ISSN 0026-8933, Molecular Biology, 2023, Vol. 57, No. 2, pp. 245–257. © Pleiades Publishing, Inc., 2023.
Russian Text © The Author(s), 2023, published in Molekulyarnaya Biologiya, 2023, Vol. 57, No. 2, pp. 254–268.

CELL MOLECULAR BIOLOGY:
FROM DNA REPAIR TO METABOLOMICS

UDC 577.29

Poly(ADP-Ribose) Polymerases 1 and 2: Classical Functions and Interaction with New Histone Poly(ADP-Ribosyl)ation Factor HPF1

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Received August 9, 2022; revised September 7, 2022; accepted September 7, 2022

Abstract—Poly(ADP-ribose) (PAR) is a negatively charged polymer, linear or branched, that consists of ADP-ribose monomers. PAR is synthesized by poly(ADP-ribose)polymerase (PARP) enzymes, which are activated upon DNA damage and use nicotinamide adenine dinucleotide (NAD^+) as a substrate. The best-studied members of the PARP family, PARP1 and PARP2, are the most important nuclear proteins involved in many cell processes, including the regulation of DNA repair. PARP1 and PARP2 catalyze PAR synthesis and transfer to amino acid residues of target proteins, including autoPARylation. PARP1 and PARP2 are promising targets for chemotherapy in view of their key role in regulating DNA repair. A novel histone PARylation factor (HPF1) was recently discovered to modulate PARP1/2 activity by forming a transient joint active site with PARP1/2. Histones are modified at serine residues in the presence of HPF1. The general mechanism of the interaction between HPF1 and PARP1/2 is a subject of intense research now. The review considers the discovery and classical mechanism of PARylation in higher eukaryotes and the role of HPF1 in the process.

Keywords: poly(ADP-ribosyl)ation, PARylation, PARP1, PARP2, poly(ADP-ribose), HPF1, histones

DOI: 10.1134/S0026893323020140

INTRODUCTION

Poly(ADP-rybosyl)ation (PARylation) is a post-translational protein modification that is catalyzed by poly(ADP-ribose) (PAR) polymerases (PARPs). PARylation regulates many key cell processes, such as DNA repair, DNA replication, structural organization of chromatin, gene expression, RNA processing, ribosome biogenesis, and translation [1–10]. PARylation is known to regulate both functions and intracellular localization of proteins and to play a role in the formation of membraneless cell compartments [4, 11–13]. Thus, crucial regulatory processes involve PARPs and PARP-catalyzed reactions of PAR synthesis and protein PARylation in higher eukaryotes. This explains why PARP1 is a primary target that the cell destroys during apoptosis [14, 15].

Abbreviations: ARH3, ADP-ribosylhydrolase 3; ART, ADP-ribosyltransferase; BER, base excision repair; HDAC, histone deacetylase; HPF1, histone PARylation factor 1; MAR, mono(ADP-ribose); MARylation, mono(ADP-ribosyl)ation; MSK1/2, mitogen- and stress-activated protein kinase 1/2; PARP1/2, poly(ADP-ribose)polymerase 1/2; PARG, poly(ADP-ribose)glycohydrolase; Polβ, DNA polymerase β; PAR, poly(ADP-ribose); PARylation, poly(ADP-rybosyl)ation; XRCC1, X-ray repair cross-complementing protein 1.

Two DNA-dependent enzymes of the PARP family, PARP1 and PARP2, are the main enzymes that catalyze PARylation and synthesis of extended PAR polymers in the nucleus [5, 16]. Overlapping functions are performed in regulating DNA repair by PARP1 and PARP2 [16]. Both of the enzymes are involved in base excision repair (BER) [17–19] and interact with DNA molecules mimicking the intermediates of the short- and long-patch BER pathways [20–24]. Protein–protein interactions with PARP1 and PARP2 were demonstrated and assessed quantitatively for the BER participants: DNA polymerase β (Polβ), X-ray repair cross-complementing protein 1 (XRCC1), and DNA ligase III [18, 19, 25]. An effect of PARP1 and PARP2 on BER was demonstrated using lesion-containing synthetic DNA duplexes and nucleosomes [17–19, 26]. In addition, PARP1 was shown to play a role in regulating double-strand break repair and elimination of bulky lesions from DNA [27–29].

Given the key role that PARP1 and PARP2 play in regulating DNA repair and other cell processes, the enzymes are thought to be promising targets in searching for new anticancer drugs and drugs to treat neurodegenerative disorders [30–34]. It should be noted that 365 reviews published in 2021 considered the

development of PARP inhibitors as potential drugs and more than 1400 original articles reported works in the field in the same year. International meetings are held annually to discuss PARPs and PARylation because the field is rapidly developing and is of immense importance for medicine. Many PARP1 inhibitors are currently tested clinically and preclinically. Olaparib, rucaparib, niraparib, and talazoparib are already used in medicine to treat cancer. Ovarian and breast cancers, especially cases with *BRCA1/2* mutations, are most intensely treated with PARP1 inhibitors as chemotherapeutic agents. Several PARP inhibitors are now at the stage of preclinical and clinical trials as drugs to treat other cancers and anti-inflammatory agents to treat myocardial infarction, stroke, and other disorders [30, 35, 36].

