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The expanding field of poly(ADP-ribosyl)ation reactions

'Protein Modifications: Beyond the Usual Suspects' Review Series

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins that is mediated by poly(ADP-ribose) polymerases (PARPs). Although the existence and nature of the nucleic acid-like molecule poly(ADP-ribose) (PAR) has been known for 40 years, understanding its biological functions—originally thought to be only the regulation of chromatin superstructure when DNA is broken—is still the subject of intense research. Here, we review the mechanisms controlling the biosynthesis of this complex macromolecule and some of its main biological functions, with an emphasis on the most recent advances and hypotheses that have developed in this rapidly growing field.

Keywords: ADP-ribose; chromatin; DNA repair; NAD^+ metabolism; transcription

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See Glossary for abbreviations used in this article.

Introduction

PARPs catalyse the transfer and polymerization of ADP-ribose units from NAD^+ to form a ramified polymer—known as PAR—which can be covalently linked mainly to glutamic residues of acceptor proteins (Fig 1). DNA-strand breakage was long considered to be the main trigger of PAR synthesis through the activation of PARP1, which led to opposite outcomes—repair of the damaged site and cell survival, or cell death—depending on the cellular context and on the intensity of the DNA insult. Poly(ADP-ribosyl)ation in response to DNA damage is highly energy-consuming, as PAR size is estimated to reach up to 200 units under stress conditions. However, the size and branching of PAR synthesized under normal conditions is still unclear. The recent discovery of novel putative PARP proteins—which have increased the number of PARP family members to 17 (Fig 2)—has considerably extended the field of study of this protein modification, which occurs only in non-yeast eukaryotes (Amé *et al*, 2004; Otto *et al*, 2005; Schreiber *et al*, 2006). It remains to be determined whether all PARP family members can effectively synthesize PAR and, if so, whether this PAR has a comparable structure to that synthesized by the founding member PARP1. In addition, some PARP family members, which lack conserved residues crucial for polymer elongation, could instead be mono(ADP-ribose) transferases. A detailed biochemical characterization of each PARP family member will allow us to answer these questions in the future. PAR can be degraded either by PARG—which is encoded by a single gene but is present in different isoforms that show various subcellular localizations (for a review, see Gagne *et al*, 2006)—or by the recently described ARH3, which shares little, if any, sequence similarity with PARG (Oka *et al*, 2006). However, if, how and when the last ADP-ribose moiety is removed from the target protein by a lyase activity remains an open question.

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Functional roles of PAR at the molecular level

Poly(ADP-ribosyl)ation modulates protein function by regulating either enzymatic activities or macromolecular interactions with proteins, DNA or RNA. On the other hand, PAR molecules can also regulate protein activity and function through non-covalent binding. This is illustrated by the exponentially growing list of protein–protein, protein–DNA and protein–RNA interactions that either require or are prevented by PAR. However, the role of non-covalent PAR-binding is likely to attract protein targets to specific subcellular sites. A somewhat conserved 20 amino-acid PAR-binding motif was initially established from the analysis of several DNA-repair and checkpoint proteins (Pleschke *et al*, 2000). More recently, some macro domains—such as those found in PARP9 or in a viral protein from the SARS coronavirus—and a C_2H_2 zinc-finger, known as the PBZ, have also been shown to bind to PAR *in vitro* (Ahel *et al*, 2008; Egloff *et al*, 2006; Karras *et al*, 2005). In some cases, a single protein target can bind to PAR non-covalently and also be an acceptor for poly(ADP-ribosyl)ation, which are processes that probably have different functional outcomes, such as recruitment to a site were PAR is produced and modification of the activity of the recruited protein, respectively. Whether the effect of PAR is due to steric hindrance or to electrostatic repulsion generated by the negatively charged ADP-ribose polymers is still an open question. Differences in the length and branching of PAR probably add another level of regulation that could allow different functional outcomes (Fahrer *et al*, 2007).

