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# Poly-ADP-Ribose Polymerase: Machinery for Nuclear Processes

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## Abstract

It is becoming increasingly clear that the nuclear protein, poly-ADP-ribose polymerase 1 (PARP1), plays essential roles in the cell, including DNA repair, translation, transcription, telomere maintenance, and chromatin remodeling. Despite the exciting progress made in understanding the ubiquitous role of poly-ADP-ribose metabolism, a basic mechanism of PARP's activity regulating multiple nuclear processes is yet to be outlined. This review offers a holistic perspective on activity of PARP1, based on empirically observable phenomena. Primary attention is given to mechanisms by which PARP1 regulates a broad range of essential nuclear events, including two complementary processes (1) regulation of protein-nucleic acid interactions by means of protein shuttling and (2) utilizing poly-ADP-ribose as an anionic matrix for trapping, recruiting, and scaffolding proteins.

### Keywords

*PARP1*; *Parg*; poly(ADP-ribose); chromatin; nucleosome; histones

## 1. INTRODUCTION

The molecular processes occurring within the eukaryotic nucleus primarily revolve around the maintenance and utilization of nucleic acid, a remarkable species of molecules having the capacity to store chemical information and consequently propagate an organism's self-sustained existence. Such maintenance and utilization of nucleic acid is based upon its interaction with proteins: another remarkable group of molecules having the ability to catalyze countless chemical reactions. Nuclear processes center upon this orchestrated protein-nucleic acid dance.

Poly-ADP-Ribose (pADPr) was first discovered 50 years ago and has been gracefully called the *third nucleic acid* (Chambon et al. 1963; D'Amours et al. 1999). The polymer is a structure consisting of ADP-ribose units and several points of branching with 20–25 residues per branch (reaching up to 200 residues in total) linked by glycosidic ribose-ribose 1″-2′ bonds (Gonzalez et al. 1987). It consists of one mole of adenosine per two moles of ribose and two moles of phosphate making pADPr twice as negatively charged as DNA or RNA (Reeder et al. 1967; Sugimura et al. 1967). Its residues are capable of base stacking and forming hydrogen bonds (Kanai et al. 1978; Sibley et al. 1986). Unlike DNA and RNA, pADPr does not have an ability to store chemical information, but rather mimic their biochemical behavior. pADPr is synthesized by poly-ADP-ribose polymerases (PARPs) using $NAD^+$, covalently attaching units of ADP-ribose to both itself and acceptor proteins via glutamic acid residues. PARPs have been shown to play essential roles in numerous nuclear processes, including chromatin remodeling, transcription, DNA repair, DNA

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synthesis, telomere maintenance, and translation. Because of likely functional redundancies, the presence of 17 paralogous PARPs in mammals (Ame et al. 2004; Tulin et al. 2003) greatly complicates their analysis. Fortunately, model organisms, such as *Drosophila*, (Adams et al. 2000) have a single nuclear PARP1 ortholog of mammalian PARP1, making this animal an invaluable model system to study PARP1 functions. Because PARP1 function in *Drosophila* system is understood most comprehancively, this review will focus primarily on findings of *Drosophila* studies, discuss how these findings complement observations made in mammalian systems, as well as their relevance and limitations to understanding poly(ADP-ribose) polymerase activity in mammals.

This review is meant to offer a holistic perspective on the mechanisms by which PARP1 activity regulates a broad range of essential nuclear processes. The effects of PARP1 activity on nuclear processes will be discussed in the context of regulating protein-nucleic acid interaction by means of protein shuttling (Chambon et al. 1963) and utilizing poly-ADP-ribose (Ame et al. 2000; D'Amours et al. 1999) as an anionic matrix for trapping, recruiting and scaffolding proteins.

## 2. BACKGROUND: ENZYMES AND ACTIVITIES

First discovered in the 1960s (Chambon et al. 1963), the role of chromatin modification by pADPr remained mysterious for a long time. The basic enzymatic reactions catalyzed by PARP1 involve transferring ADPr from NAD to either a protein acceptor or an existing pADPr chain (Fig. 1). The modification target is most commonly the COOH residue of a glutamic acid in proteins, although other amino acids may be modified as well. The average pADPr chain length is 20–25 residues (Gonzalez et al. 1987)). The PARP1 protein has three functionally defined domains, incuding 1] a DNA binding domain (DBD), encompassing DNA ligase III type Zn-fingers, which recognize DNA lesions, 2] an automodification domain (AD), which can be modified by ADPr and is also required for dimerization, and 3] a catalytic domain (CD), which performs ADPr transfer (Kameshita et al. 1984; Kameshita et al. 1986).

PARP1 is active as a dimer (Mendoza-Alvarez and Alvarez-Gonzalez, 1993; Buki et al. 1998). ADP-ribose modification of its dimerization domain through automodification causes PARP1 dimers to dissociate from both each other and active chromatin while losing enzymatic activity. A cluster of 10 to 28 Glu residues located near the center of the PARP1 AD serves as the major acceptor of this ADPr. This negative feedback loop mediated by automodification limits the time during which PARP1 molecules can remain active. In contrast, Poly(ADP-ribose) Glycohydrolase (PARG), the antagonist of PARP1, is constantly active as has been demonstrated in mammalian systems (reviewed by Davidovic et al. 2001). In *Drosophila*, PARG is the only known catalyst of pADPr degradation *in vivo*, whereas in mammals there is evidence for an additional pADPr degrading enzyme (Hanai et al. 2004; Oka et al. 2006; Tulin et al. 2006, Kotova et al. 2009) (Fig. 1). The loss of PARG reduces overall PARP1 activity by trapping a large fraction of the PARP1 protein in the inactive, automodified state, which is removed from active chromatin (Tulin et al. 2006; Kotova et al. 2009). In *Drosophila*, inactivating most PARP1 in *Parg* mutants mimics the same developmental phenotypes as observed in *PARP1* mutant homozygotes. In mice, complete inactivation of *Parg* leads to a homozygous lethal embryonic phenotype, while a partial *Parg* knockout is viable but with a phenotype distinct from the mouse *Parp1* knockout mutant (Cortes et al. 2004; Koh et al. 2004; Tulin et al. 2006).

Whereas PARG protein is located in the nucleoplasm of *Drosophila* cells (Kotova et al. 2009; Boamah et al. 2012), in mammalian systems PARG has been found to have differential localization based on the phase of cell cycle, switching between the nucleus and

cytoplasm (Winstall et al. 1999; Ohashi et al. 2003). In contrast, PARP1 is found exclusively along chromosomes and in nucleoli both in *Drosophila* (Tulin et al. 2002; Tulin et al. 2006) and in mammalian cells, although when mammalian cells were treated with RNA synthesis inhibitors, PARP1 was evenly distributed within the nucleus and not concentrated within the nucleolus (Desnoyers et al. 1996). Thus, under steady state conditions, PARG is unlikely to directly interact with PARP1 and other chromatin-associated proteins. However, following PARP1 activation and ADP-ribosylation of its targets, target proteins released from chromatin would be expected to come into contact with nucleoplasmic PARG.

