Connecting the Dots: Interplay Between Ubiquitylation and SUMOylation at DNA Double Strand Breaks

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Abstract

Protein modifications, including phosphorylation, ubiquitylation, and SUMOylation, have emerged as essential components of the response to DNA double-strand breaks (DSBs). Mutations within the genes encoding effectors of these components lead to genomic instability and in selected cases, human radiosensitivity and cancer susceptibility syndromes. In this review, we highlight recent advances in the study of DSB-associated signaling events by ubiquitylation and SUMOylation, and discuss how coordination among protein modification systems integrates components of the DNA damage response into a network that regulates DNA repair and transcriptional processes on contiguous stretches of chromatin.

Keywords

post translational modification; ubiquitylation; SUMOylation; DNA damage response

Brief introduction

DNA damage elicits a host of coordinated cellular responses, including recognition of the genomic lesion, signal transduction to halt the cell cycle, and repair of the damage. Depending on the extent of damage and the cell of origin harboring the DNA lesions, these responses influence cell fate determination, leading to survival, death or, senescence. Cellular responses triggered by DNA damage are collectively defined as the DNA damage response (DDR).

Protein posttranslational modifications (PTMs) accommodate a vast network of functions during the DDR by altering properties of target proteins, including 1) enzyme activity, 2) protein stability, 3) sub-cellular localization, and 4) abilities to interact with other proteins.[1] In response to DSBs, the phosphoinositide-3-kinase-related protein kinase (PIKK) family members, ATM, ATR and DNA-PKcs, are activated in mammalian cells, resulting in phosphorylation of more than 700 target proteins.[2] Several of these phosphorylation targets reside on chromatin, including H2AX and MDC1. PIKK dependent phosphorylation within the C-terminal tail of H2AX (γH2AX) creates a docking site for the BRCT and FHA domain containing protein MDC1, which is in turn phosphorylated by ATM, creating a recognition site by the FHA domain of the RING domain E3 ligase RNF8. Binding of phosphorylated MDC1 by RNF8 initiates an E3 ligase cascade consisting of RNF8 and a second E3 ligase

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RNF168, which is biallelically mutated in the human disease syndrome known as RIDDLE Syndrome (radiosensitivity, immunodeficiency, dysmorphic features and learning difficulties). Collectively, RNF8 and RNF168 are responsible for synthesizing the majority of the conjugated ubiquitin signal at DSBs. This signaling pathway reveals a modular assembly of the DNA damage response on chromatin, connecting DDR dependent phosphorylation to ubiquitylation at DSBs (Figure 1).

More recently, it has been shown that DSB ubiquitylation and the recruitment of BRCA1 and other repair proteins to the sites of damage is regulated by SUMOylation, placing SUMOylation as a critical component necessary for optimal ubiquitylation at DSBs.[3,4] Mounting evidence suggests that phosphorylation, ubiquitylation and SUMOylation events are important elements of the mammalian cell DDR to DSBs and coordination of these events is required to achieve an integrated DDR, including detection of DNA damage, DNA repair, checkpoint activation and transcription silencing. In this review, we discuss how ubiquitylation and SOMOylation function to protect cells from DNA damage, highlighting recent advances in the crosstalk between ubiquitylation and SUMOylation in the DDR.

Principles of protein ubiquitylation and SUMOylation

Ubiquitin and ubiquitin-like modifiers, such as SUMO and NEDD8, are small proteins (8-to 20-kDa) that are used by cells to alter the functions of target proteins via covalent attachment of the modifiers. Ubiquitin is a highly conserved 76-amino acid protein that shares 20% sequence identity with SUMO.[5,6] Vertebrates express three SUMO paralogs, SUMO-1, SUMO-2 and SUMO-3. Conjugated SUMO-2 and SUMO-3 differs only in three N-terminal amino acid residues and they share 50% sequence homology with SUMO-1.[7,8]

Conjugation of ubiquitin to a target protein via a covalent isopeptide bond is catalyzed by a highly regulated three-step enzymatic cascade that involves sequential actions of an E1 activating enzyme, an E2 conjugating enzyme and an E3 ligase.[9] The C-terminal glycine of ubiquitin is first catalyzed by E1 to form a covalent acyl-phosphate linkage with AMP. The catalytic cysteine of the E1 then attacks the linkage between ubiquitin and AMP, subsequently forming a thioester bond with the ubiquitin C-terminal glycine and releasing AMP. Through a transthiolation reaction, ubiquitin is transferred from the E1 catalytic cysteine to the E2 catalytic cysteine. The E3 ligase functions to recruit both the target protein and E2, and catalyze the transfer of ubiquitin from the E2 catalytic cysteine to a ε-amino group of the target protein lysine residue. SUMOylation requires a similar enzyme cascade to that of ubiquitylation, with the notable difference being that many less SUMO E2 and E3 enzymes have been identified.

