Hit and run versus long-term activation of PARP-1 by its different domains fine-tunes nuclear processes

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Poly(ADP-ribose) polymerase 1 (PARP-1) is a multidomain multifunctional nuclear enzyme involved in the regulation of the chromatin structure and transcription. PARP-1 consists of three functional domains: the N-terminal DNA-binding domain (DBD) containing three zinc fingers, the automodification domain (A), and the C-terminal domain, which includes the protein interacting WGR domain (W) and the catalytic (Cat) subdomain responsible for the poly(ADP ribosyl)ating reaction. The mechanisms coordinating the functions of these domains and determining the positioning of PARP-1 in chromatin remain unknown. Using multiple deletional isoforms of PARP-1, lacking one or another of its three domains, as well as consisting of only one of those domains, we demonstrate that different functions of PARP-1 are coordinated by interactions among these domains and their targets. Interaction between the DBD and damaged DNA leads to a short-term binding and activation of PARP-1. This “hit and run” activation of PARP-1 initiates the DNA repair pathway at a specific point. The long-term chromatin loosening required to sustain transcription takes place when the C-terminal domain of PARP-1 binds to chromatin by interacting with histone H4 in the nucleosome. This long-term activation of PARP-1 results in a continuous accumulation of pADPr, which maintains chromatin in the loosened state around a certain locus so that the transcription machinery has continuous access to DNA. Cooperation between the DBD and C-terminal domain occurs in response to heat shock (HS), allowing PARP-1 to scan chromatin for specific binding sites.

PARP-1 | poly(ADP-ribose) | drosophila | PARP-1 regulation | protein domains

The complexity and size of eukaryotic genomes require tight coordination between activation and repression of nuclear processes across tissues and organs (1, 2). Changes in the chromatin architecture of a eukaryotic nucleus coordinate gene expression in response to the extranuclear environment and are orchestrated by interactions between DNA and chromatin proteins (2, 3). The mechanisms that organize DNA into structural units are largely responsible for defining the specific functions and phenotypes of different cells (4–9).

After histones, the second most abundant protein in the eukaryotic nucleus is PARP-1, an effector protein that functions as a switch, controlling the activation and silencing of chromatin regions (10–14). PARP-1 enzymatic activity can be induced either by its interaction with nicked DNA or with histone H4 in a phosphorylated H2Av-histone-bearing nucleosome (15–17). When enzymatically active, PARP-1 covalently modifies itself and surrounding nuclear proteins by synthesizing strands of pADPr from the NAD substrate (10–13). Histones and DNA repair enzymes have been identified as PARP-1 modification targets (18–20). By shifting histones toward the more electronegative pADPr and away from the DNA molecule, PARP-1 activity initiates chromatin loosening, allowing transcription activation (21–24). The same process permits repair of damaged DNA and DNA replication (2, 6–9).

PARP-1 consists of three core domains: the N-terminal DNA binding domain (DBD), the middle automodification domain (A), and the C-terminal catalytic domain (C) (Fig. L4) (10, 25). The DNA-binding domain contains three Zinc fingers, ZI, ZII, and ZIII, of which only ZI and ZII are capable of interacting with DNA (10, 26–28). The third ZIII represents a protein interaction subdomain and mediates interprotein interactions of other PARP-1 domains (29, 30). During the DNA damage response, ZI, ZIII, W, and Cat domains of PARP-1 form a stable active complex around a fragment of broken DNA in vitro (Fig. L4) (29, 30). The A domain of PARP-1 is the primary target of PARP-1 activity and becomes automodified by pADPr upon PARP-1 activation (10). An automodified PARP-1 loses its ability to interact with DNA (21, 31, 32) and serves as a “shuttle” for proteins of chromatin (33). The N-terminal DBD and the C-terminal ZIII-A-W-Cat domains are responsible for PARP-1 interaction with chromatin (15, 34). Without the DBD, PARP-1 cannot bind to or be activated by DNA (15, 34). Binding of PARP-1 to histones has been shown to be regulated by its C-terminal subdomains (15, 16, 35). The presence of DBD and C-terminal domains is required for PARP-1-dependent chromatin condensation in vitro (34). Because pADPr polymers are perpetually degraded by pADPr glycohydrolase (PARG) (10–13), a sustained production of pADPr by PARP-1 is required for maintaining chromatin in its loosened state and transcription activation. Transcription silencing is prompted by PARP-1 when it binds to heterochromatin regions.

