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REVIEW

The DNA damage response pathways: at the crossroad of protein modifications

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Post-translational modifications play a crucial role in coordinating cellular response to DNA damage. Recent evidence suggests an interplay between multiple protein modifications, including phosphorylation, ubiquitylation, acetylation and sumoylation, that combine to propagate the DNA damage signal to elicit cell cycle arrest, DNA repair, apoptosis and senescence. Utility of specific post-translational modifiers allows temporal and spatial control over protein relocalization and interactions, and may represent a means for trans-regulatory activation of protein activities. The ability to recognize these specific modifiers also underscores the capacity for signal amplification, a crucial step for the maintenance of genomic stability and tumor prevention. Here we have summarized recent findings that highlight the complexity of post-translational modifications in coordinating the DNA damage response, with emphasis on the DNA damage signaling cascade.

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To ensure faithful duplication and inheritance of genetic material, the cell has evolved with the ability to detect and propagate the initial DNA damage signal to elicit cellular responses that include cell cycle arrest, DNA repair, senescence and apoptosis, which collectively have been termed the DNA damage response. Dysregulation of components involved in these processes contributes to genomic instability, which in turn leads to tumorigenesis. This is supported by the fact that clinical mutations in proteins that play a role in the DNA damage response often predispose individuals to cancer development [1]. The link between genomic instability and tumorigenesis is perhaps most exemplified by the human genetic disorder ataxia-telangiectasia (A-T). A-T is caused by mutations of the ATM gene, the product of which is intimately involved in the DNA damage signaling network. A-T patients are characterized by neurodegeneration, radiosensitivity, immunodeficiency and cancer predisposition [2, 3]. Recent studies indicate that the ATM protein kinase modulates multiple branches of signaling pathways by phosphorylating and regulating its substrates in response to DNA damage, failure of which contributes to genomic instability and tumorigenesis [4]. Like ATM, mutations in NBS1 have also been documented to predispose individuals to the genomic instability disorder Nijmegen breakage syndrome (NBS) [5]. Patients with hypomorphic mutations in NBS1 manifest microcephaly, immunodeficiency, radiation sensitivity and are prone to carcinogenesis. The close resemblance between NBS and A-T patients suggested a functional relationship between these gene products. Indeed, the MRN complex consisting of Mre11, Rad50 and NBS1 not only has been implicated as one of the initial DNA lesion sensors, but also is believed to be required for efficient ATM activation following DNA damage. As such, the understanding of molecular pathways that function to safeguard the integrity of the genetic material is critical for early detection and offers potential treatments for cancer patients.

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Protein phosphorylation as regulatory elements in response to DNA damage

DNA lesions trigger the activation of various kinases, which constitute the primary transducers in the signaling cascade. Of utmost importance are the phosphoinositide-3-kinase-related protein kinase (PIKK) family members ATM, ATR and DNA-PKcs. While ATR activation is associated with single-stranded DNA and stalled DNA replication forks, ATM and DNA-PKcs respond mainly to DNA double-strand breaks (DSBs) [6]. Given the importance of central transducer ATM in response to DNA

lesion, in particular DSBs, perhaps it is not surprising to speculate that protein phosphorylation plays a crucial role in the activation of various effector systems, which, when combined, counteract genotoxic stresses. Indeed, a recent large-scale proteomic study identified more than 700 proteins that are phosphorylated in response to DNA damage [4], signifying a highly branched network of proteins that is orchestrated and regulated by this post-translational modification. Although the functional significance of many of these phosphorylation sites remain to be revealed, it is likely that a proportion of these serves as switches to regulate protein-protein interactions. This is supported by the identification of a number of phospho-binding motifs among DNA damage responsive proteins that serve as stimulus-inducible adaptors as means to transduce the DNA damage signal [7-9]. The concerted action between phosphorylation and the ability to recognize sequence-specific phospho-proteins allows timely activation and transduction of DNA damage signals.

