Trends in Genetics

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REVIEWS

Review

Multiple Roles for Mono- and Poly (ADP-Ribose) in Regulating Stress Responses

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Although stress-induced synthesis of mono(ADP-ribose) (mADPr) and poly (ADP-ribose) (pADPr) conjugates by pADPr polymerase (PARP) enzymes has been studied extensively, the removal and degradation of pADPr, as well as the fate of ADPr metabolites, have received less attention. The observations that stress-induced pADPr undergoes rapid turnover, and that deficiencies in ADPr degradation phenocopy loss of pADPr synthesis, suggest that ADPr degradation is fundamentally important to the cellular stress response. Recent work has identified several distinct families of pADPr hydrolases that can degrade pADPr to release pADPr or mADPr into the cytoplasm. Further, many stress-response proteins contain ADPr-binding domains that can interact with these metabolites. We discuss how pADPr metabolites generated during pADPr degradation can function as signaling intermediates in processes such as inflammation, apoptosis, and DNA damage responses. These studies highlight that the full cycle of ADPr metabolism, including both synthesis and degradation, is necessary for responses to genotoxic stress.

Highlights

DNA damage promotes the rapid production of pADPr chains on damaged chromatin and is crucial for initiating recruitment of DNA repair proteins.

pADPr then undergoes rapid (seconds to minutes) degradation by PARG and related enzymes, releasing mADPr and soluble pADPr molecules into the nucleoplasm and cytoplasm.

Many stress-response proteins contain ADP-ribose-binding modules with specificity for mADPr or pADPr, such that binding of specific pADPr metabolites can regulate DNA repair proteins and apoptosis during stress.

A family of hydrolases which degrade mADPr, including NUDT5, can remove mADPr and terminate stress responses while generating useful metabolites.

pADPr and its metabolites may function as novel stress-induced signaling molecules that regulate the function of stress and DNA repair proteins.

Conjugation of ADPr to proteins plays a crucial signaling function in gene transcription, chromatin organization, and stress responses. pADPr polymerase (PARP1) was originally thought responsible for producing all cellular ADPr [1]. However, PARP1 is one of 18 human enzymes also known as diphtheria toxin-type **ADP-ribose transferases** (ARTDs; see Glossary) (reviewed in [2]) which can ADP-ribosylate proteins. Further, families of ADPr hydrolases have been identified which cleave pADPr chains, protein–ADPr bonds, or remove the terminal mADPr from pADPr chains. In addition, multiple ADPr-binding domains have been described. There are therefore both writers and erasers of ADPr as well as protein modules that can read mADPr and pADPr. Protein–mADPr and pADPr polymers (both protein-linked and soluble) as well as free mADPr may therefore represent distinct functional signaling effectors in the cell.

The PARP Family: Generating pADPr

The 18 PARP proteins share a common catalytic domain and can be classified into three groups based on their enzymatic activity. PARP1, PARP2, PARP5a, and PARP5b create branching pADPr chains ranging from 2 to 200 ADPr units in length [3]. Notably, PARP1 produces ~90% of cellular pADPr following genotoxic stress. PARP9 and PARP13 lack catalytic activity, and PARP18 is currently uncharacterized. The remaining 11 PARP enzymes add a single ADP-ribose (mADPr) unit onto their targets [3]. An early experiment in rat liver estimated that there are ~1000-fold more mADPr amino acids than pADPr amino acids [4], demonstrating the ubiquity of mADPr modification. PARP family members therefore specialize in either adding mADPr or creating pADPr, often during stress responses.

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PARPs and Stress Responses

PARP1, PARP2, and PARP3 are DNA-dependent PARPs [5] which can be activated by single- and double-strand DNA breaks (SSBs and DSBs) [6,7] or by breaks in unstable DNA structures such as **G-quadruplexes** [8]. During DSB repair, the rapid (seconds to minutes) formation of pADPr [9] serves to concentrate many chromatin regulatory proteins at the damaged sites. These include histones macroH2A1.1 and H3.3 [10–12], remodeling complexes (ALC1 and CHD2 [12,13]), and many chromatin-binding proteins [14,15]. The accumulation of proteins promotes PARP-mediated chromatin reorganization at DNA breaks and access to, and repair of, damaged chromatin [16,17]. Many of these repair proteins contain pADPr-binding modules which aid in their recruitment and retention. PARP enzymes predominantly modify acidic amino acids, including aspartate and glutamate, although serine may be the major target during DSB repair [18]. In addition, even though PARP1 generates pADPr, and PARP3 generates mADPr, they exhibit significant substrate overlap [19], suggesting that PARP3 may create the initial protein–mADPr linkage while PARP1 extends the pADPr chain. Further, the exact protein target (s) for PARP proteins during repair are unclear – histones, chromatin-binding proteins, and auto-ADPr of PARP1 at DNA breaks have all been reported [2]. DNA-dependent PARPs can also add mADPr and pADPr directly to the DNA ends [9,20–22]. Although protein–ADPr has been well studied, how cells control the extent of pADPr and remove mADPr and pADPr chains has received much less attention. We discuss here a new class of enzymes which function to degrade pADPr chains and discuss emerging evidence that pADPr and mADPr produced by degradation of protein–pADPr can function as signaling intermediates.

pADPr Hydrolases: Removing pADPr

Because ADPr conjugated to proteins can regulate activity, it is important to **dePARylate** these targets and allow a return to the basal state. pADPr is rapidly turned over during DNA repair [23], suggesting that dynamic turnover is biologically important. Further, deletion of the genes encoding PARP1 or the dePARylating enzyme PARG both have similar effects on DNA repair [24], suggesting that **poly(ADP-ribose)ylation** (PARylation) and dePARylation are tightly coupled. pADPr degradation is complex because of the difference between the chemical bonds linking ADPr to protein (via e.g., aspartate, glutamate, or serine) and those linking successive ADPr units (Figure 1, Key Figure). Several structurally distinct families of pADPr hydrolases have now been identified (Figure 1), including the macrodomain-containing enzymes MacrodD1, MacroD2, and TARG1, which cleave the protein–ADPr bond; ARH1–3 (which contain a Dra-G-like fold) and PARG, which cleave the ADPr polymer chain; and the recently identified members of the NUDIX and ENPP families, which can act directly on released mADPr. Metabolism of pADPr chains can therefore have multiple outcomes, including (i) complete hydrolysis of pADPr chains leading to free, soluble mADPr; (ii) release of mixed populations containing large, soluble nuclear or cytoplasmic pADPr polymers following endoglycohydrolase activity; and (iii) trimming of pADPr chains to leave mADPr–protein (Figure 1).

