremely delicate condition in which numerous

modulating modifications are needed to fine-tune the activity of various components or stabilize weakly associated complexes in order to maintain fork integrity. No matter whether individual factors or entire groups of proteins are concerned, there is a large gray area between those modifications that regulate unperturbed replication and those that are initiated in response to replication problems and stress conditions.

Perhaps not surprisingly, a few key replication factors emerge as nodes in a network of posttranslational modification targets around the replication fork, such as PCNA, RPA and several central recombination proteins. Complementary to these prime targets, a few conjugation and deconjugation enzymes appear to dominate the replication-associated modification landscape and might thus be critical for coordinating different pathways involved in signaling or damage processing. These include ubiquitin ligases such as Rad18, RNF4 and the $CRL4^{Cdt2}$ complex, but also prominent isopeptidases like USP1 and USP7. Undoubtedly, the range of identified targets and functions will continue to expand with the growing interest in these factors. Perhaps the biggest challenge for future research will be the interpretation of the wealth of information gathered by proteomics approaches. Substantiating and making sense of all the modification events that have by now been detected in system-wide screens, distinguishing relevant from bystander events, analyzing their regulation, and finally assigning a physiological role to them will occupy many laboratories for a long time to come.

AUTHOR CONTRIBUTIONS

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

FUNDING

Work in this lab is funded by an advanced investigator grant from the European Research Council to HU.

ACKNOWLEDGMENT

The authors would like to thank Román González-Prieto and Brian Luke for critical comments on the manuscript.

REFERENCES

Abbas, T., Sivaprasad, U., Terai, K., Amador, V., Pagano, M., and Dutta, A. (2008). PCNA-dependent regulation of p21 ubiquitylation and degradation via the CRL4Cdt2 ubiquitin ligase complex. *Genes Dev.* 22, 2496–2506. doi: 10.1101/gad.1676108

Albuquerque, C. P., Wang, G., Lee, N. S., Kolodner, R. D., Putnam, C. D., and Zhou, H. (2013). Distinct SUMO ligases cooperate with Esc2 and Slx5 to suppress duplication-mediated genome rearrangements. *PLoS Genet.* 9:e1003670. doi: 10.1371/journal.pgen.1003670

Almedawar, S., Colomina, N., Bermudez-Lopez, M., Pocino-Merino, I., and Torres-Rosell, J. (2012). A SUMO-dependent step during establishment of sister chromatid cohesion. *Curr. Biol.* 22, 1576–1581. doi: 10.1016/j.cub.2012.06.046

Alpi, A. F., Pace, P. E., Babu, M. M., and Patel, K. J. (2008). Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol. Cell.* 32, 767–777. doi: 10.1016/j.molcel.2008.12.003

Ampatzidou, E., Irmisch, A., O'connell, M. J., and Murray, J. M. (2006). Smc5/6 is required for repair at collapsed replication forks. *Mol. Cell. Biol.* 26, 9387–9401. doi: 10.1128/MCB.01335-06

Andrews, E. A., Palecek, J., Sergeant, J., Taylor, E., Lehmann, A. R., and Watts, F. Z. (2005). Nse2, a component of the Smc5-6 complex, is a SUMO ligase required for the response to DNA damage. *Mol. Cell. Biol.* 25, 185–196. doi: 10.1128/MCB.25.1.185-196.2005

Arakawa, H., Moldovan, G. L., Saribasak, H., Saribasak, N. N., Jentsch, S., and Buerstedde, J. M. (2006). A role for PCNA ubiquitination in immunoglobulin hypermutation. *PLoS Biol.* 4:e366. doi: 10.1371/journal.pbio.0040366

Arita, K., Ariyoshi, M., Tochio, H., Nakamura, Y., and Shirakawa, M. (2008). Recognition of hemi-methylated DNA by the SRA protein UHRF1 by a base-flipping mechanism. *Nature* 455, 818–821. doi: 10.1038/nature07249

Armstrong, A. A., Mohideen, F., and Lima, C. D. (2012). Recognition of SUMO-modified PCNA requires tandem receptor motifs in Srs2. *Nature* 483, 59–63. doi: 10.1038/nature10883

Avvakumov, G. V., Walker, J. R., Xue, S., Li, Y., Duan, S., Bronner, C., et al. (2008). Structural basis for recognition of hemi-methylated DNA by the SRA domain of human UHRF1. *Nature* 455, 822–825. doi: 10.1038/nature07273

Bell, S. P. (2014). DNA Replication. Terminating the replisome. *Science* 346, 418–419. doi: 10.1126/science.1261245

Bennett, C. B., Lewis, L. K., Karthikeyan, G., Lobachev, K. S., Jin, Y. H., Sterling, J. F., et al. (2001). Genes required for ionizing radiation resistance in yeast. *Nat. Genet.* 29, 426–434. doi: 10.1038/ng778

Bergink, S., Salomons, F. A., Hoogstraten, D., Groothuis, T. A., De Waard, H., Wu, J., et al. (2006). DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A. *Genes Dev.* 20, 1343–1352. doi: 10.1101/gad.373706

Bermudez-Lopez, M., Ceschia, A., De Piccoli, G., Colomina, N., Pasero, P., Aragon, L., et al. (2010). The Smc5/6 complex is required for dissolution of DNA-mediated sister chromatid linkages. *Nucleic Acids Res.* 38, 6502–6512. doi: 10.1093/nar/gkq546

Bermudez-Lopez, M., Pocino-Merino, I., Sanchez, H., Bueno, A., Guasch, C., Almedawar, S., et al. (2015). ATPase-dependent control of the Mms21 SUMO ligase during DNA repair. *PLoS Biol.* 13:e1002089. doi: 10.1371/journal.pbio.1002089

Berti, M., and Vindigni, A. (2016). Replication stress: getting back on track. *Nat. Struct. Mol. Biol.* 23, 103–109. doi: 10.1038/nsmb.3163

Bi, X., Barkley, L. R., Slater, D. M., Tateishi, S., Yamaizumi, M., Ohmori, H., et al. (2006). Rad18 regulates DNA polymerase kappa and is required for recovery from S-phase checkpoint-mediated arrest. *Mol. Cell. Biol.* 26, 3527–3540. doi: 10.1128/MCB.26.9.3527-3540.2006

Bienko, M., Green, C. M., Crosetto, N., Rudolf, F., Zapart, G., Coull, B., et al. (2005). Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. *Science* 310, 1821–1824. doi: 10.1126/science.1120615

Bienko, M., Green, C. M., Sabbioneda, S., Crosetto, N., Matic, I., Hibbert, R. G., et al. (2010). Regulation of translesion synthesis DNA polymerase eta by monoubiquitination. *Mol. Cell.* 37, 396–407. doi: 10.1016/j.molcel.2009.12.039

Bohm, S., and Bernstein, K. A. (2014). The role of post-translational modifications in fine-tuning BLM helicase function during DNA repair. *DNA Repair (Amst.)* 22, 123–132. doi: 10.1016/j.dnarep.2014.07.007

Bohm, S., Mihalevic, M. J., Casal, M. A., and Bernstein, K. A. (2015). Disruption of SUMO-targeted ubiquitin ligases Slx5-Slx8/RNF4 alters RecQ-like helicase Sgs1/BLM localization in yeast and human cells. *DNA Repair (Amst.)* 26, 1–14. doi: 10.1016/j.dnarep.2014.12.004

Bonne-Andrea, C., Kahli, M., Mechali, F., Lemaitre, J. M., Bossis, G., and Coux, O. (2013). SUMO2/3 modification of cyclin E contributes to the

control of replication origin firing. *Nat. Commun.* 4:1850. doi: 10.1038/ncomms2875

Branzei, D., Sollier, J., Liberi, G., Zhao, X., Maeda, D., Seki, M., et al. (2006). Ubc9- and mms21-mediated sumoylation counteracts recombinogenic events at damaged replication forks. *Cell* 127, 509–522. doi: 10.1016/j.cell.2006.08.050

Bravo, M., Nicolini, F., Starowicz, K., Barroso, S., Cales, C., Aguilera, A., et al. (2015). Polycomb RING1A- and RING1B-dependent histone H2A monoubiquitylation at pericentromeric regions promotes S-phase progression. *J. Cell Sci.* 128, 3660–3671. doi: 10.1242/jcs.173021

Bugreev, D. V., Yu, X., Egelman, E. H., and Mazin, A. V. (2007). Novel pro- and anti-recombination activities of the Bloom's syndrome helicase. *Genes Dev.* 21, 3085–3094. doi: 10.1101/gad.1609007

Burgess, R. C., Rahman, S., Lisby, M., Rothstein, R., and Zhao, X. (2007). The Slx5-Slx8 complex affects sumoylation of DNA repair proteins and negatively regulates recombination. *Mol. Cell. Biol.* 27, 6153–6162. doi: 10.1128/MCB.00787-07

Bursomanno, S., Beli, P., Khan, A. M., Minocherhomji, S., Wagner, S. A., Bekker-Jensen, S., et al. (2015). Proteome-wide analysis of SUMO2 targets in response to pathological DNA replication stress in human cells. *DNA Repair (Amst.)* 25, 84–96. doi: 10.1016/j.dnarep.2014.10.011

Cao, J., and Yan, Q. (2012). Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Front. Oncol.* 2:26. doi: 10.3389/fonc.2012.00026

Cazzalini, O., Sommatis, S., Tillhon, M., Dutto, I., Bachi, A., Rapp, A., et al. (2014). CBP and p300 acetylate PCNA to link its degradation with nucleotide excision repair synthesis. *Nucleic Acids Res.* 42, 8433–8448. doi: 10.1093/nar/gku533

Centore, R. C., Yazinski, S. A., Tse, A., and Zou, L. (2012). Spartan/C1orf124, a reader of PCNA ubiquitylation and a regulator of UV-induced DNA damage response. *Mol. Cell.* 46, 625–635. doi: 10.1016/j.molcel.2012.05.020

Chavez, A., George, V., Agrawal, V., and Johnson, F. B. (2010). Sumoylation and the structural maintenance of chromosomes (Smc) 5/6 complex slow senescence through recombination intermediate resolution. *J. Biol. Chem.* 285, 11922–11930. doi: 10.1074/jbc.M109.041277

Chen, Y. H., Choi, K., Szakal, B., Arenz, J., Duan, X., Ye, H., et al. (2009). Interplay between the Smc5/6 complex and the Mph1 helicase in recombinational repair. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21252–21257. doi: 10.1073/pnas.0908258106

Choi, K., Batke, S., Szakal, B., Lowther, J., Hao, F., Sarangi, P., et al. (2015). Concerted and differential actions of two enzymatic domains underlie Rad5 contributions to DNA damage tolerance. *Nucleic Acids Res.* 43, 2666–2677. doi: 10.1093/nar/gkv004

Choi, K., Szakal, B., Chen, Y. H., Branzei, D., and Zhao, X. (2010). The Smc5/6 complex and Esc2 influence multiple replication-associated recombination processes in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 21, 2306–2314. doi: 10.1091/mbc.E10-01-0050

Chou, D. M., Adamson, B., Dephoure, N. E., Tan, X., Nottke, A. C., Hurov, K. E., et al. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18475–18480. doi: 10.1073/pnas.1012946107

Ciccia, A., Nimonkar, A. V., Hu, Y., Hajdu, I., Achar, Y. J., Izhar, L., et al. (2012). Polyubiquitinated PCNA recruits the ZRANB3 translocase to maintain genomic integrity after replication stress. *Mol. Cell.* 47, 396–409. doi: 10.1016/j.molcel.2012.05.024

Clemente-Ruiz, M., Gonzalez-Prieto, R., and Prado, F. (2011). Histone H3K56 acetylation, CAF1, and Rtt106 coordinate nucleosome assembly and stability of advancing replication forks. *PLoS Genet.* 7:e1002376. doi: 10.1371/journal.pgen.1002376

Clemente-Ruiz, M., and Prado, F. (2009). Chromatin assembly controls replication fork stability. *EMBO Rep.* 10, 790–796. doi: 10.1038/embor.2009.67

Clijsters, L., and Wolthuis, R. (2014). PIP-box-mediated degradation prohibits re-accumulation of Cdc6 during S phase. *J. Cell Sci.* 127, 1336–1345. doi: 10.1242/jcs.145862

Coleman, K. E., and Huang, T. T. (2016). How SUMOylation fine-tunes the Fanconi Anemia DNA repair pathway. *Front. Genet.* 7:61. doi: 10.3389/fgene.2016.00061

Collins, S. R., Kemmeren, P., Zhao, X. C., Greenblatt, J. F., Spencer, F., Holstege, F. C., et al. (2007a). Toward a comprehensive atlas of the physical interactome of *Saccharomyces cerevisiae*. *Mol. Cell. Proteomics* 6, 439–450. doi: 10.1074/mcp.M600381-MCP200

Collins, S. R., Miller, K. M., Maas, N. L., Roguev, A., Fillingham, J., Chu, C. S., et al. (2007b). Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature* 446, 806–810. doi: 10.1038/nature05649

Coulon, S., Ramasubramanyan, S., Alies, C., Philippin, G., Lehmann, A., and Fuchs, R. P. (2010). Rad8Rad5/Mms2-Ubc13 ubiquitin ligase complex controls translesion synthesis in fission yeast. *EMBO J.* 29, 2048–2058. doi: 10.1038/emboj.2010.87

Cremona, C. A., Sarangi, P., Yang, Y., Hang, L. E., Rahman, S., and Zhao, X. (2012). Extensive DNA damage-induced sumoylation contributes to replication and repair and acts in addition to the mec1 checkpoint. *Mol. Cell.* 45, 422–432. doi: 10.1016/j.molcel.2011.11.028

Crosetto, N., Bienko, M., Hibbert, R. G., Perica, T., Ambrogio, C., Kensche, T., et al. (2008). Human Wrnip1 is localized in replication factories in a ubiquitin-binding zinc finger-dependent manner. *J. Biol. Chem.* 283, 35173–35185. doi: 10.1074/jbc.M803219200

Daigaku, Y., Davies, A. A., and Ulrich, H. D. (2010). Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature* 465, 951–955. doi: 10.1038/nature09097

Das, C., Lucia, M. S., Hansen, K. C., and Tyler, J. K. (2009). CBP/p300-mediated acetylation of histone H3 on lysine 56. *Nature* 459, 113–117. doi: 10.1038/nature07861

Das-Bradoo, S., Ricke, R. M., and Bielinsky, A. K. (2006). Interaction between PCNA and diubiquitinated Mcm10 is essential for cell growth in budding yeast. *Mol. Cell. Biol.* 26, 4806–4817. doi: 10.1128/MCB.02062-05

Davies, A. A., Huttner, D., Daigaku, Y., Chen, S., and Ulrich, H. D. (2008). Activation of ubiquitin-dependent DNA damage bypass is mediated by replication protein a. *Mol. Cell.* 29, 625–636. doi: 10.1016/j.molcel.2007.12.016

Davis, E. J., Lachaud, C., Appleton, P., Macartney, T. J., Nathke, I., and Rouse, J. (2012). DVC1 (C1orf124) recruits the p97 protein segregase to sites of DNA damage. *Nat. Struct. Mol. Biol.* 19, 1093–1100. doi: 10.1038/nsmb.2394

de Albuquerque, C. P., Liang, J., Gaut, N. J., and Zhou, H. (2016). Molecular circuitry of the SUMO pathway in controlling sumoylation homeostasis and suppressing genome rearrangements. *J Biol Chem.* [Epub ahead of print]. doi: 10.1074/jbc.M116.716399

Di Croce, L., and Helin, K. (2013). Transcriptional regulation by Polycomb group proteins. *Nat. Struct. Mol. Biol.* 20, 1147–1155. doi: 10.1038/nsmb.2669

Donzelli, M., Squatrito, M., Ganoth, D., Hershko, A., Pagano, M., and Draetta, G. F. (2002). Dual mode of degradation of Cdc25 A phosphatase. *EMBO J.* 21, 4875–4884. doi: 10.1093/emboj/cdf491

Dou, H., Huang, C., Singh, M., Carpenter, P. B., and Yeh, E. T. (2010). Regulation of DNA repair through deSUMOylation and SUMOylation of replication protein A complex. *Mol. Cell.* 39, 333–345. doi: 10.1016/j.molcel.2010.07.021

Dover, J., Schneider, J., Tawiah-Boateng, M. A., Wood, A., Dean, K., Johnston, M., et al. (2002). Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *J. Biol. Chem.* 277, 28368–28371. doi: 10.1074/jbc.C200348200

Doyle, J. M., Gao, J., Wang, J., Yang, M., and Potts, P. R. (2010). MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol. Cell.* 39, 963–974. doi: 10.1016/j.molcel.2010.08.029

Driscoll, R., Hudson, A., and Jackson, S. P. (2007). Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. *Science* 315, 649–652. doi: 10.1126/science.1135862

Drury, L. S., Perkins, G., and Diffley, J. F. (1997). The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast. *EMBO J.* 16, 5966–5976. doi: 10.1093/emboj/16.19.5966

Duan, X., Sarangi, P., Liu, X., Rangi, G. K., Zhao, X., and Ye, H. (2009). Structural and functional insights into the roles of the Mms21 subunit

of the Smc5/6 complex. *Mol. Cell.* 35, 657–668. doi: 10.1016/j.molcel.2009.06.032

Duro, E., Vaisica, J. A., Brown, G. W., and Rouse, J. (2008). Budding yeast Mms22 and Mms1 regulate homologous recombination induced by replisome blockage. *DNA Repair (Amst.)* 7, 811–818. doi: 10.1016/j.dnarep.2008.01.007

Dutertre, S., Ababou, M., Onclercq, R., Delic, J., Chatton, B., Jaulin, C., et al. (2000). Cell cycle regulation of the endogenous wild type Bloom's syndrome DNA helicase. *Oncogene* 19, 2731–2738. doi: 10.1038/sj.onc.1203595

Edmunds, C. E., Simpson, L. J., and Sale, J. E. (2008). PCNA ubiquitination and REV1 define temporally distinct mechanisms for controlling translesion synthesis in the avian cell line DT40. *Mol. Cell.* 30, 519–529. doi: 10.1016/j.molcel.2008.03.024

Eladad, S., Ye, T. Z., Hu, P., Leversha, M., Beresten, S., Matunis, M. J., et al. (2005). Intra-nuclear trafficking of the BLM helicase to DNA damage-induced foci is regulated by SUMO modification. *Hum. Mol. Genet.* 14, 1351–1365. doi: 10.1093/hmg/ddi145

Eldridge, A. G., Loktev, A. V., Hansen, D. V., Verschuren, E. W., Reimann, J. D., and Jackson, P. K. (2006). The evi5 oncogene regulates cyclin accumulation by stabilizing the anaphase-promoting complex inhibitor emi1. *Cell* 124, 367–380. doi: 10.1016/j.cell.2005.10.038

Elia, A. E., Wang, D. C., Willis, N. A., Boardman, A. P., Hajdu, I., Adeyemi, R. O., et al. (2015). RFWD3-Dependent Ubiquitination of RPA Regulates Repair at Stalled Replication Forks. *Mol. Cell.* 60, 280–293. doi: 10.1016/j.molcel.2015.09.011

Falbo, K. B., Alabert, C., Katou, Y., Wu, S., Han, J., Wehr, T., et al. (2009). Involvement of a chromatin remodeling complex in damage tolerance during DNA replication. *Nat. Struct. Mol. Biol.* 16, 1167–1172. doi: 10.1038/nsmb.1686

Fleming, A. B., Kao, C. F., Hillyer, C., Pikaart, M., and Osley, M. A. (2008). H2B ubiquitylation plays a role in nucleosome dynamics during transcription elongation. *Mol. Cell.* 31, 57–66. doi: 10.1016/j.molcel.2008.04.025

Frampton, J., Irmisch, A., Green, C. M., Neiss, A., Trickey, M., Ulrich, H. D., et al. (2006). Postreplication repair and PCNA modification in *Schizosaccharomyces pombe*. *Mol. Biol. Cell* 17, 2976–2985. doi: 10.1091/mbc.E05-11-1008

Franco, A. A., Lam, W. M., Burgers, P. M., and Kaufman, P. D. (2005). Histone deposition protein Asf1 maintains DNA replisome integrity and interacts with replication factor C. *Genes Dev.* 19, 1365–1375. doi: 10.1101/gad.1305005

Franz, A., Ackermann, L., and Hoppe, T. (2016). Ring of change: CDC48/p97 drives protein dynamics at chromatin. *Front. Genet.* 7:73. doi: 10.3389/fgene.2016.00073

Fukushima, H., Ogura, K., Wan, L., Lu, Y., Li, V., Gao, D., et al. (2013). SCF-mediated Cdh1 degradation defines a negative feedback system that coordinates cell-cycle progression. *Cell Rep.* 4, 803–816. doi: 10.1016/j.celrep.2013.07.031

Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012). RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* 26, 1179–1195. doi: 10.1101/gad.188284.112

Gali, H., Juhasz, S., Morocz, M., Hajdu, I., Fatyol, K., Szukacsov, V., et al. (2012). Role of SUMO modification of human PCNA at stalled replication fork. *Nucleic Acids Res.* 40, 6049–6059. doi: 10.1093/nar/gks256

Gallego-Sanchez, A., Andres, S., Conde, F., San-Segundo, P. A., and Bueno, A. (2012). Reversal of PCNA ubiquitylation by Ubp10 in *Saccharomyces cerevisiae*. *PLoS Genet.* 8:e1002826. doi: 10.1371/journal.pgen.1002826

Gangavarapu, V., Haracska, L., Unk, I., Johnson, R. E., Prakash, S., and Prakash, L. (2006). Mms2-Ubc13-dependent and -independent roles of Rad5 ubiquitin ligase in postreplication repair and translesion DNA synthesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 26, 7783–7790. doi: 10.1128/MCB.01260-06

Gao, Z., Zhang, J., Bonasio, R., Strino, F., Sawai, A., Parisi, F., et al. (2012). PCGF homologs, CBX proteins, and RYBP define functionally distinct PRC1 family complexes. *Mol. Cell.* 45, 344–356. doi: 10.1016/j.molcel.2012.01.002

Garcia, B. A., Hake, S. B., Diaz, R. L., Kauer, M., Morris, S. A., Recht, J., et al. (2007). Organismal differences in post-translational modifications in histones H3 and H4. *J. Biol. Chem.* 282, 7641–7655. doi: 10.1074/jbc.M607900200

Geng, L., Huntoon, C. J., and Karnitz, L. M. (2010). RAD18-mediated ubiquitination of PCNA activates the *Fanconi anemia* DNA repair network. *J. Cell Biol.* 191, 249–257. doi: 10.1083/jcb.201005101

Giannattasio, M., Zwicky, K., Follonier, C., Foiani, M., Lopes, M., and Branzei, D. (2014). Visualization of recombination-mediated damage bypass by template switching. *Nat. Struct. Mol. Biol.* 21, 884–892. doi: 10.1038/nsmb.2888

Gibbs-Seymour, I., Oka, Y., Rajendra, E., Weinert, B. T., Passmore, L. A., Patel, K. J., et al. (2015). Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. *Mol. Cell.* 57, 150–164. doi: 10.1016/j.molcel.2014.12.001

Ginjala, V., Nacerddine, K., Kulkarni, A., Oza, J., Hill, S. J., Yao, M., et al. (2011). BMI1 is recruited to DNA breaks and contributes to DNA damage-induced H2A ubiquitination and repair. *Mol. Cell. Biol.* 31, 1972–1982. doi: 10.1128/MCB.00981-10

Goldknopf, I. L., Taylor, C. W., Baum, R. M., Yeoman, L. C., Olson, M. O., Prestayko, A. W., et al. (1975). Isolation and characterization of protein A24, a "histone-like" non-histone chromosomal protein. *J. Biol. Chem.* 250, 7182–7187.

Golebiowski, F., Matic, I., Tatham, M. H., Cole, C., Yin, Y., Nakamura, A., et al. (2009). System-wide changes to SUMO modifications in response to heat shock. *Sci. Signal.* 2:ra24. doi: 10.1126/scisignal.2000282

Gonzalez-Prieto, R., Cuijpers, S. A., Luijsterburg, M. S., Van Attikum, H., and Vertegaal, A. C. (2015). SUMOylation and PARylation cooperate to recruit and stabilize SLX4 at DNA damage sites. *EMBO Rep.* 16, 512–519. doi: 10.15252/embr.201440017

Groth, A. (2009). Replicating chromatin: a tale of histones. *Biochem. Cell Biol.* 87, 51–63. doi: 10.1139/O08-102

Groth, A., Corpet, A., Cook, A. J., Roche, D., Bartek, J., Lukas, J., et al. (2007). Regulation of replication fork progression through histone supply and demand. *Science* 318, 1928–1931. doi: 10.1126/science.1148992

Guervilly, J. H., Takedachi, A., Naim, V., Scaglione, S., Chawhan, C., Lovera, Y., et al. (2015). The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. *Mol. Cell.* 57, 123–137. doi: 10.1016/j.molcel.2014.11.014

Han, J., Li, Q., Mccullough, L., Kettelkamp, C., Formosa, T., and Zhang, Z. (2010). Ubiquitylation of FACT by the cullin-E3 ligase Rtt101 connects FACT to DNA replication. *Genes Dev.* 24, 1485–1490. doi: 10.1101/gad.1887310

Han, J., Zhang, H., Zhang, H., Wang, Z., Zhou, H., and Zhang, Z. (2013). A Cul4 E3 ubiquitin ligase regulates histone hand-off during nucleosome assembly. *Cell* 155, 817–829. doi: 10.1016/j.cell.2013.10.014

Han, J., Zhou, H., Horazdovsky, B., Zhang, K., Xu, R. M., and Zhang, Z. (2007a). Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. *Science* 315, 653–655. doi: 10.1126/science.1133234

Han, J., Zhou, H., Li, Z., Xu, R. M., and Zhang, Z. (2007b). Acetylation of lysine 56 of histone H3 catalyzed by RTT109 and regulated by ASF1 is required for replisome integrity. *J. Biol. Chem.* 282, 28587–28596. doi: 10.1074/jbc.M702496200

Hanada, K., Budzowska, M., Davies, S. L., Van Drunen, E., Onizawa, H., Beverloo, H. B., et al. (2007). The structure-specific endonuclease Mus81 contributes to replication restart by generating double-strand DNA breaks. *Nat. Struct. Mol. Biol.* 14, 1096–1104. doi: 10.1038/nsmb1313

Hang, L. E., Peng, J., Tan, W., Szakal, B., Menolfi, D., Sheng, Z., et al. (2015). Rtt107 Is a multi-functional scaffold supporting replication progression with partner SUMO and ubiquitin ligases. *Mol. Cell.* 60, 268–279. doi: 10.1016/j.molcel.2015.08.023

Havens, C. G., and Walter, J. C. (2011). Mechanism of CRL4(Cdt2), a PCNA-dependent E3 ubiquitin ligase. *Genes Dev.* 25, 1568–1582. doi: 10.1101/gad.2068611

Hendel, A., Krijger, P. H., Diamant, N., Goren, Z., Langerak, P., Kim, J., et al. (2011). PCNA ubiquitination is important, but not essential for translesion DNA synthesis in mammalian cells. *PLoS Genet.* 7:e1002262. doi: 10.1371/journal.pgen.1002262

Henry, K. W., Wyce, A., Lo, W. S., Duggan, L. J., Emre, N. C., Kao, C. F., et al. (2003). Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes Dev.* 17, 2648–2663. doi: 10.1101/gad.1144003

Hishida, T., Ohya, T., Kubota, Y., Kamada, Y., and Shinagawa, H. (2006). Functional and physical interaction of yeast Mgs1 with PCNA: impact on RAD6-dependent DNA damage tolerance. *Mol. Cell. Biol.* 26, 5509–5517. doi: 10.1128/MCB.00307-06

Hoege, C., Pfander, B., Moldovan, G. L., Pyrowolakis, G., and Jentsch, S. (2002). RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419, 135–141. doi: 10.1038/nature 00991

Hoeller, D., Hecker, C. M., Wagner, S., Rogov, V., Dotsch, V., and Dikic, I. (2007). E3-independent monoubiquitination of ubiquitin-binding proteins. *Mol. Cell.* 26, 891–898. doi: 10.1016/j.molcel.2007.05.014

Horigome, C., Oma, Y., Konishi, T., Schmid, R., Marcomini, I., Hauer, M. H., et al. (2014). SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. *Mol. Cell.* 55, 626–639. doi: 10.1016/j.molcel.2014.06.027

Huang, H., Stromme, C. B., Saredi, G., Hodl, M., Strandsby, A., Gonzalez-Aguilera, C., et al. (2015). A unique binding mode enables MCM2 to chaperone histones H3-H4 at replication forks. *Nat. Struct. Mol. Biol.* 22, 618–626. doi: 10.1038/nsmb.3055

Huang, T. T., Nijman, S. M., Mirchandani, K. D., Galardy, P. J., Cohn, M. A., Haas, W., et al. (2006). Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat. Cell Biol.* 8, 339–347. doi: 10.1038/ncb1378

Hubscher, U., Maga, G., and Spadari, S. (2002). Eukaryotic DNA polymerases. *Annu. Rev. Biochem.* 71, 133–163. doi: 10.1146/annurev.biochem.71.090501.150041

Irmisch, A., Ampatzidou, E., Mizuno, K., O'connell, M. J., and Murray, J. M. (2009). Smc5/6 maintains stalled replication forks in a recombination-competent conformation. *EMBO J.* 28, 144–155. doi: 10.1038/emboj.2008.273

Jackson, P. K. (2001). A new RING for SUMO: wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. *Genes Dev.* 15, 3053–3058. doi: 10.1101/gad.955501

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell.* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jasencakova, Z., Scharf, A. N., Ask, K., Corpet, A., Imhof, A., Almouzni, G., et al. (2010). Replication stress interferes with histone recycling and predeposition marking of new histones. *Mol. Cell.* 37, 736–743. doi: 10.1016/j.molcel.2010.01.033

Jensen, R. B., Carreira, A., and Kowalczykowski, S. C. (2010). Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature* 467, 678–683. doi: 10.1038/nature09399

Jossen, R., and Bermejo, R. (2013). The DNA damage checkpoint response to replication stress: a Game of Forks. *Front. Genet.* 4:26. doi: 10.3389/fgene.2013.00026

Jung, Y. S., Hakem, A., Hakem, R., and Chen, X. (2011). Pirh2 E3 ubiquitin ligase monoubiquitinates DNA polymerase eta to suppress translesion DNA synthesis. *Mol. Cell. Biol.* 31, 3997–4006. doi: 10.1128/MCB.05808-11

Jung, Y. S., Liu, G., and Chen, X. (2010). Pirh2 E3 ubiquitin ligase targets DNA polymerase eta for 20S proteasomal degradation. *Mol. Cell. Biol.* 30, 1041–1048. doi: 10.1128/MCB.01198-09

Jung, Y. S., Qian, Y., and Chen, X. (2012). DNA polymerase eta is targeted by Mdm2 for polyubiquitination and proteasomal degradation in response to ultraviolet irradiation. *DNA Repair (Amst.)* 11, 177–184. doi: 10.1016/j.dnarep.2011.10.017

Kadyrova, L. Y., Mertz, T. M., Zhang, Y., Northam, M. R., Sheng, Z., Lobachev, K. S., et al. (2013). A reversible histone H3 acetylation cooperates with mismatch repair and replicative polymerases in maintaining genome stability. *PLoS Genet.* 9:e1003899. doi: 10.1371/journal.pgen.1003899

Kanke, M., Kodama, Y., Takahashi, T. S., Nakagawa, T., and Masukata, H. (2012). Mcm10 plays an essential role in origin DNA unwinding after loading of the CMG components. *EMBO J.* 31, 2182–2194. doi: 10.1038/emboj.2012.68

Kanu, N., Zhang, T., Burrell, R. A., Chakraborty, A., Cronshaw, J., Costa, C. D., et al. (2015). RAD18, WRNIP1 and ATMIN promote ATM signalling in response to replication stress. *Oncogene* doi: 10.1038/onc.2015.427 [Epub ahead of print].

Kao, C. F., Hillyer, C., Tsukuda, T., Henry, K., Berger, S., and Osley, M. A. (2004). Rad6 plays a role in transcriptional activation through ubiquitylation of histone H2B. *Genes Dev.* 18, 184–195. doi: 10.1101/gad.1149604

Karras, G. I., and Jentsch, S. (2010). The RAD6 DNA damage tolerance pathway operates uncoupled from the replication fork and is functional beyond S phase. *Cell* 141, 255–267. doi: 10.1016/j.cell.2010.02.028

Kashiwaba, S., Kanao, R., Masuda, Y., Kusumoto-Matsuo, R., Hanaoka, F., and Masutani, C. (2015). USP7 Is a Suppressor of PCNA ubiquitination and oxidative-stress-induced mutagenesis in human Cells. *Cell Rep.* 13, 2072–2080. doi: 10.1016/j.celrep.2015.11.014

Kegel, A., Betts-Lindroos, H., Kanno, T., Jeppsson, K., Strom, L., Katou, Y., et al. (2011). Chromosome length influences replication-induced topological stress. *Nature* 471, 392–396. doi: 10.1038/nature09791

Kim, J., Hake, S. B., and Roeder, R. G. (2005). The human homolog of yeast BRE1 functions as a transcriptional coactivator through direct activator interactions. *Mol. Cell.* 20, 759–770. doi: 10.1016/j.molcel.2005.11.012

Kim, S. H., and Michael, W. M. (2008). Regulated proteolysis of DNA polymerase eta during the DNA-damage response in C. *elegans*. *Mol. Cell* 32, 757–766. doi: 10.1016/j.molcel.2008.11.016

Kim, Y., Lach, F. P., Desetty, R., Hanenberg, H., Auerbach, A. D., and Smogorzewska, A. (2011). Mutations of the SLX4 gene in *Fanconi anemia*. *Nat. Genet.* 43, 142–146. doi: 10.1038/ng.750

Kim, Y., Spitz, G. S., Veturi, U., Lach, F. P., Auerbach, A. D., and Smogorzewska, A. (2013). Regulation of multiple DNA repair pathways by the Fanconi anemia protein SLX4. *Blood* 121, 54–63. doi: 10.1182/blood-2012-07-441212

Kim, Y., Starostina, N. G., and Kipreos, E. T. (2008). The CRL4Cdt2 ubiquitin ligase targets the degradation of p21Cip1 to control replication licensing. *Genes Dev.* 22, 2507–2519. doi: 10.1101/gad.1703708

King, R. W., Peters, J. M., Tugendreich, S., Rolfe, M., Hieter, P., and Kirschner, M. W. (1995). A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 81, 279–288. doi: 10.1016/0092-8674(95)90338-0

Klein Douwel, D., Boonen, R. A., Long, D. T., Szypowska, A. A., Raschle, M., Walter, J. C., et al. (2014). XPF-ERCC1 acts in Unhooking DNA interstrand crosslinks in cooperation with FANCD2 and FANCP/SLX4. *Mol. Cell.* 54, 460–471. doi: 10.1016/j.molcel.2014.03.015

Koken, M. H., Reynolds, P., Jaspers-Dekker, I., Prakash, L., Prakash, S., Bootsma, D., et al. (1991). Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. *Proc. Natl. Acad. Sci. U.S.A.* 88, 8865–8869. doi: 10.1073/pnas.88.20.8865

Kolesar, P., Sarangi, P., Altmannova, V., Zhao, X., and Krejci, L. (2012). Dual roles of the SUMO-interacting motif in the regulation of Srs2 sumoylation. *Nucleic Acids Res.* 40, 7831–7843. doi: 10.1093/nar/gks484

Komander, D., and Rape, M. (2012). The ubiquitin code. *Annu. Rev. Biochem.* 81, 203–229. doi: 10.1146/annurev-biochem-060310-170328

Kottemann, M. C., and Smogorzewska, A. (2013). Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature* 493, 356–363. doi: 10.1038/nature11863

Kratz, K., Schopf, B., Kaden, S., Sendoel, A., Eberhard, R., Lademann, C., et al. (2010). Deficiency of FANCD2-associated nuclease KIAA1018/FAN1 sensitizes cells to interstrand crosslinking agents. *Cell* 142, 77–88. doi: 10.1016/j.cell.2010.06.022

Krejci, L., Altmannova, V., Spirek, M., and Zhao, X. (2012). Homologous recombination and its regulation. *Nucleic Acids Res.* 40, 5795–5818. doi: 10.1093/nar/gks270

Krejci, L., Van Komen, S., Li, Y., Villemain, J., Reddy, M. S., Klein, H., et al. (2003). DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature* 423, 305–309. doi: 10.1038/nature01577

Krijger, P. H., Lee, K. Y., Wit, N., Van Den Berk, P. C., Wu, X., Roest, H. P., et al. (2011). HLTF and SHPRH are not essential for PCNA polyubiquitination, survival and somatic hypermutation: existence of an alternative E3 ligase. *DNA Repair (Amst.)* 10, 438–444. doi: 10.1016/j.dnarep.2010.12.008

Krogan, N. J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M. A., et al. (2003). The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell.* 11, 721–729. doi: 10.1016/S1097-2765(03)00091-1

Kuang, L., Kou, H., Xie, Z., Zhou, Y., Feng, X., Wang, L., et al. (2013). A non-catalytic function of Rev1 in translesion DNA synthesis and mutagenesis is

mediated by its stable interaction with Rad5. *DNA Repair (Amst.)* 12, 27–37. doi: 10.1016/j.dnarep.2012.10.003

Kubota, T., Nishimura, K., Kanemaki, M. T., and Donaldson, A. D. (2013). The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. *Mol. Cell.* 50, 273–280. doi: 10.1016/j.molcel.2013.02.012

Lachaud, C., Moreno, A., Marchesi, F., Toth, R., Blow, J. J., and Rouse, J. (2016). Ubiquitinated Fancd2 recruits Fan1 to stalled replication forks to prevent genome instability. *Science* 351, 846–849. doi: 10.1126/science.aad5634

Lanzuolo, C., and Orlando, V. (2012). Memories from the polycomb group proteins. *Annu. Rev. Genet.* 46, 561–589. doi: 10.1146/annurev-genet-110711-155603

Lau, A. W., Inuzuka, H., Fukushima, H., Wan, L., Liu, P., Gao, D., et al. (2013). Regulation of APC(Cdh1) E3 ligase activity by the Fbw7/cyclin E signaling axis contributes to the tumor suppressor function of Fbw7. *Cell Res.* 23, 947–961. doi: 10.1038/cr.2013.67

Leach, C. A., and Michael, W. M. (2005). Ubiquitin/SUMO modification of PCNA promotes replication fork progression in *Xenopus laevis* egg extracts. *J. Cell Biol.* 171, 947–954. doi: 10.1083/jcb.200508100

Lecona, E., Rodriguez-Acebes, S., Specks, J., Lopez-Contreras, A. J., Ruppen, I., Murga, M., et al. (2016). USP7 is a SUMO deubiquitinase essential for DNA replication. *Nat. Struct. Mol. Biol.* 23, 270–277. doi: 10.1038/nsmb.3185

Lee, H. S., Lee, S. A., Hur, S. K., Seo, J. W., and Kwon, J. (2014). Stabilization and targeting of INO80 to replication forks by BAP1 during normal DNA synthesis. *Nat. Commun.* 5:5128. doi: 10.1038/ncomms6128

Leman, A. R., and Noguchi, E. (2013). The replication fork: understanding the eukaryotic replication machinery and the challenges to genome duplication. *Genes (Basel)* 4, 1–32. doi: 10.3390/genes4010001

Li, Q., Zhou, H., Wurtele, H., Davies, B., Horazdovsky, B., Verreault, A., et al. (2008). Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. *Cell* 134, 244–255. doi: 10.1016/j.cell.2008.06.018

Li, X., Zhao, Q., Liao, R., Sun, P., and Wu, X. (2003). The SCF(Skp2) ubiquitin ligase complex interacts with the human replication licensing factor Cdt1 and regulates Cdt1 degradation. *J. Biol. Chem.* 278, 30854–30858. doi: 10.1074/jbc.C300251200

Liberi, G., Maffioletti, G., Lucca, C., Chiolo, I., Baryshnikova, A., Cotta-Ramusino, C., et al. (2005). Rad51-dependent DNA structures accumulate at damaged replication forks in sgs1 mutants defective in the yeast ortholog of BLM RecQ helicase. *Genes Dev.* 19, 339–350. doi: 10.1101/gad.322605

Lin, C. Y., Wu, M. Y., Gay, S., Marjavaara, L., Lai, M. S., Hsiao, W. C., et al. (2014). H2B mono-ubiquitylation facilitates fork stalling and recovery during replication stress by coordinating Rad53 activation and chromatin assembly. *PLoS Genet.* 10:e1004667. doi: 10.1371/journal.pgen.1004667

Lin, J. R., Zeman, M. K., Chen, J. Y., Yee, M. C., and Cimprich, K. A. (2011). SHPRH and HLTF act in a damage-specific manner to coordinate different forms of postreplication repair and prevent mutagenesis. *Mol. Cell* 42, 237–249. doi: 10.1016/j.molcel.2011.02.026

Lindroos, H. B., Strom, L., Itoh, T., Katou, Y., Shirahige, K., and Sjogren, C. (2006). Chromosomal association of the Smc5/6 complex reveals that it functions in differently regulated pathways. *Mol. Cell* 22, 755–767. doi: 10.1016/j.molcel.2006.05.014

Liu, G., and Warbrick, E. (2006). The p66 and p12 subunits of DNA polymerase delta are modified by ubiquitin and ubiquitin-like proteins. *Biochem. Biophys. Res. Commun.* 349, 360–366. doi: 10.1016/j.bbrc.2006.08.049

Liu, J., Doty, T., Gibson, B., and Heyer, W. D. (2010). Human BRCA2 protein promotes RAD51 filament formation on RPA-covered single-stranded DNA. *Nat. Struct. Mol. Biol.* 17, 1260–1262. doi: 10.1038/nsmb.1904

Liu, T., Ghosal, G., Yuan, J., Chen, J., and Huang, J. (2010). FAN1 acts with FANCI-FANCD2 to promote DNA interstrand cross-link repair. *Science* 329, 693–696. doi: 10.1126/science.1192656

Loeillet, S., Palancade, B., Cartron, M., Thierry, A., Richard, G. F., Dujon, B., et al. (2005). Genetic network interactions among replication, repair and nuclear pore deficiencies in yeast. *DNA Repair (Amst.)* 4, 459–468. doi: 10.1016/j.dnarep.2004.11.010

Longerich, S., San Filippo, J., Liu, D., and Sung, P. (2009). FANCI binds branched DNA and is monoubiquitinated by UBE2T-FANCL. *J. Biol. Chem.* 284, 23182–23186. doi: 10.1074/jbc.C109.038075

Lopez-Contreras, A. J., Ruppen, I., Nieto-Soler, M., Murga, M., Rodriguez-Acebes, S., Remeseiro, S., et al. (2013). A proteomic characterization of factors enriched at nascent DNA molecules. *Cell Rep.* 3, 1105–1116. doi: 10.1016/j.celrep.2013.03.009

Lu, C. Y., Tsai, C. H., Brill, S. J., and Teng, S. C. (2010). Sumoylation of the BLM ortholog, Sgs1, promotes telomere-telomere recombination in budding yeast. *Nucleic Acids Res.* 38, 488–498. doi: 10.1093/nar/gkp1008

Luke, B., Versini, G., Jaquenoud, M., Zaidi, I. W., Kurz, T., Pintard, L., et al. (2006). The cullin Rtt101p promotes replication fork progression through damaged DNA and natural pause sites. *Curr. Biol.* 16, 786–792. doi: 10.1016/j.cub.2006.02.071

MacKay, C., Declais, A. C., Lundin, C., Agostinho, A., Deans, A. J., Macartney, T. J., et al. (2010). Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell* 142, 65–76. doi: 10.1016/j.cell.2010.06.021

Maculins, T., Nkosi, P. J., Nishikawa, H., and Labib, K. (2015). Tethering of SCF(Dia2) to the replisome promotes efficient ubiquitylation and disassembly of the CMG helicase. *Curr. Biol.* 25, 2254–2259. doi: 10.1016/j.cub.2015.07.012

Marechal, A., Li, J. M., Ji, X. Y., Wu, C. S., Yazinski, S. A., Nguyen, H. D., et al. (2014). PRP19 transforms into a sensor of RPA-ssDNA after DNA damage and drives ATR activation via a ubiquitin-mediated circuitry. *Mol. Cell* 53, 235–246. doi: 10.1016/j.molcel.2013.11.002

Maric, M., Maculins, T., De Piccoli, G., and Labib, K. (2014). Cdc48 and a ubiquitin ligase drive disassembly of the CMG helicase at the end of DNA replication. *Science* 346:1253596. doi: 10.1126/science.1253596

Masumoto, H., Hawke, D., Kobayashi, R., and Verreault, A. (2005). A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. *Nature* 436, 294–298. doi: 10.1038/nature03714

Mattiroli, F., and Sixma, T. K. (2014). Lysine-targeting specificity in ubiquitin and ubiquitin-like modification pathways. *Nat. Struct. Mol. Biol.* 21, 308–316. doi: 10.1038/nsmb.2792

McDonald, W. H., Pavlova, Y., Yates, J. R. III, and Boddy, M. N. (2003). Novel essential DNA repair proteins Nse1 and Nse2 are subunits of the fission yeast Smc5-Smc6 complex. *J. Biol. Chem.* 278, 45460–45467. doi: 10.1074/jbc.M308828200

McGarry, T. J., and Kirschner, M. W. (1998). Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* 93, 1043–1053. doi: 10.1016/S0092-8674(00)81209-X

McGinty, R. K., Henrici, R. C., and Tan, S. (2014). Crystal structure of the PRC1 ubiquitylation module bound to the nucleosome. *Nature* 514, 591–596. doi: 10.1038/nature13890

McIntyre, J., Vidal, A. E., Mclenigan, M. P., Bomar, M. G., Curti, E., Mcdonald, J. P., et al. (2013). Ubiquitin mediates the physical and functional interaction between human DNA polymerases eta and iota. *Nucleic Acids Res.* 41, 1649–1660. doi: 10.1093/nar/gks1277

McIntyre, J., and Woodgate, R. (2015). Regulation of translesion DNA synthesis: posttranslational modification of lysine residues in key proteins. *DNA Repair (Amst.)* 29, 166–179. doi: 10.1016/j.dnarep.2015.02.011

Mejlvang, J., Feng, Y., Alabert, C., Neelsen, K. J., Jasencakova, Z., Zhao, X., et al. (2014). New histone supply regulates replication fork speed and PCNA unloading. *J. Cell Biol.* 204, 29–43. doi: 10.1083/jcb.201305017

Menolfi, D., Delamarre, A., Lengronne, A., Pasero, P., and Branzei, D. (2015). Essential roles of the smc5/6 complex in replication through natural pausing sites and endogenous DNA damage tolerance. *Mol. Cell* 60, 835–846. doi: 10.1016/j.molcel.2015.10.023

Mimura, S., Komata, M., Kishi, T., Shirahige, K., and Kamura, T. (2009). SCF(Dia2) regulates DNA replication forks during S-phase in budding yeast. *EMBO J.* 28, 3693–3705. doi: 10.1038/emboj.2009.320

Minesinger, B. K., and Jinks-Robertson, S. (2005). Roles of RAD6 epistasis group members in spontaneous polzeta-dependent translesion synthesis in *Saccharomyces cerevisiae*. *Genetics* 169, 1939–1955. doi: 10.1534/genetics.104.033894

Moggs, J. G., Grandi, P., Quivy, J. P., Jonsson, Z. O., Hubscher, U., Becker, P. B., et al. (2000). A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol. Cell. Biol.* 20, 1206–1218. doi: 10.1128/MCB.20.4.1206-1218.2000

Moldovan, G. L., Dejsuphong, D., Petalcorin, M. I., Hofmann, K., Takeda, S., Boulton, S. J., et al. (2012). Inhibition of homologous recombination by the PCNA-interacting protein PARI. *Mol. Cell* 45, 75–86. doi: 10.1016/j.molcel.2011.11.010

Moldovan, G. L., Pfander, B., and Jentsch, S. (2007). PCNA, the maestro of the replication fork. *Cell* 129, 665–679. doi: 10.1016/j.cell.2007.05.003

Moreno, S. P., Bailey, R., Campion, N., Herron, S., and Gambus, A. (2014). Polyubiquitylation drives replisome disassembly at the termination of DNA replication. *Science* 346, 477–481. doi: 10.1126/science.1253585

