CMLS, Cell. Mol. Life Sci. 62 (2005) 721–730
1420-682X/05/080721-10
DOI 10.1007/s00018-004-4503-3

CMLS Cellular and Molecular Life Sciences

Introduction to poly(ADP-ribose) metabolism

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Abstract. Poly(ADP-ribosyl)ation is a posttranslational modification of proteins in eukaryotic cells catalysed by a family of NAD^+ ADP-ribosyl transferases, the poly(ADP-ribose) polymerases (PARPs). PARP-encoding genes now constitute a superfamily of at least 18 members encoding proteins that share homology with the catalytic domain of the founding member, PARP-1 [1]. Poly(ADP-ribose) metabolism is of central importance in a wide variety of biological processes including maintenance of genomic stability [2–4], DNA repair [3, 5], transcriptional regulation [6, 7], centromere function [8, 9], modulation of telomere length [10–12], regulation of proteasomal protein degradation [13, 14], regulation of endosomal vesicle trafficking [15, 16] and apoptosis [17, 18]. The life cycle of poly(ADP-ribose) is discussed in the following section. In addition, an overview of the genes and proteins involved in poly(ADP-ribose) metabolism and their possible cellular function is provided.

Key words. PARP; poly(ADP-ribose); DNA damage; DNA repair; genomic instability; ageing.

Life cycle of poly(ADP-ribose)

Poly(ADP-ribosyl)ation occurs in almost all nucleated cells of mammals, plants and lower eukaryotes, but is absent in yeast. It represents an immediate cellular response to DNA damage as induced by ionizing radiation, alkylating agents and oxidants. In the absence of DNA single- and double-strand breaks, poly(ADP-ribosyl)ation is a very rare event, but it can increase over 100-fold upon DNA damage [19]. Under these conditions about 90% of poly(ADP-ribose) is synthesized by poly(ADP-ribose) polymerase 1 (PARP-1) [19]. PARP-1 is constitutively expressed but enzymatically activated by DNA strand breaks. It catalyses the formation of ADP-ribose from the oxidized form of nicotinamide adenine dinucleotide (NAD^+) by cleavage of the glycosidic bond between nicotinamide and ribose. Glutamate, aspartate and carboxyterminal lysine residues [20, 21] of target ('acceptor') proteins are then covalently modified by the addition of an ADP-ribose subunit, via formation of an ester bond between the protein and the ADP-ribose residue. Subsequently PARP-1 catalyses an elongation and branching reaction using additional ADP-ribose units from NAD^+. This generates novel ribosyl-ribosyl linkages and eventually results in the formation of polymers with chain lengths of approximately 200 ADP-ribose subunits and several branching points [22] (fig. 1).

Free ADP-ribose molecules generated by the NAD glycohydrolase (NADase) activity of PARP-1 have also been shown to react with protein lysine residues to form Schiff base adducts that can rearrange into ketoamine derivatives [23, 24]. Such protein-bound ketoamine derivatives can also be elongated by PARP-1 to generate ADP-ribose-polymers linked to proteins via lysine residues.

The most prominent target protein ('acceptor') of this poly(ADP-ribosyl)ation reaction is PARP-1 itself [25], but many other acceptor proteins have been described, such as p53 [26], both subunits of NF-κB (nuclear factor kappa B) [27], histones [28], DNA ligases [29], DNA polymerases [30], DNA-topoisomerases [31] and DNA-dependent protein kinase [32]. Due to the high negative charge of the polymer, this modification significantly alters the physical and biochemical properties of the modified proteins, such as their DNA-binding affinity, and it is likely that such alteration will have a regulatory function concerning the interaction with other proteins [33].

