BL-17A, AR-NE3A/2012G155

Structural study of the DNA replication initiation complex composed of ColE2-Rep

Hiroshi Itou[1,*]
[1]National Institute of Genetics, Mishima 411-8540, Japan

1 Introduction

ColE2-Rep is an initiator-primase of ColE2 plasmid required for the initiation of its replication. This multifunctional protein specifically binds to the replication origin of the plasmid and unwinds duplex DNA. ColE2-Rep uniquely synthesizes a three-nucleotide primer RNA, and host DNA polymerase I specifically uses the primer to start DNA synthesis. Recent progress of genome analysis showed a growing number of the plasmids, which specify ColE2-Rep-related proteins as an initiator (ColE2-Rep family of proteins). ColE2-Rep-related proteins were also found as an initiator of plasmids isolated from patients suffering from infectious gastroenteritis like cholera and considered to be involved in bacterial multidrug-resistance. Functions of the ColE2-Rep have been studied using genetic and biochemical approaches. ColE2-Rep binds to the replication origin as a monomer, and the DNA-binding domain for specific binding and unwinding of duplex DNA is in the C-terminal half of the protein. The primase domain is supposed to be in the N-terminal part of the protein. The molecular mechanisms, however, were unclear due to lack of the structural basis. Thus, we tried to determine the crystals structure of ColE2-Rep. In this study, we focused on the mechanisms of specific binding to the replication origin and unwinding of it.

2 Experiment

The DNA-binding domain of ColE2-Rep (E2Rep-DBD) was over-produced in *E.coli* cells using pET-system, and the protein was purified using column-chromatography. The purified protein was mixed with the 23 bp duplex DNA harboring the replication origin of ColE2 plasmid, and their complex was formed. The protein-DNA complex was used for crystallization. X-ray diffraction data were collected using the crystals on the beamlines BL-17A (native data) and AR-NE3A (Pt-SAD data from the Pr-soaked crystal). The data were processed using *HKL2000*, and the structure was determined using the SAD method. Substructure analysis and phasing were performed using *SOLVE* and *PHASER*, respectively. The asymmetric unit contained two protein-DNA complex molecules, and the atomic model was manually built using *COOT*. Structure refinement was performed using the native data. The process was done semi-automatically using *LAFIRE* with *REFMAC5*. The statistics of the final structure was summarized in Table 1.

3 Results and Discussion

The crystal structure of E2Rep-DBD in complex with the replication origin was determined at 2.7 Å resolution (Fig. 1). The structure showed that E2Rep-DBD composed of three structural subdomains important for specific binding to the replication origin, destabilizing and unwinding of duplex DNA, respectively. The replication origin was unwound in a site-specific manner, resulting in a conformation ready for subsequent primer RNA synthesis by the primase domain. The structure demonstrated that the functionally unknown region, which is conserved in some clade of bacterial primases, corresponds to the subdomain that plays a central role for duplex DNA unwinding. The structure suggested that the subdomain also serves as a platform for the site-specific primer RNA synthesis. Based on the structural basis, we proposed the mechanistic model of replication origin unwinding by the ColE2-Rep family of proteins. [1]

Table 1: Refinement statistics

Resolution range (Å)	36.7- 2.7
Completeness (%)	99.1
R-factor/ *R*-free-factor (%)	22.0/ 26.0
Average *B*-factor (Å^2)	34.7
RMSD bond (Å)/ angle (°)	0.015/ 2.193

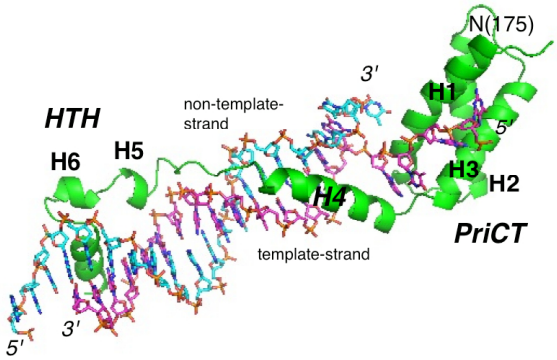

Fig. 1: The ribbon representation of the complex of E2Rep-DBD and the replication origin.

Acknowledgement

This study was performed in collaboration with Dr. Tateo Itoh at Shinsyu University.

References

[1] H. Itou *et al*., To be published。

* hitou@nig.ac.jp