Among the alternative roles proposed for PAR is the provocative but appealing idea of it being a local supply of ATP molecules, which is important in conditions of ATP shortage. PARP1 activation

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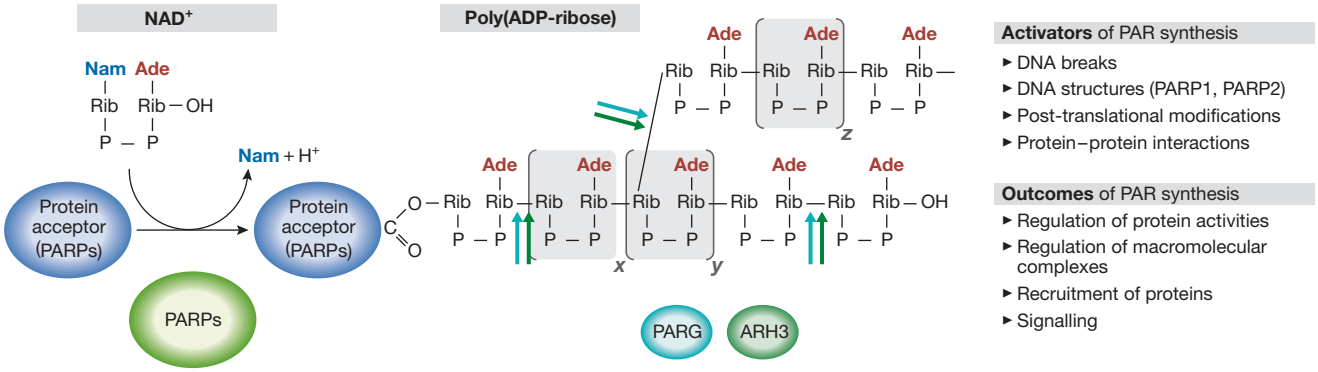

Fig 1 | Metabolism of poly(ADP-ribose). PARPs hydrolyse NAD^+ and catalyse the successive addition of ADP-ribose units mainly to glutamate residues of either acceptor proteins (heteromodification) or themselves (automodification). PAR is heterogeneous in size and complexity, as indicated by the *x*, *y* and *z* labels that represent values from 0 to >200. PARG and ARH3 can both hydrolyse PAR at the indicated positions; activators and outcomes of PAR synthesis are indicated. Ade, adenine; ARH3, ADP-ribosyl hydrolase-3; Nam, nicotinamide; PAR, poly(ADP-ribose); PARG, poly(ADP-ribose) glycohydrolase; PARP, poly(ADP-ribose) polymerase; Rib, ribose.

in response to DNA breaks could support the ATP supply necessary for ligation, which is the final step of single-strand break repair (SSBR). Converting PAR into ATP requires pyrophosphate, the by-product of nucleotide incorporation, which occurs during DNA-repair synthesis (Petermann *et al*, 2003). Supporting this idea, AMPK, which is activated when the ATP concentration is limiting, phosphorylates PARP1, thereby enhancing its automodification (Walker *et al*, 2006).

A role for PAR, and/or ADP-ribose, as a signalling molecule is also an attractive idea. In this regard, the essential contribution of PAR during the initiation of a caspase-independent cell-death pathway is described below. In addition, it has long been known that ADP-ribose acts as a second messenger that activates the TRPM2 channel to allow Ca^{2+} influx in response to oxidative stress, although a direct involvement of PARP1/PARG in producing these ADP-ribose molecules was only recently revealed (Buelow *et al*, 2008).

The extended biological outcomes of poly(ADP-ribosyl)ation

The human PARP family members that have been studied using biochemical or genetic approaches have already increased the diversity of cellular processes in which PAR or PARPs seem to have a role (Table 1). Here, we discuss some of these processes, with a particular emphasis on those that have been described recently.

The role of PAR in the regulation of chromatin structure, DNA repair, replication, transcription, insulation and telomere homeostasis has been described in several recent in-depth reviews, and is therefore only briefly mentioned here (Hsiao & Smith, 2008; Kraus, 2008; Schreiber *et al*, 2006). PAR is a crucial regulator of the DNA-damage response that involves the SSBR/BER pathway. PARP1 activation by DNA breaks leads to the relaxation of chromatin superstructure owing to poly(ADP-ribosyl)ation of histones—mainly H1—and favours the recruitment of repair factors that have a strong affinity for PAR, such as the SSBR/BER-repair scaffold protein XRCC1 (El-Khamisy *et al*, 2003; Okano *et al*, 2003). The interaction of PAR with many other repair factors and checkpoint proteins probably also contributes to the DNA-damage response (Ahel

