Biochem. J. (1985) **230**, 1–18
Printed in Great Britain

REVIEW ARTICLE

Eukaryotic nuclear ADP-ribosylation reactions

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Introduction

Eukaryotic cells contain a chromatin-bound enzyme called adenosine diphosphoribosyl transferase (ADPRT; EC 2.4.2.30) which specifically catalyses the cleavage of oxidized NAD^+ with the concomitant covalent attachment of the ADP-ribose (ADPR) moiety to acceptor proteins. These include both histone and non-histone proteins as well as the transferase itself. Proteins may be modified with a single ADP-ribose moiety, mono-(ADP-ribosyl)ation, or with a longer chain of covalently linked residues, oligo- or poly(ADP-ribosyl)ation. Most of the suggested biological functions of this protein modification process centre around the fact that the ADPRT has an absolute requirement for DNA for activity and is activated by DNA strand breaks (Hayaishi & Ueda, 1982). Thus, it is thought to play a role particularly in DNA repair processes and perhaps also in other cellular events such as cell differentiation, transformation, sister chromatid exchange and gene rearrangement and transpositions in which some cleavage and rejoining of DNA strands may occur (see below).

Considerable effort has been focused on determining the identities of acceptors of $(ADP\text{-}ribose)_n$ and of elucidating the mechanism of transferase action and the nature of the chemical links formed between ADP-ribose and its acceptors. Much of this has, of necessity, been carried out *in vitro* using reconstituted systems since there is no suitable precursor for specifically labelling ADP-ribosylated proteins *in vivo*. Use of non-specific adenosine, ribose or inorganic orthophosphate necessitates the removal of contaminants such as DNA and RNA which are present in more than a 100-fold excess over ADP-ribose protein conjugates. Many spurious results concerned with analysis *in vitro* have been reported (Adamietz, 1982). In particular, high ADPRT activity has been shown to be an artifact of the nuclear isolation procedure associated with DNA fragmentation (Halldorsson *et al.*, 1978). We must emphasize, therefore, that the chemical linkages between proteins and ADP-ribose *in vivo* have not in fact been rigorously demonstrated. Hence there is now something of a bewildering array of information on these topics which we have attempted to summarize in this article.

In addition, we include discussion on present concepts regarding putative cellular roles played by ADP-ribosylation reactions. Limited space has precluded reference to much published work, the references cited being dictated by the particular points we have chosen to emphasize for comment.

The ADP-ribosyltransferase reaction

We begin by considering the synthesis of NAD^+ in the nucleus and its attachment to the ADPRT. NMN:adenylyl transferase catalyses the formation of NAD^+ from NMN, derived from the cytosol, and ATP (reaction *a*, Fig. 1). It has been proposed that some NAD^+ is bound directly to the ADP-ribosyltransferase by substrate channelling (Uhr & Smulson, 1982), although exogenous substrate can also be used. ADPRT is inactive (E_0) unless it is bound to DNA (E_*, active enzyme; reaction *b*). In fact, Ittel *et al.* (1984) have recently isolated from calf thymus a DNA–ADPRT nucleosomal-like complex which contains a specific fraction of DNA capable of activating the enzyme more efficiently than total calf thymus DNA. Fig. 1 next shows the cleavage of bound NAD^+ with the production of ADP-ribose attached to the catalytic site of the transferase ($E_*\sim\sim ADPR$, reaction *c*). The nicotinamide is subsequently released and may serve as a negative effector of the transferase. In addition it appears that much of this released nicotinamide is converted to 1-methylnicotinamide (Shaikh *et al.*, 1980; reaction *d*) which has been shown to inhibit NAD synthesis from nicotinamide, and also to induce hepatocellular proliferation (Hoshino *et al.*, 1982).

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Abbreviations used: ADPRT, NAD^+: protein ADP-ribosyltransferase; LMG, low-mobility group; HMG, high-mobility group; BCNU, 1,3-bis-(2-chloroethyl)-1-nitrosourea; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea.

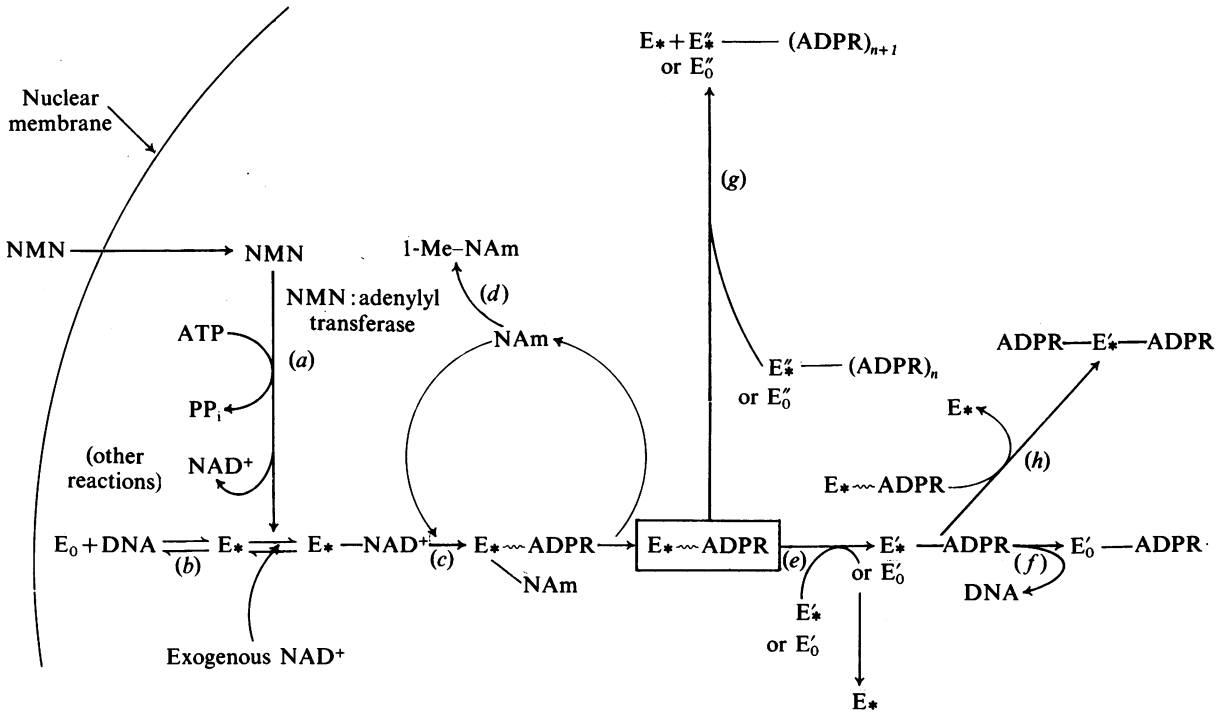

Fig. 1. *The ADP-ribosyltransferase reaction*
The diagram shows the intermediate reactions involved in forming and attaching ADP-ribose to the catalytic site (E*~~ADPR) of the transferase (*a–d*) and subsequent automodification reactions (*e–h*). E*/E$_0$, active/inactive ADP-ribosyltransferase. Different enzyme molecules are shown with a superscript (e.g. E$'_*$, E$''_*$). Abbreviations used: ADPR, ADP-ribose; NAm, nicotinamide.

Kameshita *et al.* (1984) have found that the ADPRT can be cleaved into three distinct domains of 54, 46 and 22kDa, which respectively possess the sites for binding the substrate, DNA and for accepting poly(ADP-ribose). Substantiating evidence to suggest that the catalytic site is not the acceptor site has recently been reported by Bauer & Kun (1984) who found that ADPRT which is inhibited by methyl acetimidate can still function as an ADP-ribose acceptor if an active enzyme is also present. In addition, Kawaichi *et al.* (1981) showed that the fully auto-modified transferase carries 15 ADP-ribose chains, each about 80 residues long. If the initial catalytic site served also as the acceptor site the enzyme would then be presumed to possess 15 active sites, which seems unlikely. Furthermore, Holtlund *et al.* (1983) found that a 16kDa peptide fragment, which was derived from the transferase, contained ADP-ribosylation sites after a short pulse with NAD$^+$ but was hardly modified at all as the transferase reaction proceeded, suggesting that ADP-ribose residues are transferred from one site to another during the auto-modification process. There are thus strong arguments for considering that ADP-ribose is transferred from an initial catalytic site to a different acceptor site.

The resulting molecular species shown at the centre of Fig. 1 is the activated form of the transferase bound to DNA with ADP-ribose attached to the catalytic site (E*~~ADPR). It is ADP-ribose from this site which can be donated to acceptor molecules, including other molecules of the transferase itself. Thus, the formation of E$'_*$–ADPR (reaction *e*) represents the movement of the ADPR residue from the catalytic site on the enzyme (~~) to an acceptor site (–), probably on another molecule. The enzyme may then dissociate from the DNA and thus become inactive E$'_0$–ADPR, reaction *f*). Although we have shown the requirement of DNA throughout the modification process it is unknown whether ADPRT remains active if DNA is removed subsequent to the formation of E*~~ADPR. Since it seems unlikely that the transferase is only mono(ADP-ribosyl)-ated, the ADP-ribose from the catalytic site may be covalently attached to existing ADP-ribose chains attached to an acceptor site (reaction *g*; Holtlund *et al.*, 1983).

