Role of PARP-catalyzed ADP-ribosylation in the crosstalk between DNA strand breaks and epigenetic regulation.

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Abbreviations used:

5caC, 5-carboxyl; 5hmC, 5-hydroxymethyl-cytosine; 5mC, 5-methylcytosine; ADPr, ADP-ribose; AID, activation-induced cytidine deaminase; APOBEC, apolipoprotein B mRNA-editing catalytic polypeptide-like; APLF, aprataxin and PNK-like factor; ARTD, diphtheria toxin-like ADP-ribosyl-transferases; BER, base excision repair; BRCT, BRCA1 C-terminal; CAD, caspase-activated DNase; CDK, cyclin-dependent kinase; CGI, CpG island; CHFR, checkpoint with forkhead-associated and ring domain; CSR, class switch recombination; DNMT, DNA methyltransferase; DSB, double-strand DNA break; FHA, fork head-associated; HR, homologous recombination; MAR, monomer of ADP-ribose; MMR, mismatch repair; NAD⁺, nicotinamide adenine dinucleotide; NF-κB, nuclear factor kappa B; NHEJ, non-homologous end-joining; OB fold, oligonucleotide/oligosaccharide-binding fold; PAR, polymers of ADP-

ribose; PARG, poly(ADP-ribose) glycohydrolase; PARP, Poly(ADP-ribose) polymerases; PBM, poly(ADP-ribose) binding motif; PBZ, poly(ADP-ribose) binding zinc finger; PPAR γ , peroxisome proliferator activated receptor γ ; PTM, posttranslational modification; RRM, RNA recognition motif; SSB, single-strand DNA break; ssDNA, single-stranded DNA; snoRNA, small nucleolar RNA; TET, ten-eleven translocation; UNG, uracil-N-glycosylase; TOP1, DNA topoisomerase I; TOP2, DNA topoisomerase II.

Abstract

Covalent linkage of ADP-ribose units to proteins catalyzed by poly(ADP-ribose) polymerases (PARPs) plays important signaling functions in a plethora of cellular processes including DNA damage response, chromatin organization, and gene transcription. Poly- and mono-ADP-ribosylation of target macromolecules are often responsible both for the initiation and for coordination of these processes in mammalian cells. Currently, the number of cellular targets for ADP-ribosylation is rapidly expanding, and the molecular mechanisms underlying the broad substrate specificity of PARPs present enormous interest. In this review, the roles of PARP-mediated modifications of protein and nucleic acids, the readers of ADP-ribosylated structures, and the origin and function of programmed DNA strand breaks in PARP activation, transcription regulation, and DNA demethylation are discussed.

Introduction

In most of living organisms, in response to environmental stress, cascades of molecular events are induced that involve specific changes in the cell's key macromolecules, such as proteins, nucleic acids, and lipids. These modifications are various chemical and structural transformations that enable to diversify the restrained genetic information encoded in DNA by the addition of chemical moieties, such as phosphate, acyl (methyl and acetate) groups, small peptides, and sugar residues on proteins and methyl group and its oxidized forms on nucleobases in DNA [1,2]. DNA damage poses a serious threat to genome integrity. Highly dynamic posttranslational and postreplicative modifications of proteins and DNA, respectively, are critical for DNA damage recognition and repair and for associated signaling [3]. In eukaryotes, ADP-ribosylation is a highly conserved posttranslational modification (PTM) of

proteins involved in the regulation of stress responses, cell division, transcription, and protein degradation. This reaction is catalyzed by ADP-ribosyltransferases using nicotinamide adenine dinucleotide (NAD⁺) as a source of ADP-ribose (ADPr) moiety. The largest family of ADP-ribosyltransferases in eukaryotes are poly(ADP-ribose) polymerases (PARPs) that catalyze the synthesis of monomers or polymers of ADPr (MARs or PARs, respectively) covalently attached to acceptor targets (Figure 1) [4,5]. Free nonattached PAR can also function in the cell stress responses including DNA damage, heat shock, and cytoplasmic stress response [4,6]. Importantly, the cellular turnover of PAR is regulated by PAR glycohydrolase (PARG), which degrades the polymer by hydrolysis of ribose–ribose bonds [7]. ADP-ribose (ADPr)-based modifications of target nuclear proteins including histones provide an efficient chromatin-remodeling mechanism required for efficient repair of DNA strand breaks, yet—in the case of severe genotoxic stress—PARP-catalyzed modifications of cell content may direct cell-initiated programmed cell death. Furthermore, it has been shown that PARP1 can interact with, modify, and regulate epigenetic remodeling proteins such as chromatin insulator protein CTCF (CCCTC-binding factor) and DNMT1 (DNA methylation maintenance protein); thus, the right balance between PARylation and PAR degradation by PARG is crucial for the maintenance of DNA methylation patterns in normal cells [8-10]. Of note, years ago, specific enzymes in arthropods and bacterial toxins were shown to transfer an ADP-ribosyl moiety to double- and single-stranded DNA nucleobases in a reversible manner [11,12]. Later on, it has been demonstrated that mammalian PARP1, -2, and -3 (having the ability to act as DNA break sensors) modify not only cellular proteins but also terminal phosphate residues at double-strand DNA break (DSB) and single-strand DNA break (SSB) termini [13,14]. Recently, RNA also was identified as a target of reversible mono-ADP-ribosylation [15]. At present, the biological role of PARP-mediated ADP-ribosylation of DNA strand break termini remains unclear, but might be different from that of proteins. This review focuses on the interplays among DNA breaks, ADP-ribosylation, and DNA epigenetic signatures. We also discuss the putative role of ADP-ribosylated DNA breaks in DNA metabolism and in the recruitment of specific PAR–DNA or MAR–DNA adduct–guided factors.

PARPs at the center of stress response pathways

Cellular stress responses are mediated by various sensors and effectors from multiple signaling pathways including phosphorylation, ubiquitylation, SUMOylation, methylation, acetylation, and ADP-ribosylation. PARylation and MARylation [poly- and mono(ADP-ribosyl)ation] catalyzed by PARPs alter the function of the modified proteins and provide a scaffold for the recruitment of other proteins, which in turn can also undergo ADP-ribosylation. The latter regulates a number of biological processes including the DNA damage response, chromatin reorganization, transcription, apoptosis, autophagy, mitosis, and cell metabolism and development (reviewed in [5,16-18]).

The family of PARPs, also known as diphtheria toxin–like ADP-ribosyl-transferases (ARTDs), includes 17 known members [19,20]. Highly divergent PARP homolog tRNA 2'-phosphotransferase 1 (TRPT1) is sometimes referred to as the 18th PARP family member [21]. According to their structures, PARPs can be subdivided into four subfamilies: DNA-dependent PARPs (PARP1, PARP2, and PARP3), tankyrases (PARP5a and PARP5b), CCCH (Cys–Cys–Cys–His) zinc finger and WWE (Trp–Trp–Glu) domain–containing PARPs (PARP7, PARP12, PARP13.1, and PARP13.2), and macrodomain-containing PARPs (PARP9, PARP14, and PARP15). Although PARP13 is not yet shown to have a catalytic activity [4], most of PARPs have the function of transferring PAR or MAR moieties onto their target proteins [22], DNA [13,14,23], or RNA termini [15]. PARPs play a role in a wide range of biological structures and processes, including DNA repair and maintenance of genomic stability, transcriptional regulation, centromere function, mitotic spindle formation, centrosomal function, the structure and function of vault particles, telomere dynamics, trafficking of endosomal vesicles, inflammation, apoptosis, and necrosis [6,22,24].

