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

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

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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

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

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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

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X-ray crystal structure analysis of transcriptional regulator MobR

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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-

P4

bound and unbound forms by the circular dichroism analysis. In order to investigate the molecular mechanism for recognizing its effecter specifically, the crystal structure of MobR in complex with 3-hydroxybenzoate has been determined at 2.25 Å resolution. Diffraction data were collected on the BL44XU at SPring-8. The structure showed that MobR forms homo dimer and the subunit has a winged helix-turn-helix DNA binding domain as well as the other members of the MarR family. Furthermore, the effecter-binding pocket is distant from DNA binding domain and 3-hydroxybenzoate was recognized by side chains H31, R37 and Q134 in the pocket.

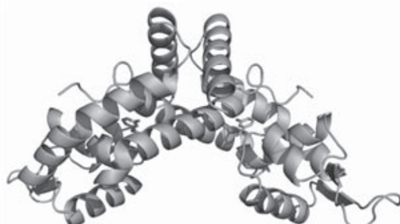
Fig. The crystal structure of MobR in complex with 3-hydroxybenzoate

Keywords: X-ray crystallography, DNA-binding proteins, transcription factor structure

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Structure of human RECQ1 helicase: Identification of a putative DNA strand separation pin

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RECQ-like helicases are ATP- and Mg^{2+}-dependent enzymes that are involved in maintaining genome integrity. The RECQ helicase family has five representatives in the human genome. Here we describe the 2Å crystal structure of human RECQ1 in complex with Mg-ADP. Overall, the structural architecture closely resembles that of bacterial RECQ albeit with altered relative domain positioning. All domains are conserved, including two RecA-like modules, the RECQ-specific zinc-binding and a winged-helix (WH) domain. The orientation of the two RecA domains, believed to harbour the helicases' ATP-dependent translocation activity, exhibits considerable variability as adjudged from the overall conformation adopted by the protein in multiple crystal forms. The C-terminal WH domain is positioned in a novel orientation in the human enzyme resulting in a more elongated molecule. This domain also exhibits a prominent beta-hairpin structural element, not seen in the bacterial enzyme, that is reminiscent of the DNA strand separation pin of other DNA helicases. The role of this pin is to act as an unwinding element by displacing the individual strands of duplex DNA. Mutation of the Tyr residue (Y564) that caps the separation pin as well as shortening the beta-hairpin abolishes DNA-unwinding activity confirming that this structural element plays a key role in DNA strand separation. The probable DNA-binding mode of RECQ1 can be inferred by comparison with other DNA-helicase complexes. The structure will be presented in detail along with implications for recognition and binding of DNA. In addition, progress on the structural characterisation of the other members of RECQ family will also be summarised.

Keywords: DNA replication, protein-DNA interactions, structural genomics

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A new nicking enzyme is developed from a mutant of the modified type II restriction enzyme scPvuII

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PvuII is the first restriction endonuclease (nuclease component of one of the type II restriction-modification systems of Proteus vulgaris) which has been converted from its wild-type (wt) homodimeric form into a single chain (sc) protein by tandemly joining the two subunits through the peptide linker GlySerGlyGly [1]. The DNA cleavage activity of the enzyme is thereby largely retained. The determined crystal structures (from twinned and un-twinned crystal forms) [2] show that the apo scPvuII adopts a more compact conformation compared to the wild-type form. Four mutants of scPvuII, which address specific aspects of its interactions with DNA have been crystallized and studied with similar results. In contrast, in equilibrium in solution, scPvuII and the mutants adopt two conformations, as proved from gel-filtration [3] and SAXS measurements. As proved from the crystal structure, the peptide linker forms new H-bonds in that area of the protein, which are possibly responsible for the two conformations of the apo enzyme. Several attempts for the co-crystallization of the scPvuII or a mutant - DNA complex were not successful, probably because of the serious aggregation problem of the proteins, as studied by Dynamic Light Scattering techniques [4]. The complex formation was also studied by SAXS method and it is actually formed in the case of scPvuII in the expected 1:1 molar stoichiometry. Later studies based on FRET technique proved that the DNA molecule in the complex is not bended [4], as it is also the case for the wtPvuII, but not for other type II restriction enzymes. The proteins - DNA interactions were also studied by EMSA techniques and the most outstanding result was that the D34G/K70A mutant of scPvuII proved to be a nicking enzyme.

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Keywords: scPvuII, nicking enzyme, mutant

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Structural characterization of ANAC019, a member of the NAC family of plant transcription factors

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The NAC proteins constitute a large group of plant specific transcription factors which play important roles in biotic and abiotic stress responses and plant development (Olsen et al, 2005, Trends in Plant Science, 10:79-87). NAC proteins consist of two regions: a conserved N-terminal region (NAC domain) with DNA binding and oligomerization abilities, and a diverse C-terminal region which functions as a transcriptional activator. We have previously determined the first crystal structure of a conserved DNA-binding