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 pro-apoptotic 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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Keywords: mst2 kinase, SARAH domain

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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

repeats this cycle (self-renewal), the GMC divides once more to produce two terminally differentiated neurons and/or glial cells. During mitosis of the neuroblasts, several molecules have been shown to be absolutely required for asymmetric division to take place. These include molecules which are asymmetrically localised and partitioned into the GMC (including Prospero mRNA and Miranda, Brat and Prospero proteins), and molecules that set up and maintain the asymmetric potential of the cell (including Inscuteable, Par-3/6, Bicaudal D, Egalitarian and Rab6). In order for correct asymmetric cell division to occur, these molecules must interact with each other. However the mechanisms underlying these protein-protein interactions are unknown.

Here we focus on gaining structural insight into the mechanisms that drive asymmetric cell division using X-ray crystallographic techniques to determine high-resolution structures of the proteins involved. We are currently making progress in expressing, purifying and crystallising the proteins Miranda, Prospero and Inscuteable. We have also solved the crystal structure of *Drosophila melanogaster* Rab6 to 1.4 Å resolution. Rab6 is known to bind many effector proteins, including Bicaudal D, and we aim to use this structure to aid determination of Rab6-effector complex structures.

Keywords: cell division, protein-protein interactions

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Structural basis of dephosphorylating activity of PIP substrate in PTPRQ

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The PTPRQ belongs to a receptor-type classical protein tyrosine phosphatase (PTP) family with one catalytic domain in the cytoplasmic region. Unlike other classical PTPs, PTPRQ has de-phosphorylating activities toward phosphatidyl-inositol (PIP) substrates. We successfully cloned, over-expressed, purified and subsequently crystallized the catalytic domain of PTPRQ. Subsequently PTPRQ was crystallized in two forms. PTPRQ crystals belonged to hexagonal space group, $P6_4$ with cell dimensions of $a = b = 78$ Å, $c = 84$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$ diffracted x-ray to 1.6 Å resolution. Structural solution was obtained by molecular replacement method using PTPRO structure. Overall, PTPRQ adopts a typical tertiary fold as other classical PTPs do. However, the disordered "E-loop" of PTPRQ surrounding catalytic core and concomitantly absence of its interactions with the residues in "P-loop" results in flat active site pocket. We propose that this structural feature might facilitate to accommodate the bigger substrates and be suitable for dephosphorylation of PIP.

Keywords: PTP, PIP, E-loop

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Structural investigation of RAP80; A novel BRCA1 interacting protein involved in the mediation of DNA damage repair function

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BRCA1 (Breast Cancer Susceptibility gene 1) is one of the central molecules that play crucial role in DNA damage repair process [1]. BRCA1 recruitment at the site of DNA damage depends on its interacting partners like RAP80 (Receptor Associated Protein 80), CCDC98, MERIT40. RAP80 comprises two tandems Ubiquitin-Interacting Motifs (UIM1, UIM2) at its N-terminus, ABRAXAS Interacting Region (AIR) at the central domain and two zinc-finger containing motifs (ZFD1, ZFD2) at C-terminus [2]. UIM motifs of RAP80 interact with K63 linked polyubiquitin chain(s) on H2AX and thereby assemble the RAP80-BRCA1 complex at the damage site for DNA repair [2]. CCDC98 (ABRAXAS) acts as a bridging molecule to mediate the interaction of RAP80 with BRCA1 [3]. MERIT40 helps in the stabilization of whole complex by favoring the interaction among various members[4].

To dissect the structural diversity, RAP80 functional domains (tandem repeat UIM, AIR) and CCDC98 were sub-cloned in bacterial expression vectors pGEX-kT and pMAL-c2 respectively. Purified recombinant protein(s) were obtained using two step purification involving affinity chromatography followed by gel filtration chromatography. Mass spectrometric analysis confirmed the identity and estimated the correct molecular mass of the purified proteins. Secondary and tertiary structures monitored using Far UV Circular Dichroism and Fluorescence spectroscopy suggested that the recombinant proteins have well folded structural conformation. Crystallization screening of RAP80 tandem UIM1 and UIM2 domain showed nucleation, further optimization is under process. CCDC98 fusion protein with Maltose binding protein tag has been purified and binding analysis using ITC and co-crystallization is under optimization. Comparative study of wild type and mutants of RAP80-BRCA1 complex will be helpful in basic understanding of DNA repair defect and hence the tumorogenesis.

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Cooperative DNA-binding mechanism of Aristaless and Clawless

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Homeodomain is one of the most widely spread superfamily of eukaryotic DNA binding domain that regulates transcriptions of various kinds of genes that are indispensable for development. Some homeodomain proteins bind cooperatively to specific DNA sequences to increase those binding affinities and site specificities for the target DNA. In this report, we examined a cooperativity of two homeodomain proteins, Aristaless (Al) and Clawless (Cll) from *Drosophila melanogaster*. These homeodomain proteins play an important role in *Drosophila* leg development. In the distal-most region, homeobox genes, *al*, *Lim1*, and *cll* are expressed to specify the region, while a pair of *Bar* homeobox genes are expressed in its immediate neighbour (distal tarsus). For the accurate differentiation of these regions, the