This mechanism could in principle be either intra- or inter-molecular. That is, poly(ADP-ribose) may be synthesized by sequential transfer of ADP-ribose units from the catalytic site to an acceptor site on the same enzyme molecule, or to

another molecule of the enzyme. Ueda *et al.* (1984) have recently reported that ADP-ribose is not transferred to either free active transferase, *N*-ethylmaleimide-treated or heat-inactivated free enzyme by immobilized calf thymus ADPRT, which itself retains the ability to auto-modify. Thus it has been suggested that auto-modification might proceed by an intramolecular mechanism. The majority of experimental data, however, suggests that the transfer of ADP-ribose is probably intermolecular. For example, transferase-derived peptide fragments, which are themselves enzymically inactive, are ADP-ribosylated *in vitro* (Holtlund *et al.*, 1983); heat-inactivated transferase accepts as much polymer as the active enzyme does and a partially heat-inactivated transferase incubated with NAD^+ and native transferase molecules is modified throughout the polypeptide. It seems highly likely, therefore, that transferase molecules are (ADP-ribosyl)ated by other transferase molecules in their vicinity, accepting only ADP-ribose from the catalytic site but not from the attachment sites, since these are not transferred to other protein molecules (Holtlund *et al.*, 1983; Kawaichi *et al.*, 1981).

An interesting property of the transferase is that it is solely responsible for catalysing both the initiating event, when the first ADP-ribose residue is attached to the acceptor site, and also the further covalent attachment to this or other ADP-ribose moieties (Kawaichi *et al.*, 1980). Furthermore, the discrepancy between the actual size and chain length of poly(ADP-ribose) led Miwa *et al.* (1979) to propose a branched structure for poly(ADP-ribose) (see Fig. 3), the formation of which was subsequently shown by Ueda *et al.* (1980) to be catalysed by the purified ADPRT.

It has been proposed that the transferase may contain two catalytic sites: one for attaching ADP-ribose initially to the acceptor, and another for subsequently elongating the ADP-ribose moiety to a polymeric chain (Jones & Skidmore, 1984). Future work will no doubt yield more information on this intriguing possibility. Reaction (*h*) in Fig. 1 illustrates that the transferase has more than a single acceptor site for the attachment of ADP-ribose. A second ADPR molecule is shown becoming bound to the already covalently modified enzyme but at a different acceptor site. The transferase molecule then contains multiple ADP-ribosylation sites (Kawaichi *et al.*, 1981; Holtlund *et al.*, 1983) and some, if not all, of the primary ADP-ribose residues may serve as sites for elongation to oligomeric or polymeric ADP-ribose as explained above (see reaction *g*).

An intriguing example of a non-protein acceptor of ADP-ribose has been reported by Yoshihara & Tanaka (1981). P^1,P^4-Bis(5′-adenosyl)tetraphosphate (Ap_4A), thought to be a positive growth signal and a trigger for DNA replication, can be modified by ADP-ribosylation *in vitro* provided that it is attached to protein at the time (reaction *i*, Fig. 2*a*).

Reaction (*j*) (Fig. 2*a*) shows the formation of free $(ADP\text{-}ribose)_n$ chains, not linked to protein (Rickwood *et al.*, 1977; Benjamin & Gill, 1980*a*). Whether these truly occur *in vivo*, or are artifacts produced by cleavage of ADP-ribose residues from proteins during experimental handling of tissues, is still a matter of contention.

Some of the links between ADP-ribose and its protein acceptors are via an ester bond between the C-1 hydroxy group of the distal ribose on an initial ADP-ribose moiety and the carboxy group of glutamic acid, or a terminal lysine residue (reaction *l*, Fig. 2*a*; Burzio *et al.*, 1979; Adamietz & Hilz, 1976). These ester linkages are labile to both neutral hydroxylamine and alkali. In contrast, some links are hydroxylamine- and/or alkali-resistant (reaction *p*, Fig. 2*a*; Kawaichi *et al.*, 1981). It has been proposed, at least for mono-(ADP-ribose) links, that these may be an ADP-ribose-guanidino linkage (Moss *et al.*, 1983). Although such bonds do react with hydroxylamine they are more stable than the ADP-ribose-glutamate bond.

Reaction (*k*) shows the formation of a hydroxylamine-resistant but acid-sensitive ADP-ribose protein conjugate via a phosphoserine (Smith & Stocken, 1973, 1975). The presence of this linkage either *in vitro* or *in vivo* has not, however, been subsequently confirmed.

Mono(ADP-ribose)–protein conjugates may arise as the result of the attachment, by the ADPRT, of a mono(ADP-ribose) residue as a preliminary event in the formation of a polymeric chain (reaction *l*, Fig. 2*a*). Alternatively, they may result from the action of poly(ADP-ribose) glycohydrolase in degrading polymeric chains to leave single ADP-ribose moieties which accumulate due to their rate-limiting removal by ADP-ribosyl protein lyase (reaction *n*).

Non-enzymic mono-(ADP-ribosyl)ation has been reported by Hilz *et al.* (1984) who found that free monomeric ADP-ribose residues can become attached to specific protein acceptors, via a hydroxylamine-resistant bond, in the absence of ADP-ribosyl transferases. The reaction, which simulates ADPRT activity, can be explained by a sequence of two reactions composed of an enzymic conversion of NAD^+ to ADP-ribose and nicotinamide, catalysed by NAD^+ glycohydrolase (reaction *r*, Fig. 2*a*), and a non-enzymic, although highly specific, ADP-ribosylation of acceptor proteins by free ADP-ribose (reaction *o*).

DNA damage is known to cause an increase in

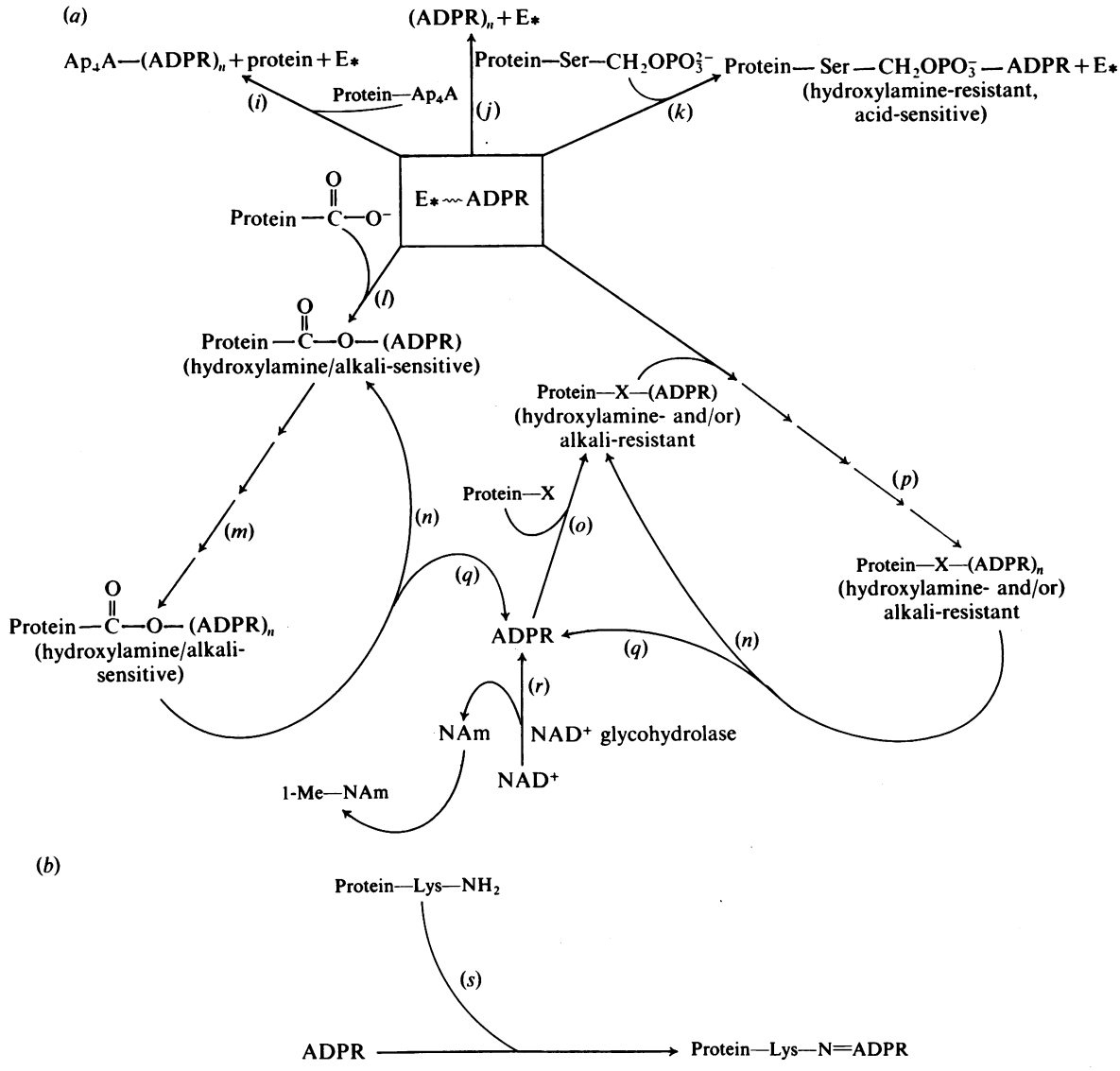

Fig. 2. *ADP-ribosylation reactions*

(*a*) Nuclear ADP-ribosylation. Reactions shown include the enzymic modification of endogenous acceptors (*i*, *k*, *l*, *m*, *p*), the formation of free ADP-ribose due to polymer turnover (*q*), the action of NAD^+ glycohydrolase (*r*) and the formation of free poly(ADP-ribose) not linked to protein (*j*), glycohydrolase degradation of polymeric (ADP-ribose) (*n*) and non-enzymic attachment of free ADP-ribose to acceptor protein (*o*). Abbreviations used: -X-, unknown linkage; E*, active ADPRT. (*b*) Non-enzymic monomeric ADP-ribosylation by Schiff's base formation. Symbols and abbreviations as for Fig. 1 and (*a*).

