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Acta Cryst. (2011) A67, C344**Structure of the N-terminal spectrin repeat of the plakin domain of BP230**

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The bullous pemphigoid autoantigen 1 (BPAG1) is a member of the plakin family of cytolinker proteins that associate with and cross-link components of the various cytoskeletal systems [1]. The epithelial isoform of BPAG1, known as BPAG1e or BP230, is a component of hemidesmosomes (HDs) that are multiprotein complexes that mediate firm adhesion of epithelial cells to the underlying basement membrane [2]. In the HDs, BP230 links the integrin $\alpha 6\beta 4$ (a laminin receptor) to the cytokeratin system. BP230 contains in its N-terminal region a plakin domain (a sequence of ~ 1000 residues conserved in the plakin family) formed by eight Spectrin Repeats (SR2 to SR9) and a SH3 domain inserted in the SR5. Upstream of the SR2 there is a 55-residues long isoform-specific tail. The N-terminal region of BP230 harbors binding site for the cytoplasmic region of the integrin $\beta 4$ subunit [3].

Here we report the crystal structure of the SR2 of BP230. The structure was solved by molecular replacement and was refined against data to 2.0 Å resolution. The structure shows the characteristic SR fold, which consists of three α -helices connected by short loops. The helices are organized in a bundle with a left-handed twist that is stabilized by coiled coil interactions. The SR2 of BP230 shares a 31% sequence identity with the equivalent region of plectin, which is another member of the plakin family present in HDs. Most of the conserved residues are located in the core of the helical bundle; thus, the polypeptide backbones of the SR2 of BP230 and plectin are almost identical. Nonetheless, there are significant differences in the solvent accessible surfaces between BP230 and plectin. Finally, we have used circular dichroism and limited proteolysis to analyze the structure of the N-terminal tail of BP230. Our data suggest that the N-terminal tail is an intrinsically disordered region.

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Acta Cryst. (2011) A67, C344**Crystal structure of PK10, unique characteristics of a MAPK from *Leishmania major***

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Ser/Thr protein kinases (STPKs) are enzymes involved in signal transduction pathways. They act as “molecular switches”, showing at least 2 conformations: the active and inactive states. We aim to understand the molecular mechanisms involved in STPKs’ catalytic regulation in *Leishmania spp.*, a protozoan parasite that causes human leishmaniasis, a neglected tropical disease. Our work has focused in

LmaPK10 a ‘mitogen activated protein kinase’ (MAPK) associated with differentiation control during the parasite’s life cycle. LmaPK10 is phosphorylated in the amastigote stage, when the parasite enters the host macrophages[1]. Extensive attempts to generate LmaPK10 knock-out parasites failed, suggesting that the protein is essential for parasite’s survival (induced complementation approaches are underway). LmaPK10 is thus a potential drug target within a rational design strategy. LmaPK10 also shows interesting sequence features, distinguishing it from human MAPKs and probably involved in catalytic regulation according to their key positions. Recombinant LmaPK10 was expressed in *E. coli* and purified to homogeneity. *In vitro* kinase activity was assayed in presence of ³²P-ATP using surrogate substrates, detecting weak auto- and substrate phosphorylation. LC/MS analysis allowed to define the autophosphorylated peptides, primarily within the activation loop, but also beyond. Crystallization conditions were found employing the full-length protein, as well as a protease-resistant C-terminal truncated construct (PK10 Δ C, lacking the final 43 amino acids). PK10 Δ C diffracted X rays to high resolution (SGP4₃2₁2) allowing its structure to be solved by molecular replacement using human p38 as search probe (*3HV3*) and refined to 1.9 Å resolution.

To the best of our knowledge, PK10 Δ C (pdb 3PG1) represents the first trypanosomatid MAPK structure to be reported. The protein crystallized in a non-phosphorylated conformation and structural analysis allowed us to identify regions and residues that are unique to this enzyme, in particular an N-terminal helix replacing conserved β -strands, and more importantly, an inserted β -hairpin just before the N-terminal lobe Δ C helix, important for catalytic regulation. PK10 Δ C appears to be trapped in a conformation that shares features with both the fully active and inactive states [2], owing to mutations in key positions otherwise absolutely conserved in STPKs. Affinity quantitation by fluorescence allowed us to determine dissociation constants for ATP, and other nucleotide substrate derivatives, as well as binding constants for the human p38-specific inhibitor SB203580. We have recently solved the structure of PK10 Δ C in complex with SB203580, revealing detailed patterns of the binding pocket, with a full structural analysis currently underway. We envisage to obtain the phosphorylated, expectedly active, conformation of LmaPK10 in order to perform biochemical and crystallographic analyses of the regulatory conformational switch, clearly relevant within the rational drug discovery strategy. Acknowledgments: LEISHDRUG , PEDECIBA, ANII

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Acta Cryst. (2011) A67, C344-C345**Crystal structures of adenomatous polyposis coli and its complex with Sam68**

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The adenomatous polyposis coli (APC) tumor suppressor protein is commonly mutated in colorectal tumors. The best-known function

of APC is the regulation of the level of β -catenin, which is a key Wnt signaling effector. Truncation mutations in APC result in the accumulation of β -catenin in the nucleus, where β -catenin binds to the T cell factor (TCF) and lymphoid enhancer factor, thereby activating the transcription of Wnt target genes.

