No.	K24-2122	
Project Title	Regulation of DNA methylation on the nucleosome	
Principal Investigator	Hironori Funabiki (The Rockefeller University・Professor)	
Project Member(s)	IMSUT Host Researcher	Makoto Nakanishi (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Isabel Wassing (The Rockefeller University・Postdoctoral Associate)
	Project Member (s)	Nicholas Prescott (The Rockefeller University・Postdoctoral Associate)
	Project Member (s)	Atsuya Nishiyama (Division of Cancer Cell Biology・Associate Professor)
	Project Member (s)	Hong Xin (Division of Cancer Cell Biology・Graduate student)
	Project Member (s)	Kyohei Arita (Yokohama City University・Professor)
	Project Member (s)	Anika Kikuchi (Yokohama City University・Graduate Student)
	Project Member (s)	Moeri Hiruta (Yokohama City University・Graduate Student)

(Form 2)

Principal Investigator
Hironori Funabiki

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

This proposal primarily aims to boost our ongoing collaboration on HELLS-CDCA7 with Dr. Atsuya Nishiyama, Associate Professor in the lab of Prof. Makoto Nakanishi at IMSUT and Prof. Kyohei Arita at Yokohama City University.

DNA methylation plays diverse epigenetic roles, including regulation of gene expression, suppression of transposable elements, and innate immunity. Changes in genomic DNA methylation profiles are associated with aging and cancers, and nucleic acid analogues to inhibit DNA methylation have been approved for cancer therapy. However, since their actions are not specific, more detailed mechanistic understanding of DNA methylation regulations is needed for invention of better therapeutic strategies.

Our laboratory have previously shown that HELLS and CDCA7, whose mutations cause Immunodeficiency, Centromere Instability and Facial Anomalies (ICF) syndrome, form a nucleosome remodeling complex to facilitate DNA methylation (Jenness 2018, PNAS, PMID 29339483). This led to a collaboration with Drs. Nishiyama, Nakanishi, and Arita, demonstrating that the zf-4CXXC_R1 domain of CDCA7 is a novel sensor for hemimethylated DNA (Wassing et al 2024, Sci Adv, PMID 39178260). Based on our findings, we hypothesized that CDCA7 senses hemimethylated CpG on the nucleosome and recruits and activates HELLS to remodel the nucleosome such that the hemimethylated CpG becomes accessible to UHRF1. However, since we and others have shown that CDCA7 can remodel nucleosome without hemimethylated CpG, it remains to be established if hemimethyalted CpG indeed facilitates nucleosome remodeling by HELLS. The structural mechanism by which CDCA7 activates HELLS is also not clear. We plan to address this question by establishing in vitro biochemical assay for nucleosome remodeling and DNA methylation as well as by obtaining cryo-EM structure of CDCA7-HELLS bound to hemimethylated nucleosome.

During the second year of the funded period, I visited IMSUT at two occasions on August 2025 and March 2026. On August 28th, where Dr. Nishiyama, Dr. Arita, two graduate students of Dr. Arita (Nao Nakamura and Amika Kikuchi) and I presented research progress. This onsite meeting was highly valuable to exchange ideas and share technical issues, coming up with a new direction of the research. The Arita lab shown their successful purification of human HELLS and UHRF1, and reconstitution of the nucleosome with H3K9me3, but they had difficulty in purifying human CDCA7. Similarly, our laboratory has been trying to purify human CDCA7, but the proteins tend to aggregate during the purification protocol. Therefore, we decided to extend our effort to purification of HELLS and CDCA7 in other species. We have previously shown that three distinct protein families (class I-III) that contain CDCA7 HMZF-like domain exist in plants. We have previously shown that the class I CDCA7 HMZF binds hemimethylated CpG, and a recent publication indicates that Arabidopsis class I CDCA7 proteins contribute to CpG methylation [PMID 41203935], as we predicted. We have obtained preliminary data indicating that class II CDCA7-like protein (DDR3) also binds hemimethylated CpG, while class III CDCA7-like protein cannot bind to any type of tested DNA regardless of methylation status. Interestingly, it has been suggested that the class II proteins (DDR1, DDR2, DDR3) bind to CHR11 and CHR17, homologs of ISWI nucleosome remodeler, and we have confirmed that DDR3 can recruit CHR17 to hemimethylated nucleosome in in vitro. Since nothing is known about potential roles of DDR1-3 and CHR11/17 in DNA methylation, structural comparison between HELLS (DDM1)-CDCA7-nucleosome complex and CHR11/17-DCR1-3-nucleosome complex will provide insights into why two distinct hemimethylation-sensing nucleosome

remodeling complexes exist in plants and if they have distinct functions coupled with their hemimethylation sensing characteristics. So far, our preliminary data indicate that class II proteins show much stricter hemimethylation-dependency on nucleosome binding than plant class I CDCA7, whose hemimethylation-specificity is reduced in the context of the nucleosome. This characteristic is also different from *Xenopus* CDCA7e, whose hemimethylation-sensing is rather enhanced by the nucleosome, though it depends on the position of hemimethylated CpG within the nucleosome. To test functionality of CDCA7-HELLS homologs that we are biochemically and structurally characterizing, Dr. Nishiyama reported a novel assay to monitor maintenance DNA methylation assay using nuclear plasmic extract (NPE) of *Xenopus* egg extract.

At my second visit to IMSUT on March 30th, 2026, we held the project meeting with the group of Drs Nishiyama and Arita, and guest participants from Dr. Yoichi Shinkai's group at RIKEN. Dr. Shinkai has independently demonstrated the capacity of CDCA7 to bind hemimethylated CpG. At this meeting Dr. Nishiyama and Amika Kikuchi, a PhD student of Dr. Arita gave talks on their work on plant DNMT1 and UHRF1 orthologs, VIM1 and MET1, respectively. Their collaboration recently led to a publication in cryo-EM structure of MET1, revealing the structural similarity and differences between MET1 and vertebrate DNMT1 (Kikuchi et al 2025, Nat Commun, PMD 41006297). The new data presented at this meeting strongly suggest that unlike human DNMT1, which is known to be activated by ubiquitylated histone H3, plant MET1 is activated by ubiquitylated H4, which is generated by VIM1. This is an exciting finding to demonstrate that even though the core players of maintenance methylation are conserved between plants and animals, the mechanistic details are distinct. I report our effort to establish a time-resolved fluorescence energy transfer (TR-FRET)-based detection of CDCA7-hemimethylated DNA interaction. We have obtained preliminary data indicating that this assay system works, and thus we are currently optimizing this assay. Our lab also further characterized the minimum hemimethylation-sensing domain of DDR3, and shared information on our initial attempt to conduct cryo-EM analysis of DDR3-nucleosome structure. We have also obtained preliminary data suggesting that CDCA7 homologs of fungi *Linderina pennispora* and *Basidiobolus ranarum* also bind to hemimethylated CpG. These fungus CDCA7 homologs are interesting, since it shares a feature of zinc finger domain of class II but overall domain composition mimics CDCA7, not plant DDR1-3 proteins. Dr. Arita shared his preliminary data suggesting that purified *Basidiobolus* CDCA7 can bind to oligo DNA with a hemimethylated CpG. They plan to characterize its cryo-EM structure, and assess if they can reconstitute the nucleosome remodeling activity with its HELLS homolog.