[Frontiers in Bioscience, Landmark, 20, 440-457, January 1, 2015]

Poly-ADP-ribosylation signaling during DNA damage repair

Barbara Golia[1], Hari R. Singh[1], Gyula Timinszky[1]

[1]Department of Physiological Chemistry, Adolf Butenandt Institute, Ludwig-Maximilians-Universität München, Butenandtstr.5, 81377 Munich, Germany

TABLE OF CONTENTS

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 (PBMs) (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

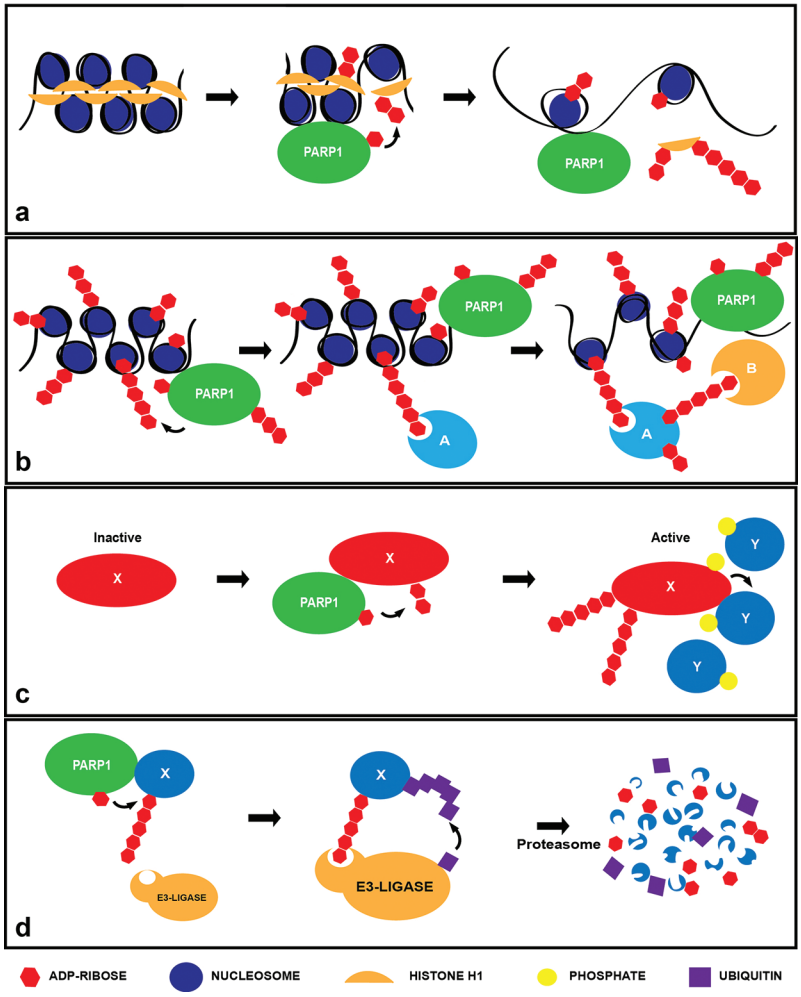

Figure 1. The molecular effects of poly-ADP-ribosylation. a) Transfer of negatively-charged ADP-ribosyl moieties on chromatin proteins. The change of sign in proteins charges induces their release from DNA and the overall chromatin relaxation. In this example, histones are modified by PARP1. b) Establishment of a complex recruiting platform, depending on ADP-ribosylated proteins offering docking sites. While at the beginning most of ADP-ribosylation is present on PARP1 itself and histones, the recruitment of early factors allow the formation of further docking sites. c) Change in the activation level or accessibility of ADP-ribosylated proteins. In the example, an enzyme is at first inactive. After PARP1 modifies it, the enzyme is activated and processes its substrates. d) Targeting for ubiquitin-mediated proteasome degradation. At first, PARP1 modifies the target protein. The PAR attractstheE3 ubiquitin ligase complex, because of its ADP-ribose binding domain. Consequently, the E3 ubiquitin ligase adds ubiquitin moieties on the target protein, which is thus committed for the proteasomal degradation.

proteins as well. PARylation of repair factors could, in fact, be a 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 promotesSMARCA5 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 *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 *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-RIBOSYLATION 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, PARP2recruits 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 increasesDDB2 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 Ku70recruit 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 BRCA-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 DSB repair 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.

7. ACKNOWLEDGEMENT

We apologize to our colleagues whose work could not be cited due to space limitations. We are grateful to Gytis Jankevicius and Corey Laverty for their helpful comments. Our work is funded by the DFG (TI 817/2-1) and DAAD (#55934632).

