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Role of Deubiquitinases in DNA Damage Response

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Abstract

DNA damage response (DDR) serves as an integrated cellular network to detect cellular stress and react by activating pathways responsible for halting cell cycle progression, stimulating DNA damage repair, and initiating apoptosis. Efficient DDR protects cells from genomic instability while defective DDR can allow DNA lesions to go unrepaired, causing permanent mutations that will affect future generations of cells and possibly cause disease conditions such as cancer. Therefore, DDR mechanisms must be tightly regulated in order to ensure organismal health and viability. One major way of DDR regulation is ubiquitination, which has been long known to control DDR protein localization, activity, and stability. The reversal of this process, deubiquitination, has more recently come to the forefront of DDR research as an important new angle in ubiquitin-mediated regulation of DDR. As such, deubiquitinases have emerged as key factors in DDR. Importantly, deubiquitinases are attractive small-molecule drug targets due to their well-defined catalytic residues that provide a promising avenue for developing new cancer therapeutics. This review focuses on the emerging roles of deubiquitinases in various DNA repair pathways.

Keywords

DNA repair; DNA Damage Response; Deubiquitination; Post-transcriptional Modification

1. Introduction

1.1 DNA damage

Eukaryotes have evolved specialized mechanisms to sense and repair unique lesion structures in the DNA induced by DNA damaging agents [1, 2]. Most lesions require specialized pathways involving sequential action of multiple proteins, whereas some lesions can be repaired directly by protein-mediated reversal. Helix-distorting DNA adducts are typically induced by ultraviolet (UV) radiation and alkylating agents and are typically repaired by the nucleotide excision repair (NER) pathway. Bases that become oxidized by

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Competing Interests

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reactive oxygen species are highly mutagenic as they often base pair with ‘incorrect’ bases and can be repaired by base excision repair (BER). Ionizing radiation (IR) and replication fork collapse induce highly toxic and mutagenic dsDNA breaks which can be repaired through either error-prone non-homologous end joining (NHEJ) or error-free homologous recombination (HR) [2, 3]. Interstrand crosslinking (ICL) is repaired through the Fanconi anemia (FA) pathway [4, 5]. If these DNA repair mechanisms fail and lesions persist during S phase, polymerases can still employ DNA damage tolerance (DDT) mechanisms. DDT can be achieved through translesion synthesis (TLS) to complete replication and leave lesions to be repaired later rather than undergo dangerous replication fork collapse and genome instability [1].

1.2 DNA damage response

DNA damage response (DDR) pathways operate both independently and together and must be tightly regulated in their own function. Many of these integrated DDR pathways are especially regulated by posttranslational modifications (PTMs), which are covalent modifications of amino-acid residues on target proteins that alter a variety of protein characteristics. Various PTMs are quick, reversible and dynamic, and allow for rapid responses to the cell’s changing status. They often work in a coordinated manner and are an advantageous way of regulating DDR [6, 7]. While phosphorylation has long been the most characterize and understood PTM in DDR, it is now becoming clear that ubiquitination is another major PTM in DDR.

Ubiquitin is a small and highly conserved regulatory protein (8.5 kDa; 76 amino acids) that is found ubiquitously in almost all eukaryotic tissues [8, 9]. Ubiquitin’s carboxy terminal glycine residue can be covalently bonded to lysine residues in the substrate protein via an isopeptide bond. Additionally, any one of the 7 internal lysine residues of the first ubiquitin molecule (K6, K11, K27, K29, K33, K48, or K63) can be used for further linkages to secondary ubiquitin molecules, forming a polyubiquitination chain. This ubiquitination process occurs through the activity of three enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase). E3 ligases are mainly responsible for giving substrate specificity to the ubiquitination process [10]. The ubiquitin chains themselves vary: some substrates receive one ubiquitin molecule (monoubiquitination), one molecule in more than one location (multi-monoubiquitination), and/or chains of multiple ubiquitin moieties (polyubiquitination) which can be branched or folded in different conformations (Figure 1). Given the great diversity in the molecular nature of this covalent linkage, it is not surprising that ubiquitination can affect proteins in different ways: altering cellular localization, altering cellular activity and/or protein interactions, or signaling for catabolic degradation through the proteasome known as Ubiquitin-Proteasome System (UPS) [11–13]. More than 80% of cellular proteins are degraded by the UPS [14].

Like other PTMs, ubiquitination is a reversible modification. Enzymes called deubiquitinases (DUBs) can oppose the action of the E3 ligases by cleaving the isopeptide bond between the C-terminal glycine on ubiquitin and lysine residues on target proteins [9, 15]. DUBs serve several other important purposes including modulating E2 activity, editing

non-proteasomal ubiquitin signals, assisting degradation machinery, ubiquitin recycling, and ubiquitin precursor processing [15, 16].

The human genome encodes approximately 100 DUBs [15, 17–19], which accounts for a major fraction of the estimated 460 proteases [20]. Seven types of DUBs are currently known and have been classified based on their active site homology (Figure 2). The DUBs of the small JAB1/MPN+/MOV34 (JAMM) family are zinc metalloproteases [21–24], the cysteine protease DUBs are the newly discovered ZUFSP/ZUP1 [25–27], and the recently discovered MIU-containing novel DUB family (MINDY) [17], ubiquitin c-terminal hydrolases (UCH) [28–30], ubiquitin specific proteases (USP, also known as UBP) [31, 32], ovarian tumor proteases (OTUs) [33, 34], and Machado-Joseph disease proteases (MJD) [35, 36].

DUB activity is commonly regulated at the levels of transcription/translation and post-translational modifications which modulate intracellular abundance and localization as well as catalytic activity [37]. As ubiquitination is known to play an important role modulating and coordinating DDR, DUBs naturally follow as important factors in regulating DDR mechanisms as well (Figure 3).

2. DUBs Role in Translesion Synthesis

2.1 Translesion synthesis

Translesion synthesis involves the use of alternate, promiscuous DNA polymerases, such as Pol η, ι, κ, and Rev1, to incorporate nucleotides opposite to damaged DNA. Usually, proliferating cell nuclear antigen (PCNA) functions as a sliding clamp, a processivity factor that anchors the replisome to the DNA template. Moreover, PCNA mediates the DNA damage tolerance pathway by recruiting TLS polymerases to sites of stalled forks when monoubiquitinated [38, 39].

