ANSC (FSTC) 607
Physiology and Biochemistry of Muscle as a Food
MUSCLE CONTRACTION

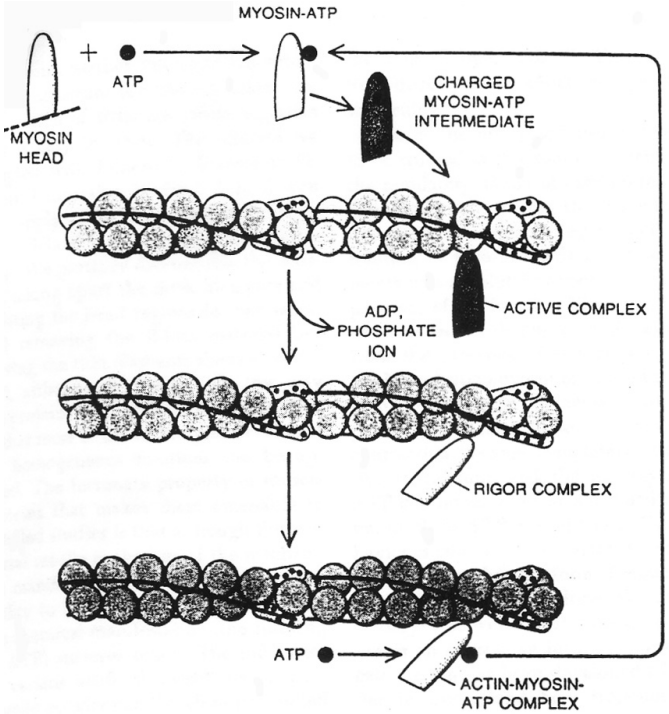
I. Basic model of muscle contraction

A. Overall

1. Calcium is released from sarcoplasmic reticulum.
2. Myosin globular head (S1) interacts with F-actin.
3. Thick and thin filaments slide past each other.
4. Calcium is resequestered in sarcoplasmic reticulum.
5. Muscle relaxes.

B. Role of ATP

1. Charges myosin heads by forming charged myosin-ATP intermediate (actually myosin-ADP).
2. Provides energy for power stroke, which allows for release of myosin head from actin (i.e., breaks the rigor bond).



CHEMICAL EVENTS of the muscle-contraction cycle are outlined as they take place in the soluble experimental system described by the authors. A myosin head combines with a molecule of adenosine triphosphate (ATP). The myosin-ATP is somehow raised to a "charged" intermediate form that binds to an actin molecule of the thin filament. The combination, the "active complex," undergoes hydrolysis: the ATP splits into adenosine diphosphate (ADP) and inorganic phosphate and energy is released (which in intact muscle powers contraction). The resulting "rigor complex" persists until a new ATP molecule binds to the myosin head; the myosin-ATP is recycled, recharged and once again undergoes hydrolysis.

II. Cooperative action of muscle proteins

A. Requirement for troponin/tropomyosin

1. ATPase in intact thin filaments *plus* myosin heads requires calcium for binding.

2. **Purified** actin plus myosin heads S1 have high ATPase activity even in the absence of calcium, so that F-actin will bind to a charged myosin head (M-ADP-P_i) in the absence of calcium.

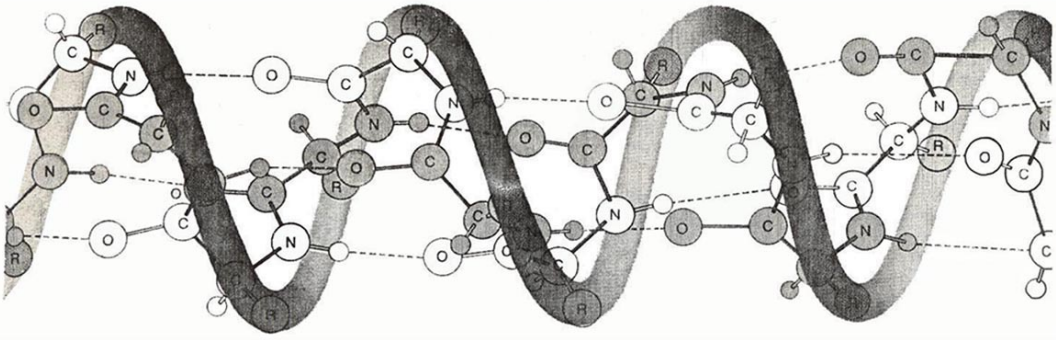
3. Regulation of contraction is provided by troponins plus tropomyosin.