Two major classes of phospho-binding modules that are intimately involved in the DNA damage response are the BRCA1 C-terminal repeat (BRCT) and forkhead-associated (FHA) domains. The BRCTs constitute a module for recognizing phospho-peptides. Its possible role in cellular processes, including cell cycle arrest and DNA repair, was deduced from its prototype BRCA1, of which its tandem BRCT repeats are essential for its tumor suppressor function. That BRCT domains are conserved across different species and are present in many proteins involved in the cellular response to DNA damage implicated a role of this motif in facilitating stimulus-inducible protein-protein interactions. Using a library of phosphopeptides that mimic amino acid sites phosphorylated by ATM/ATR, it was shown that the DNA damage responsive proteins BRCA1 and PTIP preferentially bound to phosphorylated peptides [8]. Likewise, a panel of 13 other proteins from various organisms that participate in the DNA damage response was demonstrated to bind selectively to a degenerated phosphoserine peptide library [7], supporting the general role of BRCT domains in mediating phospho-dependent interactions. The specific interaction between BRCA1 and one of its phospho-dependent interacting partner BACH1 was also shown to be required for the DNA damage-induced checkpoint control during the G2-M transition. Apart from BACH1, BRCA1 has subsequently been shown to complex with Ccdc98/Abraxas and CtIP [10-13], among others, in a phosphorylation-dependent manner (discussed below). These studies further provided a clause for the post-translational modification in the differential regulation of BRCA1 functions.

Another important protein module that serves as recognition element for phosphothreonine induced by DNA-damage-responsive kinases is the FHA domain [9, 14-16]. Proteins harboring this motif have also been implicated in cellular processes such as cell cycle regulation and DNA repair. The first evidence for the involvement of FHA domain in mediating phosphorylation-dependent interactions came from the study of the Arabidopsis kinase-associated protein phosphatase (KAPP) [17]. Through the use of interaction cloning, the kinase interaction (KI) domain was reported to associate with the serine-threonine receptor-like kinase RLK5 and was dependent on phosphorylation of the latter molecule. Hofmann and Bucher later showed that the KAPP KI domain shares sequence identity with FHA domains and demonstrated that the minimal KI domain consists of 119 residues [18]. Studies of the yeast protein Rad53 provided further support for the idea that the FHA domain facilitates phosphorylation-dependent interactions [19]. Using yeast two-hybrid screen, Rad53 was found to associate with Rad9 via one of its FHA domains. The binding between these proteins was also shown to be regulated by the TEL1/MEC1 kinases in response to DNA damage and is essential for damage-induced G2-M checkpoint arrest. Although the cellular targets for many of the FHA domain-containing proteins remain to be identified, proteomic studies have consolidated the idea of FHA domain in selecting for phosphorylated targets [16].

The requirements for these phospho-binding modules in the cellular response to DNA damage signify the versatility of damage-inducible control in the recruitment and activation of signaling pathway, which would otherwise compromise the integrity of the genetic material and contribute to genomic instability.

Emerging role of protein ubiquitylation in the DNA damage response

Ubiquitin is an essential 76-amino-acid protein that is conserved among eukaryotic species. Protein ubiquitylation proceeds via the covalent transfer of ubiquitin to one or more lysine residues of the target protein in a three-enzyme cascade that involves an E1 activating enzyme, an E2 conjugating enzyme and the specificity-conferring E3 ubiquitin ligase. An increasing number of E3 ubiquitin ligases have been implicated in processes that respond to a variety of genotoxic stresses. Moreover, these E3 ligases are known to work together with several E2 conjugating enzymes that catalyze ubiquitin conjugation via different lysine residues. Indeed, ubiquitylation is emerging as an important post-translational modification that is utilized as a regulatory mechanism in the face of DNA damage.

The most common consequence of protein ubiquitylation is protein degradation through the proteasome-mediated pathway. In support for a role of the proteasome function

in DNA damage response and tumorigenesis, the 19S proteasome subunit DSS1/SHFM1 was found to interact with fragments of human BRCA2 in a two-hybrid study [20]. Like BRCA1, germline mutations of the breast cancer susceptibility gene BRCA2 predispose individuals to early-onset breast cancer. The exact role of BRCA2 in the maintenance of genomic stability remained ill-defined until recent reports indicated a role of this nuclear protein in DNA repair by facilitating homologous recombination via loading of the recombinational repair protein Rad51 [21]. The functional involvement of DSS1 in counteracting genotoxic stress was revealed when it was shown that cells depleted of DSS1 led to failure of Rad51 assimilation onto single-stranded DNA [22], as reflected by the absence of Rad51 foci formation in response to DNA damage.

