PS04.09.21 CRYSTALLOGRAPHIC STUDY OF A FELINE CALICIVIRUS. Lan Zhou, Ming Luo, Department of Microbiology, Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, AL USA

Feline calicivirus (FCV) is a nonenveloped, spherical animal virus of the Calicivirus family. It contains a positive sense, single-stranded RNA genome and one major capsid protein. The virion has a diameter of 405 Å and exhibits T=3 icosahedral symmetry. The FCV crystals belong to an orthorhombic crystal system with unit-cell dimensions a=889.0 Å, b=998.3 Å, c=437.6 Å. Based on the Vm value (3.4 $Å^3$/Da), it was estimated that there is one FCV particle in one crystallographic asymmetric unit, which implies the presence of 60-fold noncrystallographic redundancy.

The orientations of the FCV particles in the unit cell were determined with a self-rotation as well as the locked rotation function using the GLRF program. Data between 15-7 Å and 7-4 Å, respectively, were used to search for 5-, 3- and 2-fold axes. Two sets of 5-, 3-, and 2-fold noncrystallographic symmetry axes were obtained. The orientations of the 5-, 3-, and 2-fold noncrystallographic symmetry axes of each set were those expected for an icosahedron. This indicated that two different particle orientations exist in the crystallographic asymmetric unit. Combining this with the packing consideration and the Vm value, it was concluded that there is one unique particle content from two differently oriented FCV particles in one crystallographic asymmetric unit. The precise particle orientations were refined by optimizing the fit between the directions of the observed self-rotation function peaks from one of the virus particle and the corresponding axes of a standard icosahedron.

In order to precisely define the particle positions in the unit cell, heavy atom derivatives of FCV crystals are in pursuit.

Receptors/Signal Transduction

MS04.10.01 CRYSTAL STRUCTURE OF A COMPLEX BETWEEN THE EXTRACELLULAR DOMAIN OF THE HUMAN ERYTHROPOIETIN RECEPTOR AND AN AGONIST PEPTIDE. Oded Livnah†, Enrico A. Stura†, Dana L. Johnson§, Linda K. Jolliffe§, Ian A. Wilson†, †The Scripps Research Institute, Dept. of Molecular Biology, 10666 North Torrey Pines Rd., La Jolla, CA 92037, §The R. W. Johnson Pharmaceutical Research Institute, Drug Discovery Research, 1000 Route 202, Box 300, Raritan, NJ 08869

A 20 residue cyclic peptide has been shown to be capable of effecting dimerization of a class 1 cytokine receptor and is able to mimic the biological activity of the natural hormone ligand erythropoietin (EPO). The crystal structure of the complex between the extracellular domain of the erythropoietin receptor (EPOR) and this peptide, unrelated in sequence to erythropoietin was determined at 2.8 Å resolution in an orthorhombic crystal form having the space group $P2_12_12_1$ with cell dimensions a=59.2Å, b=75.5Å, c=132.2Å with two receptor molecules in the assymetric unit. Initial MIRAS phases were calculated to a resolution of 3.1 Å using the program package PHASES and had a mean figure of merit of 0.64 (25.0-3.1 Å). Phases were refined in PHASES using the solvent flattening protocol. The structure was refined using the program package X-PLOR to an R-value of 0.21 without any solvent molecules added.

Each receptor molecule consists of two domains with fibronectin type-III folds having a 90° angle between them. The structure of the complex consists of an almost perfect two-fold 2 receptor:2 peptide assembly. The dimerization assembly differs markedly from the human growth hormone receptor complex, another class 1 cytokine receptor of known structure, and suggests that more than one type of dimerization may be sufficient to induce signal transduction and cell proliferation. The functional mimic of a biological protein ligand by an unrelated molecule has previously appeared an unreachable goal. This EPOR complex suggests future progress towards the design of a non-peptidic small molecule drugs may be possible.

MS04.10.02 THREE-DIMENSIONAL STRUCTURE OF HUMAN INTERFERON-γ COMPLEXED TO ITS CELL RECEPTOR. Daniel Thiel[1], Marie le Du[1], Richard Walter[1], Allan D'Arcy[2], Christiane Chene[2], Michael Fontoulakis[2], Gianni Garotta[2], Fritz Winkler[2] and Steve Ealick[1]. Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853 and Pharma Research, F. Hoffman-La Roche Ltd, 4002 Basel, Switzerland.

