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Mutating the Converter–Relay Interface of *Drosophila* Myosin Perturbs ATPase Activity, Actin Motility, Myofibril Stability and Flight Ability

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We used an integrative approach to probe the significance of the interaction between the relay loop and converter domain of the myosin molecular motor from *Drosophila melanogaster* indirect flight muscle. During the myosin mechanochemical cycle, ATP-induced twisting of the relay loop is hypothesized to reposition the converter, resulting in cocking of the contiguous lever arm into the pre-power stroke configuration. The subsequent movement of the lever arm through its power stroke generates muscle contraction by causing myosin heads to pull on actin filaments. We generated a transgenic line expressing myosin with a mutation in the converter domain (R759E) at a site of relay loop interaction. Molecular modeling suggests that the interface between the relay loop and converter domain of R759E myosin would be significantly disrupted during the mechanochemical cycle. The mutation depressed calcium as well as basal and actin-activated MgATPase (V_{max}) by ~60% compared to wild-type myosin, but there is no change in apparent actin affinity (K_m). While ATP or AMP-PNP (adenylyl-imidodiphosphate) binding to wild-type myosin subfragment-1 enhanced tryptophan fluorescence by ~15% or ~8%, respectively, enhancement does not occur in the mutant. This suggests that the mutation reduces lever arm movement. The mutation decreases *in vitro* motility of actin filaments by ~35%. Mutant pupal indirect flight muscles display normal myofibril assembly, myofibril shape, and double-hexagonal arrangement of thick and thin filaments. Two-day-old fibers have occasional "cracking" of the crystal-like array of myofilaments. Fibers from 1-week-old adults show more severe cracking and frayed myofibrils with some disruption of the myofilament lattice. Flight ability is reduced in 2-day-old flies compared to wild-type controls, with no upward mobility but some horizontal flight. In 1-week-old adults, flight capability is lost. Thus, altered myosin function permits myofibril assembly, but results in a progressive disruption of the myofilament lattice and flight ability. We conclude that R759 in the myosin converter domain is essential for normal ATPase activity, *in vitro* motility and locomotion. Our results provide the first mutational evidence that intramolecular signaling between the relay loop and converter domain is critical for myosin function both *in vitro* and in muscle.

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Keywords: myosin; muscle; *Drosophila*; ATPase; myofibril

The structure of the motor domain of myosin II has been determined at atomic-level resolution for skeletal muscle, smooth muscle and non-muscle isoforms.[1–4] Co-crystallization of myosin with nucleotide analogs permitted visualization of various states of the mechanochemical cycle and recon-

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Abbreviations used: AMP-PNP, adenylyl-imidodiphosphate; MHC, myosin heavy chain.

struction of myosin lever arm movement from pre-power stroke through post-power stroke.[5–7] These structural studies, along with biochemical and biophysical approaches, resulted in models for ATP's role in cocking the lever arm and for the release of ATP hydrolysis products following the lever arm power stroke.[8] Mutations in amino acid residues that serve as communication pathways between the nucleotide binding site and the lever arm show that domain interaction is critical for myosin function *in vitro* and in the cellular slime mold *Dictyostelium*.[9,10]

