Myosin-5 and Myosin-6 Differentially Detect Actin Filament Age
Dennis Zimmermann, Alicja Janik, David Kovar, Ronald Rock
1Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, USA; 2Biochemistry, University of Chicago, Chicago, IL, USA.

Unlike a static and immobile skeleton, the actin cytoskeleton is a highly dynamic network of filamentous actin (F-actin) polymers that continuously turn over. In addition to generating mechanical forces and sensing mechanical deformation, dynamic F-actin networks serve as cellular tracks for myosin motor traffic. Unfortunately, much of our understanding of processive myosins comes from in vitro studies where motility was studied on pre-assembled and artificially stabilized, static F-actin tracks. In this work, we examine the role of actin dynamics in single-molecule myosin motility using assembling F-actin. We chose the two highly processive motors, myosin-5 and myosin-6. On dynamic F-actin, the barbed-end directed myosin-5 is 1.5-fold more processive, whereas its pointed-end directed counterpart myosin-6 is 1.7-fold less processive (both relative to static F-actin). Moreover, while myosin-5 takes longer runs on ADP-Pi-rich (young) portions of the growing filament, myosin-6 takes longer runs along ADP-Pi-rich (old) F-actin. These results suggest that actin changes conformation upon Pi release, and that these two myosins respond to this change in opposite ways. Taken together, these experiments define a new mechanism of how myosin traffic may sort on different F-actin networks.

Adaptor Proteins Activate Myosin-Va during Cargo Transport
M. Yusuf Ali, Elena Kremenetsova, Maria Skolnick, David M. Warshaw, Kathleen M. Trybus.
University of Vermont, Burlington, VT, USA.

Myosin-Va (myoVa), one of the best characterized actin-based molecular motors, transports a variety of intracellular cargos. In order to bind a specific cargo, myoVa forms a tripartite complex with a Rab effector protein (i.e. adapter) and a Rab GTase protein (e.g. Rab27a) that is inserted in the granule membrane. MyoVa delivers insulin granules to the plasma membrane in pancreatic beta-cells. Interestingly, there are four known adapter proteins expressed in beta-cells, i.e., Granophilin-A/B, Rabphilin and MyRIP, all of which bind myoVa. The role of these adapter proteins in cargo transport is poorly understood.

Using TIRF microscopy, we measured the speed, run-length and stepping behavior of myoVa in presence of Qd-labeled adapter proteins. At 25 mM KCl, the adapter proteins do not show appreciable activation of the inhibited myoVa motor. However, at physiological salt concentration, the adapter proteins significantly increase the run-length and the run-frequency of myoVa on actin filaments. Specifically, in the presence of Granophilin A, the myoVa run-frequency increases ~6-fold, with an ~3.5-fold run-length enhancement as the motor steps (72nm) normally, but at half the speed. By labeling Granophilin-B and MyRIP with a Qdot, we observed binding of these adapters directly to actin filaments, suggesting that they enhance the motor’s run-length and slow speed by a tethering mechanism, similar to Melanophilin (Skolnick, et al., 2013). In contrast, Granophilin-B and Rabphilin have little binding affinity for actin. Nonetheless, they bind to and activate myoVa, because the full-length myoVa step size becomes regular like the constitutively active, truncated myoVa-HMM. A common feature of these adapter proteins is that they ensure that the motor remains active while attached to the cargo. However, only some adapter proteins have actin-tethering capacity, which may enhance the long-range vesicle transport. These functional differences may play synergistic roles in the cell.

Activation of Drosophila Melanogaster Myosin-5 Motor Function by Calcium and Cargo-Binding Protein
Huan-Hong Ji, Hai-Man Zhang, Mei Shen, Xiang-dong Li.
Institute of Zoology, CAS, Beijing, China.

In Drosophila melanogaster compound eye, myosin-5 (DmM5) plays two distinct roles in response to light stimulation: to transport pigment granules from cytosol to the rhabdomere base to decrease light exposure and to transport rhodopsin-bearing vesicles to the rhabdomere base to compensate for the rhodopsin loss during light exposure. The association of DmM5 with pigment granule and rhodopsin-bearing vesicle are mediated by two cargo-binding proteins, i.e., by dRab11 and Lin7, respectively. However, little is known how these two cargo-binding proteins affect the motor function of DmM5. Here we succeeded in expressing recombinant DmM5 and studied its regulation by calcium and cargo-binding proteins. The actin-activated ATPase activity of DmM5 is significantly lower than that of the truncated DmM5 without the C-terminal globular tail domain (GTD), indicating that the GTD is the

Myosin-5 and Myosin-6 Differentially Detect Actin Filament Age
Dennis Zimmermann, Alicja Janik, David Kovar, Ronald Rock
1Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, USA; 2Biochemistry, University of Chicago, Chicago, IL, USA.

