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Roles of Ubiquitination and SUMOylation in DNA Damage Response

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Abstract

Ubiquitin and ubiquitin-like modifiers, such as SUMO, exert distinct physiological functions by conjugating to protein substrates. Ubiquitination or SUMOylation of protein substrates determine the fate of modified proteins, including proteasomal degradation, cellular re-localization, alternations in binding partners and serving as a protein-binding platform, in a ubiquitin or SUMO linkage-dependent manner. DNA damage occurs constantly in living organisms but is also repaired by distinct tightly controlled mechanisms including homologous recombination, non-homologous end joining, inter-strand crosslink repair, nucleotide excision repair and base excision repair. On sensing damaged DNA, a ubiquitination/SUMOylation landscape is established to recruit DNA damage repair factors. Meanwhile, misloaded and mission-completed repair factors will be turned over by ubiquitin or SUMO modifications as well. These ubiquitination and SUMOylation events are tightly controlled by both E3 ubiquitin/SUMO ligases and deubiquitinases/deSUMOylases. In this review, we will summarize identified ubiquitin and SUMO-related modifications and their function in distinct DNA damage repair pathways, and provide evidence for responsible E3 ligases, deubiquitinases, SUMOylases and deSUMOylases in these processes. Given that genome instability leads to human disorders including cancer, understanding detailed molecular mechanisms for ubiquitin and SUMO-related regulations in DNA damage response may provide novel insights into therapeutic modalities to treat human diseases associated with deregulated DNA damage response.

Introduction

DNA encodes for inheritable genetic information that is not only essential to exert normal cellular function but also indispensable to maintain the human society. Thus, DNA should be stable while versatile. Although certain genetic changes are permissible to drive evolution (usually at a low mutation rate), improper damaged DNA need to be repaired timely. With the development of technology, human beings are exposed to more DNA damaging cues nowadays such as wireless internet (Wi-Fi) (Akdag *et al.*, 2016), ultraviolet (UV) radiation from sun exposure (Sinha and Häder, 2002) and even microwave ovens (Sagripanti *et al.*, 1987) used on a daily basis. If the damaged DNA is detected and repaired to a level tolerated by cells, cells will survive and may develop neoplastic transformation; otherwise cells will die and be cleared. Damaged DNA is actively monitored by DNA

damage sensors and repaired by DNA damage repair factors. Notably, in most prokaryotes such as bacteria, a SOS response is commonly triggered by DNA damage to repair damaged DNA and also contributes to anti-antibiotic features (Kreuzer, 2013). In this review, we will focus on DDR (DNA damage response) in eukaryotes given its close relationship to human physiology and pathology (Ciccia and Elledge, 2010).

In response to genotoxic challenges, eukaryotes activate DNA damage checkpoints to suppress DNA replication, arrest cell cycle, stop proliferation and meanwhile activate signal transduction pathways to directly repair damaged DNA, or promote transcription of repair enzymes. Mechanisms sensing and repairing damaged DNA are conserved in eukaryotes. Factors inducing DNA damage can be divided into two categories: intrinsic factors and exogenous factors. The most frequent sources of intrinsic DNA damage are from inaccurate DNA replication, free radicals generated *in vivo* under oxidative stress or from normal biological processes including meiotic recombination and V(D)J recombination during antibody production (Hartlerode and Scully, 2009). Strong environmental cues including UV radiation, X-ray, gamma-ray and other chemical mutagens also cause various types of DNA damage, including DSBs (double-strand breaks), SSBs (single-strand breaks), DNA base mutation, deletion, insertion, deamination, chemical modifications and formation of pyrimidine dimmers. Accordingly, distinct DNA damage responses are triggered. For example, UV-induced DNA crosslinking is resolved by NER (nucleotide excision repair) (Marteijn *et al*., 2014), unmatched, modified and damaged DNA bases are removed and refilled by the mismatch repair mechanism (Li, 2008), SSBs and DSBs are repaired by either HR (homologous recombination) (Li and Heyer, 2008) or NHEJ (non-homologous end joining) (Chang *et al*., 2017). Similar to prokaryotes, eukaryotes also utilize a SOS response in coordinating different repair pathway choices in responding to severe DNA damages (Fu *et al*., 2008). The eukaryotic DNA damage repair systems include DSB repair, inter-strand crosslink repair (ICLR), nucleotide excision repair (NER) and base excision repair (BER) (Hoeijmakers, 2001).

Cells would need acute responses to repair damaged DNA-otherwise severe unrepaired DNA damage leads to cell death (Nowsheen and Yang, 2012). The fastest reaction in cell is through biochemical reactions-indeed protein translational modifications have been observed and proven to play indispensable roles in this regard. For example, ATM, ATR or DNAPK controls phosphorylation of a large group of 'SQ/TQ' containing substrates including Chk1 and Chk2 (Chen and Poon, 2008), while as protein kinases themselves, Chk1 and Chk2 will further amplify the DNA damage signals by phosphorylating more substrates such as Cdc25A, p53, PML, Plk3 and many others (Bartek and Lukas, 2003). It is a kinase network or landscape that transduces the DNA damage signals in an acute and spatial-tempo dependent manner (Chen and Poon, 2008). In addition to extensively studied and well-characterized protein kinase cascades in DDR, ubiquitination and its close cousin, SUMOylation are other types of protein modifications that exert indispensable roles in both sensing and repairing damaged DNA (Brinkmann *et al*., 2015; Wang, Z. *et al*., 2017). In this review, we will summarize recent progress on ubiquitin and SUMO-related regulations on DDR, list all identified ubiquitination and SUMOylation events during DDR, further illustrate their physiological and pathological function and provide new insights into future research directions or therapeutic modalities targeting these identified ubiquitination or SUMOylation events.

Overview of the ubiquitin signalling

Ubiquitin is a 76 amino-acid protein highly conserved among eukaryotic species. Usually ubiquitin is considered as a modifier for proteins-attachment of ubiquitin moiety to a lysine residue on target proteins regulates important cellular processes including cellular trafficking, immune sensing, protein translation, metabolism, cell cycle and autophagy (Finley, 2009). Protein ubiquitination is a three-step enzymatic reaction requiring three types of enzymes, including E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase. In mammals, there are one major E1, forty E2s and more than 600 E3s. E3 ubiquitin ligases are mainly divided into three families based on their structures and mechanisms of ubiquitin transfer, including RING (Really Interesting New Gene), HECT (Homologous to E6-AP Carboxyl

Terminus) and RBR (RING-Between-RING) domain containing E3 ubiquitin ligase families (Zheng and Shabek, 2017). For RING and RBR families of E3 ligases, activated ubiquitin by E1 will be conjugated to E2, and it is the E2 enzyme directly transferring ubiquitin to substrates that are determined by E3 ligases. While for HECT domain containing E3 ligases, ubiquitin will be transiently transferred from E2 to E3 then transferred to substrates. In this process, E3 ubiquitin ligases determine the substrate specificity.

