CRYSTAL STRUCTURE OF THE INITIATION PROTEIN (RepE) OF DNA REPLICATION BOUND TO ORIGIN DNA

It is well known that DNA is replicated by DNA polymerases. In addition, many other proteins also play important roles in the process of DNA replication. For example, the initiation of the DNA replication, which occurs at a unique site, is controlled by several proteins. Little is known about the molecular mechanism of DNA replication including its initiation. By means of X-ray crystallography using synchrotron radiation, we have determined, for the first time, the complete three-dimensional structure of an initiator protein for DNA replication complexed with the DNA oligomer which contains the replication origin sequence recognized by this protein.

DNA replication is triggered by the binding of initiator proteins to the DNA replication origin. The replication origin has an AT-rich tract and multiple sequence repeats to which the initiator proteins bind. The binding of initiator proteins promotes the localized unwinding of the AT-rich region. Next, helicase is directed to the single-stranded region, generating a prepriming complex for priming and replication. Regulation of DNA replication by initiator protein binding is one of the most important mediators of ordered cell proliferation. Mini-F plasmid is a suitable simple model for analysis of the mechanism of DNA replication initiation. Mini-F plasmid is stably maintained in *Escherichia coli* at a copy number of one or two per host chromosome. The RepE replication initiator protein (251 residues, 29 kDa) of mini-F plasmid plays an essential role in maintaining the mini-F plasmid copy number. RepE exhibits two major functions: initiation of DNA replication (initiator function) and autogenous repression of RepE transcription (repressor function), as shown in Fig. 1 [1]. Initiation is mediated by RepE monomers that bind to direct repeats in the replication origin, whereas the autogenous repression is mediated by the RepE dimer that binds to the RepE operator. RepE exists mostly as dimer, which requires chaperones to facilitate their monomerization and initiate replication.

One mutant, RepE54, is stable in the monomeric form without aggregations, while the wild-type protein tends to aggregate easily, making crystallization difficult. We have successfully crystallized RepE54 complexed with DNA [2]. Several kinds of DNA oligomer including an iteron were surveyed for co-crystallization with the RepE54 protein. The most suitable crystals for X-ray diffraction studies were grown with a mixture of

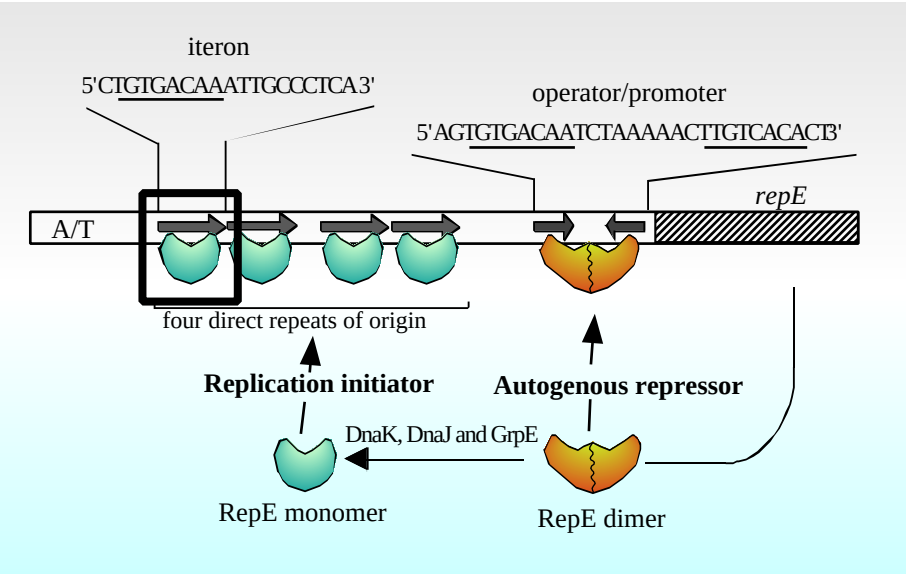

Fig. 1. Schematic drawing of the functions of the RepE initiator protein in mini-F plasmid replication. The box indicates the RepE54-DNA complex determined in this study.

the RepE54 protein and a 22-base long DNA duplex, which consists of 5' - CCTGTGACAAATTGCCCTCAG - 3' and its complement with a T-overhang at each 3' terminus. Prismatic crystals of the RepE54-DNA complex grew to approximate dimensions of 0.3 × 0.2 ×0.2 mm^3 within two weeks from a precipitating solution containing 100 mM Tris-HCl, pH 8.0, and 12% polyethylene glycol (average molecular weight is *ca*. 400) and 200 mM $MgCl_2$. The crystals belong to the monoclinic space group *C* 2, with unit cell dimensions of *a* = 108.4 Å, *b* = 81.9 Å, *c* = 73.9 Å, and β = 121.5°. One complex per asymmetric unit, the Matthews content, Vm, is calculated to be 3.12 $Å^3$/Da and the solvent content of the crystal is 60.6%.

