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Abstract of Thesis

Name (Santosh Kumar Gothwal)	
Title	Regulation of the meiotic double-strand break formation by Paf1 transcriptional elongation complex through histone H3K4-methylation dependent and independent mechanisms in *Saccharomyces cerevisiae* （出芽酵母Paf1転写伸長複合体によるヒストンH3K4メチル化依存的あるいは非依存的な減数分裂期二重鎖切断制御機構の研究）

Abstract of Thesis

Meiotic double strand break (DSB)-formation is an essential process for meiotic recombination that plays critical roles in maintenance of genetic diversity, fertility and genome evolution in sexually reproducing organisms. Histone modifications, especially Set1-dependent H3K4me3 plays a critical role in determining the locations and frequency of DSBs, at the preferred site (DSB hot spots) on the genome in budding yeast. However, in the absence of H3K4me3, another histone modification, Dot1-dependent H3K79me3 contributes for the DSBs. Meiotic chromosomes are composed of multiple loop-axis arrays. DSB sites are located on loop, while DSB factors are located on axis. Therefore, the interaction between loop and axis regions is required for efficient DSBs formation. It has been shown that Spp1/Cps40 that localizes on axis of meiotic chromosomes and physically binds to DSB factor Mer2, recognizes the H3K4me3 in the loop regions and tethers the loops towards axis to promote DSB formation. However, mutants such as *set1* or *spp1* do not abolish DSB formation completely and the DSBs in these mutants are formed on wild type hotspots, suggesting the presence of H3K4me3-independent mechanisms to regulate DSB formation. Identification of factors involved in H3K4me3-independent DSB formation is important to understand the mechanisms that determine the genome-wide landscape of meiotic recombination. Recently the influence of transcription was shown on DSB formation and distribution by controlling the assembly of axial elements, Hop1 and Red1 at the 3' convergent transcriptional sites. Given the role of Paf1C in polyadenylation of nascent mRNA at 3' ends of genes, it might be possible that Paf1C promote DSB formation and distribution in an independent manner from H3K4me3 mark.

The Paf1C is composed of five proteins in budding yeast, i.e. Rtf1, Cdc73, Leo1, Ctr9 and Paf1. Since *paf1* and *ctr9* are defective in vegetative growth, I analyzed the histone modification status and the meiotic phenotypes of *rtf1, cdc73* and *leo1* mutants. In *rtf1* mutant, both H3K4me3 and H3K79me3 were severely decreased. In contrast, *cdc73* caused significant reduction in H3K4me3, whereas did not affect H3K79me3. In *leo1* mutant, neither H3K4me3 nor H3K79me3 was altered. Consistent with the effects on H3K4me3 status, the *rtf1* caused reduction in spore viability and *cdc73* showed less but significant decrease, while *leo1* generated spore viability similar to the wild type. In addition, double mutants, *rtf1 set1* caused further decrease in the spore viability compared with single mutants, *rtf1* and *set1*, suggesting different role of Rtf1 from Set1 in meiosis.

In the study of the roles of Rtf1 and Cdc73 in DSB formation, I found that *rtf1* and *cdc73* mutants showed reduced DSB frequencies at several hotspots, indicating they are required for efficient DSB formation. Interestingly, *rtf1 set1* double mutant showed larger decrease in DSB formation than *rtf1* and *set1* single mutants, suggesting a different role of Rtf1 from Set1 in DSB formation. Importantly, chromosome-wide DSB-mapping in *rtf1* mutant showed very different distributions of DSBs from those observed in wild type and *set1* mutant. DSBs were reduced at some hotspots, whereas DSBs were promoted at some DSB-cold regions. Furthermore, DSB distributions in *rtf1 set1 dot1* mutant were different from those in *set1 dot1* double mutant, suggesting a role of Rtf1 in DSB regulation independent from those of Set1-dependent H3K4me3 and Dot1-dependent H3K79me3. Furthermore, I found that some but not all the Rtf1-enhanced DSBs are independent from Spp1, which is required for loop-axis tethering of H3K4me3, suggesting different role of Rtf1 independent from Spp1.

Cytological localization of Mre11, a component of MRX complex required for DSB formation on meiotic chromosomes, showed Mre11-focus formation was reduced by *rtf1* as well as by *set1* in the *rad50S* background. Interestingly, *rtf1 set1* double mutant caused even severer reduction in Mre11-focus formation than *rtf1* or *set1*, indicating that Rtf1 and Set1 independently contribute to the Mre11 localization. This is consistent with the notion that Rtf1 and Set1 have different roles in meiotic DSB formation.

In this study, I demonstrated that Rtf1, a component of Paf1C complex, is required for efficient DSB formation in meiosis, possibly through Set1-dependent H3K4me3 modification. Moreover, I discovered a novel role of Rtf1, which is independent from Set1 and Dot1, in DSB formation. Since *de novo* DSBs were formed at DSB-cold spot regions in *rtf1* mutant, Rtf1 has a role to repress DSBs at these sites. These findings suggest Paf1C transcriptional elongation complex contributes regulation of the genome-wide DSB landscape independently from H3K4 and H3K79 methylations.

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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

真核生物は減数分裂により次世代を担う配偶子をつくる。減数分裂において父方由来・母方由来の遺伝子を混ぜ合わせ、遺伝子の多様性を生みだす相同 DNA 組換えは必須反応である。減数分裂期相同組換えは、DNA 二重鎖切断（Double strand break; DSB）によって開始され、染色体 DNA 上の DSB 導入部位や頻度は巧妙に制御されている。これまで、染色体ヌクレオソームを構成するヒストンの修飾が DSB 形成に重要であることが報告されていたが、申請者は既知のヒストン修飾に依存しない DSB 形成制御に関わる因子を発見した。

高頻度に DSB が導入される DSB-hotspot 領域では Set1 ヒストンメチル転移酵素による H3 の 4 番目リシン（H3K4）のトリメチル化修飾(H3K4me3)が重要であり、さらに Set1 欠損株では、補完的経路として Dot1 による H3K79 メチル化(H3K79me3)が DSB 形成に寄与することが知られていた。しかしながら、上記の両メチル化が起きない条件でも低頻度で DSB が導入されるため補完的しくみの存在が想定された。申請者は、転写中の RNA ポリメラーゼと相互作用する Paf1C タンパク質複合体が、上記のメチル化修飾を誘導する経路にあり、さらにクロマチン構造にも影響することから、Paf1C の DSB 形成への寄与を、出芽酵母の遺伝子破壊変異株を用いて解析した。その結果、Paf1C 構成因子 Rtf1 は、H3K4me3 修飾に依存する DSB 形成と依存しない DSB 形成の両者に重要であることを見いだした。さらに興味深いことに、Rtf1 欠損条件では、通常は DSB が形成されない DSB cold-spot 領域で DSB が誘導されることを見いだした。これらの結果は、Rtf1 は cold-spot 領域で DSB 形成を抑制する機能を持つことを示し、Rtf1 による新規の DSB 形成制御機構の存在を示唆する。

これらの研究結果を、学術論文 "The double-strand break landscape of meiotic chromosomes is shaped by the Paf1 transcription elongation complex in *Saccharomyces cerevisiae*"として Genetics 誌(2016)に発表し、学位論文「出芽酵母 Paf1 転写伸長複合体によるヒストン H3K4 メチル化依存的あるいは非依存的な減数分裂期二重鎖切断制御機構の研究」にまとめた。よって、本論文は博士（理学）の学位論文として十分に価値があるものと認める。