The founding member of the PARP family as well as the most ubiquitous and abundant PARP, PARP1 was the most studied. This 110 kDa nuclear protein is composed of six domains essential for DNA binding, nuclear homing, automodification, protein–protein interactions, and catalytic activity. PARP1 is responsible for 80% to 90% of the PARylation activity in the cell [25]. Based on the structure–function relationship studies, PARP1 was initially characterized as a critical player in the DNA damage response and repair processes under stressful conditions. PARylation can lead to accelerated dissociation of modified proteins from DNA owing to the negative charge of the PARylated protein and steric hindrance. The best example of this dissociation is PARP1-mediated PARylation of histone H1 and PARP2-mediated PARylation of histone H2B; these modifications cause the dissociation of these histones from DNA and eventually chromatin relaxation required for replication, transcription, DNA repair [26–28]. Later on, the list of known biological functions of PARP1 has been expanded: regulation of chromatin structure, transcription, stress responses, and involvement in various physiological processes [29]. Moreover, the roles of PARP1 under normal physiological conditions have been further substantiated, e.g., the regulation of gene expression, RNA biology, and processes in cytoplasm. Recently, PARP1 has been identified as a sensor of unligated Okazaki fragments—during DNA replication in normal S phase cells—which facilitates their maturation [30]. The remaining ADP-ribosylation activity in the cell lacking PARP1 (in embryonic fibroblasts derived from PARP1^{-/-} knockout mice) falls to other active PARP members, which may or may not share structural similarities or localization with PARP1 but certainly share a highly conserved catalytic (CD) domain (PARP signature). The latter consists of a helical regulatory domain (HD) and an ADP-ribosyl transferase domain (ART) responsible for the catalytic activity, present in PARP2 and PARP3.

PARP2 was discovered as the enzyme responsible for the basal PARylation activity in PARP1-deficient cells [31] and accounts for ~10% of the PARylation activity in the cell. Just as PARP1, PARP2 recognizes and binds a DSB or SSB. The binding of PARP2 to damaged DNA structures triggers its PARylation activity. PARP2 has partially redundant functions with PARP1

that are essential for normal embryogenesis. Double-knockout *Parp1^{-/-}Parp2^{-/-}* mice show early embryonic mortality [32]. PARP2 has a function, independent of its PAR synthesis activity, which limits the accumulation of the resection barrier factor 53BP1 at DNA damage sites and directs DSBs toward resection-dependent repair pathways [33]. Aside from DNA repair, cell cycle regulation, and inflammation and metabolic regulation, PARP2 acts as a cofactor in transcription and can regulate the expression of 600 to 1,000 genes by facilitating transcription or via attraction of cofactors promoting chromatin compaction and the consequent inhibition of transcription [34-37].

PARP3 is related to PARP1 and PARP2 and its domain organization is similar to that of PARP2, but PARP3 catalyzes MARYlation instead of PARylation [20]. Similar to PARP1 and PARP2, PARP3 is an important player in cell cycle regulation and DNA repair [38]. This PARP interacts with PARP1, DNA ligase III, Ku70/80, other nonhomologous end-joining (NHEJ) proteins and promotes processing DSBs in the canonical NHEJ pathway [39]. Other than its role on the DNA damage response and repair, PARP3 has also been reported to associate with Polycomb group proteins involved in transcriptional silencing and chromatin-remodeling [40].

PARP4 (also called VPARP or ARTD4) is a component of the cytosolic ribonucleoprotein vault complex [24] and is also present in cytoplasmic clusters (vPARP rods) as well as in the nuclear matrix [5]. The conserved glutamate residue in PARP1 is replaced with isoleucine, leucine, or tyrosine in PARP4, which is associated with the absence of polymerase activity [41]. It was proposed that PARP4 may be involved in an antiviral response [42].

PARPs 5a and 5b (tankyrases) were initially identified as a part of a telomeric complex but are also located in the cytoplasm as peripheral membrane proteins localized at the Golgi complex and are associated with transport vesicles [5]. They are best known for their participation in mitosis and WNT signaling, but they also have functions in telomere and DNA damage repair [24]. Recently, a possible link between tankyrases and the DNA damage response has been proposed. On the one hand, tankyrases associate with DSBs to facilitate the recruitment of the

CtIP–BRCA1 complex to damaged chromatin and to promote DNA end resection during homologous recombination (HR); on the other hand, tankyrases associate with DSBs to stimulate the recruitment of the BRCA1A complex (consisting of RAP80–BRCA1–BRCC36–CCDC98) mediated by MERIT40 and activate the G2–M checkpoint for promoting DNA repair before mitosis [43].

PARP6 has been found to be involved in hippocampus neuronal development [24]. Moreover, it plays a role in cell cycle progression and has been associated with the progression of colorectal cancer [42]. Of note, the biological activity of PARP6 depends on its catalytic activity as well as its N-terminal cysteine-rich domain [41].

Several PARPs (PARP7, PARP10, PARP12, and PARP13) are involved in the mechanisms of posttranscriptional regulation of mRNA; the latter process is mediated either by RNA-binding domains or by ADP-ribosylation of RNA-binding proteins [24]. PARP7, present in stress granules [5], is involved in antiviral responses, cytosolic RNA processing, and transcription [34]. PARP10 is a binding protein and an inhibitor of MYC [42] and has been implicated in the regulation of nuclear factor kappa B (NF- κ B), GSK3B, and transcription [24]. PARP10 directly ADP-ribosylates NEMO. These events lead to the inhibition of nuclear localization of the p65 subunit of NF- κ B and to subsequent attenuation of NF- κ B-dependent gene expression [41]. PARP12 is a catalytically active cytosolic monoenzyme [43], which preferentially associates with the Golgi apparatus and regulates stress granule assembly, microRNA activity, and an antiviral response [42]. Intracellular expression of PARP12 increases upon stimulation by type II interferons, thereby leading to increased NF- κ B signaling, implicating PARP12 in cellular immune responses [41]. PARP13, also referred as ZAP (zinc finger antiviral protein), has been so far regarded as a catalytically inactive ART with the roles in the assembly of stress granules and regulation of microRNAs, with consequent implications in innate antiviral defense and cancer [42,43].

PARP9 and PARP14 are believed to act on transcription, especially the transcription of genes required for macrophage activation [24]. PARP9 possesses a unique MARYlating activity

specifically targeting the ubiquitin peptides and participates in the DNA damage response, transcription in lymphocytes, and in antiviral response [42]. PARP14 has been implicated in multiple cellular functions such as survival of B cells, cell migration, assembly of stress granules, transcription during inflammation processes, the DNA damage response, and an antiviral response [42]. PARP14 regulates the class distribution, affinity repertoire, and recall capacity of antibody responses, which require efficient differentiation and interactions among B cells, T helper cells, and dendritic cells [41].

Concurrently with its nuclear pore localization, PARP11 modifies targets involved in the coordination of the nuclear envelope and organization of nuclear pores and nuclear envelope biology [24,42]. PARP15 is a centrosomal PARP involved in stress granule formation, an antiviral response, cytosolic RNA processing, and tumor formation [41,42]. PARP16 is located in the endoplasmic reticulum (ER) and regulates the unfolded protein response [24,42]. Cellular localization of other PARPs (PARP8 and PARP17) and their involvement in biological processes remain unknown.

Moreover, some PARP family members can interact with each other, e.g., PARP1 with PARP2 or PARP3 as well as tankyrase 1 with tankyrase 2 [27]. This type of heterodimers may occur in different subcellular compartments and act on different substrates, which monomeric PARPs could not otherwise target individually. This observation may highlight a new organizational order for PARPs that may greatly diversify their biological responses, via combinatorial interactions.

Excessive ADP-ribosylation can lead to the activation of cell death pathways, including parthanatos, a unique form of programmed cell death that occurs independently of caspases and is distinct from necrosis and apoptosis [44]. Due to its manifold role in cell survival, the protein PARylation process is finely regulated. PAR is rapidly degraded by PARG, the main enzyme that specifically hydrolyses ribose–ribose bonds encoded by a single gene in mammals [7]. Disruption of the *PARG* gene in mice causes embryonic lethality, and studies of PARG-deficient cells have shown that accumulation of PARylated macromolecules is highly

toxic to the cell [45,46]. Nevertheless, PARG has rather limited processivity on short PAR polymers and is unable to remove MARylation marks from proteins [47]. A complete reversal of MARylation is performed in human cells by amino acid-specific ADPr-acceptor hydrolases, such as macrodomain-containing proteins MacroD1 and MacroD2, terminal ADP-ribose protein glycohydrolase 1 (TARG1), and ADP-ribosylhydrolase (ARH) family members ARH1 and ARH3 (reviewed in [48]).

