REVIEW

Poly(ADP-ribosyl)ation by PARP-1: 'PAR-laying' NAD^+ into a nuclear signal

Mi Young Kim,[1,3] Tong Zhang,[1,3] and W. Lee Kraus[1,2,4]

[1]Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853, USA; [2]Department of Pharmacology, Weill Medical College of Cornell University, New York, New York 10021, USA

Poly(ADP-ribose) (PAR) and the PAR polymerases (PARPs) that catalyze its synthesis from donor nicotinamide adenine dinucleotide (NAD^+) molecules have received considerable attention in the recent literature. Poly(ADP-ribosyl)ation (PARylation) plays diverse roles in many molecular and cellular processes, including DNA damage detection and repair, chromatin modification, transcription, cell death pathways, insulator function, and mitotic apparatus function. These processes are critical for many physiological and pathophysiological outcomes, including genome maintenance, carcinogenesis, aging, inflammation, and neuronal function. This review highlights recent work on the biochemistry, molecular biology, physiology, and pathophysiology of PARylation, focusing on the activity of PARP-1, the most abundantly expressed member of a family of PARP proteins. In addition, connections between nuclear NAD^+ metabolism and nuclear signaling through PARP-1 are discussed.

Structural and functional domains of PARP-1, the founding member of the PARP family

PARP-1 is the founding member of the PARP family, which contains as many as 18 distinct proteins in humans (Amé et al. 2004). PARPs catalyze the polymerization of ADP-ribose units from donor NAD^+ molecules on target proteins, resulting in the attachment of linear or branched polymers (Fig. 1). This enzymatic activity has been detected in organisms ranging from plants to mammals, but is apparently absent from yeast (Rolli et al. 2000; Amé et al. 2004). PARP family members share a conserved catalytic domain that contains the "PARP signature" motif, a highly conserved sequence (100% conserved in PARP-1 among vertebrates) that forms the active site (Rolli et al. 2000). Some PARP family members identified solely on homology, however, have not yet been shown to possess intrinsic PARP enzymatic activity (Amé et al. 2004). In addition to a catalytic domain, PARP family members typically contain one or more additional motifs or domains, including zinc fingers, "BRCA1 C-terminus-like" (BRCT) motifs, ankyrin repeats, macro domains, and WWE domains, each conferring unique properties on the particular PARP protein that contains them (Amé et al. 2004).

PARP-1 has a highly conserved structural and functional organization including (1) an N-terminal double zinc finger DNA-binding domain (DBD), (2) a nuclear localization signal, (3) a central automodification domain, and (4) a C-terminal catalytic domain (Fig. 2A; D'Amours et al. 1999; Rolli et al. 2000; Kraus and Lis 2003). PARP-1 binds to a variety of DNA structures, including single- and double-strand breaks, crossovers, cruciforms, and supercoils, as well as some specific double-stranded sequences (Rolli et al. 2000). PARP-1's basal enzymatic activity is very low, but is stimulated dramatically in the presence of a variety of allosteric activators, including damaged DNA, some undamaged DNA structures, nucleosomes, and a variety of protein-binding partners (D'Amours et al. 1999; Oei and Shi 2001; Kun et al. 2002, 2004; Kim et al. 2004). The targets of PARP-1's enzymatic activity include PARP-1 itself, which is the primary target in vivo, core histones, the linker histone H1, and a variety of transcription-related factors that interact with PARP-1 (Ogata et al. 1981; Huletsky et al. 1989; D'Amours et al. 1999; Kraus and Lis 2003). The automodification domain of PARP-1 contains several glutamate residues that are likely targets for automodification and a BRCT motif that functions in protein–protein interactions (D'Amours et al. 1999; Rolli et al. 2000). Collectively, the domains and activities of PARP-1 suggest important roles in a variety of nuclear functions. The activities and functions of the other PARP family members have not been studied to the same extent as PARP-1, although a clearer picture for some of the PARP family members has been emerging, as noted below and reviewed in more detail elsewhere (Smith 2001; Amé et al. 2004).

The chemical biology of PAR

The synthesis of PAR was first detected by Chambon et al. (1963) more than 40 years ago as a nicotinamide

[*Keywords*: NAD^+; PARP-1; poly(ADP-ribosyl)ation]
[3]These authors contributed equally to this work.
[4]Corresponding author.
E-MAIL wlk5@cornell.edu; FAX (607) 255-6249.
Article and publication are at http://www.genesdev.org/cgi/doi/10.1101/gad.1331805.

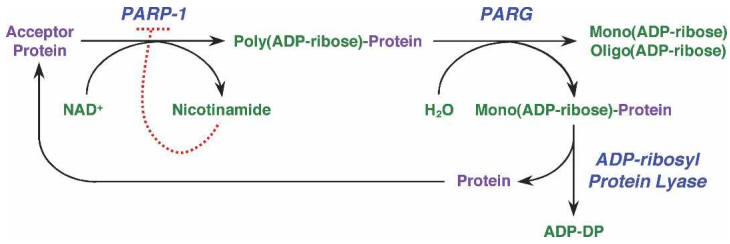

Figure 1. Synthesis and degradation of PAR on an acceptor protein. PARP-1 catalyzes the polymerization of ADP-ribose units from donor NAD^+ molecules on target proteins, resulting in the attachment of PAR. PARG catalyzes the hydrolysis of PAR producing free mono and oligo(ADP-ribose). ADP-ribosyl protein lyase cleaves the final remaining ADP-ribose monomer from the target protein, releasing ADP-3″-deoxypentose-2″-ulose (ADP-DP) (Oka et al. 1984).

mononucleotide (NMN)-induced incorporation of ^{14}C-adenine-labeled ATP into an acid-insoluble fraction in a nuclear extract. PAR is a branched polymer of repeating ADP-ribose units, which are linked via glycosidic ribose–ribose 1″ → 2′ bonds (Fig. 3). Due to its chemical composition, PAR has been referred to as the "third type of nucleic acid" (D'Amours et al. 1999), although its biological role is considerably less well understood than RNA and DNA. PAR is heterogeneous with respect to length (as many as 200 ADP-ribose units in vitro) and extent of branching (approximately one branch per 20–50 ADP-ribose units) (D'Amours et al. 1999). The significance of this heterogeneity in PAR function is unknown, but it could play a role in determining specific functional outcomes in vivo.

The consequences of linking a long negatively charged polymer to a protein are potentially profound, as numerous in vitro and in vivo studies have now demonstrated for PAR (D'Amours et al. 1999; Bürkle et al. 2000; Kraus and Lis 2003). Each residue in PAR contains an adenine moiety capable of base stacking and hydrogen bonding, as well as two phosphate groups that carry negative charges (Amé et al. 2000). PAR may form definitive structures through intramolecular interactions (Minaga and Kun 1983a,b), and these structures have the potential for noncovalent attractive (or repulsive) interactions with other molecules (Mathis and Althaus 1987; Wesierska-Gadek and Sauermann 1988; Panzeter et al. 1992). Thus, PAR may alter protein activity by functioning as a site-specific covalent modification, a protein-binding matrix, or a steric block. For example, inhibition of PARP-1's DNA-binding activity by autoPARylation may be the result of steric effects of PAR on the biochemical properties of PARP-1, perhaps masking the PARP-1 DNA-binding domain, or through charge repulsion between PAR and DNA (D'Amours et al. 1999).

The functions of PAR in vivo are likely to involve specific interactions with a variety of effector proteins. Proteomic approaches have led to the identification of a 20-amino-acid PAR-binding motif in a heterogeneous group of PAR-binding proteins, including core histones, p53, and XRCC-1, that maps to important functional domains within the proteins (Pleschke et al. 2000; Gagne et al. 2003). In addition, the "macro domain," a conserved ~190-amino-acid domain found in a wide variety of proteins, was recently shown to function as an ADP-ribose-binding module (Karras et al. 2005). This includes the macro domain of macroH2A, a histone variant thought to be involved in transcriptional repression, and the double macro domain of PARP-9, a PARP involved in leukemia (Karras et al. 2005). Some non-sequence-specific DNA-binding proteins, such as H1, may even have a greater affinity for PAR than DNA (Malanga et al. 1998). Understanding the functional consequences of PAR–protein interactions will be the key to understanding the biology of PAR itself. This is an area that will require further investigation in the future.

Catabolism of PAR by PARG

The catabolism of PAR is mediated primarily by poly(ADP-ribose) glycohydrolase (PARG), an enzyme with both exo- and endoglycosidase activities (exo ≪ endo) that hydrolyzes the glycosidic linkages between the ADP-ribose units of PAR producing free ADP-ribose (Figs. 1, 3; Amé et al. 2000; Davidovic et al. 2001). PARG proteins, which have been identified in mammals, flies, worms, and plants, have a catalytic domain, nuclear localization signal, nuclear export signal, and a putative regulatory domain (Fig. 2C; Davidovic et al. 2001; Meyer-Ficca et al. 2005). Only the catalytic domain shows a high level of homology across species, and the arrangement of the other motifs and domains within the protein varies from species to species (Amé et al. 2000).

In mammals, a single PARG gene encodes multiple PARG proteins with two predominant isoforms: a long nuclear/cytoplasmic isoform (~110 kDa) and a short cytoplasmic isoform (~65 kDa), both of which possess catalytic activity (Davidovic et al. 2001; Meyer-Ficca et al. 2005). The results of a recent study suggest that multiple long PARG isoforms may be produced by alternative mRNA splicing: a low-abundance nuclear form (110 kDa, which contains a NLS encoded by exon 1) and two high-abundance cytoplasmic forms (102 kDa lacking exon 1; 99 kDa lacking exons 1 and 2) that exhibit a perinuclear distribution (Meyer-Ficca et al. 2004). The abundance of PARG in the cytoplasm seems at odds with the fact that many of the PARP enzymes are located in the nucleus, but may indicate that low levels of PARG are sufficient for the catabolism of nuclear PAR. Generating a more comprehensive and unified picture of the nature and localization of the different PARG isoforms will require additional studies in a wider variety of cell types.

In vivo, the steady-state levels of PAR are regulated by the opposing actions of PARPs and PARG. The degradation of PAR may begin immediately upon the initiation of PAR synthesis and can be completed within minutes after the cessation of PAR synthesis has occurred (D'Amours et al. 1999; Tulin and Spradling 2003). This

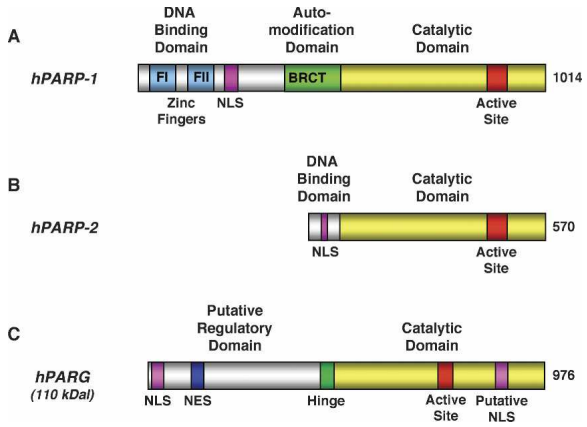

Figure 2. Structural and functional organizations of PARP-1, PARP-2, and PARG. (*A*) PARP-1 is the founding member of the PARP family, which contains as many as 18 distinct proteins in humans (Amé et al. 2004). PARP-1 has a highly conserved structural and functional organization including (1) an N-terminal DNA-binding domain with two Cys-Cys-His-Cys zinc finger motifs (FI and FII), (2) a nuclear localization signal, (3) a central automodification domain containing a BRCT protein–protein interaction motif, and (4) a C-terminal catalytic domain with a contiguous 50-amino-acid sequence, the "PARP signature" motif, that forms the active site. (*B*) PARP-2 also has a conserved structural and functional organization including (1) an N-terminal DNA-binding domain with homology to the SAP (SAF-A/B, Acinus, and PIAS) domains found in other nuclear proteins involved in chromosomal organization and DNA repair, (2) a nuclear localization signal, and (3) a C-terminal catalytic domain that shares the most similarity with the catalytic domain of PARP-1 (69% similarity). PARP-2 is the only PARP besides PARP-1 whose catalytic activity is known to be stimulated by damaged DNA. (*C*) In mammals, a single PARG gene encodes multiple PARG proteins with two predominant isoforms: a long nuclear/cytoplasmic isoform (~110 kDa; shown) and a short cytoplasmic isoform (~65 kDa; not shown), both of which possess catalytic activity. The ~110-kDa mammalian PARG protein contains a putative N-terminal regulatory domain and a C-terminal catalytic domain, as well as nuclear localization and nuclear export signals. (NLS) Nuclear localization signal; (NES) nuclear export signal.

suggests that PAR and PAR-metabolizing enzymes are highly regulated. Although PARP-1 is present at a five-fold to 20-fold molar excess over PARG in some cell types, a variety of regulatory mechanisms act to control the levels of PAR in the nucleus (Alvarez-Gonzalez et al. 1999; D'Amours et al. 1999; Davidovic et al. 2001). For example, PARP-1 has a low basal enzymatic activity, which is stimulated dramatically by PARP-1's binding partners, including various proteins and forms of DNA (Oei and Shi 2001; Kun et al. 2002; Kim et al. 2004). PARG, on the other hand, has a higher specific activity than PARP-1, and its enzymatic activity increases with increased PAR length (D'Amours et al. 1999). Furthermore, PARG activity in the nucleus may be modulated by nucleo–cytoplasmic shuttling of the protein (Bonicalzi et al. 2003; Ohashi et al. 2003), which could control the levels of nuclear PARG enzymatic activity through regulated subcellular distribution.