8. REFERENCES

1. E. Mladenov and G. Iliakis: Induction and repair of DNA double strand breaks: the increasing spectrum of non-homologous end joining pathways. Mutat Res, 711(1-2), 61-72 (2011)
DOI: 10.1016/j.mrfmmm.2011.02.005
2. S. P. Jackson and J. Bartek: The DNA-damage response in human biology and disease. Nature, 461(7267), 1071-8 (2009)
DOI: 10.1038/nature08467
3. H. Takata, T. Hanafusa, T. Mori, M. Shimura, Y. Iida, K. Ishikawa, K. Yoshikawa, Y. Yoshikawa and K. Maeshima: Chromatin compaction protects genomic DNA from radiation damage. PLoS One, 8(10), e75622 (2013)
DOI: 10.1371/journal.pone.0075622
4. I. Chiolo, A. Minoda, S. U. Colmenares, A. Polyzos, S. V. Costes and G. H. Karpen: Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell, 144(5), 732-44 (2011)
DOI: 10.1016/j.cell.2011.02.012
5. B. Schuster-Bockler and B. Lehner: Chromatin organization is a major influence on regional mutation rates in human cancer cells. Nature, 488(7412), 504-7 (2012)
DOI: 10.1038/nature11273
6. F. Carrier, P. T. Georgel, P. Pourquier, M. Blake, H. U. Kontny, M. J. Antinore, M. Gariboldi, T. G. Myers, J. N. Weinstein, Y. Pommier and A. J. Fornace, Jr.: Gadd45, a p53-responsive stress protein, modifies DNA accessibility on damaged chromatin. Mol Cell Biol, 19(3), 1673-85 (1999)
No DOI Found
7. M. De Vos, V. Schreiber and F. Dantzer: The diverse roles and clinical relevance of PARPs in DNA damage repair: current state of the art. Biochem Pharmacol, 84(2), 137-46 (2012)
DOI: 10.1016/j.bcp.2012.03.018
8. A. Pines, L. H. Mullenders, H. van Attikum and M. S. Luijsterburg: Touching base with PARPs: moonlighting in the repair of UV lesions and double-strand breaks. Trends Biochem Sci, 38(6), 321-30 (2013)
DOI: 10.1016/j.tibs.2013.03.002
9. N. Ogata, K. Ueda, M. Kawaichi and O. Hayaishi: Poly(ADP-ribose) synthetase, a main acceptor of poly(ADP-ribose) in isolated nuclei. J Biol Chem, 256(9), 4135-7 (1981)
No DOI Found
10. S. Jungmichel, F. Rosenthal, M. Altmeyer, J. Lukas, M. O. Hottiger and M. L. Nielsen: Proteome-wide identification of poly(ADP-Ribosyl)ation targets in different genotoxic stress responses. Mol Cell, 52(2), 272-85 (2013)
DOI: 10.1016/j.molcel.2013.08.026
11. Y. Zhang, J. Wang, M. Ding and Y. Yu: Site-specific characterization of the Asp- and Glu-ADP-ribosylated proteome. Nat Methods, 10(10), 981-4 (2013)
DOI: 10.1038/nmeth.2603
12. J. F. Haince, D. McDonald, A. Rodrigue,

U. Dery, J. Y. Masson, M. J. Hendzel and G. G. Poirier: PARP1-dependent kinetics of recruitment of MRE11 and NBS1 proteins to multiple DNA damage sites. J Biol Chem, 283(2), 1197-208 (2008)
DOI: 10.1074/jbc.M706734200

13. A. A. Ali, G. Timinszky, R. Arribas-Bosacoma, M. Kozlowski, P. O. Hassa, M. Hassler, A. G. Ladurner, L. H. Pearl and A. W. Oliver: The zinc-finger domains of PARP1 cooperate to recognize DNA strand breaks. Nat Struct Mol Biol, 19(7), 685-92 (2012)
DOI: 10.1038/nsmb.2335

14. M. F. Langelier, J. L. Planck, S. Roy and J. M. Pascal: Structural basis for DNA damage-dependent poly(ADP-ribosyl) ation by human PARP-1. Science, 336(6082), 728-32 (2012)
DOI: 10.1126/science.1216338

15. S. Eustermann, H. Videler, J. C. Yang, P. T. Cole, D. Gruszka, D. Veprintsev and D. Neuhaus: The DNA-binding domain of human PARP-1 interacts with DNA single-strand breaks as a monomer through its second zinc finger. J Mol Biol, 407(1), 149-70 (2011)
DOI: 10.1016/j.jmb.2011.01.034

16. M. Miwa, M. Tanaka, T. Matsushima and T. Sugimura: Purification and properties of glycohydrolase from calf thymus splitting ribose-ribose linkages of poly(adenosine diphosphate ribose). J Biol Chem, 249(11), 3475-82 (1974)
No DOI Found

17. M. Ikejima and D. M. Gill: Poly(ADP-ribose) degradation by glycohydrolase starts with an endonucleolytic incision. J Biol Chem, 263(23), 11037-40 (1988)
No DOI Found

18. S. Oka, J. Kato and J. Moss: Identification and characterization of a mammalian 39-kDa poly(ADP-ribose) glycohydrolase. J Biol Chem, 281(2), 705-13 (2006)
DOI: 10.1074/jbc.M510290200

19. M. Mashimo, J. Kato and J. Moss: ADP-ribosyl-acceptor hydrolase 3 regulates poly (ADP-ribose) degradation and cell death during oxidative stress. Proc Natl Acad Sci U S A, 110(47), 18964-9 (2013)
DOI: 10.1073/pnas.1312783110

20. G. Jankevicius, M. Hassler, B. Golia, V. Rybin, M. Zacharias, G. Timinszky and A. G. Ladurner: A family of macrodomain proteins reverses cellular mono-ADP-ribosylation. Nat Struct Mol Biol, 20(4), 508-14 (2013)
DOI: 10.1038/nsmb.2523

21. F. Rosenthal, K. L. Feijs, E. Frugier, M. Bonalli, A. H. Forst, R. Imhof, H. C. Winkler, D. Fischer, A. Caflisch, P. O. Hassa, B. Luscher and M. O. Hottiger: Macrodomain-containing proteins are new mono-ADP-ribosylhydrolases. Nat Struct Mol Biol, 20(4), 502-7 (2013)
DOI: 10.1038/nsmb.2521

22. R. Sharifi, R. Morra, C. D. Appel, M. Tallis, B. Chioza, G. Jankevicius, M. A. Simpson, I. Matic, E. Ozkan, B. Golia, M. J. Schellenberg, R. Weston, J. G. Williams, M. N. Rossi, H. Galehdari, J. Krahn, A. Wan, R. C. Trembath, A. H. Crosby, D. Ahel, R. Hay, A. G. Ladurner, G. Timinszky, R. S. Williams and I. Ahel: Deficiency of terminal ADP-ribose protein glycohydrolase TARG1/C6orf130 in neurodegenerative disease. EMBO J, 32(9), 1225-37 (2013)
DOI: 10.1038/emboj.2013.51

23. E. Barkauskaite, G. Jankevicius, A. G. Ladurner, I. Ahel and G. Timinszky: The recognition and removal of cellular poly(ADP-ribose) signals. FEBS J, 280(15), 3491-507 (2013)
DOI: 10.1111/febs.12358

24. N. Li and J. Chen: ADP-Ribosylation: Activation, Recognition, and Removal. Mol Cells, 37(1), 9-16 (2014)
DOI: 10.14348/molcells.2014.2245

25. G. G. Poirier, G. de Murcia, J. Jongstra-Bilen, C. Niedergang and P. Mandel: Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. Proc Natl Acad Sci U

