

## The diverse biological roles of mammalian PARPs, a small but powerful family of poly-ADP-ribose polymerases

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### 1. ABSTRACT

Poly-ADP-ribose metabolism plays a mayor role in a wide range of biological processes, such as maintenance of genomic stability, transcriptional regulation, energy metabolism and cell death. Poly-ADP-ribose polymerases (PARPs) are an ancient family of enzymes, as evidenced by the poly-ADP-ribosylating activities reported in dinoflagellates and archaeobacteria and by the identification of *Parp*-like genes in eubacterial and archaeobacterial genomes. Six genes encoding “bona fide” PARP enzymes have been identified in mammals: PARP1, PARP2, PARP3, PARP4/vPARP, PARP5/Tankyrases-1 and PARP6/Tankyrases-2. The best studied of these enzymes PARP1 plays a primary role in the process of poly-ADP-

ribosylation. PARP1-mediated poly-ADP-ribosylation has been implicated in the pathogenesis of cancer, inflammatory and neurodegenerative disorders. This review will summarize the novel findings and concepts for PARP enzymes and their poly-ADP-ribosylation activity in the regulation of physiological and pathophysiological processes. A special focus is placed on the proposed molecular mechanisms involved in these processes, such as signaling, regulation of telomere dynamics, remodeling of chromatin structure and transcriptional regulation. A potential functional cross talk between PARP family members and other NAD<sup>+</sup>-consuming enzymes is discussed.

## 2. INTRODUCTION

The existence of poly-ADP-ribosylation reactions has been first reported in 1963 by P Mandel's group (1) and studied now for over 40 years. Poly-ADP-ribose was postulated to serve either as reversible site-specific covalent post-translational modification or as a steric inhibitor/activator, thereby acting as a regulatory mechanism for protein substrates (reviewed in (2-4)). The attachment of negatively charged poly-ADP-ribose polymers to proteins is transient but can be very extensive *in vivo*, as polymer chains can reach more than 400 units on protein acceptors (reviewed in (2-4)). The enzyme responsible for the synthesis of poly-ADP-ribose was termed poly-ADP-ribose synthetase (PARS) or poly-ADP-ribose polymerase (PARP) (1, 5). In 1971, the poly-ADP-ribose glycohydrolase (PARG), the enzyme that cleaves the ribose-ribose bonds, was discovered by M. Miwa and T. Sugimura (6). Later on, several groups described in detail the branched structure of poly-ADP-ribose *in vitro* and *in vivo* (7-14). In 1987, the first gene encoding a poly-ADP-ribose polymerase (ADPRT, now PARP1) was identified and cloned by different labs (15-18).

PARP1 has been initially thought to be the only existing enzyme with poly-ADP-ribosylation activity in mammalian cells. However, five additional *Parp*-like genes encoding “*bona fide*” PARP enzymes were identified in recent years (19-23), indicating that PARP1 belongs to a family of “*bona fide*” PARP enzymes. The most abundant and funding member of the PARP family, PARP1 is responsible for the majority of poly-ADP-ribosylation activity. Interestingly, recent reports described more than 11 novel mammalian *Parp*-like genes (4, 24-26), which represent good candidates for a putative large family of intracellular PARP-like mono-ADP-ribose transferases. Poly-ADP-ribosylation reactions were reported to have a widespread occurrence in nucleated cells of mammals, birds, fishes, plant, fungi (including different yeasts) and protists but surprisingly, not in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* ((27-29) and reviewed in (4, 26)). For instance, orthologues of human *Parp*-like-genes were identified in the lower eukaryotes such as *Entamoeba histolytica*, *Paramecium tetraurelia*, *Trypanosoma cruzi*, *Tetrahymena thermophila* and *Dictyostelium discoideum* ((26), reviewed in (4) and own data base searches, unpublished). Recent studies provided evidence that poly-ADP-ribosylation-like reactions do even exist in eubacteria and archaeobacteria (30-32). Interestingly, orthologues of genes encoding PARP-like proteins exist in several eubacterial and archaeobacterial genomes (i.e. *Herpetosiphon aurantiacus*, *Microscilla marina*, and *Sulfolobus solfataricus*, own database searches, unpublished). Remarkably, only orthologues of the eukaryotic *Parp2* or *Parp3* genes but not any *Parp1*-like gene could be identified in bacteria (own data base searches, unpublished). Moreover, Otto *et al* recently identified *Parp*-like-genes in the genomes of the large double stranded DNA viruses *Invertebrate iridovirus 6* and *Aeromonas hydrophila* phage Aeh1 (26). More than 500 eubacterial and archaeobacterial genome sequence projects are currently under way. It will be interesting to see how

widespread “*bona fide*” poly-ADP-ribosylating enzymes indeed occur in the prokaryotic kingdoms.

The recent developments of new approaches, such as generation of different knockout mice, demonstrated that poly-ADP-ribosylation reactions play important roles in a wide range of physiological and pathophysiological processes, including maintenance of genomic stability, transcriptional regulation, centromere function and mitotic spindle formation, telomere dynamics and mitotic spindle formation during cell division, energy metabolism and cell death. However, a clear and unified picture of the physiological role of poly-ADP-ribosylation still remains to be established. In the present review we will briefly summarize the recent findings on the poly-ADP-ribosylation reactions occurring in mammalian cells and the PARP family members involved in these processes. Since the novel PARP-like ADP-ribosyltransferases appear to possess exclusively mono-ADP-ribosyltransferase activity rather than “*bona fide*” poly-ADP-ribose polymerase/transferase activities they will be not discussed here. The reader is referred to the accompanying review (Hassa *et al* Frontiers in Bioscience 2008). Moreover, studies performed exclusively with poly-ADP-ribosylation/PARP inhibitors will not be addressed in this review due to the off-target effects of poly-ADP-ribosylation inhibitors and non-specific inhibition of both poly-ADP-ribosylation and certain mono-ADP-ribosylation reactions (33-35). However, a special focus on the known off-target effects of poly-ADP-ribosylation inhibitors will be given in the last section.

## 3. POLY-ADP-RIBOSYLATION REACTION CYCLE

Poly-ADP-ribose is a heterogeneous linear or branched homopolymer of repeating ADP-ribose units linked by glycosidic ribose-ribose bonds (reviewed in (2-4, 25)). Poly-ADP-ribose synthesis requires NAD<sup>+</sup> as a precursor and immediate substrate of the reaction. Both constitutive and activated levels of poly-ADP-ribose have been suggested to be strictly dependent on the concentration of NAD<sup>+</sup> in cells (36-41). The constitutive levels of poly-ADP-ribose are usually very low in unstimulated cells (38-40, 42-45) and reviewed in (2-4)). However, in response to genotoxic stress or mitogenic stimuli, the PARP activity and the levels of poly-ADP-ribose can be increased 10–500-fold (reviewed in (46-49)). The total concentration of NAD<sup>+</sup> in undamaged proliferating mammalian cells varies between approx. 200–500 μM, and its half-life is approx. 1-2 h (50-53). When cells are exposed to high doses of genotoxic agents, sustained activation of poly-ADP-ribosylation reactions, coinciding with an increase in the levels of poly-ADP-ribose polymers, rapidly decrease the half-life of NAD<sup>+</sup> in a dose-dependent manner. The intracellular NAD<sup>+</sup> levels can undergo a decrease to 10-20% of their normal levels within 5–15 min upon exposure of cells to very high doses of DNA-damaging agents (54, 55). As NAD<sup>+</sup> is an essential coenzyme/transmitter for the generation of ATP, NAD<sup>+</sup> depletion will also result in ATP depletion. However, it is important to note, that several studies clearly indicate that under moderate levels of DNA damage, intracellular NAD<sup>+</sup>

levels undergo a decrease of only 5 to 10% (38-40, 42-45) and reviewed in (2-4)). Most free or protein-associated poly-ADP-ribose polymers, synthesized upon genotoxic stress, are rapidly degraded *in vivo* with a half-life of > 40s to 6 min, in a biphasic decay: 85% of poly-ADP-ribose polymers, synthesized upon genotoxic stress, have a half-life of less than 40s, while the residual fraction is catabolized with a half-life of approximately 6 min (38-41). The rapid turnover of poly-ADP-ribose polymers, synthesized upon genotoxic stress contrasts with the slow catabolism of the constitutive fraction of poly-ADP-ribose, exhibiting a much longer half-life of 7.7 h (41, 56). This rapid turnover strongly suggests that the degradation starts immediately upon initiation of poly-ADP-ribose synthesis. Thus, poly-ADP-ribose metabolizing enzymes are tightly regulated under physiological stress conditions.

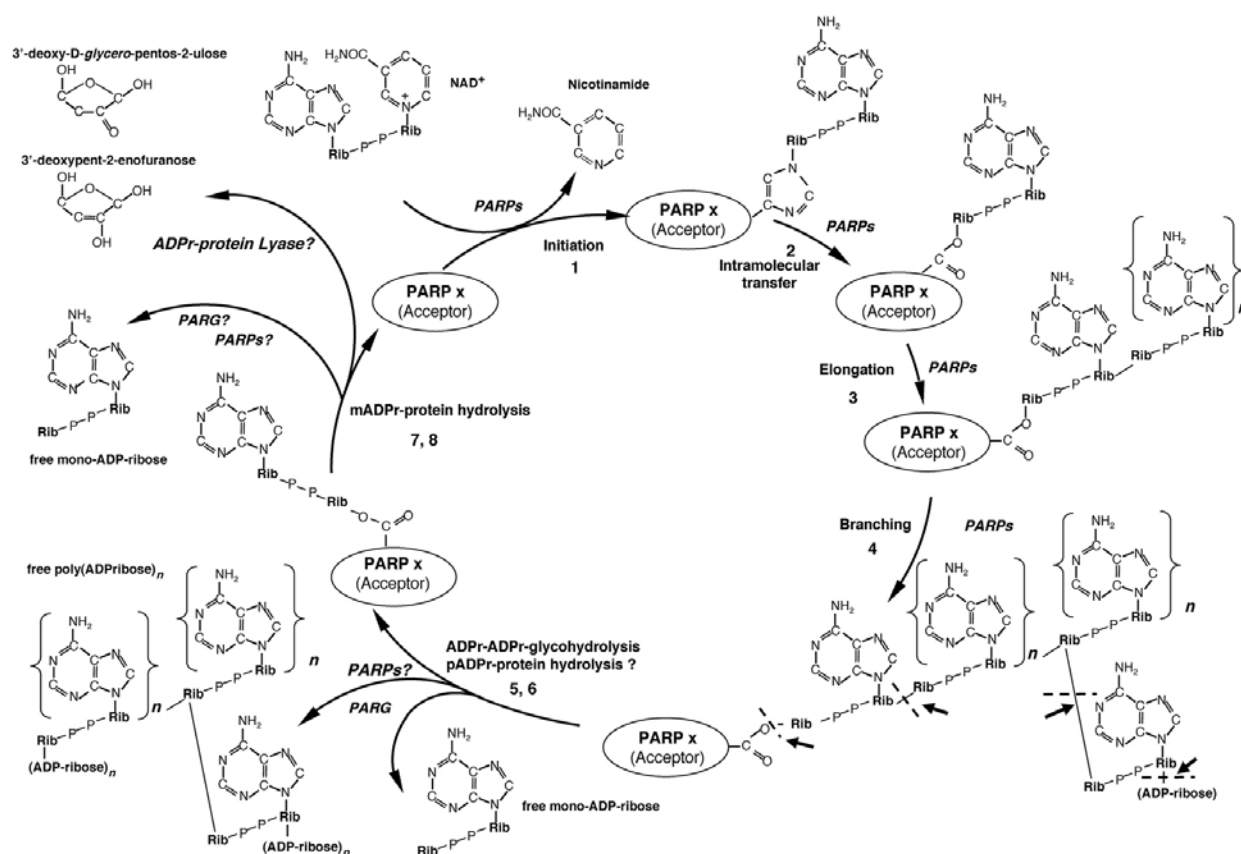
At least five distinct enzymatic activities were postulated to be required for the synthesis of free or PARP-associated linear and branched poly-ADP-ribose (2, 4, 57, 58): 1.) Initiation; covalent auto-mono-ADP-ribosylation of the catalytic domain on 2 histidine residues, which serve as initiator sites for poly-ADP-ribosylation (59). 2.) Trans-ADP-ribosylation; the instable histidine-ADP-ribose initiation adduct is immediately trans-ADP-ribosylated to the more stable glutamate carboxyl ester product (59). At least 15 glutamic acid residues reported to be present in the auto-modification domain of PARP1 have been proposed to be covalently auto-poly-ADP-ribosylated. Moreover, a total number of 28 auto-modification sites (glutamic acid and aspartic acid residues) in the DNA binding and auto-modification domains of PARP1 have been suggested to be covalently auto-mono-ADP-ribosylated (60, 61). However, no amino acid acceptor sites for poly-ADP-ribose have been identified so far. 3.) Elongation; polymer elongation involves the catalysis of a 2'-1" glycosidic bond, whereby the postulated covalently bound mono-ADP-ribose serves as a starting unit. 4.) Branching of the polymer; polymer branching occurs on average after 20 ADP-ribose units. 5.) Release of the enzyme-bound branched poly-ADP-ribose, either through poly-ADP-ribose-ribose-glycohydrolase activities (by poly-ADP-ribose glycohydrolase (PARG), see below) or a putative intrinsic poly-ADP-ribosyl-protein-hydrolase activity. Based on experimental evidence *in vitro*, it has been suggested that at least the classical PARP enzyme, PARP1 possesses auto-mono-ADP-ribosylation, elongation and branching activities. Whether poly-ADP-ribose polymerases might also possess intrinsic poly-ADP-ribose-ribose-glycohydrolase or poly-ADP-ribosyl-protein-hydrolase activity and immediately release the poly-ADP-ribose-ribose polymers remains to be investigated (4).

Two different enzymes or enzymatic activities, respectively, are known to degrade free non-protein-bound or protein-associated poly-ADP-ribose: The major enzymatic activity is the well-characterized poly-ADP-ribose glycohydrolase (PARG) activity (62, 63). The second activity, poly-ADP-ribose phosphodiesterase/ADP-ribose pyrophosphatase, was suggested to cleave the pyrophosphate linkages to release 5'-AMP from chain terminals, phosphoribosyl-AMP from internal residues and

diphosphoribosyl-AMP from branching points (64). Of these two enzymatic activities, the PARG activity is the only one, of which genes and their products are identified and characterized (62, 63, 65, 66). The major mammalian poly-ADP-ribose-ribose-glycohydrolase, PARG possesses both endoglycosidase and exoglycosidase activities (56, 62, 63, 67, 68), which are responsible for the hydrolysis of glycosidic ribose-ribose bonds within the polymer and between ADP-ribose units located at the extremity, respectively. The endoglycosidase activity releases free poly-ADP-ribose from PARPs and may provide a mechanism for the generation of various types of free poly-ADP-ribose, suggested to serve as signaling molecules involved in distinct cellular processes, such as cell-death or cell growth (4, 69). In addition, branched and short polymers are more slowly degraded by PARG(s) than long and linear poly-ADP-ribose polymers (56, 62, 63, 67, 68). This mode of action of PARG(s) may explain the very short half-life of poly-ADP-ribose synthesized in the presence of DNA damage *in vivo*. Experiments with different alkylation-damaged cultured cells demonstrated that the major fraction of poly-ADP-ribose has a half life as low as 1min while the residual fraction had a half life of 6-10 min, compared with the far longer half-life ( $\leq 7.7$ h) of constitutively synthesized poly-ADP-ribose in unstimulated cells (43-45) and reviewed in (2, 4)). Thus the biphasic degradation of poly-ADP-ribose *in vivo* clearly indicates that two major types of polymers (linear  $\leftrightarrow$  branched) with different structures and distinct half-lives may exist *in vivo* (9, 10, 56). The constitutively synthesized fraction of poly-ADP-ribose might play a role in housekeeping activities in the nucleus where as the majority of the rapidly synthesized and degraded poly-ADP-ribose must serve a more dynamic and transient function such as chromatin remodeling in DNA repair and gene expression, see also next sections (3, 4, 25, 64, 70-72). The complexity and concentration of each distinct type and structure of poly-ADP-ribose might not only vary depending on the cellular context and stimuli, but also on distinct branching activities of different PARPs *in vivo* (4). An overall view of a poly-ADP-ribosylation cycle is shown in Figure 1.

