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Multiple roles for mono- and poly(ADP-ribose) in regulating stress responses

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Abstract

While stress-induced synthesis of mono ADP-ribose (mADPr) and poly ADP-ribose (pADPr) conjugates by PARP enzymes has been studied extensively, the removal and degradation of pADPr, and the fate of ADPr metabolites has received less attention. The observations that stress-induced pADPr undergoes rapid turnover and 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 hydrolyases 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. Here, we will 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.

Conjugation of ADP-ribose (ADPr) to proteins plays a critical signaling function in gene transcription, chromatin organization and stress responses. Poly-ADPr 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 which cleave pADPr chains, protein-ADPr bonds or which remove the terminal mADPr from pADPr chains have been identified. 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, pADPr polymers (both protein-linked and soluble) and free mADPr may therefore represent distinct functional signaling effectors in the cell.

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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 poly-ADPr (pADPr) chains ranging from 2-200 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 times more mADPr amino acids than pADPr amino acids (4), demonstrating the ubiquity of mADPr modification. PARP family members therefore specialize in adding either mADPr or in creating pADPr, often during stress responses.

PARPs and stress responses:

PARP1, PARP2 and PARP3 are DNA-dependent PARPs (5) which can be activated by single and double-strand DNA breaks (SSB and DSB (6, 7)) or by breaks in unstable DNA structures such as **G-quadruplexes** (8). During DSB repair, rapid (seconds to minutes) formation of pADPr (9) serves to concentrate many chromatin regulatory proteins at the damaged sites. This includes 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 re-organization 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). Further, even though PARP1 generates pADPr and PARP3 generates mADPr, they exhibit significant substrate overlap (19), suggesting that PARP3 may create the initial protein-mADPr 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 PARP 1 at DNA breaks have all been reported (2). In addition, DNA-dependent PARPs can also add mADPr and pADPr directly to the DNA ends (9, 20–22). While protein-ADPr has been well studied, how cells control the extent of pADPr and remove mADPr and pADPr chains has received much less attention. Here, we will discuss 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 PARP 1 or the dePARylating enzyme PARG both have similar effects on DNA repair (24), suggesting that **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, **Key Figure**), including the macrodomain containing enzymes MacroD1, MacroD2 and TARG1, which cleave the protein-ADPr bond; ARH1-3 (which contain a Drag-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 by e.g. PARG, leading to free, soluble mADPr; (ii) release of mixed populations containing large, soluble nuclear or cytoplasmic pADPr polymers following endo-glycohydrolase activity; (iii) trimming of pADPr chains to leave mADPr-protein (Figure 1, **Key Figure**).

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, **Key Figure**), giving it the potential to release large branched pADPr molecules and to trim pADPr chains. PARG functions primarily as an exo-glycohydrolase, 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 bond. Instead, ARH3 preferentially cleaves ADPr attached to serine (28, 29), TARG1, MacroD1 or 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 the 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 hydrolyses 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 last ADPr. The most commonly used pADPr antibody preferentially binds long pADPr chains over short ones, and cannot detect mADPr. The rapid appearance and loss of pADPr during processes like 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 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 are critical 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.

Other 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, **Key Figure**). 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 (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) increased Glial-cell Derived Neurotrophic Factor (GDNF) in astrocytes (41). Consistent with the neuroprotective functions of GDNF, injection of pADPr into brain striatum mitigated disease phenotypes in a rodent model of Parkinson's disease. 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 as PARP enzymes are largely intracellular (2), suggesting that pADPr synthesized following oxidative stress may be released into the extracellular space. Alternatively, ecto-ADP ribosyl transferases (**ecto-ARTs**), a poorly characterized family of ribosyl transferases 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). Further work identifying extracellular enzymes that can synthesize and degrade pADPr and determining whether mADPr and pADPr are exported from cells as soluble signaling molecules is needed to address this.

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. Additionally, 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.

READERS of mADPr and pADPr BINDING DOMAINS.

