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Structure and function of the ARH family of ADP-ribose-acceptor hydrolases

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Abstract

ADP-ribosylation is a post-translational protein modification, in which ADP-ribose is transferred from nicotinamide adenine dinucleotide (NAD^+) to specific acceptors, thereby altering their activities. The ADP-ribose transfer reactions are divided into mono- and poly-(ADP-ribosyl)ation. Cellular ADP-ribosylation levels are tightly regulated by enzymes that transfer ADP-ribose to acceptor proteins (e.g. ADP-ribosyltransferases, poly-(ADP-ribose) polymerases (PARP)) and those that cleave the linkage between ADP-ribose and acceptor (e.g. ADP-ribosyl-acceptor hydrolases (ARH), poly-(ADP-ribose) glycohydrolases (PARG)), thereby constituting an ADP-ribosylation cycle. This review summarizes current findings related to the ARH family of proteins. This family comprises three members (ARH1-3) with similar size (39 kDa) and amino acid sequence. ARH1 catalyzes the hydrolysis of the *N*-glycosidic bond of mono-(ADP-ribosyl)ated arginine. ARH3 hydrolyzes poly-(ADP-ribose) (PAR) and *O*-acetyl-ADP-ribose. The different substrate specificities of ARH1 and ARH3 contribute to their unique roles in the cell. Based on a phenotype analysis of $ARH1^{-/-}$ and $ARH3^{-/-}$ mice, ARH1 is involved in the action by bacterial toxins as well as in tumorigenesis. ARH3 participates in the degradation of PAR that is synthesized by PARP1 in response to oxidative stress-induced DNA damage; this hydrolytic reaction suppresses PAR-mediated cell death, a pathway termed parthanatos.

Keywords

ADP-ribosylation; ADP-ribose-acceptor hydrolase; tumorigenesis; cholera toxin; Parthanatos; ARH

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4. Conflict of interest
The authors confirm there is no conflict of interest, financial, or otherwise.

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1. ADP-ribosylation

ADP-ribosylation is a reversible post-translational modification of proteins in which the ADP-ribose moiety of nicotinamide adenine dinucleotide (NAD^+) is transferred to acceptors such as the amino acid residues of proteins, altering their activity and thus critical cellular functions [1–3]. These modifications are broadly grouped into two categories, mono- and poly-(ADP-ribosyl)ation.

1.1. Mono-(ADP-ribosyl)ation

ADP-ribosylation was initially discovered as the mechanism by which some bacterial toxins and cytotoxins e.g., Pseudomonas exoenzyme S, pertussis toxin, cholera toxin, diphtheria toxin, exert their effects [4–7]. This family of bacterial toxins comprises mono-ADP-ribosyltransferases, which transfer a single ADP-ribose moiety to specific amino acids, e.g., arginine, cysteine, diphthamide, asparagine, of acceptor proteins in host cells, thereby disrupting host cell biosynthetic, regulatory and metabolic pathways.

Mammalian cells also contain ADP-ribosyltransferases, which catalyze reactions similar to those of the bacterial toxins [8–10]. A family of ecto-ADP-ribosyltransferases (ART1-5) from avian and mammalian tissues has been cloned and characterized [11–17]. ART1-4 are anchored to the plasma membrane through a glycosylphosphatidylinositol moiety, whereas ART5 is a secreted protein. ART1, ART2, and ART5 catalyze the stereospecific transfer of an ADP-ribose from NAD^+ to the guanidino moiety of arginine (protein), forming an α-anomeric *N*-glycosidic linkage of ADP-ribose to arginine, whereas amino acid substrates of ART3 and ART4 have not been identified. These extracellular enzymes are involved in the modification of secreted and membrane proteins, as well as cell surface receptors, including the P_2X_7 purinergic receptor, human neutrophil peptide 1 (HNP-1), integrin α7, platelet-derived growth factor-BB (PDGF-BB), and fibroblast growth factor-2 (FGF-2) [18–22]. By mono-(ADP-ribosyl)ation of target proteins, ARTs appear to regulate innate immunity and cell-cell and cell-matrix interactions. However, mono-(ADP-ribosyl)ation also occurs intracellularly. This reaction is catalyzed, in part, by members of the sirtuin family of NAD^+-dependent deacetylases (SIRT) [23–27]. The SIRT family comprises seven members (SIRT1-7), which are widely distributed in intracellular organelles. SIRT1, 2, 4, and 6 possess intrinsic mono-(ADP-ribosyl)ation activity, transferring a single ADP-ribose to an arginine residue of specific target proteins [23–26]. Glutamate dehydrogenase (GDH) is a specific target for mono-(ADP-ribosyl)ation catalyzed by SIRT4; cysteine residue at position 119 of human GDH is ADP-ribosylated [26, 28]. The mono-(ADP-ribosyl)ation of GDH negatively regulates its activity, resulting in inhibition of insulin secretion from the pancreas. In addition, some members of poly-(ADP-ribose) polymerase (PARP) family have also been reported to possess mono-ADP-ribosyltransferase activity [29–31].