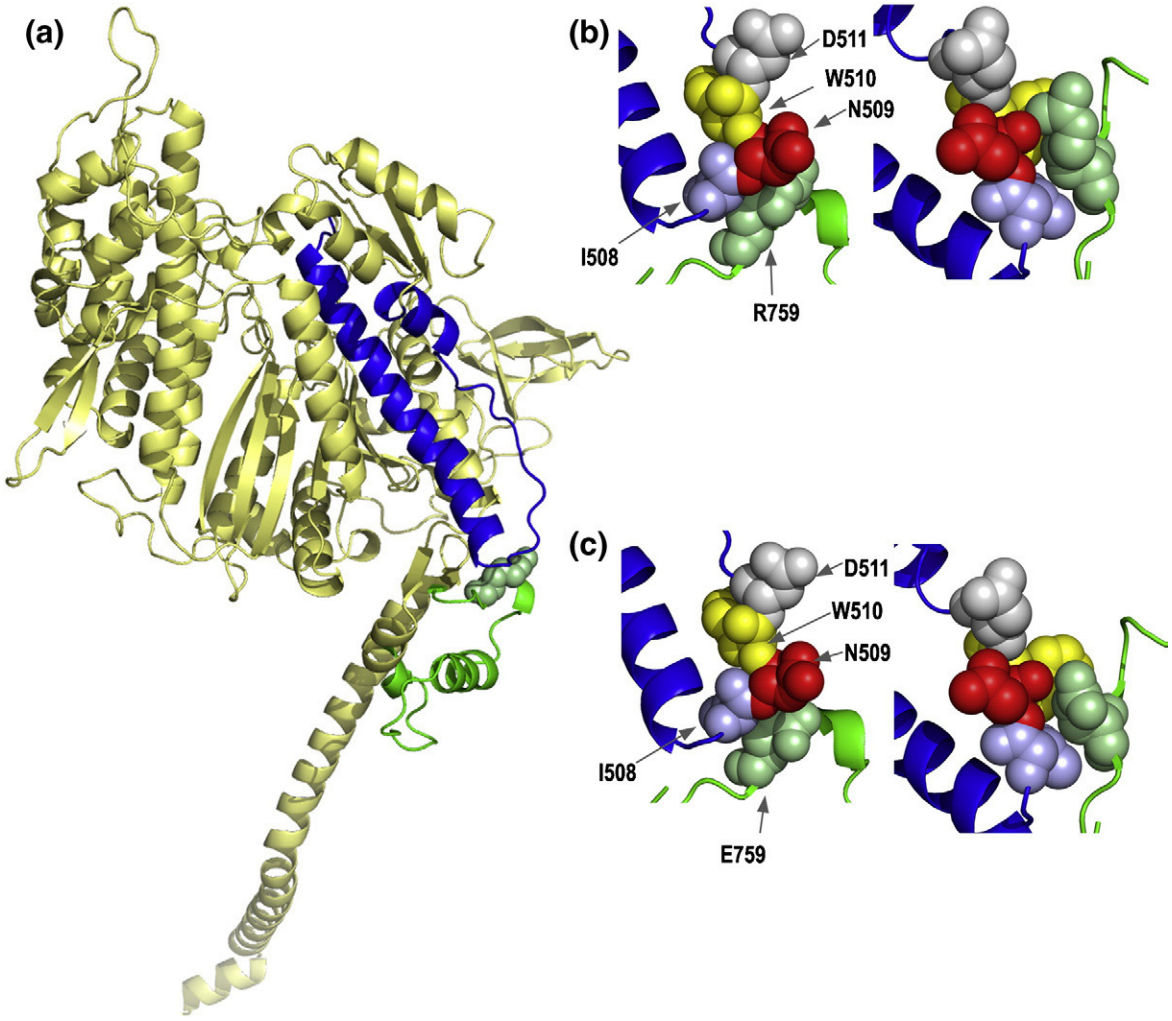

Fig. 1. Locations of the converter and relay domains of myosin and the effects of mutating the R759 converter residue. (a) Mapping of the amino acids of the *Drosophila* indirect flight muscle isoform (IFI) of myosin onto the scallop crystal structure in the pre-power stroke state [Protein Data Bank (PDB) code 1qvi]. The relay domain encoded by alternative exon 9a is highlighted in blue, whereas the central portion of the converter domain (residues 724–764) encoded by alternative exon 11e is shown in green. Converter domain residue R759 is shown as a space-filling model. The homology model was produced by fitting the *Drosophila* indirect flight muscle myosin S-1 amino acid sequence to the coordinates of scallop myosin S1 using the automated mode of the Swiss-Model homology modeling server (http://swissmodel.expasy.org/). PyMOL (http://www.pymol.org/, DeLano Scientific, Palo Alto, CA) was used to visualize the output. (b) Interaction of converter domain residue R759 (green) with amino acid residues of the relay domain [I508 (blue), N509 (red), W510 (yellow), D511 (gray)] in the pre-power stroke state (left) and the post-power stroke state (PDB code 1kk8; right). Space-filling models suggest the hydrophobic region of R759 near the peptide backbone interacts with I508 in the pre-power stroke state, while the polar terminal portion interacts with polar N509. In the post-power stroke state, interactions with I508 and N509 are retained, plus the changed orientation of the relay loop results in formation of a salt bridge between R759 and D511. See Bloemink *et al*.[19] for further modeling and discussion. (c) Interaction of mutated converter domain residue E759 with amino acid residues of the relay domain in the pre-power stroke state (left) and the post-power stroke state (right). These models were produced as described above, except that E759 replaced R759 prior to modeling. The negatively charged region of E759 is located near hydrophobic I508, eliminating the hydrophobic interaction found in wild-type myosin. The mutation also reduces interaction of residue 759 with polar N509, particularly in the pre-power stroke state (left). Disruption of the relay loop–converter domain interface is exacerbated at the post-power stroke state (right), since E759 is unable to form a salt bridge with negatively charged D511. All residue numbers correspond to those of chicken skeletal muscle myosin.[2]