

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 archaeal 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 2.1 Å. 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 hyperthermophilic marine archeon *Thermococcus* sp strain 9 N-7 has been cloned, over-expressed, 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 proof-reading 3'-5' exonuclease activities. The polymerase is a 775 amino acid, 89.6 kDa protein, which has significant sequence homology to human DNA Polymerase  $\alpha$  (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  $\alpha$  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=65 Å, 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. Re-

sponse 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  $P3_112$  with cell dimensions a=b=54.4 Å, c=130.5 Å and  $\gamma=120.00$ . 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  $P3_112$  with cell dimensions a=b=107.8 Å, c=130.5 Å and  $\gamma=120.00$ . 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 than 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.

**PS04.05.22 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 for 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 encompasses 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  $\beta$ -sheet and an  $\alpha$ -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-stranded 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