S A, 79(11), 3423-7 (1982)
DOI: 10.1073/pnas.79.11.3423

26. M. Y. Kim, S. Mauro, N. Gevry, J. T. Lis and W. L. Kraus: NAD+-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. Cell, 119(6), 803-14 (2004)
DOI: 10.1016/j.cell.2004.11.002

27. A. Huletsky, G. de Murcia, S. Muller, M. Hengartner, L. Menard, D. Lamarre and G. G. Poirier: The effect of poly(ADP-ribosyl)ation on native and H1-depleted chromatin. A role of poly(ADP-ribosyl) ation on core nucleosome structure. J Biol Chem, 264(15), 8878-86 (1989)
No DOI Found

28. R. Krishnakumar and W. L. Kraus: The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell, 39(1), 8-24 (2010)
DOI: 10.1016/j.molcel.2010.06.017

29. J. M. Pleschke, H. E. Kleczkowska, M. Strohm and F. R. Althaus: Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. J Biol Chem, 275(52), 40974-80 (2000)
DOI: 10.1074/jbc.M006520200

30. G. I. Karras, G. Kustatscher, H. R. Buhecha, M. D. Allen, C. Pugieux, F. Sait, M. Bycroft and A. G. Ladurner: The macro domain is an ADP-ribose binding module. EMBO J, 24(11), 1911-20 (2005)
DOI: 10.1038/sj.emboj.7600664

31. I. Ahel, D. Ahel, T. Matsusaka, A. J. Clark, J. Pines, S. J. Boulton and S. C. West: Poly(ADP-ribose)-binding zinc finger motifs in DNA repair/checkpoint proteins. Nature, 451(7174), 81-5 (2008)
DOI: 10.1038/nature06420

32. L. Aravind: The WWE domain: a common interaction module in protein ubiquitination and ADP ribosylation. Trends Biochem Sci, 26(5), 273-5 (2001)
DOI: 10.1016/S0968-0004(01)01787-X

33. M. Li and X. Yu: Function of BRCA1 in the DNA damage response is mediated by ADP-ribosylation. Cancer Cell, 23(5), 693-704 (2013)
DOI: 10.1016/j.ccr.2013.03.025

34. M. Li, L. Y. Lu, C. Y. Yang, S. Wang and X. Yu: The FHA and BRCT domains recognize ADP-ribosylation during DNA damage response. Genes Dev, 27(16), 1752-68 (2013)
DOI: 10.1101/gad.226357.113

35. J. P. Gagne, E. Pic, M. Isabelle, J. Krietsch, C. Ethier, E. Paquet, I. Kelly, M. Boutin, K. M. Moon, L. J. Foster and G. G. Poirier: Quantitative proteomics profiling of the poly(ADP-ribose)-related response to genotoxic stress. Nucleic Acids Res, 40(16), 7788-805 (2012)
DOI: 10.1093/nar/gks486

36. F. Zhang, Y. Chen, M. Li and X. Yu: The oligonucleotide/oligosaccharide-binding fold motif is a poly(ADP-ribose)-binding domain that mediates DNA damage response. Proc Natl Acad Sci U S A, 111(20), 7278-83 (2014)
DOI: 10.1073/pnas.1318367111

37. Q. Deng and J. T. Barbieri: Molecular mechanisms of the cytotoxicity of ADP-ribosylating toxins. Annu Rev Microbiol, 62, 271-88 (2008)
DOI: 10.1146/annurev.micro.62.081307.162848

38. G. Smeenk, W. W. Wiegant, J. A. Marteijn, M. S. Luijsterburg, N. Sroczynski, T. Costelloe, R. J. Romeijn, A. Pastink, N. Mailand, W. Vermeulen and H. van Attikum: Poly(ADP-ribosyl)ation links the chromatin remodeler SMARCA5/SNF2H to RNF168-dependent DNA damage signaling. J Cell Sci, 126(Pt 4), 889-903 (2013)
DOI: 10.1242/jcs.109413

39. T. Ruscetti, B. E. Lehnert, J. Halbrook, H. Le Trong, M. F. Hoekstra, D. J. Chen and S. R. Peterson: Stimulation of the DNA-dependent protein kinase by poly(ADP-ribose) polymerase. J Biol Chem, 273(23), 14461-7 (1998)

DOI: 10.1074/jbc.273.23.14461

40. H. C. Kang, Y. I. Lee, J. H. Shin, S. A. Andrabi, Z. Chi, J. P. Gagne, Y. Lee, H. S. Ko, B. D. Lee, G. G. Poirier, V. L. Dawson and T. M. Dawson: Iduna is a poly(ADP-ribose) (PAR)-dependent E3 ubiquitin ligase that regulates DNA damage. Proc Natl Acad Sci U S A, 108(34), 14103-8 (2011)
DOI: 10.1073/pnas.1108799108

41. C. Liu, J. Wu, S. C. Paudyal, Z. You and X. Yu: CHFR is important for the first wave of ubiquitination at DNA damage sites. Nucleic Acids Res, 41(3), 1698-710 (2013)
DOI: 10.1093/nar/gks1278

42. Z. Wang, G. A. Michaud, Z. Cheng, Y. Zhang, T. R. Hinds, E. Fan, F. Cong and W. Xu: Recognition of the iso-ADP-ribose moiety in poly(ADP-ribose) by WWE domains suggests a general mechanism for poly(ADP-ribosyl)ation-dependent ubiquitination. Genes Dev, 26(3), 235-40 (2012)
DOI: 10.1101/gad.182618.111

43. J. Oberoi, M. W. Richards, S. Crumpler, N. Brown, J. Blagg and R. Bayliss: Structural basis of poly(ADP-ribose) recognition by the multizinc binding domain of checkpoint with forkhead-associated and RING Domains (CHFR). J Biol Chem, 285(50), 39348-58 (2010)
DOI: 10.1074/jbc.M110.159855

44. Y. Zhang, S. Liu, C. Mickanin, Y. Feng, O. Charlat, G. A. Michaud, M. Schirle, X. Shi, M. Hild, A. Bauer, V. E. Myer, P. M. Finan, J. A. Porter, S. M. Huang and F. Cong: RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. Nat Cell Biol, 13(5), 623-9 (2011)
DOI: 10.1038/ncb2222