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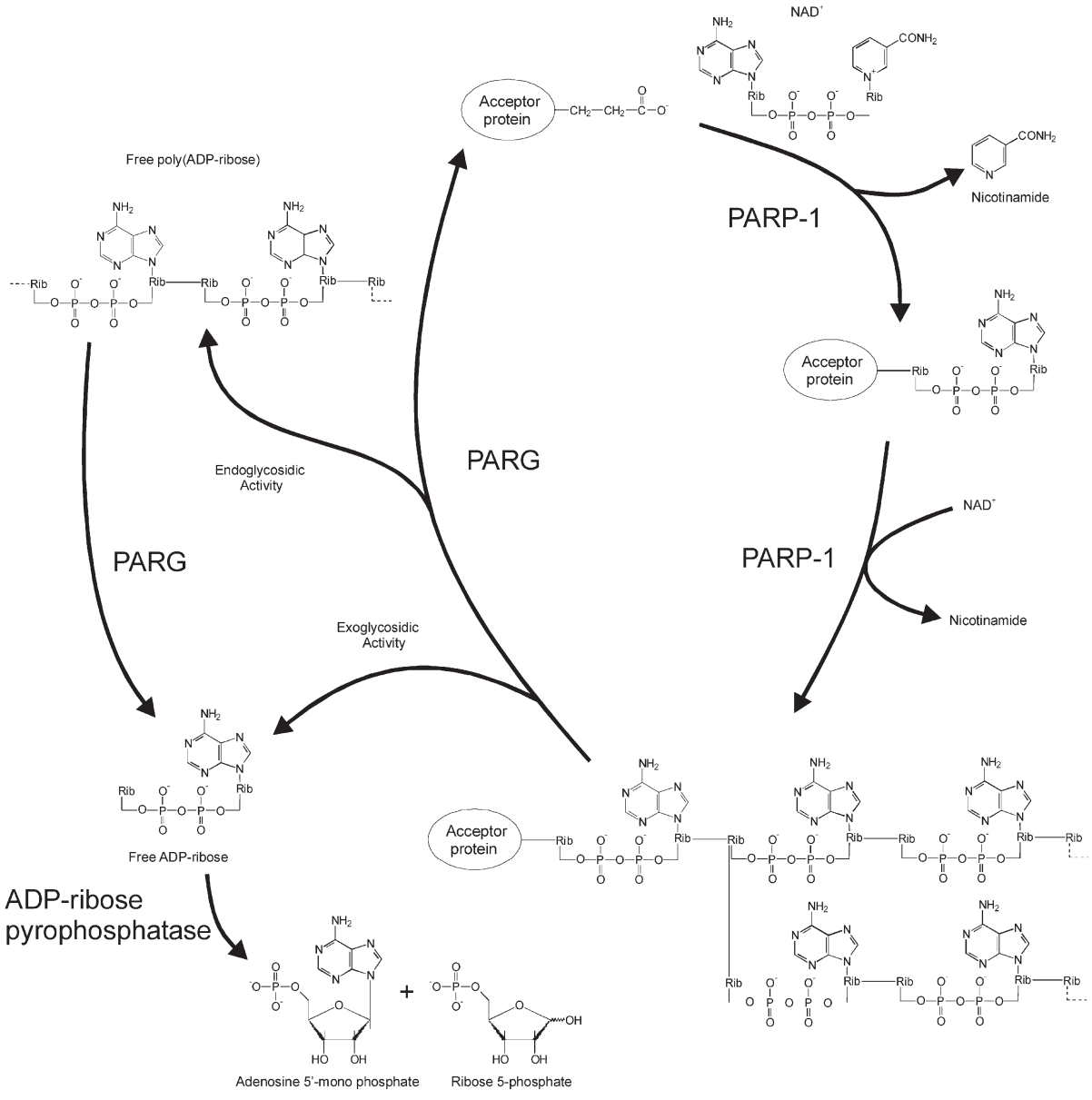

Figure 1. Metabolism of poly(ADP-ribose). PARP-1 cleaves the glycosidic bond of NAD^+ between nicotinamide and ribose followed by the covalent modification of mainly glutamate residues of acceptor proteins with an ADP-ribosyl unit, resulting in the formation of an ester bond between the protein and the ADP-ribose residue. PARP-1 also catalyses the elongation and branching reaction, giving rise to polymers with chain lengths of up to 200 ADP-ribosyl units and several branching points. PARG is the only protein known to catalyse hydrolysis of (ADP-ribose) polymers to free ADP-ribose exhibiting endoglycosidic activity along with exoglycosidic activity. PARG was reported to be able to remove even the primary ADP-ribosyl group bound to acceptor proteins [104], indicating a functional overlap with ADP-ribosyl protein lyase that was shown to remove the primary ADP-ribosyl group from acceptor proteins [38].