Proteasome inhibitors including Bortezomib have been used to sensitize tumor cells to DNA-damaging agents; however, the molecular mechanism involving its mode of action remains largely unknown. Through the use of proteasome inhibitors and RNAi knockdown, several recent reports have provided evidence for the proteasome in the activation of the Fanconi anemia pathway and DNA damage signaling pathways [23]. Interestingly, unlike DSS1, cells depleted of the 19S and 20S proteasome subunits (PSMD4, PSMD14, and PSMB3) inhibited the mono-ubiquitination and damage-induced foci formation of FANCD2, indicating that the proteasome might be differentially involved in the face of different forms of genotoxic stress. Consistent with this possibility, proteasome inhibitors suppressed DSB repair via homologous recombination but not non-homologous end joining [24]. This observation is reminiscent to those of DSS1 inactivation and might reflect a selective role of the proteasome in stimulating Rad51 nucleoprotein filament formation. Mechanistically, how the proteasome is involved in the DNA damage response remains to be unveiled; however, the fact that several of the proteasome subunits are phosphorylated by the ATM/ATR protein kinases in an IR-dependent manner suggests a potential interplay between proteasome activity and the DNA damage response [4]. Future studies will reveal how proteasome activity and, more specifically, how the DSS1-associated proteasome complex are regulated following DNA damage and contribute to DNA damage repair and the maintenance of genomic integrity.

Another role of regulated proteolysis in the maintenance of cellular homeostasis is to prevent DNA re-replication in response to DNA damage. This came from studies involving the degradation of replication licensing factor Cdt1 [25-28]. The Ddb1-Cul4 ubiquitin ligase has recently been demonstrated to regulate Cdt1 turnover. In these studies, the adaptor Cdt2 was revealed to be required for Cdt1 degradation, failure of which resulted in re-replication and G2/M DNA damage checkpoint activation. Although Cdt2 is recruited to the chromatin via Cdt1 and PCNA, which in turn enables Cdt1 ubiquitylation, the signal that targets Cdt2 to chromatin-bound PCNA during S phase or DNA damage remains to be identified.

Non-canonical ubiquitylation as a means to regulate protein-protein interactions

Apart from the conventional lysine48-linked polyubiquitylation that targets proteins for degradation, non-canonical ubiquitylation is also emerging as an important regulatory element, which governs cellular processes as diverse as gene transcription, DNA repair, receptor trafficking, endocytosis and cell cycle control. Ubiquitin conjugation via alternative lysine residues is thought to mediate specific protein-protein interactions. The fact that proteins can be modified not only with ubiquitin polymers composed of single isopeptide linkages but also with heterogeneous forked ubiquitin chains highlights the vast potential for multiple levels of regulation [29]. This is supported by the identification of a growing list of ubiquitin binding proteins that confer specificity for different ubiquitin-conjugated substrates [30, 31]. Ubiquitylation and the ability for its recognition, much like protein phosphorylation as discussed above, opens up yet another much anticipated regulatory mechanism for processes that ensures cell survival.

The multiple roles of PCNA in checkpoint control and DNA repair is exemplified by its ubiquitylation status, which determines DNA repair pathways at stalled replication forks. The Rad18-Rad6 complex mono-ubiquitylates PCNA at Lys164, which facilitates translesion synthesis (TLS) via the recruitment of translesion polymerase Rev1 [32-34]. Subsequent studies identified two ubiquitin binding domains (UBM and UBZ), which are evolutionarily conserved among Y-family TLS polymerases [35], that enable the bypass of DNA lesions by directing the replication machinery into the TLS pathway. By virtue of the interaction between Rad18 and Rad5 [36], PCNA can also be polyubiquitylated by the latter E3 ubiquitin ligase [37, 38]. In conjunction with MMS2-UBC13, Rad5 and its human functional homolog SHPRH facilitate error-free postreplicational repair through the homologous recombination repair pathway at stalled replication forks. However, how ubiquitin conjugation occurs and what factors mediate such repair remain to be identified. Intriguingly, PCNA has also been reported to be sumolyated at the same K164 residue predominantly during S phase and recruits the SRS2 helicase to sites of stalled replication forks [39]; thus, it appears that sumoylation of PCNA suppresses unscheduled recombination by disrupting Rad51 nucleofilament formation and acts as an negative regulatory element against

PCNA ubiquitylation-dependent events [40]. The capacity for multiple post-translational modifications illustrates a highly orchestrated cellular process that enables precise control of PCNA-dependent DNA repair functions.

