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PARP Goes Transcription

Review

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PARP-1, an enzyme that catalyzes the attachment of ADP ribose units to target proteins, plays at least two important roles in transcription regulation. First, PARP-1 modifies histones and creates an anionic poly(ADP-ribose) matrix that binds histones, thereby promoting the decondensation of higher-order chromatin structures. Second, PARP-1 acts as a component of enhancer/promoter regulatory complexes. Recent studies have shown that both of these activities are critical for gene regulation in vivo.

Forty years ago, Chambon and colleagues discovered that the addition of nicotinamide mononucleotide to rat liver nuclear extracts stimulated the synthesis of a polyadenylic acid, later identified as poly(ADP-ribose) (PAR) (Amé et al., 2000). This discovery began four decades of research on PAR and the enzymes that regulate its metabolism, in particular poly(ADP-ribose) polymerase (PARP). PARP, which represents a family of related proteins with a common enzymatic activity (Hassa and Hottiger, 2002; Rolli et al., 2000), covalently modifies a variety of proteins involved in the metabolism of nucleic acids and the maintenance of chromatin structure (D'Amours et al., 1999). PARP-1 is the best characterized member of this family and is the focus of this review.

PARP-1 is dramatically activated in response to extensive DNA damage, and the level of PAR increases 10- to 500-fold over that of normal cells (D'Amours et al., 1999). Studies of PARP-1 to date have focused primarily on its roles in DNA repair and cell death, while the role of PARP-1 and the modification of its targets under nonpathophysiological conditions have received less attention. Recent studies have indicated that the "background" PARP-1 activity in normal cells is an integral part of gene regulation during development and in response to specific cellular signals. This review will focus on studies that support a role for PARP-1 in modulating both chromatin structure and transcription in vivo.

PARP-1 Structure, Function, and Activity

Ubiquitous PARP activity has been found in organisms ranging from archaebacteria to mammals, although it is apparently absent in yeast (Hassa and Hottiger, 2002; Rolli et al., 2000). PARP-1, a 113 kDa protein, is the prototypical and most abundantly expressed member of a family of PARP genes that contains at least seven members in mammalian species. PARP-1 catalyzes the covalent attachment of ADP-ribose units from donor NAD^+ molecules to a variety of target proteins resulting in linear or branched polymers of PAR as large as 200 units (D'Amours et al., 1999) (Figure 1). In the absence of DNA damage, the length of the polymer is considerably shorter, ranging from single residues to oligo(ADP-ribose) units. In addition to *PARP-1*, the PARP family includes the gene encoding tankyrase (*PARP-5*), a specialized PARP enzyme involved in the maintenance of telomere length (Hassa and Hottiger, 2002; Rolli et al., 2000). The functions of the other PARP family members appear to be quite varied, but are less well characterized and are beyond the scope of this review. Although the presence of multiple PARP genes has complicated the analysis of mammalian *PARP-1* function in vivo, the biological roles of *PARP-1* may be more easily probed in organisms such as *Drosophila*, which contains just two PARP genes (a *PARP-1*-like gene, which yields three alternatively processed products [PARP-I, PARP-II, and PARP-e] and *tankyrase*) (Tulin et al., 2002). This point is emphasized by the finding that elimination of PARP-1 activity by mutation or knockout causes lethality in *Drosophila*, but not mice (Tulin et al., 2002; Wang et al., 1997).

