

The Importance of Cell Motility: Eukaryotic life would be impossible without regulated motility. In fact, most of the things that we associate with life involve motility at some level such as reproduction, gross movement, taking in food, elimination of waste, etc. The actin-myosin system transduces chemical energy in the form of ATP into mechanical energy and is a major component of cardiac, smooth and skeletal muscle. The actin-myosin system is also involved in many motile processes of non-muscle cells. We are only beginning to appreciate the involvement of the actin-myosin system in human health.

The focus of our laboratory is to uncover mechanisms of regulation of actin-myosin mediated motility that involve actin binding proteins. Different proteins are involved in regulation of striated (cardiac & skeletal) and smooth muscles.

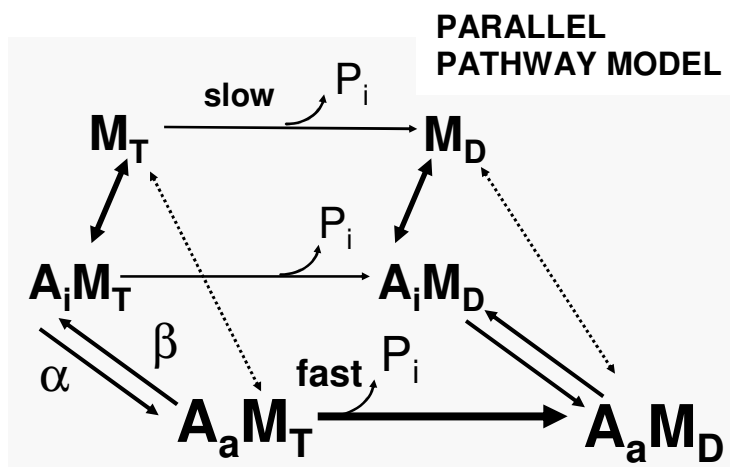
Striated Muscle Regulation by Troponin and Tropomyosin: Cardiac and skeletal muscles (STRIATED MUSCLES) are primarily under the control of the actin binding protein complex of TROPONIN and tropomyosin. Troponin consists of three subunits which bind to Ca^{++} , actin and tropomyosin. The troponin complex 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.

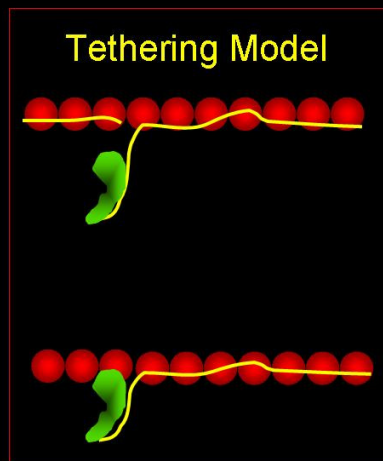
Smooth Muscle Regulation: Phosphorylation of myosin light chains is the key regulatory event in smooth muscle. However, actin binding proteins may modulate this actin directly or indirectly (by altering the organization of cellular actin). The actin-binding protein caldesmon appears to modulate smooth muscle contraction.

Caldesmon: Several mechanisms have been proposed for the function of caldesmon. We believe that the conflicting views are a result of the complex manner in which caldesmon binds to actin and the fact that caldesmon has several binding partners. Studies on the binding of caldesmon to myosin, actin, calmodulin and the competition patterns among these ligand proteins were conducted primarily by Drs. Mark Hemric, Laly Velaz, Frank Lu, Anidita Sen, and Hai Luo. Drs. Yi-Der Chen and Bo Yan wrote mathematical descriptions describing the complex equilibria. Dr. Mechthild Schroeter continues to work along with Dr. Gabriele Pfitzer's laboratory, on the caldesmon-myosin interaction in knock-out mice. Unless one is aware of these possibilities when designing experiments one may be misled.

Under some conditions caldesmon inhibits ATPase activity without displacing S1-ATP from actin. This behavior is related to several independent effects: (1) Myosin and S1 can bind to caldesmon that is attached to actin. The figure shows two possible complexes between S1, actin and caldesmon. As shown in the upper illustration, the single S1 is not necessarily bound directly to actin but may be attached to the NH₂-terminal region of caldesmon. The lower illustration is interesting in that the affinity of S1 to actin in this complex is enhanced by the

Some Modes of Caldesmon Binding

Caldesmon (yellow) binds to about 7 actin protomers (red). Although caldesmon and myosin S1 (green) compete for actin binding, caldesmon can tether myosin to the actin. A number of other possible arrangements can occur depending on the nucleotide bound to myosin.



positive effect of caldesmon. Such a complex is likely and may explain the activating effects of caldesmon at very low levels of saturation of actin with caldesmon. In studying caldesmon, it is necessary to monitor the amount of myosin and caldesmon bound to actin and the amount of caldesmon bound to myosin.

There is no guarantee that all of the proteins that participate in the regulation of smooth muscle contraction have been identified. It is therefore possible that investigations with caldesmon or other potential regulatory proteins represent

only parts of a regulatory system. Several interesting actin binding proteins have been identified. Fesselin is a novel actin binding protein that was discovered in our laboratory. While the function of fesselin has not been uncovered it is an interesting protein with interesting possibilities.

Fesselin: It is well known that actin-binding proteins are important in regulating the contractile machinery of smooth muscle cells. Actin binding proteins are also important in controlling the structural framework that surrounds and supports the contractile apparatus, the cytoskeleton. The actin-binding proteins that fall into this latter class contribute to the overall regulation of the dynamic equilibrium that exists between actin monomers (G-actin) and actin polymers or filaments (F-actin). Fesselin is thought to be a member of this class.

Fesselin was discovered in smooth muscle tissue by Dr. Barbara Leinweber in our laboratory. Fesselin has significant sequence homology to the kidney podocyte and post synaptic membrane protein synaptopodin (Mundel et al. J. Cell Biol. 139:193-204, 1997).

We have observed that fesselin binds to several contractile proteins including actin, alpha-actinin, calmodulin and myosin. Fesselin binds to F-actin with moderate affinity (2000000/M) and bundles actin filaments. Dr. Brent Beall later observed that fesselin also binds to G-actin and stimulates actin polymerization. Dr. Mechthild Schroeter discovered that Ca^{++} -calmodulin reverses the stimulatory effect of fesselin on polymerization. Ca^{++} -calmodulin affects the interaction of fesselin with G-actin but not with F-actin. Thus, while fesselin inhibits actin-activated ATPase of S1, this activity is not affected by Ca^{++} -calmodulin.

Together with Dr. Renegar's group in Anatomy and Cell Biology, we have found that fesselin is localized in dense bodies in smooth muscle cells. The location of fesselin at centers of actin organization is consistent with the observed abilities of fesselin to polymerize actin and organize actin into bundles.

Ms. Svetlana Khaymina recently obtained evidence that fesselin belongs to the family of proteins that are NATIVELY UNFOLDED. Natively unfolded proteins are less compact than typical proteins and often have multiple binding partners. Binding to a partner protein can induce folding. This observation suggests that the activity of fesselin may be regulated by the binding partner to which it is attached. We already have seen evidence of this behavior in the inhibition of actin polymerization activity of fesselin by Ca^{++} -calmodulin.

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