1.2. Poly-(ADP-ribosyl)ation

Poly-(ADP-ribosyl)ation in mammal cells has a crucial role in cellular functions including mitosis, DNA repair, and cell death [31–33]. It is initiated by transferring ADP-ribose primarily to carboxyl groups of glutamate and aspartate residues and ε–amino group of lysine residue(s) of target proteins to create *O*- and *N*-glycosidic bonds, respectively, of

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ADP-ribose to proteins; this reaction is followed by chain elongation and branching, resulting in the formation of a long, branched chain of poly-(ADP-ribose) (PAR). PAR is a polymer composed of several hundred ADP-ribose units, and thus is negatively charged; its formation is catalyzed by PARP. PARP1, the best studied protein in the PARP family, is a nuclear, chromatin-associated protein that is found in most eukaryotes except for yeast [34–36]. PARP1 is the most abundant and most active PARP and constitutes the founding member of the PARP superfamily. On the basis of sequence similarities to the catalytic domain of PARP1, seventeen PARP enzymes have been identified in the human genome [31–33]. Within this family of seventeen proteins, the enzymes capable of catalyzing poly-(ADP-ribosyl)ation are PARP1, PARP2, PARP3, PARP4, Tankyrase1 (PARP5A), and Tankyrase2 (PARP5B), whereas PARP10, PARP12, PARP14, PARP15, and PARP16 are mono-ADP-ribosyltransferases [29–31, 37–39]. PARP9 and PARP13 appear to be enzymatically inactive, because of the lack of NAD^+-binding residues. Other PARP isoforms are predicted to be mono-ADP-ribosyltransferases [31].

PARP1 is critical for cell survival under conditions in which DNA damage is induced by oxidation, alkylating agents, and ionizing radiation. Basal activity of PARP1 is increased by 500-fold in response to DNA single- and double-strand breaks by binding to DNA breaks through its zinc finger, DNA-binding domains, which initiate poly-(ADP-ribosyl)ation of glutamate, aspartate, and lysine residues of acceptor proteins [40–44]. Based on results obtained with *PARP1* and *PARP2*-deficient cells, more than 90% of PAR production results from PARP1 activity [39]. PAR with its negative charge alters the physical and biological properties of target proteins such as histones, topoisomerase I, and DNA protein kinases, resulting in DNA remodeling and repair [44–48]. PARP1 itself is also auto-modified by PAR via its auto-modification and DNA-binding domains [41, 43, 49, 50]. Its modification promotes interaction with several proteins such as XRCC1, DNA ligase III, and the Ku70 subunit of the DNA-dependent protein kinase, recruiting them to DNA-damage sites for DNA repair [34, 45, 51]. Alternatively, PAR(P) has a role in cell injury and death. A PAR-dependent death pathway has been demonstrated in several disease models including brain and myocardial ischemia–reperfusion injury, glutamate excitotoxicity, streptozotocin-induced diabetes, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism [52–58]. PAR production by excessive activation of PARP1 causes significant consumption of cellular NAD^+, followed by depletion of ATP, which results in necrotic cell death [59, 60]. In addition, non-covalent PAR itself has been reported to have a role as a death signal [54, 58, 61, 62]. PAR may induce release of apoptosis-inducing factor (AIF) from mitochondria, which is involved in caspase-independent cell death, a process termed parthanatos, which was named after Thanatos, the personification of death in Greek mythology [62]. PAR generated by PARP1 in the nucleus translocates to the cytoplasm [61]. Cytoplasmic PAR associates with apoptosis-inducing factor (AIF) anchored on mitochondrial membranes, resulting in release of AIF into the cytoplasm [58]. AIF translocates to the nucleus via its nuclear localization sequence and induces large-scale DNA fragmentation and chromatin condensation [62].