Poly(ADP-ribose) glycohydrolase (PARG) is the only protein known to catalyse the hydrolysis of (ADP-ribose) polymers to free ADP-ribose [34] (fig. 1; table 1; N.B. More details are given below). It could be demonstrated that this enzyme exhibits endoglycosidic activity along with exoglycosidic activity [35]. Its product is free poly(ADP-ribose) and ADP-ribose monomer, the latter being a potent protein-glycating sugar capable of causing protein damage [24]. ADP-ribose pyrophosphatase can convert free ADP-ribose molecules into AMP and ribose 5-phosphate, thus producing a compound much less prone to induce glycation [36] (fig. 1). The crystal structure of human ADP-ribose pyrophosphatase NUDT9 has recently been solved [37].

Furthermore, an ADP-ribosyl protein lyase has been purified and characterized that removes the protein-proximal ADP-ribosyl group from the acceptor protein and releases 5′-ADP-3′′-deoxypent-2′′-enofuranose [38].

Table 1. Enzymes involved in poly(ADP-ribose) formation or degradation and their genes.

Human gene*	Chromosomal location	Enzyme designation	Size	Refs
ADPRT	1q41-q42	NAD^+ ADP-ribosyltransferase (polymerizing); N.B. Common alternative designations are: poly(ADP-ribose) polymerase-1 **[PARP-1]**; poly(ADP-ribose) synthetase [PARS]	1014 aa (113 kDa)	44
ADPRTL1	13q11	vault poly(ADP-ribose) polymerase or NAD^+ poly(ADP-ribose) polymerase-4 [**vPARP**]	1724 aa (192.8 kDa)	70, 71
ADPRTL2	14q11.2-q12	NAD^+ poly(ADP-ribose) polymerase-2 [**PARP-2**]	583 aa (66 kDa)	64, 65
ADPRTL3	3p22.2-p21.1	NAD^+ poly(ADP-ribose) polymerase-3 [**PARP-3**]	532 aa (60 kDa); alternative splicing variant: 539 aa (60.8 kDa)	63
TNKS	8p23.1	Tankyrase-1 or NAD^+ poly(ADP-ribose) polymerase-5a [PARP-5a]	1327 aa (142 kDa)	39, 75
TNKS2	10q23.3	Tankyrase-2 or NAD^+ poly(ADP-ribose) polymerase-5b [PARP-5b]	1166 aa (126.9 kDa)	82, 83
PARG	10q11.23	poly(ADP-ribose) glycohydrolase [**PARG**]	Three different splice variants: 976 aa (111 kDa); 893 aa (102 kDa); 866 aa (99 kDa)	87

*Approved Human Gene Organization gene nomenclature committee symbol.

Proteins involved in poly(ADP-ribose) metabolism and their genes

PARP-1

Until 1998, when Tankyrase-1 (PARP-5a) was first described [39], the world of poly(ADP-ribose) metabolism was rather simple as only one enzyme was known to be responsible for the synthesis of poly(ADP-ribose). The enzyme that nowadays is referred to as PARP-1 (EC 2.4.2.30; table 1) was first discovered 4 decades ago by Chambon, Weill and Mandel in Strasbourg, France [40]. Approximately 200,000 molecules of PARP-1 are present in a HeLa cell [41], which greatly exceeds the copy numbers of other nuclear enzymes such as RNA polymerase II [42] or topoisomerase II [43]. PARP-1 is catalytically active as a dimer and is itself the major acceptor protein in intact cells [25]. It is a highly conserved enzyme displaying a characteristic three-domain structure [44], which can be further broken down into modules A–F [45] (fig. 2). The N-terminal 42-kDa DNA binding domain (DBD) also comprises the protein's nuclear localization signal (NLS) and is adjacent to a central 16-kDa automodification domain. The 55-kDa catalytic domain, which includes the active site, is located at the C-terminus.

The DBD of PARP-1 binds to single- or double-strand breaks with high-affinity via two zinc fingers [46] but has also been reported to be involved in protein-protein interactions [47]. The first zinc finger is essential for PARP-1 activation upon DNA damage, whereas the second is

