

Chromatin architecture and functions: the role(s) of poly(ADP-RIBOSE) polymerase and poly(ADPribosyl)ation of nuclear proteins¹

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Abstract: Epigenetic states that allow chromatin fidelity inheritance can be mediated by several factors. One of them, histone variants and their modifications (including acetylation, methylation, phosphorylation, poly(ADP-ribose)ation, and ubiquitylation) create distinct patterns of signals read by other proteins, and are strictly related to chromatin remodelling, which is necessary for the specific expression of a gene, and for DNA repair, recombination, and replication. In the framework of chromatin-controlling factors, the poly(ADP-ribose)ation of nuclear proteins, catalysed by poly(ADP-ribose)polymerases (PARPs), has been implicated in the regulation of both physiological and pathological events (gene expression/amplification, cellular division/differentiation, DNA replication, malignant transformation, and apoptotic cell death). The involvement of PARPs in this scenario has raised doubts about the epigenetic value of poly(ADP-ribose)ation, because it is generally activated after DNA damage. However, one emerging view suggests that both the product of this reaction, poly(ADP-ribose), and PARPs, particularly PARP 1, play a fundamental role in recruiting protein targets to specific sites and (or) in interacting physically with structural and regulatory factors, through highly reproducible and inheritable mechanisms, often independent of DNA breaks. The interplay of PARPs with protein factors, and the combinatorial effect of poly(ADPribosyl)ation with other post-translational modifications has shed new light on the potential and versatility of this dynamic reaction.

Key words: chromatin, epigenetic, poly(ADP-ribose), PARP.

Résumé : Les conditions épigénétiques qui permettent l'héritabilité de la fidélité de la chromatine requièrent la médiation de différents facteurs. Entre autres, les variants des histones et leurs modifications (acétylation, méthylation, phosphorylation, poly(ADP-ribose)ation, ubiquitination) créent différents patrons de signaux décodés par d'autres protéines, qui sont strictement reliés au remodelage de la chromatine, et qui sont nécessaires pour l'expression spécifique de gènes, la réparation d'ADN, la recombinaison et la réplication. Dans le réseau de facteurs qui contrôlent la chromatine, la poly(ADP-ribose)ation de protéines nucléaires, catalysée par les poly(ADP-ribose) polymérasés (PARPs) a été impliquée dans la régulation de phénomènes tant physiologiques que pathologiques (expression/amplification génique, division/différenciation cellulaire, réplication d'ADN, ainsi que la transformation maligne et la mort cellulaire par apoptose). Leur implication dans ce vaste scénario a soulevé quelques doutes quant à l'importance épigénétique de la poly(ADP-ribose)ation car elle est généralement activée suite à un dommage à l'ADN. Cependant, une idée qui se dégage actuellement indique que tant le produit de cette réaction, le poly(ADP-ribose), que les PARPs, spécialement PARP-1, jouent un rôle fondamental dans le recrutement des protéines cibles à des sites spécifiques et (ou) l'interaction physique avec des facteurs structurels et régulateurs, à travers ces mécanismes hautement reproductibles et héréditaires, souvent indépendants des bris dans l'ADN. Les interactions de la PARP avec des facteurs protéiques et les effets combinés de la poly(ADP-ribose)ation avec d'autres modifications post-traductionnelles font la lumière sur le potentiel et la versatilité de cette réaction dynamique.

Mots clés : chromatine, épigénétique, poly(ADP-ribose), PARP.

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Introduction

In the last decade, the genetic codes have come to be considered central to understanding the essence of life, and deciphering it has become a focus of research. The post-genomic era has introduced the concept of epigenetic, the science that studies the factors and mechanisms that allow selective access and use of gene expression, replication, and repair machineries. These mechanisms are heritable through mitosis and (or) meiosis but cause no change in the DNA sequence (Misteli 2000; Cremer and Cremer 2001; Felsfendel and Groudine 2003; Carmo-Fonseca 2004; Santos and Dean 2004).

