

Regulated conformation of myosin 5

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We have found that myosin 5, an important actin-based vesicle transporter, has a folded conformation that is coupled to inhibition of its enzymatic activity in the absence of cargo and Ca^{2+} . In the absence of Ca^{2+} , where the actin-activated MgATPase activity is low, purified brain myosin 5 sediments in the analytical ultracentrifuge at 14 S as opposed to 11 S in the presence of Ca^{2+} where the activity is high. At high ionic strength, it sediments at 10 S independent of Ca^{2+} and its regulation is poor. These data are consistent with myosin 5 having a compact, inactive conformation in the absence of Ca^{2+} and an extended conformation in the presence of Ca^{2+} or high ionic strength. Electron microscopy reveals that in the absence of Ca^{2+} , the heads and tail are both folded to give a triangular shape (Fig. 1a), very different from the extended appearance of myosin 5 at high ionic strength (Fig. 1c). Single particle image processing of these folded molecules reveals an enigmatic but rather consistent structure (Fig. 1b). A recombinant myosin 5 heavy meromyosin fragment that is missing the distal portion of the tail domain is not regulated by Ca^{2+} and has only a small change in sedimentation coefficient, which is in the opposite direction to that seen with intact myosin 5. Electron microscopy indicates that its heads are extended even in the absence of Ca^{2+} . These data suggest that interaction between the motor and cargo binding domains may be a general mechanism for shutting down motor protein activity and thereby regulating the active movement of vesicles in cells.

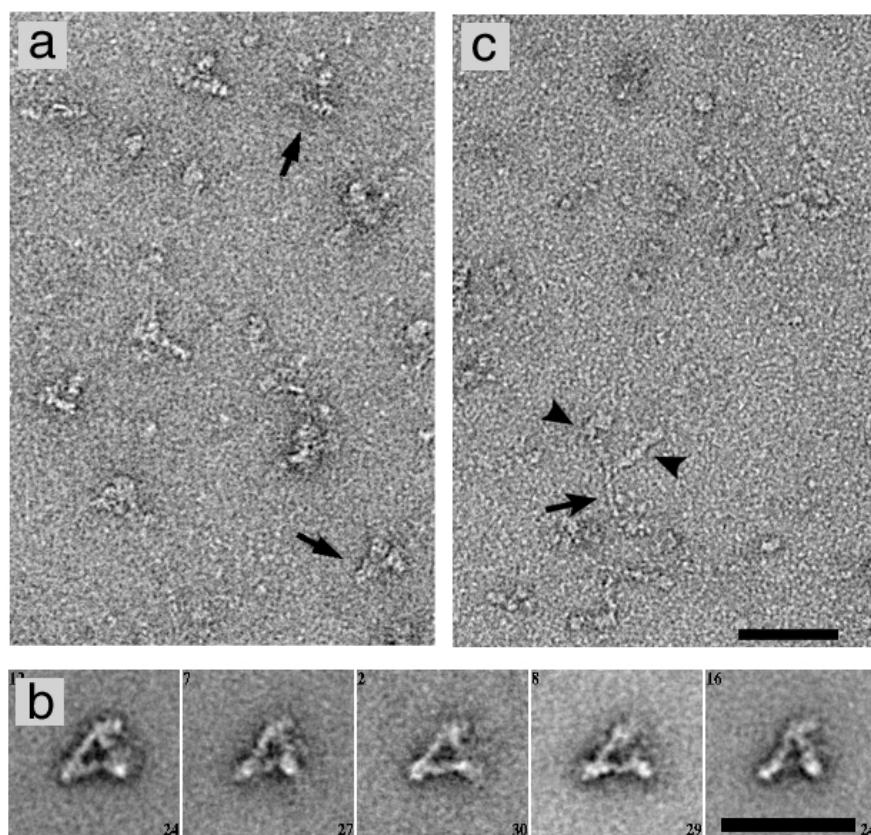


Fig. 1 Negative stain electron microscopy of myosin 5.

a, low Ca^{2+} , low ionic strength; arrows indicate two examples of the compact molecules visible in this field. **b**, typical averaged images from single particle image processing of compact molecules; class sizes are about 30 molecules. **c**, high ionic strength, low Ca^{2+} ; molecules are not compact. Instead, the two heads (arrowheads) and tail (arrow) of molecules are visible. Scale bars, 50 nm.

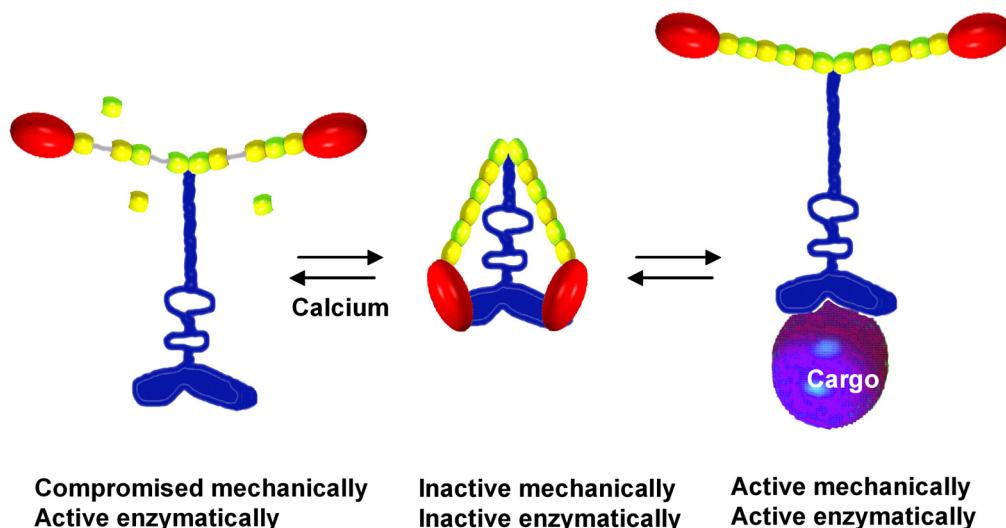


Fig. 2 Speculative model for conformation-dependent regulation in myosin 5.

The compact, shut down molecule (centre) could convert to the opened active state as a result of becoming attached to cargo (right). Elevated Ca^{2+} levels also activate the enzymatic activity, but if Ca^{2+} -binding by the calmodulin subunits of the myosin levers causes them to dissociate (left) it might compromise mechanical activity due to a consequent weakening of the lever.

An advantage of having a highly-regulated myosin 5 is that unregulated, non-cargo-bound myosin 5 in cells would needlessly hydrolyse ATP and would stay associated with actin filaments due to its processivity. We envisage that regulated myosin 5 would dissociate from its cargo on reaching its destination and then fold into the inactive form that could freely diffuse and effectively be recycled. These ideas are summarised schematically in Fig. 2.

Collaborators

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Publications

Wang, F., Thirumurugan, K., Stafford, W.F., Hammer, III, J.A., Knight, P.J. and Sellers, J.R. (2004) Regulated conformation of myosin V. *J. Biol. Chem.* **279**, 2333-2336.

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