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Parthanatos, a messenger of death

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1. ABSTRACT

Poly-ADP-ribose polymerase-1 (PARP-1)'s roles in the cell span from maintaining life to inducing death. The processes PARP-1 is involved in include DNA repair, DNA transcription, mitosis, and cell death. Of PARP-1's different cellular functions, its role in cell death is of particular interest to designing therapies for diseases. Genetic deletion of PARP-1 revealed that PARP-1 overactivation underlies cell death in models of stroke, diabetes, inflammation and neurodegeneration. Since interfering with PARP-1 mediated cell death will be clinically beneficial, great effort has been invested into understanding mechanisms downstream of PARP-1 overactivation. Recent evidence shows that poly-ADP ribose (PAR) polymer itself can act as a cell death effector downstream of PARP-1. We coined the term parthanatos after *Thanatos*, the personification of death in Greek mythology, to refer to PAR-mediated cell death. In this review, we will present evidence and questions raised by these recent findings, and summarize the proposed mechanisms by which PARP-1 overactivation kills. It is evident that further understanding of parthanatos opens up new avenues for therapy in ameliorating diseases related to PARP-1 overactivation.

2. BACKGROUND

Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear enzyme important for cellular homeostasis, and is involved in diseases such as stroke, myocardial infarction, and Parkinson disease (PD). Under physiological conditions, PARP-1 is important for DNA repair, genomic stability and transcription. In pathological conditions that cause severe genomic stress such as ischemia-reperfusion injury, inflammation, myocardial infarction, glutamate excitotoxicity, and PD, PARP-1 overactivation leads to cell death. Cell demise from PARP-1 overactivation has been attributed to depletion of cellular energy, release of the death effector apoptosis-inducing factor (AIF) from the mitochondria, and production of excess poly-ADP ribose (PAR) polymer, a novel death signal. We coined the term parthanatos from *Thanatos*, the personification of death in Greek mythology to describe cell death initiated by PAR polymer. In this review, we first summarize proposed mechanisms by which PARP-1 kills, including NAD depletion, AIF release and excess PAR. Then we present the evidence for and possible basis of parthanatos.

2.1. PARP family

Poly-ADP ribosylation is a post-translational modification catalyzed by poly-ADP ribose polymerases

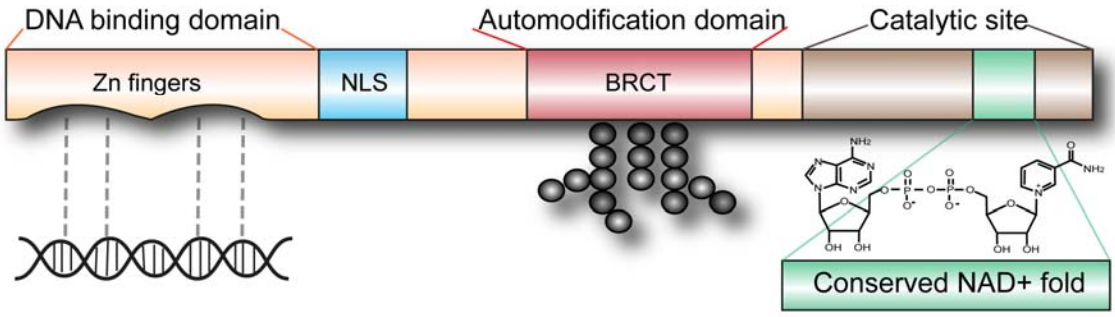

Figure 1. PARP domain. PARP-1 uses NAD^+ to form polymers of ADP-ribose on various protein acceptors. The major domains of PARP-1 include: (1) two zinc fingers responsible for PARP-1's detection of DNA breaks, (2) nuclear localization signal (NLS) containing a caspase-cleavage site, (3) an automodification domain with a BRCT motif for protein-protein interactions, (4) a catalytic site which contains the PARP signature NAD^+-fold.

PARPs). PARPs form homopolymers of PAR which can be linear or branched, and free or attached to proteins (1). Due to PAR's anionic charge, non-covalent or covalent binding of PAR to proteins alters the function of the target protein (2), which is possibly one mechanism by which PARPs modulate cellular processes such as DNA repair, DNA transcription, mitosis, and cell death.

PARP-1 is the founding member of the PARP superfamily. The crystal structure of the catalytic domain of chicken PARP-1 shows structural homology with the active site of bacterial ADP-ribosylating toxins (3). The conserved beta-alpha-loop-beta-alpha NAD^+ fold in the catalytic domain of PARP-1 was used to extensively search the NCBI non-redundant protein database for other PARPs (4). With this approach, 17 PARP family members have been identified that can potentially carry out poly-ADP ribosylation activities (5). These PARPs vary extensively in cell localization, regulation and function, but can be broadly classified into 3 groups as discussed in Hassa *et al* (1). In this review, we will focus on PARP-1, which has been strongly implicated in several experimental models of stroke, diabetes, inflammation and neurodegeneration (6, 7). For a more detailed discussion on the different members of the PARP family, please refer to excellent reviews by Schreiber *et al* (5) and by Hassa and Hottiger (8).