Two recent studies have examined the role of PARG in counteracting PARP-mediated PARylation using gene deletion in mice (genetic studies of the PAR-regulating enzymes described herein are summarized in Table 1). Mice homozygous for a targeted deletion of exons 2 and 3 ($Parg^{\Delta 2\text{-}3/\Delta 2\text{-}3}$), resulting in depletion of the 110-kDa PARG isoform, are viable, phenotypically normal, and show similar overall PAR metabolism as wild-type animals (Cortes et al. 2004). The $Parg^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ animals do, however, exhibit increased sensitivity to genotoxins and septic shock, manifested as increased lethality relative to wild-type animals in response to treatment with *N*-methyl-*N*-nitrosourea (MNU), γ-irradiation, and lipopolysaccharide (LPS) (Cortes et al. 2004). Mice homozygous for a targeted deletion of exon 4 ($Parg^{\Delta 4/\Delta 4}$), resulting in a complete depletion of all PARG isoforms (i.e., *Parg* null), die at embryonic day 3.5 (E3.5) (Koh et al. 2004). $Parg^{\Delta 4/\Delta 4}$ embryos and embryonic trophoblast stem cells accumulate high levels of PAR and undergo increased cell death by apoptosis. Likewise, *Drosophila* containing loss-of-function mutations in PARG exhibit increased lethality in the larval stages at normal developmental temperatures (Table 1; Hanai et al. 2004). Together, these studies demonstrate the importance of PAR catabolism for the maintenance of normal physiology.

The molecular and cellular biology of PAR and PARP-1

Several recent papers, highlighted below, have increased our understanding of the roles played by PAR, PARP-1, and some related PARPs in diverse molecular and cellular processes, including DNA damage detection and repair, chromatin modification, transcription, cell death pathways, insulator function, and mitotic apparatus function.

DNA damage detection and repair

The covalent modification of proteins by PARylation is an immediate and dramatic biochemical response to DNA damage induced by oxidation, alkylation, and ionizing radiation. The binding of PARP-1 to damaged DNA, including single-strand breaks (SSBs) and double-strand breaks (DSBs), through its double zinc finger DNA-binding domain potently activates PARP-1 enzymatic activity (as much as 500-fold) (D'Amours et al. 1999). As such, PARP-1 can function as a DNA damage sensor. With low levels of DNA damage, PARP-1 acts as a survival factor involved in DNA damage detection and repair. In contrast, with high levels of DNA damage, PARP-1 promotes cell death (see below) (Bürkle 2001a). PARP-1 has been implicated in multiple DNA repair pathways, including the SSB, DSB, and base excision repair (BER) pathways (Bürkle 2001b; Masutani et al. 2003). As might be expected, PARP-1 interacts physically and functionally with various proteins involved in these DNA repair pathways, and may recruit the repair proteins to sites of DNA damage (e.g., XRCC-1 in BER, DNA-dependent protein kinase in DSB repair) (Masson

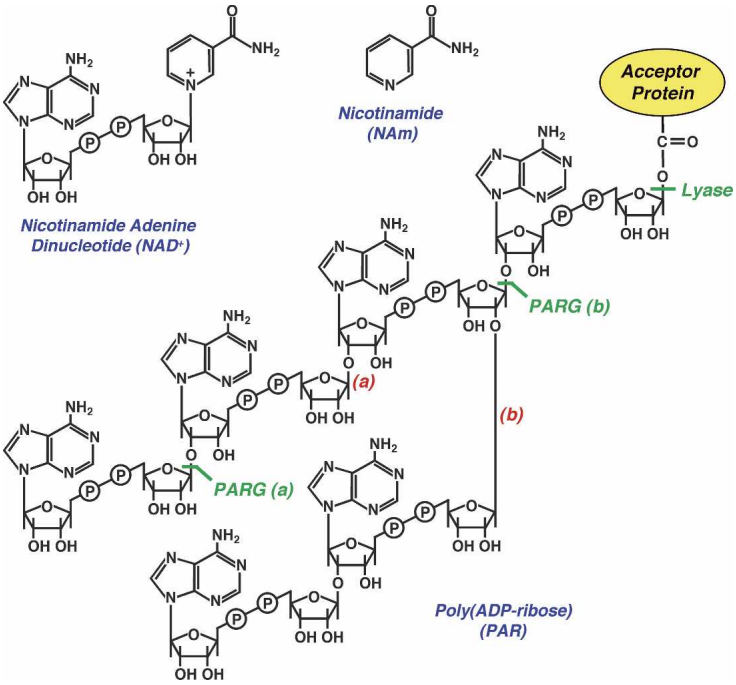

Figure 3. Chemical structures of NAD^+, nicotinamide (NAm), and PAR. PAR is a branched polymer synthesized on acceptor proteins by PARPs using NAD^+ as a donor of ADP-ribose units. The ADP-ribose units in the linear PAR chains are linked by $1'' \rightarrow 2'$ ribose–ribose glycosidic bonds [e.g., see *(a)*], whereas the ADP-ribose units at the branchpoints are linked by $1''' \rightarrow 2''$ ribose–ribose glycosidic bonds [e.g., see *(b)*]. The degradation of PAR is catalyzed by PARG, which has both exoglycosidase and endoglycosidase activities [e.g., see *PARG (a)* and *PARG (b)*, respectively] that hydrolyze the glycosidic linkages between the ADP-ribose units of PAR producing free ADP-ribose. Remaining protein-proximal ADP-ribose monomers are removed by ADP-ribosyl protein lyase.

et al. 1998; Ruscetti et al. 1998; Ariumi et al. 1999; Okano et al. 2003; Lan et al. 2004). PAR itself, as a covalent attachment of automodified PARP-1, may also act to recruit repair proteins to sites of DNA damage (Malanga and Althaus 2005). PARP-2, the only other PARP enzyme whose catalytic activity is known to be stimulated by damaged DNA, has also been implicated in BER through interactions with XRCC-1 and PARP-1 (Schreiber et al. 2002).

Two recent studies have demonstrated an interesting connection between PARP-1-dependent SSB repair and BRCA1- and BRCA2-dependent DSB repair (Bryant et al. 2005; Farmer et al. 2005). BRCA1 and BRCA2 are tumor-suppressor proteins important for DSB repair by homologous recombination, and mutation of the genes encoding these proteins causes predisposition to breast and ovarian cancers (Tutt and Ashworth 2002; Wooster and Weber 2003). Depletion or chemical inhibition of PARP-1, but not PARP-2, in BRCA1- or BRCA2-deficient cells reduces the clonogenic survival of the cells compared to PARP-1-depleted/inhibited, BRCA1- or BRCA2-sufficient cells (Bryant et al. 2005; Farmer et al. 2005). Furthermore, BRCA1- or BRCA2-deficient cells treated with a PARP-1 inhibitor show major mitotic chromosome aberrations and a loss of formation of RAD51 foci, suggesting defects in DSB repair involving RAD51-dependent homologous recombination (Bryant et al. 2005; Farmer et al. 2005). Collectively, these studies suggest that the persistent single-strand breaks formed upon PARP-1 inhibition, which promote the collapse of replication forks and would normally be repaired by homologous recombination, cannot be repaired effectively in the absence of functional BRCA1 or BRCA2. This results in the accumulation of chromosomal abnormalities, cell cycle arrest in G2/M, and apoptosis (Bryant et al. 2005; Farmer et al. 2005). Although these and other studies have demonstrated a role for PARP-1 in several DNA repair pathways, a clear picture of the exact mechanisms by which PARP-1-mediated PARylation modulates these pathways is missing.

Cell death pathways

In contrast to its role as a survival factor in the presence of low levels of DNA damage, PARP-1 acts to promote cell death in the presence of extensive DNA damage. As such, chemical inhibition or genetic deletion of PARP-1 can protect animals from several DNA-damage-dependent pathophysiological conditions leading to aberrant cell death, including (1) ischemia–reperfusion injury, (2) glutamate excitotoxicity in the central nervous system, (3) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism, (4) cardiac infarction, (5) inflammatory injury, and (6) streptozotocin (STZ)-induced diabetes (Szabo and Dawson 1998; Shall and de Murcia 2000). Although a role for PARP-1 in these conditions has been well established, the mechanisms by which PARP-1 activation leads to cell death are still under active debate in the literature. Several mechanisms have been proposed, including energy-failure-induced necrosis and apoptosis-inducing factor (AIF)-dependent apoptosis.

Necrosis is a cell death process in which a cell swells and ruptures as it dies, releasing intracellular components into the surrounding tissue, which promotes an inflammatory response (Edinger and Thompson 2004). Hypersynthesis of PAR by PARP-1 in response to extensive DNA damage can promote cell death through necrosis, which occurs as a result of the depletion of cel-

lular NAD^+ and ATP, and subsequent cellular energy failure (Decker and Muller 2002; Bouchard et al. 2003). In contrast, apoptosis is an ordered cell death process in which the cell is systematically dismantled within membrane-enclosed vesicles that are engulfed by phagocytes, preventing the release of intracellular components into the surrounding tissue (Edinger and Thompson 2004). Studies from Yu et al. (2002) show that PARP-1 can play a role in caspase-independent apoptotic cell death through AIF. AIF is a pro-apoptotic flavoprotein residing in the mitochondrial intermembrane space (like cytochrome c and other apoptotic modulators) that is among the most powerful triggers of apoptosis (Chiarugi and Moskowitz 2002). In the nucleus, AIF induces peripheral chromatin condensation and high-molecular-weight (50 kb) DNA fragmentation (Susin et al. 1999). The translocation of AIF into the nucleus is impaired in *Parp-1*$^{-/-}$ fibroblasts after treatment with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG), a DNA-alkylating agent that potently activates PARP-1 and promotes PARP-1-dependent cell death (Yu et al. 2002). Exactly how PARP-1 activation triggers the release of AIF from mitochondria is not clear, but depletion of NAD^+ following PARP-1 activation, or perhaps a product of PAR catabolism, could serve as a potential signal.

The mechanisms underlying the choice of PARP-1-dependent cell death pathways (i.e., necrosis vs. apoptosis) in response to genotoxic stimuli have not been determined, but may be influenced by the type, strength, and duration of the stimuli, as well as the cell type (Virag 2005). One feature of apoptosis is its dependency on ATP for the ordered degradation of cellular structures and maintenance of membrane integrity (Edinger and Thompson 2004). Thus, in cells whose ATP pools have been depleted due to PARP-1 activation, cell death occurs by necrosis. In addition, recent studies indicate that cellular metabolic status is a key factor in determining how ATP levels are affected by PARP-1 activation (Zong et al. 2004; Ying et al. 2005). Actively proliferating cells, such as cancer cells, are dependent on glucose catabolism through aerobic glycolysis for ATP production. Nonproliferating cells, in contrast, can catabolize a variety of metabolic substrates, including amino acids and lipids, and maintain ATP levels through oxidative phosphorylation in the mitochondria. PARP-1 activation in the nucleus preferentially depletes the nuclear and cytosolic pools of NAD^+, but not the mitochondrial pools, thereby inhibiting glycolysis, but not oxidative phosphorylation (Zong et al. 2004). Consequently, proliferating cells are more sensitive to PARP-1 activation, becoming depleted of ATP and dying by necrosis. In contrast, nonproliferating cells are resistant to ATP depletion and cell death under the same conditions (Zong et al. 2004). Similarly, in MNNG-treated astrocytes, the decision between cell death or survival is regulated by the availability of metabolic substrates. Supplying substrates that bypass cytosolic glycolysis for ATP production enhances cell survival (Ying et al. 2005). Thus, PARP-1 activity plays a central role in this form of "programmed necrosis" (Edinger and Thompson 2004), which integrates signals from cellular proliferation, metabolism, and DNA damage to determine cell fate.