Significance

Little is known about how multiple functions of a single protein are coordinated in a living cell. PARP-1 is a multidomain nuclear protein that plays a critical role in regulating developmental processes including apoptosis, DNA repair, epigenetic marking of chromatin, assembly of higher-order chromatin structures, and transcriptional activation. Using deletional isoforms of PARP-1 in vivo and in vitro experiments, we have demonstrated that the multiple domains of PARP-1 cooperate in response to interactions with different PARP-1 targets, leading either to short-term activation of the enzyme or to prolonged and sustained activity. This sustained activity produces accumulation of pADPr in the surrounding chromatin, leading to prolonged chromatin loosening.


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of chromatin via its ZI, which represses repeated mobile elements in the genome (26). Therefore, PARP-1 appears to have two antagonistic functions, acting as a transcription activator and as a repressor (33, 36), depending on which specific PARP-1 domain is involved in its interaction with the nuclear targets.

To examine how interactions of individual PARP-1 domains with different targets coordinate different nuclear functions of PARP-1, we generated deletional isoforms of PARP-1 lacking each one of its domains (Fig. 1B). Because the Drosophila genome codes for a single PARP protein (37, 38), we use Drosophila as our model organism to examine the activity and interaction of PARP-1 domains in vitro and in vivo. By studying the activity and functions of deletional isoforms of PARP-1, we show how PARP-1 localization and activity are regulated by interactions between PARP-1 domains and their targets, specifically, histones and DNA.

Results

W-Cat Domains Target Active Chromatin. It is expected that PARP-1 binds to DNA via its N-terminal domain, whereas its interaction with histones and other chromatin proteins occurs via its C-terminal domain. To examine how these interactions regulate PARP-1 positioning and function in vivo, we monitored the localization of deletional isoforms of PARP-1 fused with YFP in Drosophila (SI Appendix, Fig. S1).

In wild-type Drosophila nuclei, all PARP-1 isoforms show discrete localization in chromatin (SI Appendix, Fig. S2), confirming that each domain of PARP-1 taken on its own is sufficient for an interaction with chromatin. We tested which domains are responsible for automodification of PARP-1 and for its interaction with pADPr. Previously we reported that all proteins that have been covalently modified by pADPr (including PARP-1 itself) and proteins bound to pADPr noncovalently are subjected to turnover inside Cajal bodies (39). Because the PARG enzyme is required for clearing pADPr, all pADPr-modified proteins remain adherent to Cajal bodies in Parg null mutants (39). We compared the localization of each deletional isoform of PARP-1 in wild-type Drosophila and in Parg mutant nuclei. In the absence of the A domain, the relocation of the C-terminal catalytic domain of PARP-1 to Cajal bodies was considerably reduced (SI Appendix, Fig. S2, Parg−/−). All PARP-1 deletional isoforms were localized exclusively inside the nuclei, all PARP-1 isoforms show double mutant of PARP-1 lacking ZI and ZII demonstrate colocalization with DNA resulting in the yellow color in the overlay. All isoforms without ZI and ZII localized in active open chromatin only, resulting in the separation of red and green in the overlay. (D) PARP-1 deletional isofrom activity in vivo. PARP-1 deletional isoforms were expressed in the parp−/−; parg−/− double mutant flies. All isoforms containing the Cat domain restored pADPr accumulation. (E) Both DNA- and C-terminal domains contributed to PARP-1 protein dynamic binding to chromatin in vivo. Comparative analysis of fluorescent recovery after photobleaching (FRAP) assay for recombinant protein is shown, including ZH-YFP, ZI-A-W-Cat-YFP, full-length PARP-1-YFP, and H4-YFP. Data for the FRAP experiment show the average based on 10 replicates.
transgenic stock, nuclear proteins were extracted from third-instar larvae of the same age and size. The amounts of pADPr that accumulated in each deletional isoform were assessed on Western blots using an anti-pADPr antibody. All isoforms containing the Cat domain of PARP-1 demonstrated either complete or partial restoration of pADPr accumulation (Fig. 1D). This result supports the inference made from in vivo fluorescent imaging that C-terminal domains of PARP-1 are sufficient for DNA-independent PARP-1 activation in vivo.