Another important role of protein ubiquitylation came from the identification of FANCD2, which is mono-ubiquitylated specifically at Lys561 in response to DNA damage. The rare clinical syndrome Fanconi anemia (FA) is an autosomal-recessive or X-linked syndrome characterized by chromosome instability. FA patients are predisposed to leukemia and squamous cell carcinomas, and clinical mutations often associate with compromised FANCD2 ubiquitylation, suggesting that this modification is important for the FA complex function. Consistently, FANCD2 interaction with the breast and ovarian cancer suppressor proteins BRCA1 and BRCA2 at the chromatin requires its prior ubiquitylation [41-43]. Interestingly, the FA proteins belonging to different complementation groups are known to form a E3 ubiquitin ligase complex [44-46]. Whether the formation of the ubiquitin ligase complex is required for FANCD2 mono-ubiquitylation or simply acts as a scaffold for FANCD2 ubiquitylation by an additional E3 ubiquitin ligase is not known [47, 48]; nevertheless, the requirement for the specific modification of FANCD2 and its disease association provide yet another example of the elegance of post-translational modification in the regulation of effectors in response to DNA damage.

Recently, the Ubiquitin Interacting Motif-containing protein Rap80 was discovered to mediate the damage-induced relocalization of the Rap80-ccdc98/Abraxas-BRCA1 complex [13, 49-51]. The Rap80 UIM was unprecedentedly shown to be required and is sufficient for its localization to ionizing radiation-induced foci (IRIF), lending credence to the idea that Rap80 localizes at DNA breaks by docking at a certain ubiquitylated protein. Intriguingly, one study suggested that Rap80 is targeted to IRIF via a γ-H2AX and MDC1-dependent lysine6 and lysine63-linked ubiquitin polymers at DNA breaks [49]. Given that the Rap80 UIM binds to lysine63-linked, but not lysine48-linked, polyubiquitin chains *in vivo* and *in vitro*, it would be interesting to study how it confers substrate specificity at the molecular level. Together, the recruitment of DNA responsive proteins to sites of DNA damage via ubiquitin provides a novel mechanism to regulate protein trafficking, and suggests the possibility that mutations in these ubiquitin interacting motifs might be a casual factor for the dysregulation of the DNA damage response.

A role of ubiquitin proteases in the DNA damage response

Besides ubiquitylation, deubiquitylation has also been documented as an opposite way to regulate protein stability in response to genotoxic stress. Tandem affinity purification of the checkpoint protein 53BP1 complex identified the ubiquitin protease USP28 as a major regulator of DNA damage-induced apoptosis [52]. Using a cell line with an intact Chk2-p53-PUMA pathway, it was shown that USP28 stabilizes CHK2, 53BP1 and a number of other DNA damage responsive proteins upon irradiation. It could be that the prolonged half-life of these proteins might simply provide a fine-tuning mechanism for signaling transduction, but one could also envision the possibility that prolonged stability, and hence abundance, of various activated checkpoint proteins, in particular Chk2, might trigger apoptosis by virtue of constitutive activation and induction of proapoptotic genes.

The BRCC complex containing BRCA1, BRCA2 and Rad51 plays a role in DNA repair [53]. Like Rap80, BRCC36, a component of the complex, is required for the BRCA1 relocalization to DSBs [54]. Moreover, BRCC36-depleted cells exhibited dose-dependent IR sensitivity and did not properly arrest at the G2-M phase upon DNA damage. Interestingly, BBRC36, bearing sequence homology to the JAMM domain family of deubiquitylating enzyme, was recently shown to manifest deubiquitylase activity [49]. Exactly how the incorporation of a deubiquitylating enzyme into the BRCA1/BARD1 E3 ubiquitin ligase functions in the DNA damage response remains elusive, but the formation of a complex possessing opposing enzymatic activities highlights the importance of protein ubiquitylation and deubiquitylation as means to modulate cellular processes which are essential for cell survival.

