logically the same to one half of the DNA binding domain of the eukaryotic TATA box binding protein which has the two structural domains related by the intramolecular pseudodyad symmetry. The fold of secondary segments of the two all α-helical domains is identical with that of *E. coli* endonuclease III which acts both as an N-glycosylase, removing oxidized pyrimidine from DNA and a 3' apurinic/ apyrimidinic lyase. When mapped onto the AlkA structure, the sequences of *Bacillus subtilis* and *Saccharomyces cerevisiae* 3-methyladenine-DNA glycosylases show that sequence-conserved residues cluster in and around a cleft between domains II and III. The mutagenic and model-building studies suggest that the active site is located in the cleft and the interaction between the target base and the active site requires the target base flipping out from a double-stranded DNA. The structure of the active site shows the implication of the recognition for a diversity of substrates.

MS04.05.08 STRUCTURAL ANALYSIS OF THE TBP/TFIIA/ TATA COMPLEX. J. H. Geiger, S. Hahn, P. B. Sigler, Yale University, New Haven, CT 06510

The goal of this project is to investigate structure-function relationships of eukaryotic transcription initiation. For RNA polymerase II, there are a host of general transcription factors that must associate at the promoter with the polymerase before transcription initiation begins. Some are required for the initiation of all genes (TFIID, TFIIB) in vivo and in vitro, while some are required on a subset of promoters, or serve to activate transcription, but are not necessary for basal levels of in vitro transcription. TFIIA falls in the latter category. An essential gene in yeast, TFIIA counteracts the effects of transcriptional repressors and may be involved in the activity of transcriptional activators. It increases the affinity of TBP to the TATA box by three orders of magnitude, and extends the TBP footprint on DNA by seven base pairs in the upstream direction.

We have crystallized and solved the structure of a complex of TBP/TFIIA/TATA to 3.0 angstroms. We are using this structure to elucidate the stereochemical determinants of this assembly and to give insight into the interaction of this complex with the rest of the basal machinery and its possible interaction with activators and repressors of transcription initiation.

PS04.05.09 STRUCTURE-FUNCTION RELATIONSHIPS IN DNA LIGASE FROM THE BACTERIOPHAGE T7. Stephen R. Ashford, Aidan J. Doherty, Hosahalli S. Subramanya and Dale B. Wigley, Laboratory of Molecular Biophysics, University of Oxford, South Parks Road, Oxford, Ox1 3QU, UK

DNA ligases are ubiquitous enzymes, necessary for DNA replication and some forms of repair. The enzymes can easily be divided into two groups, and although they all catalyze essentially the same reaction - the sealing of nicks in the DNA backbone via an adenylated enzyme intermediate - they require different co-factors, and possess quite different primary sequence. The eukaryotic and viral DNA ligases require ATP, whereas the bacterial enzymes utilise NAD^+. All ATP-dependent DNA ligases share a common core structure - as shown by sequence alignments, and therefore the T7 enzyme (one of the smallest in this class) was selected for structural and biochemical analysis.

The enzyme is a monomer of 41 kDa, and the crystal structure at 2.6Å resolution has been solved, with and without ATP bound. The enzyme consists of a larger N terminal domain (residues 1 to 240) containing the co-factor binding pocket and site of adenylated enzyme intermediate formation, and a C terminal domain (240 to 349), which is remarkably similar to the oligonucleotide binding fold (OB fold) found in many proteins including staphylococcal nuclease, and bacterial cold shock protein. Further biochemical study of these two domains, in conjunction with the structure, lead to a number of conclusions about the DNA binding and specificity of the enzyme.

PS04.05.10 DNA POLYMERASE FIDELITY: A STRUCTURAL ANALYSIS. M.M. Blair, N. Mashhoon, C.R.A. Muchmore, M.F. Goodman, and W.F. Anderson, Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL 60611 and Department of Molecular Biology, USC, Los Angeles, CA 90089

E.coli DNA Polymerase II (pol II), an 89.9 kD, α-like DNA polymerase, possesses both polymerase and 3'-5' exonuclease activities on a single polypeptide chain. Pol II is induced in response to DNA damage as part of the SOS regulon in *E.coli* and is required for synthesis past abasic lesions in the absence of heat shock proteins. Recent *in vivo* studies suggest that replacing wild type with exonuclease deficient pol II leads to increased adaptive mutation frequency indicating an important role for pol II in replication fidelity in the cell. To better understand the mechanism of fidelity, we are using x-ray crystallography to study mutants of pol II and their complexes with DNA.

Mutants of pol II, D155A/E157A (exo-) and L423M, were constructed. These mutations are in highly conserved regions of Pol II involved in nucleotide/metal binding. Pol II exo- has wild type levels of polymerase activity but lacks exonuclease proofreading activity. The L423M mutant has wild type levels of polymerase and exonuclease activities but partitions preferentially towards polymerization in the presence of a mispaired primer terminus suggesting an alteration in switching between the polymerase and exonuclease sites.

Both mutants have been crystallized by vapor diffusion methods. Like Pol II, Pol II exo- crystallizes in the $P2_12_12$ spacegroup and both room temperature and low temperature data sets have been collected. Initial phasing of pol II exo- was by low resolution, modified MIR phases from room temperature pol II data. Further work is underway to improve phases via derivative searches, model building, and density modification.

Pol II wild type and exo- mutant have also been co-crystallized with synthetic double-stranded DNA oligonucleotides. Further work is in progress.

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PS04.05.11 DNA-SPECIFIC BINDING BY HIN AND FIS. Thang Chiu, Reid Johnson, Richard E. Dickerson, MBI, UCLA, CA 90095

We are interested in learning how dna-binding proteins recognize their target sequences. One system of particular interest is the Hin/Fis system of recombination. Both proteins bind their target sequences via a Helix-Turn-Helix. Hin belongs to a family of proteins that catalyses site-specific DNA inversion in enteric bacteria. Its binding site consists of a highly conserved inverted repeat of 12-bp separated by a central 2-bp 'core'. (consensus sequence: A/T G G T T T A/T G G A G/T A A) The availability of comprehensive mutagenesis data of the binding sites makes it a highly attractive system for studying protein-dna interactions. The crystal structure of a 52 aa peptide consisting the dna-binding-domain of E.coli Hin bound to the hixL half-site tGTTTTTGATAAGA/aTCTTATCAAAAAC has been solved (Feng et al, Science '94). We are interested in solving the crystal structures of Hin bound to various mutant binding sites in order to understand the mechanism by which its dna-binding specificity is determined.

Fis (Factor for Inversion Stimulation) is a recombinational activator of the Hin family of dna invertases and is also involved in phage lambda site-specific recombination, in transcriptional activation of rRNA and tRNA operons, in repression of its own synthesis, and in oriC-directed DNA replication. Although it is a