To examine how different domains contribute to PARP-1 distribution and activity in chromatin, we used a FRAP assay (15). We recorded two parameters for the recovery of fluorescence for a chromatin-associated protein: the speed of recovery based on the slope of the FRAP curve and the magnitude of recovery based on the plateau of the FRAP curve. Both parameters depend on the affinity of the protein to chromatin and the fraction of the protein that can dissociate without a deep remodeling of chromatin. Core histones are embedded in nucleosomes and, therefore, cannot be recovered without chromatin remodeling (Fig. 1E, H4-YFP). The full-length PARP-1 recovery curve plateaued after reaching 59% (Fig. 1E, PARP-1-YFP). Deleting either C-terminal (Fig. 1E, Left, ZI-ZII-YFP) or DNA-binding N-terminal (Fig. 1E, Right, ZIII-A-W-Cat-YFP) domains increased the proportion of PARP-1 recovered in 60 s. Thus, each domain contributes independently to the dynamics of PARP-1 localization and its interaction with chromatin.

**W-Cat Domains, but Not DNA-Binding Domains Bind to the hsp70 Transcriptional Start Site.** Rapid transcriptional activation of the hsp70 gene is dependent on PARP-1 activity (14, 24, 31). To confirm that the W-Cat domain primarily contributes to the transcription activation function of PARP-1 and that DNA-binding ZI and ZII are responsible for a nonspecific binding to DNA, we examined the binding patterns of each deletional isoform at the PARP-1-dependent hsp70 locus and tested the ability of each isoform to activate the transcription of hsp70 (Fig. 2). We first compared the distribution of different PARP-1 isoforms in the hsp70 locus using a chromatin immunoprecipitation assay before and after HS treatment. To eliminate any contribution from endogenous PARP-1, we expressed YFP-fused PARP-1 isoforms in the parp−/− mutant background. Before HS, full-length PARP-1-YFP predominantly accumulated at the transcription start site (TSS) and transcription termination site (TTS) (Fig. 2F, blue bars). In addition, a significant fraction of PARP-1-YFP was bound upstream from the promoter and in the coding region of the gene (Fig. 2F, blue bars). After a 30-min HS, the full-length PARP-1 was almost completely gone from the TSS and TTS, but its binding in the coding region significantly increased (Fig. 2F, red bars).

**Fig. 2.** C-terminal domains of PARP-1 are responsible for PARP-1 targeting to the TSS of the hsp70 locus, whereas DNA-binding domains target PARP-1 to areas outside of the promoter region. (A–D) The comparison of recombinant protein distribution within the hsp70 locus, ChIP assay before and after HS: PARP-1-YFP (A), ZIII-A-W-Cat-YFP (B), W-Cat-YFP (C), and ZI-ZII-YFP (D). (E) PARP-1 isoforms with C-terminal domains rescue transcription activation at the hsp70 locus following HS in parp−/− mutants. The level of hsp70 mRNA was recorded before and after 30 min of HS treatment using quantitative RT PCR (F). PARP-1 isoforms with C-terminal domains rescue histone H3 displacement from hsp70 locus, following HS in parp−/− mutants. ChIP assay compares amounts of H3 histone in the promoter region of the hsp70 locus in wild-type and parp−/− mutant animals expressing full-length PARP-1 (PARP-1-YFP) or PARP-1 deletional isoforms (ZI-ZII, ZIII-A-W-Cat, W-Cat) before (–) and after (+) 30 min of HS treatment. All error bars are based on the average of triplicates.

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transcription of the hsp70 gene, albeit not at the same level as in the presence of a full-length PARP-1 (Fig. 2E). This finding is consistent with our inference that the W and Cat domains are sufficient for PARP-1 localization in promoters and its activation there. Earlier studies (17, 24) have demonstrated that PARP-1 controls nucleosome displacement at promoters during transcription (40). We found that the isolated C-terminal domain of PARP-1 (W-Cat) also has the ability to displace histones at the hsp70 locus after HS (Fig. 2F). Thus, PARP-1 localization and activation in chromatin were sufficient to maintain active chromatin during gene transcription.

