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

Masato Mashimo, Jiro Kato, Joel Moss*

Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892-1590, United States

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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.

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,

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; GDH, glutamate dehydrogenase; HNP-1, human neutrophil peptide 1; MEFs, mouse embryonic fibroblasts; NAD^+, nicotinamide adenine dinucleotide; OAADPr, *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.

* Corresponding author at: Room 6D05, Building 10, MSC 1590, National Institutes of Health, Bethesda, MD 20892-1590, United States. Tel.: +1 301 496 1597; fax: +1 301 496 2363.
E-mail address: mossj@nhlbi.nih.gov (J. Moss).

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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 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, thereby initiating 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 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].

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) exhibits 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

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