B. Tropomyosin

1. Subunits (α and ß) are alpha-helices.

2. Subunits are entwined in opposite directions to form a coiled coil (the tropomyosin molecule).

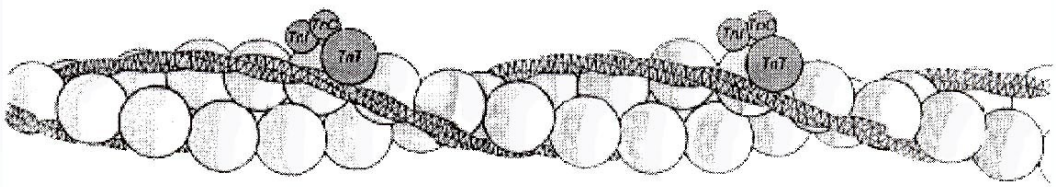
3. Each coiled-coil lies in an actin groove (coiled coiled coil).



ALPHA HELIX, a configuration that underlies the structure of many proteins, including tropomyosin, is a coiled chain of amino acid subunits. Each subunit consists of a carbon atom with a characteristic radical, or side group (R), flanked by CO and NH groups. In this drawing, successive subunits are alternately gray and white. The helix is braced by hydrogen bands (broken lines) linking the hydrogen (small circle) of each NH group with the oxygen of the fourth subunit along the chain. In a typical alpha helix there are 3.5 subunits in each complete turn of the helix. In this drawing, the colored tube connects the radicals, tracing the turns of the helix.



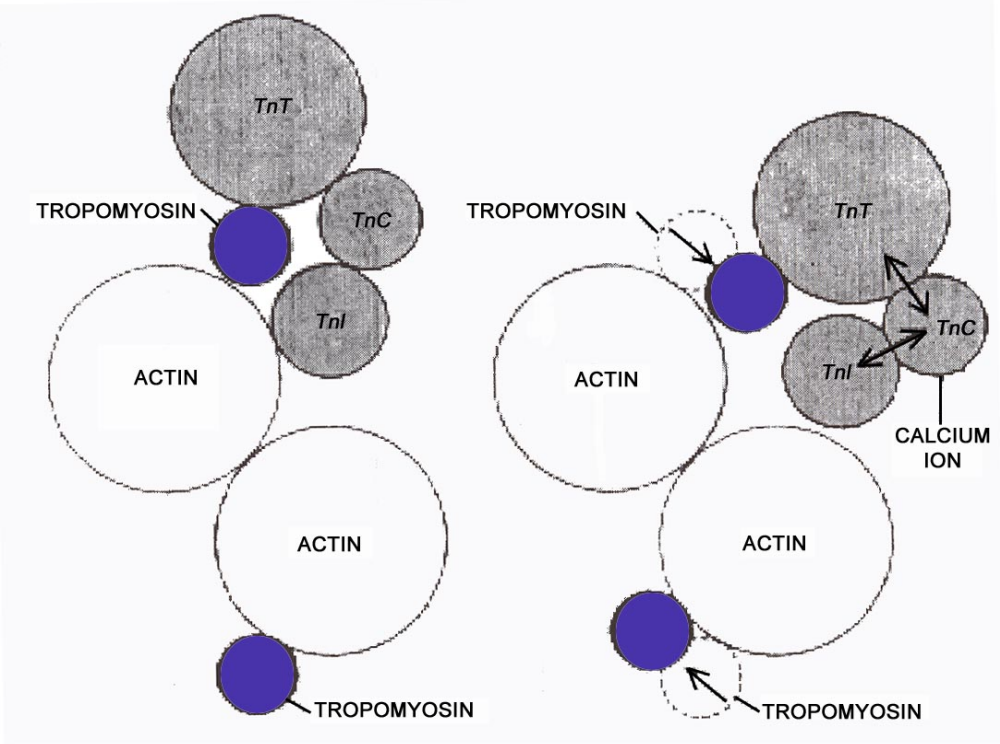
COILED COIL is formed when two alpha helixes wind around each other, side chains interlocking to form a stable structure. There are about 35 turns in an alpha helix in one turn of the larger coiled coil, but this "axial repeat" varies with small changes in the helix.