## 3. COREGULATION OF PARP1 AND NUCLEOSOMAL HISTONES

### 3.1. Histones control PARP1 protein in chromatin

After the histones, PARP1 is the most abundant nuclear protein. The pool of freely diffusible nucleoplasmic PARP1 is very small, with most of the PARP1 protein bound to chromatin and accumulated in nucleoli (Molinete et al. 1993; Dantzer et al. 1998; Tulin & Spradling 2003; Kim et al. 2004; Pinnola et al. 2007). The distribution of PARP1 in chromatin is nonrandom, occurring in characteristic profiles specific for distinct cell types. The molecular basis for PARP1 targeting to chromatin remained, for a time, poorly understood. Although Zn-fingers within the *Drosophila* PARP1 DBD can contribute to DNA binding, they specifically recognize damaged DNA *in vitro* (Gradwohl et al. 1990), and it is not clear if Zn-fingers can contribute to the association of PARP1 with intact chromatin. The Pascal lab, however, has demonstrated that PARP's Zn-finger 3 has a duel role in both regulating poly(ADP-ribose) synthesis and chromatin compaction (Langelier et al. 2010). It has also been shown in *Drosophila* that Zn-finger 1 is necessary for binding heterochromatin and silencing retrotransposable elements (Kotova et al. 2010). While mammalian PARP-2 contains no zinc finger module, and thereby no nick sensor as in PARP1, it nevertheless is able to functionally complement a PARP1 null mutant and displays a pattern of chromatin association identical to that of PARP1, although it does seem to possess a not-so obvious DNA-binding motif (Ame et al. 1999; Babinchuk et al. 1998; Meder et al. 2005). Considerable body of evidence suggests that PARP1 directly interacts with core histones (D'Amours et al. 1999; Pinnola et al. 2007). Histones H3 and H4 are preferential targets for PARP1 binding *in vitro*, and they control the enzymatic activity of PARP1 (Fig. 2) (Pinnola et al. 2007; Kotova et al. 2011).

### 3.2. Histone variants control PARP1 in chromatin

Mounting evidence supports the hypothesis that variant histone proteins provide a physical link between PARP1 and chromatin. For 20 years, activation of PARP1 and pADPr has been investigated as a sensor system for DNA damage (for review, see D'Amour et al. 1999). DNA breaks arising from genotoxic treatments immediately induce local foci of pADPr accumulation at chromatin encompassing the point of damage (Lindahl et al. 1995; Lankenau et al. 1999). Similarly, recent studies have linked phosphorylation of histone H2Ax in the same DNA damage pathways and have shown accumulation of phospho-H2Ax in the same pADPr-positive nuclear foci (Meyer-Ficca et al. 2005). In mammalian cells, a direct interaction has been shown to exist between PARP1 and H2Ax, occurring in response to ionizing irradiation. Additionally, ataxia telangiectasia mutated (ATM) has an important interplay with PARP1 involved in the phosphorylation of H2Ax. (Du et al. 2006; Haince et al. 2007). It has also been shown that chemical inhibition of PARP1 enzymatic activity triggers accumulation of phosphorylated, chromatin-bound H2Ax (Bryant et al. 2005). This last observation suggests that the PARP1-H2Ax protein complex has a broader distribution in chromatin than just at points of DNA damage and that PARP1 and H2Ax may be co-regulated by additional cell stimuli beyond DNA damage.

Convincing evidence for the interaction of PARP1 and H2Ax outside of a direct DNA repair pathway was reported recently in a study of chromatin remodeling during rat spermatogenesis (Meyer-Ficca et al. 2005). During spermatid maturation, high levels of pADPr appear precisely at the differentiation stage marked by the highest rate of chromatin protein exchanges. Local PARP1 protein activation in the spermatid nuclei completely coincided with phosphorylation of H2Ax histone (Meyer-Ficca et al. 2005). The authors of this study suggested that DNA double-strand breaks take place during those steps of differentiation. However, this work did not exclude the possibility that H2Ax phosphorylation may take place independently from DNA damage. Thus, the regulation of PARP1 interaction with histone H2Ax may provide a means for PARP1 incorporation into chromatin, as well as a way to activate poly(ADP-ribosyl)ation.

In addition to H2Ax, most eukaryotes have another histone variant, H2Az, which is highly homologous to H2Ax (Redon et al. 2002). Together, these proteins comprise roughly 10 to 20% of cellular H2As. Although H2Ax and H2Az have many common features, H2Az lacks a phosphorylation (“SQ”) domain. Also unlike H2Ax, H2Az has no functional connection to the DNA repair pathway. However, H2Az has been implicated in the maintenance of silencing (Dhillon and Kamakaka 2000), as well as transcriptional activation (Stargell et al. 1994) and maintenance of cell viability (Liu et al. 1996). Because mutation of PARP1 in *Drosophila* causes cellular defects reminiscent of the defects in H2Az, such as desilencing of heterochromatin (Tulin et al., 2002), we considered it a reasonable hypothesis that H2Az histone may also interact with PARP1.

### 3.3. Histone variant H2Av is a functional homologue of H2Ax and H2Az in *Drosophila*

*Drosophila* has a single H2A variant, H2Av, which combines specific features of the H2Az (the amino-terminal tail) and H2Ax proteins (the SQ-phosphorylation domain) (Leach et al. 2001; Madigan et al. 2002). Considerable evidence suggests that the functions of PARP1 and H2Av are related in *Drosophila*. Both PARP1 (Tulin et al. 2002) and H2Av histone (Swaminathan et al. 2005) are involved in heterochromatin formation and maintenance. Both proteins demonstrate a nonuniform pattern of chromatin association, localizing to thousands of discrete euchromatic loci, as well as to heterochromatin (Tulin et al. 2002; Leach et al. 2001). As one suggestive functional linkage, H2Av is enriched in heat shock loci (Leach et al. 2001), while PARP1 protein is responsible for chromatin opening and transcriptional induction of *hsp* genes (Tulin and Spradling 2003).

To date, studies of DNA recombination provide the best evidence for the functional linkage of H2Av and PARP1. Efficient repair of double-strand breaks by homologous recombination requires Ser137 phosphorylation of *Drosophila* H2Av. Following repair, the *Drosophila* Tip60 chromatin-remodeling complex acetylates nucleosomal Ser137-phospho-H2Av and performs ATP-dependent chromatin remodeling, which involves the exchange of phospho-H2Av for unphosphorylated H2Av (Kusch et al. 2004). The orthologous Tip60 multiprotein complex has been purified from human cells. In humans, Tip60 has also been implicated in chromatin remodeling with a role in DNA repair (Ikuka et al. 2000). As with many remodeling complexes, Tip60 is unable to work on a linker histone H1-positive template. These facts, together with the observation that activated PARP1 removes H1 from chromatin (Kim et al., 2004), allows us to propose an attractive model for H2Av – PARP1 interactions (Fig. 3). In this model, (1) a complex of H2Av and PARP1 is activated by DNA break formation; (2) phosphorylation of H2Av triggers PARP1 enzymatic activity; (3) activated PARP1 modifies H1 histone with pADPr, which removes it from damaged loci; (4) the Tip60 complex remodels local chromatin, and facilitates entry of DNA repair complexes; and, finally, (5) Tip60 removes Ser137-phospho-H2Av from repaired chromatin.

### 3.4. PARP1 activation mediates a local transient loosening of chromatin and regulates transcription

In the sections above, we summarized information about PARP1 protein interaction with chromatin and the roles of H2A histone variants. Here, we briefly summarize the current understanding of PARP1 functions in chromatin and the mechanisms of PARP1 action.

It is known that transcription induced during steroid response or the response of stress-activated genes, such as hsp70, is accompanied by a local loosening of chromatin structure that manifests in polytene chromosomes as “puffs” at the site of transcription. Chromatin in puffs is disassembled not only at the level of higher-order architecture, but also through the partial dissociation of the nucleosomal core. We have demonstrated that *Drosophila* PARP1 is necessary to maintain nucleoli and to activate genes within polytene chromosome puffs (Tulin et al. 2002; Tulin and Spradling 2003). Shortly after hormone treatment, infection or heat shock stress, pADPr polymers accumulate at the target loci. As a result of PARP1 action, the chromatin structure loosens, leading to the appearance of the visible puff as gene transcription commences. *PARP1* mutant *Drosophila* lack nucleoli, fail to form puffs, and produce greatly reduced amounts of target gene products. Additionally, in mammals, PARP1 activity has also been implicated in regulating transcription via chromatin architecture (Ju et al. 2006). These observations, along with previous studies demonstrating the roles of tankyrase-1, a member of the poly(ADP-ribose) polymerase family, in repair and telomere maintenance (Smith et al. 1998; Smith and Lange 2000; Rippmann et al. 2002), led to the suggestion that poly(ADP-ribose) polymerases acts in a wide variety of situations by locally derepressing gene transcription or other chromosomal processes that are blocked by the tight binding of histones and other inhibitory chromatin proteins.

