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SURVEY AND SUMMARY

The role of poly ADP-ribosylation in the first wave of DNA damage response

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ABSTRACT

Poly ADP-ribose polymerases (PARPs) catalyze massive protein poly ADP-ribosylation (PARylation) within seconds after the induction of DNA single- or double-strand breaks. PARylation occurs at or near the sites of DNA damage and promotes the recruitment of DNA repair factors via their poly ADP-ribose (PAR) binding domains. Several novel PAR-binding domains have been recently identified. Here, we summarize these and other recent findings suggesting that PARylation may be the critical event that mediates the first wave of the DNA damage response. We also discuss the potential for functional crosstalk with other DNA damage-induced post-translational modifications.

INTRODUCTION

The genetic information stored in the DNA is prone to damage by environmental and internal hazards such as ultraviolet light, mutagenic chemicals, ionizing radiation and reactive oxygen species (1–3). Exposure to these genotoxic stresses induces various types of DNA lesions, including DNA single-strand breaks (SSBs) and double-strand breaks (DSBs). Without repair, accumulated lesions can drastically alter the genome. Fortunately, cells have evolved a sophisticated DNA damage response system to repair these DNA lesions and maintain genomic stability.

After induction of DNA damage, the immediate reaction of a cell is to detect various DNA lesions using DNA damage sensors. These sensors are abundant in the nucleus for damage surveillance and consequent activation of the repair process. In the presence of DNA damage, these sensors initiate signals to recruit DNA damage repair (DDR) factors and activate other relevant biological processes, such as cell cycle arrest, to facilitate the repair process (4). Accumulated evidence indicates that PARP1, the founding member of PARP family, recognizes both SSBs and DSBs. PARP1 transfers the ADP-ribose moiety of NAD^+ to the side chains of asparagine, aspartic acid, glutamic acid, arginine, lysine, serine and cysteine residues on its substrates (5–16). The ribose sugar adjacent to the adenine side is linked to the next ADP-ribose residue through glycosidic bonds to form a linear PAR chain containing up to 200 ADP-ribose residues (17) (Figure 1). Branched ADP-ribose chains are also generated by α(1‴-2″)-ADP-ribose linkage (18) (Figure 1). These PAR chains form a platform to recruit DNA repair proteins via their PAR-binding domains (Figure 1). Together, these properties allow PARP1 to function as an important DNA damage sensor for both SSBs and DSBs (20).

With the identification of new proteins as 'readers' of protein PARylation, the PARylation-dependent early DNA damage response has emerged as an important aspect of the complex repair process in response to new DNA lesions. This review highlights the biological function of PARylation in response to DNA damage, focusing on the idea that PARylation may serve as an early signal to initiate the DNA damage response. Interestingly, PARylation is a transient response to DNA damage that is also controlled by dePARylation. Thus, the role of protein dePARylation, an equally important process for DDR, will also be discussed. Moreover, this review will explore the functional interactions between PARylation and other signals generated after DNA damage, including phosphorylation and ubiquitination.

PARP1 ACTS AS A SENSOR FOR BOTH SSBS AND DSBS

PARylation is a unique post-translational modification synthesized in response to DNA damage, which acts as a crucial signal after SSB or DSB induction. The basal level of PARylation is very low in cells under normal conditions (21), indicating that most PARPs are in the inactive state. However, PARP1, which catalyzes about 90% of DNA damage-induced PARylation (22), is strongly activated after binding to SSBs and DSBs. PARP1 is one of the most

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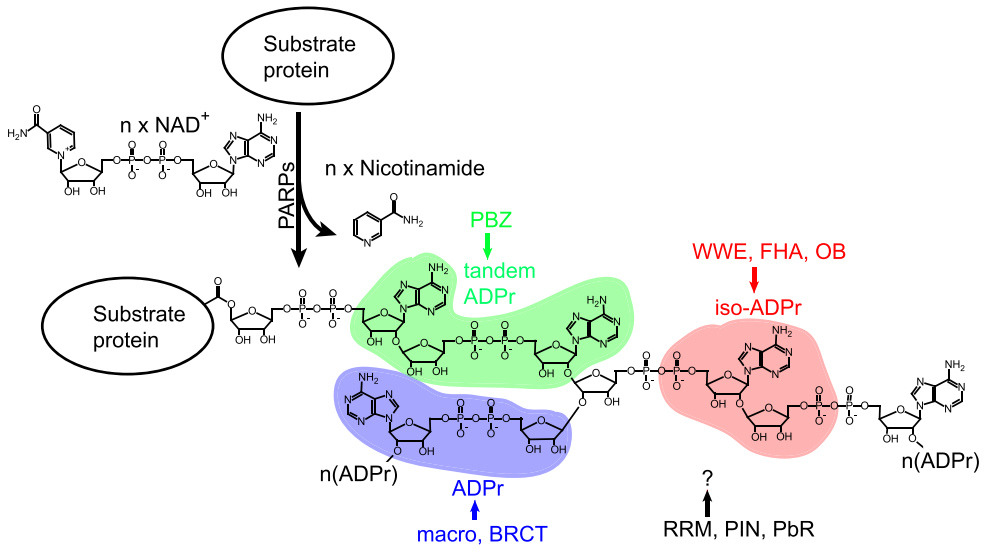

