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PS-05.01.20 DESIGN OF METALLOTHIONEIN ALPHA DOMAIN POLYMER. By J. Luo*, A. Pan, S. Yin, J. Sun, B. Kuang, L. Li, B. Ru and X. Gu, National Lab of Protein Engineering and Plant Genetic Engineering, Departemnt of Biology, Peking University, Beijing 100871, China

Metallothioneins(MTs) are low molecular weight, cysteine-rich and metal binding proteins. A native MT molecule contains two distinct domains, an α-domain which is characterized by cadmium-binding, and a β-domain which binds preferentially to zinc. Reconstruction and transformation of MTα domain polymer gene in plants may provide a valuable method for reclamation of wastelands and mine spoils rich of cadmium. Based on this conception, a gene which encodes human liver MT-IA(hMT-IA) α domain dimer was synthesized and is to be introduced into plants using the multiple copy cloning technique so that the host can yield more MT. In order to ensure the efficiency of expression of the gene in host, computer aided molecular design was employed in studying the structure and conformation both of native and mutant MT. The EMBL molecular biology data base was searched to retrieve protein sequence information of metallothionein molecules. Sequence analysis was then carried out which indicated that mammalian MTs are very conservative not only in the amino acid composition and sequence homology, but also in the domain arrangement. A three dimensional model of hMTI-A was built up taking the crystal structure of Rat MT-II and NMR data of Human MT-II, Rat MT-II and Rabbit MT-II as templates. Conformation study of this model indicates that a tripeptide of -Lys-Lys-Ser- serves as a linker between the two domains which are apparently independent. According to this investigation, a model of hMT-IAα domain dimer was constructed and the tripeptide -Lys-Lys-Ser- was used as the domain linker. A ten residue peptide -Glu-Leu-Asp-Gly-Pro-Lys-Lys-Ser-Gly-Ser- was finally chosen to link the dimers to form various α domain polymers. On designing of this linkage peptide, special consideration was given to the conformation flexibility and hydrophobicity of the peptide, the influence of the peptide on the dimer, as well as the ease of gene manipulation. This model was inspected by means of sequence analysis and molecular graphics, which gave some indication that various α domain polymers may fold in several patterns. Further experiments on expression proteins are underway.

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DS-05.02.01 STRUCTURE OF HIV REVERSE TRANSCRIPTASE by J. Wang*, S. J. Smerdon, L. A. Kohlstaedt, J. Jacger, P. A. Rice, J. M. Friedman, T. A. Steitz, Department of Molecular Biophysics & Biochemistry, Howard Hughes Medical Institute, Yale University, New Haven, CT 06511

The X-ray crystal structure of the reverse transcriptase (RT) from human immunodeficiency virus type-I co-crystallized with a non-nucleoside inhibitor, Nevarapine, has been determined at 3.2A resolution. The structure of the heterodimeric p66/p51 molecule shows a marked and somewhat unexpected degree of asymmetry with respect to domain and subdomain organization. The p66 subunit has a large cleft and resembles that of the Klenow fragment of E. coli DNA polymerase I while the equivalent cleft in p51 is filled by its connection domain. An A-form RNA-DNA hybrid can be model-built into the deep cleft that extends between the polymerase and RNase H active sites. This places the 3' end of the primer strand next to the conserved polymerase active-site carboxyls and the 3' end of the template strand 20 nucleotides upstream, next to the two metal ions of the RNase H active site. The relative positions of the metal ions and the modelled template terminus suggest a mechanism of RNA hydrolysis similar to that of the 3'-5' exonuclease of Klenow fragment. The presence of three conserved carboxylic acid residues in the pol active sites suggest that a two metal ion catalysis may also be involved in the polymerization reaction. Although these metals are not observed in electron density maps of RT at this stage, they are clearly seen in the structure of the Klenow polymerase. Solvent accessible surface calculations on the basis of a partially refined RT model indicate that interactions between the connection domains play a central role in the formation of all possible reverse transcriptase dimers (p66/p66, p66/p51, p51/p51). Furthermore, contacts with p51 involving the RNase H domain of p66 contributes substantially to the overall stability of the heterodimer. After the connection domain interactions are assumed to be in the asymmetric form seen in the X-ray structure, four main events must then take place to form the mature heterodimer which can be summarised as the unfolding and cleavage of one of the RNase H domains along with subdomain rearrangements.