The PARP-1 gene is highly conserved, especially at amino acids comprising structural motifs and functional domains. These include (1) an amino-terminal double zinc-finger DNA binding domain (DBD), (2) a central automodification domain, and (3) a carboxyl-terminal NAD^+ binding catalytic domain (Figure 2A) (Amé et al., 2000; Rolli et al., 2000). One contiguous 50 amino acid sequence in the catalytic domain, the "PARP signature" motif, which forms the active site, shows 100% conservation among vertebrates and 92% conservation among all species, suggesting a critical role for PARP-1 enzymatic activity in cellular function. The automodification domain contains a BRCT ("BRCA1 C terminus like") protein-protein interaction motif, as well as multiple glutamate residues that are likely targets for auto-poly(ADP-ribosyl)ation. Automodification can inhibit PARP-1 DNA binding, protein-protein interactions, and ADP-ribosyl transferase activity, ultimately inactivating the protein (D'Amours et al., 1999). PARP-1 is a nuclear protein by virtue of a bipartite nuclear localization signal (NLS) juxtapositioned amino terminally to the DBD. Interestingly, the conservation of PARP-1 structure is also reflected in the conservation of its physiological roles, as exemplified by the critical role of PARP-1 in NF-κB-dependent immunity gene activation in both mammals and *Drosophila* (Hassa and Hottiger, 2002; Tulin and Spradling, 2003). The structural motifs and functional domains that are critical for PARP-1's role in chromatin modification and transcription regulation will be discussed in detail below.

Regulation of PARP-1 Catalytic Activity

PARP-1's catalytic domain supports multiple distinct reactions that lead to the synthesis of PAR: (1) initiation (attachment of ADP-ribose to an acceptor protein), (2) elongation, and (3) branching (Alvarez-Gonzalez et al.,

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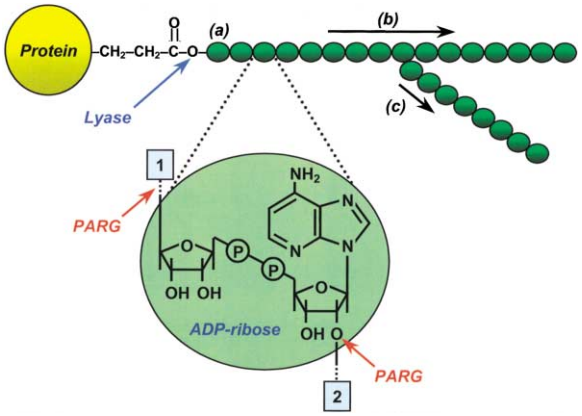

Figure 1. Structure of PAR

PAR polymers are synthesized by distinct enzymatic activities of PARP-1, including (a) initiation, (b) elongation, and (c) branching. PAR is attached to glutamate residues in target proteins via covalent linkages at the position labeled 1 in the expanded view of an ADP-ribose unit. The ADP-ribose units in the PAR polymer are linked via glycosidic ribose-ribose 1″ → 2′ bonds at the positions labeled 1 and 2 in the expanded view. The red and blue arrows indicate the sites of action of PARG and ADP-ribosyl protein lyase, respectively.

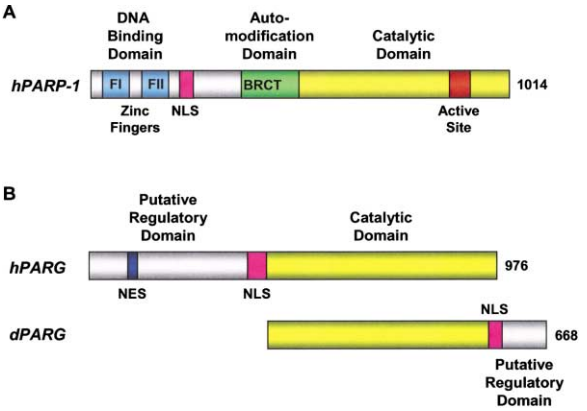

Figure 2. Structures of PARP-1 and PARG

(A) Human PARP-1 has a highly conserved structural and functional organization, including an amino-terminal DNA binding domain, a central automodification domain, and a carboxyl-terminal NAD^+ binding catalytic domain. The DNA binding domain contains two Cys-Cys-His-Cys zinc finger motifs (FI and FII). The automodification domain contains a BRCT ("BRCA1 C terminus like") protein-protein interaction motif. A contiguous 50 amino acid sequence in the catalytic domain, the "PARP signature" motif, forms the active site. NLS, nuclear localization signal. The major *Drosophila PARP-1* gene product (PARP-I, data not shown) shares the same domain structure as human PARP-1. *Drosophila* express two additional gene products from the *PARP-1* gene: an isoform produced by alternate splicing that lacks the automodification domain (PARP-II) and an isoform expressed from an alternate promoter that lacks the catalytic domain (PARP-e) (Tulin et al., 2002).

