

MS04.05.04 STRUCTURES OF NASCENT DUPLEX DNA BOUND TO A THERMOSTABLE DNA POLYMERASE AT 1.9 Å RESOLUTION. Lorena S. Beese, Chen Mao, James R. Kiefer, Stephen B. Long, and Jeff Braman*. Dept. of Biochemistry, Duke University Medical Center. *Stratagene Inc., La Jolla, CA 92037

Co-crystal structures of a thermostable DNA polymerase from a newly identified strain of *Bacillus spp* with two different DNA primer-templates bound in the active site were determined at 1.9 Å resolution. This is the first crystal structure of a DNA polymerase in the Pol I family with duplex DNA bound at the polymerase active site. We were able to deduce the direction of DNA synthesis by comparing the structures of a primer template complex and an extended primer template complex in which an additional nucleotide was added by the polymerase in the crystal. The 3' hydroxyl of the primer strand interacts with a highly conserved, catalytically important aspartate. The duplex DNA adopts a primarily B-form conformation, however the minor groove widens as the DNA enters the polymerase cleft. No bend in the DNA is necessary to reach the polymerase active site. A network of hydrogen bonds is made between the sugar-phosphate backbone of the DNA base pairs and highly conserved residues of the protein. The direction of DNA synthesis is consistent with the model proposed based on the editing complex of the *E. coli* Klenow fragment. These observations unambiguously resolve a recent controversy about the direction of DNA synthesis in the Pol I class of DNA polymerases.

The crystal structure of the apo DNA polymerase was determined by the method of multiple isomorphous replacement including the anomalous scattering data from two heavy atom derivatives. The R-factor of the refined structure is 19.6% between 8 Å and 2.2 Å resolution (R_{free}=25%) with 0.010 Å rms deviation in bond lengths and 1.5 ° rms deviation in bond angles. The structures of the DNA complexes were determined by molecular replacement using the apo structure as a starting model. These structures are refined at 1.9 Å resolution to an R factor of 19.9% (R_{free}=25%) with 0.009 Å rms deviation in bond lengths and 1.5 ° rms deviation in bond angles.

This work is supported in part by grants from the American Cancer Society (LSB) and the Searle Scholar program (LSB).

MS04.05.05 STRUCTURAL STUDIES OF TYPE I DNA TOPOISOMERASES Alfonso Mondragón, Christopher Lima, Neal Lue, Alexandra Patera, and Amit Sharma. Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208-3500, USA

DNA topoisomerases are proteins responsible for controlling and maintaining the topological state of DNA in the cell. They have been found in all cell types of both eukaryotes and prokaryotes and additionally in some viruses. They are involved in DNA replication, transcription, and genetic recombination. All topoisomerases work by forming a transient break in DNA through a phosphotyrosine bond. Type I topoisomerases break one DNA strand at a time and then pass another strand through the transient break. No external energy source is required for this reaction as the bond energy is conserved.

We have identified and crystallized a 67 kDa domain of *Escherichia coli* DNA topoisomerase I containing the catalytic tyrosine that is capable of cleaving single stranded DNA. The structure of the 67 kDa fragment has been determined and refined to 1.9 Å resolution. The fragment shows a novel structural fold, with the amino and carboxy termini forming a large, globular domain and the central region forming a torus inside which DNA may bind. We have also solved the structure of the intact *E. coli* DNA

topoisomerase III, a close relative of topoisomerase I. The two structures show clear structural similarities and probably share similar mechanism of action.

The structure of *E. coli* DNA topoisomerase I has provided a wealth of information on prokaryotic-like type I DNA topoisomerases. To further our understanding of the other major sub-family of type I topoisomerases, we have solved the structure of a 27 kDa fragment of *S. cerevisiae* DNA topoisomerase I and of a 9 kDa amino terminal fragment of vaccinia virus DNA topoisomerase I. The structure of the former shows a novel architecture with two domains linked by a pair of alpha helices forming an elbow and a polyproline helix. Together with new biochemical data, the structure suggests that this fragment is directly involved in interactions with DNA. The structure of the vaccinia virus fragment forms a five stranded beta-sheet with two short alpha helices. The lack of structural similarity with the *S. cerevisiae* structure clearly suggests that these two proteins may not be related.

MS04.05.06 THE STRUCTURE OF THE YEAST TELOMERIC PROTEIN RAPI IN COMPLEX WITH DNA: HOW TELOMERIC DNA SEQUENCES ARE RECOGNISED. D. Rhodes, P. Konig, R. Giraldo and L. Chapman, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Telomeres are the specialised nucleoprotein complexes that form the termini of eukaryotic chromosomes. These structures are essential for chromosome stability, are sites of transcriptional silencing and are required for the association and organisation of chromosomes within the nucleus.