COILED COILED COIL, is formed by the filament of tropomyosin molecules (coiled coils) wound in each of the two grooves of the active helix in a thin filament of muscle. The actin molecules (gray and white) are polar; they all point in the same direction in a double helical array. The tropolyosin filaments (color) consist of polar tropomyosin molecules, bonded head to tail, that lie near the grooves, each molecule spanning seven actin monomers. A troponin complex (dark gray) is about a third of the way from one end of each tropomyosin molecule. In this schematic diagram only the troponin complexes on one side of the actin helix are shown.

C. The troponins

1. Troponin T (TnT): binds to tropomyosin one-third from the carboxy terminus of tropomyosin.
2. Tropinin C (TnC): binds calcium.
3. Tropinin I (TnI): binds to actin and inhibits binding of myosin.



SUBUNITS OF TROPONIN may function as suggested in this tentative model of the regulation of muscle contraction. The thin filament is seen schematically, end on. In the resting state (left) the TnT subunit binds to tropomyosin and the inhibitory subunit Tnl binds actin; the calcium ion concentration is low and the links between troponin subunits are relatively loose. In the active state (right), above a critical calcium level, linkages between troponin subunits are tightened and the link between Tnl and actin is weakened; tropomyosin moves deeper into the actin groove, exposing the site at which myosin can bind.

D. Interaction between the troponins and tropomyosin (steric model)

1. The tropomyosin/troponin complex is the same side of the groove as the myosin attachment site.
2. Binding of calcium to TnC causes a change in conformation of the troponin complex.
3. This change causes a shortening (or other conformation change) in tropomyosin.
4. The tropomyosin/troponin complex shifts into the actin groove.
5. This allows the myosin S1 head to interact with actin and stimulate the release of P_1 from the AM-ADP-P_i complex.

E. Essential components of contraction for either the steric or allosteric models

1. Tension is proportional to overlap of thick and thin filaments.
2. Actin must be inextensible.
3. Speed of contraction is independent of overlap in isotonic contraction.

III. Other models of muscle contraction

A. Allosteric hindrance

1. Tropomyosin/troponin complex is on the opposite side of the groove as the myosin attachment site.
2. Binding of calcium to TnC causes a change in the conformation of actin, thus activating the myosin ATPase.

