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The Rise and Fall of Poly (ADP-ribose). An Enzymatic Perspective

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Abstract

Human cells respond to DNA damage with an acute and transient burst in production of poly(ADP-ribose), a posttranslational modification that expedites damage repair and plays a pivotal role in cell fate decisions. Poly(ADP-ribose) polymerases (PARPs) and glycohydrolase (PARG) are the key set of enzymes that orchestrate the rise and fall in cellular levels of poly(ADP-ribose). In this perspective, we focus on recent structural and mechanistic insights into the enzymes involved in poly(ADP-ribose) production and turnover, and we highlight important questions that remain to be answered.

Keywords

poly(ADP-ribose); PARP; PARG; MARG; ADP-ribose; DNA damage response

Introduction

Cells respond instantaneously to DNA damage with posttranslational modifications of proteins that repair DNA damage, alter gene expression, or control passage through the cell cycle. The covalent modification of these proteins induces a dynamic network of protein-protein interactions and regulates enzymatic activities, broadly changing cellular physiology and serving to integrate myriad responses to DNA damage that dictate outcomes for DNA repair, cell survival, and responses to chemotherapy. One of the most prodigious posttranslational modifications caused by DNA damage is the poly-(ADP-ribosylation) of proteins, catalyzed by members of the poly-(ADP-ribose) polymerase (PARP) superfamily of NAD⁺ dependent ADP-ribosyltransferases [1]. Poly-(ADP-ribose) (PAR) is a large, negatively-charged and branched polymer that can exceed the mass of the unmodified protein. PARylation creates binding sites for PAR-specific binding proteins [2,3] and

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changes the electrostatic properties of the modified protein, with the notable capacity to change DNA binding properties of enzymes, histones, and structural proteins [4]. PARP-1 itself is the target of most of the poly-(ADP-ribosylation) (PARylation) occurring in response to DNA damage. Automodification of PARP-1 increases its association with a variety of repair and signaling proteins that are recruited to sites of DNA damage by PARP-1 activity [3,5]. In turn, some of these proteins are PARylated by PARP-1.

PARP enzymes responding to damage can consume substantial amounts of cellular NAD⁺ within minutes, changing a cell's metabolic status while modifying vast numbers of proteins, many of which have been only recently identified by proteomic surveys [6,7]. For most of these proteins, the effects of PARylation remain to be functionally characterized. These studies are complicated by the fact that PAR modifications turn over rapidly due to the activity of poly-(ADP-ribose) glycohydrolase (PARG) and mono-(ADP-ribose) glycohydrolases (MARGs) [8,9]. Both the synthesis and turnover of poly-(ADP-ribose) appear to be important for normal responses to DNA damage. In this short perspective, we will review the recent literature on the structures and functions of DNA damage-dependent PARPs and PARG, and then speculate about how these activities may be tied mechanistically to various disease processes and the resulting opportunities for therapeutic intervention.

Structure and mechanism of DNA damage-dependent PARPs

Three members of the PARP superfamily are catalytically activated through interaction with DNA damage: PARP-1, PARP-2, and PARP-3. PARP involvement in the cellular response to DNA damage has long been appreciated and continues to actively develop [10,11]. A general model that has collectively emerged indicates that the DNA-damage dependent PARPs act early in the process of damage detection, which promptly results in PARP catalytic activation and a burst of PAR production. PARP presence and activity at the damage site then can contribute to the efficiency of the repair process and the repair pathway choice. A key role of the DNA-damage dependent PARPs and the PAR modification they produce is to recruit repair factors to the site of damage. Several motifs and domains have been identified in repair proteins that mediate the interaction with PAR and the recruitment to sites of PAR synthesis [12,13]. In addition to PAR serving as a recruiting platform, PAR modification of repair and chromatin-associated factors in the vicinity of a damage site is expected to change the catalytic properties of targeted proteins, and the local structure of chromatin [10]. However, detailed insights into PAR-mediated regulation of protein function are lacking in general. And although a general model for PARP contribution to the DNA damage response has formed, the molecular details of PARP involvement are not clearly established, which has limited our understanding of PARP's contribution to specific steps of repair, and the contribution of different PARPs to repair pathway choice. Over recent years, structural and biochemical studies have provided key insights into the early stages of PARP-1 involvement in DNA repair: the detection of DNA damage, and the allosteric coupling of damage detection to acute levels of PAR production. Here we will provide an overview of these important insights into PARP-1 mechanism, and we will indicate some of the key questions that remain to be answered.