Protein acceptors for ADP-ribosylation

Despite PARPs' different biological functions, over decades, little has been known about ADPr acceptors. Starting from the protein PARylation discovery, for ADP-ribosylated proteome analysis, researchers have applied chemical (high pH, hydroxylamine) or enzymatic sensitive methods (based on ARH1, PARG, ARH3, SVP, or NudT16) to quickly release the ADPr groups from modified residues. Hayaishi's group provided one of the first mechanistic insights into PAR synthesis. They demonstrated that in the rat liver, ADPr binds to histone H1 through an ester linkage with either a γ -carboxyl group of glutamic acid residue 2 or 14 or with an α -carboxyl group of C-terminal lysine residue 213 [49]. Linker histones H1 and H5 are known to be primary targets for PARP1-catalyzed PARylation, whereas PARP2 and PARP3 preferentially ADP-ribosylate core histone H2B [50-52]. PARP3 preferentially adds a mono ADPr moiety on Glu2 of histone H2B upon DNA damage [51]. Until 2017, it had been generally thought that PARP-catalyzed auto-PARylation and ADP-ribosylation of other proteins occur predominantly on aspartates, glutamates, and lysines [53-55]. Nonetheless, conventional approaches have to overcome many limitations, such as the dynamic heterogeneous nature of protein ADP-ribosylation, low abundance, lability of some sites, and chemical or enzymatic resistance of other ADPr acceptor sites. The evolution of methods for the detection of MAR/PAR attachment sites has led to the use of mutagenesis assays as well as NAD⁺ analogs, unbiased enrichment strategies, chemotherapeutic PARP inhibitors, advanced mass spectrometry (particularly based on electron-transfer higher-energy collisional dissociation), and quantitative proteomic techniques [56-59]. Investigators have uncovered modification of

more than 7000 ADP-ribosylation sites across more than 2000 ADP-ribosylation target proteins covering over one-third of the nuclear proteome under genotoxic stress conditions [59]. Upon DNA damage, serine (Ser) becomes the major ($\approx 90\%$) ADPr acceptor residue with the most easily identifiable signals related to the modification of histone proteins as well as PARP automodification [57,59]. Histones are MARylated selectively on serine residues of histone H3 (Ser10 and Ser28) and Ser6 of H2B unless the neighboring lysine residues are acetylated [57,60]. Notably, Ser ADP-ribosylation sites strongly overlap with known kinase-regulated sites (Aurora B and others) [59]. ADP-ribosylation and phosphorylation of these serine residues are considered mutually exclusive [60], suggesting a complex interplay between histone marks. The major hydrolase responsible for the reversal of the Ser-ADPr modification is ARH3 [61,62]. ARH3-deficient cells show a dramatic increase of PAR content in response to hydrogen peroxide exposure with induction of an AIF release from mitochondria and parthanatos [62,63]. Moreover, the specificity of ADP-ribosylation is regulated by different factors. HPF1 (histone PARylation factor 1) interacts with PARP1 and PARP2 and guides the ADP-ribosylation of PARP1 and high-mobility group proteins through a serine residue [56]. In HPF1's absence, acidic residues (Asp and Glu) become the main target sites for ADPr in proteins. Hundreds of ADP-ribosylation sites are also located on histidine, arginine, lysine, cysteine, and tyrosine residues [59,60,64]. It is still possible that further development of proteomic tools will allow researchers to detect new types of modifications, such as an acid-labile ADPr adduct of phosphoserine residues. This chimeric modification was noted in histones from the rat liver more than 40 years ago [65], but the enzymes responsible for its formation are still unknown. The diversity of ADPr substrate amino acids has revealed the importance of this PTM in cell signaling and survival and thus the necessity of its regulation.

Readers of ADP-ribosylated targets

ADP-ribosylation is a PTM of proteins: it induces the recruitment of the protein (such as TET [66], TP53 [67], NF- κ B [68]) or modulates its activity by covalent or noncovalent binding. Aside from protein modification, ADP-ribosylation is also involved in signaling as well as protein–

protein or protein–DNA interactions [69]. PARylation as a PTM directly regulates many cellular pathways such as transcription, chromatin modification, and DNA damage and oxidative-stress signaling. Proteins may have a PAR- or MAR-recognizing domains (Figure 2) that bind to PAR polymers or MAR moieties [29]. Depending on the nature of recognition, different proteins have different motifs. For instance, a PAR-binding motif (PBM) is believed to engage in an electrostatic interaction with negatively charged PAR chains [69,70], whereas a PAR-binding zinc finger (PBZ) recognizes two consecutive ADPr moieties although some can also recognize only one ADPr of a PAR chain [71-73]. Similarly to PBZ, WWE requires two consecutive ADPr units for its successful binding; hence, it interacts with iso-ADPr formed by both ADPr units [74]. FHA (fork head–associated) and BRCT (BRCA1 C-terminal) are protein domains that are mostly known to interact with phosphorylated peptides but also have affinity for PAR chains [75]. The latter interaction seems to be similar to that of WWE domain or PBM, respectively. Another class of ADPr-recognizing domains is macrodomains. Unlike other domains, macrodomains interact with mono-ADPr or the terminal ADPr of PAR chains in the case of H2A1.1 [76]. There are also RNA- and DNA-binding motifs that unexpectedly recognize PAR ADPr moieties [77].

The PBM

The consensus sequence of a PBM is ([HKR]-X-X-[AIQVY]-[KR]-[KR]-[AILV]-[FILPV]), i.e., approximately 20 residues. The positively-charged-amino acid content of a PBM allows for an electrostatic interaction with highly negatively charged PAR polymers. PBMs have been detected in more than 800 proteins *in silico*, and >500 hits have been obtained in proteomic analysis [69,70]. PBMs are often found in many DNA damage response proteins and other proteins that are included in the ADPrBoDB database of ADP-ribosylated proteins [78], suggesting that PAR binding promotes PARylation. For example, tumor protein p53 (p53, TP53) can bind to PAR polymers both in a covalent and in a noncovalent manner. TP53 contains multiple PBMs. Hence, Fishbach *et al.* suggest that TP53 gets covalently PARylated upon a noncovalent interaction between a PBM located in a C-terminal domain of TP53 and

PARylated PARP1 [67]. They demonstrated that noncovalent PAR binding diminishes the sequence-independent DNA-binding capacity of TP53. Nevertheless, simply having a PBM seems to be insufficient for PAR binding. For instance, the entire BRCT domain of XRCC1—rather than a short PBM—is required for its affinity for PAR [75].

The PBZ

The PBZ is a C2H2 zinc finger domain consisting of approximately 30 amino acid residues. The PBZ domain has a consensus sequence of [K/R]-X-X-C-X-[F/Y]-G-X-X-C-X-[K/R]-[K/R]-X-X-X-X-H-X-X-X-[F/Y]-X-H and so far has been discovered in three proteins: APLF (aprataxin and PNK-like factor), CHFR (checkpoint with forkhead-associated and ring domain), and CTCF [77]. Unlike CHFR, APLF contains two PBZ domains. Accordingly, one of the two PBZ domains identified in the APLF protein interacts with two consecutive ADPr units, whereas the second PBZ domain is thought to bind to the 3rd ADPr unit likely on a branched PAR polymer or terminal poly-ADPr. The PAR recognition by APLF induces its histone chaperone activity for the release of histones H3 and H4 and chromatin relaxation [79,80].