### 3.1. Structures of free and protein associated poly-ADP-ribose

The ADP-ribose units in the polymer are linked by glycosidic ribose-ribose alpha 1'-2' bonds. The chain length of poly-ADP-ribose polymers is heterogeneous. Based on gelfiltration, electrophoresis, high-performance reversed-phase liquid chromatography and electron microscopy analysis, the maximum size of poly-ADP-ribose polymers produced *in vitro* was estimated to reach up to 400 ADP-ribose units per linear polymer with an estimated average maximum size of about 350 (7-10, 41, 56, 73). Long polymers are branched in an irregular manner. Branching was calculated to occur *in vitro* with a frequency of approximately one branch per linear section of 20-50 units of ADP-ribose with an average of about 35 (7-10, 41, 56, 73-75). The average branching/elongation ratio is about 2% (76). The size of the branched polymer can vary from a few to approx. 200 ADP-ribose units. The chemical structure of the branching site of poly-ADP-ribose was determined by gas chromatography, high



**Figure 1.** Poly-ADP-ribosylation reaction cycle: Steps 1–4 and steps 5–8 of the poly-ADP-ribosylation reaction cycle represent the anabolic and catabolic reactions, respectively, in the metabolism of poly-ADP-ribose. The synthesis of poly-ADP-ribose requires three distinct PARP activities: (1) initiation or mono-ADP-ribosylation of (a) specific histidine residues (2) transfer to a glutamate (?) or aspartate residue(s) in the corresponding PARP enzyme (acceptor), (3) elongation of the polymer, and (4) branching of the polymer. The degradation requires at least 4 (alternative) PARG and (P/M)ARH activities: (5) exoglycosidase and endoglycosidase (PARG) activities, respectively, that hydrolyze the glycosidic linkages between the ADP-ribose units (6) potentially poly-ADP-ribosyl-protein hydrolase (PARH) activities and (7) mono-ADP-ribosyl-protein hydrolase (MARH) or (8) mono-ADP-ribosyl-protein lyase activities.

performance liquid chromatography, NMR and mass spectroscopy and found to be the same as those in the linear regions of the polymers (7, 8). These observations clearly indicate that the branching and elongation reactions are catalyzed by the same enzyme. The branching site of poly-ADP-ribose is *O*-D-ribofuranosyl-(1'-2')-*O*-D-ribofuranosyl-(1'-2')-adenosine-5',5',5-triphosphate (7, 8). The global structures of the branched poly-ADP-ribose are very complex. The structures of certain types of long poly-ADP-ribose chains could have helicoidal secondary structures and thus might have some similarity to the structures of RNA and DNA (77-79). Indeed, many antibodies raised against poly-ADP-ribose also recognize RNA and DNA, or *vice versa* (80-82).

Several reports suggested that the plethora of distinct types of poly-ADP-ribose structures observed *in vitro* may also exist *in vivo* (4, 9, 10, 56, 83). The chain length of poly-ADP-ribose polymers produced *in vivo* were reported to vary between approx 5-20 units of ADP-ribose under normal physiological conditions and 50–250 ADP-

ribose units under stress condition (7-10, 41, 56, 73, 84-87). The distance between two branched portions was estimated to vary between 20-60 units per branch *in vivo* (7-10, 41, 56, 73, 75, 83-87). The structures and size of poly-ADP-ribose could also be regulated by changes in poly-ADP-ribose glycohydrolase activity that occur in response to hyperthermia, metabolic changes or DNA damage in order to allow for higher levels of polymer to be maintained. For instance, a 10-fold decrease in glycohydrolase activity in intact cells subjected to hyperthermia resulted in a 5-fold increase in poly-ADP-ribose polymer levels *in vivo* (84-86). Poly-ADP-ribose polymers with an average maximum size of 67 ADP-ribose units, containing up to two points of branching per poly-ADP-ribose molecule have been observed in intact cells following treatment with the DNA alkylating agents, while cells pretreated with hyperthermia prior to DNA damage synthesized polymers with an average maximum size of 244 ADP-ribose units, containing up to six points of branching per poly-ADP-ribose molecule (56). The same study provided evidence that hyperthermia can inhibit the enzymatic activity of PARG

(56). However, it is clear that the exact size and structures of poly-ADP-ribose produced *in vivo* still remain elusive and have to be further investigated. Moreover, their exact half-life *in vivo* has to be carefully re-evaluated.

Although the functional relevance of this heterogeneity is not yet known, it could play a crucial role in determining specific functional outcomes (4). It was therefore suggested that distinct types of free poly-ADP-ribose are involved in stress-dependent signaling processes *in vivo* (3, 4, 88). The complexity and concentration of each distinct structural type of poly-ADP-ribose could vary depending on the cellular context, the stimuli and on the distinct enzymatic activities of PARG and different poly-ADP-ribose polymerases *in vivo* (3, 4). Given the complexity of poly-ADP-ribose structures and the existence of at least 6 distinct PARP enzymes, this heterogeneity of poly-ADP-ribose structures likely reflects the different signaling functions of distinct PARP family members and the specificity of poly-ADP-ribose signaling pathways. It's quite possible that a "poly-ADP-ribose code" might exist *in vivo* and dictate the outcome of distinct poly-ADP-ribose signaling pathways.

More than 30 years ago, several studies already postulated that poly-ADP-ribose might serve as a covalent post-translational modification of proteins (reviewed in (3, 4, 25, 69)). It was suggested that different poly-ADP-ribose polymerases covalently attach poly-ADP-ribose via the  $\gamma$ -carboxyl groups of glutamic acid or aspartic acid residues of putative acceptor proteins (reviewed in (4, 69)). Similar to the synthesis of free poly-ADP-ribose, ADP-ribose units might be added successively to acceptor proteins to form branched protein-bound polymers (reviewed in (3, 4, 25, 69)). This putative covalent post-translational modification was suggested to be extremely transient but very extensive *in vivo* and to reach more than 200 ADP-ribose units in length on protein acceptors (reviewed in (3, 4, 25, 69)). Up to now, more than 150 nuclear proteins, most of them chromatin associated, have been suggested to be covalently modified by poly-ADP-ribose *in vitro*. Target proteins RNA polymerases, transcription factors and high mobility group proteins (see Table 1 and reviewed in (4)). It is obvious that the association/attachment of large poly-ADP-ribose polymers on proteins will likely alter the physical and biochemical properties of the target proteins due to the high negative charge of the poly-ADP-ribose polymers. The high negative charge of poly-ADP-ribose has been initially suggested to prevent any interaction of poly-ADP-ribose-associated proteins with proteins or other anionic molecules such as DNA (57, 97). For instance, poly-ADP-ribosylation of PARP1 disrupts the interaction between PARP1 and topoisomerase I (98). Moreover, association/attachment of large poly-ADP-ribose polymers on proteins has been shown to inhibit the enzymatic activity of some DNA-dependent enzymes, including topoisomerase I or the DNA-dependent ATPase Cockayne syndrome group B protein (99). It has been also proposed that free or protein-associated poly-ADP-ribose may recruit or regulate the DNA binding and enzymatic activities of signaling proteins (i.e. in DNA repair pathways or for cell cycle progression) or could activate pro-apoptotic/necrotic

include classical PARPs, topoisomerase I and II, histones, p53 and the high-mobility-group (HMGs) proteins (reviewed in (2-4, 25, 69)). An updated list of nuclear proteins, suggested to be associated with poly-ADP-ribose *in vivo*, is given in Table 1. In intact organisms, PARP1 itself has been postulated to be the predominant acceptor of poly-ADP-ribose (89). However, the data of covalent poly-ADP-ribosylation of proteins have to be very cautiously interpreted. Despite intense studies in the last 40 years, no specific glutamate or aspartate residues functioning as poly-ADP-ribose acceptor sites could be identified *in vitro* using mass-spectrometry approaches and confirmed *in vitro* or *in vivo* by amino acid exchange analysis. On the other hand, several reports demonstrated that free poly-ADP-ribose could strongly and non-covalently bind to proteins in a salt-, acid- and detergent-resistant manner, including PARP1, PARP2 (90-95) and own observations). Remarkably, most of the postulated *in vitro* and *in vivo* poly-ADP-ribosylation substrates tested in these studies bound non-covalently to free highly charged poly-ADP-ribose *in vitro* (90-95). Covalent poly-ADP-ribosylation might occur on preformed mono-ADP-ribosylated substrates, mediated through mono-ADP-ribosylation of protein substrates by distinct mono-ADP-ribosyltransferases (4). Over 20 years ago, Tanigawa *et al.* showed *in vitro* that covalent mono-ADP-ribose adducts of histones, generated by an arginine-specific DNA-dependent mono-ADP-ribosyltransferase, can serve as initiator for poly-ADP-ribose synthesis (96). Thus, it is still not clear whether poly-ADP-ribose is covalently attached to the acceptor protein or simply associated in a non-covalent manner.

### 3.2. Functional consequences of poly-ADP-ribose-protein interactions

The physiological consequences of poly-ADP-ribosylation on the functional and physico-chemical properties of specific poly-ADP-ribose acceptor proteins are in most cases not known. Most proteins associated with poly-ADP-ribose are nuclear DNA-binding proteins, including PARPs, histones, topoisomerases, DNA and factors (i.e. nuclear translocation of AIF in cell death programs) (69, 88, 100-104). The functional consequences of poly-ADP-ribosylation on PARP1 itself are the most investigated. Poly-ADP-ribosylation of PARP1 through an intermolecular auto-modification reaction, abolishes its affinity for NAD<sup>+</sup> and DNA (105, 106). Auto-modification greatly altered both enzyme activities, decreasing both polymer synthesis and alternate NADase activity. Several studies provided evidence that the association/attachment of large poly-ADP-ribose polymers on transcription factors including TATA-binding protein (TBP) YY-1, p53, Sp1, and CREB affects their DNA-binding activities and thereby inhibits transcription *in vitro* (94, 103, 107-110). It has been suggested that association/attachment of large poly-ADP-ribose polymers may prevent the formation of active transcription complexes (103, 107, 108). A similar effect has been postulated for histones/nucleosomes. Poly-ADP-ribose polymers could function to alter chromatin conformation through non-covalent interactions with histone tails and displacement of histones from DNA (69, 88, 100, 101). A direct kinetic correlation between poly-

## The diverse biological roles of mammalian PARPs

**Table 1.** Postulated poly-ADP-ribose-associated proteins *in vivo*

Substrate	pADPr source / PARP	Functional relevance	Reference
PARPs: PARP1 and PARP1b, PARP2, PARP3,	Auto- and trans modification	Inhibition of DNA binding or enzymatic activity?	Reviewed in 2, 4, 338
PARP4, PARP5, PARP6a, PARP6b	Auto-modification only	NA	19, 23 and reviewed in 4, 338
Histones: H1.1 H1.2, H1.3, H1.4, H1.5 H2A, H2B, macroH2A1.1, macroH2A1.2 macroH2A2 H3, H3.1, H4	PARP1, PARP2, and /or PARP3	Inhibition of DNA binding activity?	Reviewed in 4, 350 351, 2
High mobility group HMG proteins: HMGA1a, HMGA1b, HMGA2, HMGB1, HMGB2, HMGN1, HMGN2	PARP1?	NA	Reviewed in 4 2, 351
Low mobility group protein LMG	PARP1?	NA	Reviewed in 2, 4, 351
Heterogeneous ribonucleoproteins hnRNPs: hnRNP A1, hnRNP A2/B1, hnRNP C1/C2, hnRNP G, hnRNP H, hnRNP K hnRNP M	PARP1 PARP2?	Inhibition of RNA/DNA binding?	121, 352, 353 and reviewed in 351
Centromer binding proteins: CENPA, CENPB Bub3	PARP1, PARP2, and /or PARP3?	NA	169, 170 and reviewed in 2, 4, 350
Lamins: Lamin A/C	NA	NA	354, 355 and reviewed in 2, 4
MARCKS proteins: MARCKS F52/Mac/MRP 50/GAP-43	PARP1, PARP2?	NA	95 and reviewed in 101
Numatrin/B23 Nucleolin/C23	PARP1, PARP2?	NA	Reviewed in 2, 4
A24 protein	PARP1	NA	356
DNA-PK	PARP1, PARP2?	Stimulation of enzymatic activity?	95, 357
Ku70/86	PARP1, PARP2?	Inhibition of DNA binding?	95
Topoisomerase I Topoisomerase II	PARP1	Inhibition of enzymatic activity?	95, 297
PCNA	PARP1?	Inhibition of DNA binding?	358
DNA polymerase $\alpha$ DNA polymerase $\beta$ , DNA polymerase $\delta$ , DNA polymerase $\epsilon$ ,	PARP1? PARP2?	Inhibition of enzymatic activity?	95 and reviewed in 2
DNA Ligases: I, II, III and IV	PARP1? PARP2?	Inhibition of DNA binding?	95 and reviewed in 2
XRCC1	PARP1? PARP2?	Inhibition of DNA binding?	95, 185, 359 136
Terminal transferase TdT	PARP1?	NA	2
Telomeric repeat binding factor-1 TRF-1	PARP5/tankyrase-1 PARP6/tankyrase-2 PARP2?	Inhibition of DNA binding?	19, 23
Telomeric repeat binding factor -2 TRF-2	PARP2?	Inhibition of DNA binding?	360
NuMA	PARP5/tankyrase-1 PARP6/tankyrase-2? PARP3?	NA	296
TAB 182	PARP5/tankyrase-1	NA	289, 290 300, 305
RNA polymerases I and II	PARP1?	Inhibition of enzymatic activity?	103, 104, 107-110
General transcription factors: TFIIF subunits RAP74 and RAP30 TBP	PARP1?	Inhibition of DNA binding?	103, 104, 107-110, 361
Transcription factors: p53 YY1 CREB SP1	PARP1?	Inhibition of DNA binding?	95, 103, 104, 107-110, 297
Transcriptional coregulators: CTCF		Inhibition of DNA binding?	227
Vault particle subunit MVP	vPARP/PARP4?	NA	22
Chromatin remodeling factors: FACT	PARP1?	Inhibition of nucleosome binding?	362

ADP-ribose incorporation, polynucleosome relaxation and histone H1 hyper-ADP-ribosylation was established *in vitro* ((111) and reviewed in (88, 100, 101). Thus, poly-ADP-ribose polymers may prevent binding of histones and transcription-associated factors to DNA through direct competition with DNA.

On the other hand, poly-ADP-ribose polymers can also promote protein-protein interactions by serving as biological glue (112-116). Several studies provided evidence that poly-ADP-ribose polymers generated by the chromatin-associated form of PARP1, can induce aggregation of modified oligonucleosomes as demonstrated by electrophoretic and sedimentation analysis (112-117). These studies demonstrated that the major products is a H1 dimer, formed by cross-linking two H1 molecules. It was suggested that these poly-ADP-ribose polymers may either directly participate in chromatin unfolding processes or coordinate them through recruitment of specific chromatin remodeling proteins and regulation of their activities, i.e. during cell cycle progression and cell survival/cell death pathways (3, 4, 100, 101, 112-116). Recent reports even speculated that a major detrimental molecular mechanism leading to cell death could be the promotion of aggregations by poly-ADP-ribose during necrosis (3, 4). A detailed list of poly-ADP-ribose associated proteins is presented in Table 1.

### 3.3. Poly-ADP-ribose-binding modules

Several reports suggested that free mono-ADP-ribose, poly-ADP-ribose and *O*-AADP-ribose are recognized by specific ADP-ribose binding motifs or modules (88, 91-95, 118-120). The Althaus lab recently provided evidence that poly-ADP-ribose can preferentially bind in a non-covalent manner to proteins that carry 20-30 amino acid long stretches containing a cluster rich in basic amino acids and a pattern of hydrophobic amino acids interspersed with basic residues (95). Pleschke J.M. *et al.* identified these poly-ADP-ribose-binding stretches in several important DNA damage checkpoint proteins including p53, XRCC1, Ku70/86 and DNA-PK (95). Remarkably, poly-ADP-ribose-binding stretches were found to partially overlap with or to be located within important functional domains responsible for DNA or RNA binding, protein-protein interactions and nuclear import and export sequences. Subsequent studies including proteomic approaches identified the most abundant poly-ADP-ribose-binding proteins as heterogeneous nuclear ribonucleoproteins (hnRNPs), a family of proteins involved in mRNA maturation and transport to the cytoplasm (121).