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

The PAR binding motif (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 besides 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, since 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 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 time points, which coincide with degradation of pADPr to mADPr, macroH2A1.2 re-accumulates to promote **homologous recombination** and chromatin re-condensation (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 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 for 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 ALC1's ATP-dependent nucleosome sliding activity (13, 66). In the basal ("off") state, ALC1's macrodomain interacts with the ATPase domain to auto-inhibit its activity. Binding of pADPr relieves inhibition of the ATPase domain and activates ALC1's **chromatin remodeling** activity (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 ALC1's 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), so 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 long with a Cys2-His2 (C2H2) zinc finger motif. PBZ domains were first described in the 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 aggregation of intrinsically disordered proteins, such as FUS and EWS (76). These aggregates of pADPr are localized regions with a high density of negative charge, and form a membrane-less 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 membrane-less 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 1 alpha (HP1 α) undergoes liquid demixing to drive heterochromatin formation (78, 79) are intriguing because HP1 α is also found to be 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 Ca²⁺-ion channel, binds mADPr via its cytoplasmic NUDT9H domain (82–84) (Figure 4). Ca²⁺ flux through the TRPM2 channel depends on the relative balance of PARP1 and PARG, with mADPr arising from pADPr degradation during oxidative stress promoting Ca²⁺ influx (85). This allows TRPM2 to function as a sensor of oxidative stress. However, high levels of mADPr can fully activate TRPM2 channels, leading to toxic intracellular Ca²⁺ levels and cell death (86). Catabolism of mADPr is therefore critical to turn off mADPr signaling via TRPM2 and other proteins.

NUDIX family 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 adenosine monophosphate (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 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 and 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 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 over-expressed 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 critical role in maintaining displacement of histones at estrogen-responsive genes and 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** (93) and repair by **non-homologous end joining** (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 hyper-activation to replenish pools of NAD⁺ (96). Further, ATP has hydrotropic properties (97) which may help redissolve hydrophobic proteins that had undergone PAR-dependent liquid demixing (76). NUDT5, and 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 which 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, which produce synthetic lethality in tumors with deficiency in homologous recombination (HR). 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 five 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 re-activation 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 led to new vulnerabilities, including enhanced sensitivity to IR and the alkylating chemotherapeutic agent temozolomide (100). Further study is needed 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). Other 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 (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 Alzheimer's and Parkinson's (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 shows 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 in 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, as well as 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 through mADPr alters target protein function (See Outstanding Questions). In particular, further studies are needed 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 critical 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 provides 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 is needed to fully appreciate the importance of this signaling pathway.

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Glossary

DNA Damage Response (DDR)

A complicated network of pathways that mediate the detection, signaling and repair of DNA damage

ARTD

ADP-ribose transferases with diphtheria toxin homology that transfer one or more ADP-ribose groups from 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

PARylation

Poly(ADP-ribose)ylation, a post-translational modification with linear or branched polymeric chains of ADPr subunits

DePARylation

Removal of poly(ADP-ribose) chains from target proteins or DNA catalyzed by poly(ADP-ribose) hydrolases

Ecto-ART/ARTC

ADP ribosyl transferases located on cell surface or extracellular compartment that transfer one ADPr group from extracellular NAD⁺ to target proteins

Parthanatos

A programmed cell death process which is mediated by pADPr

Stress Granule

Dense aggregations of proteins and RNAs formed in the cytosol under stress

Homologous Recombination (HR)

The error-free DNA repair pathway that occurs through homologous strand exchange and takes place in the late S-G2 phases of the cell cycle

Chromatin Remodeling

The dynamic rearrangement of chromatin architecture to allow access of condensed genomic DNA, including histone variant incorporation, histone depletion and/or histone sliding

Liquid Demixing

An aggregation of intrinsically disordered proteins by phase separation from the soluble intracellular space

Intrinsically Disordered Protein (IDP)

Dynamically disordered proteins that feature biased amino acid composition and low sequence complexity

Mitochondrial Dysfunction

Loss of function in mitochondria caused by the reduction of oxidative phosphorylation efficiency and ATP production

Base Excision Repair (BER)

The predominant DNA damage repair pathway for the processing of small base lesions, derived from oxidation, deamination, and alkylation damage. It is initiated by a DNA glycosylase that recognizes and removes the damaged base and completed by short- or long-patch repair

Non-Homologous End Joining (NHEJ)

The predominant pathway to repair DNA double strand breaks by religation of DNA ends with limited processing

NAD⁺ salvage pathway

Production of NAD⁺ from nicotinamide, an end product of NAD⁺ consumption, catalyzed by nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide mononucleotide adenylyltransferase (NMNAT)

Monotherapy

The treatment of disease or disorder with use of only one type of treatment such as radiation therapy. In drug therapy it refers to treatment with a single drug molecule

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HIGHLIGHTS

- DNA damage promotes rapid production of poly(ADP-ribose) (pADPr) chains on damaged chromatin and is critical for initiating recruitment of DNA repair proteins.
- pADPr then undergoes rapid (seconds-minutes) degradation by PARG and related enzymes, releasing monoADP-ribose (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, so that binding of specific pADPr metabolites can regulate DNA repair proteins and apoptosis during stress.
- A family of hydrolases, including NUDT5, which degrade mADPr, can remove mADPr and terminate stress responses while generating useful metabolites.
- pADPr and its metabolites may function as novel stress induced signaling molecules which regulate the function of stress and DNA repair proteins.

