Journal of Cell Science 102, 663-670 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

COMMENTARY

Poly ADP-ribosylation: a histone shuttle mechanism in DNA excision repair

FELIX R. ALTHAUS

University of Zürich-Tierspital, Institute of Pharmacology and Biochemistry, Winterthurerstrasse 260, CH-8057 Zürich, Switzerland

Summary

In DNA excision repair of mammalian cells, the processing of ADP-ribose by the poly ADP-ribosylation system of chromatin is stimulated several thousand-fold. Most of this turnover is associated with the automodification reaction of the nuclear enzyme poly(ADP-ribose) polymerase and the degradation of polymerase-bound polymers by the enzyme poly(ADP-ribose) glycohydrolase. The automodification cycle catalyzes a temporary dissociation from and reassociation of histones with DNA. It is proposed that this mechanism, termed "histone shuttle", may guide specific proteins to sites of repair. In addition, histone shuttling driven by the poly ADP-ribosylation system seems to be involved in nucleosomal unfolding of chromatin in DNA excision repair.

Key words: histones, DNA accessibility, NAD^+ metabolism.

Introduction: Poly ADP-ribosylation, a Post-translational Protein Modification?

Poly(ADP-ribose) is a homopolymer of repeating adenosine diphosphate ribose units. Polymers containing up to 200 ADP-ribose residues and multiple branches may possibly be found in all eukaryotic cells except yeast, where the existence of poly(ADP-ribose) remains a controversial issue (e.g. see Park et al., 1991). The reducing end of ADP-ribose polymers is covalently bound to nuclear proteins and, hence, poly(ADP-ribose) has been classified as a *post-translational protein modification* (for recent overviews see Ueda, 1987; Althaus and Richter, 1987; De Murcia et al., 1988; Jacobson and Jacobson, 1989; Poirier and Moreau, 1992). While this definition is formally correct, it may not reflect the primary function of poly(ADP-ribose) . The term "post-translational modification" implies that the modifying residue modulates protein function by *covalent* modification of the target acceptor. However, poly(ADP-ribose) is a variably sized *macromolecule* of complex structure and its molecular mass may exceed that of the protein acceptor. Thus, while the covalent bond to proteins may be a *prerequisite* for its biological activity, poly(ADP-ribose) may function primarily via *non-covalent* interactions with other macromolecules (Althaus and Richter, 1987). Several years of research focusing on this possibility and, in parallel, significant advances in the understanding of the poly ADP-ribosylation reaction have led us to propose that the poly ADP-ribosylation system of chromatin functions as a histone shuttle mechanism in DNA excision repair (Althaus et al., 1989, 1990, 1991, 1992). The present commentary focuses on recent experimental evidence relevant to this concept.

The Enzymatic Components of the Poly ADP-ribosylation System

The poly ADP-ribosylation reaction was discovered in 1966 (Chambon et al., 1966), and since then the enzymatic components involved in this reaction have been extensively characterized. Briefly, a multienzyme system, localized in chromatin, processes the ADP-ribosyl moiety of the respiratory coenzyme NAD^+ through a series of biosynthetic and catabolic steps. Three major enzymes are involved in these reactions: (i) *poly(ADP-ribose) polymerase* (EC 2.4.2.30), a DNA-binding enzyme, which utilizes NAD^+ as the substrate for the biosynthesis of protein-bound poly(ADP-ribose); (ii) *poly(ADP-ribose) glycohydrolase,* which degrades protein-bound polymers down to the protein-proximal ADP-ribose residue; and (iii) *ADP-ribosyl protein lyase*, which removes the protein-proximal ADP-ribose residue from the acceptor (for reviews see Ueda, 1987; Althaus and Richter, 1987). The total ADP-ribose-processing capacity of this system in mammalian cells is quite impressive, i.e. some 10^7 ADP-ribose residues per min per cell for poly(ADP-ribose) polymerase and likewise for poly(ADP-ribose) glycohydrolase, and about 72×10^6 residues for the enzyme ADP-ribosyl protein lyase (for reference: about 10^9 molecules of NAD^+/cell). Apart from these proteins, other enzymatic activities have been implicated in poly(ADP-ribose) catabolism. A

polymer-degrading phosphodiesterase activity has been identified in rat liver and plant tissue (Futai and Mizuno, 1967; Futai et al., 1968; Shinshi et al., 1976). ADP-ribose resulting from polymer degradation may be further processed to AMP by an ADP-ribose pyrophosphatase (Miro et al., 1989), or to ATP by an ADP-ribose pyrophosphorylase (Tanuma, 1989). Further investigations are needed to determine the significance of these other activities in poly(ADP-ribose) metabolism.

Poly(ADP-ribose) Polymerase

Purified poly(ADP-ribose) polymerase binds specifically to and is activated by single- and double-strand DNA breaks (Benjamin and Gill, 1980a,b; Ménissier-De Murcia et al., 1989). Two zinc finger motifs in the N-terminal domain of the polymerase determine these binding specificities (Mazen et al., 1989; Gradwohl et al., 1990; Ikejima et al., 1990). In DNA excision repair of living cells, such sites appear either directly as a consequence of DNA damage (e.g. ionizing radiation), or indirectly following enzymatic incision (for review see Friedberg, 1985; Lambert and Laval, 1989). Poly(ADP-ribose) polymerase is activated in vivo concomitant with the appearence of DNA strand breaks (e.g. see Cohen and Berger, 1981; Berger and Sikorski, 1981; Cohen et al., 1982; McCurry and Jacobson, 1989), and becomes a predominant acceptor for ADP-ribose polymers (Ogata et al., 1981; Adamietz, 1987). Thus, upon activation, the enzyme poly(ADP-ribose) polymerase converts into a protein carrying multiple ADP-ribose polymers of various size and branching frequencies ("automodification reaction"). Recent estimates suggest that up to 28 polymers are covalently bound to a single polymerase molecule (Desmarais et al., 1991). The functional consequences of automodification are loss of DNA binding affinity and inactivation of the polymerase (Ferro and Olivera, 1982; Zahradka and Ebisuzaki, 1982; De Murcia et al., 1983; Gaudreau et al., 1986).