Diffractions from the crystals extend to 2.0 Å resolution using synchrotron radiation of beamline **BL41XU** (Fig. 2). Diffraction data were also collected at 100 K using a flash-cooled crystal. Crystals were briefly washed in solutions containing 25% polyethylene glycol 400 and 10% glycerol as a cryoprotectant, retrieved with a nylon loop and flash-frozen in the nitrogen stream. The scaling gave a final R_{merge} of 6.6% for 104,080 reflections, of which 31,667 were unique corresponding to 88.7% completeness at 1.98 Å resolution.

The structure was solved by the multiple isomorphous-replacement method (MIR). In addition to mercury derivatives, iodine derivative crystals were prepared by co-crystallization with DNA substituted with 5-iodouracil for thymine at specific positions. The MIR phases calculated at 3.0 Å were improved by a solvent flattening. The map showed fine electron densities. The atomic model was constructed using the graphics program O. The atomic model was finally refined at 2.6 Å resolution (R = 21.3%) and is now being refined using higher resolution data.

The crystal structure of the RepE54-DNA complex showed a new type of the DNA binding motif. RepE54 is composed of two distinct N- and C-terminal domains which are structurally similar to each other and related by a non-crystallographic dyad. No such similarity was expected from its amino acid sequence. The helix-turn-helix motifs of both domains bind to the major groove of DNA with different binding affinities. The recognition helix of the C-terminal domain makes multiple specific contacts with the DNA bases, while that of the N-terminal domain has nonspecific interactions with the DNA backbones. It is also found from hypothetical model of a RepE dimer bound to the operator that the N-terminal domains of dimer face each other and thus are responsible for dimerization. The structure suggests that the C-terminal domain plays the leading role in DNA binding, while the N-terminal domain has the additional role in dimerization. This functional difference between the two domains is essential for the differential binding to the origin and the operator.

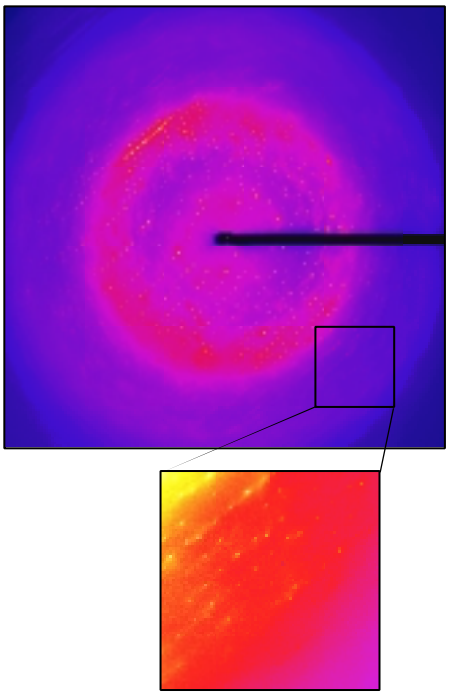

Fig. 2. X-ray diffraction image from a crystal of the RepE54-DNA complex, measured by a Rigaku R-AXIS IV oscillation diffractometer.

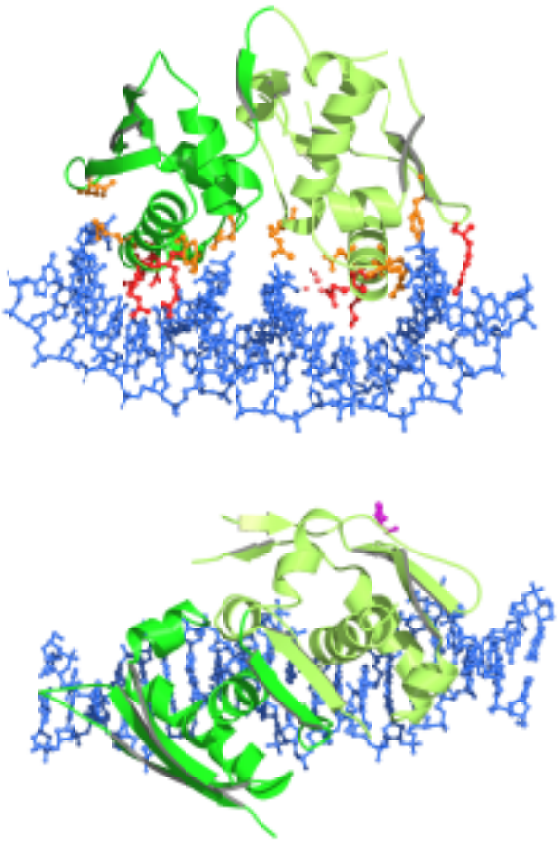

Fig. 3. Overall structure of the RepE54-DNA complex.

Hirofumi Komori and Kunio Miki

Kyoto University

E-mail : miki@kuchem.kyoto-u.ac.jp

References

[1] M. Ishiai *et al.* ,Proc. Natl. Acad. Sci. USA **91** (1994) 3839.

[2] H. Komori *et al.*, J. Biochem. **125** (1999) 24.

[3] H. Komori, F. Matsunaga, H. Yoshiki, M. Ishiai, C. Wada and K. Miki, EMBO J. **18** (1999) 4597.