The DNA-damage dependent PARPs have similar catalytic domain structures, but they differ somewhat in the domains that contact DNA damage (Figure 2)[13]. In the catalytic domain, they share a conserved structural feature known as the helical domain (HD) [14] (also referred to as the PARP regulatory domain – PRD). The HD is only found in the DNA-damage dependent PARPs, and it plays an important role in regulating PARP catalytic activity, as described later. The HD is adjacent to the ADP-ribosyl transferase (ART) fold, which is common to all PARP family members. The ART contains the binding site for NAD⁺, which donates ADP-ribose, and a second binding site for an ADP-ribose unit, which accepts the next ADP-ribose during the PAR extension reaction that can result in both linear and branched polymers [15,16](Figure 2). Detailed structural views of PAR biosynthesis (NAD⁺ binding, initiation on target protein, polymer extension) have not been obtained, thus our complete understanding of PAR synthesis is limited. The NAD⁺ binding sites for the DNA-damage dependent PARPs are similar and have the conserved His-Tyr-Glu (HYE) amino acids that define catalytically active PARP members capable of forming PAR (as opposed to mono-ADP-ribose)[17,18]. The acceptor binding sites vary between PARP-1, PARP-2, and PARP-3 and this is likely to influence the type of polymer formed (e.g. polymer length, number of branch points). For example, PARP-3 has an Arg residue in the acceptor site where PARP-1 and PARP-2 have a Met residue, which is expected to contribute to the binding pocket for the adenosine base of an acceptor ADP-ribose modification [16]. Presumably this change in sequence perturbs the binding site and contributes to the smaller size of polymer produced by PARP-3 [18]. It is not understood how the differences in the structure of PAR produced might differentially influence downstream signaling to repair pathways, and it will be important to resolve this issue.

Mechanism of PARP-1 activation

Outside of the catalytic domain, the DNA-damage dependent PARPs also have in common a Trp-Gly-Arg (WGR) domain that is essential to damage-dependent activation, and is the most defining feature of the DNA-damage dependent PARPs. A crystal structure that contained the essential domains of PARP-1 in complex with DNA damage provided the first views of the WGR domain contacts with DNA (Figure 2). The structure indicated that conserved regions of the WGR make sequence-independent contacts with the DNA backbone near the 5' terminus [19]. The importance of these contact residues to catalytic activation was confirmed through mutagenesis. Although there are no structures for PARP-2 and PARP-3 in complex with DNA damage, it is interesting to note that their activation levels are sensitive to modifications to the 5' terminus of the DNA, such as phosphorylation [20], suggesting that their WGR domains have specialized interactions with the 5' terminus. PARP-1 in contrast is relatively insensitive to the detailed composition of the break site, consistent with the PARP-1 complex structure in which the 5' terminus is not directly contacted [19]. The biochemical results for PARP-2 and PARP-3 suggest that they are most potently activated at certain stages of the repair process, for example, when a DNA break has been processed to the point of containing a 5' phosphorylated nick that is competent for DNA ligation [20]. Indeed, the efficiency of recovery from a DNA double-strand break depends on PARP-3, which is proposed to aid recruitment of the DNA ligation complex that completes the NHEJ repair pathway [21]. Hence, there are likely to be important variations

in the mechanism of activation for each of the DNA-damage dependent PARPs that contribute to their specialization toward distinct repair pathways, and the stage at which they act within a given repair pathway. Further structural and biochemical studies will help to further define the specifics of the particular PARP involvement in the DNA repair response.

