increased force production due to an increased number of myosins strongly-bound to actin during contraction. Studies on muscle fibers and animal models corroborated these results as measured by force production and stroke volume, without changes in kinetics. However, in vitro motility assays published subsequently indicated that the sliding velocity is greatly reduced in the presence of OM in this experimental geometry. We utilized single molecule optical trapping techniques to study the step size and kinetics of actively cycling, recombinant human cardiac myosin under load in the presence of OM. We measured an increase in actin-attachment lifetime of myosin and a decrease in the length of the powerstroke from 6 nm to <1 nm in the presence of 10 μM OM. The decreased actomyosin dissociation rate and unitary displacement provide a mechanism for the inhibitory action of OM in the in vitro motility assay. Additionally, these results suggest that OM inhibits a kinetic step that occurs in between phosphate release and ADP release, as solution kinetic experiments indicate that OM does not slow the rates of ADP release or ATP-induced actomyosin dissociation. Single molecule optical trapping is providing a way to observe the entire cycle of myosin in the presence of OM and is thus helping to elucidate its therapeutic mechanism.

1578-Pos Board B487

Hydration of Magnesium is Required for Myosin VI Phosphate Release

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Myosin motors utilize the free energy released by the hydrolysis of ATP to move along the filamentous actin. In order to do so, myosins undergo a chemomechanical cycle during which binding and release from actin and nucleotides (ADP and ATP) are coupled to conformational changes favoring the movement towards one end of the track. ATP binds with the phosphate groups towards the inside of the motor. After ATP hydrolysis, the motor binds actin and the phosphate is released first. Because ADP obstructs the entrance pathway, the phosphate needs to find an alternative route. Recently, a new crystal structure of myosin VI was resolved, which is believed to represent the conformation capable of releasing the phosphate from the binding site. In order to determine the structural basis of phosphate release, we performed numerous multi-microsecond-long simulations starting from the new structure. Within the time scale of our simulations, the release occurred only if we rotated the phosphate at the beginning of the simulations. The major finding is that phosphate release of always occurs after hydration of the magnesium. The simulations also predict a few different escape routes, suggesting that there is plasticity in the pathways in this critical step in the catalytic cycle of Myosin VI, and presumably in other members of this super family.

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Optical Control of Fast and Processive Engineered Myosins: Optimization and Characterization in Vitro and in Living Cells

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Spatiotemporal control of cytoskeletal transport can provide new possibilities for dissecting cellular processes or for constructing complex artificial devices. Optogenetic approaches have been used for both controlled recruitment of motors to cellular cargos [1] and direct modulation of motor speed and direction [2]. Here we have worked to create optimized and diversified engineered myosin motors with velocities that can be optically controlled using dynamic changes in lever arm geometry. Previous designs for light-activated gearshifting [2] were non-processive, and suffered from either low velocities (< 10 nm/s) or modest degrees of velocity modulation (~15%) in response to light. These limitations preclude many potential applications in cell biology, devices, and reconstituted systems. We have now engineered (i) non-processive myosin motors that combine large optical modulation depths with high velocities and (ii) processive myosin motors with optically controllable directionality. We have characterized a series of optimized constructs using in vitro motility assays of propelled actin filaments, single-molecule tracking of processive complexes, and live cell imaging of motors tagged with fluorescent protein arrays [3]. For non-processive plus-end directed myosins, we measure up to 4X speed increases in the presence of blue light, reaching velocities of 4-5 microns/s. For processive myosins, we demonstrate controllable bidirectional processive transport, and report on cellular localization under optical stimulation. [1] P. van Bergeijk, et al. (2015) Nature 518 (7537) ''Optogenetic control of organelle transport and positioning'' [2] M. Nakamura et al. (2014) Nat. Nanotechnol 9, 693 ''Remote control of myosin and kinesin motors using light-activated gearshifting'' [3] R.P. Ghosh et al. (2017) ''A Fluorogenic Array Tag for Temporally Unlimited Single Molecule Tracking'', BioRxiv doi: 10.1101/159004.