2.2 DUBs involved in TLS

Interestingly, the ubiquitin specific peptidase 1-USP1 associated factor 1 (USP1-UAF1) complex, which has been shown to be involved in the Fanconi anemia pathway, has also been shown to regulate TLS by deubiquitinating monoubiquitinated PCNA. The removal of ubiquitin from PCNA promotes switching from replicative polymerases to TLS polymerases. Elevated levels of PCNA monoubiquitination compete against its normal error-free DNA replication kinetics, as excessive and/or irregular recruitment of TLS polymerases to the replication fork leads to reduced fork speeds and is a contributing factor to genomic instability [38, 40, 41]. Additionally, ubiquitin specific peptidase 7 (USP7) has been implicated in the deubiquitination of RING-type E3 ubiquitin transferase RAD18 (RAD18) and Pol η to prevent their degradation via the UPS. Both RAD18 and Pol η proteins are destabilized as a consequence of the loss of USP7 resulting in compromised UV-induced PCNA monoubiquitination and Pol η recruitment to stalled replication forks, respectively [38, 42–44].

3. DUBs role in Fanconi anemia pathway

3.1 Fanconi anemia pathway

Fanconi anemia is a human genetic disorder characterized by a deficiency in the repair of DNA interstrand crosslinks, which leads to the blockage of DNA replication and transcription [45]. Repair of interstrand crosslinks can be facilitated by the FA pathway [4, 5]. The FA pathway consists of the upstream E3 ligase complex termed “the core FA complex” that is comprised of eight FA proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM) and other associated factors (FAAP100, FAAP24, FAAP20, MHF1, and MHF2) and the downstream Fanconi anemia complementation group D2-Fanconi anemia complementation group I (FANCD2-FANCI) complex [40, 45–50]. Monoubiquitinated FANCD2 is necessary for its localization to sites of DNA damage to facilitate the downstream repair of ICLs through the recruitment of DNA repair proteins to stalled sites of DNA replication. However, FANCD2 monoubiquitination alone is not sufficient for ICL repair. Over accumulation of monoubiquitinated FANCD2 can lead to impaired ICL repair [51].

3.2 USP1 is involved in FA pathway

FANCD2 deubiquitination by USP1, is also required for the FA pathway to function properly. The FA pathway depends on USP1 to maintain a proper equilibrium between monoubiquitinated and deubiquitinated FANCD2 [38]. USP1 forms a complex with UAF1 where its WD40 domain binds and stimulates the ubiquitin protease activity of USP1 to counteract the monoubiquitination of the FANCD2-FANCI heterodimer [52]. The whole cellular pool of FANCD2 is monoubiquitinated when USP1 is depleted, which may result in deregulated FANCD2 recruitment in cells to the damage sites [51]. This balancing act of FANCD2 monoubiquitination and deubiquitination is supported by the observation that upon DNA damage, USP1 is transcriptionally downregulated in order to likely facilitate FANCD2 monoubiquitination in the beginning [52]. Additionally, FANCI is required for foci formation of the core complex components. Ubiquitinated FANCI loses this activity, and therefore deubiquitination by USP1 is critical for efficient foci formation of the core complex. How this occurs is still mechanistically vague [46, 53].

More recently, the deubiquitination of the FANCD2-FANCI complex has been found to be influenced by the phosphorylation of FANCI. Specifically, phosphorylation in the FANCI S/TQ cluster may somehow enhance the FANCD2-FANCI interaction and hinder deubiquitination by USP1, as the monoubiquitination sites of FANCD2 and FANCI are buried in the FANCD2-FANCI interface and therefore inaccessible to USP1 [45]. How USP1 eventually deubiquitinates the FANCD2-FANCI complex following the completion of the DNA repair is still a question. A recent review speculates that the FANCD2-FANCI complex may somehow be removed from the damage sites after completion of repair and the DNA-free complex may be in a conformation that is subject to deubiquitination [46].

4. DUBs role in base excision repair

4.1 Base excision repair

Base excision repair (BER) is commonly used to repair small lesions in the genome, unlike NER which is the common pathway towards repairing bulky, helix-distorting lesions. For the repair of one nucleotide, the short-patch BER (SP-BER) pathway is incorporated. DNA glycosylases remove the damaged base, leaving only the sugar-phosphate backbone, also called an apurinic/apyrimidinic site (AP site). AP endonucleases such as apurinic/apyrimidinic endodeoxyribonuclease 1 (APE1) then cleave this site, leaving a 3' hydroxyl adjacent to a 5' deoxyribose phosphate, thereby providing a primer for DNA polymerase β (or polymerase λ in the absence of polymerase β), repairing the genome. For the repair of 2–12 nucleotides, long-patch BER (LP-BER) is incorporated. DNA synthesis is instead done by DNA Polymerase δ and Polymerase ε in conjunction with a proliferating cell nuclear antigen acting as a 'clamp' protein. These polymerases displace the downstream 5' end of DNA to form a flap intermediate. The displaced strand is removed by a nuclease such as flap structurespecific endonuclease 1 (FEN1), thereby creating a ligatable substrate. Factors such as the type of lesion, the current stage of the cell cycle, and ATP concentration will affect whether the SP or LP BER pathway is taken [54].

4.2 DUBs involved in BER

DNA polymerase β is the key polymerase that plays a role in BER. Ubiquitin specific peptidase 47 (USP47) plays a role in regulating cytoplasmic levels of newly synthesized Pol β, which is targeted for degradation if nuclear levels of Pol β are sufficient for DNA repair. It is shown that knockdown of USP47 causes an increased level of ubiquitinated Pol β, decreased levels of deubiquitinated Pol β, and a deficiency in BER. This leads to accumulation of strand breaks and ultimately a decrease in cell viability. Regulation of cytoplasmic Pol β is also important since overproduction of Pol β leads to deficient repair and an increased rate of mutagenesis, both contributing to an increase in cancer susceptibility [55].

One way that DNA can be damaged is through alkylating agents such as methyl methane sulfonate. DNA alkylation can be repaired by BER as well as by oxidative demethylases. AlkB homolog 2, alpha-ketoglutarate dependent dioxygenase (ALKBH2) and AlkB homolog 3, alpha-ketoglutarate dependent dioxygenase (ALKBH3), both oxidative demethylases, remove methyl groups from double-strand DNA as well as singlestranded DNA or RNA [56, 57]. Characterization of ALKBH3-interacting proteins show that USP7 and USP9x associate with another DUB OTU deubiquitinase 4 (OTUD4) to form a protein complex that deubiquitinates ALKBH3 [58]. Knockdown of OTUD4, USP7, or USP9x have all resulted in cells being sensitized to DNA alkylation as well as reduced levels of ALKBH3. This shows how multiple DUBs play a role in the less common DDR pathways such as base repair by oxidative demethylases. It also highlights how DUBs such as USP7 can play roles in different DDR pathways [38, 58].

Interestingly, isoforms of DUBs can also play roles in DDR pathways. For example, USP7S, a S18 containing isoform of USP7, regulates the ARF-binding protein 1 (ARF-BP1, also

known as Mule) E3 ligase. This enzyme stabilizes other enzymes that are involved in BER, the most important of which being that it regulates DNA polymerase β and λ. USP7S regulates Mule stability by reversing its self-ubiquitination [59].