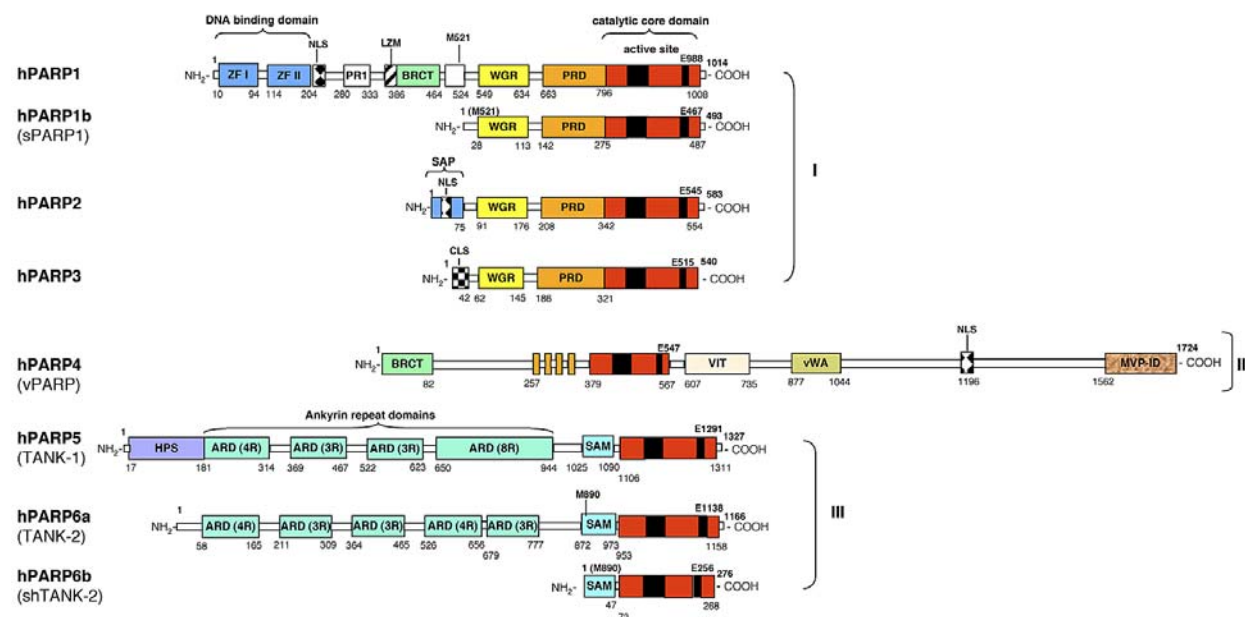
However, it is important to note that the major poly-ADP-ribose acceptor protein in the cell, PARP1 does not contain such a poly-ADP-ribose-binding motif. Indeed, no precise binding modules, functioning in a similar manner to the 14-3-3 isoforms, bromo- or chromo domains, have been identified so far for poly-ADP-ribose (4). The group of A. Ladurner recently provided biochemical and structural evidence that distinct human macro domains found in several proteins including PARP-like-mARTs, may serve as binding modules for free ADP-ribose *in vitro*

(120, 122). In addition, the macro domains from hepatitis E virus, Semliki Forest virus, and severe acute respiratory syndrome corona virus (SARS-CoV) can bind free and PARP1-bound poly-ADP-ribose *in vitro* (122-124). These studies suggest that, some macro domains may recognize free poly-ADP-ribose as a ligand (120, 122) and thus could serve as poly-ADP-ribose-binding modules. Macro domains could regulate poly-ADP-ribosylation-dependent processes, such as necrosis or SIRT-mediated gene silencing, i.e. through the depletion of poly-ADP-ribose (4, 118-120). However, there are no *in vivo* data available yet, thus it remains to be investigated whether macro domains could indeed serve as a binding module for poly-ADP-ribose under physiological conditions *in vivo* (see also over-next section).

## 4. STRUCTURES AND CLASSIFICATION OF THE PARP FAMILY MEMBERS

Poly-ADP-ribose polymerases are an ancient family of enzymes, as evidenced by the poly-ADP-ribosylating activities reported in dinoflagellates and archaeobacteria (reviewed in (4)) and the identification of *Parp*-like genes in eubacterial and archaeobacterial genomes (current bacterial genome sequencing projects). Thus, poly-ADP-ribose polymerases may have evolved directly from precursor poly?- or mono-ADP-ribosyltransferases in bacteria. Six genes encoding "bona fide" PARP enzymes have been identified in mammals: PARP1, PARP2, PARP3, PARP4/vPARP, PARP5/Tankyrases-1 and PARP6/Tankyrases-2. The best studied of these enzymes poly-ADP-ribose polymerase-1 (PARP1) plays a primary role in the process of poly-ADP-ribosylation. The PARP family members can be divided according to the sequences of their catalytic domain and type of their enzymatic activity into 3 subgroups. Figure 3 shows a slightly modified classification and schematic comparison of protein structures of the PARP family based on literature and database searches. Subgroup I includes PARP1, PARP1b (previously described as short PARP1 (sPARP1) (125), PARP2 and PARP3 (20, 21). Subgroup II is represented by a single enzyme, PARP4 (vault PARP/vPARP), the largest of the family (192.6 kDa), identified as a component of the vault complex (22). Subgroup III encompasses 2 enzymes, Tankyrase-1, tankyrase-2a and its alternatively spliced or transcribed short isoform tankyrase-2b, here referred to as PARP5 and PARP6a/b (19, 23)). Despite the limited primary sequence homology among different ADP-ribosyltransferases, the catalytic domains of prokaryotic and eukaryotic mono- and poly-ADP-ribosyltransferases are characterized by a common NAD binding fold with a conserved core consisting of a five-stranded antiparallel  $\beta$ -sheet and four-stranded mixed  $\beta$ -sheet (24, 26, 126, 127). The eukaryotic "bona fide" poly-ADP-ribosyltransferases and diphtamide-specific bacterial mono-ADP-ribosyltransferases are additionally characterized by a conserved triad motif containing histidine and the catalytic glutamate residues (HYE; reviewed in (4, 24, 26, 126, 127) and in the accompanying review (Hassa PO *et al* Frontiers in Bioscience 2008)). Moreover they share a sixth  $\beta$  strand

## The diverse biological roles of mammalian PARPS

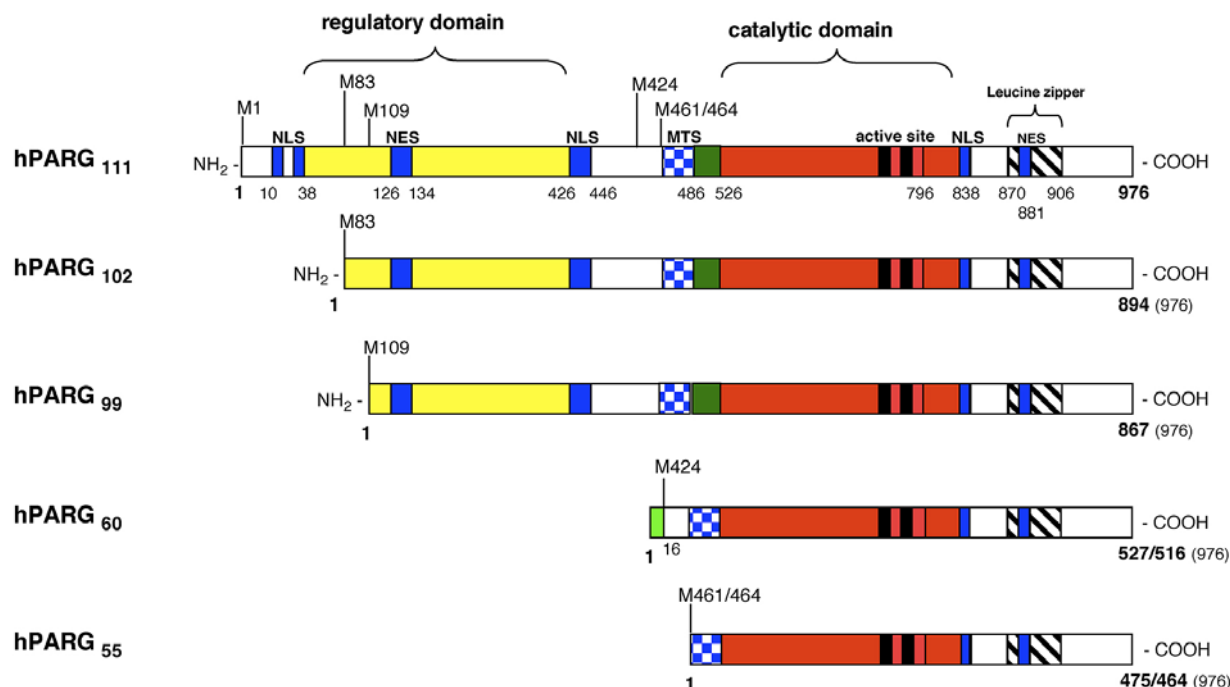


**Figure 2.** The classification and domain architecture of the human PARP family: The most significant domains detected have been indicated. Descriptions of domains: The PRD domain is called PARP regulatory domain and might be involved in regulation of the PARP-branching activity. The WGR domain is named after the most conserved central motif (W/G/R) of the domain. The WGR is found in a variety of polyA polymerases and other proteins of unknown function. The BRCT domain is named after the Breast Cancer Suppressor Protein-1 (BRCA1), carboxy-terminal domain and found within many DNA damage repair and cell cycle checkpoint proteins (180-184). The sterile alpha motif (SAM) is a widespread domain in signaling and nuclear proteins and mediates homo- or heterodimerization in many cases. The ankyrin repeat domains (ARD) mediate protein-protein interactions in very diverse families of proteins (288). The vault protein inter-alpha-trypsin (VIT) and von Willebrand type A (vWA) domains are conserved domains found in all inter-alpha-trypsin inhibitor (ITI) family members (276). Although the exact roles of the VIT and vWA domains remain unknown, they are presumed to be involved in mediating protein-protein interaction (276). ZF-I and ZF-II: PARP-1-type zinc finger domains (4). HTH: The two helix–turn–helix motifs at residues 200–220 and 280–285, respectively (hPARP1), were proposed to substantially contribute to its DNA binding activity. SAP: SAF/Acinus/PIAS-DNA-binding domain, LZM: putative leucine zipper-like motif, MVP-ID: Major-vault particle interaction domain, NLS: nuclear localization signal, CLS: centriole-localization signal, HPS: His-Pro-Ser region.

and an alpha helix between  $\beta$  strands 2 and 3. The catalytic glutamate seems to be essential for the ADP-ribosyltransferase activity but not necessarily required for the  $\text{NAD}^+$  glycohydrolysis activity (reviewed in (4, 24, 26, 126, 127) and in the accompanying review (Hassa PO *et al* Frontiers in Bioscience 2008)). The region referred to as the minimal catalytic domain, catalyzing the initiation, elongation and branching of poly-ADP-ribose polymers has been circumscribed to a 40kDa region (spanning residues 662–1014 in human PARP1). The crystal structure of the 40kDa core catalytic domain of the founding member and most catalytically active, PARP1 and subsequent amino acid replacement analysis demonstrated that the evolutionary conserved glutamate residue E988 in human PARP1 is essential for the poly-ADP-ribose chain elongation (128-132). The absence of this crucial residue in PARP1 has been shown to reduce its enzymatic activity to the level of mono-ADP-ribosylation (128-132). Structural and mutagenic studies of the  $\text{NAD}^+$ -binding core of different bacterial toxins and eukaryotic mono- and poly-ADP-ribosyltransferases identified several additional key structural features in the ADPRT catalytic domains that were suggested to be involved in NAD binding, substrate

specificity and recognition (24, 26, 133, 134). The active site, also commonly accepted as the “PARP signature”, is formed by a phylogenetically well-conserved sequence of approx. 50 amino acids (residues 859–908 of hPARP1). The ‘PARP signature’ contains the  $\text{NAD}^+$  acceptor sites and critical residues involved in the initiation, elongation and branching of poly-ADP-ribose polymers (corresponding to N868, F869, G871, L877, F897, F897, K903 and C908 in human PARP1). The catalytic core domains of PARP1, PARP2 and PARP3 are highly conserved. The “PARP signature” in PARP2 and PARP3 has been also suggested to contain most of the critical residues required for branching of ADP-ribose polymers. The blocks of sequence conservation correspond strictly to the secondary structure of the PARP1 catalytic domain, whereas, sequence variability occurs mainly in the loops (26). The catalytic domains of the other 3 remaining “*bona fide*” PARP family members, PARP4 to 6, are less conserved compared with PARP1. Remarkably, the loop between  $\beta$ -sheets 4 and 5 of the conserved PARP domain shows length differences (26). It has been suggested that this loop might be required to precisely position the catalytic glutamic acids thereby regulating the branching activity (26, 129-132). The loops





**Figure 3.** The domain architecture of the PARP isoforms: The domain architecture of the five human PARP isoforms, (modified according to (4, 160-162, 349)). hPARG60/mPARG63 contains alternative N-terminal protein sequences of 16 amino acids not found in the other isoforms. NLS: nuclear localization signals (aa10-16, aa32-38, aa421-446 and aa838-844); NES: nuclear export signals (aa126-134, aa421-446 and aa881-888) MTS: mitochondrial targeting sequence (aa461-486). Active sites: E728, E738, E756, E757 and T995.

in PARP1 to 3, which have been proposed to be associated with branching activity, are long where as the loops in PARP4 to 6, which do not synthesize branched structures are of intermediate length (26, 129-132, 135). Moreover, the “PARP signature” of PARP4 to 6 does not contain the critical residues involved in branching of poly-ADP-ribose polymers. For a more detailed description and discussion of the relationship between structure and enzymatic activity the reader is referred to the accompanying review (Hassa PO *et al.* Frontiers in Bioscience 2008).

#### 4.1. Regulation of PARP enzymatic activities

All “*bona fide*” PARP enzymes (PARP1, PARP1b, PARP2, PARP3, PARP4, PARP5 and PARP6) possess auto-modification activity, most-likely covalent auto-ADP-ribosylation activity ((19, 20, 22, 23, 125, 136-138) and reviewed in (4)). Interestingly, PARP1 showed *in vitro* the strongest auto-modification activity. Based on this unique property, PARP1 was identified as the main acceptor of poly-ADP-ribose *in vivo* (89). It was proposed that the covalently or non-covalently auto-modified form of the enzyme does not serve as an intermediate in the synthesis of poly-ADP-ribose, but plays some biological role(s) as a structural element (89). At least for PARP1 and PARP2, the auto-modified domains were mapped. Auto-modification takes place in the DNA-binding domain of PARP1 and PARP2 and in the so-called auto-modification domain of PARP1 (60, 136). Earlier studies have suggested that the auto-ADP-ribosylation activity targets 15 glutamic

acid residues in the auto-modification domain of PARP1 (139). Moreover PARP1 and PARP2 were proposed to be able to trans-ADP-ribosylate each other at multiple sites, although it is not clear whether the modification is covalent or non-covalent ((136) and own unpublished observations). Both PARP1 and PARP2 have been suggested to synthesize very complex branched poly-ADP-ribose at least *in vitro* ((20, 136) and reviewed in (2, 4)). The enzymatic activities of PARP3 and its isoforms are not yet investigated in detail. Thus, it remains to be investigated whether PARP3, have the same ability to catalyze all the reactions necessary to produce branched polymers (138). Future studies will certainly clarify whether the PARP family could be subdivided in three classes of enzymatic activities: branched polymer synthesizing PARPs (i.e. PARP1, PARP2 and PARP3?), linear polymer-synthesizing PARPs (PARP4?), and linear oligomer-synthesizing PARPs (PARP5 and PARP6; (137)). The type of branched polymers might be characteristic for each branched polymer-synthesizing PARP enzyme.