Macrodomains

Macrodomains are mono-ADPr-recognizing domains [76]. One of the well-studied macrodomains is histone variant macroH2A1.1. In addition to MAR, macroH2A1.1 can recognize PAR polymers via their terminal ADPr unit. MacroH2A1.1 participates in metabolic regulation and energy production by inhibiting PARP1 activity and decreasing its nuclear NAD⁺ consumption [81]. MacroH2A1.1–PARP1 interaction is also involved in gene regulation, for instance, in response to heat shock stress and during expression regulation of senescence-associated secretory phenotype genes or genes participating in adipocyte differentiation and metabolic regulation during muscle differentiation [82]. Another macrodomain-dependent chromatin-remodeling factor is ALC1. This is an inactive ATPase and one of the chromatin remodelers that activates upon DNA damage. During DSB repair, MacroH2A1.1 [83] and ALC1 [84] take part in chromatin remodeling in a macrodomain-dependent manner. PARP family members PARP9, PARP14, and PARP15 have both the MARYlation activity and a

macrodomain interacting with MAR [76,85,86]. PARG, TARG1, and MACROD1–3 ADPr-hydrolases also contain macrodomains [87].

The WWE domain

This domain contains two conserved tryptophans and a glutamic acid residue, hence its name, and in total is approximately 80–100 residues long [88,89]. It recognizes iso-ADPr moieties between two consecutive ADPr units. The WWE domain is reported to be present mostly in E3 ubiquitin-protein ligases (RNF146, DELTEX1, DELTEX2, DELTEX4, and HUWE1) and in two PARPs (PARP11 and PARP14) [74].

FHA and BRCT domains

These domains play a huge part in cellular responses to DNA damage by recognizing phosphorylated peptides [90,91]. It has also been discovered that FHA and BRCT domains can recognize PAR polymers. Similarly to the WWE domain, the FHA domain binds iso-ADPr of PAR chains by recognizing the two phosphate groups on ADPr moieties, whereas the BRCT domain directly recognizes ADPr of a PAR chain [75]. The latter phenomenon is possibly due to the phosphate groups on ADPr, which mimics the phosphorylated serine residue recognized by the BRCT domain [92]. ADPr recognition by an FHA or BRCT domain facilitates rapid recruitment of DNA damage response proteins: PNKP and aprataxin by the FHA domain and ligase IV, XRCC1, and the BRCA1–BARD1 complex by the BRCT domain [75,92-94].

RNA- and DNA-binding motifs (RRM, SR repeat- and KR-rich motif, OB fold, PIN domain, and GAR domain)

Of note, RNA- and DNA-binding motifs can also bind to PAR. Although this is not very surprising because PAR chains have a structure similar to that of oligonucleotides. NONO, an RNA-binding protein, is believed to increase survival during DSB repair although its function is not yet clear; its recruitment is PARP1 dependent. RRM (RNA recognition motif) of NONO recognizes PAR, thereby facilitating the recruitment of NONO to a DNA damage site [95].

It has been shown that splice factors ASF/SF2, SF3A1, SF3B1, and SF3B2 can recognize PAR chains via their SR (serine/arginine) repeats [96,97]. PAR binding to splicing factor ASF/SF2 inhibits its phosphorylation by TOPI (DNA topoisomerase I) and activity [96], indicating the involvement of PARPs and PAR chains in RNA stability and metabolism [98]. In a similar context, upon heat shock, KR (lysine/arginine)-rich repeats of the *Drosophila* Mi-2 protein bind PAR chains thus leading to Mi-2 recruitment to heat shock–responsive genes [99].

Oligonucleotide/oligosaccharide-binding fold (OB fold) is an ssDNA- and RNA-binding motif. Just as WWE and FHA domains, the OB fold of human ssDNA-binding protein 1 (hSSB1) recognizes iso-ADPr of PAR chains in addition to its ssDNA-binding ability [100].

Proteins with PIN (PiIT N terminus) domains are mostly nucleases cleaving ssDNA or ssRNA [101]. In particular, during DNA damage repair, the PIN domain of exonuclease 1 (EXO1) was found to recognize DNA damage–induced PARs, and this event is rather sufficient for its recruitment to the DNA damage site [102].

GAR (or RG/RGG box) is another PAR-binding domain and consists of a sequence enriched in arginine and glycine. There are several RNA-binding proteins with GAR domains known to recognize PAR chains during DNA damage responses, e.g., FUS/TLS, EWS/EWSR1, TAF15, and CIRBP [77,103].

Reversible ADP-ribosylation of DNA and RNA

The first evidence of DNA ADP-ribosylation was obtained 20 years ago. Watanabe's group demonstrated that a cabbage butterfly toxin, pierisin, induces apoptosis via irreversible MARYlation of a guanine base in DNA [104,105]. Later, other examples of DNA MARYlation have been demonstrated for different families of toxins: guanine MARYlation by CARP-1 from shellfish [11] and by scabin from *Streptomyces scabies* [106] as well as thymine MARYlation by DarT from bacterial toxin–antitoxin system DarTG [12].

Recently, *in vitro* studies in our laboratory have uncovered that mammalian DNA-dependent PARPs catalyze reversible modification of DNA via ADP-ribosylation of terminal phosphates at DNA strand breaks [13]. This finding provides novel molecular insights into PARPs' functions in mammalian cells. Taking into account an unsolved challenge (how to distinguish ADPr adducts on proteins and DNA in the cell), DNA ADP-ribosylation studies have been focused on *in vitro* approaches to gain knowledge about the mechanisms and specific requirements for this unusual substrate specificity of PARPs. It has been found that PARP1 preferentially PARylates DSBs containing 5'- and 3'-terminal phosphates in gapped recessed DNA duplexes, whereas PARP2 and PARP3 preferentially act on 5'-terminal phosphates at DSB and SSB termini of DNA containing multiple proximal breaks [13,14,23]. Similarly to protein modification, PARP3 produces a MAR not PAR adduct on DNA substrates, in contrast to PARP1 and PARP2 [14,23]. In addition to phosphate groups, PARP1 can PARylate 2'-OH groups of 3'-deoxynucleotide and ribonucleotides incorporated at the 3' terminus of oligodeoxyribonucleotides [13]. A recent study by Zarkovic G. et al. revealed ADP-ribosylation of ~3 kb plasmid-based DNA constructs, thus indicating DNA size limitlessness of PARP-mediated modifications of DNA break termini [14]. Moreover, PARP2 and PARP3 switch their substrate preference to DNA from protein when acting upon certain configuration of closely spaced DNA strand breaks, preferentially ADP-ribosylating DNA rather than catalyzing auto-ADP-ribosylation. Effectiveness of PARP3- and PARP2-catalyzed DNA ADP-ribosylation depends on the orientation and a distance between DNA strand breaks in a single DNA molecule [14]. According to a proposed mechanistic model, binding of a PARP to one DNA break activates the CAT domain, which in turn targets and ADP-ribosylates an acceptor group at the second breakage site of the same DNA molecule [13,14]. This process necessitates the presence of at least two DNA strand breaks separated by a distance from 1 to 2 helix turns [14]. In a DNA-bound PARP complex, this distance determines the accessibility of the DNA acceptor groups for the activated CAT domain directing ADP-ribosylation to a 5'- or 3'-terminal phosphate. At present, little is known about the mechanisms governing substrate interaction and specificity of PARP1, which accounts for most of cellular PARylation activity. Moreover, it

remains unclear of how can PARPs adopt the conformation that predisposes to DNA ADP-ribosylation activity. According to existing structural data on PARP1 and PARP2 bound to DNA breaks, the PARP's DNA-binding domain is far from its CAT domain, which is not oriented along the DNA helix [35,107,108]. Nevertheless, broad substrate specificity in *trans*, including also auto-modification of different amino acid residues in *cis*, and the multidomain structure of PARPs imply high flexibility of the CAT domain position in DNA–PARP complexes. Therefore, there may be some unexplored abilities to target substrates including formation of oligomeric protein complexes on DNA.

