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Importance of poly(ADP-ribose) polymerases in the regulation of DNA-dependent processes

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Abstract. Poly(ADP-ribosyl)ation of proteins is involved in the regulation of basal cellular processes and seems to be crucial for genomic integrity and cell survival. Several nuclear poly(ADP-ribose) polymerases (PARPs) are known which interact with various proteins involved in DNA metabolism. These proteins can be targets of poly(ADP-ribosyl)ation, which generally downregulates their activities. Accordingly, PARPs have been implicated in numerous processes involving chromosomal DNA, such as the regulation of chromatin structure, DNA repair, replication and transcription. PARP-1, the major cellular PARP, and PARP-2 are activated by DNA strand breaks. These enzymes have been shown to participate in DNA repair. PARP-1 has also been associated with DNA replication and recombination. Another outstanding feature of PARP-1 is its impact on the activities of transcription factors and on gene expression. Two other nuclear PARP enzymes, tankyrase-1 and tankyrase-2, are important for telomere maintenance.

Key words. DNA; repair; replication; transcription; telomeres.

Introduction

The covalent modification of proteins with poly(ADP-ribose) (PAR) was discovered more than 40 years ago [1]. Even though its physiological roles are still controversially discussed, there is no doubt that this reaction is involved in basal cellular processes. Extensive investigations in which the activities of PARP enzymes were inhibited or cellular PARP-1 levels were decreased revealed correlations between poly(ADP-ribosyl)ation and cellular survival. Studies using PARP-1 knockout mice provided evidence for the relevance of this enzyme for genomic integrity [reviewed in 2].

The most thoroughly characterized PARP enzyme, PARP-1, is localized in the nucleus. PARP-1 seems to be the most abundant PARP enzyme in cells and the main poly(ADP-ribose) acceptor in vivo. The catalytic activity of PARP-1 is induced by DNA strand breaks, especially by single-strand breaks (SSBs). DNA strand breaks may arise directly or during various aspects of DNA metabolism, such as DNA repair, replication, and recombination. Accordingly, numerous investigations showed an involvement of PARP-1 in these processes, presumably by functional interactions with participating proteins. Several hypotheses consider PARP-1 to be a recruitment factor for proteins involved in DNA metabolism. PARP-1 contains a BRCA1 C-terminus (BRCT) motif, a protein-protein interaction module through which the enzyme homodimerizes and interacts with the DNA repair protein XRCC1 [3]. The association between PARP-1 and XRCC1 as well as a variety of other nuclear proteins are enhanced by PARP automodification [3–5]. It was shown recently that recruitment of XRCC1 to SSBs depends on PARP activity [6]. Although PARP-1 mainly poly(ADP-ribosyl)ates itself, other proteins may be modified as well. Most of these proteins are directly involved in replication or repair of DNA (DNA polymerases, DNA topoisomerases etc.) or in transcription (several transcription factors) [reviewed in 7]. Poly(ADP-ribosyl)ation of nuclear proteins generally results in downregulation of their activities, presumably by interference of the modification with DNA binding (fig. 1). In addition to the covalent

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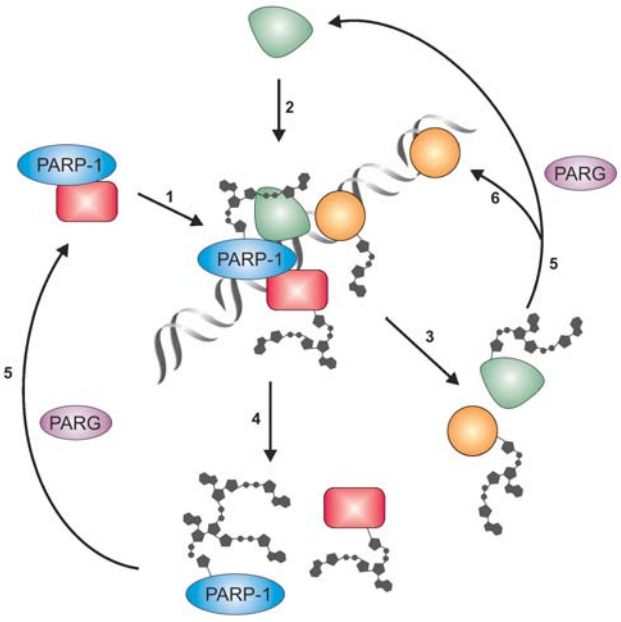