Although studies of PAR synthesis and PARPs started as early as the 1960s, a new protein cofactor of PARP1 and PARP2 was recently found and termed histone PARylation factor 1 (HPF1) [37]. HPF1 regulates the activities and specificities of PARP1 and PARP2 and forms a transient joint active site with the enzymes [38]. The interaction switches the PARylation specificity from aspartate, glutamate, and other amino acid residues to serine residues [39]. HPF1 plays an important role in histone PARylation catalyzed by PARP1 and PARP2 [40–42]. The mechanism of PARP1/PARP2 interactions with HPF1 and its functions in the chromatin context have only recently come to be investigated, and this field of research attracts great interest.

HISTORY OF POLY(ADP-RIBOSYL)ATION STUDIES

In 1963, Mandel and colleagues [43] worked in Strasbourg (France) and reported the first indications of PARylation. Nicotinamide mononucleotide (NMN) was shown to cause a 1000-fold increase in the incorporation of [^{14}C]adenine from ATP into an acid-insoluble fraction of chicken liver nuclei. Polyadenylic acid (poly(A)) was initially assumed to be an acid-insoluble reaction product. Further studies showed that PAR is produced in these conditions [44]. PAR studies were launched in the National Cancer Center in Tokyo at the same time [45]. Japanize researchers confirmed the NMN-induced accumulation of an acid-insoluble polymer in the rat liver and hepatoma cells. Interestingly, it was already hypothesized at that time that synthesis of the product is associated with cancer cell growth. The structure of the polymer and participants of its biosynthesis were established in biochemical studies. Experiments showed that NAD^+ is produced in the nucleus from NMN and ATP by NAD pyrophosphorylase and that the ADP-ribose moiety of NAD^+ is then used to synthesize PAR and to release nicotinamide [45]. Thus, the study reported by Mandel and colleagues in 1963 [43] was the first impetus and received further development in other labs worldwide.

It was long believed that PARP1 is the only enzyme that possesses PARylation activity in mammalian cells [1]. However, five different genes coding for other PAR and mono(ADP-ribose) (MAR) polymerases were identified over several years of intense research. There are 17 different proteins in the PARP family now [46–49].

PAR was found to be a linear and branched polymer that consist of ADP-ribose units linked by glycosidic bonds [16]. PAR synthesis utilizes NAD^+ as a precursor and a direct PARP substrate. Constitutive PAR levels are usually rather low in unstimulated cells [50]. However, PARP activity and the PAR level can increase by a factor of 10–500 in response to genotoxic stress (that is, when breaks appear in DNA). The PAR structure is understood well. ADP-ribose units are linked through ribose–ribose 1'-2' glycosidic bonds in PAR. The chain length varies among PAR polymers and can reach 200–400 monomeric units in vitro and in vivo [51, 52]. The majority of free and protein-bound PAR polymers synthesized in genotoxic stress is rapidly hydrolyzed by PAR glycohydrolase (PARG) and has a half-life ranging from 40 s to 6 min in vivo, pointing to a dynamic nature of the process in living cells [53]. Efficient synthesis and subsequent fast hydrolysis of PAR determine the dynamic nature of the regulation of PARP-dependent processes.

STRUCTURES OF PARP1 AND PARP2 AND SPECIFICITY OF THEIR INTERACTIONS WITH DNA

The PARP superfamily includes at least 17 enzymes, which are involved in regulating many biological processes, such as transcription, DNA repair, replication, the cell cycle, and others [10, 47, 48]. A conserved catalytic (CAT) domain is a common feature of PARP family members. The CAT domain harbors a highly conserved sequence, which is known as the PARP signature and forms the active site in PARP-family enzymes PARP [49, 54]. MARylation, rather than PARylation, is catalyzed by certain enzymes of the PARP family [48]. PARP1 was most comprehensively studied in the PARP family. PARP1 serves as a sensor of DNA lesions usually caused by ionizing radiation and oxidative stress and triggers the recruitment of necessary proteins to DNA damage sites [55, 56]. PARP2 was also identified as an enzyme that catalyzes PAR synthesis in the nucleus [57]. The role of PARP2 and its cooperation with PARP1 are a matter of intense research [10, 47, 58, 59].

The PARP1 structure is conserved to a great extent, and an amino sequence identity of approximately 62% is observed among various vertebrates. Human PARP1 is 113 kDa in molecular weight. The enzyme consists of three structural and functional domains: a N-termi-

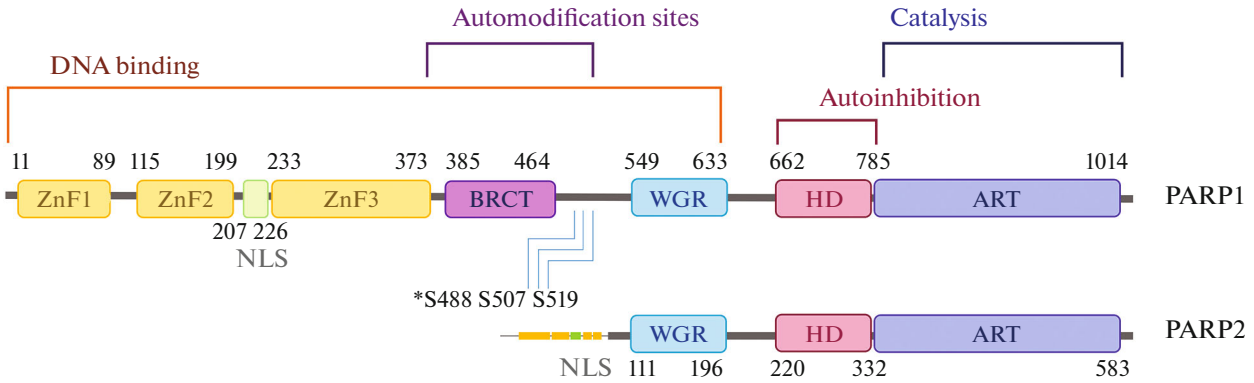

Fig. 1. Domain structures of PARP1 and PARP2. Domain functions are specified at the top and bottom. Asterisks indicate the three serine residues that act as predominant targets for PARylation by PARP1 in vivo.

