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CHARACTERIZATION OF GLYCOSYLPHOSPHATIDYLINOSITIOL-ANCHORED, SECRETED, AND INTRACELLULAR VERTEBRATE MONO-ADP-RIBOSYLTRANSFERASES[1]

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ABSTRACT

Mono-ADP-ribosylation is a posttranslational modification of proteins in which the ADP-ribose moiety of nicotinamide adenine dinucleotide is transferred to an acceptor amino acid. Five mammalian ADP-ribosyltransferases (ART1–ART5) have been cloned and expression is restricted to tissues such as cardiac and skeletal muscle, leukocytes, brain, and testis. ART1 and ART2 are glycosylphosphatidylinositol (GPI)-anchored ectoenzymes. ART5 appears not to be GPI-linked and may be secreted. In skeletal muscle and lymphocytes, ART1 modifies specific members of the integrin family of adhesion molecules, suggesting that ADP-ribosylation affects cell-matrix or cell-cell interactions. In lymphocytes, ADP-ribosylation of surface proteins is associated with changes in $p56^{lck}$ tyrosine kinase-mediated signaling. The catalytic sites of bacterial toxins and vertebrate transferases have conserved structural features, consistent with a common reaction mechanism. ADP-ribosylation can be reversed by ADP-

ribosylarginine hydrolases, resulting in the regeneration of free arginine. Thus, an ADP-ribosylation cycle may play a regulatory role in vertebrate tissues.

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INTRODUCTION

Mono-ADP-ribosylation is a posttranslational modification of proteins in which the ADP-ribose moiety of NAD is transferred to an acceptor amino acid. This reaction is catalyzed by mono-ADP-ribosyltransferases, which have been detected in viruses, bacteria, and eukaryotic cells (63). The reaction catalyzed by this family of enzymes is distinct from that catalyzed by poly(ADP-ribose) polymerase, a nuclear protein involved in DNA repair, cell differentiation, and the maintenance of chromatin structure (63). Among mono-ADP-ribosyltransferases, the bacterial toxin transferases are the best characterized in molecular structure, function, and substrate specificity (63). Cholera toxin (CT) and the similar heat-labile *Escherichia coli* enterotoxin (LT) ADP-ribosylate an arginine in the α-subunit of the adenylyl cyclase stimulatory heterotrimeric guanine nucleotide-binding (G) protein, with a resulting increased concentration of intracellular cAMP (62). Pertussis toxin (PT) modifies a cysteine in $G\alpha_i$, $G\alpha_o$, and $G\alpha_t$, which uncouples the G-protein from its receptor (95). ADP-ribosylation of diphthamide, a modified histidine, in elongation factor 2 catalyzed by diphtheria toxin (DT) or by *Pseudomonas aeruginosa* exotoxin A (ETA) results in inhibition of protein synthesis and cell death (14, 100). Several clostridial toxins, including iota and C2 toxins, ADP-ribosylate an arginine in nonmuscle actin, which prevents actin polymerization (1).

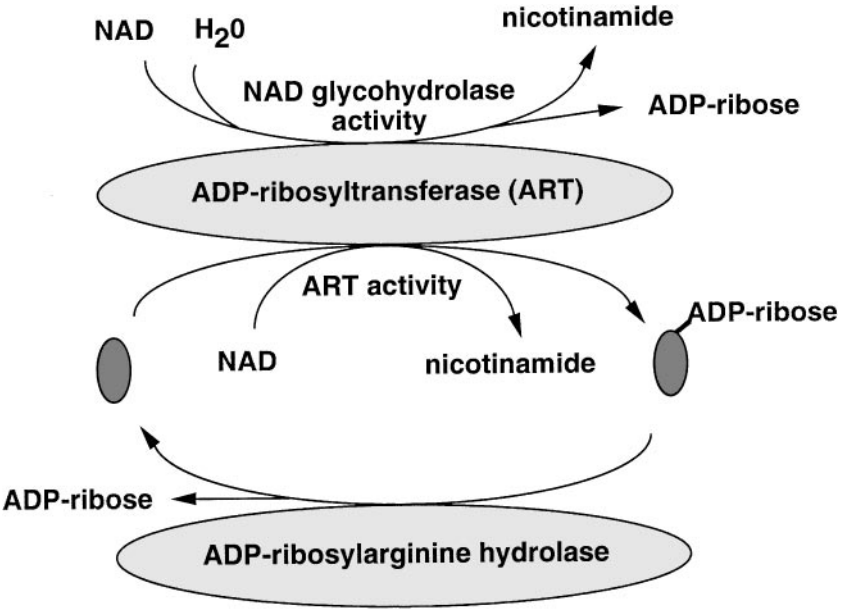


Figure 1 Enzyme activities of mono-ADP-ribosyltransferases and ADP-ribosylarginine hydrolases. ADP-ribosyltransferases transfer the ADP-ribose moiety of NAD to an acceptor protein (amino acid). In the presence of H_2O, transferases hydrolyze NAD to nicotinamide and ADP-ribose. The action of ADP-ribosylarginine hydrolases, which regenerate free arginine, is consistent with the presence of an ADP-ribosylation cycle.

It has been hypothesized that eukaryotic transferases may be mimicking the actions of bacterial toxin transferases. Vertebrate ADP-ribosyltransferase activity was first detected in turkey erythrocytes, rat liver homogenates, and *Xenopus* tissues (63). The majority of the cloned eukaryotic enzymes are arginine-specific transferases, and several potential substrate proteins have been identified (27, 97, 108). These enzymes, like the bacterial toxin ADP-ribosyltransferases, catalyze NAD hydrolysis (NAD glycohydrolase activity), which yields nicotinamide and ADP-ribose (Figure 1).

ADP-ribosylation of arginine appears to be a reversible process; free arginine can be regenerated in ADP-ribosylated proteins by the action of ADP-ribosylarginine hydrolases (101). ADP-ribosylarginine hydrolase activity was detected in the soluble fraction of turkey erythrocytes, cultured mouse cells, and rat skeletal muscle (101); rat, mouse, and human brain ADP-ribosylarginine hydrolase cDNAs were cloned and characterized (55). The presence of NAD: arginine ADP-ribosyltransferases and ADP-ribosylarginine hydrolases in cells is consistent with the existence of a regulatory ADP-ribosylation cycle (Figure 1).