(B) Human and *Drosophila* PARG share a highly conserved catalytic domain but show diversity in the sequence and organization of other domains. Human PARG contains a putative regulatory domain at its amino terminus, whereas *Drosophila* PARG contains an unrelated putative regulatory domain at its carboxyl-terminus. NLS, nuclear localization signal. NES, nuclear export signal.

1999) (Figure 1). The attachment of PAR to proteins is most likely at glutamic acid and aspartic acid residues through an ester linkage. The average branching frequency of the polymer is approximately one branch per linear repeat of 20–50 units of ADP-ribose. Polymer size and complexity (i.e., number and lengths of PAR chains, extent of branching) are determined by the relative contribution of each enzymatic activity. Interestingly, the quantity of PAR in normal tissues (i.e., tissues lacking extensive DNA damage) is relatively low (200–250 ng/g, dry weight) compared to the abundance of PARP-1 protein (200,000 to 1 million copies per cell) (D'Amours et al., 1999; Kun et al., 2002), suggesting that PARP-1 enzymatic activity is highly regulated.

A prevailing viewpoint in the literature is that PARP-1's enzymatic activity is strictly dependent on the binding of the enzyme to damaged DNA, an effect mediated by allosteric alterations in the structure of the enzyme (Amé et al., 2000; D'Amours et al., 1999). Such a requirement seems at odds with a role for PARP-1 in the regulation of cellular functions under normal physiological conditions where genome integrity is maintained. A closer inspection of the literature, however, reveals that PARP-1 can also bind with high affinity and in a cooperative manner to other DNA structures (e.g., cruciform, curved, supercoiled, and crossover, as well as some specific double-stranded sequences) (Rolli et al., 2000) and that certain undamaged linear and stem-loop DNA structures are more potent stimulators of PARP-1 enzymatic activity than damaged DNA (Kun et al., 2002). PARP-1 has the capacity to bind to two DNA helices simultaneously (Rolli et al., 2000). In this regard, it is interesting to speculate that PARP-1 might be able to bind to the dyad axis where DNA enters and exits the nucleosome, possibly providing an additional mode of allosteric regulation of PARP-1 enzymatic activity.

In addition to direct binding to DNA, PARP-1 enzymatic activity can also be stimulated by interactions with protein binding partners. For example, interaction with the DNA binding transcription factor YY1, which binds to the BRCT motif in the automodification domain of PARP-1, can stimulate PARP-1 enzymatic activity as much as 10-fold (Griesenbeck et al., 1999). These observations are especially relevant in cases where PARP-1's enzymatic activity is required for transcription regulation, since they suggest that PARP-1 could be activated for a localized effect upon recruitment to specific genes by DNA bound factors. Interestingly, the distinct enzymatic activities of PARP-1 (e.g., initiation versus polymerization) may be regulated independently. For example, the concentration of double-stranded DNA (nicked in the reported experiments) has been shown to affect the frequency of initiation, whereas the concentration of NAD^+ has been shown to affect polymer size (Alvarez-Gonzalez et al., 1999). Thus, PARP-1 enzymatic activity is likely to be (1) acutely responsive to the physiological state of the cell, as reflected by NAD^+ concentration and genome integrity, and (2) fine-tuned by its allosteric regulators to suit the specific needs of the cellular processes in which it participates.

Regulation of Chromatin Structure and Transcription by PARP-1

Roles for PARP and poly(ADP-ribosyl)ation in transcription regulation of specific genes have been demonstrated using a variety of experimental approaches,

including in vitro transcription assays, cell-based reporter gene assays, anti-sense RNA technology, gene deletion in vivo, and expression microarrays (D'Amours et al., 1999; Hassa and Hottiger, 2002). The available data suggest that PARP-1's activity in transcriptional regulation occurs by at least two mechanisms that are not mutually exclusive: (1) modifying histones to alter chromatin structure and (2) functioning as part of enhancer/promoter binding complexes in conjunction with other DNA binding factors and coactivators.