Mono-ubiquitylation of the Fanconi anemia protein FANCD2 is critical for its retention at damage-induced foci. Moreover, mutations of many FA proteins compromise FANCD2 ubiquitylation, suggesting that this modification is critical for the FA complex in DNA repair functions. Because the mono-ubiquitylated FANCD2 is transient upon DNA damage but is not subjected to proteosomal degradation, it was proposed that FANCD2 is deubiquitylated when DNA lesions are repaired. Using a library of RNAi, USP1 was identified and implicated in the regulation of FANCD2 ubiquitylation [55]. USP1 associates with FANCD2 at the chromatin, and knockdown of USP1 results in the accumulation of mono-ubiquitylated FANCD2. These results suggest that USP1 might serve to switch off the FA pathway by recycling FANCD2 during S phase exit or after DNA insult. Interestingly, the ubiquitin protease USP1 was also attributed a role in preventing TLS by deubiquitylating the DNA replication processivity factor PCNA [56]. Only in response to ultraviolet irradiation, inactivation of USP1 via an autocleavage event results in the accumulation of mono-ubiquitylated PCNA, which in turn allows TLS. Although

how USP1 level might be monitored in response to DNA damage remains a subject of research, nevertheless, the existence of ubiquitin proteases that function in response to DNA damage uncovers a means for the delicate balance between ubiquitylation and deubiquitylation.

The DNA damage response: incorporation of protein phosphorylation and ubiquitylation

One of the initial signals upon DNA damage is the phosphorylation of the H2A variant H2AX (γ-H2AX) [57, 58]. Although γ-H2AX is not essential for the initial recruitment of various signaling mediators at the damaged chromatin [59], the ATM/ATR-dependent phosphorylation of H2AX is instrumental for the accumulation of MDC1, which is the master regulator for the microenvironment at the vicinity of the damaged chromatin [60-66]. Docking of MDC1 at the modified histone subsequently allows the retention of multiple checkpoint/adaptor proteins, including NBS1, 53BP1 and BRCA1, at sites of DNA breaks [67, 68]. The accumulation of these proteins at discrete foci upon irradiation (IRIF) provides a molecular platform for the efficient amplification of the DNA damage signal, which in turn ensures prompt activation of cell cycle checkpoints. Indeed, mice deficient in MDC1 are radiation sensitive, growth retarded and immune deficient [63]. These pleiotropic phenotypes are associated with the failure to retain NBS1, 53BP1 and BRCA1 at IRIF, repair defects and genomic instability.

Lines of evidence indicate that MDC1 engages the phosphorylated Ser-139 on H2AX via its tandem BRCT domains to facilitate the IRIF localization of the MRN complex, 53BP1 and BRCA1. Although MDC1 has been demonstrated to serve as an anchor for the MRN complex directly at sites of DNA breaks [68], mechanistically how MDC1 mediates 53BP1 and BRCA1 IRIF localization only becomes apparent with the recent reports of the E3 ubiquitin ligase RNF8 as one of the missing links between MDC1 and these checkpoint proteins [69-71]. Importantly, the identification of a E3 ubiquitin ligase in the DNA damage signaling pathway also shed light on an early observation in which IRIF localization of ubiquitin conjugates can be observed. In response to DNA damage, like other DNA damage responsive proteins, the E3 ubiquitin ligase RNF8 formed discernible foci, which colocalized with γ-H2AX and MDC1. This damage-induced relocalization required the RNF8 FHA domain, which binds to MDC1 in a phosphorylation-dependent manner. IRIFs of ubiquitin conjugates, 53BP1 and BRCA1, not only required the RNF8 FHA domain but are also dependent on its RING domain, suggesting that RNF8 propagates the damage signal via ubiquitylation of protein(s) at the chromatin (Figure 1). Consistent with this idea, IR-induced ubiquitylation of H2AX requires RNF8. Whether the IRIF of ubiquitin conjugates reflects solely the ubiquitylated species of these histone molecules remains to be determined. The fact that BRCA1 has also been reported to ubiquitylate proteins at the damaged chromatin probably suggests that additional ubiquitin-conjugated proteins reside at these cytological domains [72-74]. Nevertheless, RNF8 depletion consistently abolished these IR-induced ubiquitin conjugates, indicating that RNF8 function is an early, if not the primary, ubiquitylating activity at these DSBs. That IRIF localization of the UIM-containing protein Rap80 requires RNF8-dependent ubiquitylation is consistent with the role of ubiquitylation in the transduction of the signaling event, and suggests that Rap80 might be recruited via ubiquitylated histone molecules or other yet-to-be identified RNF8 substrates.