nal DNA-binding domain (NTR), an automodification region, and a C-terminal catalytic domain (Fig. 1) [60, 61]. The DNA-binding domain harbors three zinc fingers (ZnFs): ZnF1 and ZnF2 are homologous, while ZnF3 differs from them. A mutation analysis of ZnF1 and ZnF2 showed that ZnF1 plays a key role in DNA-dependent PARP1 activity in vitro, while ZnF2 is inessential, but may play an important role in PARP1 binding to certain DNA lesions [62]. ZnF3 ensures contacts of different domains to organize the functional structure of the protein [63]. A nuclear localization signal (NLS) is additionally in the NTR domain and harbors a site at which PARP1 is cleaved by caspases during apoptosis. The central region includes the BRCT motif, which is involved in forming protein–protein contacts, and an automodification site, which contains potential PAR acceptors: 15 glutamate and three serine residues [64–66]. The C-terminal part consists of a catalytic domain (ART), a regulatory helical domain (HD), and a WGR domain [61]. The catalytic domain is nearly identical in various species [49].

PARP2 is 66 kDa protein. A substantial structural homology is characteristic of the C-terminal catalytic domains of PARP1 and PARP2, but their N-terminal regions greatly differ. While PARP1 has three ZnFs and the BRCT domain, the NTR domain of PARP2 is far shorter and is disordered in secondary structure [61, 67, 68]. The structural differences in NTR affect the interactions of the enzymes with DNA. PARP1 mostly utilizes its ZnFs to bind to damaged DNA [61]. The WGR and BRCT domains are also involved in the interaction with undamaged DNA via the monkey bar mechanism, which mediates PAPR1 migration along DNA [65, 69]. Because PARP2 lacks ZnFs, its NTR and WGR domains are involved in interacting with DNA lesions [70]. The differences in domain structure and DNA binding mechanisms are possibly responsible for differences in affinity for particular DNA lesions and catalytic activity between PARP1 and PARP2 [20, 26, 71]. In addition, PARP1 and PARP2 differ in affinity for undamaged DNA [71].

In experiments in vitro, PARP2 shows a weaker catalytic activity and synthesizes shorter PAR chains as compared with PARP1. PAPR1 efficiently binds DNA and is activated by a broad range of DNA lesions in vitro, while PARP2 is thought to be more specifically activated by DNA with breaks flanked by 5'-phosphate, including cleaved apurinic (AP) sites [70, 72–74]. PARP1 has generally higher affinity for damaged and intact DNAs as compared with PARP2, while PARP2 is more efficient than PARP1 in binding the 5'-phosphate-flanked single-strand DNA breaks [62, 75–77]. PARP1 interacts with early BER intermediates, while PARP2 most efficiently works on single-strand breaks, which are substrates of the last ligation step [56–58, 61, 76].

SEVERAL ASPECTS OF THE FUNCTIONAL ROLES OF PARP1 AND PARP2 IN THE CELL

PARP1 ensures a major part (approximately 90%) of PARP activity observed in human cells in response to DNA damage, while PARP2 accounts for 10–15% of the total activity [12]. Because PARP2 automodification is far slower, PARP2 is often considered as a catalytically less active analog of PARP1. Although PARP2 has substantially lower catalytic activity and PARP1 and PARP2 differ in affinity for DNA lesions [71], a single knockout in PARP1 or PARP2 is not lethal for the cell, while a double knockout in the two enzymes causes embryonic lethality. As a result of genetic instability, *PARP1*$^{-/-}$ and *PARP2*$^{-/-}$ cells have higher sensitivity to DNA-damaging agents [78]. Moreover, a *PARP2* knockout in mouse models leads to defects in T-cell development [79], erythropoiesis [80], and spermatogenesis [81], which are not observed in mice devoid of PARP1, suggesting unique functions for PARP2. PARP2 presumably compensates for lack of PARP1 in the response to genotoxic stress, but the mechanism of this compensation is still unclear, given the lower rela-