pADPr Hydrolases

PARG is a macrodomain-containing pADPr hydrolase that rapidly degrades pADPr chains following DNA damage. PARG exhibits both endo- (cleavage within chains) and exo- (cleavage from the end) hydrolase activity [25,26] (Figure 1), giving it the potential to release large branched pADPr molecules and to trim pADPr chains. PARG functions primarily as an exoglycohydrolase, although endo-cleavage can occur when cells contain high levels of pADPr [26], with the released pADPr acting as a signal for apoptosis (discussed further below). ARH3, which is structurally unrelated to PARG, can also degrade pADPr, although it prefers longer pADPr chains and has lower activity [27]. However, PARG cannot cleave the protein–ADPr

Glossary

ADP-ribose transferases (ARTDs): enzymes homologous to diphtheria toxin that transfer one or more ADP-ribose groups from NAD^+ to target proteins.

Base excision repair (BER): the predominant DNA damage repair pathway for the processing of small base lesions produced by oxidation, deamination, or alkylation damage. BER is initiated by a DNA glycosylase that recognizes and removes the damaged base and is completed by short- or long-patch repair.

Chromatin remodeling: the dynamic rearrangement of chromatin architecture to allow access to condensed genomic DNA, including histone variant incorporation, histone depletion, and/or histone sliding.

DePARylation: removal of poly (ADP-ribose) chains from target proteins or DNA catalyzed by poly (ADP-ribose) hydrolases.

DNA damage response (DDR): a complex network of pathways that mediate the detection, signaling, and repair of DNA damage.

Ecto-ADP-ribosyltransferases (ecto-ARTs/ARTCs): ADP ribosyltransferases located on the cell surface or in the extracellular compartment that transfer one ADPr group from extracellular NAD^+ to target proteins.

G-quadruplexes: nucleic acid secondary structures in which two or more G-tetrads (four G residues linked by the sugar–phosphate backbone and connected through Hoogsteen-type hydrogen bonds) stack on top of each other.

Homologous recombination (HR): an error-free DNA repair pathway that occurs through homologous strand exchange and takes place in the late S–G2 phases of the cell cycle.

Intrinsically disordered protein (IDP): dynamically disordered proteins that feature biased amino acid composition and low sequence complexity.

Liquid demixing: the aggregation of IDPs by phase separation from the soluble intracellular space.

Mitochondrial dysfunction: loss of function in mitochondria caused by reduction of oxidative

Key Figure

DePARylation

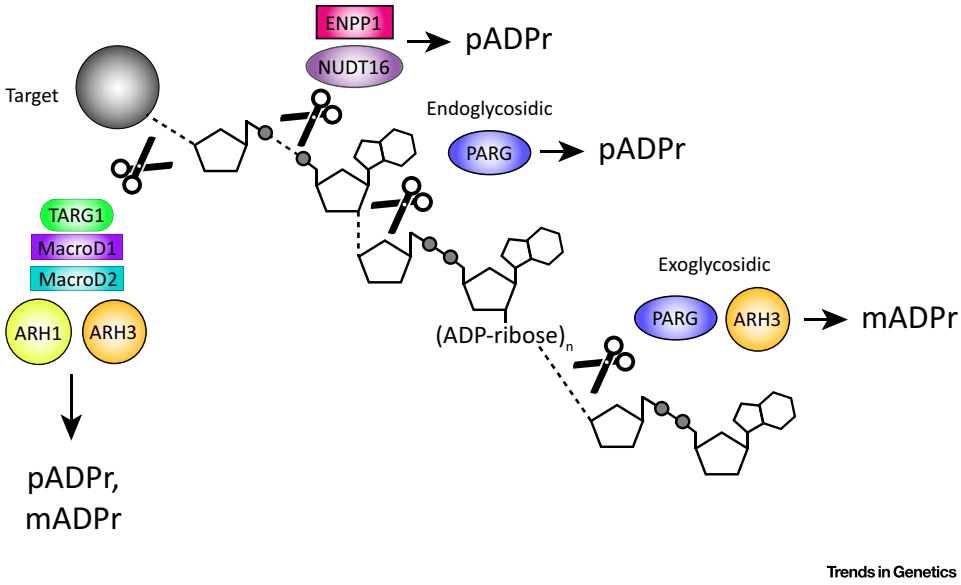

Figure 1. ADPr hydrolases cleave specific chemical linkages to release pADPr or mADPr from protein and DNA targets. PARG cleaves within the pADPr chain (endoglycosidically) to yield free pADPr, and at the end of the chain (exoglycosidically) to produce mADPr [25,26]. ARH3 cleaves exoglycosidically to produce mADPr from the end of pADPr chains and from MARylated serines [28]. ENPP1 and NUDT16 cleave phosphodiester bonds to produce protein-conjugated ribose 5′-phosphate and pADPr [37]. TARG1, MacroD1, and MacroD2 cleave the terminal ADPr bond to release pADPr or mADPr from glutamate, while ARH1 cleaves the terminal bond but only for targets MARylated on arginine [2,30]. ADP-ribose$_n$ represents chains of $n = 2–100$. Abbreviations: ADPr, ADP-ribose; dePARylation, removal of poly(ADP-ribose) chains; mADPr, mono(ADP)ribose; pADPr, poly(ADP)ribose; PARylation, addition of poly(ADP-ribose) chains.

bond. Instead, ARH3 preferentially cleaves ADPr attached to serine [28,29]; TARG1, MacroD1, and MacroD2 remove mADPr from aspartate and glutamate [30]; and ARH1 cleaves arginine–ADPr. This requirement for multiple protein–ADPr hydrolases may reflect the distinct cellular locations (e.g., MacroD1 is enriched in mitochondria [31]; Figure 2) and the need to remove ADPr from different amino acids or from DNA [31].

