Symposium 18: Calcium Flickers and Motility at the Leading Edge Membrane

3221-Symp

Imaging Neutrophil Migration in Vivo using Zebrafish Anna Huttenlocher.

Univ Wisconsin Madison, Madison, WI, USA.

Cell migration is crucial for diverse biological processes. Here we have visualized the dynamics of PI(3)K activity during neutrophil migration in intact tissues, revealing that PI(3)K activation at the leading edge is critical for neutrophil motility. Rac was activated locally in living zebrafish using genetically encoded photoactivatable Rac, demonstrating that Rac activation is sufficient to direct cell migration in vivo. In PI(3)K-inhibited cells, Rac activation at the leading edge rescued membrane protrusion but not cell migration or polarity in PI(3)K-inhibited cells. Uncoupling Rac-mediated protrusion and polarization suggests a new paradigm of two-tiered PI(3)K-mediated regulation of cell motility. This work exemplifies a broadly applicable new approach for examining spatio-temporal regulation of signaling within multicellular organisms.

3222-Symp

Second Messengers at the Leading Edge: Calcium Joins PIP3 as an Essential Signal

Joseph J. Falke.

Univ Colorado, Boulder, CO, USA.

The leading edge of a chemotaxing cell is the engine that drives the cell up an attractant gradient. In one of the most dramatic spatial redistributions in cell biology, local second messenger signals recruit dozens of regulatory proteins from cytoplasm to the leading edge membrane. In turn these proteins control the actin polymerization and membrane remodeling processes propelling migration. It has long been known that the signaling lipid PIP3 is a crucial second messenger at the leading edge where it recruits PH domain proteins. Recent evidence has revealed that calcium is also an essential second messenger at the leading edge, where the local calcium signal recruits protein kinase C and other C2 domain proteins. The leading edge PIP3, calcium, and PKC are all required components of the positive feedback loop that maintains leading edge structure and activity. This positive feedback may well play a central role in the compass that guides cell migration.

3223-Symp Calcium Flickers in Cell Migration Heping Cheng. Peking Univ, Beijing, China. No Abstract.

3224-Symp Signaling Control of Collective Cell Migration Tobias Meyer. Stanford Univ Sch Med, Stanford, CA, USA.

Symposium 19: The 'Un' in Unconventional Molecular Motors

3225-Symp

No Abstract.

Opening Up, Coming Together and Reaching Out: More Surprises from Myosin $V\!I$

Lee Sweeney.

Univ Pennsylvania Sch Med, Philadelphia, PA, USA.

Myosin VI challenges the prevailing theory of how myosin motors move on actin: the lever arm hypothesis. While the reverse directionality and large powerstroke of myosin VI can be attributed to unusual properties of a subdomain of the motor (converter with a unique insert), these adaptations cannot account for the large step size on actin. Either the lever arm hypothesis needs modification, or myosin VI has some unique form of extension of its lever arm. We determined the structure of the region immediately distal to the lever arm of the motor and show that it is a three-helix bundle. Based on C-terminal truncations that display the normal range of step sizes on actin, CD, fluorescence studies, and a partial deletion of the bundle, we have demonstrated that this bundle unfolds upon dimerization of two myosin VI monomers to generate an extension of the lever arm of myosin VI. Furthermore, based on the ability of myosin VI monomers to dimerize when held in close proximity, we postulated that cargo binding normally regulates dimerization of myosin VI. We tested this hypothesis by

expressing a known dimeric cargo adaptor protein of myosin VI, optineurin, and the myosin VI-binding segment from a monomeric cargo adaptor protein, Dab2. In the presence of these adaptor proteins, full-length myosin VI has ATPase properties of a dimer, appears as a dimer in EM, and moves processively on actin filaments. The results support a model in which cargo binding exposes internal dimerization sequences within full-length myosin VI. Since unexpectedly a monomeric fragment of Dab2 triggers dimerization, it would appear that myosin VI is designed to function as a dimer in cells.

