PS04.05.15 THE CRYSTAL STRUCTURE OF THE HUMAN PAPILLOMAVIRUS 31 E2 DNA BINDING DOMAIN IN THE ABSENCE OF DNA. V. L. Giranda, X. Kong, D. Egan, F. Lindh, T. Holzman, H. S. Yoon, and T. Robins, Abbott Laboratories, Abbott Park, IL 60064

Human papillomaviruses (HPVs) are a causative agent for proliferative epithelial lesions (e.g., warts). Certain HPV serotypes have been causally linked to the development of cervical carcinoma. The papillomavirus E2 gene product is a transacting transcriptional regulator. E2 is required for viral replication and is comprised of a DNA binding and activation domains. Previously only the structure of the bovine papillomavirus (BPV) E2 DNA binding domain bound to DNA has been reported.

The crystal structure of the HPV type 31 E2 DNA binding domain has been solved to 2.5 Å resolution in the absence of DNA. The structure is similar to the BPV E2 DNA binding domain. The monomer is comprised of four beta strands which form a beta sheet as well as two alpha helices. The first helix is the DNA recognition helix. The HPV structure, like that of BPV, shows that the DNA binding domains form a tightly associated dimer, with a four stranded beta sheet from each monomer contributing to an eight stranded beta sandwich at the dimer core. The remaining two helices (per monomer) reside on the outside of the dimer. The DNA recognition helices in the absence of DNA are similar to the those seen in the presence of DNA.

The loop between the second and third beta strand is disordered in the HPV structure (no DNA). In the BPV structure this loop is not disordered and associates with the DNA sugar phosphate backbone. Buried at the center of the eight stranded beta sandwich are four hydrophilic residues, two from each monomer, that coordinate a water or solvent ion. This differs significantly from the BPV where the core is occupied exclusively by hydrophobic residues.

This structure will provide a platform on which to base future structure aided drug design. The aim of this design is to create compounds that will inhibit the replication of HPV.

PS04.05.16 STRUCTURE OF THE LACTOSE OPERON REPRESSOR AND ITS COMPLEXES WITH DNA AND INDUCER. M. A. Kercher*, G. Chang†, N. C. Horton*, P. Lu*, J. H. Miller‡, H. C. Pace*, M. Lewis†, †Department of Biochemistry and Biophysics and *Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA and ‡Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90024, USA

In 1961 Jacob and Monod proposed a model for gene regulation which was widely adopted as a paradigm. The model was based on a "repressor" molecule that had not at that time been isolated but later became known as *lac* repressor. Determination of the 3-dimensional structures of three different forms of the *lac* repressor protein (the intact *lac* repressor, the *lac* repressor bound to the gratuitous inducer isopropyl1-ß-D-thiogalactoside (IPTG) and the *lac* repressor complexed with two 21 base-pair symmetric operator DNA duplex deoxyoligonucleotides) makes the analysis of the mechanism of gene regulation possible. Comparison of the induced and repressed forms of the protein at the dimer level show that a conformational change in the core of the structure results in changes in the distant DNA-binding areas of the protein. Examination of the protein at the tetramer level leads to a model of a 93 base-pair repression loop corresponding to the *lac* operon -82 to +11 region in which *lac* repressor acts with the catabolite activating protein is presented. A correlation of the site-specific mutational analysis with the 3-dimensional structures is also presented.(supported by NIH and US ARO)

PS04.05.17 INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE VIA A SUBSTRATE INHIBITOR COMPLEX: STRUCTURAL IMPLICATIONS. Mary L. Kopka, Mark Filipowsky and Richard E. Dickerson, Molecular Biology Institute, University of California at Los Angles, CA 90095, USA

A bis-linked distamycin drug using m-pyridyl as the linker was studied by kinetics, gel shift and nucleic acid melting point, ΔTm, analysis as an inhibitor of HIV-1 reverse transcriptase (RT). IC50 curves run with enzyme pre-incubated with template-primer (TP) show a 4-fold increase in inhibition over conditions with TP pre-incubated with drug. This fact, coupled with increased binding of TP to RT in response to drug in gel shift assays, and weak binding of the inhibitor to the RNA/DNA TP alone from ΔTm assays, indicates formation of a dead-end ternary complex of drug, enzyme and TP. The kinetics indicate that the inhibition occurs before processive synthesis begins. The drug-bound to the RNA/DNA template-primer is the inhibiting species, i.e. a substrate-inhibitor complex (SI). This SI complex is competitive with TP on the same site in a transition enzyme-TP complex and to a lesser extent on free enzyme. This dead-end ternary transition complex is the main inhibition intermediate.

