PS04.05.27 PROGRESS IN THE STRUCTURE DETERMINATION OF A HUMAN TOPOISOMERASE I - DNA COMPLEX. Matthew R. Redinbo[1], Lance Stewart[1], Ehmke Pohl[1], Xiayang Qiu[1], James J. Champoux[3], and Wim G. J. Hol[1,2], [1]Department of Biological Structure and [2]Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, [3]Department of Microbiology, University of Washington, Seattle, WA 98195

Progress in the structure determination of a complex of human topoisomerase I and double-stranded DNA will be described. Topoisomerases are a class of ubiquitous enzymes that control the level of DNA supercoiling in cells and are vital for transcription, translation and recombination. Topoisomerase I breaks a single strand of duplex DNA, forms a transient protein-DNA complex via a phosphotyrosine bond, and allows relaxation of superhelical strain about the intact DNA strand. Eukaryotic and prokaryotic topoisomerase I enzymes appear to be distinct and to have unique mechanistic characteristics. Human topoisomerase I is a 765 amino acid (91 kD) enzyme made up of four domains. Human topoisomerase I has been shown to be the sole target of the anti-cancer compound camptothecin and of camptothecin derivatives, and is thus of significant medical interest. Elevated levels of topoisomerase I have been identified in several human cancer cell types. We have obtained diffraction-quality crystals of a complex of a recombinantly expressed 70 kD portion of human topoisomerase I which includes the active site and double-stranded DNA. Data to 2.8 Å resolution has been collected at -170° C from native crystals and from crystals containing an iodinated DNA oligonucleotide. Structure determination is in progress using the multiple isomorphous replacement method.

PS04.05.28 CRYSTALLOGRAPHIC STUDIES OF THE ARCHAEAL INTRON ENCODED ENDONUCLEASE I-DmoI*I*. George H. Silva, Jacob Z. Dalgaard, Marlene Belfort, Patrick Van Roey, Wadsworth Center, New York State Dept. of Health, Albany, NY 12201-0509, USA

I-DmoI*I*, is a 22-kDa endonuclease encoded by an intron in the 23S rRNA gene of the hyperthermophilic archaeon *Desulfurococcus mobilis*. The endonuclease is thermostable with peak activity in the 65-75° C range *in vitro* and is capable of cleaving the intronless allele of the 23S rRNA gene. It recognizes an asymmetric target sequence and makes a staggered double-strand cut proximal to the intron insertion site generating 4-nucleotide 3'-OH extensions. A minimal binding site of 14-20 base pairs has been determined. I-DmoI*I*, requires a Mg^{++} co-factor for catalysis, but will readily bind DNA in its absence. I-DmoI*I*, contains the common LAGLI-DADG motif found in eukaryotic intron endonucleases. The motif consists of two twelve amino-acid sequences separated by about 100 non-essential residues. This motif is seen in proteins associated with DNA cleavage, RNA maturation and protein processing. Crystals of I-DmoI*I*, have been grown by the hanging drop method in PEG 3350, lithium sulfate and Tris.HCl pH 8.5. These crystals belong to the monoclinic space group C2 with unit cell dimensions a = 96.57 Å, b = 37.69 Å, c = 56.72 Å, β = 114.09° and V = 206,445 $Å^3$. Assuming two molecules per asymmetric unit yields a $V_M \approx 2.35$ $Å^3/D$, corresponding to about 48% solvent content. Native diffraction data have been measured to 2.3 Å resolution using flash cooling techniques. In addition, three possible derivative data sets have been obtained and are currently being used in phasing. Progress in the structure determination will be presented.

PS04.05.29 CRYSTAL STRUCTURAL ANALYSIS OF THREE SURFACE MUTANTS OF THE GENE V PROTEIN OF M13. ShaoYu Su, YiGui Gao, Howard Robinson, ++Hong Zhang, +Thomas C. Terwilliger, Andrew H.-J. Wang, Division of Biophysics & Dept. of Cell Structural Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, +Division of Life Science & Structural Biology, Los Alamos National Laboratory, Los Alamos, NM 87545

The gene V protein (GVP) from the bacteriophage M13 is a single-stranded DNA binding protein which is a homo dimer of 87 amino acids. The high resolution structure of the M13 GVP has recently been determined by the multiwavelength anomalous diffraction method. In addition, the structure of the Y41H mutant has been determined at 1.7 Å resolution. On the basis of the three dimensional structures and the crystal packing interactions of both the wild-type and Y41H GVP, a model has been proposed to explain the cooperative nature of its binding to ssDNA. The model suggests possible involvements of many surface amino acids either in the binding to DNA, or the protein-protein interactions in the GVP-ssDNA complex. In this work, we present the crystal structures of three mutants involving surface amino acids, L32R, K69H and R82C. The hydrophobic L32 residue is converted into a basic arginine, whereas the basic K69 and R82 are converted into somewhat hydrophobic histidine and cysteine respectively. The diffraction data of these three mutants have been collected to 1.9, 2.0 and 2.0 Å, respectively. The structural refinements are in progress and the results will be reported.

