REVIEW ARTICLE

Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions

Damien D'AMOURS*, Serge DESNOYERS†, Icy D'SILVA‡ and Guy G. POIRIER‡§[1]

*Wellcome/CRC Institute of Cancer and Developmental Biology, Cambridge CB2 1QR, U.K., †Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, U.S.A., ‡Health and Environment Unit, Laval University Medical Research Center, CHUQ, Québec, Canada G1V 4G2, and §Department of Medical Biology, Faculty of Medicine, Laval University, Québec, Canada G1K 7P4

Poly(ADP-ribosyl)ation is a post-translational modification of proteins. During this process, molecules of ADP-ribose are added successively on to acceptor proteins to form branched polymers. This modification is transient but very extensive *in vivo*, as polymer chains can reach more than 200 units on protein acceptors. The existence of the poly(ADP-ribose) polymer was first reported nearly 40 years ago. Since then, the importance of poly(ADP-ribose) synthesis has been established in many cellular processes. However, a clear and unified picture of the physiological role of poly(ADP-ribosyl)ation still remains to be established. The total dependence of poly(ADP-ribose) synthesis on DNA strand breaks strongly suggests that this post-translational modification is involved in the metabolism of nucleic acids. This view is also supported by the identification of direct protein–protein interactions involving poly(ADP-ribose) polymerase (113 kDa PARP), an enzyme catalysing the formation of poly(ADP-ribose), and key effectors of DNA repair, replication and transcription reactions. The presence of PARP in these multiprotein complexes, in addition to the actual poly(ADP-ribosyl)ation of some components of these complexes, clearly supports an important role for poly(ADP-ribosyl)ation reactions in DNA transactions. Accordingly, inhibition of poly(ADP-ribose) synthesis by any of several approaches and the analysis of PARP-deficient cells has revealed that the absence of poly(ADP-ribosyl)ation strongly affects DNA metabolism, most notably DNA repair. The recent identification of new poly(ADP-ribosyl)ating enzymes with distinct (non-standard) structures in eukaryotes and archaea has revealed a novel level of complexity in the regulation of poly(ADP-ribose) metabolism.

Key words: chromatin structure, DNA repair, genome integrity, poly(ADP-ribose) glycohydrolase, poly(ADP-ribose) polymerase.

POLY(ADP-RIBOSE) (pADPr): THE THIRD TYPE OF NUCLEIC ACID

Discovery and characterization of pADPr

As its name indicates, pADPr is a homopolymer of ADP-ribose (ADPr) units linked by glycosidic bonds [1–3]. The polymer was initially identified as a homopolymer of riboadenylate units (polyA) [1]. However, subsequent studies demonstrated that the polymer contained 2 mol of ribose and 2 mol of phosphate per mol of adenine, excluding the possibility that it was a polymer of riboadenylate [2–4]. In addition, it was demonstrated by the same authors that the synthesis of this homopolymer required NAD^+ as a precursor or immediate substrate of the reaction. These results strongly suggested that the polymer described by Chambon et al. [1] was in fact a homopolymer of ADPr units derived from NAD^+ hydrolysis, with the simultaneous release of nicotinamide.

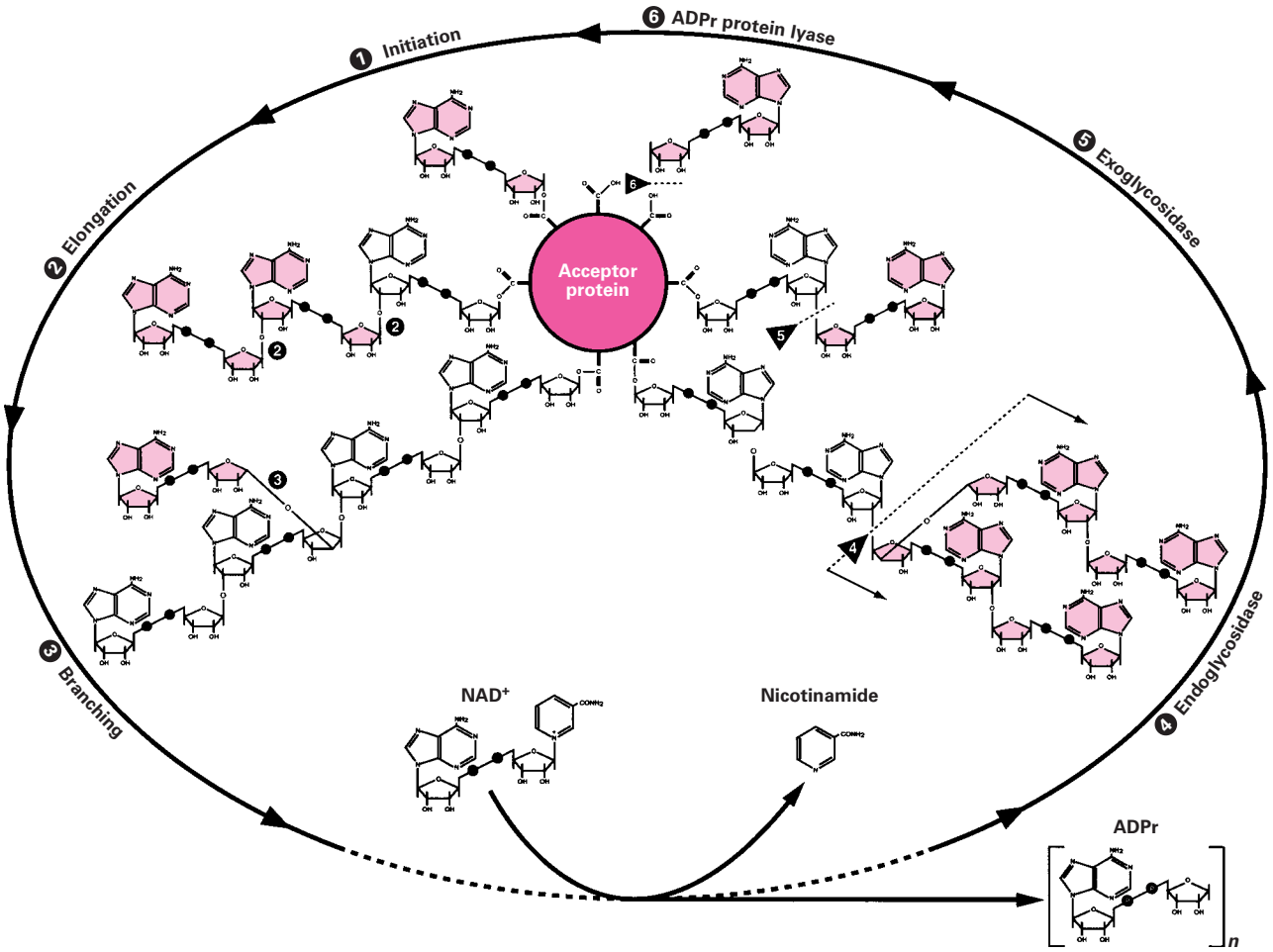
The structure of this polymer is now well known. The polymer is most probably attached on to proteins via the γ-carboxy groups of glutamic acid residues [5,6]. Although less likely, the modification of other residues, such as aspartic acid residues, is possible [7]. ADPr units in the polymer are linked by glycosidic ribose–ribose $1'' \rightarrow 2'$ bonds (Scheme 1). The chain length of polymers is heterogeneous and can reach 200 units *in vitro*. Polymers shorter in length than 11 ADPr units are referred to as oligo(ADPr) [8]. Long polymers are branched [9–13], with the linkages in the branching regions being the same as those found in the linear regions of the polymer [14]. The chemical structure of the branching site of pADPr was determined by NMR and by mass spectroscopy [14] as *O*-D-ribofuranosyl-$(1''' \rightarrow 2'')$-*O*-D-ribofuranosyl-$(1'' \rightarrow 2')$-adenosine-5′,5″,5‴-tri(phosphate), commonly known as Ado (P)-Rib (P)-Rib-P. pADPr is branched in an irregular manner [12]. The average branching frequency of the polymer is approximately one branch per linear section of 20–50 units of ADPr [12,15–17]. Minaga and Kun [18,19] postulated that long chains of ADPr have a helicoidal secondary structure. The structure of pADPr has some similarity to the structure of RNA and DNA, since antibodies raised against pADPr can recognize RNA and DNA, and vice versa [20,21].

Metabolism of pADPr *in vivo*

Scheme 1 presents an overall view of pADPr metabolism. The left (steps 1–3) and right (steps 4–6) parts of the figure represent the anabolism and the catabolism of the polymer respectively. The synthesis of pADPr requires three distinct enzymic activities: (1) initiation or mono(ADP-ribosyl)ation of the substrate, (2) elongation of the polymer, and (3) branching of the polymer (Scheme 1). The enzyme pADPr polymerase (113 kDa PARP) possesses these three activities, and is the major anabolic activity responsible for poly(ADP-ribosyl)ation in living cells [22,23].

Abbreviations used: ADPr, ADP-ribose; AP-2, activator protein-2; BER, base-excision repair; BRCT domain, BRCA1 C-terminus domain; DBD, DNA-binding domain; DSB, double-strand break; HMG, high-mobility group; MMTV, murine mammary tumour virus; pADPr, poly(ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; SSB, single-strand break; TBP, TATA-binding protein; TRF1, telomeric repeat binding factor 1; XRCC1, X-ray repair cross-complementing factor 1; YY1, Yin and Yang 1.

[1] To whom correspondence and reprint requests should be addressed: Unité de Recherche Santé et Environnement, Centre de Recherche du CHUL, CHUQ, 2705 Boulevard Laurier, Ste-Foy (Québec), Canada G1V 4G2 (e-mail Guy.Poirier@crchul.ulaval.ca).



Scheme 1 pADPr metabolism

The left-hand (steps 1–3) and right-hand (steps 4–6) parts of the cycle represent the anabolic and catabolic reactions respectively in the metabolism of pADPr. The pADPr cycle proceeds counterclockwise. The pink circle in the middle of the scheme represents a hypothetical protein acceptor modified on a glutamic acid residue (γ-COOH group shown). Refer to the text for further details.

Other PARP enzymes have recently been identified [24–26]. However, it is not yet known whether these enzymes have the ability to catalyse all the reactions necessary to produce branched polymer, or whether they can only synthesize linear polymers. The efficient degradation of pADPr requires three different enzymic activities (Scheme 1), which are carried out by two distinct enzymes: pADPr glycohydrolase (PARG) and ADP-ribosyl protein lyase. PARG possesses exoglycosidase [27] and endoglycosidase activities [28]. These activities are responsible for the hydrolysis of glycosidic bonds between ADPr units located at the extremity and within the polymer respectively. ADP-ribosyl protein lyase is the enzyme responsible for the hydrolysis of the most proximal unit of ADPr on the protein acceptor [29,30]. The latter enzymic reaction has not been studied in detail, but it does seem to be the rate-controlling step in the metabolism of pADPr [31].

The constitutive levels of polymer are usually very low in unstimulated cells [32–35]. The majority of the ADPr units found on acceptor proteins in the absence of DNA damage appear as mono- or oligo(ADPr) [32,33]. They are qualitatively different from those synthesized in the presence of DNA damage [17], and their degradation is far slower than that of polymers synthesized in response to genotoxic agents ($t_{\frac{1}{2}}$ of 7.7 h, compared with < 1 min) [34–36]. In the presence of DNA strand breaks, PARP activity and the levels of ADPr polymers can be increased by 10–500-fold [34,36,37], while cellular NAD^+ levels are correspondingly reduced [38]. In living cells, the synthesis of pADPr is directly proportional to the number of single strand breaks (SSBs) and double strand breaks (DSBs) present in the genomic DNA [22]. In addition, both constitutive and activated levels of ADPr polymers are functions of the concentration of NAD^+ in cells [39,40].

The polymers of ADPr are degraded rapidly by PARG *in vivo*, which accounts for their transient nature in living cells. The catabolism of pADPr is a function of its concentration in cells [36]. Indeed, Alvarez-Gonzalez and Althaus [36] have observed that a substantial activation of PARG requires a quantity of ADPr polymer of > 5 μM *in vivo*. This mode of action of PARG may explain the very short half-life of polymers during DNA damage *in vivo* (< 1 min) compared with the longer half-life of (constitutive) polymers in unstimulated cells [31,36,41]. In addition, branched and short polymers are degraded more slowly than long and linear polymers [41,42]. This preferential affinity of PARG for some types of chains of ADPr could also explain the

biphasic degradation of the polymer *in vivo* and the existence of two populations of polymers with distinct half-lives following DNA damage. Wielckens et al. [31] determined that the half-life of the long-lived population of polymer would be approx. 6 min in cells damaged by dimethyl sulphate.

It is very likely that the major regulatory step in the catabolism of the polymer is the release of the last ADPr unit bound to the protein. The group of Hilz [43] has observed that the level of mono(ADPr)–protein adducts increased by 9-fold in the nuclei of cells treated with DNA-damaging drugs. No increase was observed, under these conditions, in the level of cytoplasmic mono(ADPr)–protein adducts. The half-life of these adducts is far greater (8–10 min) that the half-life of pADPr, and their existence is dependent on the presence of DNA strand breaks. The sum of these observations strongly suggests that these mono(ADPr) groups are remnants of ancient polymers of ADPr, and that the major regulatory step in the metabolism of pADPr is catalysed by ADP-ribosyl protein lyase [31]. Once freed from the polymer, ADPr units are catabolized to AMP and ribose 5-phosphate by ADPr pyrophosphatases [44,45]. Free ADPr can also form ADPr–protein adducts by non-enzymic mechanisms known as glycation and glycoxidation [46,47].

Acceptors of pADPr

More than 30 nuclear substrates of PARPs have been identified *in vivo* and *in vitro* [22]. Most of the physiological substrates of poly(ADP-ribosyl)ation reactions are nuclear proteins (Table 1). Among these substrates, one finds almost exclusively proteins involved in the metabolism of nucleic acids and in the maintenance of chromatin architecture. The main acceptor of pADPr *in vivo* is PARP itself [48], as it catalyses its own automodification to complete its shuttling off DNA strand breaks (see below). However, it has to be stressed that even modest levels of poly(ADP-ribosyl)ation, such as those seen with numerous nuclear substrates, are likely to have important effects on the acceptor's properties, given the size and charge of each ADPr unit. Table 1 shows the known acceptors of pADPr according to their demonstrated or potential role in nuclear processes. Some acceptors have been classified into more than one category. The references quoted in each of these cases have been chosen to reflect the potential function of the acceptor.

The physiological consequences of poly(ADP-ribosyl)ation on the functional and physico-chemical properties of specific acceptor proteins are in most cases poorly known. However, it is clear that the addition of an anionic polymer on a DNA-binding protein will have important consequences. Indeed, as the substrate gradually accumulates more negative charges by the sequential addition of ADPr units, it will reach a point at which its net charge will prevent any interaction with other anionic molecules such as DNA [49]. Also, the addition of ADPr residues near catalytic or regulatory sites on an enzyme may modify its enzymic properties, as has been frequently established for the phosphorylation of proteins [50]. The fact that poly(ADP-ribosyl)ation of enzymes generally inhibits their catalytic activity clearly illustrates this point (Table 1).

The known effects of poly(ADP-ribosyl)ation on the functional properties of selected substrates of PARP will be discussed further below. However, it is important to note here that the poly(ADP-ribosyl)ation of a specific protein might indicate a potential function of pADPr synthesis in living cells. Now that several nuclear processes have been reconstituted *in vitro*, it will be interesting in the future to use cell-free systems to investigate the specific effects of poly(ADP-ribosyl)ation on the effectors of DNA transactions and on the whole processes themselves. Finally, one should point out the importance of non-covalent interactions between free pADPr and proteins. Indeed, it has been demonstrated that interactions between the polymer and nuclear proteins such as p53 and histones are very stable, and could modify the functional properties of these and other proteins in living cells [51–53].

Poly(ADP-ribosyl)ation, NAD^+ metabolism and cell death

NAD^+ is an essential cofactor in energy metabolism. The synthesis of ATP and the balance of the redox potential directly depend on NAD^+ levels in cells [54]. NAD^+ is also the precursor or immediate substrate for the synthesis of pADPr. The hydrolysis of the N-glycosidic bond found between the nicotinamide and the ribose moieties of NAD^+ produces a free energy of -34.3 kJ/mol (-8.2 kcal/mol) [55], which makes this bond a high-energy one. The energy liberated by the hydrolysis of this bond is used by PARPs to catalyse the synthesis of ADPr polymers.

The level of poly(ADP-ribosyl)ation in cells seems to be the most important factor for the maintenance of NAD^+ levels. Indeed, it has been demonstrated that the catabolism of NAD^+ in mammalian cells occurs mainly via poly(ADP-ribosyl)ation reactions [34,56–58]. The concentration of NAD^+ in undamaged mammalian cells is approx. 400–500 μM [39,59,60], and its half-life is approx. 1 h (D98/AH2 cells) [61]. However, sustained activation of PARP following DNA damage decreases the half-life of NAD^+ in a dose-dependent manner. Indeed, cells exposed to high doses of DNA-damaging agents (e.g. 10–12 krad of γ-rays) undergo a decrease in NAD^+ to 20 % of their normal levels within 5–15 min after the genomic insult [62,63]. Accordingly, it has been calculated that the total enzymic capacity of Ehrlich ascites cells to synthesize pADPr amounts to 10 nmol of ADPr transferred/min per 10^8 cells (at 25 °C) [31]. This consumption rate is sufficient to allow complete depletion of NAD^+ in less than 10 min, and is consistent with the kinetics of NAD^+ depletion described above. Cells treated with a variety of DNA-damaging agents, such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [64], methyl methanesulphonate [34,65], *N*-methylnitrosourea [63], triaziquonum [31], H_2O_2 [66,67], NO [68,69], peroxynitrite [70,71], calicheamicin γ_1 [72], bleomycin [73], streptozotocin [74] and neocarzinostatin [62], undergo a decrease in their levels of NAD^+ comparable with that caused by γ-irradiation, although the kinetics of the process can be slower in some cases. A quasi-total depletion of cellular NAD^+ has also been observed following treatment with high doses of these agents [62,65,75–77]. The depletion of NAD^+ induced by DNA damage follows biphasic kinetics. A decrease of 65–75 % in the levels of NAD^+ can be induced by moderate or weak doses of genotoxic agents, while depletion of the residual 25–35 % requires very high doses of the same agents [62,63,78].

This reduction in NAD^+ levels is not associated with a decrease in the biosynthesis of NAD^+ [75], or with an increase in NAD^+ glycohydrolase activity [63]. Rather, it has been clearly established that the lowering of NAD^+ levels induced by genotoxic agents is associated with an increase in PARP activity ([31,63]; see Oleinick and Evans [79] for a review) and with an increase in the levels of ADPr polymers produced in cells [33,64]. In addition, DNA-damage-induced NAD^+ depletion is associated with ATP depletion [62,80] and dGTP depletion [76], and with a substantial decrease in the levels of other deoxynucleoside triphosphates [76]. NAD^+ depletion results in ATP depletion, because NAD^+ resynthesis requires at least (depending on the biosynthesis pathway) two molecules of ATP per molecule of NAD^+ [81,82]. In addition, NAD^+ depletion blocks glyceraldehyde-3-phosphate

Table 1 Classification of substrates of the poly(ADP-ribosyl)ation reaction according to their potential or proven functions in various aspects of nucleic acid metabolism

Abbreviations: CS, cleavage specific; HSSB, human single-stranded binding; SV40, simian virus 40; hnRNP, heterogeneous nuclear RNA particles; PCNA, proliferating-cell nuclear antigen; REC, rat embryo cells.

