site-specific dna binding protein, a comparison of over 35 Fis binding sites reveals a 25-bp binding site which has, at most, a degenerate 15-bp core. Gel electrophoresis of Fis-dna complexes indicate that Fis induces a bend (in the dna) of 40-90 degree upon dna binding. This bending is required for its activity and the degree of bending (and binding) is dependent on both the sequence of the 15-bp core and the sequence of the flanking regions. Given the degenerate nature of the binding sites, the first question one might ask is "how does Fis differentiate a true binding site from a false binding site". A first step towards addressing this question is to crystallize (at least one) Fis binding site, alone and complexed to Fis protein, and solve their crystal structures. Efforts toward achieving this goal are underway.

PS04.05.12 REFINED STRUCTURE OF HIV-1 REVERSE TRANSCRIPTASE COMPLEXED WITH A DOUBLED-STRANDED DNA AND AN ANTIBODY FAB FRAGMENT AT 2.8 Å RESOLUTION. Jianping Ding[1], Kalyan Das[1], Yu Hsiou[1], Alfredo Jacobo-Molina[1], Stephen H. Hughes[2], and Edward Arnold[1]. [1]CABM & Rutgers University, Piscataway, NJ 08854; [2]ABL-Basic Research Program, NCI, Frederick, MD 21702

The structure of a ternary complex of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), a double-stranded DNA template-primer (dsDNA), and the Fab fragment of monoclonal antibody 28 (Fab28) has been refined at 2.8 Å resolution. The detailed structures of the bound DNA, the RT polymerase and RNase H active sites, and the protein-nucleic acid interactions will be described. The first 5 duplex base pairs of the DNA near the polymerase active site conform to A-form geometry; an approximately 40° bend occurs smoothly over the next 4 base pairs; and the following 9 base pairs conform to B-form geometry. The bend at the A-form/B-form junction occurs in the vicinity of the αH and αI helices of the p66 thumb, which are partly inserted into a widened minor groove. The polymerase active site is composed of structural elements from both protein and nucleic acid which can influence the recognition and incorporation of incoming dNTPs. Asp110, Asp185, and Asp186 are highly conserved residues at the polymerase active site that probably function in both dNTP binding and catalysis. Tyr183 and Met184, which are part of the YMDD motif characteristic of retroviral RTs, interact with the primer 3'-terminal nucleotide. Residues that do not directly interact with the template-primer but are expected to form part of the dNTP-binding site include Asp113, Tyr115, Phe116, Gln151, Phe160, and Lys219. The β12-β13 hairpin (also known as the "primer grip") interacts with the sugar-phosphate backbone of the primer terminus via main chain atoms of Met230 and Gly231, which are also strongly conserved in all RNA-dependent polymerases. Amino acid residues forming part of the "template grip" (that makes close contacts with the template strand) include Asp76, Glu89, Gly93, Ile94, Gln151, Gly152, Trp153, Lys154, and Pro157. The RNase H active site, defined by residues Asp447, Glu478, Asp498, and Asp549, has a structure similar to those found in crystal structures of the isolated RNase H domain. The implications of these structural findings for enzymatic reactions and structure-based drug design are discussed in light of biochemical and genetic data.

PS04.05.13 THE CRYSTAL STRUCTURE OF THE CATALYTIC CORE DOMAIN OF HIV-1 INTEGRASE IN A NEW CRYSTAL FORM. Fred Dyda, Alison B. Hickman, Timothy M. Jenkins, Alan Engelman, Robert Craigie, David R. Davies. Laboratory of Molecular Biology, National Institute of Diabetes, Digestive and Kidney Diseases

The catalytic core domain of HIV-1 integrase has been crystallized in the orthorhombic space group $P2_12_12_1$ with two molecules in the asymmetric unit. The structure was solved by molecular replacement. When compared with the previously reported trigonal form (Dyda et. al. Science 266, pp1981-1986, 1994), this orthorhombic form diffracts to higher resolution, and previously undefined parts of the structure now became ordered, probably due to the different crystal packing environment. The full active site consisting of three acidic residues is well resolved in this crystal form.

A detailed three dimensional comparison of the members of the polynucleotidyl transferase superfamily (HIV-1 integrase, Mu transposase, RNase H, RuvC resolvase, ASV integrase) shows that the closest similarity is between the integrases and the Mu transposase. This surprisingly close similarity correlates with the similarity of the chemical reactions these enzymes catalyze. When considering the entire superfamily, it is interesting to observe that the molecules display a similar disorder pattern. This is especially true in the active site region, where the location of the catalytically essential carboxylate residues varies significantly even between the two integrases. This probably indicates a conformational rearrangement taking place upon the formation of the protein-DNA complex prior to catalysis.

PS04.05.14 CRYSTAL STRUCTURE OF PU.1 ETS DOMAIN-DNA COMPLEX: A NEW PATTERN FOR HELIX-TURN-HELIX RECOGNITION: K. R. Ely, R. Kodandapani, F. Pio, C.-Z. Ni, G. Piccialli, S. McKercher, M. Klemsz and R. A. Maki, La Jolla Cancer Research Foundation, La Jolla, CA 92037 USA

The ets family of transcription factors regulate gene expression during growth and development and share a conserved ETS DNA-binding domain that binds as a monomer to the sequence 5'-C/AGGAA/T-3'. The crystal structure of the PU.1 ETS domain complexed with a 16 bp DNA oligonucleotide has been determined at 2.3-Å resolution. Crystals formed in space group *C*2 with a=89.1, b=101.9, c=55.6 Å, ß=111.2° with two complexes in the asymmetric unit. The structure was solved by the MIRAS method. The current *R*-factor=23.7 (R_{free}=29.9). The domain is similar to α+ß ("winged") helix-turn-helix DNA-binding proteins and contacts a ten bp region of duplex DNA that is bent (8°) but uniformly curved without distinct kinks. Four conserved amino acids contact DNA from a novel loop-helix-loop architecture. Arg232 and Arg235 from the recognition helix are hydrogen-bonded to the GGA bases in the major groove. These interactions represent the paradigm for ets recognition. Two loops contact the DNA backbone in the minor groove: Lys245 in the "wing" between the third and fourth ß-strands contacts the phosphate backbone of the GGAA strand upstream from the core sequence while Lys219 from the "turn" of the HTH motif contacts the phosphate backbone of the opposite strand downstream of the GGAA core. This work was supported by grants USAMRDC-DAMD17-94J-4439, NIH-CA63489-01 and NIH AI20194.