Structure and function of vascular smooth muscle

Prof. S Marston

The structure of smc and how they contract
smooth muscle contractile proteins
control of contraction by Ca^{2+} and phosphorylation

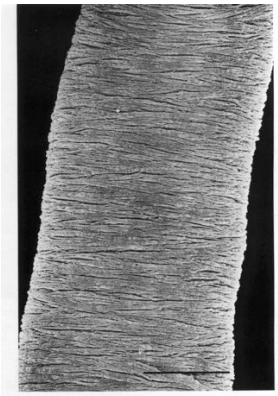
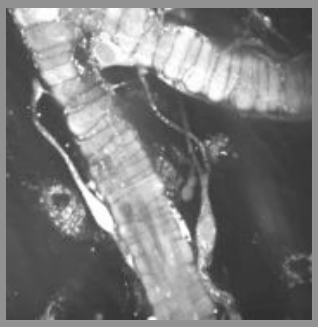


Figure 12-6. Guinea pig muscular artery about 100 μm in diameter. The media consists of a parallel array of circular muscle fibers. Bar: 50 μm.

An animation showing Z-stepping through a region of Fluo-4 loaded rat ureter, taken from top to bottom at 2 μm steps. The monolayer of circumferentially running smooth muscle cells, and then rows of endothelial cells running parallel to the blood vessel can be seen.

Burdyga T, Shmygol A, Eisner DA, Wray S. A new technique for simultaneous and in situ measurements of Ca2+ signals in arteriolar smooth muscle and endothelial cells. Cell Calcium. 2003Jul;34 (27-33)



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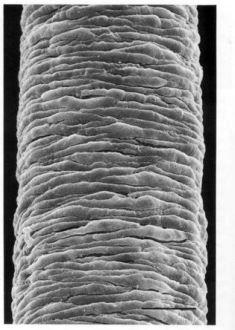

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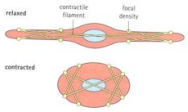

Figure 12-8. Subarachnoidal muscular arteriole of the rat. Muscle fibers are fusiform with tapered ends. They are tightly apposed to each other and are circularly oriented. Bar: 20 μm.

Smooth muscle cell contraction leads to vasoconstriction of an arteriole

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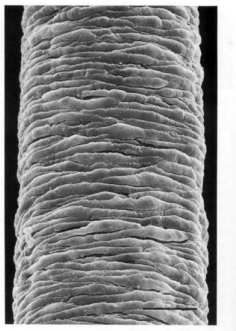

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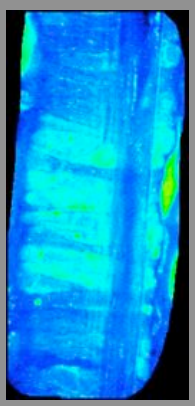
Figure 12-8. Subarachnoidal muscular arteriole of the rat. Muscle fibers are fusiform with tapered ends. They are tightly apposed to each other and are circularly oriented. Bar: 20 μm.

Smooth muscle cell contraction leads to vasoconstriction of an arteriole

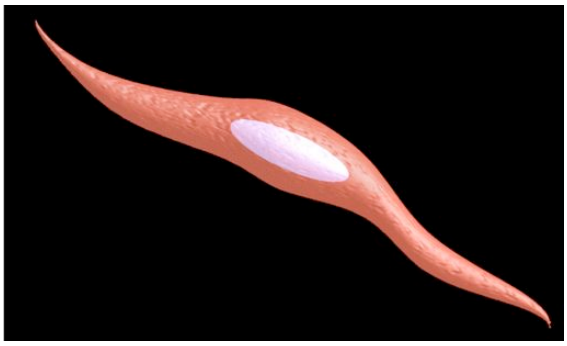
AVI movie 3. Ca^{2+} waves and contractions of individual vascular smooth muscle cells with Fluo-4 with the focal plane located in the middle of the blood vessel. It demonstrates the changes in diameter of the terminal arteriole in the area of a contracting cell.

Burdyga T, Shmygol A, Eisner DA, Wray S. A new technique for simultaneous and in situ measurements of Ca2+ signals in arteriolar smooth muscle and endothelial cells. Cell Calcium. 2003

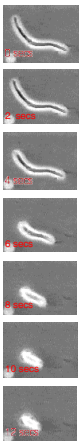
Jul;34(1):27-33.



