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.
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 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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Vipond, I.P., Baldwin, G.S. & Halford, S.E. (1995) *Biochemistry*, 34, 697-704.
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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 = 4.7$Å^3Da^{-1}$). 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-

helix DNA-binding proteins, including the C-terminal domain of procaryotic catabolite gene activator protein (CAP), the globular domain of the linker histone H5, and the DNA-binding domain of the hepatocyte nuclear factor-3 (HNF-3). By aligning the helix-turn-helix motif of Mbp1 with that of CAP and HNF-3, we can suggest the following protein DNA interactions for the Mbp1-DNA complex: major groove contacs with DNA by the recognition helix within the helix-turn-helix motif, as well as minor groove contacts by the loop of the β-hairpin.

PS04.05.35 CRYSTALLIZATION OF THE FELINE IMMUNODEFICIENCY VIRUS INTEGRASE PROTEIN. Ann E. Maris[1], Yoshio Shibagaki[2], Mary L. Kopka[3], Thang Kien Chiu[1], Samson A. Chow[2] and Richard E. Dickerson[3], [1]Department of Chemistry and Biochemistry, [2]Department of Molecular and Medical Pharmacology, [3]Molecular Biology Institute, University of California at Los Angeles, CA 90095

Integrase catalyzes integration of the cDNA copy of the viral genome into the host chromosome, a necessary step in the retroviral life cycle. Integrase processes the U5 and U3 termini of the viral DNA by cleaving off two terminal nucleotides, and leaving a recessed 3'-hydroxyl on both ends. These ends then function as the nucleophiles in a one-step transesterification reaction which leaves the viral DNA covalently joined to the host DNA.

The integrase proteins of human and feline immunodeficiency viruses (HIV and FIV) share about 86% similarity and 37% identity and have similar biochemical activities and sub-domain organization. Three domains have been identified in integrase: the core, containing catalytic activity, the N- and the C-terminus. Only the core's structure has been determined for both HIV and Rous Sarcoma Virus. The N-terminus is necessary for the joining reaction and contains a novel putative zinc-finger, which may be involved in the formation of a stable complex between integrase and viral DNA. We have purified and concentrated to 10 mg/ml a truncated FIV integrase consisting of the N-terminus and the core. Conditions were found to obtain microcrystals consistently and we are further modifying conditions to increase crystal size. In addition, we are exploring various DNA substrates to obtain integrase-DNA co-crystallization.

PS04.05.36 CRYSTALLISATION OF HPV-16 E2 DNA-BINDING DOMAIN APO- AND CO-CRYSTALS. Stephanie Roberts, Hilary Muirhead, Tony Clarke. Department of Biochemistry, University of Bristol, University Walk, Bristol BS8 1TD, UK

The DNA binding domain of the viral transcription factor E2 has been crystallised in the presence and absence of its cognate DNA. It is also hoped to co-crystallise with non-cognate DNA molecules. HPV-16 E2 requires four AT/TA base pairs at the centre of its 12 bp binding site. These are uncontacted but provide flexibility required for binding. Thus four central AT/TA base pairs in an otherwise unrelated oligonucleotide may provide a preferred non-cognate binding site, generating homogenous complexes for crystallisation.

Conditions for co-crystals have not been optimised due to problems with DNA supply, but a dataset for the apocrystals has been obtained to 2.1 angstroms. Data were obtained from a single crystal, frozen to low temperature. The space group is $P3_12$ or $P3_21$, with unit cell dimensions 44.19 x 44.19 x 76.86 and angles 90 90 120. There are 6 monomers in the unit cell, assuming a 38.4% solvent content. They are presumed dimeric, with a natural two fold symmetry. Initial analysis suggests the structure is too distinct from the published DNA-bound structure of the bovine papillomavirus E2 domain to solve by molecular replacement. Apocrystals were obtained form 3 - 8 mg/ml protein precipitated with 1.50-1.65M ammonium sulphate in 8 μl drops, at an approximate final pH of 8.2. Final concentrations of other solutes in drops was 32.5mM TRIS, 100mM sodium chloride, 0.6mM calcium chloride, 1.6mM potassium chloride. Co-crystals were obtained from 3mg/ml complex in the same salts, precipitated with 28% PEG 3500 at an approximate final pH of 7.5. The current co-crystals are too small for analysis.

PR04.05.37 REPA1, A REPLICATION CONTROL PROTEIN OF THE REPFIC REPLICON OF PLASMID ENTP307. Simon E. V. Phillips, Haiwei Song, Renata Maas*, Mark R. Parsons, Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK, *Department of Microbiology, New York University Medical Center, New York, New York 10016, USA

The large *E. Coli* plasmid EntP307 contains two replication regions or basic replicons, RepFIB and RepFIC. Replication control in RepFIC is similar to that in plasmids R1 and R100, and is effected by only two structural genes. These encode the negative regulator RepA2, and the replication initiator RepA1. In addition, constitutive transcription from another promoter produces antisense RNA that negatively regulates RepA1 production. RepA1 probably interacts directly with the replication origin, but this has been difficult to demonstrate.

We have crystallized RepA1 to establish a structural basis for its function, and provide a prototype for Rep proteins of this class. It was purified using a modification of the published procedure[1], and crystallized by hanging drop vapour diffusion. Type 1 crystals[2] grow from 2.0M ammonium sulphate solutions at pH 8.5, and are orthorhombic $P2_12_12$, with **a**=61, **b**=67 and **c**=243Å. Monoclinic Type 2 crystals grow from PEG8000 solutions at lower pH, with **a**=64, **b**=103, **c**=65Å and ß=97°. Both forms contain two 40kDa RepA1 molecules per asymmetric unit, and diffract X-rays to about 3Å resolution. Preliminary data have been collected from native type 1 crystals at 100K, and from a crystal soaked in PCMBS which shows two major mercury sites. High resolution data collection is under way using synchrotron radiation.

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2. Song et al (1996) Proteins: Struct. Funct. Genet. in press.

Protein-RNA

MS04.06.01 HOW DOES Gln-tRNA SYNTHETASE AMINOACYLATE THE CORRECT tRNA WITH THE CORRECT AMINO ACID? T. A. Steitz, L. F. Silvian, V. L. Rath, Department of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Yale University, New Haven, CT USA

To provide a structural basis for understanding the high specificity of Gln-tRNA synthetase (GlnRS) for its cognate tRNA and amino acid, structures of the enzyme cocrystallized with $tRNA^{Gln}s$ containing mutated anticodon and acceptor stem bases have been correlated with the kinetic consequences of these mutations. Further, these complexes contain a bound Gln-AMP analogue. The mutation sharing the most profound kinetic and structural change is the mutation of U35 in the anticodon to C35. The large change in k_{cat} may be a consequence of significant alterations in the structure of the anticodon and D-stem and loops and in the N-terminus of the protein.