

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. Formation of the active helicase requires loading of two essential replication proteins Cdc45 and GINS onto the Mcm2-7 helicase core complex at origins. Yeast Sld3 and its metazoan counterpart Treslin are the hub proteins mediating protein associations critical for the helicase formation. Our previous study showed that the domain conserved in the middle part of Sld3 and Treslin is responsible for the binding with Cdc45, and provided first structural basis for the essential Sld3-Cdc45 interaction [1]. Sld7 is a protein interacting with Sld3, and the complex formation is supposed to regulate Sld3 function [2]. Although Sld7 is non-essential DNA replication protein found in only a limited range of yeasts, its depletion slowed the growth of cells and causes delay in S phase. Recently, MTBP was found to bind to Treslin in human [3], and its depletion causes defects on cells similarly to the case of Sld7 in yeast, suggesting their functional kinship and the importance during the initiation step of DNA replication. To gain insight for the function, tertiary structure of yeast Sld7 was analyzed.

2 Experiment

Genetic evidence suggested that Sld7 binds to the N-terminal part of Sld3 [2]. We tried to crystallize full length Sld7 in complex with Sld3, but the attempt failed. The amino acid sequence analyses and secondary structure prediction suggested that Sld7 comprises two distinct structural domains, and thus, each domain was separately crystallized. The crystals of the N-terminal domain of *Z.rouxii* Sld7 (Sld7N; residue number 1-155) in complex with the N-terminal domain of *Z.rouxii* Sld3 (Sld3N; 1-115), and the C-terminal domain of *S.cerevisiae* Sld7 (Sld7C; 178-257) were respectively obtained.

X-ray diffraction data were collected from the crystals on the structural biology beamline BL-17A. The data for Sld7N-Sld3N complex were processed with *XDS*, and the structure was determined by the SAD method with *CRANK* in *CCP4* suite. The data for Sld7C were processed with *HKL2000*, and the structure was determined by the SAD method with *AutoSol* in *PHENIX* suite. The atomic models were refined using *LAFIRE* with *PHENIX Refine*, respectively. The statistics of the final model was summarized in Table 1.

3 Results and Discussion

The crystal structure clearly demonstrated that the N-terminal domains of Sld3 and Sld7 form a hetero-dimer (Fig. 1, magenta and green ribbons). The residues involved in the interaction between Sld3N and Sld7N were conserved among the Sld3 and Sld7 proteins from yeasts, suggesting that all the proteins form a complex similarly to the crystal structure obtained in this study. The crystal structure showed that the C-terminal domain of Sld7 forms a homo-dimer in an anti-parallel manner (Fig. 1, green and cyan ribbons). As the residues, especially the hydrophobic ones involved in the dimer formation, were conserved among the Sld7 proteins, all the Sld7 proteins suggested to form similar homo-dimer structure through their C-terminal domains. The resulting quaternary structure of the Sld3-Sld7 complexes seems to be reasonable to attach Cdc45 and GINS onto each of the two Mcm2-7 molecules aligned at the replication origin in a head-to-head orientation [4].

Table 1: Refinement statistics

	Sld7N-Sld3N	Sld7C
Resolution range (Å)	20- 2.35	34- 1.80
No. of reflection used	15,754	34,602
R-factor/R-free-factor	0.201/0.244	0.211/0.234
RMSD bound (Å)/ angle (°)	0.009/ 1.308	0.007/1.029

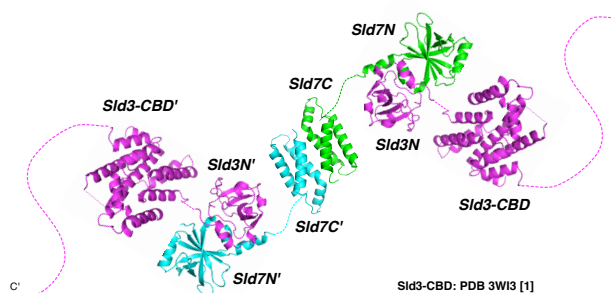


Fig. 1: Symmetric quaternary structure of the Sld3-Sld7 complexes suggested by the crystal structures.

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References

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