Figure 1. Schematic representation of PAR synthesis. PARPs hydrolyze nicotinamide from NAD^+ and covalently link the remaining ADP-ribose moieties to their substrates, forming linear or branched PAR chains. Different PAR readers recognize distinct units of the PAR chain. The PBZ motif recognizes tandem ADP-ribose. The WWE, FHA and OB domains recognize iso-ADP-ribose. The macro and BRCT domains recognize ADP-ribose. The recognition units of the RRM, PIN and PbR domains need to be identified. ADPr: ADP-ribose; iso-ADPr: iso-ADP-ribose.

abundant nuclear polypeptides, with an estimated 1–2 million molecules per nucleus (23,24). On average, one PARP1 molecule scans approximately 10 nucleosomes of chromatin (25). This scanning function enables PARP1 to quickly detect DNA damage. In laser micro-irradiation experiments, PARP1 is recruited to DNA damage sites with a $t_{1/2}$ of only ~1.6 s (Figure 2) (26).

PARP1 contains multiple domains, including three N-terminal zinc finger motifs (ZF1-ZF3), a BRCT domain, a WGR domain and a C-terminal catalytic domain. As shown in Figure 3A, once an SSB is generated, ZF2 stacks onto the 3′ terminal base pair via its hydrophobic platform. The ZF1 motif from a second PARP1 molecule dimerizes with the ZF2, and leaves an open channel to accommodate the undamaged nucleotide. This dimerization of ZF1 and ZF2 may then lead to an additional intramolecular conformational change and activation of the catalytic domain of PARP1 (27).

In contrast to SSBs, PARP1 recognizes DSBs by a slightly different mechanism. For each end generated from a DSB, the ZF1 domain recognizes the terminal base pair, whereas the ZF3 and the WGR domains contact each side of the backbone of the DNA helix and bind the minor and major grooves to stabilize the interaction (28). The WGR domain stacks to the 5′-terminus of the DNA end but the ZF2 domain is dispensable for DSB recognition (Figure 3B). This interaction with one DSB end triggers intramolecular conformational changes within the catalytic domain and activates PARylation (28). Together, these structural studies suggest that PARP1 can sense both SSBs and DSBs.

In addition to PARylation, accumulation of a phosphorylated form of histone H2AX, known as γH2AX, is one of the earliest events that occur at DSBs. H2AX differs from canonical H2A in the C-terminal tail, which contains an evolutionarily conserved serine motif in the H2AX variant. Phosphorylation at this serine residue occurs within one minute of IR treatment (Figure 2) (29,30). The accumulation of γH2AX is important for retaining numerous DDR factors at DSBs (31,32). However, in H2AX-deficient cells, most DDR factors can still transiently relocate to the sites of DSBs (31), indicating that γH2AX is not essential for their early recruitment. Moreover, the genetic deficiency in H2AX has modest effects on DSB repair *in vivo* (31–33). These results suggest that although H2AX phosphorylation is important, the precise role of γH2AX in DSB repair remains elusive.

Ataxia-telangiectasia mutated (ATM) is the major kinase responsible for H2AX phosphorylation in response to DSB induction (34–37). Accumulated evidence suggests that the MRN complex, which consists of MRE11, RAD50 and NBS1, is essential for ATM activation (38). Among these three subunits, RAD50 recognizes naked DNA ends and holds them in close proximity to each other (39,40). MRE11 is an endo- and exo-nuclease that processes DNA ends prior to religation for resection-dependent nonhomologous end joining (NHEJ) or prior to more extensive resection by other nucleases for homologous recombination (HR) (41). NBS1 mediates protein-protein interactions within the complex (42–44). The ability of the MRN complex to bind directly to the DNA ends suggests that it could be a DSB sensor (45–47). However, in laser micro-irradiation experiments, the $t_{1/2}$ for MRN recruitment is ~13 s (Figure 2) (26), which is almost 10 times slower than the $t_{1/2}$ for PARP1 recruitment. Of note, laser micro-irradiation generates mixtures of different DNA lesions, including both SSBs and DSBs. Thus, DNA damage sensors on both SSBs and DSBs will be recruited to lesions generated by laser micro-irradiation. Nevertheless, in spite of certain limitations like the presence of heterogeneous mixture of various types of DNA lesions, micro-irradiation in the UV range is the most efficient method for experimental induction of DNA damage that allows measurement in the narrow

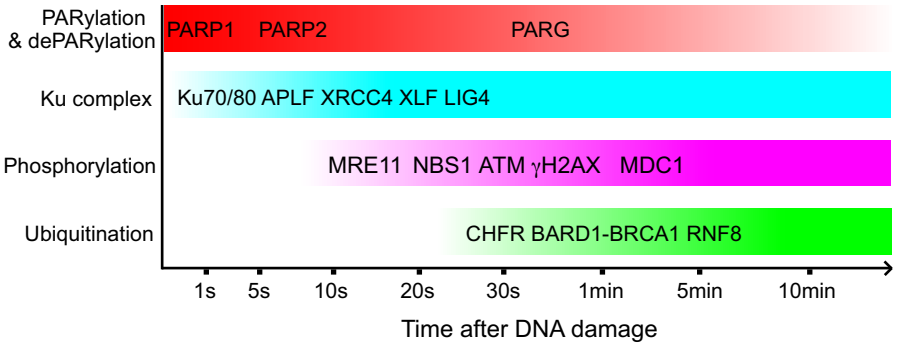

Figure 2. Schematic representation of the temporal dynamics of DDR proteins recruited to the DNA damage sites in response to laser micro-irradiation.