Interestingly, PARP-1 is inactivated during the execution phase of apoptosis (Soldani and Scovassi 2002). PARP-1 is cleaved by caspase-3 and -7, signature pro-apoptotic proteases, into an ~25-kDa N-terminal fragment containing the DBD, and an ~85-kDa C-terminal fragment that retains basal, but not DNA-damage-activated, enzymatic activity (Kaufmann et al. 1993). This cleavage eliminates PARP-1 activation in response to DNA fragmentation during apoptosis, protecting the cells from ATP depletion and subsequent necrotic death. In addition, by preventing futile attempts at DNA repair, PARP-1 cleavage may help to commit cells to the apoptotic pathway (Soldani and Scovassi 2002). PARG is also cleaved during apoptosis by caspase-3, releasing a C-terminal fragment that retains full enzymatic activity (Affar et al. 2001). Although the consequences are not clearly understood, the cleavage of both PARP-1 and PARG by caspases during apoptosis suggests an important function for PAR metabolism in regulating apoptosis.

Modification of chromatin structure

PARP-1 and PAR have been shown to play a role in regulating chromatin structure in the presence or absence of DNA damage. Early biochemical studies suggested that PARP-1 could disrupt chromatin structure by PARylating histones and destabilizing nucleosomes (Poirier et al. 1982; Mathis and Althaus 1987; Huletsky et al. 1989). Histones H1 and H2B are the main histone targets for PARylation in vivo by PARP-1 and PARP-2, respectively (Poirier et al. 1982; Huletsky et al. 1989), although the other core histones are modified as well (D'Amours et al. 1999). Non-histone chromosomal proteins, including high mobility group proteins, are also PARylated in vivo (Tanuma and Johnson 1983). PARylation of histone proteins has been implicated in the decondensation of chromatin through destabilization of nucleosomes (Poirier et al. 1982; Mathis and Althaus 1987; Kraus and Lis 2003). Note, however, that although much emphasis has been placed on histone proteins as targets for PARylation, PARP-1 itself is the primary target for PARylation in vivo, with >90% of PAR found on PARP-1 (Ogata et al. 1981; Huletsky et al. 1989; D'Amours et al. 1999). Additional biochemical studies suggested that polyanionic PAR, either free or attached to proteins such as PARP-1, may provide an attractive matrix for histones released from destabilized nucleosomes (Mathis and Althaus 1987; Realini and Althaus 1992) or even strip basic proteins, such as histones, from DNA (Mathis and Althaus 1987; Wesierska-Gadek and Sauermann 1988; Panzeter et al. 1992).

Complementing these biochemical studies, Tulin and Spradling used *Drosophila*, which has only two PARP genes (PARP-1-like, which expresses three isoforms, and tankyrase-like) (Hanai et al. 1998; Miwa et al. 1999), as a model system to examine the role of PARP-1 in the regulation of chromatin structure in vivo (Tulin et al. 2002; Tulin and Spradling 2003). Inhibition of PARP-1 enzy-

Table 1. *Summary of genetic studies on PAR-regulating enzymes noted in the text.*

Factor	Organism	Genetic manipulation	Viability	Sample phenotypes	References
PARPs	Mouse	*Parp-1*$^{-/-}$	Viable	Increased sensitivity to ionizing radiation, genotoxic agents, and environmental stress. Defects in the repair of damaged DNA.	Wang et al. 1995, 1997; Menissier de Murcia et al. 2003
				Resistance to DNA-damage-induced cell death.	Yu et al. 2002; Zong et al. 2004
				Resistance in various models of inflammation, including streptozotocin-induced diabetes and LPS-induced septic shock	Oliver et al. 1999; Mabley et al. 2001
				Increased tumor formation in some chemically induced and transgenic mouse tumor models.	Masutani et al. 2005
				Increased incidence of T-cell lymphomas in a SCID background (i.e., DNA-PK catalytic subunit mutation).	Morrison et al. 1997
				Increased spontaneous tumor formation in a *p53*$^{-/-}$ background (*Parp-1*$^{-/-}$ exon 2 deletion) *or*	Tong et al. 2001
				Increased latency of tumor formation in a *p53*$^{-/-}$ background (*Parpl*$^{-/-}$ exon 4 deletion)	Conde et al. 2001
		Parp-2$^{-/-}$	Viable	Increased sensitivity to ionizing radiation and genotoxic agents.	Menissier de Murcia et al. 2003
		Parp-1$^{-/-}$ *Parp-2*$^{-/-}$	Embryonic lethality (prior to E8.0)	Severe growth retardation and developmental abnormalities.	Menissier de Murcia et al. 2003
		Parp-1$^{+/-}$ *Parp-2*$^{-/-}$	Impaired viability in females (increased embryonic lethality)	X-chromosome instability and severe hypofertility in females.	Menissier de Murcia et al. 2003
	Drosophila	*Parp*$^{-/-}$ (deletion of all exons)	Larval lethality	Death near the end of embryogenesis	Miwa et al. 1999
		Parp$^{CH1/CH1}$ (P-element insertion that dramatically reduces expression)	Larval lethality	Death at or near second instar. Dramatically altered nuclear morphology. Defective inducible gene expression (e.g., heat shock, ecdysone). Spontaneous bacterial infection of larvae.	Tulin et al. 2002; Tulin and Spradling 2003

continued on next page

Table 1. (*continued*)

Factor	Organism	Genetic manipulation	Viability	Sample phenotypes	References
PARG	Mouse	*Parg*$^{\Delta 2-3/\Delta 2-3}$ (depletion of the 110-kDa PARG isoform)	Viable	Increased lethality in response to genotoxins and septic shock. Increased susceptibility to inflammatory responses.	Cortes et al. 2004
		Parg$^{\Delta 4/\Delta 4}$ (*Parg* null)	Embryonic lethality (at E3.5)	Increased accumulation of PAR. Increased apoptotic cell death.	Koh et al. 2004
	Drosophila	*Parg*$^{27.1}$/*Y* and *Parg*$^{27.1/27.1}$ (P-element-mediated deletion of ~2/3 of the ORF)	Temperature-dependent lethality	At 25°C, ~2/3 of larvae develop to the pupal stage, but show lethality before eclosion. At 29°C, ~1/4 of larvae develop into adult flies that exhibit progressive neurological abnormalities, reduced locomotor activity, reduced life span, and sterility.	Hanai et al. 2004

matic activity by using 3-aminobenzamide (3-AB; a general PARP inhibitor) or disruption of all PARP-1 expression blocks the accumulation of PAR, decondensation ("puffing"), and transcription at loci containing highly inducible genes (e.g., heat-shock- and ecdysone-induced genes) (Tulin and Spradling 2003). The authors proposed that in response to an external stimulus (e.g., heat shock or ecdysone), PARP-1 becomes activated and modifies chromatin proteins, resulting in chromatin decondensation and transcriptional activation (Tulin and Spradling 2003). However, the definitive targets of PARylation at the decondensed loci were not determined. Interestingly, the same group also reported that PARP-1 can play a role in promoting the formation of more compact chromatin structures (Tulin et al. 2002). Genetic disruption of PARP-1 gene expression in *Drosophila*, which causes larval lethality (Table 1; Miwa et al. 1999; Tulin et al. 2002), promotes a dramatic increase in the micrococcal nuclease sensitivity (i.e., decondensation) of heterochromatic, but not euchromatic, regions (Tulin et al. 2002). PARP-1, therefore, has opposite effects on chromatin structure (i.e., condensation vs. decondensation) depending on the type of chromatin. The mechanistic differences between these two types of regulation have yet to be elucidated.

A recent study from our laboratory has helped to clarify the mechanisms by which PARP-1 can direct the reversible modulation of chromatin structure in an NAD^+-dependent manner (Kim et al. 2004). Using a combination of biochemical, cell-based, and cytological approaches with human and *Drosophila* cells, we showed that PARP-1 incorporates into chromatin by virtue of specific nucleosome-binding properties and promotes the formation of compact, transcriptionally repressed chromatin structures. This is reminiscent of the activities of the linker histone H1. In the presence of NAD^+, PARP-1 autoPARylates and dissociates from chromatin, resulting in the formation of decondensed, transcriptionally active chromatin structures. In contrast to previous models, this process occurs in the apparent absence of histone modification. Also, we showed that nucleosomes are very potent activators of PARP-1's enzymatic activity, indicating that nucleosome-bound PARP-1 is poised for activation even in the absence of DNA damage. Finally, although PARP-1 and H1 behave similarly in several in vitro assays, they compete for binding to nucleosomes in vitro and localize to distinct chromatin domains in vivo, suggesting distinct chromatin-dependent functions for these nucleosome-binding proteins. The interplay between PARP-1 and H1 at the level of the nucleosome may also involve PARylation of H1 by PARP-1, which would presumably promote the release of H1, although this has not yet been examined directly.

Collectively, these studies support the idea that PARP-1 can facilitate both the compaction and decondensation of chromatin depending on the physiological signals available. Furthermore, these studies demonstrate that chromatin decondensation and transcriptional activation require PARylation events. The mechanisms by which cellular signals, such as hormones and stress, can trigger downstream events to activate PARP-1 are not known. In addition, it is not yet clear what signal, perhaps a specific pattern of histone modification, directs PARP-1 binding to H1-depleted regions of the genome.

A variety of in vitro and in vivo experiments have also suggested a role for PARylation in determining and maintaining the methylation patterns of genomic DNA (Zardo et al. 2003). For example, inhibition of cellular

PARP activity by 3-AB has been shown to increase the extent and alter the pattern of DNA methylation in the promoter CpG island of the Htf9 gene in L929 mouse fibroblasts (Zardo and Caiafa 1998). This may occur through decreased expression of the DNA methyltransferase DNMT1 (Zardo et al. 2002) or inhibition of DNMT1 methyltransferase activity by noncovalent binding of PAR (Reale et al. 2005). Given the important role of DNA methylation in organizing chromatin structures (Robertson 2002), PARylation-dependent alterations in the extent and patterns of DNA methylation could provide another means for PARP-1 and related enzymes to modulate chromatin structure.

Transcriptional regulation

Roles for PARP-1 and PARylation in the transcriptional regulation of specific genes have been demonstrated in several physiological contexts using a variety of experimental approaches, including in vitro transcription assays, cell-based reporter gene assays, RNAi, and gene deletion in vivo (D'Amours et al. 1999; Hassa and Hottiger 2002; Kraus and Lis 2003). From these studies, at least two different mechanisms have been proposed for the regulation of transcription by PARP-1: (1) modulating chromatin structure, as described above, and (2) acting as part of gene-specific enhancer/promoter-binding complexes (Kraus and Lis 2003). In each case, the targets of PARP-1 enzymatic activity differ. In its capacity as a component of enhancer/promoter-binding complexes, PARP-1 acts to stimulate transcription with some activators, while inhibiting transcription with others, depending on the cell type and promoter context (D'Amours et al. 1999; Hassa and Hottiger 2002; Kraus and Lis 2003). In some cases, PARP-1 enzymatic activity is not required for its transcriptional coregulator function (e.g., with NF-κB, HTLV Tax, B-Myb) (Meisterernst et al. 1997; Anderson et al. 2000; Cervellera and Sala 2000; Hassa et al. 2001). Although a growing body of work has demonstrated a role for PARP-1 as a transcriptional coregulator, mechanistic details of how PARP-1 might serve such a role have been lacking.