2.2. Synthesis of PAR

PARP-1 has several main features (Figure 1) consisting of a DNA-binding domain of two zinc zippers that recognize DNA strand breaks, a bipartite nuclear localization signal containing a caspase-cleavage site, an automodification domain with a BRCT (BRCA1 C-terminal domain) motif for protein-protein interactions, and a C-terminal catalytic site which contains the well-conserved NAD^+-fold present in mono-ADP ribosylating toxins (4). PARP-1 synthesizes PAR, a polymer of ADP-ribose linked by glycosidic bonds. PAR synthesis was first described by Chambon *et al.* (9) as nicotinamide mononucleotide (NMD)-induced and involving DNA-dependent incorporation of ATP into a nuclear extract with poly-adenine as the possible product. Further analysis revealed that poly-ADP ribose, not poly-adenine, is the product of the reaction (10-13). This is because the observed molar ratio of the product is 1 adenine:2 ribose: 2 phosphate, and that the product is susceptible to phosphodiesterase but not to alkaline hydrolysis (12, 13). In addition, it was further discovered that the ADP-ribose moiety of NAD^+, not ATP, is incorporated in the reaction which proceeds with the concomitant release of nicotinamide (12).

To form PAR, PARP-1 first forms ADP-ribose by hydrolyzing NAD^+ and releasing nicotinamide. PAR is then covalently bound to proteins through an ester bond between the first ADP-ribose and an amino acid (Glu, Asp) in the acceptor proteins. Polymerization of PAR is through catalysis of ribose-ribose 2'-1' glycosidic bonds with branching occurring on average of one branch per 20-50 ADP-ribose units (1). Depending on the stimuli, PAR formed can vary in length and in the frequency of branching. This structural heterogeneity by PAR may be in part responsible in distinguishing among the life and death functions of PARP-1 (1). The half-life of PAR within the cell depends on the activity of PARG (poly-(ADP) ribose glycohydrolase) or AH3 lyase (human ADP-ribosylhydrolase 3). Upon genotoxic stress for instance, PAR is rapidly degraded by PARG or ARH3 lyase which breaks down PAR by cleaving the glycosidic bonds between ADP-ribose units (1). Thus, given the processes by which the levels and structure of PAR can be varied, in addition to other factors regulating PARP-1 activity, PARP-1 is capable of achieving varied responses within the cell depending on the stimuli.

2.3. PARP-1 in life and death

PARP-1 function ranges from supporting survival to inducing death. One of the ways by which PARP-1 regulates its function is through regulating the formation, structure and degradation of PAR (Figure 2). In the presence of mild DNA damage, the catalytic activity of PARP-1 is increased by more than 500-fold causing ADP-ribosylation of PARP-1 and its substrates (2). Its substrates include, but are not limited to histones, DNA helicases, high mobility group proteins, topoisomerases I and II, single-strand break repair factors, base-excision repair factors and several transcription factors (reviewed in (2)). However, with excess genotoxic stress, PARP-1 is overactivated, produces excess PAR, leading to cell death.