DS-05.02.02 STRUCTURE OF HIV-1 REVERSE TRANSCRIPTASE/dsDNA/Fab COMPLEX: PROTEIN-DNA INTERACTIONS AND STRUCTURE OF THE POLYMERASE ACTIVE SITE. By J. Ding*, A. Jacobo-Molina, R.G. Nanni, X. Lu, C. Tantillo, A.D. Clark Jr., S.H. Hughes†, and E. Arnold, Center for Advanced Biotechnology and Medicine and Rutgers University Dept. of Chemistry, 679 Hoes Lane, Piscataway, NJ 08854-5638, †ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201.

The crystal structure of a ternary complex of human immunodeficiency virus type 1 reverse transcriptase (HIV-1 RT) heterodimer p66/p51, a 19 base/18 base dsDNA template-primer and a monoclonal antibody Fab fragment has been determined at 3.0 Å resolution (7 Å resolution structure reported in *Nature* 357:85-89, 1992). The polymerase domains of both p66 and p51 contain four subdomains, which are named fingers, palm, thumb and connection (Kohlstaedt, L.A., Wang, J., Friedman, J.M., Rice, P.A. & Steitz, T.A., *Science* 256:1783-1790, 1992). Although the structures of the individual subdomains within p66 and p51 are similar, their relative spatial arrangements within the two subunits are dramatically different. The template-primer binds in a large cleft formed by the fingers, palm, and thumb of p66. The structure of the template-primer is a hybrid resembling A-form DNA near the polymerase active site and B-form DNA towards the RNase H active site, with a significant bend (40-45°) at the A-/B- junction. The most numerous interactions of HIV-1 RT and the dsDNA occur primarily along the sugar-phosphate backbone and involve amino acid residues of the palm and thumb of p66. Highly conserved regions are located in the p66 palm near the polymerase active site and include a β-hairpin, denoted as

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primer grip because of its proximity to the phosphate that joins the nucleotides at the primer terminus, and a portion of the p66 palm and fingers that are closely associated with nucleotides of the template strand, therefore denoted as template grip. These structural elements, together with two α–helices of the p66 thumb, act as a clamp to position the template-primer precisely relative to the polymerase active site. The 3'-hydroxyl of the primer terminus is close to the catalytically essential Asp110, Asp185, and Asp186 residues at the active site and is in a position for nucleophilic attack on the α–phosphate of an incoming nucleoside triphosphate. Since the three-dimensional structure of the active site of nucleic acid polymerases appears to be strongly conserved, the structure of the HIV-1 RT/DNA complex may aid our understanding of the mechanism of polymerization and facilitate the design of new and improved drugs against HIV-1 infections.

DS–05.02.03 STRUCTURAL STUDIES ON A NEW CRYSTAL FORM OF HIV REVERSE TRANSCRIPTASE By R. M. Esnouf*, E. F. Garman, E. Y. Jones, D. I. Stuart, G. K. Darby, C. K. Ross, D. O'N. Somers and D. K. Stammers. Laboratory of Molecular Biophysics, Oxford University, UK and Wellcome Research Laboratories, Beckenham, Kent, UK

We have recently solved the structure of a new crystal form of HIV-1 reverse transcriptase (RT) at 3.4Å resolution. The crystals are orthorhombic, space group $P2_12_12_1$. The native crystals have cell dimensions a=147Å, b=112Å, c=79Å. However, under certain conditions a shrinkage in the a axis of 4Å is triggered. Native and heavy atom derivative data have been collected for both the large and small unit cells. The difference Pattersons for the large unit cell data were interpreted and led, with the aid of solvent flattening, to an electron density map at 6Å resolution of reasonable quality.
A polyalanine model was constructed (Esnouf, R. M., unpublished program) from the incomplete set of unrefined C^{α} coordinates deposited by the Yale group (Kohlstaedt, L. A. *et al.*, *Science*, **256**, 1783 (1992)). The molecular replacement protocols of X-PLOR (Brünger, A. T., *Acta Cryst.*, **A46**, 46 (1990)) were successful in locating this model in both the large and the small unit cells. Careful constrained refinement has revealed structural features not present in the phasing model.
The current status of the project will be reported with particular reference to the biological function of this molecule.

