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Serine ADP-ribosylation in DNA-damage response regulation

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PARP1 and PARP2 govern the DNA-damage response by catalysing the reversible post-translational modification ADP-ribosylation. During the repair of DNA lesions, PARP1 and PARP2 combine with an accessory factor HPF1, which is required for the modification of target proteins on serine residues. Although the physiological role of individual ADP-ribosylation sites is still unclear, serine ADP-ribosylation at damage sites leads to the recruitment of chromatin remodellers and repair factors to ensure efficient DNA repair. ADP-ribosylation signalling is tightly controlled by the coordinated activities of (ADP-ribosyl)glycohydrolases PARG and ARH3 that, by reversing the modification, guarantee proper kinetics of DNA repair and cell cycle re-entry. The recent advances in the structural and mechanistic understanding of ADP-ribosylation provide new insights into human physiopathology and cancer therapy.

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Introduction

Reversible post-translational modifications (PTMs) control the spatio-temporal organisation of DNA-damage response (DDR). PTMs trigger and regulate many aspects of DDR, including recognition of DNA damage sites, chromatin remodelling, recruitment of DDR factors, initiation and execution of DNA repair, as well as cell cycle arrest [1]. A variety of PTMs are involved in DDR, including selective protein phosphorylation and dephosphorylation, as well as conjugation of proteinaceous modifiers such as ubiquitin, SUMO, and Nedd8 [2]. Importantly, the different types of modification involved appear to be highly integrated and even dependent on each other. A critical example is the phosphorylation of the histone variant H2AX (termed γH2AX) by phosphatidylinositol-3-kinase-related kinases ATM, ATR, and DNA-PK that is required for DDR-linked ubiquitylation [3]. Failure to organise a proper DNA repair response strongly affects maintenance of genome integrity, thus predisposing to various human disorders, such as cancer, neurodegeneration, and immunodeficiency. Therefore, the cellular response to DNA damage as well as the PTMs involved within it have received a great deal of attention in transitional medicine [4].

Prominent among PTMs triggered by DNA damage is ADP-ribosylation, a reversible PTM of proteins that is involved in many cellular processes, such as transcription, cell division, and survival [5]. ADP-ribosylation results in the covalent attachment of a single ADP-ribose unit [namely, mono(ADP-ribosyl)ation (MARylation)] or polymers of ADP-ribose units [namely, poly(ADP-ribosyl)ation (PARylation)] to a variety of amino-acid residues on target proteins [6]. The attached ADP-ribose moieties originate from NAD^+, which is cleaved during the reaction, releasing nicotinamide. Interestingly, ADP-ribosylation can also occur on terminal DNA or RNA phosphates, thus representing a novel type of nucleic acids modification during DNA repair [7•,8–10]. Notably, pharmacological modulation of ADP-ribosylation reactions represents one of the most successful examples of anti-cancer interventions (i.e. PARP inhibitors; PARPi) [4].

Proteomic studies revealed that one third of the human nuclear proteome in the presence of DNA damage is subject to ADP-ribosylation, mainly on serine residues [11•,12••,13•]. The two types of ADP-ribosylation — MARylation and PARylation — can be recognised by different reader proteins [5], which are also capable, in some cases, of distinguishing between short and long PAR chains [14]. This realisation, together with the indications of the functional relevance of PAR chain branching [15], suggests the existence of a complex 'PAR code' [16] akin to that described for ubiquitin [17] and hints at multiple regulatory roles of ADP-ribosylation in DDR. The investigation of ADP-ribosylation functions in DDR has been facilitated by novel antibodies and antibody-like reagents, which are able to distinguish between different lengths and, in some cases, sites of the modification [18,19•].

The best-studied ADP-ribosylation 'writer' is poly(ADP-ribose) polymerase 1 (PARP1), the founding member of the PARP family of ADP-ribosyltransferases (ARTs)

which in humans consists of 17 core members (PARP1 to PARP16) [20,21].

