# Involvement of the asymmetric localization of histone H3 variants between parental genomes in mouse preimplantation development

# 博士論文　（要約）

Involvement of the asymmetric localization of histone H3 variants between parental genomes in mouse preimplantation development

（マウス 1 細胞期胚におけるヒストン H3 変異体の
局在および機能解析）

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# SUMMARY

The 1-cell embryo, formed by the fusion of a sperm and an oocyte, is the beginning of life. The maternal and paternal genomes are first enclosed in two separate compartments, referred to as maternal pronucleus and paternal pronucleus, respectively. Although they exist in the same cytoplasm, each acquire its unique chromatin structure and characteristics. First, parental asymmetry is observed in transcriptional activity, where the paternal pronucleus has a higher transcriptional activity compared to that of maternal pronucleus. In addition, DNA replication is reported to occur asymmetrically in terms of the speed of completion. These heterogeneities can be explained by the differences in chromatin structure of maternal and paternal pronuclei. The chromatin structure of the paternal pronucleus is loosened and decondensed compared to that of maternal pronucleus. However, what determines this difference in chromatin structure and the difference in characteristics seen between the pronuclei, is not yet elucidated.

Histone variants is one of the key players in determining chromatin structure. Histone H3 has three non-centromeric variants, H3.1, H3.2, and H3.3. Although they are highly similar in amino acid sequence, each of the H3.1, H3.2, H3.3 variants are known to be associated with different histone modifications and deposition pathways. For example, H3.1 and H3.2 are deposited in both heterochromatin and euchromatin; however, H3.3 are mainly deposited in euchromatin. In addition, H3.1 and H3.2 are likely to acquire histone modifications that are associated with transcriptional repression whereas H3.3 are likely to obtain those that are associated with transcriptional activation. Taken together, H3 variants are essential in determining chromatin structure.

In this study, I hypothesize that H3 variants is one of the determinants in the

difference in characteristics of maternal and paternal pronuclei at the 1-cell stage. In chapter 1, I investigated the nuclear localization of H3 variants during the preimplantation development. As a result, asymmetric nuclear localization of H3.1/H3.2 were detected in 1-cell embryos: they are localized in the perinucleolar region in the maternal pronucleus but not in the paternal one. In addition, the nuclear localization level of H3.1/H3.2 was low compared to that of later preimplantation stages and the low nuclear localization was regulated by the low mRNA expression level and low incorporation efficiency. In chapter 2, I examined the biological significance of the low nuclear localization level of H3.1/ H3.2 in 1-cell embryos, by inducing incorporation of each of the H3 variants into chromatin of 1-cell embryos and analyzed the effect of ectopic localization of each variant in preimplantation development. The results showed that when H3.1 and H3.2 were induced into chromatin in 1-cell embryos, the developmental rate of these embryos were remarkably low compared to that of H3.3-induced and control embryos. Interestingly, the H3.1/H3.2 nuclear localization of the paternal pronucleus was altered upon induced incorporation of H3.1 and H3.2, in which the H3.1/ H3.2 was detected at the perinucleolar region, similar to that of maternal pronucleus, thus leading to a lack of asymmetry. In addition, the developmental failure in H3.1 and H3.2 overexpressed embryos were caused by the delayed DNA replication at the perinucleolar region in the paternal pronucleus whereas H3.1 and H3.2 incorporation had no effect in DNA replication in the maternal one.

This study introduced a novel insight in the role of H3.1 and H3.2, which are asymmetrically localized between the maternal and paternal pronuclei in 1-cell embryos. This difference in nuclear localization regulates DNA replication, in which the DNA replication at the perinucleolar region of the paternal pronuclei is completed earlier

compared to that of maternal pronuclei. In addition, the results suggested that it is essential that H3.1 and H3.2 is not incorporated into chromatin of 1-cell embryos, as incorporation of these variants cause defects in DNA replication of the paternal pronucleus. The nuclear localization of H3.1 and H3.2 are regulated to be low at the paternal pronuclei by lowering the mRNA expression level and incorporation efficiency into chromatin at the 1-cell stage.