Activity of PARP-1 and PAR on Nucleosomes

In vitro studies of the 1980s and early 1990s first suggested mechanisms by which PARP-1 and its polymeric product PAR can disrupt chromatin structure. First, PARP-1 can directly modify the structural proteins that constitute chromatin (D'Amours et al., 1999). The poly (ADP-ribosyl)ation of chromatin proteins can have profound effects on both the packing of nucleosomes into higher-order structures and on the stability of individual nucleosomes. Histones are the main protein component of chromatin, and histones H1 and H2B show the most poly(ADP-ribosyl)ation in vivo and are the preferred targets of PARP-1 in vitro (Huletsky et al., 1989; Poirier et al., 1982), although all histones are modified to some extent (D'Amours et al., 1999). Poly(ADP-ribosyl)ation of native polynucleosomes by purified PARP-1 leads to decondensation, as seen directly by electron microscopy, mimicking the effects of H1 depletion from higher-order chromatin (Poirier et al., 1982). Second, the polyanionic PAR, attached to protein substrates or perhaps existing as free polymers, can act as an attractive local matrix for core histones released from destabilized nucleosomes (Mathis and Althaus, 1987; Realini and Althaus, 1992). The binding of histones by PAR may further expose DNA, providing the protein machinery required for transcription and other genomic processes greater access to DNA. Although the average length of PAR is much shorter in cells that are not subject to DNA damage than in cells suffering DNA damage (D'Amours et al., 1999), polymers generated at specific loci in response to particular transcriptional signals may well be sufficiently long and locally abundant to participate in chromatin decondensation. Such a general mechanism could be augmented by PARP-1 modification of nonhistone chromosomal protein substrates, including structural proteins (e.g., HMG proteins) and transcription factors (see below).

Activity of PARP-1 at Enhancers and Promoters

In addition to modulating transcription through alterations in chromatin structure, PARP-1 regulates transcription by directly altering the activity of enhancers and promoters (D'Amours et al., 1999; Hassa and Hottiger, 2002). In this mode, PARP-1 may function more like a "classical" transcriptional regulator or coregulator than a chromatin-modifying factor. PARP-1 activity at enhancers and promoters occurs in large part by functional interactions between PARP-1 and various nonhistone proteins, many of which are DNA binding transcription factors including NF-κB, B-MYB, Oct-1, nuclear receptors, and the HTLV Tax-1 protein (Anderson et al., 2000; Cervellera and Sala, 2000; Hassa and Hottiger, 2002; Miyamoto et al., 1999; Nie et al., 1998). The ability of PARP-1 to function as a transcriptional regulator or coregulator in conjunction with other DNA binding factors has been examined using a number of approaches, including in vitro and in vivo protein-protein interaction assays, promoter activity assays in PARP-1$^{-/-}$ fibroblasts with transiently transfected reporters or endogenous genes, PARP-1 antisense technology, electrophoretic mobility shift assays, and chromatin immunoprecipitation assays (D'Amours et al., 1999; Hassa and Hottiger, 2002). Although this aspect of the PARP-1 literature is too extensive to review in detail here, the salient features are described below. We caution, however, that the literature covering PARP-1 activity at enhancers and promoters presents some apparently conflicting results and, for every generalization, there are exceptions.