STRUCTURE OF THE SMOOTH MUSCLE CELL

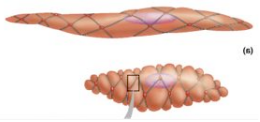


A typical smooth muscle cell



Contraction of a single smooth muscle cell at 25°C

Shortening speed is 0.2 muscle lengths per second, 10-50x slower than skeletal muscle



Idealised smooth muscle cell

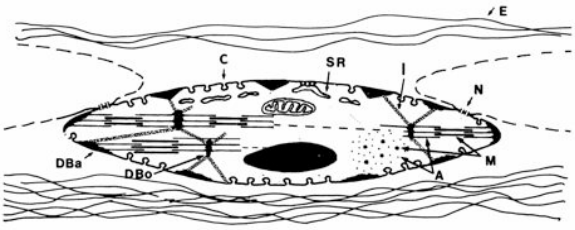
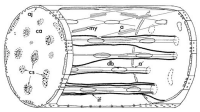


Figure 12.1 Sketch of main elements within a vascular smooth muscle cell. A, actin filaments in longitudinal and transverse view; C, caveola; DBa, DBo, dense band and dense body; E, elastic elements in parallel to cell (collagen and elastin); I, Intermediate filament; M, myosin filaments; N, nexus or gap junction; SR, sarcoplasmic reticulum. (After electron micrographs in Gabella, G. (1984) *Physiological Reviews*, **64**, 455–477)



Cytoskeletal elements of the smooth muscle cell

Intermediate filaments

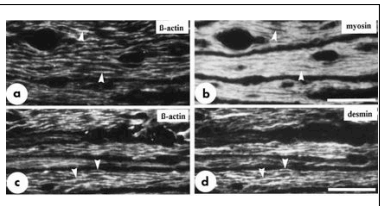
(desmin, filamin)

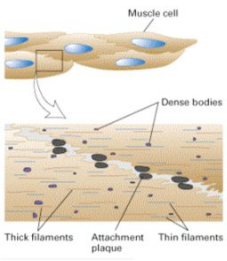
Membrane associated dense bodies

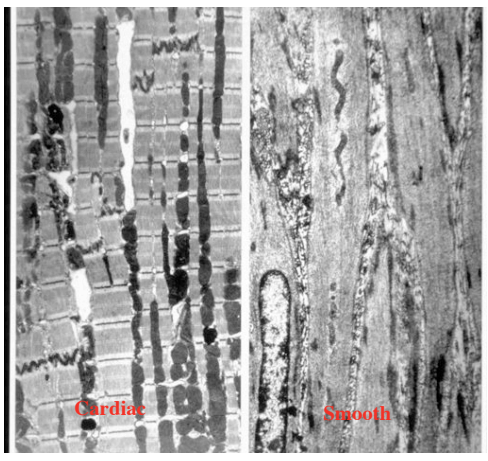
(desmin, talin, vinculin, α –actinin)

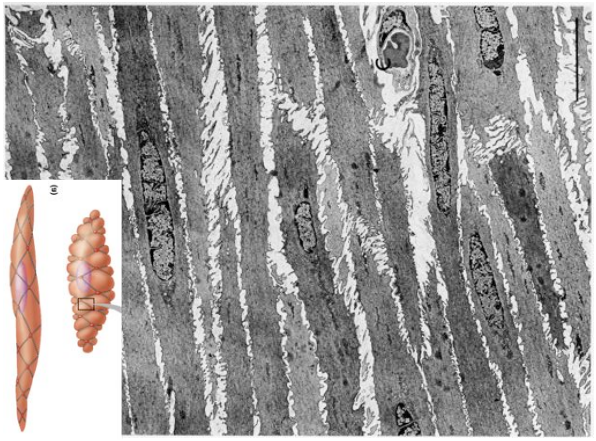
cytoplasmic dense bodies

(β–actin, α–actinin)





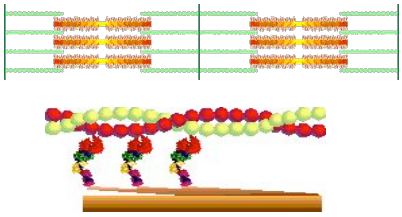




STRUCTURE OF THE SMOOTH MUSCLE CONTRACTILE APPARATUS

Basic mechanisms of muscle contraction is the same in striated and smooth muscles

