Myosin VI: How Do Charged Tails Exert Control?

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DOI 10.1016/j.str.2010.10.004

Molecular dynamics simulations and single molecule experiments are used to suggest that charged helices in the medial tail domain participate in myosin VI dimerization (Kim et al., 2010), which reinforces the mechanism that unfolding of the three helix bundle in the proximal tail serves as a lever arm extension.

Myosin VI (M6), an unusual molecular motor with a short lever arm that takes long strides along the actin network, is implicated in a number of cellular functions (Spudich and Sivaramakrishnan, 2010; Sweeney and Houdusse, 2010). Unlike other members of the myosin superfamily, M6 marches toward the (-) (or pointed) end of the actin protofilament. While the M6's movement toward the (-) end is explained in terms of a unique insert of ~40 residue hairpin turn adjacent to the converter domain, the structural origin of the ~30–36 nm step is not without controversy (Spudich and Sivaramakrishnan, 2010; Sweeney and Houdusse, 2010). In contrast, the structural basis of stepping dynamics of myosin V is well explained by swinging lever arm hypothesis in which the long lever arm swings by ~70° to cover the ~36 nm step (Spudich and Sivaramakrishnan, 2010; Sweeney and Houdusse, 2010).

The lever arm of M6 (two calmodulin bound to a unique insert and the IQ motif) is only 7 nm (Figure 1), which cannot explain the observed large step, even assuming that the lever arm swings by ~180° during each stride. How the lever arm extends by ~11–15 nm to straddle two adjacent binding sites on actin is addressed in part by Kim et al. (2010) using a combination of molecular dynamics simulation and experiments.

A geometrical requirement for 36 nm step is that the two motor domains of the M6 dimer should bind simultaneously to two actin subunits separated by 36 nm (Figure 1). The C terminus of the full length M6, which is likely to be a compact-folded monomer in isolation (Lister et al., 2004), can be partitioned into PT (proximal tail), MT (medial tail) with a large number of charged residues, and CBD (cargo-binding domain) (Figure 1). Because the lever arm is ~7 nm, the C-terminal region of M6 must extend further by ~11–15 nm to account for the ~36 nm step taken by the M6 dimer. Given that only the M6 dimer walks processively along the polar track, the origin of lever arm extension (LAE) is intimately related to the dimerization mechanism of M6.

Dimer formation, which likely occurs in vivo upon cargo binding (Yu et al., 2009), can be realized in vitro by locally enhancing the concentration of M6 on actin (Mukherjea et al., 2009). Using such a protocol in experimental single molecule studies and extensive MD simulation, Kim et al. (2010) provide compelling evidence that a set of five salt bridges between MT domains rich in ER/K residues stitch together two monomers resulting in a dimer (Figure 1; see Figure 2 in Kim et al. [2010]). The MD simulations of two isolated MT domains, which were used to obtain structural details of the dimer, showed that the formation of interhelical salt bridges requires a vertical shift (~1 nm) of one helix with respect to the other (Figure 1; see Figure 5 in Kim et al. [2010]). Although the importance of salt bridges has been predicted in the dissociation of myosin V from actin (Tehver and Thirumalai, 2010) in M6, the C terminus-charged residues appear to regulate the mechanics of the stepping process itself. The subdominant contributions of interhelical hydrophobic interactions to the stability of the isolated MT dimer discerned in MD simulations prompted additional experiments (Kim et al., 2010) to probe the processivity of the M6 dimer as a function of ionic strength. Using truncated constructs of M6 lacking the CBD, it was shown that the fraction of processive molecules decreases as ionic strength increases (Kim et al., 2010). The combined MD simulations and experiments using M6-980 and M6-940 further advance their earlier PT helix unfolding mechanism (HUM) (Mukherjea et al., 2009) that explains the ~36 nm step by demonstrating that salt bridge formation between MT helices alone suffices for dimer formation. In the absence of a high-resolution structure of the M6 dimer, the study by Kim et al. (2010) provides a structural basis for HUM (Mukherjea et al., 2009), which posited that dimerization of the MT domain results in the unfolding of PT domain three helix bundle, thus extending the lever arm. If the helices in the bundle are intact upon unfolding, as is proposed, then the lever arm would extend by about ~10–11nm, which increases the length of the lever arm to ~17–18 nm, which is sufficient to explain the ~30–36 nm step along actin.