ADP-ribosylation of cysteine was reported in human erythrocytes, and an NAD:cysteine ADP-ribosyltransferase that modified $G\alpha_i$ was purified from erythrocyte and platelet membranes (88). An NAD:cysteine ADP-ribosyltransferase (45 kDa) was purified from bovine erythrocytes (79). A 55-kDa erythrocyte membrane protein was labeled in the presence of the transferase and [^{32}P]NAD, but not in the presence of [^{32}P]ADP-ribose (79). The ADP-ribose moiety was removed from the 55-kDa protein by incubation with mercuric acetate, but not with neutral hydroxylamine (79). These data are consistent with the enzymatic formation of an ADP-ribose-cysteine linkage catalyzed by the bovine erythrocyte transferase. ADP-ribosylcysteine linkages were detected in rat liver plasma membranes by generation of free ADP-ribose following incubation of membrane proteins with mercuric ion (29). It was subsequently reported that ADP-ribosylation of cysteine can occur nonenzymatically via the reaction of ADP-ribose, generated from NAD by NAD glycohydrolases, with cysteine to form an ADP-ribosylthiazolidine, and not the thioglycoside ADP-ribosylcysteine linkage, which is formed by PT (49). The thiazolidine product was NH_2OH and $HgCl_2$ sensitive, whereas the thioglycoside linkage was sensitive to $HgCl_2$ but not to NH_2OH. Conceivably, ADP-ribose-cysteine produced by the human erythrocyte enzyme was generated nonenzymatically from free ADP-ribose. Similarly, free ADP-ribose reacted with a cysteine in aldehyde dehydrogenase (47) and actin (31). The ADP-ribose-aldehyde dehydrogenase linkage exhibited the same chemical sensitivity as a G-protein ADP-ribosylated by PT. Furthermore, nitric oxide (NO), which stimulates the ADP-ribosylation of several proteins in a variety of cellular systems, stimulated noncovalent binding of NAD to glyceraldehyde-3-phosphate dehydrogenase (48), yielding a product sensitive to $HgCl_2$. Chemical sensitivity alone, therefore, cannot distinguish between enzymatic and nonenzymatic modification with ADP-ribose of cysteine residues in proteins or distinguish ADP-ribosylation from addition of the entire NAD molecule to a protein.

Vertebrate ADP-ribosyltransferases contain regions of deduced amino acid sequence similarity to several viral and bacterial toxin transferases (17, 87). These regions form, in part, an active-site cleft, consistent with a common mechanism of NAD binding and ADP-ribose transfer shared by all transferases (17). The structure and function of vertebrate ADP-ribosyltransferases and their effects on cellular metabolism are reviewed and discussed below.

AVIAN NAD: ARGININE ADP-RIBOSYLTRANSFERASES

Turkey Erythrocyte ADP-Ribosyltransferases

The first four NAD:arginine mono-ADP-ribosyltransferases (A, B, C, and A′) were purified from turkey erythrocytes. Transferase A (58, 61), a 28-kDa protein

from erythrocyte cytosol, modified arginine and other simple guanidino compounds. Enzyme activity was stimulated by 200 mM NaCl (56), histones (20 mg/ml) (57), and, to a lesser extent, phosphatidylcholine (53). Nonionic detergents, such as Triton X-100 or Tween 20, and the zwitterionic detergent 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) enhanced transferase activity, although to a lesser extent than did NaCl, histones, or phosphatidylcholine (53). Transferase B (32 kDa) is a cytosolic enzyme that was not activated by histones and was inhibited approximately 40% by chaotropic salts (106). Transferases C and A′ were partially purified from the particulate fraction of erythrocytes (99). Transferase C, a 26-kDa protein from the plasma membrane, was unaffected by salt and histones. Transferase A′, a 25-kDa enzyme, was distinguished from transferase A in its chromatographic behavior and its localization to the nuclear fraction. Transferase A′, similar to transferase A, was stimulated by salt and histones. In contrast to the nuclear poly(ADP-ribose) polymerase, transferase A′ was not activated by DNA and used simple guanidino compounds as ADP-ribose acceptors (99).

The in vitro ADP-ribosylation of several protein substrates by turkey transferase A resulted in alteration of their functions. A critical arginine in glutamine synthetases from ovine brain (64) and *E. coli* (54) was ADP-ribosylated by transferase A, resulting in inhibition of synthetase activity. Likewise, modification of Ha-*ras*, the 20-kDa protooncogene product, by transferase A partially inhibited GTP binding and GTPase activity (91). ADP-ribosylation of the α and β polypeptide chains of chicken erythrocyte tubulin by transferase A resulted in the inhibition of microtubule assembly (74). Furthermore, incubation of bovine brain extracts with turkey transferase A similarly resulted in the ADP-ribosylation of tubulin and the high-molecular-weight microtubule-associated proteins (80). Modification of bovine brain tubulin inhibited microtubule assembly, and ADP-ribosylation of assembled microtubules resulted in microtubule depolymerization. Skeletal muscle α-actin was ADP-ribosylated on arginine-95 and arginine-372 by transferase A (31). Although both monomeric G-actin and polymerized F-actin were ADP-ribosylated by the avian enzyme, modification of G-actin merely slowed monomer polymerization and did not affect the extent of F-actin formation. Actin-catalyzed ATPase activity was likewise unaffected by transferase A–catalyzed ADP-ribosylation. In contrast, modification of arginine-177 of G-actin by *Clostridium perfringens* iota toxin (1) resulted in the inhibition of actin ATPase activity and formation of an F-actin capping protein, which prevented polymerization.

Chicken ADP-Ribosyltransferases

A chicken ADP-ribosyltransferase ($\sim$28 kDa) was isolated from hen liver and subsequently from chicken heterophil granules (50, 94); a second 28-kDa

isoform from heterophils was separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (105). The ~28-kDa transferase utilized histones, casein, protamine, and simple guanidino compounds (e.g. arginine methyl ester, agmatine) as ADP-ribose acceptors. The chicken transferase, unlike turkey transferase A, was inhibited by NaCl and lysophosphatidylcholine and stimulated by sulfhydryl reagents such as β-mercaptoethanol and by polyanions such as double-stranded DNA, RNA, or poly(L-glutamate) (50). Triton X-100 and CHAPS had no effect on enzyme activity.

Using degenerate primers based on amino acid sequences of proteolytic fragments of the purified chicken transferase, two distinct transferases (AT1 and AT2) were cloned from a chicken bone marrow cDNA library (92). AT1 and AT2 had open reading frames of 312 amino acids that were 78% identical. When the coding region of AT1 was expressed in COS-7 cells, transferase activity was detected in the culture medium. AT1 activity was thiol dependent, with optimal activation in the presence of β-mercaptoethanol, and NaCl inhibited by approximately 70%. In COS-7 cells transformed with the AT2 cDNA, transferase activity was detected in both culture medium and cell lysates. AT2 activity did not require thiol but was enhanced 100% by β-mercaptoethanol and further increased ~80% by the addition of 200 mM NaCl (92).

