T-AM-SymI-1 ELEMENTARY PROCESS IN ACTOMYOSIN ENERGY TRANSDUCTION

Toshio YANAGIDA, Dept. of Biophys., Eng., Osaka University, Toyonaka, Osaka, Japan.

Further study on a sliding distance of F-actin/one ATP cycle: We and Oono & Kodama separately have succeeded in accurate measurements of the time courses of both ADP and Pi release from rabbit psoas myofibrils during shortening when the shortening was initiated by adding ATP or Ca^{2+} (I.S. = 40mM; T = 23C). The release rates of ADP and Pi during the maximum shortening phase (v=7μm/s) were both 10 s^{-1} $head^{-1}$. Using the ATPase rate (V_a) and the sliding velocity (v), the sliding distance (d) can be obtained as $d = f \times (v / V_a)$, where f is a fraction of heads in a force generating state per total heads. The value of f was obtained from the minimum number of heads required to move continuously F-actin-NTM at maximum velocity (7μm/s) in myosin-coated surface assay , which is the number of heads interacting with the minimum length of F-actin-NTM (~40nm) to move at max. velocity without dissociation from the surface. The minimum number of heads was found to be at most 6. Therefore, f must be > 1/6 (see below discussion). Using these values, d is calculated as d > (1/6) x (7μm/s / 10ATP/s) = 120 nm /ATP (at zero-load).

Recently, Toyoshima et al. calculated "d" using v and V_a measured in the myosin-coated surface assay (I.S.=40mM, T=30C) as $d = f \times (v/V_a) = f \times (5\mu m/s / 30ATP/s) = f \times 170nm/ATP$ (Japan. B.S.). f was obtained as 1/(minimum number of heads) = 1/17 as above and they caluculated as d = (1/17) x 170nm/ATP = 10nm/ATP. However, considering that the interaction of heads with F-actin is stochastic, it is clear that the average value of f must be > 1/17. Simple calculation showed that the average f must be > 6 x (1/17) in order for at least one head to exert force on F-actin at any moment. Thus, d is not 10nm/ATP but > 60nm/ATP.

Ultrasensitive measurements on the force generation cycle: The above results lead to an interesting model that the number of power stroke (active A-M attachment-detachment) cycles per one ATP cycle is not rigidly determined as 1 but flexibly changes depending on the conditions (load, velocity,..) In order to make this mechanism clearer, development of new approaches is strongly required. We have developed a powerful instrumentation by combining the technique for measuring force by micromanipulation of a single F-actin by glass needles with the technique for measuring subnanometric displacement of the glass needles, by which the fluctuation of force produced by the small number of myosin heads (10-100) or the sliding movement can be measured.

I will show experimental data and propose a possible mechanism.

T-AM-SymI-2 TRANSLOCATION OF MICROTUBULES BY SINGLE KINESIN MOLECULES

J. Howard, R. D. Vale, and A. J. Hudspeth, Departments of Physiology and Pharmacology and Experimental Therapeutics, University of California School of Medicine, San Francisco, CA 94143-0444

Kinesin, dynein, and myosin produce forces that can be studied by adsorbing these proteins onto a surface and observing the ATP-dependent translocation of microtubules or actin filaments. Although it is generally assumed that few molecules are involved in this movement, the minimal number required has not been determined. We have addressed this issue by assaying microtubule movement as a function of the kinesin concentration on a glass surface. When kinesin is adsorbed directly onto a microscope slide, binding and movement of microtubules occur only if the kinesin density exceeds a threshold of ~2000 molecules·μm^{-2}. The steep dependence on concentration might be taken to imply that several kinesin molecules are required to translocate microtubules. An alternative possibility, however, is that most of the kinesin molecules denature when they adsorb onto the glass, and that kinesin molecules bind and maintain an active conformation only after the surface is largely covered with protein. To test this hypothesis, we pretreated glass surfaces with other proteins, such as tubulin and cytochrome *c*; microtubule movement was then observed at kinesin densities as low as ~2 molecules·μm^{-2}. At such low kinesin densities, a microtubule rarely moves more than its length and, on average, moves about half its length before dissociating. A moving microtubule maintains attachment to the substrate at only one, fixed point while the leading and trailing ends undergo Brownian motion; the microtubule dissociates when its trailing end reaches the nodal point. These behaviors are expected if a microtubule binds at a random position along its length to one kinesin, then moves until its end reaches that kinesin. Dilution experiments suggest that the number of moving microtubules is linearly proportional to the kinesin density, rather than to a higher power of the density. These experiments collectively indicate that a single kinesin molecule suffices to hold and to move a microtubule.

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T-AM-SymI-3 THE USE OF DROSOPHILA MELANOGASTER MUSCLE MUTANTS TO STUDY THE MECHANISM OF MUSCLE CONTRACTION.

D.C.S.WHITE and J.C.Sparrow, Department of Biology, University of York, York YO1 5DD, U.K.

Drosophila, with its well-studied genetics and striated thoracic muscles, is an excellent organism for such a study. The thorax has two types of muscle: (1) the fibrillar indirect flight muscle (IFMs), and (2) the non-fibrillar flight and leg muscles, of which the tergal depressor of the trochanter (TDT) is the largest. There is only a single myosin heavy chain (MHC) gene, a single IFM-specific actin gene and an actin gene expressed in the non-IFM thoracic muscles. Thus complications due to multiple isoforms of actin and myosin (a feature for example of vertebrate muscles) do not arise. In addition, most of the other myofibrillar protein genes have been cloned and sequenced. We have obtained contractile-protein mutants in two ways: (1) by chemical mutagenesis of whole flies followed by selection for flightless progeny and (2) by in vitro mutagenesis of the IFM specific actin gene, Actin88F. We obtained 20 dominant flightless mutants by chemical mutagenesis. These are mutations in 3 genes coding for IFM contractile proteins: the myosin heavy chain gene (8 mutations), the Act88F actin gene (7 mutations) and one, designated lethal(3)laker (l(3)lkr), coding for an as yet unidentified myofibrillar protein (5 mutations). I shall discuss present progress of analysing the properties of these mutations, including measurements of the mechanical kinetic responses of both the leg and flight muscles from Drosophila.

T-AM-SymI-4 PROBING THE FUNCTION OF CONTRACTILE PROTEINS IN NON-MUSCLE CELLS USING GENETIC RECOMBINATION. James A. Spudich, Dept. of Cell Biology, Stanford University Medical Center, Stanford, CA 94305.