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New and Notable

Actin Compliance: Are You Pulling My Chain?

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The cross-bridge theory of muscle contraction postulates that the globular heads of the myosin molecules (the cross-bridges) projecting from the thick filaments attach to actin in the thin filaments, pull the thin filaments toward the center of the sarcomere, and detach. The cross-bridges are thought to make repeated cycles of attachment, force generation and detachment independently and asynchronously (A. F. Huxley, 1974) during a contraction. The force or filament sliding might be generated by a tilting motion of the attached myosin head. In vertebrate skeletal muscle, cross-bridge cycling is regulated by calcium acting through troponin and tropomyosin in the thin filaments.

What proportion of the myosin heads are attached to actin during a muscle contraction? How does this fraction vary with sliding velocity? We need this information to estimate the duration of cross-bridge attachment, the force generated by each molecule and the sliding distance generated for each enzymatic cycle of the actomyosin ATPase. How can we measure the fraction of heads attached? One way, applied in many studies, is by mechanical stiffness of the muscle. If the thick and thin filaments are much stiffer than the cross-bridges, the sarcomere stiffness is proportional to the number of cross-bridges attached. Thus, interpretation of mechanical experiments depends strongly on properties of the filaments.

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The stiffness of tetanized, single, intact muscle fibers is nearly proportional to filament overlap (A. F. Huxley and Simmons, 1972; Ford et al., 1981) and changes only slightly over the plateau region of the length-tension curve (Julian and Morgan, 1981; Bagni et al., 1990), suggesting that filament stiffness is many times greater than that of the cross-bridges. These are not, however, very sensitive tests (Ford et al., 1981).

Two papers in this issue of *Biophysical Journal* (H. E. Huxley et al. (1994) and Wakabayashi et al. (1994)) address the question of filament compliance using a more direct approach. Improvements in the x-ray beams at the CHESS synchrotron at Cornell University and at the Photon Factory in Tsukuba, Japan, combined with detection of X rays on storage phosphor-imaging plates have enhanced the temporal and spatial resolution of muscle x-ray diffraction spectra. Both groups find that the 2.7 nm actin monomer spacing along the filament axis, a parameter directly related to filament strain, increases by 0.2–0.3% when muscles develop full isometric tension from the relaxed state. This tiny change is a large fraction of the total compliance (reciprocal of stiffness) of the sarcomeres in active muscle. Both groups find evidence for some mechanical compliance in the thick filaments as well. There are hints from behavior of the 5.1 and 5.9 nm layer lines in the data of Wakabayashi et al. that actin untwists slightly when it elongates, but this idea is resisted by H. E. Huxley et al. Twisting motions would relate to mechanics of the intermolecular contacts in the actin polymer as well as possible torsional forces applied by myosin (Nishizaka et al., 1993).

T. Yanagida and colleagues (Kojima et al., 1994) have found that single actin/tropomyosin filaments in vitro have about the same stiffness (65 pN/nm for 1 μm long filaments) as calculated from the present x-ray results. The bulk elastic modulus (~2 GPa) is in the range of values for silk and collagen. We also recently found sarcomere stiffness in rabbit psoas fibers in rigor to increase as striation spacing is reduced from 2.2 to 1.8 μm (Higuchi, Yanagida, and Goldman, personal communication). In this range of lengths, filament overlap in the cross-bridge region is complete and only the nonoverlap region of the thin filaments (the I-band) changes length. These x-ray, in vitro and mechanical results imply that 40–60% of the sarcomere compliance resides in the thin filaments.

Stress relaxation in the thin filaments could resolve the discrepancy between the intact fiber stiffness measurements, made with sub-millisecond length steps, and the x-ray measurements taken after the muscle has exerted tension for several hundred milliseconds. This argument is apparently ruled out by H. E. Huxley et al.'s x-ray measurements 2 ms after applied length changes, by Kojima et al.'s data in vitro implying a $\ll$0.3 ms time constant for filament elongation after sudden application of force, and by our own mechanical study on rigor fibers with stretches complete in <0.2 ms. These experiments all suggest that actin is purely elastic. To resolve the discrepancy, we should expect to see further studies of filament compliance in muscle fibers, possibly taking account of changes in lateral filament spacing with changes in sarcomere length.

