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 A and 2.2 Å resolution (Rfree=25%) with 0.010 A 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% (Rfree=25%) with 0.009 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 1.9Å 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 RAP1 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 RAP1 (1). RAP1 is a particularly interesting protein since it can bind both double stranded DNA and DNA-quadruplexes (2). In order to understand how RAP1 binds to the unusual sequences of telomeric DNA we have solved the crystal structure of the DNA-binding domain of RAP1 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-METHLYADENINE DNA GLYCOSYLASE II. [1]Y. Yamagata, [1]M. Kato, [1]Y. Tokuno, [1]Y. Nakashima, [1]K. Yasumura, [1]K Odawara, [1]N. Matsushima, [1]K. Tomita, [2]Y. Fujii, [2]K. Ihara, [2]Y. Nakabeppu, [2]M. Sekiguchi, [1]S. Fujii, [1]Faculty of Pharmaceutical Sciences, Osaka University, [2]Medical 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 α+β 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 α+β domain is topo-