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COMMUNICATION

Structural Basis for Myopathic Defects Engendered by Alterations in the Myosin Rod

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While mutations in the myosin subfragment 1 motor domain can directly disrupt the generation and transmission of force along myofibrils and lead to myopathy, the mechanism whereby mutations in the myosin rod influences mechanical function is less clear. Here, we used a combination of various imaging techniques and molecular dynamics simulations to test the hypothesis that perturbations in the myosin rod can disturb normal sarcomeric uniformity and, like motor domain lesions, would influence force production and propagation. We show that disrupting the rod can alter its nanomechanical properties and, *in vivo*, can drive asymmetric myofilament and sarcomere formation. Our imaging results indicate that myosin rod mutations likely disturb production and/or propagation of contractile force. This provides a unifying theory where common pathological cascades accompany both myosin motor and specific rod domain mutations. Finally, we suggest that sarcomeric inhomogeneity, caused by asymmetric thick filaments, could be a useful index of myopathic dysfunction.

Introduction

Muscle contraction results from repetitive cyclical interactions of myosin-containing thick filaments with actin-containing thin filaments. These filamentous systems are assembled into highly organized individual sarcomeric contractile units. Myosin, the molecular motor of muscle, contains two N-terminal subfragment 1 ("S1") globular heads followed by a long α-helical coiled-coil rod domain. The S1 heads hydrolyze ATP and generate contractile force, while the rod-like tails are essential for associating with auxiliary proteins and for the precise self-assembly and packing of myosin molecules into filaments. Much effort has been directed at understanding the role played by S1 in the contractile process and how myosin head mutations lead to skeletal and cardiac myopathies. Less attention, however, has been paid to the critical roles played by the rod in these processes.

Generation of force by the sarcomere and its transmission to the extracellular matrix are often

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Abbreviations used: S1, subfragment 1; S2, subfragment 2; LMM, light meromyosin; MD, molecular dynamics; IFM, indirect flight muscle; EM, electron microscopy; PDB, Protein Data Bank.

perturbed in muscle disease.[1] Myopathy-associated mutations in S1 can influence myosin's chemomechanical cross-bridge cycle with actin, directly affect sarcomeric force production, alter the contractile properties of myofibers and lead to disease.[2–7] In contrast, the pathogenic mechanisms associated with mutations in the rod are less clearly delineated. In fact, mutations in myosin rods have been proposed to lead to distinct pathogenic sequelae relative to those in the head.[8] Here, to probe the mechanistic basis for myopathic defects engendered by alterations in myosin's rod, we tested the hypothesis that disturbances in the rod, like S1 mutations, can influence myofibrillar force production and propagation, but do so by disrupting sarcomeric uniformity. We investigated the nanomechanical effects of alterations to the rod, how these perturbations influence *in vivo* sarcomeric symmetry and, ultimately, how they underlie the molecular basis of disease.

