Symposium 11: Cargo Transport by Single Molecular Motors

1857-Symp
Single Molecule Imaging In Live Cells Reveals Selection Of Microtubule Tracks By Kinesin Motors
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Long-distance transport of vesicular and protein cargoes in cells requires microtubules and their associated molecular motors. The basic mechanistic principles of tubulin polymerization and motor motility have been discovered from in vitro studies. The challenge is to translate the mechanics of these individual parts into the workings of ensembles of molecules inside cells. By imaging single kinesin motors in live cells, we reveal new properties about the interactions of motors and microtubules. We show that single Kinesin-1 motors move preferentially on a subset of microtubules available in COS cells. Preferential motility does not occur on dynamic microtubules marked by end binding protein 3 (EB3), which decorates the plus tips of growing microtubules. Rather, retrospective immunofluorescence staining demonstrates that single Kinesin-1 motors utilize stable microtubules marked by specific post-translational modifications. Preferential motility on stable microtubules is not a general property of kinesin motors as neither the kinesin-2 family member KIF17 nor the kinesin-3 family member KIF1A moved on a subset of microtubules. Selective transport enables Kinesin-1 to carry vesicles containing the marker protein vesicular stomatitis virus (VSV)-G along stable microtubules in COS cells whereas KIF17 transport of vesicles containing the voltage-activated potassium channel Kv1.5 occurs along both stable and dynamic microtubules in HL-1 atrial myocytes. These results support the hypothesis that a tubulin code of post-translational modifications can direct kinesin transport events in cells.

1858-Symp
Steps, Force And Motile Mechanism Of Cytoplasmic Dynein
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Native cytoplasimic dynein moves with 8-nm steps by overlapping hand-over-hand mechanism (Toba et al. PNAS 2006). The 8-nm steps were confirmed in vesicle transport using FIONA method of quantum labeled vesicles. The contribution of individual dynein head to each step of the dynein is unclear because the step size is always masked by 8-nm of microtubule pitch. To avoid the masking, a recombinant single-headed dynein was prepared and bound separately onto a bead. The movement of dynein heads on the bead was characterized by laser trap and measurement with nanometer accuracy. The two molecules of single-headed dynein move forward and backward with various step sizes. As the dynein heads on a bead can bind on the microtubule, the histograms of step sizes from -30 to +30 nm were well fitted to a single Gaussian curve. The mean step size decreased from ~5 nm to 0 nm if the stalling force is increased from 0 to 2 pN meanwhile the half width, ~11 nm, of the step size distribution was however not changed. Based on this finding, it is suggested that the directional movement is generated by a 5-nm working distance of a dynein head while the other dynein head freely diffused 11 nm. The suggestion could explain recent controversial data about step size of different preparations of dynein.

1859-Symp
Class V Myosins in Budding Yeast: Theme and Variations
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A prominent feature of most class V myosins is their ability to take multiple steps on actin without dissociating, known as processivity. Recent evidence showed that there are also non-processive class V myosins, both in humans and lower organisms. These motors need to work in ensembles to ensure continuous, unidirectional movement. The budding yeast *Saccharomyces cerevisiae* has two class V myosins, both of which are non-processive, but for different reasons. Myo2p is a dimeric motor with a low duty cycle, meaning that it spends a small portion of its cycle time strongly attached to actin. Myo4p has a high duty cycle motor, but is single-headed and thus cannot move processively as a single molecule. We propose that the association of Myo4p with its adapter protein She3p accounts for why it is single-headed. She3p is required for transport of all cargo of Myo4p (mRNA and cortical ER), and thus it has become a subunit of the motor. Myo2p, in contrast, moves many different cargoes (e.g. organelles and secretory vesicles), each with a unique adapter protein. Using a combination of *in vitro* and *in vivo* techniques, we probe how the features of each motor are uniquely suited for its particular cellular role. The involvement of other proteins that act as ''processivity factors'' will also be discussed.

1860-Symp
Regulation Of Switching Of Membrane Organelles Between Cytoskeletal Transport Systems In Melanophores
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Intracellular transport is driven by organelle-bound molecular motors that move cargo organelles along microtubules (MTs; motors of kinesin and dynein families) or actin filaments (AFs; myosin family motors). While transport along each cytoskeletal track type is well characterized, switching between the two types of transport is poorly understood. Here we used a combination of particle tracking and computational modeling approaches to measure parameters that determine how fast membrane organelles switch back and forth between MTs and AFs, and compare these parameters in different signaling states. As a model system we used melanophores, which aggregate thousands of membrane-bounded melanosomes in the cell center or redisperse them throughout the cytoplasm. Dispersion involves successive transport of melanosomes along the radial MTs and randomly arranged AFs. For aggregation, melanosomes that are transported along AFs transfer back onto MTs for movement to the MT minus ends clustered in the cell center. We performed tracking of individual pigment granules moving along MTs or AFs, determined major movement parameters (velocities and durations of runs to the plus or minus ends of MTs, and along AFs) using the Multiscale Trend Analysis, and incorporated them into a computational model for pigment transport. Comparison of the results of computational simulations of pigment distribution along the cell radius with experimentally obtained changes of pigment levels showed that regulation involves a single parameter: the transferring rate from AFs to MTs. This result suggests that MT transport is the defining factor whose regulation determines the choice of the cytoskeletal tracks during the transport of membrane organelles.

Symposium 12: Regulated Intramembrane Proteolysis (RIP)

1861-Symp
Intramembrane Proteolysis by the Rhomboid Serine Protease GlpG
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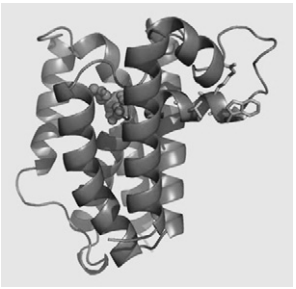
Intramembrane proteases catalyze peptide bond cleavage of integral membrane protein substrates. This activity is crucial for many biological and pathological processes. Rhomboids are evolutionarily widespread intramembrane serine proteases that cleave type I or II membrane protein substrates. In addition to high-resolution structural studies of the E. coli representative GlpG, this presentation will discuss the ability of rhomboids to cleave unfolded multi-spanning membrane proteins.



1862-Symp
Structure and mechanism of Site-2 Protease
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1863-Symp
Biochemical And Structural Characterization Of Intramembrane Proteases
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In regulated intramembrane proteolysis membrane proteins are cleaved within their transmembrane region, resulting in soluble fragments that regulate cell physiology. The intramembrane proteases responsible for cleavage are widespread with important roles in human biology and disease. Rhomboids catalyze the activation of epidermic growth factor receptor ligands in D. melanogaster. How rhomboids recognize their substrates and select which peptide bond to cleave is not understood. We have studied the substrate specificity and peptide bond selectivity of purified rhomboids from several organisms using chimeric