5. DUBs role in nucleotide excision repair

5.1 Nucleotide excision repair

Many human diseases result from complex interactions between genome and environmental agents. Mutation is a frequent consequence of unrepaired DNA damage. The nucleotide excision repair (NER) pathway plays a particularly important role in the repair of environmental mutagen-induced DNA damage. NER repairs a wide variety of helix-distorting `bulky' DNA lesions that result from damaging agents such as UV radiation, cisplatin or reactive oxygen species (ROS). It does this by cutting the damaged bases within a longer string of nucleotides out, and then resynthesizing the missing bases via the untouched template strand [60, 61].

There are two sub-pathways of NER, termed global genomic repair (GG-NER) and transcription-coupled repair (TC-NER). These pathways differ mainly in their recognition of damage. While an RNA polymerase (RNAP) blocked by a lesion initiates TC-NER, restricting recognition to the transcribed strand of active genes, GG-NER surveys the entire genome for distorting DNA lesions [3, 61]. For TC-NER, the stalled RNAP elicits the recruitment of Cockayne syndrome proteins A and B (CSA and CSB), which are both crucial in damage verification. On the other hand, GG-NER requires Xeroderma pigmentosum complementation group C (XPC) and Xeroderma pigmentosum complementation group E (XPE/DDB2) for damage recognition. XPC joins a complex with UV Excision Repair Protein RAD23 homologues and centrin 2, which scans for and recognizes lesions, and initiates NER when needed. XPC lacks the ability to recognize UV-induced lesions, which DDB2 compensates for. DDB2 can recognize these lesions and then hand the damage over to XPC to complete the NER process. TC-NER is a more complex process, yet ubiquitination plays crucial roles in both TC-NER and GG-NER [3, 60–62].

5.2 DUBs involved in GG-NER

USP24 is involved in the GG-NER pathway, specifically in the UV triggered DNA damage response. DDB2 is deubiquitinated by USP24, stabilizing the entire UV-DDB-CUL4 E3 ligase from proteasomal degradation [63]. This complex in turn ubiquitinates XPC, however it is not yet known what this linkage type and its interaction are. In addition, USP7 plays a role in regulating GG-NER. XPC, the protein which recognizes DNA damage in GG-NER, is ubiquitinated by the Cullin-RING E3 ubiquitin ligase 4 (CRL4) upon UV damage, although ubiquitination of XPC does not lead to significant proteasomal degradation. XPC can be converted back to its unmodified stage by USP7 [64]. Inhibition of USP7 increases XPC ubiquitination level, and without USP7, cells have decreased efficiency in repairing UV lesions [60, 61].

Interestingly, USP11 is another DUB that plays a part in GG-NER. USP11 knockdown in HaCaT cells increased ubiquitination of XPC as compared to control cells, suggesting that

USP11 is important in maintaining NER capacity, as USP11 mediates XPC deubiquitination at the chromatin following UVB damage [65].

5.3 DUBs involved in TC-NER

In addition to being involved in the GG-NER pathway, USP7 also plays a role in TC-NER by interacting with UV stimulated scaffold protein A (UVSSA) and controlling the stability of CSB/ERCC6 and RNA polymerase II [66–68]. UVSSA is a gene that when mutated, has been found to be responsible for causing UV-sensitive syndrome disorder. USP7 is found to interact with UVSSA, likely through UVSSA recruiting USP7 along with other TC-NER factors in a UV dependent manner. For example, upon UV induced DNA damage, UVSSA can recruit USP7 to stabilize the TC-NER master organizer ERCC6 [67, 69]. Furthermore, depletion of USP7 leads to reduced recovery of RNA synthesis and destabilization of RNA polymerase II as seen in UV-sensitive syndrome cells. This suggests that USP7 and UVSSA work together to stabilize CSB and RNA Polymerase II [61, 69].

USP45 is another DUB involved in NER. UV damage repair in USP45 knockout cells is significantly impaired compared to control cell lines, suggesting USP45's role in UV-induced damage repair. The depletion of USP45 sensitizes cells to DNA damaging agents. Specifically, the ability of cells to repair cyclobutane pyrimidine dimers (CPD), DNA lesions resulting from UV-C treatment, is greatly impaired in USP45 knockout cells. It was shown that USP45 interacts with and deubiquitinates the excision repair cross-complementation group 1 (ERCC1) subunit of the XPF-ERCC1 DNA repair nuclease which has role in the TC-NER of UV induced DNA damage [70, 71]. Evidence suggests that USP45 cleaves K48 and K63 ubiquitin linkage. Importantly, USP45 is recruited to the site of DNA damage and also controls the ability of ERCC1 to translocate to foci of DNA damage. Therefore, USP45 can affect UV damage repair by targeting ERCC1 [70].

6. DUBs role in DNA double-strand break repair pathway choice

6.1 DNA double-strand break repair

DNA double-strand breaks (DSBs) can be repaired through either error-prone non-homologous end joining (NHEJ) or error-free homologous recombination (HR) [2, 19]. Studies have shown that the appropriate choice between HR and NHEJ repair is crucial in preserving genome integrity. Ubiquitination plays a key role in the recruitment processes of these repair pathways, as such deubiquitination has been shown to influence whether the damage response favors HR or NHEJ [38, 72].

In mammalian cells, double-strand break repair begins with the recruitment and accumulation of various repair and signaling molecules by the site of the lesion. Recruitment of numerous factors to the chromatin regions surrounding DSBs requires a ubiquitination cascade [73]. The DSB sites where the molecules are recruited are commonly referred to as the ionizing radiation-induced foci (IRIF) - which protects and sequester the broken DNA-ends and modulate several cellular processes. IRIF also provide an important checkpoint signaling platform from which DNA damage-activated kinases such as ataxia telangiectasia

mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) can globally impact transcriptional processes, cell cycle transitions, and cell fate decisions [74].

Initially, ATM and related kinases phosphorylate H2A histone family member x (γH2AX) surrounding the damage site, leading to recruitment of mediator of DNA damage checkpoint 1 (MDC1), a scaffolding protein. ATM-mediated phosphorylation of which then provides a platform for ring finger protein 8 (RNF8), a RING E3 ligase, to dock. Then, RNF8-UBC13 mediates the ubiquitination of proteins at the damage site [75, 76]. RNF8-UBC13 extends the ubiquitination signal and allows the formation of K63-linked polyubiquitin chains. Then, ring finger protein 168 (RNF168), another E3 ligase, complexes with ubiquitin-conjugating enzyme E2N (UBC13, also known as UBE2N) and recognizes the RNF8-mediated ubiquitination products and ubiquitinates H2A-type histones. A major ramification of local RNF8/RNF168-mediated ubiquitination is the recruitment of checkpoint and repair factors such as breast cancer type 1 susceptibility protein (BRCA1), receptor associated protein 80 (RAP80), tumor protein P53 binding protein 1 (53BP1), RAD51 recombinase (RAD51), and RAD18 at sites of DNA damage [73]. Evidence suggests that these proteins are in a competitive balance to promote DSB repair by HR or NHEJ through DNA-end protection or DNA-end resection. For example, the clearing of RAP80 and 53BP1 from the IRIF allows nucleases to promote DNA-end resection, which in turn allows HR to proceed [37, 77–79].

