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COMMENTARY

ENDOGENOUS SUBSTRATES AND FUNCTIONAL ROLE OF EUKARYOTIC MONO(ADP-RIBOSYL)TRANSFERASES

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ADP-ribosyltransferase

ADP-ribosyltransferases are implicated widely in the post-translational processing of many proteins [1, 2]. Two quite separate classes of ADP-ribosyltransferases are recognized, namely the mono- and poly(ADP-ribosyl)transerases [also termed poly-(ADP-ribose)-polymerase]. Mono(ADP-ribosyl)-transferases hydrolyse NAD^+ to yield ADP-ribose, which associates through an *S*- or *N*-glycosidic linkage to amino acids contained within polypeptides. The other product of NAD^+ hydrolysis is free nicotinamide, which has significant inhibitory effects on the catalytic activity of mono(ADP-ribosyl)-transferases (see later). In contrast, poly(ADP-ribosyl)transferase activity yields chains of ADP-ribose units linked to each other by *O*-glycosidic linkages. The chain structures are either straight (with a $1'' \rightarrow 2'$ ribose-phosphate-phosphate backbone) or branched ($1''' \rightarrow 2''$ ribose $1'' \rightarrow 2'$ ribose).

The two enzyme activities [mono- and poly(ADP-ribosyl)transferases] can be readily distinguished on the basis of their different sensitivities to a wide range of inhibitors [3, 4]. Furthermore, hydrolysis of the ADP-ribosylated product with snake venom phosphodiesterase yields AMP from mono(ADP-ribosyl)ated proteins, and ADP-ribose units from poly(ADP-ribosyl)ated products [5].

Mono(ADP-ribosyl)transferase

Numerous bacteria including *Vibrio cholerae*, *Bordetella pertussis*, *Escherichia coli*, *Clostridium botulinum* and many others synthesize and release mono(ADP-ribosyl)transferases as exotoxins, which are implicated in the pathogenicity of these organisms [2]. The prokaryotic enzymes mediate modifications of specific G protein α-subunits, with consequent changes in the activity of the particular signal transduction pathway involved. For example, cholera toxin mono(ADP-ribosyl)ates $G_{s\alpha}$ within the intestinal mucosa, which leads to activation of adenylate cyclase, an increase in intracellular cyclic AMP concentration, and subsequent loss of Cl^- and water into the lumen of the large bowel. Transducin is also a substrate for cholera toxin, whereas pertussis toxin mono(ADP-ribosyl)ates $G_{i\alpha}$ and $G_{o\alpha}$ [1, 2].

The particular amino acid acceptor is specific to the individual prokaryotic isoenzyme, and these include Arg (cholera toxin), Cys (pertussis toxin), diphthamide (diphtheria toxin) and several others including Asp (botulinum C3 toxin) and Lys. These enzyme activities have been exploited widely in pharmacology and biochemistry to identify and elucidate pathways of signal transduction, and also to label particular G protein α-subunits with ^{32}P (derived in this case from $[\alpha^{32}P]NAD^+$).

An important development in this field of research was the identification of eukaryotic isoforms of mono(ADP-ribosyl)transferase [6–8]. Both Arg-[6–9] and Cys-specific [10] mono(ADP-ribosyl)transferases have been identified in animal tissues, and NAD^+: Arg mono(ADP-ribosyl)transferases have been purified from turkey erythrocytes [6–9] and rabbit skeletal muscle [11]. In turkey erythrocytes, there appear to be several different transferases that vary somewhat in their cellular distribution as well as kinetic, regulatory and physical properties. This contrasts with rabbit skeletal muscle mono(ADP-ribosyl)transferase, which is found in sarcoplasmic reticulum and is more restricted in its expression. To date, only one of the various eukaryotic isoenzymes has been cloned, namely the NAD^+: Arg mono(ADP-ribosyl)transferase from rabbit skeletal muscle [11]. The clone was obtained from a cDNA library, and the deduced amino acid sequence reveals a 36,134 Da protein. There was no significant homology between the deduced sequences of the rabbit enzyme and poly(ADP-ribosyl)-transferase or any of the numerous bacterial mono(ADP-ribosyl)transferases that have been cloned.