45. R. Krishnakumar, M. J. Gamble, K. M. Frizzell, J. G. Berrocal, M. Kininis and W. L. Kraus: Reciprocal binding of PARP-1 and histone H1 at promoters specifies transcriptional outcomes. Science, 319(5864), 819-21 (2008)
DOI: 10.1126/science.1149250

46. M. J. Kruhlak, A. Celeste, G. Dellaire, O. Fernandez-Capetillo, W. G. Muller, J. G. McNally, D. P. Bazett-Jones and A. Nussenzweig: Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. J Cell Biol, 172(6), 823-34 (2006)
DOI: 10.1083/jcb.200510015

47. A. J. Gottschalk, G. Timinszky, S. E. Kong, J. Jin, Y. Cai, S. K. Swanson, M. P. Washburn, L. Florens, A. G. Ladurner, J. W. Conaway and R. C. Conaway: Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. Proc Natl Acad Sci U S A, 106(33), 13770-4 (2009)
DOI: 10.1073/pnas.0906920106

48. D. Ahel, Z. Horejsi, N. Wiechens, S. E. Polo, E. Garcia-Wilson, I. Ahel, H. Flynn, M. Skehel, S. C. West, S. P. Jackson, T. Owen-Hughes and S. J. Boulton: Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. Science, 325(5945), 1240-3 (2009)
DOI: 10.1126/science.1177321

49. D. M. Chou, B. Adamson, N. E. Dephoure, X. Tan, A. C. Nottke, K. E. Hurov, S. P. Gygi, M. P. Colaiacovo and S. J. Elledge: A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. Proc Natl Acad Sci U S A, 107(43), 18475-80 (2010)
DOI: 10.1073/pnas.1012946107

50. S. E. Polo, A. Kaidi, L. Baskcomb, Y. Galanty and S. P. Jackson: Regulation of DNA-damage responses and cell-cycle progression by the chromatin remodelling factor CHD4. EMBO J, 29(18), 3130-9 (2010)
DOI: 10.1038/emboj.2010.188

51. A. Marchio, M. Meddeb, P. Pineau, G. Danglot, P. Tiollais, A. Bernheim and

A. Dejean: Recurrent chromosomal abnormalities in hepatocellular carcinoma detected by comparative genomic hybridization. Genes Chromosomes Cancer, 18(1), 59-65 (1997)
DOI: 10.1002/(SICI)1098-2264(199701)18:1<59::AID-GCC7>3.0.CO;2-0

52. K. L. Ma, X. Z. Ruan, S. H. Powis, Y. Chen, J. F. Moorhead and Z. Varghese: Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice. Hepatology, 48(3), 770-81 (2008)
DOI: 10.1002/hep.22423

53. L. Chen, T. H. Chan, Y. F. Yuan, L. Hu, J. Huang, S. Ma, J. Wang, S. S. Dong, K. H. Tang, D. Xie, Y. Li and X. Y. Guan: CHD1L promotes hepatocellular carcinoma progression and metastasis in mice and is associated with these processes in human patients. J Clin Invest, 120(4), 1178-91 (2010)
DOI: 10.1172/JCI40665

54. M. Chen, J. D. Huang, L. Hu, B. J. Zheng, L. Chen, S. L. Tsang and X. Y. Guan: Transgenic CHD1L expression in mouse induces spontaneous tumors. PLoS One, 4(8), e6727 (2009)
DOI: 10.1371/journal.pone.0006727

55. A. Kulkarni, J. Oza, M. Yao, H. Sohail, V. Ginjala, A. Tomas-Loba, Z. Horejsi, A. R. Tan, S. J. Boulton and S. Ganesan: Tripartite Motif-containing 33 (TRIM33) protein functions in the poly(ADP-ribose) polymerase (PARP)-dependent DNA damage response through interaction with Amplified in Liver Cancer 1 (ALC1) protein. J Biol Chem, 288(45), 32357-69 (2013)
DOI: 10.1074/jbc.M113.459164

56. B. Herquel, K. Ouararhni, K. Khetchoumian, M. Ignat, M. Teletin, M. Mark, G. Bechade, A. Van Dorsselaer, S. Sanglier-Cianferani, A. Hamiche, F. Cammas, I. Davidson and R. Losson: Transcription cofactors TRIM24, TRIM28, and TRIM33 associate to form regulatory complexes that suppress murine hepatocellular carcinoma. Proc Natl Acad Sci U S A, 108(20), 8212-7 (2011)
DOI: 10.1073/pnas.1101544108

57. L. Lan, A. Ui, S. Nakajima, K. Hatakeyama, M. Hoshi, R. Watanabe, S. M. Janicki, H. Ogiwara, T. Kohno, S. Kanno and A. Yasui: The ACF1 complex is required for DNA double-strand break repair in human cells. Mol Cell, 40(6), 976-87 (2010)
DOI: 10.1016/j.molcel.2010.12.003

58. K. Nakamura, A. Kato, J. Kobayashi, H. Yanagihara, S. Sakamoto, D. V. Oliveira, M. Shimada, H. Tauchi, H. Suzuki, S. Tashiro, L. Zou and K. Komatsu: Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. Mol Cell, 41(5), 515-28 (2011)
DOI: 10.1016/j.molcel.2011.02.002

59. S. Sanchez-Molina, O. Mortusewicz, B. Bieber, S. Auer, M. Eckey, H. Leonhardt, A. A. Friedl and P. B. Becker: Role for hACF1 in the G2/M damage checkpoint. Nucleic Acids Res, 39(19), 8445-56 (2011)
DOI: 10.1093/nar/gkr435

60. A. Xiao, H. Li, D. Shechter, S. H. Ahn, L. A. Fabrizio, H. Erdjument-Bromage, S. Ishibe-Murakami, B. Wang, P. Tempst, K. Hofmann, D. J. Patel, S. J. Elledge and C. D. Allis: WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. Nature, 457(7225), 57-62 (2009)
DOI: 10.1038/nature07668