6.2 DUBs involved in DSB repair

26S proteasome-associated PAD1 homolog 1 (POH1) is a JAMM/MNP+ metalloprotease [21] that cleaves K63 chains at sites of DNA damage [80], thus limiting the IR-induced formation of K63 chains at DSB sites. It is recruited to the IRIF in a BRCA1-dependent manner, and promotes the removal of RAP80, the ubiquitin chain, and 53BP1 from the IRIF [81]. One proposed model is that POH1 degrades RAP80 and the loss of RAP80-dependent protection of the ubiquitin chain promotes the removal of ubiquitin, leading to the removal of 53BP1 from the IRIF; however, the detailed molecular mechanism remains to be elucidated. RAP80 and ubiquitin chains persist at the IRIF in POH1-depleted cells, but the ubiquitin chains are removed from the IRIF when POH1 and RAP80 are simultaneously depleted, suggesting that other DUBs but not POH1 degrade ubiquitin chains. POH1 relieves the barriers imposed by 53BP1 and RAP80 in the late stages of DDR and induces the switch from NHEJ to HR. Thus, POH1 limits excessive NHEJ repair and promotes HR repair [78, 82].

OTU domain-containing ubiquitin aldehyde-binding protein 2 (OTUB2) has been identified as a suppressor of excessive ubiquitination in the early stages of DDR. OTUB2 is shown to constitutively interact with polycomb molecule lethal(3)malignant brain tumor-like protein 1 (L3MBTL1). The ubiquitination of L3MBTL1 is required for its removal from damaged chromatin in order for 53BP1 to be recruited to the damage site. However, OTUB2 deubiquitinates L3MBTL1 and suppresses the RNF8-mediated ubiquitination of L3MBTL1, which leads to less recruitment of RAP80 and 53BP1 to DSBs and therefore increases HR. Additionally, OTUB2 suppresses K63-linked ubiquitin chain formation decreasing the recruitment of RNF168 to the DSBs. Consistent with the idea that 53BP1 and RAP80 suppress HR promoting events such as DNA-end resection and RPA foci, HR repair activity

is decreased upon OTUB2 depletion [83]. In OTUB2-depleted cells, the conjugation of ubiquitin and accumulation of RNF168, 53BP1 and RAP80 at DSB sites are significantly accelerated during the beginning phases of the DDR, and total DSB repair is upregulated. However, DNA-end resection and HR are suppressed in OTUB2-depleted cells. OTUB2 enables the initiation of HR by suppressing the excessive accumulation of 53BP1 and RAP80 in an early phase of the DDR [72, 83].

BRCA1/BRCA2-containing complex subunit 36 (BRCC36) is a JAMM/MPN+ metalloprotease that cleaves the K63 chain on histone H2A [80], which may facilitate optimal recognition of RAP80. BRCC36-depletion enhances the recruitment of 53BP1 to the DSBs in RNF8-depleted cells, indicating that BRCC36 and RNF8 play opposing roles in ubiquitination-mediated DDR. Interestingly, the depletion of BRCC36 increases HR, which goes against the suppressive role of BRCC36 in RNF8-dependent DDR that should suggest that BRCC36 promotes HR. This discrepancy is probably due to inefficient accumulation of the BRCA1-A complex at DSB sites in BRCC36-depleted cells. However, the physiological role of BRCC36 in DNA repair pathway choice remains to be elucidated [72, 84].

Ubiquitin specific peptidase 11 (USP11) physically associates with H2AX and is a unique DUB for H2AX. USP11 deubiquitinates H2AX, opposing the ubiquitinating activity of the RNF8-RNF168 complex. USP11 depletion enhances the levels of ubiquitinated H2AX and is associated with prolonged 53BP1 retention and stronger ubiquitination formation at DSB sites [85]. An earlier paper identified USP11 participation in HR repair, where they proposed that the prolonged 53BP1 foci observed could be a consequence of the persistence of unrepaired DSBs or increased levels of ubiquitin-conjugation at the DSB. The increased 53BP1 retention at DSBs when USP11 is depleted could be attributed to the decrease in HR repair [86].

Ubiquitin c-terminal hydrolase L5 (UCHL5, also known as UCH37), implied through its name, contains a UCH domain [87, 88]. UCHL5 regulates DSB resection and repair by HR through protecting nuclear factor related to KappaB binding protein (NFRKB) from degradation. Specifically, UCHL5 promotes DNA-end resection and HR through regulating the stability of the NFRKB protein that is a subunit of chromatin remodeling complex INO80 [89, 90]. UCHL5 and NFRKB enhance resection by regulating the recruitment of the resection factor exonuclease 1 (EXO1) [91].

Ubiquitin specific peptidase 4 (USP4) also regulates DSB end-resection. Currently, two studies have shown USP4 positively regulates DNA-end resection in response to DNA damage, thereby promoting DSB repair by HR. USP4 interacts with the retinoblastoma binding protein 8 (CtIP, also known as RBBP8) and the MRE11RAD50-NBS1 (MRN) complex [92, 93]. Both of which have been suggested to be involved in DNA-end resection [94]. USP4 depletion has been shown to reduce the recruitment of CtIP and RAD51 to DSB sites. However, MRE11 and NBS1 recruitment to DSB sites was not affected by USP4 depletion. Additionally, in USP4-knockdown cells, DNA damage sensor replication protein A (RPA) subunit RPA2 S4/S8 phosphorylation is reduced after DNA damage [93] and RPA recruitment to DSB sites is reduced [92]. Interestingly, both studies show that USP4

counteracts its own ubiquitination by deubiquitinating itself in order to be catalytically active [92, 93].

OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) has been found to negatively regulate the DNA damage-dependent ubiquitination by RNF168 and UBC13 [95]. UBC13 is a ubiquitin conjugating enzyme that heterodimerizes with ubiquitin conjugating enzyme E2 V1 (UBE2V1, also known as UEV1a2) and synthesizes K63 polyubiquitin chains at DSB sites in concert with RNF168, thereby promoting the independent recruitment of RAP80 and 53BP1 to damaged chromatin, which are critical for DNA repair [96]. Accordingly, the overexpression of OTUB1 inhibits K63 chain accumulation and 53BP1 IRIF. Interestingly, OTUB1 suppresses RNF168-dependent K63 polyubiquitination of the chromatin surrounding DSBs independently of its catalytic activity via binding to UBC13 to inhibit its E2 activity. It seems that non-catalytic roles for DUBs are possible [95–97].

