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Regulation of poly(ADP-ribose) metabolism by poly(ADP-ribose) glycohydrolase: where and when?

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Abstract. Poly(ADP-ribose) glycohydrolase (PARG) is a catabolic enzyme that cleaves ADP-ribose polymers formed by members of the PARP family of enzymes. Despite its discovery and subsequent partial purification in the 1970s [1–3] and the cloning of its single gene in the late 1990s [4], little is known about the role of PARG in cell function. Because of its low abundance within cells and its extreme sensitivity to proteases, PARG has been difficult to study. The existence of several PARG isoforms with different subcellular localizations is still debated today after more than 30 years of intensive research. In this article, we want to summarize and discuss the current knowledge related to PARG, its different forms and subcellular distribution. We also examine the possible biological roles of PARG in modulating chromatin structure, transcription, DNA repair and apoptosis.

Key words. Poly(ADP-ribose) glycohydrolase; poly(ADP-ribose) polymerase; poly(ADP-ribose); subcellular localization; NLS; NES; DNA repair; apoptosis.

Metabolism of poly(ADP-ribose)

Poly(ADP-ribosyl)ation is a posttranslational protein modification catalyzed by members of the poly(ADP-ribose) polymerase (PARP) family. PARP enzymes utilize nicotinamide adenine dinucleotide (NAD^+) as a substrate to form long branched poly(ADP-ribose) polymers (pADPr) mostly on glutamic acid residues of target proteins [5, 6]. The covalently attached negatively charged pADPr molecules alter the physical and biochemical properties of poly(ADP-ribosyl)ated proteins [7, 8]. The best-characterized member of the PARP family is PARP-1, which catalyzes more than 90% of the pADPr synthesis that occurs in response to DNA stand breaks [7]. Many nuclear proteins modified with pADPr have been identified, including PARP-1 itself, histones, topoisomerase I, DNA polymerases α and β and p53 [9–11]. Removal of ADP-ribose polymer chains from the modified proteins is catalyzed by the enzyme poly(ADP-ribose) glycohydrolase (PARG), which is the primary enzyme responsible for pADPr degradation in vivo [12–14]. A second enzyme, ADP-ribosyl protein lyase, removes the proximal ADP-ribosyl moiety bound to the protein [15]. The importance of pADPr catabolism was previously demonstrated in yeast, which is devoid of pADPr metabolic machinery [16]. Yeast cells transformed with human PARP-1 complementary DNA (cDNA) do not survive unless they are cultured in the presence of a competitive inhibitor of PARPs (3-aminobenzamide) or co-transformed with PARG cDNA. More recently, it was demonstrated in *Arabidopsis thaliana* that gene disruption of a PARG ortholog (*tej)* has a substantial effect on circadian rhythms and expression of major circadian genes [17]. In addition, pADPr metabolism has been shown to be involved in many cellular processes such as chromatin decondensation [18, 19], DNA replication [20] and repair [21, 22], transcription [23, 24], centrosome duplication [25], regulation of telomere function [26–28], mitosis [29], necrosis [30, 31] and caspase-dependent [32–34] and -independent apoptosis [35]. It also plays an important role in the maintenance of cellular genetic integrity [7, 36, 37]. Therefore, the enzymatic activities of the various PARPs and PARG must be tightly regulated in order to respond efficiently to a given cellular context.

For more than 3 decades, it was thought that PARP-1 was the only enzyme responsible for poly(ADP-ribosyl)ation

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in living cells. In recent years, several other members of the PARP family have been characterized, namely sPARP-1, PARP-2, PARP-3, tankyrases-1 and -2, vPARP and Ti-PARP (for review see [8]). The roles of these PARPs remain ill-defined. Recent reports indicated that PARP-2 has partially redundant functions with PARP-1 in DNA repair and that tankyrase 1 plays a crucial role during cell division [29, 38]. However, we know that these PARPs show different subcellular localizations. PARP-1 has been localized to the nucleus, centromeres [39] and to the centrosome [40]. Like PARP-1, sPARP-1 is localized to the nucleus [41]. PARP-2 is also a nuclear protein and has been localized to the centromeres and telomeres [38, 42–44]. Some PARP-3 localizes preferentially to the daughter centriole [45], but a shorter form of PARP-3 is also observed in the nuclear and the cytoplasmic compartments [unpublished observations]. Most tankyrase-1 is found at telomeres at interphase but also shows a complex pattern of subcellular localization that varies across the cell cycle [26, 46, 47]. Tankyrase-2 is localized predominantly to a perinuclear region but is also observed at telomeres [48, 49]. vPARP is associated in part with the cytoplasmic vault particles, but a portion is nuclear and localizes to the mitotic spindles [50]. Because PARP family members have different subcellular localizations, it is probable that they will poly(ADP-ribosyl)ate independent target proteins and thereby influence several important but different biological processes.

