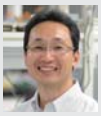
DIVISION OF CHROMATIN REGULATION



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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. SUV39H is the major H3K9-specific methyltransferase that targets the pericentric regions and is crucial for assembling silent heterochromatin. SUV39H recognizes trimethylated H3K9me3 via its CD, and enriched H3K9me3 allows SUV39H to target specific chromosomal regions. However, the detailed targeting mechanisms, especially for naïve chromatin without preexisting H3K9me3, are poorly understood. We showed that Suv39h1's CD (Suv39h1-CD) binds nucleic acids, and that this binding is important for its function in heterochromatin assembly. Suv39h1-CD had higher binding affinity for single-stranded RNA than double-stranded DNA, and its ability to bind nucleic acids was independent of its H3K9me3 recognition. Suv39h1 bound major satellite RNAs *in vivo*, and mutational studies demonstrated that both the nucleic acid–binding and H3K9me-binding activities of Suv39h1-CD were crucial for its pericentric heterochromatin assembly (Figure 1). These results suggest that chromatin-bound RNAs contribute to creating SUV39H's target specificity.

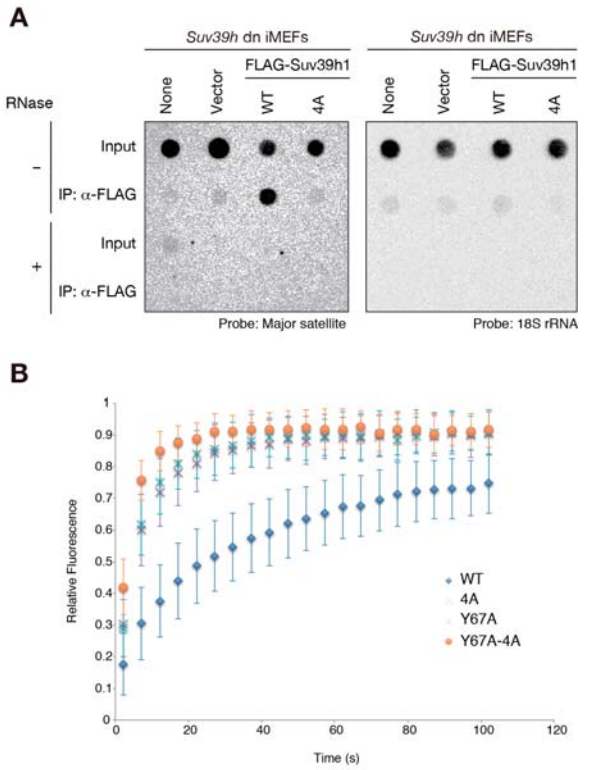

Figure 1. Suv39h1-CD's nucleic acid binding is required for its interaction with major satellite RNAs and chromatin. (A) Dot-blot analysis of immunoprecipitated RNAs. RNAs associated with WT or mutant (4A) Suv39h1 in Suv39h dn iMEFs were precipitated with the anti-FLAG M2 antibody and subjected to dot-blot analysis using a labeled probe for major satellite repeats (left) and 18S rRNA (right). (B) FRAP analysis of WT or mutant GFP-Suv39h1 in suv39h dn iMEFs.

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

2-1 Mitotic phosphorylation of HP1α regulates its cell cycle-dependent chromatin binding

HP1 is an evolutionarily conserved chromosomal protein that plays a crucial role in heterochromatin-mediated gene silencing. We previously showed that mammalian HP1α is constitutively phosphorylated at its N-terminal serine residues by casein kinase II (CK2), and that this phosphorylation enhances HP1α's binding specificity for nucleosomes containing H3K9me3. Although the presence of additional HP1α phosphorylation during mitosis was reported more than a decade ago, its biological significance remains largely elusive. Here we found that mitosis-specific HP1α phosphorylation affected HP1α's ability to bind chromatin. Using biochemical and mutational analyses, we showed that HP1α's mitotic phosphorylation was located in its hinge region and was reversibly regulated by Aurora B kinase and two serine/threonine phosphatases. In addition, chromatin fractionation and electrophoretic mobility shift assays revealed that hinge region-phosphorylated HP1α was preferentially dissoci-

ated from mitotic chromatin and exhibited a reduced DNA-binding activity. Although HP1α's mitotic behavior was previously linked to H3 serine 10 phosphorylation, which blocks the binding of HP1α's chromodomain (CD) to H3K9me3, our findings suggest that mitotic phosphorylation in HP1α's hinge region also contributes to changes in HP1α's association with mitotic chromatin.