PARP1, which is thought to account for the greatest part of detectable ADP-ribosylation in human cells under DNA damage, is one of the most abundant nuclear proteins and, in addition to DNA repair, acts as a key player in chromatin remodelling, apoptosis, transcription, telomere maintenance and DNA replication [5,6,21–23,24••]. Several PARP1 functions in DNA repair are complementary to those of PARP2 [25]. Genetic disruption of PARP1 and PARP2 simultaneously, but not individually, results in embryonic lethality in mice, demonstrating their functional redundancy [26].

PARP1 is a multi-domain protein, composed of four DNA-binding domains, namely three Zn-fingers, one tryptophan-glycine-arginine (WGR) domain, an auto-modification domain consisting of a BRCT motif, and a catalytic domain [21]. The combination of different DNA-binding domains allows recognition of various DNA breaks including single-strand and double-strand ones [27••,28••]. Interestingly, PARP1 appears to be released from a DNA end by binding to another, which could allow it to move between DNA ends in a manner that was termed the 'monkey bar' mechanism [29]. While this mechanism does not result in net dissociation of PARP1 from DNA ends, this can be achieved through PARP1 auto-modification, which is thought to counter DNA binding through electrostatic repulsion between negatively charged PAR chains and DNA backbone [30,31]. In cells, PAR chains can additionally contribute to releasing PARP1 from chromatin by recruiting factors, such as the chromatin remodeller ALC1/CHDL1 or the scaffold XRCC1, that facilitate this process [32–34]. It is largely by interfering with PARP1 auto-modification that PARP1 inhibitors induce prolonged residence of PARP1 on chromatin, a phenomenon that is known as 'trapping' and thought to be the key to cytotoxic effects of PARP1 inhibition in cancer [35].

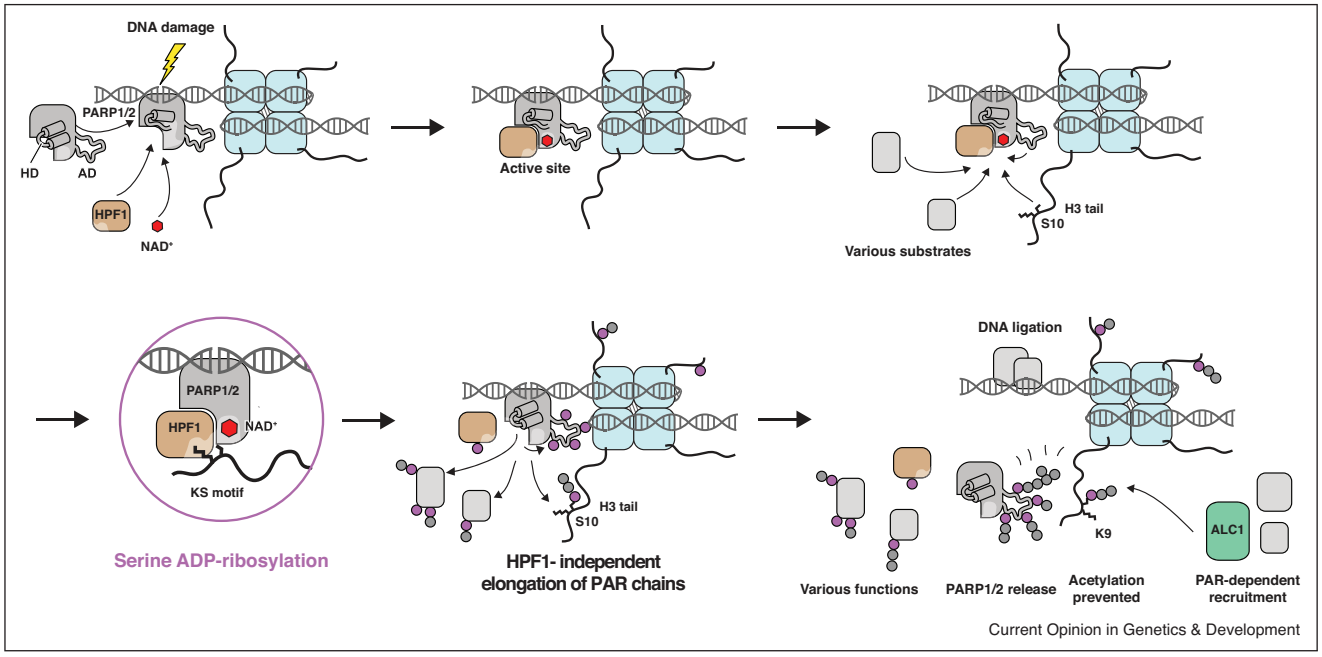
Compared with PARP1, PARP2 has a shorter N-terminal DNA-binding domain, which comprises an unstructured region and a WGR domain, while the catalytic domain is highly homologous to that of PARP1, which results in the majority of PARPi anticancer drugs targeting both PARP1 and PARP2 enzymes [27••]. While the primary role of PARP1 and PARP2 is linked to their ADP-ribosylation activity, the two proteins might also serve other, non-catalytic roles. In particular, recent PARP2 structures suggest that double-strand DNA break recognition not only activates the catalytic domain, but also aligns the two DNA ends such that the DNA appears continuous across the break, seemingly poised for ligation [36•,37••,38,39].