Outstanding Questions

1. 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?
2. Even though they have opposing activities in the cell, deletion of 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.
3. 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 that comes from recycled pADPr?
4. Are enzymes that require mobility on DNA restricted by being tethered to pADPr chains or does cleavage of the pADPr chain grant them mobility whilst maintaining the activating interaction?
5. Does the NUTD9H domain in the TRPM2 Ca²⁺ channel define another ADPr-binding domain that might be found in other proteins as well?

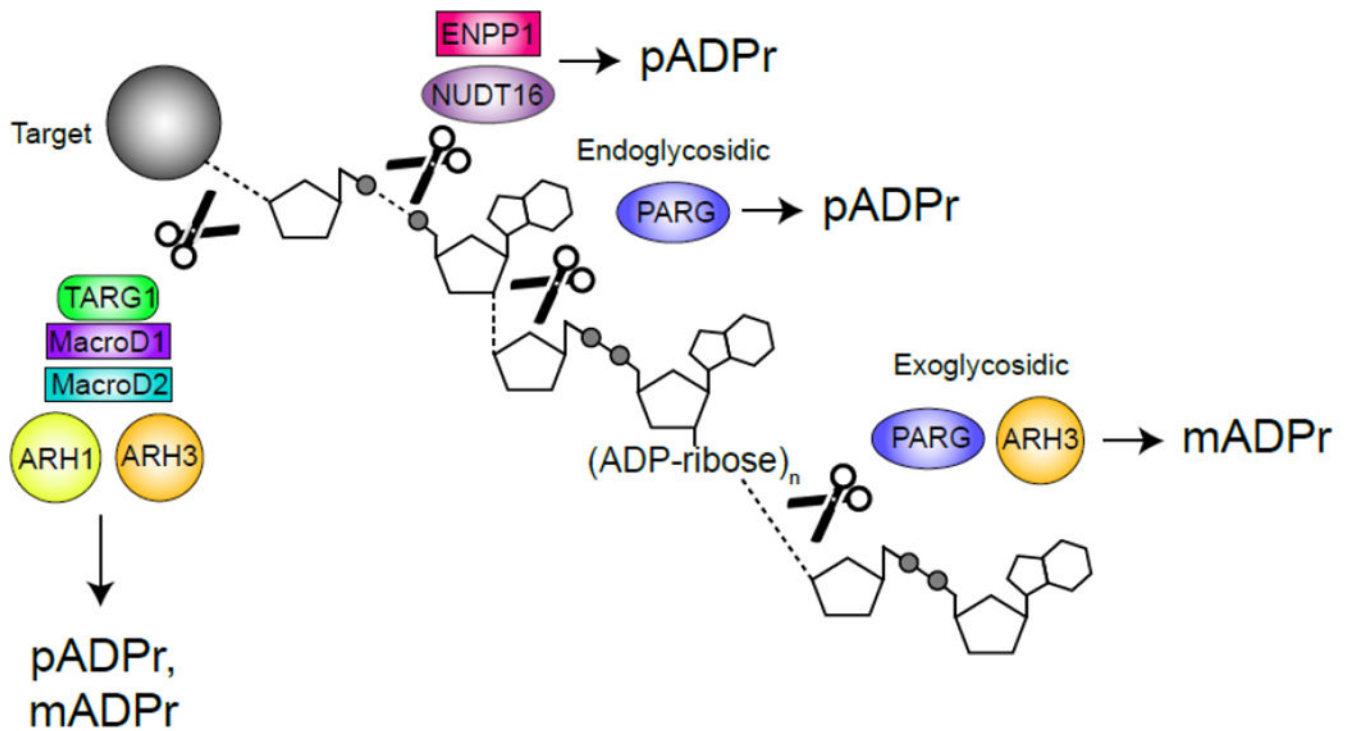


Figure 1. Key Figure – DePARylation.