61. D. Toiber, F. Erdel, K. Bouazoune, D. M. Silberman, L. Zhong, P. Mulligan, C. Sebastian, C. Cosentino, B. Martinez-Pastor, S. Giacosa, A. D'Urso, A. M. Naar, R. Kingston, K. Rippe and R. Mostoslavsky: SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. Mol Cell, 51(4), 454-68 (2013)
DOI: 10.1016/j.molcel.2013.06.018

62. Z. Mao, C. Hine, X. Tian, M. Van Meter, M. Au, A. Vaidya, A. Seluanov and V. Gorbunova: SIRT6 promotes DNA

repair under stress by activating PARP1. Science, 332(6036), 1443-6 (2011)
DOI: 10.1126/science.1202723

63. C. R. Clapier and B. R. Cairns: The biology of chromatin remodeling complexes. Annu Rev Biochem, 78, 273-304 (2009)
DOI: 10.1146/annurev.biochem.77.062706.153223

64. Y. Zhang, G. LeRoy, H. P. Seelig, W. S. Lane and D. Reinberg: The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell, 95(2), 279-89 (1998)
DOI: 10.1016/S0092-8674(00)81758-4

65. H. B. Wang and Y. Zhang: Mi2, an auto-antigen for dermatomyositis, is an ATP-dependent nucleosome remodeling factor. Nucleic Acids Res, 29(12), 2517-21 (2001)
DOI: 10.1093/nar/29.12.2517

66. M. S. Luijsterburg, K. Acs, L. Ackermann, W. W. Wiegant, S. Bekker-Jensen, D. H. Larsen, K. K. Khanna, H. van Attikum, N. Mailand and N. P. Dantuma: A new non-catalytic role for ubiquitin ligase RNF8 in unfolding higher-order chromatin structure. EMBO J, 31(11), 2511-27 (2012)
DOI: 10.1038/emboj.2012.104

67. R. Belotserkovskaya, S. Oh, V. A. Bondarenko, G. Orphanides, V. M. Studitsky and D. Reinberg: FACT facilitates transcription-dependent nucleosome alteration. Science, 301(5636), 1090-3 (2003)
DOI: 10.1126/science.1085703

68. J. Wittmeyer, L. Joss and T. Formosa: Spt16 and Pob3 of Saccharomyces cerevisiae form an essential, abundant heterodimer that is nuclear, chromatin-associated, and copurifies with DNA polymerase alpha. Biochemistry, 38(28), 8961-71 (1999)
DOI: 10.1021/bi982851d

69. C. D. Kaplan, L. Laprade and F. Winston: Transcription elongation factors repress transcription initiation from cryptic sites. Science, 301(5636), 1096-9 (2003)
DOI: 10.1126/science.1087374

70. D. V. Oliveira, A. Kato, K. Nakamura, T. Ikura, M. Okada, J. Kobayashi, H. Yanagihara, Y. Saito, H. Tauchi and K. Komatsu: Histone chaperone FACT regulates homologous recombination by chromatin remodeling through interaction with RNF20. J Cell Sci, 127(Pt 4), 763-72 (2014)
DOI: 10.1242/jcs.135855

71. K. Heo, H. Kim, S. H. Choi, J. Choi, K. Kim, J. Gu, M. R. Lieber, A. S. Yang and W. An: FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. Mol Cell, 30(1), 86-97 (2008)
DOI: 10.1016/j.molcel.2008.02.029

72. C. Dinant, G. Ampatziadis-Michailidis, H. Lans, M. Tresini, A. Lagarou, M. Grosbart, A. F. Theil, W. A. van Cappellen, H. Kimura, J. Bartek, M. Fousteri, A. B. Houtsmuller, W. Vermeulen and J. A. Marteijn: Enhanced chromatin dynamics by FACT promotes transcriptional restart after UV-induced DNA damage. Mol Cell, 51(4), 469-79 (2013)
DOI: 10.1016/j.molcel.2013.08.007

73. A. Kumari, O. M. Mazina, U. Shinde, A. V. Mazin and H. Lu: A role for SSRP1 in recombination-mediated DNA damage response. J Cell Biochem, 108(2), 508-18 (2009)
DOI: 10.1002/jcb.22280

74. G. Orphanides, W. H. Wu, W. S. Lane, M. Hampsey and D. Reinberg: The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature, 400(6741), 284-8 (1999)
DOI: 10.1038/22350

75. J. Y. Huang, W. H. Chen, Y. L. Chang, H. T. Wang, W. T. Chuang and S. C. Lee: Modulation of nucleosome-binding

activity of FACT by poly(ADP-ribosyl) ation. Nucleic Acids Res, 34(8), 2398-407 (2006)
DOI: 10.1093/nar/gkl241

76. G. L. Dianov and U. Hubscher: Mammalian base excision repair: the forgotten archangel. Nucleic Acids Res, 41(6), 3483-90 (2013)
DOI: 10.1093/nar/gkt076

77. F. Dantzer, G. de La Rubia, J. Menissier-De Murcia, Z. Hostomsky, G. de Murcia and V. Schreiber: Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. Biochemistry, 39(25), 7559-69 (2000)
DOI: 10.1021/bi0003442

78. F. Le Page, V. Schreiber, C. Dherin, G. De Murcia and S. Boiteux: Poly(ADP-ribose) polymerase-1 (PARP-1) is required in murine cell lines for base excision repair of oxidative DNA damage in the absence of DNA polymerase beta. J Biol Chem, 278(20), 18471-7 (2003)
DOI: 10.1074/jbc.M212905200

79. S. F. El-Khamisy, M. Masutani, H. Suzuki and K. W. Caldecott: A requirement for PARP-1 for the assembly or stability of XRCC1 nuclear foci at sites of oxidative DNA damage. Nucleic Acids Res, 31(19), 5526-33 (2003)
DOI: 10.1093/nar/gkg761

80. S. Okano, L. Lan, K. W. Caldecott, T. Mori and A. Yasui: Spatial and temporal cellular responses to single-strand breaks in human cells. Mol Cell Biol, 23(11), 3974-81 (2003)
DOI: 10.1128/MCB.23.11.3974-3981.2003