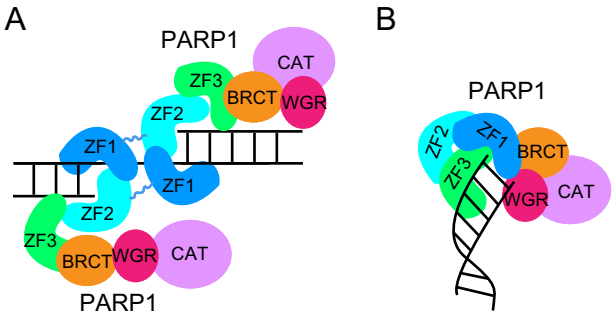

Figure 3. PARP1 is a sensor for both SSBs (**A**) and DSBs (**B**). The domains within one PARP1 molecule are colored differently. ZF1-3, N-terminal zinc finger motif 1-3; BRCT, BRCT domain; WGR, WGR domain; CAT, C-terminal catalytic domain.

time scale of few seconds (48). The relatively slow recruitment of the MRN complex suggests that this complex may serve as a secondary sensor for DSB repair. Consistently, the early recruitment of MER11 and NBS1 is mediated by PARP1 (26,49). Moreover, our recent findings indicate that NBS1 recognizes DNA damage-induced PARylation and mediates the early recruitment of the MRN complex (50). Because DNA damage-induced PARylation is mainly catalyzed by PARP1, one hypothesis is that PARP1 acts upstream of the MRN complex and γH2AX in response to DSBs. Alternatively, structural analysis indicates that the N-terminus of PARP1 favors binding to the blunt ends of DSBs (28), whereas MRE11, the catalytic subunit of the MRN complex, recognizes 5′-overhang DNA (51). Thus, it is also possible that PARP1 and the MRN complex preferentially sense different types of DSBs.

Similar to PARP1, the Ku complex, consisting of Ku70 and Ku80 subunits, also relocates to DSBs within few seconds after DNA damage and binds directly to DSBs with high affinity (Figure 2) (52–54). The Ku complex is also highly abundant, with ∼500 000 molecules per cell (55). Thus, in addition to PARP1, the Ku complex is another important DSB sensor. However, the mechanism of crosstalk between the Ku complex and PARP1 is still unclear. As DSBs can be repaired through HR, alternative NHEJ (a-NHEJ) or canonical NHEJ (c-NHEJ) pathways (56–58), it is possible that PARP1 and the Ku complex mediate DSB repair pathway choice separately through distinct mechanisms. Binding of the Ku complex to DSBs recruits and activates the DNA-PK catalytic subunit, which facilitates c-NHEJ (59). The Ku complex also interacts with numerous other c-NHEJ factors, including XRCC4 (53,60,61), APLF (54), XLF (62) and LIG4 (60). In contrast, the binding of PARP1 promotes a-NHEJ and HR repair (63–68). Thus, the competition between PARP1 and the Ku complex at DSBs may play an important role in determining the repair pathway. Moreover, these two DNA damage sensors may have complementary roles in activation of the DNA damage response. Loss of either sensor in mammals only generates minor DSB repair defects (69–73). Interestingly, double knockout of the mouse *Parp1* and *Xrcc5* (*Ku80*) genes causes early embryonic lethality (74,75), consistent with the idea that PARP1 and Ku have complementary functions. In addition, it is also possible that PARP1 and the Ku complex function together at certain steps of DSB repair. It is interesting to note that Ku70 in *Dictyostelium discoideum* even has a PAR-binding motif (76). Also, PARylation retains the Ku complex at DSBs for efficient NHEJ in *Dictyostelium discoideum* (77). Moreover, in mammals, the Ku complex is ADP-ribosylated after DNA damage induction, although the function of this ADP-ribosylation is unclear (9,16,78). Thus, future studies on the functional interaction between PARP1 and the Ku complex may reveal novel molecular mechanisms of NHEJ.

CONTRIBUTION OF PARP2 AND PARP3 TO DNA DAMAGE SIGNALING

In addition to PARP1, PARP2 also participates in the DNA damage response (79–81). Unlike PARP1, PARP2 does not contain N-terminal zinc finger motifs. However, the N-terminal region and the adjacent WGR domain of PARP2 act together to bind the ends of nucleic acid strands; this binding activates the C-terminal catalytic domain for the synthesis of PAR (82). Thus, the activation mode of PARP2 is similar to that of PARP1. Recent genetic evidence shows that *Parp1*- or *Parp2*-knockout mice has very mild DDR defects, whereas the double knockout of *Parp1* and *Parp2* arrests embryonic development at gastrulation (83), suggesting that PARP1 and PARP2 have overlapping functions. Although PARP2 is recruited to the sites of laser-induced DNA damage slower than PARP1, it persists comparatively longer (Figure 2) (84). Thus, it is likely that PARP1 and

PARP2 play slightly different roles in the DNA damage response.