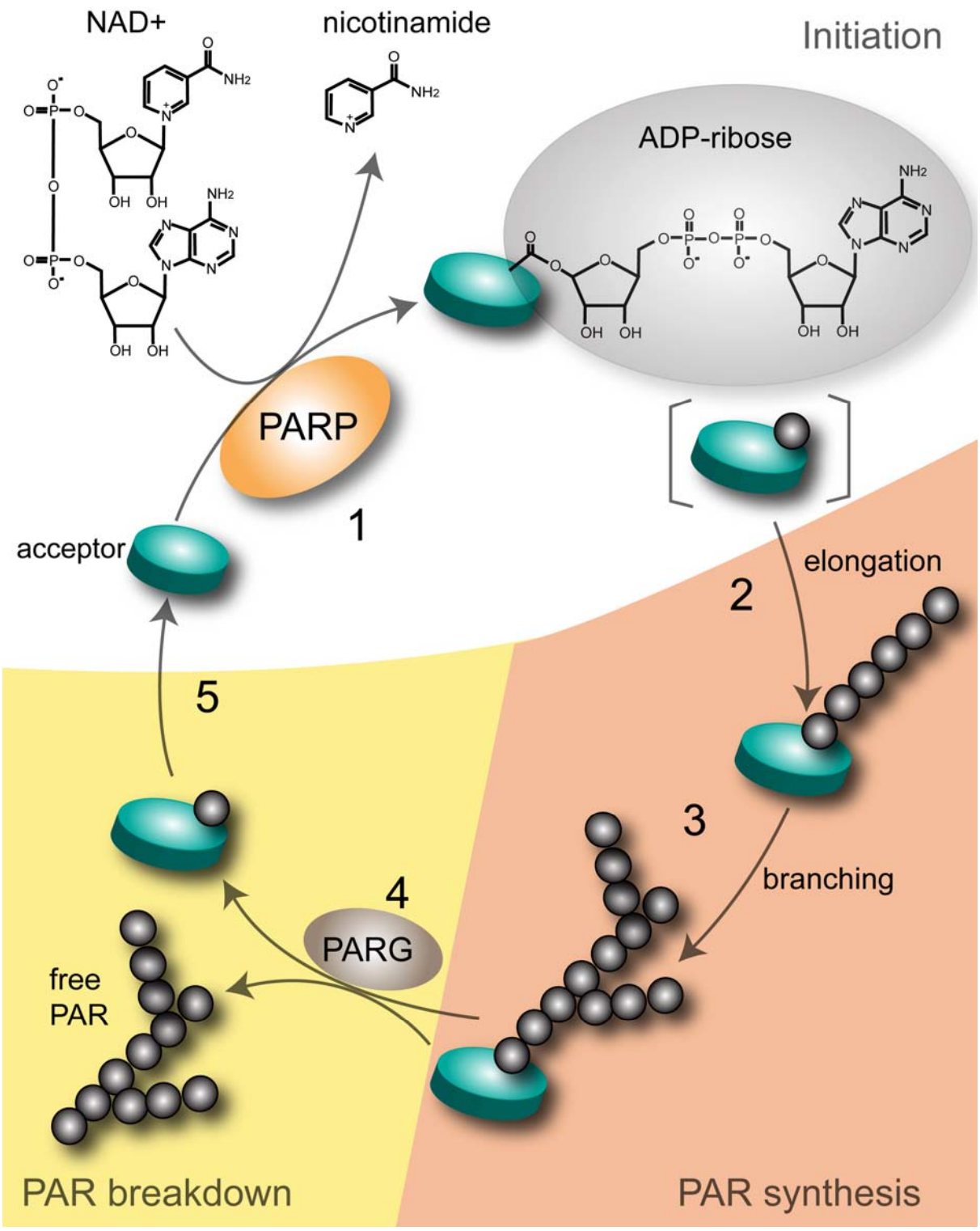

Figure 2. PAR polymer metabolism. In the initiation step of PAR synthesis (1), PARP-1 converts NAD^+ to ADP-ribose and nicotinamide. The ADP-ribose is then attached to a glutamic acid residue in the protein acceptor. Structural heterogeneity of the PAR polymer is achieved by elongation (2) and branching (3) at the 2'-OH and 2''-OH of the ribose moiety respectively. PAR breakdown (4) is catalyzed by the endoglycosidic and exoglycosidic activities of PARG. Removal of the final ADP-ribose in the protein acceptor is catalyzed by mono-ADP-ribosyl-protein lyase.

PARP-1 knockout (KO) mouse models were generated by three independent groups, and exhibited phenotypes highlighting the physiological and pathological roles of PARP-1 (14). PARP-1 KO mouse models were all found to be hypersensitive to alkylating agents and ionizing radiation, possibly due to defective DNA repair (14). In contrast, PARP-1 KO mice are protected from LPS-induced shock, ischemic injury and MPTP excitotoxicity. Thus, these genetic models highlight a dual role for PARP-1 in the cell depending on the stimuli: PARP-1 can support survival by facilitating DNA repair or can mediate pathological cascades as a result of inflammation or excitotoxicity (2). Since PARP-1's role in cell death has been found to underlie experimental models of disease, understanding how PARP-1's activity can lead to cell death in several pathological contexts will help in expanding therapeutic options for PARP-1 related diseases.

3. PARP-1 IS A REGULATOR OF CELL DEATH

3.1. PARP-1 in neuronal injury due to excitotoxicity

PARP-1 activation is downstream of overproduction of nitric oxide (NO). Neuronal damage following ischemia is known to be mediated by overactivation of N-methyl-D-aspartic acid (NMDA) glutamate receptors due to excess glutamate released by injured neurons (15). This phenomenon known as excitotoxicity underlies neuronal death and injury in experimental models of stroke, PD, and other neurodegenerative disorders (15, 16). The key steps underlying NMDA excitotoxicity have been described. Neuronal injury leads to overactivation of NMDA glutamate receptors that in turn results in an excessive influx of calcium. Excess calcium then leads to overactivation of neuronal nitric oxide synthase (nNOS). nNOS plays an important role in excitotoxicity since primary brain cultures obtained from nNOS KO mice or wild type cultures treated with NOS inhibitors are resistant to NMDA excitotoxicity (17-21). Mice lacking nNOS are also resistant against middle cerebral artery occlusion (MCAO)-induced neuronal injury or an intrastriatal injection of an excitotoxic dose of NMDA (20, 22).

NO induces ADP-(ribosyl)ation in isolated nuclei providing a mechanistic link between PARP-1 and NO (23). Excess NO by reacting with superoxide anion to produce the oxidant peroxynitrite causes DNA damage that activates PARP-1 (17, 24-27). PARP-1 activation plays a prominent role in NMDA excitotoxicity as PARP inhibitors protect neurons from NMDA excitoxicity and NO-mediated toxicity (23, 28, 29). Furthermore, PARP-1 KO mice are profoundly resistant to stroke and NMDA excitotoxicity (28-30). Interestingly, PARP-1 KO mice or wild type mice treated with PARP inhibitors are similarly neuroprotected when treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (31-33) suggesting that inhibiting PARP-1 for neuroprotective purposes is not only relevant to acute neuronal injury such as stroke, but also to neurodegenerative disorders such as PD.