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1.3. Enzymes involved in termination of ADP-ribosylation

Thus, as ADP-ribosylation participates in several important biological processes, it must be controlled both spatially and temporally. In fact, ADP-ribosylation is reversibly regulated by several enzymes including poly-(ADP-ribose) glycohydrolase (PARG), macrodomain proteins, and ADP-ribosyl-acceptor hydrolases (ARH). PARG has been thought to be the primary enzyme responsible for termination of poly-(ADP-ribosyl)ation by catalyzing the hydrolysis of the *O*-glycosidic bond of PAR chains [63, 64]. Alternative splicing of a single *PARG* gene gives rise to several PARG isoforms with different sizes, activities, and localizations; a nuclear 110-kDa protein, cytoplasmic 103-kDa, 99-kDa, and 60-kDa proteins, and a mitochondrial 55-kDa protein [65–68]. Absence of the *PARG* gene results in embryonic lethality, because of excessive accumulation of PAR in nuclei and subsequent induction of cell death [69].

However, as PARG is unable to hydrolyze the *O*-glycosidic bond of the first ADP-ribose directly attached to glutamate residues of target proteins, its activity generates mono-(ADP-ribosyl)ated proteins. Macrodomains, evolutionally conserved modules of 130–190 amino acids discovered as a domain of a core histone variant macroH2A, bind ADP-ribose monomers and polymers as well as the sirtuin product *O*-acetyl-ADP-ribose (*O*AADPr) [70, 71]. In mammalian cells, MacroD1, MacroD2, and C6orf130 which is also known as Terminal ADP-Ribose protein Glycohydrolase (TARG1), possess mono-ADP-ribosyl-acceptor hydrolase activity that cleaves the *O*-glycosidic linkage of ADP-ribose to glutamate, leading to regeneration of unmodified protein [72–75]. Homozygous mutation of the *TARG1* gene, which generates a non-functional truncated variant, results in severe neurodegeneration in humans, indicating the importance of TARG1 function on termination of poly-(ADP-ribosyl)ation [72].

The ARH family consists of three members (ARH1-3) with substantial amino acid sequence similarity (Table 1) [16, 76–80]. ARH1 and ARH3 have different substrate specificities. ARH1 cleaves mono-ADP-ribosylated substrate with the modification on an arginine, while ARH3 hydrolyzes PAR and *O*AADPr [76, 78, 81, 82]. ARH2 has not been shown to have enzymatic activities related to mono- and poly-ADP-ribose [81, 82]. The ARH family is described in more detail below.

2. ARH family

The ARH family of proteins (ARH1-3) exhibit similar size (39 kDa) and amino acid sequence [16, 76–80]. ARH1 is a mono-ADP-ribosyl-arginine hydrolase, which catalyzes the hydrolysis of the *N*-glycosidic bond linking ADP-ribose to the guanidino group of arginine, leading to release of ADP-ribose, with formation of arginine [78]. ARH3 catalyzes the hydrolysis of the *O*-glycosidic bond in PAR, generating ADP-ribose [76, 77]. These reactions are stereospecific. ARH1 and ARH3 preferentially cleave the α-anomer at the C-1″ position of ADP-ribose attached to arginine and ADP-ribose in PAR, respectively. ARH3 hydrolyzes *O*AADPr in the same manner [81, 82]. Similar to ARH3, ARH1 cleaves PAR and *O*AADPr, but ARH1 activity is less than 1% that of ARH3, indicating that ARH1 might possess weak hydrolytic activity toward the *O*-glycosidic bond [81]. *O*AADPr itself, independent of protein deacetylation, regulates SIRT activity, TRPM2 ion-channel gating,

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embryo development, and cellular redox [83–86]. Through *O*AADPr metabolism, ARH1 and ARH3 may participate in these signal transduction pathways. On the other hand, ADP-ribosylated cysteine, asparagine, and diphthamide, synthesized by bacterial toxins, are not substrates hydrolyzed by ARH1 or ARH3 [76]. ARH1 and ARH3 activities are competitively inhibited by ADP-ribose, but not ribose 5-phosphate, AMP, ADP or NAD^+ [80–82]. Thus, ARH1 and ARH3 recognize the ADP-ribose moiety of the substrate. Although ARH2 binds ADP-ribose, it has not been shown to be enzymatically active [81, 82].

Enzymatic activities of both ARH1 and ARH3 require Mg^{2+} [76–78]. According to the crystal structure of ARH3, the active site is defined by the position of two Mg^{2+} ions [77]. ARH3 contains a pair of vicinal acidic amino acids, aspartates, at positions 77 and 78, that are essential to coordinate two Mg^{2+} ions and required for its enzymatic activity [76, 77, 81]. ARH1 also contains two aspartates at positions 60 and 61 [87]. Replacement of the pair with asparagine or alanine in ARH1 or ARH3 results in a dramatic reduction in activity, although the mutant proteins are structurally intact and retain their ability to bind ADP-ribose. In ARH2, the vicinal amino acid residues at positions 60 and 61 are aspartate-asparagine, which may explain why ARH2 lacks enzymatic activity [76].