turnover of poly(ADP-ribose) (reaction *q*, Fig. 2*a*). Thus, the finding of Wielckens *et al.* (1982*a*) that nuclear bound hydroxylamine-resistant mono-(ADP-ribosyl)–protein conjugates are augmented in response to alkylation treatment can certainly be explained as a non-enzymic attachment of free ADP-ribose moieties to acceptor proteins (reaction *o*). The hydroxylamine- and/or alkali-resistant attachment of ADP-ribose chains to proteins has been reported (Kawaichi *et al.*, 1981). We propose that these result from the action of the ADPRT in elongating a non-enzymically initiated ADP-ribose chain (reaction *p*).

Whilst monomeric ADP-ribosylation appears to be quantitatively more important than polymer formation *in vivo* (Dietrich *et al.*, 1973; Wielckens

et al., 1982*b*), the majority of mono(ADP-ribosyl)-ated conjugates are located outside the nucleus (Adamietz *et al.*, 1981). This raises the intriguing possibility that mono(ADP-ribosyl)ated proteins, particularly histones, may be modified before they migrate into the nucleus by a cytosolic mono(ADP-ribosyl) transferase. Finally, Fig. 2(*b*) shows the irreversible chemical, non-enzymic formation of a Schiff's base (reaction *s*; Kun *et al.*, 1976), that is formed at pH7.4 when ADP-ribose is incubated with macromolecules such as albumin, poly(L-lysine) and especially histone Hl.

Our unified treatment of monomeric and polymeric ADP-ribosylation reactions is justified on the basis that monomeric modification is an obligate preliminary event in the formation of proteins modified by polymeric ADP-ribose chains (reaction *m*, Fig. 2*a*). Thus, ADPRT, which when purified to apparent homogeneity can still catalyse the formation of polymer chains, is by definition also a mono(ADP-ribosyl) transferase. In addition, after partial hydrolysis of poly(ADP-ribose) with glycohydrolase the polymer terminal (which may then only be a monomeric ADP-ribose unit) still has a suitable structure for the ADPRT to reinitiate chain elongation. It also seems unlikely that the ADPRT would be able to discriminate between monomeric ADP-ribose residues which were attached by a putative mono-(ADP-ribosyl) transferase, and monomeric ADP-ribose conjugates which it formed initially itself. This does not, of course, exclude the possibility that the ADPRT also catalyses the formation of significant amounts of protein conjugates modified only by monomeric ADP-ribose.

ADPRT structure and endogenous proteolytic processing

ADP-ribosyl transferase is ubiquitously distributed in the inter-nucleosomal chromatin region in nuclei from plant and animal cells. It is also found in lower eukaroytes such as the dinoflagellate *Crypthecodinium cohnii* (Werner *et al.*, 1984), and the slime moulds *Physarum polycephalum* (Brightwell *et al.*, 1975) and *Dictyostelium discoideum* (Rickwood & Osman, 1979). Enzyme activity is lacking, however, in granulocytes and mammalian erythrocytes and is greatly diminished in, if not absent from, terminally differentiated epidermal cells (Ikai *et al.*, 1981). The ADPRT from many sources has been purified and characterized (Man-

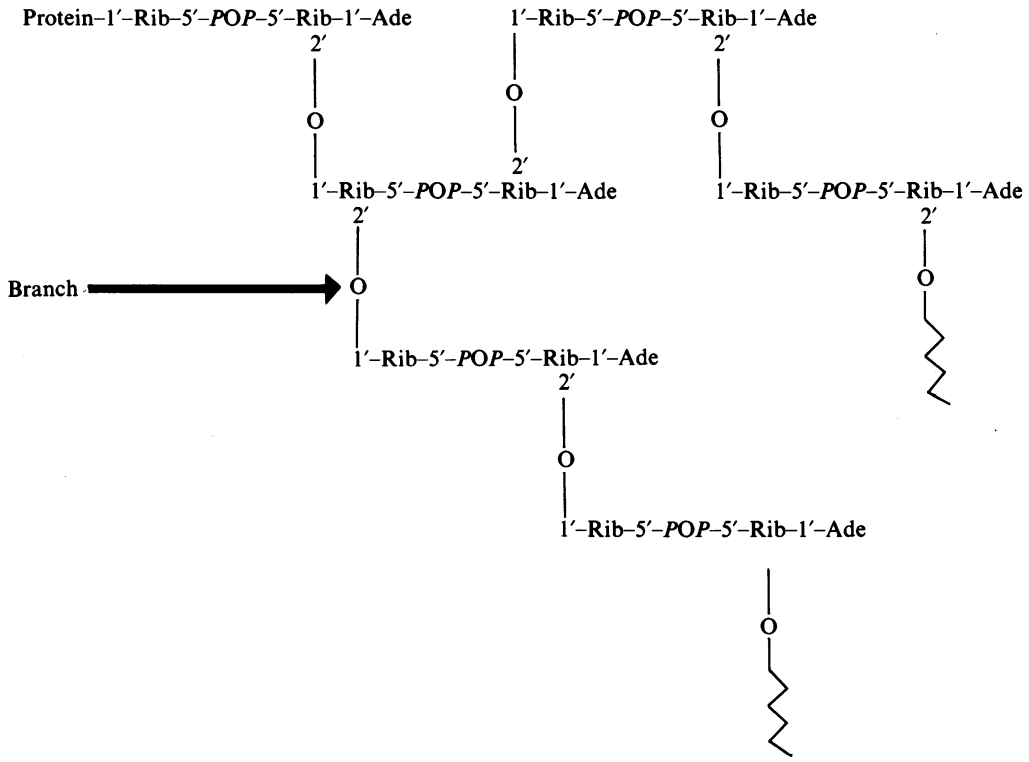

Fig. 3. *Structure of poly(ADP-ribose) showing branching*
Rib, ribose; *P*, phosphate; Ade, adenine.

del *et al.*, 1977; Okayama *et al.*, 1977; Kristensen & Holtlund, 1978; Tsopanakis *et al.*, 1978; Ito *et al.*, 1979; Niedergang *et al.*, 1979; Jump & Smulson, 1980; Agemori *et al.*, 1982; Carter & Berger, 1982, and others) and in all cases is a basic protein.

ADP-ribosyl transferases from various tissues exhibit biphasic kinetics for NAD^+ utilization (Furneaux & Pearson, 1980; Holtlund *et al.*, 1981; Jones & Skidmore, 1984), indicating possible substrate activation or inhibition, the presence of more than one transferase in a particular tissue, or the presence of more than one active site on the enzyme. Circumstantial evidence for the presence of multiple transferases was initially provided by reported differences in the molecular mass of calf thymus ADPRT purified by different laboratories (Tsopanakis *et al.*, 1978; Jongstra-Bilen *et al.*, 1981), and by the range of acceptors of ADP-ribose indicating an extraordinarily wide enzyme specificity. The metabolic consequences, established so far mostly for DNA repair, of inhibiting the transferase also differ in various tissues.

However, Jongstra-Bilen *et al.* (1981) and Holtlund *et al.* (1981) found no evidence of tissue or species specific differences in molecular mass of the transferase from amongst other tissues, including rat liver and pancreas and bovine brain and kidney, although there are discrepancies in the molecular mass of the transferase reported between these laboratories. Agemori *et al.* (1982) on the other hand suggested that species or tissue specificity of the ADPRT might exist, based on amino acid composition differences between mouse testicle and calf thymus ADP-ribosyl transferases.

Earlier enzyme purifications in the absence of proteinase inhibitors (e.g. Tsopanakis *et al.*, 1978) underestimate the molecular mass of the transferase since it has been shown that it is subject to highly specific endogenous proteolytic processing (Surowy & Berger, 1983*a*; Holtlund *et al.*, 1983). Jongstra-Bilen *et al.* (1981) concluded that transferases from calf and pig thymus and bovine and rat liver, pancreas, brain and kidney and from chicken brain all have a molecular mass of 130kDa. However, they based this estimation upon the similarity in migration of the ADPRT and β-galactosidase, the molecular mass of which they took to be 130kDa. The molecular mass of β-galactosidase is, however, known to be 116kDa (Fowler & Zabin, 1978) and hence the molecular masses of the transferases as determined by Jongstra-Bilen *et al.* (1981) are overestimated.