Many studies have shown that PARP-1, when acting at enhancers and promoters, functions as a transcriptional coactivator, stimulating the activity of DNA binding transcription factors (Anderson et al., 2000; Cervellera and Sala, 2000; Hassa and Hottiger, 2002; Nie et al., 1998). PARP-1's effect on promoter function, however, is not limited to activation, as it has been implicated in the repression of transcription in some cell and promoter contexts (Butler and Ordahl, 1999; Miyamoto et al., 1999; Soldatenkov et al., 2002). PARP-1 may be specifically recruited to target promoters by interactions with DNA binding factors (Lee et al., 2002). Alternatively, PARP-1 may contact enhancer or promoter DNA directly (e.g., by recognizing certain DNA structures or specific DNA sequences) or in conjunction with other DNA binding factors (Akiyama et al., 2001; Butler and Ordahl, 1999; Ha et al., 2002; Nie et al., 1998; Nirodi et al., 2001; Plaza et al., 1999; Rolli et al., 2000; Zhang et al., 2002). In many cases, the result of PARP-1 activity is the promotion of transcription factor binding and the assembly of enhanceosome-like complexes (Akiyama et al., 2001; Butler and Ordahl, 1999; Nirodi et al., 2001; Plaza et al., 1999). Although PARP-1 enzymatic activity is required for its transcription regulatory functions in some cases (Butler and Ordahl, 1999; Miyamoto et al., 1999; Nirodi et al., 2001), it is not (Anderson et al., 2000; Cervellera and Sala, 2000; Hassa and Hottiger, 2002; Meisterernst et al., 1997). For example, the expression of PARP-1 target genes in *Drosophila* (e.g., the *PARP-1* gene itself) is dependent on PARP-e, a PARP-1 isoform lacking the catalytic domain (Tulin et al., 2002). These results suggest that PARP-1 plays a direct role as a classical type of transcription regulator/coregulator in certain promoter contexts. One final point worth noting is that PARP-1 has been shown to poly(ADP-ribosyl)ate some transcription factors in vitro (e.g., YY1, NF-κB, TBP) and inhibit their binding to DNA (D'Amours et al., 1999; Hassa and Hottiger, 2002). The relevance of this type of activity to transcription regulation, however, needs to be evaluated in vivo, where high concentrations of other competing PARP-1 targets exist. Together, the available data demonstrate a direct role for PARP-1 in regulating the formation of transcription regulatory complexes at the promoters of certain target genes (Figure 3), in addition to its effects on chromatin structure.

Role of PARP-1 in Coordinating Chromatin Structure and Gene Expression In Vivo

A key activity of PARP-1 is its ability to modify by poly (ADP-ribosyl)ation a variety of chromatin proteins, including histones, and thereby dramatically alter chroma-

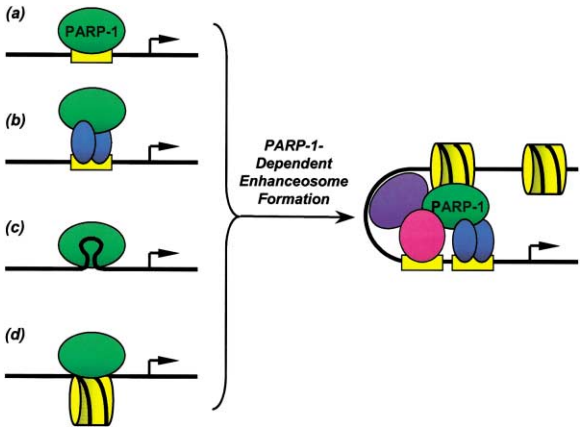

Figure 3. A Model for Activator/Coactivator Functions of PARP-1 at Enhancers and Promoters

PARP-1 can interact with the enhancers and promoters of genes by (a) direct sequence-specific binding to enhancers, (b) recruitment via DNA binding transcription factors (e.g., NF-κB), and (c) direct binding to DNA structures (e.g., cruciform, curved, supercoiled, and crossover). Given PARP-1's capacity to bind to two DNA helices simultaneously, it is interesting to speculate that PARP-1 might be able to bind to the dyad axis where DNA enters and exits the nucleosome, as shown in (d). In many cases, PARP-1 may promote the binding of other factors to DNA, stimulating the formation of enhanceosomes and the activation of transcription in a manner independent of PARP-1 enzymatic activity. The actions of PARP-1 as a transcriptional repressor are less clear, but it may use similar modes of promoter interaction to disrupt, rather than promote, enhanceosome formation.

