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The synergistic effect of p53 and PARP-1 on the nucleosome

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Motivation and Aim: Both poly (ADP-ribose) polymerase 1 (PARP-1) and p53 have been extensively studied due to their regulatory role in numerous pivotal biologic pathways. It is assumed that they are responsible for DNA damage response, differentiation, metabolism, and aging. In addition to their common functions, they interact with each other. Nevertheless, the details of PARP-1 and p53 chromatin- reorganizing activity are still uneven. The aim is to investigate the combined effect of proteins (PARP-1 and p53) on the nucleosome.

Methods and Algorithms: Fluorescently labeled mononucleosomes were assembled on a DNA template containing the strong nucleosome positioning 603 Widom flanked by two 20-bp linker DNA fragments. The templates were obtained by polymerase chain reaction using the fluorescently labeled oligonucleotides. The fluorescent pair Cy3/Cy5 was set at 10/10 bp (from the beginning of the 603 sequence) at the linker region of the nucleosome. Formation of complexes between proteins (PARP-1/p53DBD) and nucleosomes were proceeded using a potassium-containing buffer with physiologically accurate concentration of K+ (150 mM). Obtained complexes were studied by the electrophoretic mobility shift assay using native PAGE.

Results: In the present study, we conducted a series of experiments with the following structure.

During the first series of experiments, p53 was added to pre-incubated nucleosome-PARP-1 (50 nM) complexes at increasing concentrations of p53 (25-200nM). During the second series of experiments, PARP-1 (50 nM) was added to pre-incubated nucleosome-p53 (25-200nM) complexes. In both cases, a set of complexes of different stoichiometry was obtained. P53 exhibited higher activity when interacting with the nucleosome-PARP-1 complex, and PARP-1 when the reverse method of setting up the experiment was used.

Conclusion: Thus, we can assume a possible synergistic interaction of proteins during the formation of complexes with the nucleosome, as well as an independent one with displacement of one of the agents.

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