Complete removal of pADPr from proteins will therefore require the coordinated action of PARG, which hydrolyzes ADPr chains, and a specific protein–ADPr hydrolase to remove the protein–ADPr link. Currently, it is unclear if TARG1 (or other protein–ADPr hydrolases) can remove the entire pADPr chain on its own (by cleaving the link to protein) or if it requires initial trimming of pADPr by PARG to allow access to the final ADPr. The most commonly used pADPr antibody preferentially binds to long pADPr chains versus short chains, and cannot detect mADPr. The rapid appearance and loss of pADPr during processes such as DNA repair monitored using immunostaining approaches may therefore fail to detect mADPr proteins or short pADPr chains remaining following PARG processing of pADPr. Given the large number of **DNA damage response** (DDR) proteins that can associate with ADPr, residual mADPr may

phosphorylation efficiency and ATP production.
Monotherapy: the treatment of disease or disorder with a single type of treatment such as radiation therapy. In drug therapy it refers to treatment with a single drug molecule.
NAD$^+$ salvage pathway: production of NAD$^+$ from nicotinamide, an end-product of NAD$^+$ consumption, catalyzed by nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide adenylyltransferase (NMNAT).
Non-homologous end-joining (NHEJ): the predominant pathway to repair DNA double stand breaks by religation of DNA ends with limited processing.
Parthanatos: a programmed cell death process mediated by pADPr.
Poly(ADP-ribose)ylation (PARylation): a post-translational modification involving covalent addition of linear or branched polymeric chains of ADPr.
Stress granules: dense aggregates of proteins and RNAs formed in the cytosol under stress.

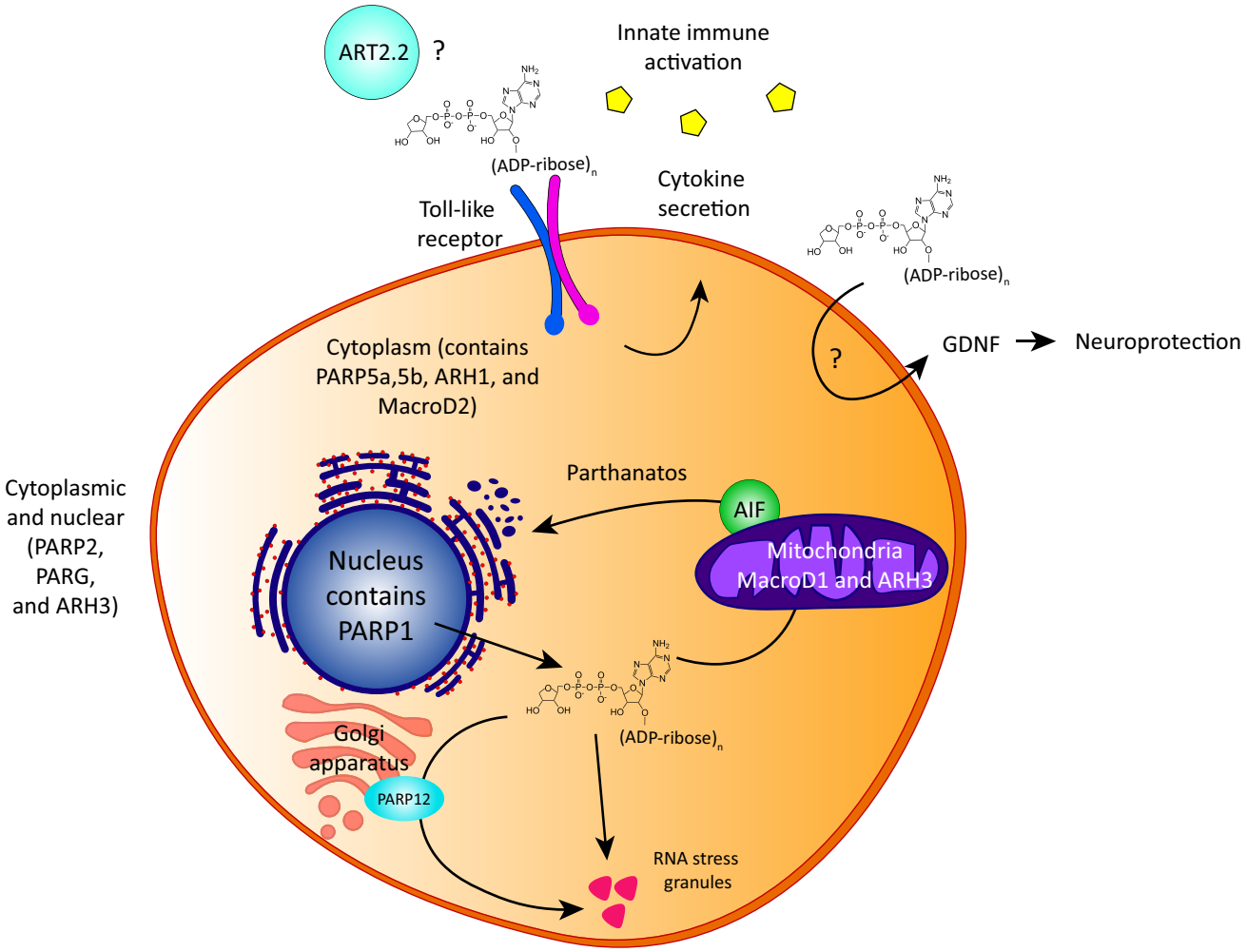

Figure 2. pADPr Signaling beyond the Nucleus. Different subsets of PARPs and pADPr hydrolases are found in different subcellular compartments including the nucleus, cytoplasm, and mitochondria. In the immune system, ART2.2 might catalyze pADPr synthesis extracellularly [43,44]. Extracellular pADPr activates TLR signaling, inducing macrophages to secrete cytokines [40]. In the nervous system, extracellular pADPr increases GDNF in astrocytes, playing roles in neuroprotection [41]. Free cytoplasmic pADPr binds to AIF, triggering its release from the mitochondria into the cytoplasm, inducing parthanatos [47]. Cytoplasmic pADPr also induces PARP12 to translocate from the Golgi apparatus to RNA stress granules, promoting stress-granule function [53]. Abbreviations: pADPr, poly(ADP-ribose); GDNF, glial cell-derived neurotrophic factor; PARP, pADPr polymerase.

recruit or retain macrodomain proteins such as macroH2A1.1 [32,33] or ALC1 [13] at sites of DNA damage. In fact, deficiency of the serine–ADPr hydrolase TARG1 is associated with increased sensitivity to DNA damage [34], and TARG1 is recruited to DNA breaks. This implies that removal of both pADPr and protein–ADPr is crucial for completion of DNA repair, and that this process may be tightly linked to specific ADPr hydrolases. However, a better understanding of the substrate specificity and cellular location of these enzymes is still needed.