Effective DNA-PAR/MARylation occurs on DNA substrates that mimic intermediate products occurring in various DNA excision repair pathways such as base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), HR, and NHEJ. For example, DNA strand break acceptor sites containing 5'-phosphates can be generated by the action of various DNA exo- and endonucleases, tyrosyl-DNA phosphodiesterase 2 (TDP2), and dRP (5'-deoxyribose phosphate) lyase activity of bifunctional DNA polymerases, whereas 3'-terminal phosphates are produced by certain bifunctional DNA glycosylases, TDP1 and MRE11 [109,110]. DNA duplexes containing a DSB and a proximal SSB can form in HR and NHEJ repair pathways. It has been reported that a stably blocked replication fork can switch the endonuclease activity of MRN–CtIP complex on and produces an internal nick located ~20 nt downstream of 5'-termini of a DSB [111], thus ensuring proximity of activating and acceptor sites required for DNA ADP-ribosylation activity of PARPs.

At present, the physiological relevance of PARP-dependent DNA ADP-ribosylation is a debated issue. Nevertheless, a dramatic substrate switch of PARPs observed *in vitro* assays, the highly efficient PARP1-catalyzed DNA PARylation in human cell-free extracts, and the presence of a PAR signal in purified genomic DNA after genotoxic treatment provide the strong albeit indirect evidence of the presence of PAR–DNA adducts in live cells [14,112]. Furthermore, it has been shown that 1 nt gapped DNA containing a MARylated 5'-phosphate residue is recognized as a 5'-adenylated DNA substrate by DNA ligase I or IIIa or by other

DNA ligases and ligated in the absence of ATP, resulting in the sealed unbroken double-stranded DNA with an aberrant abasic (AP) site-like residue [112]. Such residue can be processed further by apurinic/apyrimidinic endonuclease 1 (APE1) in BER pathway [112]. In line with these results, it has been proposed that PARP2 and PARP3 are involved in the final ligation step of NHEJ, judging by the finding that 5'-phosphorylated nicks are especially efficient activators of the auto-ADP-ribosylation activity of PARP2 and PARP3 but not that of PARP1 [36]. We can hypothesize that DNA ADP-ribosylation can promote retention of the DSB ends either until a complete repair complex is formed or until the ATP concentration required for DNA ligation is restored. Similarly, in case of SSB repair, PARP-mediated ADP-ribosylation can promote the ligation of a gap without polymerase synthesis and ATP. Of note, extensive PARP-mediated PAR synthesis leads to inhibition of hexokinase 1 activity, blockage of glycolysis, and ATP loss [113]. Thus, one of the functions of DNA ADP-ribosylation can be the patching of DNA breaks during bioenergetic collapse avoiding formation or degradation of toxic DSBs.

Recent advances in RNA biology took the phenomenon of ADP-ribosylation to another level. Studies by Shuman's group have revealed that PARP-like tRNA splicing enzymes Tpt1 (KptA) from bacteria and fungi can ADP-ribosylate RNA and DNA at 5'-monophosphate termini [114,115]. Further studies by Ahel's laboratory have shown that Tpt1 homologs in higher organisms TRPT1 (PARP18) as well as human PARP10, PARP11, and PARP15 can MARylate phosphorylated ends of RNA [15]. This 5'-phospho-ADPr modification or "capping" of RNA termini may protect RNA substrates from degradation or dephosphorylation and mediate ADP-ribosylation signaling via recruitment of specific cellular factors. Overall, these RNA related studies provide additional evidence that the phenomenon of ADP-ribosylation of nucleic acids at terminal phosphates is more widespread than previously thought.

ADPr removal from DNA and RNA termini. ADP-ribosylation of DNA and RNA termini is a fully reversible process. We have demonstrated that PARG efficiently restores native DNA structure

by hydrolyzing PAR–DNA or MAR–DNA adducts generated by PARP1, PARP2, or PARP3 [13,14]. Moreover, recently, Ahel's laboratory demonstrated that the MAR moiety that is covalently attached to oligonucleotide termini can also be removed by other cellular hydrolases, such as MACROD1, MACROD2, TARG1, and ARH3, though with much lower efficacy [23,116]. We believe that the half-life of DNA–PAR or DNA–MAR adducts is similar to that of PARylated proteins and may depend on the recruitment of PARG to the sites of ADP-ribosylation. In the absence of PARG, which is the major glycohydrolase in the nucleus, ADP-ribosylated DNA strand break termini would result in persistent DNA damage and is expected to be highly genotoxic as compared to the PARylated proteins: mainly PARPs and histones. Similarly, hydrolases PARG, TARG1, ARH3, and MACROD1 and -2 can restore intact RNA structure after ADP-ribosylation of a 5'-terminal phosphate [15]. The broad substrate specificity of glycohydrolases toward ADP-ribosylated DNA and RNA can be explained by the acid lability of the phosphodiester bond between ADPr and terminal phosphates in nucleic acids, in contrast to the bond between amino acids and ADPr in protein target [14]. Noteworthy, the replacement of a phosphate acceptor group with a thiophosphate residue has little impact on its ADP-ribosylation but makes MAR–DNA adducts resistant to PARG hydrolysis [14].

DNA damage–induced DNA demethylation, ADP-ribosylation, and cell differentiation

DNA damage response and repair proteins play a crucial role in genomic integrity. In addition, these proteins have important functions in gene expression, in particular during organism development and stem cell maintenance and differentiation. For instance, programmed DNA damage and repair are important in the immune response for B- and T-cell development and for generation of the diversity of antigen receptors [117]. In the adaptive immune system, not only DNA repair proteins but also other cell cycle regulators and checkpoint proteins play important roles. They function as regulators of proliferation, of introduction of diversity, and cell cycle arrest during V(D)J recombination and class switch recombination (CSR). Classic NHEJ

is the major pathway repairing the RAG1/RAG2-induced DSBs during V(D)J recombination, whereas in mature B cells, alternative NHEJ and other repair mechanisms such as BER also participate to repair the activation-induced cytidine deaminase (AID)-induced DNA lesions during CSR and somatic hypermutation [118]. In addition to the generation of antigen diversity, during B- and T-cell development, programmed DNA damage is a trigger for the expression of many genes including genes related to cell survival, proliferation, and development [119]. Deamination by AID occurs at the GC-rich transcriptional start sites of heavy-chain and light-chain genes where cytidine is converted into uracil. Uracil-N-glycosylase (UNG), BER, and MMR proteins detect the uracil residues in U•G mispairs. Thereafter, the nick will be introduced into DNA, and subsequent repair of the DNA lesion may lead to deletion, transition, and transversional mutations [120]. Alternatively, the repair of deaminated bases via the nick-initiated long-patch DNA excision-resynthesis mechanism during long-patch BER [121,122], NER [123], and noncanonical MMR [124] leads to replacement of neighboring 5-methylcytidine nucleotides by unmodified nucleotides. Thus, locus-specific AID targeting in transgenic mice has been reported to induce local processive DNA demethylation even ~1 kb beyond its target motif [122]. Activation and regulation of AID are not yet well characterized, although CSR is initiated by AID-induced DNA lesions at least after three cell cycles [120], and AID is more stable in the G1 phase and is regulated by the cell cycle [125]. Accordingly, replication-dependent types of DNA damage may be the prior signal for the initiation of CSR and upregulation of *AID* expression regulated by NF- κ B signaling [126,127]. AID is active on single-stranded DNA (ssDNA), indicating the presence of a secondary structure of DNA (like R-loops) during the initiation of CSR and somatic hypermutation [128]. Indeed, it has been suggested that AID recognizes G4 (quadruplex) DNA structures with at least a 5 nt ssDNA overhang prior to CSR [129].