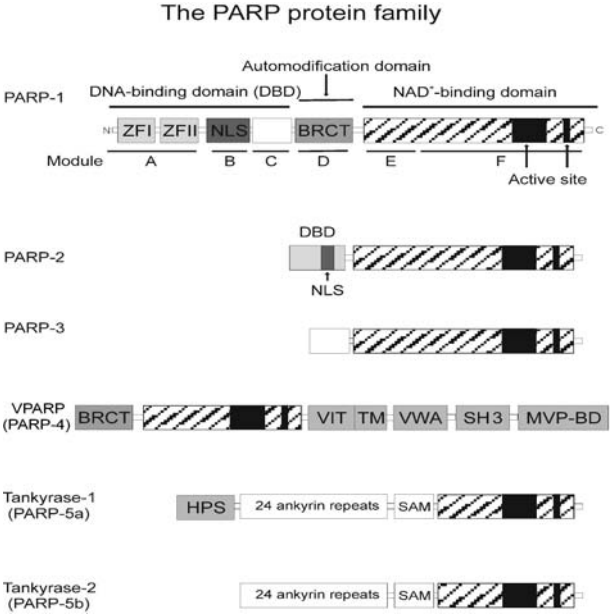

Figure 2. Structural organization of proteins belonging to the PARP family. Abbreviations: BRCT, BRCA1 C-terminus; DBD, DNA binding domain; HPS, His-Pro-Ser-rich domain; MVP-BD, major vault binding domain; NLS, nuclear localization signal; SAM, sterile alpha-module; SH3, sarc homology region; TM, transmembrane domain; VIT, vault protein inter-alpha-trypsin domain; VWA, von Willebrand factor type A domain; ZF, zinc finger.

essential for recognition of DNA single-strand breaks but not double-strand breaks [48]. Automodification was reported to be primarily induced by single-strand breaks, while histone H1 is modified mostly when PARP-1 binds to double-strand breaks [49], suggesting that activation of PARP-1 by different stimuli may lead to the modification of a different set of proteins.

The automodification domain of PARP-1 is rich in glutamic acid residues, consistent with the fact that poly(ADP-ribosy)lation occurs on such residues [21]. This domain also comprises a BRCT motif (BRCA1 C-terminus [BRCA1, breast cancer 1 protein]) that is found in many DNA damage repair and cell cycle checkpoint proteins [50].

Finally, the C-terminal 55-kDa catalytic domain contains the residues essential for NAD^+-binding, ADP-ribosyl transfer and branching reactions [51]. The crystal structure of the C-terminal catalytic fragment revealed a striking homology with bacterial toxins that act as mono-ADP-ribosyl transferases [52]. Several PARP-1 mutants have been characterized, which have either an increase in activity (gain of function [53]), a loss in activity (loss of function [54]) or an increase in branching frequency of the ADP-ribose polymer [54, 55]. Three different PARP-1 knockout mouse models have been created independently [56–58] lacking functional PARP-1 protein. These PARP-1 mutant mice showed hypersensitivity both to alkylating treatment and ionizing radiation, as well as increased genomic instability, yet protection against various pathophysiological phenomena, such as lipopolysaccharide (LPS)-induced septic shock or streptozotocin-induced diabetes [59].

Recent work showed that PARP-1 and PARP-1-mediated poly(ADP-ribosyl)ation of centrosomal proteins including p53 are also involved in the regulation of centrosome function [60, 61].

Among many other identified interaction partners of PARP-1 are also other members of the PARP-family, such as PARP-2 [5, 8, 62] and PARP-3 [63].

PARP-2

Apart from PARP-1, PARP-2 is the only other PARP known to be strongly activated by DNA strand breaks (table 1). This enzyme displays automodification properties similar to PARP-1 [64]. The protein domain structure resembles an N-terminally 'truncated' version of PARP-1 (fig. 2) with a small DBD that is very different from that of PARP-1. It consists of only 64 amino acids and does not contain any obvious DNA binding motif. It is, however, rich in basic amino acids, which might be relevant for DNA interaction. The crystal structure of the catalytic fragment of murine PARP-2 has recently been solved, thus providing a basis for development of isoform-specific inhibitors by rational drug design [65].

Despite major structural differences between PARP-1 and PARP-2, including size and the absence of zinc fingers or BRCT motif, they are both targeted to the nucleus, bind to and become activated by DNase I-treated DNA. Both PARP-2 and PARP-1 can homo- and heterodimerize, and both are involved in the base excision repair (BER) pathway, where they form a complex with XRCC1 (X-ray cross-complementing factor 1) [62].