tin structure. A recent pair of papers from the Spradling lab bring PARP-1 and its intriguing enzymatic activity to front stage in considering the relationship of transcription and chromatin architecture in vivo. Tulin and Spradling (2003) make good use of a *Drosophila Parp* mutant, PARP inhibitors, and the ability to visualize specific loci on polytene chromosomes to demonstrate that PARP-1 enzymatic activity is observed in areas of high transcriptional activity and chromatin decondensation (chromosomal "puffs," which are induced by either hormones or heat shock). Moreover, this work demonstrates that the expression of heat shock and inducible immunity genes are critically dependent on PARP-1, as a *Parp* mutant fails to induce the expression of these genes. Therefore, *PARP-1* is critical for the activation of transcription and chromatin decondensation of at least some highly inducible genes.

A second study by the same group (Tulin et al., 2002) includes results cautioning that the correlation of functional PARP-1 with decondensed chromatin and active genes is not so simple. In particular, the authors observe in a *Drosophila Parp* mutant that the chromatin of *copia,* a multicopy transposon, becomes on average dramatically more open and nuclease sensitive than in wild-type animals. In addition, the level of *copia* mRNA increases 50-fold in a *Parp* mutant. These results suggest that PARP-1, in contrast to its proposed function at the heat shock and immunity genes, may be critical for keeping the repetitive *copia* elements both condensed and transcriptionally repressed. The authors note that the chromatin structures of several other retrotransposons are also much more sensitive to micrococcal nuclease in the *Parp* mutant, even though their transcripts remain at wild-type levels. This may reflect the fact that chromatin decondensation is not sufficient for transcriptional activation of these other transposons and suggest that specific transcription factors must also be present in active form. Interestingly, the single copy *Drosophila Parp* gene itself resides within repetitive sequences of heterochromatin, and its exons also become much more accessible to nuclease in *Parp* mutant homozygotes (Tulin et al., 2002). The dichotomy of PARP-1's effects on transcription is not limited to *Drosophila*. For example, a recent expression microarray analysis using mouse *PARP-1*$^{-/-}$ fibroblasts revealed that the expression of some genes is elevated while the expression of others are depressed relative to corresponding *PARP-1*$^{+/+}$ fibroblasts (Simbulan-Rosenthal et al., 2000). Therefore, PARP-1 appears critical for the modulation of chromatin structure in vivo, but the consequences of its activity on chromatin structure and transcription can vary for genes located in different chromatin environments.

Determining which effects are primary or secondary in *PARP-1* mutants, especially with regard to the heterochromatin results discussed above, is critical for understanding the mechanisms of PARP-1 action. The argument that the effects of PARP-1 depletion are primary and a direct consequence of the loss of PARP-1 activity are strengthened in cases where effects similar to those observed in *PARP-1* mutants are also observed in wild-type cells or animals immediately following treatment with PARP inhibitors. Tulin and Spradling (2003) demonstrate the value of these complementary approaches in their analysis of heat shock gene activation in *Drosophila*. The collection of existing PARP inhibitors (Southan and Szabo, 2003), as well as the creation of new, highly specific PARP-1 inhibitors, when used as a complement to genetic approaches, should help to provide further tests of the direct role of PARP-1 activity in chromatin structure and gene regulation.

A final point worth considering in relation to the studies from the Spradling lab is that the presence and function of PARP-1 is understandably easier to demonstrate at highly transcribed loci (e.g., heat shock puffs) than those that are more modestly transcribed. At heat shock loci, the density of transcribing polymerase can be higher than the density of nucleosomes (Lis, 1998). This requires that the entire transcription unit be decondensed and that a very high ratio of disrupted to nondisrupted nucleosomes be maintained. However, most RNA polymerase II (Pol II) genes are expressed at a much lower level and have many fewer transcribing polymerases. Nonetheless, nucleosomes must still be disrupted in these cases, and the disruption is likely to be transient as higher-order chromatin structures have been observed flanking individual transcribing polymerases (Bjorkroth et al., 1988). It will be interesting to determine if PARP-1 plays an equally important role with these more modestly expressed transcription units as well.

