DIVISION OF CHROMATIN REGULATION

Professor
NAKAYAMA, Jun-ichi

Assistant Professor:	*HAMADA, Kyoko*
	KATAOKA, Kensuke
Postdoctoral Fellow:	*KAWAGUCHI, Takayuki*
	*HAMADA, Kyoko**
Visiting Undergraduate:	*OKI, Koichi*
Technical Assistant:	*TANAKA, Mayo*

The stable inheritance of gene expression or repression state is essential to cellular differentiation and development, and an epigenetic cellular-memory system derived from higher-order chromatin structure plays a fundamental role in this process. The assembly of higher-order chromatin structure has been linked to the covalent modifications of histone tails, chromatin-associated proteins, and non-coding RNAs. The exact means by which such chromatin-based epigenetic information is established and faithfully maintained across cell divisions and throughout development, however, remains incompletely understood. To try to gain a better understanding of the molecular mechanisms underlying chromatin-based epigenetic phenomena, our lab uses fission yeast, *Schizosaccharomyces pombe*, and ciliate *Tetrahymena* as model organisms for studying the molecular mechanisms of higher-order chromatin assembly. We are also attempting to determine the cellular functions of chromatin modifying factors in developing a better understanding of complicated epigenetic phenomena in higher eukaryotic cells.

I. Establishment and maintenance of higher-order chromatin structure

In eukaryotic cells, the assembly of higher-order chromatin structure, known as heterochromatin, plays an important role in diverse chromosomal processes. We have previously shown that the specific methyl modification on lysine 9 of histone H3 (H3K9me) and the binding of the chromodomain (CD) proteins such as HP1 to methylated histones are essential to the assembly of higher-order chromatin structure. In fission yeast, the H3K9me is catalyzed by the methyltransferase Clr4. It was previously shown that Clr4 forms a complex called CLRC, which includes Rik1, Raf1, Raf2, and Cul4. Since Cul4 is one of the ubiquitin ligases, and Rik1 and Raf1 show sequence similarities to mammalian DDB1 and DCAF, respectively, it is hypothesized that CLRC has E3 ubiquitin ligase activity. However, CLRC's physiological targets remained unclear. We demonstrated that affinity purified CLRC preferentially ubiquitylates histone H3. Interestingly, H3 ubiquitylation was found to promote H3K9 methylation by Clr4. These results suggested that histone ubiquitylation plays a crucial role in maintaining H3K9me. We will further characterize the crosstalk between histone methylation and ubiquitylation by defining how ubiquitylation promotes Clr4 and which domain(s) of Clr4 is responsible for binding ubiquitylated histone H3.

II. Regulation of chromodomain proteins in assembling higher-order chromatin structure

The chromodomain (CD) is a conserved sequence motif present in chromosomal proteins and functions as a binding module that targets methylated histone tails. Heterochromatin protein 1 (HP1) is an evolutionarily conserved chromodomain protein that binds to lysine H3K9me, a hallmark of heterochromatin. We previously showed that mammalian HP1α is phosphorylated at its N-terminal serine residues and that this phosphorylation contributes to HP1α's specific binding to H3K9me nucleosomes and is essential for its proper targeting to heterochromatin. We further demonstrated that Swi6, one of fission yeast HP1 homologues, is also subjected to phosphorylation at its N-terminal conserved region, and also that this region is critical for Swi6's silencing function (Figure 1). We will further characterize the mechanisms how Swi6's N-terminal region contributes to its silencing function by identifying factors that specifically or differentially interact with the Swi6's N-terminal region.

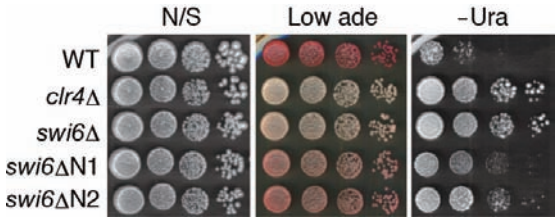

Figure 1. Swi6 N-terminal region is required for its silencing function. A 10-fold serially diluted culture of indicated strain was spotted onto nonselective medium (N/S), low adenine medium (Low Ade), or medium lacking uracil (–Ura).

