Our research objective is to understand the molecular mechanisms that control chromatin function and genome diversity & stability in mammals. To address this question, we are currently analyzing functional molecules which are expressed in the nucleus.

1) **Role of G9a KMTase in embryo proper lineage on suppression of extraembryonic genes: M. TACHIBANA and Y. SHINKAI**

Histone H3 lysine 9 (H3K9) methylation is a repressive epigenetic mark for heterochromatin formation and transcriptional silencing. Genetic loss of a H3K9 lysine methyltransferase (KMTase), G9a leads drastic reduction of H3K9me2 and exhibits embryonic lethality at mid-gestation in mice, indicating that G9a and/or G9a-mediated H3K9 methylation is essential for mouse embryogenesis. However, how the loss of G9a affects mouse embryonic development is still unclear. Since G9a suppresses tissue specific genes in ES cells via H3K9 methylation, we hypothesize that G9a may control developmentally regulated genes in mouse embryogenesis. To gain insights into the transcriptional control of G9a in vivo, we isolated RNA from embryo proper and trophoblast cells of wild-type and G9a knockout (G9a-KO) litters. We then introduced them into microarray analysis to examine the differences of expression profiles between them. Subsequently, it was revealed that specific Hox family genes (reproductive homeobox: Rhox) were ectopically reactivated in G9a-KO construct. Rhox genes, which are localized on X-chromosome, are novel Hox-family genes characterized recently (Cell, 120 p369, 2005). It is shown that Rhox genes are expressed predominantly in reproductive tissues (testis and ovary) and placenta. Rhox genes display temporal and quantitative colinear pattern of expression, however, the molecular basis which contribute the establishment and maintenance of these unique expression remains to be solved. Interestingly, it was revealed that transcriptional suppression of some Rhox genes were achieved by DNA methylation. Furthermore, the DNA methylation-dependent silencing is occurred in a lineage-specific manner (specifically occurred in embryo proper-lineage) (Genes Dev., 20, p3382, 2006). Regarding this notion, we speculate that both H3K9 methylation and DNA methylation machineries may cooperatively regulate colinear expression of Rhox genes. We are now going to set up chromatin immunoprecipitation analysis on Rhox-loci using embryo proper and trophoblasts of wild-type and G9a-KO mice.

2) **Role of G9a HMTase for pre-implantation development in mice: M. TACHIBABA and Y. SHINKAI**
The oocyte serves the distinct purpose of transmitting the maternal genome and other maternal factors that are critical for post-ovulation events, such as zygotic gene activation (ZGA). In mice, ZGA dominantly occurs during the two-cell stage. We hypothesize that maternally inherited epigenetic factors such as histone modification enzymes may play an essential role on early embryogenesis. To investigate an effect of maternal deprivation of G9a, we crossed G9a-conditioned KO mice and zona pellucida 3 (Zp3) –Cre transgenic line. The resulting Zp3-Cre, G9aF/F female mice were bred to wild-type males to test their fertility. In contrast to wild-type females that produced offspring following matings (~80%), Zp3-Cre, G9aF/F females displayed a reduced fertility (45%). In addition, the Zp3-Cre, G9aF/F females produced considerably small litters (2.2) compared to control females (7.1). To elucidate whether the ovulation is normal or not in Zp3-Cre, G9aF/F female, we accessed the numbers of oocytes from the superovulation experiment. Zp3-Cre, G9aF/F females could ovulate similar number of oocytes (~21) to those of control female (~20), indicating that maternal G9a is not necessary for this process. Then we fertilized maternally G9a-deprived oocytes with wild-type sperm and further cultured in vitro. After culturing for four-days, ~95% embryos derived from control oocytes reached blastcyst stage. In contrast, only 18% embryos could reach to this stage when they originated from maternally G9a-deplived oocytes. We concluded that maternal inheritance of G9a is necessary for normal embryonic development until implantation. One possible explanation for this phenotype is maternal G9a plays a crucial role on ZGA. To further elucidate the G9a-ZGA link, it will be important to verify the role of G9a-mediated H3K9 methylation and transcriptional suppression during early embryogenesis.

3) Expression of the mouse PR domain protein Prdm8 in the developing central nervous system: T. KOMAI and Y. SHINKAI

The PR (PRDI-BF1 and RIZ homology) domain family proteins were first noted to share the homology with the SET domain-containing histone lysine methyltransferases. Although only a few members of PR domain proteins were identified to possess histone methyltransferase activity, many of them are now well known to be key regulators for tissue development and/or cell differentiation.

We found that a member of PR domain proteins, Prdm8, is specifically expressed in the mouse brain. Immunohistochemical analysis of mouse embryos demonstrated that Prdm8 protein is expressed in particular types of neurons. In the retina, Prdm8 is specifically expressed in rod bipolar cells and a subpopulation of amacrine cells. In the developing spinal cord, Prdm8 expression is restricted to the ventral interneurons. In the brain, Prdm8 is mainly expressed in the layer 4 neurons of the neocortex. Since Prdm8 expression in tightly regulated in a spatio-temporal manner during neural development, we hypothesize that Prdm8 plays important roles in the process of neuronal
differentiation or maturation. We are now trying to examine the role(s) of Prdm8 in neuronal development by knocking out and knocking down Prdm8 in mice.

4) **Proviral silencing in embryonic stem cells requires the histone methyltransferase ESET:** T. MATSUI and Y. SHINKAI

Endogenous retroviruses (ERVs), retrovirus-like elements with long terminal repeats (LTRs), are widely dispersed in the euchromatic compartment in mammalian cells, comprising ~10% of the mouse genome. These parasitic elements are responsible for >10% of spontaneous mouse mutations. While DNA methylation plays an important role in proviral silencing in somatic and germ-lineage cells, an additional DNA methylation-independent pathway also functions in embryonal carcinoma and embryonic stem cells (ESCs) to inhibit transcription of the exogenous gammaretrovirus, murine leukemia virus (MLV). Intriguingly, a recent genome-wide study revealed that ERVs are also marked by histone H3 lysine 9 trimethylation (H3K9me3) and H4K20me3 in ESCs but not in MEFs. However, the role these marks play in proviral silencing remains unexplored. Here, we show that the H3K9 methyltransferase ESET/Setdb1/KMT1E and the Krüppel-associated box (KRAB)-associated protein 1 (KAP-1)/Trim28 are required for H3K9me3 and silencing of endogenous and introduced retroviruses specifically in ESCs. Furthermore, while ESET enzymatic activity is crucial for HP1 binding and efficient proviral silencing, the H4K20 methyltransferases Suv420h1/2 are dispensable for silencing. Strikingly, in DNA methyltransferase triple-knockout ESCs, ESET and KAP-1 binding and ESET-mediated H3K9me3 are maintained and ERVs are minimally derepressed. We propose that a DNA methylation-independent pathway involving KAP-1 and ESET/ESET-mediated H3K9me3 is required for proviral silencing during the period early in embryogenesis when DNA methylation is dynamically reprogrammed.

**LIST OF PUBLICATIONS**
**EXPERIMENTAL RESEARCH CENTER FOR INFECTIOUS DISEASES**
**LABORATORY OF MOUSE MODEL**


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