DATE: Day __09__ **Month** __05__ **Year 2025**

SUMMARY of
FY2024 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Single-Molecule Studies on the Regulation Mechanism of DNA Recombination Machinery
Applicant	**Name**	Li, Hung-Wen
	Affiliation	National Taiwan University
	Present Title	Professor
Research Collaborator (Host PI)		Akira Shinohara

Summary

In collaboration with Akira Shinohara's lab, we developed single-molecule experiments to study meiosis recombination. Interhomolog recombination in meiosis requires a meiosis-specific recombinase, Dmc1. In Saccharomyces cerevisiae, the Mei5-Sae3 complex facilitates the loading of Dmc1 onto the replication protein A (RPA)-coated single-stranded DNA (ssDNA) to form nucleoprotein filaments. In vivo, Dmc1 and Mei5-Sae3 are interdependent in their colocalization on the chromosomes. However, the mechanistic role of Mei5-Sae3 in mediating Dmc1 activity remains unclear. We used single-molecule fluorescence resonance energy transfer and colocalization single-molecule spectroscopy experiments to elucidate how Mei5-Sae3 stimulates Dmc1 assembly on ssDNA and RPA-coated ssDNA. We showed that Mei5-Sae3 stabilized Dmc1 nucleating clusters with two to three molecules on naked DNA by preferentially reducing Dmc1 dissociation rates. Mei5-Sae3 also stimulated Dmc1 assembly on RPA-coated DNA. Using green fluorescent protein-labeled RPA, we showed the coexistence of an intermediate with Dmc1 and RPA on ssDNA before RPA dissociation. Moreover, the displacement efficiency of RPA depended on Dmc1 concentration, and its dependence was positively correlated with the stability of Dmc1 clusters on short ssDNA. These findings suggest a molecular model that Mei5-Sae3 mediates Dmc1 binding on RPA-coated ssDNA by stabilizing Dmc1 nucleating clusters, thus altering RPA dynamics on DNA to promote RPA dissociation.

*Deadline: May 9, 2025
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*This summary will be published on the web.