81. J. L. Harris, B. Jakob, G. Taucher-Scholz, G. L. Dianov, O. J. Becherel and M. F. Lavin: Aprataxin, poly-ADP ribose polymerase 1 (PARP-1) and apurinic endonuclease 1 (APE1) function together to protect the genome against oxidative damage. Hum Mol Genet, 18(21), 4102-17 (2009)
DOI: 10.1093/hmg/ddp359

82. N. Noren Hooten, K. Kompaniez, J. Barnes, A. Lohani and M. K. Evans: Poly(ADP-ribose) polymerase 1 (PARP-1) binds to 8-oxoguanine-DNA glycosylase (OGG1). J Biol Chem, 286(52), 44679-90 (2011)
DOI: 10.1074/jbc.M111.255869

83. A. Hanssen-Bauer, K. Solvang-Garten, O. Sundheim, J. Pena-Diaz, S. Andersen, G. Slupphaug, H. E. Krokan, D. M. Wilson, 3rd, M. Akbari and M. Otterlei: XRCC1 coordinates disparate responses and multiprotein repair complexes depending on the nature and context of the DNA damage. Environ Mol Mutagen, 52(8), 623-35 (2011)
DOI: 10.1002/em.20663

84. A. Campalans, T. Kortulewski, R. Amouroux, H. Menoni, W. Vermeulen and J. P. Radicella: Distinct spatiotemporal patterns and PARP dependence of XRCC1 recruitment to single-strand break and base excision repair. Nucleic Acids Res, 41(5), 3115-29 (2013)
DOI: 10.1093/nar/gkt025

85. V. Schreiber, J. C. Ame, P. Dolle, I. Schultz, B. Rinaldi, V. Fraulob, J. Menissier-de Murcia and G. de Murcia: Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. J Biol Chem, 277(25), 23028-36 (2002)
DOI: 10.1074/jbc.M202390200

86. O. Mortusewicz, J. C. Ame, V. Schreiber and H. Leonhardt: Feedback-regulated poly(ADP-ribosyl)ation by PARP-1 is required for rapid response to DNA damage in living cells. Nucleic Acids Res, 35(22), 7665-75 (2007)
DOI: 10.1093/nar/gkm933

87. M. Masson, C. Niedergang, V. Schreiber, S. Muller, J. Menissier-de Murcia and G. de Murcia: XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol Cell Biol, 18(6), 3563-71 (1998)
No DOI Found

88. J. T. Heale, A. R. Ball, Jr., J. A. Schmiesing, J. S. Kim, X. Kong, S. Zhou, D. F. Hudson, W. C. Earnshaw and K. Yokomori: Condensin I interacts with the PARP-1-XRCC1 complex and functions in DNA single-strand break repair. Mol Cell, 21(6), 837-48 (2006)
DOI: 10.1016/j.molcel.2006.01.036

89. X. Kong, J. Stephens, A. R. Ball, Jr., J. T. Heale, D. A. Newkirk, M. W. Berns and K. Yokomori: Condensin I recruitment to base damage-enriched DNA lesions is modulated by PARP1. PLoS One, 6(8), e23548 (2011)
DOI: 10.1371/journal.pone.0023548

90. J. I. Loizou, S. F. El-Khamisy, A. Zlatanou, D. J. Moore, D. W. Chan, J. Qin, S. Sarno, F. Meggio, L. A. Pinna and K. W. Caldecott: The protein kinase CK2 facilitates repair of chromosomal DNA single-strand breaks. Cell, 117(1), 17-28 (2004)
DOI: 10.1016/S0092-8674(04)00206-5

91. A. A. Ali, R. M. Jukes, L. H. Pearl and A. W. Oliver: Specific recognition of a multiply phosphorylated motif in the DNA repair scaffold XRCC1 by the FHA domain of human PNK. Nucleic Acids Res, 37(5), 1701-12 (2009)
DOI: 10.1093/nar/gkn1086

92. O. J. Becherel, B. Jakob, A. L. Cherry, N. Gueven, M. Fusser, A. W. Kijas, C. Peng, S. Katyal, P. J. McKinnon, J. Chen, B. Epe, S. J. Smerdon, G. Taucher-Scholz and M. F. Lavin: CK2 phosphorylation-dependent interaction between aprataxin and MDC1 in the DNA damage response. Nucleic Acids Res, 38(5), 1489-503 (2010)
DOI: 10.1093/nar/gkp1149

93. R. D. Wood: DNA damage recognition during nucleotide excision repair in mammalian cells. Biochimie, 81(1-2), 39-44 (1999)
DOI: 10.1016/S0300-9084(99)80036-4

94. J. E. Cleaver, E. T. Lam and I. Revet: Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat Rev Genet, 10(11), 756-68 (2009)
DOI: 10.1038/nrg2663

95. M. D. Vodenicharov, M. M. Ghodgaonkar, S. S. Halappanavar, R. G. Shah and G. M. Shah: Mechanism of early biphasic activation of poly(ADP-ribose) polymerase-1 in response to ultraviolet B radiation. J Cell Sci, 118(Pt 3), 589-99 (2005)
DOI: 10.1242/jcs.01636

96. M. S. Luijsterburg, M. Lindh, K. Acs, M. G. Vrouwe, A. Pines, H. van Attikum, L. H. Mullenders and N. P. Dantuma: DDB2 promotes chromatin decondensation at UV-induced DNA damage. J Cell Biol, 197(2), 267-81 (2012)
DOI: 10.1083/jcb.201106074

97. A. Pines, M. G. Vrouwe, J. A. Marteijn, D. Typas, M. S. Luijsterburg, M. Cansoy, P. Hensbergen, A. Deelder, A. de Groot, S. Matsumoto, K. Sugasawa, N. Thoma, W. Vermeulen, H. Vrieling and L. Mullenders: PARP1 promotes nucleotide excision repair through DDB2 stabilization and recruitment of ALC1. J Cell Biol, 199(2), 235-49 (2012)
DOI: 10.1083/jcb.201112132