Reversibility: PARG and the Catabolism of PAR

Like other covalent protein modifications (e.g., phosphorylation, acetylation), poly(ADP-ribosyl)ation is re-

versible (Amé et al., 2000; D'Amours et al., 1999). In fact, the turnover of PAR in vivo is rapid, suggesting a tightly regulated and highly responsive process. For example, Tulin and Spradling (2003) showed a near complete turnover of heat-shock-induced PAR polymers and regression of puff size at *Drosophila hsp70* loci within 25 min following the end of heat shock. PAR catabolism is promoted primarily by poly(ADP-ribose) glycohydrolase (PARG), an enzyme with both endo- and exoglycosylase activities that catalyzes the hydrolysis of glycosidic linkages between the ADP-ribose units of PAR (Amé et al., 2000; D'Amours et al., 1999; Davidovic et al., 2001) (Figure 1). In addition, an ADP-ribosyl protein lyase activity catalyzes the removal of the remaining protein-proximal ADP-ribose monomers. *PARG* genes have been identified in mammals, flies, worms, and plants, but the analysis of their function has been limited (Amé et al., 2000). In mammals, the carboxyl-terminal half of PARG contains the catalytic domain, whereas the amino-terminal half of the protein contains a nuclear localization signal, a nuclear export signal, and a putative regulatory domain. In other organisms (e.g., *Drosophila*, *C. elegans*), these same domains/motifs are present, but are arranged differently (Figure 2B). Of these, only the catalytic domain shows a high level of conservation across species (Amé et al., 2000; Davidovic et al., 2001).

In vivo, the steady-state cellular levels of poly(ADP-ribosyl)ation are determined by the opposing actions of PARP and PARG (much like the levels of phosphorylation and acetylation are determined by the relative activities of the respective kinase/phosphatase and acetylase/deacetylase pairs) (Davidovic et al., 2001). Although PARP-1 may be present at a 5- to 20-fold molar excess relative to PARG in cells, factors other than the number of molecules of each enzyme may be more important in determining the extent of poly(ADP-ribosyl)ation in the nucleus (D'Amours et al., 1999; Davidovic et al., 2001). For instance, PARG has a high specific activity relative to PARP-1. Furthermore, PARG activity appears to increase proportionally with polymer size, allowing PARG to counteract the actions of PARP-1 more effectively for lengthy polymers, thus preventing the hypermodification of proteins with very long chains of PAR. Additionally, nuclear PARG activity may be modulated by a regulated nucleocytoplasmic shuttling process that distributes PARG within the cell. Finally, the enzymatically active pool of PARP-1 may be a relatively small percentage of the total until PARP-1 is allosterically activated through interactions with its DNA and protein binding partners. Thus, multiple mechanisms play a role in coordinating the actions of PARG and PARP (D'Amours et al., 1999; Davidovic et al., 2001). Although a role for PARG in transcriptional regulation has not yet been demonstrated directly, the implications from the available data are clear: PARG can counteract the chromatin-modifying actions of PARP, thus restoring chromatin structure and resetting transcriptional levels to a basal or ground state.

A Model for PARP-1 Activity in Regulating Chromatin Structure and Transcription

Eukaryotes have evolved a variety of molecular machines that chemically modify histones and alter chromatin structure (Vaquero et al., 2003). Many of these play important roles in regulating the transcription of particular sets of genes. The recent demonstration of a critical role of PARP-1 at sites in the genome experiencing high levels of transcription (Tulin and Spradling, 2003) indicate that PARP-1, PAR, and enzymes that promote PAR catabolism (e.g., PARG) also need to be considered as components of the machinery involved in gene specific transcriptional regulation.