Two recent papers have shed new light on the mechanisms by which PARP-1 can modulate signal-regulated transcription in an activator- and promoter-specific manner. Pavri et al. (2005) used a combination of biochemical and cell-based assays to demonstrate a role for PARP-1 in ligand-dependent transcription by retinoic acid receptor α (RARα)/retinoid X receptor α (RXRα) heterodimers. With chromatin templates and a purified in vitro transcription system, they found that PARP-1 is required for retinoic acid (RA)-induced transcription by RARα/RXRα. This activity does not require the catalytic domain of PARP-1, and the addition of NAD^+ leads to a loss of RA-dependence. In *Parp-1*$^{-/-}$ mouse embryo fibroblasts (MEFs), RA-induced expression of the RARα-regulated *RARβ2* gene is lost, but can be restored by complementation with full-length or catalytic-domain-deleted PARP-1. Chromatin immunoprecipitation (ChIP) assays indicate that PARP-1, RARα, and inactive Mediator (a coactivator) are bound constitutively to the *RARβ2* promoter in both *Parp-1*$^{+/+}$ and *Parp-1*$^{-/-}$ MEFs in the absence of RA. In *Parp-1*$^{-/-}$ MEFs, however, the cdk8 component of inactive Mediator fails to dissociate from the RARβ2 promoter upon treatment with RA, blocking subsequent transcription of the gene. The authors propose a model in which PARP-1 supports RA- and RARα-dependent transcription of the *RARβ2* gene by promoting the conversion of Mediator from an inactive form (+cdk8) to an active form (–cdk8). The mechanisms underlying this switch (e.g., possible PARP-1–cdk8 interactions), however, have not yet been determined. In addition, the mechanism by which nuclear NAD^+ is prevented from disrupting this regulatory system in vivo is unclear.

Ju et al. (2004) provide another perspective on how PARP-1 might function as a regulated "promoter-specific exchange factor," focusing on PARP-1-dependent dissociation of TLE (Transducin-like enhancer of Split) corepressor complexes from the proneural *MASH1* promoter. *MASH1* expression is repressed in proliferating neuronal stem cells (NSCs) by the inhibitory basic helix–loop–helix transcription factor HES1 (Hairy/Enhancer of Split-1), which recruits TLE corepressor complexes containing PARP-1. ChIP assays in NSCs indicate that treatment with PDGF, which initiates a differentiation program in the cells, promotes the dissociation of the TLE corepressor complex, but not HES1 or PARP-1, from the *MASH1* promoter. This is followed by the recruitment of HAT-containing coactivator complexes and expression of the *MASH1* gene. Interestingly, calcium/calmodulin-dependent protein kinase IIδ (CaMKIIδ) is recruited to the *MASH1* promoter and is required for PDGF-dependent release of the TLE1 corepressor complex from the promoter and the derepression of *MASH1* expression. The authors propose a model in which CaMKIIδ directs the switch from repression to activation at the *MASH1* promoter by (1) stimulating PARP-1 enzymatic activity, leading to PARylation and dissociation of the TLE repressor complex; and (2) phosphorylating HES1, thereby converting it from a TLE-dependent repressor to an activator capable of recruiting coactivator complexes.

The studies from Pavri et al. (2005) and Ju et al. (2004), both of which demonstrate roles for PARP-1 as a promoter-specific exchange factor, add to the growing literature defining roles for PARP-1 in signal-regulated transcription in the absence of DNA damage. What is perplexing is the wide variety of mechanisms for PARP-1 transcriptional coregulatory activity, which seem to vary in an activator- and gene-specific manner. What remains to be seen is if any unifying principles will be apparent when more of the mechanistic details are uncovered.

Insulator function

Recent results from Yu et al. (2004) have implicated PARylation in the regulation of CTCF, a ubiquitous DNA-binding protein, at transcriptional insulators. Insulators are elements that organize the genome into discrete regulatory domains by limiting the actions of en-

hancers and silencers through a "positional blocking" mechanism (Bell et al. 2001). In their studies, Yu et al. (2004) examined the insulator at the imprinting control region (ICR) of the *Igf2-H19* locus, an element that binds CTCF and limits the extent of expression in a parent origin-specific manner. PAR containing >10 ADP-ribose units was only detected on the maternally inherited (unmethylated) ICR. Furthermore, the presence of PAR at the ICR was dependent on functional CTCF-binding sites in the maternal allele, but not the paternal allele. ICR insulator function was shown to be sensitive to 3-AB, a general inhibitor of PARPs that rapidly reduced the levels of PAR, but not CTCF, at the ICR, and activated expression of the normally repressed maternal *Igf2* allele. The generality of these results was demonstrated using a ChIP-chip approach with a microarray containing a library of CTCF-binding sites. Nearly 80% of the CTCF target sites on the array were immunoprecipitated by antibodies to PAR and CTCF, although CTCF was not directly shown to be the PARylated protein at these sites. In an insulator trap assay, the insulator function of most of the CTCF-binding sites in the library was sensitive to 3-AB. Collectively, these results support a role for PAR in the activity of insulators, possibly as a covalent attachment to CTCF. This conclusion is extended by further studies showing that CTCF immunoprecipitated from cells is PARylated and that purified CTCF can be PARylated in vitro by PARP-1. Additional studies will be required to determine if CTCF is, indeed, the target of PARylation at insulators and, if so, exactly how PARylation affects CTCF's role in insulator function.

Mitotic apparatus function

PARP-1 and at least five other PARP family members (i.e., PARP-2, PARP-3, VPARP, tankyrase 1, and tankyrase 2) are associated with various components of the mitotic apparatus, which is required for the accurate segregation of chromosomes during cell division (Smith 2001; Amé et al. 2004). For example, both PARP-1 and PARP-2 localize to mitotic centromeres, the chromosomal regions where kinetochores form to capture microtubules from the mitotic spindle (Earle et al. 2000; Saxena et al. 2002a,b). In addition, several PARP enzymes, including PARP-1, PARP-3, and tankyrases, are associated with centrosomes, the cellular microtubule organizing center that functions as the spindle pole during mitosis (Smith and de Lange 1999; Kaminker et al. 2001; Augustin et al. 2003; Kanai et al. 2003). Furthermore, PAR and PAR-metabolizing enzymes, including VPARP and PARG, also localize to the mitotic spindle (Kickhoefer et al. 1999; Chang et al. 2004). The colocalization of PARPs and PARG to the mitotic apparatus suggests that dynamic regulation of PAR metabolism may play a key role in the control of mitotic functions. In support of this, the cellular concentration of PAR increases dramatically during metaphase and anaphase–telophase of mitosis (Bakondi et al. 2002).

Recent studies by Chang et al. (2004) suggest that the increased production of PAR during mitosis plays an essential role in the assembly and structure of bipolar spindles. Antibody staining for PAR using isolated spindles assembled in cycled *Xenopus* egg extract and in somatic cells demonstrated that PAR colocalizes with spindle microtubules, and is enriched at spindle poles and kinetochores. The addition of PARG or PAR antibody results in a rapid breakdown of spindle structure. The results of Chang et al. (2004) demonstrate a requirement for PAR in the assembly and structure of bipolar spindles, although the exact role and targets of PAR action at spindles are not known. PAR may act as a signaling component to regulate spindle proteins through covalent and noncovalent modifications. Alternatively, PAR may serve as a structural component of the spindle to provide a matrix for proper spindle assembly and function (Chang et al. 2004). In sum, a growing body of evidence has implicated roles for PAR and PAR metabolizing enzymes in the function of the mitotic apparatus. Determination of their specific roles in mitotic processes will require additional functional assays.

The physiology and pathophysiology of PAR and PARP-1

The molecular and cellular aspects of PAR and PARP-1 function underlie their roles in many physiological and pathophysiological outcomes, including genome maintenance, carcinogenesis, aging, immunity, inflammation, and neurological function. The role of PAR, PARP-1, and some related PARPs in these processes are discussed below.

Genome maintenance

Both PARP-1 and PARP-2 are important for the maintenance of genome stability, and depletion of either one alone leads to a loss of genetic stability. For example, *Parp-1*$^{-/-}$ mice or *Parp-1*$^{-/-}$ embryonic fibroblasts exhibit defective SSB repair and increased homologous recombination, sister-chromatid exchange, and micronuclei formation (Table 1; de Murcia et al. 1997; Wang et al. 1997; D'Amours et al. 1999; Bürkle 2001b). In addition, PARP-1 deficiency causes increased deletion mutations and insertions/rearrangements after treatment with alkylating agents (Shibata et al. 2005). Likewise, *Parp-2*$^{-/-}$ mice or *Parp-2*$^{-/-}$ embryonic fibroblasts exhibit increased sensitivity to ionizing radiation, increased radiation-induced chromosomal breaks, increased sister-chromatid exchange, G2/M cell cycle block, and a high level of mitotic chromosomal aberrations (Menissier de Murcia et al. 2003). Although neither PARP-1 nor PARP-2 is individually required for survival in the absence of genotoxic insults, and *Parp-1*$^{-/-}$ and *Parp-2*$^{-/-}$ mice are viable and fertile (Wang et al. 1995; de Murcia et al. 1997; Masutani et al. 1999; Menissier de Murcia et al. 2003), *Parp-1*$^{-/-}$ *Parp-2*$^{-/-}$ mice exhibit embryonic lethality prior to E8.0 (Menissier de Murcia et al. 2003). Interestingly, *Parp-1*$^{+/-}$ *Parp-2*$^{-/-}$ mice show female-specific embryonic lethality at E9.5 associated with X-chromosome instability in those females, but not in males (Table 1; Menissier de Murcia et al. 2003). Collectively, the avail-

able data indicate that PARP-1 and PARP-2 possess both overlapping and nonredundant functions that are required for the maintenance of genomic stability.

Carcinogenesis

Carcinogenesis is a multistep process involving aberrations in a variety of cellular processes, including genome maintenance, cell cycle control, proliferation, differentiation, and cell death. PARP-1 and PARylation have been implicated in all of these processes, suggesting possible connections between PARP-1 function and carcinogenesis (Masutani et al. 2003). Although *Parp-1*$^{-/-}$ mice do not exhibit a propensity for the development of early onset tumors, they do show increased tumor formation in several chemically induced and transgenic cancer models (Table 1; Masutani et al. 2005). For example, *Parp-1*$^{-/-}$ mice treated with certain alkylating agents [e.g., N-nitrosobis(2-hydroxypropyl)amine and azoxymethane] have a higher incidence of colon and liver cancers than *Parp-1*$^{+/+}$ mice (Tsutsumi et al. 2001; Nozaki et al. 2003). The incidence of tumors in response to some other DNA-damaging agents, however, is not different between *Parp-1*$^{-/-}$ and *Parp-1*$^{+/+}$ mice (Masutani et al. 2005), suggesting that the nature of the DNA damage dictates the specific contribution of PARP-1 to cancer prevention.

Other mouse models have suggested important functional interactions between PARP-1 and other genome maintenance factors in preventing carcinogenesis. For example, *Parp-1* knockout in severe combined immunodeficiency (SCID) mice, which harbor a mutant gene encoding the DNA-PK catalytic subunit, promotes a dramatic increase in the incidence of T-cell lymphoma (Table 1; Morrison et al. 1997). As noted above, PARP-1 is likely to play a role in the recruitment and activation of DNA-PK complexes at sites of DSB repair (Ruscetti et al. 1998; Ariumi et al. 1999). Simultaneous depletion of PARP-1 and p53, a tumor-suppressor gene required for cell cycle checkpoints and apoptotic cell death following DNA damage (Hofseth et al. 2004), has produced seemingly conflicting results about the role of PARP-1 in carcinogenesis in two different mouse models. Tong et al. (2001) showed that PARP-1 deficiency from biallelic deletion of exon 2 in the *Parp-1* gene accelerates spontaneous tumor formation in *p53*$^{-/-}$ mice relative to PARP-1-sufficient *p53*$^{-/-}$ mice. In contrast, Conde et al. (2001) showed that PARP-1 deficiency from biallelic deletion of exon 4 in the *Parp-1* gene increases the latency for tumor formation in *p53*$^{-/-}$ mice relative to PARP-1-sufficient *p53*$^{-/-}$ mice (Table 1). The reason for the differences in these studies is not clear, but may relate to the specific exons targeted for disruption in the PARP-1 gene or other genetic factors. Both studies do agree, however, that *Parp-1*$^{-/-}$ *p53*$^{-/-}$ cells/animals show more genomic instability than *Parp-1*$^{+/+}$ *p53*$^{-/-}$ cells/animals, pointing to functional cooperation between PARP-1 and p53 in genome maintenance, the cornerstone of cancer prevention. Collectively, the available data indicate that normal PARP-1 function is required to prevent cancer formation in response to DNA damage. Furthermore, PARP-1 is likely to cooperate with PARP-2 and other genome maintenance factors to maintain genomic integrity and prevent spontaneous tumor formation.