In addition to engaging DNA damage, the WGR domain makes important contacts with the HD, and thus physically couples damage detection to the catalytic domain, which does not bind DNA [19](Figure 2). These WGR contacts induce destabilizing structural changes in the HD that are associated with PARP-1 activation, and they are suggested to involve a change in protein dynamics [22]. However, the precise role of HD structural transitions and their potential impact on the ART are not clear and require further investigation. Understanding these structural changes is likely to be relevant to the mode of PARP inhibitor binding, since all clinical PARP inhibitors interact with the ART. WGR–HD contacts and their importance to activation are conserved in PARP-2 and PARP-3, as well as the destabilizing changes in HD, thus indicating that the allosteric coupling of damage detection to catalytic activation will proceed through similar mechanisms for the DNA damage-dependent PARPs [20]. In contrast, the DNA damage binding interfaces of PARP-1, PARP-2, and PARP-3 are likely to have significant variations based on their differences in domain composition. It will be important to understand these differences in greater detail, as it will help to clarify the specific roles that each DNA damage-dependent PARP performs in repair.

Outside of the WGR domain, there are other regulatory domains that contribute to the activation of the DNA-damage dependent PARPs. Most notably, PARP-1 has three N-terminal zinc-binding domains that contribute to DNA binding and catalytic activation in different ways (Figure 2). The crystal structure of PARP-1 essential domains in complex with a DNA double-strand break illustrated how the first zinc finger (Zn1 or F1) and the third zinc finger (Zn3 or F3) collaborate with the WGR to bind DNA damage (Figure 2). Each of these three domains is strictly required for PARP-1 catalytic activation by DNA, and the required domain-domain interfaces formed between them when engaging DNA represent novel targets for selective inactivation of PARP-1, since the zinc finger domains are unique regulatory domains only found in PARP-1 [23,24]. Zn1 plays a central role in damage detection by forming contacts with the nucleotide bases that are exposed at the DNA double strand break. This mode of interaction is consistent with earlier crystal structures of Zn1 and Zn2, each individually bound to a double strand break [25]. In all cases, Zn1 and Zn2 do not contact the 5' and 3' terminal ends of the break, and thus can allow extensions of these ends that are present in different types of DNA damage (e.g. single strand breaks). Thus, the Zn1 and Zn2 mode of engagement allows them to engage a variety of damage DNA structures. A different mode of DNA interaction was observed in a crystal structure of the Zn1-Zn2 fragment of PARP-1 in complex with a double-strand break bearing a single nucleotide overhang [26]. Although the Zn2-DNA contacts matched those seen in previous structures, the polarity of the Zn1 domain with respect to the DNA backbone was reversed. The reversed polarity was surprising given the structural homology and sequence identity between Zn1 and Zn2. Moreover, the positioning of the Zn1 in this complex sterically prevents the essential WGR domain from binding to the DNA; thus it is hard to envision how this binding mode could lead to PARP-1 activation. Lastly, the relative

positioning of the Zn1 and Zn2 domains necessitates that they originate from separate polypeptides, which is at odds with a number of recent biophysical studies indicating that PARP-1 interacts as a monomer with DNA [19,27–30]. Thus, the relevance of the reversed binding mode of Zn1 requires further investigation. Indeed, additional structural studies are needed to help clarify this discrepancy, and to fully understand how PARP-1 engages various types of DNA damage. Furthermore, it will be important to decipher how PARP interaction with DNA damage is different from its interaction with undamaged DNA, and the functional consequences of these differences [31,32]. Perhaps most notably, the structural basis for PARP-1 engaging a single-strand break is a clear gap in our understanding of PARP-1 function as a “nick” sensor. PARP-2 and PARP-3 lack the extensive regulatory domains seen in PARP-1, but still have extensions N-terminal to the WGR that at least play a role in DNA binding and activation [20]; however, there are limited structural and mechanistic insights into their N-terminal regions and how they might specialize the function of PARP-2 and PARP-3.