Like other RING-domain-containing E3 ubiquitin ligases, several lines of evidence also indicate that RNF8 acts in concert with the E2 ubiquitin conjugating enzyme UBC13 in signal transduction. First, RNF8 binds to UBC13 in a RING-dependent manner in a two-hybrid assay [72]. Second, RNF8 was shown to recruit UBC13 for lysine63 ubiquitylation [76]. Third, UBC13 deficiency recapitulated RNF8 depletion-associated deficits including abrogated ubiquitin conjugates observed at IRIF. Since UBC13 is well established as a E2 ubiquitin conjugating enzyme that mediates non-canonical lysine63-based poly-ubiquitylation chains [77-80], the notion that RNF8 restructures the local chromatin to allow concentration of repair proteins can be appreciated by its ability to attach multiple ubiquitin moieties to protein substrates that allows efficient amplification of the initial phosphorylation signal.

In addition to the E3 ubiquitin ligase RNF8 that incorporates protein phosphorylation and ubiquitylation in DNA damage-signaling cascade, such a role for BRCA1 is also becoming apparent with the recent identification of its substrates. Apart from its tandem BRCT phospho-binding motif, BRCA1 harbors an N-terminal RING domain, which is responsible for heterodimerization with its binding partner BARD1. With specificity for the E2 conjugating enzyme UbcH5c, these structurally related proteins form a complex and possess E3 ubiquitin ligase activity [81-83]. Clinical mutations affecting the RING domain indicated relevance of the ubiquitin ligase activity in the tumor suppressor function of BRCA1. One recent study identified CtIP as one of the bone fide substrates of BRCA1 important for cell cycle arrest in response to DNA damage [10, 73]. BRCA1 interacts with CtIP via its BRCT domain in a phosphorylation-dependent manner, which is required for CtIP ubiquitylation. Although the BRCA1-catalyzed CtI69-71P ubiquitylation correlated with the recruitment of CtIP to damage-induced foci, how the polyubiquitylation of CtIP

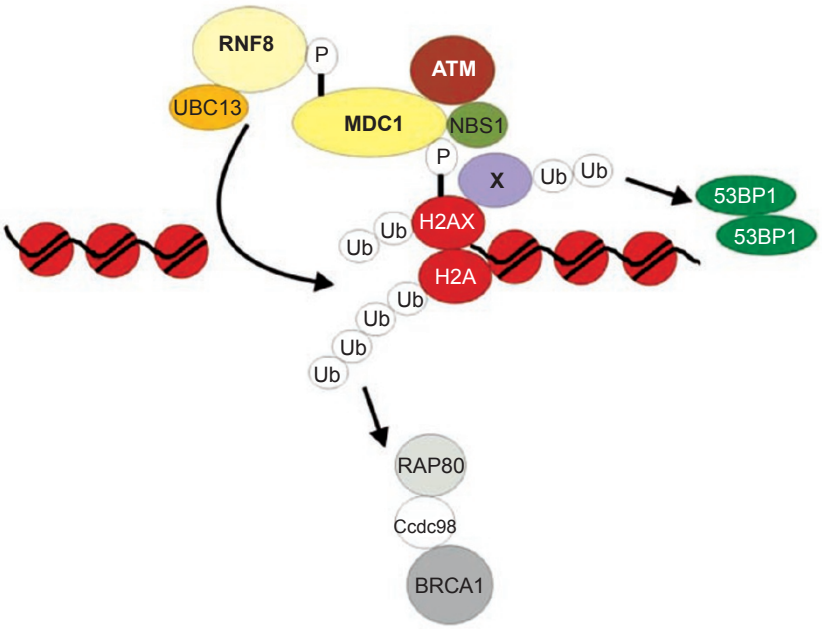

Figure 1 Schematic illustration of the DNA damage signaling pathway. A proposed model of the DNA damage signaling pathway. H2AX is phosphorylated in response to DNA damage. Binding of MDC1 serves as a molecular platform for the assembly of NBS1, ATM and RNF8 via phosphorylation-dependent interactions. RNF8 acts in concert with UBC13 to ubiquitylate H2AX and H2A, which in turn is required for the relocalization of the RAP80-Ccdc98-BRCA1 complex. 53BP1 accumulation at damage-induced foci might be stimulated by the exposure of histone 4 lysine 20 methylation subsequent to histone ubiquitylation.