Figure 1. PARP and DNA: diverse modes of action. PARP enzymes, here represented by the highly abundant PARP-1, may exert their influences on DNA-related processes in various ways. *From top left to bottom:* PARP-1 binds to DNA strand breaks and probably to certain types of undamaged DNA. (*1*) Several proteins (red rectangles; e.g. NF-*κ*B) physically interact with PARP-1 irrespective of automodification and may be recruited to DNA together with PARP-1. After binding to DNA, PARP-1 is activated and poly(ADP-ribosyl)ates itself. (*2*) Many proteins (green triangles; e.g. XRCC1) preferably interact with automodified PARP-1 and may be recruited to DNA after PARP-1 activation. Interaction partners and nearby DNA-binding proteins (orange circles; e.g. histones or transcription factors) are also poly(ADP-ribosyl)ated. (*3*) This modification may ablate DNA binding and disrupt protein complexes. If the poly(ADP-ribosyl)ated proteins are DNA-modifying enzymes or transcription factors, they may be inactivated. If the proteins are histones, chromatin structure may be remodelled. (*4*) PARP-1 itself is inactivated by automodification and subsequent dissociation from DNA. (*5*) PARG strips PARP-1 and other proteins from the newly synthesized poly(ADP-ribose), thereby generating free polymers and ADP-ribose. Several proteins may also bind to the resulting free polymers and might thus be further inhibited from DNA binding. (*6*) Proteins stripped from polymers by PARG may rebind to DNA and resume their activities.

modification of nuclear proteins by PARPs, free poly(ADP-ribose) could attract histones and several DNA damage response factors, which have been shown to non-covalently interact with this polymer [8]. Accordingly, most current models consider the poly(ADP-ribosyl)ation activity as a central aspect in cellular PARP function.

Since the above-mentioned processes involve chromosomal DNA organized into chromatin, PARPs might also act through a direct influence on chromatin structure. The impact of poly(ADP-ribosyl)ation on chromatin was impressively demonstrated in *Drosophila* polytene chromosomes. The remodeling of chromatin at sites of transcription as well as transcription itself was shown to depend on PARP activity [9]. Numerous hypotheses have been proposed regarding the influence of poly(ADP-ribosyl)ation on chromatin organization. Many models are based on the well-known 'histone shuttle' model proposed by Realini and Althaus [10], which was deduced from the fact that poly(ADP-ribose) and poly(ADP-ribosyl)ated proteins are negatively charged and thus pushed off the DNA by electrostatic repulsion [11]. It implies that histones, which have a high affinity for poly(ADP-ribose), bind to the polymers, resulting in their dissociation from DNA and thus in a transient loosening of the chromatin structure. The transience of this process is due to the activity of poly(ADP-ribose) glycohydrolase (PARG), which degrades poly(ADP-ribose) just a few minutes after its synthesis. The 'histone shuttle' model explains the role of poly(ADP-ribosyl)ation in chromatin loosening during any DNA-dependent process. Applied in other contexts, this model could also explain the observed modulating effects of PARP activity on replication, recombination, and transcription.

The transient nature of poly(ADP-ribosyl)ation makes this reaction well suited for the short-term regulation of cellular processes. Accordingly, all current models of PARP-mediated regulation implicate an essential role for the polymer-degrading PARG. Therefore, functional studies of PARG must also be considered in order to fully understand the roles of poly(ADP-ribosyl)ation [reviewed in 12].