Despite the recent advances in understanding DNA damage detection and catalytic activation, there are critical deficiencies in our understanding of how PARP modification can influence protein structure and activity. Perhaps the most prominent deficiency is our understanding of PARP-1 automodification. PARP-1 automodification has two somewhat opposing outcomes: recruitment of repair factors to PARP-1 at sites of damage, and release of PARP-1 from the site of damage. It is unclear how this transition occurs. There has been much recent progress in the identification of PARP automodification sites using mass spectrometry [33–37]. However, there is still much to learn in terms of the functional consequences of modification at a given site, and whether modification at different residues could lead to different outcomes (e.g. PARP-1 mediated recruitment *versus* PARP-1 release). Understanding the mechanism of PARP-1 release from DNA damage has relevance to the effects of certain clinical PARP inhibitors that prevent the release mechanism to varying degrees and “trap” PARP molecules on DNA damage [38,39]. Understanding PARG involvement in reversing the PAR modification and regulating PARP function will be equally important in understanding both biologically and medically relevant questions.

Turnover of poly-(ADP-ribose) is required for normal responses to DNA damage

The enzymatic synthesis of poly-(ADP-ribose) and its degradation are commensurately important for normal responses to DNA damage. In mammals, the enzyme poly-(ADP-ribose) glycohydrolase (PARG) is the main activity that removes poly-(ADP-ribose) from proteins by cleaving ribose-ribose bonds [8]. PARG is an abundant enzyme that degrades PAR by a combination of endo- and exo- glycohydrolase activity, removing most of the PAR polymer but leaving a single ADP-ribose attached to the protein. The remaining ADP-ribosyl modification can be removed by one of several recently identified mono-(ADP-ribose) glycohydrolases [33,40].

Genetic disruption of the *PARG* gene causes embryonic lethality, and decreased PARG activity sensitizes cells to a spectrum of DNA damaging agents resembling that caused by genetic knockdown of PARP-1 expression or pharmacologic inhibition of PARP activity

[41]. For example, BRCA2-deficient cells that are markedly sensitive to PARP inhibitors are also hypersensitive to PARG inhibition by the nonselective inhibitor, gallotannin [42]. These observations suggest that returning transiently PARylated proteins to their unmodified state is cytoprotective, and additionally, that the accompanying metabolic conversion of $\text{NAD}^+ \gg \text{poly}-(\text{ADP-ribose}) \gg \text{ADP-ribose}$ may be important for recovery from damage, as discussed below.

Structure and mechanism of PARG

The crystal structure of a bacterial PARG from *Thermomonospora curvata* [43] revealed an evolutionarily conserved fold that is representative of the core structures of mammalian and *Tetrahymena* PARG enzymes [44–47] (Figure 3A). The catalytic domains of these enzymes share a mixed α, β architecture resembling a Rossman fold, originally termed a macro domain in the transcriptionally repressive histone protein variant, macro-H2A [48]. The macro domain fold binds to ADP-ribose monomers and polymers [49], and it is found in mono- and poly-(ADP-ribose) glycohydrolases, PAR binding histones, and other enzymes. The macro domain of PARG has a prominent substrate binding groove that engages ADP-ribose, or the tight-binding analog ADP (hydroxymethyl)pyrrolidinediol (ADP-HPD), in the crystal structures. The active site of *T. curvata* PARG is well suited for binding to the terminal ADP-ribose of a PAR polymer, consistent with the exo-glycohydrolase activity of this enzyme [43]. The C-terminal helix of *T. curvata* PARG walls off one end of the ADP-ribose binding site, creating a pocket that can accept the terminal ADP-ribose and would interfere with binding to internal sites of the PAR polymer [43]. In contrast, the ADP-ribose binding site of mammalian PARGs is open on both ends, enabling a PAR polymer to be positioned for endo- cleavage at internal ribose-ribose bonds [44,46]. Endo- cleavage of PAR chains underlies a proposed mechanism for PARP-dependent cell death, with the generation of oligo-PAR chains that trigger mitochondrial release of the death factor, apoptosis inducing factor (AIF) [50,51].

