論文の内容の要旨

Biochemical and structural studies on the nucleosome recognition mechanism by the pioneer transcription factor p53

(パイオニア転写因子 p53 によるヌクレオソーム認識機構の生化学的・構造生物学的解析)

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In eukaryotes, the genomic DNA is accommodated into the nucleus, forming a chromatin structure with nucleosomes as a basic unit. The nucleosome is a disk-shaped structure, in which histone octamer containing two molecules each of the histone proteins H2A, H2B, H3, and H4, is wrapped by about 145 base-pairs of DNAs. The extended DNA regions connecting adjacent nucleosomes are termed linker DNA. The nucleosome structure inherently inhibits the transcription factors' binding to the genomic DNA, therefore, regulating transcription. On the other hand, a subset of transcription factors termed "pioneer transcription factors" can bind to the target DNA sequence within the nucleosome and induce chromatin opening. The ability of pioneer transcription factors to

bind to nucleosomes is indispensable in regulating cell fate, but the detailed molecular mechanism is still unknown. As a pioneer transcription factor, p53; a major protein involved in tumor suppression, induces the expression of genes involved in cell cycle arrest and apoptosis in response to various cellular stresses. However, previous crystallographic studies of the p53-DNA complexes revealed that the structure of the p53 bound DNA containing the p53 target sequence was a straight path. In this study, the mechanism of nucleosomal DNA recognition by p53 was investigated through biochemical and structural analysis using recombinant proteins.

The gel shift assay showed that linker DNAs portions are required for the formation of p53-nucleosome complexes that appeared as four discrete bands on the polyacrylamide gel. The insertion of the p53 binding sequence into the nucleosomal DNA, which mimics natural p53 binding sites in the context of chromatin, did not significantly affect the nucleosome binding efficiency, meanwhile, p53 formed an additional specific complex with the nucleosome in the presence of the p53 binding sequence. In addition, the pull-down experiment and the gel shift assay revealed that p53 directly binds to the histone H3-H4 complex via its N-terminal amino acid region. The cryo-EM analysis of the p53-nucleosome complex was then performed and revealed that p53 bound to the target sequence within the nucleosome. The hydroxy-radical footprinting experiments also support the p53-DNA interactions within the p53-nucleosome structure.

These results provide basic information on how p53 binds to the nucleosome and insight into the underlying mechanism of how pioneer transcription factors induce chromatin opening.