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 pseudo-dyad symmetry. The
two domains of the TATA box binding protein are identical to the
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 A/T-rich end of the TATA box
sequences of Bacillus subtilis 168 and Saccharomyces cerevisiae
3-methyladenine-DNA glycosylases show that sequence-conserved
residues cluster in and around a cleft between domains II and III.
The mutagenesis 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 dou­
bble-stranded DNA. The structure of the active site shows the impli­
cation of the recognition for a diversity of substrates.

**MS04.05.08 STRUCTURAL ANALYSIS OF THE TBPIFII/ 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 relations­
ships 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, TFIIH)
in vivo and in vitro, while some are required on a subset of promot­
ers, or serve to activate transcription, and 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 transcrip­
tional repressors and may be involved in activities 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 re­
pressors of transcription initiation.

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

DNA ligases are ubiquitous enzymes, necessary for DNA repli­
cation and some forms of repair. The enzymes can easily be divid­
ed 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 struc­
ture - as shown by sequence alignments, and therefore the T7 en­
zyme (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 2.6A 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 P2₁2₁2₁ 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 re­
ognize their target sequences. One system of particular interest is
the Hin/Fis system of recombination. Both proteins bind their tar­
get sequences via a Helix-Turn-Helix. Hin belongs to a family of
proteins that catalyzes site-specific DNA inversion in enteric bac­
teria. Its binding site consists of a highly conserved inverted re­
verse of A/T G G T T

We are interested in solving the crystal
structures of Hin bound to the hixL half-site
GTTTTTGTATAAG/aTCTTTATCAAAAAC 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