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論文内容の要旨

In Sacchromyces cerevisiae, it is well known that bidirectional replication initiates at sequence specific locus on chromosome, called the autonomously replicating sequences (ARSs) (Campbell and Newlon, 1991). The initiation of DNA replication starts from formation of the origin recognition complex (ORC), which contains six subunits and binds directly to the origins of replication. Then the ORC complex recruits two binding factors critical for the activation (Kelly and Brown, 2000), Cdc6 and Cdt1, which allow assembly of the mini-chromosome maintenance (MCM) complex onto the origins, completing assembly of the prereplicative complex (pre-RC) (Zou and Stillman, 2000; Jares and Blow, 2000;Walter and Newport, 2000). At the G1/S transition, the pre-RC allows replication initiation by action of two protein kinases, S-phase cyclin-dependent kinase (S-Cdk; Cdc28) and Cdc7. Then Cdc45-Sld3, Dpb11-Sld2 and GINS complex associate with origins, dependently on the pre-RC (Takayama et al., 2003; Kubota et al., 2003), and DNA polymerases(α, δ and ε) are recruited to origins to initiate DNA synthesis.

An essential protein for DNA replication, Cdc45, is known to be involved in initiation at origins and elongation of replication forks. A two-dimensional gel electrophoresis experiment has shown that a mutant cdc45-1, have a defect in the initiation step (Zou et al., 1997), while another mutant, heat-inducible degron cdc45-td cells, showed that Cdc45 plays an essential role in elongation as they cannot finish chromosome replication at the restrictive condition after early origin activation (Tercero et al., 2000). These observations suggest that Cdc45 could be a good candidate to be studied for elucidating how origin activation occurs and how elongation would be accomplished during DNA synthesis.

In S.cerevisiae, Dpb11 has been shown to play a pivotal role in loading DNA polymerases onto replication origins. The inability of DPB11 mutants to restrain mitosis in the presence of incomplete replication suggests that Dpb11 is also needed for the replication checkpoint (Araki et al., 1995; Kamimura et al., 1998; Wang and Elledge, 1999). Dpb11 has four copies of the BRCT (Brca1 C- terminal) domain (Bork et al., 1997; Callebaut and Mornon, 1997; Zhang et al., 1998), and forms a complex with Pol ε and Sld2 (Masumoto et al., 2000; Kamimura et al., 1998; Wang and Elledge, 1999). BRCT domains are autonomously folding modules consisting of about 100 amino acids that were first recognized as a repeat in the C-terminus of the Breast Cancer Susceptibility gene 1 (BRCA1). BRCT domains are shown to bind proteins that have been phosphorylated on either serine or threonine residues (Manke et al., 2003; Yu et al., 2003). Therefore, elucidation of the molecular mechanism in the interaction between BRCT domain and its physiological targets might be helpful to elucidate the cell cycle control including S phase regulation.

The Sld2-Dpb11 complex is formed in vivo and is essential for chromosomal

DNA replication (Kamimura et al., 1998). Sld2 has six closed matches to the preferred Cdk motif, S/T-P-X-K/R, S/T-P-K/R and K/R-S/T-P (X= any amino acid), and additional five S/T-P sites, which are clustered in 200-aa stretch (Pearson and Kemp, 1991). Masumoto et al showed that Sld2 is phosphorylated by S-Cdk and that this phosphorylation is necessary for the formaion of the Sld2-Dpb11 complex. Remarkably, the mutant allele of SLD2 that replaces all the serine or threonine residues in the preferred Cdk phosphorylation motif by alanine residues is inviable and is also defective in formation of Sld2-Dpb11 complex (2002). However, it is not well understood how the complex is formed between Sld2 and Dpb11.

To understand the initiation of replication regulated by Cdks, Ms. Tak has studied two events in early S phase: stable association of Cdc45 with chromatin and association of Dpb11-Sld2 complex with origins. Although the association of Cdc45 with the pre-RC has been observed in even G1 phase by chromatin immuno-precipitation (ChIP) assay, its stable association to chromatin occurs only after activation of S-Cdks in G1/S transition (Aparicio et al., 1999; Zou and Stillman, 2000; Kamimura et al., 2001). In Xenopus egg extract loading of Cdc45 onto chromatin in S phase is the last known step before origin unwinding and the commencement of DNA synthesis (Mimura et al., 2000). These results suggest that Cdc45 is limiting factor for the initiation of replication. While Dpb11 does not associate with origin without Cdk activity , Xenopus Cut5/Mus101 binds to chromatin in advance of Cdc45, rather than in a mutually dependent manner, but is needed for Cdc45 and polymerase α association steps (Hashimoto and Takisawa, 2003; Van Hatten et al., 2002).

In this study, Ms. Tak isolated series of thermosensitive allele for CDC45 defective in the initiation of DNA replication. In thermosensitive alleles for SLD2 and DPB11, drc1-1 and dpb11-26, respectively, the chromatin binding assay revealed that the chromatinbound Cdc45 is significantly reduced even after Cdk activation. A mutant allele, cdc45-26, isolated in this study, abolishes association of Sld2-Dpb11 with the early-origins, but not affect the Sld2-Dpb11 complex formation. These results suggest that S-Cdk dependent stable association of Cdc45 and association of Dpb11-Sld2 complex with the origins are mutually dependent.

Moreover, Ms. Tak defined a Dpb11 binding region in Sld2 within a 39-amino acids stretch, partly overlapping with a cluster of the phosphorylation sites. The 39-aa peptide binds to C-terminal pair of BRCT domains in Dpb11 irrespective of phosphorylation. When MS. TAK fused 39-aa to a cluster of phosphorylation sites the interaction between the fragment of Sld2 and Dpb11 was phosphorylation dependent. Therefore, Ms. Tak propose that a cluster of phosphorylation sites in Sld2 regulates the affinity of 39-aa binding domain to Dpb11. This model suggests an interesting possibility that Sld2 changes the conformation of Dpb11 to associate with other replication proteins, such as Sld3, Dpb2 and GINS.

論文の審査結果の要旨

公聴会につづいて、審査委員とTak YOn-Sooさんだけで、質疑応答が行われた。このなかで、発表されて仕事は、彼女自身の手によるもので、彼女のアイデアが主体的に生かされた仕事であることが確認できた。その分野に対する専門知識、手法に対する見識と限界の自覚、結果の解釈等博士の学位にふさわしい能力があることが確認できた。英語に対する能力は、この学位論文の記述により確かめられた。その結果、審査員全員が、学位の授与に関して賛成した。