What are the implications if these new results on filament compliance are correct? The most obvious is that they impose a strong additional reason why stiffness cannot be used safely as a measure of cross-bridge attachment. Existing hazards are the dependence of stiffness on lateral spacing between the filaments, the uncertainty whether cross-bridge stiffness is the same in single-headed and double-headed attachments and in different biochemical states, and possible nonlinearity in the cross-bridge stiffness.

A well known deviation between force (presumably proportional to the number of active cross-bridges) and stiffness is the observation that during

the onset of a tetanus, stiffness rises earlier than tension (Cecchi et al., 1982; Ford et al., 1986; Bagni et al., 1988). This is usually attributed to newly attached bridges in a state that contributes to stiffness but not to force, the lead of stiffness being related to the lifetime of this pre-force state. Bagni et al. (1988), however, showed that the effect is better described as a nonlinearity in the force-stiffness relation. The time differential between stiffness and tension varies considerably in different conditions, but the nonlinearity proposed by Bagni et al. would account for the data, even including early relaxation from a tetanus, when stiffness lags behind force.

The right sort of nonlinearity would be introduced by compliance in series with the cross-bridges, as may be seen from the following example. Suppose that when all cross-bridges are attached (e.g., in rigor), the compliance (C_s) of series structures (filaments, Z line) is equal to that of the cross-bridges. The measurable compliance of the sarcomere is the sum of the two compliances, $2 \cdot C_s$. Suppose further that during a contraction only half of the cross-bridges are attached: the cross-bridge compliance is doubled and the total sarcomere compliance is then $3 \cdot C_s$. Thus, when the number of cross-bridges is halved, the stiffness (1/total compliance) is reduced only to 2/3.

During high velocity shortening, stiffness is higher than expected on the basis of the ATPase rate and 10–20 nm of active sliding per enzymatic cycle (Irving, 1987; Higuchi and Goldman, 1991). The same type of nonlinearity between stiffness and number of attached cross-bridges may help explain this paradox, but multiple power strokes per enzymatic cycle (Harada et al., 1990; Lombardi et al., 1992) or energetically neutral attachment-detachment cycles (A. F. Huxley, 1973; Cooke et al., 1994) could also help in explaining the rather high stiffness and low energy consumption of rapidly shortening muscle.

The kinetics of tension recovery after application of quick length steps have been important in developing ideas about the force-generating mechanism since Huxley and Simmons (1971) reported that the rate constant for force recovery within 1–2 ms of a length step has distinctive, nonlinear, strain-dependent features. These properties led to the idea that the elementary force-generating mechanism is a series of discrete steps, such as tilting motions, after attachment of a cross-bridge. The suggestion of a high compliance in the actin filaments implies that stiffness of the cross-bridges themselves would be much higher than had been supposed. On the simplified theoretical picture presented by Huxley and Simmons (1971), such a high cross-bridge stiffness would have two serious consequences inconsistent with observation: a small step release would be followed by rapid tension recovery to a level well above the value immediately before the step, and the dependence of the rate of the early tension recovery on the size of a release would be much too steep. Some preliminary results of computer simulations (A. F. Huxley and Tideswell, unpublished data) have shown, however, that these alarms were largely unfounded: the overshoot of tension in the early recovery phase disappears when the cross-bridge responses are averaged over the 5.5 nm range of possible positions of the myosin molecule relative to the nearest suitably oriented actin, and the steepness of the relation between speed of recovery and size of release is greatly reduced by the direct effect of the series compliance.

A related point is that a cross-bridge stiffness of about 0.7 pN/nm, which seemed necessary to match the kinetics of the early tension recovery (Lombardi and Piazzesi, 1990), is difficult to reconcile with the values found by in vitro methods of 5 pN or more for the force generated by a single cross-bridge (Finer et al., 1994; Ishijima et al., 1994). Those values imply that at least 7 nm (5 pN/0.7 pN/nm) of shortening per half-sarcomere would be needed to bring tension from its isometric level to zero, whereas experiment shows that the actual value is 4 nm or less (Ford et al., 1977). At first sight, the existence of series compliance would seem to make this discrepancy worse, because shortening of this compliance would be added to the 7 nm in the cross-bridges themselves. However, the simulations mentioned above seem to show that the direct effects of the compliance on the kinetics allow one to assume a cross-bridge stiffness many times greater than 0.7 pN/nm. The average strain in the attached cross-bridges is then reduced from 4 nm down to less than 2 nm, and the elementary power stroke, inferred from mechanical transients, is reduced from 11–12 to 8–10 nm.