Here, we discuss the mechanisms by which PARP1 and PARP2-dependent serine ADP-ribosylation controls DDR.

Histone PARylation factor 1 (HPF1) drives DNA damage repair response by enabling PARP1 and PARP2 to catalyse serine ADP-ribosylation

PARP1 and PARP2 act as 'first responders' at DNA lesions. Both enzymes are recruited within seconds of the occurrence of various types of DNA damage and rapidly catalyse protein modification [40,41]. PARP1 and PARP2 are allosterically regulated by binding to DNA breaks, which leads to remodelling of an autoinhibitory fragment of the catalytic domain, called the helical domain (HD), which inhibits NAD^+ binding in the resting state [37••,42••]. Biochemical studies accompanied by new mass spectrometry tools have established that the main targets of ADP-ribosylation under both basal and DNA-damage conditions are serine residues in substrate proteins [11•,12••,13•,43•,44•,45]. Targeted sites are enriched for lysine-serine (KS) and, to a lesser extent, arginine-serine (RS) motifs [11•,12••,44•]. Although serine ADP-ribosylation has been detected on a large fraction of human proteins, the bulk of the modification corresponds to a limited number of key sites within PARP1 and PARP2 themselves and histone tails [12••,43•]. Intriguingly, the proximity of ADP-ribosylable serine sites (e.g. S10 of histone 3; H3S10) to lysine residues, which are known targets of acetylation (e.g. H3K9) as well as the overlap with serine phosphorylation sites (e.g. H3S10), suggests an important contribution of histone ADP-ribosylation to a multi-layered cross-talk with additional DDR-relevant histone PTMs [44•,46•,47•]. Recent studies revealed that, surprisingly, PARP1 and PARP2 are by themselves insufficient for ADP-ribosylating serine residues. Histone PARylation factor 1 (HPF1), which binds the catalytic domain of PARP1 or PARP2, is an essential participant in this process [12••,37••,43•,48••,49••]. The interaction between HPF1 and PARP1/2 — like NAD^+ binding — is negatively regulated by the HD fragment of these PARPs and is therefore enhanced upon their binding to damaged chromatin [37••,38,49••] (Figure 1). Strikingly, HPF1 together with PARP1/2 jointly forms the specific peptide-binding site, but also contributes a key residue (E284), termed the 'glutamate finger', to the catalytic core of PARP1 or PARP2 [49••]. Consistent with its catalytic role, E284 is required for *in vitro* modification of serine-containing substrates and for DNA damage-induced ADP-ribose signalling in human cells despite being dispensable for the HPF1–PARP interaction and HPF1 stability [49••,50,51•]. E284 seemingly acts as a general base that deprotonates the serine hydroxyl group to facilitate the subsequent nucleophilic attack on the 1″ carbon of NAD^+. The importance of acceptor deprotonation for the ADP-ribosylation reaction has long been postulated [52,53] and is underscored by the fact that, *in vitro* and in the absence of HPF1, PARP1 and PARP2 preferentially modify glutamate and aspartate residues, which are constitutively deprotonated at a neutral pH