Recent studies show that PARP3 also plays an important role in DDR (85–87). PARP3 has an N-terminal WGR domain and a C-terminal catalytic domain, but lacks other domains implicated in DNA binding. However, PARP3 can be activated in response to DSBs, especially by DNA breaks with 5′-phosphoryl ends (82,85,88). It is possible that the conserved WGR domain facilitates DSB recognition (89). The enzymatic activity of PARP3 is still controversial. Rulten *et al.* reported that PARP3 could generate PAR, although it was very short compared to that generated by PARP1 (85). However, Loseva *et al.* and Vyas *et al.* found that PARP3 catalyzed only mono ADP-ribosylation (MARylation) *in vitro* (88,90). PARP3 interacts with the Ku complex, DNA-PKcs, LIG4 and APLF (85,87,91), which are key factors in the c-NHEJ pathway. Also, Ku complex can be ADP-ribosylated by PARP3 (78), which is then recognized by the PAR-binding zinc finger (PBZ) motif of APLF, and thus promotes the recruitment of XRCC4 and LIG4 to enable c-NHEJ (85,87).

Specific substrates of PARP1, PARP2 and PARP3 were identified using engineered PARP mutants that only use NAD^+ analogs for PARylation (78,92,93). Although most of the substrates modified by each PARP are distinct, they can be categorized into multiple common ontological groups, such as transcription, RNA processing, chromatin organization and DNA damage repair (78). Moreover, the preferred ADP-ribosylation motifs around the ADP-ribosylation site (here indicated as E*) were also identified for each PARP. For instance, PARP1 prefers E*P, E*XP and E*E sites; PARP2 prefers EE* and GXXXXXE* sites, and PARP3 prefers K or R residues within ±8 residues of ADP-ribosylation sites (78).

Because each ADP-ribose residue contains two negatively charged phosphate groups, PARylation imparts a substantial amount of negative charge to the sites of DNA damage (19). As DNA itself is also negatively charged, PARylation may loosen the higher-order structure of chromatin due to charge-charge repulsion, which would facilitate access to the DNA repair machinery. Additionally, as PARP1 is a major substrate for PARylation (94), automodified PARP1 could disassociate from the damaged sites because of its negative charge (95,96). Histones, such as H1, H2A and H2B, are also important substrates of PARP1 (16,97,98). PARylation at the sites of DNA damage would also enhance the accessibility of large protein complexes assembled during the DDR process. Recent evidence also suggests that, in addition to chromatin remodeling, PARylation functions as a signal for recruitment of numerous DNA damage response factors to the sites of DNA damage (50,66,76,99–105). Over the past few years, several types of PAR-binding modules have been identified, including the PBZ motif; the macro, WWE, BRCT, FHA, OB-fold, RRM, and PIN domains; and PAR-binding regulatory (PbR) motif (Table 1 and Figure 1). With live cell imaging, we and other researchers have shown that the interactions between these modules and PAR mediate their recruitment to the DNA damage sites within 20–30 seconds after DNA damage (50,66,105,106). These DNA damage response factors play key roles in multiple repair mechanisms, including base excision repair (BER), SSB repair, and DSB repair. Consistently, previous analyses showed a similar role for PARPs in these repair pathways (106–108). Apart from their functions in DNA damage response, the ADP-ribosylation readers play important roles in other biological processes, such as DNA replication, cell cycle regulation, chromatin remodeling, RNA metabolism and protein turnover (109).

Although PARPs catalyze massive PARylation at the sites of DNA damage, the half-life of PAR is only a few minutes (110,111). Thus, PARylation may act as the first wave of signaling, to transiently activate DDR. Because the process of DDR continues until the lesions are fixed, especially for DSB repair, it is possible that PARylation mediates the priming of DDR by recruiting the DNA repair machinery to the region near DNA lesions through their PAR-binding modules. For example, the BRCT domain of NBS1 recognizes DNA damage-induced PAR and mediates the early recruitment of the MRN complex to DSBs within 20 seconds (50). The early recruitment of the MRN complex may prime early ATM activation and ATM-dependent cell cycle arrest. Collectively, the first wave of PAR signaling after DNA damage recruits PAR readers to the sites of DNA damage and induces DNA damage response by activating cell cycle checkpoints and DDR.

Although the cellular concentration of NAD^+ is roughly 0.3–1 mM (112–114), PARPs are able to use abundant NAD^+ to synthesize massive amounts of PAR in a very short period of time in response to DNA damage. It has been reported that when wild-type fibroblasts are treated with 0.5 mM Methylnitronitrosoguanidine (MNNG), the NAD^+ level drops 80% within 15 minutes and is no longer detectable by 30 minutes (115). Similar results were also reported when cells were treated with lower concentrations of MNNG or other DNA damage-inducing agents (116–120). Thus, DNA damage-induced PARylation may have much more far-reaching effects on cellular physiology than other post-translational modifications in cells.

These massive amounts of PAR likely provide abundant docking sites for DNA damage response factors with distinct PAR-binding modules. However, how DNA damage response factors are loaded to DNA lesions remains elusive. One possibility is that these DNA damage response factors are loaded sequentially via specific PARylation motifs. Alternatively, PARylation facilitates the recruitment of these DNA damage response factors to the proximity of DNA lesions. With dePARylation and other post-translational modifications, specific DDR machineries are retained at different types of lesions for DNA repair.

DEPARYLATION IS ALSO REQUIRED FOR DDR

Although extensive and rapid PARylation recruits DNA damage response factors to the vicinity of DNA lesions, PARylation is removed quickly. This rapid dePARylation can perhaps prevent the trapping of other factors involved in the first wave of the DNA damage response. Otherwise, trapping these DNA damage repair factor may block access of other downstream repair factors. Therefore, the timely and orderly degradation of PAR by dePARylation enzymes is the next necessary step towards DDR.