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Biochemical and Functional Characterization of the Interaction of Myo1c with 14-3-3

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Myosin-IC (Myo1c) and 14-3-3, an adaptor protein proposed to associate with Myo1c, have been implicated in regulating the delivery of GLUT4-containing compartments to the plasma membrane in response to insulin. However, molecular and functional details of the Myo1c-14-3-3 interaction have yet to be described. It has been proposed that 14-3-3 binding to Myo1c is phosphorylation-dependent with a binding site at S701, near the first calmodulin-associated, IQ-motif of the motor. Here we show that 14-3-3 binding to Myo1c increases with increasing calcium concentration. However, 14-3-3 binding does not appear to displace calmodulin from Myo1c at concentrations where calcium-bound calmodulin normally remains associated. 14-3-3 induces dissociation of actin from coverslip-bound Myo1c in the in vitro motility assay, resulting in the inhibition of directional actin gliding. Surprisingly, phosphorylation of S701 with CAM kinase-II, which has been suggested to regulate 14-3-3 binding, has little effect on the Myo1c-14-3-3 interaction or the ability of 14-3-3 to inhibit motility. Our data suggest that 14-3-3 is able to interact with Myo1c and inhibit its activity in a calcium dependent, but phosphorylation independent manner.

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Tools to Study Nonmuscle Myosin-2 Motor Function Revisited

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Nonmuscle myosin-2 is the major enzyme that powers the contractility of the actin cytoskeleton in fundamental biological processes including cytokinesis and cell motility. The protein consists of two myosin heavy chains that each associate with one essential and one regulatory (RLC) light chain. Phosphorylation of the latter on a conserved serine and threonine is regulatory for nonmuscle myosin-2 and associated with the activation of the enzymatic activity of its motor domain. Tools to generate constitutively active or inactive nonmuscle myosins-2 for cell biological experiments therefore include (i) the generation of phosphomimetic RLC mutants but also (ii) the introduction of loss-of function mutations in the myosin motor domain. Here we show that ''phosphomimetic'' RLC mutations in which we replace the phosphorylatable serine and threonine with glutamate or alanine to mimic phosphorylation or non-phosphorylation fail to recapitulate the respective RLC state. Moreover, we show that in contrast to previous studies, the frequently used disease-associated N93K mutation in nonmuscle myosin 2-A is not a complete loss-of-function mutation. Instead, the mutation modestly decreases the enzymatic activity of nonmuscle myosin-2A. The inability of RLC "phosphomimetics" and the N93K mutant to recapitulate constitutively active or inactive nonmuscle myosin-2 underline the need for new tools to control myosin motor function in cells. Based on a molecular approach to the structure-function relationship of nonmuscle myosins-2, we suggest genetic disruption of a conserved salt bridge in the myosin motor domain that is pivotal for its enzymatic activity. We find that the disruption of the salt bridge eliminates the actin-activation of myosin's motor activity. This approach is believed to be universal for all nonmuscle myosins-2 and is superior to the use of RLC ''phosphomimetics'' and the disease-associated N93K mutation to inhibit myosin motor activity.

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ATPase Activity of Diaphragm Muscle Fibres Isolated From the Rabbit Containing the R403Q Mutation in the Heart

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Introduction: Respiratory muscle weakness is present in condition of hypertrophic cardiomyopathy (HCM). The Mg2+-ATPase activity of myosin and heavy meromyosin (HMM) ultimately regulates the speed of muscle contraction and performance. In this study, we analyzed ATPase activity of myosin isolated from the diaphragm of rabbits with the R403Q mutation in beta-myosin heavy chain, which causes HCM. **Methods:** Myosin filaments were isolated from the diaphragm of rabbit with R403Q mutation and from wild-type (WT) rabbit. HMM fragments were prepared from myosin and myofibrils isolated by homogenization. The protein concentration was measured by the Bradford protein assay. The Mg2+-ATPase activity of actomyosin was reconstituted from rabbit actin, myosin or HMM in a medium