PARG is present in all eukaryotic cell types, except yeast, due to its antagonistic role with PARPs [51]. PARG possesses both exoglycosidase and endoglycosidase activities and therefore is responsible for the hydrolysis of ribose-ribosyl glycosidic bonds between ADPr units located at the extremity and within the polymer [3, 52]. The basal levels of polymer within unstimulated cells are usually very low [53–56]. However, the nuclear concentration of polymers in mammalian cells is usually within the range of once or twice the K_m value of PARG [13]. Therefore, the nuclear pADPr concentration is sufficient to maintain a constant PARG activity, even in unstimulated cells. In response to DNA strand breaks, the levels of pADPr can increase by 10–500-fold [13, 55, 57]. The synthesis of pADPr is directly proportional to the number of single stand breaks (SSBs) and double strand breaks (DSBs) present in genomic DNA [10]. Also, both constitutive and stimulated levels of ADP-ribose are directly related to the concentration of NAD^+ in cells [58, 59]. pADPr resulting from DNA damage has a very short half-life compared to constitutive polymers (< 1 min compared to 7.7 h) [13]. In fact, the K_m value of PARG for small polymers is higher (by two orders of magnitude) than that for large polymers [60]. In general, branched and short polymers are degraded more slowly than long and linear polymers [60, 61]. As a result, PARG modulates the level and complexity of polymer on different acceptor proteins [62], preventing an accumulation of highly modified nuclear proteins with very long chains of pADPr. This characteristic of PARG is particularly important to allow PARP-1 to remain active in conditions of DNA damage by degrading the polymers that prevent its interaction with DNA. Also, the concerted action of PARG and the various PARPs is critical for the maintenance of appropriate poly(ADP-ribose) levels within the cells to respond efficiently to many other cellular conditions. Furthermore, PARG subcellular distribution is most likely critical to maintain adequate pADPr steady state levels within the different cellular compartments.

Poly(ADP-ribose) glycohydrolase isoforms: various sizes

Although PARG was discovered more than 30 years ago [1], it has been difficult to study because of its low abundance within eukaryotic cells. Many groups have attempted to purify PARG from many different tissues and species and obtained varying degree of purity (table 1). It was only in 1986 that a 59-kDa PARG was purified for the first time to homogeneity from bovine thymus by the group of Ueda and Hayaishi [60]. PARG has since been purified to homogeneity by several other independent laboratories from calf thymus [63], guinea pig liver [64] and human placenta [65], to give only a few examples. However, PARGs isolated by several groups have shown considerable heterogeneity in activity, localization and molecular weight (ranging between 48 and 115 kDa) (table 1). Tanuma suggested the existence of a 75.5-kDa nuclear and a 59.3-kDa cytoplasmic PARG that he named PARG I and PARG II, respectively [66]. Other studies have shown that PARG localized in the nuclear fraction of MDBK cells [67], HeLa S3 and HL-60 cells [68] or pig testis [69]. In 1990, PARG purification was greatly improved both in terms of quantity and quality, by the utilization of an affinity matrix consisting of ADP-ribose polymers bound to dihydroxyboronyl-Sepharose [63]. In 1994, a zymogram was developed to identify functional proteins exhibiting PARG activity [70]. The zymogram consists in resolving the purified enzyme preparation on two-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) containing [^{32}P] automodified PARP-1 as substrate. After renaturation of PARG in the gel, four PARG isoforms were clearly identified with molecular weight ranging from 60 to 74 kDa. The four isoforms were named G1, G2, G3 and G4, and it was proposed at the time that the four PARG isoforms resulted from posttranslational modification. However, we now know that PARG is extremely sensitive to proteases and gets rapidly degraded unless extreme precautions are taken (see below). It is likely that many of the PARGs purified in these early studies were truncated due to proteolysis.

Table 1. Comparison of endogenous PARG isoforms identified in various mammalian tissues

Tissue	Detection	PARG	MW (localization)	References
Calf thymus	activity	PARG	– (N)	Miwa et al. [1]
Rat liver	activity	PARG	– (N)	Ueda et al. [2]
LS cells	activity	PARG	– (N)	Stone et al. [117]
Calf thymus	activity	PARG	48000 to 53000	Miwa et al. [3]
Rat testis	activity	2 variants	–	Burzio et al. [118]
Pig thymus	activity	2 variants	61500 and 67500	Tavassoli et al. [119]
Human erythrocytes	activity	PARG	56000 (C)	Tanuma et al. [84]
Guinea pig liver	activity	PARG I PARG II	75500 (N) – (C)	Tanuma et al. [64]
HeLa S3	activity	PARG I PARG II	72000 (N) 53000 (C)	Tanuma et al. [120]
Calf thymus	activity	PARG	59000	Hatakeyama et al. [60]
Guinea pig liver	activity	PARG I PARG II	75500 (N) 59300 (C)	Tanuma et al. [66]
Calf thymus	activity	PARG	59000, 60000	Thomassin et al. [63]
Human erythrocytes	activity	PARG II	59000 (C)	Tanuma et al. [85]
Guinea pig liver	activity	PARG I PARG II	75500 (N) 59500 (C)	Maruta et al. [121]
Human placenta	activity	PARG I	71000 (N)	Uchida et al. [65]
Calf thymus	zymogram	PARG G1 PARG G2 PARG G3 PARG G4	60000 60000 (doublet) 60000 74000	Brochu et al. [70]
MDBK	indirect IF Western	PARG	– (N) 59000 (N)	Desnoyers et al. [67]
Pig testis	activity	PARG TN	58000 (N)	Abe et al. [69]
HeLa S3 and HL-60	activity	PARG	– (N)	Bernardi et al. [68]
Bovine thymus	activity	PARG	59000	Lin et al. [4]
COS-7	zymogram	PARG	110000 (C)	Winstall et al. [78]
HL-60, Molt-3, Jurkat	zymogram	PARG	110000	Winstall et al. [78]
Rat testis	zymogram	PARG	60000	Shimokawa et al. [72]
Bovine tissue extracts	Western	PARG	65000, 74000, 84000, 115000	Amé et al. [122]
Jurkat	Western	PARG	111000 (C) (doublet)	Affar et al. [73]
Jurkat and HL-60	Western zymogram	PARG	111000, 85000,74000	Affar et al. [73]
HeLa	Indirect IF	PARG	– (C)	Rossi et al. [123]
HeLa	activity	PARG	– (N+C)	Rossi et al. [123]
Rat spermatocytes and spermatids	Western	PARG	110000 (N), 60000 (C)	Di Meglio et al. [86]
Neurons	Western	PARG	111000 (C), 59000 (N)	Sevigny et al. [124]
Rat C6 glioma cells and astrocytes	Western indirect IF	PARG	111000, 59000 (N+C)	Sevigny et al. [124]
HeLa S3	zymogram Western	PARG	110000 (N), 103000 (C)	Bonicalzi et al. [74]
HL-60 cells	activity	PARG	– (N+C)	Uchiumi et al. [125]