PARP-1 overactivation leads to neuronal cell death. Since PARP-1 consumes NAD^+, Berger and colleagues postulated that PARP-1 overactivation leads to massive consumption of NAD^+, followed by ATP depletion (34). However, in light of recent data, this hypothesis seems to be insufficient, as described in the next section. Hence, other mechanisms by which PARP-1 kills are proposed such as the release of the death effector, AIF (35, 36). Recent findings reveal that PAR polymer produced by PARP-1 acts as a cell death effector by inducing the release of AIF (37, 38). Thus, excess production of PAR polymer rather than consumption of NAD^+ causes cell death.

3.2. PARP-1 overactivation depletes NAD^+

According to the suicide hypothesis, PARP-1 kills primarily by NAD^+ depletion (34). The suicide hypothesis stems from studies showing that PARP-1 overactivation causes energy depletion. Indeed, inhibiting PARP-1 through pharmacological inhibitors or genetic deletion restores NAD^+ levels and viability (35). In neurons for example, different neuronal populations treated with a variety of toxic stimuli exhibit protection and concomitant energy preservation with PARP inhibitors (35, 39). Moreover, PARP-1 KO mice, which are resistant to transient cerebral ischemia-induced damage, exhibit reduced PAR and preserved NAD^+ levels (35, 39).

Based on what is known regarding bioenergetics, NAD^+ depletion causes ATP depletion; and the resulting drop in cellular energy leads to cell demise. (2, 35). NAD^+ is known to be a cofactor in several cellular metabolic processes needed for generating ATP such as glycolysis and the tricarboxylic acid cycle (2). In addition, NAD^+ resynthesis requires at least 2-4 molecules of ATP while NAD^+ depletion blocks glyceraldehyde 3-dehydrogenase activity leading to the cell investing ATP in glycolysis, but without the return in NAD^+ (2, 40, 41). Thus, in support of the suicide hypothesis, PARP-1 activation leads to a block in the glycolysis. Indeed, replenishment of glycolytic and tricarboxylic acid cycle (TCA cycle) intermediates and substrates such as alpha-ketoglutarate or pyruvate, are neuroprotective (35, 42). Also, administering NAD^+ to cells or overexpression of NAD^+ biosynthetic genes seem to rescue PARP-1 dependent cell death, suggesting that indeed, the NAD^+ decline associated with PARP-1 overactivation can cause cell demise (35). However, NAD^+ replenishment probably prevents cell death through SIRT1 (1, 43, 44).

Recent findings suggest that the compartmentalization of NAD^+ within cells also must be considered when evaluating death induced by NAD^+ depletion. The mitochondrial pool of NAD^+ appears to be more relevant for cell death rather than the cytosolic and nuclear pools, as cell death is rescued upon preservation of NAD^+ in the mitochondria by cyclosporin A, or replenishment of NAD^+ by overepression of the biosynthetic nicotinic acid mononucleotide adenylyltransferase (Namnt) (45, 46). The drop in NAD^+ levels following PARP-1 overactivation reflects whole cell NAD^+. Thus, conclusions made from these studies need to be reexamined in light of the recent finding that the mitochondrial levels of NAD^+ remain at physiological levels following genotoxic stress and can support viability

even when nuclear and cytoplasmic pools of NAD^+ are depleted (46). Whether PARP-1-dependent cell death exclusively depends on mitochondrial NAD^+ depletion remains to be determined, since overexpression of Namnt to replenish mitochondrial stores of NAD^+ leads to a partial rescue of cell death only (45). Reduction in cell death by cyclosporin A may also not be attributed solely to NAD^+ preservation in the mitochondria, as it blocks mitochondrial permeability transition, which in itself is an important player in cell death signaling.

Several studies question whether PARP-1 overactivation kills primarily by NAD^+ depletion. Paschen *et al* (47) showed that NAD^+ decline does not always correlate with ATP decline in a model of ischemia reperfusion. Moreover, Goto *et al* (48) showed that PARP-1 KO mice have reduced infarct size after MCAO ischemia compared with wild type animals, but there is no difference between groups in the changes in the energy status as measured by the water diffusion coefficient. Also, Bax and calpain KO cells are protected from N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) to the same degree as wild-type controls treated with the PARP inhibitor DPQ (49). This is in spite of a massive drop in the NAD^+ levels in Bax and calpain KO cells that is not seen in the DPQ-treated cells. These findings suggest that NAD^+ depletion due to PARP-1 activation is not sufficient to account for cell demise.

3.3. PARP-1 overactivation induces AIF release

PARP-1 overactivation leading to AIF release from the mitochondria was first demonstrated by Yu *et al* (36). In this study, PARP-1 KO mouse embryonic fibroblasts and neurons fail to release AIF. Furthermore, PARP-1 dependent cell death is reduced when AIF-depleting antibodies are delivered into the cells. Most importantly, AIF translocation has been well-documented in several experimental models of PARP-1 mediated cell death.