PARP1 is the major anabolic activity responsible for poly-ADP-ribosylation in living cells. Poly-ADP-ribose formation following DNA damage in primary mouse embryos and MEFs from *Parp1* knock-out mice were observed at levels between 2 to 50% of wild-type values, dependent on the tissue and cell type (reviewed in (4)). The enzymatic activity of PARP1 and PARP2 has been initially proposed to be exclusively dependent on the presence of

single (SSBs) and double strand breaks (DSBs) in DNA (20, 140-142). Surprisingly, studies have recently demonstrated that PARP1 can also be activated by distinct DNA stem loop/cruciform structures in a DNA damage-independent manner (106, 143-145). The PARP activities of the very closely related PARP1b (sPARP1) and PARP3 appears not to be stimulated by DNA-strand breaks (125, 138). In addition to the coenzymatic function of dsDNAs, small physiologic cellular components including polyamines,  $Mg^{2+}$ ,  $Ca^{2+}$ , and ATP can also serve as positive and negative cofactors for the catalytic activity of PARP1 (146-153). An allosteric activation of auto-poly-ADP-ribosylation of PARP1 by  $Mg^{2+}$ ,  $Ca^{2+}$ , and polyamines was demonstrated, with spermine as the most powerful activator. In contrast, physiologic concentrations of ATP can nearly completely inhibit the auto-poly-ADP-ribosylation activity of PARP1 *in vitro* (152). Remarkably the transfer of poly-ADP-ribose polymers to histones is only slightly affected by ATP, indicating that the auto-ADP-ribosylation but not trans-ADP-ribosylation activity of PARP1 is suppressed in bioenergetically intact cells (146, 147). The activity and substrate-specificity can also be regulated by poly-ADP-ribosylation stimulatory/modulatory factors (pARS/MFs), such as PARG, histones or by yet to be identified poly-ADP-ribosylation inhibitory factors (pARIFs). Recent reports provided evidence that PARG can directly interact *in vitro* and *in vivo* with PARP1 and could partially down-regulate PARP1's enzymatic activity (152, 154, 155). PARG interacts through its catalytic domain with the auto-modification domain of PARP-1 *in vitro* and *in vivo* (154). Since both PARP1 and PARG contain putative leucine zipper motifs (18, 156-162), the interaction may occur through the cryptic leucine zipper motifs. Whether the enzymatic activity of PARG is required for inhibition of PARP1 and whether PARG can also modulate the branching activity of PARP1 and PARP2 (or PARP3?) remains to be investigated. Remarkably, depletion of the nuclear full-length isoform of PARG, PARG-110 severely affects the auto-modification of PARP1 *in vivo* (163), most likely through a massive shift from branched to linear poly-ADP-ribose polymers. Furthermore, the Althaus and Kun labs previously demonstrated that histone H1 and core histones H2A, H2B and H3 could modulate the poly-ADP-ribosylation activity and shift the activity towards shorter polymers without affecting the overall poly-ADP-ribosylation activity (79, 149-151, 153, 164, 165). Histone H1 and H3 even appear to stimulate the enzymatic activity of PARP1 (79, 149-151, 153, 164, 165). Thus Histone H1 and the core histones H2A, H2B and H3 are potent regulators of both the numbers and sizes of ADP-ribose polymers through specifically regulating the polymer termination reaction of PARP1.

Two recent puzzling studies suggested that the histone variants macroH2A might function as poly-ADP-ribosylation inhibitory factors (166, 167). These authors claimed that all three macroH2A isoforms mH2A1.1, mH2A1.2 and mH2A2 could suppress the enzymatic activity of PARP1. MacroH2A mediated inhibition of PARP1 activity has been proposed to be required for X-inactivation and control of heat shock dependent gene

expression (166, 167). However, both reports have to be very cautiously interpreted. There is no *in vivo* evidence published, which would support such a model, in contrary; macroH2A1 knockout mice are viable, fertile and showed no defects in X inactivation, poly-ADP-ribosylation related increase in cell death or defects in heat shock dependent gene expression (168). Moreover, the Ladurner Lab previously demonstrated that all three macroH2A isoforms mH2A1.1, mH2A1.2 mH2A2 neither bind poly-ADP-ribose nor inhibit the poly-ADP-ribose activity *in vitro* (120). Together, there is pretty good evidence that PARPs are not regulated through the histone variant mH2A. Thus, “*bona fide*” ARIFs remain to be identified.

## 5. FUNCTIONAL CHARACTERIZATION OF THE PARP FAMILY MEMBERS

### 5.1. Subgroup I

Subgroup I encompasses three nuclear chromatin-associated enzymes PARP1, PARP1b, which seems to be a product of an alternative transcription initiation site within the *Parp1* gene (previously described as short PARP1 (sPARP1) (125), PARP2 and PARP3 (20, 21). Both PARP1 and PARP2 are playing a major role in distinct stress response pathways (4). Members of subgroup I are characterized by the WGR domain and their ability to synthesize large branched poly-ADP-ribose polymers. The 80-90 amino acid long WGR domain, found between the minimal catalytic domain and the auto-modification domain, is named after the most conserved central motif of tryptophane (W) - glycine (G) - arginine (R) residues and may be a nucleic acid binding domain (4). This region of PARP has not been extensively characterized and its function is still unknown. The WGR domain is found also in a variety of poly-A-polymerases as well as in molybdate metabolism regulators and other proteins of unknown function. In addition, PARP1, PARP2 and PARP3 contain an N-terminal DNA-binding domain. Experimental data suggest that all members of subgroup I can homo- and heterodimerize, and are localized to the nucleus (reviewed in (4)). PARP1 and PARP2 are evenly distributed in the nucleoplasm but were also shown to localize to the centromeres in a cell cycle-dependent manner. PARP1 is localized to the centrosomes and the chromosomes at the cell division phase and interphase (169-174). PARP2 was reported to accumulate at the centromeres during prometaphase and metaphase, to disassociate during anaphase, and to disappear from the centromeres by telophase (169, 170). A recent study provided preliminary evidence that PARP2 could be also localized perinuclear in unirradiated *Parp1*(+/+) MEFs cells but localized predominantly to the nucleus 6 h after irradiation at 0.5 Gy (175, 176). Surprisingly in unirradiated *Parp1*(-/-) cells, PARP2 distribution was nuclear, with no change after 0.5 Gy, indicating that PARP2 may shuttle between the cytoplasm and nucleus in a stimuli- and PARP1-dependent manner (175, 176). PARP3 is also localized to the centrosomes and resides preferentially in the daughter centriole, during the entire cell cycle (138).

#### 5.1.1. PARP1

The founding member and most abundant of the PARP family, mammalian poly-ADP-ribose polymerase 1 (PARP1) is a nuclear chromatin-associated multifunctional

enzyme. In addition to its catalytic domain, PARP1 contains a N-terminal DNA-binding domain (DBD), and an auto-modification domain (reviewed in (2, 4)). The N-terminal DBD extends from the initiator methionine to threonine-373 in human PARP1. The DBD in mammalian PARP1 contains two structurally and functionally unique zinc fingers (ZF1; aa 11-89, ZF2; aa115-199 in hPARP1) and two helix–turn–helix motifs (reviewed in (2, 4)). The DBD also contain a bipartite nuclear localization signal (NLS) of the form KRK-X(11)-KKKSKK (residues 207-226, in hPARP1 (177)). The PARP1 zinc fingers are thought to recognize altered structures in DNA rather than particular sequences but have also been reported to be involved in protein–protein interactions (reviewed in (2, 4)). The two helix–turn–helix motifs at residues 200–220 and 280–285, respectively (hPARP1), were proposed to substantially contribute to its DNA binding activity (reviewed in (2, 4)). The exact roles of each zinc finger and helix–turn–helix motif regarding their DNA binding specificity and stimulation of PARP1 catalytic activity are not yet clear and remain to be further investigated. PARP1 associates strongly with DNA SSBs and DSBs generated directly by DNA damage or indirectly by the enzymatic excision of damaged bases during DNA repair processes (reviewed in (2, 4)). Several studies indicate that the first zinc finger appears to be required for PARP1 activation by DNA single- and double-strand breaks, whereas the second zinc finger may exclusively act as a DNA single-strand break sensor (reviewed in (2, 4)). Recent studies have demonstrated that PARP1 also binds with high affinity to various physiological relevant structural discontinuities in DNA duplexes such as DNA stem/loop boundaries of cruciform hairpins, in the absence of DNA strand breaks (106, 143-145). Binding of PARP1 to this type of DNA is co-operative and induces oligomerization of PARP1. PARP1 has been suggested to represent a rare case of a protein capable of binding to the hairpin tips (106, 143-145). PARP1 binding to DNA hairpin and cruciform structures was functional and resulted in stimulation of PARP1 auto-modification and poly-ADP-ribosylation of histone H1 (106, 143-145). The physiological significance of this preference is still unclear. PARP1 has been proposed to interact with the gene regulatory sequences by binding to the promoter-localized cruciform DNA structures and thereby modulating chromatin structure and transcriptional regulation under normal physiological conditions (reviewed in (2, 4, 106, 143-145)).

The auto-modification domain of PARP1 is located in the central region of the enzyme, between residues 374 and 525 (hPARP1). This domain of PARP1 has not been extensively characterized. The auto-modification domain of PARP1 is basic and contains the majority of the 28 glutamic acid residues suggested to be involved in PARP1 auto-modification (reviewed in (2, 4)). Several recent studies identified a weak leucine-zipper motif in the N-terminal part of the auto-modification domain of PARP1 (18, 156-159, 178, 179). This leucine-zipper motif is preserved in *Drosophila*, *Xenopus*, chicken and mammalian PARPs (aa374 and 385, hPARP1) (18, 156-159, 178, 179). The presence of this motif in the auto-modification domain suggests that this motif might be

involved in homo- and/or heterodimerization with other nuclear leucine-zipper proteins ((18, 156-159, 178, 179) and reviewed in (2, 4)). The auto-modification domain of PARP1 also comprises a breast cancer 1 protein (BRCA1) C-terminus (BRCT) domain (from amino acids 386 to 464 (in hPARP1) (reviewed in (2, 4)). The BRCT domain is found within many DNA damage repair and cell cycle checkpoint proteins (180). There is a growing amount of evidence suggesting that BRCT domains constitute a module for recognizing phosphorylated peptides, and might mediate phosphorylation-dependent protein-protein interactions in processes that are central to cell-cycle checkpoint and DNA repair functions. The unique diversity of this domain super family allows BRCT modules to interact by forming homo- or hetero-BRCT multimers and phosphorylation-dependent BRCT-non-BRCT interactions (180-184). However, the PARP1 BRCT domain is a much less conserved variant of the original domain and its structure and proposed protein-protein interaction activity remains to be elucidated (reviewed in (2, 4)). Moreover, since PARP1 can bind to free poly-ADP-ribose through its auto-modification domain one cannot exclude the possibility that the BRCT domain may actually serve as a poly-ADP-ribose-binding module (4). This hypothesis is supported by the observation that the interaction between the PARP1-BRCT domain and the BRCT domains of XRCC1-and DNA topoisomerase II-beta binding protein 1 (TopBP1) is mediated through poly-ADP-ribose (136, 185, 186).

### 5.1.1.1. Functions

*Parp1* gene-disruption studies suggested that PARP1 plays diverse roles in many molecular and cellular processes including DNA damage signaling pathways, chromatin modification, transcription and cell death pathways (187, 188). These processes are critical for many physiological and pathophysiological outcomes, including maintenance of genomic integrity, regulation of cell proliferation and differentiation, neuronal function, aging, inflammation, and carcinogenesis (4). Mice lacking the gene encoding PARP1 are healthy and fertile (189-193). However, *Parp1* knockout mice exhibit hypersensitivity to ionizing radiation and more sensitive to the lethal effects of alkylating agents (189-193). Following whole body  $\gamma$ -irradiation mutant mice died rapidly from acute radiation toxicity to the small intestine. PARP1 could act as a survival factor for intestinal stem cells *in vivo* (194). Primary fibroblasts and splenocytes derived from *Parp1* knockout mice exhibited an elevated frequency of recombination, gene amplification, sister chromatid exchanges, and micronuclei formation after treatment with genotoxic agents, implicating an important role for PARP1 in the maintenance of genomic integrity (189-193). Moreover, various primary *Parp1* knockout cells show a proliferation deficiency in response to environmental stress when compared with wild type cells ((189-193) and reviewed in (187, 188)). However, the genomic instability and hypersensitivity of *Parp1* knockout mice in response to  $\gamma$ -irradiation and alkylating agents is not directly due to a defect in DNA repair itself. Cells lacking *Parp1* have a normal capacity to efficiently repair DNA damaged by UV,  $\gamma$ -irradiation and alkylating agents (189, 190). Thus, the

**Table 2.** pADPr/DNA non-bridged interaction partners of PARP1

Interaction partner	References
PARPs: PARP1, PARP2, PARP3	4, 198, 363
Histones: H1, H2A, H2B, H3, H3.1, H4	Reviewed in 2, 25
High mobility group HMG proteins: HMGA1a, HMGA1b, HMGA2,	198 reviewed in 2
Centromer binding proteins: CENPA, CENPB, Bub3	169, 170 and reviewed in 2
Numatrin/B23, Nucleolin/C23	364
DNA-dependent protein kinase complex subunits: DNA-PK DNA-dependent protein kinase Ku70/86 TRBP Thyroid hormone receptor-binding protein PC3/Topoisomerase I, Topoisomerase II TopBP1 DNA topoisomerase IIbeta binding protein I	186, 202-204, 211, 212, 357, 365, 366 and reviewed in 2, 25
Coactivators/signal integration platforms: p300/CBP β-Catenin	196, 198, 211, 212
Mediator subunits: MED14 and CDK8	198
SMRT-Corepressor complex subunits: HDAC1-3	198
General transcription factors: TFIIF subunit RAP74 PC1	198
RNA polymerases II Large subunit CTD	367
Transcription factors: RelA/p65, NFκB1/p50, Oct-1, RAR, RXRα, ERα, ERβ, GR, B-MYB p53, YY1, SP1, E2F1, AP-2α, AP-2β, AP-2γ TCF-4, TTF-1, C/EBPα, TEF-1, E47	4, 196-199, 205, 211, 212, 368-371
Kinases: ERK1/2, extracellular signal-regulated kinase 1 and 2 JNK1 c-Jun-N-terminal kinase 1 AMPK AMP-activated protein kinase Ste20-like kinase hKFC CDK2 and CDK8	198, 372-374
Enzymes of NAD/ADPr metabolism: NMNAT1 Nicotinamide mononucleotide adenylyltransferase-1 PARG	154, 155, 375

exact role of PARP1 in these processes remains to be elucidated.

On the other hand, the same *Parp1* knockout mice studies have identified various detrimental functions of PARP1 in inflammatory and neurodegenerative disorders (reviewed in (4, 187, 188)). *Parp1* gene-disruption provided protection from tissue injury in various oxidative stress-related disease models ranging from stroke, (MPTP)-induced parkinsonism, myocardial infarction, streptozotocin-induced diabetes, lipopolysaccharide-induced septic shock, arthritis, to colitis and zymosan-induced multiple organ failure (reviewed in (4, 187, 188)).

The molecular mechanisms underlying these observations could be explained by three major molecular functions of PARP1: I) it's widely accepted that PARP1 functions as an architectural nucleosome binding factor/chromatin chaperone and as chromatin modifying enzyme. PARP1 possesses the ability to non-covalently poly-ADP-ribosylate histones and thereby regulating higher-order chromatin structures. II) PARP1 can actively

participate in transcription as a component of enhancer/promoter regulatory complexes as well as in genome surveillance as a component of signalosome complexes. III) Oxidative stress-induced over-activation of PARP1 leads to NAD<sup>+</sup> /ATP depletion and/or poly-ADP-ribosylation dependent translocation of apoptosis inducing factor (AIF) to the nucleus, culminating in cell dysfunction or programmed cell death.

## 5.1.1.1. Architectural corepressor/coactivator activity

Recent reports have shown that PARP1 acts as a crucial promoter-specific coactivator of nuclear factor kappaB (NF-κB), which plays a central role in the expression of inflammatory cytokines, chemokines, adhesion molecules, and inflammatory mediators (4, 25, 195-200). Moreover, PARP1 has been shown to associate with and regulate the function of several transcription factors (reviewed in (4, 25, 201)). However, of special interest is the regulation of NF-κB-mediated transcription by PARP1 because NF-κB and PARP1 have both been demonstrated to play a pathophysiological role in a number of inflammatory disorders (4, 25, 195-200). PARP1 can physically interact with the Mediator complex, p300/CBP and with both subunits of NF-κB (p65 and p50), and synergistically coactivates NF-κB-dependent transcription (196-198). PARP1 also interacts *in vivo* with histone deacetylases (HDACs) 1-3 but not with HDACs 4-6 (198). These findings suggest a model in which PARP1 might function as a promoter-specific corepressor/coactivator exchange factor (4, 25, 201). PARP1 could promote the binding and release of components of the transcriptional machinery dependent on or independent of its enzymatic activity (reviewed in (4, 25, 201)). Although there is no clear evidence for direct and covalent PARP1-mediated trans-poly-ADP-ribosylation of transcription factors *in vivo* (4) it is still possible that free poly-ADP-ribose locally generated by PARP1 in a promoter-specific manner could indirectly associate with other transcription factors. PARP1 has numerous other protein partners in the cell and functions in a variety of other cellular processes as well. A detailed list of n/pADP-ribose /DNA non-bridged PARP1 interaction partners is shown in Table 2.