Hydrophobicity plots of the deduced amino acid sequence of rabbit skeletal muscle mono(ADP-ribosyl)transferase reveal hydrophobic N and C terminal domains. This pattern is common to a number of membrane-bound proteins that are held in the lipid bi-layer by a glycosylphosphatidylinositol anchor [12]. Two potential sites for *N*-linked

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glycosylation were identified, Asn-65 and Asn-253, the latter being the more likely in the C-terminal region of the protein [11]. Subsequent transfection of the cDNA into rat mammary adenocarcinoma cells has permitted a more thorough examination of the enzyme, and a recent preliminary report [13] confirms the presence of a glycosylphosphatidylinositol anchor. Furthermore, the anchor may be hydrolysed by PI-PLC*, which appears to release the enzyme from the plasma membrane [14].

(ADP-ribosyl)-protein hydrolases

Eukaryotic cells express (ADP-ribosyl)-protein hydrolases that serve to hydrolyse ADP-ribosylated proteins. Once again, several isoenzymes have been described with specificity for either ADP-ribosylarginine [15, 16] or ADP-ribosylcysteine [10]. Furthermore, at least one of these enzymes has been shown to be activated by increases in Ca^{2+} concentration [10]. These results suggest the possibility of post-translational cycling of certain proteins through ADP-ribosylated and non-ADP-ribosylated states of activation. The primary sequences of rat, mouse and human ADP-ribosylarginine hydrolases have been published, and they show a high degree of sequence homology [16].

Methods for measurement of mono(ADP-ribosyl)transferase activity

Measurement of the activity of prokaryotic mono(ADP-ribosyl)transferase does not usually present any problems. Catalytic activity is high, and the mono(ADP-ribosyl)ated product (either protein or an appropriate pseudo-substrate) may be readily identified. The same is not always true, however, for the eukaryotic forms of the enzyme. Considering, for example, the Arg-specific mono(ADP-ribosyl)-transferases, enzyme activity may be identified semi-quantitatively by following the mono(ADP-ribosyl)ation of numerous protein substrates (see later) by autoradiography of polyacrylamide gels used to resolve the reaction products after labelling with [α-^{32}P]NAD^+. The recent availability of [^{32}P]NAD^+ with very high specific radioactivity now makes this technique very sensitive, but detailed enzymology is laborious and the method is not precise. Numerous pseudo-substrates, however, have been synthesized, and agmatine [6, 17] or arginine-methyl ester [8, 9] has been most widely exploited in this context. However, there may still be problems with the sensitivity of the method, and the mono(ADP-ribosyl)ation of agmatine is not demonstrated easily in all tissues in which mono(ADP-ribosyl)ated proteins can be visualized autoradiographically.

Arg-specific mono(ADP-ribosyl)transferase activity may also be measured by exploiting (benzylidineamino)guanidines as pseudo-substrates [18]. These molecules absorb light in the UV range, and a simple one-step ion exchange column allows separation of the substrate and the ADP-ribosylated product. The assay is robust, and well adapted for quantification of prokaryotic enzyme activity; it is insufficiently sensitive, however, for measurement of the enzyme(s) in most eukaryotic systems.

The activity of prokaryotic mono(ADP-ribosyl)transferases may also be measured by labelling cells with [^{3}H]adenine, which leads to the metabolic labelling of the cellular pool of NAD^+. Thereafter, prokaryotic enzyme activity may be demonstrated by the labelling of proteins that are separated by SDS–PAGE [19]. In principle, this approach might be adopted to monitor the activity of eukaryotic mono(ADP-ribosyl)transferase, although in our hands the method seems insufficiently sensitive (unpublished results).

In summary, mono(ADP-ribosyl)transferase activity is very low in most eukaryotic cells. Of the available more robust analytical techniques for measurement of the activity of the Arg-specific enzyme, the [^{32}P]ADP-ribosylation of agmatine or arginine-methyl ester seems the most suitable. In many tissues, however, it appears necessary (for reasons of sensitivity) to follow the mono(ADP-ribosyl)ation of protein substrates autoradiographically [Ref. 20 and many others].

Activation of eukaryotic mono(ADP-ribosyl)-transferase

In an effort to elaborate on the physiological role(s) of mono(ADP-ribosyl)transferase, much effort has been directed to an examination of molecules that might serve a role in activating the enzyme. In rat liver, one of the many metabolites of arachidonic acid is 14,15-EET, a metabolite of NADPH-dependent cytochrome P450 monooxygenase activity. In rat liver, there is an unidentified 52 kDa substrate for mono(ADP-ribosyl)transferase, and 14,15-EET has been shown to stimulate its ADP-ribosylation in the presence of GTP [21]. There is also some evidence [20] that iloprost (a stable analogue of prostacylin) may activate mono(ADP-ribosyl)transferase in human platelets (see below), although further work is still required to establish this with certainty.