Interferon-γ (IFN-γ) was originally identified because of its ability to "interfere" with viral replication after infection. Since then IFN-γ has been shown to be responsible for many types of immune responses. IFN-γ is secreted by activated T-cells and natural killer cells and functions by binding to a specific high affinity cell receptor (Rα) and a second low affinity receptor (Rβ). We have used X-ray crystallography to determine the structure of IFN-γ complexed to its high affinity receptor (Rα) at 2.7 Å resolution. The complex crystallizes in space group C2 with a = 202 Å, b = 114 Å, c = 74 Å and β = 117° and with one complex per asymmetric unit. The structure was determined by MAD phasing in which the eight methionines of IFN-γ were substituted by selenomethionine. Data were measured at four different wavelengths using frozen crystals on CHESS beamline F-2. The complex consists of one IFN-γ dimer, two ordered receptors and one disordered receptor; the IFN-γ and ordered receptors show approximate two-fold symmetry. The IFN-γ dimer is made up of identical subunits with six α helices each. The subunits are interdigitated such that four helices from one subunit form a cleft that cradles the C-terminal helix from the other subunit. Each receptor molecule contains two 100 amino acid domains joined by a hinge. Each of these domains contains seven β strands with a fibronectin type III fold. In the crystal structure, the receptor molecules are truncated at the point where they normally attach to the cell membrane. Most of the interactions between IFN-γ and the two ordered Rα's occur between an N-terminal helix, C-terminal helix and the loop connecting helices A and B of IFN-γ and the N-terminal domain of the receptor. There is no interaction between these two Rα chains within one complex. A third partially disordered Rα chain was observed in the electron density maps. The C-terminal domain of the disordered receptor packs against the C-terminal domain of an ordered receptor chain. The N-terminal domain of the third receptor is almost completely disordered and is located near the IFN-γ molecule. Several pieces of evidence suggest that the disordered Rα chain may be mimicking an Rβ chain. This observation has lead to a hypothetical model of a ternary IFN-γ/Rα/Rβ complex.

MS04.10.03 REFINED STRUCTURES OF HETEROTRIMERIC G PROTEIN COMPLEXES AT HIGH RESOLUTION. John Sondek*, Andrew Bohm*, David G. Lambright#, Nikolai P. Skiba‡, Joseph P. Noel¶, Heidi E. Hamm‡, Paul B. Sigler*, *Dept. of Molecular Biophysics & Biochem. and Howard Hughes Medical Institute, Yale University, New Haven, CT 06510, ‡Dept. of Physiology & Biophysics, U. of Illinois, Chicago IL 60612 #Prog. in Mol. Med., U. of Massachusetts Med. Center, Worchester MA ¶Structural Bio. Lab., The Salk Institute, La Jolla, CA 92037

Many signaling cascades use heterotrimeric G proteins coupled to seven-helical transmembrane receptors to convert extra-

cellular signals into intracellular responses. Upon nucleotide exchange catalyzed by activated receptors, heterotrimers dissociate into Gα·GTP subunits and $G_{\beta\gamma}$ dimers, either of which can modulate numerous downstream effectors. The crystal structures of the trimeric ($G_{t\alpha\beta\gamma}$), dimeric ($G_{t\beta\gamma}$), and three monomeric forms ($G_{t\alpha}$·GDP, $G_{t\alpha}$·GTPγS, $G_{t\alpha}$·GDP·AlF_4^-) of transducin, the heterotrimeric G protein involved in the visual pathway, have been solved and refined to high resolution.

The heterotrimeric form of transducin, $G_{t\alpha\beta\gamma}$, reveals the mechanism of the nucleotide dependent engagement/disengagement between the α and βγ subunits that regulates their interaction with receptor and effector molecules. The interaction involves two distinct interfaces and dramatically alters the conformation of the α but not the βγ subunits. The location of the known sites for post-translational modification and receptor coupling suggest a plausible orientation for the heterotrimer with respect to both the membrane surface and the activated heptahelical receptor.

Multiwavelength anomalous diffraction data were used to solve the crystal structure of $G_{t\beta\gamma}$. The β subunit is primarily a seven-bladed β-propeller that is partially encircled by an extended γ subunit. The β-propeller, which contains seven structurally similar WD-repeats, defines the stereochemistry of the WD-repeat and the probable architecture of all WD-repeat containing domains. The structure details interactions between G protein β and γ subunits and highlights regions implicated in effector modulation for the conserved family of G protein βγ dimers.