Novel ADPr Hydrolases

Two recently identified enzymes, NUDT16 [35,36] and ENPP1 [37], can also cleave protein-linked ADPr, but leave a ribose-5-phosphate attached to the protein (Figure 1). These novel

pADPr hydrolases have not been characterized, nor has the influence of ribose-5-phosphate on protein function been explored.

Extracellular mADPr/pADPr and Signaling

ENPP1 is an extracellular protein, suggesting that pADPr signaling can occur in the extracellular space. Serum antibodies against pADPr and/or PARG have been found in patients with lupus, ulcerative colitis, and Alzheimer's disease (AD) [38,39], indicating that extracellular pADPr might play an active role in disease pathology. Extracellular pADPr (but not mADPr) can activate Toll-like receptor (TLR) signaling, which induces macrophages to secrete cytokines and promotes innate immune activation [40] (Figure 2). In the nervous system, extracellular pADPr (but not mADPr) increases glial cell-derived neurotrophic factor (GDNF) in astrocytes [41]. Consistent with the neuroprotective functions of GDNF, injection of pADPr into brain striatum mitigates disease phenotypes in a rodent model of Parkinson's disease (PD). Both studies [40,41] indicate that uptake of extracellular pADPr can regulate signaling in macrophages and neuronal cells. The origin of extracellular pADPr is unclear because PARP enzymes are largely intracellular [2], suggesting that pADPr synthesized following oxidative stress may be released into the extracellular space. Alternatively, **ecto-ADP-ribosyltransferases** (ecto-ARTs), a poorly characterized family of ribosyltransferases with extracellular catalytic domains [42], may be responsible. ART2.2, an ecto-ART anchored to the surface of T cells and lymphocytes is a potential candidate, although its ability to generate pADPr *in vivo* requires additional characterization [43,44]. To address this, further work will be necessary to identify extracellular enzymes that can synthesize and degrade pADPr as well as to determine whether mADPr and pADPr are exported from cells as soluble signaling molecules.

Cytoplasmic mADPr/pADPr and Signaling

In the cytoplasm, free pADPr is important for the **parthanatos** cell-death pathway (Figure 2), a PARP1-dependent pathway activated following ischemia–reperfusion injury [45,46]. pADPr binds directly to apoptosis-inducing factor (AIF) [47], releasing AIF from the mitochondria into the cytoplasm [45,46]. AIF then chaperones macrophage migratory inhibitory factor (MIF), a nuclease, into the nucleus, leading to MIF-dependent fragmentation of the genome and parthanatos [48]. Delivering synthetic pADPr to the cytoplasm can activate parthanatos, and free pADPr of increasing length and complexity is the most potent trigger [45,46]. Further, ARH3 and PARG function together to degrade free cytoplasmic pADPr and protect cells from parthanatos [49].

These studies imply that free pADPr is sufficient to activate parthanatos. However, whether the physiological substrate is free soluble pADPr or a (soluble) protein–pADPr remains an open question. It is also unclear how nuclear pADPr exits the nucleus. In addition, there are indications that pADPr may have cytoplasmic functions during normal cell metabolism. In mitotic and meiotic cells, cytoplasmic pADPr is important for spindle formation and positioning [50,51]. Five PARP enzymes and PARG are recruited to, and support the function of, RNA **stress granules** upon cellular stress [52]. pADPr causes PARP12 to translocate from the Golgi apparatus to RNA stress granules to promote stress-granule function [53] (Figure 2). Taken together, these data indicate that the accumulation of free pADPr in the cytoplasm can mediate cellular stress responses such as parthanatos and RNA stress-granule formation. Tight control of the synthesis and degradation of protein–pADPr chains is therefore important for regulating stress and apoptotic responses in cells.

Binding Domains: Readers of mADPr and pADPr

At least five different pADPr-binding domains have been described (Table 1). Distinct ADPr reader domains can discriminate between pADPr and mADPr as well as between soluble and

Table 1. Non-Covalent ADPr-Binding Motifs

Motif	Abbreviation	Description	Moiety read	Examples
Poly(ADP-ribose)-binding motif	PBM	~20 amino acid (aa) motif (hydrophobic aa spaced by basic aa)	Unknown	AIF [47], WRN [57], histones H2A/H2B/H3/H4 [119], p53 [59], XPA, p21, XRCC1/5/6, DNA-PK$_{cs}$, TOP1, DNA ligase 3 [54]
WWE		~80–100 aa	Iso-ADP-ribose	Iduna/RNF146 [72], HUWE1, ULF, Deltex1, Deltex2, Deltex4, PARP11 [71]
Macro		130–190 aa motif	Terminal ADP-ribose	ALC1 [63,69], MacroH2A [120], PARP9, PARP14, PARP15, PARG, TARG1, MacroD1, MacroD2 [60]
PAR-binding zinc finger	PBZ	~30 aa Cys2–His2 (C8–C8–H8–H)	Two adjacent ADP-ribose moieties	APLF [121], CHFR [122], CTCF [123]
PAR-binding regulatory motif	PbR	Cys2–His2 (C8–C6–H8–H)	Unknown	CHK1 [124]

protein-linked ADPr. pADPr and mADPr may therefore regulate distinct sets of proteins depending on the specificity of the ADPr domain on the target protein.