More recently, brefeldin A has been shown to stimulate mono(ADP-ribosyl)ation of two cytosolic proteins, one of which was identified as glyceraldehyde-3-phosphate dehydrogenase [22]. Brefeldin A is a fungal metabolite that has inhibitory effects on membrane transport systems. From this report, it appeared that there was similar specificity of the brefeldin A binding components mediating inhibition of membrane transport and stimulation of ADP-ribosylation [22]. The relationship of the effects of brefeldin A to ARF activity, and the requirement of GTP for the stimulatory effect of 14,15-EET [21] both suggest that GTP-binding proteins/ARFs may serve a role in modifying the activity of eukaryotic mono(ADP-ribosyl)transferase. More experimental data are required, however, before very firm conclusions can be drawn on this point.

Effect(s) of nitric oxide on mono(ADP-ribosyl)-transferase activity

The identification of eukaryotic mono(ADP-ribosyl)transferases and the subsequent suggestion that G protein α-subunits might be substrates for

* Abbreviations: PI-PLC, phosphatidylinositol-specific phospholipase C; 14,15-EET, 14,15-epoxyeicosatrienoic acid; and ARF, ADP-ribosylation factor.

these enzymes (see below) prompted many groups to examine the possibility that these enzymes might be involved in the regulation of signal transduction. An exciting observation in this context was the demonstration that ^{32}P-labelling of a 39 kDa substrate in the presence of [α-^{32}P]NAD$^+$ was increased dramatically by sodium nitroprusside in homogenates of human platelets, as well as rat brain, liver, intestine, heart and lung [23, 24]. Sodium nitroprusside is an NO donor in solution, but this particular effect of sodium nitroprusside is unrelated to stimulation of soluble guanylate cyclase or the production of cyclic GMP *in vitro* [23]. The primary observation was quickly confirmed by several groups [25–28], and the 39 kDa substrate was identified as glyceraldehyde-3-phosphate dehydrogenase [29–31], which is, of course, an NAD$^+$-dependent enzyme. The labelling of other proteins, including actin, was also reported to be increased by NO [26].

Further examination of the effects of NO on glyceraldehyde-3-phosphate dehydrogenase revealed not only inhibition of the enzyme activity [31], but also that the inhibitory effect was mediated by *S*-nitrosylation [32]. This issue was finally resolved when it was shown that the ^{32}P-labelling of glyceraldehyde-3-phosphate dehydrogenase following exposure to [α^{32}P]NAD$^+$ involved adduct formation with *intact* NAD$^+$, and not ADP-ribosylation at all [33]. The adduct is linked through an NO-dependent thiol intermediate, and may well be consequent upon the altered reactivity of the nitrosylated protein [33].

The formation of NAD–protein adducts in the presence of sodium nitroprusside is easily demonstrated *in vitro*, but there is no evidence at present that the reaction occurs *in vivo*. We have examined the effect(s) of i.v. infusions of sodium nitroprusside at high concentrations in the rat (sufficient to cause a fall in blood pressure). However, there was no change in the subsequent formation of NAD–protein adducts *ex vivo* (neither an increase due to tissue loading of sodium nitroprusside, nor a decrease that might have suggested previous adduct formation during the infusion; Clifford CP, unpublished results). Similarly, we have been unable to demonstrate evidence for an effect of sodium nitroprusside on the formation of NAD–protein adducts in intact NG108-15 cells in culture (Boyd RS, unpublished results).

There is at least one other NAD$^+$-dependent enzyme, namely poly(ADP-ribosyl)transferase, that is also modified by NAD$^+$ to form an NAD–protein adduct in the presence of sodium nitroprusside *in vitro* [34]. This appears as a 110–120 kDa substrate on polyacrylamide gels. Furthermore, thymidine, which is a potent inhibitor of poly(ADP-ribosyl)-transferase activity, inhibits both the catalytic activity of the enzyme and the formation of the adduct. This finding suggests that NO may well lock NAD$^+$ into the catalytic site of the enzyme [33, 34].

