Cellular Memory Laboratory (2024)
Chief Scientist: Yoichi Shinkai (Ph.D.)

(0) Research field

CPR Subcommittee: Biology

Keywords: epigenetics, transcriptional regulation, protein and DNA methylation,
retrotransposons

(1) Long-term goal of laboratory and research background

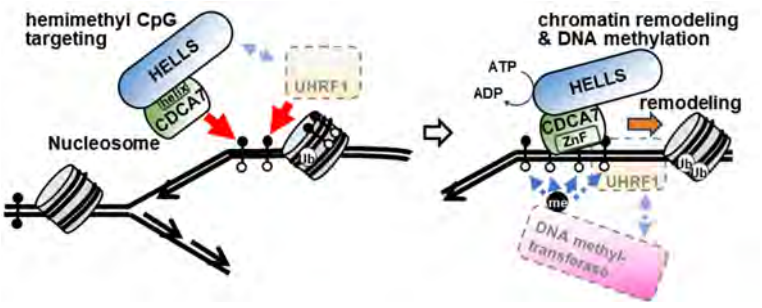
Our laboratory's principal objective is to understand the molecular mechanism of epigenetic gene regulation and the role of epigenetics in health and disease. To address these topics, we take multidisciplinary approaches, including molecular biology, biochemistry, cell biology, structural biology and mouse molecular genetics.

(2) Current research activities (FY2024) and plan

(A) Regulation of DNA methylation by chromatin remodeling enzyme HELLS-CDCA7

The C-terminal 4CXXC-type zinc finger domain of CDCA7 recognizes hemimethylated DNA and modulates activities of chromatin remodeling enzyme HELLS. Akeo Shinkai*, Hideharu Hashimoto, Chikako Shimura, Hiroaki Fujimoto, Kei Fukuda, Naoki Horikoshi, Masaki Okano, Hitoshi Niwa, Erik W. Debler, Hitoshi Kurumizaka, Yoichi Shinkai* (*corresponding author) *Nucleic Acids Research* (2024) 52:10194–10219

In higher organism, DNA methylation is involved in various biological phenomena, such as regulation of gene expression. It is also involved in several diseases such as cancer and immune deficiency because aberrant DNA methylation has been found in these patients. Thus, elucidation of the mechanism of DNA methylation can lead to understand the biological phenomena and these diseases. In higher organism, DNA methylation is generated by the reaction of DNA methyltransferase that methylates cytosine of CpG site. It is categorized into two; *de novo* DNA methylation and maintenance DNA methylation. The former is the methylation of unmethylated DNA, and the latter is the methylation of hemimethylated DNA which is methylated on one chain generated during DNA replication. In higher organism, chromosome is composed of nucleosome, that is, DNA wound around histones. Chromatin remodeling enzyme catalyzes exchange and/or movement of the histones on the nucleosome in an ATP-dependent manner. HELLS is one of the chromatin remodeling enzymes and it interacts with CDCA7 to be activated. The HELLS and CDCA7 are causable genes of ICF syndrome, an immune disease, and DNA hypomethylation is found in the patients. Thus, HELLS-CDCA7 has been thought to be involved in regulation of DNA methylation; however, the mechanism has not been elucidated so far. CDCA7 has central α-helical domain and C-terminal zinc finger domain (ZnF). In the ICF syndrome patients, mutation is found in the ZnF. We found that the central domain of CDCA7 is involved in binding and activation of HELLS, and N-terminal domain of CDCA7 negatively regulated the function of HELLS. Furthermore, we elucidated that HELLS-CDCA7 accumulates on hemimethylated DNA during DNA replication through recognition of hemimethylated DNA by the ZnF of CDCA7. These results indicates that, CDCA7 binds to hemimethylated DNA, and it recruites HELLS onto the nucleosome during DNA replication. Then, the HELLS is activated to move histones, resulting hemimethylated sites are sequentially emerged from the nucleosome, which are methylated by maintenance DNA methyltransferase.



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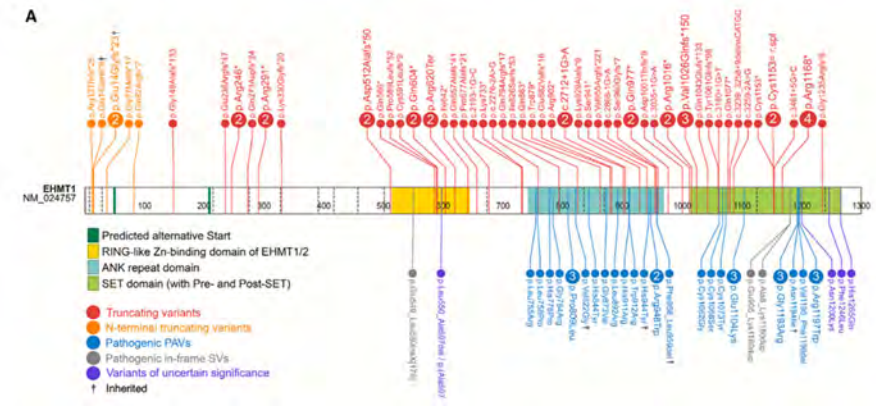
Future plan.
In ICF syndrome patients, not only aberrant DNA methylation but also chromosomal instability is found. We would like to elucidate the mechanism of stabilization of chromosome through HELLS-CDCA7 to investigate relation between DNA methylation and chromosome stability.

