Poly-ADP-ribosylation signaling during DNA damage repair

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1. ABSTRACT

Poly-ADP-ribosylation is a post-translational modification generated in high amounts by poly-ADP-ribose polymerases (PARPs) in response to cellular stress, especially genotoxic stimuli. DNA damage-induced PARylation significantly changes local chromatin structure and triggers the accumulation of several DNA damage response (DDR) proteins at the DNA lesions. In this review, we will discuss the regulation of chromatin structure and DNA damage repair machineries by DNA damage-induced poly-ADP-ribosylation.

2. INTRODUCTION

DNA lesions, if not promptly repaired, generate mutations and genomic aberrations, which can lead to genomic instability and cancer. A high number of DNA lesions form constantly as a consequence of endogenous factors, such as by-products of oxidative respiration or replication fork collapse, and exogenous physical and chemical agents, such as ionizing radiation, UV light or chemical compounds (1). To counteract this damage, mechanisms of DNA damage surveillance and repair have evolved, collectively called the DNA damage response (DDR). The response includes the DNA-repair pathways involved in repairing lesions, as well as regulatory pathways and cell-cycle checkpoints known as DNA-damage signaling (2).

In eukaryotes, the densely packed chromatin physically blocks the cellular machinery that requires access to DNA, such as the DNA repair factors. Although chromatin compaction protects the genome against DNA damage (3), recent reports also suggest that DNA repair processes are less efficient in densely packed heterochromatin (4), leading to more mutations in these areas (5). Indeed, the early steps of DNA repair may include chromatin loosening to facilitate access to the damaged DNA, since chromatin is known to become hypersensitive to nuclease digestion after DNA damage (6). Several interlinked pathways evolved to cope with the variety of DNA damage types occurring randomly any part of the genome. Therefore, it is not surprising that regulating the DNA damage response is intricate and requires a robust and plastic regulatory network. The plasticity in the DDR is established mostly though crosstalk between different DNA damage-induced post-translational modifications, such as phosphorylation, ubiquitilation, poly-ADP-ribosylation and SUMOylation. We will review the regulation of chromatin components and the repair
machinery by poly-ADP-ribosylation, focusing on the recent developments in the field.

3. ADP-RIBOSYLATION AND ITS MOLECULAR EFFECTS

One of the most dynamic DNA damage-induced post-translational modifications is poly-ADP-ribosylation (PARylation) (7). PAR is generated and transferred to the protein targets by a family of 17 enzymes known as PARPs. Of the 17 enzymes, only three have been implicated in the DDR: PARP1, PARP2 and PARP3 (8). PARP1 is alone responsible for about 90% of the DNA damage-induced production of PAR, the large majority of which is attached to PARP1 itself (9). A number of other proteins are also targets of PARylation, among them histones, other chromatin components and repair factors (10, 11). PARP1 rapidly recruits to the DNA damage site owing to its DNA-binding domain, which can recognize different kinds of DNA lesions (12). Structural studies have revealed the mode of single- and double-strand break detection by PARP1 (13-15). The binding of free DNA ends induces a conformational change in PARP1 turning on its enzymatic activity (14). Activated PARP1 transfers an ADP-ribosyl moiety – from NAD$^+$ – to mostly Glu, Asp or Lys residues of their targets, initially forming a mono-ADP-ribosylated residue, which can be extended to a long chain of ADP-ribose polymer by PARP. PAR is quickly degraded by poly-ADP-ribose glycohydrolase (PARG) leaving a single ADP-ribose on the modified amino acid (16, 17). Similarly, ADP-ribose hydrolase 3 (ARH3) can hydrolyze PAR, also leaving proteins mono-ADP-ribosylated (18, 19). The removal of the final protein-proximal mono-ADP-ribose is performed by a family of proteins called macrodomains, with three members in humans: TARG1, MacroD1 and MacroD2 (20-22). Recent reviews extensively cover our current knowledge about ADP-ribosylation metabolism (23, 24).

ADP-ribosylation can exert its biological effects in various ways: destabilizing protein-DNA interactions, regulating protein-protein interactions and protein function or labeling proteins for proteasomal degradation. Firstly, the long negatively charged ADP-ribose polymers can destabilize protein-DNA interactions directly (Figure 1a). Thus, PARylation of histones can lead to relaxation of the chromatin directly (25-27). Similarly, PARylation of the linker histone H1 also leads to the release of H1 from the nucleosomes and causes loosening of the chromatin (26, 28).