MS04.10.04 STRUCTURAL ANALYSIS OF A DIMERIC STATE OF THE FIRST INTRACELLULAR DOMAIN OF PTPa, A PROTEIN RECEPTOR-LIKE TYROSINE PHOSPHATASE. A. M. Bilwes*, J. den Hertog°, T, Hunter £. J. P. Noel*, *Structural Biology Laboratory, £Molecular Biology and Virology Laboratory, Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037, °Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

Like most of the members of the receptor-like protein tyrosine phosphatase (RPTP) family, the protein tyrosine phosphatase alpha (PTPa) is involved in key cellular functions such as cell growth division and differentiation. All exhibit common features: an extracellular N-terminal domain, a membrane-spanning helix and one or two intracellular domains (D1 and D2) displaying tyrosine phosphatase activity at different levels. Extracellular domains vary drastically in structure and are likely to bind ligands. Here we describe the X-ray structure of PTPa's domain D1. An unexpected tight, symmetric and apparently inactive dimer was present in the crystal. The amino-terminal segment of one monomer which adopts a helix-loop-helix conformation is wedged into the other monomer's active site preventing the swing of its catalytically essential loop. This amino-terminal motif resembles an elbow and the encompassed sequence is conserved amongst D1 domains of all the receptor-like PTPs (except PTPx) and not in D2 domains or non-receptor PTPs. We propose that dimerization of D1 domains is a physiological event inducing a reversible inactive state of the enzyme as a consequence of colocalization of the intracellular domains by extracellular mediated events.

MS04.10.05 PH DOMAINS: DIVERSE SEQUENCES WITH A COMMON MEMBRANE ASSOCIATION FUNCTION. Kathryn M. Ferguson#, Mark A. Lemmon†, Joseph Schlessinger†, Paul B. Sigler#, #Dept. of Molecular Biophysics and Biochemistry and the Howard Hughes Medical Institute, Yale University; †Dept. of Pharmacology, New York University Medical Center

In efforts to understand intermolecular interactions that control signal transduction, we have studied the structure and function of pleckstrin homology (PH) domains. These domains of ≈120 aa are found in some 90 different signal transduction proteins. Initially we studied the dynamin PH domain (DynPH). DynPH is a β-sandwich of two orthogonal β-sheets closed at one corner by a C-terminal α-helix. The X-ray crystal structure of DynPH confirmed that regions of sequence homology coincide with secondary structure elements, and that conserved hydrophobic residues, including the highly conserved W, form a well packed core. DynPH is electrostatically polarized, with three particularly variable loops coinciding with the positive face. These characteristics, common to all PH domains of known structure, suggest that PH domain ligands may be negatively charged, consistent with the previous suggestion that PH domains interact with anionic membrane components. The N-terminal region of phospholipase C-δ_1, (PLCδ_1) which contains a PH domain, has been implicated in binding to phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)P_2). We have shown that the isolated PH domain binds with high affinity and specificity to inositol-(1,4,5)-trisphosphate (Ins(1,4,5)P_3), and to PtdIns(4,5)P_2 in lipid vesicles. The X-ray crystal structure of PLCδPH complexed with Ins(1,4,5)P_3 shows that the ligand binds to the positive face, interacting with the three variable loops. No high affinity ligands have been identified for other PH domains. Recently, the phosphotyrosine binding (PTB) domain of the adapter protein Shc was shown to have a PH domain fold. The NPXpY binding motif, recognized by the Shc PTB domain, therefore represents a second high affinity PH domain ligand. Like PtdIns(4,5)P_2 binding to PLCδ-PH, this interaction also targets the host protein (Shc) to the cell membrane.

MS04.10.06 ARCHITECTURE AND INTRASTERIC REGULATION OF GIANT PROTEIN KINASES. B. Kobe, J. Heierhorst, S.C. Feil, M.W. Parker, G.M. Benian* and B.E. Kemp. St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia; *Dept. of Pathology, Emory University, Atlanta, Georgia 30322, USA.

Protein kinases are key regulatory enzymes in most cellular processes and must themselves be tightly regulated. Such regulation is often achieved by an intrasteric mechanism, where a part of the enzyme binds to the active site and autoinhibits its activity. The giant myosin associated proteins twitchin, projectin and titin are the largest members of the protein kinase family (600-3000 kDa) and have important roles in muscle contraction and cytoskeletal structure. Their sequences consist of numerous fibronectin type III-like and immunoglobulin (Ig)-like motifs, and a single autoinhibited protein Ser/Thr kinase structurally related to vertebrate myosin light chain kinases. We have recently shown that twitchin can be activated by the Ca^{2+}/S100$A1_2$ protein complex. To study the regulatory mechanisms of giant protein kinases, we determined the crystal structures of two recombinant fragments of twitchin. The structure of the autoinhibited twitchin kinase from *Aplysia* (A) reveals the conservation and flexibility of the autoinhibitory mechanism in giant protein kinases. The autoinhibitory sequence is positioned in the cleft between the two protein kinase lobes and inhibits the kinase by extensive contacts with residues implicated in substrate binding, ATP binding and catalysis. The structure of an extended fragment of *C. elegans*