2-2 Phosphorylation of CBX2 controls its nucleosome-binding specificity

Chromobox 2 (CBX2), a component of polycomb repressive complex 1 (PRC1), binds lysine 27–methylated histone H3 (H3K27me3) via its chromodomain (CD) and plays a critical role in repressing developmentally regulated genes. The phosphorylation of CBX2 has been described in several studies, but the biological implications of this modification remain largely elusive. We showed that CBX2's phosphorylation plays an important role in its nucleosome binding. CBX2 is stably phosphorylated *in vivo*, and domain analysis showed that residues in CBX2's serine-rich (SR) region are the predominant phosphorylation sites. The serine residues in an SR region followed by an acidic-residue (AR) cluster coincide with the consensus target of casein kinase II (CK2), and CK2 efficiently phosphorylated the SR region *in vitro*. A nucleosome pull-down assay revealed that CK2-phosphorylated CBX2 had a high specificity for H3K27me3-modified nucleosomes (Figure 2). An electrophoretic mobility-shift assay showed that CK2-mediated phosphorylation diminished CBX2's AT-hook–associated DNA-binding activity. Mutant CBX2 lacking the SR region or its neighboring AR cluster failed to repress the transcription of p21, a gene targeted by PRC1. These results suggest that CBX2's phosphorylation is critical for its transcriptional repression of target genes.

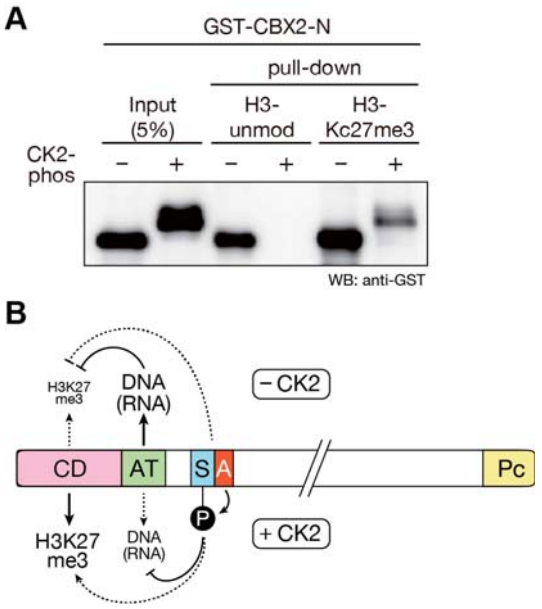

Figure 2. CK2-mediated phosphorylation of CBX2-N increases its binding specificity for H3K27me3 nucleosomes. (A) A representative nucleosome pull-down assay using synthesized nucleosomes containing unmodified H3 (H3-unmod) or H3 with a K27me3 analog (H3Kc27me3). (B) Proposed model for the role of CK2-mediated phosphorylation in the chromatin binding of CBX2.

III. Proteomic approach to identify chromatin-bound RNA-binding proteins

Recent advances in methodologies and technologies led to systematic identification of more than 1,000 RNA-binding proteins (RBPs) in mouse and human cells. Some of these RBPs are known to locate in the nucleus. Interestingly, previous studies suggest that RNAs together with their RBP partners do not only contribute to transcriptional regulation of individual genes, but also play a part in three-dimensional organization of gene loci within the nucleus and/or in regulation of the inner-nuclear structures. However, currently available information is rather fragmentary and molecular mechanisms by which RNA-RBP complexes mediate local and global effects remain largely unknown. Moreover, it is unclear if RNA-RBP interactions participate in other chromatin-based biological processes. In order to grasp the whole picture of physical or functional interactions between RNAs and RBPs in the nucleus, it is essential to identify which RNA-RBP complexes are actually bound to chromatin/nuclear substructures. We have developed a new proteomics-based method to systematically identify such RBPs as potential chromatin-/nuclear matrix-associated RBPs and successfully identified previously uncharacterized RBPs (Figure 3). We will verify their RNA-mediated chromatin interaction and investigate their roles in chromatin organization and transcriptional regulation.

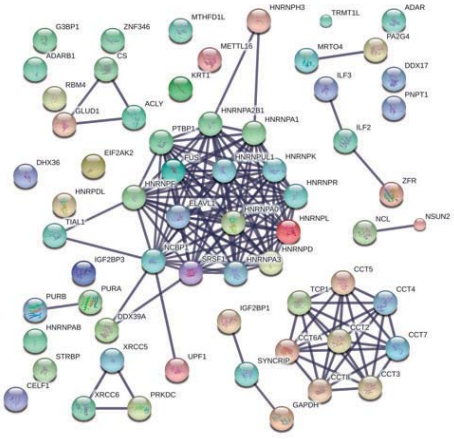

Figure 3. Network analysis of the identified RNase-sensitive nuclear proteins.

