

## Structural basis of the DNA replication initiator complex Sld3-Sld7 from budding yeast

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### 1 Introduction

Chromosomal DNA replication is tightly regulated in eukaryotic cells so that each replication origin in DNA fires just once at the correct time during the cell cycle. Formation of the active replicative helicase, which unwinds dsDNA to expose the single strand DNA for subsequent DNA synthesis, is a key step of the regulation. The active helicase is formed by loading of two essential components, Cdc45 and GINS, onto the Mcm2-7 helicase core complex at the replication origins. This process requires another set of replication proteins, and in budding yeast, Sld3 emerges as a hub mediating protein associations essential for the active helicase formation in a cell cycle dependent manner. Sld3 and Cdc45 form a complex that associates with origins in a mutually dependent manner. Association of Sld3 with origins depends on phosphorylation of the helicase core complex by Dbf4-dependent kinase. On the other hands, Sld3 is a substrate of cyclin-dependent kinase (CDK), and further phosphorylation of Sld3 by a CDK recruits GINS to origins by interacting with Dpb11, resulting in the formation of a transient intermediate, pre-initiation complex. Sld3 is well conserved in yeast and fungi and its functional counterpart Treslin was found in metazoan. These proteins differ in their molecular sizes and amino acid sequences except in the limited region called the Sld3/Treslin domain, suggesting that this domain is important for their common function, however, the role of the conserved domain remains elusive because of a lack of structural and biochemical information.

### 2 Experiment

Genetic evidence suggests that the Sld3/Treslin domain interacts with Cdc45 [1, 2]. Physical interaction between the domain and Cdc45 was analyzed biochemically, and the results showed that these proteins bind in stoichiometric 1 to 1 ratio, suggesting the Sld3/Treslin domain is sufficient to bind to Cdc45. Thus, this domain was named as the Cdc45-binding domain (Sld3-CBD).

The budding yeast Sld3-CBD (Ser148-Lys430) protein was crystallized. X-ray diffraction data were collected from the Sld3-CBD crystals on the structural biology beamline BL-NE3A. The native and SAD data were respectively collected and processed using *HKL2000*. The structure was determined using the SAD method using *SOLVE* and *PHASER* in *PHENIX* suite. The initial atomic model was obtained using *RESOLVE* and the remaining parts of the model were built manually with *COOT*. The model was refined using the native data by *LAFIRE* with

*REFMAC5* in *CCP4* suite. The statistics of the final model was summarized in Table 1.

### 3 Results and Discussion

The crystal structure of Sld3-CBD was determined (Fig. 1). The molecule showed a rhombic-shaped compact structure with 12 helices. Helix H7 passes through the center of the molecule as a backbone, and the other helices placed around the helix through hydrophobic interactions. The structure showed that the region from the amino acid position 294- 337 was structurally flexible. This region contains many basic residues, and the amino acid alignment among Sld3 and Treslin proteins showed that the basic sequence is conserved among the Sld3/Treslin proteins. Genetic and biochemical analyses demonstrated that the region is important to interact with Cdc45. Based on the results, the model of Sld3-Cdc45 complex was proposed [3].

Table 1: Refinement statistics

Resolution range (Å)	19.6- 2.4
No. of reflection used	32,583
R-factor/ R-free-factor	0.217/ 0.262
Average B-factor (Å <sup>2</sup> )	48.9
RMSD bond (Å)/ angle (°)	0.018/ 2.008

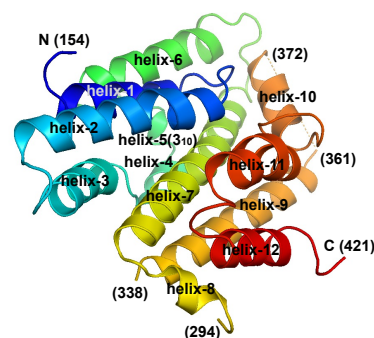


Fig. 1: The ribbon model of Sld3-CBD.

### Acknowledgement

This study was performed in collaboration with Prof. Hiroyuki Araki at National Institute of Genetics.

### References

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