Histone H4-Mediated PARP-1 Activation Is Dependent on H4 Binding to the C-Terminal W-CAT Domains of PARP-1. To examine the roles of PARP-1 domains in binding to DNA and nucleosomal histones in vitro, we generated three deletional isoforms, including ZI-ZII (consisting of ZI-ZII only), ZIII-A-W-Cat (PARP-1 lacking ZI-ZII), and W-Cat (PARP-1 lacking ZI-ZII as well as its A domain) (Fig. 3A). These deletional recombinant constructs and full-length PARP-1 were expressed and purified using the bacterial system. To test the affinity of different deletional isoforms of PARP-1 to components of nucleosomes, we performed two in vitro binding assays using DNA and H4 coupled to sepharose beads. Previously, we demonstrated that PARP-1 binds to a hydrophobic patch formed by Val61 and Leu23 amino acids of histone H4 (17). This patch is exposed on the surface of a H2Av histone-phobic patch formed by Val61 and Leu23 were replaced with glycines (H4V61G23). This mutant isoform of H4 is stable on its own and in a nucleosome (17). It interacts normally with histone H3 (Fig. 3B) but is unable to bind or activate PARP-1 (17).

Pull-down assays using DNA- and H4-coupled sepharose beads (H4-SB) (Fig. 3B) showed that ZI-ZII strongly binds to DNA but not to H4, suggesting that ZI-ZII are sufficient for DNA binding but not H4 binding. Both ZIII-A-W-Cat and W-Cat isoforms bound to H4 but not to DNA (Fig. 3B). We also found that the C-terminal but not N-terminal domains of PARP-1 recognize and bind to the H2Av-containing histone octamers with high affinity, whereas both the C- and the N-terminal domains bind to an intact mononucleosome (SI Appendix, Fig. S4).

Results reported above confirm that the two PARP-1 activators DNA and H4 bind to different domains of PARP-1 to trigger its activation. To identify which domains bind to each target, we tested the ability of DNA and H4 to activate different PARP-1 deletion and isoforms. DNA interacts with both the ZI-ZII and ZIII-A-W-Cat isoform, which lacks ZI and ZII because the first two Zn fingers are necessary for DNA-dependent PARP-1 activation (Fig. 3C). Despite lacking ZI-ZII, the ZIII-A-W-Cat isoform can be activated by H4, confirming that PARP-1 binding and activation mediated by histone H4 are dependent exclusively on the C-terminal domains of PARP-1 (Fig. 3D). Therefore, the C-terminal and N-terminal domains of PARP-1 have distinct functions that can be separated from one another by introducing mutations to the PARP-1 locus.

W-Cat Domain of PARP-1 Is Sufficient for H4-dependent PARP-1 Activation. To test the role of C-terminal subdomains during PARP-1 interaction with chromatin, we used the W-Cat deletional isoform, which lacks the DBD and A domains. We found that the W-Cat isoform does not bind to DNA (Fig. 3B). We further tested whether this isoform could still interact with nucleosomal histones and become enzymatically active. To detect the enzymatic activity of the W-Cat isoform, we needed to provide an alternative target that can be modified by ADPPr moieties because the W-Cat isoform lacks the A domain, which serves as the primary target for PARP-1 enzymatic activity (25). Previous studies have shown that PARP-1 also modifies histones H1, H2A, and H2B with pADPr in vitro (10, 21). Therefore, any of these histones could potentially serve as acceptors of pADPr. We compared H4-dependent activity of the W-Cat deletional isoform in the presence of these targets.

Following its activation by histone H4, full-length PARP-1 produces pADPr with and without other protein targets (Fig. 4A, Panel 1). When the W-Cat isoform was incubated with a NAD substrate and H4-coupled beads but without other protein targets, no pADPr was produced (Fig. 4A, Panel 2). This result demonstrates that PARP-1 cannot produce pADPr without its automodification domain and that histone H4 cannot serve as an acceptor of pADPr. We individually tested histones H1, H2A, H2B, and H2Av with pADPr in vitro (10, 21). Therefore, any of these histones could potentially serve as acceptors of pADPr. We compared H4-dependent activity of the W-Cat deletional isoform in the presence of these targets.