Two mechanisms by which PARP1 may loosen chromatin have been suggested by biochemical studies (for review, see Tulin et al., 2003). First, PARP1 may catalyze the transfer of ADP-ribose moieties from NAD to abundant nuclear proteins, such as histones. Indeed, *in vitro* modification by pADPr destabilizes the interaction of chromatin components with DNA, including histones (Poirier et al. 1982a; Aubin et al. 1983). Second, the formation of long pADPr residues on PARP1 itself may contribute to loosening (Althaus et al. 1995; Kim et al. 2004). Many chromatin proteins exhibit high affinity for pADPr and may relocate from chromatin and bind to automodified PARP1 at the sites of activation. Alternatively, the network of pADPr may generate an environment conducive to the formation of active loci, organizing the processes of transcribing, packing, and processing RNA. The observation that nucleoli disintegrate following PARP1 inhibition favors the latter model.

## 4. POLY(ADP-RIBOSE) MODULATES NUCLEAR PROTEIN FUNCTIONS

### 4.1. PARP1 covalently modifies nuclear proteins

To begin understanding the essential role of PARP1 activity in nuclear processes, it seems appropriate to begin by understanding how PARP1 activity directly modifies proteins. PARP1 can modify proteins both covalently and noncovalently; that is to say, a protein’s function or localization can be dramatically changed, either by the covalent attachment of poly-ADP-ribose or noncovalent interaction with it. Approximately thirty proteins have been identified, both *in vivo* and *in vitro*, as covalent targets of poly-ADP-ribosylation (D’Amours et al. 1999). The proteins include a broad range of components and regulators in key nuclear processes: modulating chromatin structure, DNA synthesis, DNA repair, transcription, cell cycle, and additional miscellaneous targets.

### 4.2. Poly(ADP-ribose) binds nuclear proteins

Many proteins have also been identified as having strong noncovalent affinity for pADPr proteins, including histones, hnRNPs and various others involved in DNA repair and checkpoints (Ji 2011; Panzeter et al. 1992). A relatively recent proteome-wide identification of pADPr binding proteins and pADPr-associated protein complexes highlights just how ubiquitous the role of PARP1 activity is in nuclear processes, particularly with regard to the noncovalent interaction of proteins with poly-ADP-ribose (Gagne et al. 2008). This paper provides an elegant graphical network representing the intertwining of pADPr-associated proteins into six main groups: DNA repair, DNA replication, cell cycle, chromosome organization/biogenesis, protein synthesis, and mRNA metabolism.

Additionally, noncovalent interaction with pADPr was found to be partly dependent on the poly-ADP-ribose chain length (Fahrer et al. 2007). Because pADPr is rich in phosphate, it is fair to suggest that many molecules interact with the anionic polymer via a nonspecific attraction between charges (Fig. 4). Although this may be true to some degree, substantial evidence suggests that certain proteins possess specific pADPr-binding domains.

One of the first proteins identified as having a pADPr-binding domain was the tumor suppressor protein p53 (Malanga et al. 1998). The existence of the same pADPr -binding motif has been reported in several other DNA damage/checkpoint proteins (Pleschke et al. 2000); the domain is itself a 20 amino acid motif that overlaps with functional motifs important for protein and DNA binding. It is speculated that binding to pADPr through this motif interferes with a protein's ability to bind DNA or even other proteins. Since then, two other pADPr-specific binding motifs have been identified: a 190 amino acid conserved ADP-ribose binding module known as the macrodomain (Karras et al. 2005), which has also been shown to possess affinity for single ADP-ribose moieties, and a novel poly-ADP-ribose binding zinc-finger motif (PBZ) present in many DNA damage and checkpoint proteins (Ahel et al. 2008).

### 4.3. Protein shuttling by PARP1 and poly(ADP-ribose)

The process of shuttling, or pulling proteins from nucleic acid, is a paramount mechanical process in the dynamic, complex environment of the nucleus. The role PARP1 plays in numerous nuclear activities stems from this fundamental event by which PARP1 covalently or noncovalently modifies proteins to dissociate from nucleic acid. It is believed that PARP's broad role in shuttling proteins from nucleic acid proceeds by two nonmutually exclusive events: electrorepulsion between the highly anionic poly-ADP-ribose and DNA/RNA, and the stealing/masking of DNA/RNA binding domains (D'Amours et al. 1999). Electrorepulsive shuttling involves moving, or shifting, entities based upon the repulsive force of like charges, which, in this context, is between the negatively charged phosphate of poly-ADP-ribose and nucleic acid (Ferro & Olivera 1982; Zahradka & Ebisuzaki 1982). When poly-ADP-ribose is covalently attached and elongated onto a nucleic acid-bound protein, an electrorepulsive gradient is made between the two anionic polymers. A point of repulsion then occurs between the modified protein and nucleic acid resulting in their separation.

## 5. MOLECULAR MECHANISM OF PARP1-DEPENDENT CHROMATIN OPENING (LOOSENING)

### 5.1. PARP1 modulate nucleosomal stability

One of the most dramatic effects of protein shuttling by PARP1 involves the process of opening (loosening) chromatin. Eukaryotic DNA is tightly packaged by histones into the complex chromatin. When the cell needs to access DNA for repair or transcription, the

packaged DNA needs to be opened. Substantial evidence has implicated poly-ADP-ribosylation in the process of histone shuttling and subsequent chromatin decondensation in which poly-ADP-ribose attracts histones away from DNA (Poirier et al. 1982a; de Murcia et al. 1982; Realini & Althaus 1992; Mathis & Althaus 1987; Althaus 1992). For example, the heat shock-induced puffs on the *Drosophila* polytene chromosome is a phenomenon dependent upon PARP1 activity and the accumulation of pADPr (Tulin et al. 2002; Tulin & Spradling 2003). Work published from the Lis lab has proposed a detailed mechanism for PARP-induced chromatin relaxation at the *Hsp70* locus; their data demonstrated the HSF induced spread of PARP1 throughout the locus and subsequent accumulation of pADPr (Petesch & Lis 2012). This mechanism provides a means by which the nucleosomal barrier is overcome in response to heat shock-induced transcription activation.

In order to open chromatin, PARP1 activity must modify the interaction between histone-packaged proteins and DNA. The attraction between the negatively charged phosphate backbone of DNA and alkaline residues of histones is largely responsible for their association and consequent condensation of chromatin. In this regard, experiments testing noncovalent interaction between histones and pADPr have shown that these DNA-packaged proteins have strong, and, in the case of linker histone H1, even stronger affinity for pADPr than DNA. It has also been shown that histones H2A, H2B and linker histone H1 are poly-ADP-ribosylated *in vitro*, while H1 and H2B are the main targets *in vivo* (D'Amours et al. 1999; Poirier et al. 1982b).

Additionally, histone H4-induced *Drosophila* PARP1 enzymatic activation *in vitro* was shown to strongly modify linker histone H1, but not H2A or H2B (Thomas et al. unpublished). It was also demonstrated that the poly-ADP-ribosylated H1 lost affinity for chromatin *in vitro*, suggesting a mechanism for H1 ejection from chromatin. Linker histone H1 is well associated with its contribution to chromatin condensation, and its ejection from chromatin by poly-ADP-ribosylation marks a clear mechanism for chromatin opening in response to PARP1 activation. It should also be noted that PARP1 activity has been shown to regulate lamina binding to chromatin. Lamin C has been found to be a target of poly-ADP-ribosylation (Pedraza-Reyes & Alvarez-Gonzalez 1990), resulting in its dissociation from chromatin, which, in turn, contributes to the transformation of heterochromatin into euchromatin.