There are multiple types of stem cells, which exploit endogenous types of DNA damage to regulate their differentiation. Immortalized myoblasts, which follow the fate similar to that of activated skeletal muscle stem cells, need BER factors to differentiate successfully [130]. A

while ago, endogenous DNA damage was observed immediately prior to myotube fusion [131,132]. These DNA lesions have been predicted to be SSBs or DNA nicks, which are some of the major causes of PARP activation [133]. Consistently with these data, Farzaneh *et al.* mentioned the need for PARP activity during terminal differentiation of myoblasts [131]. Later, it was also discovered that these types of DNA damage are induced by caspase-activated DNase (CAD), which in the end leads to changes in gene expression patterns, for instance, upregulation of p21, a cyclin/cyclin-dependent kinase (CDK) inhibitor protein inducing cell cycle arrest [134]. Indeed, these types of CAD-dependent DNA damage may be one of the reasons because they trigger the changes in the DNA methylation pattern of differentiating myoblasts, and these alterations eventually lead to the upregulation of myogenic factors needed for multinucleated myotube fusion [135].

ADP-ribosylation-dependent chromatin remodeling is critical for initiating the expression of genes. For example, neural activation by stimulation of immediate-early-gene expression requires a release of linker histone H1. Accordingly, Azad *et al.* have shown that a release of H1 from the promoter of an immediate early gene requires ADP-ribosylation by PARP1 and phosphorylation of the histone [136]. PARylation-dependent H1 disposition has also been observed in hormone-regulated genes [137]. A growing number of studies have revealed that PARP1 regulates gene expression through PARylation of histone methyltransferases and demethylases [66,138]. This in turn changes the histone H3 and H4 acetylation and methylation statuses and subsequently strongly alters the regulation of gene expression patterns independently of histone ADP-ribosylation, pointing to the critical role of chromatin modifying factors in transcription regulation [28]. Notably, PARylation of epigenetic regulators at DNA damage sites is very important to maintain the histone mark balance to ensure correct spatial and temporal coordination of DNA repair. In this context, ADP-ribosylation may be linked to the repair of DNA damage and chromatin remodeling; however, this mechanism may not be enough to explain the epigenetic regulation of genes responsible for the fate of the cells undergoing either B-cell or T-cell development and during myogenic cell differentiation in the

absence of ADP-ribosylation. Accordingly, ADP-ribosylation may contribute to the signaling cascade during epigenetic regulation since the methylation patterns of the cells are changing at the target sites in related genes and not globally. One of the vivid examples of this phenomenon is the concentration of programmed DNA damage at the promoter site of the *p21* gene during myogenic differentiation [134].

Active DNA demethylation as a source of programmed DNA breaks

Dynamic DNA methylation is one of the major epigenetic regulation mechanisms. DNMTs (DNA methyltransferase enzymes) transfer a methyl group onto the 5' position of cytosine. Methylated cytosine is a signature of repressed gene expression. DNA methylation regulates tissue-specific gene expression, genomic imprinting, and X-chromosome inactivation [139]. Additionally, the majority of gene promoters are located in the proximity of CpG islands, which are CG repeat-rich sequences. Unmethylated and methylated CpG islands are associated with actively transcribed and inactive genes, respectively [140]. Embryonic stem cells have approximately 4% of the CpG islands methylated [141], and this figure increases to 70–80% in somatic cells [142]. This difference can be related to the pluripotency of stem cells or the differentiated or specialized state of somatic cells. To switch pluripotency off, heterochromatinization takes place, followed by DNA methylation. Histones H3 and H4 are methylated. For instance, H3K9 residues get trimethylated by G9a, a histone methyltransferase, and are deacetylated by histone deacetylases. This event is followed by the recruitment and activity of DNA methyltransferases DNMT3 and DNMT3B [143].

DNA methylation can be modulated either for the developmental, differentiated gene expression or to avoid the hypermethylation of CpG islands, which are maintained under hypomethylation state [144]. In vertebrates, the most studied proteins involved in DNA demethylation are activation-induced deaminase/apolipoprotein B mRNA-editing catalytic polypeptide-like (AID/APOBEC) family, which are cytidine deaminases, and the ten-eleven translocation (TET) family of proteins [145]. It has been suggested that AID-catalyzed

deamination of 5-methylcytosine (5mC) to thymine results in the formation of the mismatched base pair G•T, which is repaired by methyl-CpG-binding domain protein 4 (MBD4)-initiated BER [146]. It should be noted that deamination of 5mC may be carried out not only by proteins of the AID/APOBEC family but also by DNA methyltransferases DNMT3A and DNMT3B in the absence of the methyl donor S-adenosylmethionine [147]. The participation of AID/APOBEC families has been extensively reviewed by Rebhandl *et al.*; the authors stated that AID/APOBEC takes part in many different cellular processes mostly by introducing a mutation. These enzymes play a role in B- and T-cell development by mutating immunoglobulin heavy-chain variable genes (IgV), thereby increasing the diversity of generated antibodies. The APOBEC family also participates in immune defense by targeting and mutating viral DNA [148].

TET enzymes belong to the family of 2-oxoglutarate-dependent dioxygenases. There are three members of the TET family in vertebrates: TET1, TET2, and TET3. They are all responsible for the demethylation of 5mC in DNA by oxidizing it into 5-hydroxymethyl-cytosine (5hmC), which can be followed by oxidation into 5-formyl-cytosine or 5-carboxyl-cytosine (5caC) [149,150]. It is believed that each form of oxidized cytosine has specific readers that can bind it, possibly to regulate the expression of genes. During active DNA demethylation, 5-formyl-cytosine and 5caC residues are removed from DNA by thymine-DNA glycosylase (TDG) in the BER pathway [149,151]. An alternative murine demethylation pathway involving 5mC oxidation prior to deamination has been proposed, based on a complex of GADD45A, AID, and TDG detected and the finding that SMUG1, MBD4, or TDG has a glycosylase activity on 5-hydroxymethyl-uracil [145,152].

DNA demethylation of the paternal genome in murine primordial germ cells is mechanistically linked to the emergence of SSBs, high levels of PARP1 expression, and activation of the BER pathway, accompanied by the appearance of γ H2A.X foci and nuclear PARylation [153,154]. Suppression of BER by PARP1- and APE1-specific small-molecule inhibitors increases methylation of the paternal genome both globally and in LINE1 elements [154]. Taken together,

these data indicate that DNA breaks and activation of PARPs are important for active DNA demethylation.

Interplay between PARylation and DNA methylation

PARPs-catalyzed PARylation performs diverse and important functions in the regulation of both DNA methylation and demethylation. Nucleosome-bound PARP1 is enriched in chromatin domains showing lower 5mC content within 1 kb from the transcription start sites of highly expressed genes [155,156]. For example, *Dnmt1*, *p16*, α -SMA (smooth muscle actin alpha), pluripotency-related genes, NF- κ B-dependent genes, peroxisome proliferator activated receptor γ (PPAR γ)-dependent genes, and other genes are regulated by PAR moieties and PARP1 located at the promoter site (for more detailed review see Ciccarone *et al.* [66]). DNMT1 plays an important part in DNA methylation of newly synthesized hemimethylated DNA during replication [157,158]. Zampieri and coauthors demonstrated that PARP1 is colocalized with PAR specifically at one out of the five inferred *Dnmt1* promoter regions [159]. Auto-PARylated PARP1 and PAR polymers interact with both CTCF and DNMT1, thereby preventing their access to DNA and methylation of cytosines [9,160]. DNMT1 contains two PAR-binding domains that render higher affinity of the methyltransferase to PAR polymers than to DNA [159]. Accordingly, overexpression of hydrolase PARG causes increased methylation of the *Dnmt1* promoter and repression of *Dnmt1* expression [159]. The existence of an additional layer of DNMT1 expression control should be noted: through PARP1-catalyzed PARylation of the UHRF1 factor, which mediates ubiquitination of DNMT1, to ensure its timely regulated abundance in S and G2 phases [161]. Competitive PARP inhibition leads to global genomic-DNA hypermethylation mainly concentrated in CGI (CpG island) regions *in vivo*, whereas hyperactivation of PARP1 leads to hypomethylation [159]. In the absence of ADP-ribosylation, a particular gene could also be differently affected as hypermethylated at the CGIs of the promoter site while hypomethylated within the gene body [156]. Finally, PARP1-mediated PARylation of methyl CpG-binding domain protein 2 (MeCP2) impairs its ability to bind 5mCpG sites and reorganize heterochromatin [162]. Overall, these observations suggest

that the dynamic PARylation of target proteins is involved in the crosstalk between PARP1 and DNA methylation.