Glossary

AIF	apoptosis-inducing factor
AMPK	AMP-activated kinase
ARH3	ADP-ribosyl hydrolase-3
BAL1	B-aggressive lymphoma 1
BAX	B-cell lymphoma 2-associated X protein
BER	base-excision repair
BRCA1	breast cancer 1, early onset
CBP	cyclic-AMP-responsive-element-binding protein (CREB)-binding protein
Coast6	collaborator of signal transducer and activator of transcription 6
CRM1	chromosome region maintenance protein 1
CTCF	CCCTC-binding factor
DNMT1	DNA methyltransferase 1
ERK	extracellular signal-regulated kinase
FACT	facilitates chromatin transcription
HIV-1	human immunodeficiency virus-1
HSP70.1	heat-shock 70-kDa protein 1
KIF4	kinesin superfamily 4
NF-κB	nuclear factor-κB
NMNAT1	nicotinamide mononucleotide adenylyl transferase 1
PAR	poly(ADP-ribose)
PARG	poly(ADP-ribose) glycohydrolase
PARP	poly(ADP-ribose) polymerase
PBZ	PAR-binding zinc finger
PDGF	platelet-derived growth factor
SARS	severe acute respiratory syndrome
SIRT1	sirtuin 1
TiPARP	2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible poly(ADP-ribose) polymerase
TRPM2	Transient receptor potential melastatin 2
vPARP	vault poly(ADP-ribose) polymerase
VPR	viral protein R
XRCC1	X-ray cross-complementing group 1

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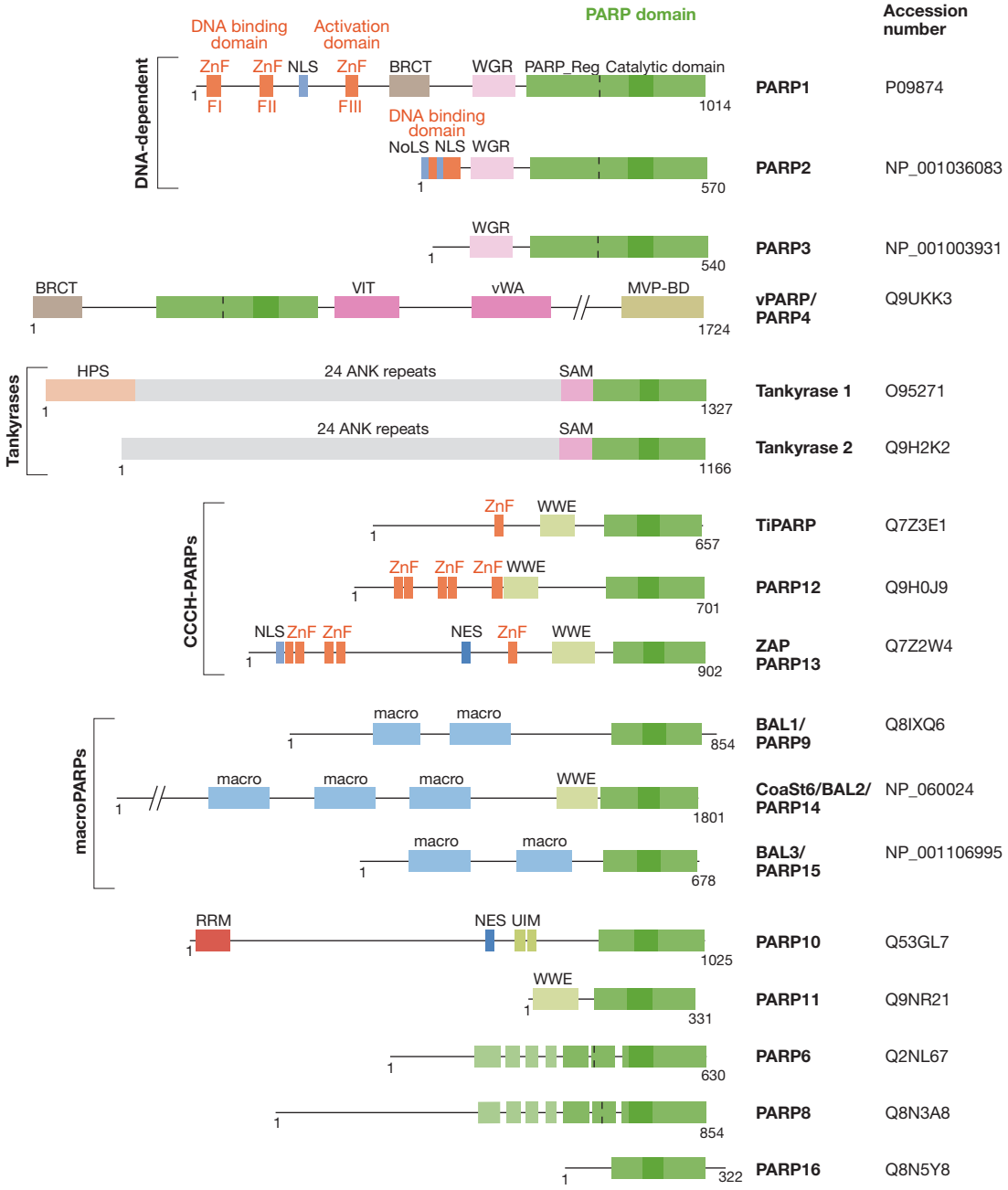

