Maharani Pertiwi Koentjoro et al., J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100



# Crystal structure of DNA-binding domain-CbnR with its promoter reveals the basis of the LysR-type transcriptional regulator recognition

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*Cupriavidus necator* NH9, which can utilize chlorocatechol as a sole carbon and energy source, degrades chlorocatechol with enzymes of the ortho-cleavage pathway. These enzymes are coded in the cbnABCD operon, of which expression is specifically regulated by a LysR-type transcriptional regulator CbnR. CbnR forms a tetramer and can be regarded as a dimer of dimers. The tetrameric CbnR has four DNA- binding domains and these DNA-binding domains recognize approximately 60 bp DNA sequence. The binding sequence is composed of two binding sites, recognition binding site and activation binding site. Each binding site seems to be recognized by two DNA-binding domains in the tetramer. While the crystal structure of the tetrameric CbnR has already been determined, the molecular mechanism of DNA recognition by CbnR remains elusive. We therefore initiated the crystal structure analysis of DNA-binding domain of CbnR in complex with RBS. The crystal structure would give an insight into the molecular mechanism of the CbnR-DNA interaction, which is the first step to understand the gene activation mechanism by LTTR. Here we report the crystal structure of CbnR(DBD) (residues 1 - 87) in complex with RBS, a 25-bp DNA fragment. The crystal structure was determined by the MR-native SAD method at 2.55 Å resolution with Rwork/Rfree of 0.221/0.264. The crystal structure shows that dimeric CbnR(DBD) interacts with RBS. The dimeric CbnR(DBD) adopts essentially the same conformation as that in the tetramic CbnR with the root mean squares deviation of 1.1 Å (174 Cα atoms). The 3α helix and the winged region of the winged-helix turn helix motif in CbnR(DBD) directly interact with the major and minor grooves of promoter sequence, respectively, and the interactions seem to bend DNA by approximately 30°. To further analyse the molecular mechanism of their interaction, biochemical analysis is in progress.

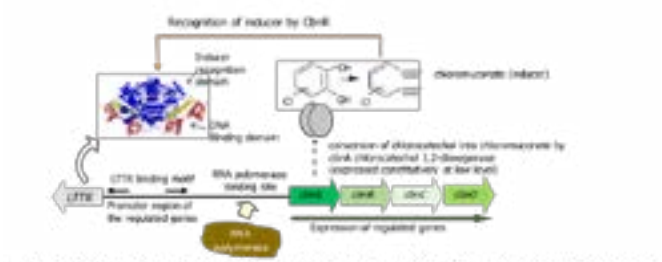

Fig. Binding of tetramer CbnR (CbnR, PDB: 1IXC) crystal structure (2.2 Å) to its promoter region lead to the transcriptional activation of the regulated genes.

## Biography

Maharani Pertiwi Koentjoro is a 3rd year PhD student and the Monbukagakusho fellow in the United Graduate School of Agricultural Science, Gifu University, Japan. She has completed her BA from Sepuluh Nopember Institute of Technology, Indonesia, and a Master in Gadjah Mada University, Indonesia. Her research interests include molecular biological and biochemical investigation on bacteria. Currently she is working on structural studies of complex molecular machines that initiates LysR-Type Transcription Regulator in bacteria.

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