It is now widely accepted that nuclear organization contributes significantly to the control of all metabolic events involving chromatin. Specific heritable mechanisms might coordinate the spatial organization of genes, transcripts, and regulatory proteins within the nucleus (Misteli 2000; Cremer and Cremer 2001; Felsfendel and Groudine 2003; Stein et al. 2003; Carmo-Fonseca 2004). Epigenetic marks include heritable covalent modifications of DNA and (or) chromatin proteins that affect gene expression in the form of histones and their modifications (Strahl and Allis 2000; Felsfendel and Groudine 2003; Henikoff et al. 2004). Histone proteins and the nucleosomes they form with DNA are the basic units of chromatin in eukaryotes. Distinct histone variants and their modifications define a histone code that is used by protein factors to regulate different nuclear events (Strahl and Allis 2000; Henikoff et al. 2004; Osley 2004; Yamashita et al. 2004). Chemical modifications to histone tails or to the body of the histone octamer serve as a signal for the binding of specific proteins, and are often associated with distinct patterns of gene expression, DNA repair, recombination, and replication (Ehrenhofer-Murray 2004). For any given gene, the timing of these events depends on both the state of chromatin architecture and the order of recruitment of chromatin-modifying factors (Cremer and Cremer 2001; Stein et al. 2003; Carmo-Fonseca 2004).

In this paper, we review the role of poly(ADP-ribose) polymerases (PARPs), mainly PARP 1, and the involvement of histone and nonhistone poly(ADP-ribosyl)ation in the modulation of chromatin dynamic states. We also question whether PARPs represent epigenetic factors in chromatin architecture/remodelling or related regulatory mechanisms.

PARP family: different enzymes and a common product, poly(ADP-ribose)

Because of DNA-break dependence on the only PARP known until a few years ago, PARP 1, the role of this enzyme, and its related product poly(ADP-ribose), has been relegated to DNA repair, overlooking the possibility of its having any epigenetic value. New insights into the possible involvement of PARP 1 in physiological processes (Kun et al. 2002; Kirsten et al. 2004) and the discovery of other members of the PARP family have led to the re-evaluation of the poly(ADP-ribosyl)ation and various molecular signals of PARPs as inheritable factors.

The synthesis of the polyanion poly(ADP-ribose) (PAR) by PARPs starts after the first ADP-ribose unit has been transferred from NAD⁺ to an acceptor protein (D'Amours et

al. 1999; Smith 2001; Amé et al. 2004). The transition from modified to unmodified targets is precipitated by poly(ADP-ribose) glycohydrolase (PARG) (D'Amours et al. 1999). PARG degrades the polymer with both endo- and esoglycosidic activity (Bonicalzi et al. 2003).

