Blitz and Blizzard: Crossbridges and Chaos

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The central goal in muscle biophysics is to detect directly the force-generating structural changes of the acto-myosin crossbridge. The principal hypothesis to be tested is that chemical transitions in the ATP hydrolysis cycle are coupled to myosin head rotation between two or more distinct orientations on actin, but a wide range of physical techniques, including electron microscopy, have not produced compelling evidence for these distinct angles (reviewed by Thomas (1987, 1993)). In the absence of nucleotide (the rigor state, probably corresponding to the AM.ADP state at the end of the ATPase cycle), there is no heterogeneity or ambiguity: myosin heads bind to actin strongly and uniformly and point away from the Z line at an angle of approximately 45° to the actin filament axis. In the presence of ATP, however, most electron microscopy (EM) studies have shown a high degree of orientational disorder, with myosin heads apparently attached to actin at widely varying angles either in solutions of actin and myosin heads (e.g., Frado and Craig, 1992) or in active muscle fibers (discussed by Hirose et al. (1993) and Hirose and Wakabayashi (1993)). One possible explanation for this observed disorder is that the active attached bridges go through a series of angular transitions, but the distinct angular states are not easily resolved in a steady-state experiment.

A report by Hirose et al. (1993), in this issue, addresses this problem by performing time-resolved EM studies during the transient phase of isometric muscle contraction. In this impressive collaboration among the laboratories of Y. Goldman, J. Murray, and C. Franzini-Armstrong, skinned fibers were activated by laser flash photolysis of caged ATP (in the presence of calcium), rapidly frozen at well-defined millisecond time points, freeze-substituted, fixed, and examined by electron microscopy. Tension was monitored during the activation of the muscle, permitting a direct correlation between the observed structures and the development of force. In agreement with previous studies, crossbridges were triangular in shape and uniformly oriented in rigor (before photolysis) but were thinner and highly disordered in the steady state of isometric contraction (300 ms after photolysis). In the transient phase, despite the initial synchronization of the crossbridges, the transition between these two populations was direct, with *no clearly defined intermediate crossbridge shape or orientation.* The most remarkable result came from the comparison of the structural and mechanical transients: *the disordering transition was slower than ATP binding but more rapid than force generation.* Even at 20 ms after the flash, when all of the myosin heads probably had ATP bound but very little force had been generated, many (but not all) bridges were already disordered. At 50 ms, when force development was only half complete, the disordering transition was complete. Thus disorder appears to be produced by an isomerization (i.e., a structural change within the myosin head) either following ATP binding or following ATP hydrolysis, but before the "weak-binding to strong-binding" transition that produces force.

Although the angular disorder observed in contraction is consistent with most other steady-state EM results, this result is in apparent conflict with that of the only other rapid-freeze EM study of crossbridge structure during the transient phase of the ATPase cycle (Pollard et al., 1993). In the latter study, involving stopped-flow experiments on actin and myosin heads in solution, most actin-bound heads were found to be in a rigor-like orientation, whether ATP was present or not. One way to reconcile these results is to take into account the known effects of the fiber lattice. In active, isometric fibers, the predominant crossbridge states are force-generating (strong-binding) states late in the ATPase cycle. In solution, the pre-force (weak-binding) states predominate, so the rigor-like bridges observed in solution (Pollard et al., 1993) might correspond to very early states in the power stroke, as observed in fibers at 20 ms but not at 50 ms (Hirose et al., 1993). In addition, the helical mismatch between actin and myosin filaments in the fiber might be expected to produce more heterogeneity in attached crossbridge angles. However, this interpretation is valid only if several other solution studies, using different fixation techniques and reporting disordered attached heads in the presence of ATP (discussed by Hirose et al. (1993) and by Pollard et al. (1993)) are invalid. Alternatively, the freeze-etch technique used by Pollard et al. (1993) might selectively preserve rigor-like bridges, or the freeze-substitution technique used by Hirose et al. (1993) might induce artifactual disorder. Although it is possible that control EM experiments and simulations (e.g., Hirose et al., 1993) can rule out some of these artifacts, the inherent limitations in EM studies of such a complex and dynamic system—fixation artifacts, low spatial and angular resolution, sampling error, ambiguity of attachment—make it unlikely that EM experiments alone can resolve these ambiguities.

X-ray diffraction and spectroscopic probes are complementary to EM—although they can not provide direct visualization, they can be performed without fixation, and spectroscopy can provide higher orientational resolution, better quantitation of minor populations, and sensitivity to dynamics. X-ray diffraction transients, initiated either by electrical stimulation of intact muscle fibers or photolysis of caged ATP, have consistently shown major changes, attributed to crossbridge binding or structural changes, that precede force development (discussed by Hirose et al. (1993)). EPR and optical probe results over the past decade have provided strong evidence for (*a*) *dynamic* (microsecond) rotational disorder of *actin-attached* myosin heads in active muscle (discussed by Thomas (1987), Hambly et al. (1992), and Berger and Thomas, (1993)), and (*b*) such a small fraction (10–20%) of rigid rigor-like heads that they would probably not be detected by either EM or x-ray diffraction (Fajer et al., 1990). Transient-phase (using caged ATP) EPR (Ostap et

al., 1993) and fluorescence polarization (Tanner et al., 1992; Allen et al., 1993) have shown that the head-disordering transition is very rapid, certainly preceding force generation and probably even preceding ATP hydrolysis. EPR has also shown that nucleotides tend to produce crossbridges bound with only one head, which may explain the thinner appearance of active crossbridges reported by Hirose et al. (1993).

Hirose et al. (1993) have established an impressive new technique for the correlation of biochemical, mechanical, and structural transitions during the transient phase of muscle contraction. Some apparent discrepancies remain to be resolved among different EM techniques, suggesting further control experiments and correlation with x-ray and spectroscopic studies. Nevertheless, two clear messages emerge: 1) Only two orientational states of attached crossbridges are observed: a well-oriented rigor-like state and a dynamically disordered state. 2) A rapid transition, shifting most of the myosin heads from the ordered to the disordered state, occurs very early in the ATPase cycle and precedes force generation. The remaining challenge—to construct and test a model in which force is generated (*a*) after the formation of a dynamically disordered state, or (*b*) by a very small fraction of rigidly oriented heads—will require further correlation of structural, spectroscopic, and mechanical transients.

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