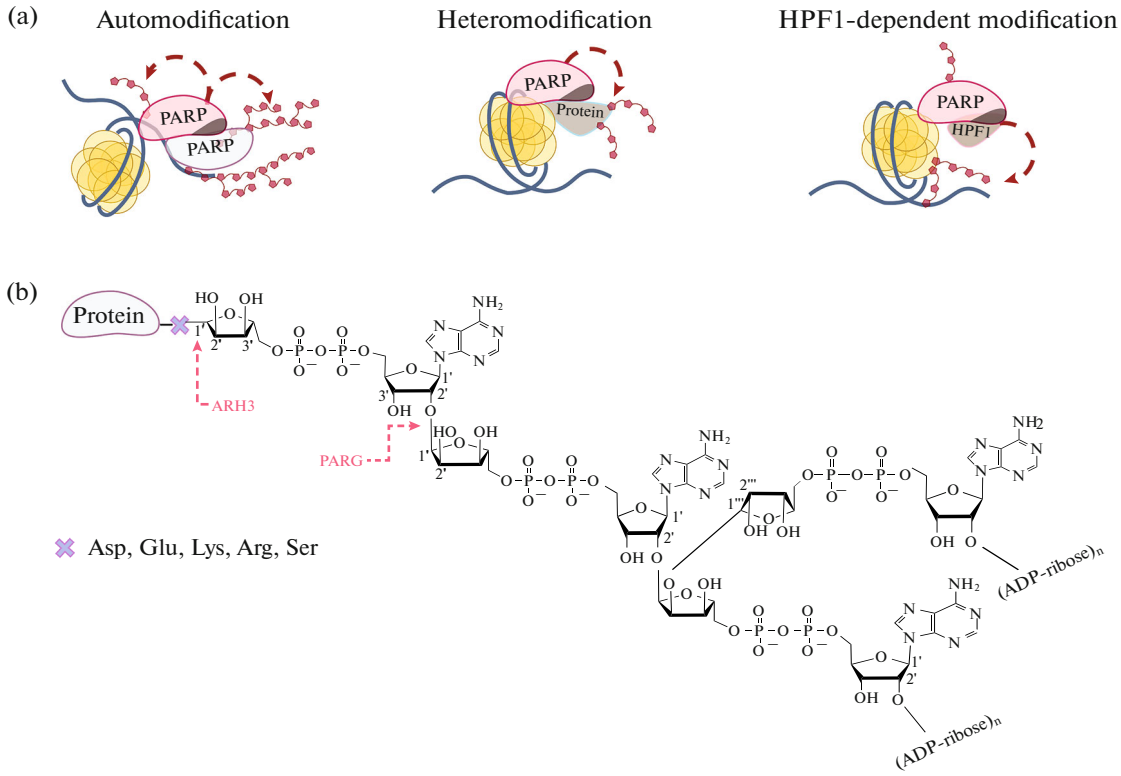

Fig. 2. Structures of (a) possible PARP complexes and (b) PAR. PAR modification of PARP (automodification), modification of target proteins (heteromodification), and HPF1-dependent modification of serine residues. ARH3, ADP-ribosylhydrolase 3; PARG, PAR glycohydrolase.

tive activity of PARP2 and differences in affinity for DNA lesions between the two enzymes. Activity stimulation by RNA-binding proteins, such as YB1 [82, 83] and SAM68 [84], and an interaction with Fus [85] were demonstrated for PARP1. As for PARP2, studies of its interactions with activity-modulating proteins are in their infancy.

DNA lesions act as activating cofactors for PARP1 and PARP2 [61, 68, 70, 86, 87]. Binding with a DNA lesion induces a chain of interdomain rearrangements in PARP1 and PARP2 and eventually changes the conformation of the autoinhibitory helical domain (HD) [61, 86, 88, 89]. HD shields domain the active center in PARP1/2. Its reorganization catalytically activates the enzymes and facilitates NAD^+ binding in the active center. Once activated, PARP1 and PARP2 synthesize and covalently attach PAR to amino acid residues of PARPs and various other target proteins. Two reaction types are recognized in this context: autoPARylation is an automodification reaction whereby a PAR molecule is covalently attached to PARP and heteroPARylation is a heteromodification whereby PAR is attached to another target protein (Fig. 2). The mechanism of autoPARylation is still an open question. On the one hand, there is evidence that a cis mechanism mediates automodification of PARP1; i.e., the same enzyme molecule synthesizes and accepts the PAR chain [90, 91]. On the other hand, dimers of PARP1 and PARP2 were reported to form and to accelerate PARylation via a contribution of a trans-modification mechanism [92, 93]. For example, light scattering data indicate that PARP1 and PARP2 occur mostly as dimers in solution [93]. The assumption that automodification of PARP1 and PARP2 occurs in an intermolecular manner within a dimer of two subunits agrees with the fact that PARP1 and PARP2 are capable of covalent modification of other proteins [52, 83]. An additional argument for a bimolecular nature of the autoPARylation reaction is provided by the stoichiometry of PARP1–DNA and PARP2–DNA complexes, which must be 2 : 1 to ensure optimal enzymatic activity [94, 95].

The roles that PARP1 and PARP2 play in the mechanisms of BER and single-strand break repair were a subject of intense research [17, 18, 23, 24, 26]. The functions of PARP1 and PARP2 in regulating these processes were established using model DNA duplexes and nucleosomes [17, 26, 72]. PARP1 and PARP2 are now known to perform both common and specific functions to maintain the genome stability and to cooperate with each other. It is possible that partner proteins contribute to the separation of PARP1 and PARP2 functions in various processes, and identification and functional studies of PARP1 and PARP2 coenzymes are therefore of particular interest.

HPF1 IS A NEW COFACTOR OF PARP1 AND PARP2

Mostly glutamate and aspartate residues and, to a lesser extent, lysine and arginine residues were initially found to undergo modification in proteins by PARP1 and PARP2 [96–98]. However, serine residues were recently identified as common PAR acceptors in human cells, in particular, in the context of double-strand break repair [39–41]. PARP1 and PARP2 were found to be necessary, but insufficient for serine PARylation [42]. Histone PARylation factor 1 (HPF1) forms unstable transient complexes with PARP1 and PARP2 as judged from their high dissociation constants: the K_d values of PARP1/2–HPF1–nucleosome complexes are 790 and 280 nM, respectively [99, 100]. A transient joint active site is formed by HPF1 with PARP1/2 to modify serine residues [37]. Human HPF1 was shown more recently to interact with PARP1 and PARP2, thus facilitating histone PARylation (PARP1 and PARP2 do not modify histones in the absence of HPF1 in vitro) [39].