Moreno, S. P., and Gambus, A. (2015). Regulation of unperturbed DNA replication by ubiquitylation. *Genes (Basel)* 6, 451–468. doi: 10.3390/genes 6030451

Morohashi, H., Maculins, T., and Labib, K. (2009). The amino-terminal TPR domain of Dia2 tethers SCF(Dia2) to the replisome progression complex. *Curr. Biol.* 19, 1943–1949. doi: 10.1016/j.cub.2009.09.062

Mosbech, A., Gibbs-Seymour, I., Kagias, K., Thorslund, T., Beli, P., Povlsen, L., et al. (2012). DVC1 (C1orf124) is a DNA damage-targeting p97 adaptor that promotes ubiquitin-dependent responses to replication blocks. *Nat. Struct. Mol. Biol.* 19, 1084–1092. doi: 10.1038/nsmb.2395

Motegi, A., Liaw, H. J., Lee, K. Y., Roest, H. P., Maas, A., Wu, X., et al. (2008). Polyubiquitination of proliferating cell nuclear antigen by HLTF and SHPRH prevents genomic instability from stalled replication forks. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12411–12416. doi: 10.1073/pnas.08056 85105

Munoz-Galvan, S., Jimeno, S., Rothstein, R., and Aguilera, A. (2013). Histone H3K56 acetylation, Rad52, and non-DNA repair factors control double-strand break repair choice with the sister chromatid. *PLoS Genet.* 9:e1003237. doi: 10.1371/journal.pgen.1003237

Murray, J. M., and Carr, A. M. (2008). Smc5/6: a link between DNA repair and unidirectional replication? *Nat. Rev. Mol. Cell Biol.* 9, 177–182. doi: 10.1038/nrm2309

Myung, K., Pennaneach, V., Kats, E. S., and Kolodner, R. D. (2003). *Saccharomyces cerevisiae* chromatin-assembly factors that act during DNA replication function in the maintenance of genome stability. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6640–6645. doi: 10.1073/pnas.1232239100

Nagai, S., Davoodi, N., and Gasser, S. M. (2011). Nuclear organization in genome stability: SUMO connections. *Cell Res.* 21, 474–485. doi: 10.1038/cr. 2011.31

Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M. B., Roberts, T. M., Brown, G. W., et al. (2008). Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 322, 597–602. doi: 10.1126/science.1162790

Nakanishi, S., Lee, J. S., Gardner, K. E., Gardner, J. M., Takahashi, Y. H., Chandrasekharan, M. B., et al. (2009). Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1. *J. Cell Biol.* 186, 371–377. doi: 10.1083/jcb.2009 06005

Nicassio, F., Corrado, N., Vissers, J. H., Areces, L. B., Bergink, S., Marteijn, J. A., et al. (2007). Human USP3 is a chromatin modifier required for S phase progression and genome stability. *Curr. Biol.* 17, 1972–1977. doi: 10.1016/j.cub.2007.10.034

Niimi, A., Brown, S., Sabbioneda, S., Kannouche, P. L., Scott, A., Yasui, A., et al. (2008). Regulation of proliferating cell nuclear antigen ubiquitination in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* 105, 16125–16130. doi: 10.1073/pnas.0802727105

Nijman, S. M., Huang, T. T., Dirac, A. M., Brummelkamp, T. R., Kerkhoven, R. M., D'andrea, A. D., et al. (2005). The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol. Cell* 17, 331–339. doi: 10.1016/j.molcel.2005.01.008

Nishiyama, A., Yamaguchi, L., Sharif, J., Johmura, Y., Kawamura, T., Nakanishi, K., et al. (2013). Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. *Nature* 502, 249–253. doi: 10.1038/nature12488

Ouyang, J., Garner, E., Hallet, A., Nguyen, H. D., Rickman, K. A., Gill, G., et al. (2015). Noncovalent interactions with SUMO and ubiquitin orchestrate distinct functions of the SLX4 complex in genome maintenance. *Mol. Cell* 57, 108–122. doi: 10.1016/j.molcel.2014.11.015

Ouyang, K. J., Woo, L. L., Zhu, J., Huo, D., Matunis, M. J., and Ellis, N. A. (2009). SUMO modification regulates BLM and RAD51 interaction at damaged replication forks. *PLoS Biol.* 7:e1000252. doi: 10.1371/journal.pbio. 1000252

Pabla, R., Rozario, D., and Siede, W. (2008). Regulation of *Saccharomyces cerevisiae* DNA polymerase eta transcript and protein. *Radiat. Environ. Biophys.* 47, 157–168. doi: 10.1007/s00411-007-0132-1

Pages, V., Bresson, A., Acharya, N., Prakash, S., Fuchs, R. P., and Prakash, L. (2008). Requirement of Rad5 for DNA polymerase zeta-dependent translesion synthesis in *Saccharomyces cerevisiae*. *Genetics* 180, 73–82. doi: 10.1534/genetics.108.091066

Palancade, B., Liu, X., Garcia-Rubio, M., Aguilera, A., Zhao, X., and Doye, V. (2007). Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. *Mol. Biol. Cell* 18, 2912–2923. doi: 10.1091/mbc.E07-02-0123

Papamichos-Chronakis, M., and Peterson, C. L. (2008). The Ino80 chromatin-remodeling enzyme regulates replisome function and stability. *Nat. Struct. Mol. Biol.* 15, 338–345. doi: 10.1038/nsmb.1413

Papouli, E., Chen, S., Davies, A. A., Huttner, D., Krejci, L., Sung, P., et al. (2005). Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell* 19, 123–133. doi: 10.1016/j.molcel.2005. 06.001

Park, J. M., Yang, S. W., Yu, K. R., Ka, S. H., Lee, S. W., Seol, J. H., et al. (2014). Modification of PCNA by ISG15 plays a crucial role in termination of error-prone translesion DNA synthesis. *Mol. Cell* 54, 626–638. doi: 10.1016/j.molcel.2014.03.031

Parker, J. L., Bielen, A. B., Dikic, I., and Ulrich, H. D. (2007). Contributions of ubiquitin- and PCNA-binding domains to the activity of Polymerase eta in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 35, 881–889. doi: 10.1093/nar/gkl1102

Parker, J. L., Bucceri, A., Davies, A. A., Heidrich, K., Windecker, H., and Ulrich, H. D. (2008). SUMO modification of PCNA is controlled by DNA. *EMBO J.* 27, 2422–2431. doi: 10.1038/emboj.2008.162

Parker, J. L., and Ulrich, H. D. (2012). A SUMO-interacting motif activates budding yeast ubiquitin ligase Rad18 towards SUMO-modified PCNA. *Nucleic Acids Res.* 40, 11380–11388. doi: 10.1093/nar/gks892

Parnas, O., Zipin-Roitman, A., Pfander, B., Liefshitz, B., Mazor, Y., Ben-Aroya, S., et al. (2010). Elg1, an alternative subunit of the RFC clamp loader, preferentially interacts with SUMOylated PCNA. *EMBO J.* 29, 2611–2622. doi: 10.1038/emboj.2010.128

Pebernard, S., Perry, J. J., Tainer, J. A., and Boddy, M. N. (2008). Nse1 RING-like domain supports functions of the Smc5-Smc6 holocomplex in genome stability. *Mol. Biol. Cell* 19, 4099–4109. doi: 10.1091/mbc.E08-02-0226

Pfander, B., Moldovan, G. L., Sacher, M., Hoege, C., and Jentsch, S. (2005). SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436, 428–433. doi: 10.1038/nature03665

Piunti, A., Rossi, A., Cerutti, A., Albert, M., Jammula, S., Scelfo, A., et al. (2014). Polycomb proteins control proliferation and transformation independently of cell cycle checkpoints by regulating DNA replication. *Nat. Commun.* 5:3649. doi: 10.1038/ncomms4649

Plachta, M., Halas, A., Mcintyre, J., and Sledziewska-Gojska, E. (2015). The steady-state level and stability of TLS polymerase eta are cell cycle dependent in the yeast *S. cerevisiae*. *DNA Repair (Amst.)* 29, 147–153. doi: 10.1016/j.dnarep.2015.02.015

Plosky, B. S., Vidal, A. E., Fernandez De Henestrosa, A. R., Mclenigan, M. P., Mcdonald, J. P., Mead, S., et al. (2006). Controlling the subcellular localization of DNA polymerases iota and eta via interactions with ubiquitin. *EMBO J.* 25, 2847–2855. doi: 10.1038/sj.emboj.7601178

Potts, P. R., and Yu, H. (2005). Human MMS21/NSE2 is a SUMO ligase required for DNA repair. *Mol. Cell. Biol.* 25, 7021–7032. doi: 10.1128/MCB.25.16.7021-7032.2005

Potts, P. R., and Yu, H. (2007). The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. *Nat. Struct. Mol. Biol.* 14, 581–590. doi: 10.1038/nsmb1259

Povlsen, L. K., Beli, P., Wagner, S. A., Poulsen, S. L., Sylvestersen, K. B., Poulsen, J. W., et al. (2012). Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitylation in DNA-damage bypass. *Nat. Cell Biol.* 14, 1089–1098. doi: 10.1038/ncb2579

Prado, F., Cortes-Ledesma, F., and Aguilera, A. (2004). The absence of the yeast chromatin assembly factor Asf1 increases genomic instability and

sister chromatid exchange. *EMBO Rep.* 5, 497–502. doi: 10.1038/sj.embor.7400128

Prudden, J., Pebernard, S., Raffa, G., Slavin, D. A., Perry, J. J., Tainer, J. A., et al. (2007). SUMO-targeted ubiquitin ligases in genome stability. *EMBO J.* 26, 4089–4101. doi: 10.1038/sj.emboj.7601838

Psakhye, I., and Jentsch, S. (2012). Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* 151, 807–820. doi: 10.1016/j.cell.2012.10.021

Qian, J., Pentz, K., Zhu, Q., Wang, Q., He, J., Srivastava, A. K., et al. (2015). USP7 modulates UV-induced PCNA monoubiquitination by regulating DNA polymerase eta stability. *Oncogene* 34, 4791–4796. doi: 10.1038/onc.2014.394

Qin, W., Wolf, P., Liu, N., Link, S., Smets, M., La Mastra, F., et al. (2015). DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. *Cell Res.* 25, 911–929. doi: 10.1038/cr.2015.72

Qing, P., Han, L., Bin, L., Yan, L., and Ping, W. X. (2011). USP7 regulates the stability and function of HLTF through deubiquitination. *J. Cell. Biochem.* 112, 3856–3862. doi: 10.1002/jcb.23317

Ramey, C. J., Howar, S., Adkins, M., Linger, J., Spicer, J., and Tyler, J. K. (2004). Activation of the DNA damage checkpoint in yeast lacking the histone chaperone anti-silencing function 1. *Mol. Cell. Biol.* 24, 10313–10327. doi: 10.1128/MCB.24.23.10313-10327.2004

Rape, M., and Kirschner, M. W. (2004). Autonomous regulation of the anaphase-promoting complex couples mitosis to S-phase entry. *Nature* 432, 588–595. doi: 10.1038/nature03023

Renard-Guillet, C., Kanoh, Y., Shirahige, K., and Masai, H. (2014). Temporal and spatial regulation of eukaryotic DNA replication: from regulated initiation to genome-scale timing program. *Semin. Cell Dev. Biol.* 30, 110–120. doi: 10.1016/j.semcdb.2014.04.014

Ricke, R. M., and Bielinsky, A. K. (2004). Mcm10 regulates the stability and chromatin association of DNA polymerase-alpha. *Mol. Cell* 16, 173–185. doi: 10.1016/j.molcel.2004.09.017

Roberts, T. M., Zaidi, I. W., Vaisica, J. A., Peter, M., and Brown, G. W. (2008). Regulation of rtt107 recruitment to stalled DNA replication forks by the cullin rtt101 and the rtt109 acetyltransferase. *Mol. Biol. Cell* 19, 171–180. doi: 10.1091/mbc.E07-09-0961

Robzyk, K., Recht, J., and Osley, M. A. (2000). Rad6-dependent ubiquitination of histone H2B in yeast. *Science* 287, 501–504. doi: 10.1126/science.287.5452.501

Roerink, S. F., Koole, W., Stapel, L. C., Romeijn, R. J., and Tijsterman, M. (2012). A broad requirement for TLS polymerases eta and kappa, and interacting sumoylation and nuclear pore proteins, in lesion bypass during *C. elegans* embryogenesis. *PLoS Genet.* 8:e1002800. doi: 10.1371/journal.pgen.1002800

Roseaulin, L. C., Noguchi, C., Martinez, E., Ziegler, M. A., Toda, T., and Noguchi, E. (2013). Coordinated degradation of replisome components ensures genome stability upon replication stress in the absence of the replication fork protection complex. *PLoS Genet.* 9:e1003213. doi: 10.1371/journal.pgen.1003213

Saha, P., Chen, J., Thome, K. C., Lawlis, S. J., Hou, Z. H., Hendricks, M., et al. (1998). Human CDC6/Cdc18 associates with Orc1 and cyclin-cdk and is selectively eliminated from the nucleus at the onset of S phase. *Mol. Cell. Biol.* 18, 2758–2767. doi: 10.1128/MCB.18.5.2758

Saponaro, M., Callahan, D., Zheng, X., Krejci, L., Haber, J. E., Klein, H. L., et al. (2010). Cdk1 targets Srs2 to complete synthesis-dependent strand annealing and to promote recombinational repair. *PLoS Genet.* 6:e1000858. doi: 10.1371/journal.pgen.1000858

Sato, K., Toda, K., Ishiai, M., Takata, M., and Kurumizaka, H. (2012). DNA robustly stimulates FANCD2 monoubiquitylation in the complex with FANCI. *Nucleic Acids Res.* 40, 4553–4561. doi: 10.1093/nar/gks053

Saugar, I., Ortiz-Bazan, M. A., and Tercero, J. A. (2014). Tolerating DNA damage during eukaryotic chromosome replication. *Exp. Cell Res.* 329, 170–177. doi: 10.1016/j.yexcr.2014.07.009

Saugar, I., Parker, J. L., Zhao, S., and Ulrich, H. D. (2012). The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Res.* 40, 245–257. doi: 10.1093/nar/gkr738

Schulz, L. L., and Tyler, J. K. (2006). The histone chaperone ASF1 localizes to active DNA replication forks to mediate efficient DNA replication. *FASEB J.* 20, 488–490. doi: 10.1096/fj.05-5020fje

Sengupta, S., Linke, S. P., Pedeux, R., Yang, Q., Farnsworth, J., Garfield, S. H., et al. (2003). BLM helicase-dependent transport of p53 to sites of stalled DNA replication forks modulates homologous recombination. *EMBO J.* 22, 1210–1222. doi: 10.1093/emboj/cdg114

Shibahara, K., and Stillman, B. (1999). Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* 96, 575–585. doi: 10.1016/S0092-8674(00)80661-3

Shima, H., Suzuki, H., Sun, J., Kono, K., Shi, L., Kinomura, A., et al. (2013). Activation of the SUMO modification system is required for the accumulation of RAD51 at sites of DNA damage. *J. Cell Sci.* 126, 5284–5292. doi: 10.1242/jcs.133744

Shimada, K., Oma, Y., Schleker, T., Kugou, K., Ohta, K., Harata, M., et al. (2008). Ino80 chromatin remodeling complex promotes recovery of stalled replication forks. *Curr. Biol.* 18, 566–575. doi: 10.1016/j.cub.2008.03.049

Siddiqui, K., On, K. F., and Diffley, J. F. (2013). Regulating DNA replication in eukarya. *Cold Spring Harb. Perspect. Biol.* 5:a012930. doi: 10.1101/cshperspect.a012930

Simpson, L. J., Ross, A. L., Szuts, D., Alviani, C. A., Oestergaard, V. H., Patel, K. J., et al. (2006). RAD18-independent ubiquitination of proliferating-cell nuclear antigen in the avian cell line DT40. *EMBO Rep.* 7, 927–932. doi: 10.1038/sj.embor.7400777

Skoneczna, A., Mcintyre, J., Skoneczny, M., Policinska, Z., and Sledziewska-Gojska, E. (2007). Polymerase eta is a short-lived, proteasomally degraded protein that is temporarily stabilized following UV irradiation in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 366, 1074–1086. doi: 10.1016/j.jmb.2006.11.093

Smogorzewska, A., Desetty, R., Saito, T. T., Schlabach, M., Lach, F. P., Sowa, M. E., et al. (2010). A genetic screen identifies FAN1, a Fanconi anemia-associated nuclease necessary for DNA interstrand crosslink repair. *Mol. Cell* 39, 36–47. doi: 10.1016/j.molcel.2010.06.023

Song, Y. H., and Ahn, S. H. (2010). A Bre1-associated protein, large 1 (Lge1), promotes H_2B ubiquitylation during the early stages of transcription elongation. *J. Biol. Chem.* 285, 2361–2367. doi: 10.1074/jbc.M109.039255

Soustelle, C., Vernis, L., Freon, K., Reynaud-Angelin, A., Chanet, R., Fabre, F., et al. (2004). A new *Saccharomyces cerevisiae* strain with a mutant Smt3-deconjugating Ulp1 protein is affected in DNA replication and requires Srs2 and homologous recombination for its viability. *Mol. Cell. Biol.* 24, 5130–5143. doi: 10.1128/MCB.24.12.5130-5143.2004

Sriramachandran, A. M., and Dohmen, R. J. (2014). SUMO-targeted ubiquitin ligases. *Biochim. Biophys. Acta* 1843, 75–85. doi: 10.1016/j.bbamcr.2013.08.022

Starostina, N. G., and Kipreos, E. T. (2012). Multiple degradation pathways regulate versatile CIP/KIP CDK inhibitors. *Trends Cell Biol.* 22, 33–41. doi: 10.1016/j.tcb.2011.10.004

Stelter, P., and Ulrich, H. D. (2003). Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature* 425, 188–191. doi: 10.1038/nature01965

Stephan, A. K., Kliszczak, M., and Morrison, C. G. (2011). The Nse2/Mms21 SUMO ligase of the Smc5/6 complex in the maintenance of genome stability. *FEBS Lett.* 585, 2907–2913. doi: 10.1016/j.febslet.2011.04.067

Stoepker, C., Hain, K., Schuster, B., Hilhorst-Hofstee, Y., Rooimans, M. A., Steltenpool, J., et al. (2011). SLX4, a coordinator of structure-specific endonucleases, is mutated in a new *Fanconi anemia* subtype. *Nat. Genet.* 43, 138–141. doi: 10.1038/ng.751

Su, X. A., Dion, V., Gasser, S. M., and Freudenreich, C. H. (2015). Regulation of recombination at yeast nuclear pores controls repair and triplet repeat stability. *Genes Dev.* 29, 1006–1017. doi: 10.1101/gad.256404.114

Sun, Z. W., and Allis, C. D. (2002). Ubiquitination of histone H_2B regulates H3 methylation and gene silencing in yeast. *Nature* 418, 104–108. doi: 10.1038/nature00883

Takayama, Y., and Toda, T. (2010). Coupling histone homeostasis to centromere integrity via the ubiquitin-proteasome system. *Cell Div.* 5:18. doi: 10.1186/1747-1028-5-18

Tan, B. C., Chien, C. T., Hirose, S., and Lee, S. C. (2006). Functional cooperation between FACT and MCM helicase facilitates initiation of chromatin DNA replication. *EMBO J.* 25, 3975–3985. doi: 10.1038/sj.emboj.7601271

Teixeira, L. K., and Reed, S. I. (2013). Ubiquitin ligases and cell cycle control. *Annu. Rev. Biochem.* 82, 387–414. doi: 10.1146/annurev-biochem-060410-105307

Terai, K., Abbas, T., Jazaeri, A. A., and Dutta, A. (2010). CRL4(Cdt2) E3 ubiquitin ligase monoubiquitinates PCNA to promote translesion DNA synthesis. *Mol. Cell* 37, 143–149. doi: 10.1016/j.molcel.2009.12.018

Thaminy, S., Newcomb, B., Kim, J., Gatbonton, T., Foss, E., Simon, J., et al. (2007). Hst3 is regulated by Mec1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. *J. Biol. Chem.* 282, 37805–37814. doi: 10.1074/jbc.M706384200

Therizols, P., Fairhead, C., Cabal, G. G., Genovesio, A., Olivo-Marin, J. C., Dujon, B., et al. (2006). Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. *J. Cell Biol.* 172, 189–199. doi: 10.1083/jcb.200505159

Thorslund, T., Mcilwraith, M. J., Compton, S. A., Lekomtsev, S., Petronczki, M., Griffith, J. D., et al. (2010). The breast cancer tumor suppressor BRCA2 promotes the specific targeting of RAD51 to single-stranded DNA. *Nat. Struct. Mol. Biol.* 17, 1263–1265. doi: 10.1038/nsmb.1905

Thu, Y. M., and Bielinsky, A. K. (2013). Enigmatic roles of Mcm10 in DNA replication. *Trends Biochem. Sci.* 38, 184–194. doi: 10.1016/j.tibs.2012.12.003

Tikoo, S., Madhavan, V., Hussain, M., Miller, E. S., Arora, P., Zlatanou, A., et al. (2013). Ubiquitin-dependent recruitment of the Bloom syndrome helicase upon replication stress is required to suppress homologous recombination. *EMBO J.* 32, 1778–1792. doi: 10.1038/emboj.2013.117

Tjeertes, J. V., Miller, K. M., and Jackson, S. P. (2009). Screen for DNA-damage-responsive histone modifications identifies H3K9Ac and H3K56Ac in human cells. *EMBO J.* 28, 1878–1889. doi: 10.1038/emboj.2009.119

Trujillo, J. P., Mina, L. B., Pujol, R., Bogliolo, M., Andrieux, J., Holder, M., et al. (2012). On the role of FAN1 in *Fanconi anemia*. *Blood* 120, 86–89. doi: 10.1182/blood-2012-04-420604

Trujillo, K. M., and Osley, M. A. (2012). A role for H_2B ubiquitylation in DNA replication. *Mol. Cell* 48, 734–746. doi: 10.1016/j.molcel.2012.09.019

Ulrich, H. D. (2009). Regulating post-translational modifications of the eukaryotic replication clamp PCNA. *DNA Repair (Amst.)* 8, 461–469. doi: 10.1016/j.dnarep.2009.01.006

Ulrich, H. D. (2012). Ubiquitin and SUMO in DNA repair at a glance. *J. Cell Sci.* 125, 249–254. doi: 10.1242/jcs.091801

Ulrich, H. D. (2014). Two-way communications between ubiquitin-like modifiers and DNA. *Nat. Struct. Mol. Biol.* 21, 317–324. doi: 10.1038/nsmb.2805

Ulrich, H. D., and Takahashi, T. (2013). Readers of PCNA modifications. *Chromosoma* 122, 259–274. doi: 10.1007/s00412-013-0410-4

Ulrich, H. D., and Walden, H. (2010). Ubiquitin signalling in DNA replication and repair. *Nat. Rev. Mol. Cell Biol.* 11, 479–489. doi: 10.1038/nrm2921

Urulangodi, M., Sebesta, M., Menolfi, D., Szakal, B., Sollier, J., Sisakova, A., et al. (2015). Local regulation of the Srs2 helicase by the SUMO-like domain protein Esc2 promotes recombination at sites of stalled replication. *Genes Dev.* 29, 2067–2080. doi: 10.1101/gad.265629.115

van Deursen, F., Sengupta, S., De Piccoli, G., Sanchez-Diaz, A., and Labib, K. (2012). Mcm10 associates with the loaded DNA helicase at replication origins and defines a novel step in its activation. *EMBO J.* 31, 2195–2206. doi: 10.1038/emboj.2012.69

Vassilev, A. P., Rasmussen, H. H., Christensen, E. I., Nielsen, S., and Celis, J. E. (1995). The levels of ubiquitinated histone H2A are highly upregulated in transformed human cells: partial colocalization of uH2A clusters and PCNA/cyclin foci in a fraction of cells in S-phase. *J. Cell Sci.* 108(Pt 3), 1205–1215.

Vassileva, I., Yanakieva, I., Peycheva, M., Gospodinov, A., and Anachkova, B. (2014). The mammalian INO80 chromatin remodeling complex is required for replication stress recovery. *Nucleic Acids Res.* 42, 9074–9086. doi: 10.1093/nar/gku605

Vaz, B., Halder, S., and Ramadan, K. (2013). Role of p97/VCP (Cdc48) in genome stability. *Front. Genet.* 4:60. doi: 10.3389/fgene.2013.00060

Veaute, X., Jeusset, J., Soustelle, C., Kowalczykowski, S. C., Le Cam, E., and Fabre, F. (2003). The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature* 423, 309–312. doi: 10.1038/nature01585

Vincent, J. A., Kwong, T. J., and Tsukiyama, T. (2008). ATP-dependent chromatin remodeling shapes the DNA replication landscape. *Nat. Struct. Mol. Biol.* 15, 477–484. doi: 10.1038/nsmb.1419

Wagner, S. A., Beli, P., Weinert, B. T., Nielsen, M. L., Cox, J., Mann, M., et al. (2011). A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. *Mol. Cell. Proteomics* 10:M111 013284. doi: 10.1074/mcp.m111.013284

Walden, H., and Deans, A. J. (2014). The Fanconi anemia DNA repair pathway: structural and functional insights into a complex disorder. *Annu. Rev. Biophys.* 43, 257–278. doi: 10.1146/annurev-biophys-051013-022737

Wallace, H. A., Merkle, J. A., Yu, M. C., Berg, T. G., Lee, E., Bosco, G., et al. (2014). TRIP/NOPO E3 ubiquitin ligase promotes ubiquitylation of DNA polymerase eta. *Development* 141, 1332–1341. doi: 10.1242/dev.101196

Watanabe, K., Tateishi, S., Kawasuji, M., Tsurimoto, T., Inoue, H., and Yamaizumi, M. (2004). Rad18 guides poleta to replication stalling sites through physical interaction and PCNA monoubiquitination. *EMBO J.* 23, 3886–3896. doi: 10.1038/sj.emboj.7600383

Waters, L. S., and Walker, G. C. (2006). The critical mutagenic translesion DNA polymerase Rev1 is highly expressed during G(2)/M phase rather than S phase. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8971–8976. doi: 10.1073/pnas.0510167103

Wei, L., and Zhao, X. (2016). A new MCM modification cycle regulates DNA replication initiation. *Nat. Struct. Mol. Biol.* 23, 209–216. doi: 10.1038/nsmb.3173

Weston, R., Peeters, H., and Ahel, D. (2012). ZRANB3 is a structure-specific ATP-dependent endonuclease involved in replication stress response. *Genes Dev.* 26, 1558–1572. doi: 10.1101/gad.193516.112

Whitehurst, C. B., Vaziri, C., Shackelford, J., and Pagano, J. S. (2012). Epstein-Barr virus BPLF1 deubiquitinates PCNA and attenuates polymerase eta recruitment to DNA damage sites. *J. Virol.* 86, 8097–8106. doi: 10.1128/JVI.00588-12

Williams, S. A., Longerich, S., Sung, P., Vaziri, C., and Kupfer, G. M. (2011). The E3 ubiquitin ligase RAD18 regulates ubiquitylation and chromatin loading of FANCD2 and FANCI. *Blood* 117, 5078–5087. doi: 10.1182/blood-2010-10-311761

Wood, A., Krogan, N. J., Dover, J., Schneider, J., Heidt, J., Boateng, M. A., et al. (2003). Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell* 11, 267–274. doi: 10.1016/S1097-2765(02)00802-X

Wu, N., Kong, X., Ji, Z., Zeng, W., Potts, P. R., Yokomori, K., et al. (2012). Scc1 sumoylation by Mms21 promotes sister chromatid recombination through counteracting Wapl. *Genes Dev.* 26, 1473–1485. doi: 10.1101/gad.193615.112

Wurtele, H., Kaiser, G. S., Bacal, J., St-Hilaire, E., Lee, E. H., Tsao, S., et al. (2012). Histone H3 lysine 56 acetylation and the response to DNA replication fork damage. *Mol. Cell. Biol.* 32, 154–172. doi: 10.1128/MCB.05415-11

Xiao, Z., Chang, J. G., Hendriks, I. A., Sigurethsson, J. O., Olsen, J. V., and Vertegaal, A. C. (2015). System-wide analysis of SUMOylation dynamics in response to replication stress reveals novel small ubiquitin-like modified target proteins and acceptor lysines relevant for genome stability. *Mol. Cell Proteomics* 14, 1419–1434. doi: 10.1074/mcp.O114.044792

Xu, X., Lin, A., Zhou, C., Blackwell, S. R., Zhang, Y., Wang, Z., et al. (2016). Involvement of budding yeast Rad5 in translesion DNA synthesis through physical interaction with Rev1. *Nucleic Acids Res.* doi: 10.1093/nar/gkw183 [Epub ahead of print].

Yang, K., Gong, P., Gokhale, P., and Zhuang, Z. (2014). Chemical protein polyubiquitination reveals the role of a noncanonical polyubiquitin chain in DNA damage tolerance. *ACS Chem. Biol.* 9, 1685–1691. doi: 10.1021/cb500133k

Yang, K., Moldovan, G. L., and D'andrea, A. D. (2010). RAD18-dependent recruitment of SNM1A to DNA repair complexes by a ubiquitin-binding zinc finger. *J. Biol. Chem.* 285, 19085–19091. doi: 10.1074/jbc.M109.100032

Ye, Y., and Rape, M. (2009). Building ubiquitin chains: E2 enzymes at work. *Nat. Rev. Mol. Cell Biol.* 10, 755–764. doi: 10.1038/nrm2780

Yu, C., Gan, H., Han, J., Zhou, Z. X., Jia, S., Chabes, A., et al. (2014). Strand-specific analysis shows protein binding at replication forks and PCNA unloading from lagging strands when forks stall. *Mol. Cell* 56, 551–563. doi: 10.1016/j.molcel.2014.09.017

Yu, Y., Cai, J. P., Tu, B., Wu, L., Zhao, Y., Liu, X., et al. (2009). Proliferating cell nuclear antigen is protected from degradation by forming a complex with MutT Homolog2. *J. Biol. Chem.* 284, 19310–19320. doi: 10.1074/jbc.M109.015289

Yuan, J., Pu, M., Zhang, Z., and Lou, Z. (2009). Histone H3-K56 acetylation is important for genomic stability in mammals. *Cell Cycle* 8, 1747–1753. doi: 10.4161/cc.8.11.8620

Zabrady, K., Adamus, M., Vondrova, L., Liao, C., Skoupilova, H., Novakova, M., et al. (2015). Chromatin association of the SMC5/6 complex is dependent on binding of its NSE3 subunit to DNA. *Nucleic Acids Res.* 44, 1064–1079. doi: 10.1093/nar/gkv1021

Zaidi, I. W., Rabut, G., Poveda, A., Scheel, H., Malmstrom, J., Ulrich, H., et al. (2008). Rtt101 and Mms1 in budding yeast form a CUL4(DDB1)-like ubiquitin ligase that promotes replication through damaged DNA. *EMBO Rep.* 9, 1034–1040. doi: 10.1038/embor.2008.155

Zeman, M. K., and Cimprich, K. A. (2014). Causes and consequences of replication stress. *Nat. Cell Biol.* 16, 2–9. doi: 10.1038/ncb2897

Zeman, M. K., Lin, J. R., Freire, R., and Cimprich, K. A. (2014). DNA damage-specific deubiquitination regulates Rad18 functions to suppress mutagenesis. *J. Cell Biol.* 206, 183–197. doi: 10.1083/jcb.201311063

Zhang, C., Roberts, T. M., Yang, J., Desai, R., and Brown, G. W. (2006). Suppression of genomic instability by SLX5 and SLX8 in *Saccharomyces cerevisiae*. *DNA Repair (Amst.)* 5, 336–346. doi: 10.1016/j.dnarep.2005.10.010

Zhang, J., and Walter, J. C. (2014). Mechanism and regulation of incisions during DNA interstrand cross-link repair. *DNA Repair (Amst.)* 19, 135–142. doi: 10.1016/j.dnarep.2014.03.018

Zhang, S., Chea, J., Meng, X., Zhou, Y., Lee, E. Y., and Lee, M. Y. (2008). PCNA is ubiquitinated by RNF8. *Cell Cycle* 7, 3399–3404. doi: 10.4161/cc.7.21.6949

Zhang, Z., Shibahara, K., and Stillman, B. (2000). PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* 408, 221–225. doi: 10.1038/35048530

Zhao, S., and Ulrich, H. D. (2010). Distinct consequences of posttranslational modification by linear versus K63-linked polyubiquitin chains. *Proc. Natl. Acad. Sci. U.S.A.* 107, 7704–7709. doi: 10.1073/pnas.0908764107

Zhao, X., and Blobel, G. (2005). A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. *Proc. Natl. Acad. Sci. U.S.A.* 102, 4777–4782. doi: 10.1073/pnas.0500537102

Zhao, X., Wu, C. Y., and Blobel, G. (2004). Mlp-dependent anchorage and stabilization of a desumoylating enzyme is required to prevent clonal lethality. *J. Cell Biol.* 167, 605–611. doi: 10.1083/jcb.200405168

Zhong, W., Feng, H., Santiago, F. E., and Kipreos, E. T. (2003). CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. *Nature* 423, 885–889. doi: 10.1038/nature01747

Zlatanou, A., Sabbioneda, S., Miller, E. S., Greenwalt, A., Aggathanggelou, A., Maurice, M. M., et al. (2016). USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. *Oncogene* 35, 965–976. doi: 10.1038/onc.2015.149

Zou, Y., Liu, Y., Wu, X., and Shell, S. M. (2006). Functions of human replication protein A (RPA): from DNA replication to DNA damage and stress responses. *J. Cell. Physiol.* 208, 267–273. doi: 10.1002/jcp.20622

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 03 May 2016
doi: 10.3389/fgene.2016.00073

Ring of Change: CDC48/p97 Drives Protein Dynamics at Chromatin

*André Franz, Leena Ackermann and Thorsten Hoppe**

Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Institute for Genetics, University of Cologne, Cologne, Germany

The dynamic composition of proteins associated with nuclear DNA is a fundamental property of chromosome biology. In the chromatin compartment dedicated protein complexes govern the accurate synthesis and repair of the genomic information and define the state of DNA compaction in vital cellular processes such as chromosome segregation or transcription. Unscheduled or faulty association of protein complexes with DNA has detrimental consequences on genome integrity. Consequently, the association of protein complexes with DNA is remarkably dynamic and can respond rapidly to cellular signaling events, which requires tight spatiotemporal control. In this context, the ring-like AAA+ ATPase CDC48/p97 emerges as a key regulator of protein complexes that are marked with ubiquitin or SUMO. Mechanistically, CDC48/p97 functions as a segregase facilitating the extraction of substrate proteins from the chromatin. As such, CDC48/p97 drives molecular reactions either by directed disassembly or rearrangement of chromatin-bound protein complexes. The importance of this mechanism is reflected by human pathologies linked to p97 mutations, including neurodegenerative disorders, oncogenesis, and premature aging. This review focuses on the recent insights into molecular mechanisms that determine CDC48/p97 function in the chromatin environment, which is particularly relevant for cancer and aging research.

Keywords: CDC48, p97, ubiquitin, SUMO, chromatin, replication, DNA repair

OPEN ACCESS

Edited by:
Ivan Dikic,
Goethe University Frankfurt, Germany

Reviewed by:
Parvin Mehdipour,
Tehran University of Medical Sciences, Iran
Stefan Jentsch,
Max Planck Society, Germany

****Correspondence:***
Thorsten Hoppe
thorsten.hoppe@uni-koeln.de

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *25 February 2016*
Accepted: *16 April 2016*
Published: *03 May 2016*

Citation:
Franz A, Ackermann L and Hoppe T (2016) Ring of Change: CDC48/p97 Drives Protein Dynamics at Chromatin. Front. Genet. 7:73. doi: 10.3389/fgene.2016.00073

INTRODUCTION

DNA is the most precious resource of an organism. Its faithful transmission to following generations is of major importance for an individual. Elaborate surveillance mechanisms are required to guard the genome, since large amounts of heterogeneous protein complexes are active at the DNA. Thus, DNA is packaged into highly dynamic chromatin structures for efficient space usage. This involves different histone variants as well as complex protein cohorts that allow for genome function (Misteli, 2007; Cutter and Hayes, 2015). Dependent on cell type, cell cycle phase, environmental cues, or aging status, multisubunit replication and transcription machineries access chromatin and thereby challenge chromosome integrity. In addition, various maintenance and repair mechanisms are active that keep chromatin intact. To ensure genome stability these processes need to be coordinated and tightly controlled in time and space. Within complex protein agglomerations specific proteins have to be recruited or removed to allow a given process to continue. The underlying molecular signaling is predominantly triggered by post-translational modifications (PTMs) of target proteins.

The ATPase CDC48/p97 (also known as VCP in human) is a central factor that integrates recognition, modification and execution of molecular processes mediated by ubiquitin (Ghislain et al., 1996; Meyer et al., 2000; Dai and Li, 2001; Wojcik et al., 2004) or ubiquitin-like molecules

(Krick et al., 2010; Bandau et al., 2012; den Besten et al., 2012; Nie et al., 2012; Bergink et al., 2013; Køhler et al., 2013, 2015). CDC48/p97 forms homo-hexameric ring-like particles, which undergo extensive conformational changes upon ATP-hydrolysis (Rouiller et al., 2002; Banerjee et al., 2016). These intramolecular changes drive the mechanistic function of CDC48/p97, which is best described as segregase activity (Rape et al., 2001; Braun et al., 2002; Shcherbik and Haines, 2007). While the precise molecular mechanism of substrate handling is controversial (Stolz et al., 2011; Barthelme and Sauer, 2015), cumulating evidence suggests that the ATP-dependent conformational rearrangements account for partial unfolding of substrates (Beskow et al., 2009; Godderz et al., 2015; Song et al., 2015), thereby promoting their segregation from multimeric protein assemblies. Following the recognition of target proteins that are marked by ubiquitin, SUMO or both, CDC48/p97 mobilizes the modified substrates from higher order protein complexes, resulting in their inactivation by breaking off the molecular context and/or promoting subsequent proteolytic turnover (**Figure 1**). The cellular processes that rely on CDC48/p97 segregase activity are diverse (Franz et al., 2014), ranging from degradation of damaged proteins associated with the endoplasmic reticulum (ER, ERAD; Ye et al., 2001; Braun et al., 2002; Jarosch et al., 2002; Rabinovich et al., 2002) or mitochondria (MAD; Heo et al., 2010; Hemion et al., 2014; Fang et al., 2015), ribosome-associated quality control (Ossareh-Nazari et al., 2010; Brandman et al., 2012; Verma et al., 2013) to lipid droplet metabolism (Olzmann et al., 2013), and lysosomal proteolysis (Ren et al., 2008; Ju et al., 2009; Krick et al., 2010; Tresse et al., 2010; Ritz et al., 2011; Buchan et al., 2013). Recently, most attention has been paid to the role of CDC48/p97 in the directed modulation of chromatin-associated protein complexes (Vaz et al., 2013; Dantuma et al., 2014). Herein, fundamental cellular processes such as DNA synthesis and DNA repair as well as transcriptional regulation require CDC48/p97.

Given the growing number of cellular pathways relying on CDC48/p97 function, it appears obvious that independent regulatory mechanisms are required to control the diverse molecular activities. In this context, cofactors provide specificity toward defined CDC48/p97 pathways (Decottignies et al., 2004; Hartmann-Petersen et al., 2004; Medicherla et al., 2004; Schuberth et al., 2004; Wang et al., 2004; Neuber et al., 2005; Park et al., 2005; Richly et al., 2005; Schuberth and Buchberger, 2005; Song et al., 2005; Ritz et al., 2011). Most cofactors interact with CDC48/p97 via conserved binding motifs and provide additional molecular properties that assist in substrate recognition, processing, or regulation of ATPase activity. Substrate recruiting cofactors harbor dedicated domains that recognize conjugated ubiquitin or SUMO, thereby facilitating substrate binding (Kloppsteck et al., 2012; Meyer and Weihl, 2014; Buchberger et al., 2015). Processing cofactors alter the length or topology of ubiquitin or SUMO marks, either by extending (E3–E4 enzymes), shortening (ubiquitin/SUMO hydrolases), or remodeling (also called editing, combined E3–E4 and hydrolase activities) the conjugates (Koegl et al., 1999; Hoppe, 2005; Rumpf and Jentsch, 2006; Jentsch and Rumpf, 2007; Kuhlbrodt et al., 2011; Heride et al., 2014). Other cofactors regulate CDC48/p97 ATPase activity (Trusch et al., 2015; Zhang et al., 2015) thus controlling substrate processing. Cofactors themselves can provide another layer of associated factors (termed accessory factors), thereby defining CDC48/p97 function (Alexandru et al., 2008; Sowa et al., 2009; Balakirev et al., 2015; Raman et al., 2015; **Figure 1**; **Table 1**).

The requirement of an organism for CDC48/p97 originates from the variety of processes that depend on its segregase activity. Hence, alterations in CDC48/p97 protein expression or mutations are associated with different diseases including neurodegeneration or premature aging (Partridge et al., 2003; Johnson et al., 2010; Nalbandian et al., 2011; Franz et al., 2014). Moreover, CDC48/p97 overexpression is associated with different cancer types connected with poor prognosis (Fessart et al., 2013). This is intelligible given the diverse chromatin related pathways, like replication or DNA repair that CDC48/p97 is associated with. Since each of these pathways is highly related to tumor formation, p97 constitutes a reasonable target for anticancer therapy (Balch et al., 2008) and first inhibitors are already tested in clinical trials (Deshaies, 2014; Chapman et al., 2015). This review provides an overview on the fundamental role of CDC48/p97 in controlling activity and dynamics of protein complexes at the chromatin. For simplicity, we will refer to spelling of conserved human orthologs throughout the article, unless otherwise stated.

DNA REPLICATION IS DRIVEN BY DYNAMIC COMPOSITION OF PROTEIN COMPLEXES

The faithful duplication of genomic information during S phase of the cell cycle is a complex biological process involving the highly ordered cascade of numerous replication factors at the chromatin (Masai et al., 2010; Fragkos et al., 2015). DNA synthesis is initiated at origins of replication, which serve as assembly platforms for DNA synthesis factories, termed replisomes. Herein, the concerted activity of origin recognition complex (ORC), CDC6, and CDT1 is required to load the replicative DNA helicase, the Mini-chromosome-maintenance (MCM) complex onto DNA. Together these factors constitute the pre-replicative complex (pre-RC). As pre-RCs do not perform helicase activity yet, pre-RC assembly is considered as licensing of DNA replication. Interestingly, inaccurately assembled pre-RCs can disassemble from DNA implicating that replication licensing involves quality control mechanisms and iterative loading events (Chen et al., 2007; Xouri et al., 2007; Frigola et al., 2013; Duzdevich et al., 2015). Subsequent to MCM assembly, the pre-RC components are dispensable and consequently inactivated. Origins actively synthesizing DNA are characterized by recruitment of further factors, including CDC45 and the go-ichi-ni-san (GINS) complex (Gambus et al., 2006; Moyer et al., 2006; Ilves et al., 2010). The presence of CDC45 and GINS thus characterizes active replisomes. During ongoing DNA synthesis and particularly close to completion of DNA replication

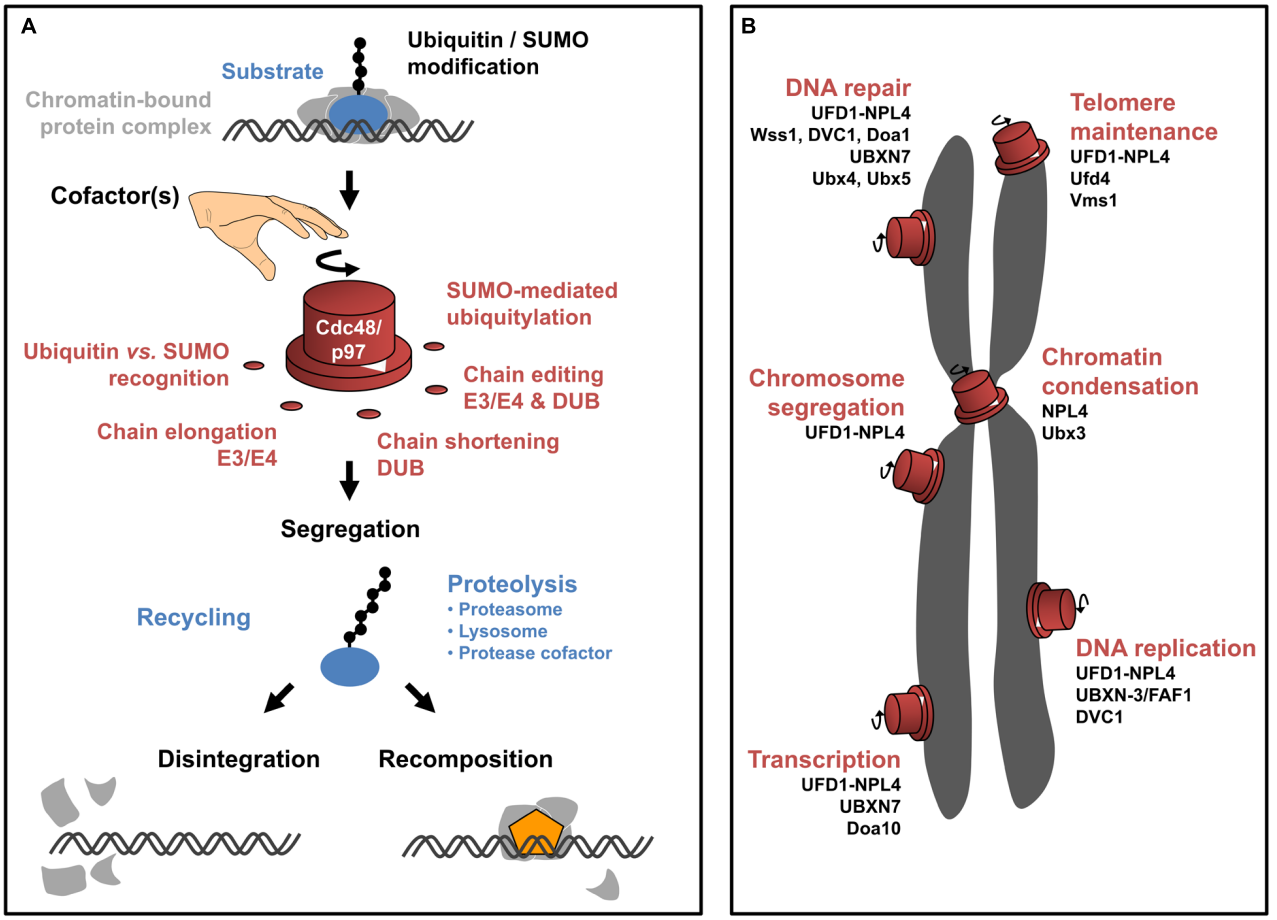

FIGURE 1 | CDC48/p97 function in chromatin-associated processes. (A) Schematic illustration of molecular mechanisms underlying CDC48/p97 activity. CDC48/p97 (red) recognizes chromatin-bound substrates (blue) that are conjugated to ubiquitin or SUMO (black circles). The modification with ubiquitin and SUMO can come in different flavors (exemplified by a chain of molecules). Both molecules can be conjugated as a monomeric moiety or a chain of several molecules. The linkage in between molecules of a ubiquitin chain is variable depending on the internal lysine-(K)-residue used for chain extension (indicated by the angle between ubiquitin molecules of a chain). As such, diverse linkages are capable of defining distinct signaling events (referred to as 'ubiquitin-code'). Moreover, SUMO-dependent ubiquitylation gives rise to hybrid chains. Depending on the exact modification of the substrates, diverse cofactors facilitate substrate recognition and/or processing of the ubiquitin/SUMO modification by extending, removing, or internal remodeling of the chain. This is probably important to define the directionality of the CDC48/p97 reaction. Eventually, CDC48/p97 segregase activity is required to mobilize the substrate from higher order protein complexes (light gray). On one hand the substrate can be recycled, probably involving hydrolysis of the modification. Otherwise, the substrate can be terminally degraded involving the proteasome, lysosome, or proteolytic cofactors. Extraction of the substrate can promote two distinct outcomes. Disintegration of the protein complex can result in its inactivation (bottom left). Alternatively, extraction of the substrate can disclose the binding site of another factor (orange) thus facilitating the directed progression of the reaction (bottom right). **(B)** Schematic overview of CDC48/p97-dependent pathways in the context of eukaryotic chromosomes (gray). CDC48/p97 (red) possesses molecular switch properties, driving molecular reactions in distinct chromatin-associated processes. The involvement of respective CDC48/p97 cofactors is listed below the indicated pathways.

converging replication factories collide and are considered to require regulated disassembly (Maric et al., 2014; Moreno et al., 2014). These processes exemplify that the composition of replication factories is highly dynamic throughout the regular replication program and, moreover, responsive to genotoxic insults that might threaten genome stability (Sirbu et al., 2013; Alabert et al., 2014; Dungrawala et al., 2015; Raschle et al., 2015). Intriguingly, CDC48/p97 has been shown to be essential for DNA replication in eukaryotes by regulating the abundance of several replication factors at distinct time points (**Figure 1**; **Table 1**).