Figure 1



Model of HPF1-dependent PARP1 and PARP2 activation and function at the DNA damage foci.
Upon DNA damage, an inactive conformation of PARP1 or PARP2 is recruited to DNA. PARP1 and PARP2 undergo a conformational change in the regulatory domain (HD) allowing NAD^+ binding. HPF1 binds to the PARP catalytic domain allowing the initiation of ADP-ribosylation of serine residues in target macromolecules, such as histone H3 tails, various DNA repair factors, and PARP1 and PARP2 themselves. Once the initial ADP-ribose is attached, PARP1/2 can catalyse the PAR chain elongation reaction without HPF1, which may dissociate from the complex. ADP-ribosylation allows recruitment of PAR-readers (i.e. ALC1) to DNA damage sites, which, in turn, trigger DNA repair and ligation. Furthermore, ADP-ribosylation at DNA damage sites contributes to modulate proximal chromatin epigenetics, regulates the activity and function of various substrates, and controls PARP1/2 release from DNA.

[54]. Indeed, while serine residues are the predominant cellular targets, the alternative activity of PARP1 and/or PARP2 towards glutamate and aspartate residues can be detected in cells, where it becomes relatively more prominent upon HPF1 knockout [43•]. In addition to activating the serine side-chains, HPF1 might use a broader negatively charged region that surrounds E284 to help recognise the lysine or arginine residue that precedes the serine in KS or RS motifs [49••].

The discovery of HPF1 sheds new light on the distinction between MARylation and PARylation. HPF1 binding to PARP1 is sterically incompatible with chain elongation [49••], which results in HPF1 having a negative effect on chain length, especially when it is present at a saturating level in an *in vitro* reaction or overexpressed in cells [48••]. When present at a high level, HPF1 instead turns PARP1 into an NAD^+ hydrolase by discouraging the attachment of ADP-ribosylation to proteins as soon as available serine sites become singly modified [51•]. However, it is unclear what the relevance of the hydrolase activity is in cells. Paradoxically, HPF1 is at the same time required for normal PARylation by contributing to the attachment of the first ADP-ribosylation unit that is a prerequisite for subsequent chain formation, and appears to be the rate-limiting step of the whole PARylation process [24••]. DNA damage induced PARylation could be seen as consisting of two distinct stages, where the first consists in the attachment of an ADP-ribosylation unit to a protein side-chain or 'chain initiation' (achieved by PARP1 or PARP2 in HPF1-depend manner) and the second in the attachment of consecutive ADP-ribosylation units or 'chain elongation' (catalysed by PARP1 or PARP2 alone and inhibited by HPF1) [24••]. The complex role of HPF1 in this process could explain why the levels of this protein in the cell are kept low [48••] — enough to efficiently initiate chains but not to preclude extension. Interestingly, as explained below, the two stages of serine ADP-ribosylation are also largely distinct when it comes to their regulation by hydrolases, with initiation being counteracted by the serine-specific ARH3 and elongation by PARG [24••]. Dysregulation of ADP-ribosylation initiation by either HPF1 knockout or ARH3 upregulation results in impaired PARP1 auto-modification and increased trapping and inhibitor sensitivity in human cells [35].

Functional consequences of PARP1/PARP2-mediated ADP-ribosylation in response to DNA damage

Functional consequences of ADP-ribosylation reactions are poorly understood and have thus received a great deal of attention in the field in recent years. PARylation has more profound consequences in the cells compared with MARylation [24••] and can even suppress DNA repair when it is excessive [55]. For these reasons, PARylation is present only transiently in response to DNA damage. *In vitro* experiments showed that, within the first ten minutes after DNA damage by H_2O_2, PARylation is converted to MARylation by the poly(ADP-ribose)glycohydrolase (PARG) [19•,56,57••]. Indeed, PARylation, if not reversed in a timely manner, appears to be very toxic to the cells [24••,58,59], for instance by impairing alternative lengthening of telomeres (ALT) and global transcription [24••]. In contrast, MARylation marks are well tolerated, and are hence compatible with recovery after DNA damage and cell division. This observation is unexpected considering that ADP-ribosylation marks were shown to be able to interfere with canonical histone modifications required for cell proliferation (i.e. H3S10 phosphorylation) [24••,43•,60].