Substrate specificity is usually provided by the E3 ligase. Consistent with the fact that a substantial fraction of the proteome is modified by ubiquitin or SUMO, human cells are predicted by bioinformatic analysis to express more than 600 E3 ligases.[10] Three major categories of E3 ligases exist: the Really Interesting New Gene (RING) domain-containing E3s, the Homologous to E6-Associated Protein (E6AP) C-Terminus (HECT) domain-containing E3s, and E3s that are not included in the previous two categories.[11–13] Despite sharing the ability to simultaneously recruit both E2 and the substrate, HECT E3 and RING domain E3 ligases use distinct molecular mechanisms to transfer activated ubiquitin from E2 to the target protein. Through its conserved cysteine residue, HECT domain E3 ligases form an intermediate thioester bond with the ubiquitin C-terminus before transferring the ubiquitin to the substrate.[12] Conversely, it is commonly accepted that RING E3 functions as a scaffold to bring together the E2 and the substrate to catalyze a direct transfer of ubiquitin from E2 to the substrate without the formation of an E3-ubiquitin thioester intermediate.[11] In contrast to the large number of ubiquitin ligases, only several SUMO E3 ligases and a single SUMO E1 (SAE1/SAE2) and SUMO E2 (Ubc9) have been identified.

All seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) and the amino-terminus of ubiquitin can be conjugated to the C-terminus of another ubiquitin molecule to form ubiquitin chains of differing structures *in vivo*.[14,15] The linkage specificity, also known as topology, of ubiquitin conjugates is essential in determining the functional outcome of ubiquitylation. Assembly of the canonical K48-linked ubiquitin chains on proteins marks them for degradation by the 26S proteasome,[16,17] whereas modification by K63-linked ubiquitin polymers does not direct substrates for degradation but usually functions in protein localization and signal transduction.[18] Ubiquitin chains containing heterogeneous isopeptide linkages may confer yet additional complexity. Mass spectrometry data indicate the existence of multiple types of isopeptide linkages in a single ubiquitin polymer *in vivo* and branched polyubiquitin chains have been shown to form *in vitro*.[14,15,19,20] It is also possible that ubiquitin can be attached to SUMO, leading to the formation of a hybrid structure composed of mixed ubiquitin-like modifiers.[21] Nevertheless, the physiologic functions of these ubiquitin structures remain to be determined. Like ubiquitin, SUMO-2/3, but not SUMO-1, can also form polymers through isopeptide linkages, yet little is known about how poly-SUMO topology relates to function.[7]

RING E3s and HECT E3s contribute in a manner that is not fully understood to the type of isopeptide linkages. A RING domain E3 ligase can pair with different E2s to effect ubiquitin chain topology, determined by the specificity of associated E2 enzyme.[20,22] For instance, the human heterodimeric RING E3 ligase BRCA1-BARD1 pairs with an E2 conjugating enzyme Ubch5c and directs the synthesis of K6-linked ubiquitin polymers.[23,24] *In vitro* evidence also show that BRCA1-BARD1 can bind to Ubc13-Mms2 or Ube2k to auto-ubiquitylate with K63-linked or K48-linked ubiquitin chains, respectively.[22] Indeed Ubc13-Mms2 is thought to be the only E2 enzyme responsible for K63-Ub synthesis in eukaryotes. Unlike the RING E3s, HECT E3 ligases are thought to specify the chain linkage of the product.[25]