CDC48/p97-mediated Control of DNA Replication Licensing and Fork Progression

The functional relevance of CDC48/p97 in DNA synthesis was first shown in *Caenorhabditis elegans* (*C. elegans*). RNAi-mediated depletion of CDC48/p97 or the dimeric cofactor UFD-1-NPL-4 caused replication defects accompanied with collapsed forks and formation of DNA repair foci (Mouysset et al., 2008). This initial observation of compromised DNA synthesis upon inactivation of the CDC48/p97$^{UFD-1-NPL-4}$ complex was

TABLE 1 | CDC48/p97 substrates in the chromatin environment.

Cdc48/p97-dependent process	Substrate(s)	Cofactor/Accessory factors	Experimental system	Reference
DNA replication				
Replication fork progression, G2/M checkpoint	n.d.	DVC1	Mammalian cells, patient cells	Lessel et al., 2014
Replication licensing	CDT-1, CDC-45-GINS	UFD-1, NPL-4, UBXN-3/FAF1	*C. elegans*, *X. laevis*, mammalian cells	Mouysset et al., 2008; Franz et al., 2011, 2016
Replication stress	FANCI, FANCD2	DVC1	Mammalian cells	Gibbs-Seymour et al., 2015
Replication stress, DNA damage tolerance (DDT)	Polη, a.o.	DVC1	Mammalian cells, *C. elegans*	Davis et al., 2012; Mosbech et al., 2012
Replication termination	MCM7	Dia2	*S. cerevisiae*, *X. laevis*	Maric et al., 2014; Moreno et al., 2014; Maculins et al., 2015
DNA repair				
Diverse genotoxic insults	SUMO/Ubiquitin-conjugates	Ufd1, Npl4	*S. cerevisiae*	Nie et al., 2012
Diverse genotoxic insults	SUMO/Ubiquitin-conjugates	Ufd1, Npl4, Rfp1, Pli1	*S. pombe*	Køhler et al., 2013
DNA damage response (DDR)	Top1, SUMO-conjugates	Wss1, Doa1	*S. cerevisiae*	Balakirev et al., 2015
DNA-double strand break repair	L3MBTL1	UFD1, NPL4	Mammalian cells, *C. elegans*	Acs et al., 2011
DNA-double strand break repair	SUMO-Rad52	Ufd1	*S. cerevisiae*, mammalian cells	Bergink et al., 2013
DNA-double strand break repair	Ubiquitin-(K48)-conjugates	UFD1, NPL4	Mammalian cells	Meerang et al., 2011
DNA-double strand break repair	DNA-PKcs	n.d.	Mammalian cells	Jiang et al., 2013
DNA-protein crosslink (DPC)	Top1, a.o.	Wss1	*S. cerevisiae*	Stingele et al., 2014
PCNA-dependent response to UV-light	n.d.	DVC1, mono-ubiquitylated PCNA	Mammalian cells	Centore et al., 2012
UV-light induced protein turnover	CSB	UFD1, UBXN7, CUL4	Mammalian cells	He et al., 2016
UV-light induced protein turnover	CDT1, SET8	UFD1, NPL4, a.o.	Mammalian cells, *X. leavis*	Raman et al., 2011
UV-light induced protein turnover	DDB2, XPC	UFD1, NPL4, UBXN7, CUL4	Mammalian cells	Puumalainen et al., 2014
UV-light induced protein turnover	Rbp1	Ufd1, Npl4, Ubx4, Ubx5, Cul3	*S. cerevisiae*	Verma et al., 2011
Transcription				
Histone ubiquitylation	Histone 2B	Ubx3	*S. cerevisiae*, mammalian cells	Bonizec et al., 2014
Mating-type switch	α2	Ufd1, Npl4, Doa10, a.o.	*S. cerevisiae*	Wilcox and Laney, 2009
Transcriptional inactivation	HIF1α	UBXN7, CUL2, VHL	Mammalian cells	Alexandru et al., 2008
Transcriptional regulation	LexA-VP16, Met4, R-Smads	Ufd1, Npl4	*S. cerevisiae*	Ndoja et al., 2014
Heterochromatin decondensation	CenH3	Ufd1, Npl4	*A. thaliana*	Merai et al., 2014
Telomere maintenance				
Telomerase efficiency	Cdc13	Vms1	*S. cerevisiae*	Baek et al., 2012
Telomerase efficiency	Est1	Ufd1, Npl4, Ufd4	*S. cerevisiae*	Lin et al., 2015
Sister-chromatid segregation				
Anaphase degradation	n.d.	Ubx4	*S. cerevisiae*	Chien and Chen, 2013
Chromatin decondensation/congression	Aurora-B	Ufd1, Npl4	*X. leavis*, mammalian cells	Ramadan et al., 2007; Dobrynin et al., 2011
Meiosis	AIR-2	n.d.	*C. elegans*	Sasagawa et al., 2012
Others				
Global analysis	SUMO-conjugates	Ufd1, STUbL	*S. pombe*	Køhler et al., 2015

The table lists the identified chromatin-associated substrates of CDC48/p97, sorted by their functional relevance in indicated cellular processes. In addition, the involvement of cofactors and/or accessory factors is displayed along with the experimental system that was used in the respective publication (a.o. and others, n.d. not determined).

further addressed in a follow-up study, identifying chromatin-associated CDT-1 is the primary substrate (Franz et al., 2011; Raman et al., 2011). Herein, the abundance of the licensing factor CDT-1 on chromatin relies on CDC48/p97 activity during initiation of DNA replication (Franz et al., 2011). In addition, CDT-1 stabilization on mitotic chromatin (correlating to G1 phase), coincides with chromatin-retention of CDC-45 and the GINS complex (Franz et al., 2011). A genetic interaction screen identified the UBX-domain protein UBXN-3 as a specialized cofactor enhancing substrate recognition by CDC48/p97 during DNA replication (Franz et al., 2016). Indeed, *in vivo* and *in vitro* protein interaction analysis confirmed that UBXN-3 provides substrate recognition toward CDT-1 and other ubiquitylated proteins (Franz et al., 2016). Analysis of individual replication forks in human cell lines revealed that siRNA-mediated depletion of the human ortholog FAF1 causes severely impaired replication fork progression associated with elevated frequency of replication fork stalling and firing of dormant origins. In fact, CDT1 protein appears to be the primary target of $CDC48/p97^{FAF1}$ also in human cells, as indicated by genetic suppression of replication defects upon codepletion and *in vivo* binding studies (Franz et al., 2016). Taken together, $CDC48/p97^{UFD1-NPL4}$, in complex with the substrate recognition module UBXN-3/FAF1, controls replication fork progression by restraining the abundance of CDT1 during replication licensing.

It should be noted that the regulatory mechanism depicted above is specific to the G1 phase of the cell cycle (Ballabeni et al., 2004; Franz et al., 2011). In contrast CDT1 protein levels are also under control during the ensuing S phase, involving PCNA and Cullin-based E3 ligases (Zhong et al., 2003; Arias and Walter, 2007; Havens and Walter, 2009; Sugimoto et al., 2009; Coleman et al., 2015). S phase degradation of CDT1 is considered as fundamental in preventing over-replication in one cell cycle as well as avoidance of chromosomal rearrangements (Davidson et al., 2006; Tatsumi et al., 2006). In response to DNA damage, CDT1 chromatin extraction and degradation also involves CDC48/p97 activity (Hu et al., 2004; Jin et al., 2006; Raman et al., 2011). In contrast to the licensing factors ORC and Cdc6, Cdt1 is required for break-induced replication in yeast (Lydeard et al., 2010). The exact requirement of CDT1 and its subsequent inactivation during DNA repair, however, remains elusive.

Interestingly, another thus far unappreciated cofactor of CDC48/p97 has attracted attention as a critical regulator in cellular pathways ensuring genome integrity (Stingele et al., 2015). Two studies could show that mutations in DVC1 [also called Spartan (SPRTN) or C1orf124 in humans, functionally related to Wss1 in *Saccharomyces cerevisiae*] are causative for genome instability phenotypes cumulating in hepatocellular carcinoma and progeria (Lessel et al., 2014; Maskey et al., 2014; **Figure 1**; **Table 1**). Patient cells expressing dysfunctional DVC1 show hallmarks of genomic instability, which is accompanied by aberrant replication fork velocity along with excessive replication stress. Furthermore, patient cell lines escape the cell cycle control by G2/M checkpoint, which usually halts the transition into mitosis until damage is repaired. Importantly, human cells exclusively expressing disease-related DVC1 mutations phenocopy the observations made in primary cells (Lessel et al., 2014). Identification of respective target substrates will decipher, which aspect of DNA replication is controlled by DVC1. Indeed DVC1 could be shown to colocalize with DNA replication factories in synchronized but otherwise untreated mammalian cells (Davis et al., 2012). Its functional relevance, however, became particularly important upon treatment with various types of genotoxic agents, which triggered the DVC1-dependent recruitment of CDC48/p97 to sites of DNA damage (Centore et al., 2012; Davis et al., 2012; Mosbech et al., 2012). Intriguing insights into the mechanistic function of DVC1's cognate Wss1 in chromatin-associated protein degradation have recently been reported in the context of replication-coupled DNA repair (Stingele et al., 2014; Balakirev et al., 2015). Herein, Wss1 protease was identified to specifically mediate the processing of DNA-protein crosslinks (DPC) in a thus far overlooked repair pathway that presumably also underlies genome instability in DVC1 mutant cells (**Figure 1**; **Table 1** and references therein). Accordingly, disease-causing mutations locate in the domain encoding the predicted DVC1 protease (SprT) domain (Lessel et al., 2014); however, a chromatin-directed protease activity of DVC1 awaits affirmation. The mechanistic details of DPC-repair will be discussed in the respective paragraph on DNA-repair pathways.

Termination of DNA Replication Requires CDC48/p97 Activity

Until recently, the molecular mechanisms underlying the termination of DNA replication in metazoans was only scarcely described (Dewar et al., 2015). Thus, the identification of CDC48/p97 in the release of the MCM helicase in complex with CDC45 and GINS (collectively termed CMG complex) at sites of replication termination was astonishing (Maric et al., 2014; Moreno et al., 2014). Yeast cells or *Xenopus* egg extracts that are depleted for CDC48/p97 are defective in the disassembly of the CMG complex at the end of the cell cycle when replication forks collide with high frequency. Both studies show that selective poly-(K48)-ubiquitylation of the MCM7 subunit is required to trigger CMG release (Maric et al., 2014; Moreno et al., 2014). Interfering with polyubiquitylation as well as CDC48/p97 activity, result in accumulation of DNA structures comparable to pharmacological inhibition of termination (Moreno et al., 2014). Moreover, ubiquitylation of MCM7 depends on active progression through S phase (Maric et al., 2014; Moreno et al., 2014). In conclusion, DNA replication requires CDC48/p97 activity to terminate DNA synthesis by unloading of active CMG complexes.

Comparing CDC48/p97-dependent regulation of replication licensing with termination of replication leaves open questions to be addressed. Moreno et al. (2014) used an experimental system in *Xenopus* egg extracts that affects polyubiquitylation in progressing S phase, thus allowing exclusive analysis of replication termination. In *S. cerevisiae*, the licensing factor Cdt1 is not regulated via proteolysis, but nuclear export, pointing at distinct regulatory mechanisms between unicellular fungi and metazoans (Tanaka and Diffley, 2002; Feng and Kipreos, 2003; Kim and Kipreos, 2007). What are the respective cofactors that

define CDC48/p97 specificity toward replication termination? In yeast, the ligase complex SCF^{Dia2} catalyzes the ubiquitylation of MCM7 and thus provides the signal for CMG disassembly (Maric et al., 2014; Maculins et al., 2015). The substrate recognition factor Dia2, however, is not obviously conserved outside of the fungi kingdom. In *Xenopus*, release of CMG complex has been linked to the E3 ligase BRCA1, however, is supposed to be specific to stalled replisomes but not termination (Long et al., 2014; Dewar et al., 2015). As such, CDC48/p97-dependent CMG release might be considered as a combined phenotype: aberrant licensing causing stalled forks, in turn requiring active CMG unloading. In *C. elegans* and *Xenopus* egg extracts, CDC48/p97 is linked to the release of CDC-45 and GINS after S phase is completed (Franz et al., 2011, 2016). Herein, CDC48/p97 cooperates with the cofactors UFD-1-NPL-4 and UBXN-3/FAF1, however, neither the depletion of *ufd-1*, *npl-4*, nor *ubxn-3* resulted in persistent chromatin-association of MCM subunits (Franz et al., 2016). These observations support the idea that CMG disassembly is more complex involving dedicated CDC48/p97 cofactors separately targeting MCM or CDC45-GINS. It has been speculated that a ring-shaped GINS molecule embraces DNA (Kubota et al., 2003; Boskovic et al., 2007), thus chromatin release might be regulated independently from MCM. Alternatively, CMG disassembly might be differentially regulated in invertebrates and vertebrates. In human cells, unloading of MCM complexes has been linked to the deubiquitylating activity of USP7 (Jagannathan et al., 2014), implicating that regulated MCM release might involve editing of ubiquitin chains. Whether USP7 activity is indeed coordinated by CDC48/p97 remains to be elucidated.

PROTEIN DYNAMICS AT SITES OF DNA REPAIR

DNA damage poses a major threat to cells. Besides defects in replication, additional intrinsic incidents may threaten chromatin integrity. Hence damage may originate from hydrolytic reactions or reactive oxygen species (ROS). However, also extrinsic and highly carcinogenic sources like UV exposure or tobacco products harm DNA. If unrepaired, DNA damage leads to accumulation of mutations or chromosomal aberrations and promotes genome instability, the cause for many diseases including cancer (Jackson and Bartek, 2009; Ciccia and Elledge, 2010). Specific to the damaging agent, the recognition of the damage and the molecular mechanism for its removal are diverse. One unifying feature of all repair pathways, however, is the progression through specific phases of damage recognition, effective repair, and finally resolution of repair intermediates. The underlying DNA damage response (DDR) triggers the dynamic and hierarchically ordered assembly and disassembly of repair factors on the chromatin (Hoeijmakers, 2001). CDC48/p97 plays a central role in various DNA repair scenarios and specialized cofactors provide mechanistic regulatory insight (**Figure 1**; **Table 1**). Please also see latest review articles on this topic (Vaz et al., 2013; Dantuma et al., 2014; Brinkmann et al., 2015; Dantuma and van Attikum, 2016).

CDC48/p97 Activity in Processing of DNA Double Strand Breaks

The most detrimental DNA lesions are double strand breaks (DSBs) since inadequate fusion of loose ends can give rise to considerable chromosome rearrangements, duplications, or deletions and hence are a severe threat for genome integrity (Hoeijmakers, 2009). Two main pathways known to repair DSBs operate differently. One is comparably simple by ligating the loose ends back together in a reaction called non-homologous end-joining (NHEJ). The other, termed homologous recombination (HR) is a much more concise pathway, using a homologous template for reestablishing the undamaged state (Hoeijmakers, 2001; San Filippo et al., 2008; Lieber, 2010; Mehta and Haber, 2014; Kowalczykowski, 2015).

CDC48/p97 was first implicated in DNA repair by finding that it gets phosphorylated at S^{784} upon DNA damage induction (Livingstone et al., 2005). Indeed DNA-dependent protein kinase, catalytic subunit (PKcs), one of the kinases mediating this PTM (Livingstone et al., 2005), directly interacts with CDC48/p97 upon ubiquitylation (Jiang et al., 2013). DNA-PKcs associates with a heterodimer of Ku70-Ku80 at DSBs and initiates NHEJ (Wang et al., 1994; Davis et al., 2014). CDC48/p97 acts here to restrict DNA PKcs occupancy on DNA by handing it over to proteasomal turnover. In this glioma cell model, loss of CDC48/p97 improves repair efficiency temporally (Jiang et al., 2013). Conversely, other studies described an increase in sensitivity toward DNA damage and subsequent genome instability when CDC48/p97 activity is limited (Acs et al., 2011; Meerang et al., 2011; Raman et al., 2011). Upon DNA DSB induction a well-studied signaling cascade is commenced, involving initial phosphorylation steps but subsequent engagement of the ubiquitin and SUMO machinery to establish binding sites for specific signaling proteins like BRCA1 and 53BP1 (Bekker-Jensen and Mailand, 2011; Polo and Jackson, 2011). This process requires tight regulation; however, ubiquitylation does not only serve as a binding platform orchestrating the recruitment of specific interaction partners. Instead also poly-(K48)-linked ubiquitin chains, which trigger proteasomal degradation (Dammer et al., 2011), were identified at DDR sites that strongly accumulate in CDC48/p97 depleted cells (Meerang et al., 2011). This observation indicates the requirement for CDC48/p97 to remove K48-ubiquitylated proteins from break sites and possibly allow recruitment of downstream factors. In fact, loss of CDC48/p97 function seems to have broad impact on recruitment of repair proteins. After treating cells with ionizing irradiation, CDC48/p97 depletion attenuates recruitment of 53BP1, BRCA1, and abolishes loading of RAD51 to repair sites (Meerang et al., 2011). Mechanistically it remained unclear how CDC48/p97 promotes recruitment of downstream proteins. A recent study highlighted that CDC48/p97 specifically enables recruitment of 53BP1 to DSBs induced by micro-irradiation. 53BP1 association with DSBs is dependent on the ubiquitin cascade but does itself not bind to ubiquitin (Botuyan et al., 2006). Here yet again a switch in signaling molecules has to be implemented. 53BP1 binds to H4K20me2, a histone mark that is initially occupied by L3MBTL1 (Min et al., 2007). Upon ubiquitylation, L3MBTL1 is primed for

extraction by CDC48/p97 together with its cofactors NPL4 and UFD1. Only then 53BP1 is able to bind its designated recruitment site and the repair process can be pursued (Acs et al., 2011). Even though different outcomes of CDC48/p97 activity at damage loci are described, both emphasize the requirement of CDC48/p97 to extract ubiquitylated target proteins from DNA damage signaling sites to facilitate further repair steps.

CDC48/p97 Functions as SUMO-dependent Segregase to Provide Genome Stability

Aside ubiquitin-conjugates CDC48/p97 and its cofactor UFD1 both recognize SUMOylated target proteins directly (Nie et al., 2012). A functional relevance of CDC48/p97 exclusively targeting SUMO-conjugates was first described in the assembly of downstream effector proteins during DNA repair (Bergink et al., 2013). During HR the essential recombinase RAD51 forms long filaments on the two single stranded loose DNA ends, which enable scanning and approaching the homologous sequence (Holthausen et al., 2010; Forget and Kowalczykowski, 2012). Its DNA association needs to be tightly controlled, since hyper-recombination is highly cytotoxic; at the same time RAD51 is essential for recombination (Tsuzuki et al., 1996; Sonoda et al., 1998; Richardson et al., 2004; Klein, 2008). In yeast, SUMO-conjugated Rad52 interacts with and aides Rad51 loading onto DNA when engaged in HR. Interestingly, CDC48/p97^{Ufd1} has direct binding affinity toward the same SUMOylated lysine on Rad52 hence counterbalancing recombination events mediated by Rad51 (Bergink et al., 2013). This finding highlights a function of CDC48/p97 independent of ubiquitin and implies that competitive binding to SUMO can promote segregation activity.

In addition to minimizing Rad51-Rad52 interaction, CDC48/p97 plays a more global role in the regulation of SUMO-conjugates at the chromatin (Køhler et al., 2015). SUMOylation was established as another layer of regulation at DSB sites that enables thorough repair (Hardeland et al., 2002; Cremona et al., 2012; Jackson and Durocher, 2013). Mutations in SUMO related proteins lead to genomic instability, but again, inappropriate retention of SUMOylated proteins on DNA also impedes accurate repair since recruitment of downstream factors is strongly reduced (Psakhye and Jentsch, 2012). Interestingly, the CDC48/p97$^{Ufd1-Npl4}$ complex has been implicated in this specific extraction as well. Ufd1 harbors a SUMO interaction motif (SIM) by which it directly binds SUMO (Nie et al., 2012). Additionally, specific ubiquitin ligases target SUMOylated proteins for ubiquitylation (SUMO-targeted ubiquitin ligases: STUbLs). CDC48/p97 together with its cofactors is thus recruited via a dual mechanism consisting of ubiquitin and SUMO, thereby facilitating extraction and possibly degradation of SUMOylated proteins at DNA damage sites (Nie et al., 2012). UFD1 takes an important role as cofactor of CDC48/p97. In addition to its direct binding to SUMO, a physical as well as functional interaction of Ufd1 with the STUbL Rfp1 (RNF4 ortholog) or the SUMO E3 ligase Pli1 (PIAS1) was shown. The concerted action of these proteins leads to ordered removal of SUMOylated proteins at damage site, again their inappropriate retention by loss of one of the factors entails genomic instability (Køhler et al., 2013). This example nicely highlights CDC48/p97s function as a molecular switch. It provides a platform for a variety of functionally distinct proteins that together lead to precisely coordinated CDC48/p97-dependent chromatin extraction of client proteins (**Figure 1**; **Table 1**).

An analogous mechanism was described for DNA repair by the Fanconi anemia pathway. After replication block, Fanconi anemia pathway becomes active to promote fork restart by initiating translesion synthesis and damage removal (Haynes et al., 2015). Two central components, FANCI and FANCD, are SUMOylated upon fork stalling. As described for other SUMOylated proteins, they are targeted for degradation by RNF4 mediated ubiquitylation and subsequent mobilization from DNA by the CDC48/p97 complex (Gibbs-Seymour et al., 2015). Here CDC48/p97 acts jointly with DVC1. Degradation of FANCI and FANCD is impaired in RNF4 mutants, highlighting that in this case ubiquitin binding of the CDC48/p97^{DVC1} complex is necessary.

Processing of DNA-protein Crosslinks

Proteins that are crosslinked to DNA or chromatin are a specialized form of chromatin modification, which can arise from metabolic sources or external insults such as reactive aldehydes, UV-light, or catalytic intermediates, e.g., upon Topoisomerase 1 inhibition (Duxin and Walter, 2015; Stingele and Jentsch, 2015; Stingele et al., 2015). DPCs result in stalling of RNA and DNA polymerases and thus impact on a variety of cellular processes. Consequently, DPCs need to be removed in a regulated manner that involves incomplete proteolytic digestion. Subsequently, the processed DPC remnant can be bypassed by a specialized translesion polymerase (Duxin et al., 2014), whereas the DPC remnant itself is considered to be eventually removed by base excision repair. Intriguingly, a DVC1-related protease acts as CDC48/p97 cofactor and harbors protease activity to catalyze DPC processing in yeast (Stingele et al., 2014, 2015; **Figure 1**; **Table 1**). Wss1 protease activity becomes particularly activated upon DNA binding, where it digests DPCs including covalently bound Topoisomerase 1, other chromatin-bound proteins as well as itself for inactivation (Stingele et al., 2014; Balakirev et al., 2015). Interestingly, Wss1 specifically targets SUMO-conjugates on chromatin via its SUMO-interaction motif (Mullen et al., 2010; Stingele et al., 2014; Balakirev et al., 2015). In contrast DVC1 is directed toward ubiquitin-conjugates and is linked to the PCNA sliding clamp (Centore et al., 2012; Davis et al., 2012; Juhasz et al., 2012; Mosbech et al., 2012; Gibbs-Seymour et al., 2015). In case of replication fork stalling-induced extraction of the Fanconi anemia ID complex, the SUMO-dependent ubiquitin E3 ligase RNF4 is central for the underlying signaling (Gibbs-Seymour et al., 2015), supporting the idea that DVC1 regulation in metazoans is multilayered involving both SUMO and ubiquitin. Although a chromatin-directed protease activity of DVC1 awaits to be verified, it is feasible that DVC1 and Wss1 represent functional equivalents. This might explain initial observations that DVC1 is required for the removal of translesion polymerase after UV-lesion bypass

(Centore et al., 2012; Davis et al., 2012; Ghosal et al., 2012; Juhasz et al., 2012; Mosbech et al., 2012; Kim et al., 2013), which is probably linked to attenuated processing of DPCs (Duxin et al., 2014; Duxin and Walter, 2015). Recently, the CDC48 cofactor Doa1 (also known as UFD3 or PLAP) was shown to be present in CDC48/p97^{Wss1} complexes (Balakirev et al., 2015). In this study, genotoxic stress resulted in nuclear GFP-Wss1 punctae, consistent with the formation of DNA repair foci. This is in line with formation of nuclear DVC1 foci upon exposure to genotoxic stress by HU, UV-light, or laser microirradiation in *C. elegans* and mammalian cells (Davis et al., 2012; Mosbech et al., 2012). Moreover, GFP-Wss1 translocates to the vacuole upon damage induction, pointing at a putative role of lysosomal degradation in Wss1-mediated DDR involving the Doa1 cofactor.

To date, the mechanistic role of CDC48/p97 in Wss1-dependent DDR is still obscure. Upon genotoxic insults DVC1/Wss1 recruits CDC48/p97 to the damaged site, pointing at an adaptor-like function promoting segregase activity (Centore et al., 2012; Davis et al., 2012; Mosbech et al., 2012; Gibbs-Seymour et al., 2015). It appears likely, that DVC1/Wss1's intrinsic protease activity is particularly important in cases of DPCs, when CDC48/p97 segregase is not capable of processing the substrate due to covalent linkage to the DNA (Stingele et al., 2014). The observation that Wss1 harbors SUMO-ligase (Balakirev et al., 2015) as well as isopeptidase activity (Mullen et al., 2010) suggests that Wss1 function might include additional layers of regulation.

CDC48/p97 Dependent Extraction in UV Induced DNA Damage Repair

Not only replication block but also obstruction of transcription poses a major threat to DNA. Frequent sources of transcription fork stalling are bulky UV lesions (Mullenders, 2015). To avoid stalling of forks, the cell probes constantly for these helix distortions via the global genome nucleotide excision repair (GG-NER) pathway that is active on both DNA templates. Here, XPC together with UV-DDB complex (UV-DNA damage binding protein) consisting of DDB1 and DDB2 detect lesions and initiate repair (Hoogstraten et al., 2008; Marteijn et al., 2014). To proceed with the excision reaction, these factors need to be removed from chromatin. CDC48/p97 in complex with NPL4-UFD1 and UBXD7 regulates the chromatin association of XPC and DDB2. Ubiquitin dependent extraction by CDC48/p97 allows their proteasomal degradation. Depletion of CDC48/p97 promotes retention of those factors and ultimately leads to genomic aberrations (Puumalainen et al., 2014). In contrast, the DUB USP7 was identified to counteract CDC48/p97 dependent extraction of XPC. It shortens the ubiquitin chain on the target protein, thereby removing the signal for extraction and possible degradation (He et al., 2014).

In case RNA Polymerase II (RNA Pol II) encounters such lesions on the actively transcribed strand, transcription coupled NER (TC-NER) is initiated (Spivak and Ganesan, 2014). During TC-NER CSB is involved in repair initiation (Fousteri et al., 2006; Anindya et al., 2010). Similar to XPC and DDB2 degradation in GG-NER, CDC48/p97-dependent proteolysis of CSB is required to facilitate progression of DNA repair upon UV-irradiation (He et al., 2016). To this end, CSB removal from chromatin is mediated by the cofactors UFD1 and UBXN7 (He et al., 2016). Both sub-pathways, GG-NER and TC-NER have different initiation signals, but merge into one mutual pathway after initial processing. When the injured DNA is excised the gap of 22–30 nucleotides needs to be sealed again. This is accomplished by replication proteins, including PCNA, a DNA polymerase, and subsequent ligation (Marteijn et al., 2014; Mullenders, 2015). In this context, CDC48/p97 is associated with removal and ensuing proteasomal degradation of CDT1 and histone methyl transferase SET8 (Raman et al., 2011). Chromatin association of the two proteins is tightly regulated not only during replication but also upon repair to avoid unscheduled replication initiation (Senga et al., 2006). Binding to the PCNA interaction protein motif degron (PIP degron) of PCNA generally primes target proteins for ubiquitylation by CRL4^{Cdt2} (Havens and Walter, 2009). Hence, after UV damage, CDC48/p97 extracts ubiquitylated CDT1 and SET8 from damaged chromatin and sends both substrates for destruction by the proteasome (Raman et al., 2011).

Normally the cell favors preserving RNA Pol II upon stalling; this is achieved by RNA Pol II backtracking, which allows repair proteins to access the lesion (Epshtein et al., 2014). As a last resort, when NER cannot be executed, RNA Pol II is removed and degraded to prevent even more severe damage (Wilson et al., 2013). Upon UV irradiation, degradation of the largest subunit of RNA Pol II Rpb1 is facilitated by ubiquitin dependent extraction. Herein, CDC48/p97 cooperates with its cofactors Ufd1 and Npl4, as well as with Ubx5 (UBXN7; Verma et al., 2011), a cofactor that is also associated with NER dependent protein extraction in humans.

ADDITIONAL FUNCTION OF CDC48/p97 IN CHROMOSOME BIOLOGY

In contrast to the load of DNA damage another determinant of cellular aging is the shortening of chromosome ends, the telomeres, with consecutive cell divisions. Interestingly, a function of CDC48/p97 in the regulation of telomere length has recently been proposed based on the identification of Cdc13 and Est1 as substrate proteins (Baek et al., 2012; Lin et al., 2015; **Figure 1**; **Table 1**). Both, Cdc13 and Est1 are key regulators of telomere replication in yeast. Baek et al. (2012) showed that CDC48/p97 cooperates with the Vms1 (ANKZF1) cofactor in the proteolytic turnover of Cdc13. Interestingly, Cdc13 destruction appears to involve both proteolytic routes, the proteasome and the lysosome. In contrast Lin et al. (2015) propose that CDC48/p97 together with Ufd1-Npl4 and the ubiquitin E3 ligase Ufd4 (TRIP12) cooperate to adjust telomere length by limiting the abundance of mono-ubiquitylated Est1. Consequently, CDC48/p97 inactivation results in shortened telomeres, presumably due to inefficient telomerase upon Cdc13 and Est1 accumulation.

In addition to DNA synthesis and repair pathways, CDC48/p97 plays pivotal roles during sister-chromatid segregation. Faulty segregation of chromatids during mitosis is a significant source of copy number variations, large

chromosomal aberrations, or chromosome destruction. In fact, a key regulator of mitosis, the kinase Aurora-B, was the first chromatin-associated substrate of CDC48/p97 to be identified (Ramadan et al., 2007; **Figure 1**; **Table 1**). Interestingly, CDC48/p97$^{Ufd1-Npl4}$ restricts Aurora-B activity to promote chromosome congression or chromatin relaxation at distinct time-points during mitosis, as well as chromosome segregation during meiotic division (Ramadan et al., 2007; Dobrynin et al., 2011; Sasagawa et al., 2012). The involvement of cofactors in the regulation of Aurora-B during meiosis I, however, remains to be defined. In yeast cells, CDC48/p97 and its cofactor Ubx4 are involved in the nuclear distribution of proteasomes, thus defining protein degradation during anaphase (Chien and Chen, 2013), implicating that additional CDC48/p97 substrates await to be identified in the context of mitosis.

Aside DNA metabolism CDC48/p97 executes critical function in the regulation of DNA-dependent RNA synthesis. Of note, failure in accurate regulation of transcription factors controlling metabolism and cell proliferation are associated with oncogenesis (Maneix and Catic, 2016). Chromatin-dependent activity of CDC48/p97 in the regulation of gene expression is especially interesting, as it does not involve subsequent protein degradation (Wilcox and Laney, 2009; Ndoja et al., 2014; **Figure 1**; **Table 1**). In yeast, CDC48/p97 controls rapid switch in gene expression through non-proteolytic release of transcription factors from the chromatin. Intriguingly, individual transcription factors that are regulated by CDC48/p97 represent transcriptional activators as well as repressors (Wilcox and Laney, 2009; Ndoja et al., 2014). Thus, CDC48/p97 is capable of initiating fast response toward transcriptional stimuli through attenuation or activation of gene expression. In *S. cerevisiae* the Ufd1-Npl4 cofactor and Doa10 are linked to transcriptional regulation by CDC48/p97 (Wilcox and Laney, 2009; Ndoja et al., 2014). In mammalian cells CDC48/p97 together with the cofactor UBXN7 and the CUL2-VHL ubiquitin ligase mediates the proteolytic inactivation of HIF1α transcription factor, suggesting a critical role of CDC48/p97 in the cellular response toward hypoxia (Alexandru et al., 2008). It remains to be shown, whether CDC48/p97-mediated regulation of HIF1α occurs on chromatin. In contrast to the extraction of transcription factors, CDC48/p97 is also implicated in chromatin remodeling. In yeast CDC48 together with Ubx3 (UBXD8) is required for the mono-ubiquitylation on histone 2B, thus controlling chromatin compaction and presumably differentiation in vertebrates (Bonizec et al., 2014). An alternative pathway controlling gene expression was described in *Arabidopsis*. Here, CDC48/p97^{NPL4} promotes chromatin de-condensation through regulated disassembly of centromeric heterochromatin, resulting in the release of rRNAs, which facilitates ribosome biogenesis (Merai et al., 2014). Here, SUMOylated centromere components including the centromeric histone variant CenH3 trigger chromatin relaxation.

CONCLUDING REMARKS

The described molecular mechanisms illustrate the central role of CDC48/p97 in the dynamic control of protein composition in the chromatin environment (**Figure 1**; **Table 1**). CDC48/p97 operates at the intersection of two major signaling pathways at the chromatin, ubiquitylation and SUMOylation. It is currently unclear whether both signaling pathways initiate separate mechanisms (Meerang et al., 2011; Bergink et al., 2013) or whether both pathways eventually converge into a common pathway (Nie et al., 2012; Køhler et al., 2013; Gibbs-Seymour et al., 2015) with CDC48/p97 as nodal point (**Figure 1**). It is feasible, however, that independent and shared signaling pathways act in parallel. Whereas ubiquitin- and SUMO-signaling are essential in mediating timely response toward genotoxic insults, both modifications need to be removed eventually to restore genome integrity. Regarding ubiquitin signaling, CDC48/p97-dependent processing of substrates on chromatin has exclusively been shown to target either K48-linked ubiquitin chains (Ramadan et al., 2007; Meerang et al., 2011; Maric et al., 2014; Moreno et al., 2014) or mono-ubiquitin (Lin et al., 2015). Whether other linkage-types are involved in CDC48/p97 regulation at the chromatin remains to be deciphered. Mono-ubiquitin and K63-linked chains are essential in the initial signaling of the molecular response to DSBs (Dantuma and van Attikum, 2016). Thus, the recognition of these modifications by CDC48/p97 may provide additional mechanistic insights in early events at DSBs. In this context, it will be of interest to address which cofactors are involved in either linkage-specific recognition or editing of linkage-types (**Figure 1**). Due to its diverse functions, global CDC48/p97 inhibition causes pleiotropic defects on chromosome biology. Thus it will be crucial to identify the cofactors that direct specificity and discriminate between distinct pathways. Although CDC48/p97 inhibitors are tested in clinical trial studies with promising properties (Anderson et al., 2015), a more specific targeting might be applicable through the selective manipulation of cofactors. The identification of substrate proteins targeted by CDC48/p97 will allow future studies to uncover the underlying molecular mechanisms in more detail, pointing out commons and differences.

AUTHOR CONTRIBUTIONS

AF and TH elaborated the concept of the manuscript. AF, LA, and TH wrote the manuscript. All authors discussed the text and commented on the manuscript.

FUNDING

TH is supported by the Deutsche Forschungsgemeinschaft (EXC229, KFO286, HO 2541/8-1, and DIP8 grant 2014376) and the European Research Council (consolidator grant 616499).

ACKNOWLEDGMENTS

We thank the members of our laboratory for critical discussion and helpful advice on the manuscript. We apologize for not having cited valuable contributions due to size limitation.

REFERENCES

Acs, K., Luijsterburg, M. S., Ackermann, L., Salomons, F. A., Hoppe, T., and Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 18, 1345–1350. doi: 10.1038/nsmb.2188

Alabert, C., Bukowski-Wills, J. C., Lee, S. B., Kustatscher, G., Nakamura, K., De Lima Alves, F., et al. (2014). Nascent chromatin capture proteomics determines chromatin dynamics during DNA replication and identifies unknown fork components. *Nat. Cell Biol.* 16, 281–293. doi: 10.1038/ncb2918

Alexandru, G., Graumann, J., Smith, G. T., Kolawa, N. J., Fang, R., and Deshaies, R. J. (2008). UBXD7 binds multiple ubiquitin ligases and implicates p97 in HIF1alpha turnover. *Cell* 134, 804–816. doi: 10.1016/j.cell.2008.06.048

Anderson, D. J., Le Moigne, R., Djakovic, S., Kumar, B., Rice, J., Wong, S., et al. (2015). Targeting the AAA ATPase p97 as an approach to treat cancer through disruption of protein homeostasis. *Cancer Cell* 28, 653–665. doi: 10.1016/j.ccell.2015.10.002

Anindya, R., Mari, P. O., Kristensen, U., Kool, H., Giglia-Mari, G., Mullenders, L. H., et al. (2010). A ubiquitin-binding domain in Cockayne syndrome B required for transcription-coupled nucleotide excision repair. *Mol. Cell* 38, 637–648. doi: 10.1016/j.molcel.2010.04.017

Arias, E. E., and Walter, J. C. (2007). Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. *Genes Dev.* 21, 497–518. doi: 10.1101/gad.1508907

Baek, G. H., Cheng, H., Kim, I., and Rao, H. (2012). The Cdc48 protein and its cofactor Vms1 are involved in Cdc13 protein degradation. *J. Biol. Chem.* 287, 26788–26795. doi: 10.1074/jbc.M112.351825

Balakirev, M. Y., Mullally, J. E., Favier, A., Assard, N., Sulpice, E., Lindsey, D. F., et al. (2015). Wss1 metalloprotease partners with Cdc48/Doa1 in processing genotoxic SUMO conjugates. *Elife* 8:4. doi: 10.7554/eLife.06763

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

Ballabeni, A., Melixetian, M., Zamponi, R., Masiero, L., Marinoni, F., and Helin, K. (2004). Human geminin promotes pre-RC formation and DNA replication by stabilizing CDT1 in mitosis. *EMBO J.* 23, 3122–3132. doi: 10.1038/sj.emboj.7600314

Bandau, S., Knebel, A., Gage, Z. O., Wood, N. T., and Alexandru, G. (2012). UBXN7 docks on neddylated cullin complexes using its UIM motif and causes HIF1α accumulation. *BMC Biol.* 10:36. doi: 10.1186/1741-7007-10-36

Banerjee, S., Bartesaghi, A., Merk, A., Rao, P., Bulfer, S. L., Yan, Y., et al. (2016). 2.3 A resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition. *Science* 351, 871–875. doi: 10.1126/science.aad7974

Barthelme, D., and Sauer, R. T. (2015). Origin and functional evolution of the Cdc48/p97/VCP AAA+ protein unfolding and remodeling machine. *J. Mol. Biol.* doi: 10.1016/j.jmb.2015.11.015 [Epub ahead of print].

Bekker-Jensen, S., and Mailand, N. (2011). The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. *FEBS Lett.* 585, 2914–2919. doi: 10.1016/j.febslet.2011.05.056

Bergink, S., Ammon, T., Kern, M., Schermelleh, L., Leonhardt, H., and Jentsch, S. (2013). Role of Cdc48/p97 as a SUMO-targeted segregase curbing Rad51-Rad52 interaction. *Nat. Cell Biol.* 15, 526–532. doi: 10.1038/ncb2729

Beskow, A., Grimberg, K. B., Bott, L. C., Salomons, F. A., Dantuma, N. P., and Young, P. (2009). A conserved unfoldase activity for the p97 AAA-ATPase in proteasomal degradation. *J. Mol. Biol.* 394, 732–746. doi: 10.1016/j.jmb.2009.09.050

Bonizec, M., Herissant, L., Pokrzywa, W., Geng, F., Wenzel, S., Howard, G. C., et al. (2014). The ubiquitin-selective chaperone Cdc48/p97 associates with Ubx3 to modulate monoubiquitylation of histone H2B. *Nucleic Acids Res.* 42, 10975–10986. doi: 10.1093/nar/gku786

Boskovic, J., Coloma, J., Aparicio, T., Zhou, M., Robinson, C. V., Mendez, J., et al. (2007). Molecular architecture of the human GINS complex. *EMBO Rep.* 8, 678–684. doi: 10.1038/sj.embor.7401002

Botuyan, M. V., Lee, J., Ward, I. M., Kim, J. E., Thompson, J. R., Chen, J., et al. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell* 127, 1361–1373. doi: 10.1016/j.cell.2006.10.043

Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C. C., Li, G. W., et al. (2012). A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell* 151, 1042–1054. doi: 10.1016/j.cell.2012.10.044

Braun, S., Matuschewski, K., Rape, M., Thoms, S., and Jentsch, S. (2002). Role of the ubiquitin-selective CDC48(UFD1/NPL4)chaperone (segregase) in ERAD of OLE1 and other substrates. *EMBO J.* 21, 615–621. doi: 10.1093/emboj/21.4.615

Brinkmann, K., Schell, M., Hoppe, T., and Kashkar, H. (2015). Regulation of the DNA damage response by ubiquitin conjugation. *Front. Genet.* 6:98. doi: 10.3389/fgene.2015.00098

Buchan, J. R., Kolaitis, R. M., Taylor, J. P., and Parker, R. (2013). Eukaryotic stress granules are cleared by autophagy and Cdc48/VCP function. *Cell* 153, 1461–1474. doi: 10.1016/j.cell.2013.05.037

Buchberger, A., Schindelin, H., and Hanzelmann, P. (2015). Control of p97 function by cofactor binding. *FEBS Lett.* 589, 2578–2589. doi: 10.1016/j.febslet.2015.08.028

Centore, R. C., Yazinski, S. A., Tse, A., and Zou, L. (2012). Spartan/C1orf124, a reader of PCNA ubiquitylation and a regulator of UV-induced DNA damage response. *Mol. Cell* 46, 625–635. doi: 10.1016/j.molcel.2012.05.020

Chapman, E., Maksim, N., De La Cruz, F., and La Clair, J. J. (2015). Inhibitors of the AAA+ chaperone p97. *Molecules* 20, 3027–3049. doi: 10.3390/molecules20023027

Chen, S., De Vries, M. A., and Bell, S. P. (2007). Orc6 is required for dynamic recruitment of Cdt1 during repeated Mcm2-7 loading. *Genes Dev.* 21, 2897–2907. doi: 10.1101/gad.1596807

Chien, C. Y., and Chen, R. H. (2013). Cdc48 chaperone and adaptor Ubx4 distribute the proteasome in the nucleus for anaphase proteolysis. *J. Biol. Chem.* 288, 37180–37191. doi: 10.1074/jbc.M113.513598

Ciccia, A., and Elledge, S. J. (2010). The DNA damage response: making it safe to play with knives. *Mol. Cell* 40, 179–204. doi: 10.1016/j.molcel.2010.09.019

Coleman, K. E., Grant, G. D., Haggerty, R. A., Brantley, K., Shibata, E., Workman, B. D., et al. (2015). Sequential replication-coupled destruction at G1/S ensures genome stability. *Genes Dev.* 29, 1734–1746. doi: 10.1101/gad.263731.115

Cremona, C. A., Sarangi, P., Yang, Y., Hang, L. E., Rahman, S., and Zhao, X. (2012). Extensive DNA damage-induced sumoylation contributes to replication and repair and acts in addition to the mec1 checkpoint. *Mol. Cell* 45, 422–432. doi: 10.1016/j.molcel.2011.11.028

Cutter, A. R., and Hayes, J. J. (2015). A brief review of nucleosome structure. *FEBS Lett.* 589, 2914–2922. doi: 10.1016/j.febslet.2015.05.016

Dai, R. M., and Li, C. C. (2001). Valosin-containing protein is a multi-ubiquitin chain-targeting factor required in ubiquitin-proteasome degradation. *Nat. Cell Biol.* 3, 740–744. doi: 10.1038/35087056

Dammer, E. B., Na, C. H., Xu, P., Seyfried, N. T., Duong, D. M., Cheng, D., et al. (2011). Polyubiquitin linkage profiles in three models of proteolytic stress suggest the etiology of Alzheimer disease. *J. Biol. Chem.* 286, 10457–10465. doi: 10.1074/jbc.M110.149633

Dantuma, N. P., Acs, K., and Luijsterburg, M. S. (2014). Should I stay or should I go: VCP/p97-mediated chromatin extraction in the DNA damage response. *Exp. Cell Res.* 329, 9–17. doi: 10.1016/j.yexcr.2014.08.025

Dantuma, N. P., and van Attikum, H. (2016). Spatiotemporal regulation of posttranslational modifications in the DNA damage response. *EMBO J.* 35, 6–23. doi: 10.15252/embj.201592595

Davidson, I. F., Li, A., and Blow, J. J. (2006). Deregulated replication licensing causes DNA fragmentation consistent with head-to-tail fork collision. *Mol. Cell.* 24, 433–443. doi: 10.1016/j.molcel.2006.09.010

Davis, A. J., Chen, B. P., and Chen, D. J. (2014). DNA-PK: a dynamic enzyme in a versatile DSB repair pathway. *DNA Rep. (Amst)* 17, 21–29. doi: 10.1016/j.dnarep.2014.02.020

Davis, E. J., Lachaud, C., Appleton, P., Macartney, T. J., Nathke, I., and Rouse, J. (2012). DVC1 (C1orf124) recruits the p97 protein segregase to sites of DNA damage. *Nat. Struct. Mol. Biol.* 19, 1093–1100. doi: 10.1038/nsmb.2394

Decottignies, A., Evain, A., and Ghislain, M. (2004). Binding of Cdc48p to a ubiquitin-related UBX domain from novel yeast proteins involved in intracellular proteolysis and sporulation. *Yeast* 21, 127–139. doi: 10.1002/yea.1071

den Besten, W., Verma, R., Kleiger, G., Oania, R. S., and Deshaies, R. J. (2012). NEDD8 links cullin-RING ubiquitin ligase function to the p97 pathway. *Nat. Struct. Mol. Biol.* 19, S511. doi: 10.1038/nsmb.2269

Deshaies, R. J. (2014). Proteotoxic crisis, the ubiquitin-proteasome system, and cancer therapy. *BMC Biol.* 12:94. doi: 10.1186/s12915-014-0094-0

Dewar, J. M., Budzowska, M., and Walter, J. C. (2015). The mechanism of DNA replication termination in vertebrates. *Nature* 525, 345–350. doi: 10.1038/nature14887

Dobrynin, G., Popp, O., Romer, T., Bremer, S., Schmitz, M. H., Gerlich, D. W., et al. (2011). Cdc48/p97-Ufd1-Npl4 antagonizes Aurora B during chromosome segregation in HeLa cells. *J. Cell Sci.* 124, 1571–1580. doi: 10.1242/jcs.069500

Dungrawala, H., Rose, K. L., Bhat, K. P., Mohni, K. N., Glick, G. G., Couch, F. B., et al. (2015). The Replication checkpoint prevents two types of fork collapse without regulating replisome stability. *Mol. Cell* 59, 998–1010. doi: 10.1016/j.molcel.2015.07.030

Duxin, J. P., Dewar, J. M., Yardimci, H., and Walter, J. C. (2014). Repair of a DNA-protein crosslink by replication-coupled proteolysis. *Cell* 159, 346–357. doi: 10.1016/j.cell.2014.09.024

Duxin, J. P., and Walter, J. C. (2015). What is the DNA repair defect underlying Fanconi anemia? *Curr. Opin. Cell. Biol.* 37, 49–60. doi: 10.1016/j.ceb.2015.09.002

Duzdevich, D., Warner, M. D., Ticau, S., Ivica, N. A., Bell, S. P., and Greene, E. C. (2015). The dynamics of eukaryotic replication initiation: origin specificity, licensing, and firing at the single-molecule level. *Mol. Cell* 58, 483–494. doi: 10.1016/j.molcel.2015.03.017

Epshtein, V., Kamarthapu, V., Mcgary, K., Svetlov, V., Ueberheide, B., Proshkin, S., et al. (2014). UvrD facilitates DNA repair by pulling RNA polymerase backwards. *Nature* 505, 372–377. doi: 10.1038/nature12928

Fang, L., Hemion, C., Bento, A. C., Bippes, C. C., Flammer, J., and Neutzner, A. (2015). Mitochondrial function in neuronal cells depends on p97/VCP/Cdc48-mediated quality control. *Front. Cell. Neurosci.* 9:16. doi: 10.3389/fncel.2015.00016

Feng, H., and Kipreos, E. T. (2003). Preventing DNA re-replication–divergent safeguards in yeast and metazoa. *Cell Cycle* 2, 431–434. doi: 10.4161/cc.2.5.527

Fessart, D., Marza, E., Taouji, S., Delom, F., and Chevet, E. (2013). P97/CDC-48: proteostasis control in tumor cell biology. *Cancer Lett.* 337, 26–34. doi: 10.1016/j.canlet.2013.05.030

Forget, A. L., and Kowalczykowski, S. C. (2012). Single-molecule imaging of DNA pairing by RecA reveals a three-dimensional homology search. *Nature* 482, 423–427. doi: 10.1038/nature10782

Fousteri, M., Vermeulen, W., Van Zeeland, A. A., and Mullenders, L. H. (2006). Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. *Mol. Cell* 23, 471–482. doi: 10.1016/j.molcel.2006.06.029

Fragkos, M., Ganier, O., Coulombe, P., and Mechali, M. (2015). DNA replication origin activation in space and time. *Nat. Rev. Mol. Cell Biol.* 16, 360–374. doi: 10.1038/nrm4002

Franz, A., Ackermann, L., and Hoppe, T. (2014). Create and preserve: proteostasis in development and aging is governed by Cdc48/p97/VCP. *Biochim. Biophys. Acta* 1843, 205–215. doi: 10.1016/j.bbamcr.2013.03.031

Franz, A., Orth, M., Pirson, P. A., Sonneville, R., Blow, J. J., Gartner, A., et al. (2011). CDC-48/p97 coordinates CDT-1 degradation with GINS chromatin dissociation to ensure faithful DNA replication. *Mol. Cell* 44, 85–96. doi: 10.1016/j.molcel.2011.08.028

Franz, A., Pirson, P. A., Pilger, D., Halder, S., Achuthankutty, D., Kashkar, H., et al. (2016). Chromatin-associated degradation is defined by UBXN-3/FAF1 to safeguard DNA replication fork progression. *Nat. Commun.* 7:10612. doi: 10.1038/ncomms10612

Frigola, J., Remus, D., Mehanna, A., and Diffley, J. F. (2013). ATPase-dependent quality control of DNA replication origin licensing. *Nature* 495, 339–343. doi: 10.1038/nature11920

Gambus, A., Jones, R. C., Sanchez-Diaz, A., Kanemaki, M., Van Deursen, F., Edmondson, R. D., et al. (2006). GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nat. Cell Biol.* 8, 358–366. doi: 10.1038/ncb1382

Ghislain, M., Dohmen, R. J., Levy, F., and Varshavsky, A. (1996). Cdc48p interacts with Ufd3p, a WD repeat protein required for ubiquitin-mediated proteolysis in *Saccharomyces cerevisiae*. *EMBO J.* 15, 4884–4899.