DS–05.02.04 INTERACTIONS OF THE CD4 AND CD8 T-CELL CO-RECEPTORS IN THE CELLULAR IMMUNE RESPONSE. Wayne A. Hendrickson, Peter D. Kwong, Daniel J. Leahy*, Seong-Eon Ryu‡, Hao Wu and Hiroto Yamaguchi. Department of Biochemistry and Molecular Biophysics and Howard Hughes Medical Institute, Columbia University, New York, NY 10032, USA. (Present addresses: *Department of Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; ‡Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138, USA).

The cellular immune response is essential in the defense against pathogens, both by assisting in the generation of antibodies and also directly in the elimination of viral infected cells. These responses are mediated by T-cells that interact with peptide antigens presented on target cells as complexes with molecules of the major histocompatibility complex (MHC). Mature T-cells are principally distinguished by the exclusive occurrence of either CD4 or CD8 glycoproteins on their surfaces. These molecules serve as co-receptors in the interaction between T-cell antigen receptors and MHC-presented peptides. The extracellular portions of CD4 and CD8 interact respectively with Class II and Class I MHC molecules, and the cytoplasmic portions of both molecules associate non-covalently with the lymphocyte kinase $p56^{lck}$.

We have determined crystal structures for extracellular fragments of both human CD4 and human CD8a. In both cases the amino-terminal domains are similar to the variable domains of immunoglobulins, but otherwise they are quite dissimilar. CD8 is a dimer, and the crystal structure reveals a mode of association like that in the Fv portions of antibodies. The whole extracellular portion of CD4 (sCD4) behaves as a monomer in solution, and a fragment comprising the first two domains (D1D2) for which the structure has been determined is also monomeric. On the other hand, the characteristics of crystals of whole sCD4 fragments suggest a specific mode of oligomerization that may be mediated by the D3D4 portion of CD4. The site on CD4 that interacts with HIV in AIDS infection has been mapped by mutation studies, and the structure of one of these mutant proteins have been determined. The structures of CD4 and CD8 fragments also provide a basis for interpreting mutational studies on the interactions between these T-cell co-receptors and their MHC targets. Work is in progress on the interactions of CD4 and CD8 with other components.

DS–05.02.05 THE CRYSTAL STRUCTURES OF HIV PROTEINASE INHIBITOR COMPLEXES. By K. Appelt, Agouron, USA

PS-05.02.06 STRUCTURAL STUDIES OF CD4: CRYSTAL STRUCTURE OF DOMAINS 3 AND 4 AND THEIR IMPLICATION FOR THE OVERALL RECEPTOR STRUCTURE by R.Leo Brady*, Gudrun Lange, Eleanor J.Dodson, A.Neil Barclay# and G.Guy Dodson Department of Chemistry, University of York YO1 5DD & # MRC Cellular Immunology Unit, South Parks Road, Oxford

CD4 is a transmembrane glycoprotein present at the surface of T lymphocytes that interacts with Major Histocompatibility Complex Class II proteins at the surface of accessory cells, and is involved in the triggering of the lymphocytes by foreign antigens. CD4 is also the major receptor for the human immunodeficiency virus. The extracellular portion has been predicted to contain 4 immunoglobulin superfamily domains and the structure of the amino terminal 2 domains has previously been determined. We now report the expression of a form of CD4 containing only domains 3 and 4, its crystallisation and analysis of its structure by X–ray crystallography with 2.8A spacing data. Both of the carboxy terminal domains are immunoglobulin related as had been predicted. The implications of the structure will be discussed with respect to the structure of the complete extracellular portion of CD4, its function and evolution as a receptor built from a concatenation of Ig superfamily domains.