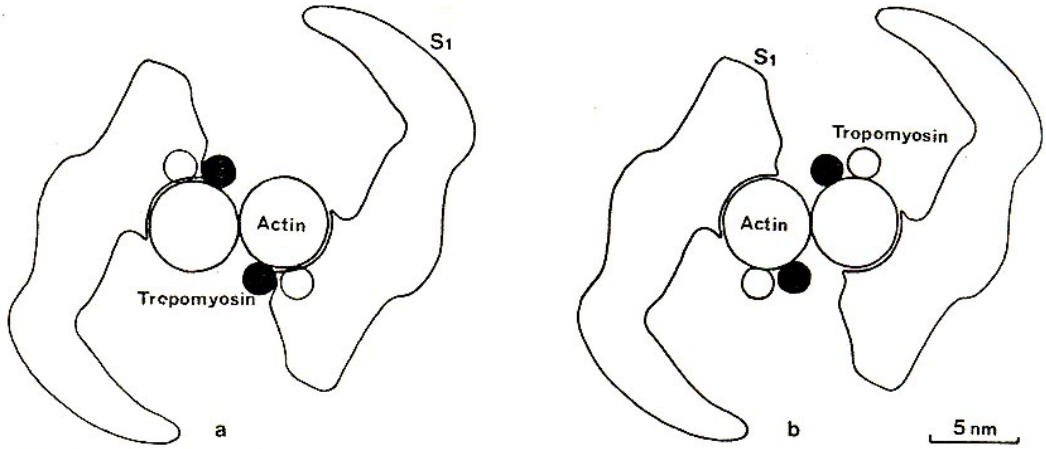
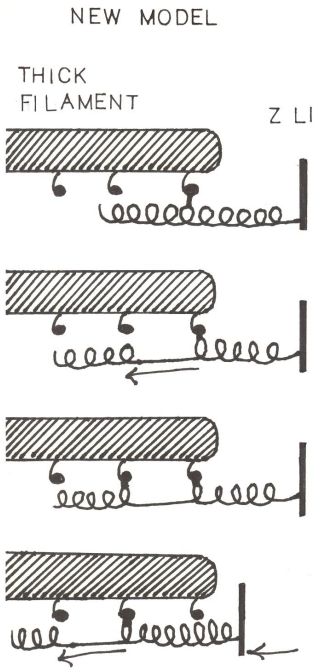
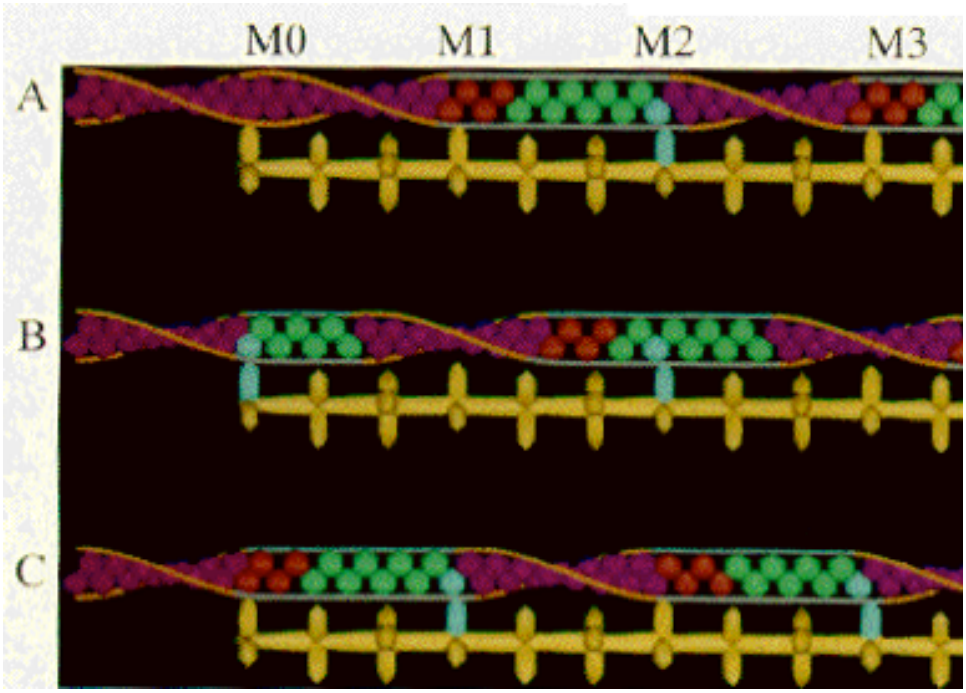


Fig. 41. (a) Steric blocking mechanism for the prevention of head attachment to actin by tropomyosin. This view is comparable to the views down the helix axis of decorated actin shown in Fig. 35 and is based on the Moore *et al.* (1970) interpretation of the arrowhead structure (Fig. 35c). Two positions for tropomyosin are shown: the open circle where tropomyosin coincides with part of the S-1-binding site on actin, and so could physically block attachment; and the filled circle where tropomyosin has moved towards the centre of the actin groove thus unblocking the binding site. (b) Similar view to (a) illustrating the alternative locations of tropomyosin, on the opposite side of the actin groove to (a). Here S-1 attachment would not be blocked. (reprinted from Seymour and O'Brien (1980), courtesy of J. Seymour and Macmillan Journals Ltd).