Potential function	Acceptors	Origin of observation	Reference
Modulation of chromatin structure	Histone H1	Multiple observations *in vivo*	See the text
	'Histones'		
	H2A	Multiple observations *in vivo*	See the text
	H2B	Multiple observations *in vivo*	See the text
	H3	Multiple observations *in vivo*	See the text
	H3d	Mouse cells C3H10T 1/2	[331]
	H4	Multiple observations *in vivo*	See the text
	H5	*In vitro*	[332]
	Variable histones CS	Sea urchin	[333]
	HMG proteins		
	1	Mouse breast tumour cells	[243]
	2	Mouse breast tumour cells	[243]
	14	34I Cells (mouse breast carcinoma)	[209]
	17	34I Cells (mouse breast carcinoma)	[209]
	T	Nuclei of trout sperm	[230]
	H6	Nuclei of trout sperm	[230]
	LMG protein	Nuclei of mouse testis	[334]
	A24 protein	CV-1 cells (monkey)	[335]
	PARP	Multiple observations *in vivo*	See the text
	Topoisomerases		
	I	Mouse epidermal cells JB6	[336]
	II	*In vitro*	[337]
DNA synthesis	HSSB		
	Subunit 34 kDa	HeLa cells	[221]
	Subunit 70 kDa	HeLa cells	[221]
	Ap4A	*In vitro*	[338]
	DNA ligases		
	I	*In vitro*	[339]
	II	*In vitro*	[339]
	DNA polymerases		
	α	*In vitro*	[339]
	β	*In vitro*	[340]
	SV40 T antigen	Monkey kidney cells	[341]
	Terminal transferase	*In vitro*	[339]
	Topoisomerase II	*In vitro*	[221]
DNA repair	PARP	Multiple observations *in vivo*	See the text
	DNA ligases		
	I	*In vitro*	[339]
	II	*In vitro*	[339]
	DNA polymerases		
	α	*In vitro*	[339]
	β	*In vitro*	[340]
	Histones	Multiple observations *in vivo*	See the text
Transcription	Histones	Nuclei of rat liver	[342]
	RNA polymerases		
	I	Nuclei of quail cells	[343]
	II	*In vitro*	[344]
	hnRNP	Nuclei of rat liver	[345]
	Topoisomerases		
	I	Mouse epidermal cells JB6	[336]
	II	Nuclei of HeLa cells	[346]
	HMG proteins		
	1 and 2	HeLa cells	[243]
	14 and 17	34I cells	[244]
	Fos	Mouse epidermal cells JB6	[347]
	p53	*In vitro* and *in vivo*	[348]
	$TF_{II}C$ (PARP)	*In vitro*	[234]
	Bovine seminal RNase	*In vitro*	[7]
	$TF_{II}F$		
	Subunit RAP30	*In vitro*	[240]
	Subunit RAP74	*In vitro*	[240]
Cell cycle	p53		
	Mutant	REC	[348]
	Wild type	*In vitro*	[329]
	Fos	Mouse epidermal cells JB6	[347]
	PCNA	HeLa and FM3A cells	[299]
Various	Endonuclease Ca^{2+}/Mg^{2+}	*In vitro*	[349]
	Micrococcal nuclease	*In vitro*	[350]
	Lamins	Nuclei of HeLa cells	[351]
	Numatrin/B23	SQ-20B cells	[316]
	Nucleolin/C23	Nuclei of HeLa cells	[317]

dehydrogenase activity, which is required to resynthesize ATP during glycolysis [62]. Indeed, lowering of ATP levels in living cells results in the induction of glycolysis and in the metabolic investment of two molecules of ATP to start the process. Under normal circumstances, these two molecules are regenerated later in the glycolytic process, along with additional molecules of ATP [54]. However, in the absence of NAD^+, glycolysis is blocked just after the investment of these two ATP molecules (at the step catalysed by glyceraldehyde-3-phosphate dehydrogenase), and this situation results in an amplification loop for the depletion of ATP [62]. It is likely that this lowering of ATP levels is responsible for the observed decrease in deoxynucleotide levels. Inhibition of PARP during DNA damage eliminates the depletion of NAD^+, ATP and deoxynucleotides [62,76,80]. It has been suggested by Althaus and co-workers [83] that the differential sensitivity of some cell lines to NAD^+ depletion is a function of the capacity of the cells to synthesize NAD^+.

The physiological consequences of NAD^+ and ATP depletion have recently been established in the context of DNA-damage-induced cell death. Indeed, it was shown recently that the completion of apoptosis is absolutely dependent on the presence of ATP and that, in the absence of this nucleotide, the type of cellular demise switches from apoptosis to necrosis [84,85]. As mentioned above, DNA-damage-induced NAD^+ depletion is a PARP-dependent process that can be completed within 15 min and, therefore, precedes by far the execution of the apoptotic process [62,63]. Since NAD^+ depletion leads to ATP depletion, and because ATP is required for the execution of apoptosis, overstimulation of PARP *in vivo* results in necrotic death in a situation where the stimulus (DNA strand break) is definitely pro-apoptotic. The cellular lysis associated with necrosis generates further damage to neighbouring cells and often results in massive inflammation (reviewed in [86,87]). This whole situation is directly responsible for the pathological complications associated with ischaemia [86,87] and with some types of diabetes in animals [88–90]. PARP is clearly instrumental in this process, since PARP-deficient mice and wild-type animals treated with PARP inhibitors are protected from post-ischaemia damage compared with animals proficient in poly(ADP-ribosyl)ation [69–71,88,91].

The amplification of the cellular damage associated with ischaemia illustrates clearly why it is preferable for multicellular organisms to favour apoptotic cell death rather than necrosis. Even in cases where DNA damage is less extensive, PARP uses substantial amounts of NAD^+ and can lower considerably the levels of ATP in cells. Such decreases in ATP levels are likely to interfere with the execution and/or completion of the apoptotic process [84,85]. This metabolic evidence, and the fact that PARP loses its structural integrity during apoptosis [92,93], suggest that eukaryotic cells have developed a mechanism to protect themselves from PARP-induced switching in the type of cell demise. Indeed, as early as 30 min after the induction of apoptosis, death proteases known as caspases cleave PARP in its bipartite nuclear localization signal ($DEVD^{214}/G^{215}$) to generate a 24 kDa DNA-binding fragment and a 89 kDa catalytic fragment [93–95,203]. This cleavage is likely to inactivate the catalytic activity of PARP, since it was shown previously that the enzyme requires a functional DNA-binding domain to perform efficient poly(ADP-ribosyl)ation [96,97]. Accordingly, the cleavage of PARP should protect apoptotic cells from switching to the necrotic pathway, by preventing the over-stimulation of PARP activity that could result from the initial (apoptosis-inducing) DNA damage or from the subsequent (apoptosis-induced) genomic degradation. This protection mechanism would only be functional in the presence of moderate levels of DNA damage, because the kinetics of NAD^+ and ATP depletion are much faster than the kinetics of PARP cleavage in the presence of elevated levels of DNA damage [62,63,94,95]. The substantiation of this interesting model will certainly increase research effort in the future. It will be important to consider the delicate balance of positive (DNA-repair-associated) and negative (pro-apoptotic and pro-necrotic) effects of PARP on cell survival in order to obtain a clear understanding of the role of this enzyme in cellular death.

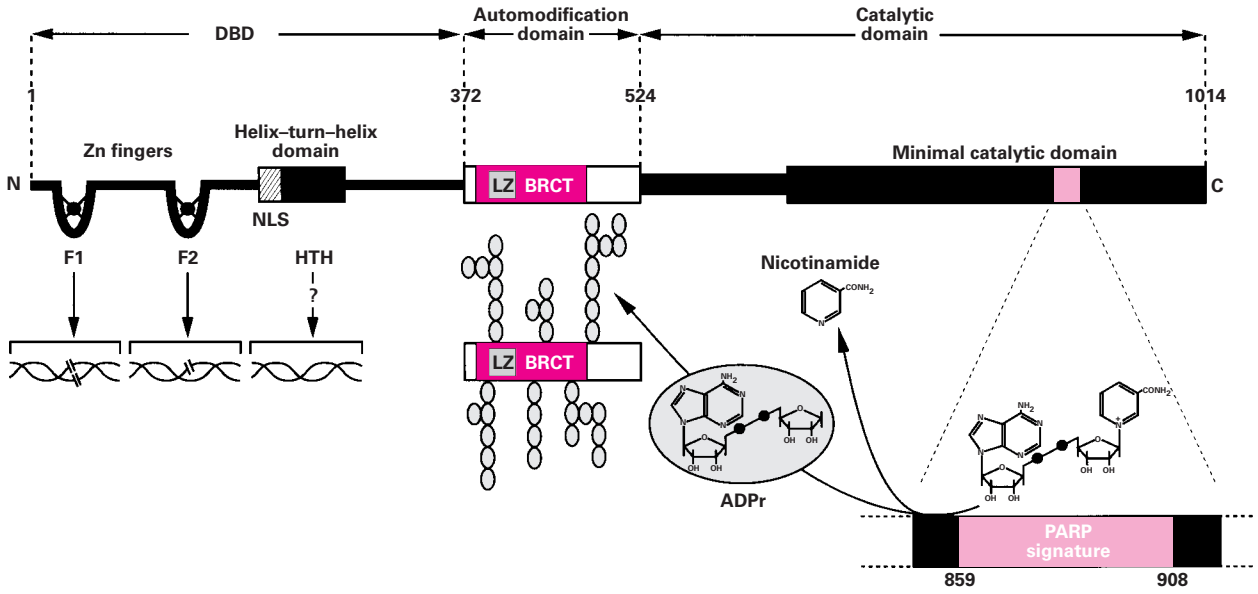
PARPs: DIVERSITY AND STRUCTURE

PARPs are enzymes catalysing the successive transfer of ADPr units on to protein acceptors to produce linear and/or branched polymers of ADPr. PARP activity has been reported in all higher eukaryotes studied so far, and in most lower eukaryotes [24,98–108] (with the notable exception of yeast [109,110]). In addition, poly(ADP-ribosyl)ating activity appears to be present in the archaeal domain, since a PARP-like enzyme has been identified in *Sulfolobus solfataricus* [26].

Until recently, it was generally accepted that there was only one type of PARP in each species. However, this view has been challenged by the identification of novel poly(ADP-ribosyl)ating enzymes [24–26]. These new PARPs are structurally distinct from the classical 113 kDa PARP enzyme, and they can be classified into two subgroups according to their size. Type II PARPs are smaller than the classical zinc-finger-containing PARP and show, in some cases, a nuclear localization [24]. Little is known about this type of PARP besides the fact that it is present in several organisms, including archea [26], plants [24] and mammals (mouse cDNAs encoding two slightly different variants of a putative PARP named PARP-2 have been isolated and deposited in GenBank under the accession numbers AF072521 and AJ007780). Type III PARPs are large proteins containing ankyrin repeats and a PARP catalytic domain [25]. Although the only known member of this subgroup is a human protein called tankyrase, this type of PARP is likely to be found in most higher eukaryotes. Human Tankyrase is localized at telomeres in living cells, and is believed to regulate telomeric function by its association with a negative regulator of telomerase activity (see below) [25].

Very limited data are available at the moment with regard to the overall contribution of non-classical PARPs to poly(ADP-ribosyl)ation reactions *in vivo*. However, it appears from the analysis of animals deficient in type I PARP (113 kDa classical zinc-finger-containing PARP) that type II and III PARPs contribute only modestly to the overall poly(ADP-ribosyl)ation potential of higher eukaryotes [23]. It is possible that these novel PARPs are involved in specific nuclear functions requiring limited levels of poly(ADP-ribosyl)ation *in vivo*. It is interesting to note that the newly identified PARPs have eluded most detection procedures for many years, maybe because their activity was masked by the very active and abundant 113 kDa PARP. Their discovery suggests that other enzymes like these may exist, and that a large family of PARP enzymes could be discovered in the future. The isolation of the cDNAs of these enzymes, coupled with the availability of PARP-deficient cell lines, will certainly help to address this point. Much work will be required in order to understand the contribution and physiological significance of these novel PARPs during poly(ADP-ribosyl)ation reactions in living cells.

The 'classical' 113 kDa PARP (type I; hereafter referred to as PARP for simplicity) is a very abundant protein [$(0.2–2.0) \times 10^6$ molecules per cell; 1.0×10^6 molecules per cell is the average amount found in most cells] [111,112] and appears to be the major poly(ADP-ribosyl)ating activity in higher eukaryotes after



Scheme 2 Molecular structure of PARP

The numbers relate to the boundaries of the domains in human PARP. NLS, nuclear localization signal; HTH, helix–turn–helix motif; LZ, leucine-zipper motif.

DNA damage [23]. Its structure has been extensively characterized and shows several unique features among eukaryotic proteins. PARP is a multifunctional enzyme formed of three domains: a DNA-binding domain (DBD), an automodification domain and a catalytic domain (Scheme 2) [96,97]. These domains were originally identified by limited proteolysis of the protein with papain and α-chymotrypsin [96,97]. Subsequently, the positions of the DBD [113,114] and the catalytic domain [113,115] of PARP have been refined to more precise regions in the protein by digestion with other proteases. Cloning of PARP cDNAs from various organisms has demonstrated that the enzyme is well conserved, especially in higher eukaryotes [116]. The most conserved regions of the molecule are the catalytic domain (near the active site) and, to a lesser extent, the DBD [116]. The functional properties and structural aspects of these domains of PARP are described below.

DBD of PARP

The N-terminal DBD extends from the initiator methionine to threonine-373 in human PARP [117,118]. This domain has a molecular mass of approx. 42 kDa and contains two zinc fingers [114,119,120] and two helix–turn–helix motifs [121–123]. The DBD of PARP also contains a high proportion of basic residues, which are probably involved in the interaction of the enzyme with DNA [121].

Structural elements

PARP is a metalloenzyme that binds zinc molecules specifically [114]. The zinc-binding sites are associated with a 29 kDa fragment of PARP derived from the limited proteolysis of the protein with trypsin [114]. The association of PARP with zinc suggested that the enzyme possesses zinc fingers, which was later confirmed by sequence analysis of the cloned cDNA [117,119,121]. Zinc finger 1 (F1) starts at cysteine-21 and ends at cysteine-56, while zinc finger 2 (F2) is found between cysteine-125 and cysteine-162 [116]. PARP zinc fingers are structurally and functionally unique, since: (i) they co-ordinate zinc molecules with a Cys-Cys-His-Cys motif, (ii) they contain 28 and 30 residues, whereas most other zinc fingers usually contain 12–13 amino acids, and (iii) they recognize altered structures in DNA rather than particular sequences [124]. The only known protein that has a zinc finger similar to those of PARP is DNA ligase III [125,126].

Two types of study have been undertaken in order to clarify the role of the zinc fingers of PARP: with the minimal DBD and with the native enzyme [114,124,127,128]. Studies by Ikejima et al. [128] on the activation of full-length PARP have revealed that both zinc fingers are necessary for the stimulation of PARP catalytic activity in response to SSBs in plasmid DNA. In addition, these authors have shown that finger F1 is the main structure responsible for the activation of the enzyme caused by DSBs [128]. These results contrast with those of de Murcia and collaborators [114,124,127], who studied the binding of an N-terminal 29 kDa fragment (containing both zinc fingers) to an oligodeoxynucleotide of 66 bp containing an SSB in its central region. Indeed, these authors observed that the affinity of the enzyme for SSBs was mainly due to finger F2, while finger F1 would only play a secondary role in the binding of the enzyme to this structure [124]. Although dealing with different outcomes, i.e. DNA binding and activation, these studies are difficult to reconcile, since PARP activation requires DNA binding. The discrepancies found in these two studies might be explained by the utilization of different types of DNA substrate, and require further investigation.

Another feature of interest in the DBD was identified in a 36 kDa fragment of PARP derived from limited proteolysis of the enzyme with plasmin [113]. This domain extends between amino acids 233 and 525, and contains the C-terminal half of the DBD of PARP and the entire automodification domain [117]. A Chou–Fasman analysis of bovine and human PARP revealed the existence of two helix–turn–helix motifs at residues $\sim$ 200–220 and $\sim$ 280–285 respectively, in the N-terminal region of the

36 kDa fragment [121,122]. This motif can mediate strong interactions between DNA and proteins, and it is the only structure responsible for the DNA-binding activity of several proteins [123,129]. In agreement with these observations, Buki and Kun [113] and Thibodeau et al. [130] have demonstrated that the 36 kDa fragment of PARP binds strongly to DNA. It seems that the contribution of the region containing the helix–turn–helix motif to DNA binding is substantial, since the elimination of a single amino acid at the N-terminus of the fragment abolishes completely its capacity to bind to DNA [131]. Moreover, Kun and collaborators [131] have observed that this fragment of PARP behaves exactly like the full-length enzyme in DNA footprinting experiments. Taken together, these observations demonstrate that the N-terminal region of the 36 kDa fragment is important for the binding of PARP to DNA, and that the helix–turn–helix motifs present in this region are the most likely structure responsible for this interaction. In addition, they indicate that the participation of the automodification domain of PARP in DNA binding is insignificant, in contrast with early observations of weak DNA-binding activity of this domain [97].

Affinity of PARP for different forms of DNA

Despite its role in DNA repair, PARP binds undamaged DNA with high affinity [132]. Binding of PARP to this type of DNA is co-operative [132,133] and allows the enzyme to oligomerize [13]. The affinity of PARP for supercoiled DNA is superior to its affinity for relaxed DNA [132], which could explain the association of the enzyme with the nuclear matrix [134–137]. Indeed, in supercoiled DNA, one finds strands that overlap and mimic the type of structure present in the nuclear matrix. It has also been demonstrated that PARP can bind to cruciform and intersecting structures in DNA [132,138]. Sastry et al. [131] have observed that the specificity of the association of PARP with internal regions of some restriction fragments correlates with the presence of A + T-rich sequences or with the presence of loops in the DNA structure [131]. The physiological significance of this preference is still unclear. The association of the enzyme with undamaged DNA depends on structures found in the 36 kDa fragment of PARP, most probably the helix–turn–helix motif. Despite all the evidence of strong interactions between PARP and undamaged linear or supercoiled DNA, it is quite clear that these forms of DNA are inefficient activators of the catalytic activity of the enzyme [33,132,139,140].