In agreement with this model, Ju *et al* recently provided evidence that PARP1 forms a coactivator/corepressor exchange complex together with nucleolin, nucleophosmin, and Hsp70 as well as topoisomerase II (TopoII β), Ku86/70 and DNA-dependent protein kinase (DNA-PK), which are normally associated with DNA damage and repair (202-204). Ku86/70 is an abundant heterodimeric nuclear protein complex, consisting of 70- and 86-kDa tightly associated subunits that comprise the DNA binding component of DNA-PK and functions in the non-homologous end joining (NHEJ) repair pathway. These authors suggested that PARP1 is a component of the TLE1 corepressor complex mediating derepression and subsequent coactivator recruitment events required for transcriptional activation of a neurogenic program during neural stem cell differentiation *in vitro* (202-204). Ju *et al* went even a step further and proposed a model where signal-dependent activation of gene transcription by nuclear

receptors and other classes of DNA binding transcription factors, including activating protein 1, requires TopoII  $\beta$ -dependent, nucleosome-specific, transient double-stranded DNA break formation with subsequent activation PARP1 enzymatic function (202-204). The subsequent poly-ADP-ribosylation activity has been suggested to be required for a nucleosome-specific histone H1-high-mobility group (HMG) B1 exchange event and for local changes of chromatin architecture (202-204). A slightly different model was recently proposed by D. Reinberg and coworkers where PARP1 could determine specificity, independent on its enzymatic activity, through regulation of the retinoid acid-induced switch of Mediator from the inactive (Cdk8+) to the active (Cdk8-) state in RAR-dependent transcription, *ex vivo* (205). However, it is important to note that the models proposed by the Rosenfeld and Reinberg labs are not supported by the corresponding phenotypes of *Parp1* and *TopoII*  $\beta$  knockout mice (206, 207). For instance, all three different *Parp1* knockout mice do not show any TLE1/HES1 or RAR/ER related developmental abnormalities or major functional deficiencies that might be expected if PARP1 would serve as an essential co-regulator under normal physiological conditions (189-193).

Several recent reports suggest that the coactivator/corepressor exchange function of PARP1 can be modulated by post-translational modifications. Phosphorylation of PARP1 by CaMKinase II $\delta$  and acetylation of PARP1 by p300/CBP lead to HES1- and NF- $\kappa$ B-mediated gene activation, respectively (198, 204). Acetylation of PARP1 by p300/CBP plays an important regulatory role in NF- $\kappa$ B-dependent gene activation by enhancing its physical interaction with p50 and functional interaction with p300/CBP and the Mediator complex in response to inflammatory stimuli (198). CaMKinase II $\delta$  mediated phosphorylation of PARP1 activates PARP enzymatic activity, which is suggested to be required for the removal of corepressor complexes from the MASH1 promoter (204).

The identification of PARP1 as a regulated promoter-specific exchange factor and component of Ku70/86-DNAPK/Rad50/ topoisomerase I and II containing complexes is likely to be prototypic of similar molecular mechanisms in genomic stability maintenance processes. Preliminary studies with cultured cells derived from *Parp1* knockout mice showed misregulation of genes involved in apoptosis, cell cycle progression, metabolism and signal transduction (208, 209). In particular, loss of PARP1 results in down-regulation of the expression of several genes involved in regulation of cell cycle control, and genomic stability maintenance processes *ex vivo* (208, 209). Moreover, increased expression of genes associated with cancer initiation or progression was induced by PARP1 deficiency. Remarkably, recent studies found that the corepressor-silencing mediator for retinoid and thyroid hormone receptor (SMRT) can associate with a DNA-PK/Ku70/Ku86/PARP1 complex (210-213). The SMRT/HDAC-3 complex was required for the transcriptional repressive property of the Ku70 subunit and for cellular recovery from DNA damage induced by

ionizing radiation or DNA damage-inducing drugs (210-213). Unliganded thyroid hormone receptor (TR) can function as a transcriptional repressor of specific cellular target genes by acting in concert with a corepressor complex harboring histone deacetylase (HDAC) activity (210-213). Thus, it has been suggested that DNA-PK promotes the establishment of transcriptionally repressive chromatin at TR target promoters by enhancing the HDAC activity of the TR-bound SMRT/DNA-PK/Ku70/86/PARP1 corepressor complex in response to DNA DSBs (210-213).

The proposed function of DNAPK-Ku70/86-PARP1 coactivator/corepressor complexes in genomic stability processes is supported by genetic approaches. Mice lacking *Ku80* are viable but growth retarded and show deficiency in V(D)J recombination (214, 215). However, homozygous disruption of both the *Parp1* and *Ku80* genes causes embryonic lethality (216, 217). In addition, *Parp1*(-/-)/*Ku80*(-/-) cultured blastocysts had an increased level of chromosomal instability and apoptosis (216, 217).

### 5.1.1.2. Modulation of the chromatin structure

In addition to its function as a coactivator/corepressor exchange factor, PARP1 has been also described to act as a structural component of chromatin and as a modulator of chromatin structure through its poly-ADP-ribosylation activity. Over 20 years ago, several studies provided evidence that poly-ADP-ribosylated chromatin adopts a more relaxed structure than its native counterpart (100, 152, 218-224). When isolated polynucleosomes of interphase chromatin were poly-ADP-ribosylated *in vitro* by a highly purified preparation of PARP1 at low and moderate ionic strengths, the solenoid structure (30-nm fiber) decondensed into the 10-nm fiber and adopted the fully extended 'beads on a string' structure characteristic for H1-depleted chromatin (100, 218, 221, 222, 224). As expected, poly-ADP-ribosylation of polynucleosomes rendered chromatin more susceptible to micrococcal nuclease digestion (152, 225). Significantly, the chromatin relaxation, induced by poly-ADP-ribosylation, was fully reversible following degradation of poly-ADP-ribose by exogenous PARG (152, 219, 220). The dual action of PARP1 and PARG in chromatin was suggested to result in reversible relaxation of chromatin *in vivo*. It remains to be further investigated whether the PARP-associated poly-ADP-ribose (auto-modified PARPs) or the histone-associated poly-ADP-ribose is responsible for the relaxation of chromatin. It has already been shown over 10 years ago by the group of F. Althaus, that even short oligomers of poly-ADP-ribose (not longer than 40 ADP-ribose units) associated with PARP1 apparently suffice to induce relaxation of the chromatin structure without any further non-covalent modification of histones (224). These studies suggest that the association/modification of nucleosomes is not required for PARP1-dependent regulation of chromatin structure, at least under the tested conditions (152, 218, 224). Based on these observations *in vitro*, various speculative models were proposed regarding the molecular mechanism and functional role of poly-ADP-ribosylation of chromatin associated PARP1 and chromatin *in vivo*.

The observed affinity of histones for free poly-ADP-ribose, led to the proposal of a “histone shuttle” mechanism for chromatin relaxation and recondensation, occurring in response to DNA damage and involving PARP1 and PARG (100) (88, 222)). According to this hypothesis, poly-ADP-ribose synthesized by PARP1, after activation by DNA strand breaks at the site of damage, could locally dissociate histones/nucleosomes from DNA, thereby granting access to damaged DNA for distinct repair machineries (88, 100, 136, 222). The free large and branched poly-ADP-ribose polymers would form an interaction matrix and serve as a scaffold onto which histones could be sequestered in order to render DNA locally accessible to the repair machinery, thus facilitating repair of damaged DNA (88, 100, 222)). Subsequent degradation of free and protein-associated poly-ADP-ribose by PARG would then allow histone-DNA complexes to reform, resulting in the refolding of the chromatin structure (88, 100, 222).

Kim *et al.* recently proposed a new model based on published data previously presented by the Althaus group and their own observation that auto-modification of PARP1 alone is sufficient to open the chromatin structure *in vitro* (100, 152, 222)). Their model suggests that PARP1, when incorporated into chromatin structures and enzymatically inactive due to the local high levels of ATP, promotes the formation of compact, transcriptionally repressed and nuclease-resistant chromatin structures. Inhibition of enzymatic activity of PARP1 is observed at higher concentrations of ATP (> 6 mM) (146, 152). They proposed that PARP1 is acutely sensitive to small changes in ATP concentration *in vivo*. Thus, reduction of local ATP concentrations by numerous transcription-related factors consuming ATP during transcription, such as chromatin remodeling factors, could increase the enzymatic activity of PARP1, which in turn results in the auto-modification and subsequent release of PARP1 from chromatin, facilitating chromatin relaxation and transcription by RNA Pol II, without any further non-covalent modifications of nucleosomes and dissociation of nucleosomes from DNA (152). The authors suggested that poly-ADP-ribosylation, catalyzed by PARP1, acts as a general chromatin structure remodeling mechanism, allowing access to specific areas of the genome especially in transcriptional processes (152).

In sharp contrast to the other models an opposite function for poly-ADP-ribosylation has been proposed by Yu *et al.* suggesting that the chromatin insulator protein CTCF might be constitutively associated with poly-ADP-ribose (226, 227), which in turn may lead to the establishment of higher order chromatin structures *in vivo*. In their model, constitutive poly-ADP-ribosylation of CTCF stabilizes the long-range interaction between *H19* ICR locus and DMR1 (differentially methylated region 1) locus on the maternal chromosome, leading to insulation at the *Igf2* gene, which is essential for the manifestation of the imprinted state of the *Igf2* gene *in vivo* (226, 227). The poly-ADP-ribose was suggested to serve as “chemical glue” between CTCF and chromatin or chromatin-associated interaction partners to render these interactions constitutive. They further suggested that poly-ADP-

ribosylation of CTCF is generally tightly linked to the various functions of CTCF and essential for developmental processes and tumor suppression (226, 227).

These models provide potential molecular mechanisms of how poly-ADP-ribosylation, and poly-ADP-ribose in particular, might influence the chromatin structure. However, the proposed fundamental role for poly-ADP-ribosylation of chromatin by PARP1 in the global organization of chromatin in mammals under normal physiological conditions is so far not supported by genetic analysis of PARP1, which represents the major poly-ADP-ribosylation activity in the cell. All three different *Parp1* knockout mice show no developmental abnormalities or major functional deficiencies that could be expected if PARP1 would function as an essential chromatin regulator under normal physiological conditions (189-193). Thus, one cannot exclude the possibility that the observations presented by Kim *et al.* (152) might merely reflect the physiological situation during early stages of apoptosis or “programmed necrosis” (228, 229). A recent study provided evidence that increased poly-ADP-ribosylation of chromatin by PARP1 in cell death processes could lead to internucleosomal DNA fragmentation (228). These processes could be prevented when cells were treated with non-specific PARP inhibitors (228). Thus, poly-ADP-ribosylation of chromatin in the early stage of apoptosis could facilitate internucleosomal DNA fragmentation by increasing the susceptibility of chromatin to cellular endonucleases. Indeed, the poly-ADP-ribosylation-induced relaxation of the chromatin structure, observed *in vitro*, might explain the internucleosomal DNA fragmentation that occurs during apoptosis (4, 156). Moreover, the suggested model in which poly-ADP-ribosylation of CTCF by PARP1 should be generally tightly linked with the functions of CTCF and be essential for developmental processes (226-228), is not at all supported by the different phenotypes observed in studies, using single knockout mice of the *Parp1*, *Parp2*, *Parp4* or *Parp6/tnks2* genes, respectively and the lethal double knockout mice of the *Parp1* and *Parp2* genes (189-193, 230-233). Neither developmental defects that are tightly linked to the functions of CTCF nor any CTCF-related imprinting disorders, such as the Beckwith-Wiedemann syndrome (234) could be observed in these different *Parp* knockout mice models (which have to be undoubtedly expected, according to the proposed model).

Taken together, based on the published data, PARP1 mediated poly-ADP-ribosylation of chromatin, seems to be required for the modulation of chromatin structures under stress conditions, but most likely not under normal physiological conditions. Alternatively, poly-ADP-ribosylation of chromatin might be mediated by PARP1 in concert with other PARPs *in vivo*, also under normal physiological conditions. Future genetic studies will certainly clarify this point.

### 5.1.1.1.3. PARP1/poly-ADP-ribosylation-mediated cell death

Over 20 years ago, N. Berger suggested first that Oxidative stress-induced over-activation of PARP1 leads to

$\text{NAD}^+$  and consequently ATP depletion, culminating in cell dysfunction or necrosis (235, 236). According to the „PARP-suicide“ model proposed by N. Berger, lethal levels of DNA damage lead to over-activation of PARP(s) and a rapid decline of cellular  $\text{NAD}^+$ , which in turn affects the activities of the enzymes involved in glycolysis, the pentose phosphate shunt and the Krebs cycle. In an attempt to restore the  $\text{NAD}^+$  pools,  $\text{NAD}^+$  is resynthesized with a consumption of 2-4 molecules of ATP per molecule of  $\text{NAD}^+$  (depending on which salvage pathway it is used in the cell (4)), and as a consequence cellular ATP levels become depleted, leading to subsequent cellular energy failure, which results in cellular dysfunction and finally in necrotic cell death (235, 236). This hypothesis could be confirmed in numerous studies on a cellular level by using novel PARP inhibitors or cells from *Parp1* knockout mice (reviewed in (4, 33, 187, 188)). Pharmacological inhibition of the enzymatic activity of PARPs or the complete absence of PARP1 significantly improved cellular energetic status and cell viability after exposure to necrosis-inducing agents. This cellular suicide mechanism has been implicated in the pathomechanisms of neurodegenerative disorders, cardiovascular dysfunction and various other forms of inflammation. Interestingly, the contribution of poly-ADP-ribosylation reactions to necrotic cell death seems to be dependent on the cell type and cellular metabolic status. PARP1 mediated poly-ADP-ribosylation plays an important role in necrotic cell death of various endothelial and epithelial cells, as well as in several types of neuronal cells, whereas necrotic cell death caused by oxidative damage in other cell types, such as hepatocytes, seems not to be dependent on poly-ADP-ribosylation reactions (reviewed in (4, 33, 229, 237-239)). Actively proliferating cells use almost exclusively glucose through aerobic glycolysis for the production of ATP and die from  $\text{NAD}^+$  and ATP depletion, as a consequence of poly-ADP-ribosylation over-activation. In contrast, non-proliferating cells can catabolize a mixture of metabolic substrates, including amino acids and lipids, and maintain ATP levels through oxidative phosphorylation in the mitochondria and are resistant or less sensitive to ATP depletion and cell death under the same conditions (229, 237).

Recently, a second model was described suggesting that over-activation of PARP1 induces translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus, causing DNA condensation and fragmentation, and subsequent cell death. Genetic studies, using *Parp1* knockout mice, provided preliminary evidence that energy depletion alone might not be sufficient to mediate poly-ADP-ribosylation-dependent cell death (240). Several groups provided evidence that PARP1-mediated hyper-poly-ADP-ribosylation upon very high insult of genotoxic stress initiates a nuclear signal that spreads to the cytoplasm and triggers the release of apoptosis-inducing factor (AIF) from the mitochondria and its translocation to the nucleus, where AIF induces apoptosis-like cell death. Pharmacological inhibition of the enzymatic activity of PARPs or the complete absence of PARP1 in different cells derived from *Parp1* knockout mice blocked the release of AIF and its translocation to the nucleus (241-244). Mitochondria play important roles in

cell death through the release of pro-apoptotic factors such as cytochrome c and AIF, which activate caspase-dependent and caspase-independent cell death, respectively (reviewed in (238)). The apoptosis-inducing factor (AIF) is a phylogenetically conserved 57kDa flavoenzyme, residing in the mitochondrial intermembrane space, which possesses both death promoting and protective functions (245-249). AIF exhibits both reactive oxygen species (ROS)-generating NADPH oxidase and monodehydroascorbate reductase activity (246-249). Under normal physiological conditions, the presence of AIF is restricted to mitochondria in almost all tissues and several cancer cell lines (reviewed in (250-253)). Recent data strongly indicate, that the redox-active enzymatic region of AIF is associated with anti-apoptotic functions, while its DNA binding region possesses pro-apoptotic/necrotic activities ((247-249, 254) and reviewed in (255, 256)). Induction of cell death by high doses of genotoxic agents or NMDA causes opening of the mitochondrial transition pore and release of AIF from mitochondria to the cytoplasm, where it combines with cyclophilin A to form an active DNase, which in turn translocates to the nucleus and contributes to nuclear DNA fragmentation into 50-kbp fragments and chromatinolysis ((242-244, 254). AIF was shown to participate in both caspase-dependent and -independent cell death processes ((242-244, 254). Translocation of AIF has been shown to occur quickly after over-activation of poly-ADP-ribosylation reactions and precedes cytochrome c release and caspase activation (241-244).