Notably, each ubiquitin contains seven lysine residues. Addition of a ubiquitin to a prior ubiquitin molecule can be linked through each of seven lysine residues in ubiquitin, or through a head-to-toe ligation, leading to formation of poly-ubiquitin chains in different linkages. According to the position of linked lysine residue, poly-ubiquitin chains can be linked through M1 (head-to-toe), K6, K11, K27, K29, K33, K48 and K63 linkages. The exact structures for poly-ubiquitin chains in a variety of linkages remain unclear, while some conformation for di-ubiquitin chains or shorter chains have been determined. K48-(Zhang, N. *et al.*, 2009) or K11-linked (Bremm *et al.*, 2010) poly-ubiquitin chains adopt compact structures (Saeki, 2017) that fit well to the 26S proteasome recently determined by Cyro-EM (Dong *et al.*, 2018). Thus, these two linkages are poised for protein degradation-an energy dependent process to destroy and recycle unwanted proteins. M1 and K63 (Weeks *et al.*, 2009) linkages are in more labile structure with a great degree of flexibility (Kulathu and Komander, 2012; Sekiyama *et al.*, 2012). These two types of ubiquitin chains usually serve as a binding platform for protein factors in various physiological conditions such as innate immunity (Xia *et al.*, 2009) and cellular trafficking (Erpapazoglou *et al.*, 2014). Recently, we found a protein modification independent function of K63-linked poly-ubiquitin chains in directly binding exposed naked DNA to facilitate DNA damage repair (Liu *et al.*, 2018). K29 (Kristariyanto *et al.*, 2015) and K33 chains adopt a zigzaging conformation (Michel *et al.*, 2015). Notably, multiple linkages of poly-ubiquitin chains have been indicated to play critical roles in DDR, including K63, K6, K27 and others (Elia *et al.*, 2015a). In addition, a given poly-ubiquitin chain can be either composed of one linkage (homotypic) or several different linkages to form chains with mixed linkages or branched chains (heterotypic) (Meyer and Rape, 2014; Ohtake and Tsuchiya, 2017). Moreover, more than one poly-ubiquitin chain can be covalently attached to the same ubiquitin molecule on different lysine residues (Suryadinata *et al.*, 2014). To make it more complicated, the ubiquitin molecule itself also undergoes various post-translational modification (PTM) events, including phosphorylation (Koyano *et al.*, 2014) and acetylation (Ohtake *et al.*, 2015), adding another layer of regulation on poly-ubiquitin chains. These distinct linkage composition and ubiquitin modifications on substrates create unique languages coding for distinct biological meanings, which have been referred to as 'ubiquitin codes' (Komander and Rape, 2012; Yau and Rape, 2016).

Ubiquitination is a reversible protein modification and a result of a balance between adding and removing ubiquitin moieties. Various deubiquitinating hydrolases or deubiquitinases (DUB) have been identified as key enzymes for the removal of ubiquitin polypeptides from target proteins (Komander *et al.*, 2009). To deal with the complicated ubiquitin system, mammalian cells develop DUBs that can be in large divided into seven families, including five families of cysteine proteases and one family of Zn-dependent metalloprotease (Komander *et al.*, 2009). Specifically, cysteine proteases include USPs (ubiquitin specific proteases), OTUs (ovarian tumour proteases), UCHs (ubiquitin carboxyl-terminal hydrolases), Joshphin family of proteases and MINDYs (motif interacting with ubiquitin containing novel DUB family)(Abdul Rehman *et al.*, 2016). The family of Zn-dependent metalloprotease consists JAMMs (JAB1/MPN/MOV34 metalloproteases), also termed as MPN+ family of DUBs (Clague *et al.*, 2013). The role of DUBs in DDR is just began to be appreciated (Kee and Huang, 2016) and there is limited knowledge about whether and how these DUBs recognize ubiquitinated proteins in a linkage-specific manner, but the general impression is that compared with E3 ubiquitin ligases, DUBs are lacking certain substrate specificity-which means that a small number of DUBs may govern deubiquitination of a large spectrum of ubiquitinated substrates. Usually, USP DUBs directly bind substrates owing to the presence of protein interacting motifs (Faesen *et al.*, 2011; Ye *et al.*, 2009), while OTU DUBs exert certain ubiquitin linkage specificity, such as targeting

the M1-linkage in LUBAC signalling (Keusekotten *et al.*, 2013) and NF-kB signalling (Rivkin *et al.*, 2013), or K63-linkage in mTOR signalling (Wang, B. *et al.*, 2017) and non-canonical NF-kB signalling (Hu *et al.*, 2013). How DUBs control DDR has been understudied.

Thus, the 'ubiquitin codes' are produced by 'ubiquitin writers', most of the time are E3 ubiquitin ligases (Natarajan and Takeda, 2017) and removed by 'ubiquitin erasers' that are DUBs. Accordingly, different 'ubiquitin code' can be read and interpreted by various 'ubiquitin readers' that carry out distinct biological functions (Pinder *et al.*, 2013). Making sure damaged DNA is repaired correctly and timely is key to maintain genome integrity, otherwise unrepaired DNA lesions may cause cells to die or accumulated DNA alternations may induce tumorigenesis (O'Connor, 2015). Thus, critical steps of DDR including the sensing of DNA damage, the recruitment of DNA damage repair factors and the repair of DNA lesions, are tightly controlled. Besides DNA repair, equally important is other cellular responses to DNA damage, such as cell cycle arrest and apoptotic cell death, if the DNA damage is very severe and unrepairable. Because of its importance to cell survival and function and its dynamic response to environment cues, DDR is tightly regulated by protein post-translational modifications. One indispensable mechanism to ensure accurate and efficient DDR is to utilize the ubiquitin signalling. Indeed, upon DNA damage, a ubiquitin landscape is quickly established to label the damage foci, recruit repair factors and regulate the entire repair process by multiple E3 ubiquitin ligases including RNF8, RNF168, BRCA1, BMI1, Ring1B, Rad18 and others (Messick and Greenberg, 2009). The efforts to investigate contribution of ubiquitin linkages start early. Initially, ectopically expressed K6 and K63-linked, but not K48-linked ubiquitin was enriched at sites of DNA damage (Sobhian *et al.*, 2007). Following studies demonstrated that K48-linked ubiquitin chains also play a critical role in removing Ku80/Ku70 complexes to facilitate the progression of NHEJ. More recent non-biased large-scale studies examining endogenous ubiquitin linkages observed a dramatic accumulated K6- and K33-linked ubiquitin chains with DDR (Elia *et al.*, 2015a). Thus, a variety of distinct linkages of ubiquitin chains may play important roles in guiding proper sensing and repair of damaged DNA under different pathophysiological conditions. Given that sensing and repair of damaged DNA are complicated processes and there is no clear boundary between these two consecutive events, in the following section, we will summarize distinct ubiquitin events and their roles in DDR in a DNA damage repair mechanism dependent manner.

Overview of the SUMO signalling

In addition to ubiquitin, there are many ubiquitin like (UBL) molecules with similar sequence/structure composition but distinct function (Hu and Hochstrasser, 2016). Small Ubiquitin-like MOdifier (SUMO) is a highly conserved (approximately 12 kDa) protein produced as an immature precursor that needs to be cleaved by sentrin/SUMO-specific protease 1 (SENP1) prior to conjugation. SUMO has similar conjugation pathways as ubiquitin, but the process is carried out by SUMO-specific enzymes. First, E1-activating enzyme (the heterodimer SAE1/SAE2) charges C-terminal di-glycine residues of mature SUMO in an ATP-dependent manner. Then the activated SUMO is transferred to the E2 conjugating enzyme UBC9 via a thioester transfer step. Next, UBC9 directly conjugates the SUMO molecule to the lysine residues of substrate proteins through an isopeptide linkage, or with the assistance of SUMO E3 ligases. The E3-ligating enzymes improve conjugation by either recognizing target lysines or enhancing SUMO discharge from the E2 to the substrate. The most well characterized SUMO E3s are Protein Inhibitor of Activated STAT (PIAS 1–4) (Rytinki *et al.*, 2009), with an SP-RING domain similar to the RING motif in many E3 ubiquitin ligases. The SUMO conjugation system has relatively fewer enzymatic components than the ubiquitin system. Compared with approximately 40 different E2-conjugating enzymes for ubiquitin, only one E2 (Ubc9) in SUMO system has been identified so far. Moreover, only a handful of SUMO E3 enzymes have been identified compared to around 600 E3 ubiquitin ligases. Notably, plants and metazoan have more enzyme isoforms of SUMO E3s compared with lower eukaryotes like in yeast.