Ghosal, G., Leung, J. W., Nair, B. C., Fong, K. W., and Chen, J. (2012). Proliferating cell nuclear antigen (PCNA)-binding protein C1orf124 is a regulator of translesion synthesis. *J. Biol. Chem.* 287, 34225–34233. doi: 10.1074/jbc.M112.400135

Gibbs-Seymour, I., Oka, Y., Rajendra, E., Weinert, B. T., Passmore, L. A., Patel, K. J., et al. (2015). Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. *Mol. Cell* 57, 150–164. doi: 10.1016/j.molcel.2014.12.001

Godderz, D., Heinen, C., Marchese, F. P., Kurz, T., Acs, K., and Dantuma, N. P. (2015). Cdc48-independent proteasomal degradation coincides with a reduced need for ubiquitylation. *Sci. Rep.* 5:7615. doi: 10.1038/srep07615

Hardeland, U., Steinacher, R., Jiricny, J., and Schar, P. (2002). Modification of the human thymine-DNA glycosylase by ubiquitin-like proteins facilitates enzymatic turnover. *EMBO J.* 21, 1456–1464. doi: 10.1093/emboj/21.6.1456

Hartmann-Petersen, R., Wallace, M., Hofmann, K., Koch, G., Johnsen, A. H., Hendil, K. B., et al. (2004). The Ubx2 and Ubx3 cofactors direct Cdc48 activity to proteolytic and nonproteolytic ubiquitin-dependent processes. *Curr. Biol.* 14, 824–828. doi: 10.1016/j.cub.2004.04.029

Havens, C. G., and Walter, J. C. (2009). Docking of a specialized PIP Box onto chromatin-bound PCNA creates a degron for the ubiquitin ligase CRL4Cdt2. *Mol. Cell* 35, 93–104. doi: 10.1016/j.molcel.2009.05.012

Haynes, B., Saadat, N., Myung, B., and Shekhar, M. P. (2015). Crosstalk between translesion synthesis, Fanconi anemia network, and homologous recombination repair pathways in interstrand DNA crosslink repair and development of chemoresistance. *Mutat. Res. Rev. Mutat. Res.* 763, 258–266. doi: 10.1016/j.mrrev.2014.11.005

He, J., Zhu, Q., Wani, G., Sharma, N., Han, C., Qian, J., et al. (2014). Ubiquitin-specific protease 7 regulates nucleotide excision repair through deubiquitinating XPC protein and preventing XPC protein from undergoing ultraviolet light-induced and VCP/p97 protein-regulated proteolysis. *J. Biol. Chem.* 289, 27278–27289. doi: 10.1074/jbc.M114.589812

He, J., Zhu, Q., Wani, G., Sharma, N., and Wani, A. A. (2016). VCP/p97 segregase mediates proteolytic processing of CSB in damaged chromatin. *J. Biol. Chem.* 291, 7396–7408. doi: 10.1074/jbc.M115.705350

Hemion, C., Flammer, J., and Neutzner, A. (2014). Quality control of oxidatively damaged mitochondrial proteins is mediated by p97 and the proteasome. *Free Radic. Biol. Med.* 75, 121–128. doi: 10.1016/j.freeradbiomed.2014.07.016

Heo, J. M., Livnat-Levanon, N., Taylor, E. B., Jones, K. T., Dephoure, N., Ring, J., et al. (2010). A stress-responsive system for mitochondrial protein degradation. *Mol. Cell* 40, 465–480. doi: 10.1016/j.molcel.2010.10.021

Heride, C., Urbe, S., and Clague, M. J. (2014). Ubiquitin code assembly and disassembly. *Curr. Biol.* 24, R215–R220. doi: 10.1016/j.cub.2014.02.002

Hoeijmakers, J. H. (2001). Genome maintenance mechanisms for preventing cancer. *Nature* 411, 366–374. doi: 10.1038/35077232

Hoeijmakers, J. H. (2009). DNA damage, aging, and cancer. *N. Engl. J. Med.* 361, 1475–1485. doi: 10.1056/NEJMra0804615

Holthausen, J. T., Wyman, C., and Kanaar, R. (2010). Regulation of DNA strand exchange in homologous recombination. *DNA Repair (Amst)* 9, 1264–1272. doi: 10.1016/j.dnarep.2010.09.014

Hoogstraten, D., Bergink, S., Ng, J. M., Verbiest, V. H., Luijsterburg, M. S., Geverts, B., et al. (2008). Versatile DNA damage detection by the global genome nucleotide excision repair protein XPC. *J. Cell Sci.* 121, 2850–2859. doi: 10.1242/jcs.031708

Hoppe, T. (2005). Multiubiquitylation by E4 enzymes: 'one size' doesn't fit all. *Trends Biochem. Sci.* 30, 183–187. doi: 10.1016/j.tibs.2005.02.004

Hu, J., Mccall, C. M., Ohta, T., and Xiong, Y. (2004). Targeted ubiquitination of CDT1 by the DDB1-CUL4A-ROC1 ligase in response to DNA damage. *Nat. Cell Biol.* 6, 1003–1009. doi: 10.1038/ncb1172

Ilves, I., Petojevic, T., Pesavento, J. J., and Botchan, M. R. (2010). Activation of the MCM2-7 helicase by association with Cdc45 and GINS proteins. *Mol. Cell* 37, 247–258. doi: 10.1016/j.molcel.2009.12.030

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078. doi: 10.1038/nature08467

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jagannathan, M., Nguyen, T., Gallo, D., Luthra, N., Brown, G. W., Saridakis, V., et al. (2014). A role for USP7 in DNA replication. *Mol. Cell. Biol.* 34, 132–145. doi: 10.1128/MCB.00639-13

Jarosch, E., Taxis, C., Volkwein, C., Bordallo, J., Finley, D., Wolf, D. H., et al. (2002). Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. *Nat. Cell Biol.* 4, 134–139. doi: 10.1038/ncb746

Jentsch, S., and Rumpf, S. (2007). Cdc48 (p97): a "molecular gearbox" in the ubiquitin pathway? *Trends Biochem. Sci.* 32, 6–11. doi: 10.1016/j.tibs.2006.11.005

Jiang, N., Shen, Y., Fei, X., Sheng, K., Sun, P., Qiu, Y., et al. (2013). Valosin-containing protein regulates the proteasome-mediated degradation of DNA-PKcs in glioma cells. *Cell Death Dis* 4, e647. doi: 10.1038/cddis.2013.171

Jin, J., Arias, E. E., Chen, J., Harper, J. W., and Walter, J. C. (2006). A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. *Mol. Cell* 23, 709–721. doi: 10.1016/j.molcel.2006.08.010

Johnson, J. O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., et al. (2010). Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* 68, 857–864. doi: 10.1016/j.neuron.2010.11.036

Ju, J. S., Fuentealba, R. A., Miller, S. E., Jackson, E., Piwnica-Worms, D., Baloh, R. H., et al. (2009). Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *J. Cell Biol.* 187, 875–888. doi: 10.1083/jcb.200908115

Juhasz, S., Balogh, D., Hajdu, I., Burkovics, P., Villamil, M. A., Zhuang, Z., et al. (2012). Characterization of human Spartan/C1orf124, an ubiquitin-PCNA interacting regulator of DNA damage tolerance. *Nucleic Acids Res.* 40, 10795–10808. doi: 10.1093/nar/gks850

Kim, M. S., Machida, Y., Vashisht, A. A., Wohlschlegel, J. A., Pang, Y. P., and Machida, Y. J. (2013). Regulation of error-prone translesion synthesis by Spartan/C1orf124. *Nucleic Acids Res.* 41, 1661–1668. doi: 10.1093/nar/gks1267

Kim, Y., and Kipreos, E. T. (2007). Cdt1 degradation to prevent DNA re-replication: conserved and non-conserved pathways. *Cell Div* 2, 18. doi: 10.1186/1747-1028-2-18

Klein, H. L. (2008). The consequences of Rad51 overexpression for normal and tumor cells. *DNA Rep. (Amst.)* 7, 686–693. doi: 10.1016/j.dnarep.2007.12.008

Kloppsteck, P., Ewens, C. A., Forster, A., Zhang, X., and Freemont, P. S. (2012). Regulation of p97 in the ubiquitin-proteasome system by the UBX protein-family. *Biochim. Biophys. Acta* 1823, 125–129. doi: 10.1016/j.bbamcr.2011.09.006

Koegl, M., Hoppe, T., Schlenker, S., Ulrich, H. D., Mayer, T. U., and Jentsch, S. (1999). A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* 96, 635–644. doi: 10.1016/S0092-8674(00)80574-7

Køhler, J. B., Jørgensen, M. L., Beinoraite, G., Thorsen, M., and Thon, G. (2013). Concerted action of the ubiquitin-fusion degradation protein 1 (Ufd1) and Sumo-targeted ubiquitin ligases (STUbLs) in the DNA-damage response. *PLoS ONE* 8:e80442. doi: 10.1371/journal.pone.0080442

Køhler, J. B., Tammsalu, T., Jørgensen, M. M., Steen, N., Hay, R. T., and Thon, G. (2015). Targeting of SUMO substrates to a Cdc48-Ufd1-Npl4 segregase and STUbL pathway in fission yeast. *Nat. Commun.* 6:8827. doi: 10.1038/ncomms9827

Kowalczykowski, S. C. (2015). An Overview of the Molecular Mechanisms of Recombinational DNA Repair. *Cold Spring Harb. Perspect. Biol.* 7, 1–37. doi: 10.1101/cshperspect.a016410

Krick, R., Bremer, S., Welter, E., Schlotterhose, P., Muehe, Y., Eskelinen, E. L., et al. (2010). Cdc48/p97 and Shp1/p47 regulate autophagosome biogenesis in concert with ubiquitin-like Atg8. *J. Cell Biol.* 190, 965–973. doi: 10.1083/jcb.201002075

Kubota, Y., Takase, Y., Komori, Y., Hashimoto, Y., Arata, T., Kamimura, Y., et al. (2003). A novel ring-like complex of *Xenopus proteins* essential for the initiation of DNA replication. *Genes Dev.* 17, 1141–1152. doi: 10.1101/gad.1070003

Kuhlbrodt, K., Janiesch, P. C., Kevei, E., Segref, A., Barikbin, R., and Hoppe, T. (2011). The Machado-Joseph disease deubiquitylase ATX-3 couples longevity and proteostasis. *Nat. Cell Biol.* 13, 273–281. doi: 10.1038/ncb2200

Lessel, D., Vaz, B., Halder, S., Lockhart, P. J., Marinovic-Terzic, I., Lopez-Mosqueda, J., et al. (2014). Mutations in SPRTN cause early onset hepatocellular carcinoma, genomic instability and progeroid features. *Nat. Genet.* 46, 1239–1244. doi: 10.1038/ng.3103

Lieber, M. R. (2010). The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* 79, 181–211. doi: 10.1146/annurev.biochem.052308.093131

Lin, K. W., Mcdonald, K. R., Guise, A. J., Chan, A., Cristea, I. M., and Zakian, V. A. (2015). Proteomics of yeast telomerase identified Cdc48-Npl4-Ufd1 and Ufd4 as regulators of Est1 and telomere length. *Nat. Commun.* 6:8290. doi: 10.1038/ncomms9290

Livingstone, M., Ruan, H., Weiner, J., Clauser, K. R., Strack, P., Jin, S., et al. (2005). Valosin-containing protein phosphorylation at Ser784 in response to DNA damage. *Cancer Res.* 65, 7533–7540.

Long, D. T., Joukov, V., Budzowska, M., and Walter, J. C. (2014). BRCA1 promotes unloading of the CMG helicase from a stalled DNA replication fork. *Mol. Cell.* 56, 174–185. doi: 10.1016/j.molcel.2014.08.012

Lydeard, J. R., Lipkin-Moore, Z., Sheu, Y. J., Stillman, B., Burgers, P. M., and Haber, J. E. (2010). Break-induced replication requires all essential DNA replication factors except those specific for pre-RC assembly. *Genes Dev.* 24, 1133–1144. doi: 10.1101/gad.1922610

Maculins, T., Nkosi, P. J., Nishikawa, H., and Labib, K. (2015). Tethering of SCF(Dia2) to the replisome promotes efficient ubiquitylation and disassembly of the CMG helicase. *Curr. Biol.* 25, 2254–2259. doi: 10.1016/j.cub.2015.07.012

Maneix, L., and Catic, A. (2016). Touch and Go -Nuclear proteolysis in the regulation of metabolic genes and cancer. *FEBS Lett.* 20, 5546–5553. doi: 10.1002/1873-3468.12087

Maric, M., Maculins, T., De Piccoli, G., and Labib, K. (2014). Cdc48 and a ubiquitin ligase drive disassembly of the CMG helicase at the end of DNA replication. *Science* 346, 1253596. doi: 10.1126/science.1253596

Marteijn, J. A., Lans, H., Vermeulen, W., and Hoeijmakers, J. H. (2014). Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat. Rev. Mol. Cell Biol.* 15, 465–481. doi: 10.1038/nrm3822

Masai, H., Matsumoto, S., You, Z., Yoshizawa-Sugata, N., and Oda, M. (2010). Eukaryotic chromosome DNA replication: where, when, and how? *Annu. Rev. Biochem.* 79, 89–130. doi: 10.1146/annurev.biochem.052308.103205

Maskey, R. S., Kim, M. S., Baker, D. J., Childs, B., Malureanu, L. A., Jeganathan, K. B., et al. (2014). Spartan deficiency causes genomic instability and progeroid phenotypes. *Nat. Commun.* 5:5744. doi: 10.1038/ncomms6744

Medicherla, B., Kostova, Z., Schaefer, A., and Wolf, D. H. (2004). A genomic screen identifies Dsk2p and Rad23p as essential components of ER-associated degradation. *EMBO Rep.* 5, 692–697. doi: 10.1038/sj.embor.7400164

Meerang, M., Ritz, D., Paliwal, S., Garajova, Z., Bosshard, M., Mailand, N., et al. (2011). The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks. *Nat. Cell Biol.* 13, 1376–1382. doi: 10.1038/ncb2367

Mehta, A., and Haber, J. E. (2014). Sources of DNA double-strand breaks and models of recombinational DNA repair. *Cold Spring Harb. Perspect. Biol.* 6:a016428. doi: 10.1101/cshperspect.a016428

Merai, Z., Chumak, N., Garcia-Aguilar, M., Hsieh, T. F., Nishimura, T., Schoft, V. K., et al. (2014). The AAA-ATPase molecular chaperone Cdc48/p97 disassembles sumoylated centromeres, decondenses heterochromatin, and activates ribosomal RNA genes. *Proc. Natl. Acad. Sci. U.S.A.* 111, 16166–16171. doi: 10.1073/pnas.1418564111

Meyer, H., and Weihl, C. C. (2014). The VCP/p97 system at a glance: connecting cellular function to disease pathogenesis. *J. Cell Sci.* 127, 3877–3883. doi: 10.1242/jcs.093831

Meyer, H. H., Shorter, J. G., Seemann, J., Pappin, D., and Warren, G. (2000). A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *EMBO J.* 19, 2181–2192. doi: 10.1093/emboj/19.10.2181

Min, J., Allali-Hassani, A., Nady, N., Qi, C., Ouyang, H., Liu, Y., et al. (2007). L3MBTL1 recognition of mono- and dimethylated histones. *Nat. Struct. Mol. Biol.* 14, 1229–1230. doi: 10.1038/nsmb1340

Misteli, T. (2007). Beyond the sequence: cellular organization of genome function. *Cell* 128, 787–800. doi: 10.1016/j.cell.2007.01.028

Moreno, S. P., Bailey, R., Campion, N., Herron, S., and Gambus, A. (2014). Polyubiquitylation drives replisome disassembly at the termination of DNA replication. *Science* 346, 477–481. doi: 10.1126/science.1253585

Mosbech, A., Gibbs-Seymour, I., Kagias, K., Thorslund, T., Beli, P., Povlsen, L., et al. (2012). DVC1 (C1orf124) is a DNA damage-targeting p97 adaptor that promotes ubiquitin-dependent responses to replication blocks. *Nat. Struct. Mol. Biol.* 19, 1084–1092. doi: 10.1038/nsmb.2395

Mouysset, J., Deichsel, A., Moser, S., Hoege, C., Hyman, A. A., Gartner, A., et al. (2008). Cell cycle progression requires the CDC-48UFD-1/NPL-4 complex for efficient DNA replication. *Proc. Natl. Acad. Sci. U.S.A.* 105, 12879–12884. doi: 10.1073/pnas.0805944105

Moyer, S. E., Lewis, P. W., and Botchan, M. R. (2006). Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10236–10241. doi: 10.1073/pnas.0602400103

Mullen, J. R., Chen, C. F., and Brill, S. J. (2010). Wss1 is a SUMO-dependent isopeptidase that interacts genetically with the Slx5-Slx8 SUMO-targeted ubiquitin ligase. *Mol. Cell. Biol.* 30, 3737–3748. doi: 10.1128/MCB.01649-09

Mullenders, L. (2015). DNA damage mediated transcription arrest: step back to go forward. *DNA Repair (Amst)* 36, 28–35. doi: 10.1016/j.dnarep.2015.09.005

Nalbandian, A., Donkervoort, S., Dec, E., Badadani, M., Katheria, V., Rana, P., et al. (2011). The multiple faces of valosin-containing protein-associated diseases: inclusion body myopathy with Paget's disease of bone, frontotemporal dementia, and amyotrophic lateral sclerosis. *J. Mol. Neurosci.* 45, 522–531. doi: 10.1007/s12031-011-9627-y

Ndoja, A., Cohen, R. E., and Yao, T. (2014). Ubiquitin signals proteolysis-independent stripping of transcription factors. *Mol. Cell* 53, 893–903. doi: 10.1016/j.molcel.2014.02.002

Neuber, O., Jarosch, E., Volkwein, C., Walter, J., and Sommer, T. (2005). Ubx2 links the Cdc48 complex to ER-associated protein degradation. *Nat. Cell Biol.* 7, 993–998. doi: 10.1038/ncb1298

Nie, M., Aslanian, A., Prudden, J., Heideker, J., Vashisht, A. A., Wohlschlegel, J. A., et al. (2012). Dual recruitment of Cdc48 (p97)-Ufd1-Npl4 ubiquitin-selective segregase by small ubiquitin-like modifier protein (SUMO) and ubiquitin in SUMO-targeted ubiquitin ligase-mediated genome stability functions. *J. Biol. Chem.* 287, 29610–29619. doi: 10.1074/jbc.M112.379768

Olzmann, J. A., Richter, C. M., and Kopito, R. R. (2013). Spatial regulation of UBXD8 and p97/VCP controls ATGL-mediated lipid droplet turnover. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1345–1350. doi: 10.1073/pnas.1213738110

Ossareh-Nazari, B., Bonizec, M., Cohen, M., Dokudovskaya, S., Delalande, F., Schaeffer, C., et al. (2010). Cdc48 and Ufd3, new partners of the ubiquitin protease Ubp3, are required for ribophagy. *EMBO Rep.* 11, 548–554. doi: 10.1038/embor.2010.74

Park, S., Isaacson, R., Kim, H. T., Silver, P. A., and Wagner, G. (2005). Ufd1 exhibits the AAA-ATPase fold with two distinct ubiquitin interaction sites. *Structure* 13, 995–1005. doi: 10.1016/j.str.2005.04.013

Partridge, J. J., Lopreiato, J. O. Jr., Latterich, M., and Indig, F. E. (2003). DNA damage modulates nucleolar interaction of the Werner protein with the AAA ATPase p97/VCP. *Mol. Biol. Cell* 14, 4221–4229. doi: 10.1091/mbc.E03-02-0111

Polo, S. E., and Jackson, S. P. (2011). Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes Dev.* 25, 409–433. doi: 10.1101/gad.2021311

Psakhye, I., and Jentsch, S. (2012). Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* 151, 807–820. doi: 10.1016/j.cell.2012.10.021

Puumalainen, M. R., Lessel, D., Ruthemann, P., Kaczmarek, N., Bachmann, K., Ramadan, K., et al. (2014). Chromatin retention of DNA damage sensors DDB2 and XPC through loss of p97 segregase causes genotoxicity. *Nat. Commun.* 5:3695. doi: 10.1038/ncomms4695

Rabinovich, E., Kerem, A., Frohlich, K. U., Diamant, N., and Bar-Nun, S. (2002). AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol. Cell. Biol.* 22, 626–634. doi: 10.1128/MCB.22.2.626-634.2002

Ramadan, K., Bruderer, R., Spiga, F. M., Popp, O., Baur, T., Gotta, M., et al. (2007). Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. *Nature* 450, 1258–1262. doi: 10.1038/nature06388

Raman, M., Havens, C. G., Walter, J. C., and Harper, J. W. (2011). A genome-wide screen identifies p97 as an essential regulator of DNA damage-dependent CDT1 destruction. *Mol. Cell* 44, 72–84. doi: 10.1016/j.molcel.2011.06.036

Raman, M., Sergeev, M., Garnaas, M., Lydeard, J. R., Huttlin, E. L., Goessling, W., et al. (2015). Systematic proteomics of the VCP-UBXD adaptor network identifies a role for UBXN10 in regulating ciliogenesis. *Nat. Cell Biol.* 17, 1356–1369. doi: 10.1038/ncb3238

Rape, M., Hoppe, T., Gorr, I., Kalocay, M., Richly, H., and Jentsch, S. (2001). Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone. *Cell* 107, 667–677. doi: 10.1016/S0092-8674(01)00595-5

Raschle, M., Smeenk, G., Hansen, R. K., Temu, T., Oka, Y., Hein, M. Y., et al. (2015). DNA repair. Proteomics reveals dynamic assembly of repair complexes during bypass of DNA cross-links. *Science* 348, 1253671. doi: 10.1126/science.1253671

Ren, J., Pashkova, N., Winistorfer, S., and Piper, R. C. (2008). DOA1/UFD3 plays a role in sorting ubiquitinated membrane proteins into multivesicular bodies. *J. Biol. Chem.* 283, 21599–21611. doi: 10.1074/jbc.M802982200

Richardson, C., Stark, J. M., Ommundsen, M., and Jasin, M. (2004). Rad51 overexpression promotes alternative double-strand break repair pathways and genome instability. *Oncogene* 23, 546–553. doi: 10.1038/sj.onc.1207098

Richly, H., Rape, M., Braun, S., Rumpf, S., Hoege, C., and Jentsch, S. (2005). A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting. *Cell* 120, 73–84. doi: 10.1016/j.cell.2004.11.013

Ritz, D., Vuk, M., Kirchner, P., Bug, M., Schutz, S., Hayer, A., et al. (2011). Endolysosomal sorting of ubiquitylated caveolin-1 is regulated by VCP and UBXD1 and impaired by VCP disease mutations. *Nat. Cell Biol.* 13, 1116–1123. doi: 10.1038/ncb2301

Rouiller, I., Delabarre, B., May, A. P., Weis, W. I., Brunger, A. T., Milligan, R. A., et al. (2002). Conformational changes of the multifunction p97 AAA ATPase during its ATPase cycle. *Nat. Struct. Biol.* 9, 950–957. doi: 10.1038/nsb872

Rumpf, S., and Jentsch, S. (2006). Functional division of substrate processing cofactors of the ubiquitin-selective Cdc48 chaperone. *Mol. Cell.* 21, 261–269. doi: 10.1016/j.molcel.2005.12.014

San Filippo, J., Sung, P., and Klein, H. (2008). Mechanism of eukaryotic homologous recombination. *Annu. Rev. Biochem.* 77, 229–257. doi: 10.1146/annurev.biochem.77.061306.125255

Sasagawa, Y., Higashitani, A., Urano, T., Ogura, T., and Yamanaka, K. (2012). CDC-48/p97 is required for proper meiotic chromosome segregation via controlling AIR-2/Aurora B kinase localization in *Caenorhabditis elegans*. *J. Struct. Biol.* 179, 104–111. doi: 10.1016/j.jsb.2012.06.009

Schuberth, C., and Buchberger, A. (2005). Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. *Nat. Cell Biol.* 7, 999–1006. doi: 10.1038/ncb1299

Schuberth, C., Richly, H., Rumpf, S., and Buchberger, A. (2004). Shp1 and Ubx2 are adaptors of Cdc48 involved in ubiquitin-dependent protein degradation. *EMBO Rep.* 5, 818–824. doi: 10.1038/sj.embor.7400203

Senga, T., Sivaprasad, U., Zhu, W., Park, J. H., Arias, E. E., Walter, J. C., et al. (2006). PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination. *J. Biol. Chem.* 281, 6246–6252. doi: 10.1074/jbc.M512705200

Shcherbik, N., and Haines, D. S. (2007). Cdc48p(Npl4p/Ufd1p) binds and segregates membrane-anchored/tethered complexes via a polyubiquitin signal present on the anchors. *Mol. Cell* 25, 385–397. doi: 10.1016/j.molcel.2007.01.024

Sirbu, B. M., Mcdonald, W. H., Dungrawala, H., Badu-Nkansah, A., Kavanaugh, G. M., Chen, Y., et al. (2013). Identification of proteins at active, stalled, and collapsed replication forks using isolation of proteins on nascent DNA (iPOND) coupled with mass spectrometry. *J. Biol. Chem.* 288, 31458–31467. doi: 10.1074/jbc.M113.511337

Song, C., Wang, Q., Song, C., and Rogers, T. J. (2015). Valosin-containing protein (VCP/p97) is capable of unfolding polyubiquitinated proteins through its ATPase domains. *Biochem. Biophys. Res. Commun.* 463, 453–457. doi: 10.1016/j.bbrc.2015.05.111

Song, E. J., Yim, S. H., Kim, E., Kim, N. S., and Lee, K. J. (2005). Human Fas-associated factor 1, interacting with ubiquitinated proteins and valosin-containing protein, is involved in the ubiquitin-proteasome pathway. *Mol. Cell. Biol.* 25, 2511–2524. doi: 10.1128/MCB.25.6.2511-2524.2005

Sonoda, E., Sasaki, M. S., Buerstedde, J. M., Bezzubova, O., Shinohara, A., Ogawa, H., et al. (1998). Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *EMBO J.* 17, 598–608. doi: 10.1093/emboj/17.2.598

Sowa, M. E., Bennett, E. J., Gygi, S. P., and Harper, J. W. (2009). Defining the human deubiquitinating enzyme interaction landscape. *Cell* 138, 389–403. doi: 10.1016/j.cell.2009.04.042

Spivak, G., and Ganesan, A. K. (2014). The complex choreography of transcription-coupled repair. *DNA Repair (Amst)* 19, 64–70. doi: 10.1016/j.dnarep.2014.03.025

Stingele, J., Habermann, B., and Jentsch, S. (2015). DNA-protein crosslink repair: proteases as DNA repair enzymes. *Trends Biochem. Sci.* 40, 67–71. doi: 10.1016/j.tibs.2014.10.012

Stingele, J., and Jentsch, S. (2015). DNA-protein crosslink repair. *Nat. Rev. Mol. Cell Biol.* 16, 455–460. doi: 10.1038/nrm4015

Stingele, J., Schwarz, M. S., Bloemeke, N., Wolf, P. G., and Jentsch, S. (2014). A DNA-dependent protease involved in DNA-protein crosslink repair. *Cell* 158, 327–338. doi: 10.1016/j.cell.2014.04.053

Stolz, A., Hilt, W., Buchberger, A., and Wolf, D. H. (2011). Cdc48: a power machine in protein degradation. *Trends Biochem. Sci.* 36, 515–523. doi: 10.1016/j.tibs.2011.06.001

Sugimoto, N., Yoshida, K., Tatsumi, Y., Yugawa, T., Narisawa-Saito, M., Waga, S., et al. (2009). Redundant and differential regulation of multiple licensing factors ensures prevention of re-replication in normal human cells. *J. Cell Sci.* 122, 1184–1191. doi: 10.1242/jcs.041889

Tanaka, S., and Diffley, J. F. (2002). Interdependent nuclear accumulation of budding yeast Cdt1 and Mcm2-7 during G1 phase. *Nat. Cell Biol.* 4, 198–207. doi: 10.1038/ncb757

Tatsumi, Y., Sugimoto, N., Yugawa, T., Narisawa-Saito, M., Kiyono, T., and Fujita, M. (2006). Deregulation of Cdt1 induces chromosomal damage without rereplication and leads to chromosomal instability. *J. Cell Sci.* 119, 3128–3140. doi: 10.1242/jcs.03031

Tresse, E., Salomons, F. A., Vesa, J., Bott, L. C., Kimonis, V., Yao, T. P., et al. (2010). VCP/p97 is essential for maturation of ubiquitin-containing autophagosomes and this function is impaired by mutations that cause IBMPFD. *Autophagy* 6, 217–227. doi: 10.4161/auto.6.2.11014

Trusch, F., Matena, A., Vuk, M., Koerver, L., Knaevelsrud, H., Freemont, P. S., et al. (2015). The N-terminal region of the ubiquitin regulatory X (UBX) domain-containing protein 1 (UBXD1) modulates interdomain communication within the valosin-containing protein p97. *J. Biol. Chem.* 290, 29414–29427. doi: 10.1074/jbc.M115.680686

Tsuzuki, T., Fujii, Y., Sakumi, K., Tominaga, Y., Nakao, K., Sekiguchi, M., et al. (1996). Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl. Acad. Sci. U.S.A.* 93, 6236–6240. doi: 10.1073/pnas.93.13.6236

Vaz, B., Halder, S., and Ramadan, K. (2013). Role of p97/VCP (Cdc48) in genome stability. *Front. Genet.* 4:60. doi: 10.3389/fgene.2013.00060

Verma, R., Oania, R., Fang, R., Smith, G. T., and Deshaies, R. J. (2011). Cdc48/p97 mediates UV-dependent turnover of RNA Pol II. *Mol. Cell* 41, 82–92. doi: 10.1016/j.molcel.2010.12.017

Verma, R., Oania, R. S., Kolawa, N. J., and Deshaies, R. J. (2013). Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. *Elife* 2:e00308. doi: 10.7554/eLife.00308

Wang, J., Satoh, M., Pierani, A., Schmitt, J., Chou, C. H., Stunnenberg, H. G., et al. (1994). Assembly and DNA binding of recombinant Ku (p70/p80) autoantigen defined by a novel monoclonal antibody specific for p70/p80 heterodimers. *J. Cell Sci.* 107(Pt 11), 3223–3233.

Wang, Y., Satoh, A., Warren, G., and Meyer, H. H. (2004). VCIP135 acts as a deubiquitinating enzyme during p97-p47-mediated reassembly of mitotic Golgi fragments. *J. Cell Biol.* 164, 973–978. doi: 10.1083/jcb.200401010

Wilcox, A. J., and Laney, J. D. (2009). A ubiquitin-selective AAA-ATPase mediates transcriptional switching by remodelling a repressor-promoter DNA complex. *Nat. Cell Biol.* 11, 1481–1486. doi: 10.1038/ncb1997

Wilson, M. D., Harreman, M., and Svejstrup, J. Q. (2013). Ubiquitylation and degradation of elongating RNA polymerase II: the last resort. *Biochim. Biophys. Acta* 1829, 151–157. doi: 10.1016/j.bbagrm.2012.08.002

Wojcik, C., Yano, M., and Demartino, G. N. (2004). RNA interference of valosin-containing protein (VCP/p97) reveals multiple cellular roles linked to ubiquitin/proteasome-dependent proteolysis. *J. Cell Sci.* 117, 281–292. doi: 10.1242/jcs.00841

Xouri, G., Squire, A., Dimaki, M., Geverts, B., Verveer, P. J., Taraviras, S., et al. (2007). Cdt1 associates dynamically with chromatin throughout G1 and recruits Geminin onto chromatin. *EMBO J.* 26, 1303–1314. doi: 10.1038/sj.emboj.7601597

Ye, Y., Meyer, H. H., and Rapoport, T. A. (2001). The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 414, 652–656. doi: 10.1038/414652a

Zhang, X., Gui, L., Zhang, X., Bulfer, S. L., Sanghez, V., Wong, D. E., et al. (2015). Altered cofactor regulation with disease-associated p97/VCP mutations. *Proc. Natl. Acad. Sci. U.S.A.* 112, E1705–E1714. doi: 10.1073/pnas.1418820112

Zhong, W., Feng, H., Santiago, F. E., and Kipreos, E. T. (2003). CUL-4 ubiquitin ligase maintains genome stability by restraining DNA-replication licensing. *Nature* 423, 885–889. doi: 10.1038/nature01747

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 28 April 2016
doi: 10.3389/fgene.2016.00068

Global-genome Nucleotide Excision Repair Controlled by Ubiquitin/Sumo Modifiers

*Peter Rüthemann, Chiara Balbo Pogliano and Hanspeter Naegeli**

Institute of Pharmacology and Toxicology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Global-genome nucleotide excision repair (GG-NER) prevents genome instability by excising a wide range of different DNA base adducts and crosslinks induced by chemical carcinogens, ultraviolet (UV) light or intracellular side products of metabolism. As a versatile damage sensor, xeroderma pigmentosum group C (XPC) protein initiates this generic defense reaction by locating the damage and recruiting the subunits of a large lesion demarcation complex that, in turn, triggers the excision of aberrant DNA by endonucleases. In the very special case of a DNA repair response to UV radiation, the function of this XPC initiator is tightly controlled by the dual action of cullin-type $CRL4^{DDB2}$ and sumo-targeted RNF111 ubiquitin ligases. This twofold protein ubiquitination system promotes GG-NER reactions by spatially and temporally regulating the interaction of XPC protein with damaged DNA across the nucleosome landscape of chromatin. In the absence of either $CRL4^{DDB2}$ or RNF111, the DNA excision repair of UV lesions is inefficient, indicating that these two ubiquitin ligases play a critical role in mitigating the adverse biological effects of UV light in the exposed skin.

Keywords: aging, cyclobutane pyrimidine dimer, DNA repair, genomic instability, photoproducts, sunburns, skin cancer, UV radiation

OPEN ACCESS

Edited by:
Ivan Dikic,
Goethe University, Germany

Reviewed by:
Howard Donninger,
University of Louisville, USA
Salvatore Piscuoglio,
University Hospital Basel – University of Basel, Switzerland

****Correspondence:***
Hanspeter Naegeli
naegelih@vetpharm.uzh.ch

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *28 February 2016*
Accepted: *12 April 2016*
Published: *28 April 2016*

Citation:
Rüthemann P, Balbo Pogliano C and Naegeli H (2016) Global-genome Nucleotide Excision Repair Controlled by Ubiquitin/Sumo Modifiers. Front. Genet. 7:68. doi: 10.3389/fgene.2016.00068

INTRODUCTION

All organisms are constantly under attack by environmental and endogenous DNA-damaging agents that endanger the sequence fidelity of their genomes. Many environmental mutagens cause "bulky" DNA adducts that destabilize the complementary pairing of bases in the native double helix (Straub et al., 1977; Knox et al., 1987). Base pair-destabilizing lesions also result from internal by-products of cellular metabolism including oxygen radicals (Brooks et al., 2000;

Abbreviations: 6-4PP, (6-4) pyrimidine–pyrimidone photoproduct; BHD, β-Hairpin domain; CETN2, centrin 2; CPD, cyclobutane pyrimidine dimer; CUL4A, cullin 4A; DDB, damaged DNA-binding; ERCC1, excision repair cross-complementing 1; GG-NER, global-genome nucleotide excision repair; MPG, methylpurine-DNA glycosylase; NER, nucleotide excision repair; NEDD8, neural precursor cell expressed developmentally down-regulated 8; Npl4, nuclear protein localization 4 homolog; Oct4, octamer binding transcription factor 4; OGG1, 8-Oxo-guanine-DNA glycosylase; OTUD4, OTU deubiquitinase 4; RAD23B, human homolog of RAD23, B; RNF111, RING finger protein 111; RPA, replication protein A; ROC1 regulator of cullins 1; RPS27A, ubiquitin-40S ribosomal protein S27A; SMUG1, single strand-selective monofunctional uracil-DNA glycosylase 1; Sox2, sex determining region Y (SRY)-box 2; Sumo, small ubiquitin-related modifier; TC-NER, transcription-coupled nucleotide excision repair; TDG, thymine-DNA glycosylase; TFIIH, transcription factor IIH; TG, transglutaminase-like; UBA52, ubiquitin A-52; UBB, ubiquitin-B; UBC, ubiquitin-C; USP7, ubiquitin-specific processing protease 7; Ufd1, ubiquitin fusion degradation 1; UV, ultraviolet; VCP, valosin-containing protein; XP, xeroderma pigmentosum.

Kuraoka et al., 2000), but the most common type of bulky DNA lesion arises from the UV spectrum of sunlight or indoor tanning devices, generating covalent crosslinks joining neighboring pyrimidines, i.e., CPDs and pyrimidine-pyrimidone (6-4) photoproducts (6-4PPs; Brash, 1988). If not readily repaired, these pyrimidine crosslinks and other bulky adducts interfere with transcription, DNA replication or cell cycle progression (Lopes et al., 2006; Brueckner et al., 2007), eventually giving rise to mutations and chromosomal aberrations that accelerate aging and culminate in cancer (Marteijn et al., 2014). Unfortunately, the incidence of skin cancer continues to increase and remains a public health concern despite widespread knowledge that excessive exposure to sunlight is the major risk factor for cutaneous neoplasms (Donaldson and Coldiron, 2011; Usher-Smith et al., 2014). This review is focused on recent advances in our knowledge of how polypeptide modifiers regulate the DNA repair response preventing sunlight-induced skin cancer.

Excision of Bulky DNA Lesions

Nucleotide excision repair is a molecular cut-and-patch machine that removes bulky base lesions by incising damaged DNA strands on either side of the injury, thereby eliminating 24- to 32-nucleotide long single-stranded segments (Huang et al., 1992; Moggs et al., 1996). Depending on their location in the genome, bulky lesions are sensed by two alternative mechanisms. The TC-NER pathway is initiated when an RNA polymerase II complex encounters obstructing base lesions (Bohr et al., 1985). Such transcriptional roadblocks trigger a stepwise reaction for the rapid removal of base lesions from transcribed strands (reviewed by Hanawalt and Spivak, 2008; Vermeulen and Fousteri, 2013; Marteijn et al., 2014). On the other hand, GG-NER activity is generally slower but detects bulky lesions anywhere in the genome independently of transcription (reviewed by Scharer, 2013; Puumalainen et al., 2016). Genetic defects in the GG-NER pathway cause XP, which is a severe cancer-prone syndrome presenting with photosensitivity, extreme sunburns and an over 1,000-fold higher risk of contracting sunlight-induced neoplasms of the skin (Hollander et al., 2005; DiGiovanna and Kraemer, 2012). Patients suffering from the XP syndrome are classified into distinct genetic complementation groups (from XP-A to XP-G) reflecting mutations in respective NER genes (Cleaver et al., 2009). A variant form of this disease (XP-V) is caused by mutations in a gene coding for DNA polymerase η that catalyzes with high nucleotide sequence fidelity the replicative bypass of UV lesions in S phase of the cell division cycle (Masutani et al., 1999).

The initial detection of bulky lesions in the GG-NER pathway is carried out by a three-subunit factor consisting of XP group C protein (XPC; Sugasawa et al., 1998; Volker et al., 2001) one of two human RAD23 homologs (predominantly RAD23B; Ng et al., 2003) and (CETN2, (Araki et al., 2001; Nishi et al., 2005; Dantas et al., 2011). The DNA-binding activity of this heterotrimeric complex resides with the XPC subunit itself. RAD23B and CETN2 contribute by supporting the proper folding of XPC protein and by protecting this DNA-binding subunit from degradation (Ng et al., 2003; Xie et al., 2004; Krasikova et al., 2012). Although RAD23B stimulates the recognition of damaged DNA by XPC protein (Sugasawa et al., 1996), it is readily released once XPC associates with DNA lesion sites (Fei et al., 2011; Bergink et al., 2012). Conversely, CETN2 remains associated with target sites (Dantas et al., 2013) where XPC provides a platform for the recruitment of TFIIH. This 10-subunit complex contains an ATPase (XPB) and a DNA helicase (XPD) that separate complementary strands to produce an unwound configuration of about 25 nucleotides around the lesion (Evans et al., 1997; Wakasugi and Sancar, 1998). Stability to the resulting open intermediate or "bubble" is conferred by XPA together with RPA, until the DNA strand containing the damage is incised by structure-specific endonucleases exactly at the double-stranded to single-stranded DNA transitions on each side of the bubble (Evans et al., 1997; Missura et al., 2001; Li et al., 2015). A protein heterodimer composed of XPF and ERCC1 introduces the incision on the 5′ side, followed by incision on the 3′ side by the endonuclease activity of XPG (Staresincic et al., 2009). After this dual incision and consequent release of the excised oligonucleotide carrying the damage, the remaining single-stranded gap is filled by DNA repair synthesis by the action of DNA polymerases η, ε, or κ (Ogi et al., 2010). Ligation by DNA ligase I and DNA ligase IIIα finally restores helix integrity (Araujo et al., 2000; Moser et al., 2007).

Structure and Interactome of the XPC Initiator

The human XPC polypeptide is made of 940 amino acids and harbors domains for binding to DNA (Hey et al., 2002; Yasuda et al., 2005; Trego and Turchi, 2006) and multiple protein partners (**Figure 1**). Its molecular structure can be extrapolated from that of Rad4 protein, the evolutionarily conserved homolog in the yeast *Saccharomyces cerevisiae* (Min and Pavletich, 2007). When undergoing co-crystals with a model bulky lesion in duplex DNA, Rad4 protein deploys four adjacent domains for substrate binding by two different modalities. One part makes use of a TG domain and a BHD1, which cooperate in associating with 11 base pairs of duplex DNA flanking the damaged site. The second part uses two further BHD2 and BHD3 to interact with four consecutive nucleotides of the undamaged DNA strand opposing the flipped-out bulky lesion. No interactions at all are formed with the lesion itself. In human XPC protein, this β-hairpin region (BHD1–3) interacting indirectly with damaged sites encompasses amino acids 637–831 (Camenisch et al., 2009).

In addition to mediating associations with substrate DNA, the TG domain is required for the interaction between Rad4 and Rad23 (Min and Pavletich, 2007), and between the corresponding human homologs XPC and RAD23B. A fraction of the human TG domain also interacts with XPA protein (Bunick et al., 2006). Another partner, known as DDB2 does not exist in lower eukaryotes like yeast. However, a transient association between DDB2 and XPC is critical for the processing of CPDs in mammals (Itoh et al., 2004) and the respective contact

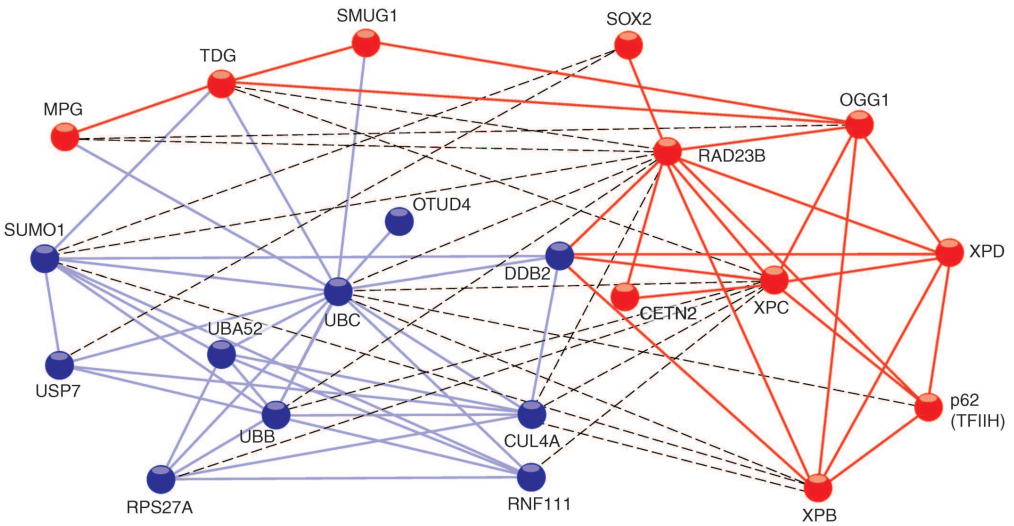

FIGURE 1 | STRING network view of XPC interactions with proteins. The connecting lines indicate proven or predicted interactions using the http://www.string-db.org information source. The different colors of the protein nodes reflect their clustering in two groups according to the KMEANS algorithm (Brohée and Helden, 2006). Blue nodes, ubiquitin-related proteins; red nodes, DNA repair proteins. Blue lines, interactions between ubiquitin-related proteins; red lines, interactions between DNA repair proteins. The dashed lines highlight interactions between the two different clusters.

sites have been mapped to the TG and BHD1 regions (Fei et al., 2011). Residues 847–863 in the carboxy-terminus of human XPC form an α-helix that binds tightly to CETN2 (Nishi et al., 2005; Yang et al., 2006). Amino acid residues 816–940 located in this carboxy-terminus and a portion of the amino-terminal region around amino acid position 334 make contacts with two members (p62 and XPB) of the 10-subunit TFIIH complex (Yokoi et al., 2000; Uchida et al., 2002; Bernardes de Jesus et al., 2008). These particular interactions reflect the actual role of XPC in recruiting the XPD helicase, another TFIIH subunit, which in turn detects lesions by scanning DNA and sequestering damaged nucleotides in a dedicated recognition pocket on its enzyme surface (Sugasawa et al., 2009; Mathieu et al., 2010). In addition, XPC protein interacts with the following base excision repair enzymes: MPG, (Miao et al., 2000), TDG, (Shimizu et al., 2003), OGG1, (D'Errico et al., 2007; Melis et al., 2011), and SMUG1, (Shimizu et al., 2010). This crosstalk with multiple DNA glycosylases indicates that XPC may adopt a more general function in recruiting diverse repair enzymes to base pair-disrupted sites in the double helix. Perhaps the most unexpected interaction of XPC protein is with the Oct4-Sox2 transcriptional activator. Indeed, the XPC complex was found to serve as a coactivator of the Oct4-Sox2-dependent expression of the Nanog pluripotency gene (Fong et al., 2011; Cattoglio et al., 2015; Zhang et al., 2015). A two-hybrid screen, which used XPC protein as the bait, revealed many further potential interaction partners involved in DNA synthesis, transcription, post-translational modification, proteolysis, signal transduction, and metabolism (Lubin et al., 2014). To date, the biological consequence of these putative associations is unknown. Finally, there are also proven interactions of XPC protein with two different deubiquitinases, i.e., OTUD4, (Lubin et al., 2014) and USP7 deubiquitinase (for *U*biquitin-*S*pecific-processing *P*rotease 7; He et al., 2014). It appears, therefore, that XPC upon ubiquitination becomes a substrate for these two deubiquitinating enzymes.

