of the N and C termini of the domain were found to participate in the domain architecture by forming an extended portion of the first helix alpha-1, and a novel looping motif that traverses straight along the domain surface, respectively. The motifs combine to increase the domain surface of WRN HRDC, which is larger than that of Sgs1 and E. coli. In WRN HRDC, neither of the proposed DNA-binding surfaces in Sgs1 or E. coli is conserved, and the domain was shown to lack DNA-binding ability *in vitro*. Moreover, the domain was shown to be thermostable and resistant to protease digestion, implying independent domain evolution in WRN. Coupled with the unique long linker region in WRN, the WRN HRDC may be adapted to play a distinct function in WRN that involves protein-protein interactions.

Keywords: protein crystallography, DNA repair enzymes, disease

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Macromolecular crystallography at the Penn State X-ray core facility

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The newly established core facility at the Huck Institutes of Life Sciences, Pennsylvania State University, is equipped with state-of-the-art crystallization equipment and X-ray instrumentation and offers crystallography services within and out of Penn State. Visit http://www.huck.psu.edu/facilities/xray-crystallography-up/ for more information. We are facilitating a number of exciting projects from various research groups at Penn State including proteins from the phage T4 DNA replisome, plant cell wall protein, expansin, RNA polymerase from Archaea, RNA dependent RNA polymerase, bacterial enhancer binding proteins, chromatin enzymes and transcription factors. We welcome collaborations from out of Penn State as well. A description of the facility research and the services offered will be presented at the meeting.

Keywords: protein crystallography, protein-DNA complexes, enzyme structure function

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Crystal structure of the Mus81-Eme1 complex

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The Mus81-Eme1 complex is a structure-specific endonuclease that efficiently cleaves the nicked Holliday junction, 3'-flap, and replication fork structures, and plays an important role in rescuing stalled replication forks and resolving the meiotic recombination intermediates in eukaryotes. We determined the crystal structure of the Mus81-Eme1 complex. Unlike previous prediction, both Mus81 and Eme1 consist of a central nuclease domain, two repeats of the helix-hairpin-helix (HhH) motif at their C-terminal region, and a linker helix that restrains the movements of each domain. We show that a flexible intra-domain linker that formed with 36 residues in the nuclease domain of Eme1 is essential for the recognition of DNA. A central groove that is sufficient to bind single-stranded DNA is formed between the nuclease domain of Mus81 and the HhH2 domains of Mus81-Eme1, and the top wall of this central groove functions as a bump for the passage of the 3'-flap or leading strand and directs it to the active site cleft in Mus81. Our structure, in conjunction with FRET and biochemical analysis, explains the basis for substrate preference, specific cleavage at several bases from the 5' end of the downstream, and provides a model for the protein-substrate DNA interaction.

Keywords: Mus81-Eme1, Holliday junction, endonuclease

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Structural studies on the promoter recognition of transcription factor HNF-6

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Hepatocyte nuclear factor-6 (HNF-6), a liver-enriched transcription factor, controls the development of various tissues, such as the pancreas and liver, and regulates the expression of several hepatic genes. This protein belongs to the ONECUT class of homeodomain proteins composed of a single cut domain and a characteristic homeodomain. This transcription factor has two distinct modes of DNA binding and transcriptional activation that use different coactivators depending on the target gene. The homeodomain of HNF-6 is involved in binding to the transthyretin (TTR) promoter, whereas it is not required for binding to the HNF-3β promoter and involved in transcriptional activation. The cut domain is involved in both DNA binding and transcriptional activation at both promoters. At first, we have analyzed the crystal structure of the DNA-binding domain of HNF-6 protein complexed with the TTR promoter DNA[1]. In the complex structure, the two domains, together with the linker region, wrap around DNA and make contact with each other. The structure revealed the DNA recognition mechanism of this protein and the structural basis for the dual mode of action of this protein. Secondly, to examine the two distinct modes of HNF-6 more in detail, we have crystallized the DNA-binding domain of HNF-6 complexed with the HNF-3β promoter.
[1] Structure. 2007 Jan;15(1):75-83.

Keywords: X-ray crystallography of biological macromolecules, DNA-binding proteins, DNA-protein interactions

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Redesign a non-specific endonuclease

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Recognition between proteins and DNA has been studied extensively

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