However, unlike only one unified ubiquitin molecule, there are more than one SUMO isoform in the SUMO system: SUMO1, SUMO2/3 and the

recently described ones including SUMO4 (Baczyk *et al.*, 2017) and SUMO5 (Liang *et al.*, 2016). SUMO proteins have high similarities to the tertiary folding structure of ubiquitin while they share limited sequence identity (less than 20%) and have different surface charge distributions (Huang *et al.*, 2004). SUMO1 was first identified as a human ubiquitin-like protein that interacts with RAD51/RAD52 proteins (Shen *et al.*, 1996), Promyelocytic leukaemia (PML) components (Boddy *et al.*, 1996), and conjugates GTPase RanGAP1 to recruit it to nuclear pore complex protein RanBP2 (Matunis *et al.*, 1996; Mahajan *et al.*, 1997). SUMO2 and SUMO3 are nearly identical in sequence (97% identity, referred to as SUMO2/3) but distinct from SUMO1 (50% identity). SUMO4 is reported as a new IκBα modifier (Guo *et al.*, 2004) but another study showed SUMO4 cannot be processed to a mature form due to its unique proline-90 residue (Owerbach *et al.*, 2005). Recently, SUMO5, previously reported as a pseudogene (Su and Li, 2002), could form novel poly-SUMO isoforms that regulate PML nuclear bodies (Liang *et al.*, 2016). In addition, SUMOylation occurs most frequently (≈75%) at a lysine residue within a consensus sequence 'ψKxE/D' (where ψ represents a hydrophobic amino acid and x any amino acid) (Bernier-Villamor *et al.*, 2002; Hendriks *et al.*, 2017; Lamoliatte *et al.*, 2017) but ubiquitination has little preference for lysine context. SUMOylation of different forms of SUMO modifiers can occur on the same or different substrates. Some proteins are preferentially modified by one type of SUMO isoform while others could be modified by different SUMO isoforms. SUMOylation can also be in the form of chains as polySUMO as in the ubiquitin system, and the chains are only generated on SUMO2/3 but not SUMO1(Sarge and Park-Sarge, 2009).

SUMOylation is also a reversible process, similar to deubiquitination, but in which deSUMOylation involves the removal of SUMO terminal glycine from the lysine residues of the substrate protein by specific proteases (Nayak and Müller, 2014). Unlike the array of proteases in the ubiquitin system, the SUMO protease family has just been found to be limited. SUMO proteases can be divided into three classes, including (1) thiol proteases, (2) cysteine proteases and (3) a mammalian specific SUMO-specific protease USPL1(Nayak and Müller, 2014). SUMO thiol proteases include six sentrin (SUMO)-specific proteases termed as SENPs including SENP-1, -2, -3, -5, -6 and -7 in mammals (Hickey *et al.*, 2012). Notably, although SENP-8 was originally identified as a deSUMOylase, later it was proven that the true substrate for SENP-8 is another ubiquitin-like molecule Nedd8 (Gan-Erdene *et al.*, 2003; Mendoza *et al.*, 2003). SENP1–3 and SENP5 are related to the yeast deSUMOylase Ulp1, and SENP6 and 7 are close to yeast deSUMOylase Ulp2. These SENPs differ in SUMO maturation (C-terminal hydrolase) and isopeptide cleavage activity. Additionally, different SENPs have their preferences for different SUMO modifier isoforms. For example, both SENP1 and SENP2 can process SUMO1and SUMO2/3, while SENP3 and SENP5 are mainly involved in SUMO2/3 deSUMOylation. PolySUMO chains of SUMO2/3 are dissociated by SENP6 and SENP7 (Hickey *et al.*, 2012). Notably, the SUMO cysteine proteases include Desi-1 and Desi-2 are only present in plants and metazoan (Nayak and Müller, 2014). Interestingly, SUMO conjugation can be achieved in both SUMO E3 ligase dependent and independent manners (Nayak and Müller, 2014).

Similar to poly-ubiquitination, poly-SUMOylation chains can also serve as a binding platform for protein factors and to date there are some SUMOylation binding domains characterized, including a hydrophobic core sequence ([V/I]-x-[V/I]-[V/I]) (Heerwagen *et al.*, 1995) surrounded by negatively charged residues, or a protein motif composed of [I/V/L]-[D/E]-[I/V/L]-[D/E]-[I/V/L] (Ouyang *et al.*, 2009) (Table 15.1). While on the other hand, there are more than 16 well-characterized ubiquitin binding domains (Grabbe and Dikic, 2009). Given that thousands of proteins have been identified to be modulated by this modification, it is not surprising that SUMOylation plays a broad spectrum of cellular functions in development, growth, metabolism, and DNA damage response (Nayak and Müller, 2014).

Ubiquitin and SUMO signalling in HR

DNA double-strand breaks are the most severe type of DNA damage, whose repair is governed by two major pathways: Homologous Recombination (HR) and Non-Homologous End-joining (NHEJ)(Lieber, 2010). The HR pathway requires

Table 15.1 Comparison of Ub and SUMO conjugation system

Components in conjugation system	Ubiquitination	SUMOylation
Conjugates	Ubiquitin	SUMO1, SUMO2/3 SUMO4, SUMO5
E1 activating enzymes	UBE1 (UBA1)	SAE1-SAE2 (UBA2)
E2 conjugating enzymes	~40 conjugation enzymes	UBE2I (UBC9)
E3 ligases	~600 E3 ligases	PIASs, RanBP2, Siz1#, CBX4
Conjugate removing enzymes	~100 DUBs	SENP1–3 and 5–7, DeSi1/2, USPL1
Conjugation sites	Little preference for lysine context	Frequent consensus sequence 'ψKxE/D'

the presence of a homologous DNA sequence as the repair template; thus, it is mainly functional in S and G2 phases (Longhese *et al.*, 2010). The NHEJ pathway, by its name, is an error-prone DNA damage repair pathway because it directly glues two broken DNA ends without caring about whether the repair products faithfully resemble their original DNA sequence. While on the other hand, as NHEJ does not require the presence of a nearby template chromatin to repair DNA lesions, it is more versatile for acute repair and to promote DNA evolution. Notably, NHEJ occurs during the entire cell cycle.

Once DSBs occur, these damaged free DNA ends can be recognized by either the Mre11/Rad50/Nbs1 (MRN) complex or the Ku70/Ku80 complex, leading to HR or NHEJ, respectively. The determining step for HR repair is DNA end resection, where broken double-strand DNA (dsDNA) will be resected into a long ssDNA (single-strand DNA) that intrudes into dsDNA to search for homologous sequence. DNA end resection is carried out by the MRN complex. MRN searches for free DNA ends-Rad50 binds dsDNA to allow perfusion of MRN complexes along DNA for this search and Mre11 carries out a nucleolytic reaction to exert two functions: (1) recruit Exo1 (Exonuclease 1) to initiate resection and (2) remove Ku70/Ku80 from binding broken DNA ends to promote HR and suppress NHEJ. This process was recently confirmed by single molecule imaging (Myler *et al.*, 2017). This process can be antagonized by BRCA1 binding to DNA (Paull *et al.*, 2001), resulting in inhibition of the nucleolytic activities of MRN and suppression of HR. In addition, Exo1 protein stability is governed by the E3 ubiquitin ligase Cyclin F (Elia *et al.*, 2015a). Upon MRN loading onto DNA, Nbs1 is poly-ubiquitinated by the E3 ubiquitin ligase Skp2 in a K63-linkage dependent manner to recruit the kinase ATM to sites of damage, where ATM phosphorylates histone H2 at Ser139, forming γ-H2Ax foci. Notably, γ-H2Ax foci serve as red flags to earmark DNA damage sites (Fig. 15.1 and Table 15.2).