ADP(ribose) (ADPr) hydrolases cleave specific chemical linkages to release poly(ADP)ribose (pADPr) or mono(ADP)ribose (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.

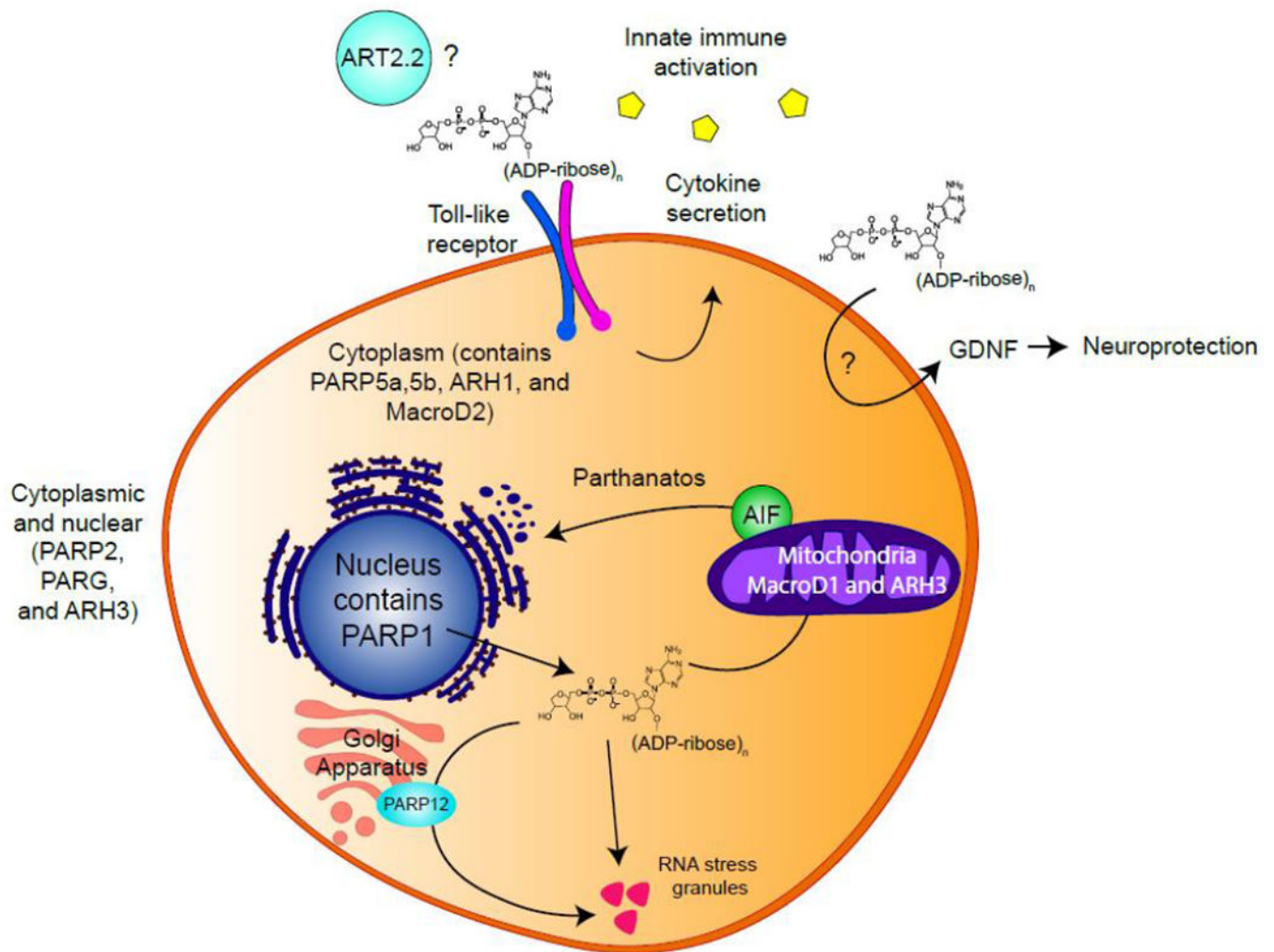


Figure 2 –. pADPr signaling beyond the nucleus.

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 increase Glial-cell Derived Neurotrophic Factor (GDNF) in astrocytes, playing roles in neuroprotection (41). Free cytoplasmic pADPr binds 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).