The catalytic schemes proposed for PARG are based on the locations of conserved active site residues and the mutational studies supporting their functional importance [43,44,46,52]. A lone glutamic acid (E756 in human PARG) is positioned where it can function as a general acid and a general base, to facilitate the exchange of the [n+1] poly-(ADP-ribose) leaving group for a water-derived hydroxyl. Additional contacts with the 2''-OH, 3''-OH, or 5''O of the ribose'' sugar may enhance the reactivity of a oxocarbenium-like intermediate for nucleophilic attack by water. Structures of PARG bound to ADP-ribose and the dinucleotide (ADP-ribose)₂ indicate that the ribose'' ring could interact with the side chain carboxylates of nearby acidic residues or with a nonbridging oxygen from the α -phosphorous of the terminal [n] ADP-ribose group, in a substrate-assisted mode of catalysis. Substrate-assisted catalysis is well documented in other glycosidases [53,54] and is a plausible mechanism for PARG, based on the structural data. Either of two bound waters observed in the crystal structure of human PARG could function as the attacking nucleophile, and their different positions with respect to the anomeric carbon would support either a retaining or inverting mechanism. Additional experimental work on the catalytic mechanism of PARG may lead to a better understanding of the nearly 1000-fold enhancement of ADP-HPD binding affinity in comparison to ADP-ribose binding [55], and

will aid in the rational development of drug-like small molecule inhibitors directed at the active site of human PARG.

PAR degradation and DNA repair

During the DNA damage response, PARG activity reverses the automodification of DNA bound PARP-1, concurrent with poly-ubiquitinylation of PARP-1 by the E3 ligase CHFR, and subsequent proteasomal degradation of PARP-1 [56]. Decreased activity of either CHFR or PARG delays repair and causes hypersensitivity to DNA damage [41,57–59], indicating that transient PARylation of PARP-1 and the subsequent removal of PARP-1 from DNA strongly contribute to the repair of DNA strand breaks. How PARP-1 turnover contributes to DNA repair is unknown, yet it is relevant to the therapeutic uses and outcomes of PARP inhibitors to treat breast and ovarian cancers, or for the development of therapeutically useful inhibitors of PARG. The regulation of chromatin-bound PARP-1 at sites of DNA damage may enable the remodeling of DNA repair complexes and/or the DNA substrate in order to complete the repair. CHFR-dependent removal of PARP-1 may promote remodeling of repair intermediates and further curtail PARP-1 enzymatic activity to preserve cellular NAD⁺ levels [56]. The exact role of PARG in this process remains to be investigated. The functionally relevant target(s) of PARG activity during DNA strand break repair may be proteins other than PARP-1, such as histones or the DNA repair scaffolding protein XRCC1, which are modified at sites of PARP-1 activity on chromatin. PARG activity also generates biologically active metabolites that may alter the fate of cells experiencing high level DNA damage, as discussed below.

PAR turnover and cell death

PAR oligomers and ADP-ribose are the products of PARG endo- and exo- glycohydrolase activities, respectively. The cellular levels of these metabolites could increase substantially when PARP-1 is hyperactive because PAR is rapidly degraded by PARG [12]. Oligo-PAR has been posited as a signaling molecule that triggers a caspase-independent pathway of programmed cell death, termed necroptosis or parthanatos [50,51]. Cell death resulting from PARP-1 hyperactivation is typically associated with the proteolytic cleavage of apoptosis inducing factor (AIF) and its translocation from mitochondria to the nucleus [60]. Under some experimental conditions, AIF translocation and cell death are observed in the absence of caspase activity, and thus, independent of the intrinsic pathway of apoptosis. Cleavage and translocation of AIF are hallmarks of the pathway and the mechanisms that trigger these events are under active investigation [61–63]. It has been proposed that oligo-PAR chains produced by the combined activities of nuclear PARP-1 and PARG could diffuse out of the nucleus and interact with the mitochondrial outer membrane to trigger AIF release [51]. A substantial body of compelling evidence for this mechanism has been reported, although there are some important details remaining to be clarified. AIF is normally associated with the inner mitochondrial membrane, so the reported localization of AIF to the outer surface of isolated mitochondria [64] is surprising and will require additional verification. Furthermore, purified PARG is predominately an exo-glycohydrolase, producing ADP-ribose from posttranslationally modified PARP-1 and from purified oligo-PAR chains. It is unclear whether oligo-PAR chains would be spared from further digestion by cytoplasmic