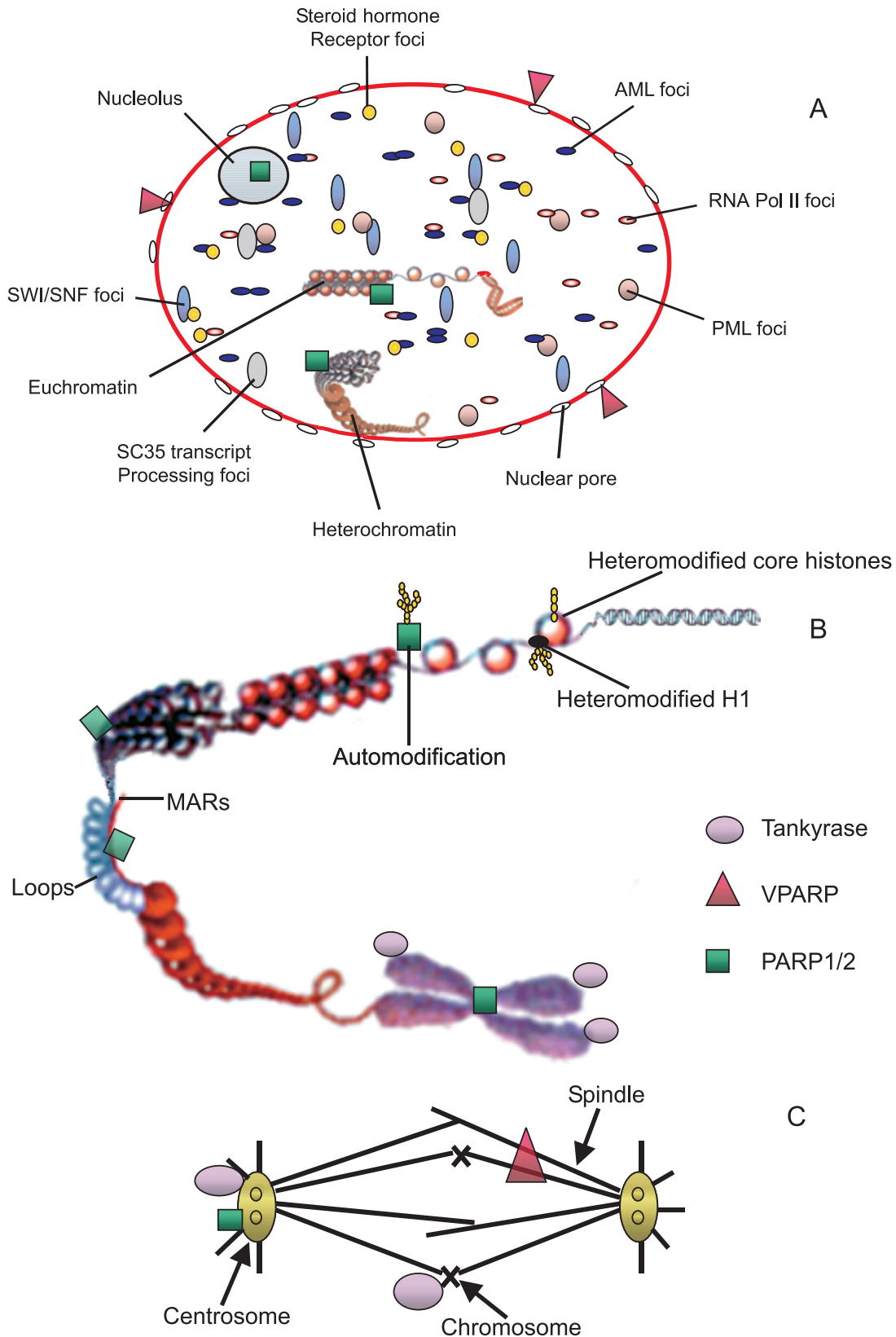
The reaction of PARP 1, regulated by the shuttling of PARG from cytoplasm to nuclei and vice versa (Bonicalzi et al. 2003), generates a series of different marks that reflect the kind (linear/branched) and length of polymers, the type of polymer binding (covalent/noncovalent), protein acceptors (structural/functional proteins), and the direction of the modification (auto-/heteromodification). In addition, the various PARPs identified to date (18 different isoforms), of which the main and best studied nuclear member is PARP 1 (Amé et al. 2004; Rouleau et al. 2004), diversify the molecular signals of the poly(ADP-ribosyl)ation system because they differ in size, modification targets, cell localization, and functions (Fig. 1). Some of the new enzymes (PARP 2, PARP 3, etc.) reside in the nucleus, and it is possible that they create an alternative, but not redundant, pathway for DNA repair, not only in PARP-1-deficient animals (Smith 2001; Huber et al. 2004; Amé et al. 2004). Specific and discrete functions are related to V PARP (a component of vaults), ribonucleoprotein complexes found in cytoplasm, the nucleus, and, at the mitotic spindle, Tankyrases 1 and 2, which are involved in telomere integrity (Smith 2001; Amé et al. 2004). A role in the maintenance of telomere length has been recently hypothesized for PARP 2; it has been found to bind with high affinity to ADP-ribosylate TRF2, the protein that remodels these special DNA structures (Dantzer et al. 2004).

PARP 1 plays more general roles (D'Amours et al. 1999; De Murcia and Shall 2000; Smith 2001). Its immediate activation after DNA damage to protect DNA breaks is only 1 of several metabolic implications that makes this enzyme, together with PARP 2, essential to DNA-repair mechanisms (Amé et al. 2004). Both enzymes are involved in the base excision repair pathway, and both act as DNA-break sensors when single-strand break repair is engaged (Huber et al. 2004). Both possess similar but not redundant functions in the maintenance of genomic stability, as demonstrated by *parp 1^(-/-)parp 2^(-/-)*-double-mutant mice, which are not viable and die at the onset of gastrulation (Menissier de Murcia et al. 2003). DNA nick sensors and DNA repair machinery recruiters work when there is either mild or extensive DNA damage; these mechanisms are recognized as having evolved to preserve genome integrity and ensure cell survival (Karagiannis and El-Osta 2004).