As mentioned above, it is only recently that ADP-ribosylation events have been mapped to defined sites in protein substrates and limited information is available on functional consequences of individual modification events. Although it is likely that PARylation affects protein functions and dynamics [61], the only understood consequence of the site specific ADP-ribosylation is the role of PARP1 auto-modification at the 3 serines residues (i.e. 499, 507, and 519). Those serine residues are the predominant *in vivo* PARP1 auto-modification sites and are crucial for PARP1 release from DNA, therefore their mutation sensitises cells to PARP inhibitors [35]. Nevertheless, the best-understood aspect of proteins' PARylation at DNA foci remains scaffolding. A number of DNA damage response proteins, including specific chromatin modifiers, are rapidly recruited through direct binding of the modification, often mediated by specific protein modules ('readers' domains), such as the macrodomain, PBZ, and WWE domains [5]. Importantly, diverse parts of the modification can be recognised by different proteins readers, thus able to distinguish between MARylation and PARylation of various lengths [5,14,62]. This observation also suggests a sort of physical and functional compartmentalisation of readers, where some factors may enrich preferentially at long PAR chains, which are mainly observed on auto-modified PARP1 and PARP2. By contrast, other factors may bind with higher affinity short PAR chains or MARylation, mainly present on histone proteins. Biochemical analysis indicates that, upon HPF1–PARP binding to nucleosomes, histone H3 is rapidly modified to saturation at the DNA break, whereas PARP2 continues to accumulate PARylation over time [37••]. This suggests that, at least for PARP2, the histone modification serves as the initial anchor for recruiting factors to sites of DNA damage. Considering the high overall similarity in the mode of action of the two proteins, PARP1 may act analogously to PARP2; nevertheless, further biochemical and ultimately cellular studies will be needed to investigate this in detail. By contrast to histone ADP-ribosylation, PARP1/PARP2 auto-modification may not present a persistent mark of DNA damage sites, as it ultimately leads to PARP release from DNA, consistent with previous observations [35,63–65]. Of note, PARP2 engages DNA ends in a manner that could prevent access of repair factor access to the break, suggesting its dissociation might be a prerequisite for subsequent repair [37••], consistent with previous observations for PARP1 [66]. In this respect, the ADP-ribosyl modification on histones might serve as a break-proximal recruiting platform and the key determinant of subsequent repair events. However, at this stage it is difficult to pronounce with confidence on the relative functional importance of histone-linked and PARP-linked modification, as both can in theory act as signals that attract DNA repair factors, many of which are recruited at DNA damage foci by ADP-ribosylation, including ALC1, APLF (aprataxin PNK-like factor), and XRCC1 [25,67,68] (Figure 1).

Among PAR readers, the helicase ALC1 possesses a macrodomain, which allows PAR binding and recruitment to DNA damage foci, thereby facilitating an open chromatin structure, and, in turn, increasing accessibility of additional repair factors to DNA [68,69]. Depletion of HPF1 abolishes recruitment of DDR factors, as illustrated for recruitment of LIG3 and XRCC1 to Okazaki fragments [70]. Similarly, the PARP1-XRCC1 axis, and possibly HPF1, is involved in short-patch single-strand break (SSB) repair physiologically occurring at enhancers as a response to neuron-specific transcriptional activity [23]. Altogether, these recent observations confirm the importance of ADP-ribosylation in triggering a proper DDR, which may be required in multiple physiological conditions.