Because the W-Cat isoform cannot be automodified in the absence of the A domain, all pADPrs produced by the W-Cat isoform upon its activation by H4 must have been attached to the H1 histone (Fig. 4A, Panel 3; SI Appendix, Fig. S5).
PARP-1 localization and activation in chromatin is necessary for maintaining active chromatin in an open state (31, 32, 35). The poly(ADP ribosyl)ation of linker histone H1 plays a crucial role in the process (32). Our results suggest that PARP-1 domains cooperatively control its activation via DNA and histone H4 binding, which leads to pADPr accumulation. We found that DNA-binding ZI and ZII are necessary for DNA-dependent short-term hit and run activation of PARP-1, which triggers the DNA-repair pathway. The C-terminal catalytic domain of PARP-1 binds to histone H4, resulting in prolonged activation of this enzyme and sustained production of pADPr. Histone H4 binds to the PARP-1 C-terminal catalytic domain and activates PARP-1 independent of the DBD. A PARP-1 W-Cat construct is targeted to the promoter region of the hsp70 gene and can activate hsp70 transcription upon HS in the PARP-1 mutant background. Therefore, the transcription activation function of PARP-1 can be mediated independently from the DBD, although the consequent level of the transcript accumulation is considerably lower than that in the presence of full-length PARP-1. The data presented here are consistent with the previously reported finding that phosphorylation of H2Av results in exposure of key epitopes of the H4 histone, leading to PARP-1 activation (17). Therefore, it seems likely that the H4-mediated mechanism is deployed to enable PARP-1 transcriptional activation in steady-state conditions in the absence of DNA damage.

Our data suggest that the DBD of PARP-1 is not strictly required for histone H4-dependent PARP-1 activation. This domain is, however, strictly necessary for DNA-mediated PARP-1 activation. Even though both ZI and ZII of the DBD have high binding affinity to DNA, it has been shown that only ZI is absolutely necessary for PARP-1 activation induced by DNA damage (29). This finding raises an interesting issue concerning additional functions of the DBD in the absence of DNA damage. We found that YFP-tagged PARP-1 isoforms with ZI and ZII colocalized with DNA in chromatin in a nonspecific genome-wide manner. A ChIP assay showed that the ZI- and ZII-bearing isoform was absent from the hsp70 TSS region but enriched outside this TSS (Fig. 2D). In addition, despite possessing the histone-binding and Cat domains, the isoforms which

**Discussion**

PARP-1 localization and activation in chromatin is necessary for maintaining active chromatin in an open state (31, 32, 35). The poly(ADP ribosyl)ation of linker histone H1 plays a crucial role in the process (32). Our results suggest that PARP-1 domains cooperatively control its activation via DNA and histone H4 binding, which leads to pADPr accumulation. We found that DNA-binding ZI and ZII are necessary for DNA-dependent short-term hit and run activation of PARP-1, which triggers the DNA-repair pathway. The C-terminal catalytic domain of PARP-1 binds to histone H4, resulting in prolonged activation of this enzyme and sustained production of pADPr. Histone H4 binds to the PARP-1 C-terminal catalytic domain and activates PARP-1 independent of the DBD. A PARP-1 W-Cat construct is targeted to the promoter region of the hsp70 gene and can activate hsp70 transcription upon HS in the PARP-1 mutant background. Therefore, the transcription activation function of PARP-1 can be mediated independently from the DBD, although the consequent level of the transcript accumulation is considerably lower than that in the presence of full-length PARP-1. The data presented here are consistent with the previously reported finding that phosphorylation of H2Av results in exposure of key epitopes of the H4 histone, leading to PARP-1 activation (17). Therefore, it seems likely that the H4-mediated mechanism is deployed to enable PARP-1 transcriptional activation in steady-state conditions in the absence of DNA damage.

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lacked the DBD (ZIIIA-A-W-Cat and W-Cat) could not fully re-
store the transcription activation function in the hsp70 gene, sug-
gesting that the DBD interaction with DNA is also required for full
transcription activation (Fig. 2E). Similar to other DNA-binding
transcription factors, such as the pioneer factor FoxA (41), the
pattern of PARP-1 binding has both specific and nonspecific prop-
certies in chromatin. We propose that the DNA-binding domain as-
ists with targeting PARP-1 to the TSS region by scanning chromatin
for binding sites (Fig. 5). Our findings suggest that the DBD and the
C-terminal catalytic domain (W-Cat) of PARP-1 represent a co-
operative mechanism that determines where and how PARP-1 in-
duces transcription.

Experimental Procedures

Flies were cultured on standard cornmeal-mollases-agar media at 22 °C
unless otherwise indicated. The fly stocks were generated by the standard

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