学 位 論 文 審 査 の 要 旨

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Development of a nucleosome assembly method using a wheat germ cell-free co-expression system
（コムギ胚芽無細胞タンパク質共翻訳によるヌクレオソーム再構成法の確立）

His Doctoral thesis is divided into four chapters and comprises 54 pages of main text, including 9 figures, 3 tables and 1 reference paper.

In the field of chromatin research, the biochemical and structural approaches have provided important evidence to fill the knowledge gap determined in physiological studies. In eukaryotes, a genome DNA is compacted in the cell nuclei as a form of chromatin. The basic building block of the chromatin is a nucleosome, composed of four histones, H2A, H2B, H3, and H4, and tightly wrapped by ~1.7 times DNA. In yeast, the correlations between chromatin structure and gene regulation are well described, in which genes in the regions with the highly compacted chromatins (closed) are depressed, while those within the less compacted chromatins (opened) are actively expressed. The recent advent of the next generation sequencing together with chromatin immunoprecipitation (ChIP-seq) using anti-histone antibodies enables the mapping of nucleosome density in a whole genome, corresponding to either silent or active gene regions in other eukaryotes, including human, animals, plants, and others. Several factors are known to contribute to the alteration of chromatin structure and function, mainly through genetic and ChIP-seq approaches. For example, a set of posttranslational modifications (PTMs) on the histone proteins serves as a marker for packed chromatin regions, noncanonical histones (histone variants) can be found in the transcriptional start sites in the opened chromatin regions, and others. Thus, somehow, regions of chromatins are assembled into either opened or closed structures by chromatin assembly factors and chromatin remodeling factors in the cells. However, molecular mechanisms of how these chromatins are assembled, how histone variants contribute to change chromatin structure, and how PTMs of dozens of combinations result in different chromatin functions are still unknown.

To understand chromatin function and structure, it is important to analyze nucleosome

biochemically. A salt dialysis-based nucleosome assembly has been widely used for structural and biochemical studies, yet several technical limitations exist. For example, histone proteins needed to be prepared using the recombinant method, which results in insoluble products and subsequent refolding is necessary. Furthermore, assembled nucleosomes are stable such that they may be irrelevant products for biochemistry. Recently, a cell-free wheat germ co-expression chromatin assembly methods were developed in our laboratory, which solved the above limitations (Okimune et al., 2020, BMC biotechnology; Endo et al., 2020, FEBS Open bio; Okimune et al., 2021, FEBS Open bio). His doctoral study expanded the method to reconstitute histone variants besides canonical ones from a model plant *Arabidopsis thaliana,* as detailed in (**1**). Furthermore, a highly purified form of assembled human nucleosomes was prepared by optimizing several purification steps, which will be subjected to Cryo-EM analysis, as described in (**2**).

(1) *In vitro* co-expression chromatin assembly and remodeling platform for plant histone variants.

Together with canonical histones, different histone variants are also reported to be involved in chromatin structure alteration and functions in *A. thaliana*. In comparison to yeast, more histone variants for H2A, H2B, and H3 (but not H4) are reported and most of them were not known whether they contribute to the formation of nucleosomes. Thus, in this study, he utilized four core canonical histones (H2A, H2B, H3 and H4), three H2A variants and two H3 variants for in vitro chromatin assembly. Out of twelve combinations of *A. thaliana* nucleosomes, seven were successfully assembled in our method and used as substrates for histone remodeling assay to ask whether different combinations of histones might be remodeled differently by the reported Arabidopsis remodeling complex, CHR11-DDR4. It was concluded that a particular remodeling complex remodels different histone variants-containing nucleosomes.

(2) Establishment of nucleosome purification method for cell-free co-expression method.

For the structural determination of the assembled nucleosome by the cell-free co-expression method, it is essential to prepare pure nucleosome samples. In the wheat germ extract used in the co-expression reaction, various wheat endogenous proteins exist, and rigorous optimization was needed to obtain highly purified forms of nucleosomes for structural study. He optimized the purification procedures of the reconstituted nucleosome and successfully achieved over 95% purity by monitoring both proteins and DNA in the complex, which is ready for the future structural determination by Cryo-EM.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Food Resources from Hokkaido University）