Secondly, PAR forms a scaffold that allows the timely recruitment of specific factors, thus enriching them at the sites of DNA damage by means of specialized ADP-ribose-binding modules (Figure 1b). To date, there are seven PAR-binding domains known that target to sites of ADP-ribosylation, with the number likely to increase in the future. The first identified ADP-ribose recognition modules were the linear PAR-binding-motifs (PBM)s (29), followed by the macrodomain (30), PAR-binding-zinc finger (PBZ) (31), the WWE domain (32), the BRCT and FHA domains (33, 34). Interestingly, this vast arsenal of ADP-ribose-binding modules is widespread among the DNA damage repair factors (35). The oligonucleotide/oligosaccharide-binding (OB) domains of SSB1, SSB2, CTC1 and MEIOB are the newest addition to the group of PAR-binding domains (36). Based on sequence homology with other OB domains, they were initially identified as single-stranded DNA binding domains; however, these domains bind PAR with higher affinity than single-stranded DNA. SSB1 and SSB2 are involved in DNA damage repair, whereas CTC and MEIOB have roles in telomere protection and meiotic recombination, which are both other activities linked to ADP-ribosylation.

Thirdly, ADP-ribosylation can influence the activity of its targets (Figure 1c). This is well known for some of the dangerous ADP-ribosylating bacterial toxins (37). However, such data are largely lacking for PARylation upon DNA damage: several proteins are modified but for most of them the modification appears to have no clear function (see (10) for a list of ADP-ribosylated proteins upon DNA damage, and (31, 38) as example of some factors whose ADP-ribosylation has no defined function). The only DNA damage-related example is the PI3K-like kinase DNA-PKcs, which has increased enzymatic activity upon ADP-ribosylation, at least in vitro (39).

Last but not least, poly-ADP-ribosylation is to mark proteins for proteasomal degradation by a subset of E3 ubiquitin ligases with the PAR-binding WWE or PBZ domains (40, 41) (Figure 1d). These E3 ligases are responsible for ubiquitylating PARylated substrates. The activation of PARP1 leads to the recruitment of the E3 ligases, RNF148/Iduna and CHFR, both of which target PARP1 to ubiquitin-mediated proteolysis (41, 42). The recruitment of RNF148/Iduna and CHFR is mediated by their WWE and PBZ domain, respectively (31, 43, 44). The mechanism of PAR-dependent ubiquitin-mediated proteolysis is likely to exist in other PARylated
proteins as well. PARylation of repair factors could, in fact, be an important way of regulation of their levels, but the experimental evidence is currently lacking.

4. THE EFFECTS OF POLY-ADP-RIBOSYLATION ON CHROMATIN STRUCTURE UPON DNA DAMAGE

The effects of PARylation on chromatin structure and dynamics were identified early on. It was observed over 30 years ago that the PARylation of histones leads to their consequent destabilization and the relaxation of chromatin structure (25-27). Later, PARP1 activity was shown to lead to linker histone H1 eviction, contributing to more a relaxed chromatin structure (28, 45). More recently, it was reported that the chromatin relaxation upon DNA damage induced by laser microirradiation depends on ATP and PARylation (38, 46). This suggests that PARylation initiates the recruitment or activation of chromatin-remodeling enzymes upon DNA damage. Indeed, a number of chromatin-remodeling enzymes
recruit to DNA damage in a PARylation-dependent manner such as Amplified in Liver Cancer 1 (ALC1), SMARCA5 and Chromodomain helicase DNA-binding protein 4 (CHD4) (38, 47-50). On the other hand, PARylation contributes to the formation and maintenance of more compact, silenced chromatin as well. The Nucleosome Remodeling Deacetylase (NuRD) complex and several Polycomb Group (PcG) proteins are recruited to DNA lesions in a PARylation-dependent manner (49, 50). The Polycomb Repressive Complex 2 generates the repressive chromatin mark H3K27me3, and the NuRD complex can deacetylate histones, both contributing to chromatin silencing.

