学位論文内容の要旨

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学位論文題名

In vitro co-expression chromatin assembly and remodeling platform for plant histone variants

（植物変種ヒストンを用いた*in vitro*共発現系によるクロマチン構造および
リモデリング機能再構築法の確立）

The eukaryotic genome is compacted and organized into chromatin; a long-range nucleoprotein complex made up of repeating units of nucleosomes. Nucleosomes consist of a histone octamer, comprising two copies each of histones H2A, H2B, H3, and H4, and an approximately 147 bp DNA wrapping around histone octamer. Nucleosomes serve not only as structural components but also as key regulators, governing various biological processes, including DNA replication, repair, transcription, and others. The incorporation of histone variants into the nucleosomes plays a central regulatory role in shaping the chromatin landscape in plants. However, the specific effects of different combinations of histone variants on nucleosome properties and dynamics remain largely unknown. To address this knowledge gap, the wheat germ extract based *in vitro* chromatin assembly platform was developed for investigating the chromatin of the model organisms *Arabidopsis thaliana*. This platform combines protein co-expression and chromatin assembly in a single reaction and facilitating the efficient screening of chromatin-related proteins and their functions. In this study, chromatin templates were reconstituted with different histone variant combinations, and chromatin remodeling and histone chaperone activities were explored.

The establishment of the chromatin assembly and remodeling platform for plants

To investigate the suitability of the previously established co-expression chromatin assembly platform [Okimune et al., BMC Biotehcnol., 2021; Endo et al., FEBS Open Bio, 2021] for chromatin reconstitution with plant histone variants, nine

representative histones were chosen from each histone family from *A. thaliana*; including four H2As (canonical H2A, H2A.X, H2A.W, and H2A.Z), one H2B (H2B.9), three H3s (canonical H3.1, H3.3, and CENH3) and canonical H4. Twelve combinations of histones were co-expressed in the presence of a circular closed plasmid DNA and seven chromatin combinations were confirmed to be reconstituted using the supercoiling assay and micrococcal nuclease (MNase) assay.

Subsequently, the assembled chromatins were employed as templates for enzyme screening, wherein a putative chromatin remodeling complex was utilized. This complex comprised CHR11, an imitation switch ATPase chromatin remodeler known for its role in promoting nucleosome sliding, and DDR4, a protein whose function has been only predicted *in vivo* [Tan, L. M. et al., Plant Cell, 2020]. CHR11 or CHR11/DDR4 were co-expressed in the chromatin assembly reaction, followed by MNase assay. The results indicated that the presence of the remodeling complex increased nucleosome repeat lengths and improved the periodicity of nucleosome spacing with varying extents for all tested combinations.

Furthermore, the five combinations, which were not reconstituted by the co-expression chromatin assembly system, were subjected as substrates for a chromatin assembly chaperone. The nucleosome assembly protein1, NAP1, is known to function in chromatin assembly in several species, here *A. thaliana* NAP1;3 was used. Two combinations, including the H2A.Z variant were rescued, suggesting that NAP1;3 facilitates the deposition of this variant.

Overall, the current method proves to be a valuable tool for assembling nucleosomes with histone variants and allowing the functional assessment of remodeling and histone chaperone activities relevant to chromatin structure and physiological functions in plants.