The molecular mechanisms underlying the PARP1-mediated shuttling of AIF are currently under debate. The published data suggest two mechanisms:  $\text{NAD}^+$  and ATP depletion, or poly-ADP-ribosylation products serving as a death signal. Several studies support a model in which  $\text{NAD}^+$  depletion, as well as ROS-induced mitochondrial dysfunction, may lead to mitochondrial permeability transition (MPT) and trigger AIF-induced cell death. These studies demonstrated that, despite an exclusive localization of PARP1 and poly-ADP-ribose in the nucleus, ATP levels first decreased in mitochondria and then in the cytoplasm of cells undergoing hyper-poly-ADP-ribosylation (257, 258). Interestingly, treatment of cells with PARP inhibitors, sub-micromolar concentrations of cyclosporin A, an inhibitor of MPT and injection of  $\text{NAD}^+$ , rescued ATP levels, blocked translocation of AIF from mitochondria to nuclei and subsequent cell death in cells undergoing hyper-poly-ADP-ribosylation (257, 258). These observations strongly suggest that  $\text{NAD}^+$  depletion and MPT are necessary intermediary steps linking poly-ADP-ribosylation to AIF translocation and cell death (257, 258). Furthermore, a very recent report provided preliminary evidence that the ATPase domain of HSP-70 is critical for sequestering AIF in the cytosol under conditions of ATP depletion (259). HSP-70 antagonizes AIF-mediated cell death by both inhibiting mitochondrial AIF release and retaining leaked AIF in the cytosol (260). The interaction between HSP-70 and AIF might be regulated through the ATP/ $\text{NAD}^+$  levels, and thus implying that HSP-70 may act as an ATP sensor under these conditions.

**Table 3.** pADPr/DNA non-bridged interaction partners of PARP2

Interaction partner	References
PARPs: PARP1, PARP2	20, 136
Histones: H1, H2A, H2B,	136
Numatrin/B23, Nucleolin/C23	136
TRF1	360
Centromer binding proteins: CENPA, CENPB, Bub3 ?	136, 138
Transcription factors: TTF-1	370

The second proposed mechanism is supported by the observation that cytoplasmic accumulation of free or protein-associated poly-ADP-ribose in *D. melanogaster* PARG loss-of-function mutants resulted in severe neurodegeneration (261). The presence of free or protein-associated poly-ADP-ribose in the cytoplasm might therefore be essential for proper cell death signaling. Furthermore, two reports provided preliminary evidence that free poly-ADP-ribose itself might translocate to the cytoplasm under cytotoxic conditions (243, 262). Although there is so far no clear evidence supporting such a hypothesis, certain poly-ADP-ribose-associated chromatin proteins, like histones or high-mobility group box proteins might also be used as AIF-releasing signals during apoptosis and programmed necrosis, respectively (4, 263). It would be interesting to investigate whether pre- or post-treatment of cells with leptomycin B (LMB), an inhibitor of CRM-dependent nuclear protein export, could inhibit the cell death process under pro-necrotic/apoptotic conditions. Remarkably, the Susin lab recently demonstrated that AIF-mediated programmed necrosis requires the sequential activation of PARP1 enzymatic activity, calpains and Bax but not p53, cathepsins or caspases (264). Calpains are calcium-dependent proteases involved in programmed cell death pathways (reviewed in (265-268)). Calpain I was previously shown to induce cleavage and release of AIF from isolated mitochondria (269). Calpain I cleaved both the precursor and mature forms of recombinant AIF near the amino terminus *in vitro* (269). Thus calpains might be good physiological targets for poly-ADP-ribose. It is quite possible that both proposed mechanisms, NAD<sup>+</sup> depletion and distinct/specific poly-ADP-ribosylation products, could even simultaneously serve as a trigger, dependent on the stimuli, metabolic status and cell type. This might occur in parallel or sequentially in waves (4). For more detailed description of the functional roles of PARP1 in cell death and cell survival processes the readers are referred to the accompanying review (Hassa PO. *Frontiers in Bioscience* 2008).

## 5.1.2. PARP2

PARP2 bears the strongest resemblance to PARP1 (60% identity in the catalytic domain) (20, 21, 135). The N-terminal part of PARP2 has no significant homology with PARP1. These structural differences between PARP1 and PARP2 strongly indicate different substrate specificities for both proteins (20, 21). The N-terminus region of human and mouse PARP2 shows high sequence variability (20). PARP2 contains a N-terminal SAP/SAF motif/module (named after scaffold-associated

protein/scaffold-associated factor SAF-A/B, Acinus and PIAS; (270)), a previously undetected eukaryotic module proposed to be involved in sequence- or structure-specific DNA and RNA binding and often associated with different domains involved in the assembly of pre-mRNA processing complexes (270). The nuclear scaffold attachment factors A and B (SAF-A/B) bind through their SAP motifs/modules to AT-rich chromosomal regions known as scaffold- or matrix-attachment regions (SAR/MAR), which are associated with transcriptionally active chromatin (270). It has been suggested that SAP motifs/modules might participate in the targeting of a variety of proteins to transcriptionally active chromatin, probably by binding to SAR regions (270). Recent studies demonstrated that PARP2 co-localize with PARP1, transcription factors (i.e., C/EBP-beta) and enzymes (DNA Topo II, DNA PK) at scaffold- or matrix-attachment regions of chromatin (271). PARP2 can homodimerize and heterotetramerize with PARP1 (136). PARP1/PARP2 heterotetramers can also localizes to mammalian active centromeres and interact with the constitutive centromere proteins Cenpa, Cenpb and Bub3 (169, 170). A detailed list of n/pADP-ribose/DNA non-bridged PARP2 interaction partners is shown in Table 3.

Similar to PARP1, PARP2 was suggested to synthesize poly-ADP-ribose polymers in a DNA-dependent manner (20). However the exact substrate for PARP2 remains to be elucidated. PARP2 displays also auto-modification properties similar to PARP1. In addition, PARP2 may account for the residual poly-ADP-ribose synthesis observed in *Parp1* knockout cells. Unlike PARP1, the physiological functions of PARP2 are not yet understood. PARP2 is mainly expressed in actively dividing tissues during mouse development, similar to PARP1, but to a much smaller extent (136, 272). Recent studies indicate that PARP1 and PARP2 could possess both overlapping and non-redundant functions. Mice carrying a targeted disruption of the *Parp2* gene are sensitive to sensitive to both ionizing radiation and alkylating agents (136). Remarkably, *Parp1*(-/-)*Parp2*(-/-) double knockout mice are not viable and die at the onset of gastrulation, suggesting that PARP1 and PARP2 are both essential during early embryogenesis (231). A recent report provided evidence that in the absence of PARP2, the survival of DP thymocytes undergoing TCR-alpha recombination is affected despite normal amounts of Bcl-xL (273). Thus, PARP2 has been suggested to be an important mediator of T-cell survival during thymopoiesis by preventing the activation of DNA damage-dependent apoptotic response during the multiple rounds of TCR-alpha rearrangements preceding a positively selected TCR (273). Moreover, PARP2 appears to have a dual function in cell death pathways: PARP2 could act as an executioner of cell death pathways in focal cerebral ischemia, but might also function as a survival factor after global ischemia (274). However, it is clear that all these preliminary observations have to be confirmed by an additional independent *Parp2* KO model. Based on the gene disruption strategies used, one cannot exclude the possibility that the observed effects are due to a dominant negative activity of a potentially expressed N-terminal DNA binding fragment of PARP2 in this *Parp2* KO mice model.



**Table 4.** pADPr/DNA non-bridged interaction partners of PARP3

Interaction partner	References
PARPs: PARP1, PARP3	138
Centromer binding proteins: CENPA, CENPB, Bub3	138
DNA-PK/Topo chromatin remodeling complexes: DNA-PK Ku70/86	275

## 5.1.3. PARP3

PARP3 is the least studied and smallest PARP so far. The protein domain structure of PARP3 is very similar to PARP2, featuring a small DNA-binding domain consisting of only 54 amino acids and apparently contains a targeting motif that is sufficient to localize the enzyme in the centrosome (21, 138)). In contrast to PARP1 and PARP2, which are ubiquitously expressed, PARP3 expression is regulated in a tissue-specific manner (138, 272). The highest expression of PARP3 was detected in the skeletal muscle, high to moderate levels were found in the lung, liver, kidney, ovary, spleen and heart, while thymus, small intestine and colon contained lower levels of the PARP3 transcripts (272). Remarkably, PARP3 seems not to be expressed in the whole brain and testis (272). PARP3 was initially identified as a core component of the centrosome (138). PARP3 is preferentially localized to the daughter centriole throughout the cell cycle (138). However subsequent studies revealed that endogenous PARP3 shows a widespread nuclear distribution, appearing in numerous small foci and a small number of larger foci (275). Since PARP3 can weakly/transiently associate with the EZH2 and Suz12 subunits of the polycomb group protein complexes PRC2 and PRC3, the larger nuclear foci have been suggested to be polycomb group bodies (275). PARP3-mediated poly-ADP-ribosylation was proposed to regulate PRC2 and PRC3 dependent gene silencing in a postiv or negative manner (275). PARP3 isoforms have been also identified as a subunit of complexes comprising DNA-PK, PARP1, Ku70, and Ku80 (275). A detailed list of n/pADP-ribose/DNA non-bridged PARP3 interaction partners is shown in Table 4.

However, there is only one preliminary report regarding the putative physiological functions of PARP3. Overexpression of PARP3 or its N-terminal domain in HeLa cells was shown to interfere with the G1/S cell cycle transition but did not influence centrosomal duplication or amplification (138). PARP3 has been shown to catalyse the synthesis of poly-ADP-ribose *in vitro* and in purified centrosome preparations. In addition, PARP3 can form stable complexes with PARP1 (138). The presence of both PARP1 and PARP3 at the centrosome was proposed to link the DNA damage surveillance network to the mitotic fidelity checkpoint. Future genetic studies will certainly clarify whether PARP3 is involved in these processes.

## 5.2. Subgroup II

Subgroup II is composed of a single, family member, PARP4 (vault-PARP), the largest of the family (193 kDa). In addition to its catalytic PARP domain PARP4 also contains a BRCT domain at the NH2 terminus,

a region similar to the inter- $\alpha$ -trypsin inhibitor protein (IHRP) and a Major Vault Protein (MVP) interacting domain at the COOH terminus (22, 230). The vault protein inter-alpha-trypsin (VIT) and von Willebrand type A (vWA) domains are conserved domains found in all inter-alpha-trypsin inhibitor (ITI) family members (276). Although the exact roles of these domains remain unknown, they are presumed to be involved in mediating protein-protein interactions (276). The catalytic domain of PARP4/vault-PARP has been shown to catalyze nADP-ribosylation reactions (22). PARP4/vault-PARP and PARP5/tankyrase-1 are the only members of the "*bona fide*" PARP family that have been localized to the cytoplasm. PARP4/vault-PARP is present in cytoplasmic ribonucleoprotein particles (vaults) and cytoplasmic clusters (vPARP-rods) as well as in the nuclear matrix (22, 230)). PARP4/vault-PARP was originally identified as a minor protein component of the vault particle, a large cytoplasmic 13-megadalton barrel-like ribonucleoprotein (RNP) assembly of unknown function, which is associated with an untranslated vault RNA sequences (vRNAs) and two other highly conserved proteins, major vault protein (MVP) and telomerase-associated protein-1 (TEP1). Vaults are found in most eukaryotic cells and have been proposed to have a role in drug resistance, nucleo-cytoplasmic transport, and regulation of signaling (22, 230, 277-283), however a clear biologic function for vaults has yet to be assigned. In addition to the association of PARP4/vault-PARP with the cytoplasmic vault particle, subpopulations of PARP4/vault-PARP localize to the nucleus, being associated with the mitotic spindle (22). Although PARP4/vault-PARP is capable of catalyzing the formation of poly-ADP-ribose polymers there is so far no evidence for branching activities. The PARP4/vault-PARP is most likely a linear-polymer-synthesizing PARP.

## 5.2.1. PARP4/vPARP

Recent studies demonstrated that PARP4/vPARP is dispensable for normal development. *Parp4/vParp*(-/-) mice were viable and fertile for up to five generations (230). Although PARP4/vPARP can interact with telomerase-associated protein 1 (TEP1) and was associated with telomerase activity in human cells, PARP4/vPARP is also dispensable for telomerase catalysis and telomere length maintenance (230). In addition, the activity of PARP4/vPARP is not essential for interaction with MVP-self-assembled vault-like particles and vault structure *in vivo* (230). Purified vaults contain poly-ADP-ribosylation activity, indicating that the assembled particle retains enzymatic activity. Vaults have been linked to multidrug resistance (MDR) of human tumors and are thought to be involved in tumor development and/or progression (277, 284)). High levels of MVP were found in tissues chronically exposed to xenobiotics and correlating with the degree of malignancy in certain cancer types. However up-regulation of MVP alone is not sufficient to confer the drug-resistant phenotype (277, 284, 285). Remarkably, the colon tumor incidence and multiplicity is higher and colon tumor latency is significantly shorter in *Parp4/vParp*(-/-) mice compared with wild-type mice (286). The increased carcinogen-induced tumorigenesis in *Parp4/vParp*(-/-)

mice is the only phenotype observed to date, and indicates that vPARP, may directly or indirectly, be involved in chemically induced colon tumorigenesis. Surprisingly, MVP was recently identified as host defense protein that contributes to resistance against *P. aeruginosa* lung infection. MVP is highly expressed in lung and intestinal epithelia, dendritic cells, and macrophages, cells that encounter xenobiotics (22, 277-283). *Mvp* knockout mice showed an increase in susceptibility to *P. aeruginosa* lung infection (287). This study also demonstrated that MVP is rapidly recruited to lipid rafts when human lung epithelial cells are infected with *Pseudomonas aeruginosa*, dependent on bacterial binding to the cystic fibrosis transmembrane conductance regulator (CFTR) (287). Since *Mvp* knockout mice have normal dendritic cell function and their general phagocytic cell response is unlikely to be altered, these authors suggested that MVP could stabilize or internalize the components of bacterial-induced lipid rafts by linking raft proteins to cytoskeletal elements such as tubulin or actin (287). MVP has been proposed to serve as a scaffold protein in signaling and intracellular transport pathways (22, 277-283). It will be of importance to investigate whether PARP4/vPARP is also involved in these processes and whether its enzymatic activity is required.

### 5.3. Subgroup III

Subgroup III encompasses 2 enzymes, tankyrase-1 (TANK1), tankyrase-2a (TANK2) and maybe the alternatively spliced or transcribed short isoform tankyrase-2b, here referred to as PARP5 and PARP6a/b, (23) (4, 19). Both PARP5 and PARP6a were identified as components of a telomeric complex (19, 23) and functions both as oligo-ADP-ribosyltransferases. As the name tankyrase indicates, PARP5 and its homologue PARP6a possess a central domain containing 24 ankyrin-type 33-amino-acid-long repeats (19, 23). The ankyrin repeat domains (ARD) mediate protein-protein interactions in very diverse families of proteins (288). The number of ankyrin repeats in a protein can range from 2 to over 20 (288). The ankyrin domain of PARP5 is flanked at the N-terminus by a region carrying homopolymeric His, Pro, and Ser residues (HPS) and at the COOH-terminus by the sterile alpha motif (SAM), which is a widespread domain in signaling and nuclear proteins and mediates homo- or heterodimerization in many cases ((19, 289-292) and reviewed in (4)). Both PARP5 and PARP6a have been shown to form homo and hetero(oligo)mers through their sterile-alpha motif domains (289-292)). PARP5 and PARP6a are suggested to function as scaffolding proteins, capable of regulating assembly of large protein complexes (291, 292). The formation of homo and hetero(oligo)mers is reversible and still allows interaction with ankyrin-domain binding proteins *in vitro*. Tankyrase oligomers are dissociated efficiently by auto-oligo-ADP-ribosylation (291, 292). PARP6a contains a unique 25-amino acid NTD that replaces the larger HPS-rich domain in PARP5 (23). In contrast to PARP5, PARP6a does not contain an NLS (293). PARP5/tankyrase-1 was initially described as a telomeric protein and localized to multiple subcellular sites, including the telomeres and mitotic centrosomes, The PARP activity was proposed to regulate telomere function (19). Subsequent data revealed that PARP5/tankyrase-1 is associated, at least in part, at

multiple subcellular sites in a cell cycle-dependent manner; at mitosis, PARP5/tankyrase-1 was found to relocate around the pericentriolar matrix of mitotic centrosomes (294-297), at the cytoplasmic side of the nuclear envelope during interphase, in a cluster near the nucleus, and specifically on the cytoplasmic fibers of nuclear pore complexes with the Golgi complex (298, 299). PARP5/tankyrase-1 has also been shown to be associated, at least in part, with the Golgi complex (299), peripherally associated with Golgi membranes, to GLUT4 (Glucose transporter) storage vesicles in the Golgi complex in adipocytes (293, 299). PARP6a localizes at several subcellular sites as the Golgi complex, endosomes, nucleus, nuclear membrane and pores, pericentriolar matrix, and telomeres (23). Studies suggested that cells contain approximately twice as much tankyrase-1 as tankyrase-2 (300).