Reversal of serine ADP-ribosylation

Two enzymes are required for reversal of serine PARylation, namely PARG and ARH3 [71]. These enzymes function in a cooperative manner; PARG very efficiently cleaves PAR chains [72] but is incapable of removing MARylation [73,74]. De-MARylation is instead catalysed by ARH3, which selectively cleaves the *O*-glycosidic linkage between the serine of target proteins and ADP-ribose [57••]. PARG is highly active in cells and accounts for the conversion in MARylation of almost all PAR chains within minutes upon DNA damage, therefore in a physiological context MARylation on serine appears to be the persistent form of ADP-ribosylation [19•,24••,57••]. PARG inhibition prevents conversion of

PARylation to MARylation [19•,75••], implying that PARG is required to keep PARylation under control and balance its 'positive' (i.e. recruitment of DNA repair factors) and 'negative' (i.e. toxic effects and pro-apoptosis) roles. ARH3 instead prevents chain formation by removing the priming unit of ADP-ribose covalently linked to modified substrates, without which a PAR chain cannot be extended [24••,57••]. Thus, PARG and ARH3, although primarily active at different stages of de-ADP-ribosylation reaction, show synergy and their joint inactivation is required to unleash widespread, and ultimately toxic, PARylation [24••].

Conclusions

The understanding of DDR control by PARP1-dependent or PARP2-dependent ADP-ribosylation at the molecular level has improved significantly in the last decade. With the development of new biochemical tools, proteomic approaches, electron microscopy, and genetic screens, new key players have been identified, providing novel mechanistic insights. Major breakthroughs in the field in the last years were the identification of HPF1 and the dissection of the molecular mechanisms of the HPF1–PARP1/2 complex catalytic activity, the characterisation of serine residues as the main targets of ADP-ribosylation in response to DDR, better elucidation of recruiting and scaffolding roles of this modification, and, not least, the cooperation between PARG and ARH3 in counteracting PARP1 and PARP2 activity.

Considering that the PARP inhibitors, currently in clinical use, were identified before the discovery of such mechanisms, it may be of interest to re-evaluate the potency and *in vitro* selectivity of existing PARPi in the context of HPF1–PARP1/2 complexes, although one must bear in mind that such complexes might be relatively scarce and short-lived in the cell, PARP1/2 presumably remaining predominantly unbound at any given moment. The emerging picture is that while HPF1 might increase affinity of PARP1 for some existing inhibitors [35,76•], this regulatory factor actually promotes resistance to PARPi in the cell by stimulating PARP1 auto-modification, which in turn counteracts inhibitor-induced trapping [35]. Future studies of existing and design of novel PARPi should take into account recent insights into allosteric communication between DNA-binding and catalytic domains of PARP1 [77••].

From the 'erasers' point of view, arising therapeutic potential of PARG inhibition (PARGi) in cancer therapy has also been investigated. PARGi is indeed very efficient in inducing toxic accumulation of cellular PARylated proteins, thus promising advantages for treatment of PARPi-resistant tumours [78••]. Moreover, our studies suggest the potential for ARH3 as a novel drug target for cancer therapy, for instance in improving PARGi cytotoxicity or in selectively killing *PARG*-null cancer cells [24••]. On the latter point, PARG depletion is a major PARPi resistance mechanism of serous ovarian and triple-negative breast cancers [75••] and could be targeted with ARH3 inhibitors. Conversely, downregulation of ARH3 confers resistance to PARPi [24••,35], which phenocopies the effect of *PARG* loss or PARG inhibition [75••,78••]. These major breakthroughs open new and fascinating scenarios regarding development of new therapeutic strategies for PARPi-resistant cancers.

As the understanding of HPF1 and ARH3 molecular functions provided insights into the elucidation of human diseases and their treatment [24••,35,48••], many other DDR factors controlled by ADP-ribosylation signalling may equally impact therapeutics and/or prognosis of human disorders. For instance, the central role of ALC1 in allowing access of DDR factors at damaged foci may explain the crucial impact of its expression levels on sensitivity to PARPi in cancer cells [32–34,79,80]. These studies provide the rationale for designing novel therapeutics focused on inhibiting the recruitment of ADP-ribosylation readers, such as ALC1, to DNA damage foci to further sensitise cancer cells to PARPi.

Altogether, ADP-ribosylation is of vital importance in DDR and its further investigation promises new insights, which may significantly contribute to the understanding of human pathophysiology and treatment of human diseases.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Luca Palazzo: Writing - original draft, Writing - review & editing. **Marcin J Suskiewicz:** Writing - original draft, Writing - review & editing. **Ivan Ahel:** Supervision, Writing - review & editing.

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