The DNA damage signal can be further amplified by a way that MDC1 (Mediator of DNA damage checkpoint protein 1) binds and protects γ-H2Ax, bringing in another MRN complex through binding Nbs1 (Stewart *et al.*, 2003; Lukas *et al.*, 2004) and a second ATM kinase [Nbs1 binds ATM (Falck *et al.*, 2005)] to phosphorylate MDC1 that is necessary to recruit a critical E3 ubiquitin ligase RNF8. MDC1 undergoes K48-linked ubiquitination as a protein turnover control with unknown E3 ligases (Shi *et al.*, 2008), a process blocked by USP7 (Su *et al.*, 2018). In addition to ubiquitination, MDC1 is also SUMOylated by PIAS4 to promote MDC1 protein turnover (Luo *et al.*, 2012). RNF8 is a key Ring-finger E3 establishing and orchestrating a ubiquitin landscape on histones at sites of DNA damage by ubiquitinating H2A or H2Ax in a K63-linkage specific manner with the help of the E2 enzyme UBC13 (Kolas *et al.*, 2007; Mailand *et al.*, 2007). In addition, RNF8 also promotes ubiquitination of Nbs1 to facilitate the MRN complex formation and HR (Lu *et al.*, 2012). The critical role of RNF8 in positively regulating DDR is evidenced by the observation that RNF8 deletion leads to cellular sensitivity to IR and arrested G2/M transition (Huen *et al.*, 2007; Kim *et al.*, 2007; Kolas *et al.*, 2007; Mailand *et al.*, 2007). Notably, the role of RNF8 in DDR is antagonized by DUBs such as USP11 (Yu, M. *et al.*, 2016) and BRCC36 through specifically removing K63-linked ubiquitin chains RNF8/UBC13 produce. In addition to establishing

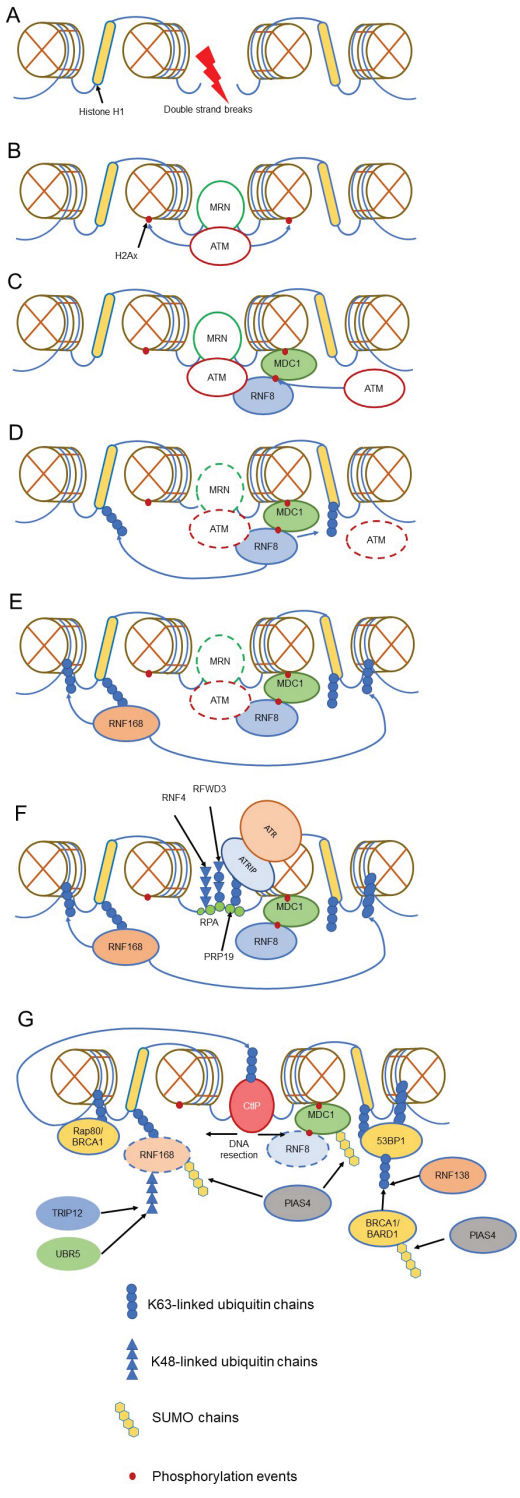

Figure 15.1 Ubiquitin and SOMO modifications in HR.

K63- and K48-linked ubiquitin chains, RNF8 also produces K11-linked ubiquitin chains on unknown substrates to inhibit transcription, and this function of RNF8 is antagonized by the DUB cezanne (Paul and Wang, 2017). Once the initial ubiquitin signal is established by RNF8/UBC13, another E3 ubiquitin ligase RNF168 recognizes ubiquitinated/SUMOylated H2A and further ubiquitinates H2A at K13-K15 residues to amplify the ubiquitin signalling (Doil *et al.*, 2009; Panier *et al.*, 2012). While other reports support the notion that although RNF168 functions depending on RNF8, RNF8 and RNF168 ubiquitinate non-histone proteins and histones, respectively, to establish the ubiquitination landscape on DNA damage (Mattiroli *et al.*, 2012; Panier *et al.*, 2012). RNF168 itself could be poly-ubiquitinated by the HECT type E3 ligases TRIP12 and UBR5, restricting the spreading of ubiquitinated γ-H2Ax and preventing genome-wide transcriptional suppression, which could be potentially detrimental to cells (Gudjonsson *et al.*, 2012). In addition, RNF168 binds and ubiquitinates PML to trigger subsequent SUMO2 modification of PML that facilitates formation of PML nuclear bodies (Shire *et al.*, 2016). While a viral E3 ubiquitin ligase ICP0 targets both RNF8 and RNF168 to negatively regulate their function (Lilley *et al.*, 2010). In addition to RNF168, RNF8-mediated ubiquitin signalling also recruits other E3 ubiquitin ligases including Rad18 (Huang *et al.*, 2009) and HERC2 (Bekker-Jensen *et al.*, 2010; Wu *et al.*, 2010) to amplify ubiquitin signalling. Beyond RNF8, another E3 ubiquitin ligase CHFR also triggers the first wave of ubiquitination events at DSBs by at least ubiquitinating PARP1 (poly-ADP-ribose polymerase I) that may regulate ubiquitination and poly-ADP-ribosylation (Fig. 15.1 and Table 15.2).

Nonetheless, established K63-linked ubiquitin chains by multiple E3 ubiquitin ligases mentioned above serve as a binding platform to recruit proper DNA damage repair factors, such as Rap80/BRCA1 and 53BP1, which determines repair by HR or NHEJ. The UIM (ubiquitin interacting motif) in Rap80 binds K63-linked poly-ubiquitin chains and promotes the assembly of the Rap80/ABRA1/BRCA1 complex (Kim *et al.*, 2007; Sobhian *et al.*, 2007; Wang, B. *et al.*, 2007; Yan *et al.*, 2007), which is essential for HR. On the other hand, RNF168 ubiquitinates 53BP1 through K63-linked poly-ubiquitination to promote 53BP1

