

MOLECULAR MECHANISM OF MYOSIN-LINKED REGULATION OF MUSCLE CONTRACTION. Raúl Padrón (1), John Woodhead (2), Fa-Qing Zhao (2), Edward Egelman (3), Lorenzo Alamo (1) and Roger Craig (2). (1) Departamento de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, VENEZUELA; (2) Department of Cell Biology, University of Massachusetts Medical School, Worcester, MA, U.S.A.; (3) Department of Biochemistry and Molecular Genetics, University of Virginia, Virginia, U.S.A. E-mail: padron@ivic.ve

We studied thick filaments by cryo-electron microscopy (EM), preserving their native structure (Fig. 1a, 1b), and carried out a 3D-reconstruction (~2.5 nm resolution, Fig. 1c) [1] using single-particle techniques [2]. The reconstruction showed new details of myosin head organization, and fitted well to the atomic structure of dephosphorylated (OFF-state) heavy meromyosin from smooth muscle obtained by EM of 2D-crystals [3] (Fig. 1e). The myosin heads interact asymmetrically, with the actin-binding region of one head (the ‘actin-blocked’ head [3] – heavy chain green in Fig. 1d,e) interacting with the converter/light chain region of the other (the ‘free’ (blue) head - Fig. 1d yellow ellipse and circle respectively). Both heads point ‘back’ towards the tail, as found previously with isolated vertebrate smooth [4] and scallop [5] muscle myosin molecules in the OFF state. Atomic fitting (Fig.1e) reveals (1) that the myosin subfragment-2 (S2) is very close to the actin-binding region of the blocked head (Fig. 1d yellow bracket), confirming single molecule EM results; (2) interactions of the lower free-head actin-binding domain with the upper blocked-head ELC (Fig. 1e yellow ellipse), and the blocked-head converter/SH3 domains with the adjacent tail (Fig. 1e yellow bracket). These interactions may help in forming/stabilizing the helices of heads. This result provides new insight into the structural basis of muscle relaxation: (1) it strongly suggests that the “interacting-heads-down” model seen with single molecules also occurs in native muscle filaments; (2) intra- and inter-molecular interactions between myosin molecules appear to block key sites required for contraction. On activation, Ca^{2+} switches ON the thick filaments by phosphorylation of the regulatory light chains, breaking the bond between the heads [3], which thus become mobile and independent of each other. This results in their disordering on the filament surface [6] and the freedom to interact with actin, leading to muscle contraction. The fit of the atomic structure of a *vertebrate, smooth* muscle myosin *molecule* into the reconstruction of an *invertebrate, striated* muscle, myosin *filament*, suggests that the “interacting-heads-down” motif may be a widespread OFF structure, present in many myosin-based contractile systems. This structure may have arisen early in evolution, before muscles evolved, as a mean of switching nonmuscle myosin off. Since nonmuscle myosin is monomeric in the off state, only intramolecular interaction between heads would have been possible, making an asymmetric structure a necessity in order to block actin-binding and converter domains. As muscles evolved, they may have retained this interaction.

