氏　　　　　名　　田中太門

学位（専攻分野）　　博士（理学）

学 位 記 番 号　　総研大甲第 1160 号

学位授与の日付　　平成２０年３月１９日

学位授与の要件　　生命科学研究科　遺伝学専攻
　　　　　　　　　　学位規則第６条第１項該当

学 位 論 文 題 目　　Proper　initiation　of　chromosomal　DNA　replication
　　　　　　　　　　requires the Sld3-Sld7 complex in budding yeast

論 文 審 査 委 員　　主　　査　　教授　　　　　　仁木　宏典
　　　　　　　　　　　　　　　　教授　　　　　　佐々木　裕之
　　　　　　　　　　　　　　　　准教授　　　　　深川　竜郎
　　　　　　　　　　　　　　　　助教　　　　　　筒井　康博
　　　　　　　　　　　　　　　　教授　　　　　　升方　久夫(大阪大学)

Eukaryotic chromosome has to be duplicated once and only once per cell cycle for maintaining the genetic information from one generation to the next. Chromosomal DNA replication initiates from replication origins. In budding yeast, replication origins are defined by specific DNA sequence motifs, which are called autonomously replicating sequence (ARS), and protein complexes. The stepwise assembly of the initiator proteins on the origins provides multiple points of control to ensure efficiency and fidelity of DNA replication. However, the mechanisms of protein assembly and origin activation remain elusive. The studies about Dpb11 and Sld proteins in budding yeast also provide the key to unravel problems in replication field. Dpb11 in budding yeast is an essential protein for the initiation of DNA replication. It was originally identified as a DNA polymerase-interacting factor. A series of the genes that named SLD were isolated as genes whose mutations are synthetically lethal with dpb11-1, the temperature sensitive mutation of DPB11. SLD1-6 genes encode Sld1-6 proteins, respectively. Sld1 (Dpb3) is one of the subunits of DNA polymerase ε. Sld4 is identical to Cdc45, essential for the initiation and progression of DNA replication. Sld6, also known as Rad53, is required for replication checkpoint. Sld2, Sld3, and Sld5 were unknown factors. These factors are essential for the initiation of DNA replication, and precise functions of individual factors in DNA replication had been elucidated. Another sld screening using dpb11-24 mutation identified the mutation in unknown gene, and it was named SLD7 (Synthetic Lethality with Dpb11-24 7). It encodes 29 KDa protein, which consists of 257 amino acids. However, this protein does not share any homology with other proteins in higher eukaryotes even in fission yeast. In addition, no functional motifs in this protein have been found so far. In this study, I examined the role of Sld7 protein in DNA replication.

The SLD7-deleted mutant (sld7Δ) was reported to be inviable in genome-wide study. In this work, however, the SLD7 gene is appeared not to be essential for cell growth, but rather required for efficient chromosomal DNA replication because sld7Δ mutant cells are viable and shows long S-phase in comparison with WT cells in flow cytometry analysis. From crude extract, the Sld7 protein is co-precipitated with the 80 kDa protein. According to the peptide-sequence determined by mass spectrometry, this protein is identical to the Sld3 protein. Furthermore, purified Sld7 and Sld3 proteins form a complex, indicating that the Sld7 protein directly binds to the Sld3 protein. This complex formation requires the N-terminal portion of Sld3 protein.

Almost all the Sld3 protein forms a complex with Sld7 protein throughout the cell cycle. It is conceivable that although Sld3 protein alone can function with reduced activity, Sld7 protein enhances the activity of Sld3 protein. As expected, SLD3 gene on multi-copy plasmid suppresses the phenotypes of sld7Δ mutant cells, but not other SLD genes. Additionally, multi-copy SLD7 gene suppresses the phenotypes of sld3-6

mutant that has a defect in the binding of Sld3 protein to Sld7 protein. Moreover, simultaneous over-expression of SLD3 and SLD7 genes in WT cells leads to severe growth defect of the cells than the over-expression of SLD3 gene alone. These results suggest that the Sld7 protein enhances the activity of Sld3 protein probably through their complex formation. Previous study showed that the increased dosage of CDC45 gene suppresses the phenotypes of sld3-4, -5, -7, and -8 mutants, but not sld3-6. In this study, over-expression of SLD7 gene from galactose-induced promoter suppresses the phenotype of sld3-6 mutant as well as SLD7 gene on multi-copy plasmid. On the other hand, over-expression of SLD7 gene aggravates the cell growth of other sld3 mutants, even at semi-permissive temperature of these mutants. Since the binding domains for Cdc45 and Sld7 proteins in Sld3 protein overlap, these aggravations seem to be caused by the competition of the Sld7 and Cdc45 proteins for binding to the Sld3 protein.

The Sld3 protein associates with early-firing origins at G1-phase. Chromatin immunoprecipitation (ChIP) assay revealed that the Sld7 protein, like the Sld3 protein, associates with replication origins at G1-phase, then gradually dissociates from the origins, and this protein does not move with replication forks. In the absence of Sld7 protein, Sld3 protein still associates with the origins. Thus, it is supposed that the association of Sld3 protein with replication origins mostly independent from the Sld7 protein, and the Sld7 protein regulates the function of Sld3 protein other than Sld3-binding to replication origins. Since the Sld3 protein binds to all the components of the Cdc45-MCM-GING (CMG) complex, and Sld7 protein enhances the activity of the Sld3 protein, it is conceivable that loss of the Sld7 protein affects the chromatin association of the components of the CMG complex. The CMG complex is thought as an active helicase at replication forks and their components are known to associate first with replication origins and more with moving replication forks. In ChIP assay, Mcm7 protein, a subunit of MCM complex, associates with the origins at G1-phase and early S-phase and then with neighboring region of the origins both in the presence and absence of Sld7 protein. However, its association signals with neighboring regions but not with origins in the absence of Sld7 protein are significantly reduced in comparison with in the presence of Sld7 protein. These results suggest that Sld7 protein helps the efficient moving of Mcm7 protein, a subunit of MCM complex, with replication forks, but not its association with the origins.