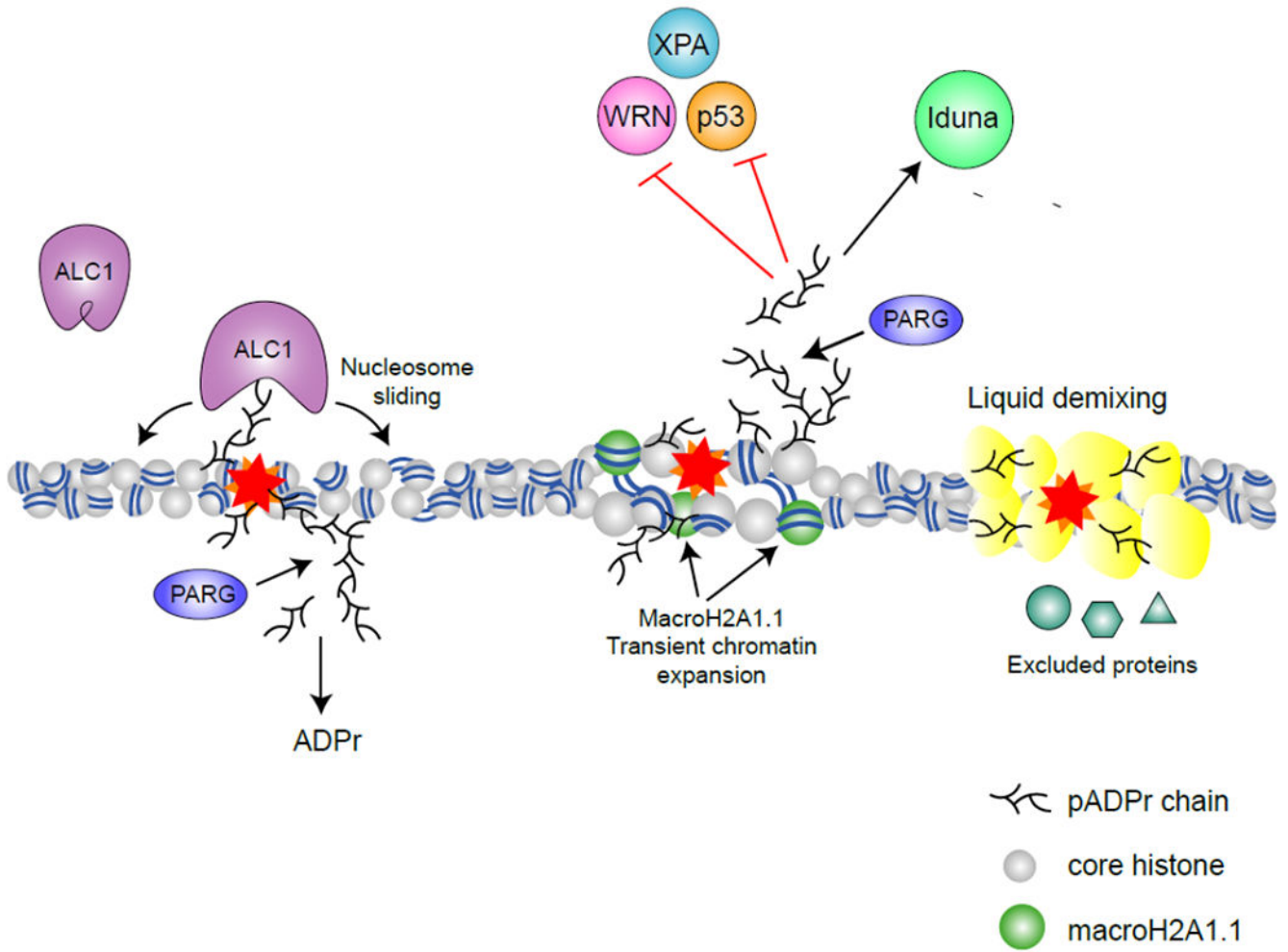


Figure 3 –. pADPr signaling in the nucleus.

Through binding of pADPr, reader proteins are recruited and functionally regulated during 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. inhibiting nuclease activity of WRN (57), NER activity of XPA (58) and DNA binding activity of p53 (59)) or positively (e.g. activating the ATPase activity of ALC1 (63) and the E3 ligase activity of Iduna (73)). pADPr can also cause liquid demixing, forming a membrane-less organelle that retain some proteins but excludes others (76).

