

sites. The structure reveals a ternary complex in which protein homo-dimerization is mediated by the specific arrangement of the two Ets-1 binding sites and demonstrates how Ets-1 transcription factor dimerizes by forming a central protein/DNA interface that involves several residues from a loop connecting the N-terminal autoinhibitory region and the ETS domain. Ets-1 variants, in which residues involved in protein-protein interaction are mutated, lose the ability for DNA-mediated dimerization and stromelysin-1 promoter transactivation. The X-ray structure of the Ets-1/DNA/Ets-1 complex formed on the stromelysin-1 promoter shows for the first time how Ets-1 transcription factor can function as a homodimer contrary to previous structures where Ets-1 was bound to DNA as a monomer or formed complexes with other transcription factors on DNA. Thus, our data unravel the molecular basis of the ability of Ets-1 to function as a facultative dimeric transcription factor and play an important role in the transcription regulation of the stromelysin-1 promoter.

Keywords: Ets-1, transcription regulation, protein-DNA complexes

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#### Structure of the FOXO3a-DBD/DNA complex suggests the effects of post-translational modification

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FOXO3a is a transcription factor of the FOXO family. The FOXO proteins participate in multiple signaling pathways, and their transcriptional activity is regulated by several post-translational mechanisms, including phosphorylation, acetylation and ubiquitination. Because these post-translational modification sites are located within the C-terminal basic region of the FOXO DNA-binding domain (FOXO-DBD), it is possible that these post-translational modifications could alter the DNA-binding characteristics. To understand how FOXO mediate transcriptional activity, we report here the 2.7 Å crystal structure of the DNA-binding domain of FOXO3a (FOXO3a-DBD) bound to a 13-bp DNA duplex containing a FOXO consensus binding sequence (GTAAACA). Based on a unique structural feature in the C-terminal region and results from biochemical and mutational studies, our studies may explain how FOXO-DBD C-terminal phosphorylation by protein kinase B (PKB) or acetylation by cAMP-response element binding protein (CBP) can attenuate the DNA-binding activity and thereby reduce transcriptional activity of FOXO proteins. In addition, we demonstrate that the methyl groups of specific thymine bases within the consensus sequence are important for FOXO3a-DBD recognition of the consensus binding site.

Keywords: FOXO3a, winged/helix, DNA complex

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#### Structural basis for human mitochondrial DNA polymerase processivity

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Replication of mitochondrial DNA (mitoDNA) is an essential process for maintenance of the molecule which encodes a subset of components for oxidative phosphorylation. The replication is conducted by a nuclear-coded DNA polymerase gamma (Polg) which also has exonuclease activity as proofreading function and dRP lyase activity as repair function. Interestingly, mutations in Polg can cause an impaired mitoDNA replication, which are implicated in human mitochondrial diseases. In addition, human Polg is the target of adverse reactions of anti-HIV reagents and is in part responsible for drug toxicities. To illustrate the structural basis for mitoDNA replication and facilitate rational design of antiviral drugs, we determined crystal structure of human Polg holoenzyme to 3.2 Å resolution. The structure revealed heterotrimer architecture of the enzyme with a monomeric catalytic subunit (Polg A) and a dimeric accessory subunit (Polg B). The two subunits form extensive interaction thereby providing a novel mechanism for high processivity of DNA replication. Polg A folds into three distinct domains, a polymerase (pol) and a nuclease (exo) domains, as well as a spacer domain sandwiched between the above two domains. While the pol and exo domains present high homology with those of other members of DNA Pol I family, the spacer domain shows a unique fold where a large area of subunit interaction are formed. The structure of the spacer domain also provides an explanation for its ability to coordinate the enzymatic activities of pol and exo domains as well as increasing processivity of Polg A. The structural information of Polg would set the stage of further understanding the mechanism for mitoDNA replication as well as mitochondrial toxicity of anti-HIV drug.

Keywords: DNA polymerase gamma, processivity, mitochondria

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#### Crystal structure of the HRDC domain of human Werner syndrome protein, WRN

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Werner syndrome is a human premature aging disorder characterized by chromosomal instability. The disease is caused by the functional loss of WRN, a member of the RecQ-helicase family that plays an important role in DNA metabolic pathways. WRN contains four structurally folded domains comprising an exonuclease, a helicase, a winged-helix, and a helicase-and-ribonuclease D/C-terminal (HRDC) domain. In contrast to the accumulated knowledge pertaining to the biochemical functions of the three N-terminal domains, the function of C-terminal HRDC remains unknown. Recently we determined the crystal structure of the human WRN HRDC domain (Kitano et al. J. Biol. Chem. 2007; 282 2717-28). The domain forms a bundle of alpha-helices similar to those of *Saccharomyces cerevisiae* Sgs1 and *Escherichia coli* RecQ. Surprisingly, the extra ten residues at each

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-3 $\beta$  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-3 $\beta$  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