were observed by negative staining EM, and their 3D reconstructions (computed by single particle methods) compared. The wild type reconstruction showed, at 4 nm resolution, the near-helical organization of the myosin heads, intramolecular head-head interactions, and immunoglobulin/fibronectin domains of titin and MyBP-C (Zoghbi et al., 2007, Biophys. J. *92*. 373a.). MyBP-C knockout filaments looked similar to the wild type in negative stain (Kensler 2007, Biophys. J. *92*. 297a.). However, the MyBP-C knockout reconstruction had a lower resolution (7 nm), suggesting that MyBP-C helps to stabilize the relaxed array of myosin heads in wild-type filaments. Titin domains were not distinguished at this resolution. Some of the intramolecular head-head interactions observed in wild-type filaments were absent in the knockout. Since such head interactions are important for the relaxed conformation of the filament, our results suggest that MyBP-C knockout filaments might not relax as fully as the wild-type. Poorer relaxation in the absence of MyBP-C may help explain compromised cardiac relaxation in MyBP-C knockout mice, and may also be related to the abnormal diastolic function in humans with HCM.

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7-Subg Computational Dissection of Intramolecular Interface Binding Energies in Various Myosin States

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Several crystal structures of the head domain of myosin are thought to represent various states in the crossbridge cycle. Here we offer an objective way to examine the various aspects of the energies involved in the myosin molecule and test hypotheses of force production and regulation. For instance, the 50k/25k cleft, the HW helix[1] and the 7 strand beta sheet are currently implicated as substructures capable of storing stress (tension) and productively releasing it as work at later points in the crossbridge cycle. We examined these energies and the implication of intramolecular interface (intraface) formation, dissolution and motion by computing ΔΔG of specific amino acids and their binding to neighboring substructures by virtual alanine scanning[2] intrafacial energies. We then compared these values in identical amino acids at various states in the crossbridge cycle. This produced a ΔΔΔG value describing how energy shifts inside the head at various states in the crossbridge cycle. These values represent the shifts in stress within myosin and within the crossbridge cycle. These intrafacial energy values and stress and strain estimates, combined with the empirical values of free energy of ATP hydrolysis, enthalpy and work output set limits for values of the energy necessary (and perhaps mechanism) to produce regulation as well as the interfacial energies associated with the actin myosin interface.

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Biological Fluorescence Subgroup

8-Subg In Vivo and In Vitro Studies of Endophilin Oligomerization

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Endophilin, which is involved in membrane vesiculation in receptor mediated endocytosis and vesicle trafficking, is a 40-kDa SH3 domain-containing protein that binds to the PRD domain of dynamin and to synaptojanin, a phosphoinositide phosphatase implicated in endocytosis. The N-terminus of endophilin contains a so-called BAR domain and the recent solution of its structure has suggested a mechanism for the ability of endophilin to induce membrane curvature. It has been suggested that dimerization of BAR domains results in a concave, positively-charged surface that can interact with, and thereby deform, membranes containing negatively charged lipids. It has been also been suggested that endophilin may be a monomer in the cytoplasm which can then dimerize upon binding to membranes or perhaps upon binding, via its SH3 domain, to dynamin's PRD domain. To clarify these issues we have studied the oligomeric state of endophilin, both in vitro (using AUC and fluorescence polarization) and in vivo. EGFP-endophilin, expressed in CV-1 cells, were studied using two-photon fluorescence correlation spectroscopy (FCS). The FCS data were analyzed using the Q-analysis method which allowed for determination of the intrinsic "brightness" of the labeled protein complexes and hence its aggregation state in the cytoplasmic regions of the cell. Despite a relatively high Kd (~5 micromolar) observed in vitro, the in vivo measurements indicate that endophilin is dimeric in the cytoplasm even at submicromolar concentrations.

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Membrane Structure and Assembly Subgroup

9-Subg Hydration of POPC Bilayers Studied by ^{1}H-PFG-MAS-NOESY and Neutron Diffraction

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Location of water molecules in POPC bilayers and the lifetime of water-lipid associations was studied by nuclear Overhauser en-