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PS04.05.30 THE THREE DIMENSIONAL STRUCTURE OF AN ATP DEPENDENT DNA LIGASE FROM BACTERIOPHAGE T7. Hosahalli S. Subramanya, Aidan J. Doherty, Stephen R. Ashford and Dale B. Wigley, Laboratory of Molecular Biophysics, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

DNA ligase is a vital enzyme which is required for important cellular processes such as DNA replication, repair of damaged DNA and recombination. The enzyme mediates the formation of phosphodiester bonds between adjacent 3'-OH and 5'phosphate termini, thereby joining the nicks in the double stranded DNA. Ligases can be classified into two groups depending on their requirement for ATP or NAD^+ as the cofactor. All eukaryotic enzymes and virally encoded enzymes are ATP-dependent, whereas prokaryotic enzymes require NAD^+ for their activity. DNA ligase from bacteriophage T7 is a monomer with a molecular weight of 41 kDa. Here we report the structure of this enzyme at 2.6 Å resolution.

The protein was crystallized by vapour diffusion method using hanging drops. Crystals belonged to the space group $P2_12_12$ with unit cell dimensions a=65.8 Å, b=86.3 Å, c=78.3 Å. The structure was solved by MIR using mercury and selenomethionine derivatives. Non-isomorphism between the crystals was a major problem in structure determination. Crystals were found to be grouped around three major forms. The non-isomorphism between the crystal forms was sufficient to allow density averaging between them to improve the electron density maps.

The structure consists of two distinct domains, a larger N-terminal domain (residues 2-240) and a C-terminal domain (residues 241-349). The N-terminal domain is an α/β structure and comprises of three mainly antiparallel ß-sheets surrounded by six α-helices. The ATP-binding site is situated in this domain in a pocket beneath one of the β-sheets. The C-terminal domain con-

sists of highly twisted antiparallel β–sheet and a single α-helix running along one edge of the sheet. The structure of this domain is remarkably similar to the oligonucleotide binding fold, observed in a number of proteins including staphylococcal nuclease, bacterial cold shock protein and gene V single-strand DNA-binding protein. The DNA-binding site is proposed to be in a groove running between the two domains.

PS04.05.31 X-RAY CRYSTALLOGRAPHIC STUDIES OF A COMPLEX OF MMLV REVERSE TRANSCRIPTASE WITH NUCLEIC ACID. Dunming Sun, Sven Jessen, Millie Georgiadis. Waksman Institute & Department of Chemistry, Rutgers University, Piscataway NJ 08855

A complex of a catalytic fragment of Moloney murine leukemia virus (MMLV) reverse transcriptase (RT) and nucleic acid was studied by X-ray crystallography. RTs, encoded by all retroviruses such as MMLV, RSV and HIV-1, have RNA- and DNA-directed DNA polymerase activities and RNase H activity. The activities of RTs are essential in the retroviral life cycle to make a double-stranded DNA from the single-stranded RNA genome of the retrovirus. Structural studies of MMLV-RT/DNA complex will help understand the mechanism of polymerization by RT and therefore contribute to RT-targeted drug design against AIDS. In this report, the 30 KDa catalytic fragment was obtained by limited trypsin proteolysis of a truncated form of the RT enzyme which lacks the RNase H domain. Complex crystals were grown in PEG4000 by hanging drop and sitting drop methods. Microseeding and macroseeding were applied to make crystals suitable for X-ray crystallographic studies. The crystals diffracted to 1.9 Å at NSLS synchrotron source. The space group was determined to be P21 and the unit cell to be a=62 Å, b=39 Å, c=136 Å, β=102°. Initial phasing has been obtained from a 5'-iodo-uracil substituted DNA derivative.

PS04.05.32 A HIGH RESOLUTION STRUCTURE OF AN *Eco*RV-DNA COMPLEX. Mark P.Thomas, E.Louise Hancox, Stephen E.Halford & R.Leo Brady.
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Structures of EcoRV complexed to substrate and product DNA have previously been refined to 2Å (Kostrewa & Winkler, 1995). On the basis of these structures and kinetic data (Vipond et al, 1995, Baldwin et al, 1995) a model of the transition state with two metals bound to the scissile phosphorane group has been proposed.