Support for the XPC Initiator from a Specialized UV Lesion Detector

Exposure of DNA to UV light results in the formation of CPDs and 6-4PPs in a stoichiometry of approximately 3:1. These two kinds of pyrimidine crosslinks differ in their biophysical properties, genomic distribution, and biological effects. First, CPD sites are characterized by a relatively minor destabilization of base pairs compared to duplex DNA containing 6-4PPs (Kim et al., 1995; Jing et al., 1998; McAteer et al., 1998). Second, CPDs are evenly distributed across the chromatin landscape, whereas 6-4PPs are formed preferentially in linker DNA segments rather than in nucleosome cores (Gale et al., 1987; Gale and Smerdon, 1990; Mitchell et al., 1990). Third, because CPDs are removed at slower rates than 6-4PPs, they display a higher mutagenic potential and are responsible for most adverse short- and long-term effects of UV radiation such as sunburns, skin aging and cutaneous cancer (Schul et al., 2002; Garinis et al., 2005).

Despite being the generic repair initiator for all bulky lesions including the slowly repaired CPDs, XPC protein does not bind CPDs in duplex DNA with any appreciable selectivity (Sugasawa et al., 2001; Hey et al., 2002; Reardon and Sancar, 2003; Wittschieben et al., 2005). This lack of specificity for CPDs is, however, compensated by DDB2 protein, which is the factor mutated in XP-E patients (Nichols et al., 2000; Kulaksiz et al., 2005). Unlike XPC, which functions as a non-specific sensor of helix-disrupting bulky lesions, DDB2 is exclusively dedicated to the detection of CPDs and 6-4PPs (Tang et al., 2000). Structural analyses of DDB2 crystals revealed a recognition hole in its

central β-propeller fold that only accommodates CPDs and 6-4PPs while excluding larger base adducts (Scrima et al., 2008; Fischer et al., 2011; Yeh et al., 2012; Osakabe et al., 2015). Notably, the complete lack of functional DDB2 protein in XP-E patients abolishes the repair of CPDs but the excision of 6-4PPs is only marginally affected (Hwang et al., 1999; Moser et al., 2005).

A generally proposed model is that DDB2 recognizes CPDs and, thereafter, delivers them to the XPC partner for initiation and execution of the GG-NER process (Tang et al., 2000; Wakasugi et al., 2001; Fitch et al., 2003). It has been demonstrated that XPC lends two of its previously mentioned DNA-binding folds (TG domain and BHD1) to interact in a transient manner with DDB2 associating with UV lesions. This dynamic DDB2-XPC-DNA intermediate at the damage site allows for the insertion, into the DNA double helix, of a β-hairpin extension protruding from BHD3, eventually competing DDB2 away from the damage (Fei et al., 2011; Mu et al., 2015). Thermodynamically, this β-hairpin insertion by XPC takes place at a considerable energetic cost for local breakage of stacking and hydrogen bond interactions between the involved bases (Mu et al., 2015). The 6-4PPs, being more base pair-disruptive, facilitate this β-hairpin insertion by reducing the helical stability at damaged sites, but XPC protein depends on DDB2 to interact in a productive manner with CPD sites. Thus, the different degree of local helical distortion explains the specific defect of XP-E cells in eliminating CPD lesions.

Polypeptide Modifiers Targeting XPC Protein

In view of the manifold implications of XPC as a generic DNA quality sensor in GG-NER that, in addition, associates with several DNA glycosylases and is responsible for non-repair functions in transcription (see above), it is not astonishing to observe that the activity, cellular level and localization of XPC protein is tightly controlled. For example, it has become clear that various polypeptide modifiers regulate the action of this versatile repair initiator during the cellular response to UV damage.

In addition to its role as a specific UV lesion detector, the DDB2 subunit cooperates with the adaptor DDB1 to recruit the CUL4A scaffold and the RING finger protein ROC1, which together build the $CRL4^{DDB2}$ ubiquitin ligase. By mediating the covalent attachment of one or more 8-kDa ubiquitin moieties to target proteins (Groisman et al., 2003), this cullin-type ligase is able to fine-tune GG-NER activity. Under steady-state conditions, the $CRL4^{DDB2}$ ubiquitin ligase is kept in an inactive form thanks to an association with the COP9 signalosome, a multi-subunit regulatory protease (Fischer et al., 2011). Following the detection of UV lesions by DDB2, COP9 is released giving way to a covalent modification of CUL4A with the ubiquitin-like polypeptide NEDD8, thus activating the ubiquitin ligase complex that, in turn modifies nearby located substrates with Lys48-linked ubiquitin chains (Scrima et al., 2008). The principal ubiquitination substrates include histones H2A, H3 and H4 as well as DDB2 itself and its DNA recognition partner XPC (Nag et al., 2001; Sugasawa et al., 2005; Kapetanaki et al., 2006; Wang et al., 2006; Guerrero-Santoro et al., 2008).

It has been proposed that the $CRL4^{DDB2}$-mediated ubiquitination of histones in response to UV radiation helps opening chromatin, thus facilitating access of the GG-NER repair machinery to damaged DNA (Wang et al., 2006). However, this view is contradicted by the finding that CUL4A conditional-knockout mice show more proficient rather than reduced GG-NER activity (Liu et al., 2009). There is, on the other hand, general agreement that the self-ubiquitination of DDB2 not only suppresses its binding to DNA but also promotes its degradation by the 26S proteasome (Sugasawa et al., 2005). The same $CRL4^{DDB2}$ ligase also ubiquitinates XPC but, unlike the fate of DDB2, XPC retains its DNA-binding property and is shielded from proteasomal breakdown (Sugasawa et al., 2005; Matsumoto et al., 2015). In addition, the XPC protein is modified with Lys63-linked ubiquitin chains by another ligase complex referred to as RNF111 or Arkadia (Poulsen et al., 2013). This extra ubiquitination reaction is strictly dependent on the prior UV-dependent modification of XPC protein with sumo, defining RNF111 as a sumo-targeted ubiquitin ligase (Wang et al., 2005).

In summary, GG-NER activity upon UV damage is coordinated by several polypeptide modifiers including NEDD8, sumo, Lys48- and Lys63-linked ubiquitin chains. Sumo and the two aforementioned ubiquitin chains decorate XPC protein at multiple covalent modification sites. Interestingly, *in situ* immunofluorescence studies indicate that a down-regulation of $CRL4^{DDB2}$ or RNF111 activity has opposite effects by inhibiting and stimulating, respectively, the accumulation of XPC in damage spots generated by UV irradiation through micropore filters. This observation raises the possibility that Lys48-linked ubiquitin chains (produced by $CRL4^{DDB2}$) and Lys63-linked counterparts (produced by RNF111) have distinct modulating roles. The function of Lys48-linked ubiquitin chains in regulating XPC is discussed in the next section below. With regard to the accompanying sumo modification, this reaction has been implicated in promoting the release of DDB2 once XPC is bound to UV lesion sites. In the absence of XPC sumoylation, both DDB2 and XPC are trapped together on damaged DNA carrying the lesion, thus posing a block to downstream NER steps (Akita et al., 2015). Since RNF111 is targeted to protein substrates by sumo residues, it is tempting to propose that the effect of sumoylation in releasing XPC may actually be executed by a subsequent attachment of Lys63-linked ubiquitin chains by RNF111. This functional link between sumo and Lys63-linked ubiquitin would explain the persistence of XPC in UV lesion spots observed by Poulsen et al. (2013) and van Cuijk et al. (2015) following RNF111 depletion.

Dynamic Relocation of XPC in Damaged Chromatin

The genome packaging in eukaryotic cells is imposed by two very diverging needs. The DNA filaments must be compressed to fit into the narrow cellular nucleus but nevertheless remain accessible to the diverse nuclear transactions. To achieve this double requirement, DNA is assembled with histones to

generate a tight but dynamic array whose repeating unit is the nucleosome (reviewed by Khorasanizadeh, 2004; Thoma, 2005). Each individual nucleosome displays a core particle, where 147 base pairs of duplex DNA are wrapped around a core histone octamer (two each of H2A, H2B, H3, and H4) and a DNA spacer or "linker" of variable length. Also, in higher eukaryotes histone H1 associates with linker DNA segments to induce further packaging allowing for increased compaction of the DNA double helix.

It is of paramount importance to address the possible regulatory role of polypeptide modifiers in the GG-NER pathway taking into account this chromatin context. New insights into the function of $CRL4^{DDB2}$-mediated ubiquitination came from the enzymatic partitioning of chromatin by incubation with micrococcal nuclease (MNase). This particular enzyme breaks down DNA in the more accessible linker segments much faster than in the less accessible nucleosome cores. As a consequence, the incubation of chromatin with MNase produces a soluble supernatant of mostly non-histone proteins that, before MNase digestion, were associated with linker DNA segments spacing the nucleosomal core particles (amounting to ∼35% of total genomic DNA). Even when saturating enzyme concentrations are used, however, MNase digestions of chromatin leave behind the vast majority of nucleosome core particles (amounting to ∼60% of total DNA) in the form of an insoluble nucleoprotein fraction (Telford and Stewart, 1989). Two previous findings led us to predict that, in response to UV irradiation, $CRL4^{DDB2}$ activity would not be uniformly distributed along nucleosome arrays. First, DDB2 protein, the DNA-binding subunit of $CRL4^{DDB2}$, associates with > 10-fold higher affinity with 6-4PPs ($K_a = 1.5 \times 10^9$ M^{-1}) relative to CPDs ($K_a = 1 \times 10^8$ M^{-1}; (Reardon et al., 1993; Wittschieben et al., 2005). Second, 6-4PPs are formed mainly in internucleosomal linker DNA (Gale and Smerdon, 1990; Mitchell et al., 1990). Therefore, we were not surprised to find that DDB2 associates preferentially, although not exclusively, with 6-4PPs situated in accessible MNase-sensitive internucleosomal segments (Fei et al., 2011). Coversely, it was believed that XPC is unable to interact with DNA assembled with histone octamers forming nucleosome cores (Yasuda et al., 2005) but, against this prevailing notion, MNase digestions of chromatin revealed that XPC protein associates rather evenly with nucleosome core particles and internucleosomal linker segments. Upon UV irradiation, this interaction of XPC protein with nucleosome core particles is stimulated (Fei et al., 2011). This latter finding is in line with structural analyses of core particle crystals containing a site-directed UV damage, which revealed that the tight wrapping around histone octamers increases the DNA flexibility at lesion sites (Osakabe et al., 2015). This higher flexibility may, in turn, explain how XPC protein is able to carry out, even in the nucleosome core context, its indirect damage sensor function by binding to the undamaged strand opposing bulky lesions.

In agreement with the selectivity of the DDB2 subunit for UV lesions in internucleosomal linker DNA, following UV radiation the whole $CRL4^{DDB2}$ ubiquitin ligase is relocated mainly to these highly amenable sites. Due to this distinctive positioning of $CRL4^{DDB2}$, the modification with Lys48-linked ubiquitin chain takes place more efficiently on XPC bound to internucleosomal DNA, whereas XPC molecules on core particles are less prone to ubiquitination (Fei et al., 2011). The role of $CRL4^{DDB2}$ in this context was confirmed by the following experimental manipulations: (i) depletion of either DDB2 or CUL4A using RNA interference, (ii) depletion of the nuclear ubiquitin pool by using the proteasome inhibitor MG132, or (iii) suppression of the ubiquitin pathway using a small-molecule E1 inhibitor. Alternatively, the ubiquitination of XPC was inhibited in mouse cells expressing a temperature-sensitive E1 mutant or with an XPC-green fluorescent fusion protein that makes the XPC protein refractory to ubiquitination. After each of these experimental manipulations, the XPC molecules were devoid of ubiquitin moieties and, as a consequence, almost completely relocated to nucleosome core particles (Fei et al., 2011). These findings demonstrate that one of the functions of $CRL4^{DDB2}$-mediated ubiquitination is to retain XPC molecules at internucleosomal sites, which constitute DNA repair hotspots for the effective recruitment of TFIIH and further downstream NER factors (**Figure 2**). In the absence of $CRL4^{DDB2}$ activity, more XPC binds to CPDs located in nucleosome core particles representing a less permissive chromatin environment with poor recruitment of downstream GG-NER factors. We concluded that the $CRL4^{DDB2}$-mediated ubiquitination serves to establish a distinctive spatiotemporal distribution of the XPC sensor during the UV damage response, in particular to optimize the recruitment of NER factors in mammalian chromatin.

Ubiquitin-dependent Extraction of DDB2 and XPC from Chromatin

Although the DDB2 damage detector is required for efficient recognition and excision of CPDs, Lys48-linked ubiquitin moieties elicit its proteolytic breakdown within few hours after exposure to UV light (Nag et al., 2001; Rapic-Otrin et al., 2002). This precipitous self-ubiquitination and degradation of DDB2 provides a time switch that limits the $CRL4^{DDB2}$ ubiquitin ligase activity, and its regulatory effect on the XPC partner, to a short period after acute UV pulses. Due to DDB2 degradation, the proportion of ubiquitinated XPC diminishes progressively and, therefore, XPC can relocate from internucleosomal DNA segments to not yet processed residual UV lesions, essentially CPDs, located within the less amenable nucleosome core particles (Fei et al., 2011). These dynamic chromatin transitions, involving degradation of DDB2 and relocation of XPC, are triggered by the ubiquitin-selective p97 segregase, also known as VCP, (Puumalainen et al., 2014). Hexameric assemblies of p97 subunits convert ATP hydrolysis into mechanical activity to liberate ubiquitinated proteins from diverse subcellular substrates (Rouiller et al., 2000; Zhang et al., 2000). That p97 hexamers recognize ubiquitinated DDB2 and XPC was first demonstrated *in situ* on UV lesions spots in the nuclei of human cells. Second, it was confirmed biochemically that Lys48-ubiquitinated DDB2, XPC, and p97 are found in the same multi-protein complex (Puumalainen et al., 2014). This p97 recruitment to ubiquitinated DDB2 and XPC depends

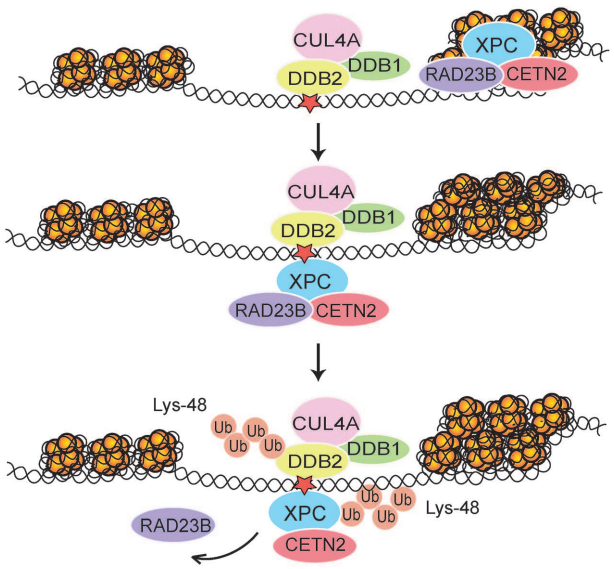

FIGURE 2 | Regulation of XPC localization in chromatin. After each UV pulse, the cullin-type CRL4^{DDB2} ligase complex (comprising *inter alia* DDB1, DDB2, and CUL4A) is recruited mostly to accessible internucleosomal sites in chromatin. The ensuing modification of XPC with Lys48-linked ubiquitin (Ub) chains leads to a temporary retention of XPC on internucleosomal DNA, thus reducing its constitutive association with nucleosome core particles (Fei et al., 2011). Subsequently, RAD23B is released and the XPC-CETN2 heterodimer provides a platform for recruitment of the TFIIH complex. The UV radiation damage is symbolized by a red star.

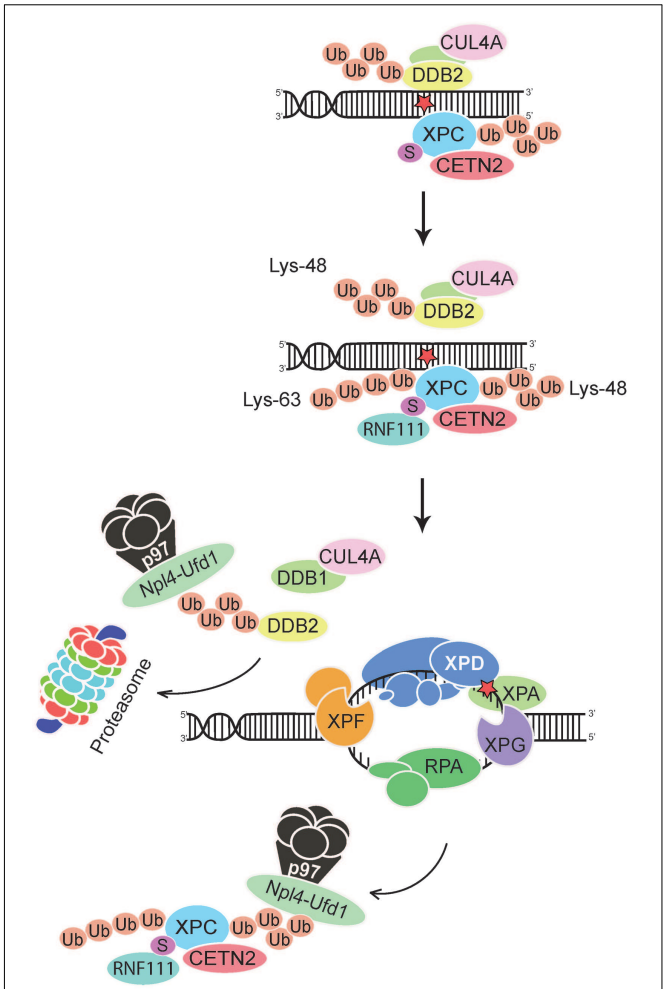

FIGURE 3 | Extraction of DDB2 and XPC from chromatin. The p97 segregase coordinates GG-NER activity by removing Lys48-ubiquitinated DDB2 and Lys48-ubiquitinated XPC from chromatin, thus promoting downstream recognition (by the XPD subunit of TFIIH in conjunction with XPA and RPA) and double DNA incision. The XPC subunit is thought to leave the preincision complex after recruitment of TFIIH but before engagement of the DNA endonucleases XPF-ERCC1 and XPG (Scharer, 2013; van Cuijk et al., 2015). Ubiquitinated DDB2 is forwarded to the proteasome for degradation, whereas XPC is recycled by de-ubiquitination (He et al., 2014; Lubin et al., 2014; Puumalainen et al., 2014). Lys63-linked ubiquitin chains on XPC may further enhance these dynamic relocations at UV lesions by favoring the dissociation of DDB2 from XPC. See text for further details on the postulated dual role of CRL4^{DDB2} (generating Lys48-linked ubiquitin chains) and RNF111 (generating Lys63-linked ubiquitin chains) in regulating GG-NER activity. Npl4-Ufd1, adaptor complex that confers specificity to the p97 segregase; the UV radiation damage is symbolized by a red star.

on adapter proteins (Meyer et al., 2000; Hänzelmann et al., 2011) known to confer substrate specificity to the p97 segregase (**Figure 3**).

Next, the p97 function was down regulated by RNA interference or, alternatively, by expression of a dominant-negative mutant (Ye et al., 2003) that still displays substrate-binding but is unable to exert segregase activity and, therefore, remains trapped on ubiquitinated proteins. The consequence of this diminished p97 activity is an enrichment of DDB2 and XPC in UV lesion spots, thus reflecting an excessive accumulation of these factors in damaged chromatin. The down-regulation of p97 inhibited the UV-induced proteolytic clearance of DDB2 and also increased the level of ubiquitinated XPC. However, despite their roles in the initiation of GG-NER activity, this induced persistence of DDB2 and XPC impaired UV lesion excision. Moreover, the compromised DNA repair efficiency resulting from p97 down regulation caused hypersensitivity to UV light and enhanced chromosomal aberrations after UV exposure.

The genome instability observed in UV-irradiated cells after p97 depletion was reversed by concurrent down-regulation of DDB2 or XPC (Puumalainen et al., 2014). These findings suggested that the uncontrolled accumulation of DDB2 or XPC is detrimental and that a tight regulation of their levels in chromatin is essential for genome stability. Elaborating on this hypothesis, one would expect that an excessive presence of one of these factors should be sufficient to destabilize the genome. In support of this hypothesis, it was found that under conditions of normal p97 activity, overexpression of wild-type DDB2 but not overexpression of a DNA-binding mutant, compromised UV lesion excision and increased the frequency of chromosomal aberrations following UV irradiation. Importantly, double overexpression experiments generating abnormally high levels of both DDB2 and p97 confirmed the expectation that the negative effects of DDB2 overexpression are reversed by concomitantly increasing p97 levels. Thus, a surplus of DDB2

enhances chromosomal aberrations only as long as its chromatin level exceeds the turnover capacity of the p97 segregase. Taken together, these findings point out that a strict spatial and temporal regulation of the chromatin homeostasis of DDB2 and its XPC partner by the p97 segregase is crucial for GG-NER activity (**Figure 3**).

CONCLUSION

The XPC complex provides the generic initiator of GG-NER activity on the basis of its ability to sense the damage-dependent disruption of base pairs in double-stranded DNA and recruit the XPD scanner for bulky lesion recognition. An intriguing peculiarity of the XPC complex is that its function in initiating the excision of UV lesions is tightly regulated by NEDD8, sumo and ubiquitin modifiers. This special regulation is apparently not needed for the recognition and excision of other bulky lesions induced by chemical carcinogens or endogenous metabolic byproducts. An evolutionary perspective may help to understand the unique need for polypeptide modifier-dependent regulation of GG-NER activity in response to UV irradiation.

Evolution of life on our planet would have failed without the emergence of an effective DNA repair function dealing with UV lesions. Indeed, a vast majority of living organisms exposed to sunlight display rapid, efficient and secure molecular tools for the repair of UV lesions consisting of DNA photolyases. By visible light-driven catalysis, these DNA photolyases revert pyrimidine dimers (CPDs and 6-4PPs) to pyrimidine monomers without excision of bases, nucleoside or nucleotide residues (Sancar, 2003; Weber, 2005). Unlike other animals, however, placental mammals are devoid of this light-dependent DNA repair reaction, possibly because they originated from nocturnal ancestors (Essen and Klar, 2006). While returning to a diurnal life under sunlight, placental mammals were left with the GG-NER pathway (also known as "dark repair") as the only means to process UV lesions in the exposed skin. In principle, many potential problems arise with this upgrade of GG-NER activity as the unique DNA repair defense against UV lesions. First, CPDs would escape repair because the XPC initiator is not able to detect this prevalent type of UV lesion. Second, once exposed to sunlight, skin cells would be faced with the simultaneous and uncontrolled cleavage of their genomic DNA at thousands or more chromosomal sites, which constitutes a striking threat to genome stability. Third, CPDs are formed evenly across the genomic DNA, including compacted chromatin sites that are poorly amenable to the GG-NER machinery.

The present review highlights NEDD8-, sumo- and ubiquitin-dependent mechanisms by which these problems related to "dark repair" by the GG-NER machinery are mitigated in human skin cells. First, the dedicated UV damage sensor DDB2 recruits its XPC partner to CPD lesions that, without DDB2, would remain undetected. Second, the GG-NER-initiating activity of XPC undergoes a tight spatial regulation. By recruitment of the $CRL4^{DDB2}$ ligase responsible for XPC ubiquitination, the GG-NER reaction is in the beginning directed to highly amenable internucleosomal DNA segments that are accessible to downstream excision factors, thus protecting more compacted chromatin sites from premature incisions that might favor the fragmentation of chromosomes. Third, the repair-initiating activity of XPC undergoes a tight temporal regulation. By means of proteolytic breakdown triggered by the $CRL4^{DDB2}$ ubiquitin ligase, the repair-stimulating action of DDB2 is self-limiting after an acute pulse of UV damage. Fourth, the physical interaction between DDB2 and XPC is counter-regulated by sumo and, presumably, the sumo-dependent RNF111 ubiquitin ligase. It is still an enigma how DDB2 and the XPC complex take advantage of histone-modifying enzymes as well as chromatin remodelers to relax chromatin regions and initiate the repair of compacted DNA substrates in a coordinated manner. It has, however, become clear that p97-mediated extraction of a surplus of ubiquitinated DDB2 and XPC is necessary to achieve optimal GG-NER activity and avoid molecular collisions with concomitant nuclear processes like transcription or DNA replication. Through addition of these NEDD8-, sumo- and ubiquitin-dependent control circuits, it has become possible during mammalian evolution to upgrade the GG-NER system as the only available DNA repair reaction protecting from UV-induced skin mutagenesis and carcinogenesis.

AUTHOR CONTRIBUTIONS

PR, CBP, and HN wrote the manuscript. PR and CP prepared the figures.

ACKNOWLEDGMENTS

Work in the authors' laboratory was supported by the Swiss National Science Foundation (Grant 143669/1), the Swiss Cancer League (2832-02-2011), and the Velux Stiftung (Project 753).

REFERENCES

Akita, M., Tak, Y. S., Shimura, T., Matsumoto, S., Okuda-Shimizu, Y., Shimizu, Y., et al. (2015). SUMOylation of xeroderma pigmentosum group C protein regulates DNA damage recognition during nucleotide excision repair. *Sci. Rep.* 5:10984. doi: 10.1038/srep10984

Araki, M., Masutani, C., Takemura, M., Uchida, A., Sugasawa, K., Kondoh, J., et al. (2001). Centrosome protein centrin 2/Caltractin 1 is part of the xeroderma pigmentosum group C complex that initiates global genome nucleotide excision repair. *J. Biol. Chem.* 276, 18665–18672. doi: 10.1074/jbc.m100855200

Araujo, S. J., Tirode, F., Coin, F., Pospiech, H., Syvaoja, J. E., Stucki, M., et al. (2000). Nucleotide excision repair of DNA with recombinant human proteins: definition of the minimal set of factors, active forms of TFIIH, and modulation by CAK. *Genes Dev.* 14, 349–359.

Bergink, S., Toussaint, W., Luijsterburg, M. S., Dinant, C., Alekseev, S., Hoeijmakers, J. H. J., et al. (2012). Recognition of DNA damage by XPC coincides with disruption of the XPC-RAD23 complex. *J. Cell Biol.* 196, 681–688. doi: 10.1083/jcb.201107050

Bernardes de Jesus, B. M., Bjoras, M., Coin, F., and Egly, J. M. (2008). Dissection of the molecular defects caused by pathogenic mutations in the

DNA repair factor XPC. *Mol. Cell. Biol.* 28, 7225–7235. doi: 10.1128/mcb.00781-08

Bohr, V., Smith, C. A., Okumoto, D. S., and Hanawalt, P. C. (1985). DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* 40, 359–369. doi: 10.1016/0092-8674(85)901503

Brash, D. E. (1988). UV mutagenic photoproducts in *Escherichia coli* and human cells: a molecular genetics perspective on human skin cancer. *Photochem. Photobiol.* 48, 59–66. doi: 10.1111/j.1751-1097.1988.tb02786.x

Brohée, S., and Helden, J. (2006). Evaluation of clustering algorithsms for protein-protein interaction networks. *BMC Bioinformatics* 7:488. doi: 10.1186/1471-2105-7-488

Brooks, P. J., Wise, D. S., Berry, D. A., Kosmoski, J. V., Smerdon, M. J., Somers, R. L., et al. (2000). The oxidative DNA lesion 8,5'-(S)-cyclo-2'-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian cells. *J. Biol. Chem.* 275, 22355–22362. doi: 10.1074/jbc.m002259200

Brueckner, F., Hennecke, U., Carell, T., and Cramer, P. (2007). CPD damage recognition by transcribing RNA polymerase II. *Science* 315, 859–862. doi: 10.1126/science.1135400

Bunick, C. G., Miller, M. R., Fuller, B. E., Fanning, E., and Chazin, W. J. (2006). Biochemical and structural domain analysis of xeroderma pigmentosum complementation group C protein. *Biochemistry* 45, 14965–14979. doi: 10.1021/bi061370o

Camenisch, U., Träuflein, D., Clement, F. C., Fei, J., Leitenstorfer, A., Ferrando-May, E., et al. (2009). Two-stage dynamic DNA quality check by xeroderma pigmentosum group C protein. *EMBO J.* 28, 2387–2399. doi: 10.1038/emboj.2009.187

Cattoglio, C., Zhang, E. T., Grubisic, J., Chiba, K., Fong, Y. W., and Tjian, R. (2015). Functional and mechanistic studies of XPC DNA-repair complex as transcriptional coactivator in embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 112, E2317–E2326. doi: 10.1073/pnas.1505569112

Cleaver, J. E., Lam, E. T., and Revet, I. (2009). Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. *Nat. Rev. Genet.* 10, 756–768. doi: 10.1038/nrg2663

Dantas, T. J., Daly, O. M., Conroy, P. C., Tomas, M., Wang, Y., Lalor, P., et al. (2013). Calcium-binding capacity of centrin2 is required for linear POC5 assembly but not for nucleotide excision repair. *PLoS ONE* 8:e68487. doi: 10.1371/journal.pone.0068487

Dantas, T. J., Wang, Y., Lalor, P., Dockery, P., and Morrison, C. G. (2011). Defective nucleotide excision repair with normal centrosome structures and functions in the absence of all vertebrate centrins. *J. Cell Biol.* 193, 307–318. doi: 10.1083/jcb.201012093

D'Errico, M., Lemma, T., Calcagnile, A., Santis, L. P. D., and Dogliotti, E. (2007). Cell type and DNA damage specific response of human skin cells to environmental agents. *Mutat. Res.* 614, 37–47. doi: 10.1016/j.mrfmmm.2006.06.009

DiGiovanna, J. J., and Kraemer, K. H. (2012). Shining a light on xeroderma pigmentosum. *J. Investig. Dermatol.* 132, 785–796. doi: 10.1038/jid.2011.426

Donaldson, M. R., and Coldiron, B. M. (2011). No end in sight: the skin cancer epidemic continues. *Semin. Cutan. Med. Surg.* 30, 3–5. doi: 10.1016/j.sder.2011.01.002

Essen, L. O., and Klar, T. (2006). Light-driven DNA repair by photolyases. *Cell Mol. Life. Sci.* 63, 1266–1277. doi: 10.1007/s00018-005-5447-y

Evans, E., Fellows, J., Coffer, A., and Wood, R. D. (1997). Open complex formation around a lesion during nucleotide excision repair provides a structure for cleavage by human XPG protein. *EMBO J.* 16, 625–638. doi: 10.1093/emboj/16.3.625

Fei, J., Kaczmarek, N., Luch, A., Glas, A., Carell, T., and Naegeli, H. (2011). Regulation of nucleotide excision repair by UV-DDB: prioritization of damage recognition to internucleosomal DNA. *PLoS Biol.* 9:e1001183. doi: 10.1371/journal.pbio.1001183

Fischer, E. S., Scrima, A., Böhm, K., Matsumoto, S., Lingaraju, G. M., Faty, M., et al. (2011). The molecular basis of CRL4DDB2/CSA ubiquitin ligase architecture, targeting, and activation. *Cell* 147, 1024–1039. doi: 10.1016/j.cell.2011.10.035

Fitch, M. E., Nakajima, S., Yasui, A., and Ford, J. M. (2003). In vivo recruitment of XPC to UV-induced cyclobutane pyrimidine dimers by the DDB2 gene product. *J. Biol. Chem.* 278, 46906–46910. doi: 10.1074/jbc.m307254200

Fong, Y. W., Inouye, C., Yamaguchi, T., Cattoglio, C., Grubisic, I., and Tjian, R. (2011). A DNA repair complex functions as an Oct4/Sox2 coactivator in embryonic stem cells. *Cell* 147, 120–131. doi: 10.1016/j.cell.2011.08.038

Gale, J. M., Nissen, K. A., and Smerdon, M. J. (1987). UV-induced formation of pyrimidine dimers in nucleosome core DNA is strongly modulated with a period of 10.3 bases. *Proc. Natl. Acad. Sci. U.S.A.* 84, 6644–6648. doi: 10.1073/pnas.84.19.6644

Gale, J. M., and Smerdon, M. J. (1990). UV-induced (6-4) photoprducts are distributed differently than cyclobutane dimers in nucleosomes. *Photochem. Photobiol.* 51, 411–417. doi: 10.1111/j.1751-1097.1990.tb01732.x

Garinis, G. A., Mitchell, J. R., Moorhouse, M. J., Hanada, K., de Waard, H., Vandeputte, D., et al. (2005). Transcriptome analysis reveals cyclobutane pyrimidine dimers as a major source of UV-induced DNA breaks. *EMBO J.* 24, 3952–3962. doi: 10.1038/sj.emboj.7600849

Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J.-I., Saijo, M., Drapkin, R., et al. (2003). The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell* 113, 357–367. doi: 10.1016/s0092-8674(03)00316-7

Guerrero-Santoro, J., Kapetanaki, M. G., Hsieh, C. L., Gorbachinsky, I., Levine, A. S., and Rapic-Otrin, V. (2008). The Cullin 4B-based UV-damaged DNA-binding protein ligase binds to UV-damaged chromatin and ubiquitinates histone H2A. *Cancer Res.* 68, 5014–5022. doi: 10.1158/0008-5472.can-07-6162

Hanawalt, P. C., and Spivak, G. (2008). Transcription-coupled DNA repair: two decades of progress and surprises. *Nat. Rev. Mol. Cell. Biol.* 9, 958–970. doi: 10.1038/nrm2549

Hänzelmann, P., Buchberger, A., and Schindelin, H. (2011). Hierarchical binding of cofactors to the AAA ATPase p97. *Structure* 19, 833–843. doi: 10.1016/j.str.2011.03.018

He, J., Zhu, Q., Wani, G., Sharma, N., Han, C., Qian, J., et al. (2014). Ubiquitin-specific protease 7 regulates nucleotide excision repair through deubiquitinating XPC protein and preventing XPC protein from undergoing ultraviolet light-induced and VCP/p97 protein-regulated proteolysis. *J. Biol. Chem.* 289, 27278–27289. doi: 10.1074/jbc.m114.589812

Hey, T., Lipps, G., Sugasawa, K., Iwai, S., Hanaoka, F., and Krauss, G. (2002). The XPC-HR23B complex displays high affinity and specificity for damaged DNA in a true-equilibrium fluorescence assay. *Biochemistry* 41, 6583–6587. doi: 10.1021/bi012202t

Hollander, M. C., Philburn, R. T., Patterson, A. D., Velasco-Miguel, S., Friedberg, E. C., Linnoila, R. I., et al. (2005). Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 102, 13200–13205. doi: 10.1073/pnas.0503133102

Huang, J. C., Svoboda, D. L., Reardon, J. T., and Sancar, A. (1992). Human nucleotide excision nuclease removes thymine dimers from DNA by incising the 22nd phosphodiester bond 5' and the 6th phosphodiester bond 3' to the photodimer. *Proc. Natl. Acad. Sci. U.S.A.* 89, 3664–3668. doi: 10.1073/pnas.89.8.3664

Hwang, B. J., Ford, J. M., Hanawalt, P. C., and Chu, G. (1999). Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc. Natl. Acad. Sci. U.S.A.* 96, 424–428. doi: 10.1073/pnas.96.2.424

Itoh, T., Cado, D., Kamide, R., and Linn, S. (2004). DDB2 gene disruption leads to skin tumors and resistance to apoptosis after exposure to ultraviolet light but not a chemical carcinogen. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2052–2057. doi: 10.1073/pnas.0306551101

Jing, Y., Taylor, J. S., and Kao, J. F. L. (1998). Thermodynamic and base-pairing studies of matched and mismatched DNA dodecamer duplexes containing cis-syn, (6-4) and Dewar photoproducts of TT. *Nucleic Acids Res.* 26, 3845–3853. doi: 10.1093/nar/26.16.3845

Kapetanaki, M. G., Guerrero-Santoro, J., Bisi, D. C., Hsieh, C. L., Rapic-Otrin, V., and Levine, A. S. (2006). The DDB1-CUL4ADDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2588–2593. doi: 10.1073/pnas.0511160103

Khorasanizadeh, S. (2004). The nucleosome. *Cell* 116, 259–272. doi: 10.1016/s0092-8674(04)00044-3

Kim, J. K., Soni, S. D., Arakali, A. V., Wallace, J. C., and Alderfer, J. L. (1995). Solution structure of a nucleic acid photoproduct of deoxyfluorouridylyl-(3'-5')-thymidine monophosphate (d-FpT) determined by NMR and

restrained molecular dynamics: structural comparison of two sequence isomer photoadducts (d-U5p5T and d-T5p5U). *Nucleic Acids Res.* 23, 1810–1815. doi: 10.1093/nar/23.10.1810

Knox, R. J., Lydall, D. A., Friedlos, F., Basham, C., and Roberts, J. J. (1987). The effect of monofunctional or difunctional platinum adducts and of various other associated DNA damage on the expression of transfected DNA in mammalian cell lines sensitive or resistant to difunctional agents. *Biochim. Biophys. Acta* 908, 214–223. doi: 10.1016/0167-4781(87)90101-1

Krasikova, Y. S., Rechkunova, N. I., Maltseva, E. A., Craescu, C. T., Petruseva, I. O., and Lavrik, O. I. (2012). Influence of centrin 2 on the interaction of nucleotide excision repair factors with damaged DNA. *Biochemistry (Moscow)* 77, 346–353. doi: 10.1134/s0006297912040050

Kulaksiz, G., Reardon, J. T., and Sancar, A. (2005). Xeroderma pigmentosum complementation group E protein (XPE/DDB2): purification of various complexes of XPE and analyses of their damaged DNA binding and putative DNA repair properties. *Mol. Cell. Biol.* 25, 9784–9792. doi: 10.1128/mcb.25.22.9784-9792.2005

Kuraoka, I., Bender, C., Romieu, A., Cadet, J., Wood, R. D., and Lindahl, T. (2000). Removal of oxygen free-radical-induced 5',8-purine cyclodeoxynucleosides from DNA by the nucleotide excision-repair pathway in human cells. *Proc. Natl. Acad. Sci. U.S.A.* 97, 3832–3837. doi: 10.1073/pnas.070471597

Li, C. L., Golebiowski, F. M., Onishi, Y., Samara, N. L., Sugasawa, K., and Yang, W. (2015). Tripartite DNA lesion recognition and verification by XPC, TFIIH, and XPA in nucleotide excision repair. *Mol. Cell.* 59, 1025–1034. doi: 10.1016/j.molcel.2015.08.012

Liu, L., Lee, S., Zhang, J., Peters, S. B., Hannah, J., Zhang, Y., et al. (2009). CUL4A abrogation augments DNA damage response and protection against skin carcinogenesis. *Mol. Cell.* 34, 451–460. doi: 10.1016/j.molcel.2009.04.020

Lopes, M., Foiani, M., and Sogo, J. M. (2006). Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Mol. Cell.* 21, 15–27. doi: 10.1016/j.molcel.2005.11.015

Lubin, A., Zhang, L., Chen, H., White, V. M., and Gong, F. (2014). A human XPC protein interactome–a resource. *Int. J. Mol. Sci.* 15, 141–158. doi: 10.3390/ijms15010141

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

Masutani, C., Kusumoto, R., Yamada, A., Dohmae, N., Yokoi, M., Yuasa, M., et al. (1999). The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase eta. *Nature* 399, 700–704. doi: 10.1038/21447

Mathieu, N., Kaczmarek, N., and Naegeli, H. (2010). Strand- and site-specific DNA lesion demarcation by the xeroderma pigmentosum group D helicase. *Proc. Natl. Acad. Sci. U.S.A.* 107, 17545–17550. doi: 10.1073/pnas.1004339107

Matsumoto, S., Fischer, E. S., Yasuda, T., Dohmae, N., Iwai, S., Mori, T., et al. (2015). Functional regulation of the DNA damage-recognition factor DDB2 by ubiquitination and interaction with xeroderma pigmentosum group C protein. *Nucleic Acids Res.* 43, 1700–1713. doi: 10.1093/nar/gkv038

McAteer, K., Jing, Y., Kao, J., Taylor, J. S., and Kennedy, M. A. (1998). Solution-state structure of a DNA dodecamer duplex containing a Cis-Syn thymine cyclobutane dimer, the major UV photoproduct of DNA. *J. Mol. Biol.* 282, 1013–1032. doi: 10.1006/jmbi.1998.2062

Melis, J. P. M., Luijten, M., Mullenders, L. H. F., and van Steeg, H. (2011). The role of XPC: implications in cancer and oxidative DNA damage. *Mutat. Res.* 728, 107–117. doi: 10.1016/j.mrrev.2011.07.001

Meyer, H. H., Shorter, J. G., Seemann, J., Pappin, D., and Warren, G. (2000). A complex of mammalian Ufd1 and Npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. *EMBO J.* 19, 2181–2192. doi: 10.1093/emboj/19.10.2181

Miao, F., Bouziane, M., Dammann, R., Masutani, C., Hanaoka, F., Pfeifer, G., et al. (2000). 3-Methyladenine-DNA glycosylase (MPG protein) interacts with human RAD23 proteins. *J. Biol. Chem.* 275, 28433–28438. doi: 10.1074/jbc.m001064200

Min, J.-H., and Pavletich, N. P. (2007). Recognition of DNA damage by the Rad4 nucleotide excision repair protein. *Nature* 449, 570–575. doi: 10.1038/nature06155

Missura, M., Buterin, T., Hindges, R., Hübscher, U., Kaspárková, J., Brabec, V., et al. (2001). Double-check probing of DNA bending and unwinding by XPA-RPA: an architectural function in DNA repair. *EMBO J.* 20, 3554–3564. doi: 10.1093/emboj/20.13.3554

Mitchell, D. L., Cleaver, J. E., and Epstein, J. H. (1990). Repair of pyrimidine (6-4)pyrimidone photoproducts in mouse skin. *J. Invest. Dermatol.* 95, 55–59. doi: 10.1111/1523-1747.ep12873312

Moggs, J. G., Yarema, K. J., Essigmann, J. M., and Wood, R. D. (1996). Analysis of incision sites produced by human cell extracts and purified proteins during nucleotide excision repair of a 1,3-intrastrand d(GpTpG)-cisplatin adduct. *J. Biol. Chem.* 271, 7177–7186. doi: 10.1074/jbc.271.12.7177

Moser, J., Kool, H., Giakzidis, I., Caldecott, K., Mullenders, L. H. F., and Fousteri, M. I. (2007). sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase IIIα in a cell-cycle-specific manner. *Mol. Cell.* 27, 311–323. doi: 10.1016/j.molcel.2007.06.014

Moser, J., Volker, M., Kool, H., Alekseev, S., Vrieling, H., Yasui, A., et al. (2005). The UV-damaged DNA binding protein mediates efficient targeting of the nucleotide excision repair complex to UV-induced photo lesions. *DNA Rep.* 4, 571–582. doi: 10.1016/j.dnarep.2005.01.001

Mu, H., Geacintov, N. E., Zhang, Y., and Broyde, S. (2015). Recognition of damaged DNA for nucleotide excision repair: a correlated motion mechanism with a mismatched cis-syn thymine dimer lesion. *Biochemistry* 54, 5263–5267. doi: 10.1021/acs.biochem.5b00840

Nag, A., Bondar, T., Shiv, S., and Raychaudhuri, P. (2001). The xeroderma pigmentosum group E gene product DDB2 is a specific target of Cullin 4A in mammalian cells. *Mol. Cell. Biol.* 21, 6738–6747. doi: 10.1128/mcb.21.20.6738-6747.2001

Ng, J. M. Y., Vermeulen, W., ven der Horst, G. T., Bergink, S., Sugasawa, K., Vrieling, H., et al. (2003). A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. *Genes Dev.* 17, 1630–1645. doi: 10.1101/gad.260003

Nichols, A. F., Itoh, T., Graham, J. A., Liu, W., Yamaizumi, M., and Linn, S. (2000). Human damage-specific DNA-binding protein p48. Characterization of XPE mutations and regulation following UV irradiation. *J. Biol. Chem.* 275, 21422–21428. doi: 10.1074/jbc.m000960200

Nishi, R., Okuda, Y., Watanabe, E., Mori, T., Iwai, S., Masutani, C., et al. (2005). Centrin 2 stimulates nucleotide excision repair by interacting with xeroderma pigmentosum group C protein. *Mol. Cell. Biol.* 25, 5664–5674. doi: 10.1128/mcb.25.13.5664-5674.2005

Ogi, T., Limsirichaikul, S., Overmeer, R. M., Volker, M., Takenaka, K., Cloney, R., et al. (2010). Three DNA polymerases, recruited by different mechanisms, carry out NER repair synthesis in human cells. *Mol. Cell.* 37, 714–727. doi: 10.1016/j.molcel.2010.02.009

Osakabe, A., Tachiwana, H., Kagawa, W., Horikoshi, N., Matsumoto, S., Hasegawa, M., et al. (2015). Structural basis of pyrimidine-pyrimidone (6-4) photoproduct recognition by UV-DDB in the nucleosome. *Sci. Rep.* 5:16330. doi: 10.1038/srep16330

Poulsen, S. L., Hansen, R. K., Wagner, S. A., van Cuijk, L., van Belle, G. J., Streicher, W., et al. (2013). RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* 201, 797–807. doi: 10.1083/jcb.201212075

Puumalainen, M.-R., Lessel, D., Rüthemann, P., Kaczmarek, N., Bachmann, K., Ramadan, K., et al. (2014). Chromatin retention of DNA damage sensors DDB2 and XPC through loss of p97 segregase causes genotoxicity. *Nat. Commun.* 5:3695. doi: 10.1038/ncomms4695

Puumalainen, M. R., Ruthemann, P., Min, J. H., and Naegeli, H. (2016). Xeroderma pigmentosum group C sensor: unprecedented recognition strategy and tight spatiotemporal regulation. *Cell Mol. Life. Sci.* 73, 547–566. doi: 10.1007/s00018-015-2075-z

Rapic-Otrin, V., McLenigan, M. P., Bisi, D. C., Gonzalez, M., and Levine, A. S. (2002). Sequential binding of UV DNA damage binding factor and degradation of the p48 subunit as early events after UV irradiation. *Nucleic Acids Res.* 30, 2588–2598. doi: 10.1093/nar/30.11.2588

Reardon, J. T., Nichols, A. F., Keeney, S., Smith, C. A., Taylor, J. S., Linn, S., et al. (1993). Comparative analysis of binding of human damaged DNA-binding protein (XPE) and *Escherichia coli* damage recognition protein (UvrA) to the major ultraviolet photoproducts: T[c,s]T, T[t,s]T, T[6-4]T, and T[Dewar]T. *J. Biol. Chem.* 268, 21301–21308.

