Conformational Changes upon Activation of a Replication Initiator Protein, RepE

DNA replication is a fundamental and essential process for lives and is stringently regulated at the stage of its initiation. In a eukaryotic cell, genomic DNA is licensed to ensure its one-time replication during one cell-cycle by several protein complexes which are activated by other cell-cycle dependent proteins, such as CDK (cyclin-dependent kinase) [1]. The DNA replication of F plasmid in a prokaryote, *Escherichia coli*, is also controlled, which maintains the copy number of F plasmid against the host chromosome at 1 or 2. An F-plasmid encoded protein, called RepE, plays a crucial role for this regulatory mechanism. RepE has two molecular association states, that is, dimeric and monomeric states, and only the monomer can work as a replication initiator. However, RepE is usually found as an inert dimeric form in the *E. coli* cell, which functions as an autogenous transcriptional repressor [2] (Fig. 1). Both the dimer and monomer are able to bind DNA at the discrete regions, that is, the *repE* operator and iteron of the origin, respectively, in which an 8-bp DNA sequence is common. One of the other interesting points of this replication control is that the DnaK molecular chaperone system of *E. coli* is required for conversion from the RepE dimer to monomers in order to activate RepE as a replication initiator.

The crystal structure of RepE54, a monomeric RepE mutant, whose initiator activity is much higher than the wild-type protein, in complex with DNA was solved in 1999 [3] as the first crystal structure of a prokaryotic replication initiator protein (Fig. 2(a)). On the basis of this structure, it is found that RepE can be divided into N- and C-terminal domains related by a non-crystallographic 2-fold symmetry. The C-terminal domain interacts with the common 8-bp region, indicating that this domain is responsible for sequence-specific DNA-binding of RepE. The N-terminal domain also interacts sequence-nonspecifically with DNA, and this domain has been expected to be a scaffold of the dimeric structure. In addition, in case of dimer-to-monomer conversion, a large conformational change in the N-terminal domain has also been predicted. To elucidate structural details of this conformational change, we have determined the crystal structure of the dimeric RepE in complex with DNA using beamlines **BL41XU**, **BL26B1**, and **BL44B2** [4].

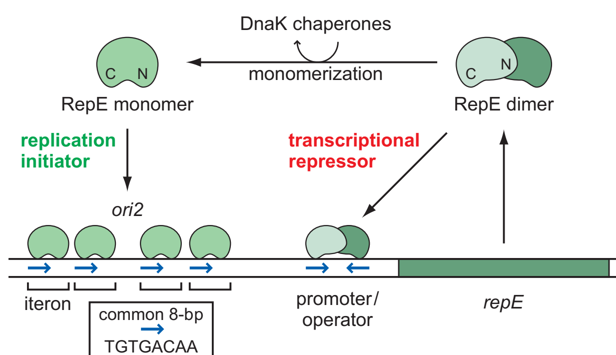

Fig. 1. Schematic draw of functions of the F-plasmid replication initiator protein, RepE. The predominant RepE dimer functions as an autogenous transcriptional repressor. RepE monomerization is mediated by the DnaK molecular chaperone system of *E. coli*. The dissociated monomer works as a replication initiator. Blue arrows indicate positions and directions of the common 8-bp DNA located in both the origin (*ori2*) and the operator region of the *repE* gene.

The crystal structure of the RepE dimer bound to the *repE* operator DNA (Fig. 2(b)) indicates structural difference as well as structural similarity between the two molecular association states of RepE. The C-terminal domain of the dimer also interacts with the common 8-bp as observed in the RepE monomer structure, while the N-terminal domain of the dimer does not contact with DNA but certainly plays a significant role for dimerization as expected. The discrete superimposition of the domain structures shows that each domain of RepE does not undergo a significant structural change during the dimer-to-monomer conversion. On the contrary, characteristic structural differences between the two molecular association states are found in a linker region tethering the two domains and in relative domain orientation. The dimer has a long α-helical structure as the domain-linker, whereas a shorter α-helix, an unstructured region and a β-strand construct the domain-linker of the monomer. This domain-linker consists of several hydrophobic residues, which should make a strong hydrophobic interaction with a hydrophobic core of the N-terminal domain. However, the domain-linker of the monomer protrudes outward from the core of the N-terminal domain, suggesting a weak interaction with the core. We think the domain-linker of the monomer can play a hinge role for alteration of the relative domain orientation compared to that in the dimeric structure.

