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-