N, nuclei; C, cytoplasm; IF, immunofluorescence; MW, molecular weight in Daltons.

The isolation of the first PARG cDNA, from bovine, revealed that a 4.1-kb cDNA encodes a PARG with a predicted molecular weight of 110 kDa, which is nearly twice the size of PARG isolated from bovine thymus and other tissues [4]. Southern analysis of bovine genomic DNA indicated that PARG is encoded by a single-copy gene (located on chromosome 10q11.23 in humans) [4, 71]. A single transcript of approximately 4.3 kb messenger RNA (mRNA) was detected by Northern blotting in bovine kidney poly(A)$^+$ RNA [4]. In fact, it was later confirmed by Shimokawa et al. that PARG is ubiquitously expressed as a single 4.0-kb mRNA in various rat tissues [72]. Despite this finding, when PARG cDNA was expressed in *Escherichia coli*, it resulted in two enzymatically active proteins of 110 kDa and 59 kDa (table 2) [4]. Many groups then suggested that the multiple PARG isoforms previously identified could have resulted from the proteolysis of a 110-kDa PARG enzyme. By using a strategy similar to that of Thomassin et al. [63] to partially purify (848-fold) PARG from a cytosolic fraction of Jurkat cells, Affar et al. noticed that a fragment of 59 kDa was produced in the later steps of purification [73]. The appearance of this fragment during the purification procedures suggests that PARG contains a site that is very sensitive to proteases. In fact, we recently showed that only the fact of using 0.05% trypsin to detach the cells from culture dishes and freezing cellular extracts at –80 °C for 16 h before gel loading results in important proteolysis of PARG [74]. Therefore, extreme precautions must be taken to preserve PARG integrity when preparing cellular extracts. Whether proteolysis of the 110-kDa PARG occurs in vivo or only during purification of the enzyme remains to be determined.

Recently, Meyer et al. characterized the human PARG gene structure [75]. They have shown that the open reading frame of PARG consists of 18 exons and 17 introns with exons 9–14 forming the catalytic center of the enzyme and exons 1–3 encoding the potential regulatory domain (fig. 1). Interestingly, expression of their PARG cDNA by in vitro coupled transcription and translation yielded several specific bands in the range of 85–111 kDa [75]. They suggested that in vitro translation may start at M^{83} in exon 2 or M^{109} in exon 3, representing the two other available ATG triplets in this open reading frame (fig.1B). A recent report by the same group describes two splice variants of human *PARG* mRNA which lead to expression of PARG isoforms of 102 kDa (hPARG102) and 99 kDa (hPARG99) in addition to the full-length PARG protein (hPARG111) [76]. These splice variants differ from hPARG111 by the lack of exon 1 (hPARG102) or exons 1 and 2 (hPARG99). Moreover, a search through the human and mouse expressed sequence tag databases using the BLAST tool of the NCBI revealed the existence of more than one PARG transcript that could not be resolved by Northern blot analysis [74, 75]. In fact, a number of PARG transcripts appear to lack the predicted exon 1 (accession numbers: human, BG719380; mouse, BB638662, CB724195, BY299765, CF903209) [74, 75]. These results indicate that previous reports of multiple PARGs can be explained by alternative splicing or promoter selection.

Table 2. Comparison of overexpressed PARG in various cell lines.