Another ADP-ribosyltransferase cDNA cloned from a chicken erythroblast library encoded a 300–amino acid protein (15) with a deduced sequence 50–52% identical to those of the two heterophil transferases. ADP-ribosyltransferase activities were also demonstrated in chicken spleen membranes (67, 93). One enzyme catalyzed the GTP-dependent ADP-ribosylation of $G\alpha_s$, resulting in increased adenylyl cyclase activity (67). Another transferase was a glycosylphosphatidylinositol (GPI)-anchored protein with an activity that was independent of both thiol and NaCl (93), thus differing from AT1 and AT2.

Several in vitro substrates of the heterophil transferase were identified; in many cases ADP-ribosylation altered substrate function. ADP-ribosylation of arginine-34 of histone H1 by the heterophil transferase inhibited subsequent phosphorylation of serine-38 by cAMP-dependent protein kinase (94). Likewise, ADP-ribosylation of critical arginines in L-type pyruvate kinase (94) and in the α and β subunits of phosphorylase kinase (94) prevented cAMP-dependent phosphorylation and subsequent modulation of kinase activities. The 33-kDa heterophil granule protein (p33), similar in amino acid sequence to the *myb*-induced myeloid protein-1 *mim-1,* was another substrate of the heterophil transferase (50, 104). Effects of ADP-ribosylation on p33 or on granule function, however, have not been determined. Arginine-28 and arginine-206 of G-actin and arginine-28 of F-actin were ADP-ribosylated by the heterophil transferase (89). Modification of arginine-206, which is located on the end of the actin molecule near a minor contact point between actin and DNase I,

inhibited actin polymerization and the interaction of G-actin with DNase I. In contrast, modification of arginine-28, which is located on the outer surface of actin, did not interfere with the association of G-actin with DNase I. ADP-ribosylation of actin by the heterophil transferase, unlike that by *C. perfringens* iota toxin (81), did not inhibit actin ATPase activity (89).

MAMMALIAN ADP-RIBOSYLTRANSFERASES

The family of mammalian ADP-ribosyltransferases comprises five enzymes (ART1–5) that share similarities in their deduced amino acid sequences.

ART1

ART1 was extensively purified from rabbit skeletal muscle as a 36-kDa protein (110). ART1 cDNA was cloned from rabbit (110) and human (70) skeletal muscle and mouse lymphoma (Yac-1) cells (68). The human ART1 gene is on chromosome 11p15 (37). Deduced amino acid sequences of the rabbit and human muscle transferases are 81% identical (70), whereas the sequence from mouse lymphoma cells is 75% and 77% identical to those of the rabbit and human muscle enzymes, respectively (68).

ART1 is a GPI-linked exoenzyme (70, 110). Rat mammary adenocarcinoma (NMU) cells transformed with rabbit or mouse ART1 cDNAs demonstrated membrane-associated transferase activity that was released into the medium by phosphatidylinositol-specific phospholipase C (PI-PLC). The transferase from transformed NMU cells and transferases from partially purified rabbit and human skeletal muscle reacted on immunoblot with antibodies that recognize the inositol-1,2-cyclic phosphate moiety, which remains after release of GPI-anchored proteins with PI-PLC (70).

In C2C12 mouse myoblasts, GPI-anchored ART1 activity increased on differentiation to myotubes (108). Incubation of intact C2C12 cells with radiolabeled NAD resulted in ADP-ribosylation of the laminin-binding protein, integrin $\alpha 7$. Modification of integrin $\alpha 7$ did not affect $\alpha 7\beta 1$ heterodimer formation or its association with the cytoskeleton or laminin. ADP-ribose-integrin $\alpha 7$ was a substrate for extracellular phosphodiesterases, which generated phosphoribosyl-integrin and 5′AMP (109). The appearance of ART1 in parallel with integrin $\alpha 7$ during skeletal muscle cell development in vitro and the ADP-ribosylation of integrin $\alpha 7$ suggested a regulatory role for this modification in myogenesis.

Although ART1 is an exoenzyme, after fractionation of skeletal muscle homogenates on sucrose gradients, transferase activity was also found associated with the sarcoplasmic reticulum (84), the cytoplasmic face of the sarcolemma (34), and transverse tubule membranes (34). The muscle-specific intermediate filament protein, desmin, which interconnects and aligns myofibrils at the

Z-line, was ADP-ribosylated in vitro on arginines-48 and -62, presumably by an ADP-ribosyltransferase indigenous to the myotube (27, 28). The arginine-specific modification of desmin was reversed by the rat brain ADP-ribosylarginine hydrolase (107), which removed the ADP-ribose moiety, regenerating free arginine. Moreover, modification of desmin was inhibited by novobiocin, a specific inhibitor of mono-ADP-ribosyltransferases (4), or by the alternative substrate meta-iodobenzylguanidine (28). ADP-ribosylation of desmin inhibited filament formation, and complete removal of ADP-ribose was required for restoration of filament-forming capability (107).

ADP-ribosyltransferase activity with properties similar to those of the cloned ART1 was detected in mouse cytotoxic T lymphocytes (CTL) (98). Incubation of CTL with 10 μM NAD resulted in the ADP-ribosylation of several membrane proteins and the inhibition of CTL proliferation; 100 μM NAD partially suppressed cytotoxic activity. A 35-kDa protein with ADP-ribosyltransferase activity was released from the membrane by incubation of intact CTL with PI-PLC, which resulted in the partial loss of the inhibitory effect of NAD on CTL proliferation and totally eliminated the suppressive effect of NAD on cytotoxicity (98). Modification by the GPI-linked lymphocyte transferase of a 40-kDa membrane protein (p40), that complexes with the tyrosine kinase $p56^{lck}$ resulted in the inhibition of $p56^{lck}$ action (97). The relationship between inhibition of $p56^{lck}$ following ADP-ribosylation of p40 and the inhibition of CTL proliferation has not been determined. In other experiments, ART1 of CTL modified arginines in the extracellular domain of the lymphocyte function–associated molecule-1, an adhesion molecule (65). ADP-ribosylation inhibited lymphocyte function–associated molecule-1–mediated generation of inositol phosphates and suppressed homotypic cell adhesion. As in C2C12 cells, 5′AMP was removed from the modified lymphocyte function-associated molecule-1 by extracellular phosphodiesterases.

ADP-ribosyltransferase activity was detected on the surface of human neutrophils, and the partial sequence of the cloned neutrophil transferase cDNA was identical to that encoding the human muscle transferase (3). The GPI-anchored neutrophil enzyme utilized the synthetic guanidino compound diethylamino(benzylidineamino)guanidine as a substrate. Analysis of chemotaxis of intact neutrophils and actin polymerization in permeabilized cells in response to N-formyl-peptide (fMLP), with or without inhibitors of mono-ADP-ribosyltransferases (e.g. novobiocin, vitamin K_1, vitamin K_3, nicotinamide) (4) or the alternative substrate diethylamino(benzylidineamino)guanidine, demonstrated close correlation between the concentrations causing 50% inhibition (IC_{50}) of ADP-ribosyltransferase activity, neutrophil chemotaxis, and actin polymerization (2). The ADP-ribosyltransferase inhibitors did not, however, affect N-formyl-peptide- or platelet activating- factor–induced increases in

intracellular Ca^{2+} concentration. In other studies, ADP-ribosylation of neutrophil β/γ-actin was enhanced by NO (12). The NO-induced modification of actin resulted in a transient inhibition of actin polymerization and adherence to fibronectin-coated surfaces.