Table 15.2 Summary of modified DDR members by ubiquitin and SUMO

Repair pathway	Substrates	E3 ligase/linkage/function	DUB/linkage/function	SUMOylase/function	DeSUMOylase/function
DDR	H1	RNF8[1–5], K63, recruits RNF168			
DDR	H2A -K13/ K15	RNF168[2,5,6], K63, recruits 53BP1, RAP80, RAD18, RNF169	USP16[7], interacts with HERC2		
			USP3[8–10], counteracts RNF168		
			USP44[11], counteracts RNF8/ RNF168-mediated histone ubiquitination		
			BRCC36[12], reverses H2A ubiquitination by RNF8/RNF168		
			POH1, negatively regulates 53BP1 accumulation		
DDR	H2A-K119/ K120	RING1B/BMI1[13–15], recruits DNA repair factors			
DDR	H2A-K127/ K129	BRCA1-BARD1[16], K6, maintain chromatin in a transcription-repressive status			
DDR	H2BK120	RNF20/RNF40[17], mono-ubiquitination, promotes HR			
DDR	BMI1			CBX4, influences DDR Ub-signalling[18]	
HR	Nbs1	Skp2[19], K63, promotes ATM binding to Nbs1 and HR			
		RNF8[20], facilitates MRN complex formation and HR			
HR	MDC1	RNF4[21], K48, MDC1 degradation		PIAS4, drives RNF4 interaction[21,22]	
HR	PARP1	CHFR[23], K63, important for first wave of ubiquitination in HR			
HR	RPA	RNF4[24], K48, promotes HR;			
		RFWD3[25,26], mixed linkages, promotes HR			
		PRP19[27], unknown linkage, binds and ubiquitinates RPA-ssDNA to bring ATRIP to ATR activation			

	HR	PML	RNF168[28], triggers PML SUMO2 modification			
	HR	BRCA1			PIAS1/4, increases BRCA1: BARD1 E3 ligase activity *in vitro*[29]	
	HR	BRCA2	HERC2[30], inhibits BRCA1 binding to BRCA2			
	HR	53BP1	RNF168[3], K63, promotes 53BP1 recruitment to the site of DNA damage		PIAS1 and PIAS4, promotes DSB repair[31]	
	HR	CtIP	promotes RNF138[32], K63, CtIP accumulation and HR activation BRCA1-BARD1[33], K63, maintain CtIP on chromatin			
	HR	RNF168	TRIP12/UBR5, K48, removes RNF168 to prevent wide-spreading histone ubiquitination		PIAS4, increases protein stability and promotes its transcription[34]	
	HR	unknown	Rad18, interacts with Rad51c to promote HR [35]			
	HR	911	Rad6-Rad18[36],			
	HR	Exo1	SCF-CyclinF[37], K48, degradation		PIAS4, reduces its stability[38]	SENP6, promotes its hypoSUMOylation[38]
	HR	PALB2	KEAP1[39], blocks PALB2/ BRCA1 complex formation and suppresses HR			
	HR	Claspin	APC/Cdh1[40], K48, degradation β-TRCP[40], K48, degradation	USP7[40], reverses β-TRCP mediated ubiquitination, stabilizes Claspin		
	HR	ERCC6	N/A	USP7[41]		
	HR	Chk1	N/A	USP7[42], stabilizes Chk1		
	HR	Mdm2	N/A	USP7[43], stabilizes Mdm2		

Table 15.2 Continued

Repair pathway	Substrates	E3 ligase/linkage/function	DUB/linkage/function	SUMOylase/function	DeSUMOylase/function
NHEJ	Ku80	RNF8[44], K48, degrades Ku80			
		RNF138[45], K48, degrades Ku80			
		F-box proteins[46], degrade Ku80 and promotes NHEJ			
		RNF126, releases Ku70/80 for NHEJ to continue			
NHEJ	DNAPK	RNF144A[47], K48, degrades DNAPK			
NHEJ	RPA70/RPA1			required for RAD51 accumulation[48]	SENP6, promotes its hypoSUMOylation[48]
NHEJ	XLF	β-TRCP[49], K48, degrades phosphorylated XLF			
NHEJ	XRCC4	Fbw7[50], K63, enhances the binding between XRCC4 and Ku70/80, promotes NHEJ repair		regulates localization[51]	
		MonoUb[52], stablizes DNA ligase IV			
Template switching/ Translesion synthesis	PCNA	RAD18[53], monoUb, facilitates TLS and stimulates the E3 activity of FANCL	USP1[54]		
			USP7[55], suppresses induced PCNA monoUb		
		Rad5, K63, promotes template switching repair in yeast			
		RNF8[56], K48, Plays a role in DNA Damage Tolerance (DDT)[56]			
Template switching/ Translesion synthesis	KAP1			auto-SUMO ligase[57], DSB-associated transcriptional repression[58]	SENP7, promoting chromatin relaxation[59]
FA	FANCD2 and FANCI	FANCL[60,61], monoubiquitination, promotes BRCA1/2 pathway			

FA	FANCG	K63Ub[62], required for binding with Rap80–BRCA1 complex and increased HR efficiency	BRCC6, the inhibition of which improved HR
NER	DDB2	DDB1[63], K48, degrade DDB2	USP24[64], degrades DDB2
NER	RNA polII	Rsp5[65], K63 or mixture of mono- and poly-Ub, prerequisite step for degradation by Elong1-Cul3	Ubp2[65], trims K63 Ub chains on RNA PolII into mono-Ub for proofreading
		Elong1-Cul3[65], K48, degrades RNA polII	Ubp3[66], reverses K48 Ub chains on RNA polII
NER	H2B	N/A	USP7[67], promotes base-excision repair
NER	XPC	UV-DDB2[68], enhances XPC binding with DNA	USP11[69], increase XPC retention on the damaged DNA
		RNF111[70], triggers XPC release from damaged DNA sites, allow binding of other NER factors	
BER	MUYH, RNA Polβ	MULE[71], K48, promotes degradation	
BER	APE1	Mdm2[72] and UBR3[73], promotes degradation	
BER	PNKP	Cul4A-DDB1-STRAP[74], promotes degradation	

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loading onto sites of damage (Bohgaki *et al.*, 2013) and subsequent repair of damaged DNA through NHEJ. BRCA1 then facilitates Rad51 loading by complexing with BRCA2/PALB2 (Sy *et al.*, 2009; Zhang, F. *et al.*, 2009a,b) and Rad51 is indispensable to search for homologous DNA sequence for HR-mediated DNA damage repair. Moreover, BRCA1 also promotes DNA end resection by recruiting the resection enzyme CtIP and excluding 53BP1 thus inhibiting NHEJ (Jiang and Greenberg, 2015). Ubiquitination of PALB2 by the E3 ligase Keap1 has been observed to specifically block the BRCA complex formation, rather than targeting PALB2 for degradation, thus suppressing HR (Orthwein *et al.*, 2015). The RING-type E3 ligase RNF138 has been shown to ubiquitinate CtIP, promoting its accumulation to the site of DNA damage, thereby activating HR repair. This ubiquitination occurs at a relatively early stage of DNA resection. On the other hand, CtIP could also be ubiquitinated by BRCA1-BARD1 E3 ligase, which serves to maintain CtIP on the chromosome after DNA damage. Another DNA repair protein under regulation of ubiquitination is RPA, which binds naked ssDNA after DNA resection. Both RNF4 (Galanty *et al.*, 2012) and RFWD3 (Elia *et al.*, 2015b) bind and ubiquitinate RPA, promoting the removal of RPA from DNA damage sites and suppressing HR repair, while PRP19 (Maréchal *et al.*, 2014) ubiquitinates RPA and brings along ATRIP, which in turn activates ATR kinase and promotes HR pathway. Moreover, RFWD3 ubiquitinates RPA to promote replication fork restart and increase HR efficiency at stalled replication forks during DNA replication (Elia *et al.*, 2015b). RPA also undergoes SUMOylation by unknown SUMOylase(s), which promotes RPA binding to Rad51 (Dou *et al.*, 2010) to facilitate HR. In addition, SUMOylation of ATRIP has also been observed to facilitate ATRIP interaction with ATR, while the identities of the SUMOylase(s) remains unknown (Wu *et al.*, 2014) (Fig. 15.1 and Table 15.2).