AIF was purified from supernatants of mitochondria induced to undergo permeability transition (50). This ubiquitously-expressed mitochondrial protein was isolated for its ability to induce nuclear fragmentation, and was shown to translocate from the mitochondria to the nucleus to induce chromatin condensation and DNA fragmentation (51). The AIF gene is localized to the A6 region of mouse X chromosome, and is composed of 17 exons (52). The AIF gene expresses a 67-kDa protein that is believed to be converted to a 57-kDa protein upon cleavage of its putative mitochondrial localization sequence (53). However, recent studies indicate that it is processed to a 62-kDa form upon cleavage of its mitochondrial localization sequence, and it is only processed to the 57-kDa form after a cell death stimulus (54, 55). Analysis of its sequence and its crystal structure reveal a glutathione reductase-like fold with an FAD-binding domain and an NADH binding domain as well as a C-terminal domain composed of five antiparallel beta-strands and two alpha-helices (56). The structure of the AIF protein reveals an overall positive electrostatic potential and homology to Bph4, a ferredoxin reductase in a dioxygenase from bacteria (56). Analysis of recombinant AIF reveals NADH-oxidase activity that can catalyze formation of superoxide anions (57) further suggesting a role for AIF in redox processes within the mitochondria.

The exact function of AIF in the mitochondria has been further clarified through the use of genetic knockdowns and conditional deletion of AIF. Gaining insights to AIF's *in vivo* function was initially precluded by AIF's essential role in embryonic development (58). Thus, models such as the Harlequin (*Hq*) mutant mouse have been useful. *Hq* mice contain a proviral insertion in the AIF gene that results in an 80% reduction of AIF protein levels (59). Neonatal *Hq* mice exhibited 18% less respiratory chain complex I and 30% less catalase compared with WT mice (60). These mice develop a late-onset degeneration in the cerebellar granule cells and retinal ganglion cells (61). In addition, there is marked oxidative stress in the brain and retina (59), suggesting that AIF acts as a free radical scavenger in the mitochondria (59). Later studies point that this increased levels of reactive oxygen radicals in *Hq* mice may be due to a role for AIF in the complex I activity of the electron transport chain (57, 62). This notion was confirmed by conditional deletion of AIF specifically in cardiac and skeletal muscle of mice, which resulted in impaired activity and decreased protein expression of mitochondrial complex I (61). In addition to a role for AIF in redox processes, conditionally deleting AIF in embryonic telencephalon produced neurons with fragmented mitochondria, abnormal cristae structure and decreased survival (63).

Similar to cytochrome c, AIF assumes a deadly role once released from the mitochondria. Whereas cytochrome c is known to kill by the canonical apoptotic pathway of apoptosome formation (64), the mechanism by which AIF kills is not clear. Translocation of AIF to the nucleus induces chromatin condensation and DNA fragmentation possibly through cyclophilin A, even in the presence of caspase inhibitors (52, 53, 58, 65). This nuclear apoptosis can be blocked by microinjecting neutralizing antibodies against AIF into the cytosol (36, 53), inhibition of cysteine proteases (66) and overexpression of hsp70 (67, 68). Translocation of AIF into the nucleus, and not its loss from the mitochondria, kills cells (63). AIF translocation to the nucleus has been observed in different cell types induced to die, including a myriad of cases of neuronal cell death (69), experimental models of Parkinson's disease (70), excitotoxicity (71), perinatal hypoxia-ischemia (72, 73), brain trauma (74), and ischemic stroke (69, 75-77). AIF is a key in neuronal cell death paradigms, since mice with reduced levels of AIF through siRNA or through the *Hq* mutation have reduced levels of AIF have decreased infarct size after ischemic injury (55, 60, 69).

4. PARTHANATOS, A MESSENGER OF DEATH

4.1. PAR polymer is toxic to cells

Neurons subjected to NMDA excitotoxicity and Hela cells subjected to genotoxic stress using MNNG display a surge of PAR polymer (37, 38). When accumulation of PAR polymer is antagonized by

immunodepletion using antiserum to PAR or when PAR polymer is degraded through PARG overexpression, cell death is decreased in both NMDA-treated neurons and MNNG-treated Hela cells (37, 38). These results suggest that PAR polymer is a key contributor to cell death. Similarly *in vivo*, PAR polymer contributes to neuronal death observed in a mouse model of stroke, since transgenic mice overexpressing PARG subjected to transient MCAO, experience smaller infarcts compared with wild-type mice (37). Conversely, mutant mice with only one copy of PARG show increased infarct volume compared with wild-type mice (37).

How does excess PAR mediate cell death? PAR is known to mediate cell death in part by inducing AIF release (38). PAR releases AIF from the mitochondria *in vitro* when PAR generated from MNNG-treated nuclei is incubated with isolated mitochondria (38). Moreover, delivery of PAR into the cell induces AIF translocation and cell death (37, 38). Cell death is mediated specifically by PAR because AIF release is abolished when PAR is catabolized by PARG or phosphodiesterase I (PDI) which degrade PAR polymers. In addition, overexpression of PARG prevents NMDA-induced AIF translocation (37, 38).

