because it plays the central role for all kinds of biological events. It is therefore of crucial importance to elucidate the underlying principles that govern the binding selectivity between proteins and DNA. ColE7 is an Escherichia coli toxin, containing the non-specific endonuclease activity with a slight preference for cleaving after thymine. Here we used the nuclease domain of ColE7 (N-ColE7) as a model system to study the interactions between a protein and DNA molecules. Based on computational methods, we constructed nuclease-ColE7 mutants, K490R, D493N, D493Q and K497R, which were predicted to bind DNA with better affinity. The fluorescence steady-state assays showed that D493N, D493Q and K497R indeed had increased DNA-binding affinity. The foot-printing assays further showed that D493N and D493Q digested DNA with a different sequence preference as compared to the wild-type enzyme. Moreover, the N-ColE7 mutant D493Q was co-crystallized with an 18-bp DNA and X-ray diffraction data were collected at NSRRC BL-13B1. The crystal structure of N-ColE7/DNA complex revealed that Q493 formed two hydrogen bonds with DNA. The extra hydrogen bond contributed to the mutant's higher DNA-binding affinity and altered sequence preference in cleavage. These results show that the combination of crystal structural analysis and computational methods is a useful device for generating a re-designed endonuclease with different properties in DNA binding and cleavage.

Keywords: proteins - DNA interaction, endonuclease, computer redesign

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Structural insights into TDP-43 in nucleic acid binding

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TDP-43 is a nucleic acid-binding protein, localized mainly in nucleus in eukaryotes. TDP-43 was firstly characterized as a transcriptional factor bound to the TAR DNA to repress the transcription of HIV-1 gene. Subsequently, TDP-43 was identified as a RNA-binding protein and plays a role as a splicing repressor in the splicing of CFTR (cystic fibrosis transmembrane conductance regulator) gene. Recently, it was further identified as a pathological protein of the pathogenic inclusions in the brains cells of FTLD-U and ALS patients. To address the role of TDP-43 in DNA/RNA binding, we characterized its nucleic-acid binding activities by filter binding assays and determined the crystal structure of a truncated TDP-43 in complex with a 10-mer DNA at a resolution of 1.65 Å. We found that TDP-43 binds both single-stranded and double-stranded DNA/RNA, and it prefers to bind TG-rich DNA and UG-rich RNA substrates. The crystal structure of the truncated TDP-43 bound to a ssDNA revealed the structural basis for its preference in TG-rich sequences. Structural comparison of TDP-43 to other RNA-binding proteins containing homologous RRM motifs further showed that the truncated TDP-43 was dimerized in a different way.

Keywords: TDP-43, DNA/RNA binding protein, structure

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Crystal structure of CRN-4: Implications for domain function in apoptotic DNA degradation

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Cell death related nuclease 4 (CRN-4) is one of the apoptotic nucleases involved in DNA degradation in Caenorhabditis elegans. CRN-4 contains a N-terminal DEDDh exonuclease domain and a C-terminal domain with unknown function. To reveal the molecular basis of DNA fragmentation in apoptosis, we determined CRN-4's crystal structures in apo-form, Mn^{2+}-bound active form and Er^{3+}-bound inactive form. Comparison of the Mn^{2+}-bound and Er^{3+}-bound CRN-4 structures revealed the geometry of the functional nuclease active site in the N-terminal DEDDh domain. The C-terminal domain bound to a structural zinc ion, termed the Zn-domain, and folded into a novel mixed α/β structure, containing basic surface residues ideal for RNA/DNA recognition. Site-directed mutagenesis further confirmed the catalytic residues in the N-terminal DEDDh domain and the DNA-binding residues in the Zn-domain. The structural comparison of CRN-4 to a number of dimeric DEDDh family nucleases, further demonstrated that CRN-4 not only dimerizes but also interacts with DNA in a unique way. Combining all these data, we suggest a structural model where DNA is bound at the Zn-domain and cleaved at the DEDDh nuclease domain in CRN-4 when the cell is triggered for apoptosis.

Keywords: apoptosis, DNA interaction, DNA-binding proteins

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Crystallization and structure of human flap endonuclease 1, FEN1, in complex with a DNA product

