immune responses. Besides the four hydrophobic transmembrane regions, CD81 hosts two extracellular domains, known as large and small extracellular loops (LEL and SEL, respectively). Human CD81 is held to act as (co)receptor for hepatitis C virus (HCV), thus a key participant in the infection.

To widen our knowledge on the roles played by CD81 LEL in binding the HCV E2 glycoprotein, the LEL crystal structure was approached. Three different crystal forms have so far been obtained. We report here on the most recently grown form (R32). Marked conformational fluctuations in the molecular regions held to be involved in binding to the viral protein, suggest rules for recognition and assembly within the tetraspan web.

Keywords: cell surface receptor, structural analysis of molecular crystals, virus receptors

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Structure of MRG15 reveals a Novel Fold and provides Insights into its Biological Functions

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MRG15(MORF4 related gene on chromosome 15) and MORF4 (mortality related gene on chromosome 4) belong to a newly identified protein family which share high sequence homology. Recently, the MRG proteins have been shown to function in transcription regulation (histone modification). We determined the crystal structures of the C terminal domains of MRG15 (MRG15C) and MORF4 (MORF4C) to 2.2 Å and 3.0 Å resolution, respectively.. A structure homology search with the DALI algorithm indicates that MRG15C and MORF4C have a novel protein fold with no obvious similarity to those of other proteins of known structures. The proteins are mainly consisted of α-helices and form homodimer in both solution and structures. Structure analysis and multiple sequence alignment indicate that there are a negatively charged hydrophilic patch at the C-terminal and a hydrophobic pocket at the dimeric interface both of which are composed of several highly conserved residues. These structural locations could be putative binding sites for other proteins or substrates. Biochemical assays indicate that the hydrophilic patch might be involved in binding with PAM14. Additionally, we have also obtained a 2.3 Å resolution diffraction data for the N-terminal domain (1-90 amino acids) of MRG15(MRG15N) which shows limited similarity to Chromo domain. Structure determination of MRG15N is ongoing. Analysis of these structures will shed new light on the biological functions of MRG15 protein and other members of the MRG protein family.

Keywords: MRG15, novel fold, chromo domain

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Crystal Structure of the Small G Protein M-Ras and its Implications

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Ras proteins are involved in a wide variety of cellular responses. They undergo conformational changes in two conserved regions, named “switch I” and “switch II” by cycling between GTP-bound active and GDP-bound inactive states, and thereby function as ‘on/off’ biological switches. In the active state, they bind to their specific effectors to initiate various signaling events.

We have determined the crystal structure of a Ras-family protein, M-Ras (residues 1-178), in complex with GDP and GTP. The overall structure of M-Ras resembles those of other Ras-family proteins excluding its characteristic conformations in switch regions. In the GTP-bound form, Ras proteins, including H-Ras and Rap2A, are known to conserve several intra-molecular interactions essential for conformational stabilization of switch I. In particular, hydrogen bonds between Thr-35 and the ã-phosphate of GTP play important roles to stabilize this effector loop, yielding a preferable conformation for effector recognition. In the case of M-Ras-GTP, the corresponding interaction through Thr-45 is completely lost, and this lack causes a distinctive switch I conformation, where switch I loop is pulled away from the guanine nucleotide and shows an open unstable conformation. In addition, the orientation of the á2-helix in switch II shows a remarkable difference from those of H-Ras and Rap2A. These structural features may provide new information to investigate effector recognition mechanisms by Ras proteins.

Keywords: Ras, switch region, crystal structure

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Structural Studies of Plant RKIP/PEBP Family Members

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Intracellular signalling is a critical cellular function, and one that is often perturbed in a variety of diseases. The RKIP/PEBP protein family is highly conserved across a wide range of organisms from humans to bacteria and plants. This family is involved in the regulation of kinase signalling pathways. Two members of this family, TFL1 and FT, from *Arabidopsis thaliana* act antagonistically to control meristem fate. However the exact mechanism of their action remains unknown.