DNA Repair

Repair of DNA SSBs

Poly(ADP-ribosyl)ation levels of undamaged cells are very low, but the introduction of DNA damage, especially of SSBs, induces PARP-1 activity instantaneously. The first studies suggesting a link between poly(ADP-ribosyl)ation and the repair of SSBs date back to 1980 [13, 14]. Several investigations using the PARP inhibitor 3-aminobenzamide followed, showing an involvement of poly(ADP-ribosyl)ation in base excision repair (BER) and single strand break repair (SSBR). The results were impressive but ambiguous, suggesting either stimulatory or inhibitory effects of PARP activity on DNA repair [reviewed in 2, 15]. Nevertheless, studies in which cellular PARP-1 activity was decreased by expression of antisense RNA or dominant negative inhibition provided further evidence for a role of PARP-1 in DNA repair [reviewed in 16]. Reduced PARP-1 levels led to enhanced sensitivities towards ionizing radiation and alkylating agents. Similar effects were observed in cell lines derived from PARP-1$^{-/-}$ mice [reviewed in 2]. In vitro studies employing recombinant proteins proved that the basal BER reaction can proceed without PARP-1 [17, 18]. Yet it was

demonstrated in 1998 that PARP-1 interacts with XRCC1, a key component of the BER complex. XRCC1 interacts preferably with automodified PARP-1 and negatively regulates its activity [3]. An impressive study revealed a role for PARP-1 in the recruitment of XRCC1 to SSBs in vivo [6]. It was shown that poly(ADP-ribose) formation is induced specifically at SSBs, and that XRCC1 recruitment to these SSBs depends strictly on PARP activity. Interestingly, PARP-deficient and XRCC1-deficient cells share some major features, i.e. increased sensitivities towards similar types of DNA damaging agents, reduced rates of SSB rejoining and increased rates of sister chromatid exchanges (SCEs), indicating that both proteins might participate in the same DNA repair pathway [reviewed in 19].

Another study revealed functional interactions between PARP-1 and the BER enzymes DNA polymerase β (Pol β) and flap endonuclease 1 [20]. Correspondingly, a positive influence of PARP-1 on long-patch DNA repair synthesis by Pol β [20] and a decrease in long-patch BER in PARP$^{-/-}$ cells were shown [21, 22]. In line with this, it was observed that lowering of cellular ATP level increases activities of PARP-1 as well as Pol β [23]. Last but not least, a physical and functional interaction between PARP-1 and the BER enzyme DNA ligase III was shown [5].

PARP-2 bears a strong resemblance to PARP-1 and is also activated by DNA strand breaks. PARP-1 and PARP-2 heterodimerize and, as PARP-1, PARP-2 interacts with XRCC1 [24]. PARP-2$^{-/-}$ mice are sensitive to genotoxic treatments. Double knockout of PARP-1 and PARP-2 in mice is embryonic lethal, a phenotype typically resulting from deficiencies in essential BER factors [25].

Besides the recruitment and stimulation of DNA repair enzymes, PARPs could fulfil a variety of additional functions in BER. Two independent studies revealed that poly(ADP-ribose) can be degraded to yield ATP [26, 27]. This ATP was shown to be used for DNA ligation in BER. It was proposed that during BER, poly(ADP-ribose) is converted to ATP by pyrophosphorylytic cleavage using pyrophosphate generated from dNTPs during DNA synthesis [27]. Furthermore, it has to be borne in mind that some of the positive effects of PARPs on BER could be due to PARP-mediated chromatin remodeling. For example, it has been reported that BER enzyme activities are reduced on nucleosome substrates [28]. The impact of chromatin structure on DNA repair still deserves study, and the results might shed a new light on PARP involvement in BER.