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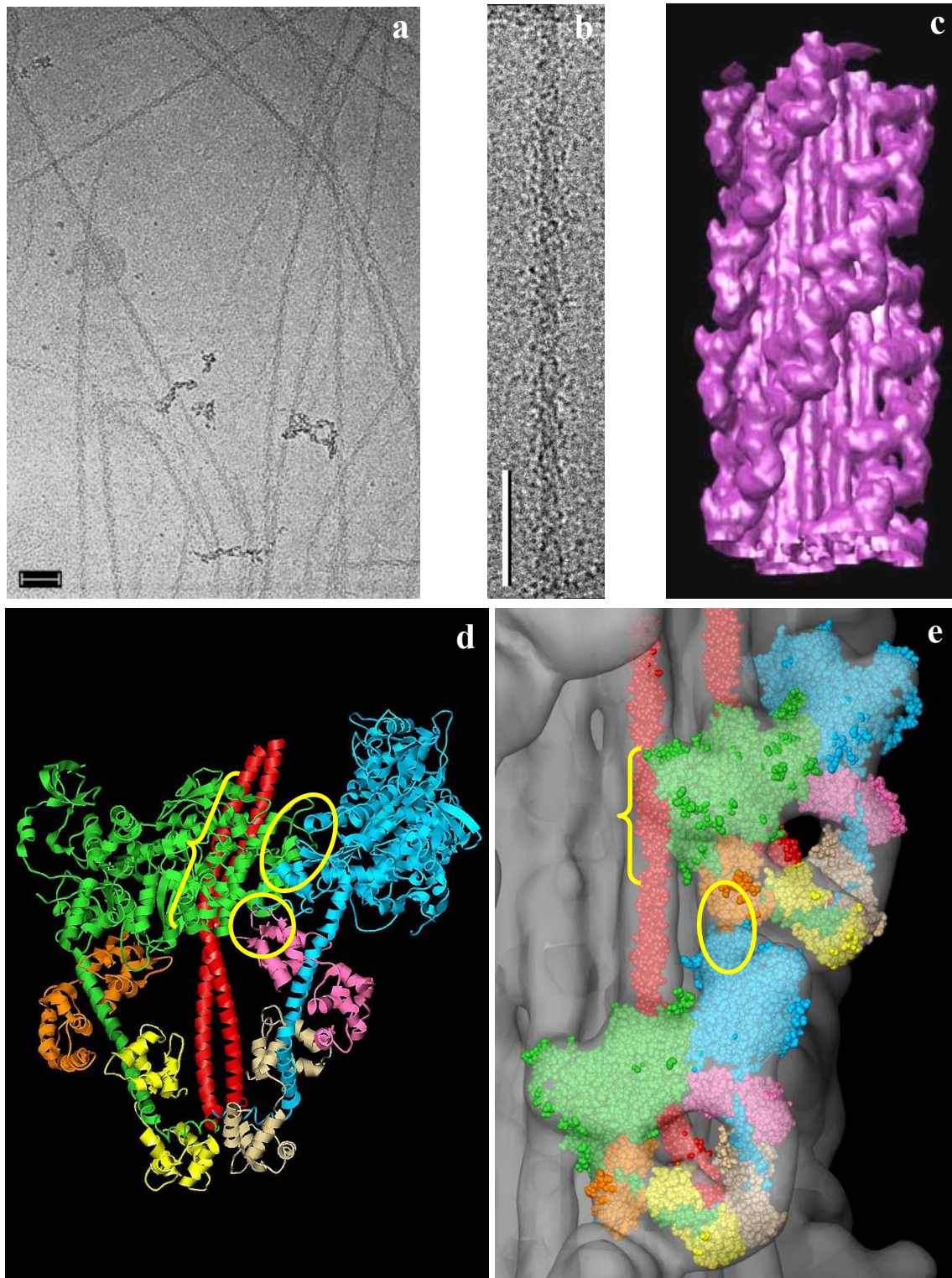


Figure 1. (a) Cryo-electron micrograph of frozen-hydrated thick filaments from tarantula striated muscle. Bar 100 nm; (b) enlarged view of a frozen-hydrated thick filament showing arrow-like structures. Bar 100 nm. (c) Surface view of a 3D reconstruction (top: bare zone); (d) Atomic model including the two myosin heads (blocked head: heavy chain green, essential light chain orange, regulatory light chain yellow; free head: heavy chain blue, essential light chain pink, regulatory light chain marble) and two segments of S2 (red). Intra-molecular interactions are marked in yellow. (e) Surface representation of the reconstruction showing atomic models of 2 heavy meromyosins fitted into adjacent motifs spaced 14.5 nm apart. Inter-molecular interactions are marked in yellow.