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.

1. Maas, R. et al (1991) Mol. Microbiol. **5**, 927-932.
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.