Sliding filaments due to interaction of myosin motor with actin filaments



But the arrangement of filaments is very different in smooth muscle

Smooth muscle is characterised by

Slow contraction speed

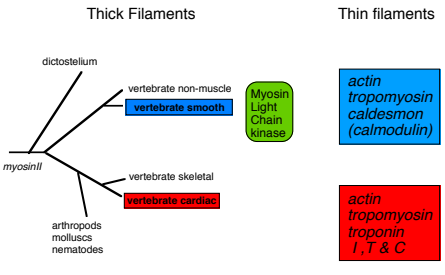
High economy of tension maintenance

Produces force over a wide range of lengths

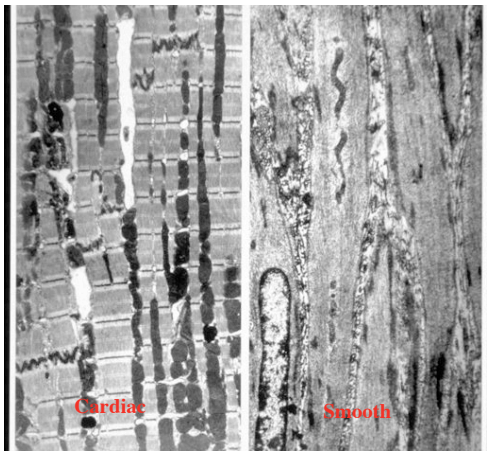
Side-polar thick filaments and thin filaments anchored in dense bodies

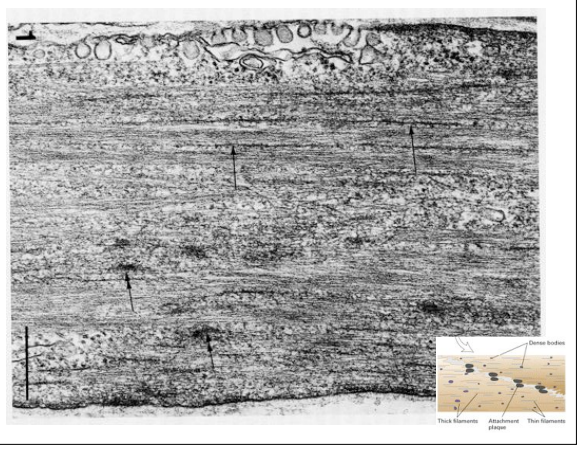
Ca^{2+}-regulation through myosin light chain phosphorylation

smooth muscle contractile proteins are closely related to non- muscle motile systems