Substrates of eukaryotic mono(ADP-ribosyl)-transferase

Most, but not all, experiments that have involved a search for the substrates of eukaryotic mono(ADP-ribosyl)transferase have been performed with purified enzyme, purified substrate or fractured cells *in vitro*. In many instances, the results are quite compelling in terms of the identification of the substrate, and in many cases modification of the function of the target protein has also been demonstrated. However, it is worth recalling that protein kinases A or C may phosphorylate a very wide range of proteins *in vitro* (particularly in the presence of high concentrations of stable cyclic AMP analogues or phorbol ester), but to infer that the same reactions occur *in vivo* (or even in intact cells in culture) may not be appropriate. Similar considerations may well apply to mono(ADP-ribosyl)ation of proteins *in vitro*, and more rigorous experimental strategies will be required to address these questions directly. Nevertheless, there are reports of many different substrates for eukaryotic mono(ADP-ribosyl)transferases, and they appear to separate into relatively few clearly defined classes of protein.

G-proteins. One of the first reports of a substrate for eukaryotic mono(ADP-ribosyl)transferase suggested the mono(ADP-ribosyl)ation of the α-subunit of G_s ($G_{s\alpha}$) in rat adipocytes [35]. Furthermore, these authors showed that sustained stimulation of G_i by adenosine enhanced the mono(ADP-ribosyl)ation of $G_{s\alpha}$, and there was prolonged activation of adenylate cyclase. Other reports at much the same time demonstrated that the enzyme activity is widely distributed and shows ADP-ribosylation of (unidentified) proteins in rat pancreatic islet cells [36] and membranes or synaptosomes from nerve terminals derived from *Torpedo* electric organ [37].

Several groups have now confirmed the mono(ADP-ribosyl)ation of $G_{s\alpha}$ by eukaryotic enzyme. The effect has been reported in whole human platelets [20] and platelet membranes [38], rat cerebral cortical homogenates [39], chicken spleen cell membranes [40] and intact NG108-15 neuronal cells [41]. The mono(ADP-ribosyl)ation of $G_{s\alpha}$ is followed by an increase in adenylate cyclase activity, and short-term exposure of the cells to polyarginine [to inhibit the mono(ADP-ribosyl)ation of $G_{s\alpha}$] reverses the effect [38].

The long-term effects of mono(ADP-ribosyl)-transferase inhibitors are, however, complex. Exposure of NG108-15 cells for 18 hr to nicotinamide [another inhibitor of mono(ADP-ribosyl)transferase] was also accompanied by inhibition of enzyme activity, but there was a parallel increase in the abundance of $G_{s\alpha}$ measured either as cholera toxin substrate or by immunoblotting [41]. Furthermore, basal adenylate cyclase activity was increased by treatment for 18 hr with nicotinamide. The increase in $G_{s\alpha}$ in NG108–15 cells is blocked by cycloheximide, and is accompanied by an increase in the abundance of mRNA encoding $G_{s\alpha}$*. This effect of nicotinamide in NG108-15 cells appears to be restricted to certain cell types, and in a thorough examination of the effects of nicotinamide on human peripheral blood lymphocytes we were unable to detect any change in the activity of G_s-mediated responses (Olmos G,

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unpublished results). The results in NG108-15 cells contrasted with those obtained with human platelets *in vitro*. Exposure of platelets to nicotinamide had no effect on adenylate cyclase activity or the abundance of immuno-reactive $G_{s\alpha}$. There was, however, an increase in the abundance of $G_{s\alpha}$ when measured as cholera toxin substrate*. The simplest interpretation of the effects of nicotinamide in platelets is once again that $G_{s\alpha}$ serves as a natural substrate for mono(ADP-ribosyl)transferase in intact cells, and furthermore that by inhibiting the activity of the endogenous enzyme, more "unlabelled" $G_{s\alpha}$ is available as substrate for cholera toxin.

A similar conclusion was drawn by Lapetina and coworkers [20] when examining the iloprost-dependent desensitization of prostacyclin receptors in platelets. This example of heterologous desensitization is associated with loss of cell surface prostaglandin I_2 receptors and also loss of "direct" $G_{s\alpha}$-dependent responsiveness (e.g. F^--sensitive adenylate cyclase). In these experiments, the abundance of immunoreactive $G_{s\alpha}$ in the plasma membrane remained unaltered [20], although cholera toxin substrate was reduced [20, 42]. Further experiments then suggested that iloprost might itself have a stimulatory effect on mono(ADP-ribosyl)transferase activity [20], although experiments by our own group have not confirmed this important observation (Donnelly LE, unpublished results). Iloprost (and indeed other prostacyclin agonists) has a significant protective effect on platelets *in vitro*, and this may have a confounding effect when measurements are made of any particular function in platelets that have been retained for many hours *in vitro*. The issue of a specific iloprost- (or even cyclic AMP-) dependent activation of mono(ADP-ribosyl)transferase is important, and has still to be resolved finally.