#### 5.3.1. PARP5/tankyrase-1

Several knockdown studies identified human PARP5/tankyrase-1 as the PARP to be required for mitosis in human cells (294-296, 301). Treatment of immortalized human cells with siRNA against *Parp5/tnks1*, resulted in mitotic arrest with aberrant chromosome configurations and abnormal spindle structures (294, 301, 302). Sister chromatids were unable to separate at their telomeres (294-296, 301). Complementation of siRNA-treated cells with a siRNA-resistant cDNA of *Parp5/tnks1* wild-type, but not an enzymatically dead mutant could rescue this phenotype (301), strongly suggesting that the PARP5/tankyrase-1-catalyzed oligo-ADP-ribosylation products are required for sister telomere resolution and mitotic progression (301). An exciting study provided evidence that poly-ADP-ribose is a non-proteinaceous, non-chromosomal component of the spindle required for bipolar spindle assembly and chromosome segregation (294-296). Accurate chromosome segregation is performed by a dynamic and complex microtubule-based superstructure known as the spindle and is absolutely essential for the viability of future cell generations. The organization of microtubules into a symmetric bipolar spindle is driven by microtubule-associated proteins, which are regulated by post-translational modifications, mainly phosphorylation, in a cell cycle-dependent manner (reviewed in (303, 304)). Chang *et al.* provided evidence that both poly-ADP-ribose and PARG localize to spindles in cells, most likely preferentially during mitosis (294-296). A certain type of poly-ADP-ribose structure might be critical for establishing and maintaining spindle bipolarity *in vivo*, perhaps through stabilizing anti-parallel microtubule interactions in the central spindle (294-296). The generation of the knockout mice of *Parp5/tnks1* and *Parp3* genes will certainly clarify whether observed effects are mediated by PARP5/tankyrase-1 alone or through the local and cell type-specific combined actions of PARP1, PARP2, PARP3 or PARP5/tankyrase-1. Several PARPs were shown to localize to the centromeres in a cell cycle-dependent manner. PARP1 seems to localize to the centrosomes and the chromosomes at cell-division phase and interphase (171-173). PARP2 was reported to accumulate at centromeres during prometaphase and metaphase, disassociate during anaphase, and disappear from the centromeres by telophase (169, 170).

**Table 5.** pADPr/DNA non-bridged interaction partners of PARPs

Interaction partner	References
PARPs: PARP5, PARP6a, PARP6b	19, 291, 292
Telomeric repeat binding factor TRF1	19
TAB 182 182-kDa tankyrase-binding protein	289, 290
IRAP insulin-responsive aminopeptidase,	289, 290
NuMA nuclear/mitotic apparatus protein	296
FBP17 formin-binding protein 17	376
Mcl-1 myeloid cell leukemia-1	377
Grb14 SH 2 domain-containing adaptor protein	378
TIN2	379

In addition to its role in mitosis PARP5/tankyrase-1 has been suggested to be required for the regulation of telomere length together with PARP6/tankyrase-2 by oligo-ADP-ribosylation of the telomeric repeat binding factor TRF-1 but not TRF-2 *in vitro* and *in vivo* (293, 297). Human TRF-1 binds to the ankyrin domain of tankyrase-1; each of the five ankyrin repeats of PARP5/tankyrase-1 can independently bind to human TRF1 ((305, 306) and reviewed in (300)). Whether TRF-1 can be directly oligo-ADP-ribosylated in a covalent manner by PARP5/tankyrase-1 and tankyrase-2 or just specifically, but non-covalently bind *in vitro* and *in vivo* to their free oligo-ADP-ribose products remains to be carefully investigated. Telomeres, the tandem-repeated hexamers at the termini of eukaryotic chromosomes, form protective complexes in association with specific proteins, such as the telomere-binding proteins Rap1, TRF-1 and TRF-2, POT-1, tankyrase-1 and tankyrase-2, thought to regulate telomere length together with telomerase ((305, 306) and reviewed in (300, 304, 307, 308)). In humans telomere length maintenance is partly controlled by a feedback mechanism, in which telomere elongation by telomerase is limited by the accumulation of the negative regulator TRF-1 complex at chromosome ends. TRF-2 serves as a protective factor at telomeres to prevent end-to-end chromosome fusions (reviewed in (307, 308)). Telomeres were suggested to be essential for genome stability in all eukaryotes. Telomere shortening correlates with cellular senescence *ex vivo*; short telomeres were shown to activate replicative senescence (reviewed in (307, 308)). Moreover, changes in telomere length and telomerase activity have been proposed to be important factors in human aging and in the pathobiology of human disease. On the other hand, many tumor cells can prevent telomere loss by aberrantly upregulating telomerase expression levels and activity (reviewed in (307, 308)). Poly-ADP-ribosylation of TRF-1 by human PARP5/tankyrase-1 and PARP6/tankyrase-2 diminished the ability of TRF-1 to bind to telomeric DNA *in vitro* (reviewed in (303, 307, 308)). Over-expression of wild type but not enzymatic mutant forms of PARP5/tankyrase-1 and PARP6/tankyrase-2 in the nucleus of human cells removed the endogenous TRF-1 complex from telomeres and promoted telomere elongation *in vivo*. SiRNA-mediated knockdown of endogenous PARP5/tankyrase-1 resulted in telomere shortening in human cells (309). A PARP5/tankyrase-1 resistant form of TRF-1 stimulated normal telomere length control, indicating that human PARP5/tankyrase-1 promoting telomere elongation *in vivo* through the removal of TRF-1 complexes from telomeres (309). Poly-ADP-ribosylation by both PARP5/tankyrase-1

and PARP6/tankyrase-2 appears to regulate access of telomerase to the telomeric complex *in vivo* ((19, 309) and reviewed in (303, 304, 307, 308)).

Remarkably the PARP5/tankyrase-1-binding motif in TRF-1 is not conserved in mice and rats. Mouse TRF-1 has no canonical PARP5/tankyrase-1-binding motif (RXXPDG) and does not bind to mouse PARP5/tankyrase-1 (309, 310). Overexpression of tankyrase1 in mouse nuclei did not diminish the ability of TRF-1 to bind to telomeric DNA (309). These preliminary observations are coincident with the much higher telomerase activity and much longer telomeres in mice and rat somatic tissues than those in humans (310). Future genetic studies with knockout mice for PARP5/tankyrase-1 will certainly clarify whether PARP5/tankyrase-1 may indeed not regulate telomere length in mice. A detailed list of n/pADP-ribose/DNA non-bridged PARP5/tankyrase-1 interaction partners is shown in Table 5.

### 5.3.2. PARP6/tankyrase-2

The observation that overexpression of PARP6/tankyrase-2 in the nucleus of human cells can lead to a release of endogenous TRF1 from telomeres suggested that also PARP6/tankyrase-2 can function as a positive regulator of telomere length in human cells, dependent on its catalytic PARP activity (293). Telomere extension by telomerase in human cells seem to be positively regulated by poly-ADP-ribosylation, mediated through PARP5/tankyrase-1 and PARP6/tankyrase-2, respectively (293, 300). However recent genetic studies could not confirm a role for PARP6/tankyrase-2 in telomere length maintenance or telomere capping in mice (232, 233). *Parp6/tnks2* knockout mice are viable and fertile but display a growth retardation phenotype. However no change in telomere length or telomere capping was observed in these mice. This observation is consistent with the lack of any canonical tankyrase-binding motif of murine TRF-1 that is required for binding to tankyrases (309, 310)). Moreover, *Parp1*, *Parp2*, *Parp4* and *Parp6/tnks2* deficiency does not alter telomere length nor telomerase activity in primary murine cells (232, 233). Since all of the different single knock-out mice generated so far (*Parp1*(-/-), *Parp2*(-/-), *Parp4*(-/-) and *Parp6/tnks2*(-/-)) did not show any abnormal telomerase activity and ageing-related phenotypes (189-193, 230-233), extensive genetic studies using combinations of double knock-out and knock-in mice (especially regarding *Parp5/tnks1*(-/-) and *Parp6/tnks2*(-/-)) will be required to give us a better insight into the potential roles of poly-ADP-ribosylation and tankyrases in telomere-related and non-related processes associated with aging and cancer *in vivo*. Mouse PARP5/tankyrase-1 and PARP6/tankyrase-2 may either have redundant functions in telomere length maintenance or completely differ from human PARP5/tankyrase-1 and PARP6/tankyrase-2 in its role in telomere length maintenance.

The growth retardation phenotype of the *Parp6/tnks2* deficient mice suggests a role for PARP6/tankyrase-2 in normal growth, development and/or metabolism (232, 233). Initial observations provided

**Table 6.** pADPr/DNA non-bridged interaction partners of PARP6

Interaction partner	References
PARPs: PARP5, PARP6a, PARP6b	23, 291, 292
Telomeric repeat binding factors TRF1	23
TAB 182 182-kDa tankyrase-binding protein	289, 290
IRAP insulin-responsive aminopeptidase,	289, 290
NuMA nuclear/mitotic apparatus protein	296
FBP17 formin-binding protein 17	376

evidence that body fat is reduced in *Parp6/tnks2* knockout mice, and thus may contribute at least in part to the observed decrease in total body weight (232, 233). Interestingly, recent studies demonstrated that the majority of PARP6/tankyrase-2 is located in the cytosol and as a peripheral membrane protein associated with the Golgi (299). PARP6/tankyrase-2 colocalizes with GLUT4 storage vesicles in the juxta-nuclear region of adipocytes. PARP6/tankyrase-2 binds specifically to a resident protein of GLUT4 vesicles, IRAP (insulin-responsive amino peptidase) and to the Src homology 2-containing adaptor protein Grb14 (299), which regulates the activity of glucose transporter GLUT4 in adipocytes (299, 311). PARP6/tankyrase-2 has been shown to be a signaling target of mitogen-activated protein kinase (MAPK) and stoichiometrically phosphorylated upon insulin stimulation (299, 311). Phosphorylation enhances the poly-ADP-ribose polymerase activity of tankyrase. Remarkably knockdown of *Parp6/tnks2*, attenuated insulin-stimulated GSV translocation and glucose uptake without disrupting insulin-induced phosphorylation cascades (311). Since PARP5/tankyrase-1 and PARP6/tankyrase-2 have been shown to form hetero(oligo)mers they could to function together as scaffolding proteins, capable of regulating assembly of large protein complexes. Both PARP5/tankyrase-1 and PARP6/tankyrase-2 may be therefore involved in the long-term effect of the MAPK cascade on the metabolism of GLUT4 vesicles and regulation of physiological GSV trafficking (299, 311). A detailed list of n/pADP-ribose/DNA non-bridged PARP6/tankyrase-2 interaction partners is shown in Table 6.

## 6. STRUCTURES, CLASSIFICATION AND FUNCTIONS OF THE PARGS

The “classical” poly-ADP-ribose glycohydrolase, PARG, represents the major PARG activity catalyzing the hydrolysis of poly-ADP-ribose polymers to free ADP-ribose in the cell (reviewed in (162, 312). PARG was suggested to modulate the level and complexity of poly-ADP-ribose on the different acceptor proteins, thereby preventing hyper-modification of nuclear proteins with very long poly-ADP-ribose chains (313). The mammalian *Parg* gene encodes for at least five isoforms; the nuclear isoforms mPARG-110/hPARG-111, the cytoplasmic and nuclear localized isoform mPARG-63/hPARG-60, the strictly mitochondrial localized mPARG-59/hPARG-55, and the two cytoplasmic isoforms PARG-102, PARG-99, mainly characterized in human (62, 63, 160, 161, 314, 315). Whether all five isoforms simultaneously exist in all mammalian species remains to be investigated. The

mPARG-110/hPARG-111 isoform represents the full-length 110/111 kDa PARG protein in human and mice, whereas hPARG-102/mPARG-101 and m/hPARG-99 are alternative splice variants, detected so far only in human and mouse cells, which lead to the expression of the 102/101 kDa and 99 kDa PARG isoforms (160, 161). These PARG isoforms differ from mPARG-110/hPARG-111 by the lack of exon 1 (hPARG-102/mPARG-101) or exons 1 and 2 (m/hPARG-99). The catalytically active 63/60 kDa PARG isoform (mPARG-63/hPARG-60) is an additional splice variant that lacks exons 1, 2, 3 and half of exon 4 and (160, 161, 163, 316, 317). In addition, human PARG60 (hPARG60) is characterized by missing exon V, making it slightly smaller in size than the mouse homolog mPARG63. Skipping of exon V appears to be specific for human and does not exist in mouse (160). The mPARG-63/hPARG-60 isoform is expressed in multiple cell compartments. The second short catalytically active PARG isoform, mPARG-59/hPARG-55 is expressed through alternative translation initiation from hPARG60 transcripts and strictly targeted to the mitochondria through its fully accessible mitochondria-targeting signal (MTS) in PARG exon IV (160). The structures of the isoforms of the “classical” PARG enzyme are schematically drawn in figure 7. The mPARG-110/hPARG-111 full-length isoform is primarily localized in the nucleus, whereas hPARG-102/mPARG-101 and m/hPARG-99 seem to be exclusively cytoplasmic, under non-stimulated conditions (318). The nuclear targeting of mPARG-110/hPARG-111 is due to two strong classical nuclear localization signals (NLS) in exon 1 (160, 161). A recent study, using living cells and overexpressed GFP-tagged PARG isoforms, demonstrated that the a cytoplasmic 102/101 kDa PARG isoform translocates into the nucleus, while the nuclear mPARG-110/hPARG-111 isoform relocalizes to the cytoplasm in response to DNA damage induced by gamma-irradiation (318). However, it remains to be further investigated whether the alternative splicing and shuttling of endogenously expressed PARG could be regulated in a cell type- and/or stimuli-specific manner. Initial studies suggested that the mPARG63/hPARG60 isoform shuttles between nucleus and mitochondria, which would be in line with a proposed function of poly-ADP-ribose in genotoxic stress-dependent, nuclear-mitochondrial crosstalk (160, 161, 163, 316). However, a recent study provided strong evidence that the mPARG63/hPARG60 isoform is mainly localized to the nucleus (315). The putative NLS sequences found at amino acid positions 421-446 and 838-844 are most likely required for the nuclear targeting of the mPARG63/hPARG60 isoform (315). Two putative nuclear export signals (NES) have been localized to amino acids 126-134 and 881-888, which might explain the preferential cytoplasmic localization of the PARG isoforms hPARG-102/mPARG-101 and m/hPARG-99, lacking exon 1 (160, 161). It will be interesting to see whether the ratio between the numbers of NLS to NES determines the localization of these isoforms. Since Na/NMNAT-2 and Na/NMNAT-3 are preferentially localized to Golgi complex and mitochondria, respectively (319), and poly-ADP-ribosylation catabolism was also shown to be associated with both compartments (reviewed in (104, 312), it was suggested that at least a subpopulation of the cytoplasmic

hPARG-102/mPARG-101 or m/hPARG-99 isoforms might also be localized to these compartments (160, 161, 312).