Intriguingly, BRCA1 itself functions as a E3 ligase by complexing with BARD1 and multiple substrates have been identified in DDR including but not limited to H2A, H2AX, RNA polII, TFIIE, NPM1, CtIP, tubulin, ER-α and claspin (Wu *et al.*, 2008; Densham and Morris, 2017). BRCA1 undergoes SUMOylation by PIAS1/4 and SUMO conjugation promotes BRCA1 E3 ligase activity *in vitro* (Morris *et al.*, 2009). The E3 ligase HERC2 negatively regulates BRCA2 protein stability by attaching K48-linked ubiquitin chains and BARD1 binding to BRCA2 protects BRCA2 from HERC2-dependent degradation (Wu *et al.*, 2010). The APC/Cdh1 E3 ligase negatively regulates DDR by targeting Claspin for K48-linked ubiquitination and degradation (Bassermann *et al.*, 2008; Gao *et al.*, 2009; Oakes *et al.*, 2014). In addition, Claspin is also targeted by anther E3 ligase β-TRCP for degradation, where USP7 specifically antagonizes β-TRCP but not Cdh1-mediated Claspin proteolysis (Faustrup *et al.*, 2009). Notably, FANCG undergoes K63-linked ubiquitination to facilitate its association with BRCA1/Rap80 to promote HR for resolving DNA crosslinks, a process that is antagonized by the DUB named BRCC36(Zhu *et al.*, 2015) (Fig. 15.1 and Table 15.2).

In addition to well-established ATM/MDC1/RNF8 signalling in response to DSBs, the BAL1/BBAP E3 ligase complex has been observed to be able to sense and transduce DNA damage signals independent of the ATM/MDC1/RNF8 signalling that is associated with PARP1 activation and BRCA1 recruitment (Yan *et al.*, 2013).

Notably, deSUMOylation by SENP7 of KAP1 (KRAB-associated protein 1) relaxes chromatin structure to promote HR (Garvin *et al.*, 2013), while SUMOylation of Tyrosyl-DNA phosphodiesterase 1 (TDP1) promotes TDP1 enrichment on damage sites although the identity of the SUMOylase(s) is elusive (Hudson *et al.*, 2012) (Fig. 15.1 and Table 15.2).

Ubiquitin and SUMO signalling in NHEJ

The NHEJ repair pathway starts with binding of damaged DNA by the Ku70/80 heterodimers through the Ring-like structure, enabling the recruitment of DNA repair factors functioning in NHEJ, including DNAPK, XLF, PAXX, XRCC4, DNA ligase IV, Artemis and DNA polymerases μ and λ (Lieber, 2010). Initially, Ku70/Ku80 needs to be loaded efficiently to ensure timely repair of damaged DNA, but during NHEJ repair Ku70/Ku80 rings need to be efficiently and timely removed. This is partially achieved by either

RNF8 (Feng and Chen, 2012) or RNF138 (Ismail *et al.*, 2015)-mediated K48-linked ubiquitination of Ku80 to remove Ku80/Ku70 complexes from DSBs to allow NHEJ to occur. On the other hand, the APC (Anaphase Promoting Complex) catalyses K48-linked ubiquitination of RNF8 to antagonize the negative regulation of Ku80 by RNF8, facilitating NHEJ (Ma *et al.*, 2018). In addition to single subunit Ring figure E3 ligases including RNF8 and RNF168, a group of F-box E3 ligases including Fbxl12, β-TRCP, Fbh1, Fbxl19, Fbxo24, Fbxo28 and Kdm2b have been observed to target Ku80 for ubiquitination and degradation, therefore facilitating NHEJ (Postow and Funabiki, 2013). RNF126 ubiquitinates and degrades Ku80 to release Ku70/Ku80 from damaged DNA to complete NHEJ. Deficiency in RNF126 leads to extended NHEJ process (Ishida *et al.*, 2017). In addition to proteasomal degradation, Ku80/Ku70 can also be removed by VCP/p97-which is important for Ku70/Ku80 extraction from DSBs on K48-linked ubiquitination in a Ufd1/Npf4 dependent manner, therefore suppressing NHEJ and facilitating HR (van den Boom *et al.*, 2016). Interestingly, Ku70 has been observed to display a DUB activity towards stabilizing the proapoptotic protein Bax, thus exerting roles in cell apoptosis in addition to DNA damage (Rathaus *et al.*, 2009). In yeast, Yku70 is SUMOylated by yeast SUMOylases including Mms21 and Siz1/2, and SUMO conjugation promotes Yku70 association with DNA (Hang *et al.*, 2014) (Fig. 15.2 and Table 15.2).

The E3 ubiquitin ligase RNF144A targets cytosolic DNAPK for K48-linked ubiquitination and degradation to promote DNA damage-induced cellular apoptotic response (Ho *et al.*, 2014). DNAPK recruits DNA damage repair factors to the site of lesions, including Artemis that trims the DNA ends with overhangs, and DNA ligase IV, which ligates blunt-ended DNA. In addition, DNAPK also phosphorylates and recruits XRCC4, PAXX, XLF to complex with DNA ligase IV to form a ligase complex with optimal activity for NHEJ. Notably, an important factor in this complex, XLF, undergoes Akt-mediated phosphorylation that triggers its association and degradation by the E3 ubiquitin ligase β-TRCP in a K48 linkage dependent manner (Gan *et al.*, 2015; Liu *et al.*, 2015) to suppress NHEJ.

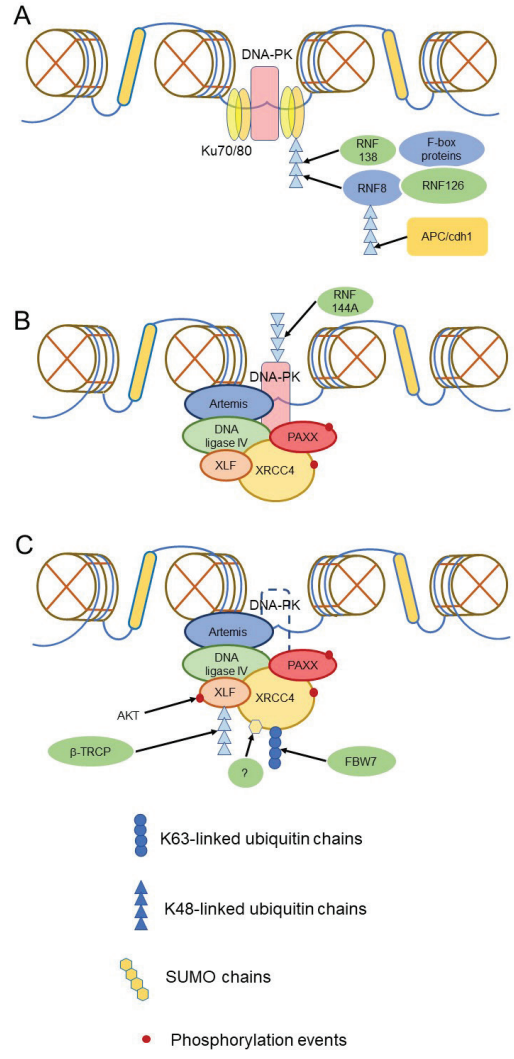

Figure 15.2 Ubiquitin and SOMO modifications in NHEJ.

XRCC4 undergoes K63-linked ubiquitination by Fbw7 to facilitate its association with Ku complexes, thus enhancing NHEJ (Zhang *et al.*, 2016). In addition, mono-ubiquitination of XRCC4 was also observed with unknown E3 ligase(s) to stabilize DNA ligase IV (Foster *et al.*, 2006). Moreover, SUMOylation of XRCC4 by PIAS retains XRCC4 in cytoplasm, thus impairing NHEJ (Yurchenko *et al.*, 2006) (Fig. 15.2 and Table 15.2). Whether and how DNA ligase IV is subjected to ubiquitin-mediated regulation remains unknown.