Enzymatically inactive PARP1, like linker histone H1, has also been shown to contribute to chromatin compaction. Both H1 and PARP1 compete for the same binding sites at the promoters of genes (Krishnakumar et al. 2008); therefore, apart from shuttling histones, PARP1 can also loosen chromatin by removing itself from DNA. PARP1 ejection from chromatin is best implicated after detecting and becoming activated by DNA strand breaks (Zahradka & Ebisuzaki 1982). The poly(ADP-ribose) covalently added to PARP1 itself creates an electrorepulsive force with DNA, resulting in PARP's dissociation from the site of damage. The chromatin loosening that subsequently follows allows space for DNA repair machinery to access the damaged DNA.

The phenomenon of PARP-induced chromatin opening does not appear completely distinct in response to either genotoxic stress or transcription. It has been shown that core histones regulate PARP1 enzymatic activity in both transcription and DNA repair, independent of broken DNA. The mammalian H2Ax histone variant, which is phosphorylated in response to genotoxic stress, is well associated with PARP1 activity. The *Drosophila* histone H2A variant (H2Av), a homologue to the mammalian H2Ax and H2Az variants, was found to regulate PARP1 enzymatic activity in response to both genotoxic stress and transcription; its phosphorylation leads to PARP1 activation. The phosphorylation of H2Av most likely

results in the exposure of histone H4 to PARP1, stimulating its enzymatic activity (Fig. 5) (Kotova et al. 2011).

The differences between histone-regulated and damaged DNA-induced PARP1 activity in loosening chromatin is not quite clear. Although automodified PARP1 loses affinity for DNA after detecting a DNA nick, it most likely modifies other proteins in the process, namely histones, while also pulling histones with it during its electrorepulsion from DNA. There is evidence to suggest that histone H4 remains directly bound to the automodified PARP1 protein after it induces PARP's enzymatic activity. If histone H4 remains bound to PARP1 after activating it, perhaps it allows a mechanism for sustained PARP1 activity and the presence of pADPr (attached to PARP), slowing its degradation by PARG. It has also been shown that histone H4 stimulates PARP1 activity four times greater than broken DNA *in vitro*. This phenomenon may suggest an interesting difference in the duration of PARP1 activity and subsequent presence of pADPr—with broken DNA stimulating a relatively temporary loosening of chromatin, as opposed to a more sustained effect by histones.

### 5.2. Molecular model of PARP1 protein regulation in chromatin

Recently published data (Pinnola et al. 2007; Kotova et al 2011; Petesch & Lis 2012) allow to propose the model of PARP1 positioning and action (Fig 6): 1) chromatin remodeling complex positions the H2A histone variant containing nucleosomes in promoter regions of genes; 2) an H2A-variant-bearing nucleosome has a greater affinity to PARP1 because of better surface representation of H3 and H4 histones, due to the nucleosome's more open configuration (Suto et al. 2000); therefore, such nucleosomes preferentially bind PARP1 and position it inside promoters; 3) kinase phosphorylates H2Ax (H2Av) C-terminal tail—in *Drosophila*, probably, a kinase implicated in maintaining open (loose) chromatin domains necessary for transcription; 4) this phosphorylation increases the strength of interaction between PARP1 and H4, thereby enzymatically activating PARP1 and initiating pADPr production; 5) pADPr induces further change in the nucleosome (relaxation) and overall surrounding chromatin structure; 6) these modifications together facilitate transcription, or genotoxic stress response, by changing the nucleosome/chromatin structure to a more open configuration.

## 6. OTHER NUCLEAR PROTEIN SHUTTLING BY PARPS

Although PARP1 activity plays a major role in chromatin loosening in response to multiple environmental or developmental cues, it is also necessary for regulating protein-nucleic acid interaction in several other nuclear processes. In eukaryotic cells, mRNA splicing involves the excision of introns in forming a mature mRNA transcript for translation. This process enables the cell to create a variety of proteins from a single gene by differentially splicing the transcribed RNA. Messenger RNA splicing is regulated, in part, by heterogeneous nuclear ribonucleoproteins (hnRNPs) which bind to the mRNA transcript and direct alternate splicing. Poly-ADP-ribosylation has been implicated in modulating mRNA splicing by regulating the binding of hnRNPs to the mRNA (Ji 2011; Ji & Tulin 2009). When an hnRNP is modified by PARP1, it dissociates from the RNA, resulting in a modulation of the transcripts splicing (Ji & Tulin 2009). PARP1 activity has also been found to regulate translation of DE-cadherin in *Drosophila* progenitor cells via the regulation of Hrp38, an orthologue of human hnRNPA1, and the 5′-untranslated region of the DE-cadherin mRNA (Ji & Tulin 2012). Poly-ADP-ribose disrupts the interaction between Hrp38 and mRNA, resulting in diminished translation of DE-cadherin.

PARP1 activity has also been found to regulate the binding of transcription factors to chromatin. Tankyrase, a PARP1 present at the telomeres of chromosomes, is essential for regulating telomere maintenance by poly-ADP-ribosylating TRF1 (Smith et al. 1998). The

TRF1 transcription factor represses a specific polymerase that elongates telomeres. By modifying TRF1 with poly (ADP-ribose), PARP1 ejects the protein from chromatin, thereby allowing the elongation of telomeres. PARP1 has also been found to poly-ADP-ribosylate TATA-binding protein (TBP), Yin Yang 1, NFkappaB, Sp1, and CREB, resulting in their inability to bind DNA (Oei et al. 1998).

The association of the FACT complex, a chromatin structure modulator, with chromatin is regulated by PARP1 activity (Huang et al. 2006). The complex is composed of two subunits: hSpt16 and SSRP1. It has been shown that hSpt16 is poly-ADP-ribosylated by PARP1, most notably after genotoxic stress treatment. The modification of hSpt16 results in the dissociation of FACT from chromatin. The nucleosome-remodeling ATPase ISWI is also poly-ADP-ribosylated by PARP1, resulting in its enzymatic inactivation, along with its dissociation from DNA and the nucleosome (Sala et al. 2008).

PARP1 can also influence chromatin structure via protein shuttling by regulating DNA methylation patterns, a critical event in epigenetics (Malanga et al. 2008; Malanga & Althaus 2004). It has been found that CCCTC-binding factor (CTCF) induces PARP1 automodification (Guastafierro et al. 2008; Caiafa et al. 2009). Subsequently, the pADPr branches attached to PARP1 trap DNA methyltransferase 1 (Dnmt1), preventing its association with DNA and thereby inhibiting its enzymatic activity. When automodified PARP1 is not present, Dnmt1 is available to methylate DNA. Dnmt1 has very strong affinity for pADPr and possesses two pADPr binding domains. Even in the presence of excess DNA, the interaction between Dnmt1 and pADPr is uninterrupted (Reale et al. 2005).

The inactivation of a chromatin-associated protein by shuttling it away from DNA and trapping it with poly-ADP-ribose is also used in DNA repair. DNA topoisomerase is a target of covalent poly-ADP-ribosylation which results in its ejection from damaged DNA (Malanga & Althaus 2004; Ferro et al. 1983). When topo I is scanning the topological state of DNA and arrives at a DNA lesion, it becomes stalled and threatens genomic stability. PARP1 activity then works to counteract stalled topo I by allowing for efficient repair of DNA strand breaks.

