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Abstract of Thesis

Name （Hana Subhan Memon Sakurai ）	
Title	A New Role of Srs2 DNA Helicase, anti-recombinase, during Meiosis (減数分裂における DNA ヘリケース、Srs2 の新しい機能)

Abstract of Thesis

Homologous recombination (HR) is involved in the repair of DNA double-strand breaks (DSBs) in both mitosis and meiosis. HR between homologous chromosomes provides a physical linkage between the chromosomes, which ensures correct segregation of the chromosomes during meiosis I (MI). In HR, Rad51, a RecA homolog, plays a key role in homology search and strand exchange by forming a helical filament on the single- stranded DNA (ssDNA). Rad51 is required for HR in both mitosis and meiosis. In addition to Rad51, a meiosis-specific RecA homolog, Dmc1, is also essential for meiotic recombination. In meiotic recombination, Dmc1 is the main strand-exchange protein for recombination between homologous chromosomes, while Rad51 assists Dmc1-assembly. Assembly and disassembly of Rad51 and Dmc1 filaments are highly dynamic and strictly controlled by several positive and negative factors. In mitosis, Srs2 DNA helicase functions as a negative regulator for HR and indeed has been shown to dislodge Rad51 filaments, thereby inhibiting Rad51-dependent strand invasion. Srs2 is also critical for meiotic recombination. However, its role in HR in meiosis remains to be elusive.

In this study, in order to analyze a role for Srs2 during meiosis, the effect of *SRS2* deletion in S. *cerevisiae* meiosis has been studied in more detail. Previous works have shown that the *srs2* deletion mutant shows reduced sporulation, lowered spore viability, and slight decrease in recombination products. However, the decrease in recombination products does not fully explain the observed low spore viability of the mutant. While the mutant shows wild-type like assembly and disassembly of Rad51 and Dmc1 at early stages of meiosis, it later exhibits a unique defect in the assembly of Rad51: the formation of a large aggregate containing Rad51, but not Dmc1. In this thesis, it was shown that, in the absence of Srs2, during meiotic DSB repair in early prophase, abnormal loading of Rad51 that could cause multiple-invasion. This early defect in Rad51 loading could be the precursor for the formation of Rad51 aggregates observed during late meiosis in the *srs2* mutant. Additionally, the depletion of Srs2 after mid-prophase I exit was sufficient to induce the formation of the Rad51 aggregates, suggesting that Srs2 plays a novel role in late prophase I.

In conclusion, Srs2 regulates the formation of Rad51 filaments separately during the meiotic DSB repair event and additionally, at the resolution step of recombination intermediates, most likely by it's translocase activity. In this thesis, the role of Srs2 in coupling the completion of recombination with consecutive chromosomal events will be further discussed.

PhD application Publication

様式 7

論文審査の結果の要旨及び担当者

氏　名	（ Hana Subhan Memon Sakurai ）	
	（職）	氏　名
論文審査担当者	主 査　教授	篠原　彰
	副 査　教授	平岡　泰
	副 査　教授	小布施　力史
	副 査　准教授	中川　拓郎

論文審査の結果の要旨

A New Role of Srs2 DNA helicase, anti-recombinase, during meiosis

減数分裂における DNA ヘリケース、Srs2 の新しい機能

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。その仕組みの解明は配偶子形成時のゲノムの安定化の分子メカニズムの解明はこれまでに知られていない組換えの機能を明らかにすることが出来るばかりでなく、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。相同組換えの中でも DNA 相同検索反応は鍵となる反応であり、この反応に関わる Rad51 タンパク質からなる構造体形成はさまざまな制御を受けることが知られている。本申請研究は減数分裂期における組換えの制御因子の１つ Srs2 ヘリケースの機能、特に Rad51 構造体形成の制御機能、に関する分子遺伝学的解析研究である。

本申請研究により、Srs2 ヘリケースが出芽酵母の減数分裂期に時間的に２段階で働くことを示すことが出来た。第一の機能は減数分裂期の DNA 相同検索反応時に、不都合な Rad51 構造体を破壊する活性であり、これはすでに試験管内で示されている Srs2 の Rad51 構造体除去機能と一致する。もう１つは減数分裂期の後期において、巨大な Rad51 の構造体形成を抑制する機能であり、Srs2 の今まで知られていない機能として、新規の発見と言える。特に遺伝的なトリックを使い、厳密な段階的 Srs2 の分解系を構築することで、Srs2 の後期機能を明らかに出来たことは特筆に値する。

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。

Sasanuma, H., Sabhan, H.M.S., Furihata, Y., Challa, K., Palmer, L. Gasser, S.M., Shinohara, M., and A. Shinohara, Srs2 helicase prevents the formation of aberrant DNA damage during late prophase I of yeast meiosis. ***Chromosoma***, 128, 453-471. 2019. doi: 10.1007/s00412-019-00709-5.

本申請研究により、減数分裂期の染色体構造についての新しい制御の仕組みを分子レベルで明らかにできた。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成果と言える。

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。