In some species, ARH1 enzymatic activity also requires thiol; rat and mouse ARH1 requires the presence of thiol, whereas human, calf and guinea pig ARH1 do not [78]. In human ARH1, a critical cysteine residue is responsible for thiol-dependence; in mouse, it is replaced by serine [88]. Likewise, human and mouse ARH3 do not require thiol, possibly because ARH3 contains valine at the position that determines thiol-sensitivity of human ARH1 [76].

Based on phenotype analysis of *ARH1*$^{-/-}$ and *ARH3*$^{-/-}$ mice and cells, both enzymes regulate several cellular responses through their activities. In this review, we highlight current knowledge of ARH1 and ARH3 related to cellular distributions and physiological and pathological functions.

2.1. ARH1 distribution and functions

ARH1 is a cytoplasmic protein ubiquitously expressed in mammalian tissues and cells [78]. In *ARH1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) and tissues, the ability to hydrolyze mono-(ADP-ribosyl)ated arginine is lost [89], suggesting that ARH1 is the only cytoplasmic enzyme that hydrolyzes the ADP-ribose-arginine bond. It appears to be a component of a mono-(ADP-ribosyl)ation cycle (Fig. 1). A role for ARH1 in disease was first found in a mouse model of cholera toxin-induced disease [89]. Cholera toxin, secreted by *Vibrio cholerae*, is a multimeric protein, which transfers an ADP-ribose to arginine residues of the α-subunit of the stimulatory guanine nucleotide-binding (Gαs) protein of the adenylyl cyclase system [6]. Its ADP-ribosylation stabilizes Gs-GTP by inhibiting the GTPase activity of Gαs, resulting in prolonged activation of adenylyl cyclase. Activation of adenylyl cyclase in intestine increases intracellular cAMP, leading to abnormalities of fluid and electrolyte transport characteristic of cholera. *ARH1*$^{-/-}$ mice and MEFs exhibit enhanced sensitivity to cholera toxin compared to their wild-type (WT) counterparts [89]. Cholera toxin increased the mono-(ADP-ribosyl)ation levels of Gαs; mono-(ADP-ribosyl)ation of

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Gαs was further increased and prolonged in *ARH1*$^{-/-}$ MEFs and mice, compared to their WT counterparts. Moreover, intestinal loops of *ARH1*$^{-/-}$ mice showed significantly enhanced fluid accumulation following exposure to cholera toxin than did those of WT mice. Thus, these data support a role for ARH1 in the intoxication process seen in cholera.

ARH1-mediated mono-ADP-ribosylarginine hydrolase activity is also involved in intracellular signal transduction. *ARH1*$^{-/-}$ and *ARH1*$^{+/-}$ mice are prone to tumor development (Table 2) [90]. Further, metastasis and multi-tumor occurrences are seen more frequently in *ARH1*$^{-/-}$ and *ARH1*$^{+/-}$ mice than in WT mice. Approximately 25% of *ARH1*$^{-/-}$ mice and 13% of *ARH1*$^{+/-}$ mice developed tumors in the age range of 3–12 months when tumors were infrequent in WT mice. In *ARH1*$^{-/-}$ mice, several types of tumors (e.g., carcinoma, sarcoma, lymphoma) developed in a variety of tissues/organs (e.g., lung, liver, spleen, lymph nodes, mammary gland, uterus, skeletal muscle) in an age-dependent manner. Thus, loss of function of ARH1 was strongly correlated with tumorigenesis. Consistent with a high frequency of tumorigenesis in *ARH1*$^{-/-}$ mice, *ARH1*$^{-/-}$ MEFs have characteristics of tumor cells; *ARH1*$^{-/-}$ MEFs proliferated faster and formed more and larger colonies in soft agar than did WT MEFs, and subcutaneous injection of *ARH1*$^{-/-}$ MEFs in athymic nude mice resulted in the formation of tumor-like masses. By stable expression of ARH1, but not an inactive ARH1 mutant, all the phenotypes seen in *ARH1*$^{-/-}$ MEFs were reversed. *ARH1*$^{-/-}$ MEFs exhibited a shorter G1 phase of the cell cycle. Abnormal cell cycle progression is possibly linked to genomic instability and uncontrolled cell growth, leading to tumorigenesis. Thus, the proper control of mono-(ADP-ribosyl)ation by ARH1 has a crucial role in cell proliferation and cancer suppression.

