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THE FERM DOMAIN, A VERSATILE PROTEIN MODULE IN SIGNAL TRANSDUCTION

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Both cell-cell and cell-extracellular matrix adhesion events accompany recruitment of several cytoplasmic proteins at the beneath of plasma membranes. These proteins contain adaptor proteins, scaffolding proteins, and linker proteins between actin cytoskeleton and adhesion molecules, as well as signaling proteins such as Rho GTPases, their regulators and effectors controlling the cell adhesion events and cytoskeleton rearrangement. Interactions of these cytoplasmic proteins with membranes and/or transmembrane proteins are critical for cell adhesion and signal transduction. The known modes of the interactions include non-covalent binding to phospholipids or to transmembrane proteins through several protein modules, in addition to covalent attachment of proteins to lipid moieties inserted in the membrane lipid bilayer. One such protein module is the FERM (4.1 and ERM) domain, which was originally found in the N-terminal regions of band 4.1 and ERM (ezrin/radixin/moesin) proteins. These proteins are known to cross-link the cytoskeletons to plasma membranes in the Rho signaling pathway. Interestingly, the FERM domain of ERM proteins interacts with phosphatidylinositol-4,5-bisphosphate (PIP2) in membranes and several proteins containing adhesion molecules such as ICAMs (intercellular adhesion molecules) and CD44 (a cell receptor protein of hyarulonic acid), scaffolding proteins such as NHERFs (Na^+/H^+ ion exchanger regulatory factor), and GDI (guanine-nucleotide dissociation inhibitor) for Rho GTPases. I will describe the crystal structures of the radixin FERM domain bound to these binding partners and discuss the molecular mechanisms by which ERM proteins accomplish the multiple molecular recognition.

Keywords: CELL ADHESION, CYTOSKELETON, SMALL GTPASE

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THE STRUCTURAL BASIS FOR LOCALISATION AND SIGNALLING BY THE FOCAL ADHESION TARGETING DOMAIN

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The localization of focal adhesion kinase (FAK) to sites of integrin clustering initiates downstream signaling. The C-terminal focal adhesion targeting (FAT) domain of FAK causes this localization by interacting with talin and paxillin. After recruitment to focal adhesions, the FAT domain also mediates signaling through Grb2 via tyrosine 925, which becomes phosphorylated by Src family kinases. We report two crystal structures of the FAT domain. Large rearrangements of the structure are indicated to allow phosphorylation of Y925 and subsequent interaction with the Src homology 2 domain of Grb2. Sequence homology and structural compatibility suggest a FAT-like fold for the C-terminal domains of the CAS family of docking proteins (CAS, Efs/Sin and Hef1), defining a superfamily of domains that may share a common mechanism for localization to focal adhesions. A structure-based alignment including these proteins and the paxillin-binding tail domain of vinculin reveals a conserved region that could play a role in focal adhesion targeting. Crystallographic and NMR analyses are in progress to allow us to characterize the molecular basis for the interaction between FAT and the so-called 'LD-motifs' of paxillin. These LD motifs are a family of recognition domains that mediate many protein-protein interactions involved in cell adhesion.

Keywords: FOCAL ADHESION KINASE, SIGNALLING, PHOSPHORYLATION

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AN ENZYME IN A SCAFFOLD: CONFORMATIONAL MODULATION OF SEROTONIN N-ACETYLTRANSFERASE BY 14-3-3

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14-3-3 proteins are a conserved family of either homo- or heterodimeric proteins. They play important biological roles in signal transduction, subcellular targeting, and cell cycle control. Their function is mediated by their ability to bind other proteins in a phosphorylation dependent manner. Pineal gland serotonin n-acetyltransferase (aanat) catalyses the key regulatory step in the biosynthesis of the circadian neurohormone melatonin that serves as a chemical feed-back signal to the circadian clock. We have previously shown using x-ray crystallography that the enzyme undergoes a major conformational change during its catalytic cycle, providing an obvious structural explanation for its observed biochemical properties. We have also shown that the enzyme is phosphorylated in the pineal gland by protein kinase at a site that is also a recognition motif for the 14-3-3 proteins. The crystal structure of the complex between aanat and 14-3-3 shows that 14-3-3 modulates the conformation of the enzyme by stabilizing the region that undergoes the conformational change upon catalysis.

This modulation changes the substrate binding properties of the enzyme, a notion that we verified using thermodynamic and kinetic measurements. We propose that one biological function of 14-3-3 binding is to modulate the conformation of its binding partner, and if the binding partner is an enzyme, this can alter the enzyme's catalytic properties.

Keywords: PROTEIN PROTEIN COMPLEX, PHOSPHORYLATION, ENZYME REGULATION

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STRUCTURE OF THE FULL-LENGTH HPR KINASE PHOSPHATASE, MIMICKING THE PRODUCT/SUBSTRATE OF THE PHOSPHO TRANSFER REACTIONS

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HPr protein kinase/phosphatase (HPrK) is a bifunctional enzyme with protein kinase and protein phosphatase activities. The balance between these two activities determines the output of the catabolite repression signaling pathway in Gram-positive bacteria. HprK from Staphylococcus xylosus consists of two clearly separated domains, which are assembled in a hexameric structure resembling a 3 bladed propeller. The C-terminal domain (kinase/phosphatase) contains two phosphate ions in the P-loop region which (by analogy with nucleoside monophosphate kinases) we propose to mimic the product-bound state.

The N-terminal domain of HPrK displays two phosphate binding sites. Under physiological conditions these sites are likely to be involved in effector binding and the modulation of the HPrK activity. Unexpectedly, the C-terminal domain of HprK is structurally related to the C-terminal domain of the phoshoenolpyruvate carboxykinase. A structure-based alignment permitted the establishment of sequence profiles to retrieve new sequences from the databases, thereby enlarging this until now one-member protein family.

Keywords: SIGNALLING, HPR-KINASE, CATABOLITE REPRESSION