The interaction of HPF1 with PARP1 is enhanced in the presence of DNA and NAD^+. As mentioned above, PARP1 and PARP2 have HD, which rapidly unfolds upon recognition of a DNA lesion to open the NAD^+-binding site [87]. A deletion of HD increases the HPF1–PARP1/2 interaction in vitro [38]. It is thought that the HD subdomain inhibits the enzyme binding with HPF1 and that DNA-induced unfolding of HD is necessary for the enzyme–cofactor interaction.

The HPF1 structure in complex with the catalytic domain of PARP2 was recently solved by X-ray analysis and cryo-electron microscopy [38, 101]. Conserved Asp283 of the C-terminal region of HPF-1 was found to be necessary for the HPF1 interaction with PARP1/2 and to form contacts with His826 of PARP1 or His381 of PARP2. It is important to note that these histidine residues of PARP1/2 are critical for PARylation elongation and extension of the PAR chain [102].

Structural and mutation analyses of the HPF1–PARP2 complex showed that introduction of the catalytic Glu284 residue of HPF1 in the joint active site may explain the HPF1-mediated switch of PARP1/2 amino acid specificity [38]. PARP1 and PARP2 contain a single glutamate residue (Glu988 and Glu545, respectively), and these residues were shown to play a crucial role in initiating PARylation [103]. However, the active-site glutamate residue alone was found to be insufficient for PARP1 and PARP2 to catalyze ADP-ribosylation of serine residues [39]. The interaction of HPF1 with PARP1/2 positions Glu284 of HPF1 in the vicinity of the catalytic glutamate of PARP1/2 and NAD^+, thus producing the active site that is capable of catalyzing the efficient transfer of ADP-ribose to serine. Mutant HPF1 with a substitution of Ala for Glu284 is devoid of the capability of switching the PARP1/2 amino acid specificity to serine residues [38]. Deprotonated Glu284 of HPF1 can act as a base in the reaction, pulling a proton from the acceptor serine residue to make it more nucleophilic. HPF1 acts essentially to increase the serine nucleophilicity for efficient catalysis. The process is similar to what occurs in active centers of serine proteases, esterases, and lipases, with the only difference that a Ser-His-Asp catalytic triad is responsible for increasing the serine nucleophilicity in the case of proteases [104]. Note that the formation of the joint active site with substrate-binding and catalytic residues of PARP1/2 and HPF1 resembles the similar functions of GTPase-activating proteins, which also introduce the catalytically essential amino acid residue in the active center of an enzyme [38].

It is of interest that HPF1 binding results in synthesis of shorter PAR polymers [37]. It was shown that Asp283 of HPF1 interacts with His381 of PARP2 (and the respective His826 of PARP1) and occupies a negatively charged binding pocket, which is necessary for recognizing the pyrophosphate group of the acceptor ADP-ribose unit during PAR chain elongation (Fig. 3) [38]. Similar results were obtained for His826 of PARP1. A PARP1 mutant with charge reversion at position 826 (His826Glu) lost the capability of forming the joint active site with HPF1. The mutant catalyzed PARylation of aspartate and glutamate residues, but synthesized only short PAR polymers. Thus, HPF1 binding with PARP1/2 blocks the histidine residue responsible for polymer extension (His826 and His381 in the active centers of PARP1 and PARP2, respectively), thus precluding PAR elongation.

A conclusion that HPF1 stimulates the initiation of PARylation was independently made by two research teams in the past years [105, 106]. Subequimolar HPF1 concentrations were observed to increase the initial rates of PARP1 and PARP2 automodification and to increase the level of PAR synthesis. At the same time, high (micromolar) HPF1 concentrations inhibited PAR synthesis [105]. Inhibition occurs because elongation is suppressed in the situation where the elongation center is occupied by HPF1 at its higher concentration that corresponds to a saturating concentration [99, 100]. The saturating HPF1 concentration is 2–5 μM. When occurring at a lower concentration, HPF1 probably dissociates from its complex with PARP after the initiation of PARylation in the joint active site as a result of its low affinity. This was confirmed in experiments that demonstrated elongation of PAR synthesis by free PARP [64, 105]. Thus, saturating HPF1 concentrations hinder elongation in the active center of PARP and decrease PAR synthesis, while lower HPF1 concentrations stimulate PAR synthesis. This is an interesting mechanism that regulates PAR synthesis.

The effects that HPF1 exerts on PAR synthesis agree with its effects on NAD^+ hydrolase activities of PARP1 and PARP2. The capability of hydrolyzing NAD^+ without synthesizing PAR concomitantly was earlier observed for PARP1 in the absence of HPF1

