JGP Journal of General Physiology

RESEARCH NEWS

How myosin II achieves total shutdown

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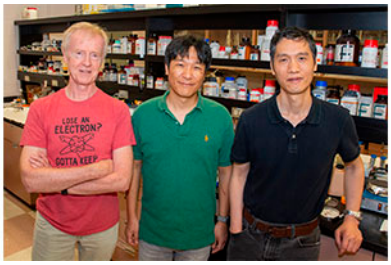
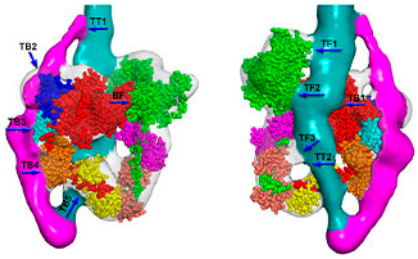
***JGP* study describes 3-D structure of the 10S form of myosin II, identifying key interactions between the head and tail domains that keep the motor protein switched off.**

The myosin II motor protein drives a wide variety of processes, from muscle contraction to cell migration, by binding to F-actin and hydrolyzing ATP. Composed of two heavy chains, two essential light chains, and two regulatory light chains (RLCs), myosin II molecules perform their functions by polymerizing into extended filaments. To conserve energy and facilitate their delivery to sites of filament assembly, however, myosin II monomers are maintained in a compact, inactive conformation named after its sedimentation coefficient of 10S. In this issue, Yang et al. provide new insights into the structure of 10S myosin II that help explain how its motor activity is completely shut off (1).

10S myosin is remarkably inactive, hydrolyzing ATP at a rate of just one molecule per hour (2). 3-D reconstructions of the 10S structure suggest that the two head domains of myosin II—comprising the light chains and the N-terminal halves of the heavy chains—inhibit each other in an asymmetric manner (3, 4). The actin-binding domain of one head is "blocked" by the other head. This second head, in contrast, may be "free" to bind actin, but its ATPase activity is inhibited because its converter domain—which helps release the products of ATP hydrolysis—is immobilized by its interaction with the blocked head.

The tail domain formed by the C-terminal halves of the heavy chains is also thought to contribute to the inhibition of 10S myosin, but this region of the molecule is largely missing from the 3-D reconstructions. 2-D electron microscopy has shown that the tail wraps around the head domains of 10S myosin II (5), but these head–tail interactions have not been observed in 3-D. "We wanted to fill in this gap," explains Roger Craig from the University of Massachusetts Medical School.





Left to right: Roger Craig, Kyoung Hwan Lee, Shixin Yang, and colleagues generate a 3-D reconstruction of the 10S conformation of myosin II that explains how interactions between the tail and head domains help to completely switch off the protein's motor activity. Key interactions include TB3, which inhibits the ATPase activity of the "blocked" head, and TF1, which masks the actin-binding domain of the "free" head.

Craig and colleagues, including co-first authors Shixin Yang and Kyoung Hwan Lee, therefore generated a 3-D reconstruction of 10S myosin using negative staining and single particle analysis of the full-length molecule. Due to their high flexibility, some parts of the tail are missing from the reconstruction, but the structure includes all of the tail regions closely associated with the heads.

The researchers were able to identify numerous interactions between the head and tail domains, many of which are likely to stabilize the asymmetric interaction of the blocked and free heads as well as the overall conformation of the 10S molecule. But several interactions appear to play a more direct role in inhibiting 10S myosin activity.

One interaction between the tail and the converter domain of the blocked head likely inhibits this head's ATPase activity, while another interaction between the tail and the actin-binding domain of the free head probably prevents this head from attaching to actin filaments. "So actin-binding and ATPase activity are switched off in both heads," Craig says. "Everything is totally shut down." In addition, interactions between different segments of the tail allow it to fold up so that it is unable to mediate myosin polymerization and filament formation.

10S myosin is activated when myosin light chain kinase phosphorylates a serine residue on each of the two RLCs. Yang et al.'s reconstruction reveals that the serine residue in the free head RLC is completely accessible. But the equivalent serine in the blocked head is masked by an interaction with the tail. The researchers suggest that phosphorylation of the first serine may slightly destabilize the 10S conformation, whereas phosphorylation of the second serine is likely to trigger the molecule's complete unfolding and activation.

"We'd now like to get a higher resolution structure using cryo-EM so that we can see exactly which residues are involved in all these interactions," Craig says.

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