Expression in	Detection	MW (localization)	References
E. coli (110.8-kDa bovine PARG in pTrcHisB)	zymogram	115000 59000	Lin et al. [4]
COS-7 cells (103-kDa bovine PARG in pRc/CMV)	zymogram	103000 (C>N)	Winstall et al. [78]
COS-7 cells (103-kDa bovine PARG in pRc/CMV)	Western blot	103000 to 110000 (C>N) (triplet)	Winstall et al. [77]
COS-7 cells (103-kDa bovine PARG in pRc/CMV)	indirect IF	– (C>N)	Winstall et al. [77]
COS-7 cells (103-kDa bovine PARG and 74-kDa bovine PARG in pEGFPC1)	GFP fusion localization	– (C>N)	Affar et al. [73]
NIH 3T3 and 293 T cells (110-kDa human PARG in pEGFPC1 or pCMV-Myc)	GFP fusion localization or indirect IF	– (N) varies with cell cycle	Ohashi et al. [83]
In vitro transcription/translation (110-kDa human PARG in pcDNA 3.1)	Western blot	110000 102000 80000	Meyer et al. [75]
COS-7 cells (103-kDa bovine PARG, 85- and 74-kDa bovine PARG in pEGFPC1)	GFP fusion localization	– (C>N) shuttling	Bonicalzi et al. [74]
HEK293 cells (111-kDa human PARG, 102- and 99-kDa human PARG in pcDNA)	indirect IF	110000 (N>C) 102000 (C>N) 99000 (C>N)	Meyer-Ficca et al. [76]

N, nuclei; C,cytoplasm; IF, immunofluorescence; MW, molecular weight in Daltons.

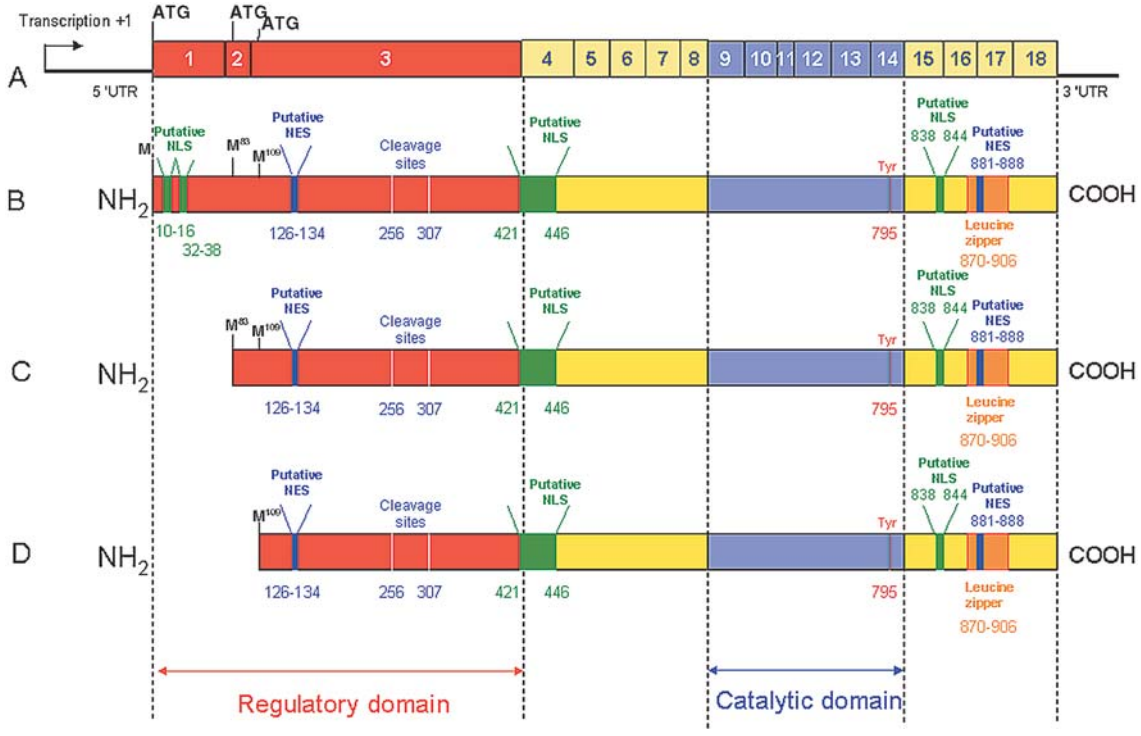

Figure 1. Schematic representation of human PARG. (*A*) Organization of the human *PARG* gene. The organization of the *PARG* gene with distribution of its 18 exons is given with the corresponding protein domains. The putative regulatory domain is formed by exons 1–3 and the catalytic domain is encoded by exons 9–14. The three putative alternative translation initiation sites are identified by ATG triplets. (*B*) Schematic diagram of the full-length 110-kDa human PARG protein and representation of its putative structural motifs. Putative NLS and NES that were proposed in rat and bovine PARG amino acid sequences are very well conserved in human PARG. The amino acid positions of the different motifs correspond to their positions in the sequence of human PARG. The caspase cleavage site at amino acid position 256 is present only in human PARG, whereas the cleavage site at amino acid position 307 is conserved in mouse, rat, bovine and human PARG. The three methionines encoded by the three ATG triplets represented in (*A*) are identified M^1, M^{83} and M^{109}. (*C*) Schematic diagram of 103 kDa human PARG and representation of its putative structural motifs. This 103-kDa PARG may result from alternative translation initiation starting at M^{83} or from alternative splicing of exon 1. (*D*) Schematic diagram of 99-kDa human PARG and representation of its putative structural motifs. This 99-kDa PARG may result from alternative translation initiation starting at M^{109} or from alternative splicing of exons 1 and 2.