The two crystal structures of RepE solved over the past decade in both molecular association states clearly demonstrated a conformational switch, in particular the domain-based structural transition, undertaken during the dimer-to-monomer conversion. We could also suggest a mechanism of the DnaK molecular chaperone system in dimer dissociation

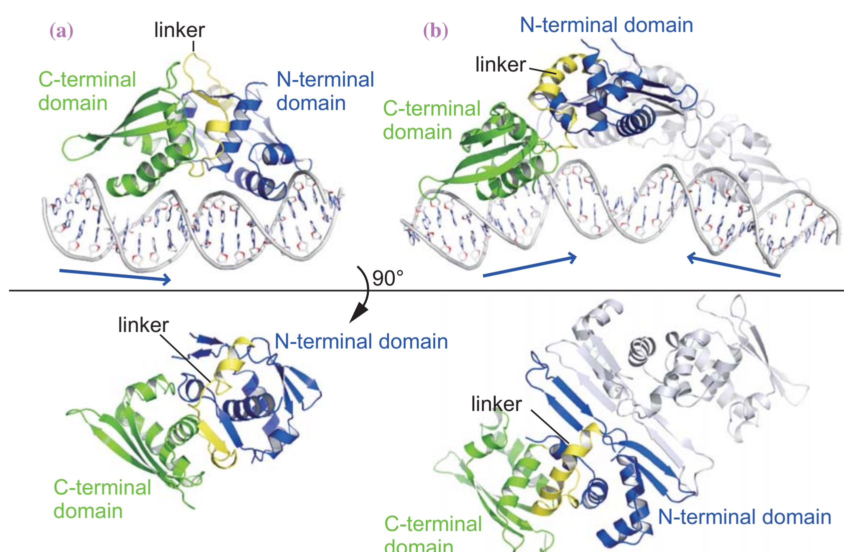

Fig. 2. Crystal structures of the RepE-DNA complexes. **(a)** Crystal structure of a monomeric RepE mutant (RepE54) in complex with the iteron DNA. **(b)** Crystal structure of the RepE dimer bound to the *repE* operator DNA. Side (upper panel) and top (lower panel) views of RepE-DNA complexes are displayed. The N-terminal domain, domain-linker, and C-terminal domain are shown in blue, yellow, and green, respectively. Blue arrows indicate the common 8-bp DNAs.

of RepE (Fig. 3). The DnaK and DnaJ proteins belonging to the DnaK system would interact with the hydrophobic region of the domain-linker and N-terminal domain of RepE, which has been indirectly indicated by sequence-dependent binding of chaperones and interaction of a homologous protein with DnaJ. The action of the DnaK chaperones may afford a structural alteration in the domain-linker of RepE, and thus variations of relative domain orientation and dissociation of the RepE dimer must be induced. With the aid of DnaK, the RepE monomer could adopt a form appropriate for origin-binding.

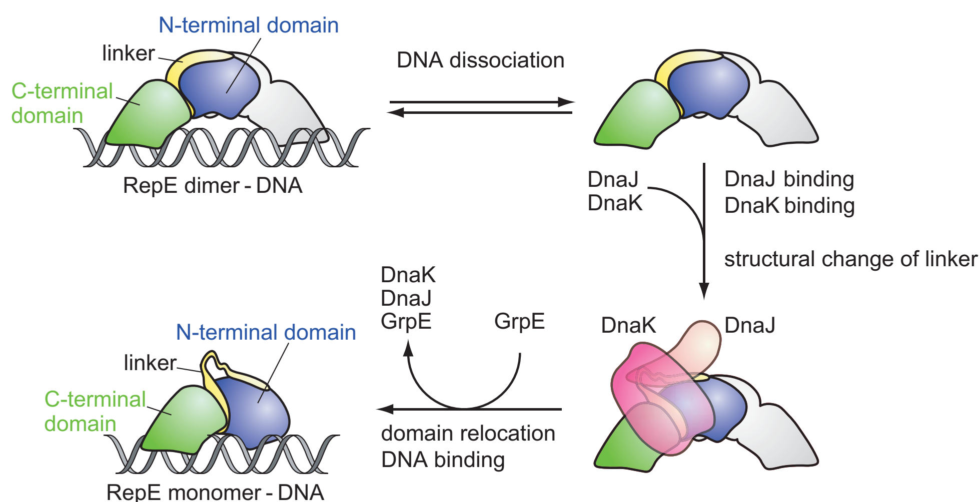

Fig. 3. Suggested activation mechanism of RepE. DnaK/DnaJ chaperone proteins would interact with the domain-linker region of RepE (upper right). Intermolecular hydrophobic interactions induce the conformational changes of the domain-linker (lower right). Structurally flexible hinge may allow domain relocation, followed by monomerization and activation of RepE.

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References

[1] J.J. Blow and A. Dutta: Nature Rev. Mol. Cell Biol. **6** (2005) 476.
[2] M. Ishiai *et al.*: Proc. Natl. Acad. Sci. USA **91** (1994) 3839.
[3] H. Komori *et al.*: EMBO J. **18** (1999) 4597.
[4] *A. Nakamura, C. Wada and K. Miki: Proc. Natl. Acad. Sci. USA* ***104*** *(2007) 18484.*