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The Structural and Mechanistic Basis of Regulation of Vesicular Transport by Rab Proteins.

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The Rab proteins are members of the Ras superfamily of small GTPases and are important regulators of intracellular vesicular transport. Like other members of this superfamily, they are involved in a cycle of GTPase and GDP exchange activity and also in a coupled cycle of reversible attachment to and detachment from membranes. These 2 cycles form the basis for coordination of a complex group of both generic and specific protein-protein as well as protein-membrane interactions. They are being studied by structural (mainly protein crystallographic) and kinetic/spectroscopic methods. Results to be discussed include those pertaining to the process of Rab prenylation, an essential requirement for their interaction with membranes, delivery of Rab proteins to membranes and the reverse process of extraction, as well as exchange of GDP for GTP catalyzed by guanine nucleotide exchange factors (GEFs). Particular emphasis will be placed on the solution of problems inherent in dealing with lipidated proteins for structural and other studies and on interpretation of the structural results to throw light onto the mechanisms underlying Rab recycling and targeting to specific membranes.

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The Structural and Mechanistic Basis of Allosteric Modulation of Myosin Motor Activity by Pharmacological Agents.

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Domain insertions and the replacement of functional modules in the myosin head fragment with synthetic sequences provide efficient means to manipulate key features of the myosin motor such as actin- and nucleotide-affinity, coupling between the actin- and nucleotide-binding sites, force production and even the direction of movement in a well defined manner. Additional, this approach facilitates the production of functional motor domains derived from a wide range of members of the myosin family. In recent work, we have combined this approach with the use of small molecule effectors of myosin motor activity. We identified pentabromopseudilin (PBP) and related halogenated alkaloids as potent inhibitors of myosin-dependent processes such as isometric tension development, unloaded shortening velocity, and *in vitro* motility. Coupling between the actin and nucleotide binding sites is reduced in the presence of these inhibitors. PBP-induced changes in rate constants for ATP-binding, ATP-hydrolysis and ADP dissociation extend the time required per actin-activated myosin ATPase cycle. Additionally, the ratio of time spent per ATPase cycle in strong and weak binding states is shifted by PBP and related compounds in favor of non-force generating states. To elucidate the binding mode of these compounds, we crystallized their complexes formed with the *Dictyostelium* myosin-2 motor domain. In every case, the electron density for the small molecule inhibitor is unambiguous. All compounds bind to a novel allosteric site near actin-binding residues at the tip of the 50-kDa domain at a distance of 16 Å from the nucleotide binding site and 7.5 Å away from the blebbistatin binding pocket. The residues involved in the binding of this new class of inhibitors are only moderately conserved between the members of the different myosin classes. This is consistent with the observed differences in IC_{50} values. The results of molecular modeling studies show that these isoform-specific variations in the extent of inhibition can be predicted at least in trend for each of the new compounds. Our results yield insights into the allosteric transmission of information between the catalytic and actin-binding sites of myosin and the occurrence of multisite allostery in single subunit systems.

Keywords: myosin; allosteric effectors; drug-protein interactions

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Structure and Functional Study of Human Retinaldehyde-bindingProtein(CRALBP).

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Cellular retinaldehyde-binding protein (CRALBP) is a 36-kD water soluble protein which is an essential chaperone in mammalian vision, found only in retina and pineal gland, and it functions in the retinal pigment epithelium (RPE) as a high affinity receptor of 11-cis-retinol in the isomerization step of the rod visual cycle and as a substrate carrier for 11-cis-retinol dehydrogenase. Human CRALBP gene defects can either tighten or abolish retinoid interactions, which in turn can compromise substrate carrier interactions with 11-cis-retinol dehydrogenase and lead to several retinal pathologies. Non functional CRALBP gene products have been reported to be associated with retinitis pigmentosa, a disease leading to blindness. To better understand CRALBP visual cycle functions, which require rapid association and dissociation of retinoid, we have successfully crystallized

the protein-ligand complex and collected data set at resolution of 3 Å, structure solution and refinement is on the way. Based on the structure further biochemistry experiments were carried out to identify the cause of the retinal degenerative disorders by mutation of CRALBP.

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Keywords: vitamin A; crystal structure; retinitis pigmentosa

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Conserved Binding Mode of Single-Stranded DNA and RNA to Cold-Shock Domains. Udo Heinemann[a], Klaas E. A. Max[a]. [a]*Max-Delbrueck Center for Molecular Medicine, Berlin, Germany.*
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Cold-shock domains occur ubiquitously in proteins from all kingdoms of life. They occur in proteins that function in transcriptional and/or translational control of gene expression. Bacterial cold-shock domains are autonomous, small proteins, whereas their eukaryal orthologs usually occur as structural units in larger proteins. Some, but not all bacterial cold-shock proteins are upregulated under cold-shock conditions and are thought to mediate cold-stress-response functions.
Already the first crystal structure of a bacterial cold-shock protein suggested a possible mode of DNA or RNA single-strand binding to a basic protein surface with conspicuously exposed aromatic side chains [1]. It was not until recently, however, that this binding mode was proven by crystal structure analysis of oligothymidine strands bound to the major cold shock proteins *Bs*-CspB of *Bacillus subtilis* and *Bc*-Csp of *Bacillus caldolyticus* [2, 3]. These structures identified seven subsites for nucleotide binding and, combined with fluorescence-based DNA binding studies, suggested the consensus sequence NTCTTTN (N = any nucleotide) for DNA binding to the *Bacillus* cold-shock proteins. This consensus was confirmed by DNA microarray studies [4].
The crystal structure of the *Bc*-Csp:dT_7 complex showed a domain-swapped dimeric structure of the cold-shock domain [3]. Domain swapping has never been observed before in a series of crystal structures of bacterial cold-shock proteins [5-9] and thus extends the range of structural polymorphs populated by cold-shock domains.
Recently, we extended the structural characterization of cold-shock domains by studying the binding of ribooligonucleotides to cold-shock domains from human Y-box factors by fluorescence-based assays and crystal structure analysis. We find a conservation of the general binding mode observed before, but there is significant variation in subsite interactions which may be functionally relevant.

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Keywords: protein-nucleic-acid crystallography; cold-shock domains; single-stranded DNA and RNA

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Decoding the Structural Basis for the *cis*-Regulatory Logic of Early Developmental Switches. Ralf Jauch[a], Andrew Hutchins[a], Nithya Baburajendran[a], Kamesh Narasimhan[a], Calista KL Ng[a], Prasanna Kolatkar[a]. [a]*Stem Cell Biology, Genome Institute of Singapore.*
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The basis for differential gene regulation is the selective recognition of /cis/-regulatory DNA sequences by sequence-specific transcription factors (TFs).TF binding sites (TFBS), however, are short, degenerate and have strong affinities for highly homologous yet functionally antagonistic proteins. Also, genome-wide TFBS mapping studies in embryonic stem cells and other tissues showed that key regulators such as Nanog, Sox2/17, Oct4 and Smad1 co-occupy thousands of binding sites and gene activity prediction based on such data is poor. How then are gene expression programs executed and how are cell fate decisions made? We use genome wide TFBS datasets to retrieve candidate motifs that may lead to the differential assembly of TFs and thereby act as, developmental enhancer'. By using biochemical and biophysical assays as well as by determining crystal structures of protein-DNA complexes we study the basis for TF function and assess contributions of individual sequence specificities, TF induced DNA deformations and co-factor recruitment. I will present structural and biochemical data of proteins from the Sox and Smad families that provide a mechanistic perspective on the decision making during the earliest stages of mammalian development.

Keywords: transcription; protein-DNA; gnomics