Reardon, J. T., and Sancar, A. (2003). Recognition and repair of the cyclobutane thymine dimer, a major cause of skin cancers, by the human excision nuclease. *Genes Dev.* 17, 2539–2551. doi: 10.1101/gad.1131003

Rouiller, I., Butel, V. M., Latterich, M., Milligan, R. A., and Wilson-Kubalek, E. M. (2000). A major conformational change in p97 AAA ATPase upon ATP binding. *Mol. Cell.* 6, 1485–1490. doi: 10.1016/s1097-2765(00)00144-1

Sancar, A. (2003). Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem. Rev.* 103, 2203–2238. doi: 10.1021/cr0204348

Scharer, O. D. (2013). Nucleotide excision repair in eukaryotes. *Cold Spring Harb. Perspect. Biol.* 5:a012609. doi: 10.1101/cshperspect.a012609

Schul, W., Jans, J., Rijksen, Y. M., Klemann, K. H., Eker, A. P., de Wit, J., et al. (2002). Enhanced repair of cyclobutane pyrimidine dimers and improved UV resistance in photolyase transgenic mice. *EMBO J.* 21, 4719–4729. doi: 10.1093/emboj/cdf456

Scrima, A., Koníčková, R., Czyzewski, B. K., Kawasaki, Y., Jeffrey, P. D., Groisman, R., et al. (2008). Structural basis of UV DNA-damage recognition by the DDB1–DDB2 complex. *Cell* 135, 1213–1223. doi: 10.1016/j.cell.2008.10.045

Shimizu, Y., Iwai, S., Hanaoka, F., and Sugasawa, K. (2003). Xeroderma pigmentosum group C protein interacts physically and functionally with thymine DNA glycosylase. *EMBO J.* 22, 164–173. doi: 10.1093/emboj/cdg016

Shimizu, Y., Uchimura, Y., Dohmae, N., Saitho, H., Hanaoka, F., and Sugasawa, K. (2010). Stimulation of DNA glycosylase activities by XPC protein complex: roles of protein-protein interactions. *J. Nucleic Acids* 2010, 805698. doi: 10.4061/2010/805698

Staresincic, L., Fagbemi, A. F., Enzlin, J. H., Gourdin, A. M., Wijgers, N., Dunand-Sauthier, I., et al. (2009). Coordination of dual incision and repair synthesis in human nucleotide excision repair. *EMBO J.* 28, 1111–1120. doi: 10.1038/emboj.2009.49

Straub, K. M., Meehan, T., Burlingame, A. L., and Calvin, M. (1977). Identification of the major adducts formed by reaction of benzo(a)pyrene diol epoxide with DNA in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 74, 5285–5289. doi: 10.1073/pnas.74.12.5285

Sugasawa, K., Akagi, J.-I., Nishi, R., Iwai, S., and Hanaoka, F. (2009). Two-step recognition of DNA damage for mammalian nucleotide excision repair: directional binding of the XPC complex and DNA strand scanning. *Mol. Cell* 36, 642–653. doi: 10.1016/j.molcel.2009.09.035

Sugasawa, K., Masutani, C., Uchida, A., Maekawa, T., van der Spek, P. J., Bootsma, D., et al. (1996). HHR23B, a human Rad23 homolog, stimulates XPC protein in nucleotide excision repair in vitro. *Mol. Cell. Biol.* 16, 4852–4861. doi: 10.1128/MCB.16.9.4852

Sugasawa, K., Ng, J. M. Y., Masutani, C., Iwai, S., van der Spek, P. J., Eker, A. P. M., et al. (1998). Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol. Cell.* 2, 223–232. doi: 10.1016/s1097-2765(00)80132-x

Sugasawa, K., Okamoto, T., Shimizu, Y., Masutani, C., Iwai, S., and Hanaoka, F. (2001). A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes Dev.* 15, 507–521. doi: 10.1101/gad.866301

Sugasawa, K., Okuda, Y., Saijo, M., Nishi, R., Matsuda, N., Chu, G., et al. (2005). UV-Induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* 121, 387–400. doi: 10.1016/j.cell.2005.02.035

Tang, J. Y., Hwang, B. J., Ford, J. M., Hanawalt, P. C., and Chu, G. (2000). Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. *Mol. Cell.* 5, 737–744. doi: 10.1016/s1097-2765(00)80252-x

Telford, D. J., and Stewart, B. W. (1989). Characteristics of chromatin release during digestion of nuclei with micrococcal nuclease: preferential solubilization of nascent RNA at low enzyme concentration. *Intern. J. Biochem.* 21, 1235–1240. doi: 10.1016/0020-711x(89)90009-8

Thoma, F. (2005). Repair of UV lesions in nucleosomes – intrinsic properties and remodeling. *DNA Rep.* 4, 855–869. doi: 10.1016/j.dnarep.2005.04.005

Trego, K. S., and Turchi, J. J. (2006). Pre-steady-state binding of damaged DNA by XPC-hHR23B reveals a kinetic mechanism for damage discrimination. *Biochemistry* 45, 1961–1969. doi: 10.1021/bi051936t

Uchida, A., Sugasawa, K., Masutani, C., Dohmae, N., Araki, M., Yokoi, M., et al. (2002). The carboxy-terminal domain of the XPC protein plays a crucial role in nucleotide excision repair through interactions with transcription factor IIH. *DNA Rep.* 1, 449–461. doi: 10.1016/S1568-7864(02)00031-9

Usher-Smith, J. A., Emery, J., Kassianos, A. P., and Walter, F. M. (2014). Risk prediction models for melanoma: a systematic review. *Cancer Epidemiol. Biomarkers. Prev.* 23, 1450–1463. doi: 10.1158/1055-9965.epi-14-0295

van Cuijk, L., van Belle, G. J., Turkyilmaz, Y., Poulsen, S. L., Janssens, R. C., Theil, A. F., et al. (2015). SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nat. Commun.* 6:7499. doi: 10.1038/ncomms8499

Vermeulen, W., and Fousteri, M. (2013). Mammalian transcription-coupled excision repair. *Cold Spring Harb. Perspect. Biol.* 5:a012625. doi: 10.1101/cshperspect.a012625

Volker, M., Moné, M. J., Karmakar, P., van Hoffen, A., Schul, W., Vermeulen, W., et al. (2001). Sequential assembly of the nucleotide excision repair factors in vivo. *Mol. Cell.* 8, 213–224. doi: 10.1016/s1097-2765(01)00281-7

Wakasugi, M., and Sancar, A. (1998). Assembly, subunit composition, and footprint of human DNA repair excision nuclease. *Proc. Natl. Acad. Sci. U.S.A.* 95, 6669–6674. doi: 10.1073/pnas.95.12.6669

Wakasugi, M., Shimizu, M., Morioka, H., Linn, S., Nikaido, O., and Matsunaga, T. (2001). Damaged DNA-binding protein DDB stimulates the excision of cyclobutane pyrimidine dimers in vitro in concert with XPA and replication protein A. *J. Biol. Chem.* 276, 15434–15440. doi: 10.1074/jbc.m011177200

Wang, H., Zhai, L., Xu, J., Joo, H.-Y., Jackson, S., Erdjument-Bromage, H., et al. (2006). Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. *Mol. Cell.* 22, 383–394. doi: 10.1016/j.molcel.2006.03.035

Wang, Q. E., Zhu, Q., Wani, G., El-Mahdy, M. A., Li, J., and Wani, A. A. (2005). DNA repair factor XPC is modified by SUMO-1 and ubiquitin following UV irradiation. *Nucleic Acids Res.* 33, 4023–4034. doi: 10.1093/nar/gki684

Weber, S. (2005). Light-driven enzymatic catalysis of DNA repair: a review of recent biophysical studies on photolyase. *Biochim. Biophys. Acta* 1707, 1–23. doi: 10.1016/j.bbabio.2004.02.010

Wittschieben, B. O., Iwai, S., and Wood, R. D. (2005). DDB1-DDB2 (xeroderma pigmentosum group E) protein complex recognizes a cyclobutane pyrimidine dimer, mismatches, apurinic/apyrimidinic sites, and compound lesions in DNA. *J. Biol. Chem.* 280, 39982–39989. doi: 10.1074/jbc.m507854200

Xie, Z., Liu, S., Zhang, Y., and Wang, Z. (2004). Roles of Rad23 protein in yeast nucleotide excision repair. *Nucleic Acids Res.* 32, 5981–5990. doi: 10.1093/nar/gkh934

Yang, A., Miron, S., Mouawad, L., Duchambon, P., Blouquit, Y., and Craescu, C. T. (2006). Flexibility and plasticity of human centrin 2 binding to the xeroderma pigmentosum group C protein (XPC) from nuclear excision repair. *Biochemistry* 45, 3653–3663. doi: 10.1021/bi0524868

Yasuda, T., Sugasawa, K., Shimizu, Y., Iwai, S., Shiomi, T., and Hanaoka, F. (2005). Nucleosomal structure of undamaged DNA regions suppresses the non-specific DNA binding of the XPC complex. *DNA Rep.* 4, 389–395. doi: 10.1016/j.dnarep.2004.10.008

Ye, Y., Meyer, H. H., and Rapoport, T. A. (2003). Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. *J. Cell Biol.* 162, 71–84. doi: 10.1083/jcb.200302169

Yeh, J. I., Levine, A. S., Du, S., Chinte, U., Ghodke, H., Wang, H., et al. (2012). Damaged DNA induced UV-damaged DNA-binding protein (UV-DDB) dimerization and its roles in chromatinized DNA repair. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2737–E2746. doi: 10.1073/pnas.1110067109

Yokoi, M., Masutani, C., Maekawa, T., Sugasawa, K., Ohkuma, Y., and Hanaoka, F. (2000). The xeroderma pigmentosum group C protein complex XPC-HR23B plays an important role in the recruitment of transcription factor IIH to damaged DNA. *J. Biol. Chem.* 275, 9870–9875. doi: 10.1074/jbc.275.13.9870

Zhang, E. T., He, Y., Grog, P., Fong, Y. W., Nogales, E., and Tjian, R. (2015). Architecture of the human XPC DNA repair and stem cell coactivator complex. *Proc. Natl. Acad. Sci. U.S.A.* 48, 14817–14822. doi: 10.1073/pnas.1520104112

Zhang, X., Shaw, A., Bates, P. A., Newman, R. H., Gowen, B., Orlova, E., et al. (2000). Structure of the AAA ATPase p97. *Mol. Cell.* 6, 1473–1484. doi: 10.1016/s1097-2765(00)00143-x

Conflict of Interest Statement: 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.

frontiers in Genetics

MINI REVIEW
published: 19 April 2016
doi: 10.3389/fgene.2016.00063

Interplay between Ubiquitin, SUMO, and Poly(ADP-Ribose) in the Cellular Response to Genotoxic Stress

*Stefania Pellegrino and Matthias Altmeyer**

Department of Molecular Mechanisms of Disease, University of Zurich, Zürich, Switzerland

Cells employ a complex network of molecular pathways to cope with endogenous and exogenous genotoxic stress. This multilayered response ensures that genomic lesions are efficiently detected and faithfully repaired in order to safeguard genome integrity. The molecular choreography at sites of DNA damage relies heavily on post-translational modifications (PTMs). Protein modifications with ubiquitin and the small ubiquitin-like modifier SUMO have recently emerged as important regulatory means to coordinate DNA damage signaling and repair. Both ubiquitylation and SUMOylation can lead to extensive chain-like protein modifications, a feature that is shared with yet another DNA damage-induced PTM, the modification of proteins with poly(ADP-ribose) (PAR). Chains of ubiquitin, SUMO, and PAR all contribute to the multi-protein assemblies found at sites of DNA damage and regulate their spatio-temporal dynamics. Here, we review recent advancements in our understanding of how ubiquitin, SUMO, and PAR coordinate the DNA damage response and highlight emerging examples of an intricate interplay between these chain-like modifications during the cellular response to genotoxic stress.

Keywords: ubiquitin, SUMO, poly(ADP-ribose), PARP, DNA damage response, DDR, genome stability, cancer

OPEN ACCESS

Edited by:
Kristijan Ramadan,
University of Oxford, UK

Reviewed by:
Nico P. Dantuma,
Karolinska Institutet, Sweden
Howard Donninger,
University of Louisville, USA

****Correspondence:***
Matthias Altmeyer
matthias.altmeyer@uzh.ch

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *28 February 2016*
Accepted: *04 April 2016*
Published: *19 April 2016*

Citation:
Pellegrino S and Altmeyer M (2016) Interplay between Ubiquitin, SUMO, and Poly(ADP-Ribose) in the Cellular Response to Genotoxic Stress. Front. Genet. 7:63. doi: 10.3389/fgene.2016.00063

INTRODUCTION

Our genetic material is under constant cellular surveillance and care. Maintaining genome stability is indeed a vital task, not only under conditions when external toxins or physical strains challenge the integrity of the genome, but also in the course of normal cellular metabolism, when reactive metabolites and physiological DNA transactions can lead to a plethora of lesions. If these damages are not detected and faithfully repaired, cells run the risk of accumulating mutations that can erode genome function, vitiate cell fate, or compromise cell survival. Faced with such threats cells have developed sophisticated mechanisms to sense and repair damaged DNA. These mechanisms, which are collectively referred to as the DNA damage response (DDR), not only ensure that most lesions are efficiently repaired, but they also coordinate genome integrity maintenance with other cellular functions such as transcription, DNA replication, and cell cycle progression (Ciccia and Elledge, 2010). The DDR is an intricate molecular network that safeguards genome integrity and helps to maintain cell identity, thus constituting a natural barrier against the development of various human diseases (Jackson and Bartek, 2009). Underpinning the crucial role of genome integrity maintenance for human health, a deteriorated DDR and signs of genome instability are typical features of many human cancers, and they represent cancer-specific vulnerabilities that can be targeted by precision therapies (O'Connor, 2015).

To fulfill its task, the DDR employs a multitude of tightly regulated posttranslational protein modifications (PTMs). In addition to modulating protein functions locally at the damage site, PTMs play important roles in spreading the DNA damage signal to the surrounding chromatin (Lukas et al., 2011; Polo and Jackson, 2011) and in activating cell cycle checkpoints (Stracker et al., 2009). Positive feedback mechanisms amplify the DNA damage signal and enable sustained accumulation of genome caretaker proteins, while antagonistic mechanisms ensure that modifications induced by DNA damage remain spatially and temporally confined (van Attikum and Gasser, 2009; Altmeyer and Lukas, 2013a,b; Panier and Durocher, 2013). Multiple PTMs cooperate in this spatio-temporal regulation and can either act in series, in parallel or in a combinatorial fashion to dynamically reshape the chromatin landscape around DNA lesions and prepare the stage for repair (Dantuma and van Attikum, 2016). This barcoding involves multi-target phosphorylation (Marechal and Zou, 2013; Shiloh and Ziv, 2013; Davis et al., 2014; Awasthi et al., 2015; Paull, 2015), as well as acetylation and methylation (Gong and Miller, 2013; Hendzel and Greenberg, 2013; Price and D'Andrea, 2013). In addition to these small moiety modifications, recent work revealed how larger PTMs, which can form extensive modification chains, coordinate the access of genome caretakers to DNA lesions and regulate repair pathway choices. Here, we briefly discuss how ubiquitylation, SUMOylation, and poly(ADP-ribosyl)ation (PARylation) are employed by the DDR, before we highlight emerging examples that have started to elucidate an intricate and still incompletely understood crosstalk between these catenarian modifications in response to DNA damage. We focus our analysis primarily on the response of mammalian cells to DNA double strand breaks (DSBs), yet an equally well-coordinated crosstalk between chromatin-based PTMs also operates in other situations of genotoxic stress (Kim and D'Andrea, 2012; Marteijn et al., 2014; Ulrich, 2014).

UBIQUITIN CONJUGATION AROUND DSB SITES

Chromosome breaks are among the most toxic DNA lesions and two major repair pathways evolved to deal with DSBs. The non-homologous end-joining (NHEJ) pathway is independent of intact template DNA sequences and can re-ligate broken DNA ends throughout the cell cycle. In contrast, faithful repair by homologous recombination (HR) depends on an undamaged template DNA and is thus restricted to the S/G2 phases of the cell cycle when sister chromatids are available. While NHEJ is generally considered error-prone due to the risk of nucleotide loss from DNA ends, HR is considered to be more accurate due to template-based repair. The choice between NHEJ and HR is tightly controlled, and imbalances in its regulation can lead to genome instability and accelerate cancer development (Chapman et al., 2012; Aparicio et al., 2014). Interestingly, the recruitment of several key repair pathway choice mediators to DNA break sites depends on local ubiquitin conjugations (Messick and Greenberg, 2009; Pinder et al., 2013). Indeed, one of the central players of repair pathway choice is the ubiquitin-sensing genome caretaker protein 53BP1, whose recruitment to DSBs requires the consecutive action of the ubiquitin E3 ligases RNF8 and RNF168 (Panier and Boulton, 2014). In a concerted manner, and initiated by upstream phosphorylation of the histone variant H2AX, RNF8, and RNF168 ubiquitylate histones H1 and H2A, respectively, and thereby provide a landing platform for 53BP1 (Mattiroli et al., 2012; Fradet-Turcotte et al., 2013; Gatti et al., 2015; Thorslund et al., 2015). 53BP1 in turn assembles the effector proteins RIF1, PTIP, Artemis, and MAD2L2/REV7 to limit the extent of DNA end resection and thereby channel repair toward NHEJ (**Figure 1A**) (Callen et al., 2013; Chapman et al., 2013; Di Virgilio et al., 2013; Escribano-Diaz et al., 2013; Zimmermann et al., 2013; Wang et al., 2014; Boersma et al., 2015; Xu et al., 2015). Of note, the functions of 53BP1 and its effectors are required for the hypersensitivity of HR-defective cancer cells to inhibitors of PAR polymerases (Lord and Ashworth, 2016), thus linking the consequences of compromised PARylation to the effects of a ubiquitin-dependent anti-resection barrier under pathological repair pathway choice conditions.

Importantly, ubiquitin conjugation is not only involved in generating the ubiquitin code that is recognized by 53BP1, but also fosters 53BP1 accumulation by RNF8/RNF168-dependent and VCP/p97-mediated removal of proteins from damaged chromatin (Acs et al., 2011; Meerang et al., 2011). As was shown for the H4K20me2-binding proteins L3MBTL1 and JMJD2A/KDM4A, this can unmask additional binding sites for the tandem tudor domain of 53BP1 (Acs et al., 2011; Mallette et al., 2012). Thus, the removal of chromatin binders seems to converge with the generation of new chromatin marks to allow for the efficient recruitment of 53BP1 and its downstream effectors (**Figure 1A**).

While these reactions build up an important anti-resection barrier that shields broken DNA ends from unscheduled nucleolytic digestion, ubiquitin conjugation also plays a role in promoting HR. For example, the ubiquitin E3 ligases TRIP12 and UBR5 cooperate to keep RNF168 levels in check and thereby prevent excessive 53BP1 function (Gudjonsson et al., 2012). More recently, the ubiquitin E3 ligase RNF138 was shown to accumulate at sites of DNA damage where it stimulates DNA end resection and promotes HR (Ismail et al., 2015; Schmidt et al., 2015). Thus, rather than channeling repair pathway choices in one direction, the ubiquitylation system employs rheostats and antagonistic sub-pathways to regulate repair decisions in a manner that likely integrates information about cell cycle phase and chromatin context.

SUMO CONJUGATION AT DSB SITES ASSISTS THE DDR

Just like ubiquitylation, also SUMOylation plays important roles for the tightly controlled protein choreography at DSB sites, and its deregulation impairs genome stability and cell proliferation (Bekker-Jensen and Mailand, 2011; Jackson and Durocher, 2013; Eifler and Vertegaal, 2015). The SUMO isoforms SUMO1 and SUMO2/3 were all found to accumulate at sites of DNA damage in a manner dependent on the SUMO E3 ligases

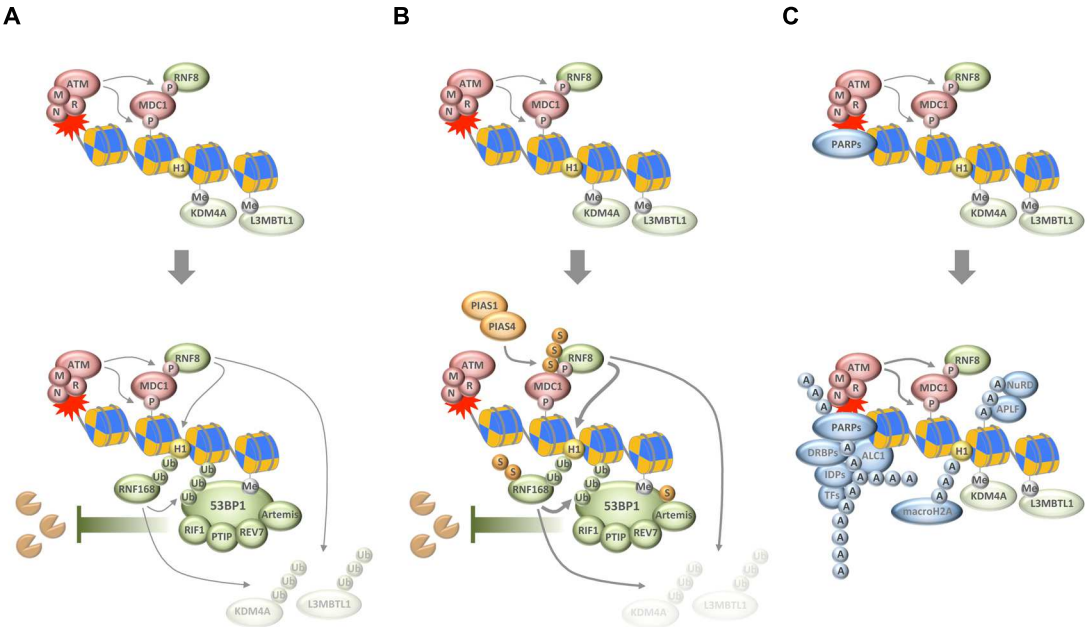

FIGURE 1 | Chain-like modifications build up dynamic DNA repair compartments that orchestrate the DNA damage response (DDR). (A) In response to DNA damage, and subsequent to the MRN/ATM/MDC1-driven phosphorylation of histone variant H2AX, ubiquitylation of H1 and H2B by RNF8 and RNF168, respectively, synergizes with ubiquitin-dependent extraction of proteins from the damaged chromatin to promote the recruitment of 53BP1 and its downstream effectors. **(B)** SUMOylation by PIAS1 and PIAS4 further enhances ubiquitin conjugation around DNA break sites. **(C)** Poly(ADP-ribosyl)ation (PARylation) by PARPs generates a recruitment platform for a plethora of PAR-binding proteins, including various transcription factors (TFs), DNA- and RNA-binding proteins (DRBPs), and a set of intrinsically disordered proteins (IDPs). P, phosphorylation; Me, methylation; Ub, ubiquitylation; S, SUMOylation; A, ADP-ribosylation; M, MRE11; R, RAD50; N, NBS1.

PIAS1 and PIAS4 (Galanty et al., 2009; Morris et al., 2009). Interestingly, the SUMO conjugation observed in response to DNA breakage promotes accumulation of ubiquitin chains on damaged chromatin and is required for the efficient recruitment of ubiquitin-dependent genome caretakers (Galanty et al., 2009; Morris et al., 2009). Among the targets of DNA damage-induced SUMOylation are MDC1, RNF168, 53BP1, BRCA1, RPA, and EXO1 (**Figure 1B**) (Galanty et al., 2009; Morris et al., 2009; Luo et al., 2012; Yin et al., 2012; Bologna et al., 2015; Hendriks et al., 2015). SUMOylation not only contributes to the recruitment of proteins to DSBs but also to their coordinated removal, and, interestingly, is required for both NHEJ and HR (Galanty et al., 2009; Morris et al., 2009; Chung and Zhao, 2015; Sarangi et al., 2015). While much remains to be learnt about the exact mechanisms how SUMOylation and SUMO chain formation in particular affect the repair of DSBs, it has become clear that the SUMOylation and ubiquitylation machineries work closely together to help restore genome integrity upon chromosome breakage (see below).

POLY(ADP-RIBOSE) CHAINS ATTRACT A DIVERSE SET OF PROTEINS TO DNA BREAK SITES

A third type of protein modification that comes in chains and ties proteins to DNA breaks sites is PARylation. Catalyzed by PAR polymerases (PARPs) in response to genotoxic stress, DNA break-associated ADP-ribose polymers provide a landing platform for a plethora of PAR-binding proteins (Teloni and Altmeyer, 2016). This includes chromatin remodelers and DNA repair factors, but also proteins involved in nucleic acid metabolism and RNA processing (Krietsch et al., 2013; Golia et al., 2015; Izhar et al., 2015; Teloni and Altmeyer, 2016). PAR-dependent events have been implicated in the cellular response to DNA single-strand breaks and in maintaining the integrity of perturbed replication forks, but also contribute to DSB repair (Beck et al., 2014). Among the proteins that respond to PAR formation are the DDR factors MRE11 and NBS1 (Haince et al., 2008), the chromatin remodeler ALC1 (Ahel et al., 2009; Gottschalk et al., 2009), the histone variant macroH2A (Timinszky et al., 2009), components of the repressive polycomb and NuRD complexes (Chou et al., 2010; Polo et al., 2010), NHEJ and HR factors (Ahel et al., 2008; Rulten et al., 2008; Li and Yu, 2013; Zhang et al., 2015), and a class of intrinsically disordered proteins that can phase separate to generate dynamic compartments (**Figure 1C**) (Altmeyer et al., 2015; Patel et al., 2015). The relative contribution of each of these recruitments for faithful DNA repair is insufficiently understood and may depend on the type of damage and its complexity as well as the overall damage load. The amount and type of damage, together with cell cycle phase and local chromatin environment, are likely to influence the number of PAR chains generated, their length and branching frequency, and may thereby impact on the protein recruitments that are driven by PAR formation.

Given that all three catenarian modifications, ubiquitylation, SUMOylation, and PARylation, orchestrate the protein accumulations around DNA break sites, significant crosstalk exists. In the following paragraphs, we highlight emerging examples of such interplay and how it affects the accrual of genome caretakers at damaged chromatin.

INTERPLAY BETWEEN UBIQUITIN AND SUMO

As noted above, the ubiquitylation and SUMOylation machineries are tightly interconnected and cooperate to reshape the chromatin landscape for proper repair (Bekker-Jensen and Mailand, 2011; Jackson and Durocher, 2013). An interesting direct link between the two systems is provided by SUMO-targeted ubiquitin ligases (STUbLs), readers of SUMO modifications that possess ubiquitin ligase activity and specifically modify SUMOylated substrates. The STUbL RNF4 is a prime example that recently emerged as important regulator of protein accumulation upon DNA breakage. RNF4 is recruited to DSBs via its SUMO interaction motifs and ubiquitylates SUMOylated DDR factors, thereby leading to their withdrawal from repair sites and initiating their proteasomal degradation (Galanty et al., 2012; Luo et al., 2012; Yin et al., 2012). Defective targeting by RNF4 enhances the retention of a subset of DDR factors and compromises the initiation of downstream events required for efficient repair. Among the proteins that are targeted by RNF4 is the adaptor protein MDC1, whose removal promotes access of the DNA end resection and HR machineries (Galanty et al., 2012; Luo et al., 2012; Yin et al., 2012). Once DNA end resection has occurred, RNF4 is again required for the extraction of the single-stranded DNA binding protein RPA, which in turn allows for the accumulation of BRCA2 and RAD51 on resected DNA (Galanty et al., 2012). Collectively, these findings suggest that SUMO-targeted ubiquitylation participates in the dismantling of the anti-resection barrier and promotes HR reactions. In support of this notion, the activity of RNF4 itself is regulated in a CDK-dependent manner, allowing it to fulfill its HR-promoting roles primarily in the S/G2 phases of the cell cycle (**Figure 2A**) (Luo et al., 2015; Kuo et al., 2016). Interestingly, the DNA damage-induced crosstalk between SUMOylation and ubiquitylation is not restricted to DSBs (Poulsen et al., 2013; Ragland et al., 2013; Gibbs-Seymour et al., 2015; van Cuijk et al., 2015), and SUMO-targeted ubiquitylation followed by targeted protein removal and/or degradation thus emerges as a common theme in the stepwise progression of DNA repair pathways.

INTERPLAY BETWEEN POLY(ADP-RIBOSE) AND UBIQUITIN

In analogy to SUMO-targeted ubiquitylation by STUbLs it was recently discovered that also PAR serves as recognition signal for selected ubiquitin ligases (Zhang et al., 2011; Zhou et al., 2011; Wang et al., 2012). The best-characterized PAR-targeted ubiquitin ligase (PTUbL) is RNF146/Iduna. By virtue of its PAR-binding WWE domain RNF146 is recruited to PARylated proteins where the WWE–PAR interaction leads to an allosteric activation of its ubiquitin ligase domain (DaRosa et al., 2015). Among the proteins that are ubiquitylated by RNF146 are PARP1, PARP2, KU70, DNA ligase III, and XRCC1 (Kang et al., 2011).

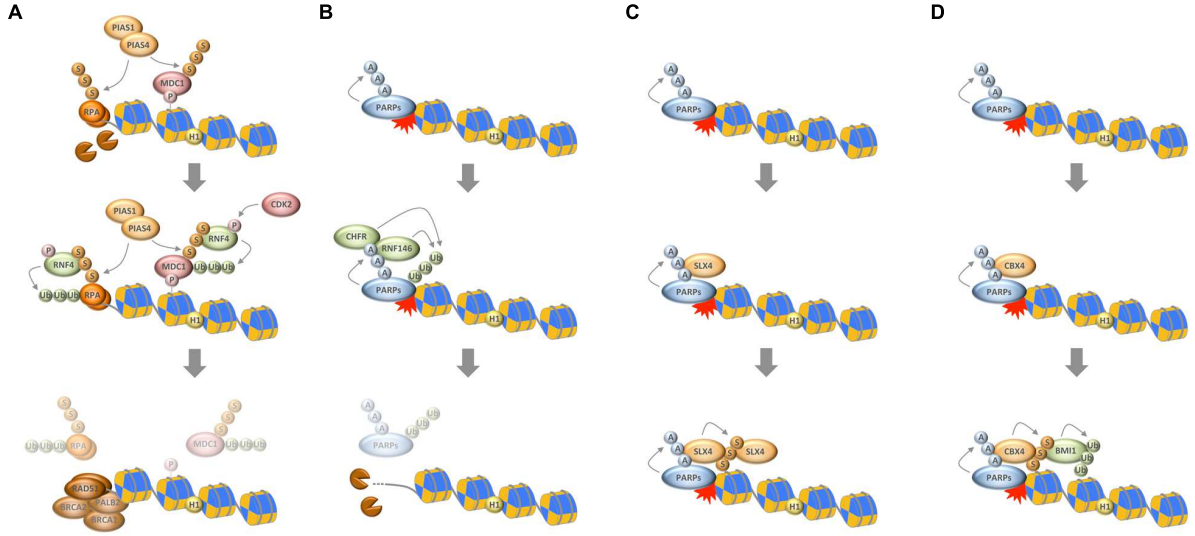

FIGURE 2 | Interplay between chain-like modifications at sites of DNA damage. (A) Interplay between ubiquitylation and SUMOylation through the STUbL RNF4, which ubiquitylates SUMOylated substrates to mediate their timely removal from repair sites. **(B)** Interplay between PARylation and ubiquitylation through the PTUbLs RNF146 and CHFR, which cooperate to dissociate automodified PARP1 from DNA break sites. **(C)** Productive interaction between PARylation and SUMOylation to stabilize the recruitment of the SLX4 complex. **(D)** Interplay between PARylation, SUMOylation, and ubiquitylation via the PAR-responsive SUMO ligase CBX4 and the SUMO-responsive ubiquitin ligase BMI1 to promote chromatin ubiquitylation in response to DNA damage. P, phosphorylation; Ub, ubiquitylation; S, SUMOylation; A, ADP-ribosylation.

Thus, and in parallel to DNA break-induced SUMOylation, also PAR participates in the targeted protein ubiquitylation and turnover at sites of genomic lesions.

While these events likely evolved to prevent excessive interactions of repair factors with DNA break sites, PAR-dependent ubiquitylation also assists the early recruitment of genome caretakers. For instance, PAR formation was shown to be required for the recruitment of the BAL1/BBAP ubiquitin ligase complex, whose activity promotes the retention of the RAP80-BRCA1 complex (Yan et al., 2013). This mechanism seems to act in parallel to the PAR-mediated recruitment of BRCA1 via the PAR-binding BRCT domains of its partner protein BARD1 (Li and Yu, 2013), and is part of the PAR-dependent selective interaction filtering that is observed almost immediately upon DNA damage induction and temporally precedes the full build-up of the RNF8/RNF168-dependent ubiquitin compartment (Altmeyer et al., 2015; Teloni and Altmeyer, 2016). Notably, even at later stages of the chromatin response to DNA damage interplay between PARylation and ubiquitylation seems to exist, because the PAR-dependent recruitment of the chromatin remodeler SMARCA5/SNF2H facilitates RNF168 accumulation and promotes efficient ubiquitin conjugation (Smeenk et al., 2013).

Another RING-type ubiquitin E3 ligase, whose role in genome integrity maintenance is linked to PAR formation, is the mitotic regulator CHFR. A PAR-binding zinc finger motif (PBZ) mediates its interaction with genotoxic stress-induced PAR and was shown to be required for the CHFR-dependent antephase checkpoint (Ahel et al., 2008; Oberoi et al., 2010). Interestingly, the functions of CHFR also feed into histone acetylation and ATM activation (Wu et al., 2011), and mediate the first wave of ubiquitylation in response to DNA damage (Liu et al., 2013). As part of this response, CHFR ubiquitylates PARP1 itself, leading to its dissociation from DNA break sites, thus representing another example of PTUbL-mediated stepwise succession of repair events (Kashima et al., 2012; Liu et al., 2013).

Taken together, PARylation assists the early recruitment of genome caretakers, including various ubiquitin ligases, which further promote chromatin modifications and lead to the formation of a dedicated repair compartment, but it also participates in temporarily restraining protein access to repair sites and in the timely and PTUbL-mediated removal of repair factors once they have fulfilled their duties (**Figure 2B**).

INTERPLAY BETWEEN POLY(ADP-RIBOSE) AND SUMO

The first direct links between PARylation and SUMOylation were described in the context of PARP1-regulated transcription (Martin et al., 2009; Messner et al., 2009), and SUMOylation of PARP1 was indeed found to be largely irresponsive to DNA damage (Zilio et al., 2013). More recently, however, a functional crosstalk between these two chain-like modifications has started to emerge also in the context of genome integrity maintenance. For instance, PARylation of tyrosyl-DNA phosphodiesterase 1 (TDP1) was shown to cooperate with TDP1 SUMOylation to stabilize the protein and promote its function in the repair of trapped topoisomerase I (TOP1) cleavage complexes (Das et al., 2014). Similarly, PARylation and SUMOylation cooperate to recruit and stabilize the SLX4 nuclease scaffold complex, itself a SUMO E3 ligase, at DNA damage sites (**Figure 2C**) (Gonzalez-Prieto et al., 2015; Guervilly et al., 2015). Finally, and as an example for productive interplay between all three chain-like modifications, PARylation is required for the recruitment of CBX4. In a pathway that functions in parallel to the PIAS1/PIAS4-mediated SUMOylation at damaged chromatin, PAR-dependent SUMOylation by CBX4 attracts the polycomb ubiquitin E3 ligase BMI1, which in turn contributes to DNA damage-induced histone ubiquitylation and promotes repair (**Figure 2D**) (Ismail et al., 2010, 2012, 2015; Ginjala et al., 2011). Thus, intriguing examples of close cooperation between catenarian modifications exist, and future findings are likely to shed more light onto the intricate interplay between ubiquitin, SUMO and PAR in the DDR.

CONCLUSION AND OUTLOOK

While distinct in their chemical nature and regulatory mechanism, ubiquitylation, SUMOylation, and PARylation share the feature of being chain-like protein modifications. The composition of modification chains, their length, linkage type and branching frequency contains information that can be used by complex regulatory circuits and signaling pathways such as the DDR. Recent work has elucidated how cells employ a sophisticated sequence of reactions with remarkable temporal and spatial resolution to shield genomic lesions and build up dynamic functional platforms that promote repair. The information content imbedded in this response is immense, and the use of modification chains may thus support the need for lesion-specific chromatin barcodes that dynamically change as repair reactions progress.

The multivalent protein recruitment polymers formed by ubiquitylation, SUMOylation, and PARylation often cooperate to achieve robust responses. To this end, they act successively or in parallel, and frequently use positive feedback loops to amplify the signal and increase its specificity. They also employ time-delayed negative feedback to terminate reactions and disassemble complexes, which are no longer needed and constitute roadblocks for downstream events. While recent work has started to elucidate the crosstalk between these modifications, how their combinatorial use and dynamic interplay reshapes the chromatin environment surrounding different types of genomic lesions, dictates repair pathway decisions, and determines repair fidelity remains incompletely understood. Moreover, almost nothing is known about mixed chain modifications, e.g., PARylation of ubiquitin or SUMO chains, and how they might be employed by the DDR. Quantitative time-resolved proteomics and imaging approaches that provide spatial information about protein redistribution and can relate this to cell cycle information are powerful tools to address these issues. The insights gained will not only deepen our understanding of the DDR, but may also provide additional clues to the mechanisms that underlie

the toxicity of inhibiting chain-like modifications in cancer treatments.

AUTHOR CONTRIBUTIONS

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

FUNDING

Research in the lab of MA is supported by the Swiss National Science Foundation (SNSF Professorship Grant PP00P3_150690) and by the University of Zurich Association Research Talent Development Fund.

ACKNOWLEDGMENTS

We apologize to authors whose work could not be cited due to space limitations. We are thankful to all members of the Altmeyer Lab and of the Department of Molecular Mechanisms of Disease for helpful discussions, and acknowledge support from the Molecular Life Sciences Program of the Life Science Zurich Graduate School.

REFERENCES

Acs, K., Luijsterburg, M. S., Ackermann, L., Salomons, F. A., Hoppe, T., and Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 18, 1345–1350. doi: 10.1038/nsmb.2188

Ahel, D., Horejsi, Z., Wiechens, N., Polo, S. E., Garcia-Wilson, E., Ahel, I., et al. (2009). Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science* 325, 1240–1243. doi: 10.1126/science.1177321

Ahel, I., Ahel, D., Matsusaka, T., Clark, A. J., Pines, J., Boulton, S. J., et al. (2008). Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. *Nature* 451, 81–85. doi: 10.1038/nature06420

Altmeyer, M., and Lukas, J. (2013a). Guarding against collateral damage during chromatin transactions. *Cell* 153, 1431–1434. doi: 10.1016/j.cell.2013.05.044

Altmeyer, M., and Lukas, J. (2013b). To spread or not to spread–chromatin modifications in response to DNA damage. *Curr. Opin. Genet. Dev.* 23, 156–165. doi: 10.1016/j.gde.2012.11.001

Altmeyer, M., Neelsen, K. J., Teloni, F., Pozdnyakova, I., Pellegrino, S., Grofte, M., et al. (2015). Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). *Nat. Commun.* 6, 8088. doi: 10.1038/ncomms9088

Aparicio, T., Baer, R., and Gautier, J. (2014). DNA double-strand break repair pathway choice and cancer. *DNA Repair (Amst.)* 19, 169–175. doi: 10.1016/j.dnarep.2014.03.014

Awasthi, P., Foiani, M., and Kumar, A. (2015). ATM and ATR signaling at a glance. *J. Cell Sci.* 128, 4255–4262. doi: 10.1242/jcs.169730

Beck, C., Robert, I., Reina-San-Martin, B., Schreiber, V., and Dantzer, F. (2014). Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. *Exp. Cell Res.* 329, 18–25. doi: 10.1016/j.yexcr.2014.07.003

Bekker-Jensen, S., and Mailand, N. (2011). The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. *FEBS Lett.* 585, 2914–2919. doi: 10.1016/j.febslet.2011.05.056

Boersma, V., Moatti, N., Segura-Bayona, S., Peuscher, M. H., van der Torre, J., Wevers, B. A., et al. (2015). MAD2L2 controls DNA repair at telomeres and DNA breaks by inhibiting 5′ end resection. *Nature* 521, 537–540. doi: 10.1038/nature14216

Bologna, S., Altmannova, V., Valtorta, E., Koenig, C., Liberali, P., Gentili, C., et al. (2015). Sumoylation regulates EXO1 stability and processing of DNA damage. *Cell Cycle* 14, 2439–2450. doi: 10.1080/15384101.2015.1060381

Callen, E., Di Virgilio, M., Kruhlak, M. J., Nieto-Soler, M., Wong, N., Chen, H. T., et al. (2013). 53BP1 mediates productive and mutagenic DNA repair through distinct phosphoprotein interactions. *Cell* 153, 1266–1280. doi: 10.1016/j.cell.2013.05.023

Chapman, J. R., Barral, P., Vannier, J. B., Borel, V., Steger, M., Tomas-Loba, A., et al. (2013). RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol. Cell* 49, 858–871. doi: 10.1016/j.molcel.2013.01.002

Chapman, J. R., Taylor, M. R., and Boulton, S. J. (2012). Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* 47, 497–510. doi: 10.1016/j.molcel.2012.07.029

Chou, D. M., Adamson, B., Dephoure, N. E., Tan, X., Nottke, A. C., Hurov, K. E., et al. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18475–18480. doi: 10.1073/pnas.1012946107

Chung, I., and Zhao, X. (2015). DNA break-induced sumoylation is enabled by collaboration between a SUMO ligase and the ssDNA-binding complex RPA. *Genes Dev.* 29, 1593–1598. doi: 10.1101/gad.265058.115

Ciccia, A., and Elledge, S. J. (2010). The DNA damage response: making it safe to play with knives. *Mol. Cell* 40, 179–204. doi: 10.1016/j.molcel.2010.09.019

Dantuma, N. P., and van Attikum, H. (2016). Spatiotemporal regulation of posttranslational modifications in the DNA damage response. *EMBO J.* 35, 6–23. doi: 10.15252/embj.201592595

DaRosa, P. A., Wang, Z., Jiang, X., Pruneda, J. N., Cong, F., Klevit, R. E., et al. (2015). Allosteric activation of the RNF146 ubiquitin ligase by a poly(ADP-ribosyl)ation signal. *Nature* 517, 223–226. doi: 10.1038/nature13826

Das, B. B., Huang, S. Y., Murai, J., Rehman, I., Ame, J. C., Sengupta, S., et al. (2014). PARP1-TDP1 coupling for the repair of topoisomerase I-induced DNA damage. *Nucleic Acids Res.* 42, 4435–4449. doi: 10.1093/nar/gku088

Davis, A. J., Chen, B. P. C., and Chen, D. J. (2014). DNA-PK: a dynamic enzyme in a versatile DSB repair pathway. *DNA Repair* 17, 21–29. doi: 10.1016/j.dnarep.2014.02.020

Di Virgilio, M., Callen, E., Yamane, A., Zhang, W., Jankovic, M., Gitlin, A. D., et al. (2013). Rif1 prevents resection of DNA breaks and promotes immunoglobulin class switching. *Science* 339, 711–715. doi: 10.1126/science.1230624

Eifler, K., and Vertegaal, A. C. (2015). SUMOylation-mediated regulation of cell cycle progression and cancer. *Trends Biochem. Sci.* 40, 779–793. doi: 10.1016/j.tibs.2015.09.006

Escribano-Diaz, C., Orthwein, A., Fradet-Turcotte, A., Xing, M., Young, J. T., Tkac, J., et al. (2013). A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol. Cell* 49, 872–883. doi: 10.1016/j.molcel.2013.01.001

Fradet-Turcotte, A., Canny, M. D., Escribano-Diaz, C., Orthwein, A., Leung, C. C. Y., Huang, H., et al. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 499, 50–54. doi: 10.1038/nature12318

Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012). RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* 26, 1179–1195. doi: 10.1101/gad.188284.112

Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009). Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* 462, 935–939. doi: 10.1038/nature08657

Gatti, M., Pinato, S., Maiolica, A., Rocchio, F., Prato, M. G., Aebersold, R., et al. (2015). RNF168 promotes noncanonical K27 ubiquitination to signal DNA damage. *Cell Rep.* 10, 226–238. doi: 10.1016/j.celrep.2014.12.021

Gibbs-Seymour, I., Oka, Y., Rajendra, E., Weinert, B. T., Passmore, L. A., Patel, K. J., et al. (2015). Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. *Mol. Cell* 57, 150–164. doi: 10.1016/j.molcel.2014.12.001

Ginjala, V., Nacerddine, K., Kulkarni, A., Oza, J., Hill, S. J., Yao, M., et al. (2011). BMI1 Is recruited to DNA breaks and contributes to DNA damage-induced H2A ubiquitination and repair. *Mol. Cell. Biol.* 31, 1972–1982. doi: 10.1128/Mcb.00981-10

Golia, B., Singh, H. R., and Timinszky, G. (2015). Poly-ADP-ribosylation signaling during DNA damage repair. *Front. Biosci.* 20:440–457. doi: 10.2741/4318

Gong, F. D., and Miller, K. M. (2013). Mammalian DNA repair: HATs and HDACs make their mark through histone acetylation. *Mutat. Res.* 750, 23–30. doi: 10.1016/j.mrfmmm.2013.07.002

Gonzalez-Prieto, R., Cuijpers, S. A., Luijsterburg, M. S., van Attikum, H., and Vertegaal, A. C. (2015). SUMOylation and PARylation cooperate to recruit and stabilize SLX4 at DNA damage sites. *EMBO Rep.* 16, 512–519. doi: 10.15252/embr.201440017

Gottschalk, A. J., Timinszky, G., Kong, S. E., Jin, J., Cai, Y., Swanson, S. K., et al. (2009). Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13770–13774. doi: 10.1073/pnas.0906920106

Gudjonsson, T., Altmeyer, M., Savic, V., Toledo, L., Dinant, C., Grofte, M., et al. (2012). TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. *Cell* 150, 697–709. doi: 10.1016/j.cell.2012.06.039

Guervilly, J. H., Takedachi, A., Naim, V., Scaglione, S., Chawhan, C., Lovera, Y., et al. (2015). The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. *Mol. Cell* 57, 123–137. doi: 10.1016/j.molcel.2014.11.014

Haince, J. F., McDonald, D., Rodrigue, A., Dery, U., Masson, J. Y., Hendzel, M. J., et al. (2008). PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. *J. Biol. Chem.* 283, 1197–1208. doi: 10.1074/jbc.M706734200

Hendriks, I. A., Treffers, L. W., Verlaan-de Vries, M., Olsen, J. V., and Vertegaal, A. C. (2015). SUMO-2 orchestrates chromatin modifiers in response to DNA damage. *Cell Rep.* doi: 10.1016/j.celrep.2015.02.033 [Epub ahead of print].