What is the molecular mode of automodification and how is it regulated? In vitro reconstitution experiments have identified the following sequence of events: after activation by DNA strand breaks, purified poly(ADP-ribose) polymerase produces a distinct pattern of polymers which are bound to a small number of polymerase molecules. As the reaction progresses, more enzyme molecules become automodified with a polymer pattern identical to the one produced in the first round of synthesis. Finally, when the reaction comes to a halt, all available polymerase molecules are automodified. Thus, in vitro, automodification of the polymerase follows a processive reaction mode (Naegeli et al., 1989). Processivity has also been observed in more complex model systems, such as in nucleosomal core particles, and in isolated nuclei, albeit with different results (Naegeli and Althaus, 1991). The numbers, size and branching frequencies of polymers were different from those synthesized by purified poly(ADP-ribose) polymerase. This led to the identification of histones H1, H2A, H2B, H3 and H4 as specific regulators of polymer patterns (Naegeli and Althaus, 1991). Histones apparently interfere with the polymer termination reaction of poly(ADP-ribose) polymerase. Thus, taking the two steps together, poly(ADP-ribose) polymerase moves processively, and when it encounters DNA-bound histones, it adapts by producing histone-type specific patterns of ADP-ribose polymers. No adaptation occurs in the presence of other DNA-binding or non-binding proteins (Naegeli and Althaus, 1991; Panzeter et al., 1992). Processivity and adaptativeness of the automodification reaction of poly(ADP-ribose) polymerase, and the inactivation and dissociation of the automodified enzyme from DNA are important features of the poly(ADP-ribose)-histone shuttle mechanism to be considered below.

Poly(ADP-ribose) Glycohydrolase

In DNA excision repair of mammalian cells, elevated poly(ADP-ribose) biosynthesis is coupled with a stimulation of polymer catabolism. At high levels of DNA damage, the catabolic half-life of poly(ADP-ribose) may be less than 40 seconds. The rapid turnover contrasts with the slower catabolism of a constitutive polymer fraction exhibiting a half-life of 7.7 hours in undamaged cells (Alvarez-Gonzalez and Althaus, 1989). What is the mechanistic basis for differential polymer catabolism in vivo? Hatakeyama et al. (1986) have shown that purified poly(ADP-ribose) glycohydrolase, a $2' \Rightarrow 1''$ exoglycosidase (Miwa et al., 1974), operates in a biphasic, bimodal reaction mode. Briefly, large polymers are degraded to smaller polymers in a fast and processive reaction. Further degradation then proceeds more slowly and in a distributive reaction mode. Rapid initial degradation of large polymers may be further accelerated by initial endoglycosidic incision (Ikejima and Gill, 1988). On the basis of these studies, polymer catabolism emerges as a highly organized process, in which the order and kinetics of degradation is largely determined by the size (and structural complexity?) of the substrate. In view of the shuttle mechanism to be discussed below, it is important to note that poly(ADP-ribose) glycohydrolase plays an important role in reversing the automodified state of poly(ADP-ribose) polymerase. This reactivates the polymerase and restores DNA binding (Zahradka and Ebisuzaki, 1982; Gaudreau et al., 1986).

The Problem of DNA Accessibility in Chromatin during DNA Excison Repair

Numerous observations support the view that chromatin structure plays an important role in DNA excision repair (for review see Friedberg, 1985; Smerdon, 1989). Most experimental data pertain to the nucleosomal level of chromatin organization and show that the tight association of DNA with histones and possibly other chromosomal proteins places constraints on the accessi-

bility of DNA repair enzymes to damaged sites in repair. Likewise, the nucleosomal organization may itself affect the distribution of damaged sites in DNA (Smerdon, 1989), and sites of base damage in nucleosomes are less accessible to various enzyme probes than sites in naked DNA (e.g. see Wilkins and Hart, 1974; Van Zeeland et al., 1981; Evans and Linn, 1984; Smerdon and Thoma, 1990). More recently, work with cell-free lysate systems for chromatin assembly and repair has shown that the assembly of DNA into nucleosomes is associated with marked suppression of nucleotide excision repair, which apparently occurs at steps preceding repair synthesis (e.g. see Wang et al., 1991). Moreover, DNA-processing enzymes and proteins involved in transcription are increasingly tested on histone-associated DNA substrates, and in several instances histones turned out to be inhibitory. Examples include DNA ligase (Ohashi et al., 1983), DNA topoisomerase I (Richter and Ruff, 1991; Richter and Kapitza, 1991), DNA helicase (Thömmes and Hübscher, 1990), RNA polymerase II (Laybourn and Kadonaga, 1991) and transcription factors (for review see Felsenfeld, 1992).

In DNA excision repair of mammalian cells, the tight association of histones with DNA is locally disrupted. Newly synthesized repair patches appear transiently in DNA regions which are accessible to chemical and enzymatic probes. In this process, nucleosomes are unfolded concomitant with incision (Smerdon, 1989) and with the activation of the poly ADP-ribosylation system (for review see Althaus and Richter, 1987). Unfolding involves DNA regions of up to 2000 bp in size (Mathis and Althaus, 1990a) and follows a characteristic periodicity (Mathis and Althaus, 1986). After repair synthesis and ligation, these domains are rapidly refolded (Smerdon, 1986), followed by a slow repositioning of core histones (for an overview see Smerdon, 1989; Sidik and Smerdon, 1992). The unfolding of nucleosomes as well as the excision of bulky DNA adducts is completely blocked in poly(ADP-ribose)-depleted cells (Mathis and Althaus, 1990b). Several parallels in these processes suggest that poly ADP-ribosylation may be a mechanism involved in nucleosomal unfolding: (i) the coincidence of highly elevated poly(ADP-ribose) turnover in chromatin and the unfolding of nucleosomes, both initiated by endonucleolytic incision of DNA strands; (ii) the absence of nucleosomal unfolding when the poly ADP-ribosylation system is completely turned off by inhibitors of poly(ADP-ribose) polymerase; and (iii) the observation that elevated poly(ADP-ribose) turnover approaches the low predamage levels with a half-life similar to that of nucleosomal refolding (Althaus et al., 1992). This raises the question of how the poly ADP-ribosylation system of chromatin could be involved in nucleosomal unfolding and refolding.