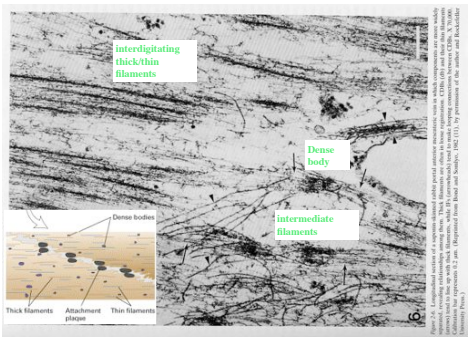


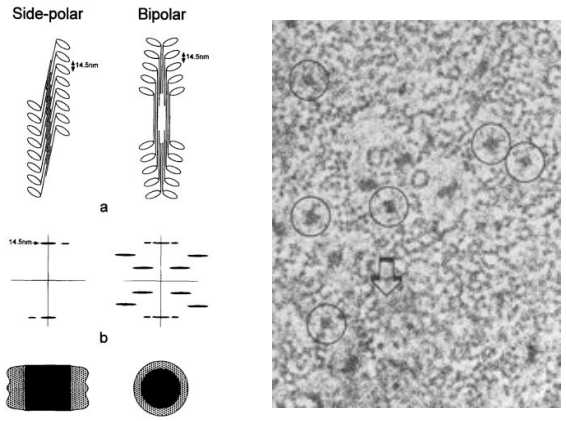
Phylogeneitic tree of sequences

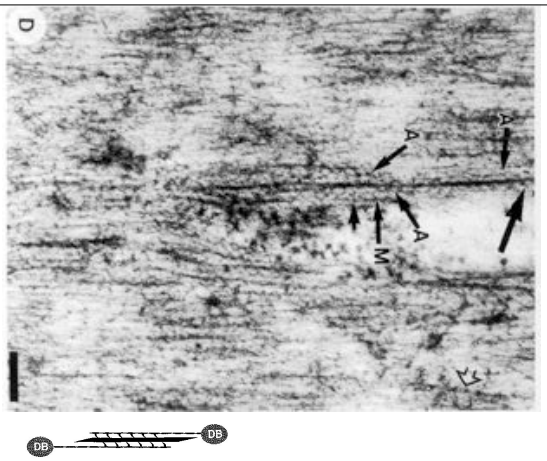


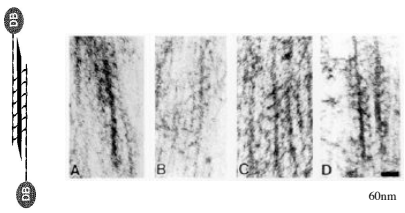


Cytoplasmic dense bodies contain α-actinin, connect to thin filaments of the contractile apparatus and intermediate filaments (desmin) of the cytoskeleton

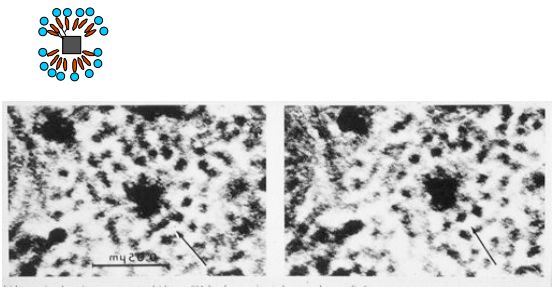






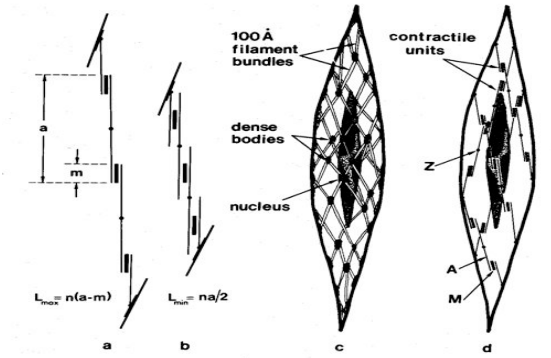


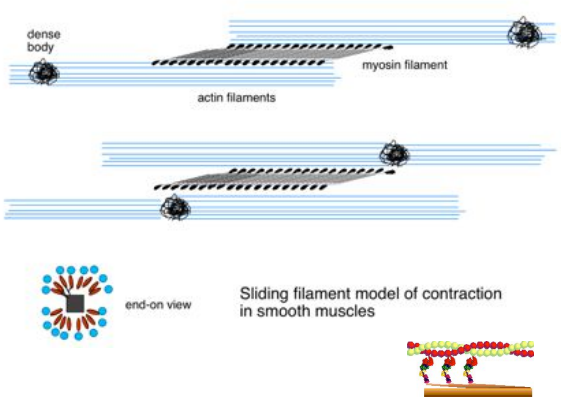
In longitudinal section the cross bridges arising from thick filaments attach to actin in rigor. Their orientation indicates side-polar arrangement



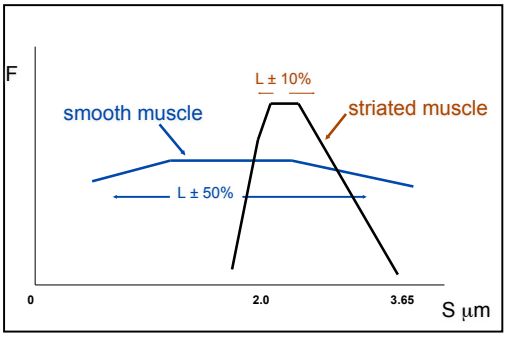
Cross section of vsm shows the contractile apparatus is made of thick myosin filaments surrounded by thin actin filaments

Smooth muscle cell structure (c,d) with contractile unit shown in (d) and (a,b) calculation of the maximum length (a) and mid-length (b) in terms of the actin (a) and myosin (m) filament lengths and the number 'n' of contractile units in series.