Interestingly, tumorigenesis seen in *ARH1*$^{-/-}$ mice and MEFs is gender-specific [91]. *ARH1*$^{-/-}$ and *ARH1*$^{+/-}$ female mice developed tumors and metastasis more frequently and at a younger age than did male mice. *ARH1*$^{-/-}$ MEFs subcutaneously injected into female nude mice formed tumor-like masses more rapidly than they did in male mice. Gender effect of ARH1 deficiency on incidence of tumor growth may result from effects of estrogen. Ovariectomized female nude mice exhibited reduced growth rate of tumors from following injection of *ARH1*$^{-/-}$ MEFs, whereas estrogen treatment of male and ovarictomized female nude mice increased the frequency and rate of tumor development. Further, estrogen promoted the survival of *ARH1*$^{-/-}$ MEFs in the circulation and thus enhanced their metastatic potential. Based on these and other data, it appeared that ARH1 is involved in estrogen-stimulated tumorigenesis.

2.2. ARH3 distribution and functions

ARH3 is ubiquitously expressed in mouse and human tissues [76]. ARH3 is mainly located in the cytoplasm (65%), followed by mitochondria (25%), and nucleus (10%) [92, 93]. ARH3 contains a mitochondrial-targeting sequence near its N-terminus, which allows ARH3 to be expressed in the mitochondrial matrix [77, 92]. Niere et al. provided evidence that ARH3 might be the primary enzyme responsible for putative PAR degradation in mitochondria [65, 92]. Overexpression of ARH3 lowered mitochondrial PAR content artificially driven by expression of the catalytic domain of PARP1 with a mitochondrial targeting sequence (mito-PARP1cd) [92]. In *ARH3*$^{-/-}$ MEFs expressing mito-PARP1cd,

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mitochondrial PAR content was greater than in WT MEFs expressing mito-PARP1cd [65]. In the presence of a PARP inhibitor, turnover of mito-PARP1cd-induced PAR in mitochondria was significantly slower in *ARH3*$^{-/-}$ MEFs than in WT MEFs. In human cultured cells (e.g., HEK 293, HepG2, HeLaS3, SH-SY5Y cells), small PARG isoforms, PARG60 and PARG55, resulting from alternative splicing of *PARG* transcripts, were expressed in cytoplasm and mitochondria, but unable to degrade PAR, because they lack exon 5, which encodes a region critical for PARG activity [65, 68]. Although some reports indicated that PAR is present in mitochondria [94], a mitochondrial PARP has not been identified and ARH3 deficiency did not increase PAR content in mitochondria under resting conditions. Therefore, the function of ARH3 in mitochondria is unclear. However, SIRT3, SIRT4, and SIRT5 are located in the mitochondrial matrix [95]. SIRT3 and SIRT5 exhibit deacetylase activity, generating *O*AADPr. By degradation of *O*AADPr, ARH3 might participate in the regulation of functions involving mitochondrial SIRTs.

ARH3 also participates in nuclear and cytoplasmic PAR degradation under oxidative stress conditions induced by hydrogen peroxide (Fig. 2) [93]. *ARH3*$^{-/-}$ MEFs were more susceptible to hydrogen peroxide-induced cytotoxicity than WT MEFs. ARH3 expression in *ARH3*$^{-/-}$ MEFs reduced their sensitivity to hydrogen peroxide. Cell death in *ARH3*$^{-/-}$ MEFs occurred in a caspase-independent manner, accompanied by nuclear shrinkage, chromatin condensation, and exposure of phosphatidylserine on the cell surface. Genetic studies using PARP1 shRNA indicated that PARP1 activation has a role as an initiation step to induce the cell death signal following oxidative stress, by synthesizing PAR in the nucleus. In *ARH3*$^{-/-}$ MEFs, nuclear PAR content in response to hydrogen peroxide was significantly increased as early as 10 min, compared to WT MEFs. After 30 min, PAR gradually translocated from nucleus to the cytoplasm in *ARH3*$^{-/-}$ MEFs, whereas PAR accumulation was less in WT MEFs, suggesting that PAR hydrolytic activity of ARH3 lowers PAR content in the nucleus and cytoplasm, preventing PAR translocation and accumulation in the cytoplasm. As suggested by the above, cytoplasmic PAR associates with AIF on the mitochondrial surface, releasing it to the cytoplasm [54, 58, 61, 62]. Although AIF is an anchored protein present in the inner mitochondrial membrane, some AIF (20–30%) is also located on the cytoplasmic side of the outer-mitochondrial membrane where it can be accessed by cytoplasmic PAR [58, 96]. Cytoplasmic PAR seen in *ARH3*$^{-/-}$ MEFs appeared to enhance AIF release from mitochondria and translocation to the nucleus, leading to large-scale DNA fragmentation and chromatin condensation. Thus, ARH3 serves as a suppressor of PARP1-dependent cell death, parthanatos, under oxidative stress.