Ubiquitin specific peptidase 34 (USP34) plays an important role in the recruitment of DNA damage checkpoint and mediator proteins at DSBs. Specifically, USP34 promotes RNF168-dependent functions at DSBs. USP34-depletion led to defective accumulation of BRCA1, 53BP1 and RAD18 to DSBs. Another DUB, USP7, has also been shown to deubiquitinate and stabilize RNF168 [98]; however, RNF168 is itself polyubiquitinated and destabilized upon IR treatment, which is reversed by USP34, suggesting that USP34 specifically stabilizes RNF168 when DNA becomes damaged [99].

Fascinatingly, a more recent study shows that USP7 complexes with ring finger protein 169 (RNF169) via crystal structure. RNF169 has antagonistic properties on the DSB signal transduction pathway possibly by restraining the ubiquitin-mediated DSB signaling events. Interestingly, this is the opposite of RNF168 which plays positive roles in amplifying ubiquitin-dependent DSB signals. RNF169 competes for RNF168-catalyzed ubiquitin adducts, thus limiting excessive loading of the DNA damage-mediator proteins 53BP1 and RAP80. As a result, the USP7–RNF169 axis promotes HR [100].

USP9x, discussed earlier under BER, has another well-known substrate, the BCL-2 family apoptosis regulator (MCL-1) which plays a role in DNA DSB repair [101]. Interestingly, knockdown of USP9x increases MCL-1 ubiquitination and turnover. It’s also been shown that increased USP9x expression is correlated with increased MCL-1 expression levels in these cancerous cells, and that patients with increased USP9x have poorer prognoses [102]. A recent study showed that MCL-1-depleted cells had a reduced frequency of IR-induced BRCA1, RPA, and Rad51 focus formation, decreased DNA end-resection, and decreased HR repair suggesting that MCL-1 may be important for DNA end resection. Additionally, the same study showed that there was an increase in 53BP1 and Rap1-interacting factor 1 homolog (RIF1) colocalization at DSB sites as a result of MCL-1 depletion, which blocks the recruitment of BRCA1 [101]. Therefore, if USP9x increases MCL-1 expression [102], then it could be suggested the USP9x indirectly promotes HR repair via MCL-1.

There is evidence that suggests that the USP1/UAF1 complex, previously discussed above to be involved in the FA pathway and TLS, promotes DSB repair via HR. A study in which $USP1^{-/-}$, $UAF1^{-/-/-}$, and $USP1^{-/-}$ $UAF1^{-/-/-}$ double-knockout DT40 cells showed

hypersensitivity to camptothecin and an inhibitor of poly(ADP-ribose) polymerase (PARP), which suggests that the USP1/UAF1 complex promotes HR. Additionally, the same study disrupted the NHEJ pathway, via knockout of Ku70 (also known as XRCC6 or X-Ray repair cross complementing 6), in UAF1$^{-/-/-}$ cells which restored the cellular resistance to camptothecin and the PARP inhibitor suggesting that the USP1/UAF1 complex promotes HR, at least in part by suppressing NHEJ. However, the direct mechanism by which USP1/UAF1 promotes HR remains to be elucidated [103].

BRCA1 associated protein 1 (BAP1) is part of the UCH DUB family [104]. BAP1 is rapidly recruited to DSB sites, and BAP1 depletion leads to reduced BRCA1, RAD51 and RPA foci and has been suggested to decrease HR. Overexpressing BAP1 reduces ubiquitinated forms of H2A and H2AX, while depletion increases them, suggesting that BAP1 is a DUB for the ubiquitinated H2A(X). The BAP1 complex may work in unison with a protein regulator of cytokinesis 1 (PRC1) complex to promote dynamic ubiquitination/deubiquitination of H2A. A possible mechanism may be that BAP1 deubiquitinates H2A in the proximity of the DSB site to increase chromatin accessibility at the damaged region to allow DNA resection during HR. This might interfere with specific chromatin and/or histone modifications events at DSBs. Consequently, BAP1 depletion increases the amount of ubiquitinated H2A and leads to reduced HR repair and sensitivity to IR [105, 106].

6. Discussion

Cells are constantly exposed to a plethora of DNA damaging agents. DNA can endure damage from normal endogenous biochemical processes, as well as exposure to exogenous genotoxic agents. If DNA lesions are neither detected, repaired, nor removed properly from the line of transmission to daughter cells, the cells will likely become mutagenic and have a highly compromised genomic stability. Cells have evolved a DNA damage response mechanism, which is a highly integrated collection of crucial pathways that detect damage and halt cell proliferation to either repair DNA lesions or to induce apoptosis if the lesions cannot be repaired.

DDR is a crucial cellular process governing cellular health and vitality. Defective DDR is a principal characteristic of tumorigenesis, as well as cancer treatment, which attempts to induce irreparable DNA damage. Dissecting the molecular biochemical pathways that function in DDR will allow for better diagnosis of neoplastic conditions and allow for the development of personalized chemo- and immunotherapeutics that can overcome hurdles of sickening side-effects and therapeutic resistance.

Lately, there has been research focused on DUBs and their interactions with cancer stem cells (CSC), a type of cancer cell that has stem-like properties, meaning they have the ability to self-renew and differentiate into different cell types. Identifying DUBs involved in regulating CSC transcription factors and proteins could provide insight into DUB regulatory mechanisms in carcinogenesis [107]. It has been shown that p53, phosphatase and tensin homolog (PTEN), and PRC1 all play roles in CSC fate. For example, other DUBs such as USP7, USP10, USP11, ataxin-3 (ATXN3), OTU deubiquitinase 1 (OTUD1) and OTU deubiquitinase 5 (OTUD5) have been found to deubiquitinate the tumor suppressor p53

[108–116]. Furthermore, USP13 and USP18 have been shown to regulate the stabilization of PTEN, another tumor suppressor whose destabilization correlates with carcinogenesis [117, 118]. Lastly, USP7 and USP11 deubiquitinate PRC1, a protein found to contribute to cancer stemness [119–121]. It is clear that DUBs play a large role in the tightly regulated pathways that CSC use to proliferate, and learning more about these interactions would allow for the production of specific DUB-targeting therapeutics [116].

There have also been studies on the interactions between DUBs and autophagy, a process necessary for homeostasis of the cell by making sure that degradation of protein aggregates and dysfunctional organelles occurs. Without proper autophagy, disease including carcinogenesis can occur. Interestingly, ubiquitination and deubiquitination are important regulatory mechanisms of autophagy machinery as well as autophagy substrates. Thus, DUBs play a crucial role in the regulation of autophagy. One example of this is ubiquitin c-terminal hydrolase L1 (UCHL1), a DUB responsible for the accumulation of α-synuclein in the brain, a hallmark of Parkinson's disease. It is thought that UCHL1 does this by negatively regulating the lysosomal degradation of α-synuclein. Additionally, UCHL1 has also been detected in various lung tumors but not in healthy lung tissue, implying a carcinogenic effect. Since one single DUB can play a role in different types of diseases, in this case a neurodegenerative disease and cancer, it is clear that they are an attractive therapeutic target, and that more research needs to be done on DUB regulatory mechanisms [122].

