CTD) closely resembles BsTopoIV-CTD, rather than its functionally equivalent BbGyrA-CTD. However, the significance of the EcGyrA-CTD structure remains to be further examined because a key motif termed GyrA box, which is indispensable for E. coli gyrase to exhibit negative supercoiling activity, is disordered. To provide more structural information for the gyrase CTDs, we have determined the crystal structure of Xanthomonas campestris gyrase CTD (XcGyrA-CTD), and the structure of EcGyrA-CTD has been re-determined in a new crystal form. Structural analyses clearly show that both XcGyrA-CTD and EcGyrA-CTD resemble BsTopoIV-CTD more closely. In addition, the position of the GyrA box is unambiguously defined in the XcGyrA-CTD structure, providing the first view of this important motif.

Keywords: type II topoisomerase, DNA gyrase, DNA bending

P04.06.237

Acta Cryst. (2008). A**64**, C305

Structural basis for hemi-methylated CpG DNA recognition by mouse Np95 SRA domain

Kyohei Arita[1], Mariko Ariyoshi[1], Hidehito Tochio[1], Yusuke Nakamura[2], Masahiro Shirakawa[1]
[1]Kyoto university / Japan, Molecular engineering, A4-132, Kyoto University Katsura, Nishikyo-ku, Kyoto, kyoto, 615-8510, Japan, [2]The university of Tokyo, Human Genome Center, Institute of Medical Science, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan, E-mail：kyouhei@z06.mbox.media.kyoto-u.ac.jp

DNA methylation of CpG dinucleotides is a major epigenetic modification of mammalian genomes and is essential for the regulations of chromatin structure, gene expression and genome stability. The epigenetic inheritance of methylation pattern of genomic DNA is carried out by DNA methyltransferase 1 (Dnmt1), which methylates newly synthesized CpG sequences during DNA replication, depending on the methylation status of the template strands. The first step of this process requires Np95 (also known as UHRF1 and ICBP90), which recognizes hemi-methylation sites via its SRA (SET and RING associated) domain and mediates correct loading of Dnmt1 to the sites. We determined the crystal structure of the unliganded SRA domain of mouse Np95 at 1.77 Å resolution. The SRA domain is folded into a single globular structure consisting of five stranded mixed and three stranded β-sheets and their associated four helices. The crystal structure allowed to identify the putative DNA binding site of the SRA domain which consists of the conserved residues among SRA proteins. Electrophoresis mobility shift assay, NMR titration experiment, and isothermal titration calorimetric measurements have shown that the SRA domain preferentially interacts with hemi-methylated DNA and has the distinct binding modes for hemi-, full, and non-methylated DNAs. Our structural and biochemical data have gained a new insight into the molecular mechanism by which Np95 SRA domain specifically recognizes the hemi-methylated sites.

Keywords: methyl DNA binding protein, protein crystallography, biochemistry

P04.06.238

Acta Cryst. (2008). A**64**, C305

Novel DNA-binding fold and DNA-recognition mode discovered in restriction enzyme PabI

Kenichi Miyazono[1,7], Miki Watanabe[2,7], Jan Kosinski[3], Ken Ishikawa[2,5], Masayuki Kamo[1], Tatsuya Sawasaki[4], Koji Nagata[1], Janusz M Bujnicki[3], Yaeta Endo[4], Masaru Tanokura[1], Ichizo Kobayashi[2,5,6]
[1]The University of Tokyo, Department of Applied Biological Chemistry, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan, [2]Department of Medical Genome Sciences, Graduate School of Frontier Science, The University of Tokyo, Tokyo, Japan, [3]Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, Poland, [4]Department of Applied Chemistry, Faculty of Engineering, Ehime University, Matsuyama, Japan, [5]Graduate Program in Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan, [6]Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [7]These authors contributed equally to this work, E-mail：kenichi.miyazono@gmail.com

PabI is a restriction endonuclease found in Pyrococcus abyssi through comparison of closely related genomes. It recognizes 5'GTAC and generates -TA3' overhang, a novel type of restriction termini. PabI was predicted to have a novel structure by analysis of amino acid sequence. Additionally, unlike most restriction enzymes analyzed, PabI is able to cleave a target DNA in the absence of added Mg^{2+}. To understand structural basis of PabI's unique properties, we determined its three-dimensional structure by X-ray crystallography. We expressed PabI and its selenomethionyl derivative in a wheat-germ cell-free translation system. The structure of PabI was solved by the SAD method at 3.0 Å resolution. Structural analysis of PabI showed that this enzyme adopts a novel protein fold as predicted. PabI forms a homodimer by formation of extended anti-parallel beta-sheet that is curved to form an extended groove, which is the unique architecture of PabI. We named this unique substructure half pipe. Mutational and in silico DNA binding analyses have assigned the groove as the double-strand DNA binding site. Our mutational analysis has revealed that there are three residues, Arg32, Glu63, and Tyr134, which are indispensable for the catalytic activity. All the three residues are located in the half pipe and may act as catalytic or DNA binding residues. These results demonstrate the value of genome comparison and the wheat germ-based expression system in finding a novel DNA-binding motif in mobile DNases and, in general, a novel protein fold in horizontally transferred genes. To our knowledge, this is the first report of determination of protein crystal structure by the wheat-germ-based cell free expression system.

Keywords: endonucleases, protein X-ray crystallography, novel structures

P04.06.239

Acta Cryst. (2008). A**64**, C305−306

X-ray crystal structure analysis of transcriptional regulator MobR

Keisuke Yokoya[1], Takeshi Hiromoto[1], Mariko Yoshida[1], Keiichi Hosokawa[2], Shinsuke Fujiwara[1], Hiroshi Yamaguchi[1]
[1]Kwansei Gakuin University, 2-1 Gakuen, Sanda, Hyogo, 669-1337, Japan, [2]Tsukuba Molecular Biology Laboratory, 3-8-1-103 Azuma, Tsukuba, Ibaraki, 305-0031, Japan, E-mail：csl26194@kwansei.ac.jp

MobR from *Comamonas testosteroni* KH122-3s is a transcriptional regulator which belongs to the MarR family and negatively regulates for the *mobA* gene that encodes a 3-hydroxybenzoate 4-hydroxylase. We have revealed that MobR is released from the operator site with the interaction of 3-hydroxybenzoate by the electrophoresis mobility shift assay. Whereas MobR does not interact with the 4-hydroxybenzoate and salicylate that are isomers of 3-hydroxybenzoate. In addition, we revealed that MobR adopted two conformational states corresponding to the effecter-