III. Characterizing nucleic acid-binding activities associated with chromatin proteins

The CD's best-characterized targets are methylated histone tails. Several lines of evidence suggest that the CD, in addition to targeting methylated histones, also binds to DNA and RNA. Although both the nucleic acid-binding and methylated histone-binding activities of CDs are known to be important for CD protein functions in several biological processes, the physical and functional relationships between these two activities are poorly understood. We previously showed that fission yeast Chp1, a CD protein functioning in the RNA silencing pathway, possesses unique nucleic acid-binding activities that are essential for heterochromatic gene silencing. To obtain further evidence that nucleic acid-binding activity associated with CD plays an important role in their chromatin targeting, we focused on mammalian CD proteins and showed that their nucleic acid-binding activity is critical for their nucleosome binding or heterochromatin assembly. We will further characterize the functional role of the CD's nucleic acid-binding activity and also try to identify chromatin factors that specifically interact with non-coding RNAs.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2016. The former title is indicated by an asterisk (*).

IV. Dynamic chromatin reorganization during sexual reproduction of *Tetrahymena*

Tetrahymena, a unicellular protozoan, shows nuclear dimorphism. They contain the transcriptionally silent, germline micronucleus (MIC) and the transcriptionally active, somatic macronucleus (MAC) in a single cell (Figure 2). During sexual reproduction, the newly synthesized MAC removes the dispensable internal eliminated sequences (IESs) from the genome and adds telomeres at the ends of fragmented chromosomes. The IES elimination requires RNA interference (RNAi)-related pathway and posttranslational histone modifications. The methylation of histone H3 lysine 9 and/or lysine 27 catalyzed by Ezl1p acts as binding site for chromodomain proteins such as Pdd1 to form heterochromatin-like structure. Although the RNAi-related pathway to target IES regions is extensively studied, the underlying molecular mechanisms for heterochromatin assembly remain poorly understood. We will focus on uncharacterized heterochromatin proteins and examine their roles in dynamic change of chromatin structure. In addition, we will try to identify factors that are involved in chromosome fragmentation and *de novo* telomere addition during MAC development.

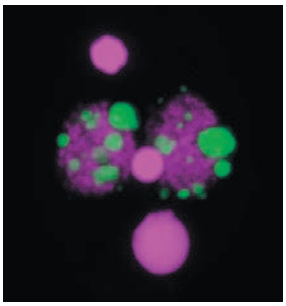

Figure 2. Developing macronuclei (MACs) during sexual reproduction of *Tetrahymena*. The cells at 14 hours post-mixing were stained with anti-Pdd1 (green) and DAPI (magenta). Pdd1 was detected exclusively in the new MACs.

Publication List:

〔Original papers〕

- Kamata, K., Shinmyozu, K., Nakayama, J., Hatashita, M., Uchida, H., and Oki, M. (2016). Four domains of Ada1 form a heterochromatin boundary through different mechanisms. Genes Cells. *21*, 1125-1136.
- Mitsumori, R., Shinmyozu, K., Nakayama, J., Uchida, H., and Oki, M. (2016). Gic1 is a novel heterochromatin boundary protein *in vivo*. Genes Genet. Syst. *91*, 151-159.
- Shimojo, H., Kawaguchi, A., Oda, T., Hashiguchi, N., Omori, S., Moritsugu, K., Kidera, A., Hiragami-Hamada, K., Nakayama, J., Sato, M., and Nishimura, Y. (2016). Extended string-like binding of the phosphorylated HP1α N-terminal tail to the lysine 9-methylated histone H3 tail. Sci. Rep. *6*, 22527.