PARG long enough to function as signaling molecules. A conceivable remedy would be an oligo-PAR binding protein functioning as a chaperone during transit of PAR chains from the nucleus to mitochondria. In support of the proposed mechanism, overexpression of a cytoplasmic isoform of PARG blocks the nuclear translocation of AIF during PARP hyperactivation, consistent with an important role for oligo-PAR chains in the necroptosis pathway [50]. Determining the exact role of oligo-PAR in the necroptosis cell death pathway will have important implications for the pathogenesis and possible treatment of disease states related to neuronal excitotoxicity and ischemia-reperfusion injury [63].

The end product of PAR hydrolysis, ADP-ribose, could also signal DNA damage and contribute to PARP-dependent cell death. The NAD⁺ metabolite 2', 3'-cyclic ADP-ribose (cADP-ribose) triggers mobilization of intracellular Ca²⁺ stores, whereas ADP-ribose and cADP-ribose both stimulate the gating activity of a nonselective plasmalemmal Ca²⁺ channel, the transient receptor potential melastatin 2 (TRPM2) channel [65]. TRPM2 channel activity contributes to the pathogenesis of ischemia-reperfusion (IR) injury, a pathological condition associated with high levels of PARP-1 activation [66,67]. A knock-down of TRPM2 expression decreases cell death and tissue injury caused by IR, as does pharmacological inhibition of PARP-1. These observations provide circumstantial evidence that TRPM2 channel activity may contribute to PARP-dependent cell death [68]. The TRPM2 protein contains a C-terminal gating domain that is homologous to NUDT9, an ADP-ribose pyrophosphatase [69,70]. In whole cell and patch clamp experiments, the application of ADP-ribose to the intracellular surface of the membrane causes an immediate stimulation of TRPM2 channel activity, which is further enhanced by low levels of Ca²⁺. These results argue for a direct role of ADP-ribose as a positive effector of TRPM2 mediated calcium fluxes.

TRPM2 channel activity and increasing intracellular Ca²⁺ could promote AIF-dependent cell death in several ways. Elevated intracellular Ca²⁺ activates the cysteine protease calpain- μ , which cleaves AIF's N-terminal membrane anchor to release AIF into the mitochondrial intermembrane space [71]. Egress of cleaved AIF from the mitochondrion requires permeabilization of the outer mitochondrial membrane, and a number of mechanisms for this have been proposed [60]. In particular, dysregulation of intracellular Ca²⁺ levels causes mitochondrial depolarization by promoting the mitochondrial permeability transition, which may facilitate the release of AIF into the cytoplasm.

Therapeutic interventions directed at poly (ADP-ribose) metabolism

A growing number of PARP inhibitors in clinical trials show promise for the treatment of cancer, although the exact mechanism(s) of their tumor-selective killing effects remain enigmatic [72]. Inhibitors targeting the active site of PARP-1 suffer from dose limiting toxicity, which may result from inadequate binding specificity and off target effects on other PARP family members. As an alternative strategy, inhibitors blocking the DNA-dependent, allosteric activation of PARP-1 enzymatic activity may be more selective for PARP-1 and therefore a safer therapeutic strategy [24]. Additionally, inhibitors of the poly (ADP-ribose) glycohydrolase PARG may also prove useful for killing repair-deficient tumors, and possibly with fewer side effects, since PARG is monogenic and without paralogs [42,73].