In the yeast *S. Cerevisiae* the telomeric DNA is packaged by the non-histone protein RAPI (1). RAPI is a particularly interesting protein since it can bind both double stranded DNA and DNA-quadruplexes (2). In order to understand how RAPI binds to the unusual sequences of telomeric DNA we have solved the crystal structure of the DNA-binding domain of RAPI bound to an 18bp telomeric DNA fragment at 2.25 Å resolution. The protein contains two similar domains that bind DNA in a tandem orientation, recognising a tandemly repeated DNA sequence. The domains are structurally related to the homeodomain and Myb motif, but show novel features in their DNA-binding mode. This structure provides the first insight into the recognition of the conserved telomeric DNA sequences by a protein (3).

1. Shore, D. (1994) Trends Genet. 10:408
2. Giraldo, R. and Rhodes, D. (1994) EMBO J. 13:2411
3. Konig, P., Giraldo, R., Chapman, L. and Rhodes, D. (1996) Cell, April 1996.

MS04.05.07 STRUCTURE AND FUNCTION OF THE DNA REPAIR ENZYME 3-METHYLADENINE DNA GLYCOSYLASE II. ¹Y. Yamagata, ¹M. Kato, ¹Y. Tokuno, ¹Y. Nakashima, ¹K. Yasumura, ¹K. Odawara, ¹N. Matsushima, ¹K. Tomita, ²Y. Fujii, ²K. Ihara, ²Y. Nakabeppu, ²M. Sekiguchi, ¹S. Fujii, ¹Faculty of Pharmaceutical Sciences, Osaka University, ²Medical Institute of Bioregulation, Kyusyu University

Escherichia coli 3-methyladenine-DNA glycosylase II, the product of the *alkA* gene, is induced during adaption to alkylation and catalyzes the release of various alkylated bases, in addition to 3-methyladenine, from DNA damaged by carcinogenic and/or mutagenic alkylating agents. We have determined the structure of 3-methyladenine-DNA glycosylase II (AlkA) at a 2.3 Å resolution by the multiple isomorphous replacement method. The enzyme consists of three domains, one $\alpha+\beta$ fold domain (domain I, residues 1 - 85) and two all α -helical domains (domains II, residues 105 - 235 and III, residues 86 - 104 and 236 - 282). The $\alpha+\beta$ domain is topo-

logically the same to one half of the DNA binding domain of the eukaryotic TATA box binding protein which has the two structural domains related by the intramolecular pseudodyad symmetry. The fold of secondary segments of the two all α -helical domains is identical with that of *E. coli* endonuclease III which acts both as an N-glycosylase, removing oxidized pyrimidine from DNA and a 3' apurinic/apyrimidinic lyase. When mapped onto the AlkA structure, the sequences of *Bacillus subtilis* and *Saccharomyces cerevisiae* 3-methyladenine-DNA glycosylases show that sequence-conserved residues cluster in and around a cleft between domains II and III. The mutagenic and model-building studies suggest that the active site is located in the cleft and the interaction between the target base and the active site requires the target base flipping out from a double-stranded DNA. The structure of the active site shows the implication of the recognition for a diversity of substrates.

MS04.05.08 STRUCTURAL ANALYSIS OF THE TBP/TFIIA/TATA COMPLEX. J. H. Geiger, S. Hahn, P. B. Sigler, Yale University, New Haven, CT 06510

The goal of this project is to investigate structure-function relationships of eukaryotic transcription initiation. For RNA polymerase II, there are a host of general transcription factors that must associate at the promoter with the polymerase before transcription initiation begins. Some are required for the initiation of all genes (TFIID, TFIIB) *in vivo* and *in vitro*, while some are required on a subset of promoters, or serve to activate transcription, but are not necessary for basal levels of *in vitro* transcription. TFIIA falls in the latter category. An essential gene in yeast, TFIIA counteracts the effects of transcriptional repressors and may be involved in the activity of transcriptional activators. It increases the affinity of TBP to the TATA box by three orders of magnitude, and extends the TBP footprint on DNA by seven base pairs in the upstream direction.

We have crystallized and solved the structure of a complex of TBP/TFIIA/TATA to 3.0 angstroms. We are using this structure to elucidate the stereochemical determinants of this assembly and to give insight into the interaction of this complex with the rest of the basal machinery and its possible interaction with activators and repressors of transcription initiation.