Fig 2 | Domain architecture of human poly(ADP-ribose) polymerase family members. Within each putative PARP domain, the region that is homologous to residues 859–908 of PARP1—the PARP signature—is indicated by a darker colour. BRCT, SAM, UIM, MVP-BD, VWA and ANK are protein-interaction modules. ANK, ankyrin; BRCT, BRCA1-carboxy-terminus; HPS, homopolymeric runs of His, Pro and Ser; macro, domain involved in ADP-ribose and poly(ADP-ribose) binding; MVP-BD, MVP-binding; NES, nuclear export signal; N(o)LS, nuclear (nucleolar) localization signal; PARP, poly(ADP-ribose) polymerase; PARP_Reg, putative regulatory domain; RRM, RNA-binding motif; SAM, sterile α-motif; TiPARP, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible poly(ADP-ribose) polymerase; UIM, ubiquitin-interacting motif; VIT, vault inter-α-trypsin; vPARP, vault poly(ADP-ribose) polymerase; vWA, von Willebrand factor type A; WGR, conserved W, G and R residues; WWE, conserved W, W and E residues; ZnF, DNA or RNA binding zinc fingers (except PARP1 ZnFIII, which coordinates DNA-dependent enzyme activation).

et al, 2008; Haince *et al*, 2008). The prevention of PAR synthesis by PARP inhibitors, or *Parp1* gene disruption in mice, leads to repair defects, cell-cycle perturbation and increased genomic instability, emphasizing the function of PARP1 as a caretaker of the genome (Masutani *et al*, 1999; Menissier de Murcia *et al*, 1997; Wang *et al*, 1997). The regulation of chromatin structure by PARP1 and PAR has been studied *in vitro* and *in vivo* at inducible mammalian promoters, and also in *Drosophila*—which contains only homologues

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of PARP1, tankyrase 1 and PARP16—at stress-activated puff loci. These studies revealed that PARP1 can regulate chromatin architecture, nucleosome removal and transcription activation in response to the appropriate stimulus (Kraus, 2008; Krishnakumar *et al*, 2008; Petesch & Lis, 2008; Tulin & Spradling, 2003).

PAR has also been shown to regulate gene expression by acting on insulators—which demarcate heterochromatin/euchromatin boundaries and prevent inappropriate cross-activation of neighbouring genes. The transcriptional insulator CTCF stimulates PARP1 activation and is poly(ADP-ribosyl)ated *in vivo*. In addition, PAR was shown to inhibit the DNA methyl transferase DNMT1. Altogether, these events contribute to chromatin insulation (Guastafierro *et al*, 2008; Yu *et al*, 2004). PARP1 could also regulate chromatin remodelling, as poly(ADP-ribosyl)ation of the FACT chromatin remodeler in reconstituted nucleosomes was shown to repress the exchange of histone H2A with the histone variant H2AX—the phosphorylation of which is a marker of double-strand DNA breaks (Heo *et al*, 2008).

The functional relationship between PARP1 and the NAD^+-consuming enzyme SIRT1—a NAD^+-dependent histone deacetylase—will probably be the subject of extensive future studies, as both activities seem to be interconnected. Local NAD^+ depletion by PARP1, together with the by-product of PAR synthesis nicotinamide—a SIRT1 inhibitor—might regulate the functionality of SIRT1. The reverse could also be true, as shown by the increased activation of PARP1 in cells that are deficient in SIRT1 deacetylase activity (Kolthur-Seetharam *et al*, 2006), with both effects synergistically favouring chromatin decondensation.