## 7. POLY-ADP-RIBOSE AS A MATRIX: RECRUITMENT AND SCAFFOLDING

The strong attraction between various proteins and poly-ADP-ribose, specific or nonspecific, is seen as having essential consequences other than mediating protein-nucleic acid interaction. As a heavily branched, anionic polymer, it is becoming increasingly clear that poly-ADP-ribose acts both as a place of origin for various nuclear processes—a point of protein recruitment—and an environment in which the processes can occur.

### 7.1. Ribosomal Biogenesis, rRNA Metabolism

A previously unreported function of PARP1 activity has recently been described in the process of *Drosophila* ribosomal biogenesis (Boamah et al. 2012). When PARP1 activity is disrupted, the nucleolus disintegrates, which results in irregular localization of nucleolar-specific proteins, the accumulation of rRNA intermediates and an overall decrease in ribosome levels. The proposed model describes how poly-ADP-ribose acts as a matrix for binding nucleolar proteins (e.g., Fibrillarin, AJ1, and CC01311) and keeping them in close proximity to precursor rRNA. It is believed that the delocalization of nucleolar proteins prevents the assembly of a functional rRNA processing complex and, subsequently, leads to overall reduction in the amount of ribosomes. In this regard, poly-ADP-ribose acts as an organizing network, or scaffolding, for the nucleolar microenvironment. It was found that a subset of nucleolar proteins preferentially binds to pADPr in wild-type cells, such as Fibrillarin, AJ1, Nucleolin, and Nucleophosmin, while other nucleolar proteins, including

CC01311, dNop5 and CKII alpha, do not (Fig. 7). The preferential binding to pADPr may thereby determine the order of steps that occur during ribosomal biogenesis. Automodified PARP1 has also been found to be responsible for shuttling protein to Cajal bodies (CBs), suborganelles implicated as possible sites for RNA-related metabolic processes—an event that combines both the shuttling and scaffolding effects of PARP1 activity (Fig. 8) (Kotova et al. 2009).

### 7.2. DNA Repair Machinery

Other than serving as a template for ribosomal biogenesis, it has also been suggested that poly-ADP-ribose functions in a similar fashion during DNA repair. Specific noncovalent interaction with pADPr was first described with proteins involved in DNA repair/checkpoint. It is certainly curious to consider the fact that PARP1 activity is dramatically increased in response to DNA damage, while several DNA repair/checkpoint proteins possess pADPr-binding domains. One example involves the nucleosomal remodeler ALC1, which has been shown to have its remodeling activity induced by automodified PARP1 (Ahel et al. 2009; Gottschalk et al. 2009). The chromatin remodeling oncoprotein protein ALC1 is recruited to poly-ADP-ribose through its macrodomain. After DNA damage and PARP1 activation, ALC1 is recruited to the site of damage resulting in the reorganization of that particular chromatin domain. ALC1 also interacts with other DNA repair proteins, such as XRCC1, perhaps resulting in an orchestrated array of events beginning with the synthesis of the poly-ADP-ribose recruiting matrix. By depleting or overexpressing ALC1, cells become especially sensitive to DNA-damaging agents. The role of ALC1 in DNA damage response would seem to be sustained by its interaction with known factors involved in DNA repair.

Additionally, a study using fluorescently tagged macrodomains from different proteins found that they accumulate at sites of laser-induced DNA damage, which, in turn, was disrupted by a mutation that prevents the macrodomain from binding poly-ADP-ribose. The histone variant macroH2A1.1 is recruited to sites of DNA damage, resulting in a change of chromatin structure. The pADPr-binding motifs of XRCC1, DNA ligase III, and DNA polymerase-E result in their recruitment and subsequent interaction at DNA strand breaks (Pleschke et al. 2000).

### 7.3. DNA Synthesis

PARP1 has also been implicated to play a major role in the assembly of an active DNA synthesome (Burzio & Koide 1970; Roberts et al. 1973; Eki & Hurwitz 1991; Dantzer et al. 1998; Simbulan-Rosenthal et al. 1998; Simbulan et al. 1993). PARP1 covalently modifies 15 of the approximate 40 proteins comprising the multiprotein DNA replication complex (MRC, DNA synthesome), including DNA polymerase and proliferating-cell nuclear antigen (PCNA) (Simbulan-Rosenthal et al. 1998). PARP1 itself is also a component of the complex. DNA polymerase has been found to stimulate PARP1 activity via interaction with the DNA polymerase catalytic subunit.

## 8. CONCLUDING REMARKS

Because PARP1 plays essential roles in regulating nuclear processes, its mechanisms of operation provide us with a platform from which to study how these processes are coordinated for normal function of a living cell. This review offers a comprehensive discussion of PARP1 functions at a fundamental level, examining the basic methods of operation by which PARP1 regulates nuclear processes. The fundamental mechanical effects of PARP1 activity have been discussed as two complementary phenomena: (1) regulation of protein-nucleic acid interaction by means of protein shuttling and (2) recruitment poly-ADP-

ribose as an anionic matrix for trapping, recruiting and scaffolding proteins. With the exciting possibilities being realized in the field of epigenetics, from new therapies for patients to the innumerable biological questions that might be answered, the importance of PARP1 functions cannot be overlooked.

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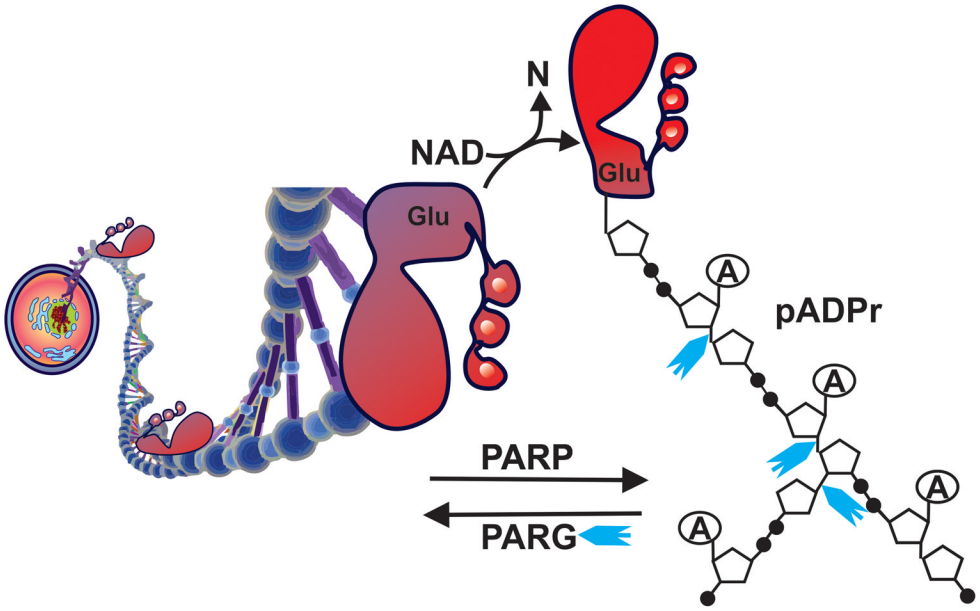


**Figure 1. Nuclear Poly(ADP-ribose) turnover**

Levels of protein pADPr reflects relative activities of the poly(ADP-ribose) polymerase (PARP) enzyme, which utilizes NAD to create pADPr-modified proteins, and the poly(ADP-Ribose) glycohydrolase (PARG) enzyme, which removes pADPr moieties. Arrowheads indicate cleavage points of poly(ADP-ribose) by PARG.