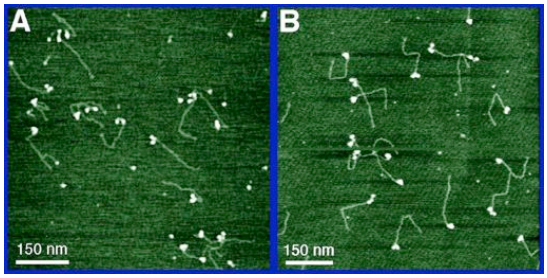


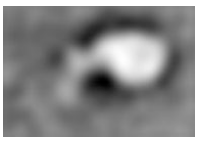


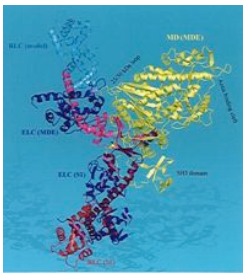
Comparative length-tension curves of smooth and striated muscles



Myosin is the motor protein





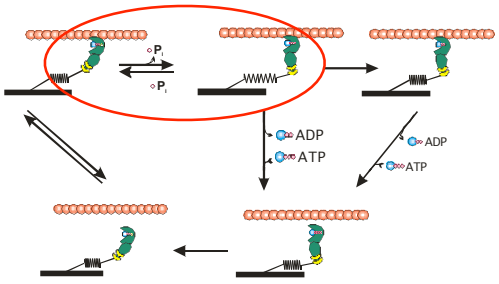


Myosin

The motor protein of muscle

ATP hydrolysis induces conformational change

The force generating step of the crossbridge cycle is Phosphate release. In smooth muscle this step is very slow

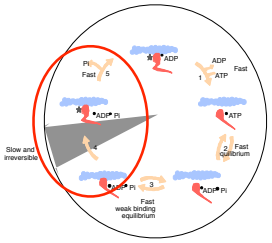


Duty Cycle= fraction of time per cycle occupied by the force generating state.

Smooth muscle is a high duty cycle motor: 30% compared with 5% for cardiac muscle

This means smooth muscle produces high force at low energy expenditure, at the expense of shortening speed

Cardiac muscle cycle



Duty Cycle= fraction of time per cycle occupied by the force generating state.

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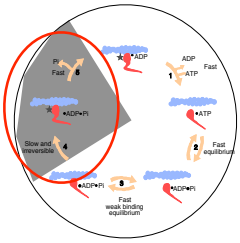
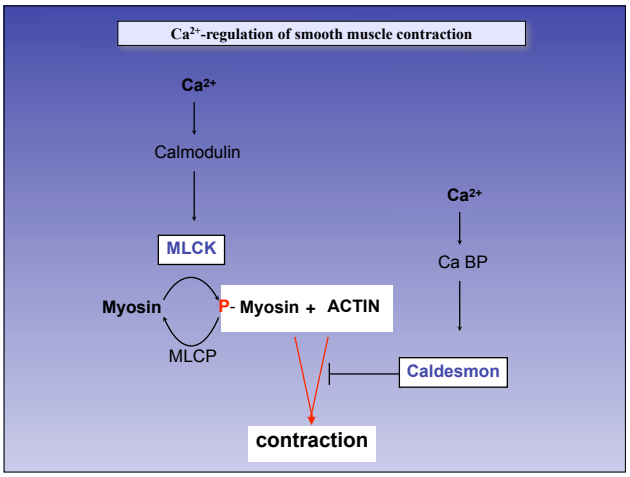
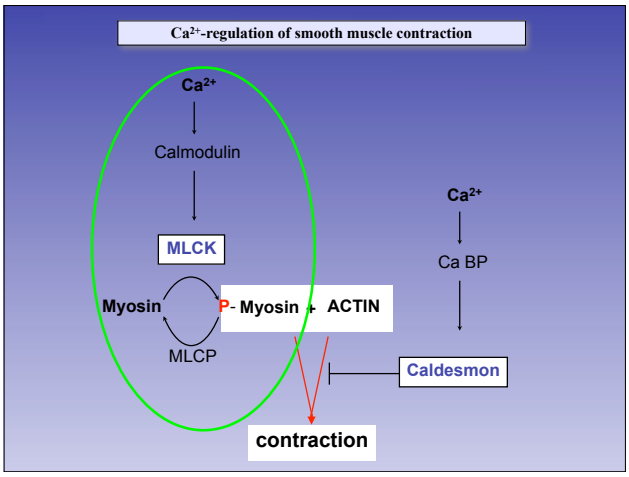
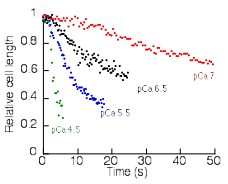
Smooth muscle cycle

