FA1-MS04-O1

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.

FA1-MS04-O2

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

FA1-MS04-O3

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