However, recent evidence has shown that PARP 1 is broadly involved in normal cell physiological functions, even in the absence of DNA-strand breaks (Yao et al. 2000; Kun et al. 2002; Kirsten et al. 2004; Cohen-Armon et al. 2004). Physiologically occurring double-stranded DNA can serve as a coenzyme for PARP 1; it catalyses, preferentially in vitro the trans-poly(ADPribosyl)ation of H1 histone (Kun et al. 2002).

It is generally accepted that PARP plays roles in both local chromatin remodelling and recruitment/modulation of the activity of various factors involved in DNA replication, repair, transcription, and recombination (El-Khamisy et al. 2003; Okano et al. 2003; Leppard et al. 2003).

Fig. 1. Nuclear and chromatin distribution of poly(ADP-ribose)polymerases (PARPs). (A) Interphase nucleus. Chromatin is interspersed within different subcompartments (foci), with heterochromatin at the periphery and euchromatin towards the center of the nucleus (interchromatin). PARPs association with different subnuclear regions is shown. (B) Unwinding a chromosome shows various organizational levels of chromatin, with some distribution of PARP 1 and 2, and of poly(ADP-ribose). Tankyrase is at the telomere ends. (C) Mitotic spindle. MARs, matrix associated regions.



Although the different roles of PARP 1 in the structural and functional features of chromatin do not necessarily exclude each other, separate discussions are needed for clarity.

PARP and chromatin remodelling

Between 1980 and 1990, PARP 1 was identified as a chromatin remodeller because its product, poly(ADP-ribose), was able to alter in vitro chromatin structure. Poirier et al. (1982) suggested that the relaxation of polynucleosomes might result from the poly(ADP-ribosylation) of the H1 histone, which mimicks depletion of the linker histone from chromatin. During that decade, electron microscopy was used to demonstrate that the relaxation of pancreatic polynucleosomes, modified with purified PARP, was a consequence of a poly(ADP-ribose)-induced weakening of the H1/DNA interaction, and that it might be reversed by the action of PARG (Poirier et al. 1982; Huletsky et al. 1989). Chromatin decondensation was also observed with the (ADP-ribosylation) of the polynucleosomes of bull testis (Faraone-Mennella et al. 1993), and when poly(ADP-ribose) was used to reduce H1-H1 cross-linking (Reale et al. 2000). Tulin and Spradling (2003) recently reported that a local loosening of the polytene chromatin structure associated with gene induction (puffing) in *Drosophila* was accompanied by elevated levels of (ADP-ribosylated) proteins. *Drosophila* histones were identified as targets of poly(ADP-ribosylation) in ecdysone-induced chromatin remodelling and modification to generate puffing (Sawatsubashi et al. 2004).

The poly(ADP-ribosylation) of chromosomal structural proteins (histones, high-mobility group proteins) deeply influences both nucleosome and higher-order chromatin organization. Nonhistone chromosomal proteins (high- and low-mobility group proteins) have been identified in vivo as the main PAR acceptors (Adamietz and Rudolf 1984; Faraone-Mennella et al. 1984).

Only 1% of all ADP-ribosylated nuclear proteins are histones (Kun et al. 2002). Among histones, H1 and H2B are the preferential ADP-ribose acceptors in vivo (Huletsky et al. 1989). Both covalent and noncovalent histone ADP-ribosylation has been described. Because N-terminal tails of core histones, which protrude outside the nucleosome, can easily become targets of modifications, core histones can be ADP-ribosylated covalently with short (3-4 unit) ADP-ribose chains, noncovalently with the binding of poly(ADP-ribose) to protein substrates, such as the auto-poly(ADP-ribosylated) PARP 1 (Fig. 1), or as free polymers (Adamietz and Rudolf 1984; Krupitza and Cerutti 1989; Saxena et al. 2002; Realini and Althaus 1992). Putative sites of noncovalent poly(ADP-ribosylation) have been identified for specific core histone variants as macro-H2A and CENP-A (Saxena et al. 2002; Rouleau et al. 2004). Both the length of polymers and the kind of modification (covalent/noncovalent) likely provide different functional signals.