Pharmacological agents targeting poly (ADP-ribose) metabolism may have other therapeutic applications as well, such as the treatment of stroke and other neurological injuries, or acute myocardial infarction [61,74].

Responses to DNA damage are highly complex and present a multitude of potential targets for therapeutic interventions aimed at selectively killing cells with dysregulated growth or sparing tissues with limited regenerative capacity from harmful insults [75]. The robust synthesis and turnover of poly (ADP-ribose) during the DNA damage response, catalyzed by PARP-1 and PARG, represents a broad paradigm for interrogating many facets of DNA repair, damage signaling, and programmed cell death through the use of small molecule ligands. Our growing understanding of the structures and catalytic mechanisms of PARP-1 and PARG will guide the rational development of pharmacological agents that be invaluable for examining the dynamic interplay of pathways that determine cell fate in normal and diseased tissues.

Abbreviations

PAR	poly(ADP-ribose)
PARP	PAR polymerase
PARG	PAR glycohydrolase
MARG	mono-(ADP-ribose) glycohydrolase
PARylation	poly(ADP-ribosylation)

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The Rise and Fall of PAR

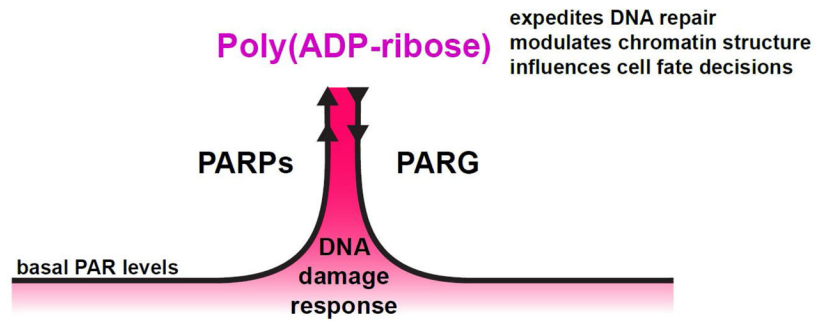


Figure 1. The rise and fall of poly(ADP-ribose)

The ADP-ribose posttranslational modification regulates many fundamental aspects of human biology. During the DNA damage response, there is an acute and transient burst of poly(ADP-ribose) production and turnover that facilitates repair and contributes to important cell fate signaling events.

DNA Damage Response PARPs

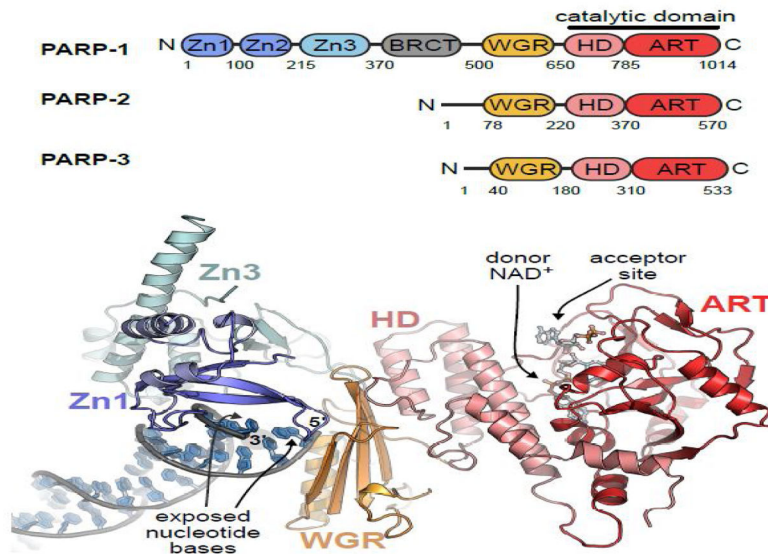


Figure 2. DNA damage response PARPs

Three human PARP enzymes are catalytically activated through binding to DNA damage: PARP-1, PARP-2, and PARP-3. The WGR domain and the HD region of the catalytic domain are defining and unique features of the DNA damage-dependent PARPs. PARP-1 consists of multiple domains that assume an active conformation upon binding to DNA damage. Zinc finger domains 1 and 3 (Zn1 and Zn3) interact with a DNA break and pack against the WGR domain, which serves as an intermediary between the C-terminal catalytic and N-terminal DNA binding domains, and allosterically couples damage detection to catalytic activation.