We are now studying EcoRV complexed with a series of altered DNA substrates. Studies of the influence of the phosphate backbone on recognition and hydrolysis of DNA by EcoRV have utilised diastereoisomeric phosphorothioate DNA analogues (Thorogood et al, 1996). The rate of hydrolysis is dependent on the position of the phosphorothioate moiety in the recognition sequence and on the diastereoisomer. By determining crystal structures of EcoRV co-crystallised with each of these phosphorothioate DNA analogues we hope to provide a firmer structural basis to explanations of the data pertaining to reaction kinetics and substrate recognition and specificity.

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PS04.05.33 CRYSTAL STRUCTURE OF THE DNA GYRASE B PROTEIN FROM *B. stearothermophilus*. F. T. F. Tsai[1], H. S. Subramanya[1], J. A, Brannigan[2], A J. Wilkinson[2],T. Skarzynski[3], O. M. P. Singh[3], A. J. Wonacott[3], and D. B. Wigley[1], [1]Laboratory of Molecular Biophysics, Rex Richards Building, Oxford University, Oxford OX1 3QU, UK, [2]Dept. of Chemistry, York University, Heslington, York YO1 5DD, UK, [3]Department of Biomolecular Structure, Glaxo Wellcome Research and Development Ltd. Medicine Research Centre, Stevenage SG1 2NY, UK

Topoisomerases are DNA-binding proteins that are found in all living organisms. They catalyse the interconversion of different topological forms of DNA by breaking, passing and resealing duplex DNA, and thereby alter the DNA superhelicity in the cell; a process which is essential in DNA replication.

Bacterial DNA gyrase is a type II DNA topoisomerase which uniquely catalyses the negative supercoiling of closed circular DNA *in vitro* utilising the free energy released by ATP hydrolysis. The protein from *B. stearothermophilus* is a heterotetrameric enzyme of 334kDa molecular weight, that consist of two pairs of subunits A (GyrA, 97kDa) and B (GyrB,70kDa). Enzymatically, the larger GyrA subunit is responsible for the DNA breakage and religation activity, while the smaller GyrB protein is associated with the ATP binding and hydrolysis activity.

The recent structural information obtained of eukaryotic and prokaryotic type II topoisomerase fragments suggested a functional mechanism for type II topoisomerases. However, it is still unclear why gyrases, in contrast to eukaryotic type II topoisomerases, are able to catalyse the negative supercoiling of closed circular DNA.

The intact GyrB protein from *B. stearothermophilus* has been purified by standard chromatographic techniques to homogeneity and has been crystallised by dialysis in the presence ADPNP. The crystals belong to the cubic space group I23, with unit cell dimensions a = 249 Å and one dimer in the asymmetric unit (Vm = 4.7Å^{3}Da^{-1}). The structure has been solved to 4.2Å resolution using molecular replacement and isomorphous replacement methods. The collection of high resolution data are currently underway.

PS04.05.34 CRYSTAL STRUCTURE OF THE DNA-BINDING DOMAIN OF MBP1, A TRANSCRIPTION FACTOR IMPORTANT IN PROGRESSION FROM G1 TO S PHASE. Rui-Ming Xu, Christian Koch*, Kim Nasmyth*, Xiaodong Cheng, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA and *Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria

In the *Saccharomyces cerevisiae* cell cycle, most genes invloved in DNA synthesis are transcriptionally activated exclusively in late G1 and early S. Their transcription depends on an 8-base pair asymmetric element (ACGCGTNA, where N=T or C) containing an *Mlu*I restriction enzyme site called the *Mlu*I cell-cycle box or MCB. A transcription factor called MBF (MCB binding factor) is implicated in driving the expression from MCB element. MBF is a heteromeric complex composed of a regulatory protein, Swi6, and a sequence-specific DNA-binding protein, Mbp1.

We have crystallized and solved the structure of the N-terminal 124-amino acid DNA-binding domain of Mbp1. The protein crystallizes in the tetragonal space group $P4_12_12$ with unit cell dimensions of 43.5 Å x 43.5 Å x 124.36 Å, and the crystals diffract X-rays to 1.7 Å resolution. The structure was solved by using the selenomethionine multiwavelength anomalous dispersion method. The structure contains a helix-turn-helix DNA-binding motif with a short β-strand N-terminal to the motif and a β-hairpin C-terminal to the motif. This arrangement of secondary structural elements is also found in other known structures of helix-turn-