PAR-Binding Motif (PBM)

The PBM features hydrophobic amino acids interspersed with charged basic residues [54,55]. The net positive charge of PBMs may promote interaction with negatively charged pADPr chains by electrostatic interaction [56]. PBMs are found in AIF, which regulates parthanatos [47] and many DNA damage-response proteins (Table 1). PBMs often overlap with other functionally important domains [54], indicating that in addition to recruiting proteins to pADPr chains they may also play roles in regulating protein function. The PBM of the Werner syndrome protein (WRN) overlaps with its exonuclease and DNA-binding domains, and its binding of short or long chains of free pADPr interferes with helicase and exonuclease activities during DSB repair [57]. Whether WRN interacts with protein-linked or free pADPr remains an open question because both forms are inhibitory in biochemical assays [57]. Similarly, the PBMs of XPA and p53 overlap with their DNA-binding domains, and interactions with pADPr abrogate their DNA-binding activity during nucleotide excision repair and transcriptional activation, respectively [58,59] (Figure 3).

Macrodomains

Macrodomains are globular pADPr-binding modules found in diverse proteins, including macroPARPs (PARP9, PARP14, and PARP15), ADPr hydrolases (PARG, TARG1, MacroD1, and MacroD2) [60], macroH2A1.1 [61,62], and the **chromatin remodeler** ALC1 [63]. Macrodomains bind mADPr or the terminal ADPr residue in pADPr chains [2]. MacroH2A, a histone variant important for maintaining chromatin organization, is encoded by two genes: *MacroH2A1* and *MacroH2A2*. *MacroH2A1* has two transcriptional variants: *macroH2A1.1* and *macroH2A1.2*. Although all three proteins contain macrodomains, only macroH2A1.1 binds to pADPr and mADPr [33]. Upon DNA damage, macroH2A1.1 undergoes rapid pADPr-dependent recruitment to damaged chromatin (Figure 3) [32,33] while macroH2A1.2 is transiently excluded [11]. At later timepoints, which coincide with degradation of pADPr to mADPr, macroH2A1.2 reaccumulates to promote **homologous recombination** (HR) and chromatin recondensation [11]. The sequential binding of macroH2A1.1 followed by macroH2A1.2 also coincides with the initial chromatin relaxation and subsequent chromatin condensation after DNA damage [11]. This has led to the idea that recruitment of macroH2A1.1 to damaged chromatin initially promotes open chromatin and facilitates repair. In support of this model, in

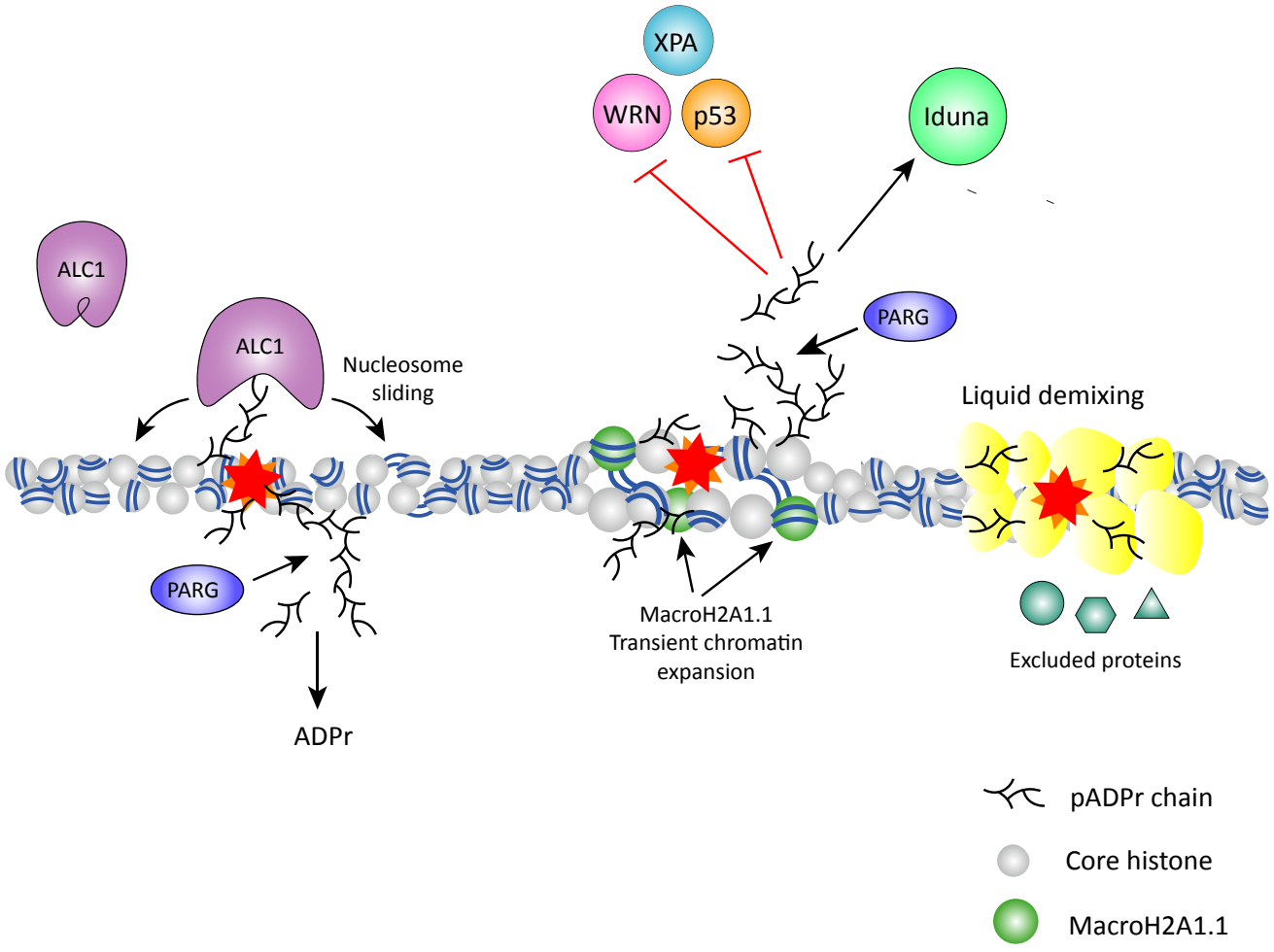

Figure 3. pADPr Signaling in the Nucleus. Through binding of pADPr, reader proteins are recruited and functionally regulated during the DNA damage response. The chromatin remodeler ALC1 is recruited and activated by pADPr binding, promoting nucleosome sliding [63]. MacroH2A1.1 is also recruited to DNA damage sites by pADPr and promotes transient chromatin expansion [32]. pADPr binding can regulate protein functions either negatively (e.g., by inhibiting the nuclease activity of WRN [57], the NER activity of XPA [58], or the DNA-binding activity of p53 [59]) or positively (e.g., by activating the ATPase activity of ALC1 [63] or the E3 ligase activity of Iduna [73]). pADPr can also cause liquid demixing, forming a membraneless organelle that retains some proteins but excludes others [76]. Abbreviations: pADPr, poly(ADP-ribose); NER, nucleotide excision repair.