Our previous efforts to clone bovine PARG always resulted in a cDNA encoding the 103-kDa PARG lacking the sequence encoded by exon 1 [77]. This bovine PARG construct could resemble the PARG product resulting from alternative translation initiation starting at M^{83}. It could as well be similar to the result of alternative splicing of the exon 1. When we transfected bovine PARG cDNA in COS-7 cells, a pattern of three distinct bands displaying apparent molecular weight between 103 and 110 kDa was detected by Western blot analysis (table 2) [77]. We then suggested that the bands with lower migrating rates could represent posttranslationally modified forms of the 103-kDa PARG. We have also looked at endogenous PARG in Jurkat cells by Western blot and observed a doublet of bands around 110 kDa [73]. Furthermore, we have performed zymograms to look at endogenous PARG activity in many different cell types such as COS-7, HL-60, Molt-3 and Jurkat cells and always detected a doublet of bands around 110 kDa [78]. We suggested that those bands could correspond to posttranslational modification or different translation origin.

Affar et al. also found that all forms of endogenous PARG were cleaved during etoposide-, staurosporine and Fas-induced apoptosis in human cells. We demonstrated that PARG cleavage by caspase-3 during apoptosis generated two C-terminal fragments of 85 and 74 kDa that retained enzymatic activity [73]. We mapped caspase cleavage sites at amino acid positions 256 (DEID) and 307 (MDVD) of the human PARG sequence (fig. 1B). One may stipulate that the previous finding of a 75-kDa isoform of PARG during purification from guinea pig liver nuclei by Tanuma et al. [64] corresponds to the generation of an active apoptotic fragment of 74 kDa. However, there are inconsistencies between this explanation and recent findings showing that both PARG apoptotic fragments fused to green fluorescent protein (GFP) localize mostly in the cytoplasmic compartment of COS-7 cells [73, 74].

It seems clear that despite many efforts to prevent PARG proteolysis during sample preparation, endogenous PARG appears as multiple bands around 110 kDa on SDS-PAGE. Whether it results from alternative splicing, posttranslational modifications, alternative origin of translation and/or proteolysis is not yet elucidated.

Poly(ADP-ribose) glycohydrolase isoforms: various subcellular localizations

Along with the numerous possible isoforms of PARG described above comes another highly debated issue, related to PARG subcellular localization. The availability of the full-length PARG cDNA facilitated the study of many unanswered questions concerning its localization within cells and its biological role in controlling pADPr metabolism. In 1997, Lin et al. reported the isolation and characterization of the cDNA encoding bovine PARG [4]. Comparison of the bovine PARG amino acid sequence with protein sequence databases did not reveal any sequence similarity with known proteins or functional domains. The amino acid sequence of bovine PARG has been examined closely for structural and functional motifs. Lin et al. have identified a putative bipartite nuclear localization signal (NLS) at amino acid positions 422–447 of the bovine PARG sequence that resembles the NLS of PARP-1 [4]. Also, residues 871–907 of bovine PARG show significant similarity to known leucine zipper dimerization sequences [79]. In 1999, a similar analysis of rat PARG sequence was published [72]. It shows a high degree of similarity with other mammalian PARGs and comprises the NLS previously reported by Lin et al. In addition, Shimokawa identified other classical type NLSs at amino acid positions 33–39 and 834–840 of rat PARG sequences as well as a potential nuclear export signal (NES) in the N-terminal half of rat PARG [72]. This NES, located at amino acid positions 124–132 of rat PARG, matches exactly the human immunodeficiency virus (HIV) Rev type NES [80, 81] and is very well conserved in other mammalian PARGs. Another putative NES was found near the C-terminal region at the amino acid position 877–884 of rat PARG, sharing homology with the leucine-rich export signal of p53 [82]. Also, a recent report demonstrated that the amino acids encoded by exon 1 of human PARG, specifically residues ^{10}CTKRPRW^{16}, comprise a functional NLS for targeting PARG to the nucleus. In the same paper, it is also demonstrated that the previously reported putative bipartite NLS at amino acid positions 415–439 does not function as a nuclear targeting sequence when fused to the GFL reporter protein [76]. Figure 1 illustrates the human PARG protein with the representation of the previously mentioned motifs identified in bovine or rat PARG sequences but very well conserved in the human PARG sequence as well.