4.2. Properties of parthanatos

Parthanatos shares cytological and morphological features of apoptosis and necrosis, but is the result of a distinct molecular mechanism. Parthanatos is caspase-independent (37, 38). Downstream of PARP-1 overactivation and complex PAR accumulation, PAR migrates from the nuclei to the cytosol, leading to AIF translocation from the mitochondria to the nucleus (37, 38, 49).

PAR toxicity is dependent on the length and complexity of the PAR polymer (37, 38). Through direct delivery of PAR polymer in neurons and Hela cells, it was found that PAR polymers of size greater than 60 ADP-ribose units are more toxic than less complex polymers (37). Delivery of >60 ADP-ribose polymer kills cells in a dose-dependent manner with toxicity observed at 20 nm PAR and near complete killing at 80 nm PAR (37). Similar levels of PAR polymer are achieved with NMDA excitotoxicity and MNNG-induced cell death (37). Delivery of equivalent concentrations of poly-adenine which has a similar negative charge to PAR polymer is not toxic to cells indicating that the toxicity is not due to the negative charge of PAR polymer (37). Since PAR complexity seems to be closely tied to cell death, it would be interesting to further study how PAR complexity is regulated, and how its production varies across the heterogeneous PARP family.

4.3. Insights from PARG (poly-(ADP) ribose glycohydrolase) deletion

The toxicity of PAR polymers is highlighted in studies of PARG KOs. Though there are several PARPs that have been characterized, there are only two proteins so far that have been identified to catabolize PAR. PARG and the recently identified ARH3 degrade PAR polymers (1, 78). The PARG gene is known to encode 4 isoforms, the nuclear 110-kDa isoform, the cytoplasmic 102-kDa and 99-kDa isoforms (detected only in humans) and the 59-kDa isoform (60-kDa in mice) (1). Mice lacking exons 2 and 3 which only leaves the PARG 59-kDa intact are viable and normal, but show an increase in PAR levels and infarct volumes when subjected to distal MCAO (79). However, a 65-kDa version of PARG accumulates in the mitochondria of these mutant mice due to an alternative translational start site (5). Since these mice do not completely lack PARG, they cannot be used as a tool to assess the effects of accumulation of PAR through PARG deletion. On the other hand, deletion of exon 4 creating a null mutation in all PARG isoforms leads to embryonic lethality at day 3.5 (80), suggesting that PARG is essential for survival (5). Trophoblasts isolated from early PARG KO embryos survive only in the presence of the PARP inhibitor, benzamide (80). This survival stems from inhibiting the accumulation of PAR, thereby highlighting the toxicity of PAR. In addition, these cells were hypersensitive to MNNG and menadione, chemicals that are known to induce PARP-1 overactivation and PAR accumulation (80). Similarly, in *Drosophila*, a loss-of-function mutation of PARG results in larval lethality, and this effect can be rescued by raising the temperature from 25 to 29°C (81). Adult PARG-deficient flies show neurodegeneration, reduced locomotor activity and a short lifespan (81), further highlighting how PAR accumulation is toxic.

4.4. Structural species of PAR and its functional relevance

Unlike most other posttranslational modifications, poly-ADP ribosylation is structurally heterogeneous in that different sizes of PAR polymer can bind to proteins either through covalent or non-covalent binding (1). The ADP-ribose units are linked by glycosidic ribose-ribose 1'-2' bonds and can stretch from 200-400 units *in vitro* and *in vivo* (1). PAR polymers may be bound or free, linear or branched, short or long. How this structural heterogeneity is controlled by the different PARPs is not known (1). Given that there are several PARPs, but only 2 PARGs identified so far, it is conceivable that the polymers produced by the PARP family members are structurally distinct and this plays a role in the diverse functions of the different PARPs. Structural heterogeneity in the PAR produced by different PARPs may account for the overlapping and non-redundant functions of PARP-1 and PARP-2. Mice lacking PARP-1 or PARP-2 are viable, but mice lacking both PARPs are embryonic lethal (82). Interestingly, knocking out PARP-1 alone is sufficient to protect completely from endotoxic and excitotoxic stimuli (14), whereas knocking out PARP-2 protects from focal but not global ischemia (83). Thus, perhaps PARP-2 is able to produce PAR polymers that are necessary for viability, but not the PAR that is cytotoxic in specific paradigms.

Though it is not known how excess PAR leads to release of AIF from the mitochondria, it is conceivable that it is through covalent and non-covalent binding of excess PAR to substrates, which ultimately results in the disruption or promotion of certain protein-protein and

DNA-protein interactions. Non-covalent binding of PAR to proteins is stable, and *in vitro* PAR binding is resistant to strong acids, chaotropes, detergents, and high salt concentrations (84, 85). PAR binding is selective depending on the PAR chain length and the presence of binding sites on the substrates (1, 85-87). For instance, PAR binds to histones with the following relative affinity: branched polymers > long, linear polymers > short, linear polymers (84). Binding affinity of PAR to different histones isoforms has this hierarchy: H1 > H2A > H2B = H3 > H4 (84). Recent studies indicate that PAR binds to a variety of proteins (1, 87). It is likely that identification of PAR substrates will be key in understanding parthanatos.

