Biological Science

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Cooperative DNA-binding and sequence-recognition mechanism of Aristaless and Clawless

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Introduction

Homeodomain is one of the most widely spread superfamily of eukaryotic DNA-binding proteins that regulates transcription of various kinds of genes which are indispensable for development. Some homeodomain proteins bind cooperatively to specific DNA sequences to increase those binding affinities and site specificities. In this study, we focused a cooperativity of two homeodomain proteins, Aristaless and Clawless from *Drosophila melanogaster*. These homeodomain proteins play an important role for *Drosophila* leg development. In the distal-most region, homeobox genes, *aristaless (al)*, *Lim1*, and *clawless (cll)* are expressed to specify the region, while a pair of *Bar* homeobox genes are expressed in its immediate neighbor (distal tarsus). For the accurate differentiation of these regions, the expression of *Bar* is repressed by the cooperative mechanism of Al and Cll in the distal-most region. These two homeodomain proteins bind cooperatively to the *Bar* enhancer element and repress its gene expression. The EMSA experiments shows that the extended conserved sequences of the Cll homeodomain are indispensable to the cooperative DNA binding. To elucidate the structural basis for the cooperative DNA binding mechanism of Al and Cll, we determined the homeodomain structures of Al and Hox11L1 (a human homologue of Cll), a binary complex structure of Al-DNA, and a ternary complex structure of Al-Cll-DNA by X-ray crystallography.

Materials and Methods

The Al homeodomain, Cll homeodomain, and Hox11L1 homeodomain proteins were overexpressed in *E. coli* using pET26b and pET28a vectors. The crystals of the Al homeodomain, Hox11L1 homeodomain, the Al-DNA complex, and the Al-Cll-DNA complex were crystallized using polyethylene glycols as precipitant, respectively. [1]

The data set of each crystal was collected at NW12A beamline in Photon Factory. The diffraction data were integrated and scaled with XDS. The structures were determined by the molecular replacement method using the program Molrep and refined with Refmac5.

Results and Discussion

The crystal structures of the Al homeodomain, the Hox11L1 homeodomain, the binary complex of Al-DNA, and the ternary complex of Al-Cll-DNA were determined at 1.00, 1.54, 2.25, and 2.70 Å resolutions, respectively [1]. In the ternary complex structure, the Al and Cll homeodomains bind DNA with a head-to-tail orientation (fig. 1). The structure of the ternary complex shows that the extended conserved region of Cll homeodomain plays a critical role for the cooperative DNA binding mechanism. In the Al-Cll-DNA complex structure, the residues in the extended regions are used not only for the intermolecular contacts between two homeodomain proteins but also for the sequence-recognition mechanism of DNA by direct interactions. The residues in the extended N-terminal arm of Cll (His-10, Tyr-8, and Arg-5) lie within the minor groove of DNA to form direct interactions with bases, whereas the extended conserved region of the C-terminus of the homeodomain interacts with Al to stabilize and localize the third α helix of the Cll homeodomain. This structure suggests a novel mode for the cooperativity of homeodomain proteins.

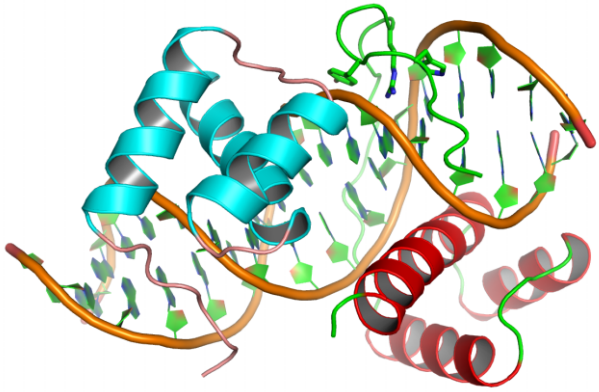

Fig.1: Crystal structure of the Al-Cll-DNA complex. (cyan; Cll homeodomain, red; Al homeodomain)

Reference

[1] K. Miyazono et al., EMBO J. 29:1613-23(2010)

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