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

basis of the interactions observed in the enzyme-inhibitor complex, implicate the side chain of Leu-272 in active expulsion of uracil from DNA by penetrating the DNA helix from the major groove, with the uracil "flipping out" via the DNA minor groove. Recent results corroborate this hypothesis and suggest a mechanism for UDG recognition of promutagenic GU mismatch base pairs within the context of double-stranded DNA.

PS04.05.23 DNA POLYMERASE II FROM *ESCHERICHIA COLI*. C.R.A. Muchmore, M.M. Blair, L. Shuvalova, M.F. Goodman, W.F. Anderson, Northwestern University Medical School, Department of Molecular Pharmacology and Biological Chemistry, Chicago, Illinois and University of Southern California, Department of Molecular Biology, Los Angeles, California.

Polymerase II from *E. coli* is a member of the group B (α-like) DNA polymerases as indicated by conserved sequence motifs. It contains both polymerase and 3'->5' exonuclease activity on a single polypeptide chain of 781 amino acid residues and is induced by DNA damage. The βγ accessory protein complex of the polymerase III holoenzyme increases polymerase II processivity, raising questions about the nature of this interaction. Information about structure and function derived from the atomic structure of *E. coli* polymerase II may be applicable to other homologous group B polymerases like mammalian cellular DNA polymerase α, eucaryotic viral polymerases and bacteriophage T4 polymerase.

Polymerase II crystallizes by sitting drop vapor diffusion with PEG 400 and citrate as precipitating agents at pH 5.8. Three heavy atom derivatives were produced under these conditions. Recently, the crystals have been successfully transferred to solutions of lower ionic strength and higher pH thus improving conditions for derivatizing crystals with heavy atom compounds.

X-ray diffraction data have been collected from the wild type polymerase II crystals at room temperature and at 103K from flash-frozen crystals. The crystals are of space group $P2_12_12$ with one monomer per asymmetric unit. Initial phases for the wild type data were obtained by MIR methods. Phases were improved and extended to higher resolution by model building, phase combination and density modification techniques.

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PS04.05.24 REFINEMENT STRATEGIES FOR PROTEIN-NUCLEIC ACID COMPLEXES. G. N. Parkinson, B. Schneider, J. Vojtechovsky, R. H. Ebright, and H. M. Berman, Department of Chemistry, Rutgers University, Piscataway, New Jersey 08855.

Protein-DNA complex crystals offer an obvious path towards visualizing modes of recognition, stabilization, interaction, and conformation of nucleic acids and proteins. We are studying a series of such crystalline complexes containing Catabolite Activating Protein (CAP) and DNA with particular emphasis on understanding the detailed interactions between the amino acid side chains and the nucleic acid bases. To do this, it is necessary to exercise special care with the refinement process.

In order to achieve improved results, we created a nucleic acid parameter file (Parkinson, et al., Acta Cryst. D52, 57 1996) for use with X-PLOR that incorporated newly determined standard values for the bond distances, bond angles, and dihedral angles. More recently we have expanded the parameter file to include dihedrals for three DNA conformational classes.

The strategies for refinement of these complexes that allow for the correct balance between the macromolecular components will be discussed.

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PS04.05.25 THE BATTLE FOR IRON - CRYSTALLOGRAPHIC STUDIES OF THE IRON DEPENDENT REPRESSOR PROTEINS FROM *C. DIPHTHERIA* AND *M. TUBERCULOSIS*. Ehmke Pohl[a], Xiayang Qiu[a], Randall K. Holmes[c] & Wim G.J. Hol [a,b]. [a]Biomolecular Structure Center and [b]Howard Hughes Medical Institute, University of Washington, Box 357742, Seattle, WA 98195, USA; [c]Department of Microbiology, University of Colorado, Health Sciences Center, Denver, CO 80262, USA.

Iron is an essential nutrient for most virulent bacteria, however the availability of free iron in the mammalian hosts is extremely limited. A number of pathogens utilize the low-iron environment as a signal for the expression of virulence factor and proteins involved in obtaining iron from the host organism.

In *Corynebacterium diphtheria* the Diphtheria Toxin Repressor (DtxR) is activated at about 2 mM Fe^{2+}. The active dimeric protein binds specifically the *tox* and *irp* operators which encode the tox gene and the irp genes. The crystal structure of DtxR has recently been solved at 2.8 Å resolution [1]. High resolution data of DtxR in complex with different divalent metals have been collected at low temperature using conventional X-ray sources and synchrotron radiation. The metal binding sites have been unraveled in greater detail than previously reported.

A functionally homologous repressor from *Mycobacterium tuberculosis* has been cloned, expressed in *E. coli* and purified. This iron dependent repressor (IdeR) shares 60% sequence identity with DtxR [2]. Crystallization and preliminary analysis by X-ray crystallography will be presented.

[1] X. Qiu, C.L.M.J. Verlinde, Z. Zhang, M.P. Schmitt, R.K. Holmes & W.G.J. Hol (1995) *Structure* **3** 87-100.

[2] M.P. Schmitt, M. Predich, L. Doukhan, I. Smith & R.K. Holmes (1995) *Infect. Immun.* **63** 4284-4289.

PS04.05.26 STUDIES TOWARDS THE X-RAY CRYSTAL STRUCTURE OF HELICASES. Christopher Putnam and John Tainer, The Scripps Research Institute, Molecular Biology, MB4, La Jolla, California 92037

DNA helicases are an important, structurally uncharacterized class of biological molecular motors that use the energy of nucleotide triphosphate hydrolysis to the unwinding of nucleic acid double helicies. The function of helicases in general are critical in the synthesis, maintence and repair of the genome of all organisms. The RuvB DNA helicase from Escherichia coli has been chosen as a starting point for investigation of this class of enzymes. RuvB is a relatively small DNA helicase that forms homohexamers around DNA. In vivo, the ATPase activity of RuvB is responsible for migration of Holliday junctions during genetic recombination. Preliminary diffraction data will be presented.