学位論文の要約

題目　Chemical Biology Study on DNA Epigenetic Modifications
（DNA エピジェネティック修飾に関するケミカルバイオロジー研究）

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序論

DNA methylation plays a role to impart additional information to DNA. In eukaryotic cells, DNA methylation mainly occurs at C5 position of cytosine. Cytosine methylation is mostly observed on CpG dinucleotide context, and about 70-80% of all CpGs are methylated in mammalian genome. DNA methyltransferase (DNMT) family proteins catalyze the methyl transfer reaction from *S*-adenosylmethionine to cytosine, forming 5-methylcytosine (mC). Generally, cytosine methylation leads to suppression of gene expression. It is known that genome-wide DNA active demethylation occurs in primordial germ cells and zygote, and locus-specific DNA active demethylation takes place in neurons and T lymphocytes. However, the precise mechanism of DNA active demethylation has yet to be revealed. In 2009, TET family proteins were discovered to possess the activity to oxidize methyl group of mC to form 5-hydroxymethylcytosine (hmC). Furthermore, in 2011 stepwise oxidation of hmC to 5-formylcytosine (fC) and 5-carboxycytosine (caC) by TET proteins was revealed. Currently, these oxidized derivatives of mC are considered to be intermediates for DNA active demethylation.

CGmCGCG is a versatile substrate with which to evaluate TET protein activity

To evaluate the ability of TET proteins to oxidize mC, 20-mer or longer DNAs containing mC have been incubated with TET protein, followed by enzymatic digestion, and TLC, mass spectroscopy, or liquid chromatography analysis. However, these methods require much steps and are time-consuming. In this study, we presented a simple and versatile method for the assessment of iterative oxidation of mC by TET protein. We demonstrated that 4- to 6-mer DNAs can be substrates of TET protein. In particular, 6-mer DNAs were much more reactive than conventional 20-mer DNAs. Although there is a report about the effects of substrate length on AlkB, ABH2, and ABH3 proteins, this is the first study which has shown the effect of substrate length on TET protein. By using 6-mer DNA (5’-dCGmCGCG-3’), it is possible to readily observe the oxidation of mC to hmC, fC, and caC with no enzymatic digestion. We also demonstrated that TET protein favors single-stranded DNA over double-stranded DNA.

Identification of sequence specificity of 5-methylcytosine oxidation by TET1 protein with high-throughput sequencing

Details of the oxidation reaction of mC by TET proteins are still poorly understood. Evaluation of genomic-level epigenetic changes by TET protein requires unbiased identification of the highly selective oxidation sites. In this study, we used high-throughput sequencing to investigate the sequence specificity of mC oxidation by TET1 protein. A 6.6×10^4-member mC-containing random DNA sequence library was constructed. The library was subjected to TET-reactive pulldown followed by high-throughput sequencing. Analysis of the obtained sequence data identified TET1-reactive sequences. We identified mCpG as a highly reactive sequence of TET1 protein. The sequence specificity of the mC oxidation reaction was lost when we treated the random DNA sequence library with higher concentration of TET1 protein.

AFM analysis of changes in nucleosome wrapping induced by DNA epigenetic modification

The wrapping and unwrapping of the nucleosome, which is a fundamental packing unit of chromatin, are tied to the regulation of gene expression. The accessibility of DNA within nucleosomes is controlled not only by chromatin-remodeling molecules, but also by chemical modifications on histones and DNA. Understanding the structural changes of a nucleosome during epigenetic modifications is a key to unravel the mechanisms of gene regulation. In this study, we reconstituted positioned mononucleosomes using methylcytosine- or hydroxymethylcytosine-substituted DNA containing the Widom 601 sequence, and analyzed their morphological features by atomic force microscopy (AFM). Our results indicate that cytosine methylation induces over-wrapping of the DNA around the histone octamer, whereas cytosine hydroxymethylation has a lesser effect on the over-wrapping of the DNA. These results suggest that two types of DNA modification yield different wrapping states of nucleosomes, which may contribute to the compaction and relaxation of the chromatin structure.

Preferential 5-methylcytosine oxidation in the linker region of reconstituted positioned nucleosomes by TET1 protein

In this study, we reconstituted positioned mononucleosomes using CpG-methylated 382 bp DNA containing Widom 601 sequence and recombinant histone octamer, and subjected the nucleosome to treatment with TET1 protein. The sites of oxidized methylcytosine were identified by bisulfite sequencing. We found a significant difference in the activity of TET proteins toward mCs located in the core and linker DNA regions. In cells, the activity of TET proteins should be stringently regulated. The inefficient oxidation by TET protein of the core DNA region may be important for preventing aberrant DNA demethylation. During the reprograming process, mCs in CpG islands are demethylated in a wide region of the genome. The assistance of chromatin remodeling factors to enable the access of TET protein to DNA seems essential for this process.