4.1. Amplified in liver cancer 1, a macrodomain-containing chromatin remodeler

ALC1 is a chromatin-remodeling enzyme of the SNF2 ATPase superfamily. The ALC1 encoding gene was identified as a frequently amplified locus in hepatocellular carcinomas (51, 52). ALC1 is involved in both DNA repair and the control of gene expression (48, 52, 53). In mice, ALC1 overexpression leads to increased tumor incidence (53, 54). ALC1 depletion sensitizes cells for DNA damaging agents and its overexpression leads to DNA repair defects (48). Unlike the large, multi-subunit chromatin remodeling complexes, ALC1 acts as a monomer and is the only chromatin remodeler described to date that requires PARylated substrates for ATPase and remodeling activity in vitro (47, 48). ALC1 interacts with and recruits the ubiquitin ligase TRIM33 to the DNA damage site, and TRIM33 itself stimulates ALC1 removal. TRIM33 overexpression was shown to reverse the ALC1 overexpression-associated DNA repair defects, supporting its role in controlling ALC1 residence time on chromatin (55). Interestingly, TRIM33 tissue-specific knockout mice have a high prevalence of liver cancers, lending further support to its antagonism with ALC1 (56).

ALC1 has two functional domains: an N-terminal SNF2-type ATPase and a C-terminal macrodomain. ALC1 recruits to the laser-induced DNA damage via its PAR-binding C-terminal macrodomain (47, 48). It was proposed that ALC1 remodels chromatin at the site of damage upon binding with PARylated nucleosomes, as it can slide nucleosomes in vitro upon PARP1 activation (47, 48). However, it has been shown that PARylated PARP1, rather than PARylated nucleosomes, is sufficient to activate of ALC1 (47). This suggests that ALC1 could play a more general role in clearing PARylated proteins from DNA, although this hypothesis has not been tested. The remodeling activity of ALC1 is enhanced in the presence of the H4 tail, in a manner similar to the ISWI remodelers (48). However, the mechanism through which the H4 tail activates ALC1 activity is not understood. Recently, the ALC1 ATPase activation was shown to require an ALC1-nucleosome-PARylated PARP1 intermediate (47). Whether the macrodomain is only a recruitment module or an allosteric regulator of the N-terminal ATPase domain is thus still to be determined.

4.2. The ISWI and NuRD chromatin-remodeling complexes

SMARCA5, also known as SNF2H, is the core ATPase subunit of several chromatin-remodeling complexes of the ISWI family, three of which were shown to recruit to DNA damage: the WICH, the CHRAC and the ACF complex (57-60). SMARCA5 depletion leads to increased IR sensitivity and DSB repair defects (38), and its recruitment to laser-induced DNA damage sites depends on PARP1 activity (38). SMARCA5 was shown to interact with RNF168, which promotes SMARCA5 accumulation at the site of DNA damage. RNF168, an E3 ubiquitin ligase specialized for Lys63-polyubiquitination-mediated signaling, is recruited upon DNA damage in a PAR-dependent manner (38). RNF168 is also PARylated, but the effect of PARylation on the E3 ligase activity of RNF168 has yet to be explored. SirT6-deficient mice show decreased chromatin association of SMARCA5 and increased sensitivity to DNA damage (61). SirT6 was shown to positively regulate PARP1 (62). The decreased SMARCA5 chromatin association in the SirT6-deficient mice could thus be explained by decreased PARP1 activity. In addition, RNF20-dependent H2B ubiquitilation was also shown to recruit SMARCA5 to sites of DNA damage, where it promotes chromatin relaxation and the recruitment of HR repair factors (58). Thus, besides PARylation, H2B ubiquitilation can also regulate SMARCA5 recruitment to sites of DNA damage.

Another chromatin remodeler similarly interacting with ADP-ribosylation is the NuRD complex. It different subunits confer diverse catalytic activities: deacetylase from HDAC1 or 2, histone chaperone from RbAP46 and 48 and chromatin remodeling from CHD3 or CHD4 (63). Both CHD3 and CHD4 were first identified as the auto-antigens for the autoimmune disease dermatomyositis, which is associated with increased cancer risk (64, 65). Moreover, knock-down of some NuRD subunits
(CHD4, HDAC1 and MTA3 increases gamma H2A.X levels, a hallmark of DNA damage repair, suggesting higher levels of DNA damage (50). CHD4 rapidly and transiently recruits to sites of laser-induced DNA damage in both a PARP- and RNF8-dependent manner (49, 50, 66). In fact, CHD4 binds PAR \textit{in vitro}, even though its PAR recognition sequence has not been identified (50). The activity of the NuRD complex plays a role in setting up a repressive chromatin structure dependent on PARP1 activity and which in turn promotes to clear RNA polymerase II from the sites of DNA damage (49). On the other hand, the RNF8-mediated recruitment of CHD4 is required for local chromatin decondensation (66). Thus, the NuRD complex appears to recruit to DNA lesions through at least two independent mechanisms. These two modes of recruitment and activation also modulate chromatin structure in different ways, with PARylation promoting the repressive function of the NuRD complex on the chromatin structure and RNF8-mediated recruitment of NuRD leading to chromatin relaxation.