Unlike a static and immobile skeleton, the actin cytoskeleton is a highly dynamic network of filamentous actin (F-actin) polymers that continuously turn over. In addition to generating mechanical forces and sensing mechanical deformation, dynamic F-actin networks serve as cellular tracks for myosin motor traffic. Unfortunately, much of our understanding of processive myosins comes from in vitro studies where motility was studied on pre-assembled and artificially stabilized, static F-actin tracks. In this work, we examine the role of actin dynamics in single-molecule myosin motility using assembling F-actin. We chose the two highly processive motors, myosin-5 and myosin-6. On dynamic F-actin, the barbed-end directed myosin-5 is 1.5-fold more processive, whereas its pointed-end directed counterpart myosin-6 is 1.7-fold less processive (both relative to static F-actin). Moreover, while myosin-5 takes longer runs on ADP-Pi-rich (young) portions of the growing filament, myosin-6 takes longer runs along ADP-Pi-rich (old) F-actin. These results suggest that actin changes conformation upon Pi release, and that these two myosins respond to this change in opposite ways. Taken together, these experiments define a new mechanism of how myosin traffic may sort on different F-actin networks.

Adaptor Proteins Activate Myosin-Va during Cargo Transport
M. Yusuf Ali, Elena Kremenetsova, Maria Skolnick, David M. Warshaw, Kathleen M. Trybus.
University of Vermont, Burlington, VT, USA.

Myosin-Va (myoVa), one of the best characterized actin-based molecular motors, transports a variety of intracellular cargos. In order to bind a specific cargo, myoVa forms a tripartite complex with a Rab effector protein (i.e. adapter) and a Rab GTase protein (e.g. Rab27a) that is inserted in the granule membrane. MyoVa delivers insulin granules to the plasma membrane in pancreatic beta-cells. Interestingly, there are four known adapter proteins expressed in beta-cells, i.e., Granophilin-A/B, Rabphilin and MyRIP, all of which bind myoVa. The role of these adapter proteins in cargo transport is poorly understood.

Using TIRF microscopy, we measured the speed, run-length and stepping behavior of myoVa in presence of Qd-labeled adapter proteins. At 25 mM KCl, the adapter proteins do not show appreciable activation of the inhibited myoVa motor. However, at physiological salt concentration, the adapter proteins significantly increase the run-length and the run-frequency of myoVa on actin filaments. Specifically, in the presence of Granphilin A, the myoVa run-frequency increases ~6-fold, with an ~3.5-fold run-length enhancement as the motor steps (72nm) normally, but at half the speed. By labeling Granphilin-B and MyRIP with a Qdot, we observed binding of these adapters directly to actin filaments, suggesting that they enhance the motor’s run-length and slow speed by a tethering mechanism, similar to Melanophilin (Skolnick, et al., 2013). In contrast, Granphilin-B and Rabphilin have little binding affinity for actin. Nonetheless, they bind to and activate myoVa, because the full-length myoVa step size becomes regular like the constitutively active, truncated myoVa-HMM. A common feature of these adapter proteins is that they ensure that the motor remains active while attached to the cargo. However, only some adapter proteins have actin-tethering capacity, which may enhance the long-range vesicle transport. These functional differences may play synergistic roles in the cell.

Activation of Drosophila Melanogaster Myosin-5 Motor Function by Calcium and Cargo-Binding Protein
Huan-Hong Ji, Hai-Man Zhang, Mei Shen, Xiang-dong Li.
Institute of Zoology, CAS, Beijing, China.

In Drosophila melanogaster compound eye, myosin-5 (DmM5) plays two distinct roles in response to light stimulation: to transport pigment granules from cytosol to the rhabdomere base to decrease light exposure and to transport rhodopsin-bearing vesicles to the rhabdomere base to compensate for the rhodopsin loss during light exposure. The association of DmM5 with pigment granule and rhodopsin-bearing vesicle are mediated by two cargo-binding proteins, i.e., by dRab11 and Lin7, respectively. However, little is known how these two cargo-binding proteins affect the motor function of DmM5. Here we succeeded in expressing recombinant DmM5 and studied its regulation by calcium and cargo-binding proteins. The actin-activated ATPase activity of DmM5 is significantly lower than that of the truncated DmM5 without the C-terminal globular tail domain (GTD), indicating that the GTD is the