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

Tetrahymena, a unicellular protozoan, shows nuclear dimorphism. It contains the transcriptionally silent, germline micronucleus (MIC) and the transcriptionally active, somatic macronucleus (MAC) in the single cell. During sexual reproduction, the newly developed MAC removes thousands of internal eliminated sequences (IESs), which are derived from transposable elements, from the genome. The IES elimination requires RNA interference (RNAi)-related pathway and posttranslational histone modifications. The methylation of histone H3 at lysine 9 and lysine 27 catalyzed by Ezl1p act as binding sites for chromodomain proteins such as Pdd1p to form heterochromatin-like structures. Although previous studies identified more than 20 factors that are linked to

heterochromatin formation and/or DNA elimination, their precise roles in chromatin reorganization remain poorly understood. We have analyzed the cellular localization of potential heterochromatin factors during MAC development and identified 3 novel proteins as heterochromatin components. We have also conducted an ectopic DNA elimination assay and demonstrated that 10 proteins are sufficient for inducing DNA elimination (Figure 4). We will further characterize these heterochromatin proteins and define their roles in chromosome reorganization and IES elimination.

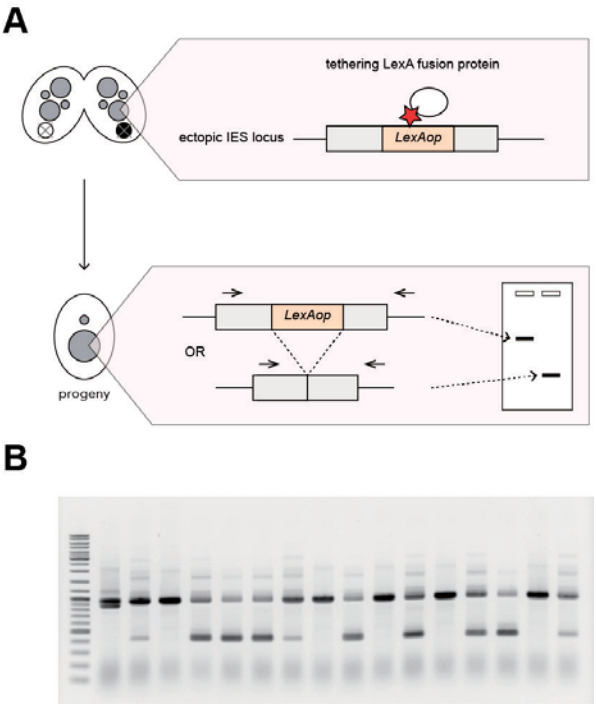

Figure 4. Identification of heterochromatin proteins sufficient to induce DNA elimination. (A) Schematics of the tethering assay. (B) A representative result of the tethering assay using one of heterochromatin proteins.

Publication List:

〔Original papers〕

- Eustache, S., Créchet, J.-B., Bouceba, T., Nakayama, J., Tanaka, M., Suzuki, M., Woisard, A., Tuffery, P., Baouz, S., and Hountondji, C. (2017). A functional role for the monomethylated Gln-51 and Lys-53 residues of the 49GGQTK53 motif of eL42 from human 80S ribosomes. Open Biochem. J. *11*, 8-26.
- Kawaguchi, T., Machida, S., Kurumizaka, H., Tagami, H., and Nakayama, J. (2017). Phosphorylation of CBX2 controls its nucleosome-binding specificity. J. Biochem. *162*, 343-355.
- Mutazono, M., Morita, M., Tsukahara, C., Chinen, M., Nishioka, S., Yumikake, T., Dohke, K., Sakamoto, M., Ideue, T., Nakayama, J., Ishii, K., and Tani, T. (2017). The intron in centromeric noncoding RNA facilitates RNAi-mediated formation of heterochromatin. PLoS Genet. *13*, e1006606.
- Shirai, A., Kawaguchi, T., Shimojo, H., Muramatsu, D., Ishida-Yonetani, M., Nishimura, Y., Kimura, H., Nakayama, J., and Shinkai, Y. (2017). Impact of nucleic acid and methylated H3K9 binding activities of Suv39h1 on its heterochromatin assembly. Elife *6*, e25317.
- Zafar, F., Okita, A.K., Onaka, A.T., Su, J., Katahira, Y., Nakayama, J., Takahashi, T.S., Nasukata, H., and Nakagawa, T. (2017). Regulation of mitotic recombination between DNA repeats in centromeres. Nucleic Acids Res. *45*, 11222-11235.