Ubiquitin and SUMO signalling in inter-strand crosslink repair (ICLR)

Inter-strand crosslinks can be induced by exposure to alkylating agents, platinum and psoralens in environment and by clinical treatments and they are toxic to cells given that they strongly prevent transcription and replication due to the inability of the dsDNA for proper separation and is strongly associated with a human disorder called fanconi anaemia (FA) (Deans and West, 2011). Thus, many members repairing ICLs are named after this disorder and the repair pathway to resolve ICLR is also called FA pathway that mainly relies on HR but with distinct sets of nucleases and other DNA processing enzymes. Mechanistically, ICLs are recognized by FANCM that recruits subsequent associated proteins such as FANCL, FANCG and others to form a core FA complex, where FANCL exerts an E3 ligase activity to mono-ubiquitinate FANCI and FANCD2 (Longerich *et al.*, 2009; Miles *et al.*, 2015). These mono-ubiquitin events serve as a binding platform for Pol V and FAN1, respectively, that will activate ATR. On the other hand, the core FA complex also recruits BTR (the Bloom's syndrome complex) and FANCJ to facilitate BRCA1-mediated HR repair. Although no poly-ubiquitination event has been reported on the FA pathway, mono-ubiquitination of FANCI and FANCD2 serves as a signalling antenna for FA repair progression. Rad18 has been observed to be critical for FANCI and FANCD2 mono-ubiquitination in its E3 ligase dependent manner (Williams *et al.*, 2011), however, whether Rad18 directly ubiquitinates FANCI and FANCD2 warrants further investigation. In addition, biallelic mutations of the RFWD3 E3 ubiquitin ligase lead to FA, supporting its critical role in FA while with the exact substrate(s) for RFWD3 in FA remain unknown (Knies *et al.*, 2017). Moreover, the E3 ubiquitin ligase Fbw7 targets the key FA pathway member FAAP20 for ubiquitination and degradation in a GSK3 phosphorylation dependent manner to clear FAAP20 on completion of FA repair (Wang *et al.*, 2016). In addition to ubiquitination, FANCI also undergoes SUMOylation by PIAS1/4 and this modification promotes FANCI protein degradation to terminate FA signalling (Gibbs-Seymour *et al.*, 2015) (Fig. 15.3 and Table 15.2).

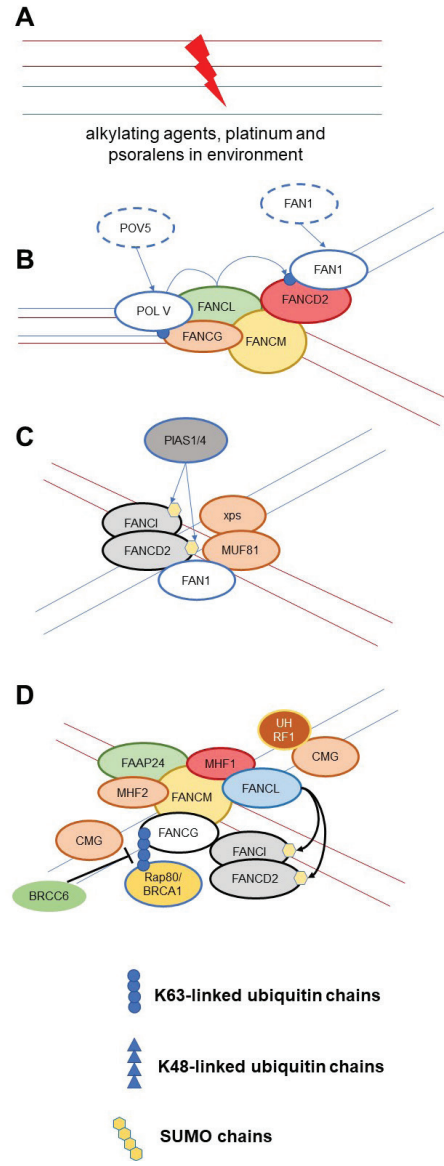

Figure 15.3 Ubiquitin and SOMO modifications in FA.

Ubiquitin and SUMO signalling in nucleotide excision repair (NER)

UV irradiation from sunlight or clinical applications trigger the formation of double thymidine dimmers, a type of DNA lesions that will be resolved by NER. NER can be divided into global genome nucleotide excision repair (GG-NER) (Yu, S. *et al.*, 2016) and

transcription coupled nucleotide excision repair (TC-NER) (Pani and Nudler, 2017). XPC is the sensor for both NERs by complexing with Rad23B and CETN2 to label damaged DNA to initiate NER. XPC undergoes UV-DDB2-mediated ubiquitination to enhance its binding to DNA (Sugasawa *et al.*, 2005), as well as SUMOylation in a DDB2 and XPA-dependent manner to prevent XPC proteasome degradation (Wang *et al.*, 2005; Wang, Q.E. *et al.*, 2007). XPC is stabilized by SUMOylation via unknown SUMOylase(s) in this process (Wang, Q.E. *et al.*, 2007). After NER initiation, RNF111 medaited ubiquitination of prior ubiquitinated or SUMOylated XPC facilitates the release of XPC from damage sites to allow binding of NER factors such as XPG and XPF (van Cuijk *et al.*, 2015). In addition, USP11 deubiquitinates XPC to extend its retention on damaged DNA, thus enhancing NER (Shah *et al.*, 2017). Consistent with this observation, reduced USP11 expression was observed in human skin cancer patients, highlighting its role as a tumour suppressor in promoting NER (Shah *et al.*, 2017). In addition, USP24 deubiquitinates and stabilizes DDB2 that promotes XPC ubiquitination and NER. The Flap endonuclease 1 (FIN1) that exerts endonuclease activity in NER is SUMOylated by unknown SUMOylase(s) and this SUMOylation event promotes FIN1 degradation to suppress NER (Guo *et al.*, 2012). In TC-NER, the RNA PolII/CSB (ATPase) complex is indispensable to fill in the DNA gaps and VCP/p97 promotes their proteolytic clearance (He *et al.*, 2016, 2017), while USP7 together with UVSSA, deubiquitinates RNA PolII and CSB to stabilize these proteins (Higa *et al.*, 2016), both of which are essential for TC-NER. In addition, SUMOylation of C-terminus of CSB by unknown SUMOylase(s) has been observed to facilitate CSB's function in NER (Sin *et al.*, 2016). Notably, NER also induces H2A ubiquitination in a manner depending on the MRN/MDC1/RNF8 signalling (Marteijn *et al.*, 2009). XPF/ERCC1 is an essential downstream factor of both GG-NER and TC-NER serving as a damage repair nuclease complex. USP45 specifically deubiquitinates XRCC1 to promote its translocation to damage sites (Perez-Oliva *et al.*, 2015) while the identity of E3 ligase(s) responsible for XRCC1 ubiquitination remains elusive (Table 15.2).

In yeast, Rad1 endonuclease cleaves ssDNAs to facilitate NER. Rad1 is SUMOylated by yeast SUMOylases Siz1/2 to release Rad1 from binding ssDNA (Sarangi *et al.*, 2014b). In addition, the nuclease complex scaffolding protein Saw1 is SUMOylated by Siz1/2 as well to attenuate Rad1 binding while meantime promotes Slx4 interaction to tone down NER (Sarangi *et al.*, 2014a). The yeast Topoisomerase II (Top2) is SUMOylated by Siz1/2 to promote Top2 centromeric localization to facilitate damage repair (Bachant *et al.*, 2002; Takahashi *et al.*, 2006; Takahashi and Strunnikov, 2008). Siz1/2 SUMOylase also SUMOylates the DNA ligase scaffolding protein Lif1, which leads to release of Lif1 from binding DNA (Vigasova *et al.*, 2013), and the DNA recombination mediator, Rad52, to reduce Rad52 binding to Ufd1 and DNA (Sacher *et al.*, 2006; Torres-Rosell *et al.*, 2007; Altmannova *et al.*, 2010; Bergink *et al.*, 2013), to terminate repair (Table 15.2).