B. Actin as a tension generator

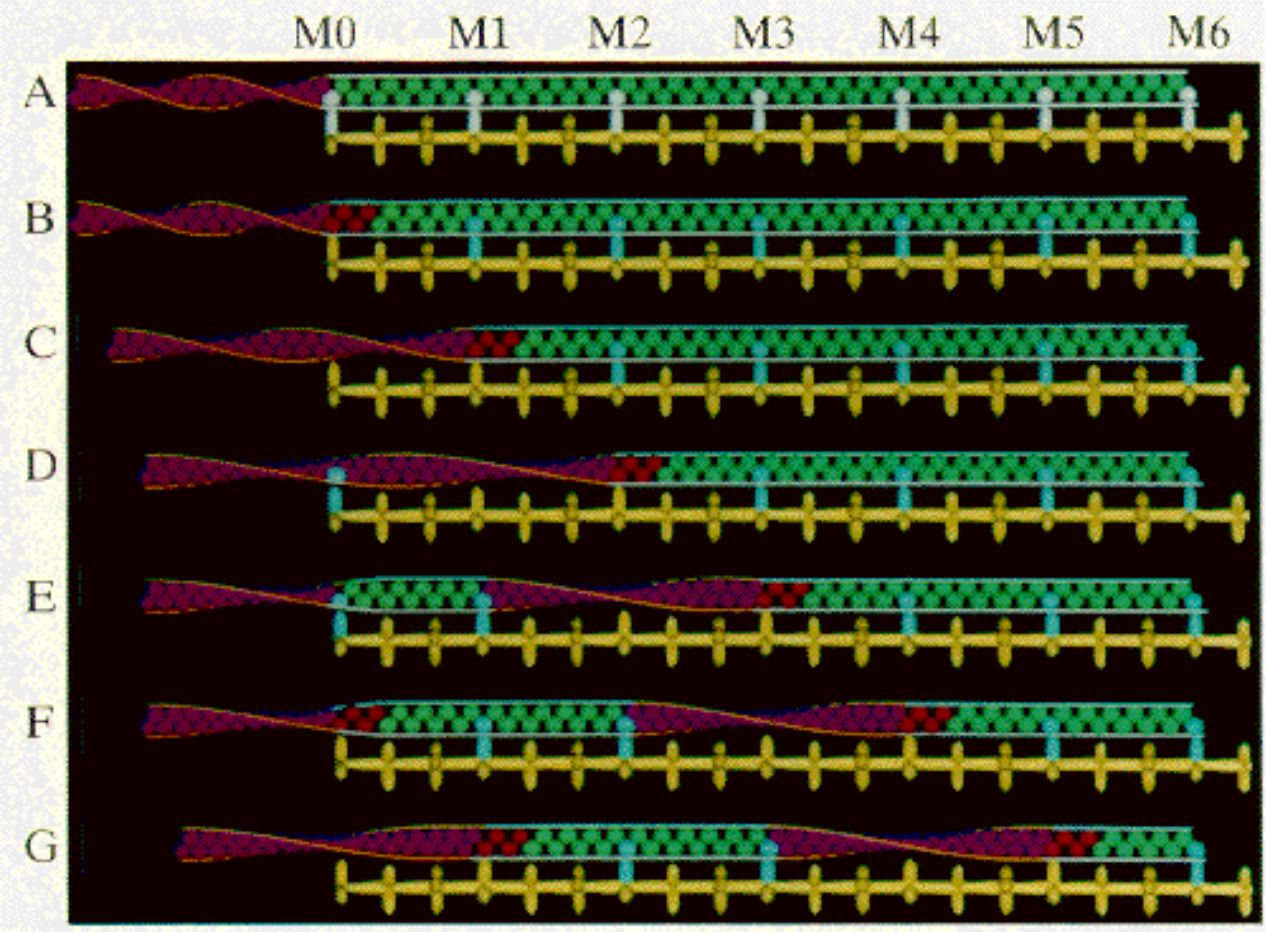


1. The binding of M-ADP-P_i to its binding site on actin induces the uncoiled or "ribbon" configuration (**r-actin**).
2. In the presence of calcium, myosin is released from actin and helicalization (return to the helical state, **h-actin**) begins.
3. The actin binding site "reaches" for next available myosin head.
4. Recoiling of actin after release causes shortening of sarcomeres.
5. The amount of tension is proportional to the number of actin monomers that make the transition from r-actin to h-actin.
6. In this model, tropomyosin is the inextensible parallel component.



