学位論文要旨

A study on the association between
DNA methylation overlapping the transcription termination sites
and transcription termination
（転写終結部位の DNA メチル化修飾と転写終結の関連性に関する研究）

氏　名　　白井　均樹

CpG methylation of genomic DNA is a well-known repressive epigenetic mark in eukaryotic transcription, and DNA methylation of promoter regions is correlated with gene silencing. In contrast to the promoter regions, the function of DNA methylation during transcription termination remains to be elucidated. A recent study revealed that DNA methyltransferase 3a (Dnmt3a) mainly functions in *de novo* methylation in the promoter and gene body regions, including transcription termination sites (TTSs), during development. To investigate the association between DNA methylation overlapping the TTSs and transcription termination, two analyses were performed. The first was a bioinformatic analysis that used the already deposited datasets of mouse cells. The second was a bioinformatic and experimental analysis of the zebrafish. In analysis of mouse, I used six pre-existing *Dnmt*$^{-/-}$ mouse cell datasets: four types of neurons (three *Dnmt3a*$^{-/-}$ and one *Dnmt1*$^{-/-}$ mutants) and two types of embryonic fibroblasts (MEFs) (*Dnmt3a*$^{-/-}$ and *Dnmt3b*$^{-/-}$ mutants). Combined analyses using methylome and transcriptome data revealed that read counts downstream of hypomethylated TTSs were increased in three types of neurons (two *Dnmt3a*$^{-/-}$ and one *Dnmt1*$^{-/-}$ mutants). Among these, an increase in chimeric transcripts downstream of the TTSs was observed in *Dnmt3a*$^{-/-}$ mature olfactory sensory neurons and *Dnmt3a*$^{-/-}$ agouti-related peptide (protein)-producing neurons, thereby indicating that read-through occurs in hypomethylated TTSs at specific gene loci. Conversely, in *Dnmt3a*$^{-/-}$ MEFs, I detected reductions in read counts downstream of hypomethylated TTSs. These results of mouse analyses indicate that the hypomethylation of TTSs can both positively and negatively regulate transcription termination, dependent on Dnmt and cell types.

During the analysis of the zebrafish, a maternal-zygotic *dnmt3aa*$^{-/-}$ mutant I was detected. Increased read counts downstream of the hypomethylated TTSs in three genes were detected via the combined analyses using methylome and transcriptome data. In addition, chimeric transcripts were also detected in one gene; similar results were experimentally obtained. These results of the zebrafish analysis indicate that transcription termination is defective in the hypomethylated TTSs in zebrafish.

Therefore, the results of the mouse and zebrafish analyses suggest that DNA methylation may be involved in transcription termination in vertebrates. This study is the first to identify the aberrant termination of transcription at specific gene loci with DNA hypomethylated TTSs attributable to Dnmt deficiency.