(B) Functional validation of the Kleefstra syndrome mutation in histone methyltransferase GLP.

Comprehensive EHMT1 variants analysis broadens genotype-phenotype associations and molecular mechanisms in Kleefstra syndrome. Dmitrijs Rots*, Arianne Bouman*, Ayumi Yamada* *et. al* (*equal contribution) ***The American Journal of Human Genetics*** (2024) 111:1605–1625

Kleefstra syndrome (KS) is a genetic neurodevelopmental disorder characterized by developmental delay and intellectual disability, caused by haplo-insufficiency of in the *EHMT1* gene, which encodes the histone methyltransferase GLP. In this study, conducted in collaboration with a research group in the Netherlands, we identified *EHMT1* mutations using samples from 209 KS patients. In addition, we performed DNA methylation signature analysis using genomic DNA isolated from patient blood samples, as well as biochemical analyses employing recombinant GLP proteins harboring patient-derived mutations. Through this analysis, we demonstrated a correlation between GLP functional impairment caused by *EHMT1* mutations and the clinical phenotypes observed in KS patients. In this study, our group was primarily responsible for the biochemical characterization of GLP point mutants. We identified several mutant GLP proteins that either completely lacked histone methyltransferase activity or exhibited reduced binding affinity to methylated histones, providing important insights into the molecular basis of KS.



Future plan.
Our previous studies have demonstrated that histone methylation activity in cells is mediated not by GLP, but by its binding partner, another histone methyltransferase, G9a. In the present analysis, we identified KS-associated GLP variants that have completely lost methyltransferase activity, highlighting the critical importance of GLP's enzymatic function. Future studies aimed at elucidating the role of GLP histone methyltransferase activity in the nervous system will further advance our understanding of the molecular mechanisms underlying this disease.

(3) Members

as of March 2025

(Chief Scientist)
Yoichi Shinkai

(Senior Research Scientist)
Tadahiro Shimazu, Akeo Shinkai, Shingo Kose

(Research Scientist)
Ayumi Yamada, Atsuko Shirai

(Postdoctoral Researcher)
Masaki Kawase

(Technical Staff)
Chikako Shimura, Kayako Nishimura
Ayane Kataoka

(Student Trainee)
Megumi Gowa, Yuto Iwaya

(Assistant)
Mika Ichihashi

(Temporary Staffing)
Yoshie Hara

(4) Representative research achievements

1. The C-terminal 4CXXC-type zinc finger domain of CDCA7 recognizes hemimethylated DNA and modulates activities of chromatin remodeling enzyme HELLS. Shinkai A*, Hashimoto H, Shimura C, Fujimoto H, Fukuda K, Horikoshi N, Okano M, Niwa H, Debler EW, Kurumizaka H, Shinkai Y*. ***Nucleic Acids Res.*** 2024 Sep 23;52(17):10194-10219. doi: 10.1093/nar/gkae677. PMID: 39142653
2. Comprehensive EHMT1 variants analysis broadens genotype-phenotype associations and molecular mechanisms in Kleefstra syndrome. Rots D#, Bouman A#, Yamada A#, … Shinkai Y*, Kleefstra T*. ***Am J Hum Genet.*** 2024 Aug 8;111(8):1605-1625. doi: 10.1016/j.ajhg.2024.06.008. Epub 2024 Jul 15. PMID: 39013458
3. Cell-type specific, inducible and acute degradation of targeted protein in mice by two degron systems. Yamashita M, Ogawa C, Zhang B, Kobayashi T, Nomura A, Barker C, Zou C, Yamanaka S, Hayashi KI, Shinkai Y, Moro K, Fargarasan S, Imami K, Seita J, Shirai F, Sawasaki T, Kanemaki MT, Taniuchi I*. **Nat Commun.** 2024 Nov 29;15(1):10129. doi: 10.1038/s41467-024-54308-9. PMID: 39613744

Supplementary

Laboratory Homepage
https://www.riken.jp/research/labs/chief/cell_mem/index.html
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