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. Dumming 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 by RT and therefore contribute to a helix-turn-helix DNA-binding motif. Current studies on the structure of a complex of RT with DNA analogues we hope to provide a firmer structural basis to DNA-directed DNA polymerase activities and RNase H activity. Microseeding and macroseeding were applied to make crystals by RT and therefore contribute to a helix-turn-helix DNA-binding motif. Current studies on the structure of a complex of RT with DNA analogues we hope to provide a firmer structural basis to DNA-directed DNA polymerase activities and RNase H activity.

**PS04.05.32** A HIGH RESOLUTION STRUCTURE OF AN EcoRV-DNA COMPLEX. Mark P.Thomas, E.Louise Hancock, Stephen E.Halford & R.Leo Brady. Department of Biochemistry, University of Bristol, University Walk, Bristol, BS8 1TD, UK.

Structures of EcoRV complexed to substrate and product DNA have previously been refined to 2.8 Å (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 metal bonds to the scissile phosphate 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.


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 33 kDa molecular weight, that consist of two pairs of subunits A (GyrA, 97 kDa) and B (GyrB, 70 kDa). 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 = 4.7 Å3Da-1). The structure has been solved to 4.2 Å 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 Korch, 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 involved in DNA synthesis are transcriptionally activated exclusively in late G1 and early S. Their transcription depends on an 8-base pair asymmetric element (ACCGGTTA, where N=7 or C containing an Mlu restriction enzyme site called the MluI cell-cycle box or MCB. A transcription factor called MBF (MCB binding factor) is implicated in driving the expression from MBP element. MBF is a heteromeric complex composed of a regulatory protein, Swi6, and a sequence-specific DNA-binding protein, Mbpl.

We have crystallized and solved the structure of the N-terminal 124-amino acid DNA-binding domain of Mbpl. The protein crystallizes in the tetragonal space group P41212 with unit cell dimensions of 43.5 Å x 43.5 Å x 124.3 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-