4.3. The histone chaperone FACT

FACT (facilitates chromatin transcription) is a histone chaperone complex with roles in transcription, replication and repair (67-73) FACT has two subunits – SPT16 and SSRP1 –, which both have roles in the DDR (73, 74). The depletion of SPT16 causes homologous recombination defects and impaired recruitment of repair factors, such as Rad51 and BRCA1 (70). SPT16 interacts with the RNF20 RING finger domain and promotes RNF20 recruitment to the site of DNA damage (70). Consequently, the SPT16-mediated recruitment of RNF20 further promotes H2B ubiquitilation, which increases the recruitment of SNF2H, the core ATPase of the ISWI remodeling complex, ultimately leading to chromatin relaxation at the site of DNA damage (58, 70).

SPT16 becomes PARylated upon DNA damage \textit{in vivo}, which causes FACT to dissociate from chromatin (75). The phosphorylation of H2A.X by DNA-PK facilitates the exchange of this histone variant from chromatin, while PARP1-mediated PARylation of SPT16 is inhibitory by releasing FACT from chromatin (71). Recently, histone exchange measurements at UV-induced DNA damage sites showed an accelerated turnover of histones H2A and H2B mediated by SPT16 (72). Contradictory to the previous findings, the authors found that small-molecule PARP inhibition slightly decreased H2A/ H2B turnover at the UV-induced DNA lesions. These differences could be due to the different experimental approaches used in the studies. Alternatively, PARP1 activity may have both stimulatory and inhibitory effects on SPT16-mediated histone exchange, which could depend on the specific H2A histone variant H2A.X.

5. POLY-ADP-RIboseylation in the different DDR pathways

In addition to regulating chromatin structure and dynamics, PARylation is a well-established regulator of the DDR (7, 8). Several factors involved in the downstream regulation of various repair pathways bind PAR, or are themselves targets of PARylation. In this section, we focus on the effects of PARylation on the different DNA repair pathways, in particular the regulation of the core factors of the repair pathways.

5.1. ADP-ribosylation during the repair of DNA single-strand damage

ADP-ribosylation is primarily linked to base excision repair (BER) and single-strand break (SSB) repair. Oxidative damage, alkylation, methylation, deamination or hydroxylation of the DNA bases can lead to base-pairing mismatch and are removed by BER. This pathway starts with the detection and subsequent excision of the damaged nucleotide by DNA glycosylases, which generate a single-strand break (76). A functional role of PARP1 in this process is demonstrated by reduced BER in PARP1 mutants and PARP1 interaction with several factors involved in BER (10, 34, 35, 77-84). PARP2 is involved in BER and SSB repair similarly to PARP1 (85). Yet, PARP2 recruits to DNA damage sites later than PARP1 and its exact role in BER is still unknown (86). A number of proteins involved in BER and SSB repair have PAR-binding modules and recruit to DNA damage in a PARP activity-dependent manner. XRCC1 (X-ray repair cross-complementing protein 1), for example, is a factor essential for assembling the repair complex in both pathways and is directly recruited to SSB foci via its BRCT domain, which recognize PAR instead of phosphorylation (34, 35, 79, 80). Moreover, XRCC1 is also PARylated by PARP1 (10, 87). PARP1 and XRCC1 are known to form a complex together with Condensin I factors upon activation of BER, but the physiological function of this interaction is still unclear (88, 89). Other examples of recruitment due to PARylation have been explored by the recent work of Li and coworkers, such as the bifunctional polynucleotide kinase/phosphatase (PNKP) and
Aprataxin (34). PNKP and Aprataxin are recruited to damage sites upon SSB-induced PARylation (34). In particular, the former makes the phosphorylation status of the DNA ends compatible for ligation, whereas the latter restores the SSB when the DNA ends are adenylated (34, 90-92). The final ligation step of BAR is carried out by Ligase III that also recruits in response to PARylation through its PBZ domain.

UV-C light-induced DNA lesions, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidine photoproducts (6-4PPs), which are repaired by nucleotide excision repair (NER), are also strong activators of PARylation (93-95). PARP1 interacts with DDB2 (damaged DNA binding 2), which recognizes CPDs and 6-4PPs (96, 97). The PARP1-DDB2 interaction facilitates chromatin rearrangement by recruiting ALC1, and increases DDB2 stability at the damage. Although DDB2 recruitment to the damage site is not affected by either PARP inhibition or knockdown of PARG, the retention time of DDB2 on chromatin is increased upon PARG knockdown. Thus, PARylation plays a role in retaining DDB2 at the damage loci (97).