This present focus on actin is timely. The x-ray, in vitro, and mechanical studies raise many questions and prod us to reinterpret earlier experiments. We need new signals to better detect cross-bridge attachment and force generation during contraction. We need explicit structural models that explain the energetic discrepancies in both fibers and in vitro data.

Oosawa (1977) related bending motions of actin, which are now well established, to the axial extensibility and suggested that thin filaments take a more active role in contraction than rigid force transmitters. Of course, the present results imply that energy can be stored in the filaments. Could storage of energy in local actin motions be essential? Many studies have shown structural or conformational changes of actin on interaction with myosin (reviewed by Naber et al., 1994), but neither spectroscopic probes on phalloidin bound to actin filaments (Naber et al., 1993) nor to a residue (Cys-374) near the myosin binding site (Ostap et al., 1992) have correlated significant rotational motions with energy transduction. However, these studies do not definitively rule out crucial motions of actin. Perhaps filament compliance "raises us up to a more sophisticated state of confusion," but few in the field will question the impact on their own hypotheses.

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Single Molecule Spectroscopies and Imaging Techniques Shed New Light on the Future of Biophysics

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Starting the mid-1970s, the development of the patch-clamp technique revolutionized molecular neurophysiology by allowing investigators to observe directly the gating of individual (i.e., single molecule) transmembrane ion channels both in vitro and in vivo. However, other fields of biomedical research could not actively participate in this revolution because the sensitivity needed to examine other classes of biomolecules did not exist. This has now changed. Technological advances in the fields of video enhanced differential interference contrast microscopy (Gelles et al., 1988; Schafer et al., 1991; Berliner et al., 1994), microscopic optical interferometry (Denk and Webb, 1990; Svoboda et al., 1994), optical trapping (Block, 1992; Kuo and Sheetz, 1993; Finer et al., 1994), ultrasensitive fluorescence detection (Bustamante, 1991), atomic force microscopy (Radmacher et al., 1994), and near-field scanning optical microscopy (Betzig and Chichester, 1993; Trautman et al. 1994; Hess et al., 1994; Xie et al., 1994; Ambrose et al., 1994) has now ushered in an age where single molecule experiments on a wide variety of biomolecules are now possible (note: reference listings are neither inclusive nor historical, simply a sampling of recent interesting results). Single-molecule studies (kinetic and structural) have numerous advantages over conventional biochemical techniques, which are restricted to examination of the population averaged properties of large molecular ensembles. It is clear that for a detailed understanding of the mechanistic steps of many molecular machines (e.g., actin-myosin and kinesin power strokes, DNA replication, DNA transcription, microtubule assembly, etc.) individual molecular events need to be monitored.

In a paper appearing in this issue, Yin et al. (1994) describe kinetic measurements of mRNA transcript elongation by a single *Escherichia coli* RNA polymerase molecule. In these experiments, biotinated DNA is tethered to a 0.23 μm polystyrene microsphere coated with avidin. Video enhanced differential interference contrast microscopy directly images the polystyrene bead. Stalled transcription complexes of *E. coli* RNA polymerase is adsorbed onto the surface of a specially treated coverslip. These stalled complexes are then incubated with avidin coated beads, which will attach to the end of the DNA. The DNAs can be made such that the bead is either "upstream" or "downstream" from the stalled site. When located upstream, the bead is drawn downward toward the coverslip as transcription proceeds, whereas for the downstream location, the length of the DNA tether increases and the bead moves farther away from the coverslip (see Fig. 1 *b* of Schafer et al. (1991) for a schematic representation of these experiments).

All of the nanometer displacements in this system are obtained by observing the extent of Brownian motion of

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