A completely different mechanism (Spink et al., 2008) suggests that interactions exclusively between two CBDs, perhaps brought into proximity upon cargo binding in vivo (Yu et al., 2009), drive dimer formation (Figure 1). In the resulting helix intact mechanism (HIM), the LAE (folded three helix bundle of the PT domain and nearly rod-like single α helix [SAH] corresponding to MT) provides the additional ~14 nm length to account for the ~36 nm step. The validity of such a model, which is more or less equivalent to the accepted lever arm picture for myosin V stepping (Spudich and Sivaramakrishnan, 2010; Sweeney and Houdusse, 2010), has been questioned (Mukherjea et al., 2009), based on the observation that dimer-forming M6 molecules lacking the CBDs also walk processively on actin. Dimer formation, with only modest yield, in M6-940 requires

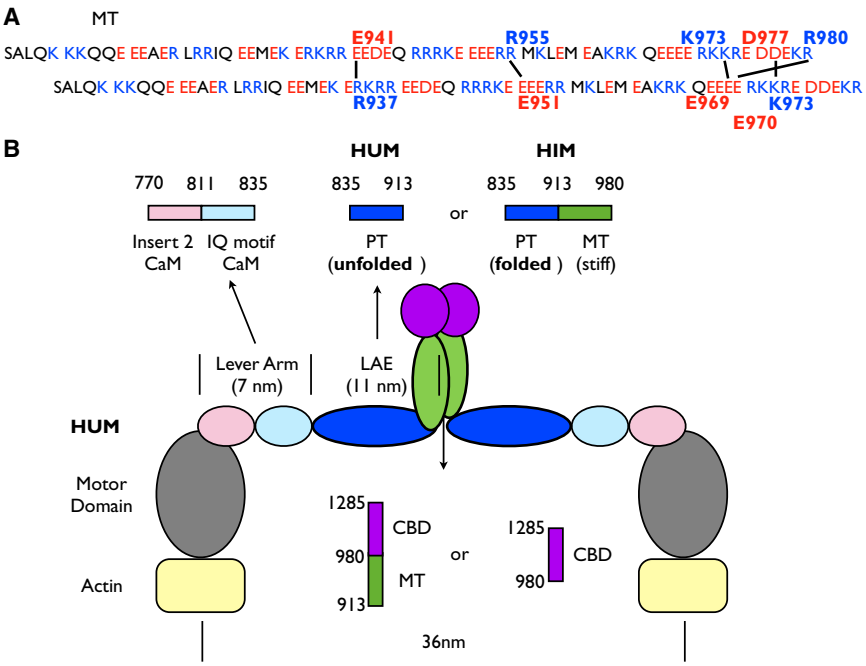

Figure 1. Models for Lower Arm Extension

(A) Schematic representation of out of registry packing of MT helices (Kim et al., 2010) stabilized by five salt bridges (solid black lines).

(B) Illustration of the role of PT and MT helices in LAE. Lever arm in M6 is only ~7 nm long and can be extended either by unfolding the PT (blue) three helix bundle (HUM) or by HIM with a folded PT and stiff SAH (blue and green in the upper right corner). In the HUM, both MT and CBD are involved in dimer formation, while only CBD drives dimer formation according to HIM (cartoon shown between the actin binding sites in yellow).

enhancement of the local M6 concentration by artificial clustering, whereas a substantial number of processive M6 form in the presence of CBD. It should be stressed that the mean step size of M6-940, with substantial dispersion, is less than that found in the processive dimers resulting from M6 that contain the CBD (Mukherjea et al., 2009). Setting aside the mechanism of dimerization of M6-940, the HIM predicts that the LAE is ~8–9 nm (~4 nm from folded PT and ~4–5 nm from the stiff truncated MT helix), which is long enough to explain the ~30 nm step. Besides, M6-940 dimers could also operate by a combination of HUM and HIM, which rationalizes the measured distribution of step sizes. Thus, in our view, the observed processive motion of M6-940 does not automatically invalidate the proposed myosin V-like picture (Spink et al., 2008) for M6.

The present study (Kim et al., 2010), the previous experimental paper in support of the HUM (Mukherjea et al., 2009), and the entirely different HIM (Spink et al., 2008) raise a number of questions, which we hope are worthy of study. (1) If HUM holds, what is the mechanism of unfolding of the PT three helix bundle? Does the ~10 pN force required to unfold the helical bundle arise from the strain in the MT dimer due to the out of registry packing (Kim et al., 2010) (Figure 1) of the MT helices? (2) Upon detachment of the motor head from actin, the three helix bundle is probably not under tension and will have a propensity to refold, during the biased diffusion stage. How is tension restored to unfold the helix bundle prior to binding of the motor head to the adjacent actin site? (3) If HIM holds, then the SAH conformation must be very sensitive to ionic strength. Is the processivity of full length M6 dimer greatly compromised at elevated salt concentration? (4) Finally, is a hybrid of HUM and HIM, which would not require complete unfolding of the helical bundle and need the SAH to be stiff, operative in providing a long enough lever arm? A combination of experiments and simulations, along the lines reported in Kim et al. (2010), will be needed to address these vexing questions.

ACKNOWLEDGMENTS

We appreciate discussions with Michael Hinczewski and Riina Tehver. This work was supported by a grant from NSF (CHE 09-14033).

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