Ubiquitination has been well established as a primary means of regulating and integrating DDR pathways, and deubiquitinases have more recently gained attention as providing additional levels of DDR regulation. Mutations and deletions in DUB genes have become increasingly associated with disease conditions, including cancer, and are now being heavily explored as drug targets for cancer and other diseases [16, 77, 123]. Unlike E3 ligases, DUBs fortunately serve as attractive small-molecule drug targets due to their well-defined catalytic residues that provide a promising avenue for developing new cancer therapeutics. However, the roles of many DUBS in DDR still have not been elucidated and warrant further investigations.

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Abbreviations

DDR	DNA damage response
UV	Ultraviolet
NER	nucleotide excision repair
BER	base excision repair
IR	ionizing radiation
NHEJ	non-homologous end joining

HR	homologous recombination
ICL	interstrand crosslink
DDT	DNA damage tolerance
TLS	translesion synthesis
PTM	post-translational modification
E1	ubiquitin activating enzyme
E2	ubiquitin conjugating enzyme
E3	ubiquitin ligase
UPS	ubiquitin-proteasome system
DUB	deubiquitinating enzyme
JAMM	JAB1/MPN+/MOV34 metalloenzyme domain DUB family
ZUFSP	zinc finger with UFM1-specific peptidase domain protein DUB family
MINDY	motif interacting with Ub-containing novel DUB family
UCH	ubiquitin c-terminal hydrolase domain DUB family
USP/UBP	ubiquitin specific protease domain DUB family
OUT	ovarian tumor domain DUB family
MJD	Machado-Joseph disease protein domain DUB family
PCNA	proliferating cell nuclear antigen
USP	ubiquitin specific peptidase
UAF1	USP1 associated factor 1
RAD18	RING-type E3 ubiquitin transferase RAD18
FA	Fanconi anemia
FANC	Fanconi anemia complementation group
FAAP	Fanconi anemia core complex associated protein
MHF	mph1-associated Histone-Fold protein
SP-BER	short-path BER
AP site	apurinic/apyrimidinic site
APE1	apurinic/apyrimidinic endodeoxyribonuclease 1

LP-BER	long-patch BER
FEN1	flap structure-specific endonuclease 1
ALKBH2	AlkB homolog 2, alpha-ketoglutarate dependent dioxygenase
ALKBH3	AlkB homolog 3, alpha-ketoglutarate dependent dioxygenase
OTUD	OTU domain-containing deubiquitinase protein
ARF-BP1/Mule	ARF-binding protein 1
ROS	reactive oxygen species
GG-NER	global genome NER
TC-NER	transcription coupled NER
RNAP	RNA polymerase
CSA	Cockayne syndrome protein A
CSB/ERCC6	Cockayne syndrome protein B
XPC	Xeroderma pigmentosum complementation group C
XPE/DDB2	Xeroderma pigmentosum complementation group E
UV-DDB-CUL4	UV-damaged DNA-binding protein-Cullin4 complex
CRL	Cullin-RING E3 ubiquitin ligases
UVSSA	UV stimulated scaffold protein A
ERCC	excision repair cross-complementation group
CPD	cyclobutane pyrimidine dimers
DSB	DNA double-strand break
IRIF	ionizing radiation-induced foci
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia and Rad3-related protein
H2A	H2A Histone Family Member
MDC1	mediator of DNA damage checkpoint 1
RNF	ring finger protein
UBC13/UBE2N	ubiquitin-conjugating enzyme E2N
BRCA1	breast cancer type 1 susceptibility protein

RAP80	receptor associated protein 80
53BP1	tumor protein P53 binding protein 1
RAD51	RAD51 recombinase
POH1	26S proteasome-associated PAD1 homolog 1
OTUB	OTU domain-containing ubiquitin aldehyde-binding protein
L3MBTL1	lethal(3)malignant brain tumor-like protein 1
BRCC36	BRCA1/BRCA2-containing complex subunit 36
UCH	ubiquitin c-terminal hydrolase
NFRKB	nuclear factor related to KappaB binding protein
EXO1	exonuclease 1
CtIP/RBBBP8	retinoblastoma binding protein 8
MRN	MRE11-RAD50-NBS1 complex
RPA	replication protein A
UBE2V1/UEV1a2	ubiquitin conjugating enzyme E2 V1
RIF	Rap1-interacting factor 1 homolog
PARP	poly(ADP-ribose) polymerase
Ku70/XRCC6	X-Ray repair cross complementing protein 6
BAP1	BRCA1 associated protein 1
PRC1	protein regulator of cytokinesis 1
CSC	cancer stem cells
PTEN	phosphatase and tensin homolog
ATXN3	Ataxin-3

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Highlights:

- DUBs are involved in translesion synthesis by regulating the ubiquitination state of PCNA influencing the recruitment of TLS polymerases.
- DUBs are involved in interstrand crosslink repair through regulating the ubiquitination state of the FANCD2-FANCI complex in the Fanconi anemia pathway.
- DUBs are involved in base excision repair by stabilizing DNA Polymerase β as well as oxidative demethylases.
- DUBs are involved in nucleotide excision repair by directly regulating the synthesis and stabilization of RNA Polymerase II and crucial damage recognition proteins.
- DUBs regulate double-stranded break repair pathway choice by either promoting the recruitment or clearance of DNA-end resecting and/or DNA-end protecting proteins.