C. Isometric contraction – tension is proportional to overlap.

1. For each actin that is changing from r-actin to h-actin, another actin is changing from h-actin to r-actin.
2. The force generated is proportional to the number of helical segments, i.e., the extent of overlap of thick and thin filaments.



D. Development of contraction

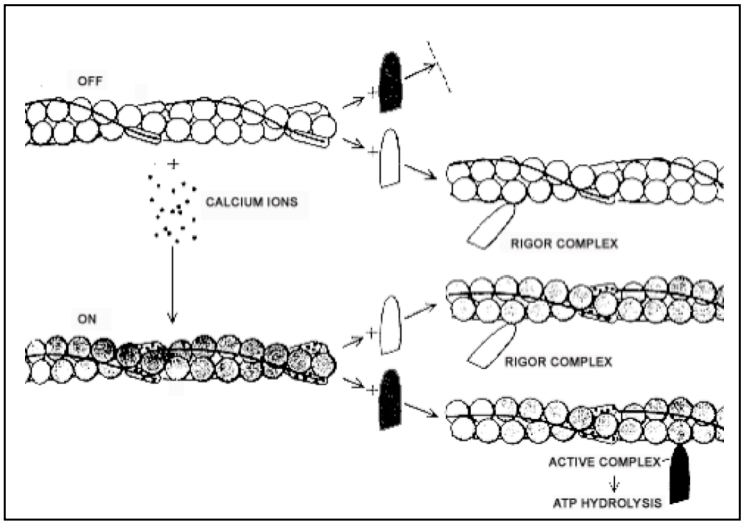
1. For each actin that is changing from r-actin to h-actin, another actin is changing from h-actin to r-actin.
2. R-actin, in the absence of Ca^{++}, is attached to myosin by weak interactions.
3. In the presence of Ca^{++}, myosin heads bind strongly to h-actin, which detaches r-actin from myosin at M0.
4. In D, the M0 myosin has released P_i and rebounded to an h-actin monomer. This starts a new ribbon of r-actin, setting the stage for the next wave of contraction.

IV. Regulation of contraction

A. Requirement for calcium

a. Calcium is required for binding of charged myosin heads.

b. Calcium is **not** required for binding of uncharged myosin heads (to form rigor bonds).

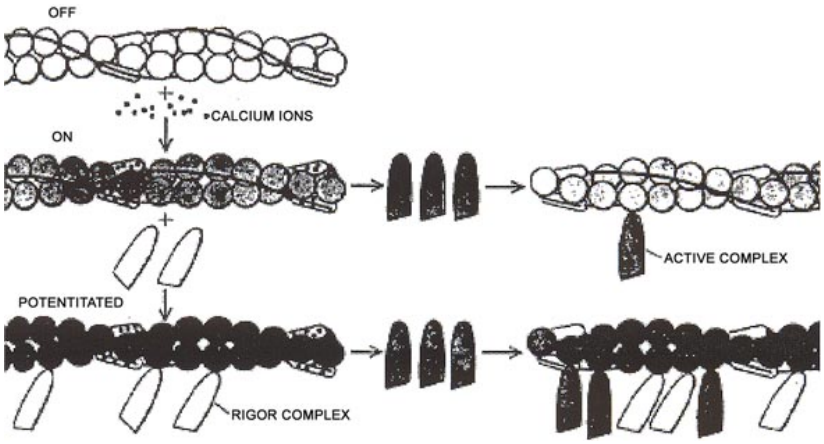


REGULATION OF MUSCLE CONTRACTION is accomplished primarily by calcium ions, which change the thin filament from an "off" to an "on" state, apparently by binding to troponin. When the thin filament is turned off (*top left*), a charged myosin-ATP intermediate cannot combine with it; when the filament has been turned on by calcium (*bottom left*), an active complex is formed (*bottom right*) and hydrolysis proceeds. Formation complexes, however, is not sensitive to calcium control, the thin filament is on or off, a myosin head can comb an actin in the filament to form a rigor complex (*middle*).