Some PARPs were reported to act as transcription cofactors. PARP1, PARP2, PARP9/BAL1 and PARP14/Coast6 have been shown to regulate transcription by influencing the formation or activity of various transcription-factor complexes, the list of which is continuously expanding (Goenka *et al*, 2007; Juszczynski *et al*, 2006; Kraus, 2008; Yelamos *et al*, 2008). However, PAR synthesis is not always necessary in this process, as observed for the PARP1-dependent but PAR-independent coactivation of NF-κB in response to inflammatory stimuli (Hassa *et al*, 2005).

A role for PARPs or PAR in the regulation of cell division, cell-cycle progression or cell proliferation has been postulated, and is supported by the function and localization of many PARPs—and PAR (Chang *et al*, 2004)—at subcellular sites that are crucial for cell-cycle regulation, such as centrosomes and mitotic-spindle poles (PARP1, PARP3 and tankyrase 1), mitotic centromeres or kinetochores (PARP1 and PARP2), mitotic spindle (vPARP and PARP2) and telomeres (tankyrase 1). Furthermore, PARPs/PAR associate with molecules that are implicated in cell-cycle control—such as mitotic-checkpoint proteins (Ahel *et al*, 2008)—and pharmacological inhibition, silencing or genetic ablation of some PARPs (tankyrase 1, PARP1 and PARP2), or overexpression of others (PARP3 and PARP10), has been shown to alter cell-cycle progression (for a review, see Hsiao & Smith, 2008; Schreiber *et al*, 2006).

One of the most exciting recent findings was that PAR synthesis can trigger cell death directly. This discovery led to a model in which PAR is a crucial regulator of cell fate in response to DNA damage, allowing a rapid cellular evaluation of the extent of damage, and controlling the decision between life and death. According to this model, PARP1 activation by tolerable amounts of DNA breaks favours DNA repair and cell survival, whereas PARP1 deficiency or inhibition affects DNA-break repair, delays cell-cycle progression and triggers apoptotic cell death—making PARP inhibitors promising therapeutic molecules to potentiate the action of genotoxic antitumoral drugs (Curtin, 2005). The overactivation of PARP1 upon severe DNA injury was shown to trigger cell death by a caspase-independent but AIF-dependent mechanism (Yu *et al*, 2006). In addition, PAR was shown to be required for the sequential activation of calpain and proapoptotic factor BAX, which leads to AIF translocation from the mitochondria to the nucleus, where it induces large-scale DNA fragmentation and subsequent PARP1 overactivation, leading to energy depletion and cell death (Moubarak *et al*, 2007; Yu *et al*, 2006). $PARP1^{-/-}$ mice and mice treated with PARP inhibitors show protection from tissue injury and organ failure in various oxidative stress-related disease models—such as acute and chronic inflammatory diseases or ischaemia—and neurodegenerative disorders (Jagtap & Szabo, 2005).

Table 1 | The diversity of cellular processes involving PARPs

Chromatin structure and DNA metabolism	
Chromatin compaction and decondensation	PARP1, PARP2?
Transcription	PARP1, PARP2, Coast6, BAL1
Chromatin domains (insulation)	PARP1
Single-strand breaks Base-excision repair	PARP1, PARP2
Double-strand breaks repair (backup pathway)	PARP1
DNA replication	PARP1
Telomere homeostasis or integrity	Tankyrase 1*, Tankyrase 2*, PARP1, PARP2
Cell division, proliferation, differentiation and death	
Centrosome, mitotic spindle pole	PARP1, PARP3*, Tankyrase 1
Centromeres, kinetochore	PARP1, PARP2
Telomere separation	Tankyrase1
Cell differentiation	PARP1, PARP2
Cell death	PARP1
Cell proliferation	PARP10
Physiology and pathologies	
Carcinogenesis, tumour progression, hypoxia or cell migration	PARP1, BAL1
Viral infection: reverse transcription, replication or transcription	ZAP, PARP1
Immune and inflammatory response	PARP1, PARP2, BAL1, Coast6
Multidrug resistance	vPARP
Intracellular transport	vPARP
Fat metabolism	PARP2
Spermatogenesis	PARP2
Vascular and skeletal development	TiPARP

See text for references. An asterisk indicates those observed in humans, not in mice. BAL1, B-aggressive lymphoma 1; Coast6, collaborator of signal transducer and activator of transcription 6; PARP, poly(ADP-ribose) polymerase; TiPARP, 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible poly(ADP-ribose) polymerase; vPARP, vault poly(ADP-ribose) polymerase; ZAP, zinc-finger antiviral protein.