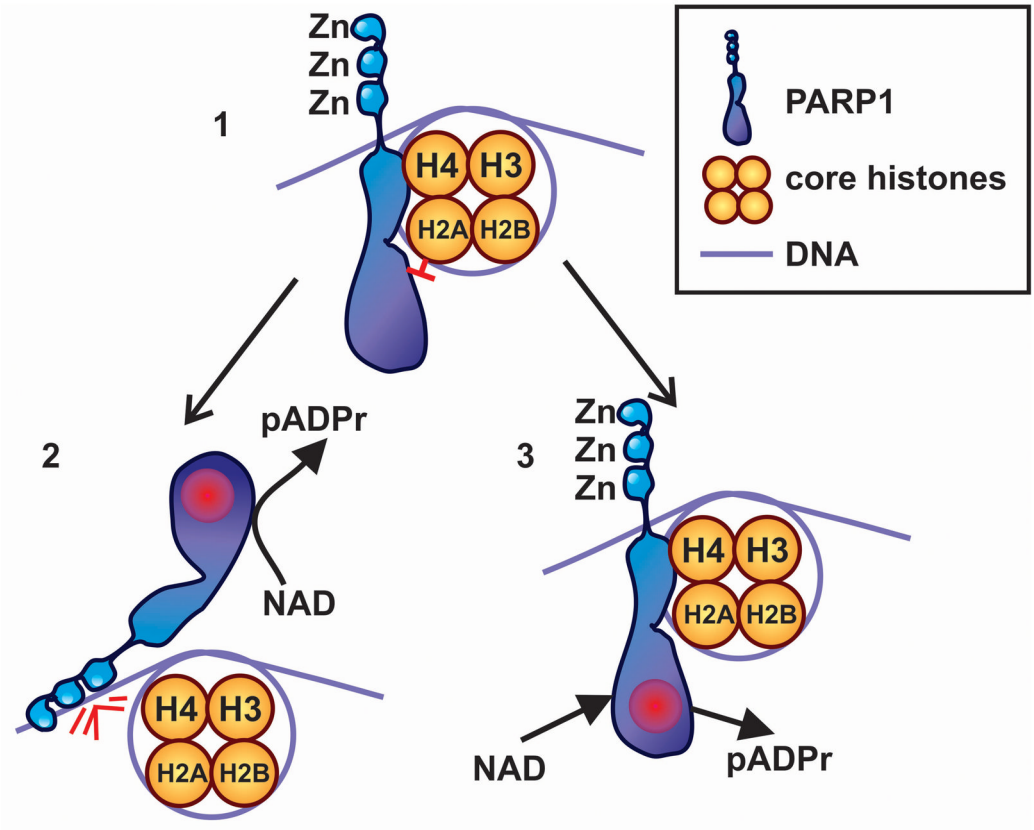


**Figure 2. Two pathways of PARP1 activation**

1. PARP1 is broadly distributed in chromatin as it interacts with core histones of nucleosomes. PARP1 is inactive in this state because of inhibitory effect of histone H2A. 2. Genotoxic stress –dependent PARP1 activation. N-terminal domain of PARP1 serves as a sensor of the double strand breaks and nicks in genomic DNA. Upon binding to damaged DNA, PARP1 mediates conformational changes in nucleosomes leading to disruption of interaction with histones and consequent activation of the PARP1 enzymatic reaction. 3. DNA-independent PARP1 activation. Developmental or environmental signals induce local changes in the “histone modification code” and subsequently expose N-tail of histone H4 and/or hide histone H2A. These structural changes are followed by H4-dependent PARP1 activation.

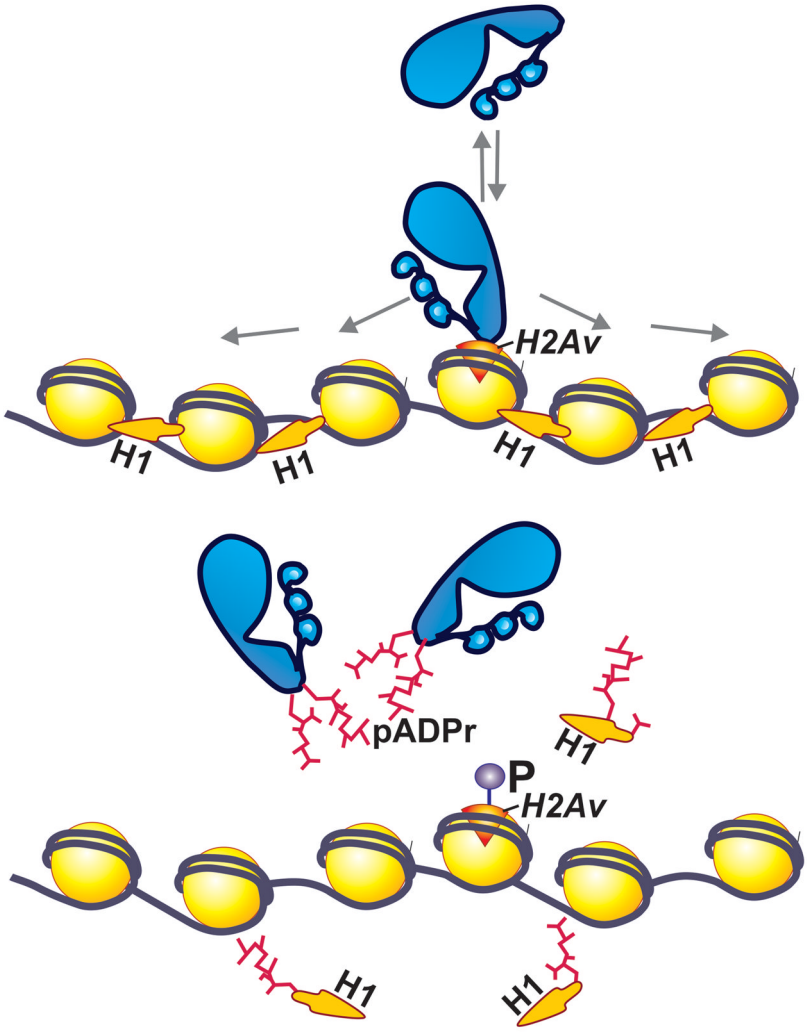


**Figure 3. Model of PARP1 interaction with histone H2Av**

Nucleosome with H2Av works as a high affinity site (red) for PARP1 (blue) binding with specific chromatin domains. While in complex with H2Av-nucleosome, PARP1 is catalytically inactive. Phosphorylation of H2Av disrupts its interaction with PARP1 and stimulates PARP1 activity (poly(ADP-ribosylation)). PARP1 modifies histone H1 (Aubin et al., 1983) and facilitates local chromatin relaxation and remodeling.

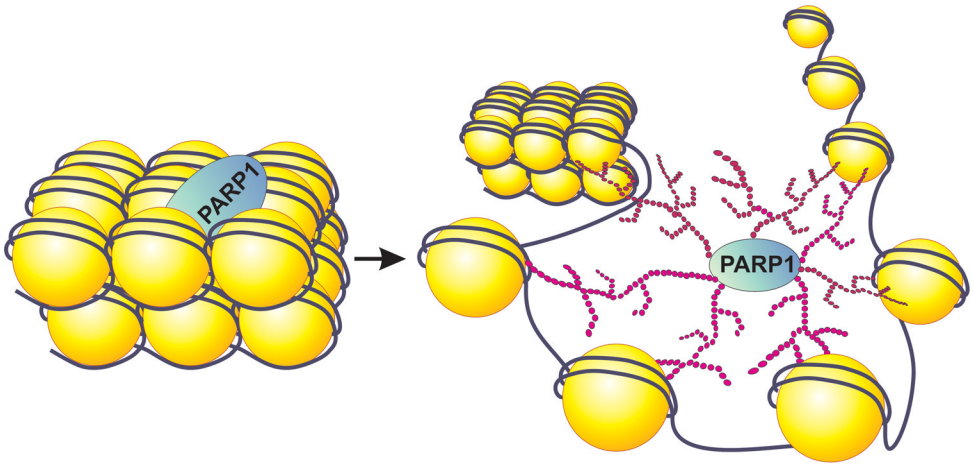


**Figure 4. PARP1 modulates chromatin structure by "electrostatic repulsion"**

Upon PARP1 activation in condensed chromatin block, this enzyme assembles poly(ADP-ribose) (pADPr), a polymer that is twice as negatively charged as the DNA molecule. Addition of pADPr to chromatin proteins likely loosens the interaction within nucleosomal arrays by electro-repulsive interactions between pADPr and DNA and removal of histones from DNA.