Table 1. Summary of PAR-binding modules

Module name	Recognition unit	Protein(s)	Reference(s)
PAR-binding zinc finger (PBZ)	Tandem ADP-ribose	APLF, CHFR	(76,161,172)
Macro domain	ADP-ribose	macroH2A1.1, ARTD7, ARTD8, ARTD9, macroD1, macroD2, macroD3, ALC1, TARG1, PARG	(108,173)
WWE domain	iso-ADP-ribose	RNF146, HUWE1, ULF, Deltex1, Deltex2, Deltex4, ARTD11	(174,175)
BRCT domain	ADP-ribose	BARD1, LIG4, NBS1, XRCC1, ECT2	(50,66,176)
FHA domain	iso-ADP-ribose	PNKP, APTX	(50)
OB-fold domain	iso-ADP-ribose	SSB1, CTC1, MEIOB, SSB2	(105)
RRM domain		NONO	(177)
PIN domain		EXO1, GEN1, SMG5	(106)
PAR-binding regulatory (PbR) motif		Chk1	(178)

To date, six human dePARylation enzymes have been identified, including poly ADP-ribose glycohydrolase (PARG), TARG1, ARH3, NUDT9 and NUDT16 and ENPP1 (Table 2, Figure 4). PARG contains a macro domain and it has exo- and endo-glycohydrolysis activity to hydrolyze the glycosidic bond between ADP-ribose units in PAR chain and release free ADP-ribose residues (121–124). TARG1 is also a macro domain-containing protein. However, TARG1 cannot hydrolyze PAR to ADP-ribose; rather, it removes the whole PAR chain from the glutamate residue on the PARylated proteins (125). TARG1 can also hydrolyze the glutamate-ADP-ribose bond and release the terminal ADP-ribose unit from MARylated proteins (125). Similar to PARG, ARH3 has exo-glycohydrolysis activity to digest PAR chains and release free ADP-ribose, although the ARH3 activity is much lower than PARG activity (126). NUDT9 and NUDT16 have nucleoside diphosphate-linked moiety X (Nudix) domains, which cleave pyrophosphate bonds, and release iso-ADP-ribose and AMP from PAR chains or AMP from MARylated proteins (127,128). It is possible that Nudix pyrophosphatases collaborate with other dePARylation enzymes to further digest ADP-ribose. ENPP1 is a newly discovered pyrophosphatase lacking a Nudix domain; it can digest PAR chains and release iso-ADP-ribose from PAR chains or AMP from MARylated proteins (129).

Recent studies have also found enzymes that remove MAR groups from proteins. These enzymes include macro D1, macro D2, ARH1 and the aforementioned TARG1, NUDT9, NUDT16 and ENPP1 (Table 2, Figure 4). Macro D1 and macro D2 are two macro domain-containing enzymes that can release ADP-ribose from ADP-ribosylated acidic residues (130). ARH1 is the only hydrolase that specifically removes MAR from arginine residues (131).

Among these deADP-ribosylation enzymes, the most potent in the context of DDR is PARG (121–124). The early recruitment of PARG to the sites of DNA damage is mediated by the interaction of its macro domain with the PAR signal (132), but PARG recruitment occurs more slowly than recruitment of PARP1, with a $t_{1/2}$ of about 50 seconds (Figure 2) (133). PARG is stably retained by proliferating cell nuclear antigen (PCNA) (133). As expected, reduction or loss of PARG expression causes a significant delay in PAR degradation and extends the half-life of PAR at DNA lesions (16,134,135). Instead of facilitating DDR, prolongation of PARylation impairs both SSB and DSB repair, and thereby inducing apoptosis (136–138). Moreover, loss of the *Parg* gene induces early embryo lethality at the gastrulation stage (136), which phenocopies the *Parp1* and *Parp2* double-knockout mouse (83). The genetic similarity further indicates that dePARylation is essential for DDR.

Besides PARG, other dePARylation enzymes are also recruited to the sites of DNA damage, suggesting that these enzymes are involved in the DNA damage response and may act sequentially following ADP-ribosylation (125,133,139). In particular, TARG1 is not only recruited to DNA lesions, but germline mutation of *TARG1* induces progressive neurodegenerative disorders (125), a phenomenon often observed when BER is impaired. Thus, these findings indicate that TARG1 participates in BER. However, the specificity of these dePARylation enzymes towards the different substrates remains elusive.

The ADP-ribose released by PAR glycohydrolases may not be merely a byproduct of the reaction, but also an important second messenger accounting for Ca^{2+} influx and caspase activation (140). Moreover, a recent study shows that ADP-ribose released from dePARylation enzymes can be digested further by NUDT5, another Nudix domain family member, to form AMP and ribose phosphate. The released AMP moiety can be used to synthesize ATP for chromatin remodeling during DDR (141). Finally, ADP-ribose can also be recycled to form NAD^+ to maintain the cellular level of NAD^+, which is a co-enzyme required for numerous biochemical reactions (142). Collectively, the digested ADP-ribose monomer may be involved in many physiological relevant processes in response to DNA damage.