Aging

The accumulation of macromolecular damage, especially damage to genomic DNA, underlies the aging process. Given their roles in cellular responses to various types of genomic insults, PARP-1 and other PARPs have been implicated in aging and longevity (Beneke and Burkle 2004; Bürkle et al. 2005). The initial connection came from studies showing that the cellular capacity of blood mononuclear cells from various mammalian species to synthesize PAR is positively correlated with the life span of the species (Grube and Burkle 1992). Recent studies have suggested important physical and functional interactions between PARP-1 and WRN, a member of the RecQ helicase family that forms part of the DNA replication complex, in DNA damage responses and in the prevention of chromosome defects and cancer (Lebel et al. 2003; von Kobbe et al. 2003). Interestingly, the gene encoding WRN is mutated in Werner Syndrome, a rare genetic disorder that induces premature aging in humans (Lee et al. 2005). In addition to their roles in counteracting DNA damage and improper chromosome segregation, as noted above, PARP-1 and other PARPs (e.g., tankyrases and PARP-2) play a role in maintaining telomere length and structure (Cook et al. 2002; Dantzer et al. 2004; Dynek and Smith 2004; O'Connor et al. 2004). Telomere maintenance is important for the prevention of cellular senescence and replicative crisis in replicating cells. Although much of the current evidence suggesting a role for PARPs in aging is correlative, the potential connections are intriguing and warrant further examination.

Inflammatory responses

PARP-1 has been implicated in pathophysiological inflammatory responses. As such, *Parp-1*$^{-/-}$ mice show resistance in various models of inflammation, including streptozotocin-induced diabetes and LPS-induced septic shock (Oliver et al. 1999; Mabley et al. 2001; Hassa and Hottiger 2002), whereas *Parg*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ mice (i.e., selective depletion of the 110-kDa isoform) show increased susceptibility (Table 1; Cortes et al. 2004). PARP-1's role in promoting pathophysiological inflammatory responses appears to be twofold. First, in response to genotoxins, PARP-1 induces necrotic cell death, which releases intracellular components into the surrounding tissue, causing tissue damage and promoting inflammation (see above). Second, PARP-1 functions as a coactivator of transcription factors that regulate immune and inflammatory response genes (e.g., NF-κB and AP-1) (Oliver et al. 1999; Hassa and Hottiger 2002; Andreone et al. 2003). In fact, NF-κB- and AP-1-regulated pro-inflammatory genes are down-regulated in *Parp-1*$^{-/-}$ mice or *Parp-1*$^{-/-}$ immune cells (Zingarelli et al. 1998; Shall and de Murcia

2000; Hassa and Hottiger 2002) and up-regulated in immune cells treated with a PARG inhibitor (Rapizzi et al. 2004). Collectively, the available data indicate that PARP-1 plays an important role in pathophysiological inflammatory responses, associated primarily with the dysregulation of NF-κB function. The mechanisms by which PARP-1 functions as a coactivator of NF-κB are not clear, but are likely to involve direct interactions with NF-κB and may not require PARP-1's enzymatic activity (Oliver et al. 1999; Hassa and Hottiger 2002).

Neuronal function

PARP-1 has been implicated in neuronal pathology and, more recently, in normal neuronal function as well. Most of the work in this area has focused on PARP-1's role in inducing necrotic cell death upon neuronal injury, including excitotoxicity (i.e., glutamate-mediated neuronal death), ischemia/reperfusion, and traumatic injury (Szabo and Dawson 1998; Ha and Snyder 2000; Cole and Perez-Polo 2004). Neuronal injury generates significant amounts of oxygen- and nitrogen-derived free radicals (e.g., superoxide, hydroxyl radical, nitric oxide), which are particularly toxic to neuronal cells (Cole and Perez-Polo 2004). These reactive oxygen species set off a cascade of DNA damage, PARP-1 activation, and necrosis in neuronal cells. Chemical inhibition or genetic depletion of PARP-1 in mammalian systems blocks these neurotoxic effects (Zhang et al. 1994; Ha and Snyder 2000; Mandir et al. 2000; Cole and Perez-Polo 2004). In contrast, loss-of-function mutation of PARG in *Drosophila* promotes progressive neurodegeneration and shortened life span in surviving adult animals (Table 1; Hanai et al. 2004). These results suggest that regulated PAR metabolism is important in maintaining normal neuronal functions.

Recent studies have also suggested a role for PARP-1 in learning and memory (Satchell et al. 2003; Cohen-Armon et al. 2004). Cohen-Armon et al. (2004) used the marine mollusk *Aplysia* as a model system to examine the role of a PARP-1 homolog in long-term memory formation. Interestingly, they found that PARP-1 enzymatic activity is increased in *Aplysia* neuronal cells in two different learning paradigms: response to an aversive stimulus and feeding-related learning. Treatment with the PARP-1 inhibitor 3-AB during training blocked long-term memory, whereas treatment with serotonin, which facilitates long-term memory formation, increases PARylation of histone H1. The authors speculate that PARylation of nuclear proteins that regulate the expression of genes involved in long-term memory formation might mediate the effects they observed. More extensive studies, however, are needed to determine how PARP-1 is activated and the specific role it plays during long-term memory formation.

Nuclear NAD$^+$ metabolism and the regulation of PARP-1 activity

The enzymatic activity of PARPs requires a ready supply of NAD$^+$, which is hydrolyzed to produce ADP-ribose units for the PARylation of protein targets. Unlike cellular redox reactions that use NAD$^+$ as a cofactor without a net loss of pyridine nucleotide, PARPs and some other nuclear enzymes (e.g., the protein deacetylase Sir2/SIRT1) cleave the glycosidic bond between nicotinamide and the ADP-ribose moiety of NAD$^+$ (Fig. 4). Consequently, the resynthesis of NAD$^+$ is essential for maintaining PARP functions. In fact, the regulated (and per-

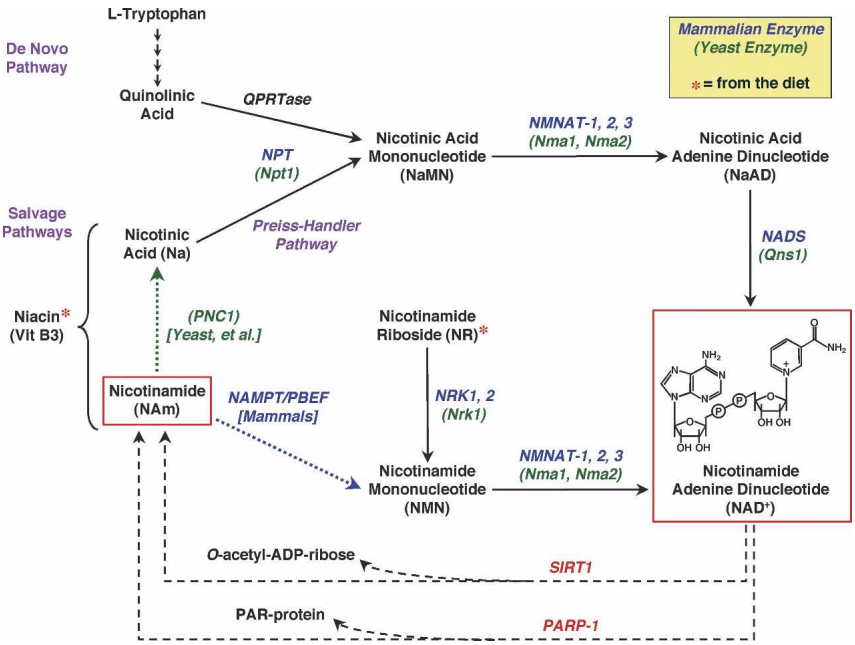

Figure 4. NAD$^+$ biosynthetic pathways. The biosynthesis of NAD$^+$ occurs through both salvage and de novo pathways (Rongvaux et al. 2003). The salvage pathways begin with either nicotinamide or nicotinic acid, collectively referred to as niacin or vitamin B3. One salvage pathway leading from nicotinic acid (Na) to NAD$^+$, known as the Preiss-Handler pathway, goes through two intermediates, nicotinic acid mononucleotide (NaMN) and nicotinic acid adenine dinucleotide (NaAD). A parallel salvage pathway leading from nicotinamide (NAm) to NAD$^+$ goes through one intermediate, nicotinamide mononucleotide (NMN). The de novo pathway leads from tryptophan to quinolinate, which connects to the Preiss-Handler salvage pathway through NaMN. Recently, nicotinamide riboside (NR) was also shown to be a precursor for NAD$^+$ synthesis, connecting to the NAm salvage pathway through NMN (Bieganowski and Brenner 2004). The enzymatic actions of PARP-1 and SIRT1 release the NAm moiety from NAD$^+$ to produce ADP-ribose-protein and *O*-acetyl-ADP-ribose, respectively.

haps localized) availability of NAD^+ may represent a key point of control for PARP-1 and other PARPs. For example, the concentration of NAD^+ has been shown to affect the length of PAR synthesized by PARP-1 in vitro (Alvarez-Gonzalez and Mendoza-Alvarez 1995). Furthermore, in biochemical assays, the availability of NAD^+ regulates PARP-1's effects on chromatin structure and transcription (Kim et al. 2004). How NAD^+ functions as a nuclear signal to regulate PARP-1 activity in vivo, however, is unclear. Studies examining the biosynthesis of NAD^+ have begun to provide some clues.

The biosynthesis of NAD^+ occurs through both salvage and de novo pathways (Fig. 4; Rongvaux et al. 2003). The salvage pathways begin with either nicotinamide or nicotinic acid, collectively referred to as niacin or vitamin B3. The de novo pathway leads from tryptophan to quinolinate, which connects to the nicotinic acid salvage pathway through nicotinic acid mononucleotide (NaMN). In most mammalian tissues, nicotinamide, a product of NAD^+ hydrolysis by PARP-1 and SIRT1, is likely the most relevant NAD^+ precursor for the regulation of PARP-1 functions (Rongvaux et al. 2003). Nicotinamide is first converted to NMN by nicotinamide phosphoribosyltransferase (NAMPT). The production of NAD^+ from NMN and ATP is then catalyzed by a family of NMN adenylyltransferases (NMNATs) (Magni et al. 2004). The enzymes possessing NMNAT activity also have NaMN adenylyltransferase (NaMNAT) activity and are, therefore, required for all NAD^+ biosynthetic pathways. Interestingly, the nicotinamide salvage pathway is not well conserved. In lower eukaryotes—including *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans*—no NAMPT activity has been found (Rongvaux et al. 2003). In these species, nicotinamide is converted to nicotinic acid, which then enters the parallel nicotinic acid ("Preiss-Handler") salvage pathway found in all eukaryotic species. Recently, nicotinamide riboside was also shown to be a precursor for NAD^+ synthesis, connecting to the nicotinamide salvage pathway through NMN (Fig. 4; Bieganowski and Brenner 2004).

Given that many PARPs are nuclear proteins with nuclear functions, they require a source of nuclear NAD^+. Although the nuclear and cytoplasmic pools of NAD^+ are thought to be freely exchangeable in animals, the predominant form of NMNAT in mammals, NMNAT-1, is a nuclear protein (Schweiger et al. 2001), while other forms, NMNAT-2 and NMNAT-3, are extranuclear (i.e., cytoplasmic and mitochondrial, respectively) (Berger et al. 2004). The existence of both nuclear and extranuclear forms of NMNAT suggests that localized production of NAD^+ is important for the NAD^+-dependent processes in those compartments. In fact, overexpression of NMNAT-1 in mammalian cells does not increase total cellular NAD^+ levels, but is still able to regulate NAD^+-dependent nuclear processes (Mack et al. 2001; Araki et al. 2004). Although detailed studies examining potential functional interplay between PARP-1 and NMNAT-1 are lacking, preliminary results suggests that these enzymes interact and might modulate each other's enzymatic activities (Ruggieri et al. 1990; Schweiger et al. 2001).