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Sidebar A | In need of answers

(i) Are some poly(ADP-ribose) polymerase (PARP) family members really mono(ADP-ribose) transferases? If so, what determines their different enzymatic activity?

(ii) How are all PARPs and poly(ADP-ribose) glycohydrolase (PARG) subtly regulated *in vivo*?

(iii) Why are poly(ADP-ribose) (PAR) length and complexity heterogeneous, and how is this regulated *in vivo*?

(iv) Does protein-bound PAR, free PAR or the PAR degradation product ADP-ribose act as a signalling molecule, for example in apoptosis-inducing factor (AIF)-dependent cell death?

(v) Can a PAR-binding protein discriminate between PAR synthesized by different PARPs?

Many—sometimes conflicting—studies have attempted to decipher the complex functional relationship between PARP1 and another guardian of the genome, p53. Poly(ADP-ribosyl)ation has been proposed to positively regulate p53, by impeding its interaction with the nuclear-export receptor CRM1, resulting in nuclear accumulation of p53 and transactivation of target genes (Kanai *et al*, 2007). This result is, however, difficult to reconcile with data showing that non-covalent PAR binding to p53 impairs its DNA binding to consensus sequences (Malanga *et al*, 1998).

Many physiological and pathophysiological roles have been ascribed to PAR at a systemic level. Animal models that are genetically deficient in PARP family members have allowed the discovery of new and diverse physiological roles of PARPs and PAR, for example, in fat metabolism, spermatogenesis and T-cell development (PARP2$^{-/-}$; Yelamos *et al*, 2008) or in PDGF signalling (TiPARP$^{-/-}$; Schmahl *et al*, 2007). Double-knockout mice have also underscored the possible partial redundancy between some PARP family members, such as PARP1 and PARP2, or tankyrase 1 and tankyrase 2 (Chiang *et al*, 2008; Menissier de Murcia *et al*, 2003). Interestingly, PARP1$^{-/-}$ mice have mainly revealed phenotypes in physiopathological situations, such as sensitivity towards genotoxic agents, and protection from inflammatory and neurological disorders, as mentioned above. The role of PARPs/PAR in carcinogenesis is less clear. PARP9/BAL1—which is overexpressed in some high-risk B lymphomas and able to promote cell migration—is suspected to participate in tumour progression (Juszczynski *et al*, 2006), whereas vPARP$^{-/-}$ mice show increased sensitivity to carcinogen-induced colon tumours (Raval-Fernandes *et al*, 2005). The role of PARP1 in carcinogenesis is also still ambiguous; the susceptibility of PARP1$^{-/-}$ mice to chemically induced and spontaneous tumour development seems to depend not only on the carcinogen and the target organ, but also on which one of the three existing PARP1$^{-/-}$ mice models is used (Martin-Oliva *et al*, 2006; Miwa & Masutani, 2007; Tong *et al*, 2007). Hypoxia—a phenomenon associated with tumour growth—was shown to be counteracted by PARP inhibitors or by genetic ablation of PARP1 (Martin-Oliva *et al*, 2006). However, inhibition of PARP activity has promising outcomes in cancer treatment, as PARP inhibitors—which are non-specific to PARP1 and can inhibit other PARP family members—can potentiate the effect of chemotherapeutic drugs and radiotherapy, and have been shown to selectively kill tumour cells with deficiencies in BRCA1, BRCA2 and other factors involved in double-stranded DNA-break repair through homologous recombination (Turner *et al*, 2008). Some of these inhibitors have already entered clinical trials for cancer treatment (Curtin, 2005).

Several PARPs also actively participate in the host response to viral infection. The role of PARP1 in host–virus interactions is, however, ambiguous, as there are studies showing both proviral and antiviral effects of PARP1 on viral replication and transcription (Kameoka *et al*, 2004; Parent *et al*, 2005). PARP13/ZAP is a suppressor of retrovirus infection that induces viral RNA degradation (Kerns *et al*, 2008) and BAL1/PARP9—which acts in the interferon-γ signalling pathway (Juszczynski *et al*, 2006)—is also likely to influence host–virus interactions. It will be useful to evaluate whether these PARP family members could constitute potential new therapeutic targets in viral infections.