PARP-2 together with PARP-1 has been detected at centromeres [5, 66], where they both interact with centromeric proteins [9]. Whereas PARP-2 localization is discrete at the centromer, PARP-1 shows a broader centromeric and pericentromeric distribution [8]. Possible diverse roles of PARP-2 and PARP-1 in modulating the structure and checkpoint functions of the mammalian centromere, in particular during radiation-induced DNA damage, have been suggested by an increase in centromeric chromatid breaks observed in PARP-2 knockout mice subjected to γ-irradiation and by the observed female-specific lethality associated with x chromosome instability in PARP-$1^{+/-}$ / PARP-$2^{-/-}$ mice [5]. PARP-$1^{-/-}$ / PARP-$2^{-/-}$ double mutant mice are not viable and die at the onset of gastrulation, demonstrating that the expression of both PARP-1 and PARP-2 and/or DNA strand break-dependent poly(ADP-ribosyl)ation is essential during early embryogenesis [5].

A functional role of PARP-2 in the maintenance of telomere integrity is supported by the co-localisation of PARP-2 and TRF2 (telomeric-repeat binding factor 2), a negative regulator of telomere length [67]. PARP-2 activity regulates the DNA binding activity of TRF2 via covalent heteromodification of the dimerization domain of TRF2 as well as via non-covalent binding of poly(ADP-ribose) to the myb domain of TRF2 [1]. This protein interaction may be involved in modulating t-loop formation in response to DNA damage.

In conclusion, there is evidence for functional interaction between PARP-1 and PARP-2 possessing both overlapping and non-redundant functions in the maintenance of genomic stability and during embryonic development [5].

PARP-3

PARP-3 was identified as a core component of the centrosome localizing preferentially to the daughter centriole throughout the cell cycle [63]. It was shown that human PARP-3 negatively influences cell cycle progression at the G_1/S border without interfering with centrosome duplication [63]. The protein domain structure is very similar to PARP-2 (table 1, fig. 2), with a small DNA binding domain that is different from PARP-1. It consists of only 54 amino acids and contains a targeting motif that is sufficient to localize the enzyme to the centrosome [63]. Overexpression of PARP-3 or its N-terminal domain in HeLa cells interfered with the G_1/S cell cycle

transition. PARP-3 catalyses the synthesis of poly(ADP-ribose) in vitro and in purified centrosome preparations and forms a stable complex with PARP-1, in agreement with reports of PARP-1 localization at the centrosome [60, 61]. It is tempting to speculate that the presence of both PARP-1 and PARP-3 at the centrosome may link the DNA damage surveillance network to the mitotic fidelity checkpoint.

PARP-4 or Vault PARP (VPARP)

Vault particles are cytoplasmic ribonucleoprotein (RNP) particles composed of several small untranslated RNA molecules and three proteins 100, 193 and 240 kDa in size, respectively. With a total mass of 13 MDa vaults are the largest RNP complexes found in the cytoplasm of mammalian cells. Although the cellular function of vaults is as yet unknown, their subcellular localization and distinct morphology point to a role in intracellular, particularly nucleo-cytoplasmic, transport [68]. It was reported that vault particles may also be involved in intracellular detoxification, as all three vault proteins showed increased expression levels in many multi-drug-resistant human cell lines [69].

The 193-kDa vault protein comprises a region of 350 amino acids that shares 28% identity with the catalytic domain of PARP-1 [70, 71]. The p193 protein or VPARP [vault poly(ADP-ribose) polymerase] (table 1) indeed exhibits PARP activity and can poly(ADP-ribosyl)ate the p100 subunit or major vault protein (MVP) within the vault particle and to a lesser extent itself [70]. The N-terminal region of VPARP contains a BRCT domain similar to the automodification domain of PARP-1, suggestive of a related function. Immunofluorescence and biochemical data show that VPARP is not exclusively associated with the vault particle, but can also localize to the nucleolus, the nuclear spindle and to nuclear pores [68, 70].