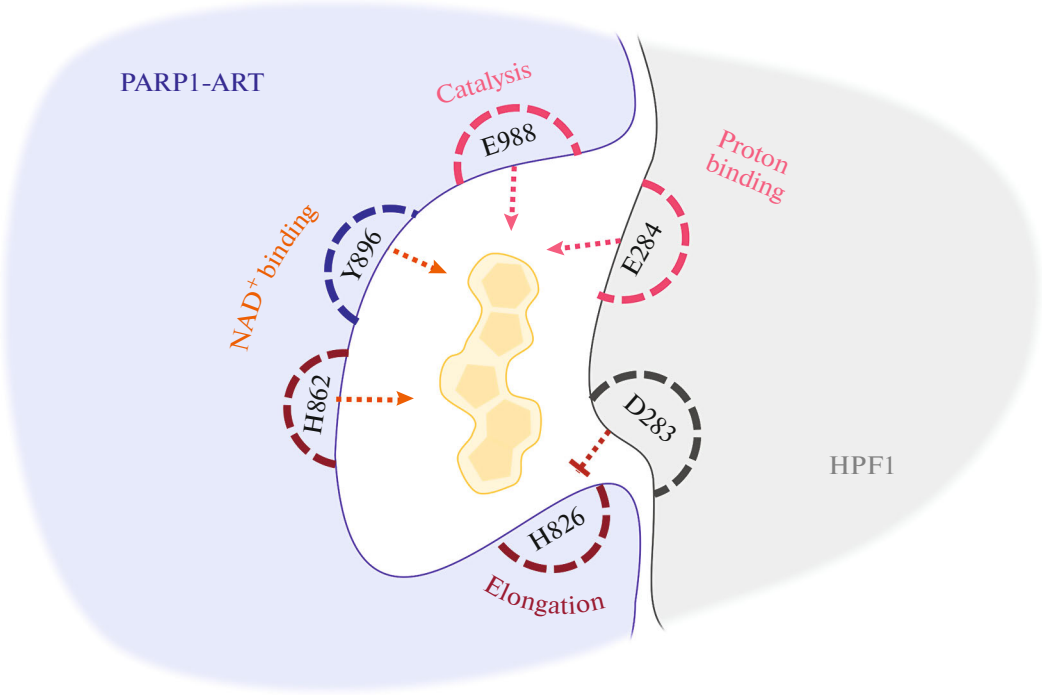

Fig. 3. Scheme of the joint active site of PARP1 and HPF1 and the functions of amino acid residues. The H-Y-E triad (H826, Y896, E988) is involved in positioning NAD^+ in the ADP-ribosyltransferase center (ART) and catalysis. HPF1 provides a glutamate residue (E284), which ensures deprotonation of a serine residue to render it more nucleophilic. E988 of PARP1 is involved in the nucleophilic attack of the nicotinamide–ribose bond by the deprotonated serine residue to complete the initiation step. H826 of PARP1 is necessary for elongation and is shielded by D283 of HPF1 in the joint active site.

[107]. This activity is stimulated by HPF1 used at higher concentrations relative to PARP1 (a 20-fold excess) [102]. Two factors were assumed to explain the PARP1 switch to NAD^+ hydrolysis and production of free ADP-ribose: PAR elongation is impossible at saturating HPF1 concentrations, and when sites for synthesis initiation are exhausted, PARP utilizes water as an acceptor of ADP-ribose, hydrolyzing NAD^+. However, a HPF1-dependent decrease in "idle" NAD^+ hydrolysis was observed in PARylation-stimulating conditions in our experiments [105]. It seems that subequimolar HPF1 concentrations do not saturate all PARP active sites. PARP molecules free of HPF1 may both provide PARylation sites and catalyze elongation [105]. It should be noted that the HPF1 concentration is far lower than the PARP1 concentration and comparable with the PARP2 concentration in the cell [37]. Thus, the relative HPF1 concentration is far lower than necessary for saturating the PARP1/2 active sites according to the dissociation constants established for PARP1/2–HPF1–nucleosome complexes (790 and 280 nM, respectively) [99, 100]. This ensures the optimal conditions for the stimulation of initiation without suppressing elongation and increasing NAD^+ hydrolysis and testifies again that PARP1/2 automodification occurs in dimers in the presence of HPF1, one subunit being associated with HPF1 to form the transient joint active site and the other serving as a PAR acceptor [105].

Thus, opposite effects are exerted by HPF1 on the initiation and elongation of PAR synthesis. The initiation is most likely stimulated by introduction of the additional catalytic glutamate residue in the active center of the enzyme. The elongation is inhibited because HPF1 interacts with PARP1 His826, which is involved in the active center and is important for elongation. A balance between the two effects is mostly determined by the HPF1–PARP concentration ratio in both in vitro and in vivo experiments [99, 100, 105].

RECRUITMENT OF PARP1, PARP2, AND HPF1 TO DNA DAMAGE SITES

Data obtained in early studies of HPF1 gave grounds to assume that HPF1 is involved in DNA repair together with PARP1. For example, Gibbs-Seymour et al. [37] showed that HPF1 limits hyperautomodification of PARP1 in vivo and in vitro and is recruited to DNA lesions. A HPF1 knockout substantially increases the cell sensitivity to DNA-alkylating agents, such as methyl methanesulfonate (MMS), and increases the cell sensitivity to PARP inhibition. A *HPF1*$^{-/-}$*PARP1*$^{-/-}$ double knockout slightly decreases the cell sensitivity to MMS and PARP inhibitors as

compared with the single knockouts, thus partly restoring the wild-type phenotype [37].

When PARP1 is recruited in complex with HPF1 to DNA lesions, HIF1 remains at the damage sites longer than PARP1 [37, 38, 64]. PARP1 is recruited to DNA damage sites within the first 30 s. HPF1 is recruited to DNA damage sites together with PARP1, but the HPF1 recruitment dynamics is independent of PARylation and is most likely mediated by protein–protein interactions [37]. PARP1 seems to leave the damage sites within 2 min [64], while HPF1 remains at the sites for up to 5 min [37]. Treatment with PARP1 inhibitors retains both PARP1 and HPF1 at the damage sites [37, 108]. A *HPF1* similarly affects the duration of PARP1 retention on DNA damage sites; i.e., the duration increases in this case [64]. This seems to affect the dynamics of protein recruitment to DNA lesions, supporting the HPF1 role in transmitting the DNA damage signal. These findings make it possible to assume that catalytic activity of PARP1 is necessary for its dissociation from damage sites and that HPF1 accelerates the process, most likely, by stimulating the initiation of PARylation [105].