Previously, genome-wide study showed that Sld7-GFP protein localizes to nuclei and spindle poles, and mutation in SLD7 gene results in aberrant mitochondria morphology. These results imply a role of the Sld7 protein other than DNA replication in nucleus. In this study, sld7Δ mutant cells are shown sensitive to nocodazole and thiabendazole, inhibitors for polymerization of microtubules, and multi-copy SLD3 gene does not restore these sensitivities to wild-type level. These results imply that the loss of Sld7 protein leads to the defect in some other processes rather than DNA replication, such as cell division, and further suggest that Sld7 protein does not work

with Sld3 protein at all times for these functions.

In conclusion, the Sld7 protein plays roles in several cellular events, and especially for efficient chromosomal DNA replication, it forms a complex with Sld3 protein, associates with replication origins, and then facilitates the dissociation of replication machinery from origins.

真核生物における染色体 DNA の複製は、細胞周期を通じ、S 期に一度だけ起こる。これまでの研究から複製開始時には、複製開始点上に結合した複製装置前駆体から、Cdc45 タンパク質を含む複製装置が離れ DNA 上を移動できるようになり、これに続き DNA 鎖の合成が起こるということが明らかになっている。そして、複製開始を制御しているタンパク質は、複製開始領域の上の複製装置前駆体の形成を制御することで、過剰な染色体 DNA の複製を抑えている。田中太門さんは、この複製開始制御する分子機構の全貌を明らかにすることを目的に研究を進め、出芽酵母の新規のタンパク質因子 Sld7 が、Sld3 タンパク質と複合体を形成し、複製装置前駆体と開始領域の結合あるいは複製装置の解離を制御していることを本研究で解明した。

　Dpb11 タンパク質は DNA 合成酵素と相互作用し、増殖に必須の因子である。増殖可能な *dpb11* 変異株にさらに新たに突然変異を導入し、その結果増殖不能になるような突然変異遺伝子の分離、いわゆる合成致死遺伝子スクリーニングが行われた。そして、*sld* と名付けられた一連の遺伝子変異が見つかっている。Sld タンパク質は、DNA 複製と関連することが予想されるが、その一つである Sld7 タンパク質の DNA 複製に関する機能は長らく不明であった。そこで、本研究ではその機能の解明に取り組んだ。Sld7 の欠損では DNA 複製期の遅延が生じ、細胞増殖速度が低下していることから、Sld7 タンパク質が複製開始の制御に重要な役割をしていることがまず確認された。次に酵母細胞抽出液から Sld7 タンパク質と共沈殿する因子を分離し、質量分析からこれが Sld3 タンパク質であることを見いだした。Sld3 タンパク質はリン酸化による活性制御を受け、複製装置前駆体の形成を調整する重要な役割を担っていることがすでに知られている。さらに、田中さんは精製タンパクを用いて、Sld7 タンパク質が Sld3 タンパク質に直接結合し、複合体を形成することを確かめた。また、酵母ツーハイブリッド法により、結合に必須な領域を明らかにした。この複合体は細胞周期の全体を通じて形成され、細胞内ではほぼすべての Sld3 タンパク質が Sld7 タンパク質との複合体として存在していた。他方、遺伝学的な実験結果からも、Sld7 タンパク質と Sld3 タンパク質の相互作用を明らかにした。すなわち、それぞれの変異を、それぞれのタンパク質の過剰生産で相補することができた。また、この結果は Sld7 タンパク質が結合することにより Sld3 の活性を上げていることを示していた。また一方で、Sld7 タンパク質の過剰生産が特定の *sld3* 変異体において細胞増殖速度の低下をもたらすことを見いだした。Sld3 タンパク質上の Sld7 タンパク質の結合部位が Cdc45 タンパク質の結合部位と一部重なることから、Sld7 タンパク質は Sld3 タンパク質への Cdc45 タンパク質の結合を制御しているのではないかと考えるに至った。クロマチン免疫沈降法により、複製開始領域とその周辺領域での複製装置構成因子の挙動を測定したところ、複製開始領域への結合は Sld7 タンパク質の欠損下でも特に変化はないものの、周辺領域ではその結合が著しく低下していた。これは、複製開始頻度の減少ないしは複製装置が開始領域から離れ DNA 上を移動する過程に異常が生じていること意味する。この原因の一つとして、Cdc45 タンパク質による複製開始の制御の不能が挙げられる。これは、Sld7 タンパク質が Sld3 タンパク質と Cdc45 タンパク質の結合を制御しているという上記のモデルを支持するものである。

本研究は複製の開始制御の新たな因子を見いだし、Sld3-Sld７タンパク質複合体の分子機能を明らかにし、これまでの複製開始を制御の分子機構モデルを拡張して示した点で画期的である。この知見がきっかけとなり、複製開始を制御の分子機構に関する研究はさらにその理解が深まるものと期待される。以上の理由で田中太門さんの学位提出論文は博士号授与の要件を満たすと審査員全員一致で判断した。