The iloprost-dependent loss of F^--sensitive adenylate cyclase activity in platelets is not inhibited by nicotinamide,* from which we conclude that heterologous desensitization with loss of $G_{s\alpha}$-dependent responses is not a consequence of the mono(ADP-ribosyl)ation of $G_{s\alpha}$ (at least in platelets).

In summary, the best evidence for mono(ADP-ribosyl)ation of a G protein implicates $G_{s\alpha}$. Numerous research groups have now shown independently that $G_{s\alpha}$ may be a substrate for mono(ADP-ribosyl)transferase [20, 38–41], and furthermore have confirmed that modification of $G_{s\alpha}$ may alter adenylate cyclase activity [38, 40]. However, other G proteins have also been identified as substrates for mono(ADP-ribosyl)transferase, including transducin in homogenates of rod outer segment of retina [43] and $G_{i\alpha}$ in human erythrocyte vesicles [44]. The stability of the ADP-ribosyl linkage in (ADP-ribose)-$G_{i\alpha}$ to hydroxylamine suggested a linkage to a Cys residue (cf. pertussis toxin). Furthermore, the capacity of $G_{i\alpha}$ to serve as a substrate for the eukaryotic enzyme was reduced by prior exposure of the vesicles to pertussis toxin [44]. $G_{o\alpha}$ has also been reported to serve as a substrate for a mono(ADP-ribosyl)transferase purified from rat brain [39].

ADP-ribosylation of elongation factor 2, which comprises one of the G protein family, by diphtheria toxin or *Pseudomonas* toxin A inhibits its activity and blocks protein synthesis [1, 2]. A similar eukaryotic enzyme activity has also been demonstrated in intact pyBHK polyoma virus-transformed baby hamster kidney cells [45] and bovine hepatic homogenates [46].

Cytoskeletal proteins. Another major group of substrates for mono(ADP-ribosyl)transferases appears to include several proteins involved in cytoskeletal microfilaments. Non-muscle actin from rat brain was the first of these to be identified as a substrate for mono(ADP-ribosyl)transferase [39]. Thereafter, several actins, including chicken non-muscle β/γ-actin, skeletal muscle α-actin, and smooth muscle α-actin were all identified as substrates [47]. It has also been shown that turkey erythrocyte mono(ADP-ribosyl)transferase will exploit numerous substrates in bovine brain extracts, including both the α and β chains of tubulin, tubulin dimer, MAP-2, and possibly MAP-1 as well [48]. Lastly, B-50/GAP-43 has been identified as a substrate of mono(ADP-ribosyl)transferase in rat brain homogenate [49]. B-50/GAP-43 is a neuronal phosphoprotein associated with growth and regeneration within the nervous system.

Others. The Ca^{2+}-dependent ATPase of rabbit skeletal muscle sarcoplasmic reticulum appears also to be a substrate for mono(ADP-ribosyl)transferase [50]. Furthermore, there is NAD^+-dependent suppression of ATPase activity, which may be reversed by the addition of free arginine. From these results, it was suggested that Ca^{2+} transport in the sarcoplasmic reticulum may be regulated through changes in the rate of ADP-ribosylation of the Ca^{2+}-dependent ATPase [50].

There is evidence for mono(ADP-ribosyl)ation of the 78 kDa glucose-regulated protein in mouse hepatoma cells [51]. It is proposed that this may be involved in the responses to nutritional stress, since starvation of the cultured hepatic cells is followed by increased mono(ADP-ribosyl)ation of the glucose-regulated protein [51]. Finally, other substrates of mono(ADP-ribosyl)transferase have been reported, including p33 [52] and a 22 kDa rho-like protein [53].

Biological effects of mono(ADP-ribosyl)transferases revealed by inhibitors

There are as yet no specific inhibitors of mono(ADP-ribosyl)transferases, and assignment of a biological role to this enzyme activity on the basis of the effects of a single inhibitor may not be secure. At present, the data are rather limited in which it is possible to compare the rank order of potencies for inhibition of the enzyme and inhibition of the particular biological response. Nevertheless, studies on the inhibitory effects of several compounds suggest interesting possibilities for this enzyme in signal transduction.

Meta-iodobenzylguanidine or its precursor guanyltyramine, or nicotinamide all inhibit histamine-induced production of inositol phosphates and

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prostacyclin in endothelial cells [54]. The effects of the inhibitors appeared to show selectivity for responses to histamine, with no alteration in responses mediated by leukotriene C_4 [54]. A role for mono(ADP-ribosyl)transferases in PLC activity has also been suggested by other groups. Nicotinamide or 3-aminobenzamide inhibit T cell-receptor-mediated inositol phosphate production and Ca^{2+} mobilization in cytotoxic T cells [55].