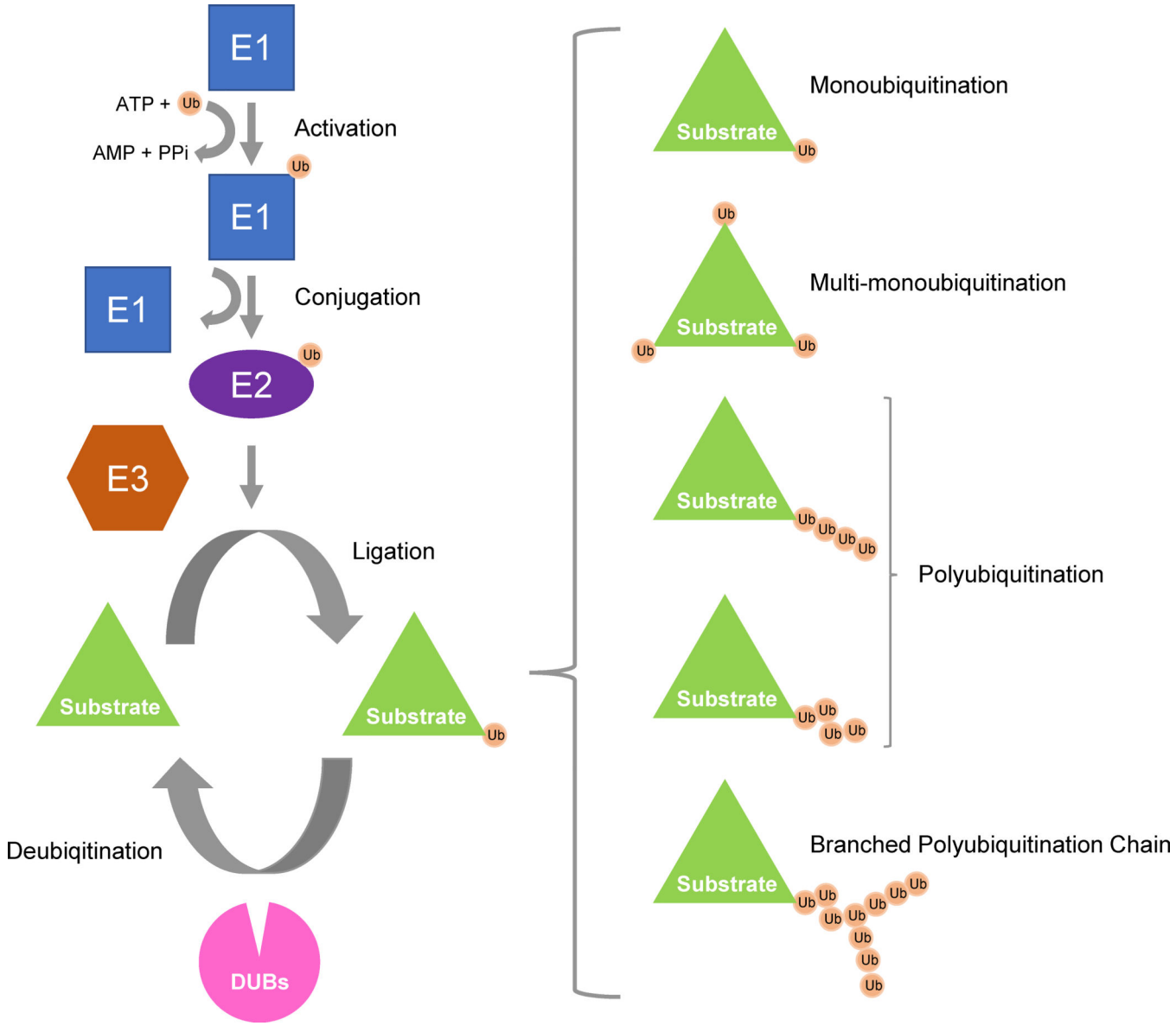

Figure 1. Ubiquitin Catalytic Process.

Ubiquitin (Ub) is first activated by ATP-dependent ubiquitin activating enzyme (E1), which results in the ubiquitin being thioester conjugated first to E1. Then the Ub is transferred from E1 to ubiquitin conjugating enzyme (E2). E2-Ub interacts with a ubiquitin ligase (E3), which then facilitates the transfer of ubiquitin to the substrate protein. Ubiquitin contains seven lysine residues that can be used to link ubiquitin molecules together. Additional ubiquitin molecules can be linked to the first one to form a polyubiquitin chain. Polyubiquitin chains linked at different positions alters the fate of the target protein. In addition, substrates can be monoubiquitinated or monoubiquitinated at multiple substrate sites (multi-monoubiquitination). Ubiquitin can be removed from the substrate by deubiquitinating enzymes (DUBs).

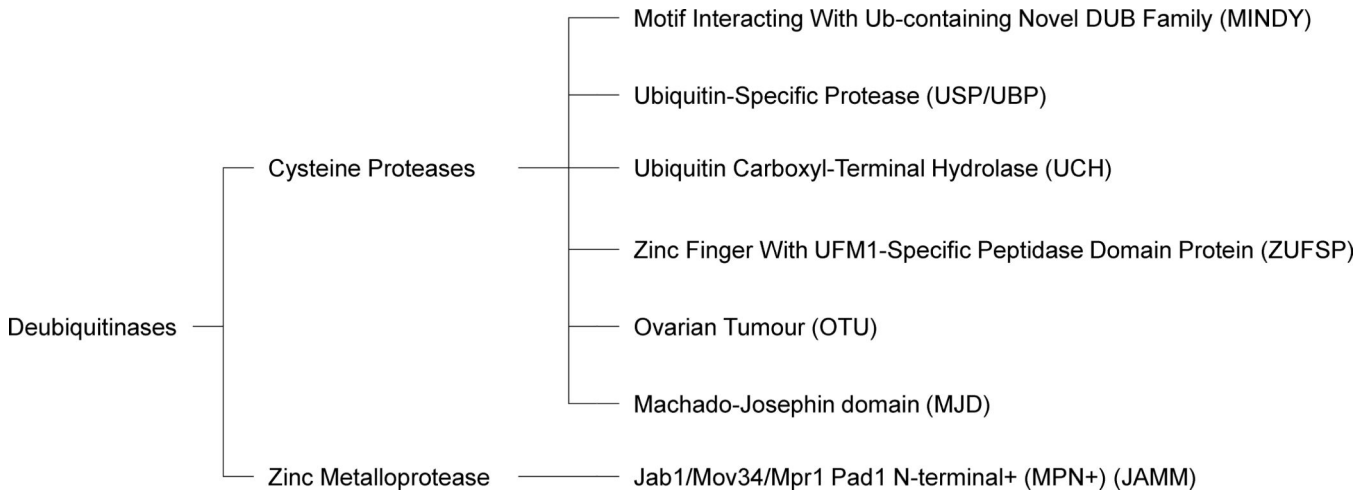

Figure 2. Deubiquitinase Classes and Families.

Deubiquitinating enzymes (DUBs) can be classified based on their active site homology into two main classes: cysteine proteases and zinc metalloproteases. Of these two classes, there are five families of cysteine protease DUBs and one family of zinc metalloprotease DUBs. The five families of cysteine protease DUBs consist of motif interacting with Ub-containing novel DUB family (MINDY), ubiquitin-specific protease (USP/UBP), ubiquitin carboxyl-terminal hydrolase (UCH), zinc finger with UFM1-specific peptidase domain protein (ZUFSP), ovarian tumor (OTU), and Machado-Josephin domain (MJD). The one family of zinc metalloprotease DUBs consist of Jab1/Mov34/Mpr1 Pad1 N-terminal+ (MPN+) (JAMM).