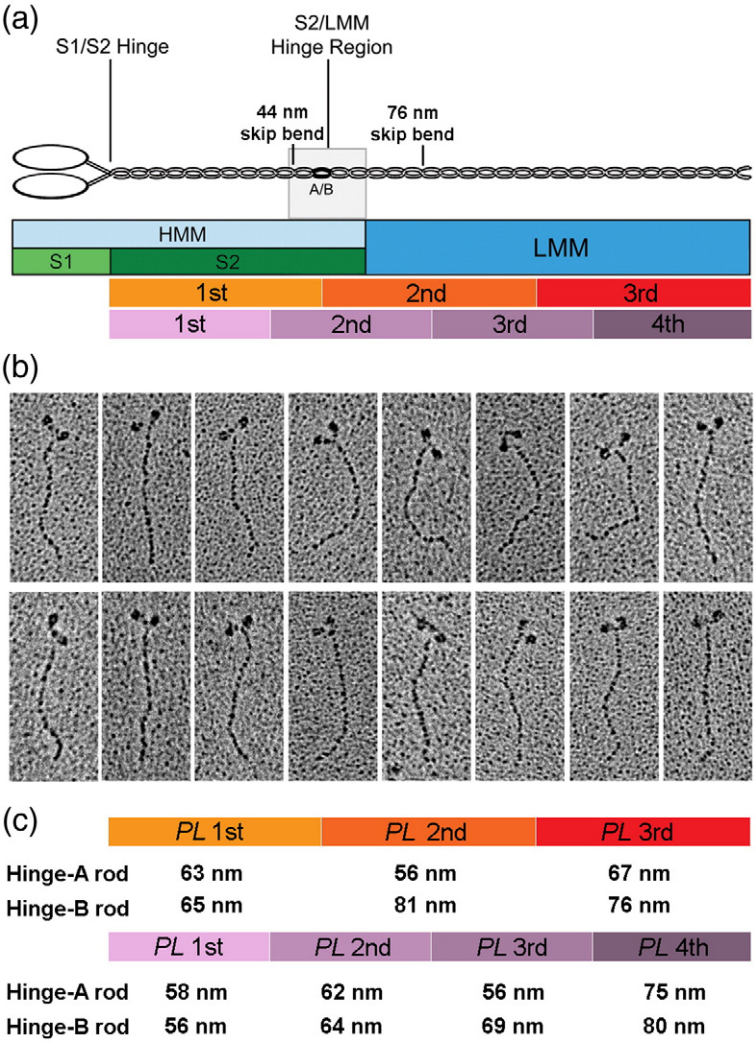

Fig. 1. S2/LMM hinge variants contribute to myosin rod stiffness. *yw* (hinge-A) and *15b-47* (hinge-B) myosin molecules were purified, rotary shadowed and imaged at 23 °C with a FEI Tecnai 12 transmission electron microscope operating at 120 kV at a magnification of 21,000× as previously described.[10] Digital images were taken with a TVIPS (Tietz) TemCam-F214 high-resolution digital camera. The ~ 150-nm hinge-A or the ~ 155-nm hinge-B myosin rods were divided into three ~ 50-nm segments and into four ~ 38-nm segments that were skeletonized after manual selection of points every 4–5 nm along the center of the molecule's longitudinal axis[13–15] directly from the micrographs. The persistence length (*PL*) was calculated via the tangent correlation method, after θ (the deviation angles along the myosin rod from an idealized straight rod) was determined for each tail segment. Previously developed algorithms to determine *PL* and θ were used.[13–15] Plots relating the inverse slope of $\ln\langle\cos(\theta(s))\rangle$ to the segment length yielded the *PL* values, where the factor of 2 in $\langle\cos(\theta(s))\rangle = e^{-s/2(PL)}$ accounts for the two dimensionality of electron micrographs. (a) Transgenic *Drosophila* myosins that differ only in 19 amino acids within hinge-A or hinge-B (A/B) of the S2/LMM hinge were expressed in the IFM. The limited myosin proteolysis products known as S1, S2, HMM (*h*eavy *m*ero*m*yosin) and LMM and the approximate location of "skip bends" are indicated.[16] 1st, 2nd, 3rd or 4th refer to the various rod sections that were independently evaluated in each segmental-based analysis. *PL* values (in nanometers) were calculated for each segment for hinge-A- and hinge-B-expressing molecules. (b) Myosin rod segment persistence lengths (*PL*s) were determined from rotary shadowed images of hinge-A-expressing molecules (top row) and of hinge-B-expressing molecules (bottom row). (c) The tangent correlation method used to calculate *PL* plots $\ln\langle\cos(\theta(s))\rangle$ as a function of arc length along the samples (where θ is the deviation angle from a straight rod). Measurements from rotary shadowed molecules suggest that hinge-B rods are less flexible than hinge-A rods. *PL* measurements reveal minor rigidity increases of the initial 50 nm (first one-third) and of the second quarter of hinge-B rods consistent with a local stiffening contribution from the transgenic hinge. The remaining downstream segments of hinge-B rods are substantially more rigid relative to the same fragments of hinge-A tails. This suggests propagation of the mechanical effects of amino acid substitutions in the hinge region, which lead to overall stiffness increases along hinge-B-expressing myosin rods.