The combinatorial effects of (ADP-ribosylation) and other post-translational modifications have also been described. (ADP-ribosylation) can occur on acetylated core histones (Malik and Smulson 1984). Acetylated H4 subspecies are predominantly tri- and tetra-(ADP-ribosylated) (Golderer and Grobner 1991). Newly synthesized core histones can be reversibly acetylated and oligo(ADP-ribosylated), probably

to facilitate their assembly into histone complexes and their deposition onto DNA at the replication fork (Boulikas 1995). n-butyrate-induced hyperacetylation of core histones increases the incorporation of ADP-ribose into highly acetylated H4 subspecies (Golderer and Grobner 1991). These observations have led to 2 hypotheses: that a concerted action of the 2 postsynthetic modifications triggers (ADP-ribosylation); or that the recruiting role of histone acetylation triggers (ADP-ribosylation). The latter is currently thought to be the most likely. Many modifications are close enough to each other on the histone tails to influence the ability of enzymes to further modify target sites (Strahl and Allis 2000).

Poly(ADP-ribosylation) of linker histone H1 has been generally reported to drastically alter DNA conformation, destabilizing DNA/histone interactions (Poirier 1982; Huletsky et al. 1989).

However, the relative affinities of poly(ADP-ribose), described for different somatic and tissue-specific H1 histone variants, modulate their ability to stabilize DNA higher-order structure (Khadake and Rao 1995; De Lucia et al. 1994; Faraone-Mennella et al. 1999). The noncovalent binding of polymers, preferentially long and branched, with different H1 variants is highly competitive and takes place in the presence of a large excess of DNA (Malanga et al. 1998; Reale et al. 2000). The testis-specific H1t, the least condensing linker histone variant, is also the most poly(ADP-ribosylated), both covalently, with short chains of ADP-ribose (Faraone-Mennella et al. 1999), and noncovalently, with long and branched polymers (Malanga et al. 1998).

Most observations suggest that PAR attached to H1 histones facilitates histone removal from DNA, thus modulating chromatin decondensation and affecting nucleosome stability. PAR/H1 noncovalent interactions not only modulate chromatin decondensation (Reale et al. 2000), but also affect chromatin dynamics, either by limiting the accessibility of other DNA-binding proteins or by acting as a signal to other remodeling proteins.

In contrast, evidence of chromatin condensation has been obtained by observing the cross-linking of ADP-ribosylated H1, and explains its presence in inactive DNA (Wong et al. 1983) and upon covalent and length-specific (4-5 ADP-ribose units) in vitro oligo(ADP-ribosylation) of the testis variant H1t (Faraone-Mennella et al. 1999). H1t induces a dose-dependent compaction of rat testis H1-depleted chromatin. It is hypothesized that oligo(ADP-ribose) plays a role in the structure of the arginine-rich C-terminus of the histone, by reducing the net positive charges and favouring the beta-structure needed for interaction with DNA. C-terminus is required for the high-affinity chromatin binding of somatic histone H1 variants, but these variants lack the arginine cluster at the end and possess specific DNA-binding motifs (Wellman 1996; Hendzel et al. 2004). Thus, modifications, such as the phosphorylation of somatic histones, can disrupt binding by affecting the secondary structure of this region (Hendzel et al. 2004).

The apparently conflicting effects of PARP on chromatin structure (condensation/decondensation) might correlate with the nature of particular chromatin environments (hetero-, euchromatin), and might depend on the different distribution of the enzyme on chromosomes, which allows modulation of the chromatin structure at many chromosomal positions in

response to a variety of specific signals arising at these regions (Kraus and Lis 2003).

PARP and chromatin functions

PARPs are involved in chromatin functions, and various members of the family play roles at the nuclear and chromatin levels. Most information deals with PARP 1; however, because of the intricate network of processes involving this enzyme, no clear picture of its functions and their interplay has been established. It is generally accepted that PARP 1 activation provides a rapid post-translational signal that controls the transcription and replication machineries and mobilizes the DNA repair apparatus (Amé et al. 2004; Rouleau et al. 2004).