PARP associates strongly with DNA SSBs and DSBs generated directly by DNA damage [140,141,193] or indirectly by the enzymic excision of damaged bases during DNA repair. Indeed, it has been observed by electron microscopy that PARP associates with SSBs [142] and DSBs [132] on oligonucleotides and linear DNA fragments respectively. This observation was later confirmed by Smulson and collaborators [143] using atomic force microscopy. PARP binds electrostatically to DNA ends and covers a region of seven nucleotides on each side of SSBs [124,127]. This symmetrical protection of SSBs in DNA footprinting experiments suggests that PARP binds DNA strand breaks as a dimer. In general, PARP binds to DNA strand breaks generated by oxidation, alkylation, deamination, depurination, ionizing radiation (X- and γ-rays) and a great variety of anti-cancerous agents [62,73,74,144]. The enzymic activity of PARP is strongly stimulated in response to its interaction with DNA strand breaks [33,139]. The activation of PARP induced by DNA DSBs is significant, but less important than the activation due to DNA SSBs [140,144,193]. This might indicate that the repair of SSBs and of DSBs requires different levels of pADPr synthesis *in vivo*.

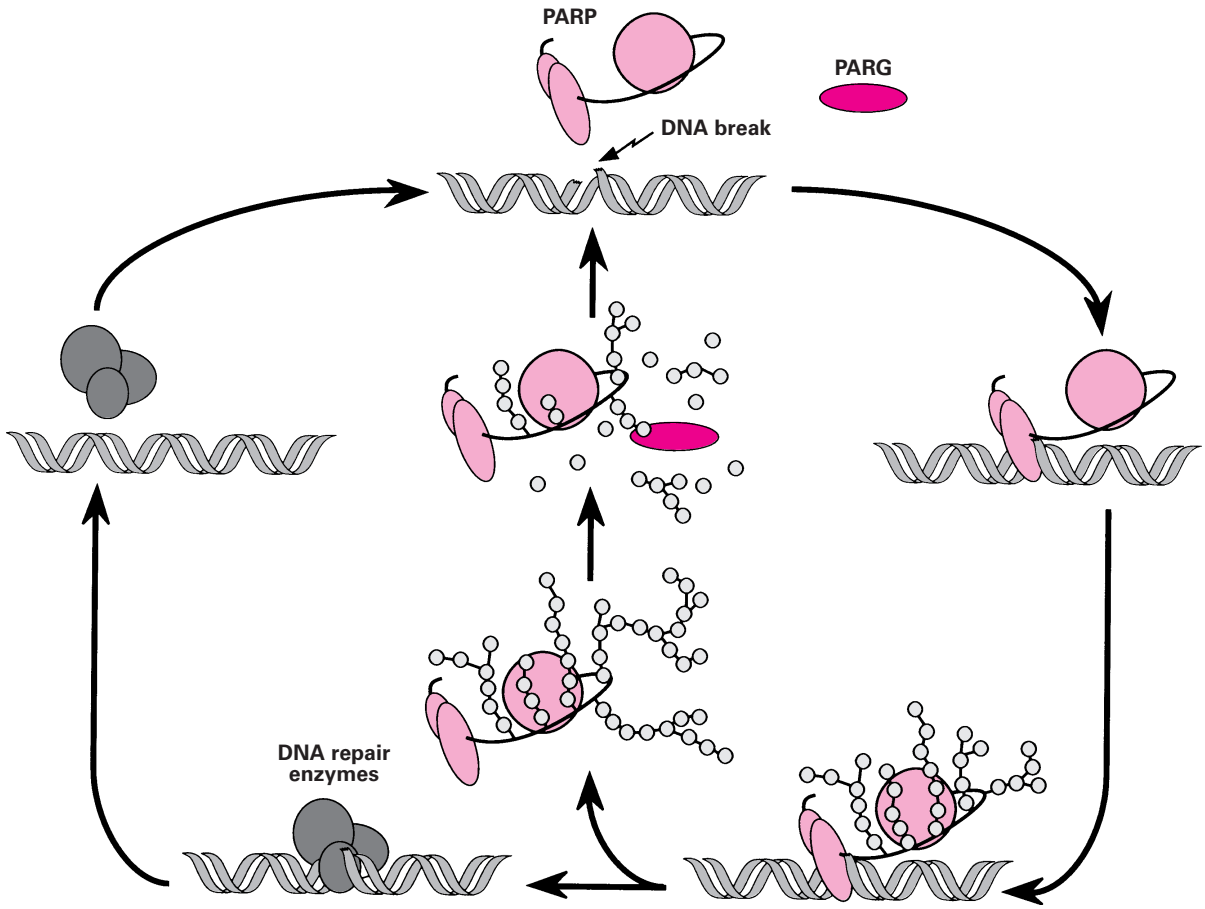
Automodification domain

The automodification domain of PARP is located in the central region of the enzyme, between residues 374 and 525 (human protein) [117,118]. The automodification domain of PARP has not been extensively characterized. The analysis of the primary structure of the enzyme reveals that this domain is basic and that it contains the majority of the 15 glutamic acid residues that would be involved in PARP automodification [16,119,121]. Antibodies directed against this domain inhibit the automodification reaction by 50 %, which confirms that at least half of the residues serving as acceptors of ADPr chains are found in this domain [145]. These antibodies also recognize the automodified enzyme, which indicates that the epitopes recognized by the antibodies are exposed in the automodified enzyme [145]. As expected, the peptides used to generate the antibodies cannot serve as substrates for poly(ADP-ribosyl)ation. This result is in agreement with the work of Kameshita et al. [146], who have shown that the automodification domain in itself cannot serve as an acceptor for ADPr chains unless it is associated with the catalytic domain or the DBD of the enzyme.

Structural elements

The groups of Miwa and Sugimura have identified a leucine-zipper motif in the N-terminal part of the automodification domain of *Drosophila melanogaster* PARP [104]. This motif is preserved in chicken and mammalian PARPs, but not in *Sarcophaga peregrina* PARP [104,147]. It has been proposed by Uchida et al. [104] that this motif could be responsible for the homo- and/or hetero-dimerization of PARP. Indeed, it has been shown previously that protein–protein interactions can be mediated through leucine zippers [148]. This model is in agreement with some observations suggesting that PARP can dimerize [131,149] and that this homodimerization correlates with the stimulation of the catalytic activity of the enzyme [149,150]. This model also implies that poly(ADP-ribosyl)ation of PARP in the automodification domain could regulate the interactions of the enzyme with other leucine-zipper-containing proteins. Several proteins have been shown to interact with PARP through its automodification domain. These include the ribosomal proteins L22 and L23a from *D. melanogaster* [151], the transcription factors Yin and Yang 1 (YY1) [152] and Oct-1 [153], the human ubiquitin-conjugating enzyme hUBC9 [154], XRCC1 (X-ray repair cross-complementing 1) [126,155] and histones [156]. However, none of these interactions appear to be mediated through leucine zippers.

The automodification domain of PARP also contains a BRCT (BRCA1 C-terminus) domain (from amino acids 384 to 479) [157]. This domain consists of approx. 95 (weakly conserved) amino acids found in several proteins that regulate cell-cycle checkpoints and DNA repair [157]. There is a growing amount of evidence suggesting that BRCT domains are protein–protein interaction modules that allow BRCT-motif-containing proteins to establish strong and specific associations [158]. The PARP BRCT domain is a variant of the original domain, along with the BRCT domains of replication factor-C and bacterial ligases, since their C-termini are less conserved than the C-termini of the rest of the family members [157]. Neverteless, the PARP BRCT domain appears to be a fully functional protein-interaction motif, since the region of the enzyme containing this motif was shown to interact directly with the N-terminal BRCT domain of the XRCC1 protein [155]. A similar BRCT-mediated interaction between the BRCT domain of DNA ligase III and the second



Scheme 3 Shuttle model of PARP

Poly(ADP-ribosyl)ation of PARP (or other DNA-binding proteins such as histones) in response to DNA strand breaks results in a gradual loss of affinity of the protein for DNA. This loss of affinity results ultimately in the release of automodified PARP from the DNA strand break and in the subsequent inactivation of the enzyme. The DNA-binding activity of PARP is re-activated following the degradation of the polymer by PARG. The 'beads on a string' structures on PARP represent pADPr. For simplicity, PARP is shown in this scheme as a monomeric enzyme acting before the DNA repair factors. This does not exclude the possibility that PARP acts as a dimer and that the DNA repair machinery accesses the DNA lesion simultaneously with PARP (see recruiting model in the text). Modified from Trends Biochem. Sci.; vol. 20; Lindahl, T., Satoh, M. S., Poirier, G. G. and Klungland, A.; pp. 405–411;

(C-terminal) BRCT domain of XRCC1 has been recently identified [158,160]. These interactions are responsible for the formation of a DNA-base-excision-repair (BER)-specific protein complex, and further support the importance of PARP in this repair pathway [155].

Interestingly, Miwa and collaborators [161] have isolated a cDNA from *D. melanogaster* that encodes a truncated variant of PARP. This variant lacks an automodification domain and, accordingly, should not interact with the proteins described above. It will be interesting to see if such variants exist in higher eukaryotes, and how the absence of an automodification domain will impact on the function of PARP and on the regulation of DNA repair.

Model of 'PARP shuttling'

DNA damage is the most important element in the regulation of poly(ADP-ribosyl)ation reactions. DNA strand breaks stimulate the catalytic activity of PARP 500 times over its basal level [37]. The automodification that results from this enzymic activation regulates PARP activity and binding on DNA strand breaks. The totality of enzymic changes brought about by the automodification of the enzyme and the sequence of events that follow *in vivo* can be explained by the PARP shuttling model (Scheme 3). This model implies that, during PARP automodification, the enzyme becomes gradually more charged, since each residue of ADPr adds two negative charges on to the molecule. An electrorepulsive gradient is established between the polymers of ADPr covalently linked to the enzyme and DNA during the automodification reaction. Eventually, the reaction reaches a 'point of repulsion', when the net charge of PARP is too negative to allow an interaction between the enzyme and DNA [49]. The poly(ADP-ribosyl)ated PARP molecule is consequently freed from the DNA strand break and its catalytic activity is inactivated [162]. Subsequently, PARG hydrolyses the polymers present on PARP, thus allowing the enzyme to resume a new cycle of automodification in response to DNA damage (Scheme 3). Several lines of evidence support this model. Notably, it has been demonstrated using purified enzymes and substrates that the automodification of PARP inhibits its catalytic activity, and that the automodified enzyme loses its affinity for DNA [41,127]. Moreover, the presence of PARG during PARP automodification allows PARP to recover both its affinity for DNA and its catalytic activity [127]. The shuttling model has also been

demonstrated during the poly(ADP-ribosyl)ation of histones and during chromatin decondensation ([163,164]; see [165] for a review).

Catalytic domain

Structure–function relationship

The catalytic domain of human PARP is located in the C-terminal part of the enzyme. It has a molecular mass of approx. 55 kDa and spans residues 526–1014 in human PARP [117,118]. The catalytic activity of this fragment cannot be stimulated by DNA strand breaks, and corresponds to the basal activity of the native enzyme [37,97,146]. The ADPr transferase activity has been further circumscribed to a 40 kDa region at the extreme C-terminus of the enzyme. This region is referred to as the minimal catalytic domain, and can catalyse the initiation, elongation and branching of ADPr polymers independently of the presence of DNA [37]. The loss of the last 45 amino acids at the C-terminal end of this domain completely abolishes enzyme activity [166]. Residues spanning positions 859–908 in human PARP are phylogenetically well conserved [167] and comprise what is commonly accepted as the 'PARP signature' [116]. The 17 kDa region found between the minimal catalytic domain and the automodification domain possesses an antigenic structure that is recognized by antibodies found in some rheumatoidal diseases [168]. This region of PARP has not been extensively characterized and its function is still unknown.

The catalytic domain of PARP shares several structural features with mono(ADP-ribosyl)ating enzymes. Indeed, comparative analysis of mono(ADPr) transferases and PARP has suggested that Glu-988 might be important for PARP activity [169]. Site-directed mutagenesis of this residue in PARP decreased the elongation of the polymer by 2000 times and also reduced, albeit more modestly, the initiation of new chains of ADPr [169,170]. Recently, the catalytic domain of chicken PARP complexed to an inhibitor analogous to nicotinamide has been crystallized and its structure resolved by X-ray diffraction [171]. The active site of this domain consists of a β-α-loop-β-α structural motif which is responsible for NAD^+ binding and is found in several mono(ADPr) transferases [171]. This motif differs significantly from the Rossman fold (β-β motif) found in other NAD^+-utilizing enzymes [165], and appears to be representative of a new family of ADP-ribosyltransferases [167,169]. A G-rich segment in the 40 kDa fragment of PARP contains two potential sites for the association of dinucleotides, namely Gly-Lys-Gly and Gly-Lys-Thr [166]. It has been effectively demonstrated by photoinsertion of an NAD^+ analogue that Lys-893 ($G^{892}KG^{894}$ motif) and Trp-1014 associate with NAD^+ [172]. However, site-directed mutagenesis of Trp-1014 suggests that this residue is not critical for PARP enzymic activity [172]. The relationship between mono- and poly-(ADP-ribosyl)ating enzymes has been further substantiated by the identification of three consensus sequences that would be involved in the formation of an NAD^+-binding site and that are conserved in all ADP-ribosylating enzymes [173].

Poly(ADP-ribosyl)ation reaction

The molecular mechanism of the poly(ADP-ribosyl)ation reaction has been addressed in several studies. It has been shown by cross-linking experiments [174] and by analysis of the kinetics of automodification of PARP [150] that the catalytically active species of the enzyme is a homodimer. This conclusion is supported by DNase footprinting experiments showing that PARP protects DNA from nucleolytic degradation in a symmetrical manner at DNA SSBs [124,127]. It has also been reported that pADPr chains are elongated at their protein-distal extremities [175,176], which suggests that the poly(ADP-ribosyl)ation reaction is intermolecular and is consistent with PARP acting as a catalytic dimer [150].

The K_m of PARP for NAD^+ varies as a function of the type of DNA co-activator used to characterize the enzyme and as a function of the automodification status of the enzyme. Indeed, it has been effectively demonstrated that, unlike the unmodified enzyme, automodified PARP has a weak affinity for NAD^+ [162]. Values for the K_m of purified PARP vary between 20 μM and 80 μM [178], and the V_{max} is approx. 2000 nmol of ADPr transferred/min per mg of protein [177]. The k_{cat} of PARP decreases by 25 % after 1 min of reaction, which could be due to a decrease in the affinity of PARP for DNA [178]. Mendoza-Alvarez and Alvarez-Gonzalez [179] have recently shown that the mono(ADP-ribosyl)ation (initiation) reaction catalysed by PARP is 230 times slower and 50-fold less efficient than the polymerization (elongation) reaction. Histones stimulate PARP activity up to 20-fold [180–183]. They act as allosteric activators of the enzyme by decreasing the K_m of PARP for NAD^+ and by increasing its V_{max} [181]. Interestingly, several transcription factors have been reported to stimulate PARP activity [184,185]. The biochemical basis for the latter stimulatory effect is still unknown.

PARG

PARG is the most important enzyme for the catabolism of pADPr. Its existence was first demonstrated by Miwa and Sugimura [186] and by Ueda et al. [187]. These authors observed that homogenates of various mammalian cells generate AMP and ADPr from polymers of ADPr. This observation could not be explained by a phosphodiesterase activity, since phosphodiesterase hydrolyses pADPr into phosphoribosyl-AMP [186,187]. Human and bovine PARG cDNAs have been cloned, and were shown to encode proteins of 111 kDa [188]. Little is known about the molecular organization of PARG, except that several isoforms of the enzyme can be purified in mammals [42,189–192]. Since only one gene encoding PARG has been identified per genome so far [188], it appears likely that the smaller isoforms are generated by the proteolytic processing of the full-length enzyme [191]. Whether this processing is an experimental artifact or has some physiological relevance remains to be determined.

PARG degrades long polymers extremely rapidly *in vitro*, whereas short polymers are processed much less efficiently under the same conditions. This is well illustrated by the fact that the K_m of PARG is low for long polymers (< 0.3 μM) and increases significantly for shorter polymers (10 μM) [42,194,195]. This difference in the degradation kinetics of long and short polymers can be explained in two ways. First, PARG could have a processive activity on long polymers that becomes distributive on short polymers. Alternatively, the existence of a highly active endoglycosylase activity acting in concert with an exoglycosylase could explain the enzymic behaviour of PARG, as initially suggested by Ikejima and Gill [28]. It appears that the latter possibility might explain, at least partly, the kinetics of pADPr degradation. Indeed, when the mechanism of action of PARG purified to homogeneity was studied, approx. 8–10 % of endoglycohydrolase activity was detected on long polymers [192,195]. The endoglycolytic activity appears to act randomly at points of branching or elsewhere in the polymer of ADPr. This activity of PARG has also been observed during polymer turnover following

DNA damage [196], in *in vitro* turnover systems with purified enzymes [178] and with chromatin [164]. Another factor affecting polymer degradation by PARG is association on an acceptor protein. Indeed, pADPr is degraded more rapidly when it is covalently associated with proteins such as PARP and histone H1 [194].

The endoglycosylase activity of PARG is physiologically important, because it is responsible for the generation of protein-free ADPr polymers that can interact with histones and other nuclear proteins [51,144,197]. In addition, it is important to note that even a low level of endoglycolytic activity, such as 10 %, would result in a significant increase in the degradation kinetics of polymers (by supposing that this activity has a K_m identical with that of the exoglycolytic activity). Indeed, under such conditions, a polymer of 300 units would be reduced to a length of 90 units after only 10 % degradation by PARG (27 exoglycosidic and three endoglycosidic cuts). Therefore the endoglycosidic hydrolysis of long polymers would efficiently prevent the hyper-modification of nuclear proteins with very long chains of ADPr. In addition, this type of hydrolysis could allow PARP to remain active, by loosening the polymers that prevent its interaction with DNA [162]. At the metabolic level, the previous system would behave as a sophisticated NADase activity resulting from the simultaneous formation and degradation of pADPr (as illustrated in Scheme 1), or from the abortive NADase activity of PARP [198,199].

IMPORTANCE OF POLY(ADP-RIBOSYL)ATION IN THE METABOLISM OF NUCLEIC ACIDS

As suggested by Lindahl et al. [125], there is currently no exclusive consensus on the biological role of PARP. However, several observations implicate poly(ADP-ribosyl)ation reactions in DNA repair, transcription, replication, recombination and the modulation of chromatin structure. The evidence supporting the involvement of poly(ADP-ribosyl)ating enzymes in these processes is discussed in detail below.