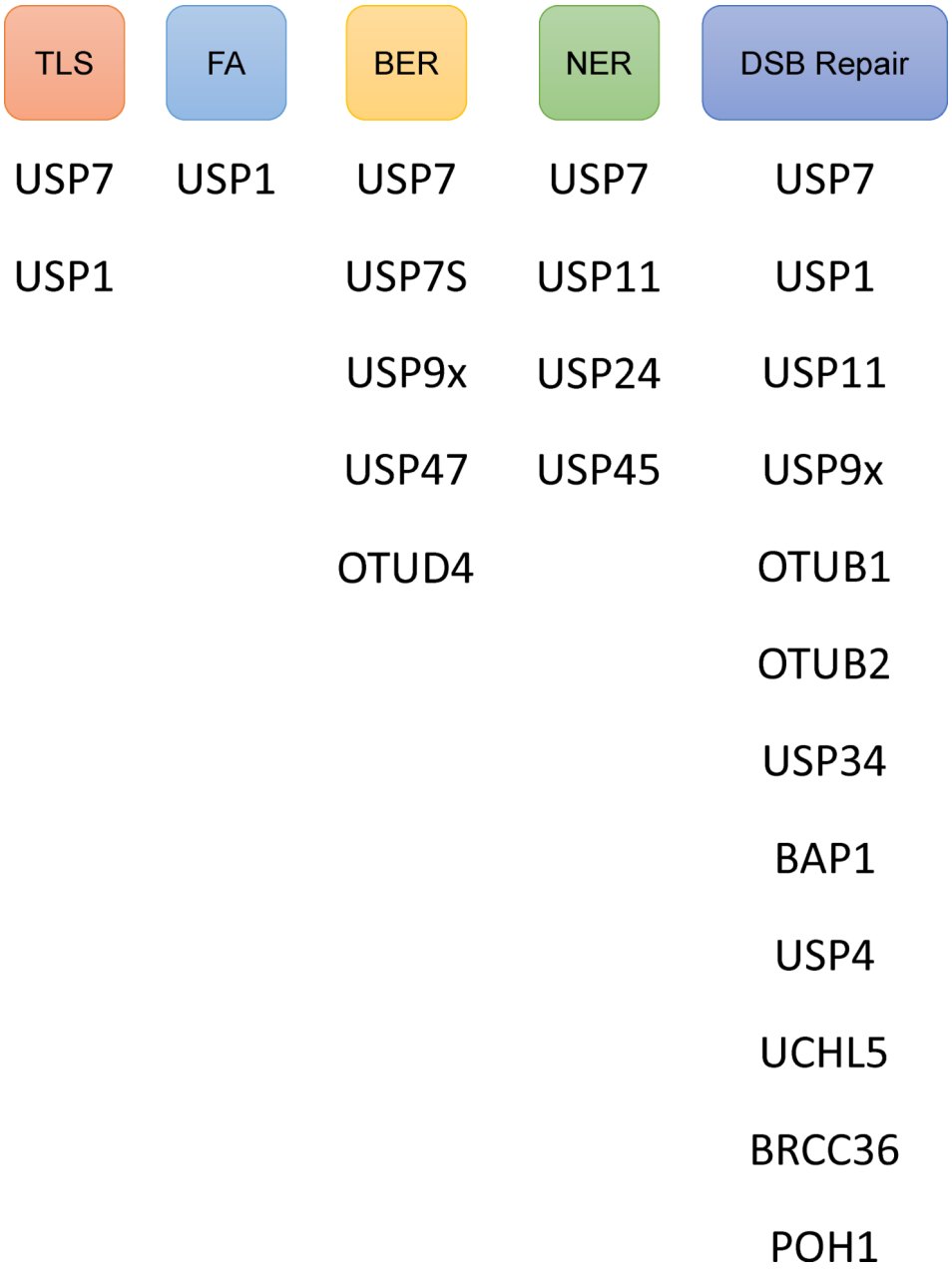

Figure 3. Deubiquitinases Involved in DNA Repair Pathways.

DNA damaging agents induce unique lesions. Shown are identified DUBs involved in the DNA repair pathways. See text for explanations of their role in each particular pathway. Note that specific DUBs can be involved in multiple pathways.

Table 1.

DUBs that Regulate the DDR Pathways.

DUB	Substrate/Target	Process	Functional Consequence	References
USP7	XPC	NER	May regulate the stabilization and chromatin loading of XPC for GG-NER	[60, 61, 64]
	UVSSA & ERCC6/CSB	NER	Regulates the stability of ERCC6/CSB for TC-NER	[61, 66–69]
	ALKBH2 & ALKBH3	BER	Promotes alkylation damage resistance & regulates sensitivity to alkylating agents	[58]
	RNF168 & RNF169	DSB	Stabilizes RNF168 and may regulate BRCA1 recruitment at DSB sites; HR repair increased via USP7-RNF169 axis	[98, 100]
	PCNA	TLS	Stabilizes RAD18 and Pol η	[42–44]
USP7S	Mule/ARF-BP1	BER	Stabilizes DNA Polymerase β and λ	[59]
USP24	DDB2	NER	Allow proper detection of UV-induced damage by stabilizing E3 ligase	[60, 61, 63]
USP45	ERCC1	NER	Promotes translocation of ERCC1 to foci of DNA damage and ability of cells to repair CPDs	[70, 71]
USP11	XPC	NER	Proper damage recognition of lesions	[65]
	H2AX	DSB	HR repair increased	[85, 86]
USP9x	ALKBH2 & ALKBH3	BER	Promotes alkylation damage resistance & regulates sensitivity to alkylated agents	[58]
	MCL-1	DSB	Stabilizes MCL-1 & HR repair increased; NHEJ repair decreased	[101, 102]
USP47	Polymerase β	BER	Synthesize nicked DNA	[55]
OTUD4	USP7 & USP9x, ALKBH2 & ALKBH3	BER	Promotes alkylation damage resistance via stabilization of AlkB homologues	[58]
OTUB1	UBC13	DSB	HR repair decreased	[95–97]
OTUB2	L3MBTL1 & K63-linked ubiquitin chains	DSB	HR repair increased; NHEJ repair decreased	[72, 83]
USP34	RNF168	DSB	HR repair decreased	[99]
BAP1	H2A	DSB	HR repair increased	[104–106]
UCHL5	NFRKB	DSB	HR repair increased	[87–91]
USP4	CtIP & MRN	DSB	HR repair increased	[92–94]
BRCC36	K63	DSB	HR repair increased; NHEJ repair decreased	[72, 80, 84]
POH1	K63	DSB	HR repair decreased	[21, 78, 80–82]
USP1	FANCD2/FANCI	FA	Efficient foci formation at sites of DNA Damage	[40, 45–53]
	PCNA	TLS	Promotes recruitment of TLS polymerases	[41]
	???	DSB	HR repair increased; NHEJ decreased	[103]