Mono(ADP-ribosyl)transferases are also implicated in protein synthesis. Exposure of RAW 264.7 mouse macrophages to nicotinamide in culture prevents the increased activity of NO synthase that follows exposure of these cells to interferon-γ or lipopolysaccharide. Furthermore, the effect was accompanied by a reduction in mRNA coding for NO synthase [56]. There is also evidence for inhibition of the induction of fibronectin gene expression by 3-aminobenzamide in bronchial epithelial cells, following exposure to $TGF_{\beta 1}$ [57].

Finally in this context, (i) the differentiation and proliferation of embryonic skeletal myoblasts in culture are inhibited by *meta*-iodobenzylguanidine [58], and (ii) the proliferation of human or rat vascular smooth muscle cells is inhibited by hexamethylenebisacetamide [as well as two other inhibitors of mono(ADP-ribosyl)transferase activity] [59].

Conclusion

The discovery of eukaryotic mono(ADP-ribosyl)transferases has been followed by much interest and speculation on their possible role in cellular metabolism. It is quite clear that there are numerous enzyme isoforms, but to date we have the structure of only one of them. However, the Arg-specific mono(ADP-ribosyl)transferase from rabbit skeletal muscle appears to be membrane bound, and many of its (possible) substrates appear to be involved in the complex pathways of signal transduction, cell movement and gene regulation. The finding that at least one of the mono(ADP-ribosyl)transferases is anchored to membranes by a glycosylphosphatidylinositol anchor that may be hydrolysed by PI-PLC suggests any number of possible regulatory functions for this enzyme in receptor-mediated signal transduction. First, it allows for the possibility that the enzyme might be activated by PI-PLC, or even translocated to intracellular substrates that are otherwise inaccessible. Second, there is now increasing evidence for the involvement of glycosylinositolphosphates as intracellular mediators [60].

However, the identification of the substrates for these enzymes has proven to be a very much harder task than was first apparent. Measurement of the catalytic activity of the enzymes in intact cells is difficult. To date the approaches have been either (a) to label the cells with [^{32}P]orthophosphate, or (b) to permeabilize the cells to [^{32}P]NAD with polyethylene glycol or by electroporation. Thereafter, the modification produced may be confirmed as mono(ADP-ribosyl)ation by exposure of the products to snake venom phosphodiesterase. This yields AMP from mono(ADP-ribosyl)ated proteins (and incidentally from NAD^+-protein adducts as well—see above). Refinement of these protocols and, above all, the development of specific inhibitors are still required to establish with more confidence the *in vivo* substrates and physiological roles of these enzymes. The identification of (ADP-ribosyl)-protein hydrolases that appear to be involved with mono(ADP-ribosyl)transferases in the re-cycling of selected proteins through states of being ADP-ribosylated or non-ADP-ribosylated is, in itself, suggestive of the involvement of these enzymes in cell regulation.

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Note added in proof—Since submission of this short review, we have become aware of a most important recent contribution from Zolkiewska and Moss [61]. For the first time a substrate for mono(ADP-ribosyl)transferase has been identified with the same orientation on the cell surface as the cloned Arg-specific enzyme. Almost without exception, it has been noted that glycosylphosphatidylinositol (GPI)-linked proteins are located on the external aspect of the cell surface [12], and the recent identification of Arg-specific mono(ADP-ribosyl)transferase as a GPI-linked enzyme [11, 13] suggested that its substrates should be similarly located. In differentiated mouse skeletal muscle cultures, the major substrate was identified as integrin $\alpha 7$, and, furthermore, partial proteolytic digestion of the product identified the ADP-ribosylation site on integrin $\alpha 7$ to be located on the extracellular domain of the protein [61].

It is clear that there are different mono(ADP-ribosyl)transferase isoenzymes, and it has yet to be established which of these are similarly anchored to the plasma membrane. It seems probable that other substrates of the GPI-anchored isoforms of the enzyme will also be found on the outer aspect of the plasma membrane. However, there may be exceptions to this, since inositol-containing lipid-anchored proteins have been identified on the inner aspect of the plasma membrane [62], and even on the luminal surface of secretory granules [63]. It seems very likely that the recent expansion in our knowledge of ADP-ribosyltransferases will continue, and many of these uncertainties will be resolved in the near future.

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