Hendzel, M. J., and Greenberg, R. A. (2013). Conversations between chromatin modifications and DNA double strand break repair: a commentary. *Mutat. Res.* 750, 1–4. doi: 10.1016/j.mrfmmm.2013.08.003

Ismail, I. H., Andrin, C., McDonald, D., and Hendzel, M. J. (2010). BMI1-mediated histone ubiquitylation promotes DNA double-strand break repair. *J. Cell Biol.* 191, 45–60. doi: 10.1083/jcb.201003034

Ismail, I. H., Gagne, J. P., Caron, M. C., McDonald, D., Xu, Z., Masson, J. Y., et al. (2012). CBX4-mediated SUMO modification regulates BMI1 recruitment at sites of DNA damage. *Nucleic Acids Res.* 40, 5497–5510. doi: 10.1093/nar/gks222

Ismail, I. H., Gagne, J. P., Genois, M. M., Strickfaden, H., McDonald, D., Xu, Z., et al. (2015). The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. *Nat. Cell Biol.* 17, 1446–1457. doi: 10.1038/ncb3259

Izhar, L., Adamson, B., Ciccia, A., Lewis, J., Pontano-Vaites, L., Leng, Y., et al. (2015). A systematic analysis of factors localized to damaged chromatin reveals PARP-dependent recruitment of transcription factors. *Cell Rep* 11, 1486–1500. doi: 10.1016/j.celrep.2015.04.053

Jackson, S. P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature* 461, 1071–1078. doi: 10.1038/nature08467

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Kang, H. C., Lee, Y. I., Shin, J. H., Andrabi, S. A., Chi, Z., Gagné, J. P., et al. (2011). Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14103–14108. doi: 10.1073/pnas.1108799108

Kashima, L., Idogawa, M., Mita, H., Shitashige, M., Yamada, T., Ogi, K., et al. (2012). CHFR protein regulates mitotic checkpoint by targeting PARP-1 protein for ubiquitination and degradation. *J. Biol. Chem.* 287, 12975–12984. doi: 10.1074/jbc.M111.321828

Kim, H., and D'Andrea, A. D. (2012). Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. *Genes Dev.* 26, 1393–1408. doi: 10.1101/gad.195248.112

Krietsch, J., Rouleau, M., Pic, E., Ethier, C., Dawson, T. M., Dawson, V. L., et al. (2013). Reprogramming cellular events by poly(ADP-ribose)-binding proteins. *Mol. Aspects Med.* 34, 1066–1087. doi: 10.1016/j.mam.2012.12.005

Kuo, C. Y., Li, X., Stark, J. M., Shih, H. M., and Ann, D. K. (2016). RNF4 regulates DNA double-strand break repair in a cell cycle-dependent manner. *Cell Cycle* 15, 787–798. doi: 10.1080/15384101.2016.1138184

Li, M., and Yu, X. (2013). Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation. *Cancer Cell* 23, 693–704. doi: 10.1016/j.ccr.2013.03.025

Liu, C., Wu, J., Paudyal, S. C., You, Z., and Yu, X. (2013). CHFR is important for the first wave of ubiquitination at DNA damage sites. *Nucleic Acids Res.* 41, 1698–1710. doi: 10.1093/nar/gks1278

Lord, C. J., and Ashworth, A. (2016). BRCAness revisited. *Nat. Rev. Cancer* 16, 110–120. doi: 10.1038/nrc.2015.21

Lukas, J., Lukas, C., and Bartek, J. (2011). More than just a focus: the chromatin response to DNA damage and its role in genome integrity maintenance. *Nat. Cell Biol.* 13, 1161–1169. doi: 10.1038/ncb2344

Luo, K., Deng, M., Li, Y., Wu, C., Xu, Z., Yuan, J., et al. (2015). CDK-mediated RNF4 phosphorylation regulates homologous recombination in S-phase. *Nucleic Acids Res.* 43, 5465–5475. doi: 10.1093/nar/gkv434

Luo, K., Zhang, H., Wang, L., Yuan, J., and Lou, Z. (2012). Sumoylation of MDC1 is important for proper DNA damage response. *EMBO J.* 31, 3008–3019. doi: 10.1038/emboj.2012.158

Mallette, F. A., Mattiroli, F., Cui, G., Young, L. C., Hendzel, M. J., Mer, G., et al. (2012). RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J.* 31, 1865–1878. doi: 10.1038/emboj.2012.47

Marechal, A., and Zou, L. (2013). DNA damage sensing by the ATM and ATR kinases. *Cold Spring Harb. Perspect. Biol.* 5:a012716. doi: 10.1101/cshperspect.a012716

Marteijn, J. A., Lans, H., Vermeulen, W., and Hoeijmakers, J. H. (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, N., Schwamborn, K., Schreiber, V., Werner, A., Guillier, C., Zhang, X. D., et al. (2009). PARP-1 transcriptional activity is regulated by sumoylation upon heat shock. *EMBO J.* 28, 3534–3548. doi: 10.1038/emboj.2009.279

Mattiroli, F., Vissers, J. H., van Dijk, W. J., Ikpa, P., Citterio, E., Vermeulen, W., et al. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150, 1182–1195. doi: 10.1016/j.cell.2012.08.005

Meerang, M., Ritz, D., Paliwal, S., Garajova, Z., Bosshard, M., Mailand, N., et al. (2011). The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks. *Nat. Cell Biol.* 13, 1376–1382. doi: 10.1038/ncb2367

Messick, T. E., and Greenberg, R. A. (2009). The ubiquitin landscape at DNA double-strand breaks. *J. Cell Biol.* 187, 319–326. doi: 10.1083/jcb.200908074

Messner, S., Schuermann, D., Altmeyer, M., Kassner, I., Schmidt, D., Schar, P., et al. (2009). Sumoylation of poly(ADP-ribose) polymerase 1 inhibits its acetylation and restrains transcriptional coactivator function. *FASEB J.* 23, 3978–3989. doi: 10.1096/fj.09-137695

Morris, J. R., Boutell, C., Keppler, M., Densham, R., Weekes, D., Alamshah, A., et al. (2009). The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. *Nature* 462, 886–890. doi: 10.1038/nature08593

Oberoi, J., Richards, M. W., Crumpler, S., Brown, N., Blagg, J., and Bayliss, R. (2010). Structural basis of poly(ADP-ribose) recognition by the multizinc binding domain of checkpoint with forkhead-associated and RING Domains (CHFR). *J. Biol. Chem.* 285, 39348–39358. doi: 10.1074/jbc.M110.159855

O'Connor, M. J. (2015). Targeting the DNA damage response in cancer. *Mol. Cell* 60, 547–560. doi: 10.1016/j.molcel.2015.10.040

Panier, S., and Boulton, S. J. (2014). Double-strand break repair: 53BP1 comes into focus. *Nat. Rev. Mol. Cell Biol.* 15, 7–18. doi: 10.1038/nrm3719

Panier, S., and Durocher, D. (2013). Push back to respond better: regulatory inhibition of the DNA double-strand break response. *Nat. Rev. Mol. Cell Biol.* 14, 661–672. doi: 10.1038/nrm3659

Patel, A., Lee, H. O., Jawerth, L., Maharana, S., Jahnel, M., Hein, M. Y., et al. (2015). A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. *Cell* 162, 1066–1077. doi: 10.1016/j.cell.2015.07.047

Paull, T. T. (2015). Mechanisms of ATM activation. *Annu. Rev. Biochem.* 84, 711–738. doi: 10.1146/annurev-biochem-060614-034335

Pinder, J. B., Attwood, K. M., and Dellaire, G. (2013). Reading, writing, and repair: the role of ubiquitin and the ubiquitin-like proteins in DNA damage signaling and repair. *Front. Genet.* 4:45. doi: 10.3389/fgene.2013.00045

Polo, S. E., and Jackson, S. P. (2011). Dynamics of DNA damage response proteins at DNA breaks: a focus on protein modifications. *Genes Dev.* 25, 409–433. doi: 10.1101/gad.2021311

Polo, S. E., Kaidi, A., Baskcomb, L., Galanty, Y., and Jackson, S. P. (2010). Regulation of DNA-damage responses and cell-cycle progression by the chromatin remodelling factor CHD4. *EMBO J.* 29, 3130–3139. doi: 10.1038/emboj.2010.188

Poulsen, S. L., Hansen, R. K., Wagner, S. A., van Cuijk, L., van Belle, G. J., Streicher, W., et al. (2013). RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* 201, 797–807. doi: 10.1083/jcb.201212075

Price, B. D., and D'Andrea, A. D. (2013). Chromatin remodeling at DNA double-strand breaks. *Cell* 152, 1344–1354. doi: 10.1016/j.cell.2013.02.011

Ragland, R. L., Patel, S., Rivard, R. S., Smith, K., Peters, A. A., Bielinsky, A. K., et al. (2013). RNF4 and PLK1 are required for replication fork collapse in ATR-deficient cells. *Genes Dev.* 27, 2259–2273. doi: 10.1101/gad.223180.113

Rulten, S. L., Cortes-Ledesma, F., Guo, L., Iles, N. J., and Caldecott, K. W. (2008). APLF (C2orf13) is a novel component of poly(ADP-ribose) signaling in mammalian cells. *Mol. Cell. Biol.* 28, 4620–4628. doi: 10.1128/MCB.02243-07

Sarangi, P., Steinacher, R., Altmannova, V., Fu, Q., Paull, T. T., Krejci, L., et al. (2015). Sumoylation influences DNA break repair partly by increasing the solubility of a conserved end resection protein. *PLoS Genet.* 11:e1004899. doi: 10.1371/journal.pgen.1004899

Schmidt, C. K., Galanty, Y., Sczaniecka-Clift, M., Coates, J., Jhujh, S., Demir, M., et al. (2015). Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. *Nat. Cell Biol.* 17, 1458–1470. doi: 10.1038/ncb3260

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

Smeenk, G., Wiegant, W. W., Marteijn, J. A., Luijsterburg, M. S., Sroczynski, N., Costelloe, T., et al. (2013). Poly(ADP-ribosyl)ation links the chromatin remodeler SMARCA5/SNF2H to RNF168-dependent DNA damage signaling. *J. Cell Sci.* 126, 889–903. doi: 10.1242/jcs.109413

Stracker, T. H., Usui, T., and Petrini, J. H. (2009). Taking the time to make important decisions: the checkpoint effector kinases Chk1 and Chk2 and the DNA damage response. *DNA Repair (Amst.)* 8, 1047–1054. doi: 10.1016/j.dnarep.2009.04.012

Teloni, F., and Altmeyer, M. (2016). Readers of poly(ADP-ribose): designed to be fit for purpose. *Nucleic Acids Res.* 44, 993–1006. doi: 10.1093/nar/gkv1383

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., et al. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393. doi: 10.1038/nature15401

Timinszky, G., Till, S., Hassa, P. O., Hothorn, M., Kustatscher, G., Nijmeijer, B., et al. (2009). A macrodomain-containing histone rearranges chromatin upon sensing PARP1 activation. *Nat. Struct. Mol. Biol.* 16, 923–929. doi: 10.1038/nsmb.1664

Ulrich, H. D. (2014). Two-way communications between ubiquitin-like modifiers and DNA. *Nat. Struct. Mol. Biol.* 21, 317–324. doi: 10.1038/nsmb.2805

van Attikum, H., and Gasser, S. M. (2009). Crosstalk between histone modifications during the DNA damage response. *Trends Cell Biol.* 19, 207–217. doi: 10.1016/j.tcb.2009.03.001

van Cuijk, L., van Belle, G. J., Turkyilmaz, Y., Poulsen, S. L., Janssens, R. C., Theil, A. F., et al. (2015). SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nat. Commun.* 6, 7499. doi: 10.1038/ncomms8499

Wang, J., Aroumougame, A., Lobrich, M., Li, Y., Chen, D., Chen, J., et al. (2014). PTIP associates with Artemis to dictate DNA repair pathway choice. *Genes Dev.* 28, 2693–2698. doi: 10.1101/gad.252478.114

Wang, Z., Michaud, G. A., Cheng, Z., Zhang, Y., Hinds, T. R., Fan, E., et al. (2012). Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WWE domains suggests a general mechanism for poly(ADP-ribosyl)ation-dependent ubiquitination. *Genes Dev.* 26, 235–240. doi: 10.1101/gad.182618.111

Wu, J., Chen, Y., Lu, L. Y., Wu, Y., Paulsen, M. T., Ljungman, M., et al. (2011). Chfr and RNF8 synergistically regulate ATM activation. *Nat. Struct. Mol. Biol.* 18, 761–768. doi: 10.1038/nsmb.2078

Xu, G., Chapman, J. R., Brandsma, I., Yuan, J., Mistrik, M., Bouwman, P., et al. (2015). REV7 counteracts DNA double-strand break resection and affects PARP inhibition. *Nature* 521, 541–544. doi: 10.1038/nature14328

Yan, Q., Xu, R., Zhu, L., Cheng, X., Wang, Z., Manis, J., et al. (2013). BAL1 and its partner E3 ligase, BBAP, link Poly(ADP-ribose) activation, ubiquitylation, and double-strand DNA repair independent of ATM, MDC1, and RNF8. *Mol. Cell. Biol.* 33, 845–857. doi: 10.1128/mcb.00990-12

Yin, Y. L., Seifert, A., Chua, J. S., Maure, J. F., Golebiowski, F., and Hay, R. T. (2012). SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. *Genes Dev.* 26, 1196–1208. doi: 10.1101/gad.189274.112

Zhang, F., Shi, J., Bian, C., and Yu, X. (2015). Poly(ADP-Ribose) mediates the BRCA2-dependent early DNA damage response. *Cell Rep.* 13, 678–689. doi: 10.1016/j.celrep.2015.09.040

Zhang, Y., Liu, S., Mickanin, C., Feng, Y., Charlat, O., Michaud, G. A., et al. (2011). RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. *Nat. Cell Biol.* 13, 623–629. doi: 10.1038/ncb2222

Zhou, Z. D., Chan, C. H., Xiao, Z. C., and Tan, E. K. (2011). Ring finger protein 146/Iduna is a poly(ADP-ribose) polymer binding and PARsylation dependent E3 ubiquitin ligase. *Cell Adh. Migr.* 5, 463–471. doi: 10.4161/cam.5.6.18356

Zilio, N., Williamson, C. T., Eustermann, S., Shah, R., West, S. C., Neuhaus, D., et al. (2013). DNA-dependent SUMO modification of PARP-1. *DNA Repair (Amst.)* 12, 761–773. doi: 10.1016/j.dnarep.2013.07.001

Zimmermann, M., Lottersberger, F., Buonomo, S. B., Sfeir, A., and de Lange, T. (2013). 53BP1 regulates DSB repair using Rif1 to control 5′ end resection. *Science* 339, 700–704. doi: 10.1126/science.1231573

Conflict of Interest Statement: 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.

frontiers in Genetics

REVIEW
published: 19 April 2016
doi: 10.3389/fgene.2016.00061

How SUMOylation Fine-Tunes the Fanconi Anemia DNA Repair Pathway

*Kate E. Coleman and Tony T. Huang**

Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA

Fanconi anemia (FA) is a rare human genetic disorder characterized by developmental defects, bone marrow failure and cancer predisposition, primarily due to a deficiency in the repair of DNA interstrand crosslinks (ICLs). ICL repair through the FA DNA repair pathway is a complicated multi-step process, involving at least 19 FANC proteins and coordination of multiple DNA repair activities, including homologous recombination, nucleotide excision repair and translesion synthesis (TLS). SUMOylation is a critical regulator of several DNA repair pathways, however, the role of this modification in controlling the FA pathway is poorly understood. Here, we summarize recent advances in the fine-tuning of the FA pathway by small ubiquitin-like modifier (SUMO)-targeted ubiquitin ligases (STUbLs) and other SUMO-related interactions, and discuss the implications of these findings in the design of novel therapeutics for alleviating FA-associated condition, including cancer.

Keywords: Fanconi anemia (FA), SUMOylation, SUMO-targeted ubiquitin ligases (STUbLs), SUMO proteases (SENPs), SUMO-interaction motifs (SIMs), SUMO-like domains (SLDs), deubiquitinases (DUBs), DNA interstrand crosslinks (ICLs)

OPEN ACCESS

Edited by:
Kristijan Ramadan, University of Oxford, UK

Reviewed by:
Richard Chahwan, Albert Einstein College of Medicine, USA
Andrew N. Blackford, University of Cambridge, UK

****Correspondence:***
Tony T. Huang
tony.huang@nyumc.org

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *03 March 2016*
Accepted: *01 April 2016*
Published: *19 April 2016*

Citation:
Coleman KE and Huang TT (2016) How SUMOylation Fine-Tunes the Fanconi Anemia DNA Repair Pathway. Front. Genet. 7:61. doi: 10.3389/fgene.2016.00061

FANCONI ANEMIA (FA) DNA REPAIR PATHWAY

Fanconi anemia is a very rare autosomal recessive disease (occurring in just 1 of every 100,000 births), typically distinguished by bone marrow abnormalities, cancer predisposition, and congenital anomalies (Kee and D'Andrea, 2012). Patients with this disease exhibit defects in the repair of interstrand crosslinks (ICLs), DNA lesions which hook bases of opposing DNA strands together, and are consequently hypersensitive to crosslinking agents such as cisplatin, diepoxybutane (DEB), and mitomycin C (MMC) (Noll et al., 2006). Despite the low prevalence of the FA disease, the complex pathway involved in the recognition and repair of ICLs orchestrates multiple DNA repair processes, such as homologous recombination (HR) and translesion synthesis (TLS), making FA an important model in the study of DNA repair signaling.

To date, 19 FANC proteins have been identified in the FA pathway, which fall into three distinct functional groups. One group is the FA core complex, consisting of FANCA, B, C, E, F, G, L, and M, which together with three Fanconi associated proteins (FAAP20, FAAP24, and FAAP100) functions as an E3 ubiquitin ligase (Garcia-Higuera et al., 2001; Meetei et al., 2003; Ciccia et al., 2007; Ling et al., 2007; Leung et al., 2012). Upon pathway induction by DNA damage or replication stress, the targeting components of the FA core, FANCM and FAAP24, bind chromatin and recruit the FA core complex (Qiao et al., 2001; Kim et al., 2008). A histone-fold-containing complex consisting of MHF1-MHF2 proteins facilitates the recruitment of FANCM to chromatin to enhance

pathway activation (Singh et al., 2010; Yan et al., 2010). The FA core complex subsequently monoubiquitylates group II proteins, FANC**I** and FANC**D2**, which associate to form a heterodimer called the ID complex (Garcia-Higuera et al., 2001; Sims et al., 2007; Smogorzewska et al., 2007). This monoubiquitylation of the ID complex localizes it to chromatin, where it recruits the group III effector proteins to initiate downstream ICL repair.

Following ID monoubiquitylation, a complex series of steps ensues to complete ICL repair. First, the mono-ubiquitinated ID complex serves as a scaffold for recruitment of several nucleases, which make nucleolytic incisions flanking the ICL to unhook the crosslink. These nucleases include SLX4 (discussed in more detail below), ERCC1-XPF1, and MUS81-EME1 structure-specific endonucleases (Hanada et al., 2006; Knipscheer et al., 2009; Stoepker et al., 2011; Klein Douwel et al., 2014). Cross-linked nucleotides on the complementary strand are subsequently bypassed by the process of TLS, which involves specialized Y-family polymerases such as polζ and REV1 (Räschle et al., 2008; Kim et al., 2012; Budzowska et al., 2015). The nucleolytic incisions result in a double strand break (DSB) in the DNA, which is repaired by HR (discussed below), and nucleotide excision repair (NER) fills the remaining gap (Räschle et al., 2008; Kim and D'Andrea, 2012). Finally, in the last step, the deubiquitinating (DUB) complex USP1-UAF1 removes monoubiquitin from the ID complex, allowing for pathway regeneration (Nijman et al., 2005; Cohn et al., 2007) (**Figure 1**). For more extensive reviews on the steps of the FA pathway, please refer to the following reviews: (Kim and D'Andrea, 2012; Walden and Deans, 2014; Duxin and Walter, 2015). Despite extensive study, it is likely that additional aspects of FA pathway activation and regulation remain unidentified, prompting recent studies of other regulatory proteins and post-translational modifications (PTMs) involved in the FA pathway.

CONTROL OF SIGNALING PATHWAYS BY SUMOylation

In addition to ubiquitin signaling, modification of proteins by small ubiquitin-like modifier (SUMO) has been implicated in several aspects of cellular signaling. Similar to protein ubiquitylation, the process of activating and conjugating SUMO modifications to substrates involves an E1-E2-E3 enzyme cascade. SUMO proteins are initially translated as immature precursors that must be processed by proteases to a mature form containing a C-terminal diglycine motif. This mature form of SUMO subsequently becomes bound by an E1 enzyme that activates SUMO through sequential adenylation and thioester bond formation. SUMO is then passed to a single E2 conjugating enzyme, ubiquitin-like conjugating enzyme 9 (Ubc9), that transfers the SUMO to substrate accepter lysine residues with the help of E3 protein ligases. Protein SUMOylation is mediated by several SUMO E3 ligases in mammalian cells, including those of the PIAS family: PIAS1, PIAS2 (PIASxα/β), PIAS3, and PIAS4 (PIASy). These E3 enzymes facilitate SUMO conjugation either by promoting specificity to substrate recruitment by E2∼SUMO, or by stimulating the discharge of SUMO to substrates (Gareau and Lima, 2010). The E2 enzyme Ubc9 recognizes its substrates through the consensus SUMOylation motif, ΨKxE/D, where Ψ is a large hydrophobic residue (Rodriguez et al., 2001). Conversely, SUMO can also be de-conjugated from substrates through the activity of SUMO specific proteases (SENPs), making this a highly dynamic modification analogous to ubiquitylation and phosphorylation (Kumar and Zhang, 2015).

Another family of proteins called SUMO-targeted ubiquitin ligases (STUbLs) has also been characterized recently that connects the processes of SUMOylation with ubiquitylation. Uniquely, STUbL enzymes recognize their substrates through SUMO-interaction motifs (SIMs), short hydrophobic peptide sequences that mediate non-covalent attachments with SUMO (Perry et al., 2008). Often, STUbL enzymes promote the specific recruitment of proteins containing both ubiquitin-interacting motifs (UIMs) and SIMs through the synthesis of SUMO-ubiquitin conjugate chains. As an example, the RAP80 subunit of the BRCA1 complex uses both its SIM and UIM motifs to bind SUMO-ubiquitin conjugate chains synthesized by the STUbL RNF4 for efficient recruitment to DSBs immediately following DNA damage (Guzzo et al., 2012; Hu et al., 2012). By promoting the degradation of previously SUMOylated target proteins, STUbL enzymes play important roles as global regulators of SUMOylation levels. Imbalances in global protein SUMOylation levels can have several adverse cellular consequences, including genome instability and sensitivity to genotoxic stress (Perry et al., 2008).

Small ubiquitin-like modifier and SUMO-like modifications can influence a wide variety of cellular signaling pathways by directing changes in protein-protein interactions, altering protein intracellular localization, directing protein turnover (via STUbL enzymes described above), or changing protein activity. Despite its importance in these other signaling pathways (reviewed in Gareau and Lima, 2010), a functional role for ubiquitin-SUMO crosstalk in the Fanconi Anemia pathway has not been defined until recently. This review highlights new evidence of FA pathway modulation by SUMO modifications and SUMO-like interactions, and describes the impact of these observations on our understanding of FA pathway regulation and disease treatment.

ROLE OF FANCA SUMOylation

One example of how SUMOylation contributes to FA pathway regulation is through promoting the polyubiquitylation of FA core complex member FANCA. A recent study from the D'Andrea lab identified a patient with a point mutation in FANCA ($FANCA^{I935S}$) that fails to bind the FAAP20 subunit of the FA core complex, leading to decreased FANCA protein levels. In uncovering the mechanism behind this decreased stability of FANCA, the authors discovered that defective FAAP20 binding by the FANCA mutant leads to increased exposure of a SUMOylation site on FANCA at residue K921, which in turn promotes UBC9-mediated SUMOylation, polyubiquitylation by the STUbL RNF4, and proteasome-dependent degradation of

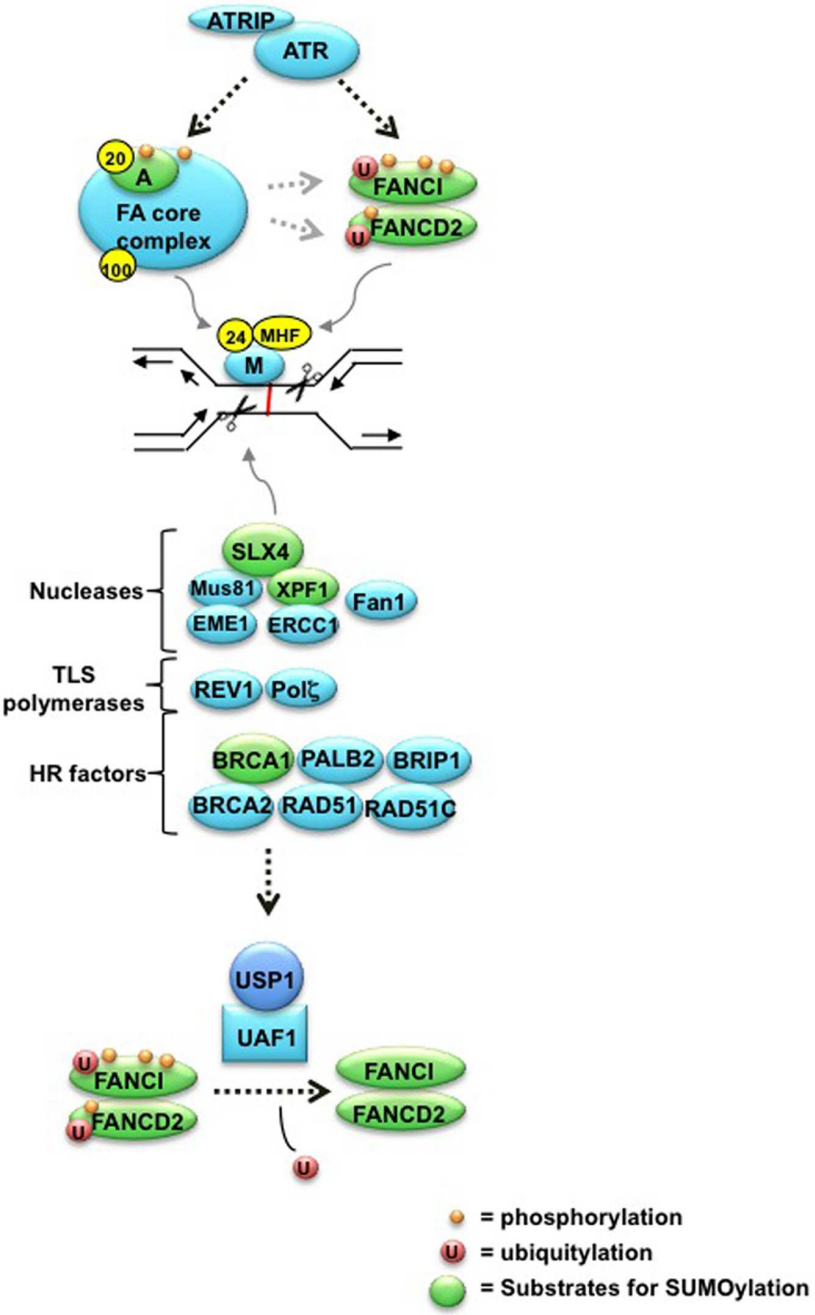

FIGURE 1 | The ICL repair pathway. The FANCM-FAAP24 complex first recognizes stalled replication structures, and recruits the FA core complex to the ICL. ATR-Chk1 phosphorylates and activates multiple components, including FANCA, E, I, and D2, which is required for monoubiquitylation of the ID complex by the FA core complex. Upon monoubiquitylation, the activated ID complex localizes to chromatin and coordinates the activities of downstream nucleases that create incisions around the crosslink. The resulting structure is then repaired by multiple processes, involving lesion bypass by TLS polymerases, HR, and NER. Finally, the USP1-UAF1 DUB complex removes monoubiquitin from the ID complex to complete repair. See accompanying text for more details. Yellow spheres indicate Fanconi Anemia-associated proteins (FAAPs) FAAP20, FAAP24, and FAAP100. Targets for SUMOylation highlighted in this review are denoted in green.

FANCA. Failure of the FANCA mutant to bind FAAP20 still allows efficient FANCD2 monoubiquitylation, but leads to an inability to properly recruit the REV1 translesion polymerase (Kim et al., 2012), contributing to lower rates of TLS-mediated mutagenesis. On the other hand, the authors showed that wild-type FANCA is also subject to SUMOylation and RNF4-mediated polyubiquitylation, but to a lesser degree, and depletion of RNF4 contributed to increased MMC sensitivity

(Xie et al., 2015) (**Figure 2A**). Taken together, these results indicate that the regulated release of FAAP20 from FANCA is a normal critical step in the FA pathway and suggest that failure to properly release FANCA from the FA core complex could contribute to pathway disruption and genome instability.

SUMOylation OF THE ID COMPLEX FOR THE FA PATHWAY

As discussed previously, ID complex monoubiquitylation is removed by the USP1-UAF1 (WDR48) DUB complex, allowing for FA pathway regeneration (Nijman et al., 2005; Cohn et al., 2007). Evidence shows that depletion of USP1 in either murine models or chicken DT40 cells enhances chromatin loading of the ID complex in the absence of exogenous DNA damage (Oestergaard et al., 2007; Kim et al., 2009), but only to similar levels as observed following mitomycin C (MMC) treatment, suggesting the existence of alternative mechanisms to restrain ID complex loading at DNA lesions. Work by Gibbs-Seymour et al. (2015) demonstrates that direct SUMOylation of the ID complex can also stimulate the removal of activated ID complexes at sites of damage, thereby controlling ID complex dosage at DNA lesions. ID complex SUMOylation is dependent on the ATR kinase and two SUMO E3 ligases, PIAS1 and PIAS4, and is antagonized by the SUMO protease SENP6. Following SUMOylation, the STUbL RNF4 targets the ID complex for polyubiquitylation, ultimately promoting ID complex chromatin extraction from DNA lesions by the DVC1-p97 ubiquitin segregase complex. This ubiquitin-SUMO network thereby helps fine-tune ID complex recruitment to DNA damage sites. The authors further show that dysregulation of this process through expression of a SUMOylation-deficient FANCI mutant results in increased DNA damage and MMC sensitivity, illustrating the importance of this mechanism for limiting ID complex dosage at DNA lesions (Gibbs-Seymour et al., 2015) (**Figure 2B**).

An obvious follow-up question to this study is: why is it so important to precisely regulate ID complex dosage at DNA damage sites by this elaborate mechanism? The authors suggest that this regulation by ubiquitin-SUMO crosstalk could help prevent excessive nucleolytic processing of DNA, downstream of ID complex monoubiquitylation. Like USP1-mediated deubiquination, SUMOylation of ID proteins could also be an important mechanism allowing for ID complex recycling and FA pathway regeneration. Future work will be needed to further understand mechanistically how dysregulation of ID complex dosage at DNA damage sites contributes to genome instability.

SLX4 ACTS AS A SUMO E3 LIGASE

One of the key steps in ICL repair is nucleolytic excision of the cross-link and downstream repair of the resulting DSB by HR. A key player that coordinates these complex repair processes is the SLX4 protein. Together with its activating subunit, SLX1, SLX4 associates with XPF-ERCC1 and MUS81-EME1 structure-specific endonucleases to cleave branched DNA structures (Andersen et al., 2009; Munoz et al., 2009; Stoepker et al., 2011). SLX4 additionally interacts with the mismatch repair proteins MSH2-MSH3 and is required for telomere stability through association with TRF2 (Wan et al., 2013). SLX1-SLX4 complexes serve as HJ resolvases and process multiple recombination intermediates (Fekairi et al., 2009). Thus, SLX4 serves as a scaffold for many different nucleases involved in ICL and HR repair, but our understanding of how these interactions are orchestrated to direct specific repair outcomes has been limited.

New studies from the Gaillard, Vertegaal, and Zou labs have shed light on SLX4 regulation, showing that SUMO and ubiquitin modifications are involved in directing the different activities of the SLX4 complex. Guervilly et al. (2015) made the surprising discovery that the SLX4 complex is a SUMO E3 ligase that SUMOylates SLX4 itself. SUMOylation by SLX4 is dependent on an interaction with the charged UBC9∼SUMO E2 enzyme as well as newly identified SIMs in SLX4. Studies from the Vertegaal lab identified three such SIMs in the SLX4 protein and showed that these motifs were critical for proper ICL repair and targeting of SLX4 to PML nuclear bodies and laser-induced DNA damage sites (González-Prieto et al., 2015). In addition to SUMOylating itself, SLX4 also targets the XPF subunit of the repair endonuclease XPF-ERCC1 for SUMO modification (Guervilly et al., 2015). Unexpectedly, the BTB domain of SLX4, which facilitates XPF targeting (Andersen et al., 2009), was specifically required for SUMOylation of XPF *in vivo* and *in vitro*, showing an additional role for the BTB domain outside of XPF binding (**Figure 2C**). At one extreme, chronic overexpression of SLX4 induces global replication stress and is extremely cytotoxic, which the authors suggest are consequences of extensive nucleolytic processing and chromatid breakage. On the other hand, the SUMO E3 ligase activity of SLX4 facilitates expression of common fragile sites (CFS), unstable genomic loci that are difficult to replicate. In fact, failure to localize SLX4 to CFS is associated with increased anaphase bridges and mitotic catastrophe. Therefore, on a global scale, increased SUMO ligase activity of SLX4 is detrimental as it contributes to replication stress, but is necessary to prevent mitotic catastrophe following CFS expression (Guervilly et al., 2015).

Ouyang et al. (2015) made similar findings about the involvement of SUMOylation in SLX4 activity. Namely, they also discovered that SLX4 binds SUMO2/3 chains via SIMs in a manner dependent on charged UBC9, and that SUMO interactions are important for suppressing fragile site instability and processing of CPT-induced replication intermediates. However, the authors extend these findings by directly comparing SLX4 targeting via its ubiquitin-binding zinc finger (UBZ) domains versus its SIM domains. While the UBZs of SLX4 are critical in ICL repair, the SIMs of SLX4 are instead more important for binding DNA damage sensors such as RPA, the MRN complex, and the telomere binding protein TRF2. Thus, the UBZs and SIMs

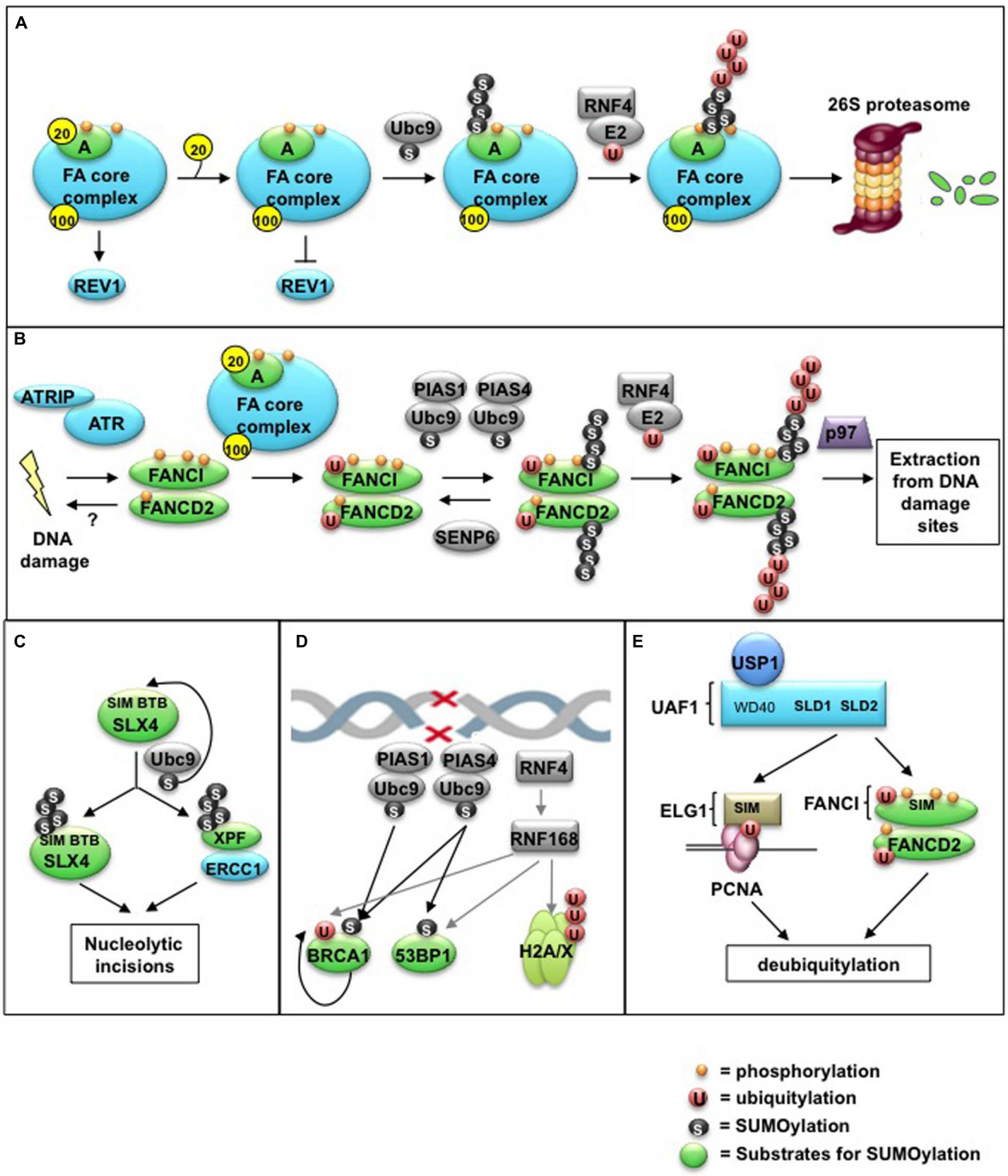

FIGURE 2 | Control of the FA pathway by SUMO and SUMO-like modifications. (A) Regulated release of the FAAP20 subunit from FANCA is required for SUMOylation of FANCA via the E2 conjugating enzyme Ubc9. Removal of FAAP20 inhibits the recruitment of the downstream TLS polymerase REV1 during ICL repair, resulting in decreased TLS-mediated mutagenesis. SUMOylation of FANCA subsequently triggers RNF4-mediated polyubiquitylation and proteasome-mediated degradation. **(B)** The ID complex is targeted for SUMOylation in a manner dependent on the activities of the ATR kinase, the FA core complex, and the SUMO E3 ligases PIAS1/PIAS4. Alternatively, SUMOylation can be removed by the SUMO protease SENP6. SUMOylation of the ID complex allows for recognition by RNF4, leading to ID complex polyubiquitylation and removal from damage sites via the DVC1-p97 segregase complex. **(C)** The SLX4 complex acts as a SUMO E3 ligase by triggering its own SUMOylation in addition to that of the DNA repair/recombination endonuclease XPF. This SLX4-dependent SUMOylation is dependent on the E2 Ubc9, as well as its own SIMs and BTB domains, and is important to prevent mitotic catastrophe following CFS expression. **(D)** The SUMO E3 ligases PIAS1 and PIAS4 promote DSB repair by localizing to damage sites and SUMOylating multiple target proteins, including BRCA1 and 53BP1. This SUMOylation is required for proper ubiquitin-adduct formation mediated by RNF8, RNF168, and BRCA1 to initiate a DNA damage response (DDR). **(E)** The SUMO-like domain SLD2 of UAF1 directs USP1-UAF1 targeting to its substrates via SIMs located within FANCI and the PCNA-interacting protein hELG1. These SLD-SIM interactions regulate deubiquitylation of FANCD2 and PCNA-Ub substrates, respectively, to coordinate HR and TLS activities during FA DNA repair.

of SLX4 are functionally distinct, providing a mechanism for targeting SLX4 in different repair contexts (Ouyang et al., 2015).

ROLE OF SUMOylated PCNA IN MAMMALIAN CELLS

The FA pathway is important for activation of HR-mediated repair, a DSB repair mechanism that uses an intact sister chromatid as a repair template (review in Moynahan and Jasin, 2010). While HR has the advantage of being an error-free mode of repair as opposed to the non-homologous end joining (NHEJ) pathway, inappropriate hyperrecombination is associated with genomic instability and cancer (Martin et al., 2007). How the FA pathway coordinates crosslink repair and HR appropriately has been a poorly understood topic. It is known that in budding yeast, the UvrD domain helicase Srs2 limits inappropriate HR by removing RAD51 nucleofilaments from ssDNA at an early step in the HR pathway (Papouli et al., 2005). A homolog for Srs2 with similar antirecombinase activity had not been identified in human cells, however, prompting a recent study by the D'Andrea lab to identify novel HR regulators in mammalian cells. The authors identified C12orf48 as an anti-recombinase in human and chicken DT40 cells that specifically interacts with a PCNA-SUMO fusion protein *in vitro*. They named this protein PCNA associated recombination inhibitor (PARI). Similar to Srs2 in yeast, PARI restricts recombination by interfering with RAD51-DNA HR structures and is required for genome stability. Interestingly, PARI knockdown in FA cells, which are deficient in HR (Nakanishi et al., 2005), improves genome instability by increasing recombination frequency (Moldovan et al., 2012). These results indicate that manipulation of PARI levels could be an effective chemoprotective approach in HR-deficient cancers. Nevertheless, several key questions still remain pertaining to the role and recruitment of PARI to the replication fork, including whether SUMOylation of mammalian PCNA exists and whether PARI is truly the long sought-after functional human homolog of budding yeast Srs2.

ROLE OF SUMOylation IN HR REPAIR

Following unhooking of an ICL by nucleolytic incisions, a DSB is generated that must be repaired by HR. Current evidence demonstrates a role for SUMOylation in coordinating this DSB repair step. SUMO E3 ligases PIAS1 and PIAS4 accumulate at DNA damage sites in mammalian cells, where they target multiple substrates for SUMO modification, including 53BP1 and BRCA1, to damage foci. SUMOylation facilitates the localization of these DNA repair proteins to DSB sites and also is required for RNF8 and RNF168-mediated ubiquitylation of target proteins (including H2A/X) to signal downstream repair (Galanty et al., 2009). In particular, SUMOylation of BRCA1 by PIAS SUMO E3 ligases increases its ubiquitin ligase activity, identifying it as a STUbL (Morris et al., 2009). In addition to BRCA1, another STUbL, RNF4, was identified that promotes DSB repair by regulating the turnover of DSB-responsive factors MDC1 and replication protein A (RPA), allowing for recruitment of factors necessary for DSB repair by HR (Galanty et al., 2012). Through the combination of these mechanisms (**Figure 2D**), SUMO-ubiquitin crosstalk amplifies DSB signaling to promote efficient DSB repair. Although likely, it is currently unclear whether regulation of BRCA1 by SUMOylation plays a role in the repair of ICLs as part of the Fanconi Anemia pathway.

ROLE OF SUMO-LIKE DOMAINS (SLDs) IN DUB-SUBSTRATE INTERACTIONS

While the importance of USP1/UAF1-mediated deubiquitylation of FANCD2 has been clearly established (Nijman et al., 2005; Oestergaard et al., 2007; Kim et al., 2009), the question of how USP1/UAF1 is targeted to the FANCI/FANCD2 heterodimer has remained elusive until recently. Yang et al. (2011) discovered that this targeting mechanism involves SUMO-like domains (SLD1 and SLD2) at the C-terminus of UAF1, which bind directly to SIM motifs of FANCI. Likewise, the SLD2 domain of UAF1 also binds to the SIM on hELG1 to direct USP1/UAF1 binding to another important substrate, PCNA-Ub (Huang et al., 2006). This SLD-SIM interaction is critical for FA pathway function, as deletion of the SLD2 sequence of UAF1 or the SIM of FANCI leads to deficient FANCD2 monoubiquitylation and DNA repair. Thus, SLD-SIM interactions provide a means for the regulated delivery of USP1/UAF1 DUB complex to its substrates for efficient ICL repair (Yang et al., 2011) (**Figure 2E**). As UAF1 is a highly abundant protein with many diverse binding partners, this study also points to the possibility that SLD-SIM targeting may also play broader roles not only in DNA repair, but as a general means to sort intracellular proteins involved in other processes.

FUTURE PERSPECTIVES

In summary, the above studies clearly demonstrate the importance of SUMO and SUMO-like modifications in fine-tuning FA pathway activation and DNA repair (summarized in **Figure 2**). It is probable that findings reported here represent a small portion of targets controlled by SUMOylation in the FA pathway, and future work should uncover other unidentified substrates and SUMO ligases/proteases critical to this process. These studies also point to the possibility of pharmacologically targeting SUMO ligases, SIM-SUMO interactions, and SENPs as a way to manipulate FA pathway activity in FA cells and HR-defective cancers. Consistent with this notion, several studies have reported the overexpression of SENPs in various disease conditions and cancers, prompting recent advancements in the development of small molecule inhibitors of SENPs with therapeutic potential (review in Kumar and Zhang, 2015). On the other hand, aggressive modulation of SUMO-

ubiquitin signaling could also pose a risk for inefficient repair by the FA pathway and other DNA repair mechanisms. Thus, future studies are highly necessary to further understand the proper balance of SUMOylation and ubiquitination activity necessary for proper FA pathway function, and how dysregulation of these ubiquitin and ubiquitin-like post-translational modifiers underlies genome instability.

AUTHOR CONTRIBUTIONS

KC wrote the manuscript with advice from TH.

FUNDING

Support was provided by the National Institutes of Health (NIH), American Cancer Society (ACS), NYU Laura and Isaac Perlmutter Cancer Center Support Grant's Developmental Project Program, and Irma T. Hirschl Career Scientist Award.

ACKNOWLEDGMENT

We also thank K. Burns-Huang and members of the Huang lab for critical reading of the manuscript.

