

Studies on Structure-Function Relationship of DNA Replication Control Proteins by Means of X-ray Crystallography

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The RepE initiator protein of mini-F plasmid plays an essential role to control the initiating replication from origin, *ori2*. RepE exhibits two major functions: the initiation of DNA replication from *ori2* (initiator function) and the autogenous repression of *repE* transcription (repressor function). The initiation is mediated by the RepE monomers that bind to the *ori2* iterons (direct repeats), whereas the autogenous repression is mediated by the dimers that binds to the *repE* operator, which contains an inverted repeat sequence related to the iterons (M. Ishiai, C. Wada, Y. Kawasaki & T. Yura, *Proc. Natl Acad. Sci. USA*, **91**, 3839-3843 (1994)).

One of the RepE mutants (RepE54) produced hyperactive RepE that cannot form dimers, unlike the wild-type protein that is found mostly as dimers. The RepE54 monomers bind with a very high efficiency but hardly bind to the operator. The wild-type protein tends to form aggregations so extremely that it is difficult to crystallize. On the other hand, the RepE54 hardly forms such aggregations. It is important to elucidate the crystal structure of RepE54-DNA complex at the atomic level for detailed insight into the molecular mechanism by which this protein recognizes the iteron and initiate replication. We have undertaken the crystallization of the RepE54 protein in complex with various DNA oligomers to determine the three dimensional structure of this protein-DNA complex and to clarify the structure-function relationship.

Prismatic crystals of the complex between the RepE protein and an iteron DNA were obtained. The crystals belong to the monoclinic system with the space group *C2*.

Diffraction intensities recorded on the imaging plates at the BL41XU beamline extend to about 2.5 Å resolution as shown in Figure. A series of diffraction images was collected using the crystal with an approximate dimensions of 0.2×0.2×0.1 mm with the oscillation range of 2 deg. and the exposure time of 32 sec. The processing of the images is in progress.

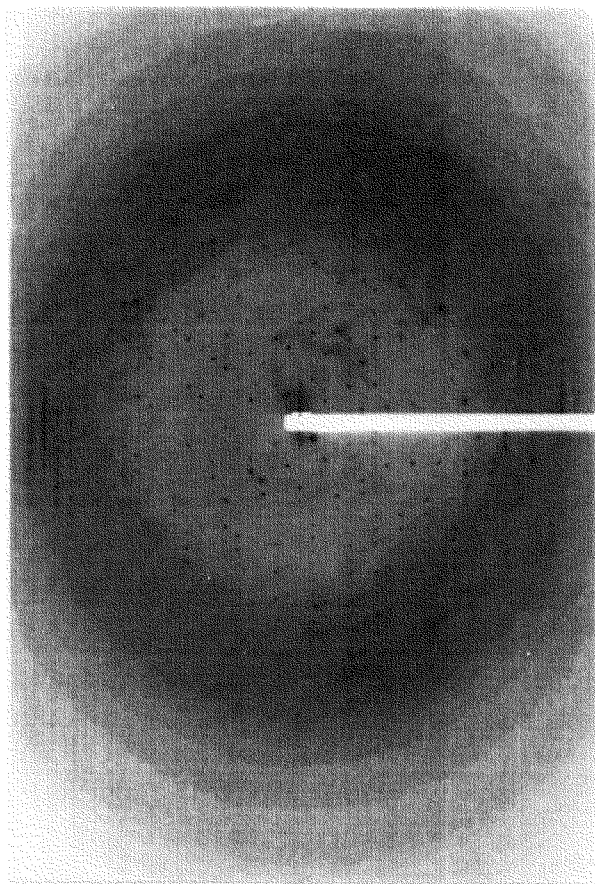


Figure. X-ray diffraction pattern of the crystal of RepE protein-DNA complex at the BL41XU beamline.