Holtlund *et al.* (1981) compared the moleculer masses of ADP-ribosyl transferases (purified in the presence of proteinase inhibitors) from EAT and HeLa cells and pig thymus, and found them to be identical at 112kDa, although there are slight discrepancies in kinetic parameters between these enzymes despite their similar amino acid compositions. Thus it appears that the molecular mass of the purified ADPRT is about 112kDa, a view supported by Kawaichi's finding that the purified rat liver enzyme is 110kDa (Kawaichi *et al.*, 1980).

Holtlund *et al.* (1983) reported that in the absence of proteinase inhibitors enzymically inactive transferase-derived peptides co-purify with calf thymus ADP-ribosyl transferase on DNA–agarose and Blue Sepharose, and are as efficient at accepting ADPR as the intact enzyme. Shizuta's group (Nishikima *et al.*, 1982) found that the transferase can be cleaved, by a mild papain digestion, into 46 and 74kDa fragments. Subsequent work (Kameshita *et al.*, 1984) demonstrated the presence of three distinct domains respectively capable of binding NAD^+, DNA and of accepting ADP-ribose. Labelling experiments with [^{32}P]NAD^+ were carried out at a very low NAD^+ concentration, however, and the possibility that the whole transferase molecule might be modified at higher NAD^+ concentrations has not been excluded.

Surowy & Berger (1983*b*) found that normal human lymphocytes process automodified transferase molecules to specific lower molecular weight products, and that this processing is stimulated by Ap_4A. The processing is not, however, specific for AP_4A but is stimulated by other nucleotides containing pyrophosphate bonds, such as ATP, as well as by pyrophosphate itself (Berger & Surowy, 1984). The effect of Ap_4A on auto-processing is quite complex but it appears to cause modification of fragments of 96, 79 and 62kDa, which have been identified by pulse–chase experiments as proteolytic degradation products of the transferase. It is interesting that levels of Ap_4A are reduced in resting cells but that treatment of non-proliferating cells with exogenous Ap_4A results in the appearance of an ADP-ribosylated peptide of molecule mass 96kDa which characteristically occurs in phytohaemagglutinin-stimulated cells (Surowy & Berger, 1983*a*).

The transferase then is probably regulated *in vivo* by highly specific proteolysis. In fact, since proteolytic cleavage fragments can themselves be ADP-ribosylated the most physiologically important species may be a transferase-derived peptide. In this respect the transferase is no different from other chromatin-bound or -associated enzymes such as DNA polymerases α and β, deoxynucleotidyl terminal transferase, and DNA topoisomerase, in which highly specific proteolysis is known to occur (Chang *et al.*, 1982; Nakamura *et al.*, 1981; Liu & Miller, 1981). Furthermore, there is little evidence of tissue- or species-specific differences in molecular masses of the ADPRT derived from various

sources, indicating that the structure–function relationship of the transferase has been well preserved during the course of evolution. In this sense also it is similar to other nuclear enzymes such as RNA polymerase, DNA polymerases α and β, and to the histones, the major structural proteins of chromatin.

Poly(ADP-ribose) degradation

Only two kinds of poly(ADP-ribose)-degrading enzymes are known, poly(ADP-ribose) glycohydrolase and phosphodiesterase from rat liver, snake venom and tobacco cells. Poly(ADP-ribose) glycohydrolase, an exoglycosylase, hydrolyses ribose–ribose bonds and also the branched region of poly(ADP-ribose) containing ribose–ribose–ribose bonds, to yield ADP-ribose (Miwa & Sugimura, 1982) (Fig. 4).

Phosphodiesterases may be endonucleolytic or exonucleolytic (Miwa & Sugimura, 1982). They produce 2′-(5-phospho-β-D-ribosyl)adenosine 5′-phosphate, abbreviated as *P*-Ado(*P*-Rib), 5′-AMP and ribose 5-phosphate as final products. A small amount of 2′-[5-phospho-β-D-ribosyl-2′-(5-phospho-β-D-ribosyl]adenosine 5′-phosphate, abbreviated as *P*-Ado(*P*-Rib)(*P*-Rib), is also produced from branched poly(ADP-ribose) (Fig. 4). The pH optimum for poly(ADP-ribose) glycohydrolase is 7.6 and its apparent K_m for poly(ADP-ribose) is 0.58 μM, whereas the pH optimum for phosphodiesterase is about 10 and the apparent Michaelis constant for poly(ADP-ribose) is about 28 μM (Miwa & Sugimura, 1982). Poly(ADP-ribose) glycohydrolase, then, is almost certainly the more physiologically important enzyme for degrading poly(ADP-ribose).

The enzyme which specifically cleaves the ester bond between ADP-ribose and histone or other proteins is ADP-ribosyl protein lyase. This enzyme is a single polypeptide of 80 kDa which recognises the whole ADP-ribose unit remaining following glycohydrolase digestion. It is of major physiological significance in view of the fact that monomeric ADP-ribosylation is quantitatively more important than polymer formation. Thus, it seems plausible that the degradation of protein-bound poly(ADP-ribose) is carried out by a co-operation of poly(ADP-ribose) glycohydrolase and ADP-ribosyl protein lyase (Oka *et al.*, 1982).

Poly(ADP-ribose) acceptor proteins

There are many difficulties involved in studying ADP-ribosylated proteins. The lack of a specific precursor suitable for analysis *in vivo* has already been mentioned. The inconvenient susceptibility to alkali of the majority of ADP-ribose–acceptor linkages is another factor which hinders ready analysis. Electrophoretic fractionation followed by autoradiography is often used to detect ADP-ribosylated proteins. Unfortunately there is often a wide diversity of electrophoretic mobility, of perhaps a single acceptor species, due to modification to varying extents by the attachment of polymer chains of different lengths.

These and other experimental difficulties are reflected in the vast amount of frequently conflicting data accumulating concerning the identities of acceptors of ADP-ribose. Many ADP-ribose acceptors exist only *in vitro* and are probably artifacts due to unphysiological transferase activity in DNA damaged cells or nuclei (see Table 1). There is increasing evidence that ADPRT is inactive in normally growing terminally differentiated undamaged cells (Ueda & Hayaishi, 1982). Many analyses, however, are performed in DNA-damaged cells, as damaging DNA has been shown to activate the transferase. If ADPRT activity is involved in cellular events other than repairing DNA damage, however, then the maximally stimulated transferase activity cannot represent or elucidate the true physiological role of ADP-ribosylation. Thus, more sensitive methods need to be applied to analysis *in vivo* in normal DNA-undamaged cells.

Prentice & Gurley (1983) have recently shown that nuclei isolation procedures affect nuclease digestibility of chromatin. Thus, new ADP-ribose acceptors may be exposed and/or lost due to the conditions of nuclei isolation. Nuclei isolated from rat liver can modify all four core histones with monomeric ADP-ribose, but only histone H2B if the nuclei are prepared according to the method of Blobel & Potter (1966), which includes a centrifugation step through iso-osmotic sucrose. In addition, differences are reported, in terms of ADP-ribosylation, for nuclei isolated from rat liver, mouse liver, mouse testis, Ehrlich ascites carcinoma, etc. (see Table 1). Whilst these discrepancies may reflect true species and tissue specific differences in ADP-ribosylation, they are equally likely to result from different conditions employed in the preparation of cells and nuclei.

The biological functions of ADP-ribose depend, at least partially, upon the specific proteins modified *in vivo*. It is proposed that ADP-ribosylation of structural chromatin proteins, such as the histones, causes a transient alteration in poly-nucleosomal construction (Ueda *et al.*, 1982; see also later section below). On the other hand, ADP-ribosylation of functional proteins is thought to play a role in modulating their activity. It is this latter aspect of ADP-ribosylation which we now