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In lagging strand DNA synthesis and long-patch repair, primers of Okazaki fragments are displaced to form a single-stranded 5'-flap structure by DNA polymerase. Flap endonuclease-1 (FEN1) is a key enzyme to cleave these flaps specifically at the junction between single- and double-stranded regions, thereby producing the nick for ligation. This reaction is an essential step in replication and repair for maintaining genome stability. However, the mechanism by which FEN1 produces a precise nick is unknown. Here we report crystallization and structure determination of human FEN1 in complex with a 3'-flap nicked DNA product. DNA lengths and FEN1 constructs were systematically screened to find crystals suitable for X-ray works. Furthermore, we designed a DNA-protein complex crystallization screening (DPCS) kit for efficient screening. The kit consists of 338 PEG and MPD solutions collected from commercial crystallization screening kits without similar or overlapping conditions. Solutions containing acidic buffers (pH < 5) or divalent ions (zinc, copper and cadmium) were also excluded since FEN1 tends to denature or aggregate under these conditions. The kit was

successful to produce crystals in our screening. Initial diffraction tests were performed using a home-source X-ray generator (Rigaku FR-E) equipped with a Rigaku R-AXIS detector at 100 K. The best crystal was found to diffract up to 2.75 Å resolution. The structure was determined by molecular replacement using our previous structure of human FEN1 in the FEN1-PCNA complex crystal (PDB code 1UL1) as a search model. The structure shows that the enzyme holds both the upstream and downstream duplexes and induces a sharp kink of the DNA by embedding the kinked template strand in a basic cleft.

Keywords: DNA replication, DNA-protein complexes, endonucleases

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Structural studies of the multidrug-responsible transcriptional repressor protein CgmR

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The CgmR protein from Corynebacterium glutamicum is a multidrug-responsible transcriptional repressor belonging to the TetR-family. The crystal structure analyses of CgmR in its three different functional conformations, a DNA-binding form, drug-binding forms, and a drug-free form revealed this protein is inherently in a DNA-releasing conformation. Repressor proteins are molecular switches and dissociate from their bound operator in inducer-binding dependent manner. There are two possible two-state mechanistic models explaining functional cycle of repressors. These models are composed of two functional protein conformations, DNA-binding form and DNA-releasing form. In the first model (Inducing model), the default conformation of its inducer-free condition is the DNA-binding form, and binding of inducers provokes its structural change to dissociate from operator. In an alternative model, the default conformation is the DNA-releasing form, and binding of inducers stabilizes it to prevent changing to the DNA-binding form (Stabilizing model). Functional appearances of these models as transcriptional repressors are identical, however their mechanistic aspects as molecular switches are different each other. Repressors adopting the former inducing model have been known (e.g. TetR), while another model has not been confirmed yet. Our structural study revealed that CgmR adopts the latter stabilizing model, and this is the first example experimentally demonstrating the unconfirmed model. From structural viewpoints, this model adopted by CgmR has innate ability to respond against divergent ligand molecules. The model is consistent with molecular function of the CgmR protein, and also explains functional propensity of other TetR-family proteins as multidrug responsible repressors.

Keywords: crystal structure analysis, multidrug-binding protein, transcriptional regulation

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The C-terminal extension in archaeal and eukaryotic DNA ligases modulates the DNA binding activity

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ATP-dependent DNA ligase encoded by the hyperthermophilic archaeon Pyrococcus furiosus (PfuLig) seals single-strand breaks (nicks) in DNA duplex substrates. The thermostability of this enzyme is exploited in the ligase chain reaction and the ligase detection reaction to distinguish single base mutations associated with genetic diseases. Here we report the identification of an essential residue responsible for the improvement of the ligation activity. PfuLig comprises the N-terminal DNA-binding domain, the middle adenylation domain, and the C-terminal OB-fold domain. The architecture of each domain resembles those of human DNA ligase I (hLig I). The closed form (PfuLig) compared to the substrate-bound open form (hLig I), the helical extension conserved at the C termini in archaeal and eukaryotic DNA ligases appears to play crucial role in stabilizing a closed form without DNA substrate, therefore this helical moiety might affect the substrate-DNA-binding activity. The deletion of extensional helix caused the increased DNA binding activity. But the ligation activity of this mutant became lower than wild type. After the several mutational experiments on the C-terminal helical moiety, we demonstrated that Asp540, one of the selected amino acid residues, is accounted for the improved DNA binding and ligation activity over the optimum temperature.

Keywords: protein engineering and biotechnology, DNA-protein interactions, mutational analysis

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Structural basis for transcriptional regulation mechanisms by the transcription factor Ets2

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The Ets transcription factor family has been known to regulate gene transcription related to cellular differentiation, proliferation, senescence, apoptosis and oncogenic transformation. All family members have highly conserved DNA binding domain, termed the Ets domain (ETSD). To date, three-dimensional structures of some Ets transcription family members complexed with DNA containing their target sequences have been determined. However, the specificity and selectivity of DNA recognition and subsequent transcription by each family member are poorly understood. In this study, we focused on Ets2. Firstly, in order to elucidate DNA recognition mechanism of Ets2, we determined the three-dimensional structure of the ETSD from Ets2 complexed with the DNA containing the Ets2 target sequence. Secondly, we evaluated the kinetic parameters to DNA binding by Ets2ETSD and Ets2ΔN307 including the inhibitory domains flanking at both N- and C-termini of ETSD by the surface plasmon resonance (SPR) method and clarified transcriptional regulation by auto-inhibition mechanism to DNA