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A Closer Look at a Molecular Motor by Atomic Force Microscopy

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Myosin, the prototype chemomechanic energy converter in biology, has been subject to numerous functional and structural analyses. A breakthrough in understanding the mechanics of this amazing machine came by the atomic model of the actin-myosin complex derived from x-ray and electron microscopy data (Rayment et al., 1993). More recently, imaging intermediate states in the energy conversion cycle has provided support for the lever model developed from this structure (Whittaker et al., 1995). In spite of this progress, many open questions have remained and call for further studies. The S1 fragment and S1-decorated actin filaments, but not the entire myosin molecule, have been analyzed to atomic detail. Because both heads and a segment of the tail are required for full biological activity of the smooth muscle myosin under physiological conditions, a closer analysis of the entire myosin dimer is of acute interest.

Electron microscopy of negatively stained (Walker and Trinick, 1989) or heavy-metal-shadowed myosin molecules (Winkelmann et al., 1984) has provided detailed insight into the architecture of this biomolecule, although the heavy-metal deposits are likely to obscure the finer features of the native structure. Scanning transmission electron microscopy (STEM) of unstained molecules, which does not rely on metal coats, has made it possible to determine mass maps (Walzthöny et al., 1984). However, the signal of unstained biomolecules is small, even in the STEM dark-field mode, thus limiting the resolution of single particle images. Therefore, the images acquired by Zhifeng Shao and collaborators (this issue) of dehydrated myosin molecules with a cryo-atomic force microscope (cryo-AFM) represent a breakthrough, as they show the molecules with an unprecedented clarity. Accordingly, myosin heads were observed not only to assume a compact or an extended conformation, but to exhibit substructure that confirms the projections recorded by electron microscopy and which can be interpreted in terms of the heavy chain and regulatory domains. Moreover, the tails, which are often kinked, show distinct elevations repeated every 7 nm, consistent with the cross-over distance of coiled-coils, 14 nm.

The superior quality of these topographs called for a detailed comparison of nonphosphorylated and thiophosphorylated myosins. Amazingly, the tails turned out to be about 5 nm shorter in the phosphorylated state, with the foreshortening identified to occur between the two major bends. This led to the interesting hypothesis that phosphorylation of the regulatory chain might induce an α-helix–random coil transition of a tail segment between the major bends. This is indeed a new finding that may explain an induced flexibility of the tail required for docking of the phosphorylated head domain to the actin filament. In addition, a distinct difference in the population of extended and close-packed heads upon thiophosphorylation was found, suggesting an effect of phosphorylation on the flexibility of the head-tail junction.

Cryo-AFM has thus demonstrated its power in providing new and exciting data. The stability of individual biomolecules at low temperature and the preservation of their structure during the rather simple preparation steps allow high-resolution images of superb clarity to be recorded. This advantage is paid for with the requirement to dehydrate the sample, which prevents the observation of biomolecules in buffer solution. However, myosin molecules have been imaged at room temperature in liquid with the AFM (Hallett et al., 1995), suggesting that dynamic measurement under physiological conditions may become feasible.

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