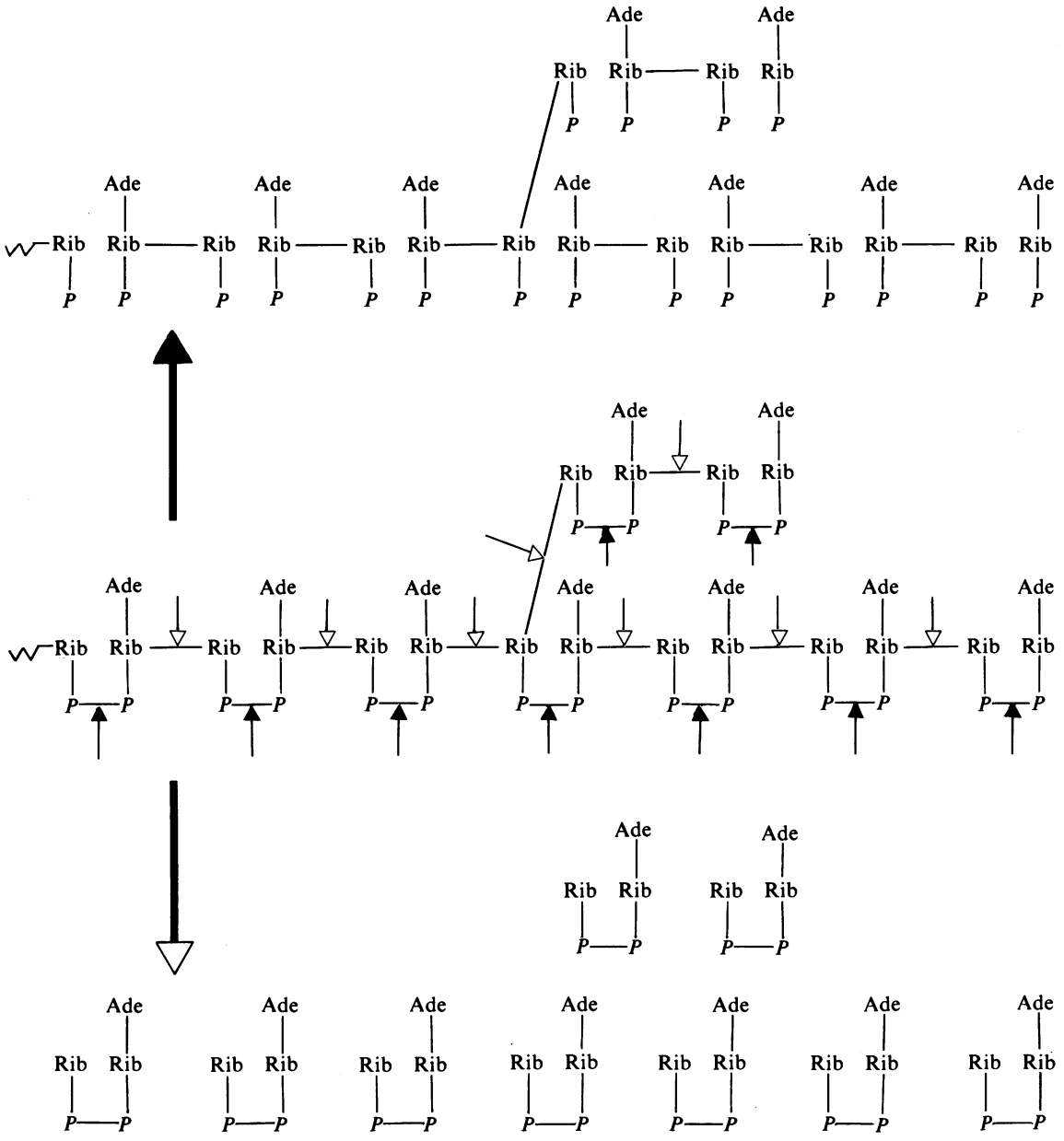

Fig. 4. *Enzymic degradation of poly(ADP-ribose)*

Poly(ADP-ribose) can be hydrolysed by poly(ADP-ribose) glycohydrolase (⏚) to produce ADP-ribose and by phosphodiesterase (⏚) to produce *P*-Ado(*P*-Rib) and *P*-Ado(*P*-Rib)(*P*-Rib) from the branch region, 5′-AMP from the terminus distal to the protein attachment site and Rib-*P* in free poly(ADP-ribose), i.e. from the hitherto protein attachment region.

turn to, paying attention to the suggested mechanisms of enzymic regulation by covalent attachment of polymeric-ADP-ribose.

Enzymic regulation by poly(ADP-ribosyl)ation

Purified DNA polymerases α and β, DNA ligase II, deoxynucleotidyl terminal transferase, Ca^{2+}/Mg^{2+}-dependent endonuclease, DNA topoisomerase I and the ADP-ribosyl transferase itself are known to be modified by covalent attachment of poly(ADP-ribose) (Yoshihara *et al.*, 1984; Tanaka *et al.*, 1984; Ferro & Olivera, 1984; Jongstra-Bilen *et al.*, 1983; Kawaichi *et al.*, 1981). In all cases the enzymes are inhibited by the modification process. Since all of these enzymes

Table 1. *Acceptor proteins*

Cell or nuclei type	ADP-ribose conjugation	Reference
Intact rat liver cells	Mono(ADP-ribose) attached to histone H1 via a hydroxylamine-resistant but acid-sensitive serine phosphate.	Smith & Stocken (1973)
	99% of the naturally occurring $(ADP\text{-}ribose)_n$ $(4<n<34)$ covalently bound to non-histone proteins.	Minaga *et al.* (1979)
	ADP-ribose attached to histones as oligomers and monomers.	Ueda *et al.* (1975)
	All four core histones are modified. Extensive modification of 63 and 71 kDa acceptors. Minor modification of a 47 and 52 kDa acceptor. Transferase automodification excluded.	Adamietz (1982)
	70–80% of ADP-ribose covalently bound in monomeric form.	Dietrich *et al.* (1973)
Isolated rat liver nuclei	ADPRT is the major acceptor of poly(ADP-ribose).	Adamietz (1982)
	The four classes of core histones are modified by mono(ADP-ribose) but only histone H2B if nuclei are prepared according to the method of Blobel & Potter (1966).	Adamietz (1982)
	ADPRT is the major acceptor of ADP-ribose	Ogata *et al.* (1981)
	Mono and poly(ADP-ribosyl)ation of histones reported.	Nishizuka *et al.* (1968)
Isolated rat liver mitochondria	50–55 kDa acceptor non-enzymically modified by hydroxylamine-resistant monomeric ADP-ribose.	Hilz *et al.* (1984)
	100 kDa acceptor (which dissociates into two 50 kDa molecules) is reversibly modified by mono and oligomeric ADP-ribose.	Kun *et al.* (1975)
Submitochondrial particles	Mono(ADP-ribosyl)ation of a 30 kDa inner membrane protein via a hydroxylamine-resistant linkage.	Richter *et al.* (1983)
Isolated beef heart mitochondria	30 kDA acceptor non-enzymically modified by hydroxylamine-resistant monomeric ADP-ribose.	Hilz *et al.* (1984)
Intact mouse testis	LMG proteins are the major acceptors of ADP-ribose. HMG proteins are also modified but to a lesser extent.	Leone *et al.* (1984)
	HMG and LMG proteins modified. Slight modification of histone H1 and very slight modification of histones H2A and H3.	Faraone-Menella *et al.* (1982)
Mouse testis nuclei	LMG proteins preferentially modified by $(ADP\text{-}ribose)_n$ $(n = 4\text{–}6)$. HMG proteins are modified but to a lesser extent.	Faraone-Menella *et al.* (1984)
Intact mouse liver	Histones ADP-ribosylated preferentially. Major acceptor histone H1, moderate acceptor H2 and minor acceptor H3. Slight modification of non-histone proteins.	Faraone-Menella *et al.* (1982)
Isolated mouse liver nuclei	Free polymer formed.	Rickwood *et al.* (1977)
Intact EAT cells	Pattern of ADP-ribosylation *in vitro* differs from that *in vivo*. Many acceptors exist only *in vitro*. 57 and 62 kDa acceptors exclusively modified *in vivo*. All other proteins including histones and the ADPRT are modified only *in vitro*. Treatment with DNA-damaging agents induces histone modification.	Adamietz (1982)
Isolated EAT cell nuclei	Major acceptor of poly(ADP-ribose) is the transferase.	Adamietz (1982)
	Poly(ADP-ribosyl)ation of all four core histones, although H3 is only modified slightly.	Adamietz (1982)
	Presence of multiple acceptor sites on histone H1.	Braeuer *et al.* (1981)
Permeabilized human lymphocytes	Proteins of 116, 72, 42, 32, and 21–15 kDa modified by poly(ADP-ribose).	Surowy & Berger (1983*a*)
	Upon MNNG treatment exclusive modification of a 62 kDa ADP-ribose acceptor.	Surowy and Berger (1983*a*)
	Upon mitogen stimulation a protein of 96 kDa is exclusively modified.	Surowy & Berger (1983*a*)
	Extensive transferase automodification.	Ogata *et al.* (1981)
Isolated human lymphocyte nuclei	Extensive transferase automodification	Ogata *et al.* (1981)
Intact HeLa cells	Histone H1 dimer excluded *in vivo*	Adamietz *et al.* (1978)
	ADP-ribose ten times lower *in vivo* than *in vitro*.	Adamietz (1982)
	Immunological evidence presented for the occurrence *in vivo* of a histone H1 dimer.	Wong *et al.* (1983)

