Abstract of Thesis

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Title	Role of Mei5-Sae3 complex in Dmc1 assembly in yeast meiosis （酵母減数分裂期のDmc1アセンブリーにおけるMei5-Sae3複合体の機能）

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During meiosis, homologous recombination between maternal and paternal homologous chromosomes takes place. This establishes a physical link between the chromosomes, which is essential for proper segregation of chromosomes in meiotic prophase I. During homologous recombination, after the formation of Double stranded breaks (DSBs), the resected DNA is coated by a single strand binding protein, Replication protein A (RPA). After RPA, two bacterial RecA homologs, Rad51 and Dmc1 are recruited on to the single stranded DNA. Both the homologs form a helical filament on the single stranded DNA. Rad51 plays an important role in homology search and strand exchange between sister chromatids during mitosis. However, during meiosis, Dmc1 mediates the strand exchange and promotes inter-homolog recombination. However, this preference of Dmc1 towards homologous chromosomes over sister chromatids as donor template, called inter-homolog bias, remains to be answered. In budding yeast meiosis-specific proteins, Mei5 and Sae3, are involved in Dmc1 assembly by forming a ternary complex with Dmc1.

Previous studies have shown that, Mei5 and Sae3 deletion mutants show similar defects in meiotic recombination as the Dmc1 deletion mutant. Both the mutants show a defect in Dmc1 foci formation. Also, Mei5-Sae3 and Dmc1 are inter-dependent on each other for foci formation and show co-localization with each other on meiotic chromosomes. Mei5-Sae3 complex is also conserved from yeast to mammals. In this study, I am interested in understanding the molecular mechanism behind Mei5-Sae3 facilitating the Dmc1 assembly on single stranded DNA.

In my thesis, I will introduce the structure of the Mei5-Sae3 complex in *S. cerevisiae*. The crystal structure was obtained by my lab from collaborative research with Dr. Atsushi Nakagawa at the Institute of Protein Research, Osaka university. Based on the structure and the conserved nature of charged amino acids in Mei5-Sae3, I created various Mei5 and Sae3 mutants and characterized the meiotic phenotypes in detail. I found a highly conserved region consisting of 3 amino acids, 95K-96W-97R (positively charged –Lysine, neutral amino acid- Tryptophan and positively charged-Arginine) located in a conserved structure of Mei5-Sae3 complex which is documented in studies of orthologs of Mei5-Sae3.

Additionally, conserved sites in Sae3 were also studied which included a region with possible interaction with

the Mei5 K-W-R region consisting of four amino acids, Y-N-E-L: Tyrosine-56, Asparagine-57, Glutamic acid-58 and Leucine-59. The meiotic phenotypes of the Sae3 point mutants were similar to the Mei5 K-W-R point mutants.

In this study, I discuss how 96W of Mei5, in particular, plays an important role in the function of Mei5-Sae3 complex. Despite being proficient in recruiting Dmc1, Mei5, Rad51 and RPA to ssDNA, *mei5-W96A* shows an arrest in meiosis. This mutant also shows a defect in DSB repair, which is similar to *mei5Δ*. However it is not defective in Mei5-Sae3 complex formation. It also shows interaction with the C-terminal region of Sae3. Using this residue, I will further discuss a new role of Mei5-Sae3 complex in Dmc1-mediated homologous recombination.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Role of Mei5-Sae3 complex in Dmc1 assembly in yeast meiosis
酵母減数分裂期の Dmc1 アセンブリーにおける Mei5-Sae3 複合体の機能

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ることが知られているが、そ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。

Sawant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Mei5-Sae3複合体に着目して、その機能解析を実施した。相同染色体間の相同検索を担うDmc1フィラメント形成に必須のMei5-Sae3複合体の構成要素であるMei5, Sae3に複数のアミノ酸置換を導入して解析した結果、Mei5, Sae3で保存されている残基、Mei5-KWR, Sae3-YNEがMei5-Sae3複合体機能、つまりDmc1の集合反応に必要であり、さらにDmc1フィラメント形成後の機能にも重要な役割を持つことを見出した。その成果をもとに、Mei5-Sae3複合体によるDmc1フィラメント形成の新規モデルを提唱することが出来た。

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共筆頭著者として発表している。
Sawant P., Wagui S.M., Fujita Y., Ito, M., Furukohori A., and Shinohara A, The role of conserved amino acid residues of Sae3 in Mei5-Sae3 complex for Dmc1 assembly in meiotic recombination. Genes & Genetic Systems, in press