To help elucidate the means by which these proteins act, crystallographic studies are being undertaken. While previous work has determined the structures of TFL1 and FT alone, current efforts are focused on extending the structural information to include protein:ligand complexes. This includes complexes of these proteins with phosphorylated amino acid, and attempts to co-crystallise these proteins with their respective protein ligands.

Because of the high level of conservation within the RKIP/PEBP protein family it is hoped that studying the functional mechanism of plant members will help elucidate the action of these proteins in humans, for which crystallisation of their complexes has proven intractable.

Keywords: signal transduction, kinase regulation, RKIP/PEBP

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Inwardly rectifying Potassium Channels

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Inwardly rectifying potassium (Kir) channels are integral membrane proteins selective to conducting K^+ ions into cells. These channels are found in many organisms and are involved in a wide range of physiological processes such as propagation of action potential and hormone regulation. The basic topology of Kir channels consist of a tetramer of two transmembrane helices, and a loop containing the selectivity filter. Both the N and C termini of each monomer in the tetramer are intracellular, and these interact to form a large globular domain, which is involved in gating. One of the difficulties in solving the structure of membrane proteins such as Kir channels is obtaining significant quantities of highly purified protein. Rather than relying on the isolation of soluble proteins from natural sources, structural biologists are focused on producing large amounts of target proteins heterologously. Therefore, a number of strategies in this project have been employed to overcome this problem. Murine Kir2.1 has been successfully cloned and overexpressed in the membrane of the methylotrophic yeast *Pichia pastoris*. This yeast was chosen as an expression host as it has many advantages of higher eukaryotic expression systems but it is as easy to manipulate as *E.coli* or *Saccharomyces cerevisiae.* Further investigations into purification and characterisation of the expressed Kir2.1 are currently being undertaken. The intracellular extramembranous domains of the murine Kir2.1 channel have been overexpressed in *E.coli.* This protein forms a stable tetramer and can be purified to a very high level. Crystallisation trials with this pure protein are in progress, as are

functional and interaction studies.
Keywords: kir, potassium channels, membrane proteins

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Structural and Functional Analysis of SHPS-1, a Receptor-type Membrane Protein
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Src homology 2 domain-containing protein tyrosine phosphatase [SHP] substrate 1 (SHPS-1), a receptor-type transmembrane glycoprotein whose cytoplasmic region binds and activates the protein tyrosine phosphatases SHP-1 and SHP-2, and thereby modulates multiple cellular functions. Its extracellular region regulates intercellular communication in the neural and immune systems through its association with CD47 on adjacent cells. Interactions between CD47 and SHPS-1 are implicated in multiple cellular processes, including cell motility [1], neutrophil transmigration, phagocytosis of red blood cells by splenic macrophages [2], and T cell activation. Although the roles of the CD47-SHPS-1 system has been presented, little is known about the cell surface organization of these ligand/receptor complexes and the structural basis for signal transduction. To gain new insights into the physiological and biological roles of the CD47-SHPS-1 system, we determined the crystal structure of the SHPS-1 extracellular domain. The domain adopts a classical immunoglobulin (Ig) fold that was observed to form an antiparallel dimer. A dimeric form of SHPS-1 was observed *in vivo*, and our structural and biophysical data shows that the extracellular domain of SHPS-1 is dimeric in solution, compatible with the view of SHPS-1 acting as a *cis*-dimeric adhesion receptor. Previous investigations showed native CD47 formed *cis*-dimers. These features suggest that both CD47 and SHPS-1 *trans*-interact each other by the formation of *cis*-dimers and offer perceptions into interactions of related Ig superfamily receptors.