Recombination and repair of DNA double strand breaks

As mentioned above, cells from PARP-1$^{-/-}$ mice exhibit genomic instability, such as elevated SCE after genotoxic treatments [2], and several investigations indicated that PARP-1 decreases SCE levels [reviewed in 29]. SCEs are believed to arise from the repair of DNA damage by homologous recombination (HR) during S phase. The central HR factor Rad51 accumulates in nuclear foci after DNA damage [30], and while PARP-1 does not colocalize with Rad51 foci, spontaneous Rad51 foci formation is significantly increased in PARP-1$^{-/-}$ cells as compared to wild-type cells [31]. These observations suggest an anti-recombinogenic function of PARP-1, a view supported by the observation that overexpression of PARP-1 reduces alkylation-induced SCE while slightly enhancing cytotoxicity of alkylating agents [32]. Satoh et al. proposed that poly(ADP-ribosyl)ation at sites of DNA strand breaks might cause a negative charge repulsion of DNA, thereby preventing accidental homologous recombination within tandem repeat sequences [33]. However, it is important to note that increased recombination activities could also reflect increased levels of DNA damage such as SSBs encountered by replication forks. Indeed, SCEs have been described as a typical outcome of the HR-mediated repair of SSBs during replication [34]. Increased SCEs in a PARP-1-deficient background might thus not reflect a deregulation of recombination events but a defect in SSBR.

Finally, a functional interaction between PARP-1 and DNA-PK$_{CS}$ has been reported in several independent studies [reviewed in 29]. Both enzymes are DNA strand-break sensors, and DNA-PK$_{CS}$ is involved in the repair of double strand breaks (DSBs) through non-homologous end joining (NHEJ). Deficiency in DNA-PK$_{CS}$ results in defective V(D)J recombination and severe combined immunodeficiency (SCID) in mice, a defect which could be reversed by generation of PARP-1 and DNA-PK$_{CS}$ double knockout mice. The authors concluded that PARP-1 and DNA-PK$_{CS}$ cooperate to minimize genomic damage caused by DNA strand breaks [35]. It was demonstrated that PARP-1 and the DNA binding subunit of DNA-PK$_{CS}$ form a complex even in the absence of DNA [36]. Multiple sequence motifs for non-covalent interaction with poly(ADP-ribose) were found in subunits of DNA-PK$_{CS}$ [8]. In NHEJ, DNA-PK$_{CS}$ associates with DNA-bound Ku70/Ku80 to form the active DNA-PK holoenzyme. PARP-1 has been described to associate with a Werner syndrome protein/Ku70/Ku80 complex and to poly(ADP-ribosyl)ate Ku70/Ku80, thereby decreasing their DNA binding abilities [37]. The implications of these observations remain to be clarified.

DNA Replication

It has been postulated for a long time that PARP could be involved in DNA replication. Previous studies reported that PARP activity is enhanced in proliferating cells [38,

39], at replication forks [40], and in newly replicated chromatin [41]. Cultured fibroblasts from PARP-1$^{-/-}$ mice posses a slower growth rate compared to wild-type cells [42]. PARP-1 was shown to interact and co-localize with DNA polymerase α-primase [43, 44] and was co-purified with a multiprotein complex containing central DNA replication proteins such as DNA polymerases α and δ, helicases, topoisomerases and proliferating cell nuclear antigen (PCNA) [45]. Many of these proteins were poly(ADP-ribosyl)ated [45]. Dantzer et al. proposed that PARP may participate in a survey mechanism controlling replication fork progression in the presence of DNA damage [44]. This hypothesis might be supported by the findings that PARP-1 interacts with the G1 checkpoint protein p21$^{waf1/cip1}$ [46] and the RecQ helicase Werner syndrome protein (WRN) [47, 48]. WRN is a component of the DNA replication complex which appears to be crucial for dealing with DNA damage during replication. Its absence leads to the genomic instability disorder Werner syndrome [49]. WRN has been reported to stimulate the DNA damage-induced poly(ADP-ribosyl)ation of proteins other than PARP-1 [50]. Recently, a role for PARP-1 in the cellular response to replication fork arrest was suggested [51]. After treatment with hydroxyurea, which stalls replication forks by depleting deoxynucleotide pools, PARP-1$^{-/-}$ cells displayed lower survival rates, elevated Rad51 foci and SCE formation as well as delayed progress from S into G2/M phase. Although the underlying molecular mechanisms are still obscure, these observations, taken together with results described in the other chapters, might suggest a role of PARP-1 at the intersections between DNA damage repair, DNA replication stalling and restart, and checkpoint signaling.