These results will be interpreted considering the relevant conformational changes the enzyme undergoes immediately prior to and during reverse transcription.

PS04.05.18 CRYSTAL STRUCTURE OF KLENOW-ANALOGOUS FRAGMENT OF *THERMUS AQUATICUS* DNA POLYMERASE I AT 2.5Å RESOLUTION. Sergey Korolev, Murad Nayal, Wayne M. Barnes, Enrico Di Cera, and Gabriel Waksman. Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St Louis, MO 63110, USA

The crystal structure of the large fragment of the *Taq* DNA polymerase (Klentaq1), determined at 2.5Å resolution, demonstrates a compact, two domain architecture. The C-terminal domain is identical in fold to the equivalent region of the Klenow fragment of *Escherichia coli* DNA polymerase I (Klenow Pol I). Although the N-terminal domain differs greatly in sequence from its counterpart in Klenow Pol I, it has clearly evolved from a common ancestor. The structure of Klentaq1 reveals the strategy utilized by this protein to maintain activity at high temperatures and provides the structural basis for future improvements of the enzyme.

Ref:
S. Korolev et al., (1995) Proc. Natl. Acad. Sci. USA 92, 9264-9268.

PS04.05.19 STRUCTURAL ANALYSIS OF THE TBP/TFIIB/DNA COMPLEX FROM THE HYPERTHERMOPHILIC ARCHAEA PYROCOCCUS WOESEI. P. Kosa, B. DeDecker, G. Ghosh, P. B. Sigler, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510

Eukaryotic transcription Factor IIB is necessary for basal or activated transcription. TFIIB forms a complex with TATA-binding protein (TBP) and DNA. The crystal structure of this complex has been solved. TFIIB has been shown to directly interact with TBP, TFIIF, and RNA polymerase II, and is proposed to be involved in mediating activated transcription through an interaction with activators.

Homologs of TBP and TFIIB have been found in several Archaea species, suggesting that Archaea have a eukaryotic like pol II transcriptional apparatus. *Pyrococcus woesei,* an archaea from deep sea thermal vents which exhibits a maximum growth temperature of 110°C, has an A/T rich, TATA-like promoter se-

quence. The amino acid sequence of PwTBP is 40% identical to that of all eukaryotic species, and the TFIIB homolog (TFB) is 30% identical to eukaryotic factors. The homology extends throughout both sequences and the domain architecture of both proteins appears conserved.

In order to extend our structural understanding of the transcriptional preinitiation complex, we have grown crystals of PwTFB in a complex with PwTBP and an archael TATA-box for x-ray analysis. We have generated an MIR map, and we are in the process of tracing and refining the structure of the complex, with data to 2.1Å. Analysis of the Archaea complex may provide insight into the evolution of the transcriptional apparatus, the mechanism of thermostability, and the specificity of assembly of the ternary complex.

PS04.05.20 CRYSTALLIZATION AND PRELIMINARY CHARACTERIZATION OF A HYPERTHERMOSTABLE ARCHEAL DNA POLYMERASE. Chen Mao*, Min Zhou*, James R. Kiefer*, Robecca Kucera**, Maurice Southworth**, Francine Perler**, Lorena S. Beese*. *Duke University Medical Center, Durham NC 27710, **New England Biolabs, Beverly, MA 01915, USA

A DNA polymerase from a hyperthermophillic marine archeon Thermococcus sp strain 9 N-7 has been cloned, overexpressed, and characterized (Southworth, M.W., Kong , H., Kucera, R.B., Ware, J., Jannasch, H.W., and F.B. Perler, P.N.A.S, 1996 in press). The organism was isolated from a hydrothermal vent chimney scraping on the East Pacific Rise near Galapagos Island at 9 N latitude. The purified polymerase has a half life of 6.7 hours at 95 C and can replicate DNA at temperatures in excess of 85 C in vitro. The enzyme has both DNA polymerase and proofreading 3'-5' exonuclease activities. The polymerase is a 775 amino acid, 89.6 kDa protein, which has significant sequence homology to human DNA Polymerase a (Genbank U47108). Structural elucidation of 9 N-7 DNA polymerase may contribute to our understanding of how DNA is replicated with high fidelity at extremely high temperatures. In addition, it may provide the first structural information about a member of the human Pol a class of DNA polymerases.

