Striated Muscle Regulation by Troponin and Tropomyosin: Cardiac and skeletal muscles are controlled by regulatory complexes composed of three troponin subunits and tropomyosin. The troponin complex is unique to striated muscles. Mutations in any of the regulatory subunits (troponin I, troponin T, troponin C and tropomyosin) may result in myopathies or cardiomyopathies.

The interactions among the troponin subunits, tropomyosin, and actin are Ca\(^{++}\) dependent. Ca\(^{++}\) binding to troponin C alters the interactions among the three troponin subunits and also changes the relative stability of the three positions of tropomyosin on actin. Actin is incapable of stimulating the ATPase activity of myosin when tropomyosin is outside of the actin helical groove (B state) or when tropomyosin is situated near the groove (C state, the most stable state at saturating Ca\(^{++}\)). Movement of tropomyosin into the helical groove (M state) results in stimulation of ATP hydrolysis by myosin. That active M state is most highly stabilized by rigor myosin binding to actin. The increased rate of ATP hydrolysis by myosin results in force production or movement.

Several hypotheses have been proposed for the mechanism by which this movement of tropomyosin regulates contraction. In the Steric Blocking hypothesis the position of tropomyosin in relaxed muscle (low free Ca\(^{++}\)) blocks the binding of myosin to actin. This model also makes sense from the standpoint that relaxed muscle has little resistance to stretch as would be expected if the contractile proteins were uncoupled.

We have found that the story is somewhat more complex than this although in a manner of speaking steric blocking does occur. The complexity that we observed is that while Ca\(^{++}\) has a large effect on the binding of myosin-ADP to actin it has almost no effect on the binding of myosin-ATP to actin. Myosin passes through several chemical states during the hydrolysis of ATP. Some of the states bind to actin in a Ca\(^{++}\) dependent manner while others bind in a Ca\(^{++}\) independent manner. The low resistance to stretch in relaxed muscle does not occur because myosin cannot bind to actin but because the binding (attachment and detachment) of myosin-ATP to actin is so fast that it offers little resistance.

In the early 1980’s a Parallel Pathway Model of regulation was proposed (Hill, Greene and Eisenberg (1980) PNAS 77, 3186). That model predicts the correct kinetics of ATP hydrolysis in solution and simulates the regulation observed in muscle fibers. Those fiber studies were done with Drs. Bernhard Brenner and Terry Kraft at the University of Hannover; and Drs. Leepo C. Yu and Yi-der Chen at the National Institute of Health.

The basis of the 1981 Hill et al. model is that actin exists in two functional states
(inactive and active) which are in rapid equilibrium with each other. The rate constants $\alpha$ and $\beta$ (see figure below) define the distribution between the inactive states $A_1$ and the active states $A_a$. These states are dictated by the binding of Ca++ to troponin and the resulting position of tropomyosin on the actin filament. The active state of the actin filament binds more tightly to Ca++ and to myosin-ADP than does the inactive state of actin. Thus, both Ca++ and myosin-ADP (or rigor myosin) tend to stabilize the active form of the actin filament. The rate of phosphate release from myosin is very slow when myosin is detached from actin. One of the functions of actin is to accelerate the rate of phosphate release.

In the parallel pathway model actin can catalyze phosphate release only when it is in the active state. We propose that the binding of Ca++ to troponin causes a shift in the position of tropomyosin that places more of the actin in the active state so that various steps in the ATP hydrolysis pathway can occur rapidly. A more pronounced change in tropomyosin position is required for myosin in the force producing states (such as myosin-ADP) to bind properly to regulated actin. Thus, myosin-ADP and rigor myosin stabilize the active state of regulated actin.

Although the model was successful in describing the Ca++ effect on the equilibrium binding of myosin S1 to regulated actin and the activation of ATPase activity, it was supposed that such model with only two actin states could not explain the effect of Ca++ on the KINETICS of binding of myosin S1 that was observed by Trybus & Taylor (PNAS 77, 7209 (1980)) and McKillop & Geeves (Biochem. J. 279, 711 (1991)). Using Monte Carlo methods and additional experimentation we have shown the parallel pathway model correctly predicts binding kinetics. However, that is not the end of the story. Rather, studies of mutations in troponin that lead to cardiomyopathies gave us another clue.

Drs. Scott Fredricksen and Boris Gafurov showed that two troponin T ($\Delta$14 and R92Q) mutations that increased actin-activated ATPase rates stabilized the active state of actin. One of the mutants had an ATPase rate that exceeded that observed in the absence of the inhibitory proteins. Therefore, the regulatory proteins activate as well as inhibit actin-myosin activity. The parallel pathway model can explain activation as well as inhibition so it is well suited for studying these disorders. However, studies with other mutants supported the idea of Trybus & Taylor and Geeves and others that there were three positions of the regulatory switch and not two.

Dr. Tomoyoshi Kobayashi supplied us with phosphomimetic mutations of Tnl for study. Dr. Mohit Mathur found that these mutants stabilized the inactive state of regulated actin. Dr. Mathur studied other mutants of troponin and tropomyosin, the latter from Dr. Bryant Chase. These mutants all appeared to change the distribution between inactive and active states in both EGTA and Ca++. Our hypothesis is that many disease causing mutations and post-translational modifications of troponin alter the rate constants $\alpha$ and $\beta$ (Fig. 1) and thus change the predominant pathway for ATP hydrolysis and the rate of activation and inactivation.

We were somewhat surprised by the R146G TnI mutation supplied by Dr. Kobayashi.
Dr. Mathur and Mr. Dylan Johnson found that this and similar mutations stabilized a state distinct from the inactive or active stats described in the Hill model. This was our first observation of a functional intermediate state.

The Hill model states that Ca** binding was not sufficient to fully activate contraction. Thus, that model included an inactive Ca**-free state and an inactive Ca**-bound state as well as active states. However, those two inactive states were treated as a single meta-state. The R146G TnT data could only be described by treating the two inactive states as separate entities.

Several researchers used fluorescent probes, electron microscopy or other structural techniques to examine changes in actin filament structure as it was activated by Ca** or rigor S1 binding. They concluded that there were 3 metastable positions of tropomyosin on actin and they had argued that it was necessary to consider those 3 states in schemes of regulation. They were correct on that point.

<table>
<thead>
<tr>
<th>States of Regulated Actin</th>
<th>B</th>
<th>C</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation of myosin ATPase</td>
<td>inactive</td>
<td>inactive or marginal</td>
<td>active</td>
</tr>
<tr>
<td>Binding to myosin S1-ATP</td>
<td>binds with rapid on/off kinetics</td>
<td>binds similarly to B state</td>
<td>binding 2-fold stronger than B</td>
</tr>
<tr>
<td>Binding to S1-ADP or S1-AMP-PNP</td>
<td>affinity about 1/20th of the M state.</td>
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The Δ14 TnT mutant continues to fascinate us because it demonstrates surprising features of the COOH terminal 14 residues of troponin T. Work continues with Dr. William Angus and Mr. Dylan Johnson with collaborations with Drs. Yumin Li, Colin Burns and Anne Spuches at ECU and Drs. Jose Pinto (Florida State Univ.), Dr. Robert Stehle (University of Cologne) and Dr. John Robinson (Univ. South Dakota):

1. Actin filaments containing Δ14 TnT cannot enter the inactive B state.
2. Actin filaments containing Δ14 TnT are hyperactive at saturating Ca**. Their activity approaches that of actin filaments containing bound rigor type myosin.
   a. Troponin is an activator as well as an inhibitor.
   b. The COOH terminal of TnT attenuates the degree of activation.
   c. The degree of activation is probably regulated.