論文の内容の要旨

Structural basis of centromeric chromatin formation involving the CENP-A nucleosome
（CENP-A ヌクレオソームによるセントロメアのクロマチン形成の構造基盤）

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The genomic DNA of the eukaryotic cell is packed into the cell nucleus by forming chromatin, which consists of nucleosomes as the fundamental unit. The nucleosome consists of DNA wrapped around the histone octamer, formed from 2 copies of histone H2A, H2B, H3, and H4. The centromere chromatin is a special region of chromatin that is epigenetically defined by CENP-A, a histone H3 variant, and is responsible for chromosome segregation. In this study, I investigated 2 epigenetic features of the CENP-A nucleosome: the flexible DNA ends and the mono-methylation of H4K20 (H4K20me1).

To investigate the effect of the flexible DNA ends of the CENP-A nucleosome on higher-order chromatin structure, tri-nucleosomes with a center-positioned CENP-A nucleosome or H3 nucleosome were reconstituted *in vitro* to mimic the centromeric chromatin. Subsequently, cryo-EM analyses of the reconstituted tri-nucleosomes were performed. From the cryo-EM structures, it can be seen that

the tri-nucleosomes with a center positioned CENP-A nucleosome are less twisted. This may enable the CENP-A nucleosomes to be exposed from surrounding H3 nucleosomes in a poly-nucleosome fiber and contribute to kinetochore assembly.

On the other hand, it has been shown that the H4K20 in the CENP-A nucleosome is mono-methylated in cells, and that the structural characteristics of the CENP-A nucleosome may lead to higher H4K20 mono-methylation rates compared to the canonical H3 nucleosome. The H4K20 mono-methylation reaction is solely catalyzed by the methyltransferase SET8 (also named PR-Set7/KMT5A). However, how SET8 recognizes the nucleosomal H4K20 substrate, and why CENP-A nucleosome increases the mono-methylation rates of the H4 tail remain elusive. To address these issues, the SET8-CENP-A nucleosome complex and the SET8-H3 nucleosome complex were reconstituted *in vitro* and cryo-EM analyses were performed.

The cryo-EM structures of the SET8-CENP-A nucleosome complex and the SET8-H3 nucleosome complex share a similar appearance. In both structures, SET8 is located above the nucleosome disc and interacts directly with the acidic patch of nucleosome, which is formed by a cluster of negatively charged residues from H2A and H2B. Apart from the acidic patch, SET8 can also be seen interacting with the histone H4 tail. The H4 tail extends from the nucleosome disc surface into the SET domain of SET8.

Importantly, I discovered that the H4 tail of the H3 nucleosome is repositioned upon the binding of SET8. Interestingly, this new conformation of the H4 tail in the SET8-bound nucleosome is the conformation that has been suggested to be preferred by the free CENP-A nucleosome. Therefore, the H4 tail conformation in the free CENP-A nucleosome may be preferred by SET8, leading to a higher methylation rate.

This study provides the basis for understanding how the structural features of the CENP-A nucleosome may affect the higher-order structure and histone modification of centromeric chromatin.