The Poly ADP-ribosylation System: a Histone Shuttle Mechanism

Results obtained in model systems of various complexities suggest that the automodification reaction of poly(ADP-ribose) polymerase serves to shuttle histones off and back onto DNA (Althaus et al., 1989, 1990, 1991, 1992). The first model system involves a preparation of nucleosomal core particles which retain endogenous poly(ADP-ribose) polymerase in active form. Automodification of the polymerase causes the dissociation of the 146 bp core DNA fragment from core particles as detectable by reversed mobility shift gel electrophoresis (Mathis and Althaus, 1987). DNA dissociation and polymer synthesis follow parallel time courses and inhibition of polymer synthesis with competitive polymerase inhibitors, or in the presence of α-anomeric NAD (which is not a substrate for the enzyme), prevents the release of DNA. This demonstrates the potential of the automodification reaction of poly(ADP-ribose) polymerase to interfere with histone-DNA interactions at the nucleosomal level of chromatin.

Automodified poly(ADP-ribose) polymerase dissociates from DNA (Ferro and Olivera, 1982; Zahradka and Ebisuzaki, 1982; Gaudreau et al., 1986). The polymers attached to the polymerase are more acidic than DNA (two negative charges per monomer unit as compared to only one per nucleotide of DNA) and therefore should compete with DNA for histone binding. In fact, filter binding studies suggest competition of free ADP-ribose polymers with DNA for binding of histones H1, H3, and H4 (Wesierska-Gadek and Sauermann, 1988). Thus, electrostatic interactions of polymerase-bound polymers with basic proteins such as histones could account for the release of DNA from nucleosomal core particles. However, the experimental evidence shows that the interaction of histones with ADP-ribose polymers is far stronger and more specific than would be expected on the basis of electrostatic interactions (Panzeter et al., 1992). Complexes of ADP-ribose polymers with histones H1, H2A, H2B, H3 and H4 resist phenol partitioning, strong acids, detergents, and high salt concentrations. The following rules define these interactions: branched polymers exhibit the highest binding affinities, followed by long linear polymers and short linear polymers, and among histones the hierarchy of binding is H1 > H2A > H2B = H3 > H4 (Panzeter et al., 1992). For histone H1, the primary site of polymer binding is the carboxy-terminal domain, which is also the domain most effective in inducing higher order structure of chromatin (Thoma et al., 1983). Panzeter et al. (1992) have also examined the specificity of these non-covalent interactions using a high-stringency binding assay. Surprisingly, among 28 basic as well as acidic DNA-binding and non-binding proteins tested, only histones bound to ADP-ribose polymers. Thus, the polymers of automodified poly(ADP-ribose) polymerase have the capacity to target histones selectively for dissociation from DNA. In fact, ADP-ribose polymers covalently bound to the polymerase are about 10 times more potent in dissociating histone H2B from H2B-DNA complexes as compared to free polymers (Realini and Althaus, 1992; Althaus et al., 1992). Conversely, histones bind with

strong preference to ADP-ribose polymers when automodified poly(ADP-ribose) polymerase and DNA are presented as binding partners. Under these conditions, histone-DNA complexes are formed only after all polymer binding sites are saturated. The branching points of ADP-ribose polymers turned out to be the sites with highest binding affinities for histones, as detected by nuclease protection analysis (Realini and Althaus, 1992; Althaus et al., 1992).

Like the automodification reaction of poly(ADP-ribose) polymerase, the dissociation of histone-DNA complexes proceeds in a processive manner, i.e. in the first round of automodification, a small number of histone-DNA complexes is completely stripped of histones, and as more automodified polymerase molecules are formed, another subset of complexes is stripped until finally all complexes are dissociated. Intermediates typical of a distributive reaction, i.e. complexes partially depleted of histones, have not been observed (Realini, 1991; Realini and Althaus, 1992; Althaus et al., 1992). Calculations of the reaction stoichiometry revealed that 40 ADP-ribose residues bound to the polymerase can dissociate the entire histone complement of a chromatosome (i.e. a chromatin particle containing 165 bp DNA, eight core histones, and one molecule of histone H1; Simpson, 1978). Compared to other naturally occurring polyanions, such as poly(A), tRNA and heparin, poly(ADP-ribose) is a 100 to 1000 times more potent in dissociating histone-DNA complexes when calculated for equivalent numbers of negative charges (Panzeter et al., 1992; Realini, 1991; Realini and Althaus, 1992; Althaus et al., 1992).

The automodification reaction of poly(ADP-ribose) polymerase affects histone-DNA complexes in other ways. The DNA becomes susceptible to micrococcal nuclease digestion, and this phenomenon parallels the increase in automodified polymerase molecules. Like histone-DNA complex dissociation, development of micrococcal nuclease susceptibility occurs processively and is inhibited by inhibitors of poly(ADP-ribose) polymerase. Similar results have been obtained with DNase I as the probing enzyme (Realini, 1991; Realini and Althaus, 1992; Althaus et al., 1992). Finally, DNA released from histone-DNA complexes is accessible to other DNA binding proteins including histones H1, H2A, H2B, H3 and H4. Interestingly, this is also true for purified DNA helicase from calf thymus, an enzyme implicated in DNA replication and repair (Thömmes and Hübscher, 1990). The strand separation reaction catalyzed by DNA helicase is completely inhibited when histone H1 or core histones are present on the DNA template. Sequestration of histones by ADP-ribose polymers establishes template accessibility and reactivation of DNA helicase (Thömmes et al., 1992). Thus, the automodification of poly(ADP-ribose) polymerase may directly affect histone-DNA interactions so that DNA becomes locally accessible to other proteins (Realini, 1991; Realini and Althaus, 1992; Althaus et al., 1992).