Interestingly, PAR export from the nucleus may result from the action of nuclear 110-kDa PARG, which releases protein-free PAR from acceptor proteins such as PARP1. PARG depletion by shRNA resulted in increased and prolonged poly-(ADP-ribosyl)ation of PARP1, inhibited PAR translocation from the nucleus to cytoplasm, and attenuated AIF-mediated cell death [93]. These findings are consistent with the facts that selective knockout of nuclear 110-kDa *PARG* has a protective effect against renal and intestinal injury induced by ischemia/reperfusion in mice [36, 97, 98]. PARG has both endo- and exo-glycosidase activities that hydrolyze the glycosidic linkage between ADP-ribose moieties of PAR chains [99–101]. Endo-glycosidase activity of PARG might account for production of protein-free

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PAR, which can pass through nuclear pores and then bind to AIF on mitochondrial membranes. This model is consistent with biochemical studies that show that (1) PARG preferentially cleaves long, rather than short PAR chains, (2) the K_m value for long PAR chains is approximately ~1% of that for small ones, and that (3) PARG hydrolyzes covalently protein-bound PAR more rapidly than it does protein-free PAR [63, 102]. On the other hand, according to the three-dimensional structure of ARH3, the cavity of ARH3 docks only on the terminal ADP-ribose moiety of the PAR chain [77], suggesting that ARH3 has exoglycosidase activity, and thus is unable to substitute for PARG. Despite that the fact that ARH3 has lower PAR-degrading activity than PARG [76], the different substrates for PAR degradation and the cellular localization may contribute to their unique roles in the regulation of parthanatos.

3. Concluding remarks

We describe the properties of ARH1 and ARH3, focusing on their enzymatic activities, cellular distributions, and physiological and pathological functions. Based on phenotype analysis of *ARH1*$^{-/-}$ and *ARH3*$^{-/-}$ mice, we found that mono-ADP-ribosyl-acceptor hydrolase activity of ARH1 is involved in reversing the response to cholera toxin, possibly serving as a novel host defense mechanism [89]. Moreover, *ARH1* appears to act as a tumor suppressor gene [90]. In contrast, ARH3 has poly-ADP-ribosyl-acceptor hydrolase activity and is involved in PAR degradation and the induction of parthanatos under oxidative stress conditions [65, 92, 93]. Thus, although ARH1 shares substantial amino acid sequence similarity with ARH3, they have different enzymatic activities.

We demonstrate that, through their substrate-specific hydrolase activities, both ARH1 and ARH3 regulate ADP-ribose levels; abnormalities in the regulation of either of these enzymes may lead to defective regulation of metabolism and potentially to disease. Both mono- and poly-(ADP-ribosyl)ation may thus have significant roles in health and disease. Understanding these functions may result in novel therapeutic targets.

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Abbreviations

MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
Gαs	α-subunit of the stimulatory guanine nucleotide-binding protein
ARH	ADP-ribosyl-acceptor hydrolase
AIF	Apoptosis-inducing factor
mito-PARP1cd	Catalytic domain of PARP1 with a mitochondrial targeting sequence
ART	ADP-ribosyltransferase
FGF-2	Fibroblast growth factor-2

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GDH	Glutamate dehydrogenase
HNP-1	Human neutrophil peptide 1
MEFs	Mouse embryonic fibroblasts
NAD$^+$	Nicotinamide adenine dinucleotide
***O*AADPr**	*O*-acetyl-ADP-ribose
PDGF-BB	Platelet-derived growth factor-BB
PARG	Poly-(ADP-ribose) glycohydrolase
PARP	Poly-(ADP-ribose) polymerase
TARG1	Terminal ADP-ribose protein glycohydrolase
WT	Wild-type

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Highlights

- The ADP-ribose-acceptor hydrolase (ARH) family consists of three 39-kDa proteins.
- ARH1 is involved in tumorigenesis and the response to bacterial toxin transferases.
- ARH3 participates in the induction of parthanatos, PARP1-mediated cell death.

