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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Keywords: damage, repair, ubiquitin

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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

expression of Bar is repressed by the cooperative mechanism of Al and Cll in the distal-most region. These two homeodomain proteins bind cooperatively to the *Bar* enhancer element and repress its gene expression.

To elucidate the structural basis of the cooperative DNA binding mechanism of Al and Cll, we determined the minimal region of Al and Cll that are indispensable for the cooperative DNA binding mechanism of these proteins at first. Then, we tried to determine the homeodomain structures of Al and Cll, a binary complex structure of Al-DNA, and a ternary complex structure of Al-Cll-DNA to analyse the structural basis of the cooperativity of these proteins. In this study, we determined four structures by X-ray crystallography: the structure of Al homeodomain, the structure of Hox11L1 homeodomain (human homolog of Cll), the binary complex structure of Al-DNA, and the ternary complex structure of Al-Cll-DNA. Our results show a novel ternary complex structure formation of homeodomain proteins. In the ternary complex structure, the extended conserved region of Cll homeodomain plays a critical role for the cooperative DNA binding mechanism. The extended conserved residues located in the N-terminal to the Cll homeodomain are well ordered in the ternary complex structure and inserted into a minor groove of DNA. We show that three residues (His-10, Tyr-8, and Arg-5 of Cll homeodomain) are indispensable for both sequence recognition and cooperative DNA binding mechanism of Al and Cll. On the other hand, the extended conserved residues located in the C-terminal to the Cll homeodomain form intermolecular interactions with Al to increase binding affinity of Cll homeodomain. The structural analysis of Al-DNA and Al-Cll-DNA complexes shows that the cooperative DNA binding of Al and Cll is caused by a structural modification of DNA by the binding of Al homeodomain to DNA. Our result provides a novel possibility of cooperative DNA binding of homeodomain proteins for accurate gene regulations.

Keywords: biology, complex, transcription

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Purification and crystallization of a Protein-DNAComplex

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Our project deals with a transcription factor (25 kDa) in complex with a DNA that harbours the cognate binding sequence. The protein was fused to a C-terminal His_6 tag by genetic engineering and overexpressed under standard conditions (37ºC, 225rpm, LB medium, IPTG induction) in the BL21(DE3)pLysS E.coli strain. The protein purification was done in two steps, which consisted in an affinity column (His-Trap Chelating, *GE Healthcare*) followed by a gel filtration (Superdex 75, *GE Healthcare*).

For the DNA-protein complex different oligonucleotides were designed based on the specific DNA-binding sequence and taking into account a more general binding sequence described for the protein superfamily. The oligos, of blunt or cohesive ends, included sequences of different number of base pairs.

In order to understand the behaviour of the complex and to know the correct ratio between DNA and protein, electrophoretic mobility shift assays (EMSA) were performed at different protein and DNA stoichiometries. Two retardation bands systematically appeared at higher protein ratios, suggesting the formation of a dimer onto the DNA. This oligomerisation state was confirmed by ultracentrifugation assays.

Crystallization was tried by the vapour diffusion method at 20ºC and 4ºC, dispensing both hanging and sitting drops with the protein alone or the DNA-protein complex. The initial crystallization conditions were subsequently optimised. The best crystals obtained belonged to the orthorhombic space group P222 (cell parameters a=56.12; b=118.41; c=120.05; $\alpha=\beta=\gamma=90º$) and diffracted to 4 Å resolution.

In order to improve the crystal quality and the resolution several methods were tried, like micro and macroseeding [1] or dehydration methods [2]. Additional methods will be tried in combination with different types of oligos.

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Keyword: protein-DNA complex, purification, crystallization

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An unfolding model for the GDP/GTP conformational switch of the GTPase Arf6

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Small G proteins of Arf family (ADP ribosylation factor) are ubiquitously implicated in membrane trafficking. Arf1 and Arf6, two members of the family, are highly similar: they have over 60% sequence identity, and structural studies have shown that the surfaces they use to interact with regulators and effectors are essentially identical in sequence and structure [1]. Yet, they have different functions in cells. Arf1 is a major regulator of most aspects of vesicular traffic. Indeed, Arf1 regulates the recruitment of coated vesicles of the Golgi apparatus, while Arf6 is restricted to the plasma membrane where it acts at the crossroads of trafficking and cytoskeleton functions [2]. Consistent with their cellular specificities, Arf1 and Arf6 also have distinctive biochemical properties *in vitro*, for which no straightforward structural explanation has been put forward.

Arf proteins alternate between a GDP-bound inactive form in the cytosol and a GTP-bound active form, which is bound to membranes and able to interact with effectors to induce a cellular response. We show that a truncated Arf6 mutant, which mimics membrane-bound Arf6-GDP is partially unfolded in the crystal compared to cytosolic, full-length Arf6. This unusual conformation is the major Arf6-GDP species in solution, as shown by synchrotron SAXS analysis [3]. In contrast, the equivalent Arf1 mutant is essentially identical to full-length, cytosolic Arf1-GDP, as shown by NMR analysis [4]. Taken together, these experiments suggest that the structural routes for the activation of Arf1 and Arf6 diverge at the step where GDP-bound Arf is recruited to membranes prior to nucleotide exchange. These differences may account for the biochemical differences between Arf1 and Arf6, and yield their functional specificities.

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Keywords: GTPases, crystallography, SAXS