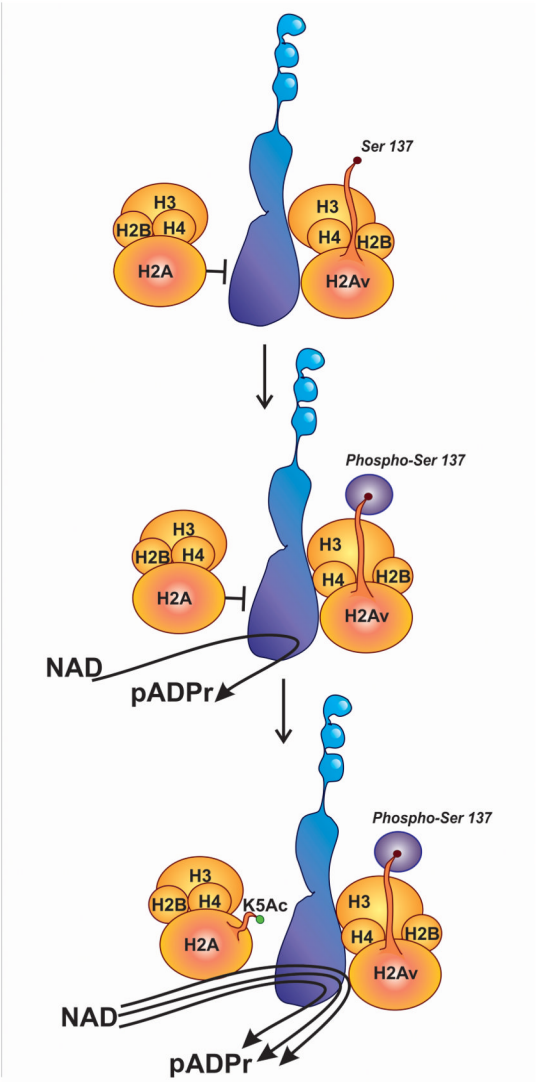


**Figure 5. Molecular model of PAPR-1 activation by nucleosomal histones**

An H2Av-bearing nucleosome has a greater affinity to PARP1 because of better surface representation of H3 and H4. Terefore, such nucleosome preferentially binds PARP1 and positions it inside promoters (Kotova et al. 2011). A neighboring H2A-nucleosome inhibits PARP1 via H2A – PARP1 interaction (Pinnola et al. 2007). Phosphorylation of H2AvSer137 increases the strength of interaction between PARP1 and H4 within H2A-nucleosome, thereby initiating pADPr production. The acetylation of H2A Lys 5 in H2A nucleosome disrupts inhibitory effect of H2A on PARP1, thus, enhance pADPr synthesis.

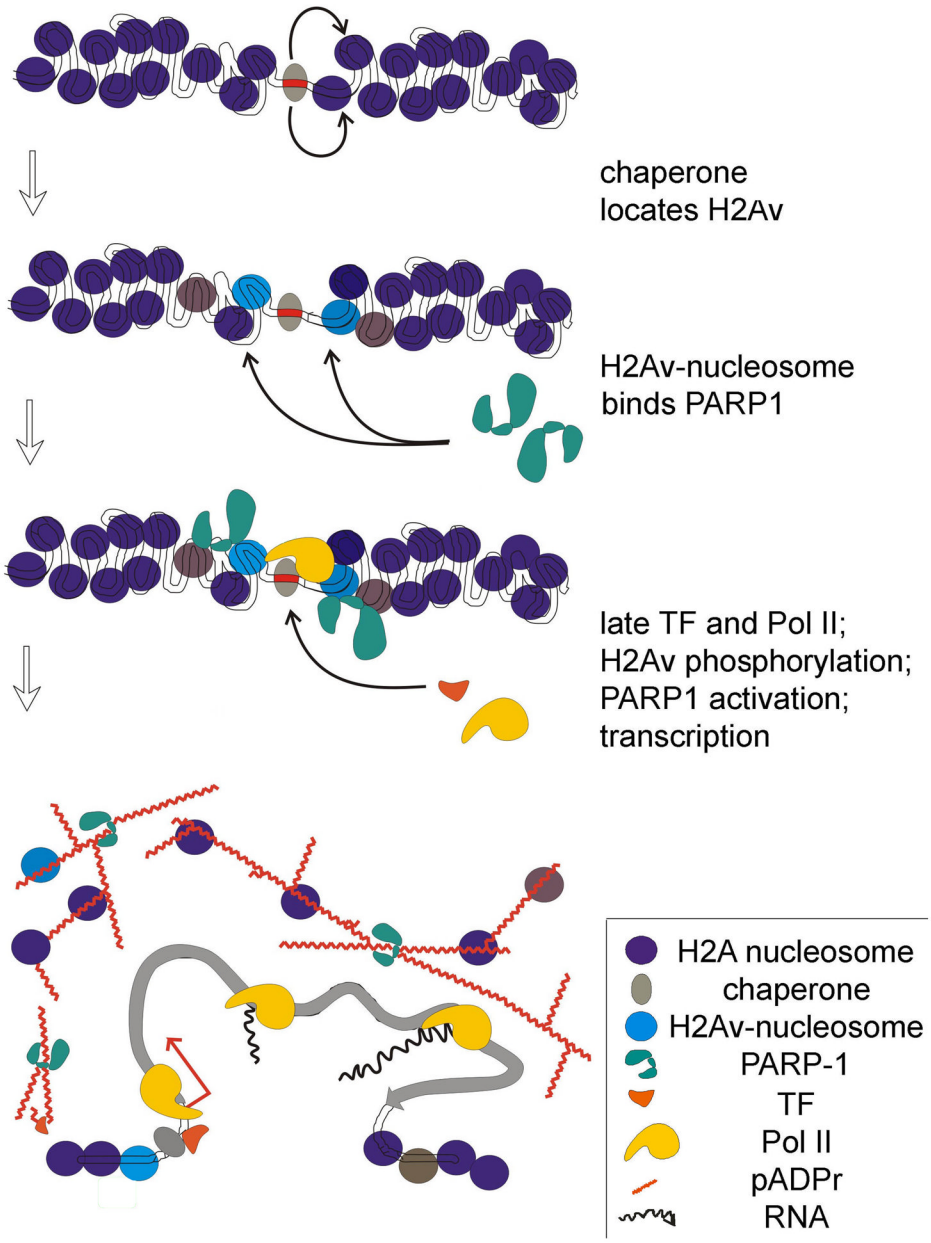


**Figure 6. Molecular model for PARP1 dependent transcription in development**

1) chromatin remodeling complexes position H2A variants-bearing nucleosomes inside promoter regions; 2) an H2A variants-bearing nucleosome has a greater affinity to PARP1 because of a better surface representation of H3 and H4; therefore, such nucleosome preferentially binds PARP1 and positions it inside promoters; 3) transcriptional factors and/or RNA Polymerase II stimulate phosphorylation of H2AvSer137, thereby activating PARP1 and initiating pADPr production; 4) pADPr induces relaxation in the nucleosome; 5) together, these modifications facilitate transcription through changes in nucleosome structure and facilitate transcription by Pol II.