Consistent with the diversity of ubiquitin structures, more than twenty ubiquitin binding domains (UBDs) have been identified to date.[26] Indeed, it is thought that specific recognition by ubiquitin binding domain- containing proteins confers functional specificity of different ubiquitin chain topologies. Such interactions would enable sorting of ubiquitylated proteins to different locales within the cell. The majority of UBDs, including ubiquitin-interacting motif (UIM), motif interacting with ubiquitin (MIU, or inverted UIM), ubiquitin-binding zinc finger (UBZ) and ubiquitin-associated domain (UBA), use a single or tandem α-helices to bind a hydrophobic patch of ubiquitin that is centered around Ile44.[27,28] To bind the same ubiquitin surface around Ile44, some proteins instead employ β-sheet structures that are present in the domains of ubiquitin proteases.[29] Since the Ile44-containing surface is crucial for the binding of ubiquitin by UBDs, it is likely that different UBDs compete for the Ile44-containing surface and the transduction of the ubiquitin signal may therefore be controlled by the amount and the binding affinity of the UBDs available.

Because the specificity of ubiquitin chains imparts distinct roles in cellular functions, it is not surprising that an increasing number of UBD-containing proteins display specificity in their recognition of ubiquitin linkages. RAD23A, a human homologue of radiation sensitivity abnormal 23, has a UBA domain at its C-terminus that specifically binds to K48-linked but not K63-linked ubiquitin chains and targets modified substrates to the 26S proteasome.[30] Other UBDs containing proteins, including ubiquitin specific peptidase 2 (USP2),[31] Npl4 zinc finger domain of Tak1 binding protein 2,[31] and Receptor Associated Protein 80 (RAP80),[32] preferentially bind to K63-linked instead of K48-linked ubiquitin chains. RAP80 specifically binds to K63-linked ubiquitin chains through two tandem UIMs and targets a BRCA1 containing protein complex to DNA damage-induced foci.[20,32,33] Structural studies of RAP80 revealed that the region linking the two UIMs ensures that the RAP80 UIM1 and UIM2 α-helices are arranged to specifically bind the proximal and distal ubiquitin moieties across a single K63 linkage.[34,35] Contrary to RAP80, ataxin-3, an ubiquitin specific protease associated

with the development of spinocerebellar ataxia type 3, demonstrates K48 linkage specificity with two UIMs and a two-residue linker region.[36] Remarkably, swapping the linker between the two UIMs of RAP80 with the two-residue linker of ataxin-3 results in a 6-fold increase of K48 preference of the Rap80 UIMs.[35]

Modification by ubiquitin or SUMO is reversible through the action of deubiquitylating enzymes (DUBs). DUBs are proteases that hydrolyze ubiquitin or ubiquitin-like proteins and their functions include activating ubiquitin pro-proteins, reversing modifications by ubiquitylation or ubiquitin-like modifiers, and recycling ubiquitin prior to proteolytic degradation of an ubiquitylated substrate by the proteasome. Nearly 100 DUBs are encoded by the human genome to account for the diversity of ubiquitin and ubiquitin-like protein structures and structure specificity of DUBs, while it is estimated that there are 6 human deSumoylating enzymes.[37,38] DUBs are divided into two categories based on the catalytic mechanism, the papain-like cysteine protease family that uses an active site cysteine for nucleophilic attack of the ubiquitin isopeptide bond and the JAB1/MPN/Mov34 metalloenzyme (JAMM) domain zinc-dependent metalloprotease family that hydrolyzes the ubiquitin conjugates using Zn^{2+} dependent hydroxide delivery to hydrolyze the scissile ubiquitin isopeptide bond.[39,40] To date, only active site cysteine containing proteases have been implicated in deSUMOylation.[41] DUB activity is tightly regulated so that cells have fine-tuned control over ubiquitin cleavage. For example, DUBs are normally constitutively inactive and their activity is regulated by PTMs of the enzyme,[42–44] the binding to substrates [45,46] or association with specific cellular organelles.[47]

Ubiquitylation and SUMOylation in the protection of genome integrity

Emergence for diverse ubiquitin and SUMO landscapes at DNA damage-induced foci

DSBs are potentially cytotoxic and pose a significant threat to genome integrity. DDR proteins respond to DNA damage within minutes, resulting in rapid accumulation of signaling and repair factors at the sites of damage. The accumulation and retention of DDR factors, the number of which is estimated to be more than 1000 molecules of a given DNA repair protein at a single DSB,[48] can be visualized as repair foci by fluorescent microscopy.