FUNCTIONAL INTERACTION BETWEEN DNA DAMAGE-INDUCED PARYLATION AND PHOSPHORYLATION

Besides PARylation, other post-translational modifications, like DSB-induced phosphorylation and ubiquitination, are also induced by DNA damage and function as signals to mediate the recruitment of DNA damage machinery (143,144). The phosphorylation cascade in response to DNA damage is initiated by a group of well-documented PI3-like kinases, including ATM, ATM- and Rad3-related (ATR), and/or DNA-dependent protein kinase (DNA-PKcs) (145–147). These kinases are mainly activated in response to DSBs, and some redundancy of these kinases in DSB-induced phosphorylation events has been shown. Among these kinases, ATM is considered the primary inducer of the phosphorylation cascade in response to DSBs

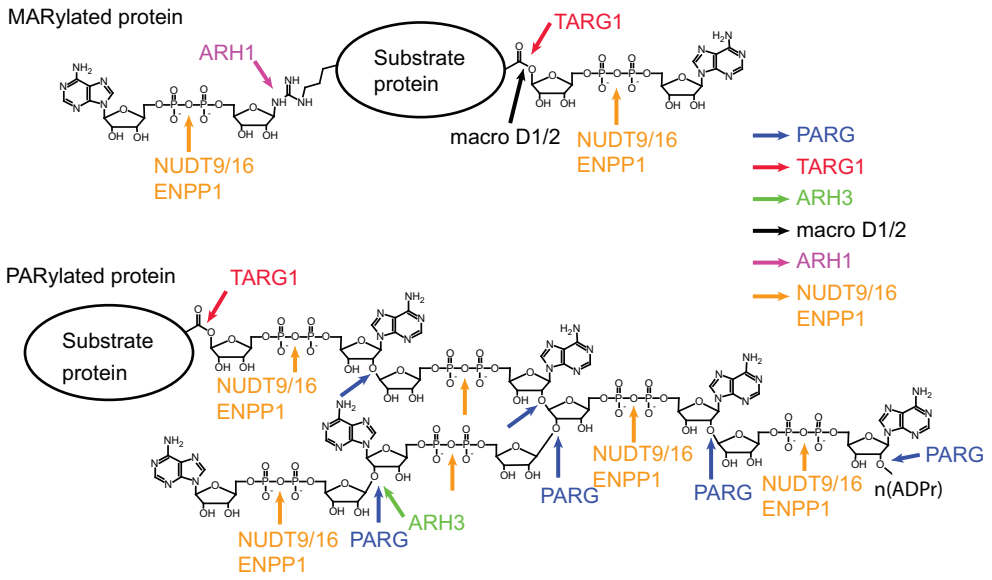

Figure 4. Schematic representation of the deADP-ribosylation process. The cutting sites of each enzyme are shown by the arrows with indicated colors. ADPr: ADP-ribose.

Table 2. Summary of deADP-ribosylation enzymes

Name	Synonyms	Subcellular location	Key domain	Substrates and activity	Reference(s)
PARG		Nucleus, cytoplasm, mitochondria	Macro domain	PAR chain, exo- and endo-glycohydrolysis to produce ADP-ribose and short PAR chain.	(179,180)
TARG1	C6orf130 OARD1	Nucleus, cytoplasm	Macro domain	Mono ADP-ribosylated protein, cleaving the bond between acidic residues and ADP-ribose; poly ADP-ribosylated protein, releasing the whole PAR chain from protein; deacylation of O-acetyl-ADP-ribose, O-propionyl-ADP-ribose, and O-butyryl-ADP-ribose to produce ADP-ribose and acetate, propionate, and butyrate, respectively.	(125,130,181)
MacroD1	LRP16	Nucleus	Macro domain	Mono ADP-ribosylated protein, cleaving the bond between acidic residues and ADP-ribose; deacetylation of O-acetyl-ADP-ribose.	(130)
MacroD2	C20orf133	Nucleus, cytoplasm	Macro domain	Mono ADP-ribosylated protein, cleaving the bond between acidic residues and ADP-ribose; deacetylation of O-acetyl-ADP-ribose.	(130)
ADPRH	ARH1	Nucleus, cytoplasm		Mono ADP-ribose-arginine protein, cleaving the N-glycosidic bond of ADP-ribose attached to an Arg residue of a protein to produce free ADP-ribose and unmodified protein.	(131)
ADPRHL2	ARH3	Nucleus, cytoplasm, mitochondria		Deacetylation of O-acetyl-ADP-ribose; PAR chain, exo-glycohydrolysis to produce ADP-ribose.	(126,182,183)
NUDT9		Cytoplasm	Nudix hydrolase	Cleaving ADP-ribose and IDP-ribose to form the corresponding nucleoside 5′-monophosphates and ribose 5-phosphate; cleaving O-acetyl-ADP-ribose to form AMP and acetylated ribose 5′-phosphate; low activity to digest PAR by cleaving the pyrophosphate bonds.	(127,128,184)
NUDT16		Nucleus, cytoplasm	Nudix hydrolase	Cleaving m7G or m227G caps from U8 snoRNA or mRNA and leaving a 5′-monophosphate-RNA; Poly/mono ADP-ribosylated protein, cleaving the pyrophosphate bonds of ADP-ribose.	(128,185)
ENPP1		Extracellular, lysosome, plasma membrane		Cleaving the phosphodiester bonds in (d)NTP, (d)NDP, NAD, ADP-ribose, FAD, diadenosine polyphosphates, UDP sugars, PAR chains and mono ADP-ribosylated proteins.	(129)

(148). The rapid accumulation and activation of the ATM kinase cascade results in Ser or Thr phosphorylation of several hundreds of proteins, including effectors of the DNA damage response such as BRCA1, CHK2 and p53 (146), which further activate cell cycle checkpoints and DDR.