ART2

The ART1 enzymes have significant amino acid sequence identity to the RT6 (ART2) family of rodent T cell alloantigens (86, 110). In rats, $RT6^a$ and $RT6^b$ are alleles of a single gene that encodes the corresponding alloantigens RT6.1 and RT6.2 (36). The RT6 proteins appear only in postthymic lymphocytes and are restricted to peripheral T cells and intestinal intraepithelial lymphocytes. The variably glycosylated RT6.1 and nonglycosylated RT6.2 are 25–30-kDa GPI-anchored proteins that differ by only 10 amino acids. In mice, there are two functional copies of the RT6 gene (*Rt6-1* and *Rt6-2*) located on chromosome 7 that are closely linked to the *H1* minor histocompatibility locus. The deduced amino acid sequences of mouse Rt6-1 and Rt6-2 are 79% identical, whereas those of mouse Rt6-1 and rat RT6.2 proteins are 71% identical. In humans and chimpanzees, the ART2 gene contains three premature stop codons corresponding to amino acids 47, 141, and 193 of the rat RT6 protein. The human ART2 pseudogene is on chromosome 11q13 (36).

In diabetes-prone bio-breeding/Worcester rats, the absence of $RT6^+$ T cells is associated with a profound lymphopenia and the development of an autoimmune-mediated diabetes (23). Diabetes can be prevented in these rats by transfusion and long-term engraftment of $RT6^+$ lymphocytes; but not of thymocytes or bone marrow cells, which are lacking in $RT6^+$ cells (8). In contrast, diabetes-resistant bio-breeding/Worcester rats, depleted of $RT6^+$ T cells following infusion of anti-RT6 monoclonal antibody, develop an increased incidence of diabetes (24). Nonobese diabetic mice have relatively low levels of Rt6-specific mRNA and are prone to develop an immune-mediated diabetes (36). Likewise, in (NZB $\times$ NZW) F1 hybrid and BSXB mice, low levels of Rt6 mRNA are associated with an autoimmune lupus-like glomerulonephritis (36).

NMU cells transformed with RT6.2 cDNA synthesized a GPI-linked NAD glycohydrolase (86). NAD glycohydrolase activity from EpSM30 and EpD3 lymphocytes, which express RT6.1 and RT6.2, respectively, was immunoprecipitated after release of the RT6 proteins from the membrane with PI-PLC (25). Both RT6.2 and RT6.1 were capable of auto-ADP-ribosylation (25, 44). Cross-linking of RT6.2 in intact lymphocytes with anti-RT6.2 antibody-enhanced auto-ADP-ribosylation (77). In addition, cross-linking of RT6.2 in the presence of phorbol ester and recombinant interleukin (IL)-2 resulted in the enhanced proliferation of T cells and an increase in expression of the α subunit of IL-2 receptor (76). Furthermore, members of the *src* tyrosine kinase family, $p60^{fyn}$

and p56^{lck}, coimmunoprecipitated with anti-RT6 antibodies (76). This data is similar to that obtained from mouse CTL (97), and therefore is consistent with the notion that GPI-anchored ART proteins affect immune function through a common signaling mechanism.

Mouse Rt6-1, synthesized in insect cells by use of the baculovirus system, possessed auto-ADP-ribosyltransferase activity and catalyzed ADP-ribosylation of added proteins, for example of histones (77). As free ADP-ribose did not inhibit the ADP-ribosylation of histones, this was apparently an enzymatic modification and not a nonenzymatic reaction of ADP-ribose generated by hydrolysis of NAD by a NAD glycohydrolase. Mouse Rt6-1 protein, expressed in NMU as well as in insect cells, used agmatine as an ADP-ribose acceptor (59). Unlike the rat RT6 proteins, the mouse Rt6-1 was primarily an ADP-ribosyltransferase that demonstrates a relatively low level of NAD glycohydrolase activity (38, 59). The determination of the precise role of ART2 enzymes in immune modulation is an area of active investigation.

ART3 and ART4

ART3 and ART4 cDNAs were recently cloned from human testis (40) and spleen (35), respectively. The deduced amino acid sequences of ART3 and ART4 are 14% and 31% identical, respectively, to that of the human ART1. The ART3 gene was localized to chromosome 4q13-q21 (40) and ART4 to chromosome 12q13.2-q13.3 (35). On Northern RNA blotting analysis, a 1.8-kb poly(A)$^+$ RNA from human skeletal muscle and testis and a weaker 1.6-kb poly(A)$^+$ RNA from heart hybridized with an ART3 cDNA probe (40). An ART4 probe hybridized with 1.4-, 2.4-, and 5.5-kb poly(A)$^+$ RNAs from spleen, ovary, and intestine (35). The hydrophobicity profiles of the deduced amino acid sequences of ART3 and ART4 contain hydrophobic amino and carboxy termini, consistent with posttranslational processing of the nascent protein and, perhaps, the addition of a GPI-anchor, similar to that observed in ART1. Enzymatic activities of ART3 and ART4 have not been determined.

ART5

The ART5 cDNA was cloned from Yac-1 murine lymphoma cells (69). Its deduced amino acid sequence is 32% identical to that of mouse ART1, approximately 30% identical to those of mouse ART2, and 29% and 25% identical to the human ART3 and ART4 proteins, respectively. Unlike ART1, ART5 had significantly more NAD glycohydrolase than ADP-ribosyltransferase activity; although it catalyzed auto-ADP-ribosylation, ADP-ribosylation of other proteins substrates was relatively poor. On Northern blot analysis, a labeled ART5 cDNA probe hybridized with 1.6- and 2.0-kb bands in poly(A)$^+$ RNA from testis, where it was most abundant, and with a 1.6-kb poly(A)$^+$ RNA from cardiac and skeletal muscle. In NMU cells transformed with the ART5 cDNA,

transferase and NAD glycohydrolase activities were membrane associated but apparently not GPI anchored (69).

Other Mammalian ADP-Ribosyltransferases

Four distinct 66-kDa ADP-ribosyltransferases (I–IV) were purified from rat brain (46). Phospholipids and the soluble ADP-ribosylation factors (sARF) I and II, 20-kDa GTP-binding proteins from bovine brain involved in vesicular trafficking, had differing effects on the activities of the four enzymes. All were inhibited by ADP-ribose and ADP but not nicotinamide. Nonmuscle β/γ-actin and smooth muscle γ-actin were ADP-ribosylated in vitro by all four transferases, whereas skeletal muscle α-actin was a substrate only for transferases I and IV. In addition, transferases I, II, and IV ADP-ribosylated $G\alpha_s$; transferases I, III, and IV modified $G\alpha_o$ (46).

