binding, which is proposed in Ets1. Finally, in order to illustrate binding specificity against gene promoter, we measured DNA binding affinity of Ets2ETSD to various DNA sequences using the SPR method. Additionally, we investigated alterations of DNA binding mode by building up model structures based on the Ets2ETSD/DNA structure determined in this work. In conclusion, this research indicates specificity and selectivity of DNA binding by Ets2 in the three-dimensional level and explains some transcriptional regulating mechanisms of Ets2.

Keywords: transcription factor structure, DNA-protein complexes, DNA recognition

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Comparison of crystal structures of NF-kB p50/RelB/DNA and p52/RelB/DNA complexes

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NF-kBs constitute a family of transcription activators that modulate the expression of a large number of genes that are responsible for key cellular functions including development, proliferation, survival and inflammation. This family consists of five members, p50, p52, RelA, c-Rel and RelB which share a ~300 residues segment of high sequence homology at or near their N-terminus. This homologous segment, known as the Rel Homology Region (RHR) is critical for nearly all the functions, including DNA binding, dimerization, inhibitor binding and nuclear localization. Unlike the other NF-kB proteins, p50 and p52 lack a transactivation domain but contain inserts within and outside the RHR. These two subunits associate with RelA, c-Rel and RelB to form the predominant NF-kB dimers responsible for gene activation. RelB does not stringently follow the NF-kB family rules. It is the only member that is not known to form a homodimer and has restricted ability to heterodimerize. RelB preferentially forms heterodimers with p50 and p52 in vivo. We describe here the X-ray crystal structures of NF-kB p50/RelB heterodimer and p52/RelB heterodimer bound to the same 10-bp kB DNA. Although p50 and p52 have identical DNA contacting amino acids, these two complexes reveal distinctive base-specific contacts. In the p50/RelB complex, the p50 subunit contacts GGG in the 5 bp half-site and RelB subunit contacts GG in the 4 bp half-site. In the p52/RelB complex, p52 subunit contacts CGG with H62 interact overhang cytosine while RelB subunit bound GGG. The specific binding in these two complexes suggests that RelB may allow the recognition of more diverse kB sequences. Our studies thus provide a basis as to why RelB/p50 and RelB/p52 heterodimers display differential biological regulations.

Keywords: DNA-protein complexes, transcription factor, DNA-packing

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Structural basis for regulation of bifunctional roles of the F-plasmid replication initiator RepE

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RepE is an essential protein for the initiation of the F-plasmid replication, and its initiator activity is fundamentally controlled by interconversion of two molecular association states (i.e., monomer and dimer). The RepE monomers are the replication initiators, binding to iteron DNA sequences of the replication origin. In contrast, the dominant dimeric form of RepE has no initiator activity but functions as an autogenous transcriptional repressor, binding to the promoter/operator region of the *repE* gene, which shares an 8-bp sequence with the iteron. The RepE dimer therefore requires the DnaK molecular chaperone system to be dissociated into monomers and to be activated as initiators. During the past decade, our group determined the crystal structures of RepE in both association forms as RepE-DNA complexes (Komori *et al.*, *EMBO J.* 1999; Nakamura *et al.*, *PNAS* 2007). RepE can be divided into two domains, the N-terminal and C-terminal domains, with a linker connecting them. Both domains include a winged helix-turn-helix motif, and the specific 8-bp of DNA is recognized by the C-terminal domain. Although the N-terminal domain of the monomer also interacts with DNA, that of the dimer does not participate in DNA binding but is involved in RepE dimerization. Surprisingly, the conformations of each domain are similar between the monomer and dimer, while the secondary structure of the domain linker and relative domain orientation differ significantly from each other. Furthermore, there would be interacting areas of DnaK/DnaJ chaperones nearby the domain linker. These structural features suggest that actions of the DnaK system may induce a structural transition to the domain linker and cause a domain rearrangement of RepE, and thereby the dimer must be converted to monomers.

Keywords: DNA replication, conformational change, protein structure and function

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Structural study of the C-terminal domain of DNA gyrase

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Most bacteria harbor two essential type IIA DNA topoisomerases, DNA gyrase and topoisomerase IV (TopoIV). While these two enzymes are highly homologous, they exhibit distinct activities. DNA gyrase supports transcription and replication by introducing negative supercoils into DNA, whereas TopoIV preferentially relaxes positive supercoils and serves as the main decatenating enzyme to facilitate chromosome segregation. Based on crystal structures of the C-terminal domains (CTDs) from Borrelia burgdorferi gyrase (BbGyrA-CTD) and Bacillus stearothermophilus TopoIV (BsTopoIV-CTD), it was proposed originally that the functional divergence of these two enzymes can be attributed to differences in the surface contour of their respective CTDs. Specifically, the DNA-binding surface of gyrase CTD has a steeper curvature and is thus more affective in bending DNA. Surprisingly, later determined crystal structure of the CTD of Escherichia coli gyrase (EcGyrA-

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-