Table 1.–*continued*

	Most histone H1 conjugates contain only single ADP-ribose residues, some of which are alkali- and hydroxylamine-resistant.	Wong *et al.* (1983)
	Treatment with MNU preferentially produces polymeric chains at the expense of monomeric or oligomeric chains.	Thraves & Smulson (1982)
Isolated HeLa cell nuclei	ADPRT is the main acceptor of poly(ADP-ribose).	Jump & Smulson (1980)
	$\frac{2}{3}$ ADP-ribose found as H1–$(ADP\text{-ribose})_{15}$–H1 complex.	Stone *et al.* (1977)
	Histone H1 carries predominantly polymeric ADP-ribose residues.	Adamietz *et al.* (1978)
	Transferase modification is increased 3-fold by MNU treatment. Histone modification is increased 2-fold by MNU treatment.	Thraves & Smulson (1982)
Intact Yoshida AH974 hepatoma cells	Histone H1 is a minor acceptor of polymeric and monomeric ADP-ribose even in DNA-damaged cells. Dimethyl sulphate treatment increases histone H1 monomeric ADP-ribosylation 30-fold but does not affect H1 polymeric modification.	Kreimeyer *et al.* (1984)
	Histones H3/H2B are major acceptors of monomeric and polymeric ADP-ribose following dimethyl sulphate treatment. ADPRT is a poor acceptor of ADP-ribose following dimethyl sulphate treatment.	Kreimeyer *et al.* (1984)
	Histone H2B is a major acceptor of monomeric and polymeric ADPR in DNA-damaged cells. Bonds are hydroxylamine-resistant. Histone H4 is also modified in dimethyl sulphate treated cells. Proteins of 100–116kDa and 170kDa carry substantial amounts of mono and poly(ADP-ribose) in DNA-damaged cells.	Adamietz & Rudolph (1984)
Intact mouse 34I cell line	HMG 1, 2, 14 and 17 and histone H1 modified by poly(ADP-ribose). HMG 14 and 17 modification is more affected than the other proteins by the addition of 3-aminobenzamide.	Tanuma & Johnson (1983)
Detergent-lysed mouse L, HeLa and BSCl cells	Free $(ADP\text{-ribose})_n$ formed.	Benjamin & Gill (1980*a*,*b*)
Isolated rat islets of Langerhans	A membrane protein is modified by an endogenous ADPRT.	Bernofsky & Amanoo (1984)
Sarcoplasmic reticulum	ADPRT activity demonstrated toward synthetic guanidino analogues. Major endogenous 83kDa acceptor reported.	Soman *et al.* (1984)
Intestinal epithelial cell nuclei	In nuclei of dividing cells 80% of ADP-ribose is in polymeric form. 60% of the total labelled proteins are acid-soluble.	Porteous *et al.* (1979)
Permeabilized pancreatic cells	Histone H1 is the major ADP-ribose acceptor.	Dam *et al.* (1981)
Isolated pancreatic cell nuclei	HMG 14 and 17 are extensively modified. HMG 1 and 2 and histone H1 are moderately ADP-ribosylated.	Dam *et al.* (1982)
Isolated pancreatic nucleosomes	Histone H1 is the single ADP-ribose acceptor and is hypermodified.	Dam *et al.* (1982)
Macronuclei from *Tetrahymena thermophila*	ADP-ribosylation of histones H1, H2A, H2B and H3 and of HMG-C.	Levy-Wilson (1983)
Purified ADPRT incubated with proteins or enzymes	Modification of a purified (Ca^{2+}/Mg^{2+})-dependent endonuclease from rat liver. The enzyme is inhibited by the modification process.	Tanaka *et al.* (1984)
	Transferase automodification reported.	Kawaichi *et al.* (1981)
	Histone H1 modified by oligomeric ADP-ribose. H1 dimer also observed. H5 dimer observed at high NAD^+ concentrations. HMG 14 and HMG 17 only slightly ADP-ribosylated. HMG 1 and HMG 2 also modified.	Poirier *et al.* (1982*a*)
	Histone H1 is the major ADP-ribose acceptor when present in sufficient quantity.	Kawaichi *et al.* (1980)
	Co-purification with the ADPRT of a minor topoisomerase activity which is inhibited by poly(ADP-riboysl)ation.	Ferro & Olivera (1984); Jongstra-Bilen *et al.* (1983)

Table 1.–*continued*

Poly(ADP-ribosyl)ation of purified DNA polymerases α and β, DNA ligase II, and deoxynucleotidyl terminal transferase. Modified enzymes are strongly inhibited (50–75%).	Yoshihara *et al.* (1984)

require DNA for activity, it has been suggested that inhibition is due to an electrostatic repulsion between the natural substrate, DNA, and the ADP-ribose chains covalently bound to the enzymes (Tanaka *et al.*, 1984; Ferro & Olivera, 1982, 1984). Evidence to support this hypothesis is provided by the findings that ADP-ribosylated endonuclease and ADPRT have a reduced affinity for DNA, that free poly(ADP-ribose) is non-inhibitory, and that positively charged DNA-binding proteins enhance endonuclease activity (Tanaka *et al.*, 1984; Ferro & Olivera, 1982). Yamada *et al.* (1974) and Nomura *et al.* (1981) have, however, reported that rat liver nuclear exonuclease and a Mg^{2+}-dependent endonuclease from adult hen liver nuclei are inhibited by free poly(ADP-ribose). Enzyme inhibition is probably reversible due to the short half-life of the polymer *in vivo*.

The report by Yoshihara *et al.* (1984) that purified DNA ligase II is inhibited by ADP-ribosylation is of special significance, in view of the findings of Creissen & Shall (1982, 1983) who suggested that DNA ligase II may be activated by binding ADP-ribose. These latter workers based this view on the observation that exposure of L1210 cells to dimethyl sulphate increases ligase II activity, that this increase can be prevented by ADPRT inhibitors, and that radioactivity derived from [^{3}H]NAD^+ comigrates with partially purified ligase II on hydroxyapatite. Furthermore, following alkylation treatment, the ADP-ribose material associated with DNA ligase II increases, along with a proportionate increase in enzyme activity.

Ohashi *et al.* (1983) have provided an alternative view of the involvement of ADP-ribose in activating ligase II. They suggest that poly(ADP-ribose) loosens histone–DNA interactions, allowing ligase to act upon normally inaccessible intranucleosomal DNA. Furthermore, ADP-ribose may sterically locate DNA repair enzymes at the sites of DNA strand breaks since it is known that ligase has a high affinity for $(ADP\text{-}ribose)_n$ and that poly(ADP-ribose) synthesized *in vitro* is bound to the ADPRT, which is itself located at the sites of DNA damage.

The activation of an endogenous endonuclease causes random DNA degradation following DNA damage (Tanaka *et al.*, 1984). The blocking of endonuclease activity by attachment of poly(ADP-ribose) may thus be a vital process in maintaining cells in a viable state during DNA repair. This agrees with the suggestion of Yoshihara *et al.* (1984) that activation of the ADPRT following DNA damage results in a kind of emergency halt of chromatin functions, probably at the damaged site.

ADPRT activity is modulated by DNA strand breakage

Roitt (1956) showed that treatment of cells with alkylating agents induces cellular NAD^+ depletion and therefore prevents glycolysis. Subsequent to this depletion in NAD^+ is a dramatic decrease in ATP levels causing a reduction in ATP-dependent functions leading, eventually, to cell death (Sims *et al.*, 1983). Activation of the ADPRT by DNA-damaging agents, as a causative factor in decreasing cellular NAD^+, was originally reported independently by Whish *et al.* (1975) and Smulson *et al.* (1975) and subsequently confirmed by several groups (Juarez-Salinas *et al.*, 1979; Jacobson *et al.*, 1983; Sims *et al.*, 1983). Wielckens *et al.* (1982*a*), however, found a significantly retarded temporal relationship between the decrease in NAD^+, following DNA damage with triaziquonum, and ADPRT activation, suggesting the activation of degradative enzymes involved in NAD^+ metabolism other than ADP-ribosyl transferase. In contrast a simultaneous fall in NAD^+ concentration and rise in ADPRT activity (prevented by adding ADPRT inhibitors) was reported by Jacobson *et al.* (1980), who further found that the increase in the transferase activity roughly corresponded with the amount of NAD^+ removed in Balb 3T3/A31 fibroblasts.

The tremendous difference between endogenous amounts of polymeric ADP-ribose in comparison with ADP-ribosyl units in the substrate NAD^+, coupled to the rapid turnover of cellular NAD^+ following DNA damage, led Wielckens *et al.* (1982*a*) to propose that the polymer had a rapid turnover. This was previously suggested by Juarez-Salinas *et al.* (1979), and more recently a high rate of poly(ADP-ribose) turnover *in vivo* has been demonstrated (Jacobson *et al.*, 1983). The report of Wielckens *et al.* (1982*b*), however, that elevated levels of poly(ADP-ribose) persist long after NAD^+ depletion, argues against a rapid turnover of at least some ADP-ribose units, and the temporally displaced kinetics of ADPRT activation and NAD^+ depletion remain an enigma.

Berger *et al.* (1979) found that in either growth-

arrested Chinese hamster ovary cells at 37°C, or in cold-sensitive DNA synthesis arrest CHO cell mutants at 33°C, DNA synthesis ceased and was accompanied by the development of DNA strand breaks. Concomitant with the development of these strand breaks is the activation of the ADPRT.

The dependence of poly(ADP-ribose) synthesis on DNA strand breakage has been extensively reported (Gill *et al.*, 1974; Janakidevi & Koh, 1974; Berger *et al.*, 1979; Benjamin & Gill, 1980*a*; Shall, 1982; Jacobson *et al.*, 1983), although no support has been obtained for the hypothesis that predicts that DNA fragmentation is a singularly rate-determining factor in controlling the activity of ADP-ribosyl transferase. In fact, there is increasing evidence that factors other than DNA fragmentation may also be involved in modulating ADPRT activity (Jackowski & Kun, 1981; Althaus *et al.*, 1982; Hacham & Ben-Ishai, 1984; Wallace *et al.*, 1984).