Four 69-kDa ADP-ribosyltransferases with distinct chromatographic properties were purified from rat adrenal medulla (22). Similar to the purified brain transferases, the adrenal enzymes ADP-ribosylated an arginine in β/γ-actin but, unlike the brain transferases, all four enzymes from the adrenal gland modified γ-actin, $G\alpha_s$, $G\alpha_i$, and $G\alpha_o$. The small differences in estimated molecular weight among transferases from brain and adrenal medulla may depend on tissue specificity or differential processing in the two organs. Other investigators detected mono-ADP-ribosyltransferase activity in the nuclear fraction of rat testis from 3-week-old rats, where histones H2b and H3 served as ADP-ribose acceptors (39). Treatment of the rats with testosterone, luteinizing hormone, or follicle-stimulating hormone dramatically reduced the in vitro [^{32}P]ADP-ribosylation of nuclear histones. This transferase activity appears distinct from that of ART5, based on its localization to the nuclear fraction of testis.

Incubation of rat brain homogenates with radiolabeled NAD and 5′-guanylylimidodiphosphate resulted in the ADP-ribosylation of $G\alpha_s$, ARF, and an unidentified 50-kDa protein (21). Addition of LiCl (0.3–1 mM) to extracts of rat frontal cortex inhibited ADP-ribosylation of $G\alpha_s$ and GAP-43, a 43-kDa growth-associated protein (66), even in the presence of sodium nitroprusside, an NO donor that stimulated endogenous ADP-ribosylation (21). Administration of Li^+ to rats for 6 days, however, had no effect on endogenous ADP-ribosylation, despite therapeutic serum Li^+ levels; whereas chronic (4 weeks) Li^+ administration increased levels of ADP-ribosylation in the frontal cortex (66). The functional correlates of this effect of Li^+ on ADP-ribosylation of brain proteins are unknown.

ADP-ribosyltransferase activity was detected in compact myelin and other myelin-containing white matter fractions from human brain (7). Arginine-9 and arginine-54 of the 20-kDa GTP-binding myelin basic protein (MBP) were modified by the myelin transferase. Moreover, GTPγS caused a concentration-

dependent increase in ADP-ribosylation of MBP. ADP-ribosylation of MBP, its binding to GTP, and the demonstration that MBP stimulates PI-PLC purified from human myelin led to the suggestion that MBP may be a guanine nucleotide-binding protein involved in signal transduction (7).

Other brain proteins, for example MARCKS, an 80- to 90-kDa myristoylated, alanine-rich, protein kinase C substrate protein (10), and the GAP-43-related protein neurogranin (13), are substrates of endogenous ADP-ribosyltransferases. In addition, arginine and cysteine linked to cyanogen bromide-activated Sepharose beads were ADP-ribosylated in the presence of rat hippocampal homogenates (82). Furthermore, incubation of hippocampal slices with vitamin K_1 and nicotinamide, inhibitors of mono-ADP-ribosyltransferases (4), blocked long-term potentiation without affecting basal excitatory synaptic transmission, inhibitory postsynaptic potential, or N-methyl-D-aspartate (NMDA) receptor-mediated transmission (82).

ADP-ribosyltransferase activity has been detected in other cell systems. Examples include the endogenous ADP-ribosylation of $G\alpha_s$, with resulting stimulation of adenylyl cyclase activity in cardiac muscle membranes, platelets, and NG108-15 cells (50a, 20, 73). ADP-ribosyltransferases from rat basophilic leukemia and Fischer rat thyroid line 5 cells modified glyceraldehyde-3-phosphate dehydrogenase and BARS-50, a novel heterotrimeric GTP-binding protein that is a substrate for brrefeldin A (BFA)-stimulated ADP-ribosylation (16). BFA inhibits constitutive protein secretion and disrupts the structure and function of organelles involved in protein trafficking (33, 71). ADP-ribosylation of BARS-50 was correlated with BFA-induced disassembly of the Golgi. In these experiments, the ADP-ribose linkage was sensitive to HCl and NaOH but not $HgCl_2$ or NH_2OH, which does not correspond to sensitivities described for products of known arginine-, cysteine-, and asparagine-specific transferases.

INHIBITORS OF MONO-ADP-RIBOSYLTRANSFERASES

In general, three enzymes, poly(ADP-ribose) polymerase, mono-ADP-ribosyltransferase, and NAD glycohydrolase, appear to be affected by a common group of inhibitors (4). Some compounds are more selective in their inhibition of one or more of these enzymes. Inhibitors of mono-ADP-ribosyltransferase and poly(ADP-ribose) polymerase activities are shown in Table 1. The effective concentration of some of the inhibitors that preferentially block poly(ADP-ribose) polymerase activity is two or more orders of magnitude lower than that required to block mono-ADP-ribosyltransferase activity. For example, the IC_{50} concentration that inhibited polymerase activity for benzamide and its derivatives was 3–6 μM, whereas the IC_{50} for turkey transferase A was 2700–4100 μM (75). Similarly, 5′-bromodeoxyuridine had IC_{50} values of 15 and 590 μM for the polymerase and transferase A, respectively. These differences

Table 1 Concentrations of compounds that cause 50% inhibition (IC_{50}) of mono-ADP-ribosyltransferases and poly(ADP-ribose) polymerase[a]

Compound	IC_{50} transferase (μM)	IC_{50} polymerase (μM)
5′-Bromodeoxyuridine	590 ± 94	15 ± 1.3
Thymidine	1900 ± 300	43 ± 5.2
3-Methoxybenzamide	2700 ± 250	3.4 ± 0.31
Theophylline	2800 ± 460	46 ± 15
3-Aminobenzamide	3000 ± 970	5.4 ± 0.40
Nicotinamide	3400 ± 410	43 ± 5.2
Benzamide	4100 ± 220	3.3 ± 0.28
Arachidic acid (C20:0)	4.4	>1000
Stearic acid (C18:0)	6.1	>1000
Palmitic acid (C16:0)	16	>1000
Arachidonic acid (C20:4)	66	44
Linoleic acid (C18:2)	90	48
Linolenic acid (C18:3)	110	110
Palmitoleic acid (C16:1)	200	95
Vitamin K_1	1.9	520
Vitamin $K_{2(20)}$	13	ND
Novobiocin	280	2200

[a]ND, Not determined. (From References 4, 75.)

may be useful in differentiating pathways involving poly(ADP-ribosyl)ation from those dependent on mono(ADP-ribosyl)ation.