Phosphorylation of H2AX is observed about 1 minute after DNA damage (29,30), a time scale that is considerably slower than the PARP1 recruitment (Figure 2). It is possible that the PI3-like kinase-induced phosphorylation cascade is the second wave of signaling during the DNA damage response. As mentioned earlier, PARylation can prime the activation of the ATM-dependent phosphorylation cascade via recruitment of the MRN complex (50). In our previous study, we showed that the BRCT domain of NBS1, one of the components of the MRN complex, recognizes PARP1-dependent PARylation at DNA lesions, which recruits and activates ATM (50). PARylation may thus serve as the first wave of signaling at DNA lesions, thereby facilitating ATM-dependent phosphorylation as a second wave of signaling.

One of the prominent phosphorylation targets of ATM is H2AX. Following DNA damage, ATM-induced γH2AX can be observed within one minute in the range of ∼1 kb of the chromatin flanking the DNA lesion. γH2AX can spread up to ∼500 kb of the flanking chromatin regions in a few hours, and is important for anchoring numerous DDR factors surrounding DNA lesions (31,32). Interestingly, PARylation, the first wave of signals, may negatively regulate this H2AX phosphorylation event. Mass spectrometry analysis indicates that H2AX is quickly PARylated at E141 after DNA damage (16). Because PARylation at E141 brings a large amount of negative charge close to Ser139, it is possible that PARylation of E141 suppresses the phosphorylation of Ser139, which may subsequently delay the recruitment of DDR factors or destabilize some DDR factors at DNA lesions. However, because the PARylation on H2AX is also quickly removed by dePARylation enzymes, the Ser139 motif can be re-exposed to PI3-like kinases. Such transient delay of H2AX phosphorylation adds another layer of regulation for the recruitment of DDR factors. The wave of PARylation mediates the recruitment of numerous DDR complexes, whereas dePARylation and subsequent phosphorylation of H2AX may selectively stabilize certain DDR factors at DNA lesions to fulfill the repair function.

One typical example of two-stage recruitment is the recruitment of the BRCA1/BARD1 complex, a key complex for DSB repair. The recruitment of this complex involves both PARylation and the γH2AX-dependent pathway. Both BRCA1 and BARD1 have N-terminal Ring domains, through which they bind to each another (149). They also have C-terminal BRCT domains. The BRCT domain of BRCA1 is a phospho-Ser binding domain (150,151), whereas the BRCT domain of BARD1 is a PAR-binding domain (66). After the induction of DSBs, BARD1 recognizes the PAR signal at the sites of DNA damage through its BRCT domain, which mediates the quick mobilization of BRCA1 to DNA lesions, thereby recruiting a functional BRCA1/BARD1 complex at the sites of DNA damage (Figure 5A). After the dePARylation, retention of the BRCA1/BARD1 complex is mediated through a γH2AX-dependent pathway (31,66) (Figure 5B). The BRCT domain of BRCA1 has a phospho-serine binding domain

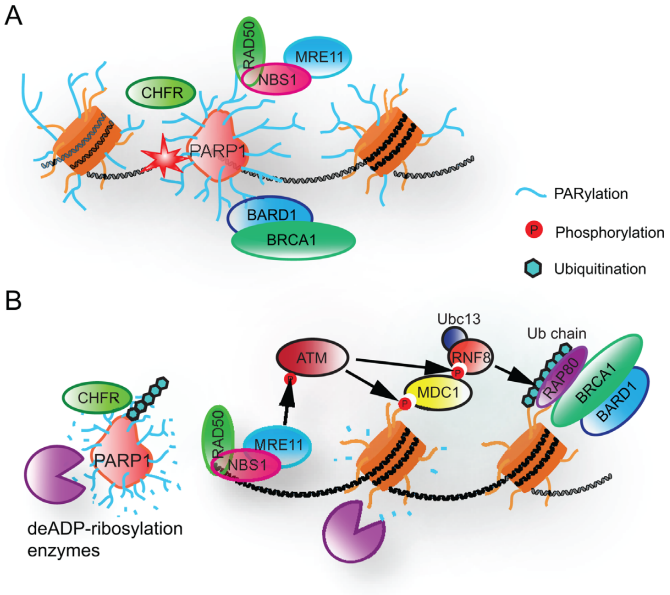

Figure 5. Functional interactions between DNA damage-induced PARylation and other post-translational modifications. (**A**) PARP1-mediated PARylation facilitates the early recruitment of DNA damage factors (e.g. NBS1, BARD1 and CHFR). (**B**) In response to PARylation, other post-translational modifications (e.g. phosphorylation and ubiquitination) stabilize the DDR machinery.

through which it recognizes a phospho-Ser motif in the RAP80 complex, which is anchored at the DNA lesions via a γH2AX-dependent pathway (152–155). Thus, both PARylation and phosphorylation play important roles in recruiting and retaining the BRCA1 complex at DSBs for lesion repair. The synergistic effects of PARylation and phosphorylation on DSB repair are further supported by mouse genetic studies. *In vivo* studies show that mice lacking the *Parp1*, *H2ax,* or *Atm* genes have minor DNA repair defects (33,69,70,72,156), whereas the loss of both *Parp1* and *H2ax* (or *Atm*) leads to early embryonic lethality at the gastrulation stage (157,158). Again, this embryonic lethal phenotype of the *Parp1* and *H2ax* (or *Atm*) double-mutants is similar to that of HR-deficient mice, including the *Brca1* knockout mouse, which is consistent with the notion that both PAR and γH2AX are required for the stable recruitment of BRCA1 (159).

