論文の内容の要旨

Crystal structure analysis of DnaB-DnaC complex involved in DNA replication in *E. coli*

(大腸菌の DNA 複製に関わる DnaB-DnaC 複合体の結晶構造解析)

氏名 岡田晃季

DNA replication is a fundamental process of biological inheritance that occurs in all living organisms for the passage of their DNA to the next generation. In this process, double-stranded (ds)DNA is split into single strands so that DNA polymerase can read the template and synthesize complementary strands. This unwinding of dsDNA into two single strands is performed by a family of proteins called helicases. In *Escherichia coli*, DnaB protein is the helicase that plays this crucial role. DnaB disrupts the hydrogen bonds of dsDNA, resulting in the initiation of DNA replication. The recruitment of DnaB to the origin of replication (oriC) of the genomic DNA is performed by another protein, DnaC.

In this study, the initiation of replication by DnaB and DnaC is studied from the point of view of the molecular structure. The crystal structure of DnaCn-bound DnaBc is reported here (Fig. 1.) and provides the first insight into the binding and the release of DnaC and DnaB.

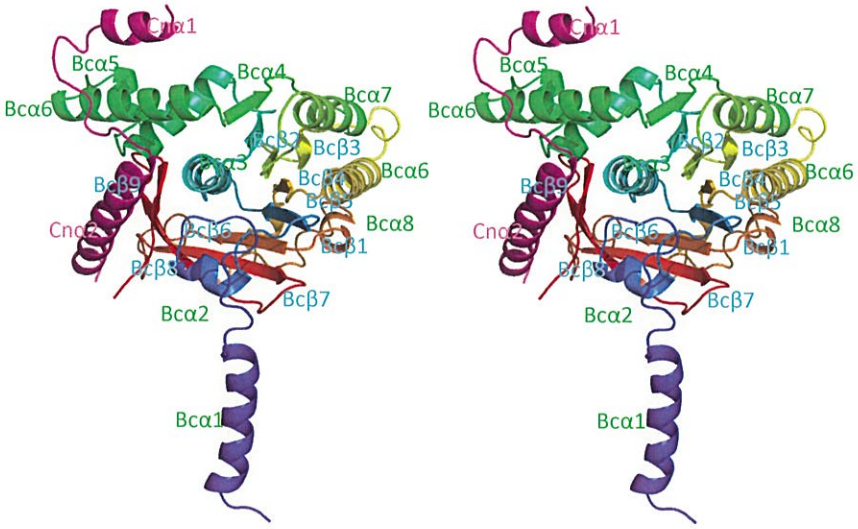

Fig 1. The overall structure of DnaBc and DnaCn in stereo view.
DnaCn is shown in magenta, DnaBc is shown in blue to red from its N-terminus to C-terminus. DnaCn alpha helices are labeled in pink, DnaBc alpha helices are labeled in green, and DnaBc beta sheets are labeled in cyan.

The crystal structure of the DnaBc–DnaCn complex shows that two helical regions in DnaCn are important for its binding to DnaB. The first helical region (CnHelix1) lies on one side of two alpha helices (BcHelix1′ and BcHelix6) of neighboring DnaBc proteins. CnHelix1, BcHelix1′, and BcHelix6 play a key role in DnaC release on DnaG binding to DnaB. The second helical region (CnHelix2) has a longer helix than CnHelix1 and is present on one side of one DnaBc protein, thereby making contact with only one DnaBc molecule per CnHelix2. Thus, by solving the crystal structure of the DnaBc–DnaCn complex the mechanism of DnaB and DnaC binding was elucidated.

To confirm the mechanism of complex formation, additional mutagensis analysis of the crystal structure of the DnaBc–DnaCn complex was conducted. An earlier random mutational analysis study of DnaC had shown that the mutations of residues mainly in CnHelix2, located close to DnaBc, decreased the binding affinity of DnaB for DnaC. An additional mutational analysis was conducted for the CnHelix1 residues and some CnHelix2 residues that were considered to be in

contact with DnaBc in DnaBc–DnaCn structure thus confirming the crystal structure of DnaBc–DnaCn is likely to be the structure in the DnaB–DnaC complex.

DnaBc ATPase activity against the DnaBc–DnaCn structure is also discussed. The structure revealed that ATPase site of DnaBc consists of Walker A and Walker B motifs, which is a classicial ATP binding motif among DnaB proteins. DnaCn binding to DnaBc did not seem to cause a structure change in DnaBc that causes the deficiency in ATP binding. This does not contradict the fact that DnaC–ATP complex inhibits the ATPase activity of DnaB but DnaC–ADP. That is, ATPase domain of DnaC is located in DnaCc whereas the domain solved in the crystal structure of this study was DnaCn. Therefore, DnaCn will not itself bind to ATP and hence it can be assumed that DnaBc–DnaCn will be an ATPase active form of DnaB, which was seen in the crystal structure of DnaBc–DnaCn.

Furthermore, small-angle X-ray scattering (SAXS) analysis for DnaC, DnaB, and the DnaB–DnaC complex was conducted. SAXS results for DnaC revealed that DnaC in solution exists in a conformation such that DnaCn and DnaCc are divided by a linker region. Hence, it is suggested that the structure of DnaC changes in the linker region of DnaCn and DnaCc, allowing the DnaC complex to bend in a hinge motion and place DnaCn beside DnaBc hexamer and DnaCc below DnaBc hexamer (opposite to DnaBn). Overall, our study of DnaB and DnaC structures presents the first insight into the initiation of replication mediated by these two proteins.