No. 77

Functional analysis of nucleoid associated protein HU from *Thermus thermophilus* HB8

高度好熱菌由来ヌクレオイド構成タンパク質 HU の機能解析

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Eukaryotic genomic DNA with histones forms nucleosomes, which further form highly organized chromatin structures in a nucleus. One of the features of histones is various post-translational modificatios such as phosphorlation, acetylation and methylation. These modifications regulate the DNA-binding affinity of histones and influence not only the conformation of DNA, nucleosomes and chromatin but also the functions of DNA such as replication, repair and transcription. On the other hand, prokaryotic cells lack the nuclear membrane and nucleosome architecture unlike eukaryotic cells. Aggregated structure of prokaryotic genomic DNA was discovered in 1970s and named as “nucleoid”. Subsequent study revealed that nucleoid is composed of DNA and some proteins which is termued as nucleoid associated proteins (NAPs). Although nucleoid and NAPs in prokaryotes are predicted to have the same roles as chromatin and histones in eukaryotes, the function of each NAP is still unclear. Investigation of NAPs is essential to unravel the prokaryotic genome architecture.

Heat-unstable nucleoid protein (HU) is one of NAPs and more highly conserved than other NAPs. HU is a small protein consisting of about 90 amino acids, and exists as dimer in solution. X-ray crystal structures of HU, containing DNA-complexed forms, from *Escherichia coli* and *Bacillus subtilis* have been determined. However, the overall conformation of a nucleoid as a chromatin is still unclear. It was reported that the interaction between HU and DNA seems to be nonspecific and HU is highly abundant in *E. coli* cells, about 30,000 molecules per cell (about 60 μM). *Thermus thermophilus* HB8 (*Tt*HB8) is an extreme thermophile with an optimal growth temperature of 80°C, and the high stability of the proteins from *Tt*HB8 is suitable for *in vitro* analysis. Since *Tt*HB8 has only 2,200 genes, much less than human 22,000 genes and *E. coli* 4,300 genes, essential genes for organisms might represent a major proportion of the *Tt*HB8 overall genes. Also, in the case of NAPs, *Tt*HB8 has only three NAPs containing HU, in contrast to *E. coli,* which has twelve NAPs. This suggests that HU in *Tt*HB8 (TtHU) works to substitute for all other NAPs. These makes TtHU suitable to unravel the bacterial nucleoid. In this study, we characterized TtHU by biochemical and the cytological approaches. The biochemical approach was addressed to study the DNA-binding affinity of TtHU and the incluence of TtHU to the affinity of other DNA-binding proteins to DNA. The cytological approach was addressed to search the TtHU-interacting proteins and post-transcriptional modifications and to study the influence of TtHU to the nucleoid.