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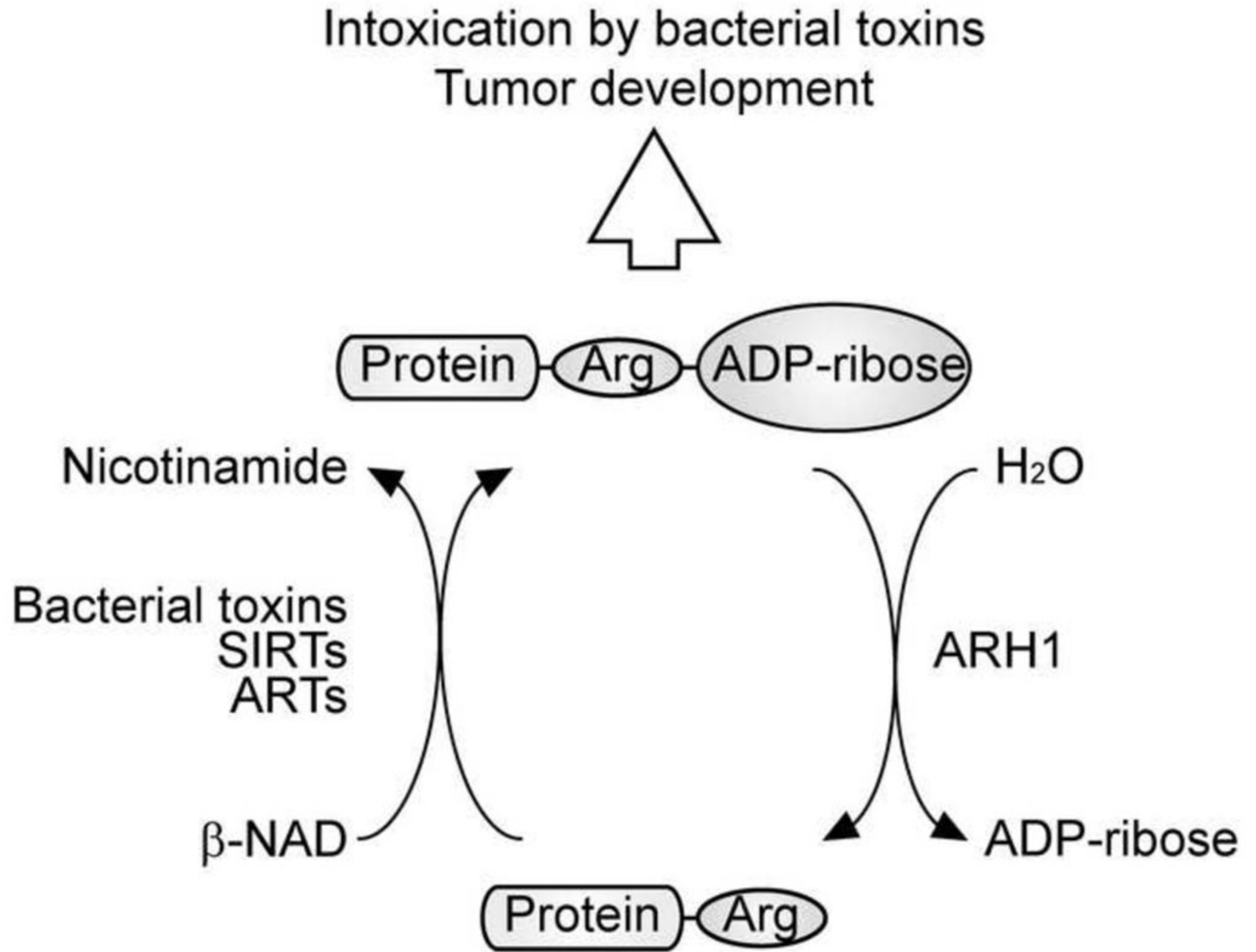

Fig. 1. Arginine-specific mono-(ADP-ribosyl)ation

Bacterial toxins, ARTs, and some members of the SIRT family transfer the ADP-ribose moiety of NAD^+ to arginine residues of acceptor proteins with release of nicotinamide [6, 7, 9, 24]. Mono-(ADP-ribosyl)ation may be involved in tumorigenesis as well as in disruption of host regulatory biosynthetic and metabolic pathways by bacterial toxins [89, 90]. ARH1 is responsible for catalyzing the hydrolysis of the *N*-glycosidic bond linking ADP-ribose to the guanidino group of arginine, which occurs at the C-1″ position of ADP-ribose. Hydrolysis leads to release ADP-ribose with regeneration of arginine. ARH1 deficiency results in enhanced sensitivity to cholera toxin as well as other toxins and tumor development.