[1] Motegi S., et al., *EMBO J*., 2003, **22**, 2634. [2] Oldenborg P.A., et al., *Science*, 2000, **288**, 2051.
Keywords: SHPS-1, CD47, intercellular communication

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Interaction between Raf Kinase and Raf Kinase Inhibitor Protein
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Human Raf Kinase Inhibitor Protein (hRKIP) has been shown to negatively regulate the Mitogen-Activated Protein Kinase (MAPK) signalling cascade by forming an inhibitory complex with the serine/threonine kinase Raf-1. All existing crystal structures of proteins from the RKIP family feature a highly conserved surface pocket, and it has been postulated that this region forms the primary binding interface with phosphorylated forms of Raf-1. Binding studies using randomised libraries of phosphorylated peptides indicate RKIP preferentially binds peptides containing phosphotyrosine. In this study, we have attempted to introduce phosphoserine, phosphthreonine and phosphotyrosine to crystals of hRKIP, by both co-crystallisation and soaking methods. A stable complex could only be formed with phosphotyrosine. The structure of the hRKIP-phosphotyrosine complex was solved, and confirms phosphotyrosine binds within the conserved pocket. These studies are being extended to study the binding of involving a synthetic 12-mer peptide, incorporating a tyrosine residue known to be phosphorylated in Raf-1 and its adjoining sequence. These experiments aim to provide a model for the interaction of RKIP and Raf-1, aiding our understanding of the molecular control of the MAPK signalling cascade.
Keywords: signal transduction proteins, macromolecular crystallography, ligand-protein interactions

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Structural Studies of Mycobacterial Protein Kinases and Phosphatases
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Mycobacterium tuberculosis pathogenicity relies on the peculiar ability of this microorganism to survive and replicate in human macrophages, establishing persistent infection. To understand the pathogen response to the host's environment, we are studying signaling proteins that are presumed to play an important role in the processes that regulate the complex life cycle of mycobacteria.

Two genes encoding the Ser/Thr kinases PknA and PknB, which have been described as essential by saturation mutagenesis [1], are found in a single conserved operon that also includes the gene pstp encoding the only Ser/Thr phosphatase in the mycobacterial genome. The crystal structures of PknB [2] and PstP have recently been determined in our laboratory. Both structures confirm the extraordinary conservation of the protein folds and catalytic mechanisms across the evolutionary distance between eukaryotes and prokaryotes.

We will present a comparative study of these proteins and further characterisation of protein-protein and protein-ligand interections that could be involved in a putative signaling pathway of *M .tuberculosis.*

[1] Sassetti, et al., *Mol. Microbiol*. 2003, **48**, 77-84. [2] Ortiz-Lombardia, et al., *J. Biol. Chem.*, 2003, **278**, 13094-13100.
Keywords: mycobacteria, signal transduction proteins, protein interactions

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Banana Lectin, a β-prism I Fold Lectin with Two Carbohydrate-binding Sites
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The β-prism I as a lectin fold was first characterised in this laboratory in jacalin from jackfruit seeds. This lectin fold was then thought to be characteristic of the *Moraceae* family. Subsequently, the fold was found in lectins from other families as well. We have now determined the structure of banana lectin, which adopts this fold, complexed with methyl-α-D-mannose. The X-ray analysis has been carried out on a trigonal crystal form at two temperatures. As in the case of other lectins with the same fold, the structure consists of three Greek keys, which form the faces of a pseudo-threefold symmetric prism. In other lectins, the primary carbohydrate-binding site is made up of the loop in one of the Greek keys. In banana lectin, however, two nearly identical binding sites are generated using the loops in two Greek keys. Interestingly, it turns out that while there is no noticeable reflection of threefold symmetry in the sequences of other lectins, some vestiges of this symmetry is seen in the sequence of banana lectin. This may have some evolutionary implications as well.

A comparison of the structures of banana lectin with those of other similar lectins provides insights into the variability in the oligomerization of lectins with β-prism I fold. The plasticity of the subunits in such proteins appears to be related to this variability.
Keywords: lectin crystallography, β-prism I fold, protein-carbohydrate interactions