The reverse reaction, i.e. the reassembly of dissociated histone-DNA complexes is effected by poly(ADP-ribose) glycohydrolase (Realini, 1991; Realini and Althaus, 1992; Althaus et al., 1992). Several aspects of this reaction are noteworthy. The reassembly of histone-DNA complexes is a two-step reaction, the first one leading to the formation of complexes which, by mobilitiy shift analyses, are indistinguishable from the complexes prior to dissociation. This reaction is complete after partial degradation of polymerase-bound polymers, but the DNA of these complexes is not yet fully protected from micrococcal nuclease or DNase I digestion. The second step involves establishment of full nuclease resistance and this requires additional polymer degradation. Several aspects of the poly(ADP-ribose) glycohydrolase reaction are noteworthy. Initially, polymers are degraded in an endoglycosidic and exoglycosidic manner, and linear polymers are degraded faster than branched polymers (Braun, 1991; Braun and Althaus, unpublished observations). The slower degradation of branched polymers is attributable to the strong binding of histones to the sites of branching (Braun, 1991; Realini, 1991). Thus, the degradation of polymerase-bound polymers and the concomitant reassembly of histone-DNA complexes emerges as a highly organized process which could determine the order of reassociation of different histone species with DNA (Althaus et al., 1992). Various factors, such as the numbers, sizes and complexities of polymers, the differential polymer binding affinities of different histone species, and the differential processing of polymers by poly(ADP-ribose) glycohydrolase seem important in this process.

In summary, poly(ADP-ribose) polymerase and poly(ADP-ribose) glycohydrolase may act in concert to remove histones temporarily from sites of DNA strand breaks and put them back on. This allows other proteins to gain access to DNA temporarily at the site of shuttling. The shuttle mechanism operates in a processive, adaptive, target-specific and reversible manner.

Evidence for Histone Shuttling in DNA Excision Repair in Vivo

In DNA excision repair of mammalian cells, the tight association of histones with DNA is locally disrupted and domains engaged in repair become accessible to chemical and enzymatic probes (for review see Smerdon, 1989). In poly(ADP-ribose)-depleted cells, this process is blocked and bulky DNA adducts are no longer excised (Mathis and Althaus, 1990b). These results are compatible with the view that histone shuttling is involved in nucleosomal unfolding. The molecular steps leading to activation of poly(ADP-ribose) polymerase after DNA damage have also been demonstrated in vivo (cf. Skidmore et al., 1979, 1980; Juarez-Salinas et al., 1979; Durkacz et al., 1980). Expression of the subcloned DNA-binding domain of poly(ADP-ribose) polymerase in transfected monkey cells blocks postincisional activation of the resident enzyme following carcinogen treatment (Küpper et al.,

1990). This indicates that in vivo, poly(ADP-ribose) polymerase is indeed specifically targeted to sites of DNA strand breaks. Moreover, poly(ADP-ribose) polymerase forms branched polymers in vivo (Juarez-Salinas et al., 1982) and sequential automodification of the polymerase ("processivity") has also been observed in living cells (Cole et al., 1991). The polymer pattern synthesized in mammalian cells in response to carcinogen treatment is similar to that formed in nuclei and isolated core particles (Malanga and Althaus, 1992), suggesting that histones are also the predominant regulators of polymer sizes and branching in vivo ("adaptiveness"). Finally, degradation of ADP-ribose polymers in repairing cells (Alvarez-Gonzalez and Althaus, 1989) exhibits the biphasic pattern characteristic of poly(ADP-ribose) glycohydrolase action in vitro (Hatakeyama et al., 1986), and the extent of stimulation of polymer turnover depends on the level of DNA damage (Alvarez-Gonzalez and Althaus, 1989). Likewise, refolding and repositioning of nucleosomes after ligation of repair patches is a biphasic reaction (Smerdon, 1989; Sidik and Smerdon, 1992).

Conclusions

Two types of conclusions emerge from the evidence discussed above. First, the in vitro reconstitution approach has clearly demonstrated the *potential* of the poly ADP-ribosylation system to function as a histone shuttle mechanism on DNA. In addition, this approach has brought to light several key features of the mechanisms involved, such as processivity, adaptiveness, specificity and reversibility. With this information, we can specifically ask whether or not the poly ADP-ribosylation system exhibits any of these features when it operates in vivo. Thus, the second type of conclusion pertains to the in vivo evidence, which indeed suggests that several key features of the shuttle mechanism are expressed in living mammalian cells: (1) *Processivity*, i.e. sequential addition of polymers to poly(ADP-ribose) polymerase in combination with a highly conserved pattern of polymers. (2) *Adaptiveness*, i.e. a polymer size pattern very similar to the one produced by poly(ADP-ribose) polymerase when adapting to the presence of histones in vitro (suggesting that histones are also the predominant regulators in vivo). (3) *Reversibility*, i.e. the rapid catabolism of ADP-ribose polymers, which is quantitativly and qualitatively similar to the reaction catalyzed by poly(ADP-ribose) glycohydrolase in vitro.

Apart from these features, a number of striking parallels have been noted, such as the coincidence of postincisional activation of the poly ADP-ribosylation system and the onset of nucleosomal unfolding, and the coincidence of nucleosomal refolding with the deactivation of the poly ADP-ribosylation system. Moreover, the stimulation of poly(ADP-ribose) turnover and nucleosomal unfolding are both dependent on the level of DNA damage. Finally, inhibition of poly(ADP-ribose) polymerase in mammalian cells blocks nucleosomal unfolding coincident with the repair of bulky DNA adducts, suggesting that the poly ADP-ribosylation system is indeed involved in dissociating histones from nucleosomal DNA.