98. Y. G. Yang, A. Saidi, P. O. Frappart, W. Min, C. Barrucand, V. Dumon-Jones, J. Michelon, Z. Herceg and Z. Q. Wang: Conditional deletion of Nbs1 in murine cells reveals its role in branching repair pathways of DNA double-strand breaks. EMBO J, 25(23), 5527-38 (2006)
DOI: 10.1038/sj.emboj.7601411

99. S. J. DiBiase, Z. C. Zeng, R. Chen, T. Hyslop, W. J. Curran, Jr. and G. Iliakis: DNA-dependent protein kinase stimulates an independently active, nonhomologous, end-joining apparatus. Cancer Res, 60(5), 1245-53 (2000)
No DOI Found

100. P. Frit, N. Barboule, Y. Yuan, D. Gomez and P. Calsou: Alternative end-joining pathway(s): Bricolage at DNA breaks. DNA Repair (2014)

DOI: 10.1016/j.dnarep.2014.02.007

101. H. E. Bryant, N. Schultz, H. D. Thomas, K. M. Parker, D. Flower, E. Lopez, S. Kyle, M. Meuth, N. J. Curtin and T. Helleday: Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature, 434(7035), 913-7 (2005)
DOI: 10.1038/nature03443

102. H. Farmer, N. McCabe, C. J. Lord, A. N. Tutt, D. A. Johnson, T. B. Richardson, M. Santarosa, K. J. Dillon, I. Hickson, C. Knights, N. M. Martin, S. P. Jackson, G. C. Smith and A. Ashworth: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature, 434(7035), 917-21 (2005)
DOI: 10.1038/nature03445

103. A. G. Patel, J. N. Sarkaria and S. H. Kaufmann: Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc Natl Acad Sci U S A, 108(8), 3406-11 (2011)
DOI: 10.1073/pnas.1013715108

104. A. S. Waldman and B. C. Waldman: Stimulation of intrachromosomal homologous recombination in mammalian cells by an inhibitor of poly(ADP-ribosylation). Nucleic Acids Res, 19(21), 5943-7 (1991)
DOI: 10.1093/nar/19.21.5943

105. A. Semionov, D. Cournoyer and T. Y. Chow: Inhibition of poly(ADP-ribose) polymerase stimulates extrachromosomal homologous recombination in mouse Ltk-fibroblasts. Nucleic Acids Res, 27(22), 4526-31 (1999)
DOI: 10.1093/nar/27.22.4526

106. N. Schultz, E. Lopez, N. Saleh-Gohari and T. Helleday: Poly(ADP-ribose) polymerase (PARP-1) has a controlling role in homologous recombination. Nucleic Acids Res, 31(17), 4959-64 (2003)
DOI: 10.1093/nar/gkg703

107. V. Schreiber, D. Hunting, C. Trucco, B. Gowans, D. Grunwald, G. De Murcia and J. M. De Murcia: A dominant-negative mutant of human poly(ADP-ribose) polymerase affects cell recovery, apoptosis, and sister chromatid exchange following DNA damage. Proc Natl Acad Sci U S A, 92(11), 4753-7 (1995)
DOI: 10.1073/pnas.92.11.4753

108. J. F. Haince, S. Kozlov, V. L. Dawson, T. M. Dawson, M. J. Hendzel, M. F. Lavin and G. G. Poirier: Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. J Biol Chem, 282(22), 16441-53 (2007)
DOI: 10.1074/jbc.M608406200

109. D. J. Richard, E. Bolderson, L. Cubeddu, R. I. Wadsworth, K. Savage, G. G. Sharma, M. L. Nicolette, S. Tsvetanov, M. J. McIlwraith, R. K. Pandita, S. Takeda, R. T. Hay, J. Gautier, S. C. West, T. T. Paull, T. K. Pandita, M. F. White and K. K. Khanna: Single-stranded DNA-binding protein hSSB1 is critical for genomic stability. Nature, 453(7195), 677-81 (2008)
DOI: 10.1038/nature06883

110. D. J. Richard, L. Cubeddu, A. J. Urquhart, A. Bain, E. Bolderson, D. Menon, M. F. White and K. K. Khanna: hSSB1 interacts directly with the MRN complex stimulating its recruitment to DNA double-strand breaks and its endo-nuclease activity. Nucleic Acids Res, 39(9), 3643-51 (2011)
DOI: 10.1093/nar/gkq1340

111. D. J. Richard, K. Savage, E. Bolderson, L. Cubeddu, S. So, M. Ghita, D. J. Chen, M. F. White, K. Richard, K. M. Prise, G. Schettino and K. K. Khanna: hSSB1 rapidly binds at the sites of DNA double-strand breaks and is required for the efficient recruitment of the MRN complex. Nucleic Acids Res, 39(5), 1692-702 (2011)
DOI: 10.1093/nar/gkq1098

112. Y. Shiloh and Y. Ziv: The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol

Cell Biol, 14(4), 197-210 (2013)
DOI: 10.1038/nrm3546

113. Y. Ariumi, K. Ueda, M. Masutani, T. D. Copeland, M. Noda, M. Hatanaka and K. Shimotohno: *In vivo* phosphorylation of poly(ADP-ribose) polymerase is independent of its activation. FEBS Lett, 436(2), 288-92 (1998)
DOI: 10.1016/S0014-5793(98)01144-2

114. Y. Ariumi, M. Masutani, T. D. Copeland, T. Mimori, T. Sugimura, K. Shimotohno, K. Ueda, M. Hatanaka and M. Noda: Suppression of the poly(ADP-ribose) polymerase activity by DNA-dependent protein kinase in vitro. Oncogene, 18(32), 4616-25 (1999)
DOI: 10.1038/sj.onc.1202823

115. S. Galande and T. Kohwi-Shigematsu: Poly(ADP-ribose) polymerase and Ku autoantigen form a complex and synergistically bind to matrix attachment sequences. J Biol Chem, 274(29), 20521-8 (1999)
DOI: 10.1074/jbc.274.29.20521

116. M. Wang, W. Wu, B. Rosidi, L. Zhang, H. Wang and G. Iliakis: PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic Acids Res, 34(21), 6170-82 (2006)
DOI: 10.1093/nar/gkl840