ultimately regulates G2/M transition remains to be examined. Since CtIP ubiquitylation does not affect its turnover rate, these non-canonical ubiquitin chains formed on CtIP molecules, when recruited to the chromatin, might serve as a signal for Chk1 activation and cell cycle arrest.

As part of the cellular signaling for global DNA repair, BRCA1 was previously shown to directly ubiquitylate stalled RNAPII at DNA lesions [84, 85]. RNAPII is phosphorylated, ubiquitylated and degraded in response to DNA damage. Although these reports revealed another potentially important role of BRCA1 in the detection of DNA damage, further studies will be required to elucidate how the BRCA1-dependent ubiquitylation of RNAPII plays a role in genome surveillance.

Crosstalk between post-translational modifications

A growing list of proteins is being identified to be post-translationally modified by several different enzymes and play important roles in response to genotoxic stress. Among all, the tumor suppressor protein p53 has been shown to be phosphorylated, acetylated, sumolyated, methylated and ubiquitylated, with the ultimate outcome to stabilize and activate its role as a transcription factor for upregulating the expression of a series of proteins involved in cell cycle control, apoptosis and senescence [86, 87]. p53 is present at low levels in unperturbed cells. This is achieved by the MDM2 ubiquitin ligase that ubiquitylates and targets p53 for degradation [88, 89]. Upon stress stimulation, a series of post-translational modifications, some of which alters the interaction between MDM2 and p53, enable the accumulation of p53. Like many other DNA damage responsive proteins, p53 is phosphorylated at multiple sites by the ATM kinase upon irradiation [90-92]. These ATM-dependent phosphorylations have been suggested to stabilize p53, much like the proposed role of USP28, in response to genotoxic stress. The damage-induced phosphorylation of p53 also enhances its association with the CBP/p300 transcriptional coactivators, which results in increased p53 acetylation and further stability [93-95]. Recent evidence also revealed a role of the methyltransferase Set8/Pr-Set7 in the suppression of p53 function [96]. Set8 expression is downregulated upon DNA damage, and, in conjunction with other stress-induced modifications, allows p53-mediated transcription activation of proapoptotic genes and checkpoint activation. Interestingly, the Set7/9 methyltransferase also targets p53 and was shown to be a prerequisite for subsequent acetylation that stabilizes p53 [97]. Together with a host of other enzymes that modify p53 in response to a variety of stimuli, these dynamic post-translational modifications enable p53 functions to be regulated in a precise manner to exert its role in tumor suppression.

As mentioned earlier, the MRN complex is required for S phase checkpoint and serves as a sensor for the detection of DNA lesion [98, 99]. NBS1 constitutes the primary modulator for the function of the complex. The acetylation of NBS1 was recently reported to be regulated by protein deacetylase SIRT1 [100]. It was proposed that NBS1 is kept hypoacetylated, which in turn is required for the efficient IR-induced phosphorylation and cell survival.

Another example of trans-regulatory modifications in response to DNA damage came from recent evidence that

suggested acetylation of the histone variant H2AX. This damage-induced Tip60-mediated histone acetylation of H2AX is required for its subsequent ubiquitylation, which together promotes the release of the modified histone from the chromatin [101]. Although these modifications do not require prior phosphorylation on H2AX, the acetylation-dependent ubiquitylation of the histone molecule might represent a way to remodel the chromatin to facilitate DNA repair.

Post-translational modifications allow proteins to be regulated in a temporal and spatial manner, and recent studies have provided ample evidence for trans-regulatory mechanisms that further enable the fine-tuning and precise control over protein stability and activity. Although the physiological relevance of a number of post-translational modifications have been addressed, further studies will be required to unravel how the multitude of protein modifications combine to ensure efficient activation of cellular processes in response to genotoxic stress.

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