Relevance to Previous Models for the Role of Poly ADP-ribosylation in DNA Excision Repair

The work of Shall and associates (Durkacz et al., 1980; Shall, 1984) and, subsequently, several hundred reports have established and confirmed that the poly ADP-ribosylation participates in DNA excision repair (for reviews see Althaus and Richter, 1987; Jacobson and Jacobson, 1989; Poirier and Moreau, 1992), but the molecular mechanism(s) involved have remained elusive. On the basis of the evidence available in the early eighties, Shall and associates proposed an involvement of poly ADP-ribosylation in the ligation of DNA strand breaks (Shall, 1984). At first glance, the concept of a histone shuttle mechanism driven by the poly ADP-ribosylation system (Althaus et al., 1992) seems to contradict the notion that poly ADP-ribosylation is involved in the regulation of ligation activity in DNA excision repair (Shall, 1984). However, experimental evidence obtained by Ohashi et al. (1983) provides an important link between the two concepts. They demonstrated that the apparent stimulation of DNA ligase activity by poly ADP-ribosylation results from a reversal of DNA ligase inhibition by histone H1. Thus, the activation of DNA ligase can be rationalized by the histone shuttle mechanism, and the function of the poly ADP-ribosylation system is to help DNA ligase gain access to DNA repair sites by removing histones. Likewise, the action of poly(ADP-ribose) polymerase inhibitors on DNA damage processing, described in numerous reports (for review see Shall, 1984; Althaus and Richter, 1987; Jacobson and Jacobson, 1989; Poirier and Moreau, 1992) can also be explained on the basis of the shuttle mechanism.

Perspectives

It is conceivable that histone shuttling by the poly ADP-ribosylation system is involved in other chromatin functions apart from DNA repair. Shall and associates (Bertazzoni et al., 1989) have proposed that poly ADP-ribosylation may be involved in all cases of DNA breakage and rejoining, such as in DNA recombinations, sister-chromatid exchange, plasmid insertion into the genome, and gene rearrangements. These possibilities have to be addressed. Furthermore, several aspects of poly ADP-ribosylation are not explained by the shuttle mechanism. For example, a measurable portion of ADP-ribose polymers in vivo is not covalently bound to poly(ADP-ribose) polymerase ("automodification") but to other proteins ("heteromodification"), such as histones and various nuclear enzymes (for review see Ueda, 1987; Althaus and Richter, 1987). The molecular conditions for heteromo-

dification are poorly understood and the biological role of this reaction remains to be elucidated. On the other hand, molecular genetic approaches have refined our understanding of the function of poly(ADP-ribose) polymerase (for review see De Murcia et al., 1991), and will soon be applicable to the study of poly(ADP-ribose) glycohydrolase and hopefully ADP-ribosyl protein lyase, both of which are not easily accessible to biochemical characterization in tissues and cells. Finally, specialized techniques, such as the incorporation of photoreactive substituents in ADP-ribose polymers, could prove useful for the analysis of macromolecular interactions of ADP-ribose polymers with chromatin proteins in vivo.

Addendum

In a report appearing after submission of this manuscript, Satoh and Lindahl (1992) propose that the binding of poly(ADP-ribose) polymerase to DNA strand breaks blocks access for DNA repair enzymes and that this block is released by the automodification of the polymerase. The experimental evidence is as follows: (i) stimulation of overall repair activity in NAD^+-supplemented cell extracts using naked plasmid DNA as a substrate (histones are absent in this extract); (ii) no stimulation of repair activity by NAD^+ in the presence of 3-aminobenzamide, an inhibitor of poly(ADP-ribose) polymerase; (iii) absence of repair stimulation by NAD^+ in extracts treated to remove poly(ADP-ribose) polymerase; and (iv) the demonstration that these cell extracts form acid-precipitable material from NAD^+ (which could be attributable to mono ADP-ribosylation or poly ADP-ribosylation or both). This evidence should be viewed complementary to the work of Zahradka and Ebisuzaki (1982), who actually measured the interaction of unmodified and automodified poly(ADP-ribose) polymerase with DNA and who proposed a similar mechanism. Thus, extending this concept to a repair situation, Satoh and Lindahl (1992) suggest that poly(ADP-ribose) polymerase could play a non-obligatory role in DNA repair by regulating the accessibility of some (unidentified) repair enzymes to naked DNA. However, the absence of histones in their repair system precludes detection of histone shuttling, which is the salient feature of the mechanism presented in this commentary. Nevertheless, the models agree with regard to the automodifcation of poly(ADP-ribose) polymerase and the functional consequences of automodification on the interaction with DNA.

I am grateful to the reviewers for thoughtful comments and the opportunity to comment on Satoh and Lindahl (1992) after submission of this manuscript. My thanks go to Rafael Alvarez, Susanne Bachmann, Stefan Braun, Margaret Collinge, Ralph Eichenberger, Liane Höfferer, Hanna Kleczkowska, Pius Lötscher, Maria Malanga, Georg Mathis, Marianne Mattenberger, Hanspeter Naegeli, Phyllis Panzeter, Claudio Realini, Marie-Claude Richard, Stefan Waser and Barabara Zweifel, who contributed their work and ideas to the development of the concepts presented. Research performed in our laboratory was supported by the Swiss National Foundation for Scientific Research, the Krebsliga, and the Kanton Zürich.

References

Adamietz, P. (1987). Poly(ADP-ribose) synthase is the major endogenous nonhistone acceptor for poly(ADP-ribose) in alkylated rat hepatoma cells. *Eur. J. Biochem.* **169**, 365-672.

Althaus, F. R., Bachmann, S., Braun, S. A., Collinge, M. A., Höfferer, L., Malanga, M., Panzeter, P. L., Realini, C., Richard, M. C., Waser, S. and Zweifel, B. (1992). The poly(ADP-ribose)-protein shuttle of chromatin. In *ADP-Ribosylation Reactions* (ed. Poirier, G.G. and Moreau, P.), Springer, New York (in press).