Modulation of chromatin structure

Poly(ADP-ribosyl)ation of chromatin protein causes major changes in nucleosomal architecture. Indeed, we have shown that polynucleosomes could be completely decondensed upon poly(ADP-ribosyl)ation by purified PARP [163]. The polymer itself (or automodified PARP) seems unable to modify chromatin structure on polynucleosomes, which suggests that a covalent interaction between the polymer and histones is required to induce decondensation [197]. However, protein-free polymer can destabilize the nucleosome core and even cause the dissociation of the chromatin depleted in histone H1 [200,201]. These observations indicate that, following the covalent modification of histone H1, pADPr may open the chromatin structure by covalent as well as non-covalent interactions. Poly(ADP-ribosyl)ated decondensed chromatin can be refolded by the addition of PARG [164]. Indeed, the dual action of PARP and PARG in chromatin results in histone exchange reactions that appear to be reversible. This conclusion is also supported by the work of Althaus and associates [202], who have shown that pADPr turnover renders DNA accessible to nuclease when core histones are incubated with DNA; upon re-hydrolysis of the polymer by PARG, the DNA becomes resistant to nuclease activity. The modulation of chromatin architecture by poly(ADP-ribosyl)ation reactions is very important, because chromatin structure regulates several nuclear processes, including DNA transcription, replication and repair. The well established role of histones as pADPr acceptors *in vivo* [34,204,205] indicates that the physiological functions of PARP are likely to be mediated through the reorganization of chromatin structure in living cells.

Adamietz and Rudolph [206] have shown that histones H1 and H2B are the main histones poly(ADP-ribosyl)ated *in vivo*. Similarly, we have been able to show that, during alkylation-induced damage, the main histone acceptor on simian virus 40 mini-chromosomes is histone H2B [207]. Krupitza and Cerutti [208] have found that, during free-radical-induced damage, 2–3 % of histones H1, H3, H2B and H4 are modified. This level of poly(ADP-ribosyl)ation may appear proportionally low, but actually represents a very high level of poly(ADP-ribosyl)ation given the natural abundance of histones in living cells. Several other chromatin proteins are poly(ADP-ribosyl)ated *in vivo*. Indeed, protamines and high-mobility group (HMG) proteins 1, 2, 14 and 17 have been shown to be modified by PARP under various conditions in living cells [209–211]. A correlation has been established between the level of poly(ADP-ribosyl)ation of chromatin protein, especially HMG proteins, and the level of transcription of certain genes (see below). Chromatin can also be poly(ADP-ribosyl)ated by non-covalent interactions [197,212]. Indeed, it has been shown that protamine and histones can bind branched polymers of ADPr in a non-covalent manner, with the following relative affinity: H1 > H2B > H2A > H3 > H4 [212]. The poly(ADP-ribosyl)ation of histones has also been studied in cell-free systems. It has been shown that histones H2A, H2B and H1 are the main histone substrates of poly(ADP-ribosyl)ation reactions *in vitro* [213].

It appears that the relative levels of poly(ADP-ribosyl)ation of various chromatin proteins are shifted in the presence of PARG. Indeed, in *in vitro* turnover experiments, PARP becomes a relatively less important acceptor as the polymer becomes shorter in length [214–216]. On the other hand, proteins such as A24, histones H2A (u.H2A), H2B (u.H2B), H3 and especially histone H1 become proportionally more modified compared with PARP under the same conditions [215] (u means ubiquitinated). During very rapid turnover (when polymers shorten to oligomers of 4–20 ADPr units in length), histones can carry more than 60 % of the polymer [215]. Therefore it is most likely that there is a biphasic response during chromatin poly(ADP-ribosyl)ation *in vivo*. Initially, both PARP and histones are modified, with a clear preference for PARP. However, the relocalization of PARG to sites of DNA damage (since it is mainly a perinuclear enzyme [217]) should create a turnover situation where histones and other heteromodified proteins [206] would become relatively more modified, thus reducing the relative levels of PARP modification while maintaining the changes established initially in chromatin architecture.

The revised model for the process by which PARG and PARP modify chromatin structure during DNA repair is as follows. (1) Binding of PARP to DNA strand breaks and enzymic activation. (2) Rapid shuttling of PARP from DNA strand breaks because of the low nuclear concentration of PARG, and opening of the chromatin structure following the modification of histones. (3) Entry of PARG in the nucleus; rapid turnover of the polymer and a shift in the relative substrate preference of PARP. The chromatin structure is maintained in a decondensed state. (4) DNA strand breaks are repaired; polymer levels are rapidly lowered because of the large excess of PARG activity, and chromatin returns to its initial configuration.

From these results, Althaus [165] has postulated that, similarly to PARP shuttling on DNA breaks, there is a shuttle mechanism for chromatin that relaxes its structure and facilitates DNA BER (discussed in further detail below).

Poly(ADP-ribosyl)ation and DNA replication

A number of studies in the early 1970s suggested that poly(ADP-ribosyl)ation plays an active role during the process of DNA replication [218,219]. These studies were later supported by experimental evidence obtained with cell-free systems. Indeed, the work of Hurwitz and collaborators [220] indicated that PARP slowed down DNA replication catalysed by the mono-polymerase system *in vitro*. However, it was later demonstrated that PARP is not necessary for the processivity of DNA polymerases, and that it has no role to play during replication catalysed by the dipolymerase system [221]. It now appears that the effect observed with the monopolymerase system was caused by an interaction between PARP and DNA strand breaks. This interpretation of the results is further supported by the fact that the effects described above could also be obtained with a truncated variant of PARP corresponding to the DBD of the enzyme [222]. It is difficult to understand the role that PARP might play in these *in vitro* systems, since single-stranded DNA appears to be occupied by DNA-binding proteins other than PARP during DNA replication.

More recently, direct evidence supporting a role for PARP in DNA replication has been obtained. Indeed, PARP has been shown to interact physically with the catalytic subunit of DNA polymerase α–primase tetramer in several distinct systems [223–226]. First, the two enzymes were shown to co-localize in specific regions of the nucleus, and the specificity of this interaction was confirmed by co-immunoprecipitation experiments [223–225]. PARP was also shown to be associated with the core components of a multiprotein DNA replication complex (DNA synthesome) containing DNA polymerase α [223,226]. The presence of PARP in this complex supports the idea that PARP might regulate the composition or activity of the DNA synthesome via poly(ADP-ribosyl)ation reactions. One important regulatory member of this DNA replication complex, proliferating-cell nuclear antigen (PCNA), has indeed been shown to be poly(ADP-ribosyl)ated by PARP [226]. Furthermore, the sole presence of PARP stimulates the catalytic activity of purified DNA polymerase α *in vitro* [225]. This effect does not appear to be completely dependent on the binding of PARP to DNA, since a fragment of the enzyme containing only the DBD of PARP cannot fully recapitulate the stimulatory effect seen with the full-length enzyme [225]. Taken together, these observations strongly suggest that PARP is associated with the DNA replication machinery *in vivo* and that it might regulate the activity of some key components of the DNA synthesome by poly(ADP-ribosyl)ation. This conclusion is also supported by *in vivo* studies showing that chemical inhibition of PARP interferes with the conversion of the 10-kb DNA replication intermediates to mature large-molecular-size DNA [227].

Additional evidence for a role for poly(ADP-ribosyl)ation reactions during DNA replication comes from the observation that a novel type of PARP called tankyrase is specifically associated with telomeres [25]. Telomeres are the physical ends of chromosomes; they are formed of short sequence repeats and they terminate with 3′ overhanging single-stranded DNA [228]. This structure cannot be replicated by the conventional DNA replication machinery (because of the $5' \rightarrow 3'$ polarity in the synthesis of DNA), and telomeres would become progressively shorter following each cellular cycle in the absence of a special type of DNA replication [228]. A reverse transcriptase called telomerase is the DNA polymerase responsible for the maintenance of telomere length in living cells. This enzyme is negatively regulated by a second protein called telomeric repeat binding factor 1 (TRF1), which is a target for poly(ADP-ribosyl)ation by tankyrase *in vitro* [25]. Poly(ADP-ribosyl)ation of TRF1 reduces its ability to bind to telomeric repeats and is therefore likely to alter its ability to regulate telomere length [25]. The fact that tankyrase and TRF1 interact physically makes it very probable that tankyrase will specifically poly(ADP-ribosyl)ate TRF1 *in vivo*. Nevertheless, much work will be required in order to elucidate the role of tankyrase at telomeres and how it may potentially regulate telomere replication (or other telomeric functions) through TRF1. It is interesting to note that tankyrase is susceptible to inhibition by 3-aminobenzamide, a typical inhibitor of PARP [25]. It is therefore more than likely that the effects seen with this chemical in previous studies were due to the inhibition of both PARP and tankyrase. It will be important in the future to revisit some of the data generated with 3-aminobenzamide in order to clarify the respectives roles of PARP and tankyrase and to define ways of specifically inhibiting these poly(ADP-ribosyl)ating enzymes.

Poly(ADP-ribosyl)ation and DNA transcription

Association of PARP with actively transcribed chromatin

Several studies have been undertaken in order to clarify the relationship between poly(ADP-ribosyl)ation and transcription. Mullins et al. [229] and Levy-Wilson [230] have demonstrated that PARP activity is associated preferentially with regions of chromatin that are transcriptionally active. These results have been confirmed by De Lucia [231]. Accordingly, Hough and Smulson [232] observed that poly(ADP-ribosyl)ated nucleosomes were also preferentially associated with actively transcribed chromatin. However, the association between these regions of chromatin and poly(ADP-ribosyl)ated proteins is not exclusive to other (transcriptionally silent) regions, which excludes the possibility that the function of poly(ADP-ribosyl)ation is solely connected to transcription [232]. It has been demonstrated previously that poly(ADP-ribosyl)ation could induce decondensation of chromatin structure *in vitro* [163] and that this decondensation was associated with an increased sensitivity of nucleosomal DNA to nucleolytic degradation [202]. Correspondingly, the results described above have to be interpreted with caution, since in several of these studies the criterion used to define actively transcribed chromatin was its susceptibility to digestion by nucleases. In several cases, the decondensation of chromatin induced by poly(ADP-ribosyl)ation is dependent on DNA damage and, obviously, independent of transcription. Under these conditions, the relationship between poly(ADP-ribosyl)ated chromatin and actively transcribed chromatin, as defined by its susceptibility to nucleolytic degradation, might be circumstantial and non-indicative of a direct causal relationship between transcription and poly(ADP-ribosyl)ation.

Stimulation of transcription by PARP and poly(ADP-ribosyl)ation

During the purification of transcription factors, Roeder and collaborators [233,234] identified a factor that could increase the specificity of the initiation of RNA polymerase II transcription. This factor, initially named $TF_{II}C$, was purified, characterized and identified as being PARP. In reconstituted systems, PARP eliminated the non-specific transcription resulting from the presence of SSBs in the transcription template without altering specific (promoter-driven) transcription [234]. The function of PARP in this system does not require enzymic activation, since the effect observed by Slattery et al. [234] occurred in the absence of NAD^+. However, the presence of both SSBs in the transcription substrate and NAD^+ in the transcription reaction

resulted in the automodification of PARP, its dissociation from DNA and the abrogation of the effects on transcription [49,162]. Ohtsuki et al. [235] demonstrated that a fragment of PARP containing the DBD of the enzyme could inhibit the non-specific transcription with the same efficiency as the intact enzyme. The physiological interpretation of these observations is not simple because, in the absence of DNA damage *in vivo*, one always finds a saturating concentration of NAD^+ (400–600 μM) [39,59,60] for the catalytic activity of PARP (K_m for NAD^+ of approx. 50 μM). Nevertheless, it seems that the inhibition of non-specific transcription by PARP is an universal regulatory mechanism for transcription, at least *in vitro*, since the same mechanism has been observed with genes transcribed by RNA polymerase I [236] or RNA polymerase III [234].

More recently, Meisterernst et al. [237] found that PARP may play additional roles during transcription in cell-free systems. Indeed, it seems that PARP could stimulate transcription carried out by RNA polymerase II by being part of a cofactor activity that acts during the formation of the pre-initiation complex. As described previously, the co-activator functions of PARP are inhibited by automodification of the enzyme, and can be totally mediated by the DBD of PARP [237]. However, the positive effect of PARP seen in this system is independent of DNA damage, since no differences were observed when supercoiled and nicked plasmids were used as transcription templates [237]. These results are consistent with a recent study showing that PARP increases the rate of protein-complex formation on the *PAX-6* gene enhancer (EP) *in vitro* [238]. The physiological relevance of this observation was confirmed by using PARP inhibitors in living cells and by showing that these chemicals inhibit EP enhancer activity *in vivo* [238].

Additional *in vivo* evidence for a role for PARP as a transcriptional co-activator was revealed during the search for proteins interacting with the transcription factor activator protein-2 (AP-2). In this study, PARP was shown to interact with the AP-2 family of transcription factors and to enhance AP-2-mediated transcription *in vitro* and *in vivo* [239]. It appears that PARP stimulates transcription in this system by suppressing AP-2 self-inhibition [239]. It is not known if this stimulatory effect is dependent on the poly(ADP-ribosyl)ation of AP-2 or on PARP activity. Interestingly, the other AP-2 interactor identified in this study, a subunit of $TF_{II}F$ (RAP74), was shown previously to be a substrate for poly(ADP-ribosyl)ation *in vitro* [240]. It is therefore possible that the positive effect of PARP on AP-2-mediated transcription is due to the poly(ADP-ribosyl)ation of RAP74. In a different study, Butler and Ordahl [241] observed an *in vivo* poly(ADP-ribosyl)ation-dependent stimulation of muscle CAT1 (MCAT1)-element-mediated transcription. These authors showed that the inhibition of PARP activity in living cells represses MCAT1-element-driven transcription when using reporter assays. Moreover, they showed that PARP interacts with a cellular factor, transcription enhancer factor 1, that is necessary for MCAT1-element-mediated transcription, and that this factor can be poly(ADP-ribosyl)ated *in vitro* [241]. Therefore it appears possible that poly(ADP-ribosyl)ation of transcription enhancer factor 1 might modulate its enhancer ability *in vivo*.

Poly(ADP-ribosyl)ation-dependent inhibition of transcription

Several lines of evidence support a role for PARP and poly(ADP-ribosyl)ation in transcription. However, the nature of this role remains to be defined, because no unified picture has emerged from the experimental observations described above. The total dependence of the catalytic activity of PARP on DNA strand breaks argues that the major function of poly(ADP-ribobsyl)ation is unlikely to be at the level of transcription. However, it appears possible that poly(ADP-ribosyl)ation could regulate transcription during DNA damage. Indeed, it has been demonstrated that cells treated with DNA-damaging agents undergo a substantial decrease (70–75%) in their RNA polymerase activity, which brings about an inhibition of mRNA and rRNA synthesis [242]. This decrease can be completely blocked by 3-aminobenzamide, a powerful inhibitor of PARP, thereby confirming the specificity of the relationship between poly(ADP-ribosyl)ation and transcription.

A poly(ADP-ribosyl)ation-dependent inhibition of transcription has also been observed by Tanuma et al. [243] in living cells. These authors showed that suppressing the poly(ADP-ribosyl)ation of some chromatin proteins, presumably HMG 14, HMG 17 and histone H1, stimulates glucocorticoid-induced expression of murine mammary tumour virus (MMTV) RNA in 34I cells [209,210]. Conversely, inhibition of PARG activity resulted in a drastic reduction in the expression of the MMTV RNA [244,245]. These results are in agreement with the important role played by HMG proteins in the expression of several genes [246,247]. Poly(ADP-ribosyl)ation of HMG proteins 14 and 17 might modify their functional properties, and it seems likely that this mechanism could explain, at least partly, the inhibition of the expression of MMTV RNA. These observations are also consistent with the recent identification of PARP as an inhibitor of nuclear-receptor-dependent transcription. Indeed, Miyamoto et al. [248] have shown that, when PARP is recruited to promoters *in vivo*, either as a fusion protein or when it binds to retinoid X receptors, it can inhibit transcription in a poly(ADP-ribosyl)ation-dependent manner.

An NAD^+-dependent silencing of RNA polymerase II-dependent transcription has also been observed using cell-free systems [249]. The inhibition seen in these systems appears to be due, at least partly, to the poly(ADP-ribosyl)ation of the TATA-binding protein (TBP) and of the transcription factor YY1. Indeed, it was shown with purified enzymes and cellular extracts that the poly(ADP-ribosyl)ation of these proteins abrogates their DNA-binding activities and results in the suppression of their positive effects on transcription [185]. These observations are consistent with experiments showing that direct or indirect recruitment of PARP to promoters reduces their activity in living cells [248]. Interestingly, it was shown that poly(ADP-ribosyl)ation of YY1 and TBP cannot be performed when these factors are bound to DNA, and that transcription complexes bound to their templates are immune to the inhibitory effects of poly(ADP-ribosyl)ation. This might explain why TBP was not identified as a substrate for poly(ADP-ribosyl)ation in previous screens [240] and why an NAD^+-dependent inhibition of transcription was not observed in previous studies using cell-free systems [237]. Surprisingly, YY1 was shown to interact preferentially with poly(ADP-ribosyl)ated PARP and to stimulate its catalytic activity [184]. Other transcription factors, such as p53, TBP and $TF_{II}B$, were shown to stimulate PARP activity, albeit more modestly than YY1 [185]. The transcriptional activity of the p53 tumour suppressor has also been shown to be regulated by poly(ADP-ribosyl)ation *in vitro* and *in vivo* [250–252]. The importance of poly(ADP-ribosyl)ation reactions for the DNA-damage-induced p53 response will be discussed further below.

Taken together, these observations demonstrate that poly(ADP-ribosyl)ation can regulate DNA transcription both positively and negatively. It appears likely that the mechanism by which PARP regulates transcription in the absence of DNA damage will be case-specific, and that a universal mechanism for such regulation will be difficult to identify. On the other hand, a universal mechanism such as the poly(ADP-ribosyl)ation of

basal transcription factors may explain the way in which poly(ADP-ribosyl)ation generally inhibits transcription in the presence of DNA lesions.

DNA repair and the maintenance of genomic integrity

DNA damage and the synthesis of pADPr

Early evidence supporting a role for PARP in the process of DNA repair came from the work of Roitt in 1956 [253], who observed that cells treated with a DNA-damaging agent showed a decrease in their levels of NAD^+. However, it was only towards the end of the 1970s that poly(ADP-ribosyl)ation was shown to be responsible for NAD^+ depletion in cells suffering DNA damage [62,63]. The specificity of the relationship between the activation of PARP and the decrease in NAD^+ has been firmly established by the use of specific inhibitors of PARP [62,63]. The subsequent development of a procedure allowing the precise quantification of pADPr further confirmed that the formation of lesions in DNA was associated with a major increase in pADPr synthesis [64]. It was also demonstrated that the reduction seen in the cellular levels of NAD^+ in cells treated with DNA-damaging agents was not associated with a decrease in NAD^+ biosynthesis [75].