5.2. The role of ADP-ribosylation in double-strand break repair

The most deleterious DNA lesions are double-strand breaks (DSBs), because the unrepaired DNA ends can induce genomic rearrangements, such as translocations or deletions. There are two major repair pathways against DSBs: homologous recombination (HR, also called homology directed repair) and non-homologous end-joining (NHEJ) (2). HR requires a homologous sequence, typically present after replication (late S and G2), in order to restore the original DNA sequence. NHEJ, on the other hand, anneals the broken ends without using a homologous template and operates throughout the cell-cycle. However, NHEJ introduces mutations upon repair. Although it is being error-prone, NHEJ is a simpler and faster process in comparison to the laborious and slower HR. The decision between HR and NHEJ repair is regulated at multiple stages. The first decision is at the recognition and binding of the break: the MRN complex (MRE11, Rad50 and NBS1) initiates HR, and the DNA-PK holoenzyme (DNA-PKcs, Ku70, Ku80) initiates NHEJ. The deletion of NBS1 in murine cells reduces DSB repair by HR and increases NHEJ (98). Conversely, the loss of the Ku proteins or DNA-PKcs impairs NHEJ (1, 99). The failure of classical NHEJ can lead to the repair of DSBs by the alternative NHEJ, a backup pathway, which involves SSBR pathway components and also PARP1 (100).

PARP1 binds to DSBs, so it is not surprising that PARP1 activity modulates DSB repair as well. A milestone in the research of PARP1 and DSB repair came in 2005. Two laboratories reported that PARP inhibitors efficiently kill cell lines deficient in BRCA1 or BRCA2, both proteins important in HR (101, 102). It was hypothesized that PARP inhibition leads to the accumulation of single-strand breaks – due to impaired BER and SSBR – that turn into DSBs, which accumulate due to impaired repair and eventually cause cell death. However, impaired BER does not lead to lethality in BRCA deficient cells; it is DNA-PK hyperactivation that kills the BRCA-deficient cells upon PARP inhibition (103). The molecular basis and the targets of PARP1, which suppress DNA-PK overactivation in the HR-deficient cell lines, are unknown. Nevertheless, PARP1 activity limits DNA-PK activation, at least, if HR is defective.

There have been several reports of PARP1 negatively regulating HR by counteracting the accumulation of Rad51, a protein required for the homology search during HR, into foci and decreasing sister chromatid exchange and intra- and extra-chromosomal homologous recombination (104-107). A closer look at the proteins that are known to recruit to DNA damage in a PARylation-dependent manner or known to be PARylated reveals that a surprisingly large number of them are involved in HR. The list is growing and certainly incomplete: MRE11, RAD50, NBS1, ATM, BARD1, RNF168, SSB1 and SSB2 are among the HR-related proteins associated with PARylation (12, 31, 33-36, 38, 41-44, 108). For example, SSB1 and SSB2 rapidly recruit to sites of DNA damage in response to PARylation, which they recognize with their PAR-binding OB domains (36, 109). SSB1 is one of the earliest proteins arriving to DNA damage and it interacts with the MRN complex, facilitating the recruitment of the MRN complex to DSBs (110, 111). MRE11 also has a PAR-binding motif, confirmed with in vitro pull downs, whereas NBS1 has a FHA-BRCT fusion domain with a double specificity for PARylation and phosphorylation. The early phase of NBS1 recruitment is PARylation-dependent and the late phase is mediated by DNA damage-induced phosphorylation (34). Ataxia telangiectasia mutated (ATM) is a kinase crucial for the activation and recruitment of HR factors, as well as cell-cycle checkpoint activation (112). ATM has
two PAR-binding motifs, which might explain the way ATM recruits to DSBs during the early phase of the DDR (108). The BARD1/BRCA1 heterodimer also recruits to DNA damage in part by PARylation (33). In particular, the BRCT domain BARD1 is a high-affinity PAR-binding domain (33), and the PAR-mediated recruitment of BARD1 is important for both the recruitment and the function of BRCA1. Thus, PARylation appears to play a positive role in HR in most cases.

