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Project Title	Regulatory mechanism of maintenance DNA methylation	
Principal Investigator	Heinrich Leonhardt (Ludwig Maximilians University Munich・Professor)	
Project Member(s)	**IMSUT Host Researcher**	Makoto Nakanishi (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Atsuya Nishiyama (Cancer Cell Biology・Associate Professor)
	Project Member (s)	Kyohei Arita (Yokohama City University/Structural Biology Laboratory, Graduate School of Medical Life Science・Professor)
	Project Member (s)	Christopher B. Mulholland (Ludwig-Maximilians-Universität München/Faculty of Biology, ・Postdoctoral fellow)
	Project Member (s)	Weihua Qin (Ludwig-Maximilians-Universität München/Faculty of Biology, ・Postdoctoral fellow)

(Form 2)

Principal Investigator
Heinrich Leonhardt

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Report

Maintenance DNA methylation in mammalian cells depends on UHRF1, which recognizes hemimethylated CpG sites and recruits DNMT1 through histone H3 and PAF15 ubiquitination. In early development, this pathway must be transiently suppressed to enable global DNA demethylation. In previous work, we identified the mammal-specific factor DPPA3 as a key inhibitor of UHRF1. The goal of this project is to define the molecular mechanism of this inhibition and determine how it is regulated in embryonic stem cells.

During the current funding period, we have made substantial progress by defining the molecular basis of DPPA3-mediated UHRF1 inhibition. We identified a dual-interface mechanism in which DPPA3 binds both the PHD finger and the SRA domain of UHRF1. Importantly, we discovered that the SRA interaction is mediated by an intermolecular zinc-dependent interface, in which conserved cysteine residues in DPPA3 coordinate a Zn^{2+} ion together with UHRF1. Functional analyses in mouse embryonic stem cells demonstrate that this zinc-clasp is essential for DPPA3 activity, as cysteine mutants fail to inhibit UHRF1 chromatin binding and instead display increased DNA methylation. These findings establish the zinc-clasp as a central regulatory element controlling UHRF1 function.

Building on this mechanistic insight, our recent work has focused on testing whether zinc availability modulates UHRF1 behavior in living cells. Using UHRF1-GFP knock-in ESCs, we manipulated intracellular zinc levels through $ZnCl_2$ supplementation and chelation with TPA. Zinc supplementation resulted in a pronounced redistribution of UHRF1-GFP toward the cytosol and a strong increase in its mobility, as measured by FRAP, consistent with reduced chromatin binding. In contrast, zinc depletion produced the opposite effect, leading to a loss of cytosolic UHRF1 and decreased mobility, indicative of increased chromatin association comparable to that observed in Dppa3 knockout cells. FluoZin-based measurements confirmed that these treatments effectively modulate intracellular zinc levels.

Importantly, these zinc-dependent effects were abolished in Dppa3 knockout and Dppa3-3Cys mutant ESCs, in which UHRF1 remained tightly chromatin-bound and exclusively nuclear under all conditions. This demonstrates that the response to zinc availability requires DPPA3 and its zinc-coordinating cysteine residues, providing strong evidence that the DPPA3-UHRF1 zinc-clasp functions as a tunable sensor of intracellular zinc levels.

We are now extending these findings to determine how zinc-dependent changes in UHRF1 chromatin binding affect DNA methylation. Targeted bisulfite libraries have been prepared to assess DNA methylation at LINE-1 elements following prolonged zinc perturbation in wild-type and mutant ESCs, and we are establishing hairpin bisulfite sequencing to quantify hemimethylation and directly measure maintenance methylation fidelity at selected repetitive elements. In parallel, we have implemented a long-read Nanopore-based approach to assess genome-wide methylation changes, enabling direct detection of DNA methylation on native genomic DNA without bisulfite conversion. Initial Nanopore-based analyses confirmed the hypermethylation phenotype of Dppa3-3Cys mutants previously observed using TaBA-seq, and this approach will now be applied to

quantify genome-wide effects of zinc modulation.

In addition, we have initiated experiments addressing the remaining aims of the project by examining whether human DPPA3 exhibits zinc-dependent activity and by testing how cellular redox conditions influence the zinc-clasp interaction. These studies are currently underway and will extend our findings to species-specific regulation and to the chemical control of the DPPA3-UHRF1 interaction.