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