How does PARP 1 function in these processes? PARP 1 can operate as a component of enhancer/promoter binding complexes, as a recruiter of factors and cofactors, or as a catalyst of their poly(ADP-ribosyl)ation (Kraus and Lis 2003; Rouleau et al. 2004).

PARP may interact with partner proteins mainly through its DNA binding automodification domains, which contain a BRCT (breast cancer susceptibility protein, BRCA-1, C-terminus) motif that is involved in specific protein-protein contacts (Amé et al. 2004). In the case of tumor suppressor p53, PARP/p53 complex formation is regulated by p53 phosphorylation (Wesierska-Gadek et al. 2003). An association between PARP and p21waf1/cip1 through the C-terminal domain is related to their cooperation in regulating the functions of proliferating cell nuclear antigen (PCNA) during DNA replication/repair (Frouin et al. 2003). Ku autoantigen is the DNA-binding subunit of DNA-protein kinase (DNA-PK) that forms a molecular complex with PARP in vivo and in vitro in the absence of DNA; as a functional consequence, the affinity between the Ku autoantigen and base unpairing regions is synergistically enhanced (Galande and Kohwi-Shigematsu 1999). Tulin and Spradling (2003) demonstrated that in *Drosophila*, even when enzymatically inactive, PARP molecules remain associated with many chromosome regions and may play essential structural roles; during *Drosophila* development, the enzymatically inactive PARP-e isoform auto-regulates *Parp* transcription by influencing the chromatin structure of its heterochromatic environment (Tulin et al. 2003).

Chromatin structure organization by *Drosophila Parp* is also regulated by the ADP-ribosylation of chromosomal proteins; it exerts influence on both the expression and silencing of particular euchromatic and heterochromatic sequences at different times during *Drosophila* development. Many of the detectable modified proteins with antibodies that recognize protein-(ADP-ribosyl) groups are located along chromosomes, and are particularly enriched in nucleoli and in the heterochromatic chromocenter, regions strongly affected by *Parp* mutations (Tulin and Spradling 2003).

The list of targets of PARP 1 activity is very long and not yet exhaustive. It includes proteins involved in different processes, such as DNA polymerases, topoisomerases, and ligases; high-mobility group proteins and transcription factors, such as FOS; and p53, XRCC-1 (X-ray complementing factor-1), PCNA, hUbc9, the human homolog of the yeast ubiquitin conjugating enzyme Ubc9, and the DNA-dependent

protein kinase catalytic subunit (D'Amours et al. 1999; Pleschke et al. 2000; Nakajima et al. 2004; Amé et al. 2004).

Checkpoint proteins, such as p53, p21^{CIP1/WAF1}, xeroderma pigmentosum group-A-complementing protein, MSH6, DNA ligase III, XRCC1, DNA polymerase ϵ , DNA-PK_{CS}, Ku70, NF- κ B, inducible nitric-oxide synthase, caspase-activated DNase, and telomerase possess a poly(ADP-ribose)-binding motif that overlaps 5 functional domains responsible for protein-protein interactions, DNA binding, nuclear localization, nuclear export, and protein degradation (Pleschke et al. 2000). Reale et al. (2005) report a PARP 1/DNA methyltransferase heterodimeric complex mediated by poly ADP-ribose that silences the DNA methyltransferase.

Direct association of PARP and (or) poly(ADP-ribosyl)ation play a role in the recruitment to certain sites of specific proteins, such as PCNA and topoisomerase I, to the DNA synthesome during the early S phase, or of signal proteins, such as p53 and MARCKS protein, to sites of DNA breakage. Extensive poly(ADP-ribosyl)ation of p53, topoisomerase I, and topoisomerase II beta in vivo is also implicated in the accumulation and destabilization of these acceptor proteins in cells (reviewed in Simbulan-Rosenthal et al. 2001; Rouleau et al. 2004; Yung et al. 2004). PARP 1 and PARP 2 induce the efficient repair of DNA-strand breaks associated with stalled topoisomerase I, which, if unrepaired, could lead to genomic instability or cell death. ADP-ribose polymers target specific domains of topoisomerase I, reprogram the enzyme to remove itself from cleaved DNA, and close the resulting gap (Malanga and Althaus 2004).