Upon DSB induction, ubiquitin species with different linkage types emerge at the sites of damage, albeit with different kinetics.[49] The accumulation of these ubiquitin conjugates at the sites of DNA damage depends on phosphorylation events mediated by PIKKs (Figure 1). DSBs activate ATM and related PIKKs, which phosphorylate H2AX and lead to recruitment of MDC1 to the sites of damage. The phosphorylation of MDC1 by ATM is required for the recruitment of RNF8, the first of the three ubiquitin E3 ligases that sequentially localize to damage-induced foci. The RING E3 ligase RNF8 binds to phosphorylated MDC1 via its forkhead-associated (FHA) domain. The FHA domain of RNF8 has recently been reported to bind to the HECT domain of HERC2 following its ionizing radiation (IR)-induced phosphorylation at Thr4827. Interestingly, although RNF8 uses the same FHA domain for binding phosphorylated MDC1 and HERC2, these interactions are not mutually exclusive.[50] The interaction between HERC2 and RNF8 facilitates the assembly of the E2 ubiquitin conjugating enzyme Ubc13 with RNF8 and promotes the synthesis of DNA damage-induced K63-linked ubiquitin chains on H2A and its variants.[50] Another RING domain ubiquitin E3 ligase RNF168 recognizes and binds to K63-linked ubiquitin chains on H2A and H2AX through its two MIUs, amplifying the local concentration of K63-linked ubiquitin together with Ubc13 and resulting in the recruitment and retention of 53BP1 and BRCA1 at the sites of lesions. Collectively, RNF8 and RNF168 are responsible for the majority of conjugated ubiquitin signal at DSBs[51,52] BRCA1 is yet another ubiquitin E3 ligase that localizes to damage-induced foci. Unlike RNF8 or RNF168, BRCA1 localization requires its interaction with a complex composed of BRCC36, BRCC45, Abraxas, MERIT40, and RAP80 [53–55]

RAP80 specifically recognizes K63-linked ubiquitin conjugates through its two UIMs and recruits BRCA1 and the other members of the BRCA1-RAP80 complex to DNA damage sites. [32,33,56] BRCA1 functions as a heterodimer with the RING domain protein BARD1 and has been proposed to synthesize K6-linked ubiquitin at sites of DNA damage. Indeed, it has been shown to be autoubiquitylated by K6-linked ubiquitin in the presence of Ubch5c *in vitro* and the accumulation of K6-linked ubiquitin at damage-induced foci depends on both BRCA1 and Ubch5c.[23,24,57] In response to DNA damage, BRCA1 has also been reported to ubiquitylate BRCA1 carboxy-terminal interacting partner, CtBP-interacting protein (CtIP) in a manner that depends on a phosphorylation-mediated interaction between CtIP and BRCA1 BRCT domains. [58] The E3 ligase activity of BRCA1 through ubiquitylation of CtIP was suggested to be required for its control over a transient G2/M checkpoint.[58,59] To address the role of BRCA1 E3 ligase activity in a clean genetic system, a hypomorphic variant of BRCA1 with respect to E3 ligase activity was knocked into murine ES cells. This rationally designed mutation reduces BRCA1 RING domain association with Ubch5c limiting BRCA1 in vitro E3 ligase activity.[60] To the surprise of many, BRCA1I26A did not show obvious deficits in homologous recombination mediated repair of a nuclease induced DSB, suggesting that either compensatory mechanisms exist within the cell or that BRCA1 E3 ligase activity plays a heretofore unappreciated role in a process other than the repair of DSBs by HR.[61] An alternative possibility is that additional regulatory factors enable the BRCA1I26A mutant to be an active E3 ligase in vivo. Recent evidence that BRCA1 E3 activity is increased 10–20 fold in vitro by SUMOylation,[4] raises the possibility that BRCA1 SUMOylation could surmount the reduced affinity of BRCA1I26A for E2 enzymes, thereby maintaining BRCA1 dependent DSB ubiquitylation. A detailed analysis of BRCA1I26A knockin cells will be necessary to address these possibilities.