Althaus, F. R., Collinge, M., Loetscher, P., Mathis, G., Naegeli, H., Panzeter, P. and Realini, C. (1989). The poly ADP-ribosylation system of higher eukaroytes: How can it do what? In *Chromosomal Aberrations - Basic and Applied Aspects* (ed. Obe, G. and Natarajan, A.T.), pp. 22-30. Springer, Berlin, Heidelberg.

Althaus, F. R., Loetscher, P., Mathis, G., Naegeli, H., Panzeter, P. and Realini, C. (1991). Poly ADP-ribosylation in DNA damage processing. *Light Biol. Med.* **2**, 477-484.

Althaus, F. R., Naegeli, H., Realini, C., Mathis, G., Loetscher, P. and Mattenberger, M. (1990). The poly ADP-ribosylation system of higher eukaroytes: A protein shuttle mechanism in chromatin. *Acta Biol.* **41**, 9-18.

Althaus, F. R. and Richter, C. (1987). *ADP-Ribosylation of Proteins: Enzymology and Biological Significance.* Springer Verlag, Berlin, Heidelberg, New York, Tokyo.

Alvarez-Gonzalez, R. and Althaus, F. R. (1989). Poly(ADP-ribose) catabolism in mammalian cells exposed to DNA-damaging agents. *Mutat. Res.* **218**, 67-74.

Benjamin, R. C. and Gill, D. M. (1980a). ADP-ribosylation in mammalian cell ghosts. Dependence of poly(ADP-ribose) synthesis on strand breakage in DNA. *J. Biol. Chem.* **255**, 10,493-10,501.

Benjamin, R. C. and Gill, D. M. (1980b). Poly(ADP-ribose) synthesis in vitro programmed by damaged DNA. A comparison of DNA molecules containing different types of strand breaks. *J. Biol. Chem.* **255**, 10,502-10,508.

Berger, N. A. and Sikorski, G. W. (1981). Poly(adenosine diphosphoribose) synthesis in ultraviolet-irradiated xeroderma pigmentosum cells reconstituted with micrococcus luteus UV endonuclease. *Biochemistry* **20**, 3610-3614.

Bertazzoni, U., Scovassi, A. I. and Shall, S. (1989). Fourth European Meeting on ADP-ribosylation of Proteins, Pavia (Italy), 20-23 April 1989. *Mutat. Res.* **219**, 303-307.

Braun, S. (1991). Molecular mechanisms of poly(ADP-ribose) glycohydrolase action. Thesis, University of Zürich, Switzerland.

Chambon, P., Weill, J. D., Doly, J., Strosser, M. T. and Mandel, P. (1966). On the formation of a novel adenylylic compound by enzymatic extracts of liver nuclei. *Biochem. Biophys. Res. Commun.* **25**, 638-643.

Cohen, J. J. and Berger, N. A. (1981). Activation of poly(adenosine diphosphate ribose) polymerase with UV irradiated and UV endonuclease treated SV40 minichromosomes. *Biochem. Biophys. Res. Commun.* **98**, 268-274.

Cohen, J. J., Catino, D. M., Petzold, S. J. and Berger, N. A. (1982). Activation of poly(adenosine diphosphate ribose) polymerase by SV40 minichromosomes: Effects of deoxyribonucleic acid damage and histone H1. *Biochemistry* **21**, 4931-4940.

Cole, G. A., Bauer, G., Kirsten, E., Mendeleyev, J., Bauer, P. I., Buki, K. G., Hakam, A. and Kun, E. (1991). Inhibition of HIV-1 IIIb replication in AA-2 and MT-2 cells in culture by two ligands of poly(ADP-ribose) polymerase: 6-amino-1,2-benzopyrone and 5-iodo-6-amino-1,2-benzyopyrone. *Biochem. Biophys. Res. Commun.* **180**, 504-514.

De Murcia, G., Huletsky, A. and Poirier, G. G. (1988). Review: Modulation of chromatin structure by poly(ADP-ribosyl)ation. *Biochem. Cell Biol.* **66**, 626-635.

De Murcia, G., Jongstra-Bilen, J., Ittel, M. E., Mandel, P. and Delain, E. (1983). Poly(ADP-ribose) polymerase automodification and interaction with DNA: electron microscopic visualization. *EMBO J.* **2**, 543-548.

De Murcia, G., Ménissier-De Murcia, J. and Schreiber, V. (1991). Poly(ADP-ribose) polymerase: molecular biological aspects. *BioEssays* **13**, 455-462.

Desmarais, Y., Ménard, L., Lagueux, J. and Poirier, G. G. (1991). Enzymological properties of poly(ADP-ribose) polymerase: characterization of automodification sites and NADase activity. *Biochim. Biophys. Acta* **1078**, 179-186.

Durkacz, B., Omidiji, O., Gray, D. A. and Shall, S. (1980). [ADP-ribose]n participates in DNA excision repair. *Nature* **283**, 593-596.

Evans, D. H. and Linn, S. (1984). Excision repair of pyrimidine dimers from simian virus 40 minichromosomes in vitro. *J. Biol. Chem.* **259**, 10252-10259.

Felsenfeld, G. (1992). Chromatin as an essential part of the transcriptional mechanism. *Nature* **355**, 219-224.

Ferro, A. M. and Olivera, B. M. (1982). Poly(ADP-ribosylation) in vitro. Reaction parameters and enzyme mechanism. *J. Biol. Chem.* **257**, 7808-7813.

Friedberg, E. C. (1985). *DNA Repair.* W.H. Freeman, New York.

Futai, M. and Mizuno, D. (1967). A new phosphodiesterase forming nucleoside 5'-monophosphate from rat liver. *J. Biol. Chem.* **242**, 5301-5307.