B. Potentiation of contraction

a. Uncharged myosin heads can be bound to actin (at low ATP!).

b. Addition of charged myosin heads now give a greater rate of ATP hydrolysis than is seen in absence of rigor bonds.



POTENTIATED STATE is the third state of actin. Molecules in the off state are turned on by calcium ions and in the on state they can form active complexes (*right*). At low ATP concentration rigor complexes are formed. This raises actins that have also turned on to a potentiated state.

V. Kinetics of muscle contraction

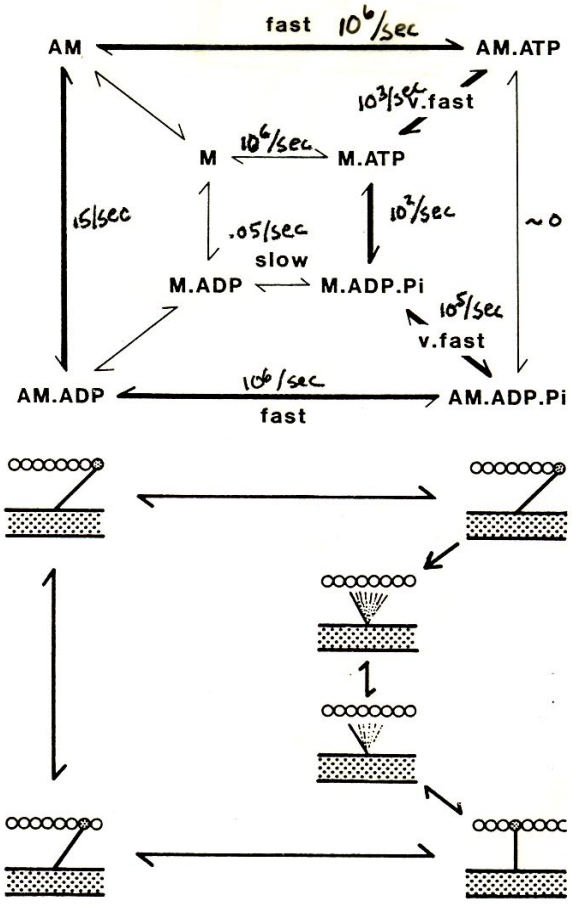


Fig. 32. Correlation between Mg-ATPase cycle and cross-bridge cycle. (Top) Simplified scheme of the myosin and actomyosin Mg-ATPases, shown in the form of two concentric cycles. A is F-actin; M is myosin, HMM or S-1. For clarity, indication of the uptake or release of actin, ATP, ADP or inorganic phosphate (Pi) has been omitted from the scheme. Heavy arrows indicate the predominant pathway *in vitro*. (Bottom) Hypothetical structural stages in the cross-bridge cycle. The scheme would be appropriate for a muscle shortening under no load. The situation in an isometric muscle (where no filament translation occurs) may be more complex. The Z-line would be to the left of the diagram.

A. AM → AM-ATP
   a. 10^6/sec
   b. Fast!

B. AM-ATP → M-ATP
   a. 10^3/sec
   b. Pretty fast.

C. M-ATP → M-ADP-P_i
   a. 10^6/sec (fast!)
   b. 10^2/sec (slower)

D. M-ADP-Pi → AM-ADP-P_i
   a. 10^5/sec
   b. Fast.

E. AM-ADP-P_i → AM-ADP
   a. 10^6/sec
   b. Fast!

F. AM-ADP → AM
   a. 15/sec
   b. Rate-limiting step for contraction.