With regard to the role of SUMOylation in the DDR, it has been shown to participate in base excision repair, regulation of translesion DNA synthesis, and both homologous recombination and nonhomologous end joining repair of DSBs.[3,4,62,63] A base excision repair protein, thymine-DNA glycosylase (TDG) plays a central role in the cellular defense against mutations caused by the spontaneous deamination of 5-methylcytosine and cytosine. TDG removes the resultant uracil or thymine that mispairs with guanine, and leaves an abasic site for the ensuing actions of BER.[64,65] DNA bound TDG can be SUMOylated, which alters the conformation of TDG and dissociates the glycosylase from the abasic site.[66] Another example with regard to roles of SUMOylation in DNA repair is RAD52, which plays a central role in homologous recombination. Rad52 promotes strand annealing by exchanging RPA with Rad51 on single stranded DNA. SUMOylation of Rad52 sustains its activity and protects Rad52 from proteaosomal degradation, implicating a role of SUMOylation in the homologous recombination pathway for the protection of genome integrity.[67] SUMOylation also participates in nonhomologous end joining. Repair proteins involved in nonhomologous end joining, including Ku70-Ku80 and XRCC4, and 53BP1 have also been found to be modified by SUMOylation and failure to execute SUMOylation reactions at DSBs reduces NHEJ mechanisms of repair.[3,4,68–70].

Circumstantial evidence exists for a far broader role of SUMO in the DDR. Sequence alignments showed that out of the 700 proteins that are potential phosphorylation targets of ATM/ATR,[2] 618 of them contains at least one SUMO modification motif ψKxE (our analysis), suggesting that DNA damage may target PIKK substrates for SUMOylation.

DNA damage-associated ubiquitylation and SUMOylation in the protection of genome integrity

Disruption of the formation, recognition and clearance of ubiquitin or SUMO conjugates at DSBs lead to genomic instability and in selected cases human radiosensitivity and cancer susceptibility syndromes. Mutations in BRCA1 and BRCA2 occur in about 20% of the families

with inherited breast cancers.[71,72] The RING domain of BRCA1 is the catalytic domain for its ubiquitin E3 ligase activity. Mutations in the RING domain, which accounts for approximately 20% of the clinical BRCA1 missense mutations, disrupts the E3 ligase activity of BRCA1 by altering its interaction with ubiquitin-conjugating enzymes.[73,74] In addition, RNF168, an ubiquitin E3 ligase, was found mutated in the recently discovered RIDDLE syndrome, which manifested as immunodeficiency and radiosensitivity. Disruption of RNF168 dependent conjugation of K63-linked ubiquitin results in impaired localization of 53BP1 and BRCA1 to the sites of DSBs.[51,52]

Disruption of the SUMO pathway also results in decreased repair and sensitizes cells to DNA damaging agents. *S. cerevisiae* cells expressing a temperature-sensitive SUMO E2 mutant Ubc9 display hypersensitivity to the alkylating agent MMS and to UV radiation at a semi-permissive temperature.[75] Furthermore, over-expression of a dominant-negative mutant of Ubc9 in the human breast cancer cell line, MCF-7 resulted in increased sensitivity to anticancer agents requiring DSB repair by homologous recombination.[76] Furthermore, studies in both yeast and human cells revealed that deficiency of the SUMO ligase, MMS21/hMMS21 resulted in decreased capacity to repair DNA lesions.[77,78]

Besides the synthesis of ubiquitin conjugates, the specific recognition of ubiquitin structures at the sites of damage is also important for preserving genome integrity and preventing the onset of familial breast cancer. Through its tandem UIMs, RAP80 specifically recognizes K63-linked ubiquitin chains synthesized at the sites of DNA damage and mediates the recruitment of BRCA1 to DSBs. Sequencing for mutations in the RAP80 gene in BRCA1/BRCA2 mutation-negative Finnish breast cancer families identified a single amino acid deletion (delE81) in one of the highly conserved UIMs of RAP80.[79] This mutant protein showed severely impaired ability to bind to ubiquitin and to localize itself and BRCA1 to DSBs. In addition, expression of the mutant protein significantly increased the level of chromosomal aberrations.[79] Interestingly, a screen for mutations in MERIT40 and Abraxas, core components of the BRCA1-RAP80 complex, in breast and ovarian cancer families did not find disease related sequence variations, suggesting that loss of function mutations within the BRCA1-RAP80 complex will not be a common genetic cause of inherited breast cancer.[80,81] However, given that multiple recognition events for linkage specific ubiquitin species are required for the proper execution of DDR signaling and tumor suppression,[82–85] it is possible that mutations in genes central to these pathways will contribute to the onset of familial cancer syndromes. RNF8, RNF168, and other components of the ubiquitin and SUMO DDR pathways are new candidate genes worthy of investigation in breast cancer and in other malignancies.