Three different crystal forms of this polymerase have been grown. All crystals belong to the orthorhombic Laue group Pmmm, but differ significantly in their cell dimensions. Crystal form I (a=65Å, b= 111 Å, c= 152 Å) diffracts to 3.5 Å resolution and is extremely mosaic (more than 2 degrees). Crystal form II (a= 79 Å, b= 84 Å, c= 134 Å) and crystal form III (a= 97 Å, b= 103 Å, c= 113 Å) both diffract to better than 3 Å resolution and have low mosaicity (less than 0.3 degrees). The estimated solvent content of all three crystal forms is between 50-60% with one molecule per asymmetric unit. Native data sets have been collected from each crystal form. Heavy atom derivative screening is currently in progress.

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PS04.05.21 DNA-BINDING DOMAIN OF OmpR, A DIFFICULT STRUCTURE DETERMINATION. Erik Martínez-Hackert*, Helen Berman* and Ann Stock&#, CABM, *Dept. of Chem., Rutgers. Univ., &Dept. of Biochem., UMDNJ & #HHMI

Escherichia coli respond to changes in osmotic pressure by altering the expression of two porin proteins, OmpF and OmpC, located in the outer membrane. The differential expression of the *ompF* and *ompC* genes is regulated by two proteins that belong to the ubiquitous two component family of regulatory proteins, the histidine kinase, EnvZ, and the response regulator, OmpR. Response regulator proteins are characterized by a conserved regulatory domain of approximately 130 amino acids and often contain a C-terminal effector domain. The N-terminal domain catalyzes transfer of a phosphoryl group from the histidine kinase. The activity of the C-terminal or downstream effector domain is controlled by the phosphorylation state of the regulatory domain. Response regulators are in many cases transcription factors with the C-terminal end comprising a DNA recognition domain.OmpR belongs to a family of at least 40 homologous response regulators with a DNA-binding domain of approximately 110 amino acids. This group of transcription factors does not have sequence homology with DNA-binding proteins of known three-dimensional structure, thus a novel DNA-binding motif might be expected. The structure determination of the C-terminal domain of OmpR, OmpRc, should define a protein fold and provide insight regarding DNA recognition and perhaps the mechanism of transcriptional regulation for a large family of transcription factors.

We have obtained two new crystal forms of an OmpR C-terminal fragment that includes amino acids 130-239. Crystals belong to the trigonal spacegroup $P3_n12$ with cell dimensions a=b=54.4 Å, c=130.5 Å and γ=120.00. A second crystal form has been obtained by soaking this crystal form in a cryo-buffer and flash-cooling to 108 K in a liquid nitrogen stream. Crystals belong to the trigonal spacegroup $P3_n12$ with cell dimensions a=b=107.8 Å, c=130.5 Å and γ=120.00. Both crystal forms diffract to at least 2.3 Å at a synchrotron light source.

We have encountered several difficulties during this structure determination: 1) An exhaustive search for heavy atom derivatives did not produce useful derivatives. 2) Cell axis c can vary more then 2%. 3) SeMet data was collected, but Se could not be positioned. We report a strategy to introduce single-site cysteine mutations for use in heavy atom derivatization. We have successfully collected one heavy atom dataset. Structure determination is in progress.

PSO4.05.22 STRUCTURAL BASIS FOR THE REMOVAL AND EXCLUSION OF URACIL FROM DNA. Clifford D. Mol, Geir Slupphaug, Andrew S. Arvai, and John A. Tainer, Dept. of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Rd, La Jolla, CA 92037, Bodil Kavli, and Hans E. Krokan UNIGEN Center for Molecular Biology, University of Trondheim, N-7005 Trondheim, Norway

High resolution crystal structure analysis of human uracil-DNA glycosylase (UDG) in complex with the uracil glycosylase inhibitor (Ugi) protein from the Bacillus subtilis bacteriophage PBS2 reveals the structural basis for the very strong association of Ugi with UDG and suggests a possible mechanism fro UDG binding to uracil-containing DNA. The crystal structure of the UDG-Ugi complex, determined at 1.90 Å resolution, delineates an extensive intermolecular interface that is quite polar and contains a number of buried, bridging water molecules. Ugi does not interact directly with the uracil-binding pocket of UDG, but instead inserts a beta-strand in the DNA-binding groove of UDG and encompassess a protruding, sequence-conserved enzyme loop. Within this enzyme loop, a conserved leucine residue, Leu-272, protrudes out from the enzyme surface and inserts into a hydrophobic pocket between the five-stranded anti-parallel ß-sheet and an α-helix of Ugi.

The central role played by Leu-272 in the enzyme-inhibitor interface suggested a biologic role for Leu-272 in UDG-DNA interactions. We proposed that Ugi is a protein mimic for the DNA substrate upon which UDG acts: a double-straded DNA helix containing an abasic site created when the uracil base "flips-out" into the enzyme active site. A UDG-dsDNA model, created on the