PARP-5a or Tankyrase-1

Tankyrase-1 was initially identified through its interaction with the telomeric-repeat binding factor 1 (TRF1), a negative regulator of telomere length [39] (table1). The N-terminus of Tankyrase-1 contains a so-called HPS domain consisting of stretches of consecutive histidine, proline and serine residues, followed by 24 ankyrin (ANK) repeats, which is a structural feature only found in Tankyrase-1 and Tankyrase-2 within the known members of the PARP-family (fig. 2). Ankyrin repeats display a 33-amino acid motif which mediates protein-protein interactions [72]. Adjacent to the ANK domain is another protein interaction motif, the sterile alpha-module (SAM) [73]. The C-terminus of Tankyrase-1 displays homology to the PARP-1 catalytic region. Due to the absence of a DNA binding domain, Tankyrase-1 activity does not depend on the presence of DNA strand breaks but seems to be regulated by the phosphorylation state of the protein [15]. About 10% of cellular Tankyrase-1 protein is recruited to telomeres through binding of its ANK domain to TRF1 [74], which together with TRF2 functions in telomere length regulation [75]. Long-term overexpression of TRF1 in a telomerase-positive tumour-cell line resulted in a gradual and progressive telomere shortening, which could be reversed by expression of a dominant-negative TRF1 mutant that inhibited binding of endogenous TRF1 to telomeres. Thus the binding of TRF1 controls telomere length in cis by inhibiting the action of telomerase at the ends of individual telomeres [76]. Poly(ADP-ribosyl)ation of TRF1 by Tankyrase-1 inhibits its binding to telomeric DNA whereby Tankyrase-1 regulates telomere length through modulation of the poly-(ADP-ribosyl)ation status of TRF1 [77]. Furthermore, the intranuclear overexpression of Tankyrase-1, but not that of a PARP-deficient derivative, causes the lengthening of telomeres. Tankyrase-1 was therefore proposed to reverse the negative effect of TRF1 on telomere length [77]. Additional proteins, however, are also involved in TRF1 regulation, such as TIN2 (TRF1-interacting nuclear protein 2), which was reported to protect TRF1 from poly(ADP-ribosyl)ation by Tankyrase-1 by forming a ternary complex together with TRF1 and Tankyrase-1 without affecting Tankyrase-1 automodification [12].

Despite the effect of Tankyrase-1 on telomeres, most of the protein is found in the cytoplasm. It can either be detected at centrosomes, where it seems to interact with nuclear mitotic apparatus protein (NuMa) [74, 78], or in association with nuclear pore complexes [74], or at Golgi-associated GLUT4 vesicles [15]. Tankyrase-1 was also reported to interact with **ta**nkyrase-**b**inding protein of 182 kDa (TAB182), a newly identified protein that displays a heterochromatin-like staining pattern in the nucleus and co-stained with cortical actin in the cytoplasm [79]. Tankyrase-1 appears to be an important insulin signalling target, as the protein not only interacts with insulin-responsive amino peptidase (IRAP) located to GLUT4 storage vesicles in the Golgi, but upon insulin stimulation is also stoichiometrically phosphorylated by mitogen-activated protein kinase (MAPK). Tankyrase-1 poly(ADP-ribosyl)ates IRAP, as well as itself, and this activity is enhanced by MAPK-mediated phosphorylation, indicating that Tankyrase-1 may mediate the long-term regulation of GLUT4 vesicles by the MAPK cascade [15, 16].

PARP-5b or Tankyrase-2

Tankyrase-2 was originally described as a tumour antigen that elicits an antibody response in certain tumour patients [80, 81] (table 1). Later, Tankyrase-2 was reported

to interact with several other proteins such as TRF1 [82], IRAP [16], or Grb14, an SH2 domain-containing adaptor protein that binds to the insulin and fibroblast growth factor receptors [83]. Tankyrase-2 displays a domain structure similar to Tankyrase-1 except for the N-terminal HPS domain, which is missing in Tankyrase-2 [82, 83] (fig. 2). Tankyrase-1 and Tankyrase-2 show a significant functional overlap. Both proteins possess PARP activity and poly(ADP-ribosyl)ate some of their interaction partners (IRAP [15, 16], TAB182 [79], TRF1 [15, 16], but not TRF2 [11]) as well as themselves, whereas Tankyrase-2 displays preferential automodification activity [11, 16]. Chicken cells were shown to contain about twice as much Tankyrase-1 as Tankyrase-2 [84]. Overexpression of either protein in the nucleus released endogenous TRF1 from the telomere, suggesting partially redundant function [11, 77]. On the other hand, overexpression of Tankyrase-2, but not Tankyrase-1, caused rapid poly(ADP-ribosyl)ation-dependent cell death, demonstrating that the two proteins differ in regulation of activity and substrate specificity [82]. Both Tankyrases can self-associate via the SAM domain to form high-molecular-mass complexes, indicating a function as master scaffolding molecules in organizing protein complexes [84].

In summary, Tankyrase-1 and Tankyrase-2 are two highly related PARPs that interact with a wide variety of cytoplasmic and nuclear proteins and display extensive functional overlap.