In addition to the synthesis and recognition of ubiquitin, evidence has emerged in the last several years that clearance of the ubiquitin by DUBs is an indispensible element of the DDR. Similarly, clearance of γH2AX phosphorylation is required for a full recovery from the DDR. [86,87] Following IR, USP3 depleted cells showed persistent γH2AX and ubiquitin conjugates foci and a prolonged G2/M checkpoint.[88] On the other hand, DUBs may protect the integrity of the genetic information of an organism by eliminating the cells with extensive DNA damage through apoptosis. For example, USP28 has been shown to be required for DNA damage-induced apoptosis mediated by the Chk2-p53-PUMA pathway through stabilizing Chk2 and 53BP1.[89] The temporal control of DUBs activity and the functions of target protein altered by deubiquitylation are also critical determinants of the role of DUBs in preserving genome stability. For instance, the Fanconi Anemia Syndrome proteins, FANCD2 and FANCI are each monoubiquitylated and this modification is critical for their localization to DSBs.[90,91] USP1 binds to FANCD2 and the inhibition of USP1 leads to the accumulation of monoubiquitylated FANCD2 and mitomycin C hypersensitivity, suggesting that failure to deubiquitylate FANCD2, like impaired ubiquitylation, inhibits its function in repairing DNA interstrand cross-links.[92,93] As with deubiquitylation, regulated cleavage of SUMO from target proteins

is also important for the proper execution of DDR. Inhibition of Ulp2, SUMO homolog Smt3-deconjugating enzyme, in S. cerevisiae as well as mutations in Ulp1 in S. pombe led to hypersensitivity to hydroxyurea and UV radiation, respectively.[94,95]

SUMOylation dependent Ubiquitylation

Since a specific lysine residue in a protein can be subject to either ubiquitylation or SUMOylation, it is not surprising that a single protein can perform distinct functions based on the PTM mark it bears. For example, PCNA can be modified either by monoubiquitin, K63-linked ubiquitin chains, or SUMO on the same lysine residue (K164).[96] During S phase, in response to stalled replication forks, monoubiquitylation of PCNA on K164 directs cells for translesion synthesis, whereas polyubiquitylation leads to recombination-related error-free repair, of which the detailed mechanism remains unclear.[96–98] SUMOylation of PCNA on K164 in yeast specifically recruits Srs2 to prevent unwanted recombination between newly formed sister chromatids following DNA replication.[99,100]

DSBs trigger a DDR that is characterized by the synthesis of ubiquitin conjugates at the sites of damage-induced repair foci. Sequential recognition of the K63-linked ubiquitin structures eventually leads to the recruitment of BRCA1 and 53BP1. Two recently published studies report that the synthesis of ubiquitin conjugates mediated by BRCA1 depends on two SUMO E3 ligases, PIAS1 and PIAS4.[3,4] Depletion of PIAS1 or PIAS4 significantly reduced the level of FK-2 recognized ubiquitin conjugates at the sites of IR-induced foci or micro-irradiation stripes.[3,4] Although it has been shown that the disruption of PIAS1 or PIAS4 activity leads to impaired recruitment of BRCA1,[3,4] it remains unclear how the SUMO E3 ligases participate in the recruitment of BRCA1. In these studies, depletion of PIAS4 has been shown to affect the sequential recruitment of DNA damage responsive proteins up to RNF168, whereas depletion of PIAS1 only affects the accumulation of RAP80 and BRCA1, suggesting that the two SUMO E3 ligases affect the recruitment of BRCA1 through distinct mechanisms.[3,4] BRCA1 has two SUMO target motifs (ψKxE) immediately following its N-terminal RING domain, and mutation of the lysine residue (K119R) of one of these motifs is associated with decreased ubiquitin accumulation at the site of BRCA1 foci following IR.[4] *In vitro* experiments showed that SUMOylated BRCA1-BARD heterodimer has approximately 10–20 fold increased ubiquitin ligase activity compared to the unmodified heterodimer.[4] Together, these results suggest that PIAS1 or PIAS4 dependent synthesis of ubiquitin conjugates at the sites of DNA damage might be mediated through the regulation of BRCA1 ligase activity via SUMO modification.