The finding of putative NLSs and NESs in mammalian PARG sequences suggests that PARG may reside in both cytoplasm and nucleus. A systematic analysis of various PARG constructs will be necessary to define the functional localization signal sequences. However, recent reports of the subcellular distribution of several PARG constructs hint at how some conflicting results regarding PARG localization may be reconciled. Overexpression of our 103-kDa bovine PARG (lacking the sequence encoded by exon 1) in COS-7 cells showed a preferential cytoplasmic localization [77]. In fact, densiometric scanning of Western blots revealed that PARG detected in the cytoplasmic fraction of cells overexpressing PARG accounted for 85% of the total amount of cellular PARG [77]. These results were confirmed by analyzing the cellular localization of a GFP fusion protein of 103-kDa bovine PARG. This fusion protein localized predominantly in the cytoplasm of COS-7 cells [73, 74]. However, another recent study that looked at the cellular distribution of transiently expressed GFP- or Myc-tagged human full-length 110-kDa PARG in mammalian cells revealed that GFP-hPARG is found almost exclusively in the nucleus during interphase, although its cellular distribution changes dramatically during the cell cycle [83]. It is likely that full-length bovine PARG localizes to the nucleus during interphase such as full-length human [83] and mouse PARG [unpublished observations]. The recent observations that the NLS at amino acid positions 10–16 in human PARG is functional and that overexpressed hPARG111 is nuclear while hPARG102 and hPARG99 are cytoplasmic suggest that the first 82 amino acids of the PARG sequence, encoded by exon 1, comprise crucial sequences for the regulation of PARG localization [76]. It was previously hypothesized that the N-terminal domain of PARG was likely to be responsible for the regulation of PARG function in vivo [75]. The N-terminal of PARG might have a crucial role to play in maintaining full-length PARG in the nucleus of cells in normal condition. As we mentioned above, we systematically detected endogenous PARG as a doublet of bands of about 110 and 103 kDa following SDS-PAGE and Western blotting or zymograms. Interestingly, the band with the fastest migrating rate is more abundant than the slowest PARG isoform [73, 74, 77, 78]. We have recently analyzed the subcellular distribution of endogenous PARG by isolating different fractions of Jurkat [73] or HeLa S3 cells [74]. We confirmed that PARG is predominantly found in the soluble fraction of the cytoplasm. The observation that enucleated human erythrocytes contain poly(ADP-ribose) glycohydrolase activity supports the finding of a cytoplasmic form of PARG [84, 85]. We also found that PARG localizes in other cell compartments that are mainly concentrated in the Golgi and endoplamic reticulum enriched fractions [74]. In fact, we have shown colocalization of PARG with FTCD, a protein specifi-

cally associated with the Golgi. In this same study, we observed that the 103-kDa PARG isoform is the predominant form in all fractions except in the nuclear fraction where the relative amount of 110 and 103 kDa PARG is similar [74]. These results were recently confirmed by Meyer et al. that have shown that endogenous PARG is detectable mainly in the cytoplasm due to the predominance of the hPARG102 and hPARG99 isoforms [76]. Also, these results are consistent with those observed by another group showing that full-length endogenous 110-kDa PARG is mainly localized in the nucleus of rat primary spermatocytes [86].

For many years, we thought that pADPr synthesis was a phenomenon that occurred exclusively in the nuclei of mammalian cells. Therefore, the biological functions of a nuclear PARG had a great deal of interest. However, with the recent findings of cytoplasmic PARPs, the biological significance of a cytoplasmic PARG become obvious. We have recently demonstrated that the cytoplasmic PARG isoform is the most abundant PARG isoform in quantity and activity [74]. Also, experiments using leptomycin B, a drug that specifically inhibits NES-dependent nuclear export, allowed us to confirm that cytoplasmic PARG is engaged in CRM1-dependent nuclear export and shuttles between the nuclear and cytoplasmic compartments [74]. At the moment, little is known regarding the biological significance of the proposed nucleocytoplasmic shuttling and the presence of different PARG isoforms. We may hypothesize that the nucleocytoplasmic shuttling kinetics of cytoplasmic PARG might be affected by genotoxic stress and the relative amount of enzymatically active PARG in both compartments might be changing accordingly. Therefore, nucleocytoplasmic shuttling of PARG might be essential for a tight control of pADPr catabolism. Further experiments will be aimed at defining the concerted action of the various PARG isoforms and PARPs in different cellular compartments and in diverse cellular contexts. These experiments should shed some light on the biological functions of the different PARG isoforms.

Poly(ADP-ribose) glycohydrolase isoforms: defining their biological functions

In recent years, progress has been made in elucidating the mechanism of poly(ADP-ribose) catabolism. The biological functions of this process are still the subject of intensive study. Clearly, the scientific community will need powerful tools to study and define the precise function of poly(ADP-ribose) catabolism in metazoan. Investigations carried out with genetic alterations or pharmacological inhibition of PARG will give important clues to elucidate the prevalent role of poly(ADP-ribose) catabolism.

Genetic alterations of PARG enzyme

Only a limited number of genetic models have been developed to study PARG functions. Nevertheless, it has been shown in *Arabidopsis thaliana* that a single point mutation within the PARG catalytic domain of *tej* gene product (the plant ortholog of *parg*) alters circadian rhythms [17]. This was the first report of a loss-of-function mutant of PARG. The mutant was found after screening for plants with altered circadian period length. Characterization of this *tej* mutant revealed that the deficiency of PARG activity results in the perpetual automodification of PARP and in the modulation of transcription rates of clock-controlled genes [17]. The phenotype of this PARG mutant strongly supports the importance of tightly regulated poly(ADP-ribose) levels for the regulation of gene transcription. In the context of this study, the extent of poly(ADP-ribosyl)ation by the concerted action of PARPs and PARG could determine the circadian period length.