More conclusive evidence for the regulation of NAD^+-dependent nuclear enzymes by NAD^+ biosynthetic pathways comes from studies on the activity of yeast Sir2/mammalian SIRT1, an NAD^+-dependent nuclear protein deacetylase whose activity is modulated by PNC1, NMNATs, and NAMPT (Anderson et al. 2002, 2003; Araki et al. 2004; Revollo et al. 2004). These same studies suggest that removal of nicotinamide, an inhibitor of both Sir2/SIRT1 and PARP-1 (Hageman and Stierum 2001; Bitterman et al. 2002), by enzymes in the salvage pathways (e.g., PNC1, NAMPT) may be as important for the activation of Sir2/SIRT1 (and perhaps PARP-1) as the production of NAD^+. An interesting possibility that has not yet been explored is the potential recruitment of NAD^+ biosynthetic enzymes to sites of PARP-1 action, providing tightly controlled local NAD^+ production as a means to regulate PARP-1 functions. Finally, PARPs and other NAD^+-dependent nuclear enzymes, such as SIRT1, may compete for a common pool of NAD^+ that could become limiting under conditions of severe DNA damage (Zhang 2003). Therefore, NAD^+ metabolism in the nucleus may play a key role in coordinating multiple aspects of nuclear functions through the regulation of NAD^+-dependent nuclear enzymes.

How can PARP-1 and PAR have so many different functions?

The current evidence clearly indicates that PARP-1 and PAR play important roles in many different cellular processes under both physiological and pathophysiological conditions (Fig. 5). The key question that has not been addressed, however, is how these ubiquitous factors can have so many different functions. Although PARP-1

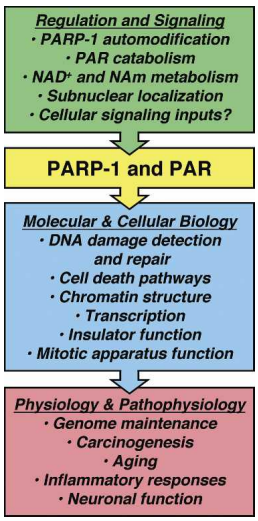

Figure 5. Functions of PARP-1 and PAR. PARP-1 and PAR play diverse roles in many molecular and cellular processes, as indicated. These processes are critical for many physiological and pathophysiological outcomes, including genome maintenance, carcinogenesis, aging, inflammation, and neuronal function. See the text for details.

shares its cellular PARylation duties with other PARP family members, which may help to explain the viability and subtle phenotypes of *Parp-1*$^{-/-}$ mice, this is probably only part of the answer. Perhaps more important is the specific regulation of PARP-1 sub-nuclear localization and activity, which is likely to be tied to a plethora of cellular signaling pathways, including the regulated production of nuclear NAD$^+$. Currently, we know much more about PARP-1 and other PARPs as effector molecules than we know about the signaling pathways leading to their regulated participation in specific cellular processes, especially in the absence of cellular stress. This is an area that will require further elucidation if we are to make sense of the diverse functions of PARP-1 and PAR.

Acknowledgments

We thank Gilbert de Murcia, John Lis, Steve Mauro, and members of the Kraus lab for providing critical comments on this review.

References

Affar, E.B., Germain, M., Winstall, E., Vodenicharov, M., Shah, R.G., Salvesen, G.S., and Poirier, G.G. 2001. Caspase-3-mediated processing of poly(ADP-ribose) glycohydrolase during apoptosis. *J. Biol. Chem.* **276:** 2935–2942.

Alvarez-Gonzalez, R. and Mendoza-Alvarez, H. 1995. Dissection of ADP-ribose polymer synthesis into individual steps of initiation, elongation, and branching. *Biochimie* **77:** 403–407.

Alvarez-Gonzalez, R., Watkins, T.A., Gill, P.K., Reed, J.L., and Mendoza-Alvarez, H. 1999. Regulatory mechanisms of poly-(ADP-ribose) polymerase. *Mol. Cell. Biochem.* **193:** 19–22.

Amé, J.-C., Jacobson, E.L., and Jacobson, M.K. 2000. ADP-ribose polymer metabolism. In *From DNA damage and stress signalling to cell death: Poly ADP-ribosylation reactions* (eds. G. de Murcia and S. Shall), pp. 1–34. Oxford University Press, New York.

Amé, J.C., Spenlehauer, C., and de Murcia, G. 2004. The PARP superfamily. *Bioessays* **26:** 882–893.

Anderson, M.G., Scoggin, K.E., Simbulan-Rosenthal, C.M., and Steadman, J.A. 2000. Identification of poly(ADP-ribose) polymerase as a transcriptional coactivator of the human T-cell leukemia virus type 1 Tax protein. *J. Virol.* **74:** 2169–2177.

Anderson, R.M., Bitterman, K.J., Wood, J.G., Medvedik, O., Cohen, H., Lin, S.S., Manchester, J.K., Gordon, J.I., and Sinclair, D.A. 2002. Manipulation of a nuclear NAD$^+$ salvage pathway delays aging without altering steady-state NAD$^+$ levels. *J. Biol. Chem.* **277:** 18881–18890.

Anderson, R.M., Bitterman, K.J., Wood, J.G., Medvedik, O., and Sinclair, D.A. 2003. Nicotinamide and PNC1 govern lifespan extension by calorie restriction in *Saccharomyces cerevisiae*. *Nature* **423:** 181–185.

Andreone, T.L., O'Connor, M., Denenberg, A., Hake, P.W., and Zingarelli, B. 2003. Poly(ADP-ribose) polymerase-1 regulates activation of activator protein-1 in murine fibroblasts. *J. Immunol.* **170:** 2113–2120.

Araki, T., Sasaki, Y., and Milbrandt, J. 2004. Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science* **305:** 1010–1013.

Ariumi, Y., Masutani, M., Copeland, T.D., Mimori, T., Sugimura, T., Shimotohno, K., Ueda, K., Hatanaka, M., and Noda, M. 1999. Suppression of the poly(ADP-ribose) polymerase activity by DNA-dependent protein kinase in vitro. *Oncogene* **18:** 4616–4625.

Augustin, A., Spenlehauer, C., Dumond, H., Menissier De Murcia, J., Piel, M., Schmit, A.C., Apiou, F., Vonesch, J.L., Kock, M., Bornens, M., et al. 2003. PARP-3 localizes preferentially to the daughter centriole and interferes with the G1/S cell cycle progression. *J. Cell Sci.* **116:** 1551–1562.

Bakondi, E., Bai, P., Szabo, E.E., Hunyadi, J., Gergely, P., Szabo, C., and Virag, L. 2002. Detection of poly(ADP-ribose) polymerase activation in oxidatively stressed cells and tissues using biotinylated NAD substrate. *J. Histochem. Cytochem.* **50:** 91–98.

Bell, A.C., West, A.G., and Felsenfeld, G. 2001. Insulators and boundaries: Versatile regulatory elements in the eukaryotic. *Science* **291:** 447–450.

Beneke, S. and Burkle, A. 2004. Poly(ADP-ribosyl)ation, PARP, and aging. *Sci. Aging Knowledge Environ.* **2004:** re9.

Berger, F., Ramirez-Hernandez, M.H., and Ziegler, M. 2004. The new life of a centenarian: Signalling functions of NAD(P). *Trends Biochem. Sci.* **29:** 111–118.

Bieganowski, P. and Brenner, C. 2004. Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD$^+$ in fungi and humans. *Cell* **117:** 495–502.

Bitterman, K.J., Anderson, R.M., Cohen, H.Y., Latorre-Esteves, M., and Sinclair, D.A. 2002. Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1. *J. Biol. Chem.* **277:** 45099–45107.

Bonicalzi, M.E., Vodenicharov, M., Coulombe, M., Gagne, J.P., and Poirier, G.G. 2003. Alteration of poly(ADP-ribose) glycohydrolase nucleocytoplasmic shuttling characteristics upon cleavage by apoptotic proteases. *Biol. Cell* **95:** 635–644.

Bouchard, V.J., Rouleau, M., and Poirier, G.G. 2003. PARP-1, a determinant of cell survival in response to DNA damage. *Exp. Hematol.* **31:** 446–454.

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J., and Helleday, T. 2005. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* **434:** 913–917.

Bürkle, A. 2001a. PARP-1: A regulator of genomic stability linked with mammalian longevity. *Chembiochem.* **2:** 725–728.

———. 2001b. Physiology and pathophysiology of poly(ADP-ribosyl)ation. *Bioessays* **23:** 795–806.

Bürkle, A., Schreiber, V., Dantzer, F., Oliver, F.J., Niedergang, C., de Murcia, G., and Ménissier-de Murcia, J. 2000. Biological significance of poly(ADP-ribosyl)ation reactions: Molecular and genetic approaches. In *From DNA damage and stress signalling to cell death: Poly ADP-ribosylation reactions* (eds. G. de Murcia and S. Shall), pp. 80–124. Oxford University Press, New York.

Bürkle, A., Brabeck, C., Diefenbach, J., and Beneke, S. 2005. The emerging role of poly(ADP-ribose) polymerase-1 in longevity. *Int. J. Biochem. Cell Biol.* **37:** 1043–1053.

Cervellera, M.N. and Sala, A. 2000. Poly(ADP-ribose) polymerase is a B-MYB coactivator. *J. Biol. Chem.* **275:** 10692–10696.

Chambon, P., Weill, J.D., and Mandel, J.L. 1963. Nicotinamide mononucleotide activation of a new DNA-dependent polyadenylic acid synthesizing enzyme. *Biochem. Biophys. Res. Comm.* **11:** 39–43.

Chang, P., Jacobson, M.K., and Mitchison, T.J. 2004. Poly(ADP-ribose) is required for spindle assembly and structure. *Nature*

432: 645–649.

Chiarugi, A. and Moskowitz, M.A. 2002. Cell biology. PARP-1—A perpetrator of apoptotic cell death? *Science* **297:** 200–201.

Cohen-Armon, M., Visochek, L., Katzoff, A., Levitan, D., Susswein, A.J., Klein, R., Valbrun, M., and Schwartz, J.H. 2004. Long-term memory requires polyADP-ribosylation. *Science* **304:** 1820–1822.

Cole, K. and Perez-Polo, J.R. 2004. Neuronal trauma model: In search of Thanatos. *Int. J. Dev. Neurosci.* **22:** 485–496.

Conde, C., Mark, M., Oliver, F.J., Huber, A., de Murcia, G., and Menissier de Murcia, J. 2001. Loss of poly(ADP-ribose) polymerase-1 causes increased tumour latency in p53-deficient mice. *EMBO J.* **20:** 3535–3543.

Cook, B.D., Dynek, J.N., Chang, W., Shostak, G., and Smith, S. 2002. Role for the related poly(ADP-ribose) polymerases tankyrase 1 and 2 at human telomeres. *Mol. Cell. Biol.* **22:** 332–342.

Cortes, U., Tong, W.M., Coyle, D.L., Meyer-Ficca, M.L., Meyer, R.G., Petrilli, V., Herceg, Z., Jacobson, E.L., Jacobson, M.K., and Wang, Z.Q. 2004. Depletion of the 110-kilodalton isoform of poly(ADP-ribose) glycohydrolase increases sensitivity to genotoxic and endotoxic stress in mice. *Mol. Cell. Biol.* **24:** 7163–7178.

D'Amours, D., Desnoyers, S., D'Silva, I., and Poirier, G.G. 1999. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem. J.* **342 (Pt 2):** 249–268.

Dantzer, F., Giraud-Panis, M.J., Jaco, I., Ame, J.C., Schultz, I., Blasco, M., Koering, C.E., Gilson, E., Menissier de Murcia, J., de Murcia, G., et al. 2004. Functional interaction between poly(ADP-ribose) polymerase 2 (PARP-2) and TRF2: PARP activity negatively regulates TRF2. *Mol. Cell. Biol.* **24:** 1595–1607.

Davidovic, L., Vodenicharov, M., Affar, E.B., and Poirier, G.G. 2001. Importance of poly(ADP-ribose) glycohydrolase in the control of poly(ADP-ribose) metabolism. *Exp. Cell Res.* **268:** 7–13.

Decker, P. and Muller, S. 2002. Modulating poly (ADP-ribose) polymerase activity: Potential for the prevention and therapy of pathogenic situations involving DNA damage and oxidative stress. *Curr. Pharm. Biotechnol.* **3:** 275–283.

de Murcia, J.M., Niedergang, C., Trucco, C., Ricoul, M., Dutrillaux, B., Mark, M., Oliver, F.J., Masson, M., Dierich, A., LeMeur, M., et al. 1997. Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *Proc. Natl. Acad. Sci.* **94:** 7303–7307.