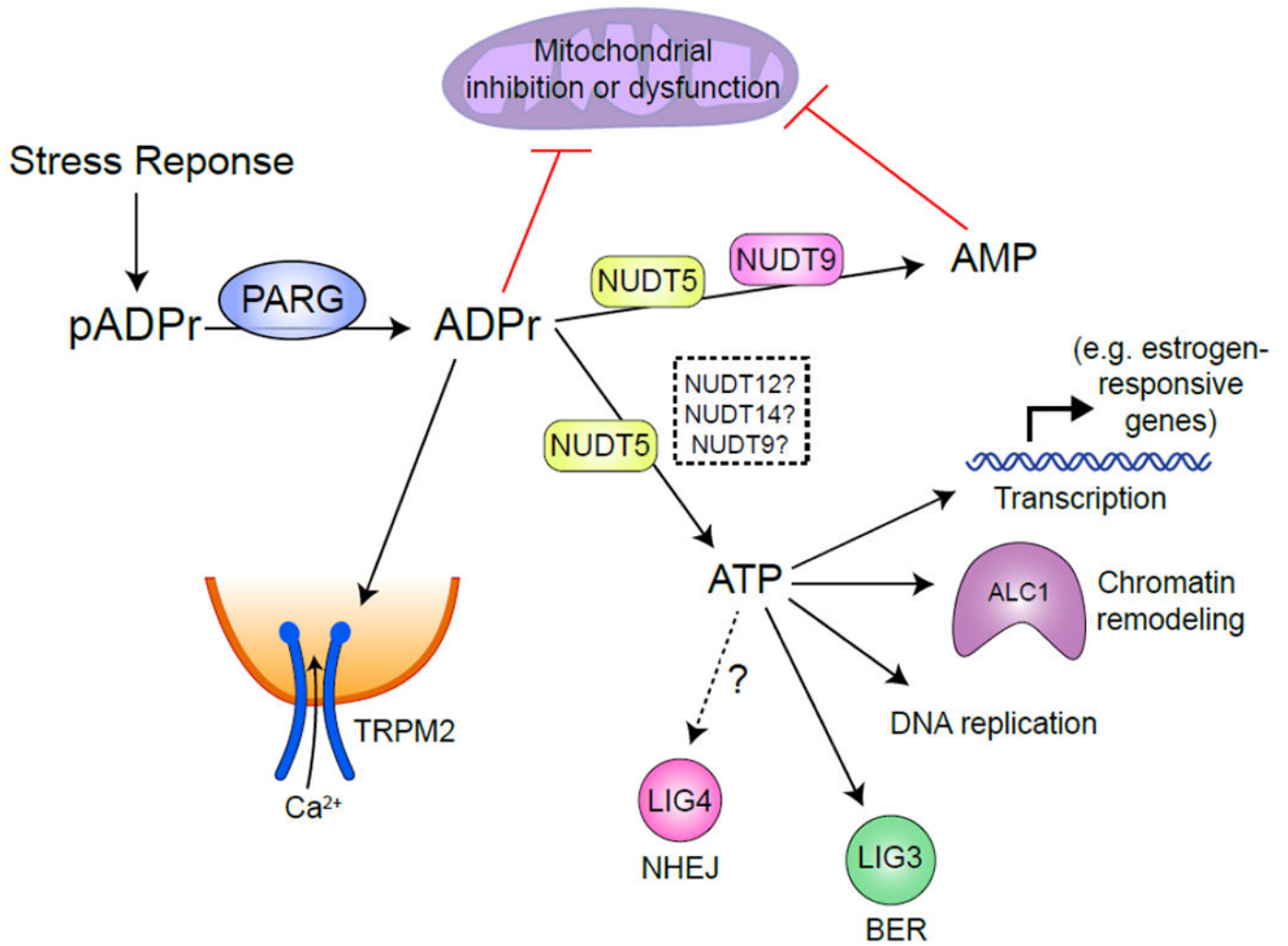


Figure 4 –. The fate of free mADPr.

Following 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 TRPM2 cation channel, inducing Ca^{2+} influx into cells (84). NUDT9 catalyzes mADPr to AMP (91), while NUDT5 possess two catalytical activities to convert mADPr to either AMP or ATP (94). Over-production 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, chromatin remodeling during DNA replication or repair.

Table 1 –

Non-covalent ADPr binding motifs

Motif	Abbreviation	Description	Moiety Read	Examples
Poly(ADP-ribose)-binding motif	PBM	~20 aa motif (hydrophobic aa spaced by basic aa)	Unknown	AIF (47), WRN (57), histone 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 amino acid 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)