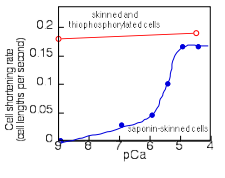


CA^{2+}-REGULATION OF THE SMOOTH MUSCLE CONTRACTILE APPARATUS







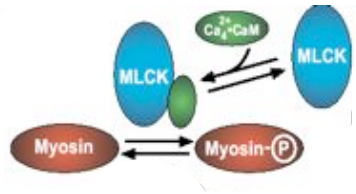


Ca^{2+} controls contraction via myosin phosphorylation

Contraction of a permeabilised smooth muscle cell requires only ATP as substrate and Ca^{2+} as activator.

If myosin is pre-phosphorylated contraction is independent of Ca^{2+}

Ca^{2+}-CALOMODULIN ACTIVATION OF MYOSIN LIGHT CHAIN KINASE



How a kinase/phosphatase pair regulate the level of myosin phosphorylation. In vascular smooth muscle the level of phosphorylation is about 60% when activated

i.e kinase is 160% of phosphatase activity.

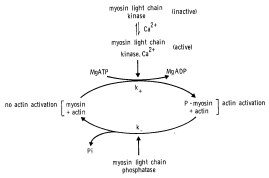


FIG. 6(a). Diagrammatic representation of the myosin phosphorylation hypothesis. k_+ is the rate constant of phosphorylation and k_- is the rate constant of dephosphorylation.

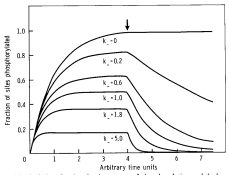
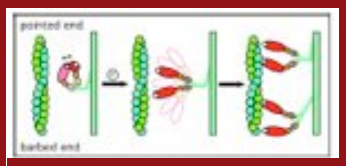
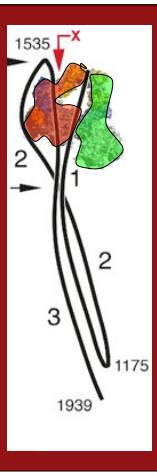


FIG. 6(b). Model calculation showing the time course of phosphorylation and dephosphorylation and the regulation of the steady state level of myosin phosphorylation by opposing phosphorylation and dephosphorylation reactions. k_- was set at 1(time unit)$^{-1}$ and k_- at 0·5(time unit)$^{-1}$. The time course of phosphorylation was worked out by numerical integration. At 4 time units k_+ was set to zero, the trace now gives the time course of dephosphorylation. The steady-state level of phosphorylation is given by $k_+/k_- + k_+$ and the observed rate constant of phosphorylation $k_+ + k_-$ (time units)$^{-1}$. Thus increase in k_+ gives a more rapid approach to the steady-state and a lower steady-state level of phosphorylation.

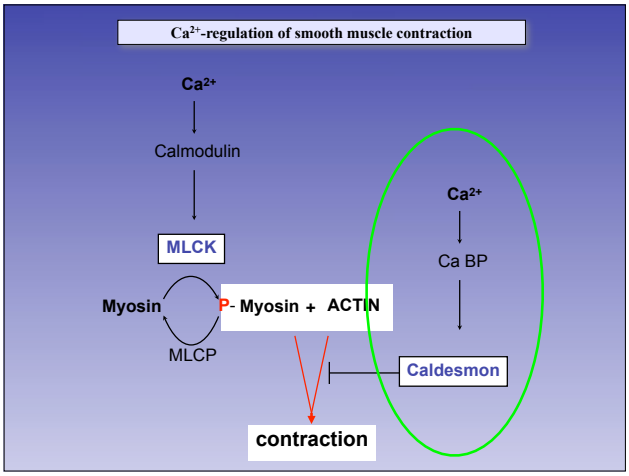


When myosin is unphosphorylated myosin is folded up with the tail looped round the head.

The two heads of myosin bind to each other and cannot contact actin. Phosphorylation frees the heads.