Vitamins K_1 (phylloquinone) and $K_{2(20)}$ (menaquinone) and the saturated long-chain fatty acids arachidic, stearic, and palmitic acids were specific inhibitors of mono-ADP-ribosyltransferase activity, with IC_{50} values between 1.9 and 16 μM (4). The unsaturated long-chain fatty acids, on the other hand, did not differentially inhibit the heterophil transferase and the poly(ADP-ribose) polymerase. Novobiocin (Table 1) and the flavanoid sylbin (19) are two other natural compounds that specifically inhibit the transferase. These studies were conducted with a limited number of ADP-ribosylating enzymes, and effects of inhibitors on other members of these families may well differ (e.g. IC_{50} specificity). Nonhydrolyzable substrates [e.g. meta-iodobenzylguanidine and diethylamino(benzylidineamino)guanidine)] for mono-ADP-ribosyltransferases have been shown to inhibit ADP-ribose transfer reaction in a competitive fashion.

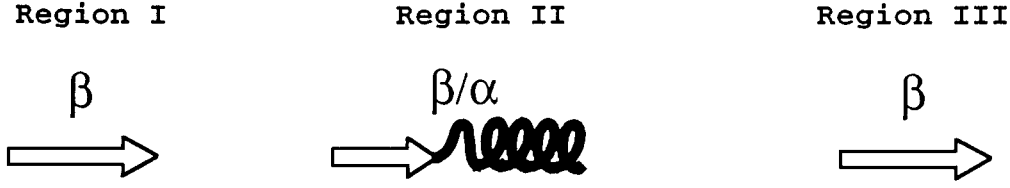
CONSERVED REGIONS AMONG ADP-RIBOSYLTRANSFERASES

Despite a lack of overall identity of deduced amino acid sequences, the bacterial toxin ADP-ribosyltransferases possess regions of sequence similarity that

appear to form, in part, the catalytic site (18). Based on crystallography and computer modeling, the NAD-binding cleft of the bacterial toxins as well as the eukaryotic transferases appear to be composed of an α-helix bent over a β-strand. An arginine or histidine, and an active-site glutamate, which are critical for enzymatic activity, are located on two β-strands flanking the NAD-binding cavity (18).

Two types of active sites have been proposed for bacterial toxin transferases (18) (Figure 2). In the first group, which includes DT and ETA, the catalytic cleft is formed by a histidine-containing region, an aromatic and hydrophobic amino acid-rich segment, and the region containing the active-site glutamate. In region 1, His21 of DT is important for both NAD binding and ADP-ribose transfer to elongation factor-2. Replacement of His21 with other amino acids, with the exception of Asn, markedly reduced ADP-ribosyltransferase activity (18). The His21 to Asn mutation, however, resulted in retention of NAD binding and ADP-ribosyltransferase activity, which is consistent with the hypothesis that the π-nitrogen of the imidazole group of histidine is important in orienting NAD in the catalytic site by hydrogen bonding to the carboxamide group of nicotinamide. Based on the crystal structure of DT bound to NAD, His21 appears to maintain the integrity of the catalytic pocket by forming an additional hydrogen bond with Tyr54, the side chain of which is involved in an aromatic stacking interaction with the nicotinamide ring of NAD (5). In ETA, replacement of His440, which is analogous to His21 of DT (18), with Ala, Phe, or Asn dramatically reduced ADP-ribosyltransferase, but not NAD glycohydrolase, activity or NAD binding. The crystal structure of ETA complexed with a less-hydrolyzable NAD analogue, β-methylene-thiazole-4-carboxamide adenine dinucleotide, revealed that the side chain of His440 interacts with the adenine ribose and also with Tyr470, an amino acid involved in van der Waals interactions with the thiazole-ribose moiety of β-methylene-thiazole-4-

Figure 2 Regions of similarity among ADP-ribosyltransferases. Bacterial toxin ADP-ribosyltransferases were grouped according to three-dimensional structure (18). In the group exemplified by heat-labile enterotoxin of *Escherichia coli* (LT), region 1 contains a critical arginine (*asterisk*) on a β-strand, region 2 contains an aromatic amino acid-hydrophobic amino acid-Ser(*asterisk*)-X-Ser-hydrophobic amino acid motif, and region 3 contains the active-site glutamate (*asterisk*) on a β-strand. In the group exemplified by diptheria toxin (DT), region 1 contains a histidine (*asterisk*) on a β-strand; region 2 contains a Tyr-X_{10}-Tyr motif within a hydrophobic-rich segment of amino acids; and region 3 possesses the catalytic glutamate (*asterisk*) on a β-strand. Amino acid position is indicated to the *right* of each amino acid segment. CT, cholera toxin; PT, pertussis toxin; iota, *Clostridium perfringens* iota toxin; ETA, *Pseudomonas aeruginosa* exotoxin A; rART1, rabbit ART1; mRt6-1, mouse Rt6-1; RT6.2, rat RT6.2; hART3, human ART3; hART4, human ART4; mART5, mouse ART5; hPARP, human poly(ADP-ribose) polymerase; chAT1, AT1 from chicken heterophils.



LT	1	NGDKLYRADS	58	GYVSTSLSLR	107	HPYEQEVSAL
CT	1	NDDKLYRADS	58	GYVSTSISLR	107	HPDEQEVSAL
PT	3	PPATVYRYDS	49	AFVSTSSSRR	124	ATYQSEYLAH
iota	289	SNLIVYRRSG	335	NFISTSIGSV	375	YAGEYEVLLN
rART1	173	RCRQVFRGVH	199	GFASASLKNV	235	FPGEEEVLIP
mRt6-1	140	GCRSVYRGTN	164	HFASSSLNRS	204	YTHEEEVLIP
RT6.2	140	DCHSVYRGTK	164	QFTSSSLSKK	204	RPDQEEVLIP
hART4	136	CYEVHYRTKD	160	QFLSTSLLKE	193	FSLKKEVLIP
mART5	155	PGEVVFRGVG	181	QFTSSSVDER	217	FPEEREVLIP
chAT1	158	RCYYVYRGVR	183	QFTSTSLRKE	219	FPSEDEVLIP
DT	15	ENFSSYHGTK	50	WKGFYSTDNKYDAAGY	145	SSVEYINNWE
ETA	434	YVFVGYHGTF	466	WRGFYIAGDPALAYGY	550	GRLETILGWP
hART3	88	MNFKDNHGIA	126	REDYIYGFQFKAFHFY	242	KESERITLIP
hPARP	856	NRRLLWHGSR	89	GKGIYFADMVSKSANY	985	LYNEYIVYDI

carboxamide adenine dinucleotide (41). In this model, His440 is involved in the binding of NAD, but it appears too distant from the N-glycosidic bond of ribose to affect ADP-ribose transfer directly.