PARP2 is recruited to DNA damage sites later than PARP1 [108, 109]. For example, catalytic activation of PARP1 was shown to accelerate the PARP2 recruitment. However, its effect is not critical because PARP2 is still recruited to DNA damage sites in the absence of PARP1, although with a certain lag [109]. It is possible to assume that PARP1 in complex with HPF1 catalyzes PARylation and that newly synthesized PAR accelerates the PARP2 recruitment to damaged DNA. Thus, PARP1, HPF1, and, later, PARP2 colocalize at DNA damage sites in chromatin. In this context, the in vitro finding that HPF1-dependent histone modification by PARP2 is more efficient than by PARP1 is possible to consider as indirect evidence that HPF1 plays a role in the response to DNA damage at the chromatin level [105].

DYNAMICS OF SERINE-LINKED PAR SYNTHESIS AND DEGRADATION

As mentioned above, PARylation is a reversible posttranslational modification. PAR-degrading enzymes exist along with the enzymes that synthesize and interact with PAR polymers [6]. PARG is an important PAR-degrading enzyme. Its mechanism of action is such that the first ADP-ribose residue bound with an amino acid residue of a target protein is not removed by PARG [110, 111]. A MARylated protein is a product of the PARG-catalyzed reaction and serves as a substrate of MAR-removing enzymes. The PARG function is critical to the cell because a double knockout in *PARG* leads to PAR accumulation and early apoptosis in human cell lines and embryonic lethality in mice [112]. Thus, efficient PARG-mediated PAR turnover is necessary for the function of cell systems after DNA damage [17, 85].

MAR arises as a product of MARylation or PARG action and is removed by several enzymes that belong to the families of (ADP-ribosyl)hydrolases (ARH1, ARH2, and ARH3) and macrodomain-containing (ADP-ribosyl)hydrolases (MacroD1, MacroD2, and TARG1) [113–115]. Of these, ARH3 is the only (ADP-ribosyl)hydrolase that removes MAR from serine residues [116, 117]. The other enzymes hydrolyze MAR bound with aspartate, glutamate, and arginine residues.

Newly synthesized PAR polymers seem to be rapidly degraded by PARG. The stability of MAR that remains covalently linked to PARP1/2 is determined primarily by the nature of the MAR-carrying amino acid residue. Proteomic studies of PARylation sites showed that modified serine residues are the most prevalent and most stable in vivo. The data are supported by the fact that aspartate and glutamate ribosylation is detected only in *PARG* knockout cells [97]. At the same time, serine MARylation is reliably observed in wild-type cells [112]. The stability of this modification is regulated predominantly by ARH3 [112]. Thus, catalytic activity of the HPF1–PARP1/2 complex, synthesizing PAR, is counteracted by the PAR-eliminating enzymes PARG and ARH3 [116–118].

HISTONE PARYLATION AND CANONICAL HISTONE MARKS

The functional significance of histone PARylation is related to chromatin state control. Histones H2B and H3 or, more exactly, their amino acid residues H2BS6, H3S28, and H3S10 were identified as preferential substrates of PARP1/2–HPF1 [39]. H3S10 is a more efficient acceptor of ADP-ribose than H3S28 [40]. Note that the above histone PARylation sites act as phosphorylation sites as well [41, 119]. H2BS6 is phosphorylated in early mitosis and totally dephosphorylated once mitosis is complete. H2BS6 phosphorylation of is especially high in centromeric and pericentric regions, and its blockage distorts anaphase and leads to incomplete cytokinesis [120]. H3S10 phosphorylation is initiated in pericentric heterochromatin in late-interphase G2 cells. Once initiated, H3 phosphorylation seems to spread throughout chromatin. There is generally an exact spatiotemporal correlation between H3 phosphorylation and early chromatin condensation [121]. PARylation of H2BS6 and H3S10 may exert a regulatory effect and arrest the cell cycle in the case of DNA damage. Hananya et al. [122] showed that H2BS6 PARylation inhibits chromatin condensation and organization of higher-order structures until repair is complete, thus supporting the above hypothesis.

Phosphorylation of H3S28 together with H3S10 is involved in regulating transcription [123]. A combination of H3S10 phosphorylation with H3K9 or H3K14 acetylation most likely plays an important role in regulating transcription [124–126]. In particular, fast temporal phosphorylation of histone H3 at Ser10 and Ser28 by mitogen- and stress-activated kinases 1 and 2

