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

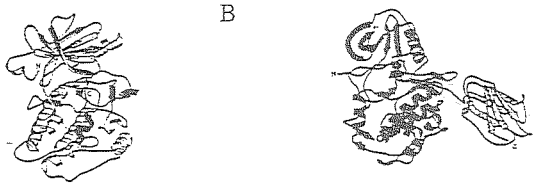
In efforts to understand intermolecular interactions that control signal transduction, we have studied the structure and function of pleckstrin homology (PH) domains. These domains of ≈120 aa are found in some 90 different signal transduction proteins. Initially we studied the dynamin PH domain (DynPH). 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*

twitchin (B) containing the autoinhibited kinase domain and a C-terminal Ig-like domain shows that the Ig-like domain extends from the kinase domain opposite to the active site and exposes possible myosin interacting surfaces. Our studies of the regulation and domain architecture of giant protein kinases jointly with an analysis of autoinhibitory sequences of other autoinhibited kinases point to possible common and diverse features of the intrasteric regulatory mechanisms.

A B



MS04.10.07 CRYSTAL STRUCTURE OF A HISTIDINE KINASE DOMAIN OF THE ANAEROBIC SENSOR PROTEIN ARCB FROM *E. COLI*. Toshio Hakoshima[1], Masato Kato[1], Toshiyuki Shimizu[1], Kazuya Ishige[2], and Takeshi Mizuno[2]. [1]Department of Molecular Biology, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma, Nara 630-01, Japan and; [2]School of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

The *Escherichia coli* ArcB protein is the anaerobic sensor and a member of the large family of the so-called two-component signal transduction proteins. ArcB comprising 778 amino acid residues possesses three phosphorylation sites (His-292, Asp-576, His-717). These multi-phosphorylation sites were suggested to make a phosphotransfer circuit that is crucial for the mechanism underlying signal transduction through ArcB. The C-terminal histidine kinase domain is composed of 125 amino acid residues, in which the active His-717 is located. Here, three-dimensional structure of this domain has been determined at 2.06Å resolution. The crystal belong to the space group $P2_12_12_1$ with unit cell dimensions, a=30.56Å, b=34.93Å, c=1 10.78Å. The structure was solved by MIR methods and was refined. The current model has an R-factor of 23%. The kinase domain of ArcB is found to form an all-α structure consisting of six helices (H1 to H6); 75% of its residues locates on the α helices. The molecules has a kidney-like shape with dimensions, 30Å by 30Å by 45Å. Two helices H3 and H6 are 7- and 8-turns long, respectively, and are bent at the centers of the helices. The helices H4 and H5 form a four-helix bundle subdomain together with the C-terminal half of the helix H3 and the N-terminal half of the helix H6. The active residue, His-64 (corresponding to His-717 in the intact ArcB), locates at the surface of the helix H4 and is surrounded with Glu61, Lys-65 and Lys-67 from the same helix and with Gln-83 and Gln-86 from the helix H5. This active site lies in the internal curvature of the kidney-shaped molecule. The helices H1 and H2 form another subdomain with the N-terminal half of the helix H3 and the C-terminal half of the helix H6.

MS04.10.08 STRUCTURE OF THE PHOSPHORYLATED FORM OF THE CDK2:CYCLIN-A COMPLEX. Philip D. Jeffrey, Alicia A. Russo, Nikola P. Pavletich, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA

Progression through the eukaryotic cell cycle is controlled by the cyclin-dependent kinases (CDKs), which are in turn under the control of multiple levels of regulation. The isolated CDK is inactive, becoming partially activated upon binding of the corresponding cyclin subunit. Full activation of the kinase complex is achieved upon phosphorylation at a site on the CDK subunit (Thr 160 in CDK2).

We have previously determined the structure of a CDK2:CyclinA complex in its unphosphorylated state at 2.3 Angstrom resolution (ref. 1). Comparison of the CDK2 subunit with the structure of the uncomplexed CDK2 (ref. 2) revealed extensive conformational changes in two regions (the PSTAIRE helix and activation loop). In contrast, comparison of the cyclinA subunit with the uncomplexed cyclinA structure (ref. 3) did not indicate any conformational change on complex formation.

Subsequently, we have crystallized the fully active complex containing CDK2 that was phosphorylated at Thr 160. The structure of this complex reveals further conformational changes caused by phosphorylation. The contribution of these changes to the full activation of the kinase will be discussed.

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MS04.10.09 HORMONE BINDING TO THE THYROID HORMONE RECEPTOR. R. Fletterick, R. Wagner, J. Baxter, A. Shiau, J. Apriletti. University of California, San Francisco, Dept. of Biochemistry/Biophysics, San Francisco, CA 94143-0448, USA

This receptor is a central metabolic regulator and is a member of the family of nuclear receptors that regulate transcription of responsive genes. The structures of two isoforms α and β were determined to understand the mechanism of recognition of hormone and the changing relationship of the receptor to its receptor, repressor and activator partners. The ligand binding domain (LBD) of the receptor comprises 12 helices and employs the hormone as a component of the hydrophobic core of the domain. The structures of the LBD with hormone and hormone analogs suggest means for precise recognition. The LBD can form dimers with homologs of the receptor or homodimers. The structures of two different homodimers have been determined to learn whether the means of association is used in transcriptional control.

PS04.10.10 CRYSTAL STRUCTURE OF mTNF. K.J. Baeyens, P. Brouckaert*, A. Raeymaekers*, W. Fiers*, C.J. De Ranter & H.L. De Bondt, Laboratory for Analytical Chemistry and Medicinal Physicochemistry, K.U.Leuven, Leuven, Belgium; *Laboratory of Molecular Biology, VIB and U. of Ghent, Belgium

TNF is a trimer consisting of 17 kDa subunits. The 3D-structure of human (h) TNF at 2.6 Å resolution has been reported. TNF interacts with two types of receptor, TNF-R55 and TNF-R75. Cellular signaling occurs by triggering one or the other, or both receptors, depending on the cell type and conditions. hTNF in the mouse only interacts with TNF-R55, and hence is a specific ligand for the latter. Remarkably, in the normal mouse, hTNF is 50-fold less toxic as compared to murine (m) TNF, indicating that the