Consistent with this interpretation, BRCA1 has been shown to be modified by SUMO in response to DNA damage,[3,4] yet it is not clear whether it is modified by SUMO-1 or SUMO-2/3, or both. PIAS1 has been demonstrated to be responsible for SUMO-2/3 conjugation, whereas PIAS4 is mainly responsible for the synthesis of SUMO-1 and to a less extent for the synthesis of SUMO-2/3.[3] However, it has also been shown that depletion of either PIAS1 or PIAS4 impaired the pulling down of BRCA1 by both anti-SUMO-1 and anti-SUMO-2/3 antibodies.[4]

Consistent with the roles of PIAS1 and PIAS4 in the DDR, depleting either of them resulted in decreased DSB repair by homologous recombination and nonhomologous end joining.[3,4] Together, these studies established SUMOylation as a key element in the DDR.

DSB-induced transcription silencing; functions of DSB associated ubiquitin beyond DNA repair protein recruitment

The ultimate goal of DDR is to protect the integrity of genetic information and its faithful transmission either to DNA by replication or to mRNA by transcription. Although physiological DSBs have been shown to elicit global transcriptional activation and chromatin relaxation in an ATM dependent manner,[101,102] little is known about how DDR influence the transcription of genes local to the sites of DNA damage. A common modification displayed at both DSBs and transcriptionally silent chromatin is ubiquitylated histone H2A (uH2A) [49, 103]. However, until recently, it has been unclear if uH2A could mediate crosstalk between these processes. Elucidation of the relationship between the DDR and transcriptional silencing local to the sites of DSBs has recently been reported.(Niraj Shanbhag, 2010) By modifying a system that was previously used for visualizing gene transcriptional in a single living cells,[104], this study investigated how the DDR influence the transcription of the genes on the same stretch of chromatin distal to DSBs. The system was developed by integrating transgene array integrated into a single location in the genome of human U2OS cells on chromosome 1p3.6.[104] Each unit of the transgene array is composed of multiple DNA sequence elements including 256 copies of the lac operator, 96 copies of tetracycline response elements (TRE), a minimal CMV promoter, CFP fused to the peroxisomal targeting signal SKL and 24 copies of the MS2 RNA stem loop sequence (Figure 2A). FokI endonuclease is fused to the lac repressor-mCherry fusion protein to introduce DSBs at least 4kb away from the CMV promoter. Doxycycline can be used to activate the transcription of the genes downstream of the TRE. Nascent transcription can be visualized by YFP-MS2 that binds to MS2 stem loops and translation can be indicated by the accumulation of CFP-SKL in the cytoplasm.

Using the single cell reporter system, a novel DDR-associated transcriptional silencing program, double strand break induced silencing cis (DISC), has been identified.(Shanbhag et al., 2010 Cell in press) FokI endonuclease-induced DSBs resulted in transcription inhibition of distal genes on the contiguous stretch of DNA as demonstrated by decreased YFP-MS2 signal close to the sites of damage as well as decreased MS2 transcripts levels measured by qRT-PCR. DISC has been shown to be a local cellular response to the damage and is dependent on the activity of ATM, as ATM inhibition reverses the damage-induced transcriptional silencing. Similar to polycomb repressive complex 1 (PRC1) related transcription repression, [105−107] DSBs-associated transcription silencing is also correlated with the ubiquitylation of H2A, but not with DSB associated K63-Ub levels (Figure 2B). Mechanistically, DISC may be achieved through hypophosphorylation of RNAPII at the Serine 2 residue of the c-terminal domain repeat region, thereby preventing RNAPII dependent transcriptional elongation. ATM dependent inhibition of RNAPII elongation during DISC prevented transcription associated chromatin decondensation, a particularly interesting observation in light of the strong connection between transcription and aberrant chromosomal translocations found in malignancy.[108,109] Perhaps ATM dependent silencing prevents large scale chromatin movements that could disrupt synapsis of chromosomal termini at a DSB. Indeed, ATM deficiency is highly correlated with transcription-associated chromosome translocations.[108, 110,111]