REFERENCES

Andersen, S. L., Bergstralh, D. T., Kohl, K. P., LaRocque, J. R., Moore, C. B., and Sekelsky, J. (2009). *Drosophila* MUS312 and the vertebrate ortholog BTBD12 interact with DNA structure-specific endonucleases in DNA repair and recombination. *Mol. Cell* 35, 128–135. doi: 10.1016/j.molcel.2009.06.019

Budzowska, M., Graham, T. G., Sobeck, A., Waga, S., and Walter, J. C. (2015). Regulation of the Rev1-pol ζ complex during bypass of a DNA interstrand cross-link. *EMBO J.* 34, 1971–1985. doi: 10.15252/embj.201490878

Ciccia, A., Ling, C., Coulthard, R., Yan, Z., Xue, Y., Meetei, A. R., et al. (2007). Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Mol. Cell* 25, 331–343. doi: 10.1016/j.molcel.2007.01.003

Cohn, M. A., Kowal, P., Yang, K., Haas, W., Huang, T. T., Gygi, S. P., et al. (2007). A UAF1-containing multisubunit protein complex regulates the Fanconi anemia pathway. *Mol. Cell* 28, 786–797. doi: 10.1016/j.molcel.2007.09.031

Duxin, J. P., and Walter, J. C. (2015). What is the DNA repair defect underlying Fanconi anemia? *Curr. Opin. Cell Biol.* 37, 49–60. doi: 10.1016/j.ceb.2015.09.002

Fekairi, S., Scaglione, S., Chahwan, C., Taylor, E. R., Tissier, A., Coulon, S., et al. (2009). Human SLX4 Is a Holliday junction resolvase subunit that binds multiple DNA repair/recombination endonucleases. *Cell* 138, 78–89. doi: 10.1016/j.cell.2009.06.029

Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012). RNF4, a SUMO-rageted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* 26, 1179–1195. doi: 10.1101/gad.188284.112

Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009). Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* 462, 935–939. doi: 10.1038/nature08657

Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M. S., Timmers, C., Hejna, J., et al. (2001). Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* 7, 249–262. doi: 10.1016/S1097-2765(01)00173-3

Gareau, J. R., and Lima, C. D. (2010). The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat. Rev. Mol. Cell Biol.* 11, 861–871. doi: 10.1038/nrm3011

Gibbs-Seymour, I., Oka, Y., Rajendra, E., Weinert, B. T., Passmore, L. A., Patel, K. J., et al. (2015). Ubiquitin-SUMO circuitry controls activated fanconi anemia ID complex dosage in response to DNA damage. *Mol. Cell* 57, 150–164. doi: 10.1016/j.molcel.2014.12.001

González-Prieto, R., Cuijpers, S. A. G., Luijsterburg, M. S., van Attikum, H., and Vertegaal, A. C. O. (2015). SUMOylation and PARylation cooperate to recruit and stabilize SLX4 at DNA damage sites. *EMBO Rep.* 16, 512–519. doi: 10.15252/embr.201440017

Guervilly, J. H., Takedachi, A., Naim, V., Scaglione, S., Chawhan, C., Lovera, Y., et al. (2015). The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. *Mol. Cell* 57, 123–137. doi: 10.1016/j.molcel.2014.11.014

Guzzo, C. M., Berndsen, C. E., Zhu, J., Gupta, V., Datta, A., Greenberg, R. A., et al. (2012). RNF4-dependent hybrid SUMO-ubiquitin chains are signals for RAP80 and thereby mediate the recruitment of BRCA1 to sites of DNA damage. *Sci. Signal.* 5:ra88. doi: 10.1126/scisignal.2003485

Hanada, K., Budzowska, M., Modesti, M., Maas, A., Wyman, C., Essers, J., et al. (2006). The structure-specific endonuclease Mus81-Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks. *EMBO J.* 25, 4921–4932. doi: 10.1038/sj.emboj.7601344

Hu, X., Paul, A., and Wangs, B. (2012). Rap80 protein recruitment to DNA double-strand breaks requires binding to both small ubiquitin-like modifier (SUMO) and ubiquitin conjugates. *J. Biol. Chem.* 287, 25510–25519. doi: 10.1074/jbc.M112.374116

Huang, T. T., Nijman, S. M. B., Mirchandani, K. D., Galardy, P. J., Cohn, M. A., Haas, W., et al. (2006). Regulation of monoubiquitinated PCNA by DUB autocleavage. *Nat. Cell Biol.* 8, 339–347. doi: 10.1038/ncb1378

Kee, Y., and D'Andrea, A. D. (2012). Molecular pathogenesis and clinical management of Fanconi anemia. *J. Clin. Invest.* 122, 3799–3806. doi: 10.1172/JCI58321

Kim, H., and D'Andrea, A. D. (2012). Regulation of DNA cross-link repair by the Fanconi anemia/BRCA pathway. *Genes Dev.* 26, 1393–1408. doi: 10.1101/gad.195248.112

Kim, H., Yang, K., Dejsuphong, D., and D'Andrea, A. D. (2012). Regulation of Rev1 by the Fanconi anemia core complex. *Nat. Struct. Mol. Biol.* 19, 164–170. doi: 10.1038/nsmb.2222

Kim, J. M., Kee, Y., Gurtan, A., and Andrea, A. D. D. (2008). Cell cycle – dependent chromatin loading of the Fanconi anemia core complex by FANCM / FAAP24. *Blood* 111, 5215–5222. doi: 10.1182/blood-2007-09-113092

Kim, J. M., Parmar, K., Huang, M., Weinstock, D. M., Ruit, C. A., Kutok, J. L., et al. (2009). Inactivation of murine Usp1 results in genomic instability and a Fanconi anemia phenotype. *Dev. Cell* 16, 314–320. doi: 10.1016/j.devcel.2009.01.001

Klein Douwel, D., Boonen, R. A., Long, D. T., Szypowska, A. A., Räschle, M., Walter, J. C., et al. (2014). XPF-ERCC1 Acts in Unhooking DNA Interstrand Crosslinks in Cooperation with FANCD2 and FANCP/SLX4. *Mol. Cell* 54, 460–471. doi: 10.1016/j.molcel.2014.03.015

Knipscheer, P., Räschle, M., Smogorzewska, A., Enoiu, M., Ho, T. V., Schärer, O. D., et al. (2009). The Fanconi anemia pathway promotes replication-dependent DNA interstrand cross-link repair. *Science* 326, 1698–1701. doi: 10.1126/science.1182372

Kumar, A., and Zhang, K. Y. J. (2015). Advances in the development of SUMO specific protease (SENP) inhibitors. *Comput. Struct. Biotechnol. J.* 13, 204–211. doi: 10.1016/j.csbj.2015.03.001

Leung, J. W. C., Wang, Y., Fong, K. W., Huen, M. S. Y., Li, L., and Chen, J. (2012). Fanconi anemia (FA) binding protein FAAP20 stabilizes FA complementation group A (FANCA) and participates in interstrand cross-link repair. *Proc. Natl. Acad. Sci. U.S.A.* 109, 4491–4496. doi: 10.1073/pnas.1118720109

Ling, C., Ishiai, M., Ali, A. M., Medhurst, A. L., Neveling, K., Kalb, R., et al. (2007). FAAP100 is essential for activation of the Fanconi anemia-associated DNA damage response pathway. *EMBO J.* 26, 2104–2114. doi: 10.1038/sj.emboj.7601666

Martin, R. W., Orelli, B. J., Yamazoe, M., Minn, A. J., Takeda, S., and Bishop, D. K. (2007). RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. *Cancer Res.* 67, 9658–9665. doi: 10.1158/0008-5472.CAN-07-0290

Meetei, A. R., de Winter, J. P., Medhurst, A. L., Wallisch, M., Waisfisz, Q., van de Vrugt, H. J., et al. (2003). A novel ubiquitin ligase is deficient in Fanconi anemia. *Nat. Genet.* 35, 165–170. doi: 10.1038/ng1241

Moldovan, G. L., Dejsuphong, D., Petalcorin, M. I. R., Hofmann, K., Takeda, S., Boulton, S. J., et al. (2012). Inhibition of homologous recombination

by the PCNA-interacting protein PARI. *Mol. Cell* 45, 75–86. doi: 10.1016/j.molcel.2011.11.010

Morris, J. R., Boutell, C., Keppler, M., Densham, R., Weekes, D., Alamshah, A., et al. (2009). The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. *Nature* 462, 886–890. doi: 10.1038/nature08593

Moynahan, M. E., and Jasin, M. (2010). Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nat. Rev. Mol. Cell Biol.* 11, 196–207. doi: 10.1038/nrm2851

Munoz, I. M., Hain, K., Declais, A. C., Gardiner, M., Toh, G. W., Sanchez-Pulido, L., et al. (2009). Coordination of structure-specific nucleases by human SLX4/BTBD12 is required for DNA repair. *Mol. Cell* 35, 116–127. doi: 10.1016/j.molcel.2009.06.020

Nakanishi, K., Yang, Y., Pierce, A. J., Taniguchi, T., Digweed, M., Andrea, A. D. D., et al. (2005). Human Fanconi anemia monoubiquitination pathway promotes homologous DNA repair. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1110–1115. doi: 10.1073/pnas.0407796102

Nijman, S. M. B., Huang, T. T., Dirac, A. M. G., Brummelkamp, T. R., Kerkhoven, R. M., D'Andrea, A. D., et al. (2005). The deubiquitinating enzyme USP1 regulates the Fanconi anemia pathway. *Mol. Cell* 17, 331–339. doi: 10.1016/j.molcel.2005.01.008

Noll, D. M., Mason, T. M., and Miller, P. S. (2006). Formation and repair of interstrand cross-links in DNA. *Chem. Rev.* 106, 277–301. doi: 10.1021/cr040478b

Oestergaard, V. H., Langevin, F., Kuiken, H. J., Pace, P., Niedzwiedz, W., Simpson, L. J., et al. (2007). Deubiquitination of FANCD2 is required for DNA crosslink repair. *Mol. Cell* 28, 798–809. doi: 10.1016/j.molcel.2007.09.020

Ouyang, J., Garner, E., Hallet, A., Nguyen, H. D., Rickman, K. A., Gill, G., et al. (2015). Noncovalent interactions with SUMO and ubiquitin orchestrate distinct functions of the SLX4 complex in genome maintenance. *Mol. Cell* 57, 108–122. doi: 10.1016/j.molcel.2014.11.015

Papouli, E., Chen, S., Davies, A. A., Huttner, D., Krejci, L., Sung, P., et al. (2005). Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell* 19, 123–133. doi: 10.1016/j.molcel.2005.06.001

Perry, J. J. P., Tainer, J. A., and Boddy, M. N. (2008). A SIM-ultaneous role for SUMO and ubiquitin. *Trends Biochem. Sci.* 33, 201–208. doi: 10.1016/j.tibs.2008.02.001

Qiao, F., Moss, A., and Kupfer, G. M. (2001). Fanconi anemia proteins localize to chromatin and the nuclear matrix in a DNA damage- and Cell cycle-regulated manner. *J. Biol. Chem.* 276, 23391–23396. doi: 10.1074/jbc.M101855200

Räschle, M., Knipscheer, P., Knipsheer, P., Enoiu, M., Angelov, T., Sun, J., et al. (2008). Mechanism of replication-coupled DNA interstrand crosslink repair. *Cell* 134, 969–980. doi: 10.1016/j.cell.2008.08.030

Rodriguez, M. S., Dargemont, C., and Hay, R. T. (2001). SUMO-1 conjugation in vivo requires both a consensus modification motif and nuclear targeting. *J. Biol. Chem.* 276, 12654–12659. doi: 10.1074/jbc.M009476200

Sims, A. E., Spiteri, E., Sims, R. J., Arita, A. G., Lach, F. P., Landers, T., et al. (2007). FANCI is a second monoubiquitinated member of the Fanconi anemia pathway. *Nat. Struct. Mol. Biol.* 14, 564–567. doi: 10.1038/nsmb1252

Singh, T. R., Saro, D., Ali, A. M., Zheng, X. F., Du, C. H., Killen, M. W., et al. (2010). MHF1-MHF2, a histone-fold-containing protein complex, participates in the Fanconi anemia pathway via FANCM. *Mol. Cell* 37, 879–886. doi: 10.1016/j.molcel.2010.01.036

Smogorzewska, A., Matsuoka, S., Vinciguerra, P., McDonald, E. R., Hurov, K. E., Luo, J., et al. (2007). Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. *Cell* 129, 289–301. doi: 10.1016/j.cell.2007.03.009

Stoepker, C., Hain, K., Schuster, B., Hilhorst-Hofstee, Y., Rooimans, M. A., Steltenpool, J., et al. (2011). SLX4, a coordinator of structure-specific endonucleases, is mutated in a new Fanconi anemia subtype. *Nat. Genet.* 43, 138–141. doi: 10.1038/ng.751

Walden, H., and Deans, A. J. (2014). The Fanconi anemia DNA repair pathway: structural and functional insights into a complex disorder. *Annu. Rev. Biophys.* 43, 257–278. doi: 10.1146/annurev-biophys-051013-022737

Wan, B., Yin, J., Horvath, K., Sarkar, J., Chen, Y., Wu, J., et al. (2013). SLX4 assembles a telomere maintenance toolkit by bridging multiple endonucleases with telomeres. *Cell Rep.* 4, 861–869. doi: 10.1016/j.celrep.2013.08.017

Xie, J., Kim, H., Moreau, L. A., Puhalla, S., Garber, J., Abo, M. A., et al. (2015). RNF4-mediated polyubiquitination regulates the Fanconi anemia/BRCA pathway. *J. Clin. Invest.* 125, 1523–1532. doi: 10.1172/JCI79325

Yan, Z., Delannoy, M., Ling, C., Daee, D., Osman, F., Muniandy, P. A., et al. (2010). A histone-fold complex and FANCM form a conserved DNA-remodeling complex to maintain genome stability. *Mol. Cell* 37, 865–878. doi: 10.1016/j.molcel.2010.01.039

Yang, K., Moldovan, G. L., Vinciguerra, P., Murai, J., Takeda, S., and D'Andrea, A. D. (2011). Regulation of the Fanconi anemia pathway by a SUMO-like delivery network. *Genes Dev.* 25, 1847–1858. doi: 10.1101/gad.17020911

Conflict of Interest Statement: 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.

frontiers in Genetics

MINI REVIEW
published: 11 April 2016
doi: 10.3389/fgene.2016.00058

Real Estate in the DNA Damage Response: Ubiquitin and SUMO Ligases Home in on DNA Double-Strand Breaks

Nico P. Dantuma and Annika Pfeiffer*

Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

Ubiquitin and the ubiquitin-like modifier SUMO are intimately connected with the cellular response to various types of DNA damage. A striking feature is the local accumulation of these proteinaceous post-translational modifications in the direct vicinity to DNA double-strand breaks, which plays a critical role in the formation of ionizing radiation-induced foci. The functional significance of these modifications is the coordinated recruitment and removal of proteins involved in DNA damage signaling and repair in a timely manner. The central orchestrators of these processes are the ubiquitin and SUMO ligases that are responsible for accurately tagging a broad array of chromatin and chromatin-associated proteins thereby changing their behavior or destination. Despite many differences in the mode of action of these enzymes, they share some striking features that are of direct relevance for their function in the DNA damage response. In this review, we outline the molecular mechanisms that are responsible for the recruitment of ubiquitin and SUMO ligases and discuss the importance of chromatin proximity in this process.

Keywords: DNA damage, DNA double-strand breaks, SUMO, ubiquitin, chromatin

OPEN ACCESS

Edited by:
Kristijan Ramadan, University of Oxford, UK

Reviewed by:
Richard Chahwan, Albert Einstein College of Medicine, USA
Thorsten Hoppe, University of Cologne, Germany

****Correspondence:***
Nico P. Dantuma
nico.dantuma@ki.se

Specialty section:
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Genetics

Received: *09 February 2016*
Accepted: *26 March 2016*
Published: *11 April 2016*

Citation:
Dantuma NP and Pfeiffer A (2016) Real Estate in the DNA Damage Response: Ubiquitin and SUMO Ligases Home in on DNA Double-Strand Breaks. Front. Genet. 7:58. doi: 10.3389/fgene.2016.00058

INTRODUCTION

The cellular response to compromised genome integrity is a vital process that is tightly regulated by a number of post-translational modifications (PTMs) that dictate the course of action at the sites of DNA damage. While ensuring that proper action will be taken to eliminate the threat, these regulatory circuits at the same time avoid unnecessary and potentially hazardous activation of DNA repair pathways. In this review, we will focus on ubiquitin and the small ubiquitin-like modifiers (SUMO)-1, -2, and -3, which are central players in this process, where they in tight conjunction with other PTMs – most notably phosphor-modifications but also another ubiquitin-like protein modifier Nedd8 – activate signaling cascades and coordinate mobilization of the proper DNA repair machinery (Bekker-Jensen and Mailand, 2011; Jackson and Durocher, 2013). Rather than providing a complete overview of the rapidly expanding number of ligases that are involved in this process, we will focus on a limited set of ligases that illustrates the importance of proximity to DNA lesions in DNA damage-induced ubiquitylation and SUMOylation.

Abbreviations: DSB, DNA double-strand break; IRIF, ionizing radiation-induced foci; MIU, motif interacting with ubiquitin; PAR, poly(ADP-ribose); PTM, post-translational modification; PRC, polycomb recessive complex; SIM, SUMO-interacting motif; STUbL, SUMO-targeted ubiquitin ligase; ZnF, zinc finger.

Modification of chromatin and chromatin-associated proteins by these PTMs in response to DNA double-strand breaks (DSBs) results in the formation of the characteristic ionizing radiation-induced foci (IRIF) that mark the sites of DNA damage (Lukas et al., 2011). In contrast to phosphorylation at IRIF, which is primarily facilitated by the PI3K-like kinase ATM with the variant histone H2AX being the predominant target (Shiloh, 2003), decoration of the chromatin with ubiquitin and SUMO is attributed to several enzymes that differ in their specificity for substrates at the chromatin (Bekker-Jensen and Mailand, 2011; Jackson and Durocher, 2013). Despite the many differences between the ubiquitin and SUMO ligases involved in the DNA damage response, they share a number of characteristics such as the critical role of chromatin recruitment for their functionality and their tendency to target multiple substrates at the DSBs.

REGULATION BY PROXIMITY

An important mechanistic difference between the DSB-induced phosphorylation and ubiquitin/SUMO response at IRIF lies in the way their activity is regulated. While the activity of ATM is kept dormant in undamaged cells only to be unleashed upon the detection of DSBs (Bakkenist and Kastan, 2003), most of the enzymes that are responsible for conjugation of ubiquitin and SUMO at sites of DSBs lack direct activation mechanisms. Despite the fact that additional regulatory mechanisms may be in play, a general concept appears to be the DNA damage-induced translocation of ligases to the DSBs as a primary determinant for directing the activity of these enzymes toward chromatin and chromatin-associated proteins (**Figure 1**).

Various PTMs and also the exposure of single-stranded DNA (ssDNA) triggers the accrual of ubiquitin and SUMO ligases (**Figure 2**). RNF8 and RNF168 are two RING ubiquitin ligases that play an important role in the DSB-induced ubiquitylation response and act downstream of the ATM-dependent phosphorylation triggered by DNA damage (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007; Doil et al., 2009; Stewart et al., 2009). RNF8 interacts with its FHA domain to ATM-phosphorylated MDC1, which in turn binds ATM-phosphorylated variant histone H2AX (γH2AX), a hallmark of IRIF (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). A dual recruitment mechanism is involved in accrual of RNF168, which has one binding module that facilitates interaction with linker histone H1 modified with non-proteolytic lysine 63 (K63)-linked ubiquitin chains (Thorslund et al., 2015) and a second binding module that recruits it to the core histone H2A/H2AX ubiquitylated at lysine residues K13/K15

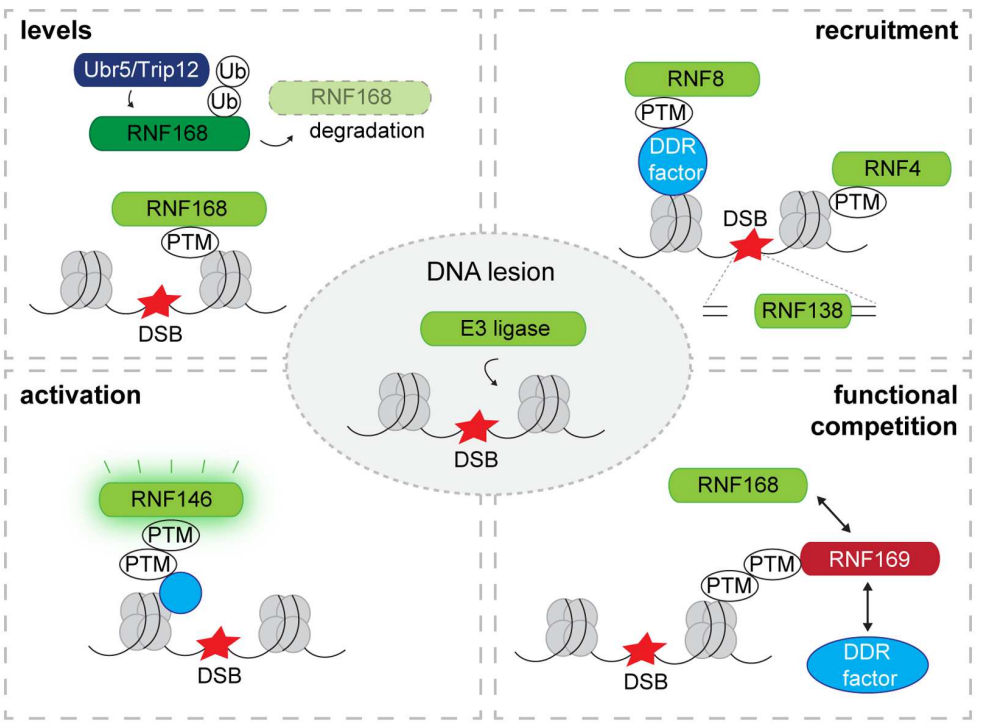

FIGURE 1 | Recruitment and regulation of E3 ligases at DNA lesions. Levels: E3 ligases are targeted by other E3 ubiquitin ligases for proteasomal degradation. Proteasomal degradation of E3 ligases limits the quantity of E3 ligases that translocate to DNA lesions as has been observed for RNF168 which is targeted for degradation by TRIP12 and UBR5. Recruitment: The recruitment of E3 ligases to DSBs is often mediated by PTMs that are attached to the chromatin or chromatin-associated proteins. Some E3 ligases are recruited by directly binding to free DNA ends exposed at DSBs. Activation: While most E3 ligases in complex with their E2 possess constitutive enzymatic activity, a PTM can activate E3 ligase activity as has been observed for the PAR-dependent ubiquitin ligase RNF146. Competition: Competitors negatively regulate the recruitment of E3 ligases by binding to PTMs that facilitate the binding of E3 ligases as has been observed for RNF168 and its competitor RNF169. DSB, DNA double-strand break; Ub, ubiquitin; PTM, post-translational modification; DDR, DNA damage response.

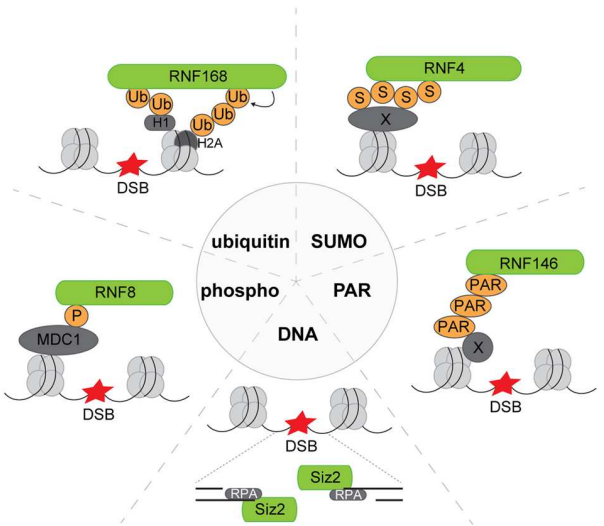

FIGURE 2 | Recruitment modes of exemplary E3 ligases to DNA lesions. Phosphor: phosphorylated MDC1 serves as the recruitment platform for RNF8 to DNA lesions. Ubiquitin: RNF168 binds to both ubiquitylated linker histone H1 and ubiquitylated core histone H2A. SUMO: RNF4 harbors SUMO-interacting motifs by which it can bind to SUMOylated substrates, e.g., MDC1 at DNA lesions. PAR: The E3 ligase RNF146 translocates to DNA breaks and is activated by binding to PARylated substrates. DNA: By binding to ssDNA and RPA, the yeast SUMO ligase Siz2 is recruited to DNA lesions. DSB, DNA double-strand break; Ub, ubiquitin; S, SUMO, small ubiquitin-like modifier; PAR, poly(ADP-ribose); P, phosphate group; X, variable or unspecified protein.

(Panier et al., 2012). The latter modification is generated by RNF168 and provides a positive feedback loop that amplifies RNF168-mediated ubiquitylation (Mattiroli et al., 2012). The RNF8/RNF168-mediated ubiquitylation response results in the recruitment of proteins involved in the repair of DSBs, such as BRCA1 and 53BP1, to the chromatin at sites of DNA damage.

Artificial tethering of these two ubiquitin ligases to the chromatin is sufficient to locally reconstitute the DNA damage response to a large extent without inflicting actual DSBs (Acs et al., 2011; Luijsterburg et al., 2012). Interestingly, sequestration of RNF8 at chromatin resulted in the formation of foci that displayed many of the hallmarks observed at IRIF such as ubiquitylation of histone H2A, formation of K63-linked ubiquitin chains and recruitment of RNF168 and BRCA1 (Luijsterburg et al., 2012). While tethering of RNF168 did not result in accrual of RNF8, consistent with the notion that it acts downstream of RNF8, it also gave rise to H2A ubiquitylation and BRCA1 recruitment (Luijsterburg et al., 2012). Thus the mode of action of these two ubiquitin ligases illustrates that chromatin retention plays an important role in their regulation. It is tempting to speculate that the fact that they operate downstream of ATM and hence rely on activation of ATM provides sufficient safety measures to prevent random erroneous activation of the pathway at chromatin. Moreover, their constitutive activity may have advantages in the sense that it may allow these proteins to have other functions in the absence of DNA damage as has been documented for RNF8 (Takano et al., 2004).

Amplification of the ubiquitylation response at sites of DNA damage by RNF168 is essential for a robust DNA damage response and is hence tightly regulated at various levels (**Figure 1**). Two ubiquitin ligases, UBR5 and TRIP12, target RNF168 for proteasomal degradation and depletion of these ligases results in supraphysiological steady-state levels of RNF168 giving rise to superfluous activation of the ubiquitylation response at DSBs (Gudjonsson et al., 2012).

In addition, chromatin accrual of RNF168 is kept under control by its paralog RNF169 (Chen et al., 2012; Poulsen et al., 2012), which also binds to RNF168-generated ubiquitin chains but does not amplify the signal (Panier et al., 2012; **Figure 1**). Also activation of the DNA damage response by herpes simplex virus type 1 is prevented by the viral ubiquitin ligase ICP0 targeting RNF8 and RNF168 (Lilley et al., 2010). It is striking that these regulatory mechanisms target the steady-state levels and chromatin accrual of RNF168 and not its activity underscoring the importance of localization of this ubiquitin ligase in DNA damage signaling.

An exceptional case is the poly(ADP-ribose) (PAR)-dependent ubiquitin ligase RNF146, also known as Iduna, since DNA damage-induced PARylation not only induces its translocation but also releases its ubiquitin ligase activity (Kang et al., 2011; **Figure 1**). RNF146 selectively interacts with PARylated proteins at DSBs resulting in their ubiquitylation. Although its activity is not confined to PARylated proteins at DSBs (Zhang et al., 2011), the PAR-dependent recruitment of RNF146 is important for efficient DNA repair. Structural analysis revealed that interaction between PAR and the WWE domain of RNF146 switches its RING domain into an active state that promotes conjugation of ubiquitin to PARylated proteins (DaRosa et al., 2015). Thus, RNF146 is kept in a dormant state only to be activated upon interaction with PARylated substrates.

In contrast to the large number of ubiquitin ligases in metazoan cells, SUMO ligation is mediated by a selective set of dedicated enzymes. In particular SUMO ligases belonging to the PIAS family – Siz2 in yeast and PIAS1-4 in mammalian cells – have been implicated in the cellular response to DSBs. Also these enzymes modulate substrates at the chromatin as a direct consequence of their DNA damage-induced translocation to breaks (**Figure 1**). For yeast Siz2 it has been shown that once recruited it SUMOylates chromatin-associated proteins at DNA damage in a rather promiscuous fashion, a process that has been referred to as group modification (Psakhye and Jentsch, 2012). Strikingly, artificial tethering of proteins to the chromatin is sufficient to turn them into substrates for DNA damage-recruited Siz2 underscoring its ability to modify proteins primarily based on their proximity (Psakhye and Jentsch, 2012). Since the individual contribution of the SUMO modifications is limited while at the same time the presence of an active SUMOylation response is critical for homologous recombination, it has been proposed that the SUMO modifications may provide a "glue" that stabilizes local interactions by binding to SUMO-interacting motifs (SIMs), which are commonly found in DNA repair proteins (Jentsch and Psakhye, 2013). It is important though to mention that modification of specific substrates can also be highly relevant as has been shown for PCNA, which is SUMOylated at a

specific lysine residue by Siz1, dictating the preferred mechanism for dealing with lesions that block replication forks (Mailand et al., 2013). DSBs in mammalian cells recruit the SUMO ligases PIAS1 and PIAS4 where they modify BRCA1, 53BP1 and other substrates with SUMO1 and SUMO2/3 conjugates (Galanty et al., 2009; Morris et al., 2009). The PIAS1/4-facilitated SUMOylation is critical for a functional DNA damage response and impediment of this process compromises recruitment of RNF168, 53BP1, and BRCA1 (Galanty et al., 2009; Morris et al., 2009). Although the general underlying molecular mechanism for the critical role of SUMO in DNA damage-induced ubiquitylation remains elusive, it has been shown that the ubiquitin ligase activity of the BRCA1/BARD1 complex is enhanced by SUMOylation, which may in part explain its stimulatory effect (Morris et al., 2009). It is not known whether similar group modifications are involved in this process but it is noteworthy that both in yeast and mammalian cells the role of SUMOylation is complex and can stimulate recruitment, retention or extraction depending on the nature of substrate and the context of the modification.

TRACING DNA LESIONS

The central role of the recruitment of ubiquitin/SUMO ligases in activation of DNA repair pathways also implies that their translocation to DSBs has to be tightly regulated. Notably, while lack of activation of DNA repair mechanisms or DNA damage signaling cascades in the presence of DSBs is dangerous for cells, inappropriate or superfluous activation of these systems form an equally serious threat. It is interesting that some of the ubiquitin ligases that are implicated in this process use analogous mechanisms for their recruitment and combine motifs that bind to specific DNA damage-induced PTMs with domains that interact with chromatin ensuring that these modifications will only be recognized as valid signals in the context of chromatin.

Proper accrual is of particular importance for the RNF168 ubiquitin ligase which is responsible for the amplification of the DNA damage-induced ubiquitylation response initiated by RNF8. While RNF168 is essential for recruitment of 53BP1 and BRCA1 and the actual repair of DSBs, excessive levels of RNF168 also compromise DNA repair (Gudjonsson et al., 2012). RNF168 specifically ubiquitylates histone H2A(X) in the context of the nucleosome by interacting through a basic region within its RING domain with an acidic patch that is present at the interface of the H2A/H2B dimer (Leung et al., 2014; Mattiroli et al., 2014). Binding of RNF168 to the nucleosome allows its cognate ubiquitin conjugase to transfer the ubiquitin to the target lysine residues within H2A(X). However, this direct interaction with the nucleosome is not sufficient for establishing chromatin retention since RNF168 has to selectively interact with ubiquitylated linker histone H1 (Thorslund et al., 2015) or the H2AK13,15ub mark (Mattiroli et al., 2012). Notably, RNF168 contains two recognition modules both involving motif-interacting with ubiquitin (MIU) domains that are specific for these modifications (Panier et al., 2012). Interestingly, its paralog RNF169, which suppresses DNA damage-induced ubiquitylation (Chen et al., 2012; Poulsen et al., 2012), only contains the module that facilitates interaction with the RNF168-generated H2AK13,15ub mark allowing it to inhibit the amplification of the signal by tempering with the initial activating response (Panier et al., 2012).

It is noteworthy that the BMI1/RING1b ligase, which is part of the polycomb recessive complex 1 (PRC1) that facilitates the canonical ubiquitylation of histone H2A at residue K119 (Sparmann and van Lohuizen, 2006), employs the same acidic patch to faithfully interact with the nucleosome (Leung et al., 2014; McGinty et al., 2014). Although it had been proposed that the BMI1/RING1b ligase interacts with nucleosomal DNA in a sequence-independent manner (Bentley et al., 2011), structural analysis showed that its cognate E2 UbcH5 facilitates this interaction (McGinty et al., 2014). Importantly, this ubiquitin ligase complex has also been linked to the DNA damage response, both at DSBs and UV lesions, where it monoubiquitylates histone H2AX and promotes the DNA damage response (Ismail et al., 2010). PRC1 accumulates at DSBs by a mechanism that is different from its well-established chromatin retention by PRC2-generated H3K27me^3 and does not require DNA damage-induced γH2AX. PRC1 is also required for DNA damage-induced silencing at DSBs but this activity requires the presence of PRC2 suggesting that it is more similar to the canonical role of these complexes in suppression of transcription (Kakarougkas et al., 2014).

RNF4 is a SUMO-targeted ubiquitin ligase (STUbL) that selectively ubiquitylates proteins that have been modified by chains consisting of the highly related SUMO2 and SUMO3 modifiers, in particular under conditions of proteotoxic or genotoxic stress (Kosoy et al., 2007; Sun et al., 2007). In response to DSBs, RNF4 translocates to sites of DNA damage by interacting with its SIMs with chromatin-associated proteins that are subject to DNA damage-induced SUMOylation (Galanty et al., 2012; Luo et al., 2012; Yin et al., 2012). RNF4-mediated ubiquitylation of MDC1 and RPA results in removal of these proteins from DSBs and plays an important regulatory role. In addition to its interaction with the SUMO conjugates, the RING domain of RNF4 contains a nucleosome-interacting motif that is structurally related to the motifs in RNF168 and RING1b and which is required for targeting RNF4 to chromatin (Groocock et al., 2014). Although the nucleosome-interacting motif binds DNA, as in the case for RING1b, it is not clear whether DNA binding and/or histone interaction are responsible for its nucleosome targeting activity (Groocock et al., 2014).

Another recruitment mechanism is employed by the ubiquitin ligase RNF138, which stimulates repair of DSBs by homologous recombination. This ubiquitin ligase contains three zinc finger (ZnF) motifs that specifically interact with ssDNA overhangs at lesions (Ismail et al., 2015; Schmidt et al., 2015). Accordingly, RNF138 acts downstream of the Mre11 nuclease that is responsible for the generation of ssDNA at DSBs. RNF138-mediated ubiquitylation fulfills a dual role at DSBs since it facilitates the removal of the Ku70–Ku80 heterodimer (Ismail et al., 2015) and stimulates the recruitment of CtIP resulting in resection of DNA ends (Schmidt et al., 2015) and repair of the lesions by homologous recombination. Thus, unlike the above mentioned ubiquitin ligases, RNF138 localizes to the actual

break and not to the chromatin in proximity of the DSBs and uses a dedicated recruitment motif to accomplish this. Also RNF111, which modifies histone H4 with chains of the ubiquitin-like modifier Nedd8, interacts with naked DNA and it has been proposed that this may serve to secure its recruitment to DSBs (Ma et al., 2013). Confusingly, the same ubiquitin ligase has also been reported to localize to UV damage in a SUMO-targeted fashion where it modifies its target XPC with ubiquitin chains instead of Nedd8 (Poulsen et al., 2013), resulting in chromatin extraction of XPC raising questions both about RNF111's mechanism for accrual and mode of action (van Cuijk et al., 2015).

The generation of ssDNA is also important for the recruitment of the budding yeast SUMO ligase Siz2 to DSBs. Siz2 belongs to the family of PIAS ligases which have been found to be involved in the DNA damage response not only in yeast but also in human cells, in particular PIAS1 and PIAS4. Originally it was proposed that the conserved SAP domain in these SUMO ligases facilitates recruitment by binding to ssDNA which triggers a wave of early SUMOylation at DSBs (Psakhye and Jentsch, 2012). However, a recent study revealed that while the ssDNA is critical for translocation of Siz2, it does so by binding the ssDNA-binding complex RPA (Chung and Zhao, 2015). Siz2 interacts with this trimeric complex that coats ssDNA resulting in SUMOylation of RPA and other chromatin-associated targets. Also the PIAS1 and PIAS4 SUMO ligases interact with the same RPA subunit (Chung and Zhao, 2015) and accrual has been shown to be dependent on their N-terminal SAP domains (Galanty et al., 2009), suggesting that similar recruitment mechanisms may be in play in human cells. SUMOylation of RPA followed by ubiquitylation catalyzed by STUbLs results in chromatin eviction of these proteins and plays a critical role in regulating the repair of DSBs (Galanty et al., 2012).

CONCLUDING REMARKS

The detailed insights in the recruitment mechanisms that regulate chromatin association of DNA damage ubiquitin and SUMO ligases and the important role of proximity in DNA damage-induced protein modifications stands in sharp contrast to our modest understanding of how these PTMs regulate the fate of the modified proteins. Importantly, ubiquitylation and SUMOylation have been shown to be stimulators of protein recruitment, retention and extraction, supposing opposite actions that are hard to reconcile in one mechanistic paradigm, raising questions of what determines the final biological outcome of these modifications. For example, RNF8-mediated ubiquitylation forms the docking platform for critical DNA repair proteins (Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007), while at the same time it has been shown to promote ubiquitin-dependent chromatin extraction of proteins (Acs et al., 2011; Meerang et al., 2011; Feng and Chen, 2012; Mallette et al., 2012). Also the STUbL RNF4 has been shown to select SUMOylated chromatin-associated proteins for eviction (Galanty et al., 2012; Luo et al., 2012; Yin et al., 2012) but has also been implicated in the recruitment of proteins to DSBs (Hendriks et al., 2015). The picture is further complicated by the notion that SUMO modifications can by themselves target proteins for extraction (Bergink et al., 2013), whereas at the same time SUMO group modification has been proposed to play a general role in stabilizing chromatin association of proteins (Psakhye and Jentsch, 2012). Decrypting the ubiquitin/SUMO code in the DNA damage response will be a major challenge for the future and may shed light not only on the molecular mechanisms that dictate the behavior of proteins at DNA damage but also other processes that have the chromatin environment as their central stage, such as transcription and replication.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENT

Our research is financially supported by grants from the Swedish Research Council and the Swedish Cancer Society.

REFERENCES

Acs, K., Luijsterburg, M. S., Ackermann, L., Salomons, F. A., Hoppe, T., and Dantuma, N. P. (2011). The AAA-ATPase VCP/p97 promotes 53BP1 recruitment by removing L3MBTL1 from DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 18, 1345–1350. doi: 10.1038/nsmb.2188

Bakkenist, C. J., and Kastan, M. B. (2003). DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 421, 499–506. doi: 10.1038/nature01368

Bekker-Jensen, S., and Mailand, N. (2011). The ubiquitin- and SUMO-dependent signaling response to DNA double-strand breaks. *FEBS Lett.* 585, 2914–2919. doi: 10.1016/j.febslet.2011.05.056

Bentley, M. L., Corn, J. E., Dong, K. C., Phung, Q., Cheung, T. K., and Cochran, A. G. (2011). Recognition of UbcH5c and the nucleosome by the Bmi1/Ring1b ubiquitin ligase complex. *EMBO J.* 30, 3285–3297. doi: 10.1038/emboj.2011.243

Bergink, S., Ammon, T., Kern, M., Schermelleh, L., Leonhardt, H., and Jentsch, S. (2013). Role of Cdc48/p97 as a SUMO-targeted segregase curbing Rad51-Rad52 interaction. *Nat. Cell Biol.* 15, 526–532. doi: 10.1038/ncb2729

Chen, J., Feng, W., Jiang, J., Deng, Y., and Huen, M. S. (2012). Ring finger protein RNF169 antagonizes the ubiquitin-dependent signaling cascade at sites of DNA damage. *J. Biol. Chem.* 287, 27715–27722. doi: 10.1074/jbc.M112.373530

Chung, I., and Zhao, X. (2015). DNA break-induced sumoylation is enabled by collaboration between a SUMO ligase and the ssDNA-binding complex RPA. *Genes Dev.* 29, 1593–1598. doi: 10.1101/gad.265058.115

DaRosa, P. A., Wang, Z., Jiang, X., Pruneda, J. N., Cong, F., Klevit, R. E., et al. (2015). Allosteric activation of the RNF146 ubiquitin ligase by a poly(ADP-ribosyl)ation signal. *Nature* 517, 223–226. doi: 10.1038/nature13826

Doil, C., Mailand, N., Bekker-Jensen, S., Menard, P., Larsen, D. H., Pepperkok, R., et al. (2009). RNF168 binds and amplifies ubiquitin conjugates on damaged chromosomes to allow accumulation of repair proteins. *Cell* 136, 435–446. doi: 10.1016/j.cell.2008.12.041.

Feng, L., and Chen, J. (2012). The E3 ligase RNF8 regulates KU80 removal and NHEJ repair. *Nat. Struct. Mol. Biol.* 19, 201–206. doi: 10.1038/nsmb.2211

Galanty, Y., Belotserkovskaya, R., Coates, J., and Jackson, S. P. (2012). RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev.* 26, 1179–1195. doi: 10.1101/gad.188284.112

Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009). Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* 462, 935–939. doi: 10.1038/nature08657

Groocock, L. M., Nie, M., Prudden, J., Moiani, D., Wang, T., Cheltsov, A., et al. (2014). RNF4 interacts with both SUMO and nucleosomes to promote the DNA damage response. *EMBO Rep* 15, 601–608. doi: 10.1002/embr.201338369

Gudjonsson, T., Altmeyer, M., Savic, V., Toledo, L., Dinant, C., Grofte, M., et al. (2012). TRIP12 and UBR5 suppress spreading of chromatin ubiquitylation at damaged chromosomes. *Cell* 150, 697–709. doi: 10.1016/j.cell.2012.06.039

Hendriks, I. A., Treffers, L. W., Verlaan-De Vries, M., Olsen, J. V., and Vertegaal, A. C. (2015). SUMO-2 Orchestrates Chromatin Modifiers in Response to DNA Damage. *Cell Rep.* 10, 1778–1791. doi: 10.1016/j.celrep.2015.02.033

Huen, M. S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., et al. (2007). RNF8 transduces the DNA-damage signal via histone ubiquitylation and checkpoint protein assembly. *Cell* 131, 901–914. doi: 10.1083/jcb.201003034

Ismail, I. H., Andrin, C., Mcdonald, D., and Hendzel, M. J. (2010). BMI1-mediated histone ubiquitylation promotes DNA double-strand break repair. *J. Cell Biol.* 191, 45–60. doi: 10.1083/jcb.201003034

Ismail, I. H., Gagne, J. P., Genois, M. M., Strickfaden, H., Mcdonald, D., Xu, Z., et al. (2015). The RNF138 E3 ligase displaces Ku to promote DNA end resection and regulate DNA repair pathway choice. *Nat. Cell Biol.* 17, 1446–1457. doi: 10.1038/ncb3259

Jackson, S. P., and Durocher, D. (2013). Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell.* 49, 795–807. doi: 10.1016/j.molcel.2013.01.017

Jentsch, S., and Psakhye, I. (2013). Control of nuclear activities by substrate-selective and protein-group SUMOylation. *Annu. Rev. Genet.* 47, 167–186. doi: 10.1146/annurev-genet-111212-133453

Kakarougkas, A., Ismail, A., Chambers, A. L., Riballo, E., Herbert, A. D., Kunzel, J., et al. (2014). Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. *Mol. Cell.* 55, 723–732. doi: 10.1016/j.molcel.2014.06.028

Kang, H. C., Lee, Y. I., Shin, J. H., Andrabi, S. A., Chi, Z., Gagne, J. P., et al. (2011). Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14103–14108. doi: 10.1073/pnas.1108799108

Kolas, N. K., Chapman, J. R., Nakada, S., Ylanko, J., Chahwan, R., Sweeney, F. D., et al. (2007). Orchestration of the DNA-damage response by the RNF8 ubiquitin ligase. *Science* 318, 1637–1640. doi: 10.1126/science.1150034

Kosoy, A., Calonge, T. M., Outwin, E. A., and O'connell, M. J. (2007). Fission yeast Rnf4 homologs are required for DNA repair. *J. Biol. Chem.* 282, 20388–20394. doi: 10.1074/jbc.M702652200

Leung, J. W., Agarwal, P., Canny, M. D., Gong, F., Robison, A. D., Finkelstein, I. J., et al. (2014). Nucleosome acidic patch promotes RNF168- and RING1B/BMI1-dependent H2AX and H2A ubiquitination and DNA damage signaling. *PLoS Genet.* 10: e1004178. doi: 10.1371/journal.pgen.1004178

Lilley, C. E., Chaurushiya, M. S., Boutell, C., Landry, S., Suh, J., Panier, S., et al. (2010). A viral E3 ligase targets RNF8 and RNF168 to control histone ubiquitination and DNA damage responses. *EMBO J.* 29, 943–955. doi: 10.1038/emboj.2009.400

Luijsterburg, M. S., Acs, K., Ackermann, L., Wiegant, W. W., Bekker-Jensen, S., Larsen, D. H., et al. (2012). A new non-catalytic role for ubiquitin ligase RNF8 in unfolding higher-order chromatin structure. *EMBO J.* 31, 2511–2527. doi: 10.1038/emboj.2012.104

Lukas, J., Lukas, C., and Bartek, J. (2011). More than just a focus: the chromatin response to DNA damage and its role in genome integrity maintenance. *Nat. Cell Biol.* 13, 1161–1169. doi: 10.1038/ncb2344

Luo, K., Zhang, H., Wang, L., Yuan, J., and Lou, Z. (2012). Sumoylation of MDC1 is important for proper DNA damage response. *EMBO J.* 31, 3008–3019. doi: 10.1038/emboj.2012.158

Ma, T., Chen, Y., Zhang, F., Yang, C. Y., Wang, S., and Yu, X. (2013). RNF111-dependent neddylation activates DNA damage-induced ubiquitination. *Mol. Cell.* 49, 897–907. doi: 10.1016/j.molcel.2013.01.006

Mailand, N., Bekker-Jensen, S., Faustrup, H., Melander, F., Bartek, J., Lukas, C., et al. (2007). RNF8 ubiquitylates histones at DNA double-strand breaks and promotes assembly of repair proteins. *Cell* 131, 887–900. doi:10.1016/j.cell.2007.09.040

Mailand, N., Gibbs-Seymour, I., and Bekker-Jensen, S. (2013). Regulation of PCNA-protein interactions for genome stability. *Nat. Rev. Mol. Cell Biol.* 14, 269–282. doi: 10.1038/nrm3562

Mallette, F. A., Mattiroli, F., Cui, G., Young, L. C., Hendzel, M. J., Mer, G., et al. (2012). RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J.* 31, 1865–1878. doi: 10.1038/emboj.2012.47

Mattiroli, F., Uckelmann, M., Sahtoe, D. D., Van Dijk, W. J., and Sixma, T. K. (2014). The nucleosome acidic patch plays a critical role in RNF168-dependent ubiquitination of histone H2A. *Nat. Commun.* 5, 3291. doi: 10.1038/ncomms4291

Mattiroli, F., Vissers, J. H., Van Dijk, W. J., Ikpa, P., Citterio, E., Vermeulen, W., et al. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150, 1182–1195. doi: 10.1016/j.cell.2012.08.005

McGinty, R. K., Henrici, R. C., and Tan, S. (2014). Crystal structure of the PRC1 ubiquitylation module bound to the nucleosome. *Nature* 514, 591–596. doi: 10.1038/nature13890

Meerang, M., Ritz, D., Paliwal, S., Garajova, Z., Bosshard, M., Mailand, N., et al. (2011). The ubiquitin-selective segregase VCP/p97 orchestrates the response to DNA double-strand breaks. *Nat. Cell Biol.* 13, 1376–1382. doi: 10.1038/ncb2367

Morris, J. R., Boutell, C., Keppler, M., Densham, R., Weekes, D., Alamshah, A., et al. (2009). The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. *Nature* 462, 886–890. doi: 10.1038/nature08593

Panier, S., Ichijima, Y., Fradet-Turcotte, A., Leung, C. C., Kaustov, L., Arrowsmith, C. H., et al. (2012). Tandem protein interaction modules organize the ubiquitin-dependent response to DNA double-strand breaks. *Mol. Cell.* 47, 383–395. doi: 10.1016/j.molcel.2012.05.045

Poulsen, M., Lukas, C., Lukas, J., Bekker-Jensen, S., and Mailand, N. (2012). Human RNF169 is a negative regulator of the ubiquitin-dependent response to DNA double-strand breaks. *J. Cell Biol.* 197, 189–199. doi: 10.1083/jcb.201109100

Poulsen, S. L., Hansen, R. K., Wagner, S. A., Van Cuijk, L., Van Belle, G. J., Streicher, W., et al. (2013). RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* 201, 797–807. doi: 10.1083/jcb.201212075

Psakhye, I., and Jentsch, S. (2012). Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* 151, 807–820. doi: 10.1016/j.cell.2012.10.021

Schmidt, C. K., Galanty, Y., Sczaniecka-Clift, M., Coates, J., Jhujh, S., Demir, M., et al. (2015). Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. *Nat. Cell Biol.* 17, 1458–1470. doi: 10.1038/ncb3260

Shiloh, Y. (2003). ATM and related protein kinases: safeguarding genome integrity. *Nat. Rev. Cancer* 3, 155–168. doi: 10.1038/nrc1011

Sparmann, A., and van Lohuizen, M. (2006). Polycomb silencers control cell fate, development and cancer. *Nat. Rev. Cancer* 6, 846–856. doi: 10.1016/j.cell.2008.12.042

Stewart, G. S., Panier, S., Townsend, K., Al-Hakim, A. K., Kolas, N. K., Miller, E. S., et al. (2009). The RIDDLE syndrome protein mediates a ubiquitin-dependent signaling cascade at sites of DNA damage. *Cell* 136, 420–434. ddoi: 10.1016/j.cell.2008.12.042.

Sun, H., Leverson, J. D., and Hunter, T. (2007). Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. *EMBO J.* 26, 4102–4112. doi: 10.1038/sj.emboj.7601839

Takano, Y., Adachi, S., Okuno, M., Muto, Y., Yoshioka, T., Matsushima-Nishiwaki, R., et al. (2004). The RING finger protein, RNF8, interacts with retinoid X receptor alpha and enhances its transcription-stimulating activity. *J. Biol. Chem.* 279, 18926–18934. doi: 10.1074/jbc.M309148200

Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., et al. (2015). Histone H1 couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393. doi: 10.1038/nature15401

van Cuijk, L., Van Belle, G. J., Turkyilmaz, Y., Poulsen, S. L., Janssens, R. C., Theil, A. F., et al. (2015). SUMO and ubiquitin-dependent XPC exchange drives nucleotide excision repair. *Nat. Commun.* 6, 7499. doi: 10.1038/ncomms8499

Yin, Y., Seifert, A., Chua, J. S., Maure, J. F., Golebiowski, F., and Hay, R. T. (2012). SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. *Genes Dev.* 26, 1196–1208. doi: 10.1101/gad.189274.112

Zhang, Y., Liu, S., Mickanin, C., Feng, Y., Charlat, O., Michaud, G. A., et al. (2011). RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. *Nat. Cell Biol.* 13, 623–629. doi: 10.1038/ncb2222

Conflict of Interest Statement: 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.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read, for greatest visibility

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

FAST PUBLICATION
Average 85 days from submission to publication (across all journals)

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

SUPPORT
By our Swiss-based editorial team

IMPACT METRICS
Advanced metrics track your article's impact

GLOBAL SPREAD
5'100'000+ monthly article views and downloads

LOOP RESEARCH NETWORK
Our network increases readership for your article

Frontiers
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
www.frontiersin.org

Find us on