

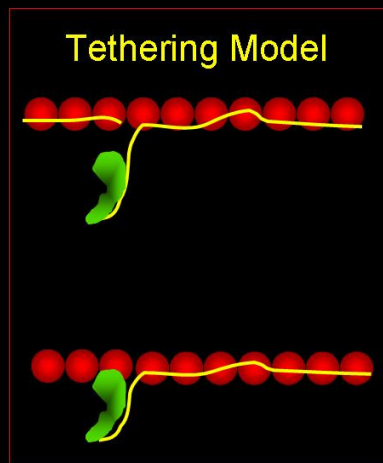
Smooth Muscle Regulation: Phosphorylation of myosin light chains is the key regulatory event in smooth muscle. However, actin binding proteins may modulate this actin directly or indirectly (by altering the organization of cellular actin). The actin-binding protein caldesmon appears to modulate smooth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