However, PARylation may not always function together with phosphorylation. The Ser139 motif in H2AX is a typical phosphorylation motif for the PI3-like kinases, as the consensus phosphorylation motif is S/T-Q-D/E (146). Interestingly, PARylation is usually observed at aspartic acid and/or glutamic acid residues on the substrates (9,16). Because the consensus motif of the PI3-like kinases contains aspartic acid and/or glutamic acid at the +2 position, these motifs can also be potentially PARylated. Additionally, competition between the phosphorylated and PARylated states can regulate the subsequent choice of repair pathways. Thus, PARylation can also act as a competitor for DNA damage-induced phosphorylation at certain loci.

FUNCTIONAL INTERACTION BETWEEN DNA DAMAGE-INDUCED PARYLATION AND UBIQUITINATION

Ubiquitination is another significant post-translational modification linked with DDR. Similar to phosphorylated proteins, ubiquitinated proteins begin to accumulate at the sites of DNA damage several minutes after DSB damage (160,161), indicating that ubiquitination can also serve as a secondary wave of signaling in response to DNA damage. Both H2A and H2AX have been identified to be ubiquitinated primarily at the K13/15 and K119/120 residues on their tails (162–164), and ubiquitination at K13/15 is induced by DNA damage. Interestingly, recent evidence shows that lysine residues can also be PARylated by PARP1 (5,12). Thus, PARylation may also compete with ubiquitination to modify histones at DNA lesions. Although it is unclear if H2A and H2AX are PARylated at K13/15 in response to DNA damage, PARylation on any adjacent residue could be sufficient to suppress DNA damage-induced ubiquitination. However, further studies are needed to elucidate if PARylation and ubiquitination act in concert during sequential steps of DDR.

In addition, PARylation can prime the ubiquitination at DNA lesions. Two ubiquitin E3 ligases, CHFR and RNF146, are recruited to DNA lesions by PARylation (76,161,165). The C-terminus of CHFR harbors a PBZ motif that recognizes PAR chains (76,161). Once DNA damage occurs, CHFR is quickly recruited to the sites of DNA damage via the PBZ motif and initiates ubiquitination (Figure 5A). However, dePARylation mediates quick release of CHFR from DNA lesions. Thus, the role of CHFR-dependent ubiquitination may be regulated during DDR. Moreover, our studies have shown that CHFR-dependent ubiquitination may have overlapping function with RNF8-dependent ubiquitination during DDR (166). In addition to CHFR, RNF146 contains a WWE domain that also interacts with PAR (165). Although RNF146 is a cytoplasmic protein, it relocates to the nucleus and is enriched at DNA lesions in response to genotoxic stress. The enrichment of RNF146 at DNA lesions is mediated by DNA damage-induced PARylation. However, the substrates of RNF146 at the sites of DNA damage remain elusive. Nevertheless, these studies on CHFR and RNF146 further support a model wherein PARylation, the first wave of DNA damage signaling, activates ubiquitination, the later wave of signaling at DNA lesions.

CONCLUDING REMARKS

PARylation is a dynamically regulated post-translational modification, which plays a versatile role in the early steps of DDR. It creates the first wave of DNA damage response through recruitment of numerous DNA damage response factors to the regions near DNA lesions. PARylation also regulates other late post-translational modifications, such as phosphorylation and ubiquitination, at the sites of DNA damage. The additive effect of these post-translational modifications leads to stable retention of the DNA repair machinery. PARylation also affects chromatin remodeling directly through the negative charge in each ADP-ribose unit. The coupling of PARylation and dePARylation can also provide a ready and abundant source of energy for DDR.

However, there are still a lot of interesting questions about the synthesis, recognition and degradation of PAR, which need to be addressed to fully understand the function of PARylation in DNA damage response. The biological function of branched PAR chains remains unclear. With the advancements in proteomics, more site-specific PARylation events can be studied, shedding light on their potential roles in the subsequent steps of DNA repair. Additionally, the roles of the dePARylation enzymes other than PARG are yet to be deciphered. It is possible that different dePARylation enzymes could be directed at specific removal of different PARylation targets or activated in other physiological conditions. Besides DDR, PARylation also participates in many other biological processes, which have been comprehensively summarized in other reviews (167–169). Studies of the molecular mechanism of other PARylation-dependent cellular functions may also provide novel clues to understanding the first wave of DNA damage response.

Another interesting feature of PARPs is that only four out of 16 PARP family enzymes catalyze PARylation, whereas others catalyze MARylation. To date, several lines of evidence suggest that similar to PARylation, MARylation plays very important roles in DDR (89,170). In particular, PARP3 is recruited to the sites of DNA damage and facilitates chromatin remodeling (85,87). PARP3-dependent MARylation is important for both DSB and SSB repair (85,91). Similarly, PARP10, another mono ADP-ribosyltransferase is also involved in DDR (171). In particular, PARP10 has tandem ubiquitin-interacting motifs, which may recognize ubiquitin signals at the sites of DNA damage. Future characterization of these mono ADP-ribosyltransferases may reveal the versatile role of ADP-ribosylation in the DNA damage response.

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