The role of PARP during DNA repair was clearly established by the studies of Shall and collaborators [254–256]. These authors demonstrated that chemical or nutritional inhibition of PARP slows down DNA repair and increases considerably the cytotoxicity of DNA-damaging agents. The cytotoxicity of PARP inhibitors is due to an increase in the half-life of DNA strand breaks [254,256–259]. It was later demonstrated that the inhibition of PARP results in genomic instability, as expected from the longer half-life of DNA strand breaks *in vivo*, and in the accumulation of damaged cells at the G_2/M boundary of the cell cycle [260,261]. The genomic instability caused by the suppression of poly(ADP-ribosyl)ation reactions is characterized by increased levels of both sister chromatid exchanges [262–265] and homologous recombination [266–269], the potentiation of carcinogen-induced gene amplification [270,271] and the loss of a number of amplified genes [272–274]. Interestingly, abrogation of poly(ADP-ribosyl)ation in living cells does not stimulate (as seen with homologous recombination), but rather reduces, the levels of illegitimate recombination (random integration of exogenous DNA) [275–277]. Inhibition of PARP also results in an increased rate of apoptosis [278] and in an important reduction in cellular death by necrosis [86,87]. As mentioned above, the decrease in the rate of necrosis is due mainly to the maintenance of NAD^+ levels during cellular demise, and results in an apparent increase in the levels of apoptosis [86,87]. In addition, it appears likely that the increased rate of apoptosis seen in the absence of poly(ADP-ribosyl)ation can be partially explained by the reduced ability of cells to repair DNA damage [279].

Several lines of evidence suggest that poly(ADP-ribosyl)ation reactions are involved in the DNA BER pathway. First, the types of DNA damage that induce poly(ADP-ribosyl)ation *in vivo*, mainly base damage and DNA SSBs, are usually repaired by BER [62–74]. Secondly, PARP could be an integral component of a multiprotein BER complex containing XRCC1, DNA ligase III and DNA polymerase β (discussed in more detail below) [126,155]. Thirdly, inhibition of PARP activity results in the inhibition of BER *in vivo* and *in vitro*. Indeed, Satoh and Lindahl [280] showed that, in a cell-free DNA BER assay, the repair of SSBs is dependent on the presence of NAD^+, the substrate of PARP. Inhibition of poly(ADP-ribosyl)ation in this system inhibited the repair of SSBs [280]. This observation was later extended to the *in vitro* repair of DNA bases damaged by alkylation and by oxidative insults [281]. In addition, the involvement of PARP in the BER pathway received support from the use of transdominant and chemical inhibition of PARP in living cells [254,257–259,282,283]. As mentioned above, the presence of these inhibitors results in a specific increase in the half-life of DNA lesions that are usually repaired by the BER pathway.

The limited specificity of the chemical inhibitors of PARP, when used at high concentrations, has considerably complicated the interpretation of studies in which they have been used [284,285]. Lindahl and colleagues [280,281] have also shown that a quasi-total inhibition of PARP is necessary to inhibit BER significantly. Indeed, these authors have observed that the automodification of a single PARP enzyme with only 25 residues of ADPr is sufficient for BER to be completed [196]. This observation suggests that inhibition of at least 95% of PARP activity has to be obtained in order to observe inhibition of DNA repair. At concentrations that specifically inhibit PARP *in vivo* [196], 3-aminobenzamide or more powerful inhibitors (e.g. phenanthridinone) inhibit only 50% of the repair, which demonstrates the resistance of PARP activity to competitive inhibitors [196,287–289]. Despite these considerations, most results obtained with chemical inhibitors of PARP were later confirmed by a molecular genetics approach that mimics chemical inhibition without its potential side-effects (see below). Therefore it appears that the use of chemical inhibitors of PARP at moderate concentrations *in vivo* is a reliable way to specifically inhibit poly(ADP-ribosyl)ation. New highly effective chemical inhibitors of PARP have been developed recently, and show very promising results in tests *in vitro* and *in vivo* [290–292].

One interesting observation from the *in vitro* BER assay is that the repair of damaged plasmids can also be completed after the removal of PARP from the cellular extracts used in the assay [280]. Furthermore, BER of uracil in oligonucleotides was reconstituted with purified enzymes in the absence of PARP [160]. These observations suggest that PARP might be involved only in a subset of BER events and/or that its role in this pathway is dependent on the conformation of the repair substrate. On the other hand, Lindahl and co-workers [125,280] concluded from these observations that PARP is not required for DNA BER and that, on the contrary, the enzyme is an inhibitor of BER *in vivo*. It is clear that the development of *in vitro* systems has been instrumental in our understanding of the basic mechanisms of the BER pathway [160]. However, the investigation of the role of poly(ADP-ribosyl)ation in DNA repair by the use of these cell-free repair systems presents some theoretical problems as to how closely they represent the actual situation *in vivo*. For example, in the *in vitro* repair system of Satoh and Lindahl [280], it seems that all PARP molecules were modified during the course of repair, which is not the case during DNA repair *in vivo*, where there is a large excess of PARP molecules [31,111]. This indicates that the level at which the injury is analysed in cell-free systems does not represent the real stoichiometry prevalent in intact cells. This is also illustrated by the fact that endogenous levels of NAD^+ are usually not limiting for poly(ADP-ribosyl)ation reactions with moderate levels of DNA damage [39,59,60]. It is therefore unlikely that PARP will act as an inhibitor of DNA BER *in vivo* under normal circumstances. Even more important is the fact that the DNA used in the *in vitro* system is not structured in chromatin [160,280], whereas it clearly is in living cells. The strong evidence implicating poly(ADP-ribosyl)ation in the reorganization of chromatin structure clearly indicates that the role of PARP might be at this level during BER in living cells (discussed in more detail below). In conclusion, it is unclear at the moment if further insight into the role of

poly(ADP-ribosyl)ation can be obtained only by the use of simplified *in vitro* DNA repair systems. It will be necessary in the future to develop new BER assays that can monitor the repair of DNA substrates assembled in chromatin in order to address some of the points described above.

Transdominant inhibition of PARP

More recently, a new approach has been developed to inhibit PARP *in vivo* [282,283,289]. This approach is based on the overexpression of the DBD of PARP in living cells. In this system, the DBD of PARP associates constitutively with DNA strand breaks [143] and, consequently, blocks the access of these lesions to the resident enzyme. The transdominant inhibition that results from this system is extremely efficient in inhibiting the synthesis of pADPr, as determined by immunological and biochemical methods [282,283,289]. Moreover, this new approach has allowed analysis of the effects of PARP on BER without having to consider the potentially non-specific effects associated with the use of chemical inhibitors. Expression of the dominant-negative DBD sensitizes cells to alkylating agents and γ-irradiation, but not to UV irradiation, as would be expected for a protein involved in BER [278,283]. Furthermore, microinjection of the DBD of PARP into living cells blocks alkylation-induced, but not UV-induced, DNA repair synthesis (unscheduled DNA synthesis), again supporting a role for PARP in BER [282]. Cell lines constitutively expressing the DBD of PARP have been developed [278]. These cells show interesting features, such as increased levels of spontaneous and DNA-damage-induced sister chromatid exchanges, accumulation at the G_2/M phases of the cell cycle and an increased rate of cell death by apoptosis [278]. Moreover, conditional expression of the DBD of PARP was shown to stimulate carcinogen-induced gene amplification, as observed previously with chemical inhibitors of PARP [293]. In fact, most features associated with the transdominant inhibition of PARP have been observed previously when using chemical inhibitors of this enzyme. Therefore the transdominant approach to inhibiting PARP has confirmed that most, if not all, effects observed with chemical inhibitors of poly(ADP-ribosyl)ation (e.g. 3-aminobenzamide) are genuine, and not due to unspecific side effects.

Nevertheless, it is necessary to note that transdominant, chemical or nutritional inhibition of PARP creates a situation that does not exist in the cell. Indeed, under normal circumstances PARP associates with DNA strand breaks for a very short period of time *in vivo*. However, during the inhibition of PARP by the approaches described above, the DBD of the enzyme or the catalytically inactive PARP remains associated with DNA strand breaks for a substantial length of time. It is clear that the occupation of the damaged site by PARP or by its shorter variants prevents the repair enzymes from having access to DNA strand breaks, and thus increases the half-life (and cytotoxicity) of these lesions [254,259].

Animals and cells deficient in PARP

Mice deficient for the *PARP* gene have been developed in several laboratories [88,279,294]. Surprisingly, initial experiments using cells isolated from these animals did not support a role for PARP in DNA repair [294]. However, it appears from recent studies that the experiments used to investigate DNA repair in the initial study were not adequate to illustrate the role of PARP in response to DNA damage. Indeed, the system used by Wang and co-workers [294] was based on the capacity of cells to repair and actively transcribe alkylated plasmids. Alkylation-induced damage is repaired by two pathways in eukaryotic cells: BER and an alternative repair pathway using methyltransferases [295]. The utilization of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, an alkylating agent, in these experiments could have downplayed the contribution of PARP to repair, because it has been demonstrated that the activity of O^6-methylguanine-DNA transferase is stimulated in the absence of poly(ADP-ribosyl)ation [296]. It is therefore possible that the repair of alkylated plasmids observed by Wang et al. [294] in $PARP^{-/-}$ cells was due to partial compensation by the methyltransferase pathway rather than to the proficiency of the BER pathway [292]. In addition, the plasmid DNA used in these studies does not show the typical chromatin structure characteristic of chromosomes, and therefore lacks an important level of organization in which PARP appears to play a significant role. Finally, the fact that the plasmid has to be transcribed in order to monitor repair activity is likely to minimize the contribution of PARP to DNA repair, because it has been shown previously that PARP plays a role mainly in the repair of non-transcribed genes [297].

de Murcia and colleagues have recently developed $PARP^{-/-}$ mice [279]. These mice are extremely sensitive to γ-rays and to *N*-methylnitrosourea and show genomic instability, as indicated by increases in the levels of both sister chromatid exchanges and chromatid breaks, following DNA damage [279]. Correspondingly, cell lines derived from $PARP^{-/-}$ mice accumulate at the G_2/M phases of the cell cycle after being treated with DNA-damaging agents and undergo rapid apoptosis, again confirming the observations obtained with the chemical inhibitors of PARP and with the transdominant approach [224,279]. The reduced viability of these cells following DNA damage appears to be due to an important delay in DNA strand break rejoining, and can be restored by re-introducing PARP cDNA into the cells [298]. Smulson and collaborators [299] have developed an antisense system that allows one to inhibit PARP expression by up to 90 % in living cells. Expression of the antisense RNA of PARP in mammalian cells caused a considerable decrease in the repair of DNA lesions produced by methyl methanesulphonate and HN_2, particularly during the period that immediately followed the induction of the damage in DNA [300,301]. Mutant cell lines lacking PARP activity have also been isolated [302–306] and their response to genotoxic agents has been characterized. These cells demonstrate similar sensitivity to DNA-damaging agents as cells overexpressing the antisense mRNA of PARP [302,304,306].

Taken together, these studies demonstrate that PARP plays an important and positive role in the response to DNA damage and in the maintenance of genomic integrity. More recently, Wang et al. [307] reported that their $PARP^{-/-}$ mice are very sensitive to alkylating agents and γ-irradiation, thereby supporting the results obtained by de Murcia's group [279,298].

Roles of poly(ADP-ribosyl)ation during DNA repair

DNA repair is known to occur via several pathways in living cells [295]. PARP plays a role in the DNA BER pathway [282]. Although the precise function of PARP in BER is not known, preliminary experiments suggest that poly(ADP-ribosyl)ation is not involved in the excision of the damaged bases [308–310] or in the resynthesis of DNA after excision [311,312]. Recent observations suggest that PARP might be involved in more than one way in BER and in other types of DNA repair. The models that have been proposed to explain the role of poly(ADP-ribosyl)ation in the maintenance of genomic integrity are described below. These models are not mutually exclusive, and it is very likely that they can be integrated in a more general scheme to explain the role of PARP in living cells.

1. The recruiting model. PARP is one of the first nuclear

factors to recognize lesions in DNA, and it is therefore in an ideal position to directly recruit the DNA (base excision) repair machinery to the site of DNA damage in living cells [125]. This model was supported by the identification of a BER complex comprising PARP, XRCC1, DNA ligase III and DNA polymerase β [126,155,160]. Indeed, the presence of PARP in this multiprotein complex suggests that this enzyme can recruit the DNA repair apparatus directly to the sites of DNA damage *in vivo* and facilitate DNA repair in this way. XRCC1 protein acts as a molecular scaffold that nucleates the formation of this BER complex by interacting with all components of the complex individually [126,155]. Since XRCC1 can be poly(ADP-ribosyl)ated *in vitro*, it seems possible that PARP could regulate the activity of the complex by modifying XRCC1 *in vivo* and altering its ability to interact with other components of the complex. It is not known at the moment if other members of the BER machinery are targets for direct (covalent) or indirect (non-covalent) modification by poly(ADP-ribosyl)ation.

It appears that the stoichiometry of the BER complex is important for the regulation of PARP activity. Indeed, it has been shown that the overexpression of XRCC1 reduces PARP activity in living cells [155]. Similarly, DNA ligase III and XRCC1 were shown to inhibit PARP activity *in vitro* when present in excess over PARP [126,155]. Under these conditions, DNA ligase III is likely to inhibit PARP activity by a competitive mechanism, since the two enzymes share similar zinc fingers and DNA-nick-binding activity [126]. PARP may also recruit DNA repair factors through the modification of chromatin proteins. The long chains of pADPr could indeed target repair enzymes to the sites of DNA strand breaks much faster that they would normally do if they had to find the damage themselves throughout the nucleus. The recruiting model is compatible with PARP acting before and/or after the excision of damaged bases. However, these two possibilities might reflect different contributions of PARP to DNA repair, and it will be important in the future to clarify the timing of PARP action in the sequence of BER events.

2. The anti-recombination model. A model describing the potential role of PARP in BER has been proposed by Satoh and Lindahl [280]. According to this model, PARP inhibits DNA repair and prevents DNA recombination. Indeed, via its association with DNA strand breaks and the poly(ADP-ribosyl)ation of neighbouring proteins, PARP could specifically protect DNA ends from nuclease and/or DNA strand exchange activities which are required for recombination reactions. This model has received some support from the analysis of *scid*/*PARP*$^{-/-}$ double-knockout animals, which revealed that the absence of PARP stimulates recombination and results in an increased incidence of lymphomas [313]. However, it has to be noted that the inhibition of BER observed in an *in vitro* system by Lindahl and colleagues and the inhibition of recombination proposed by these same authors are two different processes [125,280]. The anti-recombination model is at odds with several studies showing that chemical inhibition of PARP increases the levels of homologous recombination [266–269]. In addition, PARP-deficient cells are not characterized by increased levels of extrachromosomal recombination [314]. At least for the BER pathway, the inhibition (if present) would be extremely transitory in intact cells, since PARP automodification and shuttling of DNA strand breaks is extremely rapid due to the high cellular concentration of NAD^+ [280].

PARP has also been found in a multiprotein complex capable of some DNA recombination activities [315]. It is exceptional that three of the four components of this complex (namely numatrin/B23 [316], nucleolin/C23 [317] and PARP itself) are known to be modified by poly(ADP-ribosyl)ation. In addition, the fact that these proteins can be poly(ADP-ribosyl)ated *in vivo* {nucleolin/C23 was shown to be poly(ADP-ribosyl)ated in isolated nuclei [317]} further supports the specificity of the interaction seen between these proteins, and suggests that PARP may regulate the activity of the complex by poly(ADP-ribosyl)ating its components. The specific role of PARP (positive or negative) in the regulation of this B-cell-specific complex remains to be defined. However, it is interesting to note that chemical inhibitors of PARP were shown to increase the rate of antibody class switching in B cells [318].

3. The chromatin-dependent repair model. The kinetics of DNA repair are greatly influenced by the presence of histones on damaged DNA [286]. The well established poly(ADP-ribosyl)ation of histones in response to DNA damage [35,204,205] strongly suggests that PARP plays a much more important role in DNA repair when DNA is structured in chromatin. Indeed, because PARP activity is induced by DNA strand breaks [33,139], chromatin structure should be opened in the immediate vicinity of DNA lesions [due to the poly(ADP-ribosyl)ation of histone H1 and core histones] [163], and this local disruption of chromatin configuration is very likely to be critical for the repair of highly condensed chromatin. This is well illustrated by the fact that, in the absence of PARP activity, DNA repair in non-transcribed regions of the genome is significantly less efficient than in transcribed regions [297]. Non-transcribed regions often demonstrate a condensed chromatin structure, while those that are actively transcribed generally demonstrate an open chromatin structure that is accessible to proteins such as transcription factors and, presumably, DNA repair proteins [319]. Also supporting this hypothesis is the observation that the poly(ADP-ribosyl)ation of chromatin makes it reversibly competent to enzymic processing by heterogeneous nucleases [202]. In a similar manner, PARP could facilitate DNA repair by alleviating the steric congestion caused by densely packed chromatin. This could allow DNA repair factors to access a subset of DNA lesions that are otherwise irreparable. This hypothesis is consistent with the fact that several DNA repair and DNA-damage-signalling factors act as large multisubunit protein complexes (e.g. $DNApk_{cs}$ and the BER machinery). It is not difficult to imagine how these large complexes could be hindered in the presence of highly condensed chromatin and how chromatin decondensation could help these factors to perform their functions. Taken together, these observations could explain the extreme sensitivity of *PARP*$^{-/-}$ mice to alkylating agents and γ-irradiation [279,307].

It is still an open debate whether poly(ADP-ribosyl)ation affects DNA repair by changing chromatin structure, as discussed recently by Smerdon and Conconi [320]. In order to address this point, DNA BER studies should be performed with *bona fide* chromatin substrates, as carried out by Smerdon and Thoma [321] for nucleotide excision repair.

4. The signalling model. As mentioned previously, poly(ADP-ribosyl)ation is an immediate response to DNA damage, which indicates that PARP is one of the first proteins to be associated with DNA strand breaks *in vivo*. One way in which PARP could stimulate repair and/or ensure genomic stability might be through signalling the presence of DNA lesions to downstream effectors involved in co-ordinating the cellular response to DNA damage. Indeed, the extensive modification of histones and of PARP itself at sites of DNA strand breaks could act as a strong signal that activates the repair machinery or other signalling molecules. Although sharing some similarities, the signalling and recruiting models are conceptually distinct and could clearly reflect two different functions of PARP.

This model has been substantiated by the observation that poly(ADP-ribosyl)ation reactions are involved in the regulation of p53 functions. Indeed, chemical inhibition of PARP was shown to suppress significantly the accumulation of p53 in response to ionizing radiation [250–252]. This observation has been corroborated in several cell lines, including cell lines derived from PARP-deficient mice [322,323]. As expected, the suppression of p53 accumulation resulting from the absence of PARP activity abrogates p53-induced transactivation of *MDM2* and $p21^{WAF1}$ gene expression (and possibly other p53-responsive genes) [251,252,324]. The absence of p53 transcriptional activity correlates with the absence of irradiation-induced sequence-specific DNA-binding activity on p53 consensus DNA substrates in nuclear extracts obtained from cells treated with a chemical inhibitor of PARP [252]. Similarly, partial inhibition of PARP expression ($>90\%$) in a cell line expressing PARP antisense RNA results in a significant delay in p53 induction in response to γ-irradiation [324]. In addition, p53 levels were shown to be considerably decreased, even in the absence of DNA damage or other types of cellular insults, in PARP-deficient cells [322,323].

