Conserved structure of compact myosin 2 in species separated by at least 600 million years of independent evolution

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Introduction

The myosin 2 family not only comprises those isoforms found in muscle cells that drive muscle contraction, but also those responsible for intracellular movements such as cytokinesis or neuronal dynamics. Myosin 2 forms filaments from which myosin heads interact cyclically with actin, powered by hydrolysis of ATP. The C-terminal halves of the two heavy chains comprising each myosin molecule associate to form an α-helical coiled-coil tail while the N-terminal halves fold separately to form the two heads. Each head has a motor domain, containing actin and ATP binding sites, connected to the tail by an α-helical lever that is stabilised by an essential light chain (ELC) and a regulatory light chain (RLC). Across the Animal Kingdom, myosin 2 includes isoforms whose activity is regulated in different ways. Vertebrate smooth muscle myosin (SmM) is activated by phosphorylation of the RLC whereas scallop striated adductor muscle myosin (ScM) is activated by calcium binding to its ELC. The paired heads of inhibited molecules from myosins regulated by phosphorylation have an asymmetric arrangement with motor-motor interactions. Both myosins can form compact molecules distinct from the extended molecules found at high salt concentrations. For SmM from turkey gizzard, our recent electron microscopy had shown that the compact conformer has the tail folded back close to the heads, and we obtained a detailed structure of the head region and the path of the folded-up tail. It was unknown whether such interactions were a common motif for inactivation used in other forms of myosin-linked regulation. Therefore we have compared structures of the compact molecules of SmM and ScM, using negative-stain electron microscopy and single particle image processing.

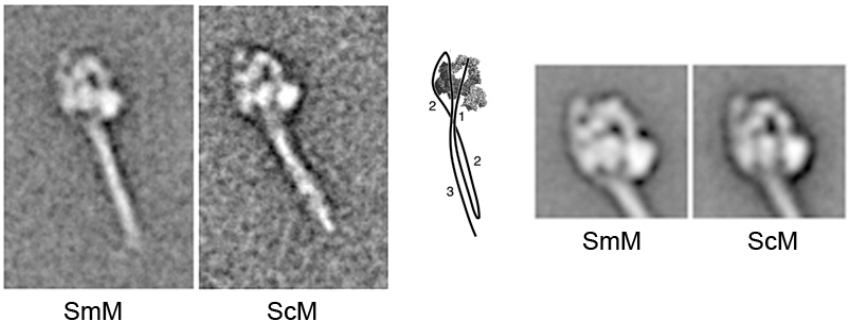

Figure 1 - Close similarity of compact molecules of turkey gizzard smooth muscle myosin (SmM) and scallop striated muscle myosin (ScM). The central diagram explains how the appearances originate from the two heads and the three segments of the folded-up tail.

Myosin 2 forms the same compact structure in scallop and turkey

At the resolution of the electron microscope images (~2 nm), these two remote members of the myosin 2 family are indistinguishable (Fig. 1). Not only are the shapes and asymmetric dispositions of the two heads the same, but the tail folds at apparently identical sites and wraps around the left head in an identical way. A test of whether two datasets are indeed indistinguishable is to combine them and use image processing to align them together and group similar molecules together into classes. If the datasets are indistinguishable, both will contribute roughly equally to the classes. On this basis too, we find no differences in structure. Thus these myosins which have different regulatory mechanisms and which diverged from a common ancestral myosin at least 600 Myr ago have retained the same quaternary structure for this inhibited state. Conservation across such a large evolutionary distance suggests that this conformation is of fundamental functional importance, though we do not yet understand the role that it plays in the life of the muscle cell.

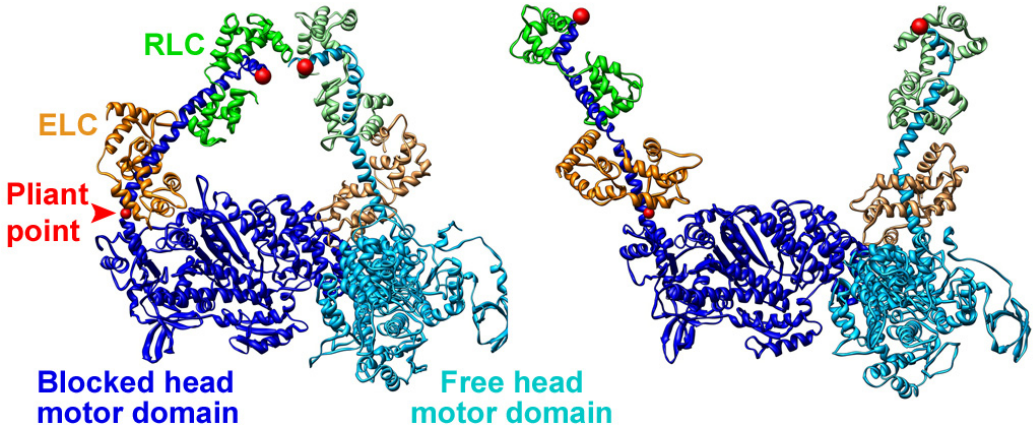

Figure 2 - Structure of scallop myosin heads inferred from our images of the inhibited state (left), contrasted with available scallop head structures (right). The red spheres at the ends of both the two levers have to join onto a single tail: easy for the left-hand model; impossible for the right-hand one.

Scallop head has to bend to form the compact structure

The close similarity of the compact scallop molecules to the smooth muscle ones implies that the heads have the same shape in both species. It is therefore interesting to note that there is a disruption of the heavy chain α-helix at the motor-lever junction in the SmM head (the 'Pliant point'; Fig. 2) that has so far not been seen in any ScM head crystal structure. This bend brings the ELC into new contacts with the motor domain, but the importance of these contacts in the regulatory mechanism remains to be discovered.

Collaborators

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Publications

Jung, H.S., Burgess, S.A., Billington, N., Colegrave, M., Patel, H., Chalovich, J.M., Chantler, P.D. & Knight, P.J. (2008). Conservation of the regulated structure of folded myosin 2 in species separated by at least 600 million years of independent evolution. *Proc. Natl. Acad. Sci. USA*. **105**, 6022-6026.

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