The hydrophobic amino acids in region 2, postulated to be involved in hydrophobic interactions with the nicotinamide and adenine rings of NAD, appear to be conserved in DT and ETA: Trp50, Phe53, Tyr54, and Tyr65 of DT correspond to Trp466, Phe469, Tyr470, and Tyr481 of ETA (18). Trp50 of DT and Trp466 of ETA, however, have not been observed in crystal structures to make such interactions, although replacement of Trp50 with Ala eliminated NAD binding and markedly reduced (5000-fold) transferase activity (102). Likewise, replacement of Tyr65 of DT with Ala reduced transferase activity 350-fold (6). As replacement of Tyr65 with Phe or Ala did not affect binding of adenosine to DT, it was hypothesized that Tyr65 interacted with the nicotinamide and not with adenine or ADP-ribose moieties (9). The three-dimensional structures of the hydrophobic region of DT and ETA revealed that a Tyr on a β-strand is separated from a second tyrosine on an α-helix by 10 amino acids (5, 18).

Photoaffinity labeling, site-directed mutagenesis, and crystallography have identified a region 3 active-site glutamate that is critical for enzyme activity. Replacement of Glu148 and Glu553 in DT and ETA, respectively, inhibited ADP-ribosylation without affecting NAD binding (5). The carboxylate group of Glu148 of DT virtually superimposes on Glu553 of ETA and is in close proximity to the N-glycosidic bond of NAD (5). Photoaffinity labeling of DT with NAD resulted in lysis of the nicotinamide-ADP-ribose bond of NAD, decarboxylation of Glu148, and formation of a new bond between the γ-methylene carbon of Glu148 and carbon-6 of the nicotinamide ring (9). A similar photoproduct was generated with ETA. Replacement of Glu553 with Gln dramatically reduced ADP-ribosyltransferase activity, consistent with the notion that the carboxylate group of the active-site Glu is critical for catalysis (103).

In another group of bacterial toxins exemplified by CT, LT, and PT, region 1 contains an Arg located on a β-strand, region 2 is identified by the consensus sequence aromatic residue-X-Ser-Thr-Ser-hydrophobic residue that is positioned on the floor of the catalytic cleft, and region 3 contains the active-site Glu on a β-strand (18).

Based on the crystal structure of LT (83), Arg7 in region 1 could potentially bind to the adenine or phosphate portions of NAD. Arg7 also forms a hydrogen bond with the backbone carbonyl of Ser61, located in region 2. Replacement of Arg7 by Lys in LT and CT abolished transferase activity (18), whereas replacement of Ser61 with Thr minimally reduced ADP-ribosyltransferase activity and did not affect NAD binding (11). Ser61 apparently maintains proper conformation of the active site of LT and does not play an essential role in catalysis.

Likewise, in the crystal structure of PT, the side chain of Arg9 extended into the active site and formed a hydrogen bond with Ser52, maintaining proper conformation of the catalytic cleft, whereas mutation of Arg9 to Lys resulted in the complete loss of activity (18).

In region 3, the carboxylate group of Glu112 of LT and of CT superimposes on that of Glu148 of DT and Glu553 of ETA and is in close proximity to the N-glycosidic bond of NAD (5). Substituting Asp for Glu112 of LT completely inhibited enzymatic activity but had little effect on binding of NAD or agmatine (11). On the other hand, a Glu110-to-Asp mutation reduced transferase activity by a factor of approximately 20, consistent with the hypothesis that Glu112 may be more critical than Glu110 for catalytic activity. Similar properties were demonstrated for Glu129 of PT (18). Replacement of Glu129 of PT with Asp inhibited NAD glycohydrolase activity with minimal effect on NAD binding. In *C. perfringens* iota toxin, mutation of Arg295, Glu378, or Glu380 dramatically inhibited ADP-ribosyltransferase and cytotoxic activity (72). The Glu-X-Glu sequence at the catalytic site of iota toxin is identical to that of CT and LT (Figure 2).

Other bacterial toxin ADP-ribosyltransferases possess active-site glutamates identified by photoaffinity labeling and site-directed mutagenesis. *Clostridium botulinum* exoenzyme C3 and the related *Clostridium limosum* C3-like toxin ADP-ribosylate an asparagine in GTPases of the Rho family; they contain an active-site Glu173 and Glu174, respectively (18, 38, 87). Glu381 of *P. aeruginosa* exoenzyme S, which has been implicated as a virulene factor in burn and chronic lung infections, is critical for enzymatic activity (42).

The regions of amino acid sequence similarity among bacterial toxin transferases are also apparent in alignments of the vertebrate ADP-ribosyltransferases (38, 87). In computer modeling studies of the mouse ART2, Arg126 on a β-strand (region 1), Ser147 on a β-strand followed by an α-helix (region 2), and the active site Glu184 on a β-strand (region 3) are positioned in the catalytic cleft in a manner similar to that found in the crystal structure of the bacterial toxins: in particular, LT, CT, and PT (18, 38). Based on alignment of deduced amino acid sequences, Arg179 and Arg174 of the rabbit and mouse ART1, respectively, Arg161 of ART5, and Arg164 of the chicken heterophil transferases are conserved and hypothesized to play a role similar to that of Arg7 and Arg9 of LT and PT, respectively (Figure 2). Although His114 of the rabbit ART1 aligns with His21 of DT, its replacement with Asn did not abolish enzymatic activity (87). A recombinant mouse ART1 protein synthesized in *E. coli* in which the amino-terminal 121 amino acids were deleted possessed NAD glycohydrolase, but not ADP-ribosyltransferase, activity (32). The truncated transferase lacks His110 (analogous to His114 of rabbit ART1) but does possess Arg174, which is consistent with the hypothesis that, as in LT and PT,

the conserved Arg may play a role in maintaining active site conformation and NAD binding.

The rabbit ART1 sequence contains three Glu residues at positions 238–240. Replacement of Glu240 with Asp or Ala resulted in the loss of transferase activity; substitution of Asp for Glu239 did not (87). An Asn or Gln replacement of Glu238, however, inactivated the transferase. Glu238 and Glu240 of ART1 were postulated to be analogous to Glu110 and Glu112 of CT and LT. The Glu-rich region of ART1 is aligned with Glu207, Glu208, and Glu209 of mouse ART2 sequences (Rt6-1 and Rt6-2), whereas in the rat ART2 sequences (RT6.1 and RT6.2), Glu207 is replaced by Gln (38). Replacement of Gln207 of the rat ART2 (RT6.1) enzyme, which possesses predominantly NAD glycohydrolase activity, with Glu resulted in the generation of an arginine-specific ADP-ribosyltransferase and increased auto-ADP-ribosyltransferase activity, with little effect on NAD binding or NAD glycohydrolase activity (26, 43). After introduction of a Glu-X-Glu motif, the rat ART2 protein exhibited enzymatic activity, such as mouse ART2 or ART1 transferases.

