

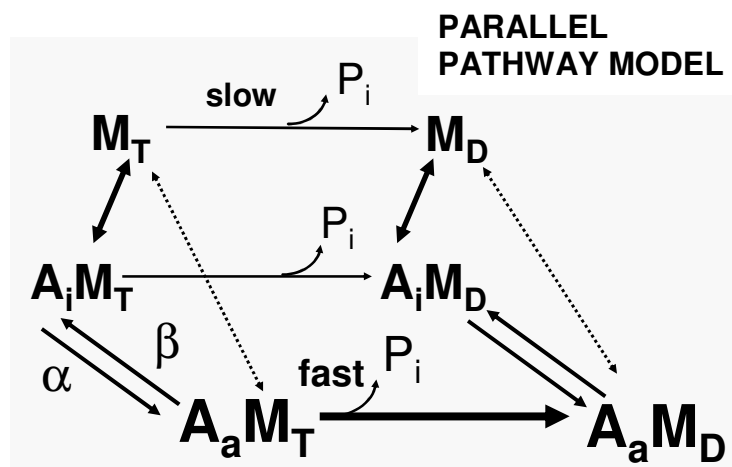
Striated Muscle Regulation by Troponin and Tropomyosin: Cardiac and skeletal muscles (STRIATED MUSCLES) are primarily under the control of the actin binding protein complex of TROPONIN and tropomyosin. Troponin consists of three subunits which bind to Ca^{++} , actin and tropomyosin. The troponin complex is unique to striated muscles. Mutations in any of the regulatory components (troponin I, troponin T, troponin C and tropomyosin) may result in myopathies or cardiomyopathies.

The interactions among troponin T, troponin C, tropomyosin, and actin are Ca^{++} dependent. When Ca^{++} binds to specific regulatory sites of TnC there is a tighter association among the troponin subunits and a change in the position of tropomyosin on the actin filament. This change in binding of tropomyosin on actin results in the ability of actin to accelerate the ATP hydrolysis of myosin and results in force production. Several hypotheses have been proposed for the mechanism by which this movement of tropomyosin allows contraction. The first hypothesis to gain wide acceptance was the steric blocking hypothesis. This is a simple model in which the position of tropomyosin in relaxed muscle (low free Ca^{++}) blocks the binding of myosin to actin. In the absence of binding of myosin to actin there is no activation of ATP hydrolysis and no movement. 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. Thus during the hydrolysis of ATP, myosin passes through several chemical states and some of them 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 regulation of striated muscle was proposed (Hill, Greene and Eisenberg (1980) PNAS 77, 3186). That model is noteworthy since it does predict the correct kinetics of ATP hydrolysis in solution (some models avoid this critical test) and it also successfully simulates the regulation observed in muscle fibers. The more recent muscle fiber studies were done with Drs. Bernhard Brenner and Terry Kraft at the University of Hannover; and Drs. Leopo C. Yu and Yi-der Chen at the National Institutes and Health. The bases of the 1981 Hill et al. model is that actin exists in two states which are in rapid equilibrium with each other. The rate constants α and β (see figure below) define the distribution between the inactive states A_i 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.

We have been studying mutations in troponin that result in cardiac disorders such as Familial Hypertrophic Cardiomyopathy. Drs. Scott Fredricksen and Boris Gafurov showed that two troponin T 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. We have also observed that some mutations of troponin I that result in lower ATPase rates stabilize the inactive state of regulated actin. That work is being done by Dr. Mohit Mathur in collaboration with Dr. Tomoyoshi Kobayashi. Our hypothesis is that many disease causing mutations and post-translational modifications of troponin alter the rate constants α and β (Fig. 1) and thus change the predominant pathway for ATP hydrolysis and the rate of activation and inactivation. We are continuing to examine other mutants in collaboration with Drs. Tomoyoshi Kobayashi and

Bryant Chase. We are also examining the effects of mutations on the changes in the interactions among the troponin components in collaboration with Dr. Yumin Li (chemistry, ECU) and Ms. Xialan Dong. Finally, we are studying the mechanism of interconversion between the active state and inactive state of regulated actin. That work is being led by Dr. Emma Borrego-Diaz.