学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

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）

In eukaryotes, genome DNA, enclosed within the nucleus, is packaged into nucleoprotein complex, chromatin. The fundamental unit of chromatin is the nucleosome, which consists of approximately 150 bp DNA wrapped around a histone octamer, comprising two copies each of the four core histones, H2A, H2B, H3, and H4. The assembly or disassembly of nucleosomes by histone chaperones and chromatin remodeling factors, the alternation of nucleosome composition through the incorporation of histone variants and the installing of post-translational modification, and their crosstalk induce dynamic structural changes in chromatin to regulate DNA repair, DNA replication, and gene expression. Thus, understanding the detailed molecular mechanisms of chromatin dynamics is an important challenge in life science, and biochemical approaches utilizing well-controlled reconstituted nucleosome substrates could help directly reveal the functions of chromatin-associated factors, such as histone variants and histone chaperones. The salt dialysis method is most widely used for the preparation of reconstituted nucleosomes, but the assembly reaction is performed under artificial conditions, making it hard to resembling highly coordinated histone deposition onto DNA in vivo.

I previously established a novel nucleosome reconstitution method using a wheat germ cell-free co-expression system to overcome the limitation of salt dialysis. This method enables the nucleosome assembly by co-expressing histone proteins and histone chaperones in the presence of template DNA, resembling the histone deposition process that occurs in vivo. Drosophila and human canonical nucleosomes were confirmed to be reconstituted by

the cell-free co-expression method.

In this study, I aimed to expand this method further to enable the reconstitution of plant and histone variant-containing nucleosomes and also perform functional assessments of chromatin remodeling factors. Furthermore, I attempted to establish the nucleosome purification method suitable for our reconstitution method to determine the structural characteristics of both canonical- and histone variant-incorporated nucleosomes assembled by our method.

In Chapter 2, I investigated the reconstitution of plant histone variant-containing nucleosomes. Histone H2A variants (H2A.X, H2A.W, and H2A.Z) and histone H3 variants (H3.3 and CENH3) derived from the model plant, Arabidopsis thaliana were examined for their incorporation into nucleosomes using cell-free co-expression method. Out of 12 nucleosome combinations, seven combinations were assembled by our method and subjected to the chromatin remodeling assay. The remodeling activity of the imitation switch (ISWI) chromatin remodeler, CHR11, and the complex of ISWI and DDT-related subfamily, CHR11-DDR4, were evaluated by co-expressing in the nucleosome assembly reaction. From these results, it was shown that CHR11-DDR4 remodeling complex has a preference for the nucleosomes that contain certain histone variants.

In chapter 3, I established the nucleosome purification method for the cell-free co-expression method, aiming to determine the three-dimensional structure of nucleosomes assembled by our method. Human histone H3.1 and histone H3 variants (H3.3 and CENP-A) were investigated for their incorporation into nucleosomes, and purified products were evaluated. The novel primate-specific histone H3 variant, H3.X whose structure and in vivo functions remain elusive, was also examined for its assembly into nucleosomes, which will be readily utilized for the future structural study.

In summary, I have further developed the nucleosome reconstitution method to enable the assembly of histone variant-containing nucleosomes and functional assessments of chromatin remodeling factors. In addition, I have outlined future directions for the determination of the structural characteristics of nucleosomes.