4.5. PAR and mitochondria

Downstream of PARP-1 overactivation is AIF release from the mitochondria (36), indicating that there is nuclear-mitochondrial crosstalk that occurs in PARP-1 mediated cell death (Figure 3). Upon PARP-1 overactivation, excess PAR, free or bound, shuttles outside of the nucleus and binds to specific cytosolic or mitochondrial proteins. PAR binding to these proteins ultimately leads to AIF release from the mitochondria. Targets modified by PAR possibly include proteins with roles in AIF release, mitochondrial membrane permeabilization or mitochondrial function.

Mitochondria maintain physiological integrity of cells through their important role in energy generation. However, upon cellular stress, mitochondria become permeabilized, and participate in cell death signaling by releasing death effectors such as cytochrome c, AIF, second mitochondria-derived activator of caspase/ direct inhibitor of apoptosis binding protein with a low pI (Smac/DIABLO), and Omi stress-regulated endoprotease/ high temperature requirement protein A2 (Omi/HtrA2) (88). Release of these proapoptotic factors is thought to occur through outer membrane permeabilization (OMP) or opening of the permeability transition pore (PTP) (88).

Mitochondrial permeability transition (PT) and loss of mitochondrial membrane potential are early events in PARP-1 dependent cell death (36). PT follows the formation of mitochondrial permeability transition pore (PTP). Although there are conflicting reports about the composition of the PTP, it is thought that the PTP is a proteinacious pore that is composed of adenine nucleotide translocase (ANT) from the inner mitochondrial membrane, voltage dependent anionic channel (VDAC) and cyclophilin D from the mitochondrial matrix (88). PTP can be inhibited by the immunophilin cyclosporin A (89) or bongkrekic acid (90). Cyclosporin A inhibits PARP-1 dependent cell death in astrocytes following MNNG treatment (91). In addition, blocking PTP by cyclosporin A can preserve mitochondrial NAD^+ pools (91). Moreover, mice lacking cyclophilin D are protected against ischemia-reperfusion injury (92-94). These results taken together suggest that PT may underlie PARP-1 mediated cell death. However, additional studies are needed to determine whether inhibiting PT is sufficient to prevent AIF release and cell death.

OMP involves the Bcl-2 family of proteins with members classified into pro-apoptotic or anti-apoptotic. One of the proapoptotic members, Bax, has been implicated in AIF release (49). Upon death stimuli, Bax translocates from the cytosol to the mitochondria, where it form pores on the outer mitochondrial membrane through oligomers of itself or through association with other Bcl-2 members (88). Fibroblasts lacking Bax show compromised AIF release from the mitochondria, and are protected against PARP-1 mediated cell death (49). Thus, Bax activation is downstream of PARP-1 overactivation and induces OMP needed for AIF release (49). However, it is possible that Bax deficiency merely delayed the onset of cell death. This was not clear from this study, since it did not investigate events beyond 12 hours. Previous data from our laboratory, indicates that Bcl-2 overexpression does not completely protect cells against PARP-1 dependent cell death, but only delays the onset of cell death (36). A deficiency in Bax may produce a similar effect by preserving mitochondrial integrity following PARP-1 activation. Thus, further studies are needed to explore in detail the role of Bax and other Bcl-2 proteins in PARP-1 dependent cell death.

In addition to OMP, evidence shows that cleavage of AIF by calpains is needed for AIF to be completely released from the mitochondria (49, 95). Calpains are calcium-dependent proteases that are believed to cleave AIF causing its release from the inner mitochondrial membrane (49). Mutation of AIF on its calpain-cleavage site protects against oxygen-glucose deprivation induced neuronal death (55). Moreover, overexpressing calpastatin, a calpain inhibitor, protected neurons against cell death from transient global ischemia (55). In MNNG-treated fibroblasts, calpain activation was found to be downstream of PARP-1 overactivation and upstream of Bax activation (49). These findings hint at an active role for calpains downstream of PARP-1 overactivation, but it is not clear how these events are interconnected. Specifically, what is the direct relationship among excess PAR, calpain activation and Bax translocation (49)? In addition, it remains to be identified whether calpain inhibition or genetic knockdown is indeed a factor for cell survival in PARP-1 dependent cell death, or whether calpain inhibition simply delays the onset of cell death and AIF-translocation.

5. CONCLUSION

Parthanatos underlies PARP-1 mediated cell death through the actions of PAR polymer inducing AIF translocation from the mitochondria to the nucleus. The identification of PAR polymer as a novel death signal opens up new avenues for therapy in ameliorating diseases related to PARP-1 overactivation. How PAR polymer relates to other events implicated in AIF release such as mitochondrial membrane permeabilization, calpain activation, and Bax translocation requires further study.