Transcription

Plentiful data regarding the relationship between PARP and RNA transcription can be found in the literature. First of all, PARP-1 was identified as a transcription factor, associated with the RNA polymerase II system and classified as TF II C [52]. 14 years later, the identical enzyme was rediscovered as a co-activator of Pol II-dependent transcription [53]. Thereafter, various studies were published describing regulatory roles of PARP-1 in transcription generally or in transcription of specific genes [reviewed in 54]. Some investigations demonstrated that PARP-1 participates directly in the assembly of transcription complexes at enhancers and promoters, thereby acting as a transcriptional regulator and predominantly as a transcriptional co-activator [reviewed in 55]. PARP-1 may specifically recognize promoter structures or may be recruited to target promoters by interaction with DNA-binding transcription factors [56].

Most of the published studies report the enzymatic activity of PARP-1 to be involved in transcriptional regulation. Especially poly(ADP-ribosyl)ation of transcription factors, which prevents them from binding to their specific recognition sites, seems to be important for the regulation of gene expression [57]. For example, it has been reported that a key regulatory transcription factor, the tumor suppressor p53, interacts with PARP-1. p53 is a target of modification, and is unable to bind to its DNA recognition site if poly(ADP-ribosyl)ated [58]. Additionally, it was shown that p53 has a high affinity to poly(ADP-ribose). Consequently, even a low concentration of free polymers can inhibit DNA binding by p53 efficiently [59]. Furthermore, a functional link between NF-κB and PARP-1 was demonstrated [reviewed in 55, 60]. Both subunits of NF-κB can be poly(ADP-ribosyl)ated in vitro, which markedly suppresses its DNA binding activity [61]. This implies that NF-κB-dependent transcription might be inhibited by poly(ADP-ribosyl)ation. The number of transcription factors which are interacting with PARP-1 or are targets of poly(ADP-ribosyl)ation is still growing [55, 62]. Ongoing transcription seems to be facilitated by the presence of PARP-1, but when activated, PARP-1 may inhibit initiation of new transcription cycles [53, 63]. When DNA damage occurs and PARP-1 is thereby activated, PARP-1 activity could enable the prevention of de novo transcription [62]. In contrast, ongoing transcription at damaged genes is apparently accelerated by PARP-1 activity [64]. A transient in vivo poly(ADP-ribosyl)ation of the transcription factors YY1 and p53 immediately after genotoxic treatment of cells was indeed observed [65, 66]. It is also important to note that interaction with transcription factors, for example YY1 or p53, stimulates the activity of PARP-1 manifold [67, 68].

Recently, Tulin and Spradling demonstrated a direct involvement of PARP activity in transcription by in vivo studies on *Drosophila melanogaster* [9]. In *Drosophila* polytene chromosomes, transcription is associated with puffing, a local loosening of the chromatin. The authors demonstrated that PARP activity is required for the generation of normal-sized puffs in response to transcription-activating signals. In this study, PARP activity was also crucial for gene transcription at the induced puffs. The conclusion was drawn that in *Drosophila,* poly(ADP-ribosyl)ation is activated by environmental signals to remodel the chromatin structure and facilitate transcription. Strikingly, the same authors reported that even an allegedly inactive form of *Drosophila* PARP, PARP-e, might influence chromatin structure and transcription [69]. The mechanisms which activate PARP in response to gene expression signals but in the absence of damaged DNA still remain to be clarified. Also how PARP may influence chromatin independent of its catalytic activity is not readily understood yet.

