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# Nucleosome reorganization by PARP-1

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*Introduction*: Human poly (ADP-ribose) polymerase (PARP) family consists of 17 proteins that are involved in many cellular processes, including transcription, modulation of chromatin structure, genome maintenance, replication, recombination, and DNA damage repair [1]. PARP-1 is the best structurally and functionally characterized protein of this family, since it is a key player in almost all nuclear and some cytoplasmic events in the cell. The diverse spectrum of PARP-1 activity in the cell is due to its enzymatic and DNA-binding activities. The multidomain structure of PARP-1 includes a DNA-binding region with three zinc fingers localized at the N-terminal end and an enzymatic active site localized at the C-terminal region of the protein. PARP-1 has distinct catalytic activity-dependent and independent functions during DNA transactions [2]. First, upon binding to a DNA lesion, PARP-1 becomes catalytically active, thus acting as a DNA damage sensor enzyme, which hydrolyzes $NAD^+$ and forms long, branched chains of negatively charged polyADP-ribose (PAR) on a variety of nuclear proteins, including histones and PARP-1 itself [3]. PARP-1 is responsible for the production of approximately 90 % of PAR in the cell [4]. The catalytic activity-independent function is realized during interaction of PARP-1 with intractable chromatin regions where it makes protein-binding sites on DNA available for interactions with pioneer factors [2]. Impaired PARP-1 metabolism is associated with the development of various cancers, as well as cardiovascular and neurodegenerative diseases [5]. Accordingly, PARP-1 inhibitors are used as antitumor drugs, and the applicability to the treatment of other metabolic diseases is being investigated [6]. Despite the intensive study of PARP-1, many aspects of its interaction with chromatin at the nucleosomal level are not fully understood; in particular, it is not clear how PARP-1 contributes to the accessibility of DNA regions hidden in nucleosomes to pioneer factors. How is the catalytically independent mode of action of PARP-1 realized on nucleosomes? To answer these questions, we developed sensitive sensors using fluorescent nucleosomes to probe the conformational changes in nucleosomal DNA induced by PARP-1.

*Results*: Uniquely positioned nucleosomes were reconstituted on the 603 DNA templates *in vitro*. To make the nucleosomes suitable for analysis by single-particle Förster resonance energy transfer (spFRET) microscopy, a single pair of fluorescent dyes Cy3 (donor) and Cy5 (acceptor) was introduced in different, close positioned regions of the nucleosomal DNA [7]. The efficient FRET was observed between Cy3 and Cy5 without disturbance of histone-DNA contacts in the assembled nucleosomes. Labeled DNA

regions localized 13 and 135 bp from the nucleosome boundary are involved in so called DNA "breathing" in the nucleosome. Labels at 35 and 112 bp are located near the DNA contacts with H2A/H2B dimers, whereas labels at 57 and 91 bp are near the H3/H4 tetramer. To study PARP-1 induced changes in the linker DNA region, both linker DNA helixes were labeled at positions of 18 bp from the boundaries of the core nucleosome. Electrophoretic mobility shift assay revealed that PARP-1 can form three distinct complexes with linker nucleosomes. It was found that these complexes differed in a number of PARP-1 molecules bound to a nucleosome. First PARP1-nucleosome complex is observed at a low PARP1 concentration (10 nM). Second and third complexes having a lower mobility in the gel are formed at 20 nM and 50 nM PARP1 respectively. A similar concentration-dependent effect was observed during nucleosome reorganization by PARP-1. After binding of the first PARP-1 molecule, no pronounced structural changes were observed in nucleosomes, probably because of PARP-1 binding at the DNA ends localized at a distance from the nucleosome. With an increase in the concentration of PARP-1 to 20 and 50 nM, the distance between nucleosomal DNA gyres increased along the entire DNA length, while linker DNA helixes converged. Last observation indicates that one of the PARP-1 molecules can interact with the nucleosome in the H1-like manner by binding near the nucleosome dyad. Moreover, we have shown that PARP-1 was able to displace H1.0 from nucleosomes in the absence of polyADP-ribosylation. To understand the effect of PARP-1 on nucleosome structure, 25 ns molecular dynamics of nucleosome complexes with PARP-1 was performed. Analysis showed that two PARP-1 molecules bound to DNA ends can form intermolecular bonds between the catalytic domains. This interaction, in turn, induced a distortion of the linker DNA accompanied by an increase in the distance between the gyres of nucleosomal DNA.

*Conclusion*: Our data suggest that PARP-1 can induce formation of an alternative nucleosome state that is likely involved in gene regulation and DNA repair.

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