Dynek, J.N. and Smith, S. 2004. Resolution of sister telomere association is required for progression through mitosis. *Science* **304:** 97–100.

Earle, E., Saxena, A., MacDonald, A., Hudson, D.F., Shaffer, L.G., Saffery, R., Cancilla, M.R., Cutts, S.M., Howman, E., and Choo, K.H. 2000. Poly(ADP-ribose) polymerase at active centromeres and neocentromeres at metaphase. *Hum. Mol. Genet.* **9:** 187–194.

Edinger, A.L. and Thompson, C.B. 2004. Death by design: Apoptosis, necrosis and autophagy. *Curr. Opin. Cell Biol.* **16:** 663–669.

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., et al. 2005. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434:** 917–921.

Gagne, J.P., Hunter, J.M., Labrecque, B., Chabot, B., and Poirier, G.G. 2003. A proteomic approach to the identification of heterogeneous nuclear ribonucleoproteins as a new family of poly(ADP-ribose)-binding proteins. *Biochem. J.* **371:** 331–340.

Grube, K. and Burkle, A. 1992. Poly(ADP-ribose) polymerase activity in mononuclear leukocytes of 13 mammalian species correlates with species-specific life span. *Proc. Natl. Acad. Sci.* **89:** 11759–11763.

Ha, H.C. and Snyder, S.H. 2000. Poly(ADP-ribose) polymerase-1 in the nervous system. *Neurobiol. Dis.* **7:** 225–239.

Hageman, G.J. and Stierum, R.H. 2001. Niacin, poly(ADP-ribose) polymerase-1 and genomic stability. *Mutat. Res.* **475:** 45–56.

Hanai, S., Uchida, M., Kobayashi, S., Miwa, M., and Uchida, K. 1998. Genomic organization of *Drosophila* poly(ADP-ribose) polymerase and distribution of its mRNA during development. *J. Biol. Chem.* **273:** 11881–11886.

Hanai, S., Kanai, M., Ohashi, S., Okamoto, K., Yamada, M., Takahashi, H., and Miwa, M. 2004. Loss of poly(ADP-ribose) glycohydrolase causes progressive neurodegeneration in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **101:** 82–86.

Hassa, P.O. and Hottiger, M.O. 2002. The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-κB in inflammatory disorders. *Cell Mol. Life Sci.* **59:** 1534–1553.

Hassa, P.O., Covic, M., Hasan, S., Imhof, R., and Hottiger, M.O. 2001. The enzymatic and DNA binding activity of PARP-1 are not required for NF-κ B coactivator function. *J. Biol. Chem.* **276:** 45588–45597.

Hofseth, L.J., Hussain, S.P., and Harris, C.C. 2004. p53: 25 years after its discovery. *Trends Pharmacol. Sci.* **25:** 177–181.

Huletsky, A., de Murcia, G., Muller, S., Hengartner, M., Menard, L., Lamarre, D., and Poirier, G.G. 1989. The effect of poly(ADP-ribosyl)ation on native and H1-depleted chromatin. A role of poly(ADP-ribosyl)ation on core nucleosome structure. *J. Biol. Chem.* **264:** 8878–8886.

Ju, B.G., Solum, D., Song, E.J., Lee, K.J., Rose, D.W., Glass, C.K., and Rosenfeld, M.G. 2004. Activating the PARP-1 sensor component of the groucho/TLE1 corepressor complex mediates a CaMKinase IIδ-dependent neurogenic gene activation pathway. *Cell* **119:** 815–829.

Kaminker, P.G., Kim, S.H., Taylor, R.D., Zebarjadian, Y., Funk, W.D., Morin, G.B., Yaswen, P., and Campisi, J. 2001. TANK2, a new TRF1-associated poly(ADP-ribose) polymerase, causes rapid induction of cell death upon overexpression. *J. Biol. Chem.* **276:** 35891–35899.

Kanai, M., Tong, W.M., Sugihara, E., Wang, Z.Q., Fukasawa, K., and Miwa, M. 2003. Involvement of poly(ADP-ribose) polymerase 1 and poly(ADP-ribosyl)ation in regulation of centrosome function. *Mol. Cell. Biol.* **23:** 2451–2462.

Karras, G.I., Kustatscher, G., Buhecha, H.R., Allen, M.D., Pugieux, C., Sait, F., Bycroft, M., and Ladurner, A.G. 2005. The macro domain is an ADP-ribose binding module. *EMBO J.* **24:** 1911–1920.

Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E., and Poirier, G.G. 1993. Specific proteolytic cleavage of poly-(ADP-ribose) polymerase: An early marker of chemotherapy-induced apoptosis. *Cancer Res.* **53:** 3976–3985.

Kickhoefer, V.A., Siva, A.C., Kedersha, N.L., Inman, E.M., Ruland, C., Streuli, M., and Rome, L.H. 1999. The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase. *J. Cell Biol.* **146:** 917–928.

Kim, M.Y., Mauro, S., Gevry, N., Lis, J.T., and Kraus, W.L. 2004. NAD^+-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. *Cell* **119:** 803–814.

Koh, D.W., Lawler, A.M., Poitras, M.F., Sasaki, M., Wattler, S., Nehls, M.C., Stoger, T., Poirier, G.G., Dawson, V.L., and Dawson, T.M. 2004. Failure to degrade poly(ADP-ribose) causes increased sensitivity to cytotoxicity and early embry-

onic lethality. *Proc. Natl. Acad. Sci.* **101:** 17699–17704.
Kraus, W.L. and Lis, J.T. 2003. PARP goes transcription. *Cell* **113:** 677–683.
Kun, E., Kirsten, E., and Ordahl, C.P. 2002. Coenzymatic activity of randomly broken or intact double-stranded DNAs in auto and histone H1 *trans*-poly(ADP-ribosylation), catalyzed by poly(ADP-ribose) polymerase (PARP I). *J. Biol. Chem.* **277:** 39066–39069.
Kun, E., Kirsten, E., Mendeleyev, J., and Ordahl, C.P. 2004. Regulation of the enzymatic catalysis of poly(ADP-ribose) polymerase by dsDNA, polyamines, Mg^{2+}, Ca^{2+}, histones H1 and H3, and ATP. *Biochemistry* **43:** 210–216.
Lan, L., Nakajima, S., Oohata, Y., Takao, M., Okano, S., Masutani, M., Wilson, S.H., and Yasui, A. 2004. In situ analysis of repair processes for oxidative DNA damage in mammalian cells. *Proc. Natl. Acad. Sci.* **101:** 13738–13743.
Lebel, M., Lavoie, J., Gaudreault, I., Bronsard, M., and Drouin, R. 2003. Genetic cooperation between the Werner syndrome protein and poly(ADP-ribose) polymerase-1 in preventing chromatid breaks, complex chromosomal rearrangements, and cancer in mice. *Am. J. Pathol.* **162:** 1559–1569.
Lee, J.W., Harrigan, J., Opresko, P.L., and Bohr, V.A. 2005. Pathways and functions of the Werner syndrome protein. *Mech. Ageing Dev.* **126:** 79–86.
Mabley, J.G., Suarez-Pinzon, W.L., Hasko, G., Salzman, A.L., Rabinovitch, A., Kun, E., and Szabo, C. 2001. Inhibition of poly (ADP-ribose) synthetase by gene disruption or inhibition with 5-iodo-6-amino-1,2-benzopyrone protects mice from multiple-low-dose-streptozotocin-induced diabetes. *Br. J. Pharmacol.* **133:** 909–919.
Mack, T.G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., et al. 2001. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. *Nat. Neurosci.* **4:** 1199–1206.
Magni, G., Amici, A., Emanuelli, M., Orsomando, G., Raffaelli, N., and Ruggieri, S. 2004. Enzymology of NAD^+ homeostasis in man. *Cell Mol. Life Sci.* **61:** 19–34.
Malanga, M. and Althaus, F.R. 2005. DNA damage signaling through poly(ADP-ribose). In *Poly(ADP-ribosyl)ation* (ed. A. Burkle), pp. 41–50. Landes Bioscience, Georgetown, TX.
Malanga, M., Atorino, L., Tramontano, F., Farina, B., and Quesada, P. 1998. Poly(ADP-ribose) binding properties of histone H1 variants. *Biochim. Biophys. Acta* **1399:** 154–160.
Mandir, A.S., Poitras, M.F., Berliner, A.R., Herring, W.J., Guastella, D.B., Feldman, A., Poirier, G.G., Wang, Z.Q., Dawson, T.M., and Dawson, V.L. 2000. NMDA but not non-NMDA excitotoxicity is mediated by poly(ADP-ribose) polymerase. *J. Neurosci.* **20:** 8005–8011.
Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier de Murcia, J., and de Murcia, G. 1998. XRCC1 is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. *Mol. Cell. Biol.* **18:** 3563–3571.
Masutani, M., Suzuki, H., Kamada, N., Watanabe, M., Ueda, O., Nozaki, T., Jishage, K., Watanabe, T., Sugimoto, T., Nakagama, H., et al. 1999. Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. *Proc. Natl. Acad. Sci.* **96:** 2301–2304.
Masutani, M., Nakagama, H., and Sugimura, T. 2003. Poly(ADP-ribose) and carcinogenesis. *Genes Chromosomes Cancer* **38:** 339–348.
Masutani, M., Gunji, A., Tsutsumi, M., Ogawa, K., Kamada, N., Shirai, T., Jishage, K., Nakagama, H., and Sugimura, T. 2005. Role of poly-ADP-ribosylation in cancer development. In *Poly(ADP-ribosyl)ation* (ed. A. Burkle), pp. 205–219. Landes Bioscience, Georgetown, TX.
Mathis, G. and Althaus, F.R. 1987. Release of core DNA from nucleosomal core particles following (ADP-ribose)n-modification in vitro. *Biochem. Biophys. Res. Commun.* **143:** 1049–1054.
Meisterernst, M., Stelzer, G., and Roeder, R.G. 1997. Poly(ADP-ribose) polymerase enhances activator-dependent transcription in vitro. *Proc. Natl. Acad. Sci.* **94:** 2261–2265.
Menissier de Murcia, J., Ricoul, M., Tartier, L., Niedergang, C., Huber, A., Dantzer, F., Schreiber, V., Ame, J.C., Dierich, A., LeMeur, M., et al. 2003. Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J.* **22:** 2255–2263.
Meyer-Ficca, M.L., Meyer, R.G., Coyle, D.L., Jacobson, E.L., and Jacobson, M.K. 2004. Human poly(ADP-ribose) glycohydrolase is expressed in alternative splice variants yielding isoforms that localize to different cell compartments. *Exp. Cell Res.* **297:** 521–532.
Meyer-Ficca, M.L., Meyer, R.G., Jacobson, E.L., and Jacobson, M.K. 2005. Poly(ADP-ribose) polymerases: Managing genome stability. *Int. J. Biochem. Cell Biol.* **37:** 920–926.
Minaga, T. and Kun, E. 1983a. Probable helical conformation of poly(ADP-ribose). The effect of cations on spectral properties. *J. Biol. Chem.* **258:** 5726–5730.
———. 1983b. Spectral analysis of the conformation of polyadenosine diphosphoribose. Evidence indicating secondary structure. *J. Biol. Chem.* **258:** 725–730.
Miwa, M., Hanai, S., Poltronieri, P., Uchida, M., and Uchida, K. 1999. Functional analysis of poly(ADP-ribose) polymerase in *Drosophila melanogaster*. *Mol. Cell. Biochem.* **193:** 103–107.
Morrison, C., Smith, G.C., Stingl, L., Jackson, S.P., Wagner, E.F., and Wang, Z.Q. 1997. Genetic interaction between PARP and DNA-PK in V(D)J recombination and tumorigenesis. *Nat. Genet.* **17:** 479–482.
Nozaki, T., Fujihara, H., Watanabe, M., Tsutsumi, M., Nakamoto, K., Kusuoka, O., Kamada, N., Suzuki, H., Nakagama, H., Sugimura, T., et al. 2003. Parp-1 deficiency implicated in colon and liver tumorigenesis induced by azoxymethane. *Cancer Sci.* **94:** 497–500.
O'Connor, M.S., Safari, A., Liu, D., Qin, J., and Songyang, Z. 2004. The human Rap1 protein complex and modulation of telomere length. *J. Biol. Chem.* **279:** 28585–28591.
Oei, S.L. and Shi, Y. 2001. Transcription factor Yin Yang 1 stimulates poly(ADP-ribosyl)ation and DNA repair. *Biochem. Biophys. Res. Commun.* **284:** 450–454.
Ogata, N., Ueda, K., Kawaichi, M., and Hayaishi, O. 1981. Poly(ADP-ribose) synthetase, a main acceptor of poly(ADP-ribose) in isolated nuclei. *J. Biol. Chem.* **256:** 4135–4137.
Ohashi, S., Kanai, M., Hanai, S., Uchiumi, F., Maruta, H., Tanuma, S., and Miwa, M. 2003. Subcellular localization of poly(ADP-ribose) glycohydrolase in mammalian cells. *Biochem. Biophys. Res. Commun.* **307:** 915–921.
Oka, J., Ueda, K., Hayaishi, O., Komura, H., and Nakanishi, K. 1984. ADP-ribosyl protein lyase. Purification, properties, and identification of the product. *J. Biol. Chem.* **259:** 986–995.
Okano, S., Lan, L., Caldecott, K.W., Mori, T., and Yasui, A. 2003. Spatial and temporal cellular responses to single-strand breaks in human cells. *Mol. Cell. Biol.* **23:** 3974–3981.
Oliver, F.J., Menissier de Murcia, J., Nacci, C., Decker, P., Andriantsitohaina, R., Muller, S., de la Rubia, G., Stoclet, J.C., and de Murcia, G. 1999. Resistance to endotoxic shock as a consequence of defective NF-κB activation in poly(ADP-ribose) polymerase-1 deficient mice. *EMBO J.* **18:** 4446–4454.
Panzeter, P.L., Realini, C.A., and Althaus, F.R. 1992. Noncova-

lent interactions of poly(adenosine diphosphate ribose) with histones. *Biochemistry* **31:** 1379–1385.