Similarities may exist between DISC and polycomb dependent transcriptional silencing.[112] Polycomb repressive complex 2 (PRC2) related transcription silencing is centered at trimethylation of H3K27, which has been reported to be enriched at DSBs.[113] PRC1 complexes cooperate with PRC2 to mediate silencing through uH2A dependent RNAPII inhibition at PRC repressed genes.[106,114,115] Although it has been suggested that uH2A prevents RNAPII phosphorylation by chromatin transcription complex (FACT), consequently blocking RNAPII elongation,[116,117] how the DDR signals to RNAPII hypophosphorylation and inhibition of elongation remains to be explored. Identification of DISC for the first time demonstrated a

direct regulation of the DDR on the transcription of genes distal to the sites of DNA damage on a contiguous stretch of chromatin. Several important question remains to be answered, including the precise mechanism of DSB silencing, how far silencing extends along chromatin from a single DSB, and what role silencing plays in maintaining genome integrity. An interesting question for future investigations is whether DSB silencing contributes to epigenetic changes emanating from persistent DSB responses following IR or telomere dysfunction, as these lesions are known to lead to the formation of senescence associated heterochromatin bodies. The tools are now in place to begin addressing these important questions.

Final words

PTMs, including phosphorylation, ubiquitylation and SUMOylation are key elements of the DDR that provide cells multiple means to fine-tune cellular responses to DNA damage. Although the roles of ubiquitylation and SUMOylation are the emphasis of this review, compelling evidence exists that other PTMs, such as acetylation, methylation, mono- and poly-ADP-ribosylation, also actively participate in the DDR, creating a dynamic network of molecular actions that cells use to protect their genetic information.

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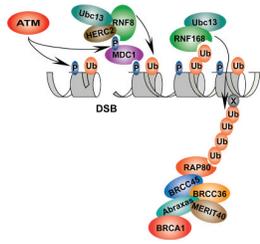
Figure 1.
The DDR pathway. In response to DNA DSBs, ATM and related PIKKs phosphorylate H2AX (γH2AX), creating a platform for the assembly of DDR proteins. Localization of MDC1 to sites of damage by binding to γH2AX and its subsequent phosphorylation by ATM is required for the recruitment of the ubiquitin E3 ligase RNF8. RNF8, together with the E2 ubiquitin conjugating enzyme Ubc13 and HERC2, synthesize K63-linked ubiquitin on histone H2A and its variants. RNF168, another RING domain ubiquitin ligase, recognizes the ubiquitin conjugates synthesized by RNF8/Ubc13, leading to its recruitment to sites of damage and further synthesis of K63-linked ubiquitin. RAP80 specifically binds to K63-linked ubiquitin, recruiting a protein complex including Abraxas, MERIT40, BRCC36, BRCC45 and BRCA1 to sites of damage.
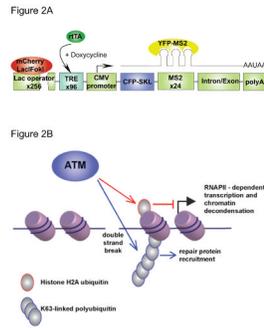
Figure 2.
DSB-induced transcriptional silencing. (A) Schematic of the transcription reporter locus. The lac operator (256 copies) is followed by the tetracycline response element (96 repeats) and a CMV promoter driven reporter gene that codes for the CFP-SKL protein and the MS2 RNA stem loop (24 repeats). Expression of the mCherry-Lac repressor-FokI nuclease domain fusion protein induces DSBs within the lac operator region. Transcription of the report gene can be induced by doxycycline and visualized by YFP-MS2 that binds to the MS2 stem loops. (B) DSB-induced local transcriptional silencing. DSBs induce the synthesis of histone H2A ubiquitin and K63-linked poly-ubiquitin chains at sites of damage. While histone H2A ubiquitin is responsible for DSB-induced ATM dependent transcriptional silencing, K63-linked poly-ubiquitin chains are responsible for the recruitment of repair factors.