More recently, a loss-of-function mutant of PARG in *Drosophila* was reported [87]. The mutant lacks the entire conserved catalytic domain of PARG and exhibits lethality in the larval stage at the normal development temperature of 25 °C. The fact that the *Drosophila parg*$^{-/-}$ mutants could not develop into adult flies indicates that PARG is essential for development of the fruit fly [87]. However, one-fourth of the mutants progressed to adult stage at 29 °C but showed progressive neurodegeneration with reduced locomotor activity and a short lifespan. Moreover, an important accumulation of poly(ADP-ribose) could be detected in the central nervous system of the adult PARG mutants. These results suggest that poly(ADP-ribose) metabolism is required for maintenance of the normal function of neuronal cells. Another important observation reported by Hanai and co-workers is the cellular distribution of the accumulated poly(ADP-ribose). In fact, they observed a wide accumulation of poly(ADP-ribose) throughout the cells. [87]. For the first time, an accumulation of poly(ADP-ribose) outside of the nuclear compartment could be demonstrated, suggesting a crucial role of an active PARG isoform to regulate poly(ADP-ribose) levels in the cytoplasm. Hanai et al. suggested that the phenotype observed in *Drosophila* PARG mutant might be useful to better understand neurodegenerative conditions such as Alzheimer and Parkinson diseases.

In a recent review, Masutani and co-workers reported a *parg*$^{-/-}$ embryonic stem (ES) cell line generated by gene targeting [88]. This cell line was reported to survive but presented an increased sensitivity to methylmethanesulfonate, as well as to γ irradiation and featured increased basal poly(ADP-ribose) levels and early apoptosis [88].

Pharmacological inhibition of PARG enzyme activity

A specific and potent inhibitor for PARG would be a great tool for understanding the physiological role of poly(ADP-ribose) catabolism. So far, no good inhibitor of PARG has been found useful to characterize its biological functions. It was previously suggested that tannins (gallotannin, ellagitannin or tannic acid), extracted from green tea leaves, could be potent inhibitors of PARG [89–91]. Previous studies indicated that oxidative and excitotoxic neuronal death could be inhibited following suppression of PARG activity by gallotannin [92, 93]. Interestingly, it was observed that gallotannin slows down the ADP-ribose polymer turnover and thus limits the NAD^+ depletion that would otherwise lead to cell death [94]. However, recent studies have suggested that pADPr accumulation following a gallotannin treatment could be resulting from a combination of non-specific effects [95, 96]. Novel convincing data demonstrated that the cytoprotective effect of gallotannin is likely to be more a general phenomenon of anti-oxidant compounds rather than a true PARG inhibition effect [95, 96]. More important, a recent publication demonstrates that anti-oxidative properties of tannins may change to pro-oxidative activities at higher concentrations [97]. These results reported by Labieniec et al. clearly demonstrate that tannic acid can contribute to DNA damage and thus leads to PARP-1 activation. PARP-1 activation will inevitably result in an increase of pADPr cellular levels, a phenomenon that could be mistaken for a consequence of PARG inhibition by gallotannin. These new observations on tannins suggest that studies that have used tannins as PARG inhibitors should be re-evaluated critically.

Another PARG inhibitor is the adenosine diphosphate-(hydroxymethyl)-pyrrolidinediol (ADP-HPD), an analogue of ADP-ribose. It was identified as a partial noncompetitive PARG inhibitor with an IC_{50} (Concentration needed for 50% inhibition) of approximately 0.33 μM [98]. A direct interaction between the adenine ring of ADP-HPD and a conserved residue of PARG enzyme across organisms, the tyrosine 795 (Y795) of human PARG (fig. 1), results in PARG inhibition [99]. The potency of inhibition by ADP-HDP suggests that it could be useful for studying pADPr metabolism. However, ADP-HDP is not cell permeable, which is critical for cell culture-based experiments. Furthermore, this ADP-ribose analogue still contains the adjoining phosphate linkages that could be cleaved by phosphodiesterases into ADP and HDP moieties. Each of these separate moieties cannot by itself inhibit PARG [100]. The lack of specific or membrane-permeable compounds to inhibit PARG activity is a real problem, and it puts pressure on scientists to develop new PARG inhibitors to further determine the precise biological functions of PARG.