PARG Structure and Catalytic Mechanism

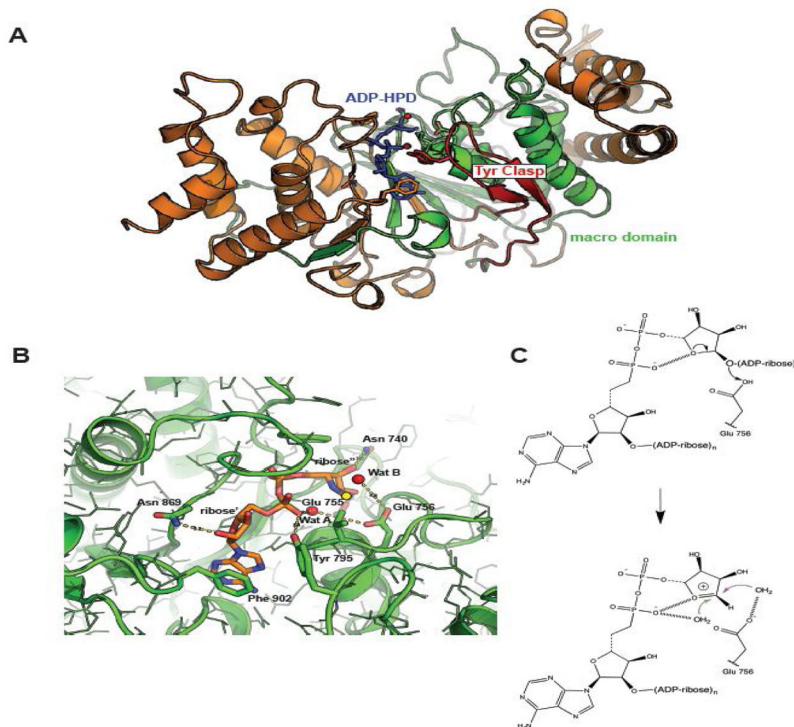


Figure 3. PARG structure and catalytic mechanism

A. The catalytic domain of human poly (ADP-ribose) glycohydrolase PARG (residues 448-976) consists of a macro domain (green; residues 611-812) flanked by N-terminal and C-terminal helical bundles (orange). The high affinity inhibitor adenosine diphosphate hydroxymethyl(pyrrolidinediol) (ADP-HPD; blue) is bound in the active site cleft, flanked by a β -hairpin structure termed the tyrosine clasp (red). Tyrosine 795 from the tyrosine clasp interacts with the α -phosphate of ADP-HPD and ADP-ribose (see panel B). **B.** The active site of PARG features a catalytic glutamate (Glu 756) and polar residues that engage the ribose and pyrrolidine hydroxyl groups of ADP-HPD and two bound water molecules (red spheres). The bound waters are positioned on either face of the carbon corresponding to the anomeric position of a poly (ADP-ribose) substrate (yellow circle), where they could function as the attacking nucleophile in a retaining (Wat A) or inverting (Wat B) mechanism of hydrolysis. **C.** Proposed catalytic mechanisms for PARG [43,46] assign Glu 756 as the catalytic acid that protonates the ADP-ribose leaving group, and as the catalytic base that activates a water nucleophile for attack of the anomeric carbon of ribose". An interaction between the α -phosphorous and the O4' of ribose (N of the pyrrolidine ring shown here) may stabilize the carbenium intermediate to assist catalysis.