Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Studies on multifunctional genome elements functioning in mouse spermatogenesis
（マウス精子形成で機能する多機能性ゲノムに関する解析）

Mammalian spermatogenesis is a sequential continuation of cellular differentiation to generate functional sperm from an initially undifferentiated germ cell. This process is regulated by different types of genes especially in producing primary spermatocytes, but the molecular mechanism of gene activation during spermatogenesis is not fully understood. Our group found a dual promoter-enhancer (DPE) element which have both promoter and enhancer activity in mouse primary spermatocytes. Here I hypothesized that more DPEs function in spermatogenesis and performed genome-wide analyses to identify DPE candidates. By chromatin immunoprecipitation-sequencing (ChIP-seq), I initially identified three genome regions marked with both H3K4me1 and H3K4me3, but they did not show promoter activity. Then, I searched for genome regions around transcriptional start sites (TSSs) that were associated with H3K4me1 by ChIP-seq and RNA-sequencing. It was able to identify 337, 2176 and 69 DPE candidates in spermatogonia, primary spermatocytes, and GC-2spd(ts) cells, respectively. I selected 13 candidates and checked transcriptional activity using reporter gene analysis. The reporter gene assay revealed that 8 out of 13 candidates showed significant enhancer activity while 10 showed promoter activity.

In order to identify the *in vivo* mechanism of these regions I have deleted 5 DPE sequences in GC-2spd(ts) cells by CRISPR/Cas9 genome editing. Then I used quantitative real time polymerase chain reaction (qRT-PCR) with the deleted and control cells and demonstrated that all of them function as promoters of

respective genes and 3 of them enhance the expression of a neighboring gene. Notably, one region that did not exhibit enhancer activity by reporter gene assay acted as an enhancer with the genome editing. Therefore, my current results strongly suggest that genome regions with H3K4me1 around TSSs of expressed genes are DPEs and that the number of DPEs dramatically increases on the onset of meiosis from spermatogonia to primary spermatocytes. These point to the significance of DPE in transcriptional activation during spermatogenesis.

Apart from that I identified a DES-K16 region which has both enhancer and silencer activity. An intron of the *Kctd16* gene and this region shows chromatin marked with specific methylation patterns for both enhancers (H3K4me1 and H3K27ac) and silencers (H3K27me3) in mouse spermatocytes. *In vitro* reporter gene assay demonstrated that DES-K16 exhibited significant enhancer activity in spermatocyte-derived GC-2spd(ts) and hepatic tumor-derived Hepa1-6 cells, and a deletion of this sequence in GC-2spd(ts) cells resulted in a decrease and increase of expression levels *Yipf5* and *Kctd16* genes, respectively. This was consistent with increased and decreased expression of *Yipf5* and *Kctd16*, respectively, in primary spermatocytes during testis development. While known dual enhancer-silencers exert each activity in different tissues, this is the first report on a dual enhancer- silencer element which activates and suppresses gene expression in a single cell type simultaneously.