In the ART5 (Glu220, Arg221, and Glu222) (69) and chicken heterophil (Glu222, Asp223, and Glu224) (92) ADP-ribosyltransferases, a Glu-X-Glu–containing region aligns with the active-site residues of ART1 and ART2 (Figure 2). The deduced amino acid sequences of human poly(ADP-ribose) polymerase (18), and perhaps ART3, appear to have regions of similarity that can be aligned with DT and ETA (Figure 2). Moreover, crystal structure of the chicken PARP (78) and mutagenesis of human PARP (90) demonstrated that Glu988, which is essential for ADP-ribose chain elongation, is positioned in a catalytic groove similar to that found in the bacterial toxins. These data are consistent with the hypothesis that several of the bacterial toxin and vertebrate transferases possess a common mechanism of NAD binding and ADP-ribose transfer and that at least some of the differences observed in three-dimensional structures may reflect differences in substrate proteins.

ADP-RIBOSYLARGININE HYDROLASES

ADP-ribosylarginine hydrolases remove the ADP-ribose moiety from transferase-catalyzed ADP-ribosylated proteins. Hydrolases have been identified in mammalian, avian, and bacterial systems and appear to be ubiquitous in eukaryotic tissues.

Turkey Erythrocyte ADP-Ribosylarginine Hydrolase

The turkey erythrocyte hydrolase is a soluble, 39-kDa monomeric protein (60). The ADP-ribose, but not the arginine moiety, was critical for substrate recognition and degradation by the hydrolase (52). Hydrolase activity was

competitively inhibited by ADP-ribose > ADP > AMP; arginine, agmatine, and guanidine had no effect. Arginine, generated by the hydrolase-catalyzed degradation of ADP-ribosylarginine, served as substrate for the purified turkey ADP-ribosyltransferase (51), demonstrating preservation of the guanidino moiety during hydrolysis. Furthermore, the turkey ADP-ribosyltransferase catalyzes the synthesis of the α-anomer of ADP-ribosylarginine, whereas the hydrolase utilizes α-ADP-ribosylarginine but not the β-anomer as substrate, demonstrating that the stereospecificity of the hydrolase matches that of the turkey ADP-ribosyltransferase (52). This finding is compatible with the hypothesis that the ADP-ribosyltransferases and ADP-ribosylarginine hydrolases act as opposing arms of an ADP-ribosylation cycle (101).

Mammalian ADP-Ribosylarginine Hydrolases

An ADP-ribosylarginine hydrolase was purified ~20,000-fold from rat brain (55). The hydrolase cDNA cloned from a rat brain cDNA library contains an open reading frame of 1089 bp (55). The rat brain hydrolase expressed as a glutathione S-transferase–linked fusion protein in *E. coli* had Mg^{2+}- and dithiothreitol-dependent hydrolase activity. A hydrolase-specific oligonucleotide probe hybridized on Northern blot with a 1.7-kb mRNA in total RNA from all rat tissues (55).

The mouse hydrolase cDNA, cloned by polymerase chain reaction (PCR) with oligonucleotide primers generated from the rat brain hydrolase cDNA, was 92% and 94% identical in nucleotide and deduced amino acid sequences, respectively, to those of the rat (85). On the other hand, nucleotide and deduced amino acid sequences of the human and rat hydrolases were 82% and 83% identical, respectively (85). In the rat and mouse hydrolases, the positions of five cysteines were identical, whereas in the human hydrolase, only four of the five cysteines were present (85). Cysteine 108 in the rat and mouse enzymes was replaced by a serine at position 103 in the human sequence. As the rat, but not the human, hydrolase was activated by dithiothreitol, site-directed mutagenesis of the rat and human hydrolases was utilized to determine whether this difference was related to the fifth cysteine (85). A mutant human hydrolase expressed in *E. coli* in which cysteine was substituted for serine at position 103 (Ser103Cys) produced a thiol-dependent enzyme similar to the native rat hydrolase. Wild-type recombinant human hydrolase, however, retained its thiol independence. On the other hand, the mutated rat hydrolase that contained a serine at position 108 instead of cysteine (Cys108Ser) demonstrated thiol independence, in contrast to the similarly expressed thiol-dependent, wild-type rat hydrolase (85).

Other experiments utilizing recombinant ADP-ribosylargininehydrolases demonstrated the reversibility of arginine-specific ADP-ribosylation. A rat

ADP-ribosylarginine hydrolase produced in *E. coli* released the ADP-ribose moiety from $G\alpha_s$ that had been ADP-ribosylated by cholera toxin; it also released the ADP-ribose moiety from the auto-ADP-ribosylated A_1 subunit of cholera toxin (45). Nonmuscle actin ADP-ribosylated by botulinum C2 toxin (96) also served as a substrate for the hydrolase. $G\alpha_o$ ADP-ribosylated by PT, DT-catalyzed modification of EF-2, and the GTP-binding protein rho modified by C3 exoenzyme, on the other hand, were not affected by the recombinant hydrolase (45), consistent with the specificity of the hydrolase for the ADP-ribose-arginine bond.

SUMMARY

Eukaryotic ADP-ribosyltransferase activity has been detected in diverse tissues, including turkey erythrocytes, rabbit skeletal muscle, and human testis. These enzymes possess structural features that are similar to those of several of the bacterial toxin transferases, which suggests a common mechanism of substrate binding and enzyme catalysis. Data from several investigators demonstrate that ADP-ribosylation in eukaryotic systems can alter cellular metabolism. A regulatory ADP-ribosylation cycle in eukaryotic cells (Figure 1) has been proposed (101) based on the presence of NAD:arginine ADP-ribosyltransferases and ADP-ribosylarginine hydrolases, which remove ADP-ribose, regenerating free arginine (protein). For the mammalian ART proteins that are GPI-anchored exoenzymes, however, processing of the modified arginine by extracellular phosphodiesterases followed by phosphatases generates phosphoribosylarginine and ribosylarginine, respectively, with no evidence that the original protein structure is restored by an ADP-ribosylarginine hydrolase-like activity.

The functional effects of ADP-ribosylation appear to differ depending on the tissue in which the ART proteins are expressed. In human neutrophils, ADP-ribosylation of actin was associated with the inhibition of neutrophil chemotaxis. The transferases from turkey erythrocytes and chicken heterophils also modify actin and may similarly influence cell function. The fact that integrins in muscle cells and CTL are ADP-ribosylated is consistent with the idea that this modification may affect cell-cell or cell-matrix interactions. In addition, T cell proliferation appears to be modulated by GPI-anchored ART1 and ART2 proteins, perhaps through their interaction with specific *src* tyrosine kinases. There are also numerous examples of in vitro ADP-ribosylation of G-proteins, particularly by transferases from brain, providing exciting implications for neuronal signal transduction.

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