PS04.05.09 STRUCTURE-FUNCTION RELATIONSHIPS IN DNA LIGASE FROM THE BACTERIOPHAGE T7. Stephen R. Ashford, Aidan J. Doherty, Hosahalli S. Subramanya and Dale B. Wigley, Laboratory of Molecular Biophysics, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

DNA ligases are ubiquitous enzymes, necessary for DNA replication and some forms of repair. The enzymes can easily be divided into two groups, and although they all catalyze essentially the same reaction - the sealing of nicks in the DNA backbone via an adenylated enzyme intermediate - they require different co-factors, and possess quite different primary sequence. The eukaryotic and viral DNA ligases require ATP, whereas the bacterial enzymes utilize NAD⁺. All ATP-dependent DNA ligases share a common core structure - as shown by sequence alignments, and therefore the T7 enzyme (one of the smallest in this class) was selected for structural and biochemical analysis.

The enzyme is a monomer of 41 kDa, and the crystal structure at 2.6Å resolution has been solved, with and without ATP bound. The enzyme consists of a larger N terminal domain (residues 1 to 240) containing the co-factor binding pocket and site of adenylated enzyme intermediate formation, and a C terminal domain (240 to 349), which is remarkably similar to the oligonucleotide binding fold (OB fold) found in many proteins including staphylococcal nuclease, and bacterial cold shock protein. Further biochemical study of these two domains, in conjunction with the structure, lead to a number of conclusions about the DNA binding and specificity of the enzyme.

PS04.05.10 DNA POLYMERASE FIDELITY: A STRUCTURAL ANALYSIS. M.M. Blair, N. Mashhoon, C.R.A. Muchmore, M.F. Goodman, and W.F. Anderson, Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL 60611 and Department of Molecular Biology, USC, Los Angeles, CA 90089

E. coli DNA Polymerase II (pol II), an 89.9 kD, α -like DNA polymerase, possesses both polymerase and 3'-5' exonuclease activities on a single polypeptide chain. Pol II is induced in response to DNA damage as part of the SOS regulon in *E. coli* and is required for synthesis past abasic lesions in the absence of heat shock proteins. Recent *in vivo* studies suggest that replacing wild type with exonuclease deficient pol II leads to increased adaptive mutation frequency indicating an important role for pol II in replication fidelity in the cell. To better understand the mechanism of fidelity, we are using x-ray crystallography to study mutants of pol II and their complexes with DNA.

Mutants of pol II, D155A/E157A (exo-) and L423M, were constructed. These mutations are in highly conserved regions of Pol II involved in nucleotide/metal binding. Pol II exo- has wild type levels of polymerase activity but lacks exonuclease proofreading activity. The L423M mutant has wild type levels of polymerase and exonuclease activities but partitions preferentially towards polymerization in the presence of a mispaired primer terminus suggesting an alteration in switching between the polymerase and exonuclease sites.

Both mutants have been crystallized by vapor diffusion methods. Like Pol II, Pol II exo- crystallizes in the P2₁2₁2 spacegroup and both room temperature and low temperature data sets have been collected. Initial phasing of pol II exo- was by low resolution, modified MIR phases from room temperature pol II data. Further work is underway to improve phases via derivative searches, model building, and density modification.

Pol II wild type and exo- mutant have also been co-crystallized with synthetic double-stranded DNA oligonucleotides. Further work is in progress.

This work is supported by NIH Grant GM15075, GM48569 and ES05355.

PS04.05.11 DNA-SPECIFIC BINDING BY HIN AND FIS. Thang Chiu, Reid Johnson, Richard E. Dickerson, MBI, UCLA, CA 90095

We are interested in learning how dna-binding proteins recognize their target sequences. One system of particular interest is the Hin/Fis system of recombination. Both proteins bind their target sequences via a Helix-Turn-Helix. Hin belongs to a family of proteins that catalyses site-specific DNA inversion in enteric bacteria. Its binding site consists of a highly conserved inverted repeat of 12-bp separated by a central 2-bp 'core'. (consensus sequence: A/T G G T T T A/T G G A G/T A A) The availability of comprehensive mutagenesis data of the binding sites makes it a highly attractive system for studying protein-dna interactions. The crystal structure of a 52 aa peptide consisting of the dna-binding-domain of *E. coli* Hin bound to the hixL half-site tGTTTTTGATAAGA/aTCTTATCAAAAAC has been solved (Feng et al, Science '94). We are interested in solving the crystal structures of Hin bound to various mutant binding sites in order to understand the mechanism by which its dna-binding specificity is determined.

Fis (Factor for Inversion Stimulation) is a recombinational activator of the Hin family of dna invertases and is also involved in phage lambda site-specific recombination, in transcriptional activation of rRNA and tRNA operons, in repression of its own synthesis, and in oriC-directed DNA replication. Although it is a