



Lincoln's Inn Fields

Structural Biology

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The Structural Biology Laboratory uses X-ray crystallography and biochemical methods to uncover allosteric mechanisms regulating enzyme catalysis and to understand how multi-subunit protein complexes are assembled and control function. Our current research investigates components of growth factor-dependent signalling pathways, the molecular function of inter-strand crosslink repair endonucleases, and the architecture of molecular assemblies involving actin and actin-related proteins. Each area has direct relevance to understanding cancer and developing new cancer therapeutics. This year we have uncovered a structural and functional connection between an F-box component of an E3 ubiquitin ligase and an inhibitory subunit of the proteasome (*J.Biol.Chem.* 2008 823:22325-35) and describe how RPEL motifs from a co-activator of serum response factor engage G-actin (*EMBO J.* 2008, 27:1-11).

Structure and function of the putative oncoprotein Fbxo7

The levels of many regulatory and misfolded proteins are

controlled by the ubiquitin-proteasome system (UPS). Recruitment of protein substrates to the UPS machinery is frequently carried out by the multi-subunit E3 ubiquitin ligases, the largest group of which is the SCF family (Skp1-Cullin1-F-box). Within SCF E3 ligases, it is the F-box subunit that mediates the recognition of substrates for ubiquitination. Previous work with our collaborator Dr. Heike Laman (Cambridge University) identified the F-box protein, Fbxo7, as a putative oncoprotein that uniquely distinguishes between the G1 cdk (cyclin-dependent kinases) and specifically enhances the levels of D-cyclin/cdk6 complexes (*EMBO J.* 2005, 24: 3104-16). More recently, Fbxo7 has been suggested to undergo auto-ubiquitination and was found by linkage analysis to be mutated in Parkinsonian-Pyramidal Syndrome.

To further characterise Fbxo7, we have used a combination of structural, biochemical and genetic approaches to identify and characterise regions involved in protein interaction as a means to identify potential substrates for SCF^{Fbxo7}. Both affinity purification and yeast-two hybrid experiments identified the proteasome inhibitor-31 (PI31), a regulatory subunit of the immunoproteasome, as a putative interaction partner for Fbxo7. We then uncovered an unexpected structural and evolutionary link between Fbxo7 and PI31 by using crystallographic and bioinformatic analysis of a dimerisation domain unique to both proteins (*J.Biol.Chem.* 2008 823:22325-35). The structure of this domain (FP domain) was subsequently used to guide the design of site-specific mutants defective in their ability to either homo-dimerise and/or hetero-dimerise Fbxo7 and PI31. These mutants are being used to assess the functional consequences of ablating the Fbxo7-PI31 interaction *in vivo*. Although PI31 appears not to be a substrate for SCF^{Fbxo7}, we are currently exploring whether PI31 could modulate SCF^{Fbxo7} function by antagonising Fbxo7 homo-dimerisation.

Macromolecular assemblies involving actin

Actin is a major component of the cytoskeleton of all eukaryotic cells and plays a fundamental role in a wide

variety of cellular processes including cell morphology and cell motility. Monomeric actin (G-actin) can polymerise to form helical actin filaments (F-actin), whose organisation contributes to cellular mechanical strength. A wide variety of proteins modulate the assembly, rearrangement and disassembly of F-actin including the mammalian enabled (Mena)/vasodilator-stimulated phosphoprotein (VASP) family. These tetrameric proteins alter the geometry of assembling F-actin filaments by antagonising capping proteins and bundling actin filaments. Last year, we and our collaborators in the Cell Motility Laboratory showed that Mena function is regulated by the tumor suppressor Tes. Tes binds the EVH1 domain of Mena through a non-canonical Lim-domain mediated interaction, thereby blocking a crucial protein binding site on Mena (*Mol. Cell* 2007 28, 1071–1082). We are continuing our collaboration with the Cell Motility Laboratory by investigating the molecular interactions made by Tes with other associated cytoskeletal protein partners including actin-related proteins.