There are several reports of an interaction between DNA-PK and PARP1 (29, 39, 113-117). Because of their PBMs, DNA-PKcs and Ku70 recruit to DNA damage in a PAR-dependent manner and form a complex with PARP1 (29). The effect of their interaction is rather controversial: PARylation of DNA-PK was shown to stimulate DNA-PK activity but others did not observe the stimulatory effect of PARylation (39, 113, 114). Nevertheless, PARP1 and DNA-PK appear to cooperate in the DNA damage repair as revealed by experiments in double-knockout mice and by their role in V(D)J recombination in B cells (118-120). Also, depletion of APLF (Aprataxin and PKNF like factor), a nuclease involve in both BER and NHEJ, leads to similar sensitization to DNA damaging agents, like that seen in PARP1-deficient cells (121, 122). APLF recruits to sites of laser-induced DNA damage in response to PARylation via its PBZ motifs (121, 123). In particular, early APLF recruitment depends on PARP3 activity, whereas ATM-mediated phosphorylation is important for APLF retention on the damage site (124-126). The XRCC4/Ligase IV complex, which carries out the final ligation of the DNA ends in NHEJ, is also recruited to damaged DNA through the PAR-binding BRCT domain of Ligase IV (34). Therefore, PARylation positively regulates NHEJ repair proteins, rather than being inhibitory as was the case for BRCA1-deficient cells (103).

All in all, PARP1 appears to be a “pro-DSB repair” factor in normal cells, promoting both HR and NHEJ. The role of PARP1 becomes significant upon the failure of one or more of the pathways. In fact, the main role of PARP1 in NHEJ appears to be in alternative NHEJ, a highly error-prone repair pathway that can function in the absence of classical NHEJ (116). Several laboratories observed that PARP1 inhibits classical NHEJ factors, namely the heterodimer Ku70/Ku80, from the recognition of the free ends (127-130). This inhibition leads to the recruitment of the XRCC1/Ligase III complex, which carries out DNA ligation (34, 35, 79, 131).

6. CONCLUSIONS

PARylation has long been appreciated as an important regulator of both chromatin structure and DNA damage repair (28). The best understood role of PARylation is as a platform for recruiting and concentrating repair factors at the sites of DNA damage (7, 8). This is well established, and is evidenced by the frequent presence of PAR binding domains in repair proteins (35). Much less is known about how PARylation modulates protein activity or how PAR-mediated ubiquitilation regulates protein levels. Hopefully, our understanding of the effects of protein PARylation will advance quickly with the recent developments in the identification of ADP-ribosylation sites within proteins using mass spectrometry.

DNA damage-induced PARylation leads to local chromatin relaxation (45); however, it is not the only way PARylation affects chromatin. PARylation recruits a number of chromatin remodeling enzymes, which relax chromatin at the sites of DNA damage, but PARylation can also silence chromatin by recruiting of the NuRD complex and PRC2 (38, 47-49). This duality is also observed in the connections between PARP and the DDR. The role of PARP1 in repairing single-strand DNA lesions is well established but its involvement in the repair of DSBs is less well understood (7, 8). PARylation appears to mostly stimulate both HR and NHEJ in normal cells (12, 31, 33-36, 38, 39, 41-44, 108, 118-120); however, there are also observations of PARylation negatively regulating HR or NHEJ (103-107, 127-130). The importance of PARP activity becomes obvious when some of the repair pathways fail as highlighted by the toxicity of PARP inhibitor in BRCA-deficient cells (101, 102). That PARylation concurrently causes apparently conflicting biological effects could be the consequence of the ways that the DNA damage response is studied. Most of the approaches to induce DNA damage, such as ionizing radiation, drugs or laser microirradiation, generate a variety of DNA lesions ranging from base damage to DSBs. Furthermore, these lesions occur simultaneously and randomly throughout the genome in both silent and active chromatin regions. DSBs generated by restriction endonucleases are well defined within the genome, however the activity of the restriction endonucleases in compacted parts of the chromatin might be limiting. And the experimental detection of the repair process is also less accurate as the response arising from multiple DNA lesions is observed and often in a population of cells.
The local chromatin environment, the level of transcription and the stage of the cell cycle are all likely to modulate important factors in DNA damage repair. Mutations accumulate within heterochromatic parts of the genome with higher frequency than in euchromatin, suggesting less efficient repair in compacted chromatin (4, 5). Local transcription, in fact, affects HR-NHEJ pathway choice (132, 133). The role of PARylation might be clearer for a particular DNA break at a given locus at a given time. Our understanding of the DDR and the roles of PARylation in the process would greatly benefit from future technology development to allow the induction of DNA lesions with high accuracy at specific parts of the genome and detection of DNA being repaired at a nucleotide resolution within single cells.

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