PARG

While several genes are known to encode different enzymes that catalyse the synthesis of (ADP-ribose) polymers, there is only one single gene known to encode an enzyme catalysing the hydrolysis of (ADP-ribose) polymers to free ADP-ribose: the *PARG* gene encoding poly(ADP-ribose) glycohydrolase (PARG) [34, 85, 86]. In 1997 a bovine complementary DNA (cDNA) encoding a protein possessing PARG activity was identified and characterized [87]. The human *PARG* gene consists of 18 exons and shares a 470-bp bidirectional promoter with the gene encoding the translocase of the inner mitochondrial membrane 23 (TIM23). Promoter activity is severalfold higher for TIM23 than for PARG, indicating that the two genes are expressed at different levels [88]. Three splice variants of the human *PARG* gene were shown to give rise to PARG isoforms targeted either to the nucleus or to the cytoplasm [89]. Overexpression and immunofluorescence analysis revealed that the largest isoform of PARG is targeted to the nucleus, while the two smaller isoforms show mostly cytoplasmic localization [89]. These observations led to the identification of a strong NLS in the region of the protein coded for by exon 1.

PARG contains a caspase 3 cleavage site that is used during apoptosis [90] and has been reported to contain a putative nuclear export signal [91].

Recently in *Drosophila melanogaster* a loss-of-function mutant was described that lacks the conserved catalytic domain of PARG. This mutant exhibits lethality in the larval stages at the normal developmental temperature of 25 °C [92]. However, about 25% of the mutants progress to the adult stage at 29 °C but showed progressive neurodegeneration with reduced locomotor activity and a shortened lifespan. In association with this, extensive accumulation of poly(ADP-ribose) could be detected in the central nervous system. These results suggest that poly(ADP-ribose) metabolism is required for maintenance of the normal function of neuronal cells.

The structure and biological roles of PARG are discussed in detail by Bonicalzi et al. in this issue of the journal.

ADP-ribosyl protein lyase

Two decades ago an enzyme was isolated from rat liver that was shown to remove the protein-proximal ADP-ribosyl residue [38]. This protein was named ADP-ribosyl protein lyase (formerly termed ADP-ribosyl histone-splitting enzyme). It was shown to liberate a dehydrated form of ADP-ribose (5′-ADP-3″-deoxypent-2″-enofuranose) from the acceptor protein. Its role in poly(ADP-ribose) metabolism is poorly understood as yet.

Biological roles of poly(ADP-ribosyl)ation

DNA repair and maintenance of genomic integrity

A plethora of studies have firmly established that poly(ADP-ribosyl)ation significantly contributes to cellular recovery from cytotoxicity in proliferating cells inflicted with low or moderate levels of DNA damage by alkylation, oxidation or ionising radiation, thus establishing PARP-1 as a ‘survival factor’ [2, 4]. Furthermore, the positive correlation between cellular poly(ADP-ribosyl)ation capacity and life span of mammalian species [93] is in line with the observation that proteins involved in poly(ADP-ribosyl)ation play a crucial role in regulating telomere length [10].

As mentioned above, the expression of both PARP-1 and PARP-2 is essential during early embryogenesis as Parp-1$^{-/-}$ / Parp-2$^{-/-}$ double mutant mice are not viable [5]. PARP-1 and PARP-2 have both been shown to play a crucial role in the BER pathway [3, 5].

Regulation of transcription

It has long been postulated that poly(ADP-ribosyl)ation, as a regulator of chromatin remodelling [33, 94], could influence the regulation of gene expression. Indeed, numerous physical and functional interactions of PARP-1

with transcription factors have been described [95]. For example, PARP-1 plays a pivotal role in NF-κB-dependent gene expression, which makes it an important cofactor in immune and inflammatory responses [6, 7, 96].

Regulation of centromere function

Both constitutive and transient centromere proteins interact with PARP-1 and PARP-2 and undergo poly(ADP-ribosyl)ation, indicating that poly(ADP-ribosyl)ation might act as a regulator of both constitutive kinetochore proteins and those involved in spindle checkpoint control [8, 9]. The absence of any drastic centromeric phenotype in PARP-1 knockout mice is suggestive of some functional redundancy for PARP-1 at the centromere [5].