Nevertheless, the origin of PARP1 activation on those promoter regions remains poorly understood. Based on *in vitro* data, it has been proposed that CTCF directly interacts with PARP1 and induces PARP1 automodification in a DNA-independent manner [9]. Nevertheless, no solid evidence available that this mechanism is responsible for the observed PAR synthesis in the cell. *In vitro* data show that the level of PARP1 activity was ~10-fold higher in the presence of both activated DNA and CTCF as compared to just DNA and CTCF alone [9]. In this regard, the combined action of both DNA breaks and CTCF seems preferable for the induction of PARP1 automodification during PARP1-mediated regulation of gene expression at certain promoter sites in the genome. Moreover, several studies indicate that even though CTCF plays certain roles in the regulation of gene expression, DNMT1 [159] and under certain circumstances p16 can still be expressed in the absence of CTCF but not that of PAR polymers [163]. In the context of genotoxic stress-induced enzymatic modifications of DNA, CTCF is actually recruited to the DNA damage site once there is PAR in the proximity of the lesion. CTCF has 11 zinc finger domains that can bind to DNA and among them zinc finger 4–6 motifs have been reported to recognize and bind PAR chains, which are structurally similar to ssDNA [164]. CTCF can also be PARylated by the PARP proteins. This PARylation seems to be cell-cycle-dependent; proliferating cells hardly contain any ADP-ribosylated CTCF, whereas the cells under cell cycle arrest contain a large amount of modified CTCF. This PARylation also correlates with upregulation of many genes responsible for cell cycle arrest, differentiation, and development [165]. It is noteworthy that Klenova and colleagues have provided evidence that CTCF dissociates from majority of sites on chromatin after PARylation [165]. This idea also supports the notion of CTCF binding to ADPr motifs instead of intact DNA [164].

Moreover, the physical and functional interplays among CTCF, DNMT1, and PARP1 also involve TET proteins. On the one hand, CTCF interacts with and recruits TET proteins to genomic DNA, thus promoting oxidation of 5mC [166]; on the other hand, TET-catalyzed

formation of 5hmC and especially 5caC strengthens CTCF's association with DNA [167,168]. TET expression is positively regulated by the PARylation activity of PARP1, which leads to DNA demethylation [169]. Additionally, covalently PARylated TET enzymes convert 5mC into its oxidized forms more efficiently, although as negative feedback, noncovalent binding of PAR to a TET protein represses its activity [66]. Accordingly, a noncovalent PAR-binding motif has been identified in the catalytic domain of TET1 [170]. It should be emphasized here that 5caC residues can serve as a potential source of SSB and subsequent PARP activation, when they are processed by TDG-dependent BER. Alternatively, TET1 can also activate PARP1 *in vitro* in the absence of nicked DNA [170], making their interactions even more complex. Accordingly, TET1-dependent activation of PARP1 would lead to covalent PARylation of TET1 and induction of its hydroxylase activity, which can be blocked at some point by non covalent interaction of TET1 with PAR. Nevertheless, inactive TET1-PAR complex still can bind DNA, block the access to DNMT1, and act as an anchor for the transcription factors via PAR interaction [170]. Moreover, it was shown that TET1 is recruited by PARP1-dependent PARylation at the PPAR response elements and promotes the local production of 5hmC during adipocyte differentiation [171].

Crosstalk of transcription, DNA breaks, PARylation, and epigenetic regulation

Regardless of exogenous or endogenous DNA-damaging agents, during DNA transcription and replication, different regions of genomic DNA show increased susceptibility to DNA breakage owing to multiple factors such as noncanonical DNA secondary structures (G-quadruplexes, hairpins, or R-loops) or a chromatin context (chromosomal fragile sites) (reviewed in [172,173]). Secondary structures of DNA increase the risk of DNA breaks: either targeted ones such as G4 structures recognized by AID during B-cell development or off-targeted G4s in active gene regions [129]. Another vivid example of transcription-associated DNA damage is the damage due to formation of R-loops: a naturally occurring reannealing of the nascent RNA

transcript with the template DNA strand, with the accompanying displacement of the opposite ssDNA strand. Unscheduled formation of R-loops is associated with DNA breaks, replication fork stalling, and transcription blockage [174]. Notably, aside from transcription pausing, R-loops are also observed in unmethylated CGI sequences in the proximal promoter regions during development, possibly regulating transcription activation and restricting DNA methylation by DNMT3B1 [175]. Formation of R-loops is favored by negative supercoiling behind RNA polymerase II and by a specific sequence context (reviewed in [176]). TOP1 counteracts both positive and negative DNA supercoiling during transcription by introducing SSBs accompanied with the formation of transient TOP1 cleavage complexes (TOP1ccs) at their 3' termini [177]; this event leads to a decrease in the formation of DNA:RNA hybrids [178]. TOP1ccs are readily "trapped" on chromatin and become irreversible covalent adducts, which, if not repaired, can lead to transcription and replication blockage and formation of SSBs and DSBs. It has been reported that cleavage of the ssDNA strand of R-loops by endonucleases, including XPF, XPG, and FEN1, results in TOP1cc-induced DSB production during transcription in nonreplicating cells [179].

Nevertheless, the DNA damage in promoter and enhancer regions upstream of transcription start sites can stimulate transcription instead of blocking it by switching on DNA damage response pathways including PAR signaling (Figure 3). Indeed, activation of estrogen-responsive gene transcription of pS2 is dependent on the β isoform of DNA topoisomerase II (TOP2 β)-induced DSBs in the promoter region [180]. This phenomenon has also been observed in androgen receptor, retinoic acid receptor, and activator protein 1-dependent types of gene transcription [180]. In general, TOP2 has a role of introducing transient DSBs to resolve the concatenated DNA and supercoiling for transcription regulation (TOP2 β) and topological stress during chromosomal segregation and DNA replication (TOP2 α) [181]. The repair of TOP1- and TOP2 β -dependent DSBs is mediated by both NHEJ and HR pathways and is often accompanied by PARP1 recruitment to the DNA damage site and induction of PAR synthesis (reviewed in [182,183]). This leads to modulation of gene expression at different levels

including changes in chromatin structure, DNA methylation, and transcription factor recruitment and regulation of their activity (reviewed in [17,184]). For example, the aforementioned ADP-ribose readers (chromatin remodelers such as ALC1 [84], APLF [79,80]; transcription factors NF- κ B [185] and TAF15 [186]; and DNA damage repair proteins, such as XRCC4, Ligase IV, and MRN [75]) are recruited to the DSB sites by noncovalent PAR binding, frequently becoming a substrate of subsequent PARP1-dependent PARylation as well. Recent research revealed that RNA polymerase II and PARP1-dependent NELF (negative elongation factor) complex recruitment is required for DSB-induced transcriptional repression [187], but concurrently, PARP1-catalyzed PARylation of NELF-E and NELF-A inhibits NELF and promotes transcription elongation [188]. Another example of topoisomerase-dependent and PARP1-dependent gene expression is believed to be *TP53* expression. *TP53* is split into two epigenetic domains depending on its chromatin organization and methylation [189]. Accordingly, the first domain—specifically the promoter region of *TP53*—is hypomethylated, despite being rich in epigenetic histone markers H3K9ac, H3K4me2, H3K4me3, and H4K16ac, which are involved in the regulation of active gene transcription [189]. Furthermore, TOP2, PARP1, HDAC, DNMT1, and CTCF have been found to be localized to this region and additionally with TOP1 and BRG1 (chromatin-remodeling ATPase) in the hypermethylated 2nd domain [189]. One possibility is that the hypomethylated status of the promoter region and epigenetic regulation are maintained by the inhibitory effect of PARP1-dependent ADP-ribosylation on DNMT1 as well as by recruitment of CTCF via its PBZ domain [77] after TOP2-coupled DNA damage [9,160].