The dual role played by PARP and the role of PAR in nuclear processes depend on the level of the substrate NAD⁺ and the presence of PARP-activating DNA breaks. In DNA replication, in the absence of NAD⁺, the physical interactions of PARP with DNA polymerase- α activates polymerase- α ; the addition of NAD⁺ to MRC (17S multiprotein DNA replication complex or synthesome) inhibits polymerase- α catalytic activity (Simbulan-Rosenthal et al. 2001).

Similarly, in the absence of NAD⁺, PARP may exert its transcription function through direct binding, either to the gene-regulating sequences or to transcription factors. In the presence of the substrate, the modification of transcription factors by poly(ADP-ribosyl)ation prevents their binding to consensus sequences (Kraus and Lis 2003; Rouleau et al. 2004).

Because ADP-ribosylation can activate or inhibit protein function, depending on the acceptor substrate, nuclear PARP 1 transduces different signals to cellular machineries that regulate gene expression, cell-cycle progression, and DNA repair. PARP 1 uses endothelial cells to regulate the expression of genes in both a positive and negative fashion; the final effects depend on the target gene (Carrillo et al. 2004). In the transcription of eukaryotic genes, the poly(ADP-ribosyl)ation of transcription factors (TFIIF and TEF-1, TATA-box-binding protein, YY1, SP-1, cAMP-response element-binding protein, p53, and NF κ B) prevents their binding to specific promoter sequences (Rawling and Alvarez-Gonzalez 1997; Oei et al. 1998; Butler and Ordahl 1999; Anderson et al. 2000). PARP itself may interact directly with gene promoters, binding, for instance, the DNA sequences within the MCAT1 regulatory element (Butler and Ordahl 1999) to the DF4

protein-binding site of the *Pax-6* gene neuroretina-specific enhancer (Plaza et al. 1999). Furthermore, PARP 1 is involved in the active transcriptional DNA-protein complex formation on *Reg* promoter (Akiyama et al. 2001).

Some data support the notion of PARP protein as a potent regulator of transcription, including the downregulation of its own promoter (Soldatenkov et al. 2002). PARP can bind to DNA secondary structures (hairpins) in heteroduplex DNA in a DNA end-independent fashion; the automodification of PARP in the presence of NAD⁺ inhibits its hairpin-binding activity (Soldatenkov et al. 2002). The transcriptional activity of the PARP promoter is 4–5 times greater in PARP knock-out cells than in wild-type fibroblasts (Soldatenkov et al. 2002).

PARP 1 is also important for suppressing recombination at DNA ends (Simbulan-Rosenthal et al. 2001) and participating in anchoring chromatin to the nuclear matrix (Quesada et al. 2000; Pandita and Dhar 2000), where certain DNA repair and recombination processes appear to occur (Sakkers et al. 1999). Nuclear matrix is a fraction greatly enriched in PARP, PAR, and transcriptional activity (Alvarez-Gonzalez and Ringer. 1988; Faraone-Mennella et al. 2003), and in transcription factors (i.e., C/EBP-beta) and enzymes (DNA Topo II, DNA PK) that colocalize with PARP 1 and 2 at the matrix associated regions of chromatin. PAR contributes to PARP 1 localization at the nuclear matrix (Tramontano et al. 2005).

Within matrix associated regions, base unpairing region are sites of binding for PARP 1, and for other proteins, such as SAF-A, HMG(Y), nucleolin, p53, and Ku autoantigen. The role of these sites is dual: their interaction provides not only an architectural core but also helps recruit functional multimolecular complexes at the base of chromatin loops, which affect multiple distant genes (Galante 2002). Some of these proteins, such as Ku autoantigen, are either partners of PARP 1 or targets of poly(ADP-ribose) (Galante and Kohwi-Shigematsu 1999). The role of both PARP and Ku autoantigen in chromatin remodelling (Ku is involved in chromosome condensation during the G₂ and M phases of the cell cycle) supports a mechanistic link between chromatin structure and various other functions involving DNA.