These effects of PARP on p53 function could explain the well established accumulation of cells at the G_2 phase of the cell cycle in response to chemical or transdominant inhibition of PARP [278]. Indeed, p53 has been shown to be a key effector of the G_1 arrest and is also involved, although to a much lesser extent, in the G_2 arrest [325]. In the absence of PARP activity, some lesions could remain undetected before DNA replication, and these would result in further damage during S phase and in a subsequent arrest at the G_2 phase of the cell cycle. Induction of the p53-induced cell cycle arrest by poly(ADP-ribosyl)ation may provide the time necessary for these lesions to be repaired, and could certainly contribute to the maintenance of genomic integrity. The reduced activation of p53 in the absence of PARP could also account for the abrogation of the apoptotic response observed under certain conditions, but not under others, in PARP-deficient cells [307,326,327]. Indeed, p53 has been shown to be a critical factor in the induction of apoptosis in response to a subset of apoptotic stimuli [325]. Despite the clear evidence supporting a role for poly(ADP-ribosyl)ation in the regulation of p53 functions, alternative DNA-damage-signalling pathways must operate in the absence of poly(ADP-ribosyl)ation, since PARP-deficient mice do not show the high cancer rate typically found in p53-deficient mice [279,294]. In addition, p53 induction can be observed to some extent in $PARP^{-/-}$ animals treated with high doses of DNA-damaging agents [279]. Interestingly, p73 levels are increased in PARP-deficient cells, which might explain, at least partially, the observations described above [328].

How might PARP regulate p53? This important question has not yet been answered, but a number of hypotheses can be proposed to explain the observations decribed. Poly(ADP-ribosyl)ation of p53 has been demonstrated *in vitro*, but it appears that this modification cannot account for the effects observed *in vivo*, because no poly(ADP-ribosyl)ation of wild-type p53 can be detected in living cells [329,330]. However, p53 can interact strongly and non-covalently with polymers of ADPr [53]. It is likely that the extensive nature of poly(ADP-ribosyl)ation reactions (>200 units of ADPr/chain) at sites of DNA strand breaks would provide a strong signal to direct p53 or other signalling factors to the sites of DNA damage in the nucleus. The rapid degradation of pADPr by PARG would also provide a convenient pADPr-unloading mechanism and would allow p53 to perform efficiently its DNA-damage-signalling functions at the site of DNA damage. PARP has also been shown to interact physically with p53 [251,330]. This interaction may provide an alternative, or maybe an additional, way by which PARP could regulate p53 activity, either by directly modifying its functional properties or by recruiting the protein to DNA strand breaks. Clearly, further experimental work will be required to test these working models and to see if other DNA-damage-signalling proteins, such as the ATM (ataxia telangiectasia mutated) protein, are affected by poly(ADP-ribosyl)ation reactions.

FUTURE DIRECTIONS

As discussed in this review, poly(ADP-ribosyl)ation is involved in several nuclear transactions, and the absence of this process leads to pleiotropic effects *in vivo*. Despite the clear evidence for a role for poly(ADP-ribosyl)ation in the metabolism of nucleic acids, no unified picture for the role of this protein modification has yet emerged. However, recent advances have greatly clarified the ways in which several nuclear processes are (or might be) regulated by poly(ADP-ribosyl)ation, most notably DNA repair. It will be very important in the future to integrate this knowledge with the potential functions of poly(ADP-ribosyl)ation in the reorganization of chromatin architecture. Although technically difficult, the long-awaited demonstration of chromatin restructuring in response to poly(ADP-ribosyl)ation *in vivo* will be of great interest, given the strong biochemical evidence supporting this phenomenon and the implications it has for other nuclear processes.

The recent discovery of non-classical PARPs in eukaryotes and in one species of archaea has generated renewed interest in the field of poly(ADP-ribosyl)ation. The investigation of the roles of these novel PARPs in living cells will certainly represent an intense and exciting new field of research on its own. Since at least one of these PARPs is sensitive to inhibition by 3-aminobenzamide, it will be important to revisit some of the data obtained with this inhibitor of poly(ADP-ribosyl)ation reactions. Poly(ADPr) synthesis has also been shown to play important roles in cellular processes such as differentiation and cellular death. Future research should be directed at integrating the pro-survival (DNA repair), pro-apoptotic (DNA damage signalling) and pro-necrotic (overstimulation of PARP activity) effects of poly(ADP-ribosyl)ation in order to fully understand the roles of PARP cleavage and pADPr synthesis in the cellular response to DNA damage. The availability of new tools, such as cell lines deficient in PARPs, transdominant and chemical inhibitors of poly(ADP-ribosyl)ation and probes to detect pADPr in cells, will contribute greatly to the future understanding of the complex and fascinating functions of poly(ADP-ribosyl)ation in living cells.

REFERENCES

1 Chambon, P., Weil, J. D. and Mandel, P. (1963) Biochem. Biophys. Res. Commun. **11**, 39–43
2 Chambon, P., Doly, J. D., Strosser, M. T. and Mandel, P. (1966) Biochem. Biophys. Res. Commun. **25**, 638–643
3 Reeder, R. H. (1967) J. Biol. Chem. **242**, 3172–3179
4 Sugimura, T. (1967) Biochim. Biophys. Acta **138**, 438–441
5 Riquelme, P. T., Burzio, L. O. and Koide, S. S. (1979) J. Biol. Chem. **254**, 3018–3028
6 Burzio, L. O., Riquelme, P. T. and Koide, S. S. (1979) J. Biol. Chem. **254**, 3029–3037
7 Suzuki, H., Quesada, P., Farina, B. and Leone, E. (1986) J. Biol. Chem. **261**, 6048–6055
8 Tanaka, M., Miwa, M., Hayashi, K., Kubota, K. and Matsushima, T. (1977) Biochemistry **16**, 1485–1489
9 Tanaka, M., Hayashi, K., Sakura, H., Miwa, M., Matsushima, T. and Sugimura, T. (1978) Nucleic Acids Res. **5**, 3183–3194
10 Kanai, Y., Tanuma, S. and Sugimura, T. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 2801–2804

11 Kanai, M., Miwa, M., Kuchino, V. and Sugimura, T. (1982) J. Biol. Chem. **257**, 6217–6223
12 Hayaishi, K., Tanaka, M., Shimada, T., Miwa, M. and Sugimura, T. (1983) Biochem. Biophys. Res. Commun. **112**, 102–107
13 de Murcia, G., Jongstra-Bilen, J., Ittel, M. E., Mandel, P. and Delain, E. (1983) EMBO J. **2**, 543–548
14 Miwa, M., Ishihara, M., Takishima, S., Takasuka, N., Maeda, M., Yamaizumi, Z., Sugimura, T., Yokoyama, S. and Miyazawa, T. (1981) J. Biol. Chem. **256**, 2916–2921
15 Miwa, M., Saikawa, N., Yamizuni, Z., Nishimira, S. and Sugimura, T. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 595–599
16 Kawaichi, M., Ueda, K. and Hayaishi, O. (1981) J. Biol. Chem. **256**, 9483–9489
17 Alvarez-Gonzalez, R. and Jacobson, M. K. (1987) Biochemistry **26**, 3218–3224
18 Minaga, T. and Kun, E. (1983) J. Biol. Chem. **258**, 725–730
19 Minaga, T. and Kun, E. (1983) J. Biol. Chem. **258**, 5726–5730
20 Kanai, Y., Sugimura, T. and Matsushima, T. (1978) Nature (London) **274**, 809–812
21 Sibley, J. T., Braun, R. P. and Lee, J. S. (1986) Clin. Exp. Immunol. **64**, 563–569
22 Althaus, F. R. and Richter, C. (1987) ADP-Ribosylation of Proteins: Enzymology and Biological Significance, Springer-Verlag, Berlin
23 Shieh, W. M., Ame, J. C., Wilson, M. V., Wang, Z. Q., Koh, D. W., Jacobson, M. K. and Jacobson, E. L. (1998) J. Biol. Chem. **273**, 30069–30072
24 Babiychuk, E., Cottrill, P. B., Storozhenko, S., Fuangthong, M., Chen, Y., O'Farrel, M., van Montagu, M., Inze, D. and Kushnir, S. (1998) Plant J. **15**, 635–645
25 Smith, S., Giriat, I., Schmitt, A. and De Lange, T. (1998) Science **282**, 1484–1487
26 Faraone-Mennella, M. R., Gambacorta, A., Nicolaus, B. and Farina, B. (1998) Biochem. J. **335**, 441–447
27 Miwa, M., Tanaka, M., Matsushima, T. and Sugimura, T. (1974) J. Biol. Chem. **249**, 3475–3482
28 Ikejima, M. and Gill, D. M. (1988) J. Biol. Chem. **263**, 11037–11040
29 Okayama, H., Honda, M. and Hayaishi, O. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 2254–2257
30 Oka, J., Ueda, K., Hayaishi, O., Komura, H. and Nakanishi, K. (1984) J. Biol. Chem. **259**, 986–993
31 Wielckens, K., Schmidt, A., George, B., Bredehorst, R. and Hilz, H. (1982) J. Biol. Chem. **257**, 12872–12877
32 Ferro, A. M. and Oppenheimer, N. J. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 809–813
33 Benjamin, R. C. and Gill, D. M. (1980) J. Biol. Chem. **255**, 10502–10508
34 Wielckens, K., George, E., Pless, T. and Hilz, H. (1983) J. Biol. Chem. **258**, 4098–4104
35 Kreimeyer, A., Wielckens, K., Adamietz, P. and Hilz, H. (1984) J. Biol. Chem. **259**, 890–896
36 Alvarez-Gonzalez, R. and Althaus, F. R. (1989) Mutat. Res. **218**, 67–74
37 Simonin, F., Poch, O., Delarue, M. and de Murcia, G. (1993) J. Biol. Chem. **268**, 8529–8535
38 Singh, N. S., Poirier, G. G. and Cerutti, P. A. (1985) EMBO J. **4**, 1491–1494
39 Loetscher, P., Alvarez-Gonzalez, R. and Althaus, F. R. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 1286–1289
40 Coppola, S., Nosseri, C., Maresca, V. and Ghibelli, L. (1995) Exp. Cell Res. **221**, 462–469
41 Malanga, M. and Althaus, R. (1994) J. Biol. Chem. **269**, 17691–17696
42 Hatakeyama, K., Nemoto, Y., Ueda, K. and Hayaishi, O. (1986) J. Biol. Chem. **261**, 14902–14911
43 Hilz, H., Wielkens, K. and Bredehorst, R. (1982) in ADP-Ribosylation Reactions: Biology and Medicine, pp. 305–321, Academic Press, New York
44 Miro, A., Costas, M. J., Garcia-Diaz, M., Hernandez, M. T. and Camesell, J. C. (1989) FEBS Lett. **244**, 123–126
45 Fernandez, A., Ribeiro, J. M., Costas, M. J., Pinto, R. M., Canales, J. and Cameselle, J. C. (1996) Biochim. Biophys. Acta **1290**, 121–127
46 Cervantes-Laurean, D., Minter, D. E., Jacobson, E. L. and Jacobson, M. K. (1993) Biochemistry **32**, 1528–1534
47 Cervantes-Laurean, D., Loflin, P. T., Minter, D. E., Jacobson, E. L. and Jacobson, M. K. (1996) J. Biol. Chem. **271**, 10461–10469
48 Ogata, N., Ueda, K., Kawaichi, M. and Hayaishi, O. (1981) J. Biol. Chem. **256**, 4135–4137
49 Ferro, A. M. and Olivera, B. M. (1982) J. Biol. Chem. **257**, 7808–7813
50 Hunter, T. (1998) Philos. Trans. R. Soc. London B Biol. Sci. **353**, 583–605
51 Panzeter, P. L., Realini, C. A. and Althaus, F. R. (1992) Biochemistry **31**, 1379–1385
52 Nozaki, U. T., Masutani, M., Akagawa, T., Sugimura, T. and Esuni, H. (1994) Biochem. Biophys. Res. Commun. **198**, 45–51
53 Malanga, M., Pleschke, J. M., Kleczkowska, H. E. and Althaus, F. R. (1998) J. Biol. Chem. **273**, 11839–11843
54 Voet, D. and Voet, J. G. (1995) Biochemistry, 2nd edn., Wiley, New York
55 Zatman, L. J., Kaplan, N. O. and Colowick, S. P. (1953) J. Biol. Chem. **200**, 197–212
56 Hillyard, D., Reichsteiner, M., Manlapaz-Ramos, P., Imperial, J. S., Cruz, L. J. and Olivera, B. M. (1981) J. Biol. Chem. **256**, 8491–8497
57 Carson, D. A., Seto, S., Wasson, D. B. and Carrera, C. J. (1986) Exp. Cell Res. **164**, 273–281
58 Schraufstatter, I. U., Hyslop, P. A., Hinshaw, D. B., Spragg, R. G., Sklar, L. A. and Cochrane, C. G. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 4908–4912
59 Williams, G. T., Shall, S. and Ford, C. C. (1983) Biochim. Biophys. Acta **762**, 272–280
60 Williams, G. T., Lau, K. M. K., Coote, J. M. and Johnstone, A. (1985) Exp. Cell Res. **160**, 419–426
61 Rechsteiner, M., Hillyard, D. and Olivera, B. M. (1976) Nature (London) **259**, 695–696
62 Goodwin, P. M., Lewis, P. J., Davies, M. I., Skidmore, C. J. and Shall, S. (1978) Biochim. Biophys. Acta **543**, 576–582
63 Skidmore, C. J., Davies, M. I., Goodwin, P. O. M., Halldorson, H., Lewis, P. J., Shall, S. and Zia'ee, A. (1979) Eur. J. Biochem. **101**, 135–142
64 Juarez-Salinas, H., Sims, J. L. and Jacobson, M. K. (1979) Nature (London) **282**, 740–741
65 Stubberfield, C. R. and Cohen, G. M. (1988) Pharmacology **37**, 3967–3974
66 Schraufstatter, I. U., Hinshaw, D. B., Hyslop, P. A., Spragg, R. G. and Cochrane, C. G. (1986) J. Clin. Invest. **77**, 1312–1320
67 Junod, A. F., Jornot, L. and Peterson, H. (1989) J. Cell. Physiol. **140**, 177–185
68 Zhang, J., Dawson, V. L., Dawson, T. M. and Synder, S. H. (1994) Science **263**, 687–689
69 Radons, J., Heller, B., Bürkle, A., Hartmarin, B., Rodriguez, M. L., Kröncke, K. D., Bunkart, V. and Kolb, H. (1994) Biochem. Biophys. Res. Commun. **199**, 1270–1277
70 Szabo, C., Zingarelli, B., O'Connor, M. and Salzman, A. L. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 1753–1758
71 Zingarelli, B., O'Connor, M., Wong, H., Salzman, A. L. and Szabo, C. (1996) J. Immunol. **156**, 350–358
72 Zhao, B., Konno, S., Wu, J. M. and Oronsky, A. L. (1990) Cancer Lett. **50**, 141–147
73 Hoyt, D. G. and Lazo, J. S. (1993) Biochem. Pharmacol. **46**, 1819–1824
74 Whish, W. J. D., Davies, M. I. and Shall, S. (1975) Biochem. Biophys. Res. Commun. **65**, 722–730
75 Jacobson, M. K., Levi, V., Juarez-Salinas, H., Barton, R. A. and Jacobson, E. L. (1980) Cancer Res. **40**, 1797–1802
76 Das, S. K. and Berger, N. A. (1986) Biochem. Biophys. Res. Commun. **137**, 1153–1158
77 Jonsson, G. G., Jacobson, E. L. and Jacobson, M. K. (1988) Cancer Res. **48**, 4233–4239
78 Rankin, P. W., Jacobson, M. K., Mitchell, V. R. and Busbee, D. L. (1980) Cancer Res. **40**, 1803–1807
79 Oleinick, N. L. and Evans, H. H. (1985) Radiat. Res. **101**, 29–46
80 Sims, J. L., Berger, S. J. and Berger, N. A. (1983) Biochemistry **22**, 5188–5194
81 Jacobson, E. L., Lange, R. A. and Jacobson, M. K. (1979) J. Cell. Physiol. **99**, 417–425
82 Bernofsky, C. (1980) Mol. Cell. Biochem. **33**, 135–143
83 Alvarez-Gonzalez, R., Eichenberger, R. and Althaus, F. R. (1986) Biochem. Biophys. Res. Commun. **138**, 1051–1057
84 Eguchi, Y., Shimizu, S. and Tsujimoto, Y. (1997) Cancer Res. **57**, 1835–1840
85 Leist, M., Single, B., Castoldi, A. F., Kühnle, S. and Nicotera, P. (1997) J. Exp. Med. **185**, 1481–1486
86 Szabo, C. (1998) Eur. J. Pharmacol. **350**, 1–19
87 Szabo, C. and Dawson, V. L. (1998) Trends Pharmacol. Sci. **19**, 287–298
88 Masutani, M., Suzuki, H., Kamada, N., Watanabe, M., Ueda, O., Nozaki, T., Jishage, K., Watanabe, T., Sugimoto, T., Nakagama, H., et al. (1999) Proc. Natl. Acad. Sci. U.S.A. **96**, 2301–2304
89 Burkart, V., Wang, Z. Q., Radons, J., Heller, B., Herceg, Z., Stingl, L., Wagner, E. F. and Kolb, H. (1999) Nat. Med. (N.Y.) **5**, 314–319
90 Pieper, A. A., Brat, D. J., Krug, D. K., Watkins, C. C., Gupta, A., Blackshaw, S., Verma, A., Wang, Z. Q. and Snyder, S. H. (1999) Proc. Natl. Acad. Sci. U.S.A. **96**, 3059–3064
91 Eliason, M. J. L., Sampei, K., Mandir, A. S., Hurn, P. D., Traystman, R. J., Bao, J., Pieper, A., Wang, Z. Q., Dawson, T. M., Snyder, S. H. and Dawson, V. L. (1997) Nat. Med. (N.Y.) **3**, 1089–1095
92 Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E. and Poirier, G. G. (1993) Cancer Res. **53**, 3976–3985
93 Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G. and Earnshaw, W. C. (1994) Nature (London) **371**, 346–347
94 Casiano, C. A., Martin, S. J., Green, D. R. and Tan, E. M. (1996) J. Exp. Med. **184**, 765–770
95 Greidinger, E. L., Miller, D. K., Yamin, T. T., Casciola-Rosen, L. and Rosen, A. (1996) FEBS Lett. **390**, 299–303
96 Nishikimi, M., Ogasawara, K., Kameshita, I., Taniguchi, T. and Shizuta, Y. (1982) J. Biol. Chem. **257**, 6102–6105
97 Kameshita, I., Matsuda, Z., Taniguchi, T. and Shizuta, Y. (1984) J. Biol. Chem. **259**, 4770–4776