Notably, DNA repair and/or processing of transcription and/or topoisomerase-induced DNA damage lead to the formation of terminally phosphorylated DNA breaks that can be targeted for PARP-dependent DNA ADP-ribosylation as discussed earlier. Thus, TOP1cc removal depends primarily on the TDP1-driven excision of the 3'-tyrosine adduct that generates a 3'-phosphorylated terminus [190]. It has been reported that TDP1 directly interacts with PARP1 and that they are epistatic for the repair of Top1ccs [191]. DSB processing during alternative

NHEJ or HR pathways goes through formation of 5' protruding ends also shown to be effective substrates for PARP1-catalyzed PARylation [13,14]. Moreover, PARP1 is activated by DNA secondary structures like DNA hairpins, G4, and stably unpaired regions [192], whose presence at promoter and enhancer sites in the proximity of a DSB will create alternative PARP1 activation or binding sites for the modification of DNA termini. Notably, PARP3-dependent suppression of G4 in response to DNA damage was recently reported [193]. PARP3 deficiency leads to an increase in the content of G4 in G4-rich regions at the sites flanking DSBs, but the molecular mechanism of PARP3 activity at G4 sites remains unknown [193]. It is possible that ADP-ribosylation of DSB termini in these cases will temporarily block their processing or degradation and modulate DNA break signaling, coordinating the choice of a repair mechanism and transcription.

Concluding Remarks

Besides the well-known biological roles of PARPs such as DNA repair, chromatin modification, and gene expression regulation, these enzymes might also be involved in the programmed DNA strand break-dependent regulation of transcription and replication. The majority of genes regulated by PARPs belong to stress-responsive and differentiation-related genes. This observation suggests that DNA strand breaks may be required also for the regulation of expression of some genes among others listed earlier. The recruitment of effectors—whether via nonspecific surface charge binding or through specific interaction with PAR or MAR adducts covalently linked to protein or DNA—may initiate a signaling cascade of epigenetic changes in chromatin, which result in modulation of the patterns of gene expression (Figure 4).

It is tempting to speculate that the PARP-dependent regulation of gene expression involves transient DNA strand breaks at specific promoter sites, which induce ADP-ribosylation of not only of PARPs themselves and other nuclear proteins, but also DNA strand breaks termini, therefore leading to the recruitment of specific DNA repair and transcription factors to the

methylation-free gene promoters. However, this hypothesis awaits confirmation from further technically advanced studies. In the meantime, PARylated DNA along with auto-PARylated PARP1 in proximity may block the access of DNMT1 to DNA and maintain the hypomethylated status, which is prerequisite to gene expression. This idea may also be valid to the regulation of expression of *p16* and *RASSF1A*, which are dependent on the presence of PAR [163]. One of the common features of *DNMT1*, *p16*, and *RASSF1A* is that they are all CTCF target genes [66,163]; in addition, both *p16* and *RASSF1A* take part in cell cycle regulation and arrest during DNA damage response [194-196]. Alternatively, covalent ADP-ribosylation of DNA strand break termini may retard the repair, and this in part may explain the phenomenon of the long-lived DSBs produced by TOP2 in complex with PARP1 at the promoters of genes, which are regulated by the signal-dependent mechanism, such as PSA, RAR β , DIO1 and MMP12, and ER α (estrogen receptor- α) target genes [180]. Notably, androgen-induced TOP2 β -mediated DSBs could be detected even 6 h after hormone stimulation [197].

In addition, new roles of PARPs and PARylation in the RNA biology are emerging. Very recently, using RNA immunoprecipitation it was shown that PARP1 binds thousands of mRNAs and long noncoding RNAs as well as hundreds of small nuclear RNAs and small nucleolar RNAs (snoRNAs). PARP1 binding to snoRNAs leads to its activation and autoPARylation, recruitment, and PARylation of DDX21, an RNA helicase, which, in turn, promotes ribosomal DNA transcription when ADP-ribosylated [198]. Noteworthy, activation of PARPs when bound to RNA or DNA substrates creates a possibility that terminal phosphate residue in proximity to their CAT domain can be ADP-ribosylated. Whether noncanonical RNAs are naturally occurring ADPr acceptors in a living cell remains to be examined in future studies.

At present, it is not clear how specificity of PAR and MAR readers is affected by the nature of ADP-ribosylated target protein residue. Similarly, the specific binding factors of DNA–MAR or DNA–PAR adducts are not yet known, but it is tempting to speculate that they should recognize both the DNA/RNA and ADPr-phosphate moiety. One of the possible readers of ADP-ribosylated DNA strand breaks termini could be aprataxin, which recognizes an ADP residue

on 5' termini of a DNA break formed during an abortive DNA ligation reaction and catalyzes the release of the AMP group [199]. Aprataxin interacts with PARP1, which promotes the recruitment of aprataxin to the sites of DNA breaks [200,201]. The PAR-binding affinity of the FHA domain of aprataxin may also contribute to its recruitment to the site of nicked DNA. Hence, due to structural similarities, the MAR unit at a DNA strand break end [14,112] may also be a good substrate for aprataxin activity along with recruitment of XRCC1 or XRCC4, depending on the nature of the DNA strand break, to finalize the repair of DNA damage via BER or NHEJ, respectively [199].

In conclusion, recent biochemical, structural, and cellular studies have expanded our understanding of the diverse roles of ADP-ribosylation. However, new technologies and tools are required for future studies to reliably distinguish ADP-ribosylated proteins and ADP-ribosylated DNA or RNA products in a live cell, to identify target- and site-specific ADPr readers, and to reveal the functional relevance of site-specific ADP-ribosylation on individual targets [14,41,202].

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FIGURE LEGENDS

Fig. 1. An overview of substrates, sites and products of ADP-ribosyltransferases.

Fig. 2. ADPr recognizing domains

Fig. 3. Schematic representation of the DNA damage–induced ADP-ribosylation and transcription. Prior to transcriptional initiation, DNA is nicked by topoisomerases and/or nucleases introducing SSBs and DSBs. Upon DNA damage, PARP1 (possibly PARP2, PARP3, or other PARPs) will be activated and recruited to the damage site. PARPs ADP-ribosylate themselves, potentially DNA termini, histones, and other proteins, block DNMT1 activity, and recruit DNA repair proteins and transcription factors to the DNA damage sites in the promoter or enhancer region. Then, the affected chromatin decondenses, DNA gets demethylated, and transcription starts. After restoration of DNA integrity and potentially of the methylation pattern, transcription can be blocked again.

Fig. 4. Schematic representation of the cellular responses to programmed and random DNA strand breaks.

Figure 1
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Figure 2

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ADPr recognizing domains

(ADPr)_n
PBM

Terminal ADPr
Macro

iso-ADPr
FHA, WWE, OB-fold

Phosphate of (ADPr)_n
BRCT

Two consecutive ADPr
PBZ

(ADPr)_n as DNA, RNA or a
nucleotide counterpart
RRM, GAR, PIN

Target-specific ADPr readers

Protein-(ADPr)_n
?

Amino acid-(ADPr)_n
?

DNA-phosphate-(ADPr)_n
?

DNA-base-ADPr
?

RNA-phosphate-(ADPr)_n
?

Figure 3

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Figure 4
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