undamaged cells, binding of macroH2A1.1 to pADPr facilitates its localization to acetylated chromatin and even promotes H2B acetylation, a mark associated with relaxed chromatin [64]. Subsequent removal of pADPr and release of macroH2A1.1 following repair then allows chromatin repacking.

ALC1/CHD1L is an ATP-dependent chromatin remodeler that functions during DNA repair (Figure 3) [65]. ALC1 contains a macrodomain that has two distinct functions: recruitment of ALC1 to damage sites and activation of the ALC1 ATP-dependent nucleosome sliding activity [13,66]. In the basal ('off') state, the macrodomain of ALC1 interacts with the ATPase domain to autoinhibit its activity. Binding of pADPr relieves inhibition of the ATPase domain and activates the chromatin remodeling activity of ALC1 [63,67]. ALC1 binds pADPr chains rather than the

mADPr recognized by canonical macrodomains [68]. This difference may reside in a stretch of basic amino acids adjacent to the ADPr-binding pocket [69], suggesting that electrostatic interactions, such as those seen in PAR motifs, may contribute to ALC1–pADPr interaction. Further, nucleosome sliding by ALC1 involves significant motion relative to the underlying DNA. If ALC1 is tethered to protein–pADPr on the nucleosome, this may restrict its motion, and therefore the extent of nucleosome sliding relative to the site of damage. Alternatively, binding of free pADPr to the ALC1 macrodomain would allow ALC1 to move freely along the chromatin. Switching between these two modes of operation may allow cells to control the extent of chromatin remodeling during DNA repair.

WWE and PBZ Domains

The E3 ligase Iduna/RNF146 binds pADPr through its WWE domain (Figure 3). WWE domains recognize the base–ribose link between two ADPr units, termed iso-ADPr [70,71], such that WWE domains require pADPr for binding. Iso-ADPr binding induces a conformational change in Iduna which converts it to an active E3 ligase. Iduna then ubiquitinates DNA repair factors, including PARP1, XRCC1, KU70, and LIG3 [72,73]. Because several E3 ligases contain WWE domains, this may represent a general mechanism of activation for these enzymes [71]. The PAR-binding zinc-finger (PBZ) domain is ~30 amino acids in length with a Cys2–His2 (C2H2) zinc-finger motif. PBZ domains were first described in the **non-homologous end-joining** (NHEJ) factor APLF [74] in which tandem PBZ domains are required for phosphorylation by ATM [75], indicating that pADPr signaling can drive crosstalk with other post-translational modifications.

Liquid Demixing

Local increases in pADPr during stress responses or DNA repair can trigger **liquid demixing**. In liquid demixing, pADPr (either protein–pADPr or soluble pADPr chains released by PARG) initiates the formation of liquid droplets by seeding the aggregation of **intrinsically disordered proteins** (IPDs) such as FUS and EWS [76]. These aggregates of pADPr are localized regions with a high density of negative charge and form a membraneless organelle that retains some proteins but excludes others [76]. The ability of pADPr-dependent liquid droplets to preferentially solubilize certain proteins (e.g., MDC1) may explain the striking number of proteins (>100) that accumulate on damaged chromatin in a PARP-dependent manner [77]. Soluble pADPr can nucleate droplet formation *in vitro* [76], raising the question of whether free or protein-bound pADPr (or both) can promote liquid demixing *in vivo*. Liquid demixing by pADPr may compartmentalize damaged chromatin, maintaining damaged DNA ends in close proximity and promoting repair (Figure 3). Once repair is complete, degradation of pADPr dissolves these transient, membraneless organelles to relieve the temporal exclusion. Liquid demixing has also been invoked to describe pADPr aggregation at spindles [50] and in RNA stress granules [52]. Moreover, the recent findings that heterochromatin protein 1α (HP1α) undergoes liquid demixing to drive heterochromatin formation [78,79] are intriguing because HP1α is also PARylated [80] and recruited to chromatin in a PAR-dependent manner [81].

The Fate of mADPr

TRPM2 and Cell Death

pADPr degradation by PARG following DNA damage creates a large pool of free mADPr (Figure 4) which can function as a signaling molecule. Transient receptor potential melastatin 2 (TRPM2), a Ca^{2+} ion channel, binds to mADPr via its cytoplasmic NUDT9H domain [82–84] (Figure 4). Ca^{2+} flux through the TRPM2 channel depends on the relative balance of PARP1 and PARG, and mADPr arising from pADPr degradation during oxidative stress promotes Ca^{2+} influx [85]. This allows TRPM2 to function as a sensor of oxidative stress. However, high levels

