学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study on factors related to DNA replication in cell cycle
(細胞周期における DNA 複製に関連する因子の研究)

The cell cycle is a series of events, including interphase (G1 phase, S phase and G2 phase) and cell division (M phase). The cell cycle begins with cell growth in the G1 phase, followed by DNA replication and organelles duplication in the S phase. DNA replication is performed as the double helix is separated into two single strands that serve as templates for the synthesis of new complementary strands, resulting in the product of two identical DNA molecules (semi-conservative replication). The precise process of DNA replication ensures the accurate transmission of genetic information to daughter cells which is essential for maintaining cellular integrity and function. Then, after chromosome formation in the G2 phase, the cytoplasm is divided into two daughter cells in the M phase. There are two principal types of cell division in eukaryotes: mitosis, producing daughter cells genetically identical to the parent cell; meiosis, reducing the number of chromosomes from two of each type in the diploid parent cell to one of each type in the daughter cells. Meiosis is essential for sexual reproduction as it produces gametes with half the number of chromosomes, allowing genetic diversity and the formation of new traits in offspring.

Our study focused on DNA replication initiation in the S phase and telomere bouquet formation in meiosis.

Study on Cdc45 recruitment via Sld7-Sld3 for DNA helicase CMG complex formation at DNA replication initiation

Eukaryotic chromosomal DNA replication in the cell cycle begins with the unwinding of dsDNA into ssDNA, including an active helicase Cdc45–MCM–GINS (CMG) complex formation. First, in CMG formation, two inactive helicase MCM hexamer rings are loaded onto autonomously replicating sequences (ARS) on dsDNA and form a double hexamer (MCM DH) at the replication origin. After MCM DH on dsDNA is phosphorylated by Dbf4-dependent protein kinase (DDK), the initial factor Sld3 and its partner Sld7 recruit Cdc45 (Sld7-Sld3-Cdc45) to each MCM. Subsequently, the phosphorylation of Sld3 by cyclin-dependent kinase (CDK) regulates the assembly of GINS onto MCM DH with Dpb11, CDK-phosphorylated Sld2, and DNA polymerase ε to form the active DNA helicase CMG dimer. Finally, the factors Sld2, Sld3, Sld7 and Dpb11 are released from the active CMG by elusive mechanisms, and then facilitate bidirectional replication by translocating along the 3' to 5' direction of DNA. Although the structural and functional studies of the Sld7CTD dimer, Sld7NTD–Sld3NTD, Sld3CBD, MCM DH, and CMG have been performed

previously, how Sld3–Sld7 brings Cdc45 into the MCM for CMG formation to regulate the initiation of DNA replication remains unclear.

In this study, we determined a structure of the Sld3 Cdc45-binding domain (Sld3CBD) in complex with Cdc45 (Sld3CBD–Cdc45), revealing detailed interaction between Sld3CBD and Cdc45. A helix of Sld3CBD was formed upon binding to Cdc45 and also conformational changes of the Cdc45 DHHA1 domain were found when binding to Sld3CBD together. The mutant analysis indicated that the binding between Sld3CBD and Cdc45 could be broken easily, as disrupting only one binding site among interactions induced the separation of Sld3CBD and Cdc45. Together with the previous studies of Cdc45, our Sld3CBD-Cdc45 structure revealed that Sld3CBD binding sites on Cdc45 are spatially distinct from that of GINS and MCM bonding on Cdc45, indicating that the Sld3CBD, Cdc45, GINS could bind to MCM together, confirmed by modelling of Sld3CBD-CMG (SCMG). Therefore, we proposed that after recruiting Cdc45, Sld7–Sld3 could remain in Cdc45–MCM until GINS recruitment to form CMG. Furthermore, the consistency between the particle size of Sld7–Sld3–Cdc45 obtained by DLS and the distance of Sld3CBDs in the model of Sld3CBD-Cdc45–MCM dimer, indicated the binding manner of the Cdc45–Sld3–$[Sld7]_2$–Sld3–Cdc45 off/on MCM DH. DNA-binding assay of Sld3 and its complexes with single-stranded ARS1 fragments (ssDNA) show a reduction in ssDNA binding affinity of Sld3CBD-Cdc45 compared to Sld3CBD-ssDNA, but recovery when complexes retain Sld7. This serial binding assay implied a relationship between the dissociation of Sld7–Sld3 from CMG and the unwound single-stranded DNA. Overall, our findings contribute to a deeper understanding of the molecular basis of CMG complex formation regulated by Sld3.

Study on the interaction of proteins involved in telomere bouquet formation in meiosis

During meiosis, chromosomes are replicated, recombined and distributed to daughter cells through meiosis I, and II stages. For the distribution process to proceed correctly, the meiotic cell pairs its chromosomes during meiosis I. This paring requires telomere cluster formation at the nuclear membrane called “telomere bouquet”. In *Saccharomyces cerevisiae*, telomeres are anchored to the nuclear envelope by interaction with SUN protein Mps3 which is located on the nuclear envelope and can be moved with the telomere to form the telomere bouquet. Recently, ribosome assembly factors Ebp2 and Rrs1 were found to work as essential initiators to start the movement of telomere by binding to the N-terminal of Mps3(Mps3NTD).

In this study, we performed a series of binding assays and found that Ebp2 binds to Mps3NTD via Rrs1 and Rrs1-partner ribosome assembly factor Rpf2. Together with mass photometry and SEC-SAXS results, we proposed an important bridging role of Rrs1 in Ebp2-Rrs1-Mps3 binding. This will deepen our understanding of the telomere bouquet formation mechanism.