Ubiquitin and SUMO signalling in base excision repair (BER)

BER repairs damaged DNA bases in a highly coordinated order with a rapid speed. Recognition of the damaged bases is carried out by DNA glycosylases such as Msh2, Mlh1 and MutYH. Upon excision of the damaged DNA bases by AP endonucleases (such as APE1), the gaps will be filled by PNKP and XRCC1/DNA ligase III. Ubiquitination mediated protein stability control of BER components was firstly observed in early 2000s (Hernandez-Pigeon *et al.*, 2004). Soon afterwards, MutYH (Dorn *et al.*, 2014) and RNA Polβ (Parsons *et al.*, 2009) levels were found to be negatively regulated by the E3 ligase Mule. In addition, both Mdm2 (Busso *et al.*, 2009) and UBR3 (Meisenberg *et al.*, 2012) target APE1 for proteasomal degradation to restrain APE1 expression and activity in BER. PNKP is recognized and degraded by a E3 ligase complex composed of Cul4A–DDB1–STRAP, a process that can be antagonized by ATM-mediated phosphorylation of PNKP (Parsons *et al.*, 2012). As a PARP-dependent E3 ligase, RNF146 ubiquitinates XRCC1 and DNA ligase III to facilitate BER (Kang *et al.*, 2011; Zhou *et al.*, 2011). Moreover, the E3 ligase CHIP was observed to govern the protein turnover of a handful of BER members, including XRCC1, OGG1 and RNA Polβ (Parsons *et al.*, 2008). In addition, Cullin 1 and Cullin 4-based E3 ligases have also been implicated in degrading BER

components UNG and SMUG1 induced by Vpr (Schröfelbauer *et al.*, 2005). Interestingly, Rad7 and San1 E3 ligases target variants or mutated, but not WT-Msh2 for proteasomal degradation, suggesting that in addition to control of normal BER process, certain ubiquitin signalling may also govern aberrant protein turnovers for BER members under pathophysiological conditions. In yeast, SUMOylation of the DNA glycosylase TDG attenuates TDG binding to DNA to negatively regulate BER (Hardeland *et al.*, 2002; Steinacher and Schär, 2005; Baba *et al.*, 2005, 2006; Smet-Nocca *et al.*, 2011) (Fig. 15.4 and Table 15.2).

Discussion and future perspectives

Genome stability is essential for normal cell physiology such as development, metabolism, proliferation in individuals, and also indispensable to faithfully pass genetic information to next generation. While certain flexibility is also allowed to gain advantages to adapt to environment or for evolution for better survival. In this review, we focus on DNA damage repair regulations in individuals rather than across different generations.

The tight while tempo and spatial control of genome stability is achieved by a delicate DNA damage sensing, initiating, repair and termination system, mechanisms of which are conserved evolutionarily from yeast to human. Although distinct types of DNA damages are repaired by a variety of mechanisms, all key components in these repair pathways are controlled at their cellular levels – both protein abundance and enzyme activity. Although DNA damage induced transcriptional regulation of certain genes is also present (Elkon *et al.*, 2005; Alvarez-Fernandez *et al.*, 2010), as an acute response, protein post-translational regulations play a more important role. In addition to protein phosphorylation that can amplify signals quickly towards a large-scale, protein ubiquitination and SUMOylation provide a powerful approach to properly earmark unnecessary proteins for degradation, alter protein cellular localization, and more importantly, provide a platform for protein binding to recruit necessary DNA damage repair factors. This is partially achieved by the uniqueness of the ubiquitin code. The ubiquitin code is composed of types of ubiquitin modifications

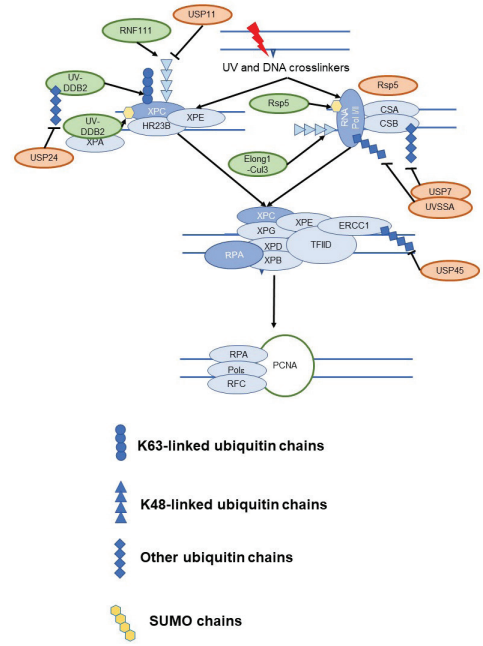

Figure 15.4 Ubiquitin and SOMO modifications in BER.

(mono-ubiquitination or polyubiquitination), versatile ubiquitin linkages that are gaining more and more attention due to their underappreciated physiological functions (Swatek and Komander, 2016), composition of polyubiquitin chains (homogenous or branched chains), post-translational modifications of a single ubiquitin molecule and complexity in ubiquitination accepting sites on substrates. Different combinations of these ubiquitin codes provide distinct biological meanings that can be interpreted by different ubiquitin code reader proteins. Compared with ubiquitin, less is known about SUMO, and it remains to be determined whether SUMO molecules are also undergoing posttranslational modifications and whether branched SUMO chains are present.

In addition to ubiquitin or SUMO molecules, cellular levels, cellular location and activities of ubiquitin or SUMO enzymes are also tightly controlled to ensure proper repair of damaged DNA. If not, unfaithful repair of damaged DNA, delayed repair and insufficient repair will lead to genome instability. Genome instability has been linked and shown as the cause for a variety of human disorders, including Xeroderma pigmentosum, Cockayne

syndrome, Fanconi anaemia, Bloom syndrome, Ataxia telangiectasia, Hutchinson–Gilford Progeria syndrome, other rare genetic diseases and cancer (Watanabe and Maekawa, 2013). These diseases are resulted from DNA nucleotide changes, nucleotide insertion, deletion, translocation and changes or exchanges at chromosomal levels. Thus, it is not surprising that dysregulation of key ubiquitin E3 ligases, deubiquitinases, SUMOylases and deSUMOylases are observed in cancer. For example, cancer patient derived Fbw7 mutations occur in its substrate binding region, leading to inability for cancerous mutated Fbw7 to target its physiological substrates for degradation. Thus, aberrantly accumulated Fbw7 substrates [FAAP20 in ICLR (Wang *et al.*, 2016)] lead to improper ICLR facilitating tumorigenesis. Another example is the E3 ubiquitin ligase SPOP. Mutations in substrates binding regions of SPOP in cancer similarly impair DDR by disrupting normal substrate degradation process, while the exact identity of the SPOP substrates in DDR remains unknown (Boysen *et al.*, 2015). Compared with ubiquitin system, whether and how SUMO modifying enzymes (including both SUMOylases and deSUMOylases) contribute to human diseases are just began to be appreciated and further thorough investigations are warranted.

Although E3 ubiquitin ligases usually do not display enzymatic activity but rather facilitate ubiquitin transfer from E2 enzymes to substrates, inhibitors targeting E3/substrate interactions have been developed such as Skp2 inhibitors (Wu, L. *et al.*, 2012; Chan *et al.*, 2013). These inhibitors demonstrate potential in treating cancer, however, they have not been applied in DNA damage studies. Similarly, only a few DUB inhibitors have been developed with promises in cancer therapy (Kategaya *et al.*, 2017; Turnbull *et al.*, 2017), although their function in DDR is understudied. Advances of detailed molecular understanding of the ubiquitin and SUMO-mediated regulatory signalling events will pave the foundation to identify new ubiquitin and SUMO modifying enzymes as potential drug targets to alter or correct defective DDR to treat human genetic diseases caused by genome instability.

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