98 Scovassi, A. I., Izzo, R., Franchi, E. and Bertazzoni, U. (1986) Eur. J. Biochem. **159**, 77–84
99 Brightwell, M. D., Leech, C. E., O'Farrell, M. K., Whish, W. J. and Shall, S. (1975) Biochem. J. **147**, 119–129
100 Poirier, G. G., Côté, S. and Pallotta, D. (1987) Biochem. Cell. Biol. **65**, 81–85
101 Kofler, B., Wallraff, E., Herzog, H., Schneider, R., Auer, B. and Schweiger, M. (1993) Biochem. J. **293**, 275–281
102 Werner, E., Sohst, S., Gropp, F., Sinon, D., Wagner, H. and Kroger, H. (1984) Eur. J. Biochem. **139**, 81–86
103 Burtscher, H. J., Klocker, H., Auer, B., Hirsch-Kaufmann, M. and Schweiger, M. (1987) Biochem. J. **248**, 859–864
104 Uchida, K., Hanai, S., Ishikawa, K., Ozawa, Yl., Uchida, M., Sugimura, T. and Miwa, M. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 3481–3485
105 Desnoyers, S., Shah, G. M., Poirier, G. G. and Hengartner, M. O. (1996) AACR Conference on Programmed Cell Death, Bolton Landing, Lake George, NY, October 19–23 (Abstract A45)
106 Laroche, A., Plante, J., Beaumont, G. and Poirier, G. G. (1980) Can. J. Biochem. **58**, 692–695
107 Chen, Y. M., Shall, S. and O'Farrell, M. (1994) Eur. J. Biochem. **224**, 135–142
108 Lepiniec, L., Babiychuk, E., Kushnir, S., Van Montagu, M. and Inze, D. (1995) FEBS Lett. **364**, 103–108
109 Ruggieri, S., Gregori, L., Natalini, P., Vita, A., Emanuelli, M., Raffaelli, N. and Magni, G. (1990) Biochemistry **29**, 2501–2506
110 Park, J. K., Kim, W. J., Park, Y. S., Choi, H. S., Yu, J. E., Han, D. M. and Park, S. D. (1991) Cell. Mol. Biol. **37**, 339–344
111 Yamanaka, H., Penning, C. A., Willis, E. H., Wasson, D. B. and Carson, D. A. (1988) J. Biol. Chem. **263**, 3879–3883
112 Ludwig, A., Behnke, B., Holtlund, J. and Hilz, H. (1988) J. Biol. Chem. **263**, 6993–6999
113 Buki, K. G. and Kun, E. (1988) Biochemistry **27**, 5990–5995
114 Mazen, A., Menissier-de Murcia, J., Molinete, M., Simonin, F., Gradwohl, G., Poirier, G. G. and de Murcia, G. (1989) Nucleic Acids Res. **17**, 4689–4698
115 Froelich, C. J., Hanna, W. L., Poirier, G. G., Duriez, P. J., D'Amours, D., Salvesen, G. S., Alnemri, E. S., Earnshaw, W. C. and Shah, G. M. (1996) Biochem. Biophys. Res. Commun. **227**, 658–665
116 de Murcia, G. and Ménissier-de Murcia, J. (1994) Trends Biochem. Sci. **19**, 172–176
117 Kurosaki, T., Ushiro, H., Mitsuuchi, Y., Suzuki, S., Matsuda, M., Matsuda, Y., Katunuma, N., Kangawa, K., Matsuo, H., Hirose, T. et al. (1987) J. Biol. Chem. **262**, 15990–15997 [additions and corrections (1988) J. Biol. Chem. **263**, 7437]
118 Alkhatib, H. M., Chen, D., Cherney, B., Bathia, K., Notario, B., Giri, G., Slattery, E., Roeder, R. G. and Smulson, M. E. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 1224–1228
119 Cherney, B., McBride, O. W., Chen, D., Alkahatib, H., Bhatia, K., Hensley, P. and Smulson, M. E. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 8370–8377
120 Berg, J. M. (1990) J. Biol. Chem. **265**, 6513–6516
121 Uchida, K., Morita, T., Sato, T., Ogura, T., Yamashita, R., Nogushi, S., Suzuki, H., Nyunoya, H., Miwa, M. and Sugimura, T. (1987) Biochem. Biophys. Res. Commun. **148**, 617–622
122 Saito, I., Hatakeyama, K., Kido, T., Ohkubo, H., Nakanishi, S. and Ueda, K. (1990) Gene **90**, 249–254
123 Brennan, R. G. and Mathews, B. W. (1989) J. Biol. Chem. **264**, 1903–1906
124 Gradwohl, G., Ménissier-de Murcia, J., Molinete, M., Simonin, F., Koken, M., Hoeijmakers, J. H. J. and de Murcia, G. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 2990–2994
125 Lindahl, T., Satoh, M. S., Poirier, G. G. and Klungland, A. (1995) Trends Biochem. Sci. **20**, 405–411
126 Caldecott, K. W., Aoufouchi, S., Johnson, P. and Shall, S. (1996) Nucleic Acids Res. **24**, 4387–4394
127 Ménissier-de Murcia, J., Molinete, M., Gradwohl, G., Simonin, F. and de Murcia, G. (1989) J. Mol. Biol. **210**, 229–233
128 Ikejima, M., Noguchi, S., Yamashita, R., Ogura, T., Sugimura, T., Gill, D. M. and Miwa, M. (1990) J. Biol. Chem. **265**, 21907–21913
129 Harrison, S. C. and Aggarwal, A. K. (1990) Annu. Rev. Biochem. **59**, 933–969
130 Thibodeau, J., Potvin, F., Kirkland, J. B. and Poirier, G. G. (1993) Biochim. Biophys. Acta **1163**, 49–53
131 Sastry, S. S., Buki, K. G. and Kun, E. (1989) Biochemistry **28**, 5670–5680
132 Gradwohl, G., Mazen, A. and de Murcia, G. (1987) Biochem. Biophys. Res. Commun. **148**, 913–919
133 Sastry, S. S. and Kun, E. (1988) J. Biol. Chem. **263**, 1505–1512
134 Kaufmann, S. H., Brunet, G., Talbot, B., Lamarre, D., Dumas, C., Shaper, J. and Poirier, G. G. (1991) Exp. Cell Res. **192**, 524–535
135 Fakan, S., Leduc, Y., Lamarre, D., Brunet, G. and Poirier, G. G. (1988) Exp. Cell Res. **179**, 517–525
136 Desnoyers, S., Kirkland, J. B. and Poirier, G. G. (1996) Mol. Cell Biochem. **159**, 155–161
137 Mosgoeller, W., Steiner, M., Hozak, P., Penner, E. and Wesierska-Gadek, J. (1996) J. Cell Sci. **109**, 409–418
138 Sastry, S. S. and Kun, E. (1990) Biochem. Biophys. Res. Commun. **167**, 842–847
139 Ohgushi, H., Yoshihara, K. and Kamiya, T. (1980) J. Biol. Chem. **255**, 6205–6211
140 Weinfeld, M., Chaudhry, M. A., D'Amours, D., Pelletier, J. D., Poirier, G. G., Povirk, L. F. and Lees-Miller, S. P. (1997) Radiat. Res. **148**, 22–28
141 Bramson, J., Prevost, J., Malapetsa, A., Noe, A. J., Poirier, G. G., Desnoyers, S., Alaoui-Jamali, M. and Panasci, L. (1993) Cancer Res. **53**, 5370–5373
142 Le Cam, E., Fack, F., Menissier-de Murcia, J., Cognet, J. A., Barbin, A., Sarantoglou, V., Revet, B., Delain, E. and de Murcia, G. (1994) J. Mol. Biol. **235**, 1062–1071
143 Smulson, M. E., Pang, D., Jung, M., Dimtchev, A., Chasovskikh, S., Spoonde, A., Simbulan-Rosenthal, C., Rosenthal, D., Yakovlev, A. and Dritschilo, A. (1998) Cancer Res. **58**, 3495–3498
144 Panzeter, P. L. and Althaus, F. R. (1994) Biochemistry **33**, 9600–9605
145 Duriez, P. J., Desnoyers, S., Hoflack, J. C., Shah, G. M., Morelle, B., Bourassa, S., Poirier, G. G. and Talbot, B. (1997) Biochim. Biophys. Acta **1334**, 65–72
146 Kameshita, I., Matsuda, M., Nishikimi, M., Ushiro, H. and Shizuta, Y. (1986) J. Biol. Chem. **261**, 3863–3868
147 Masutani, M., Nozaki, T., Hitomi, Y., Ikejima, M., Nagasaki, K., De Prati, A. C., Kurata, S., Natori, S., Sugimura, T. and Esumi, H. (1994) Eur. J. Biochem. **220**, 607–614
148 Alber, T. (1992) Curr. Opin. Gen. Dev. **2**, 205–210
149 Bauer, P. I., Buki, K. G. and Kun, E. (1990) FEBS Lett. **273**, 6–10
150 Mendoza-Alvarez, H. and Alvarez-Gonzalez, R. (1993) J. Biol. Chem. **268**, 22575–22580
151 Koyama, Y., Katagiri, S., Hanai, S., Uchida, K. and Miwa, M. (1999) Gene **226**, 339–345
152 Oei, S. L., Griesenbeck, J., Schweiger, M., Babich, V., Kropotov, A. and Tomilin, N. (1997) Biochem. Biophys. Res. Commun. **240**, 108–111
153 Nie, J., Sakamoto, S., Song, D., Qu, Z., Ota, K. and Taniguchi, T. (1998) FEBS Lett. **424**, 27–32
154 Masson, M., Menissier-de Murcia, J., Mattei, M. G., de Murcia, G. and Niedergang, C. P. (1997) Gene **190**, 287–296
155 Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J. and de Murcia, G. (1998) Mol. Cell. Biol. **18**, 3563–3571
156 Buki, K. G., Bauer, P. I., Hakam, A. and Kun, E. (1995) J. Biol. Chem. **270**, 3370–3377
157 Bork, P., Hofmann, K., Bucher, P., Neuwald, A. F., Altschul, S. F. and Koonin, E. V. (1997) FASEB J. **11**, 68–76
158 Zhang, X., Morera, S., Bates, P. A., Whitehead, P. C., Coffer, A. I., Hainbucher, K., Nash, R. A., Sternberg, M. J., Lindahl, T. and Freemont, P. S. (1998) EMBO J. **17**, 6404–6411
159 Reference deleted
160 Kubota, Y., Nash, R. A., Klungland, A., Schar, P., Barnes, D. E. and Lindahl, T. (1996) EMBO J. **15**, 6662–6670
161 Kawamura, T., Hanai, S., Yokota, T., Hayashi, T., Poltronieri, P., Miwa, M. and Uchida, K. (1998) Biochem. Biophys. Res. Commun. **251**, 35–40
162 Zahradka, P. and Ebisuzaki, K. (1982) Eur. J. Biochem. **127**, 579–585
163 Poirier, G. G., de Murcia, G., Niedergang, C., Jongstra-Bilen, J. and Mandel, P. (1982) Proc. Natl. Acad. Sci. U.S.A. **79**, 3423–3427
164 de Murcia, G., Huletsky, A., Lamarre, D., Gaudreau, A., Pouyet, J., Daune, M. and Poirier, G. G. (1986) J. Biol. Chem. **261**, 7011–7017
165 Althaus, F. R. (1992) J. Cell Sci. **102**, 663–670
166 Simonin, F., Menissier-de Murcia, J., Poch, O., Muller, S., Gradwohl, G., Molinete, M., Penning, C., Keith, G. and de Murcia, G. (1990) J. Biol. Chem. **265**, 19249–19256
167 Ruf, A., Ménissier-de Murcia, J., de Murcia, G. and Schulz, G. E. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 7481–7485
168 Yamanaka, H., Willis, E. H., Penning, C. A., Peebles, C. L., Tan, E. M. and Carson, D. A. (1987) J. Clin. Invest. **80**, 900–904
169 Marsischky, G. T., Wilson, B. A. and Collier, R. S. (1995) J. Biol. Chem. **270**, 3247–3254
170 Ruf, A., Rolli, V., de Murcia, G. and Schultz, G. E. (1998) J. Mol. Biol. **278**, 57–65
171 Ruf, A., de Murcia, G. and Schulz, G. E. (1998) Biochemistry **37**, 3893–3900
172 Kim, H., Jacobson, M. K., Rolli, V., Ménissier-de Murcia, J., Reinbolt, J., Simonin, F., Ruf, A., Schultz, G. and de Murcia, G. (1997) Biochem. J. **322**, 469–475
173 Domenighini, M. and Rappuoli, R. (1996) Mol. Microbiol. **21**, 667–674
174 Bauer, P. I., Buki, K. G., Hakam, A. and Kun, E. (1990) Biochem. J. **270**, 17–26
175 Alvarez-Gonzalez, R. (1988) J. Biol. Chem. **263**, 17690–17696
176 Taniguchi, T. (1987) Biochem. Biophys. Res. Commun. **147**, 1008–1012