The type of break introduced into the DNA duplex is important in regulating ADPRT activity. Double-stranded restriction fragments with flush ends are reported by Benjamin & Gill (1980*b*) as being most effective, three times more so than DNA fragments with 3′ extensions which are more than three times as effective as DNA with unpaired nucleotides extending from the 5′ termini, or DNA with single strand breaks. Apurinic and methylated DNA are not activating co-factors for the transferase (Shall, 1982), and neither is covalently closed double- or single-stranded DNA unless it is fragmented.

Effects of ADPRT inhibitors on DNA repair

There is now a plethora of literature on the proposed relationship between ADP-ribosylation reactions and DNA repair (see Shall, 1982 and other articles in the book). We have restricted our discussion to one key aspect of this, namely, the use of ADPRT inhibitors.

A role for poly(ADP-ribose) in the repair of DNA has been suggested by Durkacz *et al.* (1980*a*,*b*) who found that inhibitors retarded rejoining of DNA strand breaks induced by dimethyl sulphate in mouse L1210 cells. ADPRT inhibitors were also found to inhibit the decrease in cellular NAD^+ levels normally seen following dimethyl sulphate treatment in the absence of inhibitors.

Bohr & Klenow (1981) found that ADPRT inhibitors stimulate DNA repair in human lymphocytes following treatment with dimethyl sulphate or u.v. light. This suggests that DNA might be repaired more quickly in the absence of poly(ADP-ribose) than in its presence. This is consistent with the findings of Yoshihara *et al.* (1984) who found that various DNA repair enzymes are inhibited when they are poly(ADP-ribosyl)ated *in vitro*.

There are clearly differences in the ability of ADPRT inhibitors to inhibit DNA excision repair in different cell types. It may be that the influence of such inhibitors on DNA repair is dependent on the agent used initially to stimulate the repair synthesis. Also, in addition to possible differences in cell membrane permeability to the inhibitors, there may be substantial differences in the kinetic parameters of the several steps in DNA repair which contribute to the observed differences in responsiveness to ADP-ribosyl transferase inhibitors in various cell lines. It must be stressed therefore, that, whenever ADPRT inhibitors have been used to demonstrate a particular consequence of ADPRT activity, the proposed biological function deduced could be due to factors unrelated to poly(ADP-ribose) synthesis. This is illustrated further in the next section.

ADPRT inhibitors are non-specific

ADPRT inhibitors, such as benzamide, 3-aminobenzamide and nicotinamide are not completely specific and exhibit some side effects, although these are generally only found at relatively high concentrations. Borek *et al.* (1984) have only recently shown that benzamide and 3-aminobenzamide inhibit both purine synthesis and methylation mediated by *S*-adenosylmethionine. The effects of these inhibitors on differentiation in intact cells may be due to glucose starvation, since Grunfeld & Shigenaga (1984) found that nicotinamide, benzamide and bromodeoxyuridine inhibit deoxyglucose uptake in differentiated 3T3-L1 fat cells, OK opossum kidney cells and UMR bone cells. In addition, deoxyglucose uptake is also inhibited by ADPRT inhibitors in an undifferentiated 3T3-L1 fibroblast cell line. These observations complement those of Milam & Cleaver (1984) who found that benzamide inhibited the rate of glucose oxidation in a lymphoid cell line and increased lactate dehydrogenase release, which has been used as an index of cytotoxicity. The data of Grunfeld & Shigenaga (1984) also support those of Schechter (1984), i.e. that nicotinamide inhibits basal and insulin-stimulated lipogenesis in rat fat cells, since most lipid found in adipocytes is synthesized from glucose (Kuri-Harcuch *et al.*, 1978). In addition Johnson (1981) has reported that ADPRT inhibitors also inhibit other enzymes involved in NAD^+ metabolism, particularly the nicotinamide 1-methyltransferase. Hence other actions of ADPRT inhibitors may confound experimental interpretation.

ADP-ribosylation and cellular differentiation

That ADPRT activity changes during normal progressive differentiation has been extensively reported (Claycomb, 1976; Ghani & Hollenberg, 1978; Farzaneh & Pearson, 1979; Porteous *et al.*, 1979; Rickwood & Osman, 1979; Farzaneh *et al.*, 1982; Hacham & Ben-Ishai, 1984, and others). It appears that high poly(ADP-ribose) levels and ADPRT activity are apparent during the stage of development called commitment, but that low poly(ADP-ribose) levels exist during the overt expressional phase of differentiation (Cherney *et al.*, 1982; Porteous & Pearson, 1982; Hacham & Ben-Ishai, 1984). It is not certain whether DNA strand breaks, or chromatin structural changes resulting in an increased availability of ADP-ribose acceptors, are responsible for these changes in enzyme activity. Farzaneh *et al.* (1982) and Johnstone & Williams (1982) concluded that spontaneous DNA strand breaks are responsible for activating the ADPRT during early differentiation events in chick myeloblasts and human lymphocytes respectively. This contrasts with the observations of Hacham & Ben-Ishai (1984) and of Althaus *et al.* (1982), who found that transient and spontaneous alterations in ADPRT activity in primary hepatocyte cultures are not causally related to DNA fragmentation. Furthermore Jackowski & Kun (1981) found age-dependent variations in ADPRT activity in cardiocyte nuclei in the absence of measurable DNA fragmentation.

Similar discrepancies concerning the ability of ADPRT inhibitors to induce (Terada *et al.*, 1979) or prevent (Brac & Ebisuzaki, 1984) erythroid differentiation in Friend virus infected erythroleukaemic cells have been reported. These discrepancies can possibly be explained by the finding of Morioka *et al.* (1980) that different culture conditions employed, with or without daily medium change, have profound effects on ADPRT activity in differentiated and undifferentiated cell lines.

Recently Caplan & Midwa (1984) suggested an indirect transferase involvement in differentiation events via modulation of cellular nicotinamide levels. In this respect nicotinamide analogues injected into individual fertilized chicken eggs are subsequently transported to limb regions where they cause limb malformation (Cherney *et al.*, 1982). This effect can be blocked by coinjection with nicotinamide. In *Drosophila melanogaster*, ADPRT inhibitors have been shown to retard development, kill larvae in a dose-dependent fashion and induce mitotic recombination in response to γ-irradiation (Ferro *et al.*, 1984). Non-inhibitory analogues such as 3-aminobenzoic acid have no observable physiological effect, further postulating a role for poly(ADP-ribose) during development *in vivo*.

Tanuma & Johnson (1983) have proposed a role for ADP-ribosylation of chromosomal proteins in the regulation of gene expression. Glucocorticoid treatment of mouse mammary 34I tumour cells decreases ADP-ribosylation of various nuclear proteins but especially of HMG 14 and 17, concurrent with the induction of mouse mammary tumour virus gene expression. A decrease in ADP-ribosylation of HMG 14 and 17 could cause subtle changes in chromatin structure making promoter regions more accessible to RNA polymerase II.

Initiation of transcription catalysed by RNA polymerase II is important in the regulation of eukaryotic gene expression. Slattery *et al.* (1983) have recently shown that ADPRT suppresses random transcription initiation in a reconstituted system on DNA templates in which nicks were introduced with DNAase. This suppression occurred in the absence of NAD^+, the substrate for the transferase. Automodified transferase, on the other hand, resulting when NAD^+ was present in the incubation, increased random transcription initiation, since there is presumably electrostatic repulsion between the phosphate groups in DNA and those in the poly(ADP-ribose) covalently attached to the transferase, favouring dissociation of the enzyme–DNA complex.

Ohtsuki *et al.* (1984) showed that it is the DNA-binding domain of the transferase which is responsible for preferentially inhibiting the random transcription initiation in a HeLa-cell lysate, resulting in the production of run-off RNA initiated from the correct initiation site on truncated DNA.

Steroid hormones are clearly established as influencing gene expression and their effects on ADP-ribosylation reactions are also, therefore, of interest in this regard. Testosterone deficiency, induced by castration, was found to lead to a decrease in total ADP-ribose residues in mouse kidney restorable upon daily injection with the hormone (Gartemann *et al.*, 1981); oestrogen was found to stimulate the ADPRT in quail oviduct (Muller *et al.*, 1984); and progesterone was found to induce ADPRT activity in *Xenopus* oocytes (Burzio & Koide, 1977). ADP-ribose has been implicated, through its steric role in the formation of histone dimers, in the condensation and relaxation of lampbrush chromosomes induced by progesterone treatment (Shimoyama *et al.*, 1982).