Telomere functions

The ends of chromosomes are organized into unique structures known as telomeres. A multi-protein complex including the enzyme telomerase is responsible for the replication of telomeric DNA and for its protection from degradation and fusion. The integrity of telomeres is essential for chromosome stability. Telomere shortening leads to senescence and may induce cell death. It has been speculated that poly(ADP-ribosyl)ation might control chromosome stability by regulating the maintenance of telomeric DNA. Among others, it was demonstrated that mice lacking PARP-1 display telomere shortening compared to wild-type mice [70]. Besides telomerase, specific telomere-DNA-binding proteins termed TRF1 and TRF2 are needed for the regulation of telomere length. In this context, one of the most important accomplishments in the research on PARPs was the discovery of tankyrase, a new member of the PARP family. Tankyrase-1 was identified through its interaction with TRF1, a negative regulator of telomere elongation [71]. TRF1 is poly(ADP-ribosyl)ated by tankyrase and thereby released from telomeres [71]. Thus, tankyrase-1 may regulate telomere length via modification of TRF1. It was demonstrated that overexpression of tankyrase-1 promotes telomere elongation in vivo, supposedly by preventing TRF1, but not TRF2, from accumulation on telomeres [72]. Moreover, using a small interfering RNA (siRNA) approach, Dynek and Smith recently presented convincing evidence that the catalytic activity of tankyrase 1 is specifically required to resolve sister chromatid cohesion at telomeres, which is essential for progression through mitosis [73]. Admittedly, it has been reported that tankyrase-1 is not exclusively localized at telomeres. It can also be found at nuclear pore complexes in a cell cycle-dependent manner [74] or at Golgi-associated vesicular compartments [75]. This indicates that the functions of tankyrases are not exclusively limited to telomere maintenance. A few years later a homologue, tankyrase-2, was characterized, which resembles tankyrase-1 very closely in its properties [76, 77]. Further analyses revealed that both tankyrases contain a sterile alpha motif (SAM) domain, which is important for self-association [78]. In the center of tankyrases functional studies revealed highly conserved ankyrin-repeat-clusters (ARCs) responsible for interactions with partner proteins like TRF1 [79]. It was demonstrated that each of the five ARCs of tankyrase-1 may function independently as a binding site for TRF1, and ARC V seems to be crucial for the TRF1-releasing activity of tankyrase 1 [80].

Interestingly, a functional interaction between PARP-2 and TRF2 was described recently [81]. The two proteins associate with high affinity. Furthermore, PARP-2$^{-/-}$ mice display a partial loss of telomeric repeats, suggesting a functional role of PARP-2 in the maintenance of telomere integrity. The binding of TRF2 to telomeres was shown to be regulated by both covalent modification of TRF2 with poly(ADP-ribose) and non-covalent binding of poly(ADP-ribose) polymers to TRF2 [81].

Conclusions

Similar to the pleiotropic consequences of other covalent protein modifications such as phosphorylation or acetylation, poly(ADP-ribosyl)ation may affect numerous processes, including the DNA-dependent processes transcription, replication, recombination and DNA repair. Overlapping capabilities of different PARPs have been described, and for the most prominent enzymes, functional 'backups' such as PARP-2 and tankyrase-2 seem to exist. Thus, plenty of information about PARP functions can be found in the literature, making it hard to condense and generalize in order to make unambiguous and valid statements. The majority of the known roles of PARP are still related to the regulation of nuclear functions, although the PARP enzymes vPARP and tankyrase are predominantly localized outside nuclear compartments. Since PARP-1 is responsible for the majority of poly(ADP-ribose) detected in cells, most observed effects of poly(ADP-ribosyl)ation seem to be caused by PARP-1. In resting cells the natural content of detectable poly(ADP-ribose) is rather low, therefore the fundamental question remains how and when PARP enzymes are activated. The induction of PARP-1 activity in response to DNA damage is well understood. With regard to the activation of tankyrase, it can be suspected that this signal is cell-cycle dependent. The activation of *Drosophila* PARP seems to be independent from DNA strand breaks. The characterization of the mechanisms of activation of the other PARPs will yield further insights into the biological roles of poly(ADP-ribosyl)ation. Another significant aspect is the identity of the relevant substrates of modification. Automodification seems to be common to all known PARP enzymes. Generally, the modification with poly(ADP-ribose) may lead to a dissociation of protein complexes or interfere with DNA binding of modified proteins (fig. 1). Alternatively, diverse target proteins may be attracted or recruited through non-covalent interactions with synthesized poly(ADP-ribose). Since diverse proteins interacting with PARPs and/or PAR have been discovered, manifold reactions can be affected by poly(ADP-ribosyl)ation. This mechanistic feature can result, not mutually exclusively, in loosening of chromatin, facilitation of DNA repair, prevention of de novo transcription, inhibition of illegitimate recombination events or promotion of telomere elongation.

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