Pavri, R., Lewis, B., Kim, T.K., Dilworth, F.J., Erdjument-Bromage, H., Tempst, P., de Murcia, G., Evans, R., Chambon, P., and Reinberg, D. 2005. PARP-1 determines specificity in a retinoid signaling pathway via direct modulation of mediator. *Mol. Cell* **18:** 83–96.

Pleschke, J.M., Kleczkowska, H.E., Strohm, M., and Althaus, F.R. 2000. Poly(ADP-ribose) binds to specific domains in DNA damage checkpoint proteins. *J. Biol. Chem.* **275:** 40974–40980.

Poirier, G.G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C., and Mandel, P. 1982. Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. *Proc. Natl. Acad. Sci.* **79:** 3423–3427.

Rapizzi, E., Fossati, S., Moroni, F., and Chiarugi, A. 2004. Inhibition of poly(ADP-ribose) glycohydrolase by gallotannin selectively up-regulates expression of proinflammatory genes. *Mol. Pharmacol.* **66:** 890–898.

Reale, A., Matteis, G.D., Galleazzi, G., Zampieri, M., and Caiafa, P. 2005. Modulation of DNMT1 activity by ADP-ribose polymers. *Oncogene* **24:** 13–19.

Realini, C.A. and Althaus, F.R. 1992. Histone shuttling by poly(ADP-ribosylation). *J. Biol. Chem.* **267:** 18858–18865.

Revollo, J.R., Grimm, A.A., and Imai, S. 2004. The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. *J. Biol. Chem.* **279:** 50754–50763.

Robertson, K.D. 2002. DNA methylation and chromatin—Unraveling the tangled web. *Oncogene* **21:** 5361–5379.

Rolli, V., Ruf, A., Augustin, A., Schulz, G.E., Ménissier-de Murcia, J., and de Murcia, G. 2000. Poly(ADP-ribose) polymerase: Structure and function. In *From DNA damage and stress signalling to cell death: Poly ADP-ribosylation reactions* (eds. G. de Murcia and S. Shall), pp. 35–79. Oxford University Press, New York.

Rongvaux, A., Andris, F., Van Gool, F., and Leo, O. 2003. Reconstructing eukaryotic NAD metabolism. *Bioessays* **25:** 683–690.

Ruggieri, S., Gregori, L., Natalini, P., Vita, A., Emanuelli, M., Raffaelli, N., and Magni, G. 1990. Evidence for an inhibitory effect exerted by yeast NMN adenylyltransferase on poly(ADP-ribose) polymerase activity. *Biochemistry* **29:** 2501–2506.

Ruscetti, T., Lehnert, B.E., Halbrook, J., Le Trong, H., Hoekstra, M.F., Chen, D.J., and Peterson, S.R. 1998. Stimulation of the DNA-dependent protein kinase by poly(ADP-ribose) polymerase. *J. Biol. Chem.* **273:** 14461–14467.

Satchell, M.A., Zhang, X., Kochanek, P.M., Dixon, C.E., Jenkins, L.W., Melick, J., Szabo, C., and Clark, R.S. 2003. A dual role for poly-ADP-ribosylation in spatial memory acquisition after traumatic brain injury in mice involving NAD^+ depletion and ribosylation of 14–3–3γ. *J. Neurochem.* **85:** 697–708.

Saxena, A., Saffery, R., Wong, L.H., Kalitsis, P., and Choo, K.H. 2002a. Centromere proteins Cenpa, Cenpb, and Bub3 interact with poly(ADP-ribose) polymerase-1 protein and are poly(ADP-ribosyl)ated. *J. Biol. Chem.* **277:** 26921–26926.

Saxena, A., Wong, L.H., Kalitsis, P., Earle, E., Shaffer, L.G., and Choo, K.H. 2002b. Poly(ADP-ribose) polymerase 2 localizes to mammalian active centromeres and interacts with PARP-1, Cenpa, Cenpb and Bub3, but not Cenpc. *Hum. Mol. Genet.* **11:** 2319–2329.

Schreiber, V., Ame, J.C., Dolle, P., Schultz, I., Rinaldi, B., Fraulob, V., Menissier de Murcia, J., and de Murcia, G. 2002. Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1. *J. Biol. Chem.* **277:** 23028–23036.

Schweiger, M., Hennig, K., Lerner, F., Niere, M., Hirsch-Kauffmann, M., Specht, T., Weise, C., Oei, S.L., and Ziegler, M. 2001. Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis. *FEBS Lett.* **492:** 95–100.

Shall, S. and de Murcia, G. 2000. Poly(ADP-ribose) polymerase-1: What have we learned from the deficient mouse model? *Mutat. Res.* **460:** 1–15.

Shibata, A., Kamada, N., Masumura, K., Nohmi, T., Kobayashi, S., Teraoka, H., Nakagama, H., Sugimura, T., Suzuki, H., and Masutani, M. 2005. Parp-1 deficiency causes an increase of deletion mutations and insertions/rearrangements in vivo after treatment with an alkylating agent. *Oncogene* **24:** 1328–1337.

Smith, S. 2001. The world according to PARP. *Trends Biochem. Sci.* **26:** 174–179.

Smith, S. and de Lange, T. 1999. Cell cycle dependent localization of the telomeric PARP, tankyrase, to nuclear pore complexes and centrosomes. *J. Cell Sci.* **112 (Pt 21):** 3649–3656.

Soldani, C. and Scovassi, A.I. 2002. Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: An update. *Apoptosis* **7:** 321–328.

Susin, S.A., Lorenzo, H.K., Zamzami, N., Marzo, I., Snow, B.E., Brothers, G.M., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., et al. 1999. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* **397:** 441–446.

Szabo, C. and Dawson, V.L. 1998. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia–reperfusion. *Trends Pharmacol. Sci.* **19:** 287–298.

Tanuma, S. and Johnson, G.S. 1983. ADP-ribosylation of nonhistone high mobility group proteins in intact cells. *J. Biol. Chem.* **258:** 4067–4070.

Tong, W.M., Hande, M.P., Lansdorp, P.M., and Wang, Z.Q. 2001. DNA strand break-sensing molecule poly(ADP-ribose) polymerase cooperates with p53 in telomere function, chromosome stability, and tumor suppression. *Mol. Cell. Biol.* **21:** 4046–4054.

Tsutsumi, M., Masutani, M., Nozaki, T., Kusuoka, O., Tsujiuchi, T., Nakagama, H., Suzuki, H., Konishi, Y., and Sugimura, T. 2001. Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity. *Carcinogenesis* **22:** 1–3.

Tulin, A. and Spradling, A. 2003. Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at *Drosophila* puff loci. *Science* **299:** 560–562.

Tulin, A., Stewart, D., and Spradling, A.C. 2002. The *Drosophila* heterochromatic gene encoding poly(ADP-ribose) polymerase (PARP) is required to modulate chromatin structure during development. *Genes & Dev.* **16:** 2108–2119.

Tutt, A. and Ashworth, A. 2002. The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. *Trends Mol. Med.* **8:** 571–576.

Virag, L. 2005. PARP-1 and the shape of cell death. In *Poly(ADP-ribosyl)ation* (ed. A. Burkle), pp. 141–154. Landes Bioscience, George-
town, TX.

von Kobbe, C., Harrigan, J.A., May, A., Opresko, P.L., Dawut, L., Cheng, W.H., and Bohr, V.A. 2003. Central role for the Werner syndrome protein/poly(ADP-ribose) polymerase 1 complex in the poly(ADP-ribosyl)ation pathway after DNA damage. *Mol. Cell. Biol.* **23:** 8601–8613.

Wang, Z.Q., Auer, B., Stingl, L., Berghammer, H., Haidacher, D., Schweiger, M., and Wagner, E.F. 1995. Mice lacking ADPRT

and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. *Genes & Dev.* **9:** 509–520.

Wang, Z.Q., Stingl, L., Morrison, C., Jantsch, M., Los, M., Schulze-Osthoff, K., and Wagner, E.F. 1997. PARP is important for genomic stability but dispensable in apoptosis. *Genes & Dev.* **11:** 2347–2358.

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

Wooster, R. and Weber, B.L. 2003. Breast and ovarian cancer. *N. Engl. J. Med.* **348:** 2339–2347.

Ying, W., Alano, C.C., Garnier, P., and Swanson, R.A. 2005. NAD^+ as a metabolic link between DNA damage and cell death. *J. Neurosci. Res.* **79:** 216–223.

Yu, S.W., Wang, H., Poitras, M.F., Coombs, C., Bowers, W.J., Federoff, H.J., Poirier, G.G., Dawson, T.M., and Dawson, V.L. 2002. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science* **297:** 259–263.

Yu, W., Ginjala, V., Pant, V., Chernukhin, I., Whitehead, J., Docquier, F., Farrar, D., Tavoosidana, G., Mukhopadhyay, R., Kanduri, C., et al. 2004. Poly(ADP-ribosyl)ation regulates CTCF-dependent chromatin insulation. *Nat. Genet.* **36:** 1105–1110.

Zardo, G. and Caiafa, P. 1998. The unmethylated state of CpG islands in mouse fibroblasts depends on the poly(ADP-ribosyl)ation process. *J. Biol. Chem.* **273:** 16517–16520.

Zardo, G., Reale, A., Passananti, C., Pradhan, S., Buontempo, S., De Matteis, G., Adams, R.L., and Caiafa, P. 2002. Inhibition of poly(ADP-ribosyl)ation induces DNA hypermethylation: A possible molecular mechanism. *FASEB J.* **16:** 1319–1321.

Zardo, G., Reale, A., De Matteis, G., Buontempo, S., and Caiafa, P. 2003. A role for poly(ADP-ribosyl)ation in DNA methylation. *Biochem. Cell Biol.* **81:** 197–208.

Zhang, J. 2003. Are poly(ADP-ribosyl)ation by PARP-1 and deacetylation by Sir2 linked? *Bioessays* **25:** 808–814.

Zhang, J., Dawson, V.L., Dawson, T.M., and Snyder, S.H. 1994. Nitric oxide activation of poly(ADP-ribose) synthetase in neurotoxicity. *Science* **263:** 687–689.

Zingarelli, B., Salzman, A.L., and Szabo, C. 1998. Genetic disruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. *Circ. Res.* **83:** 85–94.

Zong, W.X., Ditsworth, D., Bauer, D.E., Wang, Z.Q., and Thompson, C.B. 2004. Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes & Dev.* **18:** 1272–1282.

Poly(ADP-ribosyl)ation by PARP-1: `PAR-laying' NAD^+ into a nuclear signal

Mi Young Kim, Tong Zhang and W. Lee Kraus

Genes Dev. 2005, **19:**
Access the most recent version at doi:10.1101/gad.1331805