Futai, M., Mizuno, D. and Sugimura, T. (1968). Mode of action of rat liver phosphodiesterase on a polymer of phosphoribosyl adenosine monophosphate and related compounds. *J. Biol. Chem.* **243**, 6325-6329.

Gaudreau, A., Ménard, L., De Murcia, G. and Poirier, G. G. (1986). Poly(ADP-ribose) accessibility to poly(ADP-ribose) glycohydrolase activity on poly(ADP-ribosyl)ated nucleosomal proteins. *Biochem. Cell Biol.* **64**, 146-153.

Gradwohl, G., Ménissier-De Murcia, J., Molinete, M., Simonin, F., Koken, M., Hoeijmakers, J. H. J. and De Murcia, G. (1990). The second zinc-finger domain of poly(ADP-ribose) polymerase determines specificity for single-stranded breaks in DNA. *Proc. Nat. Acad. Sci. USA* **87**, 2990-2994.

Hatakeyama, K., Nemoto, Y., Ueda, K. and Hayaishi, O. (1986). Purification and characterization of poly(ADP-ribose) glycohydrolase. Different modes of action on large and small poly(ADP-ribose). *J. Biol. Chem.* **261**, 14902-14911.

Ikejima, M. and Gill, D. M. (1988). Poly(ADP-ribose) degradation by glycohydrolase starts with an endonucleolytic incision. *J. Biol. Chem.* **263**, 11,037-11,040.

Ikejima, M., Noguchi, S., Yamashita, R., Ogura, T., Sugimura, T., Gill, D. M. and Miwa, M. (1990). The zinc fingers of human poly(ADP-ribose) polymerase are differentially required for the recognition of DNA breaks and nicks and the consequent enzyme activation. *J. Biol. Chem.* **265**, 21,907-21,913.

Jacobson, M. K. and Jacobson, E. L., eds (1989). *ADP-ribose Transfer Reactions: Mechanisms and Biological Significance.* Springer, New York.

Juarez-Salinas, H., Levi, V., Jacobson, E. L. and Jacobson, M. K. (1982). Poly(ADP-ribose) has a branched structure in vivo. *J. Biol. Chem.* **257**, 607-609.

Juarez-Salinas, H., Sims, J. L. and Jacobson, M. K. (1979). Poly(ADP-ribose) levels in carcinogen-treated cells. *Nature* **282**, 740-741.

Küpper, J. H., De Murcia, G. and Bürkle, A. (1990). Inhibition of poly(ADP-ribosyl) ation by overexpressing the poly(ADP-ribose) polymerase DNA-binding domain in mammalian cells. *J. Biol. Chem.* **265**, 18,721-18,724.

Lambert, M. W. and Laval, J., eds (1989). *DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells.* Plenum Press, New York.

Laybourn, P. J. and Kadonaga, J. T. (1991). Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science* **254**, 238-245.

Malanga, M. and Althaus, F. R. (1992). Analysis of poly(ADP-ribose) molecules during DNA repair in living cells. *Experientia* **48**, A82.

Mathis, G. and Althaus, F. R. (1986). Periodic changes of chromatin organization associated with the rearrangement of repair patches accompany DNA excision repair of mammalian cells. *J. Biol. Chem.* **261**, 5758-5765.

Mathis, G. and Althaus, F. R. (1987). Release of core DNA from nucleosomal core particles following (ADP-ribose)$_n$ modification in vitro. *Biochem. Biophys. Res. Commun.* **143**, 1049-1057.

Mathis, G. and Althaus, F. R. (1990a). Isolation of 8-methoxypsoralen-accessible DNA domains from chromatin of intact cells. *Cell Biol. Toxicol.* **6**, 35-46.

Mathis, G. and Althaus, F. R. (1990b). Uncoupling of DNA excision repair and nucleosomal unfolding in poly(ADP-ribose)-depleted mammalian cells. *Carcinogenesis* **11**, 1237-1239.

Mazen, A., Ménissier-De Murcia, J., Molinete, M., Simonin, F., Gradwohl, G., Poirier, G. G. and De Murcia, G. (1989). Poly(ADP-ribose) polymerase: a novel finger protein. *Nucl. Acids Res.* **17**, 4689-4698.

McCurry, L. S. and Jacobson, M. K. (1981). Poly(ADP-ribose) synthesis following DNA damage in cells heterozygous or homozygous for the Xeroderma pigmentosum genotype. *J. Biol. Chem.* **256**, 551-553.

Ménissier-De Murcia, J., Molinette, M., Gradwohl, G., Simonin, F. and De Murcia, G. (1989). Zinc-binding domain of poly(ADP-ribose) polymerase participates in the recognition of single strand breaks on DNA. *J. Mol. Biol.* **210**, 229-233.

Miro, A., Coistas, M. J., Garcia-Diaz, M., Hernandez, M. T. and Cameselle, J. C. (1989). A specific, low K_m ADP-ribose pyrophosphatase from rat liver. *FEBS Lett.* **244**, 123-126.

Miwa, M., Tanaka, M., Matsushima, T. and Sugimura, T. (1974). Purification and properties of a glycohydrolase from calf thymus splitting ribose-ribose linkages of poly(adenosine diphosphate ribose). *J. Biol. Chem.* **249**, 3475-3482.

Naegeli, H. and Althaus, F. R. (1991). Regulation of poly(ADP-ribose) polymerase. Histone-specific adaptations of reaction products. *J. Biol. Chem.* **266**, 10,596-10,601.

Naegeli, H., Loetscher, P. and Althaus, F. R. (1989). Poly ADP-ribosylation of proteins. Processivity of a posttranslational modification. *J. Biol. Chem.* **264**, 14,382-14,385.

Ogata, N., Ueda, K., Kawaichi, M. and Hayaishi, O. (1981). Poly(ADP-ribose) synthetase, a main acceptor of poly(ADP-ribose) in isolated nuclei. *J. Biol. Chem.* **256**, 4135-4137.