Evidence for the involvement of PARG in chromatin structure and transcription

Several lines of evidence suggest that poly(ADP-ribosyl)ation cycling is required for structural chromatin remodelling during base excision repair (BER) [21, 22], transcription [23, 24], DNA replication [20] and numerous cell death pathways [30–35]. It has been reported that pADPr synthesis during active transcription contributes to decondensation of chromatin structure [101]. Thus, remodelling of chromatin structure through pADPr synthesis gives open access to DNA for the transcription machinery. Moreover, the contribution of the scaffolding properties of poly(ADP-ribose) and the synergy between PARPs and PARG to regulate or modulate chromatin structure suggest a mechanism that could be very similar to the chromatin remodelling orchestrated by the SWI/SNF complex. PARP-1 binding to damaged DNA results in its activation and in the generation of a poly(ADP-ribose) network. Poly(ADP-ribosyl)ation of DNA-associated proteins causes the decondensation of chromatin and provides DNA access to repair enzymes [101]. Degradation of pADPr through PARG action releases pADPr-bound or modified proteins, which lead to recondensation of chromatin structure. This represents a unified model where PARP-1 and PARG act together to remodel chromatin by regulating pADPr metabolism. Despite the fact that a precise role for PARG in transcriptional regulation has not yet been demonstrated directly, interesting data from recent studies suggest that PARG, with its unique pADPr catabolism properties, could counteract the chromatin-modifying actions of PARPs and thus modulate the transcriptional levels [17, 87, 101–104]. As mentioned earlier, this modulation of transcription level by the PARG ortholog *tej* was also observed for clock-controlled genes in *Arabidopsis thaliana* [17]. Additionally, it was demonstrated that in the absence of PARG activity, an extensive accumulation of poly(ADP-ribosyl)ated proteins leads to abnormal chromatin remodelling and disordered transcription during first stage of development of *Drosophila* larvae [87]. This strongly suggests that poly(ADP-ribose)-dependent modulation of chromatin structure might be tightly regulated by an active PARG in the nucleus.

Contribution of PARG in the DNA repair

Many studies have shown that DNA damage caused by alkylating or oxidative agents results in considerable synthesis of pADPr near the site of damaged DNA. PARG is predicted to participate in the DNA repair cycle by removing the poly(ADP-ribose) from poly(ADP-ribosyl)ated proteins and automodified PARP-1, leading to recondensation of chromatin structure [102]. Also,

Maruta et al. suggested that the concerted action of PARG and ADP-ribose pyrophosphorylase is capable of generating ATP from ADP-ribose units [105]. This process is likely nuclear, leading to the presumption that an active nuclear PARG isoform will participate in producing an increased level of ATP around the site of damaged DNA. An additional study, conducted by Oei and Ziegler [106], has proposed that ATP required for the DNA ligation step in base excision repair is generated by poly(ADP-ribose) cycling. Overall, it seems that PARG acts in concert with PARP-1 to modulate the chromatin structure in order for the DNA repair machinery to access the site of damaged DNA. By recycling the pADPr into ATP, PARG participates in the regeneration of a local ATP pool that is useful for ligation of damaged DNA. Through these actions, PARG is taught to participate in the maintenance of genome integrity. Also, it is interesting to note that the enzymes ADP-ribose pyrophosphatases generate important concentrations of AMP from ADP-ribose units [107, 108]. Moreover, AMP is known to stimulate AMP-kinase, which is involved in lipid and glucose metabolism [109, 110].

Role of PARG in apoptosis and cell death

Cells undergoing apoptosis show only a transient synthesis of pADPr. Once more, it seems that a nuclear active form of PARG is implicated in the proper control of pADPr levels during apoptosis and cell death pathways. As mentioned above, it was demonstrated that human PARG is cleaved by caspase 3 during apoptosis [73]. The early cleavage of both PARP-1 and PARG by caspases indicates that regulation of poly(ADP-ribose) metabolism is primordial to cell death pathways. Following PARP-1 activation, high amounts of both free ADP-ribose units and ADP-ribose oligomers are generated by PARG activity [67]. We already know that free ADP-ribose units interact via non-covalent associations with key proteins that modulate chromatin structure and DNA repair [111, 112]. Therefore, it is tempting to speculate that protein-free poly(ADP-ribose) generated through PARG activity might act as a death signal. We may hypothesize that when the full-length nuclear PARG is cleaved by caspases and relocalized to the cytoplasm, it may carry oligomers of ADP-ribose in the cytoplasmic compartment. Two recent studies have suggested physiological roles for pADPr in the cytoplasm. First, Dumitriu et al. [113] have shown that degradation of pADPr by PARG, following ultraviolet irradiation, generated a relatively high concentration of ADP-ribose in the nucleus. Since ADP-ribose is an analogue of ADP and ATP, it can diffuse from the nucleus to the cytoplasm. Therefore, as soon as the level of cytoplasmic ADP-ribose exceeds a certain threshold, it will compete with ATP for binding to the ABC transporters located at the plasma membrane and cause their inhibition [113]. Similarly, Yu and co-workers have recently suggested that pADPr can serve as a death signal in the cytoplasm by triggering AIF release from the mitochondria in a PARP-1-dependent, caspase-independent fashion [35, 114]. Therefore, regulation of pADPr metabolism by PARPs and PARG in the nucleus could modulate the levels of cytoplasmic pADPr and therefore indirectly induce apoptotic events in the cytoplasm. This could explain why, in response to DNA damage, the accumulation of pADPr in *parg*$^{-/-}$ ES cell line leads to early apoptosis [88]. Clearly, further studies will be needed to elucidate the physiological function of cytoplasmic pADPr.

In this review, we have discussed evidence linking PARG to many fundamental processes such as chromatin remodelling, transcription, DNA repair and apoptosis. The presence of the majority of PARG activity in the cytoplasmic compartment of cells raises interesting new questions concerning the function of PARG in the cytoplasm. Similar to the cellular distribution of p53 that changes upon cellular situation [115, 116], we think that PARG activity might be required in different cellular compartments to respond efficiently to various cellular stimuli.

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