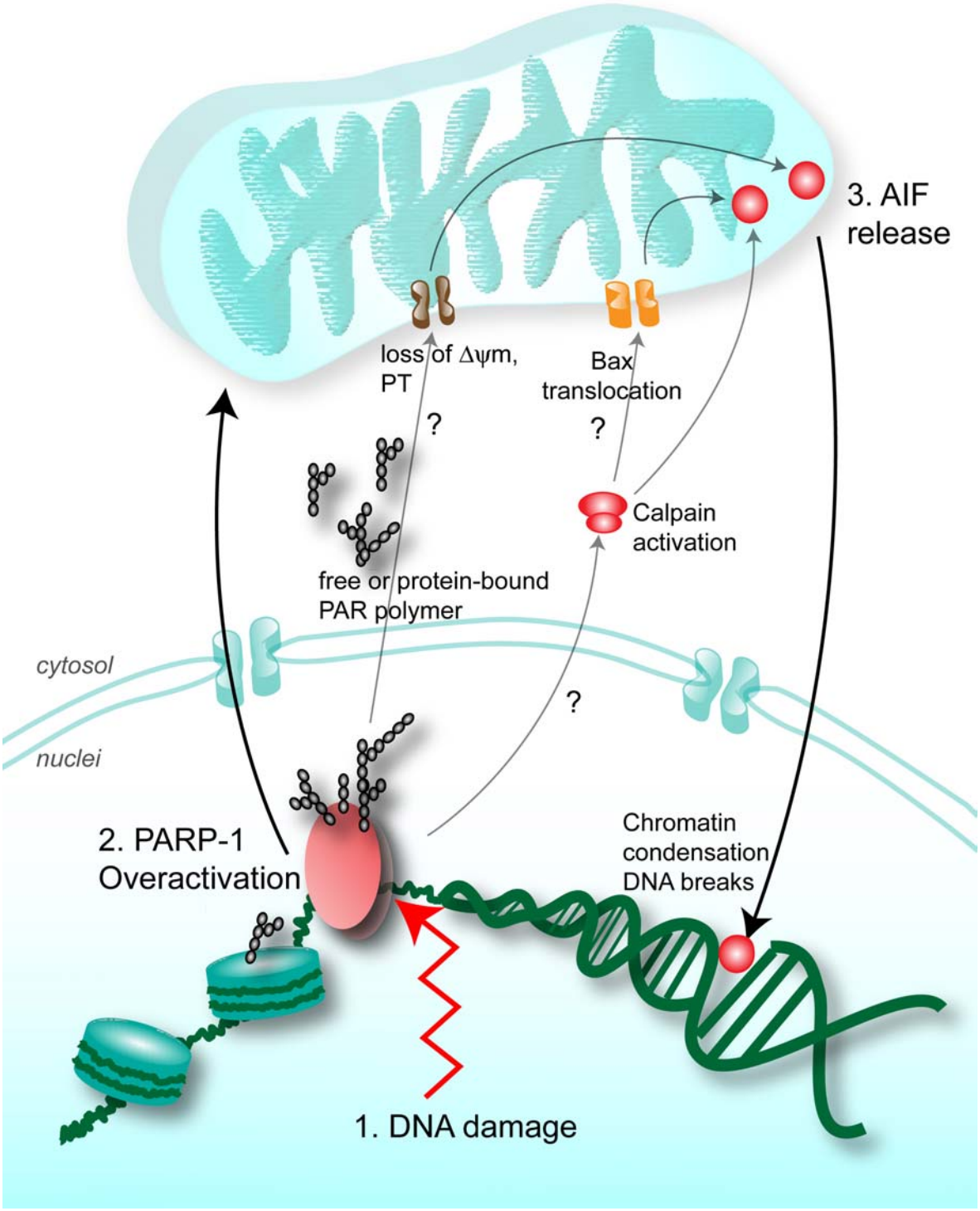

Figure 3. PARP-1 overactivation leads to cell death. In the presence of death stimuli such as excessive DNA damage (1), PARP-1 overactivation (2) leads to the release of the death effector AIF from the mitochondria (3). The biochemical events mediating this nuclear-mitochondrial crosstalk are not completely known. Excess free or protein-bound complex PAR polymer may move from the nuclei to the cytosol where it disrupts protein-protein interactions. Since loss of mitochondrial membrane potential was observed in PARP-1 mediated cell death, PAR possibly binds cytosolic or mitochondrial proteins with roles in AIF release, mitochondrial membrane permeabilization or mitochondrial function. Other events downstream of PARP-1 overactivation include calpain activation, and Bax translocation to the mitochondria. These events appear to be important for AIF release: calpain is hypothesized to cleave AIF which is then released from mitochondria through pores formed by Bax.

Since PAR mediates binding, the next steps involve the identification of specific PAR targets and to determine how binding by PAR leads to cytotoxicity.

6. ACKNOWLEDGEMENTS

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Abbreviations: PARP: poly-(ADP) ribose polymerase; PAR: poly-(ADP) ribose; PARG: poly-(ADP) ribose glycohydrolase; AIF: apoptosis-inducing factor; MNNG: N-methyl-N'-nitro-N-nitrosoguanidine; NMDA: N-methyl-D-aspartic acid; NAD: nicotinamide adenine dinucleotide; KO: knock-out; Namnt: nicotinic acid mononucleotide adenylyltransferase; PTP: permeability transition pore

Key Words: PARP-1, PAR polymer, PARG, Parthanatos, Cell Death, AIF, Review

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