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Crystallographic analysis of Methyl-CpG-binding protein (MECP2)-DNA complex

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Introduction

DNA methylation is one of the most common epigenetic mechanisms for regulating gene expression. DNA methylation occurs at 5' cytosine of CpG dinucleotides, most of which are clustered in the CpG islands. Methylation of CpG islands leads to transcriptional silencing of the gene located downstream of methyl CpG.

The mechanism for the gene silencing involves proteins with high affinity for methylated CpGs, such as the methyl-CpG-binding protein MECP2. MECP2 contains methylated CpG binding domain (MBD) and a transcriptional repression domain, which interacts with various co-repressor complexes. MECP2 also binds to four-way junction DNA through its MBD, whether it includes methyl CpG or not. It suggests that MECP2 also has higher-order structure-specific binding ability to DNA or chromatin.

Mutations in MECP2 cause Rett syndrome, which is a progressive neurodevelopmental disorder that occurs almost exclusively in females, indicating that MECP2 has a crucial role in neurodevelopment. Understanding of the regulation of gene expression by MECP2 is beginning to be gained at molecular level. Our aim is to elucidate a phenotype genotype relationship by crystallographic analysis of MECP2 and its DNA complex.

Experiments and discussion

The purified MECP2 MBD was mixed with equal-molar DNA including methylated CpG and transcription factor C/EBP binding sequence, followed by addition of the caat enhancer binding protein (C/EBP). The C/EBP, which is a bzip type transcriptional regulatory protein, was utilized for effective crystal packing [1]. For the Multi-wavelength Anomalous Diffraction (MAD) method, a protein-DNA complex including Br-labeled DNA was prepared. The resultant complex has been crystallized. Diffraction images were collected with synchrotron radiation at the beam lines BL-5A and NW12A in Photon Factory and processed using HKL2000. The crystal belongs to the space group $C222_1$ and diffracted at 2.4 Å resolution. The phase of the MAD data was calculated with SOLVE/RESOLVE. The structural refinement was calculated by CNS.

Although electron density of C/EBP and DNA was observed, that of MECP2 was not clear. This might be due to disorder of the MECP2 molecule in the crystal. We are trying to optimize the condition for crystallization including the designs of MECP2 fragment and DNA. In addition, we prepared four-way junction DNA binding to MECP2, and got crystals of the complex, but it diffracted to very low resolution using PF beam lines. We are trying to find out the condition for crystallization of the complex as well as optimization of MECP2 fragment.

References

[1] Tahir H. Tahirov et al., Cell. 104, 755-767 (2001).

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