Modulation of telomere length

Poly(ADP-ribosyl)ation plays a crucial role in telomere length regulation, because nuclear overexpression of Tankyrase-1, but not that of a PARP-deficient derivative, causes the lengthening of telomeres. Tankyrase-1 was therefore proposed to regulate access of telomerase to the telomeric complex through modulation of TRF1 by poly(ADP-ribosyl)ation [11].

Regulation of protein degradation

The proteasome is a large multi-catalytic protease that exists in all eukaryotic cells and is responsible for a major part of intracellular proteolysis. This comprises physiological turnover of a variety of short-lived proteins as well as stress responses [97]. Automodified PARP-1 was reported to activate the proteasome to facilitate selective degradation of oxidatively damaged histones, suggesting a joint role of PARP-1 in the removal of oxidized nucleoproteins and in restoring native chromatin structure following oxidative stress [13, 14].

Regulation of endosomal vesicle trafficking

Endocytotic vesicles in myocytes and adipocytes contain the glucose transporter GLUT4 and IRAP. Reversible translocation of GLUT4 between these GLUT4 vesicles in the Golgi and the plasma membrane allows insulin to regulate glucose utilization [98]. Tankyrase-1 appears to be an important insulin-signalling target, as the protein not only interacts with IRAP in GLUT4 storage vesicles in the Golgi but is also stoichiometrically phosphorylated by MAPK upon insulin stimulation. Tankyrase-1 poly(ADP-ribosyl)ates IRAP, as well as itself, and this activity is enhanced by MAPK-mediated phosphorylation, indicating that Tankyrase-1 may mediate the long-term regulation of GLUT4 vesicles by the MAPK cascade [15, 16].

Regulation of centrosome function

Regulation of centrosome function is crucial to accurate transmission of chromosomes to the daughter cells in mitosis. PARP-1 and PARP-3 have been identified at centrosomes, where they form a stable complex [63] and poly(ADP-ribosyl)ate p53 [61], which has been shown to localize at centrosomes as well and to control centrosome duplication [99]. Thus, both PARP-1 and PARP-3 seem to be involved in centrosome duplication by modulating p53 activity via poly(ADP-ribosyl)ation.

Tankyrase-1 is another member of the PARP family that localizes to the centrosome in a cell cycle-dependent manner. During mitosis, Tankyrase-1 co-localizes with nuclear mitotic apparatus protein (NuMa) [74], with which it was shown to form a stable complex at the centrosome [78]. When NuMa returns to the nucleus after mitosis [100], this co-localization terminates and Tankyrase-1 associates with GLUT4 vesicles that coalesce around centrosomes [15] and function in insulin-dependent glucose utilization [98]. Thus, spindle poles and Golgi alternately contain most of cellular Tankyrase-1, whereas only a small fraction functions at telomeres.

Implications for apoptosis

Specific cleavage of PARP-1 by caspase-3 within the NLS of PARP-1 has long been known to be a hallmark of apoptosis. This proteolytic event is thought to cause a loss of DNA strand break-induced stimulation of the catalytic activity of PARP-1. Nevertheless, during the early stages of apoptosis massive formation of poly(ADP-ribose) can be observed, indicating that PARP-family proteins are involved in this process [101]. Furthermore, it could be shown that PARP-1 activation is required for translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus and that AIF is necessary for PARP-1-dependent cell death [18]. These data support a model, in which PARP-1 activation signals AIF release from mitochondria, resulting in a caspase-independent pathway of programmed cell death [17].

Necrotic cell death and associated pathologic states

Two decades ago a mechanism of cell death depending on overactivation of PARP and severe and irreversible depletion of its substrate NAD^+ was first proposed [102]. In recent years this mechanism has been demonstrated to play a major role in a wide variety of pathophysiological conditions, including ischaemia-reperfusion damage and various inflammatory conditions (for review, see [103]). Based on this mechanism, PARP-inhibitory compounds are currently being developed as novel therapeutics to treat such diseases.

Conclusion

Over the last decade, the poly(ADP-ribose) field has diversified enormously and in multiple respects. Not only has the number of enzymes catalysing poly(ADP-ribosyl)ation increased from one to over a dozen, but also a much wider range of sub-cellular compartments where poly(ADP-ribose) can be found as well as cellular events that seem to be regulated via poly(ADP-ribosyl)ation have emerged. Last but not least, it is very exciting to see that the field has moved away from its exclusive basic-science orientation and now also possesses a great potential for medical application.

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