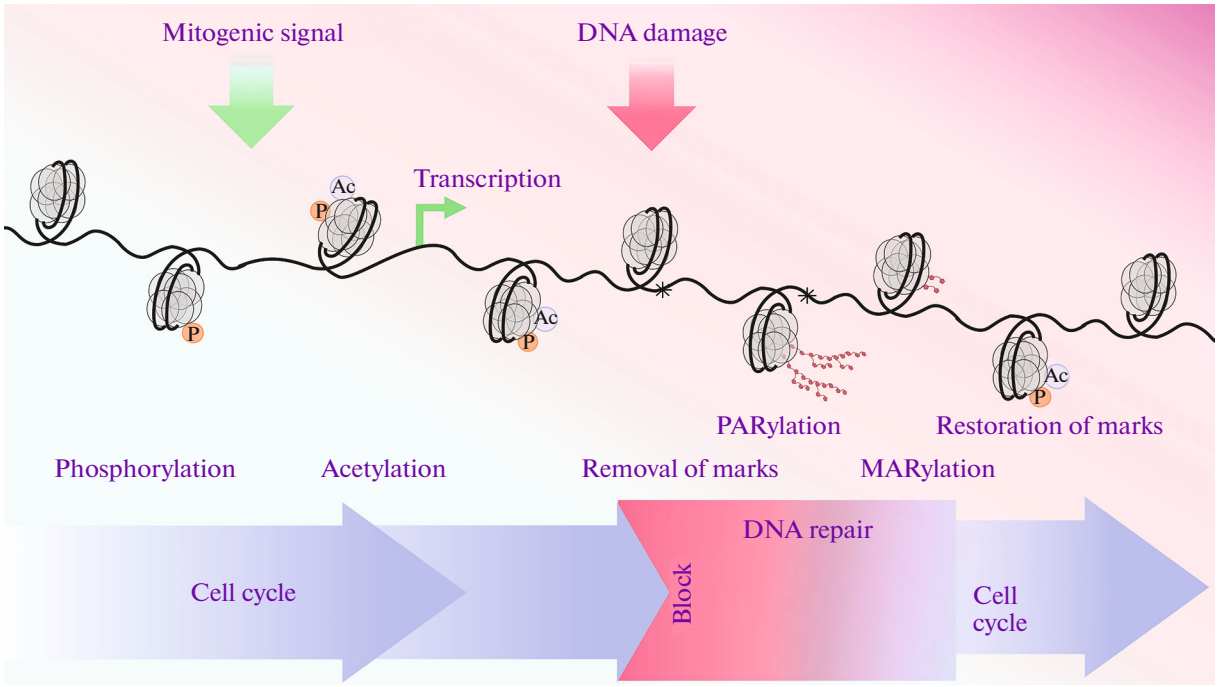

Fig. 4. Changes in the pattern of posttranslational modification of histones during the cell cycle and in response to DNA damage.

(MSK1 and MSK2) is observed upon transcriptional activation of early response genes [125]. MSK1/2-mediated phosphorylation of H3S28 on stress-responsive promoters was found to facilitate dissociation of HDAC corepressor complexes, thus increasing local histone acetylation and subsequent transcriptional activation of stress-induced genes [123]. It was demonstrated that PARylation of histone H3 at serine residues prevents its acetylation and, vice versa, acetylation of H3K9 is sufficient for blocking PARylation of H3S10 [127]. In other words, phosphorylation and acetylation suppress HPF1-depenent histone PARylation. This explains the contribution of histone deacetylase in regulating DNA repair [128, 129]. Deacetylation of H3K9 and H3K14 was observed in response to DNA damage [130, 131]. Acetylation restricts the spreading of histone PARylation at the same time, thus preventing a too broad spreading of the DNA damage signal, and is restored when DNA repair is complete. In ARH3 knockout cells, the level of H3K9 acetylation in response to DNA damage remains lower for a longer period of time than in wild-type cells. Stable PARylation of histones seems to prevent restoration of their acetylation level [112]. This may render H3K9 and H3K14 acetylation and histone PARylation mutually exclusive (Fig. 4).

Thus, a balance between PARylation and phosphorylation of serine residues is regulated by kinases, deacetylases, and PAR-eliminating enzymes. While phosphorylation and acetylation are thought to be markers of transcription and cell progress through the cell cycle, PARylation most likely regulates an arrest of these processes to give the cell time to restore the DNA structure in the case of DNA damage.

CONCLUSIONS

(ADP-ribosyl)ation is a posttranslational modification and consists in the addition of ADP-ribose in a monomeric (MARylation) or polymeric (PARylation) form. Although PARylation has been studied for 60 years, new details are continuously learned in its mechanism. The discovery of the enzymes responsible for PAR synthesis and degradation was followed by identification many proteins that interact with PAR. The range of cell processes found to involve PARylation increases every year, while DNA repair certainly remains the main of them.

Both model DNAs and nucleosome systems are used to study PARPs. Nucleosome systems make it possible to detect the PARP–histone interactions and to assess their effects on the nucleosome structure [26, 132–134]. The discovery of serine PARylation and HPF1 as a cofactor of the process marked a new period of PARylation studies. It seems expedient now to always study the PARP functions in chromatin in the presence of HPF1. Certain regularities are already known for the HPF1–PARP1/2 interactions, although some discrepancies are still found in the available data and should be resolved to better understand the mechanism of action of HPF1. It is noteworthy that a deletion of *HPF1* does not abolish serine PARylation [40]. The finding makes it possible to assume that other, yet unknown PARP1/2 cofactors change the PARylation

specificity as well as HPF1. The effect of HPF1 on PARP1/2 activity was demonstrated both in vivo and in vitro and is important to understand in the context of studies of PARP1/2 inhibitors, some of which are used as anticancer drugs in medicine. Recent studies implicated HPF1 in the cell response to PARP1/2 inhibitors [100]. The PARP1/2 interactions with HPF1 are therefore necessary to consider when further searching for PARP1/2 inhibitors.

ACKNOWLEDGMENTS

We are grateful to N.I. Rechkunova for fruitful discussion of the review.

FUNDING

This work was supported by the Russian Science Foundation (project no. 22-14-00112) and a state contract with the Institute of Chemical Biology and Fundamental Medicine (no. 121031300041-4, part "Several Aspects of the Roles of PARP1 and PARP2 in the Cell").

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human subjects performed by any of the authors.

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Translated by T. Tkacheva