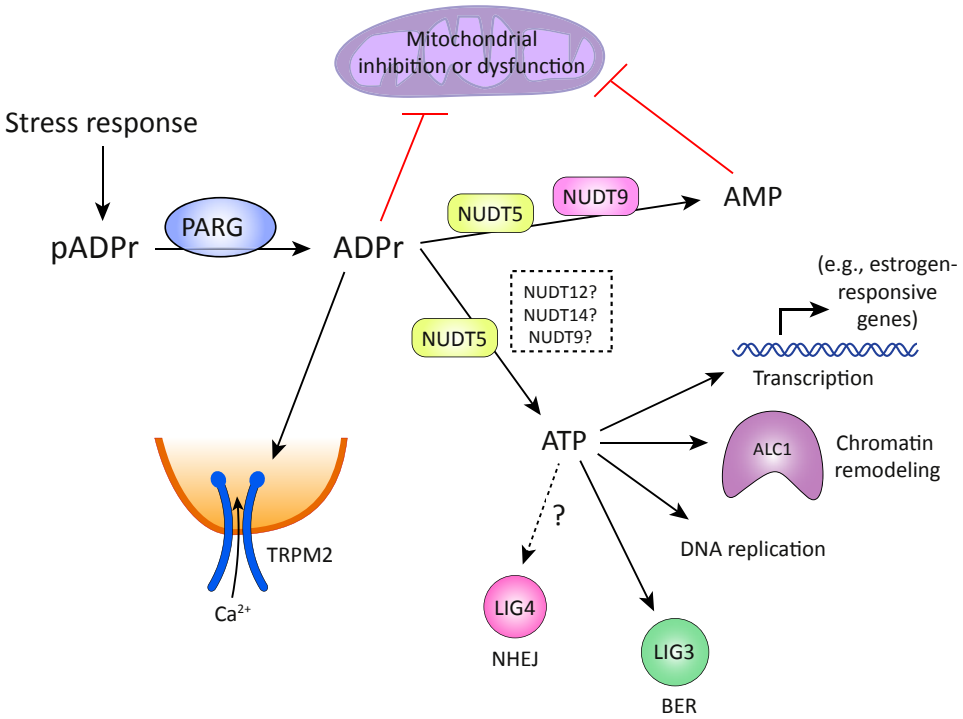

Figure 4. The Fate of Free mADPr. During a stress response a large pool of free mADPr is generated by PARP- and PARG-catalyzed PAR polymerization and degradation in the nucleus. Free mADPr activates the TRPM2 cation channel, inducing Ca^{2+} influx into cells [84]. NUDT9 catalyzes mADPr conversion to AMP [91], while NUDT5 possesses two catalytic activities that convert mADPr to either AMP or ATP [94]. Overproduction of mADPr or AMP can lead to mitochondrial dysfunction [92]. ATP generated by NUDT5 (whether other NUDIX family proteins also catalyze this process is undetermined) may be used locally for transcription and chromatin remodeling during DNA replication or repair. Abbreviation: BER, base excision repair; mADPr, mono(ADP-ribose); NHEJ, non-homologous end-joining; pADPr, poly(ADP-ribose).

of mADPr can fully activate TRPM2 channels, leading to toxic intracellular Ca^{2+} levels and cell death [86]. Catabolism of mADPr is therefore crucial to turn off mADPr signaling via TRPM2 and other proteins.

NUDIX Family Proteins and mADPr Degradation

The rapid synthesis and degradation of pADPr can deplete ATP and NAD^+ in cells [87]. pADPr can also bind to and inhibit hexokinase, which catalyzes the initial step of glycolysis [88,89]. Two members of the NUDIX (nucleoside diphosphates linked to other moiety, X) hydrolase family, NUDT5 [90] and NUDT9 [91], can further hydrolyze mADPr to AMP and D-ribose 5-phosphate. Elevated AMP can lead to **mitochondrial dysfunction** [92]. Thus, sustained activation of PARP during stress responses and degradation of pADPr can lead to bioenergetic collapse through ATP and NAD^+ depletion and ultimately to cell death. Interestingly, NUDT5 (Figure 4) also possess mADPr pyrophosphorylase activity which can convert mADPr into ATP [93]. This provides a potential mechanism for regenerating or maintaining local ATP levels, thereby limiting the rapid depletion of ATP which is a hallmark of sustained PARP activation.

NUDT5 forms phosphorylated dimers which predominantly convert mADPr to AMP and pyrophosphate. However, dephosphorylation of NUDT5 destabilizes the dimer and favors

the synthesis of ATP, suggesting that NUDT5 metabolism of mADPr to AMP or ATP is dictated by phosphorylation [94]. However, the NUDT5 kinase or phosphatase responsible for this functional switch is not known. PARylation is also important for regulating chromatin structure and gene transcription. In breast cancer cells, NUDT5 is frequently overexpressed and promotes estrogen-dependent chromatin remodeling and transcription by increasing local ATP levels [94]. NUDT5 associates with PARP1 and PARG, and coupled synthesis and catabolism of pADPr by PARP1/PARG/NUDT5 complexes can generate a local source of ATP for hormone-induced chromatin remodeling. The ATP generated by NUDT5 plays a crucial role in maintaining displacement of histones at estrogen-responsive genes, thereby supporting transcription and cell growth. NUDT5 can therefore provide ATP to support chromatin reorganization by remodeling ATPases which promote transcription. Many aspects of the DDR also require energy. For example, ATP-dependent chromatin remodelers such as ALC1 or p400 [13,16,17] reorganize chromatin during DNA repair. ATP from pADPr has also been reported to fuel DNA replication [95], the ligation step of **base excision repair** (BER) [93], and repair by NHEJ [5]. In addition, cells lacking NUDT5 show increased sensitivity to DNA damage [94]. The ability of NUDT5 to convert mADPr to ATP during DNA repair may maintain local ATP concentrations for kinase or ligase activity and ATP-dependent chromatin remodeling. However, the recent discovery that pADPr can be directly coupled to DNA [9] indicates that there are significant complexities to mADPr metabolism during DNA repair which require further experimental investigation. Locally generated ATP may also be funneled to the **NAD^+ salvage pathway** during PARP1 hyperactivation to replenish pools of NAD^+ [96]. Further, ATP has hydrotropic properties [97] which may help to redissolve hydrophobic proteins that have undergone PAR-dependent liquid demixing [76]. NUDT5, NUDT12, and NUDT14, which may also use ADPr as a substrate [98], may remove mADPr to terminate signaling and recycle a fraction of mADPr to increase local ATP levels that were depleted by the initial pADPr generation (Figure 4). Understanding how NUDT5 and other proteins participate in pADPr metabolism during both transcriptional activation and DNA repair is an exciting area for further study.

