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Abstract of Thesis

Name （ Stephen Mwaniki Wangui）	
Title	The analysis of Rad51/Dmc1 regulators, Rad52 and Mei5, in yeast meiotic recombination. （酵母の減数分裂組換えにおける Rad51/Dmc1 調節因子 Rad52 および Mei5 の分析。）

Abstract of Thesis

Homologous recombination (HR) during meiosis is crucial for the proper segregation of homologous chromosomes and the generation of genetic diversity during gametogenesis through DNA exchange. Meiotic recombination is characterized by the deliberate formation of DNA double-stranded breaks (DSBs). DSBs are then resected to form single-stranded DNA, ssDNA. Consequently, the ssDNA-binding protein, Replication protein A (RPA), coats the ssDNA to form RPA filaments. Bacterial RecA homologues, Rad51 and Dmc1, then replace RPA on the ssDNA to catalyse homology search and strand exchange leading to DSB repair. In mitosis, Rad51 filaments are sufficient to catalyse homology search and DNA exchange using the sister chromatid as a repair template. In contrast, homology search and strand exchange in meiotic recombination is catalysed by Dmc1 and uses the homologous chromosome as a template, with Rad51 playing an auxiliary role.

Replacement of RPA on ssDNA with Rad51 and Dmc1 is the rate-limiting step during meiotic recombination. The assembly of Rad51 and Dmc1 on the ssDNA is dynamic and highly regulated by both positive and negative factors. Positive Rad51 factors, “mediators”, facilitate the formation of Rad51 filaments on RPA-coated ssDNA. The budding yeast contains Rad52, Rad55-57 and PCSS/Shu complex. Negative Rad51 regulatory factors, such as Srs2 helicase in the yeast, dismantle Rad51 filaments. In budding yeast, Dmc1-filament formation is promoted by the meiosis-specific Mei5-Sae3 complex. Previous studies showed that mei5 and sae3 mutants are defective in Dmc1-filament formation. However, the role and regulation of Mei5 in meiosis remain unclear.

In my thesis, to know the regulation and role of Mei5 in meiosis as a Dmc1 mediator, various *mei5* point mutants were constructed and studied in detail. A previous study showed that the *mei5-R117A* mutant showed an arrest in meiosis. In this study, I characterized the *mei5-R117A* mutant in meiotic recombination in more detail. Interestingly, *mei5-R117A* cells expressed a shorter Mei5 protein than wild-type Mei5. I also found that two additional amino acid substitutions F124A and R134A, produced the shorter Mei5 protein like *Mei5-R117A*. The detailed analyses of the *mei5-R117A* mutant suggest the post-translational processing of Mei5 protein in meiotic cells. I also identified key residues, whose substitution suppressed the formation of the shorter-sized *Mei5-R117A* protein. I speculate a previously undescribed Mei5 processing during meiosis. R117A, F124A, and R134A substitutions could enhance the processing, probably by changing the interaction with a putative processing protein.

In parallel, to elucidate the role of Rad52 in post-assembly of Rad51-filament, the effect of acute depletion of Rad52 was studied. Previous studies showed the role of Rad52 in the assembly of Rad51 filament; the rad52 deletion mutants are unable to form Rad51 assembly called Rad51 foci detected by indirect immuno-staining of Rad51 on meiotic chromosome spreads. However, the role of Rad52 following Rad51 filament assembly remains unknown. To investigate the postassembly stage, I combined RAD52-AID (auxin-inducible degron) system which allows acute depletion of Rad52 and dmc1 deletion mutation which stops Rad51 foci turn-over. Using this system, I

depleted Rad52 after the Rad51 assembly and found that Rad51 foci disappeared soon after the depletion, suggesting the role of Rad52 in the stabilization of Rad51 filaments at the post-assembly stage. By using the AID system, I also studied the functional relationship of Rad52, RPA, and Srs2 in the assembly and disassembly of Rad51 filament during meiotic recombination. These results provide a new view on an active protein machinery containing Rad51 filament for homology search and strand exchange.

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論文審査の結果の要旨及び担当者

氏　名	（ Stephen Mwaniki Wangui ）	
論文審査担当者	（職）	氏　名
	主 査　教授	篠原　彰
	副 査　教授	中川　敦史
	副 査　教授	原田　慶恵

論文審査の結果の要旨

The analysis of Rad51/Dmc1 regulators, Rad52 and Mei5, in yeast meiotic recombination.
酵母の減数分裂組換えにおける Rad51/Dmc1 調節因子 Rad52 および Mei5 の分析。

減数分裂期の組換えはゲノムの多様性の産生と、配偶子形成に必須の役割を果たしている。その仕組み、配偶子形成時のゲノムの安定化の分子メカニズムを解明することは、これまでに知られていないゲノム安定化の仕組みを明らかにするばかりでなく、配偶子の機能不全などの医学的側面の理解に繋がることが期待できる。特に減数分裂期組換えは体細胞分裂期組換えとは異なり、相同染色体間で起き、その相同検索にはRad51/Dmc1が関わることが知られているが、これらタンパク質のDNA上への集合・解離の制御の分子メカニズムについてはほとんど解明されていない。

Stephen Mwaniki氏は、学位の申請研究として、減数分裂期の組換えの仕組みを明らかにするために出芽酵母の２つのRad51/Dmc1、Rad52とMei5に着目して、その機能解析を実施した。Mei5のアミノ酸置換R117AによりMei5が翻訳後修飾として減数分裂期特異的な切断を受けること、その切断に必要な領域（5アミノ酸）を見出し、Mei5機能の負の制御システムの存在を示した。また、オーキシン誘導型デグロン系を用い、条件特異的な分解系により、減数分裂期の段階ごとのRad52の機能の解析を実施した。相同鎖交換反応を担うRad51-１本鎖DNAの集合反応のみならず、Rad51複合体の安定化に必要であることを見出した。この発見は、これまで、Rad51-１本鎖DNA複合体が相同鎖検索反応能の分子実態であること考えられてきた従来の考えとは異なり、Rad51-Rad52-１本鎖DNA複合体がその分子実態であるという新しいモデルの提案に繋がると期待できる。

本研究により、減数分裂期組換えの新しい制御の仕組みを明らかにした点において、学位に値する成果と言える。今後の進展により、当該分野での研究の発展も大きく期待できる。

よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。

博士研究の一部は下記の国際誌の論文の筆頭著者として発表している。

Stephen Mwaniki, Priyanka Swant, Osarentin P. Osemwenkhaee, Yuruka Fujita, Masaru Ito, Asako Furukohori, and Akira Shinohara. Mutational analysis of Mei5, a subunit of Mei5-Sae3 complex, in Dmc1-mediated recombination during yeast meiosis. ***Genes Cells***, 29, 650-666, 2024. doi: 10.111/gtc13138.