177 Ueda, K., Kawaichi, M., Inada, S. and Hayaishi, O. (1982) Curr. Top. Cell. Regul. **21**, 175–187
178 Lagueux, J., Ménard, L., Candas, B., Brochu, G., Potvin, F., Verreault, A., Cook, P. F. and Poirier, G. G. (1995) Biochim. Biophys. Acta **1264**, 201–208
179 Mendoza-Alvarez, H. and Alvarez-Gonzalez, R. (1999) Biochemistry **38**, 3948–3953
180 Okayama, H., Edson, C. M., Fukushima, M., Ueda, K. and Hayaishi, O. (1977) J. Biol. Chem. **252**, 7000–7005
181 Ito, S., Shizuta, Y. and Hayaishi, O. (1979) J. Biol. Chem. **254**, 3647–3651
182 Petzolt, S. J., Booth, B. A., Leimbach, G. A. and Berger, N. A. (1981) Biochemistry **20**, 7075–7081
183 Carter, S. G. and Berger, N. A. (1982) Biochemistry **21**, 5475–5481
184 Griesenbeck, J., Ziegler, M., Tomilin, N., Schweiger, M. and Oei, S. L. (1999) FEBS Lett. **443**, 20–24
185 Oei, S. L., Griesenbeck, J., Schweiger, M. and Ziegler, M. (1998) J. Biol. Chem. **273**, 31644–31647
186 Miwa, M. and Sugimura, T. (1971) J. Biol. Chem. **246**, 6362–6364
187 Ueda, K., Oka, J., Naruniya, S., Miyakawa, N. and Hayaishi, O. (1972) Biochem. Biophys. Res. Commun. **46**, 516–523
188 Lin, W., Amé, J. C., Aboul-Ela, N., Jacobson, E. L. and Jacobson, M. K. (1997) J. Biol. Chem. **272**, 11895–11901
189 Burzio, L. O., Riquelme, P. T., Ohtsuka, E. and Koide, S. S. (1976) Arch. Biochem. Biophys. **173**, 306–319
190 Tanuma, S., Kawashima, K. and Endo, H. (1986) Biochem. Biophys. Res. Commun. **135**, 979–986
191 Winstall, E., Affar, E. B., Shah, R., Bourassa, S., Scovassi, I. and Poirier, G. G. (1999) Exp. Cell Res. **246**, 395–398
192 Brochu, G., Duchaine, C., Thibault, L., Lagueux, J., Shah, G. M. and Poirier, G. G. (1994) Biochim. Biophys. Acta **1219**, 342–350
193 D'Silva, I., Pelletier, J. D., Lagueux, J., D'Amours, D., Chaudhry, A., Weinfeld, M., Lees-Miller, S. P. and Poirier, G. G. (1999) Biochim. Biophys. Acta **1430**, 119–126
194 Uchida, K., Suzuki, H., Maruta, H., Abe, H., Aoki, K., Miwa, M. and Tanuma, S. (1993) J. Biol. Chem. **268**, 3194–3200
195 Braun, S. A., Panzeter, P. L., Collinge, M. A. and Althaus, F. R. (1994) Eur. J. Biochem. **220**, 369–375
196 Satoh, M. S., Poirier, G. G. and Lindahl, T. (1994) Biochemistry **33**, 7099–7106
197 Thibault, L., Hengartner, M., Lagueux, J., Poirier, G. G. and Muller, S. (1992) Biochim. Biophys. Acta **1121**, 317–324
198 Desmarais, Y., Ménard, L., Lagueux, J. and Poirier, G. G. (1991) Biochim. Biophys. Acta **1078**, 179–186
199 Kirsten, E., Bauer, P. I. and Kun, E. (1991) Exp. Cell Res. **194**, 1–8
200 Mathis, G. and Althaus, F. R. (1987) Biochem. Biophys. Res. Commun. **143**, 1049–1054
201 Huletsky, A., de Murcia, G., Muller, S., Hengartner, M., Lamarre, D. and Poirier, G. G. (1989) J. Biol. Chem. **264**, 8878–8886
202 Realini, C. A. and Althaus, F. R. (1992) J. Biol. Chem. **267**, 18858–18865
203 Germain, M., Affar, E. B., D'Amours, D., Dixit, V. M., Salvesen, G. S. and Poirier, G. G. (1999) Exp. Cell. Res., in the press
204 de Murcia, G., Huletsky, A. and Poirier, G. G. (1988) Biochem. Cell Biol. **66**, 626–635
205 Thraves, P. J., Kasid, U. and Smulson, M. E. (1985) Cancer Res. **45**, 386–391
206 Adamietz, P. and Rudolph, A. (1984) J. Biol. Chem. **259**, 6841–6846
207 Poirier, G. G., Brown, T. C. and Cerutti, P. A. (1985) Carcinogenesis **6**, 283–287
208 Krupitza, G. and Cerutti, P. (1989) Biochemistry **28**, 4054–4060
209 Tanuma, S., Johnson, L. D. and Johnson, G. S. (1983) J. Biol. Chem. **258**, 15371–15375
210 Johnson, G. S. and Ralhan, R. (1986) J. Cell. Physiol. **129**, 36–42
211 Wong, N., Poirier, G. G. and Dixon, G. H. (1977) Eur. J. Biochem. **77**, 11–21
212 Althaus, F. R., Hofferer, L., Kleczkowska, H. E., Malanga, M., Naegeli, H., Panzeter, P. and Realini, C. (1993) Environ. Mol. Mutagen. **22**, 278–282
213 Huletsky, A., Niedergang, C., Fréchette, A., Aubin, R., Gaudreau, A. and Poirier, G. G. (1985) Eur. J. Biochem. **146**, 277–285
214 Ménard, L., Thibault, L. and Poirier, G. G. (1990) Biochim. Biophys. Acta **1049**, 45–58
215 Thomassin, H., Ménard, L., Hengartner, C. and Poirier, G. G. (1992) Biochim. Biophys. Acta **1137**, 171–181
216 Lagueux, J., Shah, G. M., Ménard, L., Thomassin, H., Duchaine, C., Hengartner, C. and Poirier, G. G. (1994) Mol. Cell. Biochem. **138**, 45–52
217 Winstall, E., Affar, E. B., Shah, R., Bourassa, S., Scovassi, I. and Poirier, G. G. (1999) Exp. Cell Res., in the press
218 Burzio, L. and Koide, S. S. (1970) Biochem. Biophys. Res. Commun. **40**, 1013–1020
219 Roberts, J. H., Stark, P. and Smulson, M. (1973) Biochem. Biophys. Res. Commun. **52**, 43–50
220 Lee, S.-H., Ishimi, Y., Kenny, M. K., Bullock, P., Dean, R. B. and Hurwitz, J. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 9469–9473
221 Eki, T. and Hurwitz, J. (1991) J. Biol. Chem. **266**, 3087–3100
222 Eki, T. (1994) FEBS Lett. **356**, 261–266
223 Coll, J. M., Hickey, R. J., Cronkey, E. A., Jiang, H. Y., Schnaper, L., Lee, M. Y., Uitto, L., Syvaoja, J. E. and Malkas, L. H. (1997) Oncol. Res. **9**, 629–639
224 Dantzer, F., Nasheuer, H. P., Vonesch, J. L., de Murcia, G. and Menissier-de Murcia, J. (1998) Nucleic Acids Res. **26**, 1891–1898
225 Simbulan, C. M., Suzuki, M., Izuta, S., Sakurai, T., Savoysky, E., Kojima, K., Miyahara, K., Shizuta, Y. and Yoshida, S. (1993) J. Biol. Chem. **268**, 93–99
226 Simbulan-Rosenthal, C. M., Rosenthal, D. S., Boulares, A. H., Hickey, R. J., Malkas, L. H., Coll, J. M. and Smulson, M. E. (1998) Biochemistry **37**, 9363–9370
227 Lonn, U. and Lonn, S. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 104–108
228 Cech, T. R. and Lingner, J. (1997) in Telomere and Telomerase (Chadwick, D. J. and Cardew, G., eds.), pp. 20–34, Wiley, Chichester
229 Mullins, D. W., Giri, C. P. and Smulson, M. (1997) Biochemistry **16**, 506–513
230 Levy-Wilson, B. (1981) Arch. Biochem. Biophys. **208**, 528–534
231 De Lucia, F. (1996) J. Cell. Biochem. **63**, 334–341
232 Hough, C. J. and Smulson, M. E. (1984) Biochemistry **23**, 5016–5023
233 Matsui, T., Segall, J., Weil, P. A. and Roeder, R. G. (1980) J. Biol. Chem. **255**, 11992–11996
234 Slattery, E., Dignam, D. J., Matsui, T. and Roeder, R. G. (1983) J. Biol. Chem. **258**, 5955–5959
235 Ohtsuki, M., Sekimizu, K., Agemori, M., Shizuta, Y. and Natori, S. (1984) FEBS Lett. **168**, 275–277
236 Kurl, R. N. and Jacob, S. T. (1985) Nucleic Acids Res. **13**, 89–101
237 Meisterernst, M., Stelzer, G. and Roeder, R. G. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 2261–2265
238 Plaza, S., Aumercier, M., Bailly, M., Dozier, C. and Saule, S. (1999) Oncogene **18**, 1041–1051
239 Kannan, P., Yu, Y., Wankhade, S. and Tainsky, M. A. (1999) Nucleic Acids Res. **27**, 866–874
240 Rawling, J. M. and Alvarez-Gonzalez, R. (1997) Biochem. J. **324**, 249–253
241 Butler, A. J. and Ordahl, C. P. (1999) Mol. Cell. Biol. **19**, 296–306
242 Taniguchi, T., Agemori, M., Kameshita, I., Nishikimi, M. and Shizuta, Y. (1982) J. Biol. Chem. **257**, 4027–4030
243 Tanuma, S., Yagi, T. and Johnson, G. S. (1985) Arch. Biochem. Biophys. **237**, 38–42
244 Tsai, Y. J., Aoki, T., Maruta, H., Abe, H., Sakagami, H., Hatano, T., Okuda, T. and Tanuma, S. (1992) J. Biol. Chem. **267**, 14436–14442
245 Aoki, K., Maruta, H., Uchiumi, F., Hatano, T., Yoshida, T. and Tanuma, S. (1995) Biochem. Biophys. Res. Commun. **210**, 329–337
246 Singh, J. and Dixon, G. H. (1990) Biochemistry **29**, 6295–6302
247 Ding, H.-F., Rimsky, S., Batson, S. C., Bustin, K. and Hansen, U. (1994) Science **265**, 796–799
248 Miyamoto, T., Kakizawa, T. and Hashizume, K. (1999) Mol. Cell. Biol. **19**, 2644–2649
249 Oei, S. L., Griesenbeck, J., Ziegler, M. and Schweiger, M. (1998) Biochemistry **37**, 1465–1469
250 Whitacre, C. M., Hashimoto, H., Tsai, M. L., Chatterjee, S., Berger, S. J. and Berger, N. A. (1995) Cancer Res. **55**, 3697–3701
251 Vaziri, H., West, M. D., Allsopp, R. C., Davison, T. S., Wu, Y. S., Arrowsmith, C. H., Poirier, G. G. and Benchimol, S. (1997) EMBO J. **16**, 6018–6033
252 Wang, X., Ohnishi, K., Takahashi, A. and Ohnishi, T. (1998) Oncogene **17**, 2819–2825
253 Roitt, I. M. (1956) Biochem. J. **63**, 300–307
254 Durkacz, B. W., Omidiji, O., Gray, A. and Shall, S. (1980) Nature (London) **283**, 593–596
255 Durkacz, B. W., Shall, S. and Irwin, J. (1981) Eur. J. Biochem. **121**, 65–69
256 Durkacz, B. W., Irwin, J. and Shall, S. (1981) Biochem. Biophys. Res. Commun. **101**, 1433–1441
257 Zwelling, L. A., Kerrigan, D. and Pommier, Y. (1982) Biochem. Biophys. Res. Commun. **104**, 897–902
258 Morgan, W. F. and Cleaver, J. E. (1983) Cancer Res. **43**, 3104–3107
259 Ahnstrôm, G. and Ljungman, M. (1988) Mutat. Res. **194**, 17–22
260 Jacobson, E. L., Meadows, R. and Measel, J. (1985) Carcinogenesis **6**, 711–714
261 Nozaki, T., Masutani, M., Akagawa, T., Sugimura, T. and Esumi, H. (1994) Jpn. J. Cancer Res. **85**, 1094–1098
262 Oikawa, A., Tohda, H., Kanai, M., Miwa, M. and Sugimura, T. (1980) Biochem. Biophys. Res. Commun. **97**, 1311–1316
263 Morgan, W. F. and Cleaver, J. E. (1982) Mutat. Res. **104**, 361–366
264 Morgan, W. F. and Wolff, S. (1984) Cytogenet. Cell. Genet. **38**, 34–38
265 Schwartz, J. L., Morgan, W. F. and Weichselbaum, R. R. (1985) Carcinogenesis **6**, 699–704
266 Ferro, A. M., McElwain, M. C. and Olivera, B. M. (1984) Cold Spring Harb. Symp. Quant. Biol. **49**, 683–690

267 Magnusson, J. and Ramel, C. (1990) Mutagenesis **5**, 511–514
268 Waldman, A. S. and Waldman, B. C. (1991) Nucleic Acids Res. **19**, 5943–5947
269 Puchta, H., Swoboda, P. and Hohn, B. (1995) Plant J. **7**, 203–210
270 Bürkle, A., Meyer, T., Hilz, H. and zur Hausen, H. (1987) Cancer Res. **47**, 3632–3636
271 Bürkle, A., Heilbronn, R. and zur Hausen, H. (1990) Cancer Res. **50**, 5756–5760
272 Shima, H., Nakayasu, M., Aonuma, S., Sugimura, T. and Nagao, M. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 7442–7445
273 Nagao, M., Nakayasu, M., Aonuma, S., Shima, H. and Sugimura, T. (1991) Environ. Health Perspect. **93**, 169–174
274 Lunel-Orsini, C., Buttin, G. and de Saint Vincent, B. R. (1996) Mutat. Res. **349**, 63–75
275 Farzaneh, F., Panayotou, G. N., Bowler, L. D., Hardas, B. D., Broom, T., Walther, C. and Shall, S. (1988) Nucleic Acids Res. **16**, 11319–11326
276 Waldman, B. C. and Waldman, A. S. (1990) Nucleic Acids Res. **18**, 5981–5988
277 Waldman, B. C., O'Quinn, J. R. and Waldman, A. S. (1996) Biochim. Biophys. Acta **1308**, 241–250
278 Schreiber, V., Hunting, D., Trucco, C., Gowans, B., Brunwald, D., de Murcia, G. and de Murcia, J. M. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 4753–4757
279 Ménissier-de Murcia, J., Niedergang, C., Trucco, C., Ricoul, M., Dutrillaux, B., Mark, M., Olivier, F. J., Masson, M., Dierich, A., LeMeur, M. et al. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 7303–7307
280 Satoh, M. S. and Lindahl, T. (1992) Nature (London) **356**, 356–358
281 Satoh, M. S., Poirier, G. G. and Lindahl, T. (1993) J. Biol. Chem. **268**, 5480–5487
282 Molinete, M., Vermeulen, W., Bürkle, A., Menissier-de Murcia, J., Küpper, J. H., Hoeijmakers, J. H. and de Murcia, G. (1993) EMBO J. **12**, 2109–2117
283 Küpper, J.-H., Müller, M., Jacobson, M. K., Tatsumi-Miyajima, J., Coyle, D. L., Jacobson, E. L. and Bürkle, A. (1995) Mol. Cell. Biol. **15**, 3154–3163
284 Milam, K. M. and Cleaver, J. E. (1984) Science **223**, 589–591
285 Milam, K. M., Thomas, G. H. and Cleaver, J. E. (1986) Exp. Cell Res. **165**, 260–268
286 Reference deleted
287 Rankin, P. W., Jacobson, E. L., Benjamin, R. C., Moss, J. and Jacobson, M. K. (1989) J. Biol. Chem. **267**, 1569–1575
288 Shah, G. M., Poirier, D., Desnoyers, S. P., St-Martin, S., Hoflack, J. C. and Poirier, G. G. (1996) Biochim. Biophys. Acta **1312**, 1–7
289 Küpper, J. H., de Murcia, G. and Bürkle, A. (1990) J. Biol. Chem. **265**, 18721–18724
290 Griffin, R. J., Srinivasan, S., Bowman, K., Calvert, A. H., Curtin, N. J., Newell, D. R., Pemberton, L. C. and Golding, B. T. (1998) J. Med. Chem. **41**, 5247–5256
291 Bowman, K. J., White, A., Golding, B. T., Griffin, R. J. and Curtin, N. J. (1998) Br. J. Cancer **78**, 1269–1277
292 Schlicker, A., Peschke, P., Bürkle, A., Hahn, E. W. and Kim, J. H. (1999) Int. J. Radiat. Biol. **75**, 91–100
293 Küpper, J. H., Müller, M. and Bürkle, A. (1996) Cancer Res. **56**, 2715–2717
294 Wang, Z. Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M. and Wagner, E. F. (1995) Genes Dev. **9**, 509–520
295 Friedberg, E. C., Walker, G. C. and Siede, W. (1995) DNA Repair and Mutagenesis, ASM Press, Washington, DC
296 Lefebvre, P. and Laval, F. (1989) Biochem. Biophys. Res. Commun. **163**, 599–604
297 Ray, L. S., Chatterjee, S., Berger, N. A., Grishko, V. I., Le Doux, S. P. and Wilson, G. L. (1996) Mutat. Res. **363**, 105–114
298 Trucco, C., Oliver, F. J., de Murcia, G. and Menissier-de Murcia, J. (1998) Nucleic Acids Res. **26**, 2644–2649
299 Simbulan-Rosenthal, C. M., Rosenthal, D. S., Ding, R., Jackman, J. and Smulson, M. E. (1996) Prog. Nucleic Acid Res. Mol. Biol. **55**, 135–156
300 Ding, R., Pommier, Y., Kang, V. H. and Smulson, M. (1992) J. Biol. Chem. **267**, 12804–12812
301 Stevnsner, T., Ding, R., Smulson, M. and Bohr, V. A. (1994) Nucleic Acids Res. **22**, 4620–4624
302 Chatterjee, S., Petzold, S. J., Berger, S. J. and Berger, N. A. (1987) Exp. Cell Res. **172**, 245–257
303 Chatterjee, S., Cheng, M. F., Trivedi, D., Petzold, S. J. and Berger, N. A. (1989) Cancer Commun. **1**, 389–394
304 MacLaren, R. A., Witmer, M. V., Richardson, E. and Stamato, T. D. (1990) Mutat. Res. **231**, 265–274
305 Yoshihara, K., Itaya, A., Hironaka, A., Sakuramoto, S., Tanaka, Y., Tsuyuki, M., Inada, Y., Kamiya, T., Ohnishi, K., Honma, M., Kataoka, E., Mizusawa, H., Uchida, M., Uchida, K. and Miwa, M. (1992) Exp. Cell Res. **200**, 126–134
306 Witmer, M. V., Aboul-Ela, N., Jacobson, M. K. and Stamato, T. D. (1994) Mutat. Res. **314**, 249–260
307 Wang, Z. Q., Stingl, L., Morrison, C., Jantsch, M., Los, M., Schulze-Osthoff, K. and Wagner, E. F. (1997) Genes Dev. **11**, 2347–2358
308 Durrant, L. G., Margison, G. P. and Boyle, J. M. (1981) Carcinogenesis **2**, 1013–1017
309 Cleaver, J. E., Bodell, W. J., Morgan, W. F. and Zelle, B. (1983) J. Biol. Chem. **258**, 9059–9068
310 Klocker, H., Burtscher, H. J., Auer, B., Hirsch-Kauffmann, M. and Schweiger, M. (1983) Naturwissenschaften **70**, 93–94
311 Walker, I. G., Th'ng, J. P. H., Schrader, T. J. and Norry, T. W. (1984) Can. J. Biochem. Cell Biol. **62**, 329–334
312 Cleaver, J. E. (1985) Cancer Res. **45**, 1163–1169
313 Morrison, C., Smith, G. C. M., Jackson, S. P., Wagner, E. F. and Wang, Z. Q. (1997) Nat. Genet. **17**, 479–482
314 Morrison, C. and Wagner, E. (1996) Nucleic Acids Res. **24**, 2053–2058
315 Borggrefe, T., Wabl, M., Akhmedov, A. T. and Jessberger, R. (1998) J. Biol. Chem. **273**, 17025–17035
316 Ramsamooj, P., Notario, V. and Dritschilo, A. (1995) Radiat. Res. **143**, 158–164
317 Leitinger, N. and Wesierska-Gadek, J. (1993) J. Cell. Biochem. **52**, 153–158
318 Shockett, P. and Stavnezer, J. (1993) J. Immunol. **151**, 6962–6976
319 Smerdon, M. J. (1991) Curr. Opin. Cell Biol. **3**, 422–428
320 Smerdon, M. J. and Conconi, A. (1999) Prog. Nucleic Acid Res. Mol. Biol. **62**, 227–255
321 Smerdon, M. J. and Thoma, F. (1990) Cell **61**, 675–684
322 Agarwal, M. L., Agarwal, A., Taylor, W. R., Wang, Z. Q., Wagner, E. F. and Stark, G. R. (1997) Oncogene **15**, 1035–1041
323 Wesierska-Gadek, J., Wang, Z. Q. and Schmid, G. (1999) Cancer Res. **59**, 28–34
324 Simbulan-Rosenthal, C. M., Rosenthal, D. S., Ding, R., Bhatia, K. and Smulson, M. E. (1998) Biochem. Biophys. Res. Commun. **253**, 864–868
325 Giaccia, A. J. and Kastan, M. B. (1998) Genes Dev. **12**, 2973–2983
326 Simbulan-Rosenthal, C. M., Rosenthal, D. S., Iyer, S., Boulares, A. H. and Smulson, M. E. (1998) J. Biol. Chem. **273**, 13703–13712
327 Leist, M., Single, B., Kunstle, G., Volbracht, C., Hentze, H. and Nicotera, P. (1997) Biochem. Biophys. Res. Commun. **233**, 518–522
328 Schmid, G., Wang, Z. Q. and Wesierska-Gadek, J. (1999) Biochem. Biophys. Res. Commun. **255**, 399–405
329 Wesierska-Gadek, J., Schmid, G. and Cerni, C. (1996) Biochem. Biophys. Res. Commun. **224**, 96–102
330 Kumari, S. R., Mendoza-Alvarez, H. and Alvarez-Gonzalez, R. (1998) Cancer Res. **58**, 5075–5078
331 Singh, N. and Cerutti, P. A. (1986) Biochem. Biophys. Res. Commun. **132**, 811–819
332 Poirier, G. G., Niedergang, C., Champagne, M., Mazen, A. and Mandel, P. (1982) Eur. J. Biochem. **127**, 437–442
333 Imschenetzky, M., Morin, V., Carvajal, N., Montecino, M. and Puchi, M. (1996) J. Cell. Biochem. **61**, 109–117
334 Faraone-Mennella, M. R., Quesada, P., Farina, B., Leone, E. and Jones, R. (1982) Biochem. J. **205**, 245–248
335 Okayama, H. and Hayaishi, O. (1978) Biochem. Biophys. Res. Commun. **84**, 755–762
336 Krupitza, G. and Cerutti, P. (1989) Biochemistry **28**, 2034–2040
337 Darby, M. K., Schmitt, B., Jongstra-Bilen, J. and Vosberg, H.-P. (1985) EMBO J. **4**, 2129–2134
338 Yoshihara, K. and Tanaka, Y. (1981) J. Biol. Chem. **256**, 6756–6761
339 Yoshihara, K., Itaya, A., Tanaka, Y., Ohashi, Y., Ito, K., Teraoka, H., Tsukada, K., Matsukage, A. and Kamiya, T. (1985) Biochem. Biophys. Res. Commun. **128**, 61–67
340 Ohashi, Y., Itaya, A., Tanaka, Y., Yoshihara, K., Kamiya, T. and Matsukage, A. (1986) Biochem. Biophys. Res. Commun. **140**, 666–673
341 Goldman, N., Brown, M. and Khoury, G. (1981) Cell **24**, 567–572
342 Yu, F.-L. (1985) FEBS Lett. **190**, 109–114
343 Muller, E. G. and Zahn, R. K. (1976) Mol. Cell. Biochem. **12**, 147–159
344 Taniguchi, T., Suzuki, S. and Shizuta, Y. (1985) Biochem. Biophys. Res. Commun. **127**, 526–532
345 Kostka, G. and Schweiger, A. (1982) Biochim. Biophys. Acta **969**, 139–44
346 Scovassi, A. I., Mariani, C., Negroni, M., Negri, C. and Bertazzoni, U. (1993) Exp. Cell Res. **206**, 177–181
347 Amstad, P. A., Krupitza, G. and Cerutti, P. A. (1992) Cancer Res. **52**, 3952–3960
348 Wesierska-Gadek, J., Bugajskaschretter, A. and Cerni, C. (1996) J. Cell. Biochem. **62**, 90–101
349 Tanaka, Y., Yoshihara, K., Itaya, A., Kamiya, T. and Koide, S. S. (1984) J. Biol. Chem. **259**, 6579–6585
350 Ohashi, Y. (1986) J. Biochem. (Tokyo) **99**, 971–979
351 Adolph, K. W. and Song, M. K. H. (1985) Biochemistry **24**, 345–352