Targeting pADPr Metabolism for Therapy

The key role of PARP enzymes in DNA repair motivated the development of PARP inhibitors, small molecules that produce synthetic lethality in tumors with HR deficiency. Inhibition of PARP enzymes shows promise as a **monotherapy** in DNA repair-deficient (BRCA1/BRCA2) tumors and can sensitize cells to ionizing radiation (IR) [99]. The past 5 years have seen the first FDA approvals of the PARP inhibitor olaparib for patients with germline *BRCA1/BRCA2* mutations. However, one of the central challenges of PARP inhibitors is therapeutic resistance that develops either as a result of reactivation of the HR pathway or unknown mechanisms. A genetic screen identified loss of PARG as a mechanism to confer PARP inhibitor resistance in $BRCA2^{-/-}$ cells, and demonstrated mosaic loss of PARG in human breast and ovarian cancers, suggesting that $PARG^{-/-}$ clones might be selected by treatment with PARP inhibitors. Despite conferring PARP inhibitor resistance, loss of PARG leads to new vulnerabilities, including enhanced sensitivity to both IR and the alkylating chemotherapeutic agent temozolomide [100]. Further study will be necessary to determine whether PARG suppression is a physiological mechanism of PARP inhibitor resistance in human tumors.

Both synthesis and degradation of pADPr are important for functional DNA repair processes [101,102]. Therefore, targeting PARG or other enzymes involved in pADPr degradation may also have clinical applications. PARG inhibitors also sensitize cells to IR, suggesting an additional strategy to disrupt pADPr homeostasis [103,104]. The PARG dependency of HR-deficient tumors is less straightforward. Recent reports examining genetic susceptibilities

between PARG and BRCA1/2 have shown contradictory results, suggesting that the broader cellular context or PARG expression level may be important. [105–107]. Further, PARG inhibitor sensitivity was independent of BRCA1/BRCA2 status, suggesting that PARG inhibitors could have different clinical applications from PARP inhibitors [108]. For example, PARG inhibitors synergize with ibrutinib, a clinically useful kinase inhibitor, suggesting the possibility of new vulnerabilities [108]. Additional possible PARG inhibitor synergies await discovery. Other therapeutic strategies to disrupt pADPr homeostasis are in earlier stages of development. A novel screening platform identified inhibitors of MacroD1 [109] which may be effective in treating lung, breast. and pancreatic cancers which overexpress MacroD1 [110]. A NUDT5 inhibitor which inhibits formation of ATP from mADPr blocks the proliferation of breast cancer cells [111], providing a third potential approach for interfering with pADPr metabolism.

Finally, small molecules that disrupt pADPr metabolism may have applications beyond oncology (reviewed in [112]). For example, PARP inhibitors are being examined for prevention of reperfusion injury following heart attack and pulmonary hypertension [113,114], and may have applications in stroke and neurodegeneration [112]. Recent studies have identified familial mutations in *TARG* and *ARH3* that lead to accumulation of pADPr and cause rare neurodegenerative diseases [34,115,116]. Several more common neurodegenerative diseases including AD and PD are characterized by hyperactivation of PARP1 leading to pADPr accumulation in neurons. Interestingly, neuronal cells are particularly vulnerable to mitochondrial toxicity associated with PARP1 hyperactivation and pADPr accumulation. Recent data from *C. elegans* and mice show that inhibiting pADPr accumulation, either by inhibiting PARP enzymes or expressing PARG, improves neuronal regenerative potential [117]. Further, in mouse models of AD and PD, PARP1 deficiency mitigates the disease phenotype [118]. Taken together, these data suggest that disrupting pADPr metabolism is neurodegenerative diseases may be a promising strategy.

Concluding Remarks

Since the discovery of ADPr addition to proteins there have been significant advances in our understanding of the role of this modification in cell function and cell stress. In addition to a family of PARPs that can write this modification, a second diverse group of erasers has been identified. The coupled action of these readers and writers leads to both the rapid accumulation of complex pADPr chains on proteins and the degradation of these chains into both mADPr and large soluble pADPr complexes. Much work remains to be done to determine how modification of different amino acid residues by mADPr alters target protein function (see Outstanding Questions). In particular, further studies will be necessary to reveal the roles and regulation of different mADPr hydrolases which remove ADPr from specific residues in response to stress. In addition to writers and erasers of ADPr, there are several families of ADPr readers which can bind to mADPr or pADPr, providing the potential for regulation of protein targets, either by direct protein tethering to soluble mADPr/pADPr or by interaction with ADPr bound to proteins. Finally, turnover of free mADPr in the cell has received the least attention, but removal of mADPr is crucial for limiting apoptotic events and negative impacts on ATP production in the cell. Further, the potential that mADPr can be recycled back to ATP provides a novel method for maintaining local ATP levels during energy-intensive pADPr signaling and stress. ADPr provides a rich signaling mechanism for controlling stress responses, from directly modifying protein function to promoting protein interaction and the potential for pADPr degradation to create a new signaling molecule, mADPr. pADPr and its metabolites therefore represent both a flexible post-translational modification and a new signaling molecule which provide rapid and dynamic regulation of cell function during cellular stress and related events. Unraveling the specificity of this signaling pathway and the function of its diverse writers, readers. and erasers during stress will be necessary to fully appreciate the importance of this signaling pathway.

Outstanding Questions

NUDT5 is not the only NUDIX enzyme that could convert ADPr into AMP or ATP. What are the cellular roles of NUDT9, 12, and 14, and could they also participate in stress response?

Even though they have opposing activities in the cell, deletion of the genes encoding PARP1 or PARG results in similar phenotypes of sensitivity to genotoxic stress. It remains to be explored whether PARP1 and PARG act in concert in some way that contributes to the similarity of the phenotype.

Are there other ATP-dependent chromatin remodelers that derive ATP from similar recycling mechanisms? For example, FACT and ISWI have genetic interactions with PARP1 – could they also use ATP generated from recycled pADPr?

Are enzymes that require mobility on DNA restricted by being tethered to pADPr chains, or does cleavage of the pADPr chain grant them mobility while maintaining the activating interaction?

Does the NUTD9H domain in the TRPM2 Ca^{2+} channel define another ADPr-binding domain that might also be found in other proteins?

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