3226-Symp

Novel Functions for Myosin-I in Microvillar Membrane Motility and Mechanics

Matthew J. Tyska.

Vanderbilt Univ Med Ctr, Nashville, TN, USA.

Class I myosins are evolutionarily ancient, monomeric unconventional myosins that interact with highly charged acidic phospholipids in cellular membranes. Myosin-1a, one of eight class I myosins expressed in vertebrates, is found almost exclusively in epithelial cells that line the intestinal tract (also known as enterocytes). Here, myosin-1a resides in the brush border - an array of actin-rich microvilli that extend from the enterocyte apical surface. Within the microvillus, myosin-1a links the inner leaflet of the plasma membrane to a supporting parallel bundle of actin filaments. Although the functional consequence of myosin-1a/membrane interactions remained unclear for many years, our recent studies indicate that this motor can power the plus-end directed movement of microvillar membrane along core actin bundles. This novel form of subcellular motility leads to the shedding of small vesicles from microvillar tips, into the intestinal lumen. Careful analysis of vesicle components suggests that shedding activity may represent a previously unidentified aspect of epithelial cell biology, which serves to protect enterocytes from microbes in the intestinal lumen. Parallel biophysical studies indicate that myosin-1a (and other class I myosins) also contributes to membrane-cytoskeleton adhesion, which enables the apical membrane to resist deformation, and in turn, allows a single enterocyte to stabilize a staggering ~1000 microvilli on its apical surface. Together, these findings position myosin-1a as an important player in microvillar membrane motility and mechanical stability. Because they also highlight the unique multifunctional nature of this motor, our current efforts are focused on defining the molecular mechanisms and regulatory switches that enable cells to coordinate these functions.

3227-Symp

Mechanism and Regulation of Cytoplasmic Dynein Julie Huang¹, Julia Kardon², Carol Cho², Ronald Vale²,

Samara Reck-Peterson¹.

¹Harvard Med Sch, Boston, MA, USA, ²UCSF, San Francisco, CA, USA. Cytoplasmic dynein is a molecular motor responsible for nearly all minus-end directed microtubule-based transport in cells. We use the model system S. cerevisiae to purify recombinant cytoplasmic dynein and its associated complexes and subunits (dynactin, lis1 and nudel). Using this recombinant system we are able to easily engineer point mutations, truncations and genetic tags that allow us to add a variety of handles that can be used for purification or fluorescent labeling. We then measure the activity of these complexes using a number of assays including single molecule fluorescence microscopy. Our results reveal that there are a variety of mechanisms that can be used to regulate the motor activity of cytoplasmic dynein including two mechanisms that enhance dynein processivity. Using purified recombinant dynein-dynactin complexes, we find that dynactin is a dynein processivity factor, but does not use its microtubule-binding domain to enhance processivity. We also find that regulation of ATPase activity is another possible mechanism of regulation, as mutants that cannot hydrolyze ATP at one of dynein's four ATP (AAA4) binding sites are also more processive. Current studies in the lab are focused on determining additional points of regulation in the dynein mechanochemical cycle.

3228-Symp

Unconventional Model for Dynein-Driven Movement

Keiko Hirose¹, Hironori Ueno¹, Takuo Yasunaga², Chikako Shingyoji³. ¹Natl Inst Adv Ind Sci Tech, Tsukuba Ibaraki, Japan, ²Kyushu Inst Tech, Fukuoka, Japan, ³Univ Tokyo, Tokyo, Japan.

Previous electron microscopy (EM) and FRET analyses have shown that the tail of dynein swings with respect to the head and the stalk between the two nucleotide states that are thought to correspond to the pre- and post-powerstroke states (Burgess et al., 2003; Kon et al., 2005). However, how these structural changes observed in isolated dynein molecules are related to the minus-end-directed movement along a microtubule has not been clear. To understand how dynein changes with respect to microtubules, we studied the structures of sea urchin, outer-arm dynein bound to microtubules in the two nucleotide