APC is a multidomain protein that interacts with a variety of proteins. The armadillo repeat (Arm) domain of APC facilitates the interaction of APC with numerous proteins. It was recently found that the Arm domain of APC interacts with the tyrosine-rich (YY) domain of Sam68, the Src-associated in mitosis, 68 kDa protein. It was suggested that the complex between APC and Sam68 negatively regulates TCF-1 alternative splicing, thereby inhibiting Wnt signaling.

In this study, we determined the crystal structures of the APC-Arm fragment, consisting of residues 396–732, and its complex with the Sam68-YY fragment, consisting of residues 365–419. APC-Arm forms a superhelix with a positively charged groove. We mapped the locations of the missense mutations in APC-Arm, identified in familial adenomatous polyposis patients, onto the structure of APC-Arm. The Arg414 and Lys516 residues that are targeted for missense mutations to Cys and Asn residues, respectively, are located on the surface of APC-Arm, and may impair ligand binding.

Sam68-YY adopts a bent conformation, and forms numerous interactions with the residues in the positively charged groove of APC-Arm. To confirm the significance of the crystallographically determined binding sites, we performed isothermal titration calorimetry (ITC) on wild-type APC-Arm and Sam68 mutants, and on APC-Arm mutants and wild-type Sam68. Point mutations were sufficient to abrogate complex formation. To assess whether phosphorylation affects Sam68-YY binding to APC-Arm, we also performed ITC on wild-type APC-Arm and phosphorylated Sam68. Phosphorylation of Sam68 Tyr387, at the apex of the bend, completely abolished binding to APC-Arm.

We compared the structures of the Arm domains of APC and other Arm-containing proteins, such as β -catenin and p120 catenin. We found that while the overall structures of APC-Arm and β -catenin-Arm are similar, their ligand recognition modes differ significantly. On the other hand, both the overall structures and ligand recognition modes are similar between APC-Arm and p120 catenin-Arm. However, the conformations of Sam68-YY and the juxtamembrane domain of E-cadherin, the ligand of p120 catenin-Arm, are different, in that the former is bent while the latter is elongated. We speculate that ligand bending may be a structural determinant for binding to APC-Arm.

Keywords: cancer, complex, crystallography

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Structural insight into the SARAH domain from Mst2 kinase in the apoptosis pathway

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Mst1 and Mst2 (Mammalian Sterile 20-like kinase 1 and 2) are proapoptotic protein kinases and involved in cell proliferation and survival. The C-termini of Mst1 and Mst2 contain a common protein-protein interaction domain, named SARAH (Sav/Rassf/Hpo), which is found in three classes of eukaryotic tumour suppressors, Salvador, Rassf, and Hippo. The interaction of these SARAH domains controls apoptosis and cell cycle arrest. Moreover, Mst2 SARAH domain is known to interact with Raf, resulting in the suppression of apoptosis [1]. The SARAH mediated homo- and heterodimerization are crucial in the pathways

that induce apoptosis and cell cycle arrest. In this study, we describe the sample preparation and x-ray crystal structure of SARAH domain from Mst2 kinase. The gel-filtration chromatography shows that the SARAH domain of Mst2 forms a homodimer in solution. These results provide a useful information for the structural and functional study of Mst2 SARAH domain in the apoptosis pathway.

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Intermolecular communication between DnaK and GrpE in the DnaK chaperone system

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DnaK is a homolog of the highly conserved ATP-dependent Hsp70 family of chaperone proteins in prokaryotes. The mechanism of peptide association and dissociation in this chaperone protein DnaK is regulated by concerted and consecutive association of co-chaperone proteins DnaJ (Hsp40) and GrpE (nucleotide exchange factor) in the chaperone cycle. Our understanding of detailed mechanism for the interaction among these proteins towards peptide folding remains vague due to insufficient structural information. Here we report the crystal structure of a full-length GrpE homodimer in complex with a functionally intact DnaK chaperone from *Geobacillus kaustophilus HTA426* at 4.1Å resolution. The overall structure represents the nucleotide-free and substrate-bound conformation. The structure demonstrates that the conserved linker region of DnaK is stabilized by the N-terminal long α -helices of the GrpE dimer during ternary complex formation with a hydrophobic peptide. Furthermore we show the possible interaction between substrate-binding domain of DnaK with the N-terminal disordered region of GrpE in accelerating bound substrate release and complex stabilization. These findings provide a molecular mechanism for client substrate binding, processing and release during Hsp70 chaperone cycle.

Keywords: chaperone cycle, heat shock protein 70, protein-protein interaction

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Control of asymmetric cell division in developing *Drosophila* Neuroblasts

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Asymmetric cell division enables small groups of multipotent stem cells to produce a vast range of terminal cells with diverse functions. It plays a key role in the development of higher organisms. In the developing *Drosophila* embryo, the multipotent stem cells, which generate all of the neuronal and glial cells of the adult central nervous system, are known as neuroblasts. Neuroblasts divide asymmetrically along their apico-basal axis to produce a new neuroblast (apical) and a smaller ganglion mother cell (GMC, basal). Whilst the new neuroblast