ADP-ribosylation and neoplastic transformation

Neoplastic and rapidly proliferating cells have significantly higher ADPRT activities than have normal or resting cells (Hayaishi & Ueda, 1977). Of all nucleated eukaryotic cells only mature

granulocytes lack ADPRT activity. Granulocytes from patients with acute myeloblast leukaemia, and leukaemic cells undergoing blastic crisis in chronic myeloid leukaemic patients, however, retain high ADPRT activities. Aberrant poly(ADP-ribose) metabolism has also been implicated in the development of skin, cervical and colorectal cancers (Ueda & Hayaishi, 1982; Hirai *et al.*, 1981). Briefly, epidermal and cervical epithelial cells differentiate into enucleated highly keratinized exofoliative cells in squamous layers, concomitant with the loss of RNA, DNA and poly(ADP-ribose) synthesizing activities. ADP-ribosyl transferase activities are greatly elevated, however, in tumour cells of basal cell epithelioma, malignant melanoma, and cervical and colorectal carcinomas. Even in pre-malignant colorectal polyps ADPRT activity is significantly increased (Hirai *et al.*, 1981). Thus, immunostaining of smear cells for poly(ADP-ribose) may be a useful tool for identifying cervical malignancy and other morbidities before any pathological changes are detectable using conventional cytological analyses.

It is now well established that most, if not all, carcinogenic agents affect DNA structure (and are therefore possibly mutagenic). The consensus of opinion seems to favour the notion that DNA strand breaks, produced as a consequence of carcinogenic action, are followed by a quick and extensive response by ADPRT to restore chromatin structure. Thus, ADPRT modulators affect the actions of DNA damaging agents and it therefore seems conceivable that ADPRT inhibitors play a part in promoter action on chemical oncogenesis.

What then is the sort of evidence that ADPRT plays a role in cell transformation?

Borek *et al.* (1984) describe malignant transformation induced by various agents as a multistage process initiated by the induction of DNA damage and fixation of this damage by DNA and cellular replication. We know that ADPRT inhibitors potentiate the cytotoxic effects of *N*-methyl-*N*-nitrosourea, dimethyl sulphate, γ-irradiation, BCNU, and bleomycin. For example Kawamitsu *et al.* (1981) observed that 3-aminobenzamide markedly potentiates the action of bleomycin to suppress the growth of EAT cells transplanted intraperitoneally into mice. The suppression index of bleomycin was 5% whereas that of bleomycin and 3-aminobenzamide together was 83%. As a consequence of this it might be expected that ADPRT inhibitors would inhibit cancer formation by preventing the potential proliferation of transformed cells.

As regards the initial transformation events, work from Konishi's laboratory (Takahashi *et al.*, 1982; Konishi *et al.*, 1984) showed that ADPRT inhibitors enhanced induction of γ-glutamyl transpeptidase-positive foci in livers of rats treated with diethyl nitrosamine. In addition, Lubet *et al.* (1984) reported that enhanced transformations, as well as toxicity, resulted when cultured Balb/3T3 cells were treated with 3-aminobenzamide and short-chain alkylating agents together.

In contrast to these reports Kun *et al.* (1983) found that ADPRT inhibitors block neoplastic transformation in human fibroblasts subjected to transforming but non-toxic doses of carcinogens. This finding has been extended by Borek *et al.* (1984) who found that benzamide and 3-aminobenzamide, at doses low enough to prevent side effects, inhibit malignant transformation *in vitro* in hamster embryo and mouse C3H10T½ cells. The suppression of this transformation is in sharp contrast to the effects of ADPRT inhibitors in enhancing sister chromatid exchange and killing of alkylated cells.

The above discussion then leaves something of a dilemma as regards the reported opposing effects of ADPRT inhibitors on cell transformation induced by chemical carcinogens. A number of factors may be responsible for these apparent discrepancies.

(1) Cells are reported to possess both ADP-ribose dependent and -independent DNA repair pathways (Bohr & Klenow, 1981; Zwelling *et al.*, 1982) and the independent pathway is suggested to be more rapid and error-prone than the dependent pathway. This may not be the case, however, in the putative independent pathway in all cell types. Therefore, when ADPRT is inhibited, cells presumably switch to an ADP-ribose-independent pathway and, depending on the characteristics of this in various tissues, different results may be observed.

(2) Contrasting results may be due simply to the different concentrations of ADPRT inhibitor used (Kun *et al.*, 1983). The transferase may be incompletely inhibited in some cases, since the extent of activation of this enzyme in the first place is dependent on the initial extent of DNA damage caused by the carcinogens.

(3) The cellular response to different extents of transferase activation *per se* may also be important. For example, when extensive DNA damage results in highly activated ADPRT this may deplete NAD and ATP levels to such an extent that cell mortality results (Sims *et al.*, 1983).

Finally, it should be noted that there is no evidence that transformation results from inaccurate repair of strand breaks for any DNA-damaging agent. Indeed there is evidence that alkylating agents, for example, induce tumours by the formation of O^6-alkylguanine, which miscodes during replication (Goth & Rajewsky, 1974; Grover, 1979).

Effect of ADP-ribosylation on chromatin structure

Since dynamic changes in chromatin structure may be expected to accompany, and indeed influence, all the major chromatin-associated events such as DNA replication, repair and transcription, we complete this article with some final considerations on the involvement of protein ADP-ribosylation in these changes.

Much of the work on this topic comes from the laboratory of Mark Smulson, who established in earlier work that intrinsic ADPRT was chromatin-bound and that it was maximally active *in vitro* when associated with an isolated chain of 8–10 nucleosomes (summarized by Butt & Smulson, 1982). The transferase activity decreased with further increasing nucleosome chain length but stabilized at $n = 13$. They reported the crosslinking of ADP-ribosylated histone H1 molecules, dependent on NAD^+ concentration and incubation time, and proposed a role for this complex in chromatin condensation. It was considered that the highly basic ends of histone H1 probably interacted solely with DNA (Allan *et al.*, 1980) and that poly(ADP-ribosyl)ation of the *C*- and *N*-termini led to changes in histone H1–DNA and H1–H1 interactions with facilitated chromatin condensation. The report that the melting temperature of such an ADP-ribosylated chromatin increases supports this notion (Janakidevi & States, 1980).

The observations of Poirier *et al.* (1982*b*), however, lead apparently to contrary conclusions in that they found a relaxation of chromatin structure when isolated nucleosomes were ADP-ribosylated with purified calf thymus ADPRT. Although these workers also detected poly(ADP-ribose)–H1 complexes in their nucleosomal fraction they did not observe the nucleosomal aggregation described from Smulson's laboratory.

It is difficult at this time to reconcile these discrepancies. The use of added purified ADPRT (Poirier *et al.*, 1982*b*) compared with that of the intrinsic enzyme (Smulson's group) may be a contributory factor. Additionally, Poirier *et al.* (1982*b*) proposed that relaxed domains of chromatin structure induced by poly(ADP-ribosyl)ation could be present within the nucleosomal aggregates described by Smulson and co-workers.

This is a topic which clearly has yet to be resolved.

Subsequent work has since demonstrated the occurrence *in vivo* of cross-linked ADP-ribosylated H1 dimers (Wong *et al.*, 1983). In this dimer the *C*-terminus of one histone is modified predominantly by monomeric ADP-ribose and elongation (up to 15 residues) occurs from the *N*-terminus of the other histone molecule (Wong *et al.*, 1984).

It is proposed that the role of the H1 complex *in vivo* may involve the cross-linking of specific chromatin domains while they undergo DNA repair or replication (Thraves & Smulson, 1982). In this context we can speculate further. During chromatin replication, or indeed transcription, the nucleosomal histone octamers probably dissociate from at least one strand of the DNA. Their binding to poly(ADP-ribose) would provide a means for maintaining the histones as octamers ready to rebuild the solenoid after the passage of the replication or transcription polymerases and associated proteins. In addition, the sequestration of positively charged nucleosomal histones by negatively charged poly(ADP-ribose) may also facilitate the dissociation of nucleosomes from the DNA.

Current work on histone post-translational modifications, including phosphorylation and acetylation (Malik & Smulson, 1984; Whitlock *et al.*, 1980), suggests that similar domains of chromatin may be jointly accessible to various modifications of histones (Wong & Smulson, 1984). What the temporal and topographical relationships are between these modifications and how they work to regulate various biological functions has yet to be determined.

Concluding remarks

Covalent modification of nuclear proteins by ADP-ribosylation is a major post-translational modification which is gaining an increasing amount of attention, particularly due to its postulated role in DNA repair processes. Because of its proposed involvement in this and other major cellular events such as cell proliferation, differentiation and transformation, the ADP-ribose field is currently attracting many new workers, principally from established mammalian DNA repair laboratories. Therefore, in the next few years we can look forward to applying our growing understanding of precise molecular mechanisms regarding the role of poly(ADP-ribosyl)ation in metabolic control processes.

Perhaps the most exciting development, at least from a clinical standpoint, is the use of ADP-ribosyl transferase inhibitors in potentiating the cytotoxicity of antitumour agents, such as bleomycin and BCNU. Further investigation of the metabolic events affected by ADPRT inhibitors, and in particular the identification of critical proteins whose modification is affected by these inhibitors, should help to elucidate the mechanisms of transformation and carcinogenesis *in vitro*. If information obtained from such approaches can be successfully exploited in the situation *in vivo* they might lead to a method of blocking oncogenesis, the practical import of which cannot be overstated.

We thank the Science and Engineering Research Council for financial support, and Professor H. M. Keir for his encouragement. We are indebted to Professor Sydney Shall and Dr. Farzin Farzaneh, Universities of Sussex and London, for their helpful comments during the preparation of this article. We also acknowledge with much gratitude the patient efforts of Miss Fiona Grant in typing this manuscript.

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