117. L. Spagnolo, J. Barbeau, N. J. Curtin, E. P. Morris and L. H. Pearl: Visualization of a DNA-PK/PARP1 complex. Nucleic Acids Res, 40(9), 4168-77 (2012)
DOI: 10.1093/nar/gkr1231

118. M. S. Henrie, A. Kurimasa, S. Burma, J. Menissier-de Murcia, G. de Murcia, G. C. Li and D. J. Chen: Lethality in PARP-1/Ku80 double mutant mice reveals physiological synergy during early embryogenesis. DNA Repair (Amst), 2(2), 151-8 (2003)
DOI: 10.1016/S1568-7864(02)00199-4

119. J. Mitchell, G. C. Smith and N. J. Curtin: Poly(ADP-Ribose) polymerase-1 and DNA-dependent protein kinase have equivalent roles in double strand break repair following ionizing radiation. Int J Radiat Oncol Biol Phys, 75(5), 1520-7 (2009)
DOI: 10.1016/j.ijrobp.2009.07.1722

120. C. Morrison, G. C. Smith, L. Stingl, S. P. Jackson, E. F. Wagner and Z. Q. Wang: Genetic interaction between PARP and DNA-PK in V(D)J recombination and tumorigenesis. Nat Genet, 17(4), 479-82 (1997)
DOI: 10.1038/ng1297-479

121. N. Iles, S. Rulten, S. F. El-Khamisy and K. W. Caldecott: APLF (C2orf13) is a novel human protein involved in the cellular response to chromosomal DNA strand breaks. Mol Cell Biol, 27(10), 3793-803 (2007)
DOI: 10.1128/MCB.02269-06

122. S. Bekker-Jensen, K. Fugger, J. R. Danielsen, I. Gromova, M. Sehested, J. Celis, J. Bartek, J. Lukas and N. Mailand: Human Xip1 (C2orf13) is a novel regulator of cellular responses to DNA strand breaks. J Biol Chem, 282(27), 19638-43 (2007)
DOI: 10.1074/jbc.C700060200

123. S. Eustermann, C. Brockmann, P. V. Mehrotra, J. C. Yang, D. Loakes, S. C. West, I. Ahel and D. Neuhaus: Solution structures of the two PBZ domains from human APLF and their interaction with poly(ADP-ribose). Nat Struct Mol Biol, 17(2), 241-3 (2010)
DOI: 10.1038/nsmb.1747

124. C. J. Macrae, R. D. McCulloch, J. Ylanko, D. Durocher and C. A. Koch: APLF (C2orf13) facilitates nonhomologous end-joining and undergoes ATM-dependent hyperphosphorylation following ionizing radiation. DNA Repair (Amst), 7(2), 292-302 (2008)
DOI: 10.1016/j.dnarep.2007.10.008

125. S. L. Rulten, A. E. Fisher, I. Robert, M. C. Zuma, M. Rouleau, L. Ju, G. Poirier, B. Reina-San-Martin and K. W. Caldecott: PARP-3 and APLF function together to accelerate nonhomologous end-joining.

Mol Cell, 41(1), 33-45 (2011)
DOI: 10.1016/j.molcel.2010.12.006

126. A. L. Fenton, P. Shirodkar, C. J. Macrae, L. Meng and C. A. Koch: The PARP3- and ATM-dependent phosphorylation of APLF facilitates DNA double-strand break repair. Nucleic Acids Res, 41(7), 4080-92 (2013)
DOI: 10.1093/nar/gkt134

127. R. Perrault, H. Wang, M. Wang, B. Rosidi and G. Iliakis: Backup pathways of NHEJ are suppressed by DNA-PK. J Cell Biochem, 92(4), 781-94 (2004)
DOI: 10.1002/jcb.20104

128. H. Hochegger, D. Dejsuphong, T. Fukushima, C. Morrison, E. Sonoda, V. Schreiber, G. Y. Zhao, A. Saberi, M. Masutani, N. Adachi, H. Koyama, G. de Murcia and S. Takeda: Parp-1 protects homologous recombination from interference by Ku and Ligase IV in vertebrate cells. EMBO J, 25(6), 1305-14 (2006)
DOI: 10.1038/sj.emboj.7601015

129. F. Fattah, E. H. Lee, N. Weisensel, Y. Wang, N. Lichter and E. A. Hendrickson: Ku regulates the non-homologous end joining pathway choice of DNA double-strand break repair in human somatic cells. PLoS Genet, 6(2), e1000855 (2010)
DOI: 10.1371/journal.pgen.1000855

130. M. N. Paddock, A. T. Bauman, R. Higdon, E. Kolker, S. Takeda and A. M. Scharenberg: Competition between PARP-1 and Ku70 control the decision between high-fidelity and mutagenic DNA repair. DNA Repair (Amst), 10(3), 338-43 (2011)
DOI: 10.1016/j.dnarep.2010.12.005

131. M. Audebert, B. Salles and P. Calsou: Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. J Biol Chem, 279(53), 55117-26 (2004)
DOI: 10.1074/jbc.M404524200

132. A. Gunn, N. Bennardo, A. Cheng and J. M. Stark: Correct end use during end joining of multiple chromosomal double strand breaks is influenced by repair protein RAD50, DNA-dependent protein kinase DNA-PKcs, and transcription context. J Biol Chem, 286(49), 42470-82 (2011)
DOI: 10.1074/jbc.M111.309252

133. F. Aymard, B. Bugler, C. K. Schmidt, E. Guillou, P. Caron, S. Briois, J. S. Iacovoni, V. Daburon, K. M. Miller, S. P. Jackson and G. Legube: Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. Nat Struct Mol Biol, 21(4), 366-74 (2014)
DOI: 10.1038/nsmb.2796

Key Words: Poly-ADP-ribose, PARP, chromatin, DNA damage response, Review

Send correspondence to: Gyula Timinszky, Department of Physiological Chemistry, Adolf Butenandt Institute, Ludwig-Maximilians-UniversitätMünchen, Butenandtstrasse 5B, 81377 Munich, Germany, Tel: 49-0-89-2180 77100, Fax: 49-0-89-2180 77093, E-mail: gyula.timinszky@med.lmu.de