PARP 1 associates with normal mammalian centromeres and human neocentromeres. It interacts with, and poly(ADP-ribosyl)ates, 2 constitutive centromere proteins (Cenpa, Cenpb) and a spindle checkpoint protein (Bub3) upon induction of DNA damage, suggesting a role for PARP 1 in centromere assembly/disassembly and checkpoint control (Saxena et al. 2002). Classic and nonclassic PARPs (V PARP, Tankyrases) may share a role in centrosome or spindle function, or a common storage site during mitosis (Fig. 1). Because telomeres are DNA ends that are anchored to the nuclear matrix (de Lange 1992; Pandita and Dhar 2000), and because they appear to elicit a DNA-damage response when dysfunctional, PARPs are also thought to participate in telomere maintenance and (or) the transmitting of signals generated by dysfunctional telomeres (Kaminker et al. 2001), and thus play a role in regulating cellular senescence and genomic stability (Burkle 2001). Both PARP 1 and Tankyrase 1 (TANK1) can, at least in some circumstances, influence telomere length. Germ-line inactivation of PARP 1 in mice modestly reduces telomere length (d'Adda di Fagagna et al. 1999), whereas

the overexpression of nuclear-targeted TANK1 in human tumor cells modestly increases telomere length (Smith and de Lange 2000). Substrates have been identified for TANK1; these are limited to TRF1 and TANK1 itself (Smith 2001). Interestingly, Tankyrase 2 (TANK2) was recently identified as an autoantigen in several cancer patients (Kuimov et al. 2001; Monz et al. 2001), although the significance of this finding is not yet clear. Because both TANKs localize to the nuclear periphery, they may participate in DNA- or telomere-damage signaling, as do the classic PARPs. On the other hand, their association with Golgi and other cytoplasmic vesicles raises the possibility of nonnuclear functions.

A particular role for poly(ADP-ribosylation) as a protector from proteolysis of specific proteins, during sea urchin fertilization, has been described (Morin et al. 1999). In contrast to automodified poly-(ADP-ribose), PARP 1 seems to activate the nuclear 20S proteasome to facilitate selective degradation of oxidatively damaged histones (Arnold and Grune 2002). Here, a joint role for PARP 1 in the removal of oxidized nucleoproteins and in DNA repair is suggested, with PARP 1 providing a co-ordinative link between 2 nuclear antioxidant defence systems. The concerted activation of these systems would produce a fast and efficient restoration of the native chromatin structure following oxidative stress (Arnold and Grune 2002).

Conclusions

The objective of this review is not to give an exhaustive list of different implications of PARP and PAR. In fact, it does not discuss much of the literature dealing with the involvement of PARP and PAR in cell-death events that result from extensive DNA damage and lead to NAD⁺ depletion (Chiarugi 2002), or in pathological states, which are thoroughly reviewed by Virag and Szabo (2002). The suggestion that PARP can be considered, if not a killer, then a “subtle key participant in the conspiracy of death” (Chiarugi 2002), clouds the physiological roles of PARP and poly(ADP-ribosylation).

It is not easy to draw a clear picture of the modes of action of PARP because it is widely distributed within the intricate network of chromatin architecture. Localization of PARPs in different nuclear subcompartments of interphase nuclei and in mitotic chromatin and structures, as spindles (Fig. 1), reflects different functional roles, depending on specific states and the requirements of cells. Thus, the well-documented role of PARP 1 in the cellular response to DNA damage is primarily physiological and essential to control the performance of delicate processes, such as recombination, chromosome segregation, and all the events where DNA breakage might occur (Amé et al. 2004), although PARP 1 can become dangerous in cases of acute injury.

However, confining PARP 1 to the role of repairing enzymes minimizes its functional meaning. PARP promotion of chromatin remodelling correlates with gene induction, and controls other events involved in chromatin structure and organization. Besides enzyme activity, in many processes PARP co-operates by physically interacting with other proteins. Although many facets of its involvement and their link(s) are still unknown, the dual role of PARP as a protein partner of transcriptional factors and (or) coactivators, and

its ability to generate local alteration of chromatin by auto- or heteromodification, support the idea of PARP as a normal and routine component of cell machinery.

Epigenetic modifications are defined marks of genomic regions acting as heritable and stable instructions for the specification of chromatin organization and structure that dictate transcriptional states (Santos and Dean 2004). It appears that this definition fits well with the involvement of PARP and its reaction in the physiological processes covered in this review.

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