How are PARPs activated and regulated?

Among the 17 members of the PARP family, PARP1 and PARP2 were the only PARP enzymes reported to be highly stimulated by DNA interruptions. The discovery that PARP1 could also be activated by particular types of DNA structure—such as hairpins, cruciforms and supercoiled DNA (Kim *et al*, 2004; Lonskaya *et al*, 2005)—invalidated the dogma that DNA breaks are essential for activation. Similarly, it was subsequently observed that PARP1 could even be activated by protein–protein interactions in the absence of DNA *in vivo* and *in vitro*, as reported for phosphorylated ERK and for CTCF (Cohen-Armon *et al*, 2007; Guastafierro *et al*, 2008). These findings strongly support the emerging idea that PARP1 could have other physiological functions besides DNA nick-sensing.

PARP1 inactivation was observed upon binding to the histone variant macroH2A1.1 at the unstimulated HSP70.1 promoter (Ouararhni *et al*, 2006) and to the microtubule-based molecular-motor KIF4 in the absence of neuron stimulation (Midorikawa *et al*, 2006). In *Drosophila*, core histones have been shown to regulate Parp1 activation differentially, with H2A and the amino-terminal tail of histone H4 having an inhibitory and stimulatory effect, respectively (Pinnola *et al*, 2007). Another possible PARP1 regulator that should be considered is the nuclear NAD$^+$ biosynthetic enzyme NMNAT1, which, in addition to providing PARP1 with its substrate NAD$^+$, is able to associate with automodified PARP1. This physical interaction is sufficient to stimulate PARP1 activity *in vitro*, independently of NMNAT1 enzymatic activity (Berger *et al*, 2007).

PARP1 function can also be modulated by post-translational modifications. Phosphorylation by ERK1/2 provides maximal activation after DNA damage (Kauppinen *et al*, 2006), and acetylation by the histone acetyl transferases p300 and CBP enhances the synergistic cooperativity between PARP1, p300/CBP and the mediator complex in NF-κB-dependent gene activation in response to inflammatory stimuli (Hassa *et al*, 2005). PARP1 activity can also be controlled by its mislocalization, as is the case during HIV-1 infection, when VPR sequesters PARP1 in the cytoplasm. This strategy allows the virus to alter the PARP1 functions that could interfere with viral pathogenesis, such as NF-κB coactivation (Muthumani *et al*, 2006). Finally, little is known about the regulation of the other PARP family members and of the highly active PAR-degrading enzyme PARG, which also need to be tightly controlled and are therefore probably subject to stringent regulation.

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Do new PARPs and functions mean more complexity?

The importance of PAR in the regulation of both physiological and pathophysiological processes has been widely illustrated, whereas the molecular mechanisms implicated have not yet been clearly defined. This fact is emphasized by the frequent difficulty to reconcile data obtained either *in vitro* or from cellular models with the apparent lack of a corresponding phenotype in genetically altered mouse models. It is interesting to note that one of the first roles of PARP1 and PAR to be discovered—the regulation of chromatin structure—was neglected when the appearance of mice and cellular models opened the way to physiological and pathophysiological studies. However, this function has once again come to the forefront and has been re-examined as a possible way in which to reconcile the pleiotropic functions described for PARP1 so far. In addition, the complex structural nature of the PAR produced is not yet fully understood. We still do not know if and how polymer length and ramification are regulated *in vivo*, and for what purpose. Many other obvious questions are still waiting for answers (Sidebar A). Why is it worthwhile for a cell to synthesize this highly energy-consuming molecule? Why does evolution keep selecting this post-translational protein modification, and driving the appearance of additional PARP family members in higher eukaryotes and of functional splicing variants for many PARPs (Otto *et al*, 2005; Schreiber *et al*, 2006)? The members of the PARP family studied so far have already emphasize the wide diversity of cellular processes in which this post-translational protein modification seems to have a crucial role. Although we have come a long way since the times when poly(ADP-ribosyl)ation was regarded as a mere curiosity, a considerable amount of time and effort will probably be required to answer all of the remaining questions regarding PAR functions and regulation (Sidebar A).

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