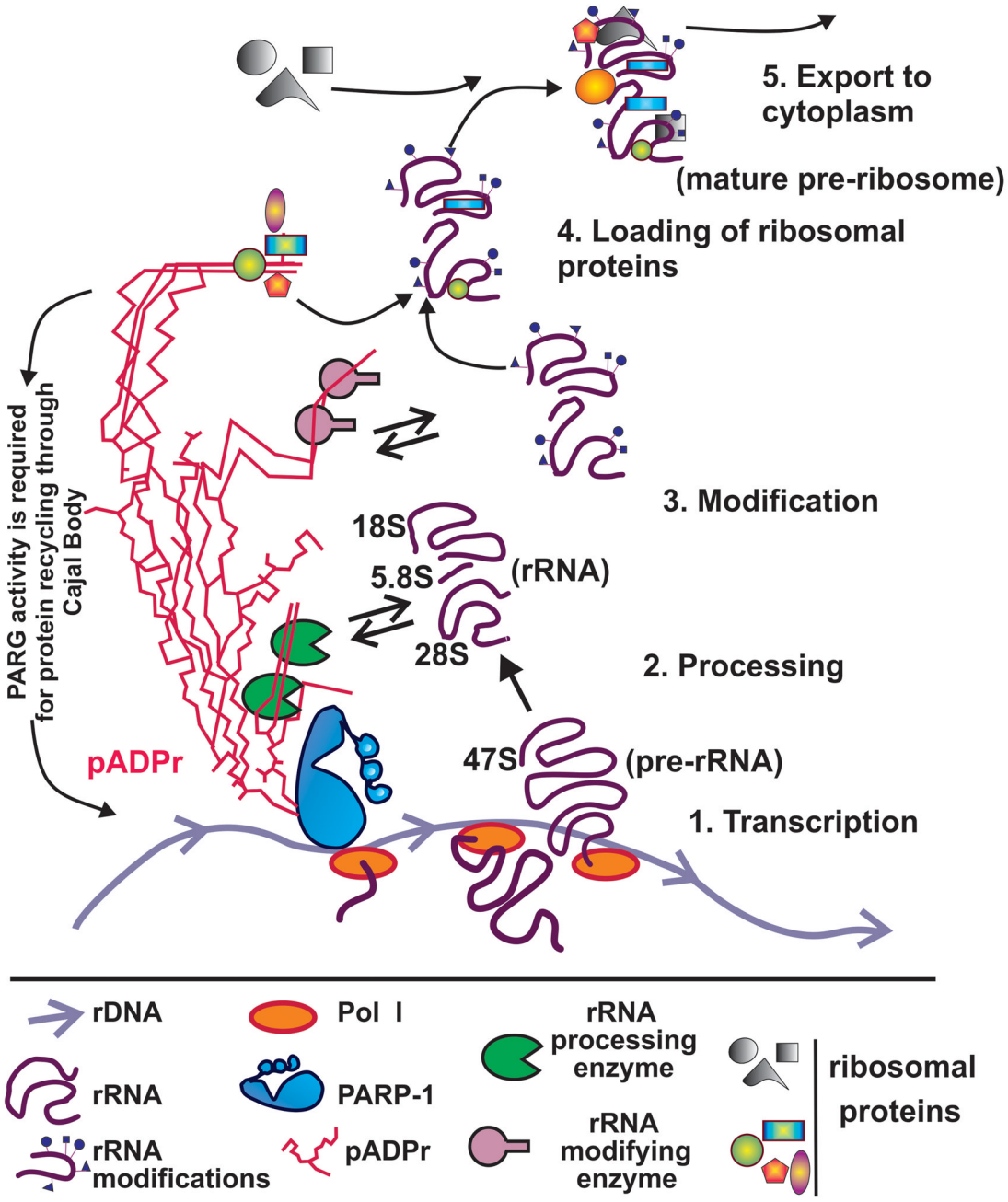


**Figure 7. Nuclear PARP1 facilitates ribosomal biogenesis**

PARP1 becomes automodified upon each act of transcriptional start within rDNA gene and serves as a chaperoning machine during the entier cycle of ribosome maturation in nucleolus. The dynamic Poly(ADP-ribose) tree forms a network, which organizes specific nucleolar microenvironment, brings a subset of nucleolar proteins -- such as Fibrillarin and AJ1 -- to the proximity of precursor rRNA, and coordinates the order of events of rRNA processing, modification, and loading of subsets of ribosomal proteins. Depletion of PARP1 leads to removal of pADPr-binding proteins from nucleoli, which disrupts processing, modification, and folding of ribosomal RNA. PARG is required to 1) restart the system and 2) recycle protein components after completion of one cycle of ribosomal subunit synthesis.

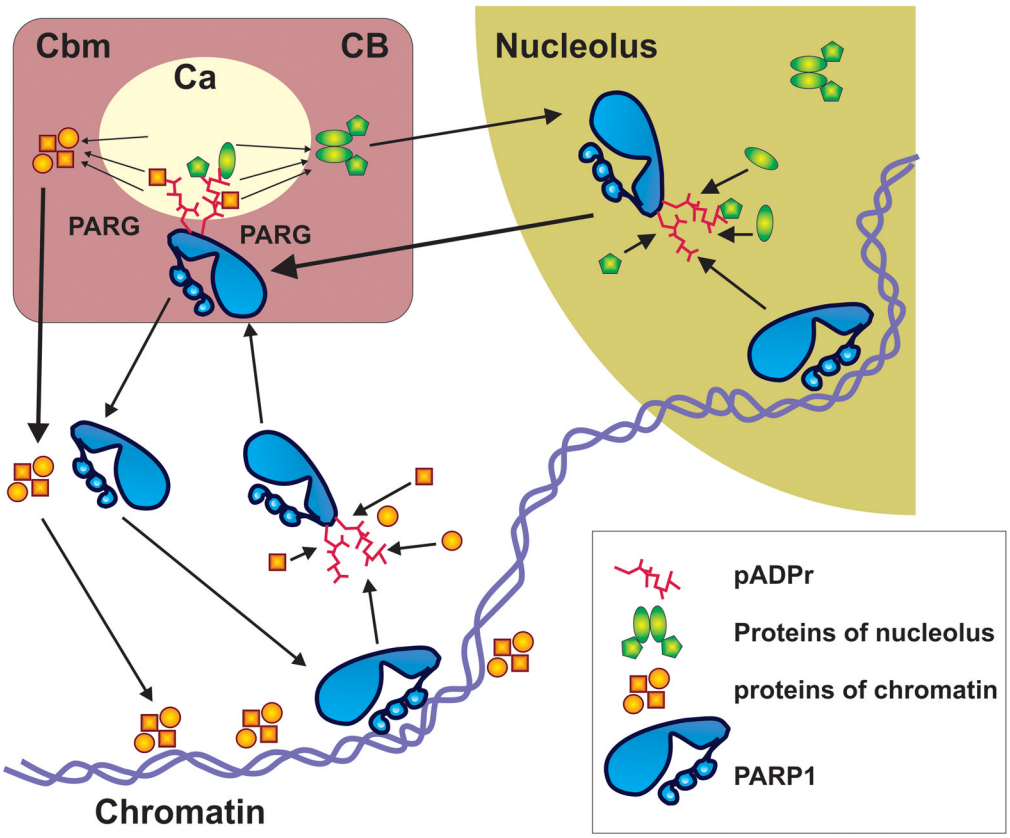


**Figure 8. A model of protein delivery to Cajal body by PARP1 shuttling**

(1) PARP1 is localized in chromatin and nucleoli. (2) Upon activation, PARP1 automodifies and (3) gains the ability to bind by pADPr a number of proteins with pADPr-binding domain. (4) Whole complex consisting of automodified PARP1 and proteins seated on pADPr migrates into Cajal bodies. (5) In CB, complex is disassembled as a result of cleavage of pADPr and released proteins are recycled. PARP1, pADPr protein-complexes of chromatin and nucleolus are shown. CBm = Cajal body matrix, and Ca = Cajal body cavity.