Abstract of Thesis

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Title	Importance of centromere chromatin in regulation of homologous recombination in fission yeast （分裂酵母の相同組換え制御におけるセントロメアクロマチンの重要性）

Abstract

Eukaryotic centromeres viewed as a constriction on mitotic chromosomes are indispensable for faithful segregation of chromosomes. Errors in centromere function result in aneuploidy that may lead to genetic diseases, and cancer. A group of proteins including histone H3 variant CENP-A and histone fold containing CENP-TWSX, both of which are essential for proper microtubule attachment during mitosis, specifically localize to the centromere forming unique chromatin structure called kinetochore. This chromatin is flanked by the pericentric heterochromatin that is marked by the methylation of histone H3 on 9th lysine by the Clr4/Suv39 methyltransferase. It provides a platform for Swi6/HP1 that stabilizes cohesin proteins that is important for sister chromatid attachment and bi-polar attachment of kinetochore to microtubules. Another conserved feature of the centromere is presence of repeats sequences that are prone to rearrangement. Interestingly, gross chromosomal rearrangement (GCR) mediated by the centromere repeats is increased by a deletion of *rad51* in fission yeast, showing that homologous recombination (HR) is important for maintaining the structural integrity of centromere. However, the precise regulatory mechanism of recombination in centromeres remains elusive.

To gain insight of recombination in centromere, I determined the spontaneous recombination that occurs between the *ade6B/ade6X* heteroalleles integrated at the inverted repeats of centromere 1 (*cen1*) and compared it with a non-centromeric *ura4* locus in fission yeast, *Schizosaccharomyces pombe*. In the centromere, Rad51-dependent HR that requires Rad51, Rad54, and Rad52 was predominant, whereas Rad51-independent HR that requires Rad52 also occurred in the non-centromeric region. Moreover, crossovers (CO) between inverted repeats were suppressed in the centromere as compared to the non-centromeric region. Thus, the mechanism of HR is differently regulated in centromere from that of non-centromeric *ura4* locus. Remarkably, the choice of recombination pathway is important to maintain integrity

of centromeres. To see if the centromere chromatin is responsible for the specific regulation of recombination, I examined the effect of several factors of heterochromatin and kinetochore on recombination. I found that the deletion of *clr4*, *swi6* and a temperature sensitive mutation in *rad21* subunit of cohesin increased the spontaneous rate of recombination. However, *clr4* deletion did not increase the proportion of COs. These results suggest that the heterochromatin affects the initial events of recombination but does not play a role in the formation of recombination products. A mutation in CENP-A and several other kinetochore factors did not change the proportion of COs in centromere, suggesting that they do not play a role in regulation of HR in centromere. CENP-S, CENP-X histone-fold proteins, form CENP-TWSX and also CENP-SXSX complex, which can bind Fml1/FANCM helicase that is involved in DNA repair. Mhf1/CENP-S, Mhf2/CENP-X and Fml1 were required to suppress COs. Interestingly, a mutation in Mhf1, *mhf1-LR* that disrupts the tetramer complex is mildly sensitive to genotoxins such as MMS, CPT and HU unlike *mhf1*Δ and *fml1*Δ. However, *mhf1-LR* suppressed COs and GCRs in the centromere, similar to *fml1*Δ. Thus, it is likely that MHF tetramers are particularly important in the CO suppression in centromere. When replication forks stall in centromere, the unique chromatin may prevent excessive branch migration of joint molecules that can lead to COs. Instead, MHF tetramer in concert with Fml1 bind such branched DNA structures and dissociate the joint molecules to drive synthesis-dependent strand annealing (SDSA) pathway of HR that always results in noncrossovers (NCOs). These data for the first time uncovered the regulation of mitotic recombination between DNA repeats in centromeres and its physiological role in maintaining genome integrity.

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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

申請者 Zafar 氏は、染色体不安定化要素である反復配列により構成されるセントロメア領域での DNA 相同組換え制御機構に着目し、クロマチン構成因子の寄与を、モデル生物である分裂酵母を用いて研究した。セントロメア反復配列中に挿入した ade6 ヘテロアリル間の組換えを測定する解析系を用いて、セントロメアでは、染色体腕部 ura4 座位に比べて Rad51-Rad54 に依存する相同組換えが主要であり、また遺伝子再編をもたらす交叉型組換えが強く抑制されることを発見した。さらにセントロメア機能に関与する種々のクロマチンタンパク質の寄与を解析した結果、セントロメアではヌクレオソーム様複合体 CENP-TWSX 複合体の構成要素である CENP-SX と、CENP-SX と相互作用する FANCM/Fml1 ヘリカーゼが交叉型組換えの抑制に重要であることを発見した。同氏の研究成果は、反復配列における染色体異常の発生を抑制する制御機構の解明に重要な進展をもたらすものである。

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。