Mice with a targeted deletion of exons 2 and 3 of the *Parg* gene, resulting in depletion of the nuclear PARG-110 protein and the cytoplasmic isoforms PARG-101 and PARG-99, are viable and phenotypically normal but show an increased sensitivity to alkylating agents and ionizing radiation (163, 316). In addition these mice were susceptible to streptozotocin-induced diabetes or endotoxic shock and showed an enhancement of ischemic brain injury, most likely due to dysregulation of the nuclear and cytoplasmic poly-ADP-ribosylation metabolism and accumulation of poly-ADP-ribose (163, 316). The PARG activity was greatly reduced in the cytoplasmic and nuclear fractions of *Parg-Δ2-Δ3/Δ2-Δ3*-knock-out cells. The mainly nuclear isoform PARG-59 is still expressed in the *Parg-Δ2-Δ3/Δ2-Δ3*-knock-out mice (163, 316), indicating that this isoform is responsible for the residual poly-ADP-ribose-degrading activity present in these mutant cells. On the other hand, mice with a targeted deletion of exons 3 and 4 of the *Parg* gene, resulting in a complete depletion of all isoforms, show early embryonic lethality and increased sensitivity to alkylating agents and ionizing radiation (317). The lethality results from the failure to hydrolyze poly-ADP-ribose. *Parg* null embryonic day (E) 3.5 blastocysts and embryonic trophoblast stem cell lines derived from early *Parg* null embryos are viable only when cultured in medium containing the broad range non-specific PARP/mART inhibitor 3-amino-benzamide (317). These PARG-deficient cells accumulate very high levels of poly-ADP-ribose and undergo increased cell death in absence of 3-amino-benzamide. The short PARG isoforms lack the proposed N-terminal PARG regulatory domain, suggesting that the mPARG-63/hPARG-60 and mPARG-59/hPARG-55 isoforms are constitutively active (315) and may be required for the degradation of the constitutively synthesized poly-ADP-ribose in unstimulated cells. These data clearly indicate that only the cytoplasmic/nuclear localized mPARG-63/hPARG-60 and the strictly mitochondrial localized mPARG-59/hPARG-55 isoforms are required for embryonic development. Remarkably, the activity of both mPARG-63/hPARG-60 isoform and PARPs in intact cells is increased in *Parg-Δ2-Δ3/Δ2-Δ3*-knock-out cells upon DNA damage (315). The degree of PARG activation is greater than PARP1 and PARP2, resulting in decreased PARP1 auto-modification and poly-ADP-ribose accumulation. These genetic studies clearly demonstrate that a precise coordination of PARP and PARG activities is important for cellular responses under normal physiological as well as cytotoxic stress conditions.

The unexpected finding that the mitochondria-localized ADP-ribose-protein-hydrolase-3 (ARH3) also possesses intrinsic PARG activity (320), strongly indicates that mammalian cells may contain several additional structurally unrelated PARG-like gene products that have not yet been detected. Interestingly, in the genome of the cress plant *Arabidopsis thaliana*, several not yet characterized genes exist (321), which show some similarity with the TEJ protein, a plant homologue of the mammalian PARG (322).

## 7. CROSS-TALK AMONG PARPs, MARTS AND OTHER ADP-RIBOSYLATING ENZYMES

Growing genetic evidence is arising for distinct intracellular cross talks among PARPs, PARP-like-mARTs (PI-mARTs) and other ADP-ribosylating enzymes involved in the regulation of cell proliferation, differentiation, cell survival and cell death. For instance, animal models using *Parp1* and *Parp2* knockout mice demonstrated that these two enzymes cooperate and possessing both overlapping and non-redundant essential functions in the maintenance of genomic stability and during early embryonic development. *Parp1*(-/-)/*Parp2*(-/-) double mutant mice are not viable and die at the onset of gastrulation (231). The molecular mechanism remains to be investigated. Moreover, PARP5/tankyrase-1 appears to protect cells from genotoxin induced cell death and injury through inhibition of PARP1-mediated NAD depletion and cell death (311). Surprisingly, PARP6/tankyrase-2 caused rapid necrotic cell death when highly over-expressed in human immortalized cells (23). Thus, Both PARP5/tankyrase-1 and PARP6/tankyrase-2 may modulate PARP1 or PARP2 in an antagonistic manner. Three independent studies provided preliminary evidence for a functional link between SIRT1 and PARP1 (323-326). The first study showed a drastic increase in PAR synthesis in SIRT1-deficient cells upon DNA damage (326). These authors proposed that the unbalanced regulation of PARP1 in the absence of SIRT1 could result in AIF-mediated cell death (326). The second report demonstrated that, increased activity of PARP1 upon genotoxic stress, was associated with depletion of cellular NAD<sup>+</sup> levels and reduced SIRT1 deacetylase activity in myocyte cells, contributing to myocyte cell death during heart failure (323-325). These authors suggested that SIRT1 is a downstream target of NAD replenishment leading to cell protection (323-325). These findings provide the first evidence that the two NAD<sup>+</sup>-dependent enzymes SIRT1 and PARP1 might modulate each other's enzymatic activity under physiological conditions. However, the exact molecular mechanisms underlying this antagonistic cross talk remain to be elucidated. Several recent reports proposed that PARPs, PI-mARTs and other NAD<sup>+</sup> consuming ADP-ribosylating enzymes could either directly regulate each other through trans-ADP-ribosylation or indirectly through modulation of the NAD<sup>+</sup> levels and/or ADP-ribose metabolites (4, 118, 119, 327)).

### 7.1. Regulation through NAD levels

Mono- and poly-ADP-ribosylating enzymes consume the same substrate, NAD<sup>+</sup>, NAD<sup>+</sup>/nicotinamide levels could therefore serve as converging points for interactions of PARP/poly-ADP-ribosylation reactions and PI-mART/SIRT/CD38-dependent mono-ADP-ribosylation-ADP-ribose cyclation pathways (4, 118, 119, 327)). For instance, different poly- and mono-ADP-ribosylation reactions could modulate the NAD<sup>+</sup>-dependent deacetylation of proteins by SIRTs via the NAD<sup>+</sup>/nicotinamide connection. The decline of NAD<sup>+</sup> levels and the rise of nicotinamide on activation of poly-ADP-ribosylation reactions may down regulate the activity of SIRTs under stress conditions due to deacetylation and

mono- ADP-ribosylation being dependent on high concentrations of  $\text{NAD}^+$  and inhibited by low physiological levels of nicotinamide (50% inhibitory concentration of  $<50 \mu\text{M}$ ) (reviewed in (36, 328, 329)). On the other hand, PARPs might be indirectly regulated by the ADP-ribosyl cyclase/cyclic-ADP-ribose hydrolase CD38 through the modulation of  $\text{NAD}^+$  levels under normal physiological conditions. Indeed, several studies demonstrated that tissue levels of  $\text{NAD}^+$  are significantly increased in *Cd38* knockout mice (330-332). Moreover, the endogenous activity of SIRT1 was several-fold higher in nuclear extracts from *Cd38* knockout mice when compared to wild-type nuclear extracts, strongly indicating that a nuclear localized CD38 isoform (333) is a major regulator of cellular/nuclear  $\text{NAD}^+$  level, and SIRT1 activity (331, 332). Moreover, the increase in  $\text{NAD}^+$  levels leads to  $\text{NAD}^+$ -dependent activation of the SIRT-PGC1 $\alpha$  axis a well-established cascade, involved in the regulation of mitochondrial biogenesis and energy homeostasis (334). As a consequence, *Cd38*-deficient mice are protected against high-fat diet-induced obesity, which demonstrates that CD38 is necessary for the development of diet-induced obesity (334). An additional study provided evidence that the ecto-mono-ADP ribosyltransferase (e-mART2) can sense and translate the local concentration of ecto- $\text{NAD}^+$  into corresponding levels of mono-ADP-ribosylated cell surface proteins, while CD38, through its enzymatic activity, can control the level of e-mART2-catalyzed ADP-ribosylation of cell surface proteins by limiting the substrate availability for e-mART2 (335).

### 7.2. Regulation through trans-ADP-ribosylation

PARPs, PI-mARTs and SIRTs could also modulate each other in a positive and/or negative manner through direct interaction and trans-ADP-ribosylation (4). These modifications could occur simultaneously through heteromerization or stepwise without heteromerization events (4). PARPs, PI-mARTs and SIRTs may also cross-talk through their overlapping substrates. Over 20 years ago, Tanigawa *et al.* showed *in vitro* that covalent mono-ADP-ribose adducts of histones, generated by an arginine-specific DNA-dependent mono-ADP-ribosyltransferase(s), can serve as initiators for poly-ADP-ribose synthesis (96). Moreover, the DNA-binding domain poly-ADP-ribose polymerase-1 (PARP1) was found to be an efficient protein acceptor for the arginine-specific ADP-ribosylation reaction catalyzed by cholera toxin. This study suggested that the enzymatic activity of PARP1 might be repressed by arginine-specific mono-ADP-ribosylation mediated by cholera toxin (336). A recent report demonstrated that during the exposure of activated T-cells to  $\text{NAD}^+$ , the ADP-ribosyl cyclase/cyclic-ADP-ribose hydrolase CD38 is modified by ecto-mono-ADP-ribosyltransferases specific for cysteine and arginine residues. E-mART-mediated mono-ADP-ribosylation of CD38 on arginine residues inactivates both cyclase and hydrolase activities and causes a decrease in intracellular cyclic-ADP-ribose and a subsequent decrease in  $\text{Ca}^{2+}$  influx, resulting in apoptosis of the activated T-cells (337).

### 7.3. Cross-talk of PARPs and mARTs through ADP-ribose metabolites

A recent study provided evidence that the macro domains of PI-mART10 (BAL1/PARP9) may serve as

high-affinity binding modules for different classes of free ADP-ribose (i.e., mono-ADP-ribose, poly-ADP-ribose) (Karras EMBOJ 2005). These data strongly indicate that the macro-PI-mARTs may interact with PARPs through poly-ADP-ribose, suggesting a  $\text{NAD}^+$ /ADP-ribose-metabolite-mediated cross talk between macro domain-containing PI-mARTs and SIRTs or PARPs, respectively (4). Moreover, the potential ADP-ribose phosphatase/phosphoesterase activity associated with the macro domains in macro-PI-mARTs, could function on poly-ADP-ribose polymers formed by PARP. It was speculated that macro domain-containing PI-mARTs may control polymer size by the generation of poly-ADP-ribose polymer ends that could not be elongated by PARPs (4, 118-120, 338)).

## 8. PHARMACOLOGICAL INHIBITORS

The first non-selective broad range non-specific inhibitor of ADPRT activities, 3-aminobenzamide was reported over 25 years ago (reviewed in (33)). During the last two decades of intensive research, over 50 potential PARP inhibitors were developed (reviewed in (33)). However, all PARP inhibitors examined to date directly compete with the ADPRT substrate  $\text{NAD}^+$ . The vast majority belongs to the class of nicotinamide-based inhibitors and mimics the nicotinamide moiety of  $\text{NAD}^+$ . Given the similarity of the  $\text{NAD}^+$ -binding site sequences and the number of PARP-like enzymes existing in cells of higher eukaryotes broad-range inhibitory effects of these ADPRT inhibitors are expectable. Inactivation of PARP1 by gene disruption or inhibition with 5-iodo-6-amino-1,2-benzopyrone ( $\text{INH}_2\text{BP}$ ) protects mice from multiple-low-dose-streptozotocin (MLDS)-induced type I diabetes. However, treatment of *Parp1* knockout mice with  $\text{INH}_2\text{BP}$ , increased the onset of diabetes, demonstrating the potent off-target effect of PARP inhibitors (339).

Initial studies suggested that they don't affect the N-linked mono-ADP-ribosyltransferase reactions in higher eukaryotes and might be a specific for PARP-like enzymes or O-linked mono-ADP-ribosyltransferase reactions (reviewed in (4, 33, 35)). Indeed, most of them mainly target poly-ADP-ribose polymerases and PARP-like mono-ADP-ribosyltransferases (reviewed in (4, 33, 35)). On the other hand a recent study reported the crystal structure of the catalytic domain of ETA in complex with PJ34, the first example of a bacterial mono-ADP-ribosyltransferase in complex with an inhibitor of poly-ADP-ribose polymerases. Diphtheria toxin and exotoxin A are virulence factors produced by the pathogenic bacteria *Corynebacterium diphtheriae* and *Pseudomonas aeruginosa*. The same study demonstrated that many so-called potent PARP1 inhibitors could even serve as perfect pharmacological inhibitors of members of the bacterial diphtamide specific ADP-ribosyltransferase-toxin family (35). The water-soluble PARP inhibitors PJ34 from Inotek Inc and the PARP inhibitors from Guilford Pharmaceuticals could act as a potent inhibitor of the catalytic domain of Diphtheria toxin and exotoxin A with unexpectedly low  $\text{IC}_{50}$  values ranged from  $0.17 \mu\text{M}$  for GP-D to  $0.28 \mu\text{M}$  for PJ34 (35). Remarkably, treatment of Rabbits with PJ34 was

shown to reduce gut inflammation and limit bacterial translocation of *Pseudomonas aeruginosa* (340). A similar observation was reported for the PARP inhibitor INO-1001 from Inotek Inc in an ovine model of *Pseudomonas aeruginosa*-induced sepsis (341). Thus, together with the high-resolution structural data of the complex of the P. aeruginosa toxin ETA with PJ34 raise the question whether PJ34 and potentially also INO-1001 might indeed directly target bacterial virulence factors and not the classical PARP1 or PARP2, suggested to play a crucial role in these processes.

It is of great importance to find new approaches that can avoid the off target effects towards poly-ADP-ribose polymerases, but at the same time increase the selectivity for each PARP-like mono-ADP-ribosyltransferases member. During the last five years, new classes of benzimidazoles, pyrrolocarbazoles, phthalazinones, quinazolinones, quinazolidinones, and quinoxalines as more specific PARP inhibitors were developed by some of the leading industry groups. However, with a few exceptions, little progress has been made in developing isozyme-specific PARP inhibitors (342-348). For instance, structure-based drug design screens identified two classes of quinazolinone derivatives and quinoxaline derivatives as potent and selective PARP1 and PARP2 inhibitors, respectively (343-345). Using recombinant PARP1 and PARP2, the quinazolinone derivatives displayed high selectivity for PARP1 whereas the quinoxaline derivatives showed superior selectivity for PARP2 in PARP enzyme assays (343-345). Unfortunately, the newest generations of PARP inhibitors claimed to be PARP1 specific are not yet available for the scientific community. Thus, there is no report published by independent groups, which would confirm the claims made by the pharmaceutical companies. It is an absolute necessity that all ADPRT inhibitors have to be thoroughly tested *in vitro* and *in vivo* against the whole panel of poly-ADP-ribose polymerases, PARP-like mono-ADP-ribosyltransferases, and bacterial mono-ADP-ribosyltransferases. The potential off target effects could be addressed by using single knockout mice and knock-in mice expressing enzymatic-inactive PARP or PI-mART forms and combinations thereof. In addition, long-term studies are required to calculate the risks and benefits associated with therapeutic PARP and/or PARG inhibition.

## 9. SUMMARY AND FUTURE PERSPECTIVES

Since poly-ADP ribose was discovered over 40 years ago, a tremendous amount of work has been done to decipher the physiological and pathophysiological roles of ADP-ribosylation reactions on the molecular level. During the last decade, it became clear that poly-ADP-ribosylation reactions play important roles in a wide range of physiological and pathophysiological processes, including intracellular signaling, transcriptional regulation, telomere dynamics and maintenance of genomic stability, cell differentiation and proliferation as well as cell death. However, despite the progress made in recent years no unified picture for the role of poly-ADP-ribosylation reactions has yet emerged. Obviously, we are just

beginning to gain insight into the biochemistry of poly-ADP-ribosylation reactions and the ways in which cellular processes are regulated by poly-ADP-ribosylation. The most basic questions remain unanswered. Does poly-ADP-ribose serve as a covalent post-translational modification? A recent report suggested that a "poly-ADP-ribose code" could exist *in vivo* and determine specific functional outcomes of distinct poly-ADP-ribose signaling pathways (4). The occurrence of such a "poly-ADP-ribose code" would imply that distinct binding modules for free and protein-bound poly-ADP-ribose exist *in vivo* and function in a similar manner to the 14-3-3 isoforms, bromo, chromo, PHD or Tudor domains.

The use of novel mass spectrometry techniques in combination with newly developed antibodies which specifically recognize distinct free or protein-bound poly-ADP-ribose structures would allow large-scale identification of poly-ADP-ribose-binding proteins and thus decipher the putative "poly-ADP-ribose-glycomes" potentially existing in mammals. The combined application of new *in vitro* reconstitution systems with mass spectrometry and genome-wide chromatin immunoprecipitation methods used to operate at a resolution of less than one nucleosome will provide insight into the putative poly-ADP-ribosylation mediated chromatin remodeling in specific DNA damage pathways and transcriptional processes. In addition, the recent identification of a new family of putative PARP-like mono-ADP-ribosylating enzymes in eukaryotes has revealed a novel level of complexity in the regulation of the mono- and poly-ADP-ribose metabolism. Thus, the development of novel highly PARP family member-specific chemical inhibitors of oligo- and poly-ADP-ribosylating activities and genetic tools including knock-in mice expressing enzymatic-inactive PARP and PI-mART forms and combinations thereof, will greatly contribute to the future understanding of the complex functions of poly-ADP-ribosylation in living cells.

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