Actin has unconventional functions outside of the cytoskeleton in processes such as gene transcription, chromatin remodelling and signal transduction. In one documented example relevant to transcriptional regulation,

G-actin binds to the serum response factor co-activator MAL (also known as MRTF-A, myocardin-related transcription factor A). This interaction determines MAL subcellular localisation by preventing its nuclear accumulation as well as repressing transcriptional activation by the MAL-SRF complex. The G-actin binding site resides within the MAL amino-terminus which contains three copies of the RPEL motif (Arg-Pro-X-X-X-Glu-Leu, where X is any residue), quite distinct from other actin-binding proteins. Mutations at invariant positions within each RPEL motif have been shown to impair interaction with G-actin and de-repress MAL activity.

We are investigating the structural basis for MAL:G-actin interaction in close collaboration with the Gene Transcription Laboratory. This year we reported the crystal structures of two different 32-residue RPEL peptides from MAL (RPEL1^{MAL} and RPEL2^{MAL}) bound to G-actin:latrunculin B:ATP (*EMBO J.* 2008, 27:1-11). Each RPEL peptide presents two consecutive helices and a helical cap to bind the G-actin hydrophobic cleft and a ledge on subdomain 3, regions known to be involved in actin polymerisation (Figure 1a). Although many G-actin binding proteins engage the hydrophobic cleft of actin (Figure 1b), only the vitamin-D binding protein binds G-actin in an equivalent 'cleft-and-ledge' binding mode as MAL. Our RPEL^{MAL}:G-actin structures explain the sequence conservation defining the RPEL motif, including the invariant arginine that lies within the R-loop connecting both helices (Figure 1a). Characterisation of the RPEL^{MAL}:G-actin interaction with fluorescence anisotropy and cell reporter-based assays by our collaborators has further validated the significance of actin-binding residues within an RPEL motif for proper MAL localisation and regulation *in vivo*. Our future efforts will focus on the architecture of the RPEL domain (containing the three tandem RPEL motifs) bound to multiple G-actin molecules and how this assembly affects MAL function and localisation.

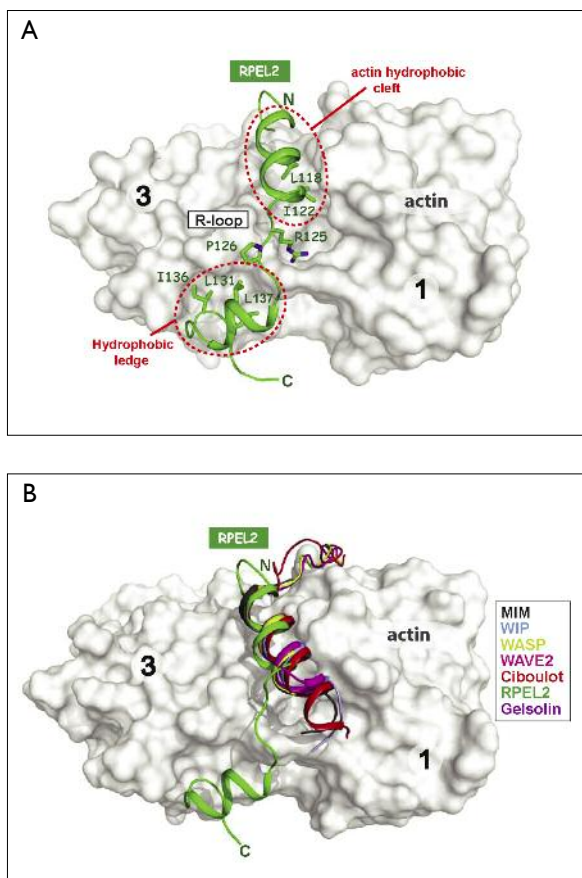


Figure 1. a) molecular interaction of RPEL2^{MAL} peptide (green) with G-actin (white/grey surface). b) similarity between RPEL2^{MAL} (green) and other actin-binding proteins (see box for colour codings for individual actin-binding proteins).

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