Ohashi, Y., Ueda, K., Kawaichi, M. and Hayaishi, O. (1983). Activation of DNA ligase by poly(ADP-ribose) in chromatin. *Proc. Nat. Acad. Sci. USA* **80**, 3604-3607.

Panzeter, P., Realini, C. and Althaus, F. R. (1992). Noncovalent interactions of poly(adenosine diphophate ribose) with histones. *Biochemistry* **31**, 1379-1385.

Park, J. K., Kim, W. J., Park, Y. S., Choi, H. S., Yu, J. E., Han, M. and Park, S. D. (1991). Inhibition of topoisomerase I by NAD and enhancement of cytotoxicity of MMS by inhibitors of poly(ADP-ribose) polymerase in Saccharomyces cerevisiae. *Cell. Mol. Biol.* **37**, 739-744.

Poirier, G. G. and Moreau, P., eds (1992). *ADP-Ribosylation Reactions.* Springer, New York (in press).

Realini, C. (1991). Poly ADP-ribosylation: A protein shuttle mechanism in chromatin. Thesis, University of Zürich, Switzerland.

Realini, C. and Althaus, F. R. (1992). Histone shuttling by poly ADP-ribosylation. *J. Biol. Chem.*, in press.

Richter, A. and Kapitza, M. (1991). Histone H1 inhibits eukaryotic DNA topoisomerase I. *FEBS Lett.* **294**, 125-128.

Richter, A. and Ruff, J. (1991). DNA topoisomerase I cleavage sites in DNA and nucleoprotein complexes. *Biochemistry* **30**, 9741-9748.

Satoh, M. S. and Lindahl, T. (1992). Role of poly(ADP-ribose) formation in DNA repair. *Nature* **356**, 356-358.

Shall, S. (1984). ADP-ribose in DNA excision repair: A new component of DNA excision repair. *Advan. Radiat. Biol.* **11**, 1-69.

Shinshi, H., Miwa, M., Kato, K., Noguchi, M., Matsushima, T. and Sugimura, T. (1976). A novel phophodiesterase from cultured tobacco cells. *Biochemistry* **15**, 2185-2190.

Sidik, K. and Smerdon, M. J. (1992). Correlation between repair patch ligation and nucleosome rearrangement in human cells treated with bleomycin, UV radiation or methyl methane sulfonate. *Carcinogenesis* **13**, 135-138.

Simpson, R. T. (1978). Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry* **17**, 5524-5531.

Skidmore, C. J., Davies, M. I., Goodwin, P. M., Halldorsson, H., Lewis, P. J., Shall, S. and Zia'ee, A. (1979). The involvement of poly(ADP-ribose) polymerase in the degradation of NAD caused by gamma-radiation and N-methyl-N-nitrosourea. *Eur. J. Biochem.* **101**, 135-142.

Skidmore, C. J., Davies, M. I., Omidiji, O., Zia'ee, A. A. and Shall, S. (1980). Poly(ADP-ribose) polymerase - An enzyme responsive to DNA damage. In *Novel ADP-ribosylations of Regulatory Enzymes and Proteins* (ed. Smulson, M. E. and Sugimura, T.), pp. 197-205. Elsevier, Amsterdam, New York.

Smerdon, M. J. (1986). Completion of excision repair in human cells. Relationship between ligation and nucleosome formation. *J. Biol. Chem.* **261**, 244-252.

Smerdon, M. J. (1989). DNA excision repair at the nucleosome level of chromatin. In *DNA Repair Mechanisms and Their Biological Implications in Mammalian Cells* (ed. Lambert, M. W. and Laval, J.), pp. 271-294. Plenum Press, New York, London.

Smerdon, M. J. and Thoma, F. (1990). Site-specific DNA repair at the nucleosomal level in a yeast chromosome. *Cell* **61**, 675-684.

Tanuma, S. (1989). Evidence for a novel metabolic pathway of (ADP-ribose)$_n$: Pyrophosphorolysis of ADP-ribose in HeLa S3 cell nuclei. *Biochem. Biophys. Res. Commun.* **163**, 1047-1055.

Thoma, F., Lose, R. and Koller, T. (1983). Involvement of the domains of histones H1 and H5 in the structural organization of soluble chromatin. *J. Mol. Biol.* **167**, 619-640.

Thömmes, P. and Hübscher, U. (1990). DNA helicase from calf thymus. Purification to apparent homogeneity and biochemical characterization of the enzyme. *J. Biol. Chem.* **265**, 14,347-14,354.

Thömmes, P., Strack, B., Ferrari, E., Realini, C., Althaus, F. R. and Hübscher, U. (1992). Eukaryotic DNA helicases. *Experientia* **48**, A82.

Ueda, K. (1987). Nonredox reactions of pyridine nucleotides. In *Pyridine Nucleotides* (ed. Dophin, D., Poulson, R. and Avranovic, O.), pp. 549-598. Wiley, New York.

Van Zeeland, A. A., Smith, C. A. and Hanawalt, P. C. (1981). Sensitive determination of pyrimidine dimers in DNA of UV-irradiated mammalian cells. Introduction of T4 endonuclease V into frozen and thawed cells. *Mutat. Res.* **82**, 173-189.

Wang, Z., Wu, X. and Friedberg, E. C. (1991). Nucleotide excision repair of DNA by human cell extracts is suppressed in reconstituted nucleosomes. *J. Biol. Chem.* **266**, 22472-22478.

Wesierska-Gadek, J. and Sauermann, G. (1988). The effect of poly(ADP-ribose) on interactions of DNA with histones H1, H3, and H4. *Eur. J. Biochem.* **173**, 675-679.

Wilkins, R. J. and Hart, R. W. (1974). Preferential DNA repair in human cells. *Nature* **247**, 35-36.

Zahradka, P. and Ebisuzaki, K. (1982). A shuttle mechanism for DNA-protein interactions. The regulation of poly(ADP-ribose) polymerase. *Eur. J. Biochem.* **127**, 579-585.