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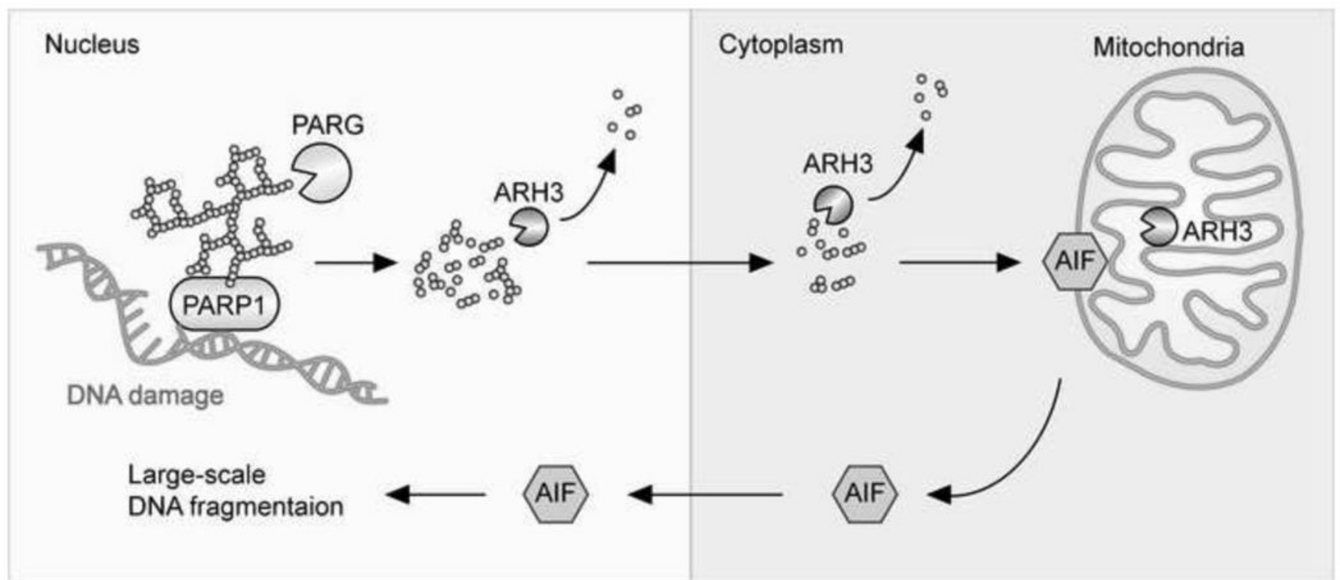

Fig. 2. Regulation of parthanatos by PARP1, PARG, and ARH3

DNA damage secondary to oxidative stress activate PARP1, results in increased PAR synthesis in the nucleus. Protein-associated PAR is cleaved by the endoglycosidic activity of nuclear PARG, generating protein-free PAR, which then translocates to the cytoplasm. Cytoplasmic PAR releases AIF attached to the outer surface of mitochondrial membranes. AIF released into the cytoplasm then translocates to the nucleus and induces large-scale DNA fragmentation and chromatin condensation. This figure was modified from that in Mashimo et al. [93].

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Table 1

Similarity of amino acid sequences and relative enzymatic activities of the ARH family

Amino acid sequences of ARH2 and ARH3 were compared to ARH1 sequence [76]. Their relative enzymatic activities were quantified using a five-grade scale. "-" indicates that no activity was detected. ARH1 is the only enzyme responsible for the hydrolysis of ADP-ribosylated arginine [89]. ARH3 hydrolyzes the *O*-glycosidic bond of PAR chains and *O*AADPr as does ARH1. ARH1, however, has < 1% of the specific activity of ARH3 [76, 81, 82]. Enzymatic substrates of ARH2 have not been identified.

	aa	Similarity to ARH1 (%)	ADP-ribosyl-arginine	PAR	OAADPr
ARH1	357		+++++	+	+
ARH2	354	68	−	−	−
ARH3	363	41	−	+++	++++

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Table 2

Effect of ARH1 genotype on incidence of tumors

Incidence of tumors (%) in ARH1 genotype was evaluated. Metastasis and multi-tumor occurrences were seen more frequently in $ARH1^{-/-}$ and $ARH1^{+/-}$ mice than in WT mice. In $ARH1^{-/-}$ and $ARH^{+/-}$ mice, several types of tumors (e.g., carcinoma, sarcoma, lymphoma) developed at a younger age than was seen with WT mice. Lymphoma was seen most frequently in $ARH1^{-/-}$ and $ARH^{+/-}$ mice.

		Carcinoma		Lymphoma	Sarcoma		Total
Age	**Genotype**	**Adeno-**	*** HC-**		**Hemangio-**	**Rhabdomyo-**	
3–12 months	+/+	0	0	0	0	0	0.0
	+/–	4.1	1.1	7.9	0.2	0.1	13.4
	–/–	6.2	2.1	12.9	4.0	0	25.2
13–20 months	+/+	0	0	2.9	0.6	0	3.5
	+/–	3.0	0.6	7.1	0.6	0	11.2
	–/–	5.8	0.6	8.9	5.0	0	20.3

* Hepatocellular (HC). This table was modified from Kato et al. [90].