New and Notable

Muscle Contraction: Actin Filaments Enter the Fray

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To make sense of complex biological systems, biophysicists often devise methods to reduce the number of interacting components so that underlying molecular mechanisms are revealed: The ultimate form of this reductionist approach is to work with single molecules. On the other hand, physiologists usually prefer to work with intact isolated systems that closely mimic live conditions. To integrate information obtained by both approaches it is useful to have techniques that bridge the gap between ''physiology'' and ''molecular biophysics''. Suzuki, Fujita, and Ishiwata (in this issue) report a new technique to study the mechanism of force generation in a semi-intact muscle preparation. Their experiment involves threading an individual actin filament into a chemically treated muscle myofibril so that the forces between native myosin filaments and the single actin filament can be measured. The muscle lattice structure is preserved and the experimental conditions are close to physiological. What can we learn from such a new approach and how will it help answer some of the thorny issues surrounding the detailed mechanism of force generation by actomyosin in muscle fibers?

We now know that there are at least 18 different myosin families (1) and they are responsible not just for muscle contraction but also for a wide variety of other cell motilities. In recent years, much interest has turned to the newly discovered, nonmuscle myosins. However, the mechanism of muscle contraction remains of central interest to the field and muscle myosin remains the ''gold standard'' in our quest to understand the detailed mechanism of force generation by actomyosin. In general, we know that force production in muscle is due to the cyclical interaction of myosin heads with actin, coupled to the hydrolysis of ATP to ADP and Pi and this is known as the cross-bridge cycle. We believe that one mechanical ''kick'' is produced for each ATP molecule consumed.

MUSCLE FIBERS

The near crystalline arrangement of interdigitating thick and thin filaments within the sarcomeres of a muscle cell (or fiber) cause individual myosin motors to work as a team and generate huge forces and rapid velocities of shortening. Early researchers exploited the natural diversity of muscle types present in different organisms. Some muscles are very fast, others are slow, some are adapted for self-sustaining oscillatory contractions, and others lock up in a ''catch state'' and consume little ATP. Comparative studies using different muscle types have led to important insights into the general contractile mechanism. Using vertebrate striated muscle fibers, we know that a single nerve impulse will activate billions of myosins in concert and that a sudden change in muscle length will partially synchronize the myosin ATPase cycles. In fact, much of what we know about the mechanism of force generation derives from sudden ''length-step'' experiments made using activated single muscle fibers from frog (2) or rabbit combined with a variety of other, synchronized measurements. One of the beauties of these studies is that they can be performed at close-to-physiological conditions. In recent years, use of synchrotron radiation (3) and fluorescent optical probes (4), together with our knowledge of the actin and myosin atomic structures, has given new insights into the way myosin generates force in muscle.

NOISE ANALYSIS

Part of the central dogma of the cross-bridge cycle is that each myosin should work independently and generate force as a square-wave pulse each time it cycles with actin. The amplitude of force fluctuations produced by these stochastic interactions will depend upon the square root of the number of myosins, whereas total force is linear with number. Statistical analysis of noise can give insights into the underlying molecular mechanisms and this approach was first pioneered in nerve research (5). However, because a single muscle fiber contains $\sim 10^{12}$ molecules the expected fluctuations in force would be just one millionth of the total force. Early attempts to make these very challenging measurements in muscle failed (6,7) and it was only recently that successful noise measurements have been made using single actin filaments (8). Notably, no one has yet measured noise produced by cross-bridge cycling in intact muscle.

SINGLE MOLECULES

Over the past 10 years, single-molecule mechanical studies have enabled direct observation of individual power strokes by single myosin heads (9). In these experiments, an individual actin filament is attached between two microbeads that are held and manipulated using optical tweezers. By position ing the actin close to a single myosin molecule the stochastic mechanical interactions can be measured using a position-sensitive photodetector to

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monitor the microbead positions. These highly reductionist, single-molecule experiments have given great insight into the mechanism of force generation by actomyosin. The techniques have also been invaluable for studying nonmuscle myosins and the natural variation between myosin families has given new clues about the underlying molecular mechanism of force generation. However, many questions remain about how myosin works in intact muscle fibers and there are still significant gaps in our understanding: For instance, we still need to know more about the role played by muscle structure in the regulation of contraction in striated, smooth, and cardiac muscles; whether individual myosin heads really behave as independent force generators in the intact muscle lattice; what processes are responsible for rapid tension recovery after a muscle length step; and how insect flight muscles are ''stretch-activated''.

HYBRID SYSTEM

To uncover the secrets that lie hidden within the intricate structure of muscle, Ishiwata's group have developed a hybrid system that lies halfway between muscle-fiber and single-molecule studies. They hold a single actin filament at one end by attaching it specifically to a plastic microbead that is held and manipulated using optical tweezers. They then take a muscle myofibril from which the native actin filaments have been specifically removed (by treatment with gelsolin) and position it so that the actin filament can be threaded into the frayed end of the myosin filament lattice. Since the preparation is immersed in an MgATP-containing solution, the actin is pulled in toward the M-line by the myosin heads. This motion is monitored using a videocamera system. Movement of the actin filament can be arrested and even reversed by adjusting the power of the laser tweezer. By stopping the motion at various predetermined positions, the researchers were able to measure the relationship between force and filament overlap. Their results seem to match the classical results obtained from intact muscle fiber preparations: The average force is directly proportional to overlap between actin and myosin filaments and hence is determined by the number of myosin heads available to bind.

The results so far seem consistent with our classical understanding of the muscle contractile mechanism but the experiment remains tantalizing as the time response of the recording equipment is currently insufficient to analyze the stochastic noise produced by the relatively small number ($\sim$100) of myosins. In the future, experiments made with increased time resolution should give important additional information and permit more advanced statistical analysis (10). One hopes that such studies will shed light on some of the mechanistic questions raised earlier. We also eagerly await studies of the diverse muscle types that were used early on in our quest to ''solve muscle'', for instance, insect flight and scallop muscle fibers. The possibility also exists to mix and match different thin-filament regulatory systems with different myosin thick-filament lattices. The new ability to look at small ensembles of myosins in their native lattice arrangement and also vary the type of actin or myosin used means we can let the frog see the rabbit.

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