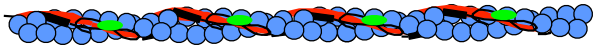


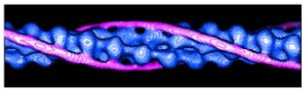
Structures of Smooth Muscle Myosin and Heavy Meromyosin in the Folded, Shutdown State
Stan A. Burgess1, 2, Shuzo Yu1, 2, 1, Matt L. Walker1, 2, 1, Rhoda J. Hawkins2, 3, 1, Joseph M. Chalovich4 and Peter J. Knight
doi:10.1016/j.jmb.2007.07.014

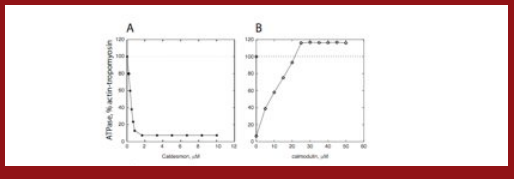


Striated muscle thin filament regulated by troponin and tropomyosin

Smooth muscle thin filaments are regulated by caldesmon, calmodulin and tropomyosin

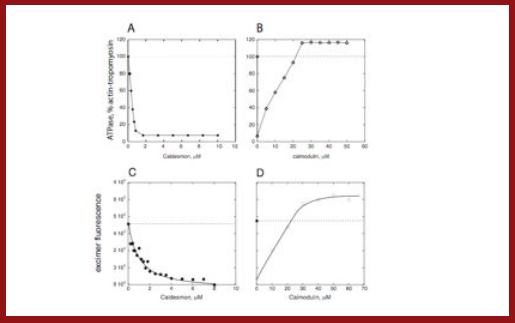






Caldesmon binds to actin-tropomyosin and inhibits crossbridge cycling.

Ca^{2+}-calmodulin binds to caldesmon and reverses the inhibition

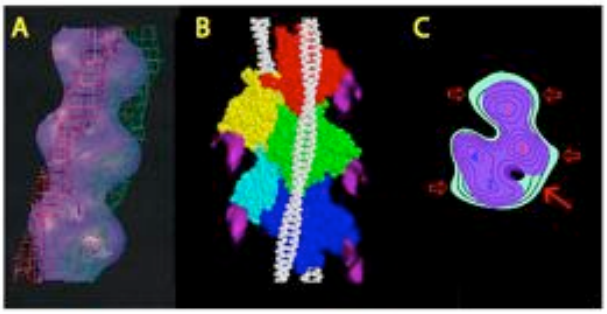


Caldesmon and Ca^{2+}-calmodulin switch thin filaments between 'on' and 'off' states, like troponin, as shown by a fluorescent probe attached to tropomyosin that is sensitive to this structural transition

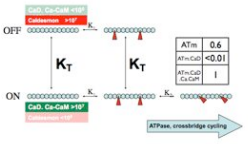
A- tropomyosin and caldesmon located on the actin filament

B- atomic model of actin-tropomyosin shows location of inhibitory domain of caldesmon

C- cross section shows where caldesmon inhibitory domain is located on actin



Smooth muscle thin filaments are an allosterically regulated cooperative sytem. Caldesmon is an allosteric ionhibitor and Ca^{2+}-CaM. Caldesmon is an allosteric activator



Activation

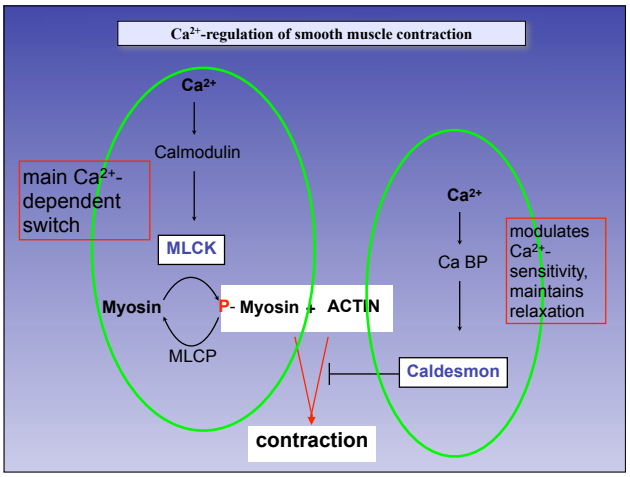
Ca^{2+}-CaM. Caldesmon binds only to ON state

