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estimated the energy difference between the pre- and post-state of the lever arm to be 3.0 kBT (that is, maximum work of the lever arm). This was far less than the 13 kBT work we observed during the Brownian search-and-catch. We furhter discuss theories on how the Brownian search-and-catch produces mechanical work.

2885-Pos Board B655

Cargo Activation of Full Length Myosin Va by Melanophilin Observed at the Single Molecule Level

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Full-length myosin Va (myoVa) is auto-inhibited via a motor domain-globular tail interaction, unlike the truncated constitutively active myoVa-HMM. One potential mechanism to activate the full-length motor is cargo binding to the tail, which would compete with the head-tail interaction and trigger the molecule to extend and be activated for transport.

In the absence of cargo, it was recently shown that full-length myoVa has two modes of interaction with actin in the presence of MgATP (Armstrong et al.). Most motors bind to actin but do not move, while the remainder show processive motion, but with a variable stepping pattern and altered gating. Here we investigate how binding of melanophilin (Mlph), which links the melanocyte-specific isoform of myoVa to the Rab27a(GTP)-melanosome complex, affects the properties of myoVa at the single-molecule level.

In the absence of Mlph at 150mM KCl, a subset of Quantum dot labeled full-length myoVa moved at a median velocity of 566nm/s with the variable stepping pattern previously described, suggesting altered gating under these conditions. Addition of Mlph recruited 7-times more motors to move processively, consistent with a simple model of cargo activation. The myoVa-Mlph complex also showed increased run lengths, with many traveling to the ends of the actin filament. In the presence of Mlph, myoVa moved much more slowly (median velocity=76nm/s), leading to longer travel times on actin. When Mlph was bound to the motor the step sizes were normally distributed around 60 $\pm$ 14nm (SD) steps. Therefore, while myoVa moves more slowly along actin in the presence of the cargo adapter protein Mlph, it covers a greater distance with a more uniform and efficient stepping pattern. This slower processive movement could potentially facilitate binding of the Rab27a(GTP)-melanosome complex.

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Stepping Dynamics of Myosin Va Motors Physically-Linked through a Common Qdot-cargo

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Myosin Va is a processive molecular motor that transports intracellular cargo along actin filament tracks. In vivo, multiple myosin Va motors, attached to the same cargo, must interact but the mode of interaction is far from certain. We have shown that oppositely-directed myosins synchronized their stepping while engaged in a tug of war (Ali et al. 2011). Therefore, to understand the mechanical interactions between multiple motors of the same type, we have developed a simplified in vitro model in which two individual myosin Va were linked via a Qdot-cargo. To monitor each motor's stepping dynamics, one head of each motor was labeled with either a red or a green Qdot. For this two motor complex, velocity was reduced 1.3 - fold while run length increased 1.6 - fold. The leading motor must experience a resistive load from the trailing motor to account for the velocity reduction and why the leading motor has an 11% back step probability. When motors in the complex were close together (~36 nm), their stepping appeared independent. However, when the distance between them grew larger (>72 nm), they began to synchronize their stepping as the tension in the linkage between them presumably rose. We relate the findings with a model of two coupled stochastic steppers in which the stochasticity of motor steps stretches the linkage, while the stiffness of the linkage limits the intermotor distance and synchronizes their stepping. Even in this simplified model system, mechanical interactions between two identical motors are complex but will help define the collective mechanics of larger motor ensembles.

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Interacting Behavior of Two Myosin Va Motors Coupled via a DNA Scaffold

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The number of processive motors attached to a cellular cargo influences its transport behavior. Altering either motor number or the ratio of different classes of motors can therefore be a mechanism to regulate intracellular transport. Jamison et al. recently showed that two kinesin-1 motors coupled by a DNA scaffold have transport properties that are often dominated by one of the motors. Here we perform a similar experiment with myosin Va (myoVa), which has a larger step size (~36nm) and walks on a smaller track than kinesin. A heterodimeric myoVa was labeled on only one head with either a red or green quantum dot (Qdot). Two myoVa molecules were then linked to an ~50 nm long double-stranded DNA scaffold. Only complexes with one red and one green Qdot were analyzed. Our results show that the complex has increased run length (~1μm) compared to a single myoVa (~0.6μm). Average run lengths are, however, smaller than those predicted for two myosins assuming motor stepping, binding, and detachment is unaffected by intermotor interactions. Furthermore, the motor complex moved with reduced velocity (0.19 μm/s versus 0.27 μm/s for the single motor case). A histogram of the distances between the labeled heads of the two motors contains multiple peaks at ~85, 130 and 165 nm, indicating the system is flexible. The distance between motors changes in time and the stepping pattern of the two motors are variable, suggesting asynchronous motor stepping. After the first motor binds to actin, the second motor binds at ~10 s-1. Our findings suggest that the walking behavior of two myoVa molecules is altered when they are coupled mechanically, but perhaps in a different way than multiple kinesins. Our technique constitutes a unique tool to understand collective motor behavior.

2888-Pos Board B658

Processivity Determinants of Engineered Myosins

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Myosin superfamily proteins have diverse mechanical properties adapted for specific cellular tasks. Specialized dimeric myosins are capable of processive hand-over-hand motion along actin filaments. We have used protein engineering to explore structural requirements for processivity. Previous work has shown that processivity can be retained with artificial lever arms, without requiring detailed tuning of lever arm structure or mechanics[1-4]. However, we have found that myosins engineered for desired characteristics such as bidirectionality[4] are often less processive than natural motors. We asked whether processivity could be enhanced using two strategies: (1) multimerization to form three-headed and four-headed myosins, and (2) introduction of flexible spacers in lever arms. Models of uncoordinated stepping predict that trimeric and tetrameric myosins should be much more processive than their dimeric counterparts. However, coordinated stepping mechanisms may be disrupted by the presence of additional heads. Similarly, the introduction of flexible spacers may increase the accessibility of actin binding sites for suboptimal lever arm geometries, but is also expected to abrogate strain-mediated coordination between heads. After characterizing a panel of myosins with engineered lever arms and multimerization domains, we have found that (1) trimers and tetramers show large improvements in processivity over dimers, and (2) the addition of flexible regions greatly enhances the processivity of constructs with short lever arms. Our findings reinforce the the idea that gating is dispensable for processivity in high duty ratio myosins[3] and yield general strategies for increasing processivity in engineered myosins for use in synthetic biology or nanotechnology applications.

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Optical Control of Speed and Directionality in Engineered Myosin Motors

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Engineering molecular motors with dynamically controllable properties will allow selective perturbation of mechanical processes in vivo. We are developing a set of engineered actomyosin motors in which external signals trigger changes in lever arm geometry and mechanics, with predictable effects on motor properties such as directionality, step size, and processivity. Building on earlier protein engineering studies of directionality determinants [1-2], we previously constructed myosin motors that respond to a change in [Ca++] by reversing their direction of motion along the polarized actin filament [3]. Our designs relied on triggering rigid-to-flexible transitions in chimeric lever arms. We have now extended this work by constructing myosins that respond to optical signals, rather than metal ions. Light is a versatile control signal that can be readily modulated in time and space, and is generally orthogonal to cellular signaling. Using structure-guided protein engineering, we have incorporated photoreceptor domains into the lever arms of chimeric myosin motors. We have generated