An attractive model emerges from the Spradling lab studies where PARP-1's broad distribution on chromosomes allows it to modulate chromatin structure at many chromosomal positions, in response to a variety of specific signals that arise at these positions. Moreover, the ultimate effects of PARP-1 on chromatin structure may depend on the nature of the particular chromatin environment (e.g., euchromatin versus heterochromatin) (Figure 4). The consequences of PARP-1 action in euchromatin include opening chromatin structure at promoters and facilitating Pol II transcription through barriers such as nucleosomes or higher-order chromatin structures. The well-characterized role of DNA damage in stimulating PARP activity during DNA repair would simply represent one manifestation of this more global activity. Because PARP-1's distribution in *Drosophila*

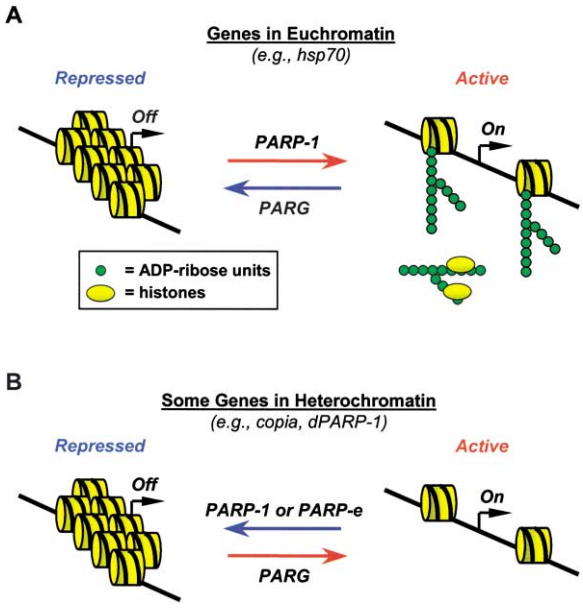

Figure 4. A Model for the Regulation of Chromatin Structure and Transcription by PARP-1 In Vivo

PARP-1's broad distribution on chromosomes allows it to modulate chromatin structure at many chromosomal positions in response to a variety of signals. The specific changes in chromatin structure depend on the nature of the particular chromatin environment (e.g., euchromatin versus heterochromatin).

(A) In euchromatin, PARP-1 preferentially modifies H1 and H2B to decondense higher-order chromatin structure and disrupt nucleosomes. In addition, polyanionic PAR, either free (as shown) or attached to proteins, can serve as a histone acceptor. The resulting increase in DNA accessibility facilitates activated transcription.

(B) In heterochromatin, as shown by Tulin et al. (2002) for *Drosophila*, either PARP-1 or PARP-e, a truncated form lacking the catalytic domain, is critical for maintaining heterochromatic structures and repressing transcription of some genes.

nuclei is broad (Tulin and Spradling, 2003), the model only requires the activation of PARP-1's enzymatic activity at specific loci. However, PARP-1's distribution on chromosomes is also subject to regulation, as PARP-1 is recruited to specific genes or loci as part of enhancer/promoter binding complexes. Interestingly, PARP-1 function is not limited to Pol II-transcribed genes, but is also required for maintenance of nucleolar structure which is dependent on Pol I transcription (Tulin et al., 2002). Thus, PARP-1 is a multifunctional protein whose activities are harnessed to regulate transcription in a variety of ways.

In closing, it is interesting to note that multiple parallels can be drawn between PARP-1 and other histone- and chromatin-modifying enzymes. For example, like histone acetyltransferases (HATs) and ATP-dependent chromatin remodelers (Vaquero et al., 2003), PARP-1 can affect multiple genomic DNA-dependent processes including transcription, replication, repair, and recombination. In addition, like other histone-modifying coactivators (Kraus and Wong, 2002), PARP-1 can participate directly in the assembly of transcription complexes at enhancers and promoters. Furthermore, PARP-1 is likely to operate in a synergistic manner in combination with other chromatin-related factors. In this regard, note that nucleosomes can be simultaneously poly(ADP-ribosyl)ated and acetylated (Malik and Smulson, 1984). The interplay of PARP-1 with other molecular complexes such as histone modifiers (e.g., HATs), nucleosome remodelers (e.g., SWI/SNF), or nucleosome disrupters (e.g., FACT) remains an exciting and challenging area for future investigation.

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