Myosin binds only to ON state, therefore myosin crossbridge cycling is activated

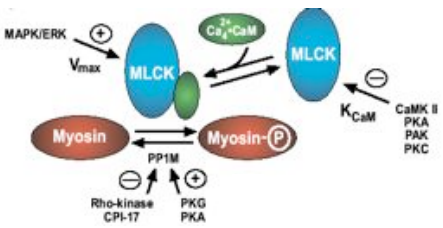
Inhibition

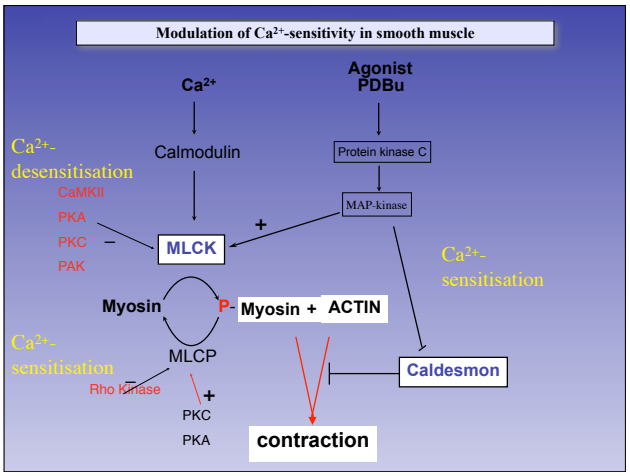
Caldesmon binds only to OFF state

Myosin binds only to ON state, therefore myosin crossbridge cycling is cooperatively inhibited

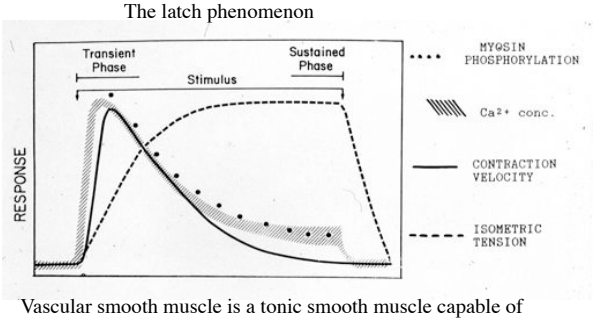


Modulation of MLCK and MLC-P by cell signalling kinases





The latch phenomenon



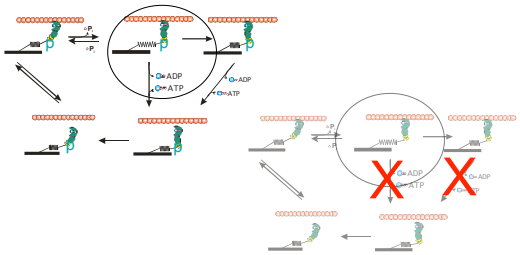
Vascular smooth muscle is a tonic smooth muscle capable of maintaining force with low energy expenditure.

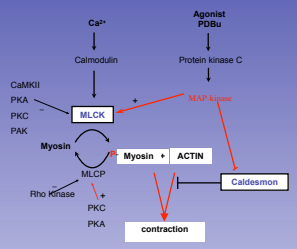
Phosphorylation of CaD and MLCP increases Ca^{2+}-sensitivity

Crossbridges cycle slowly but remain attached and bearing tension: myosin is dephosphorylated whilst attached?

Molecular basis of latch in smooth muscles

Dephosphorylation whilst attached locks crossbridge in long-lived tension generating state





Summary

The basic contractile mechanism of smooth and striated muscles is the same.

Smooth muscles contract slowly but maintain force with a low tension cost especially in latch.

Ca2+ controls the contractile apparatus through myosin phosphorylation and through caldesmon

Cell signalling kinases modulate this process

Reading:

Marston (1982) *The regulation of smooth muscle contractile proteins.* Prog Biophys Mol Biol 41 , 1-41

Kamm et al. (1985) *The function of myosin and myosin light chain kinase phosphorylation in smooth muscle.* Annu Rev Pharmacol Toxicol 25 , 593-620

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Kamm and Stull (2001) *Dedicated myosin light chain kinases with diverse cellular functions.* J Biol Chem vol. 276 (7) pp. 4527-30

Marston and EL-Mezgueldi (2008) *Role of tropomyosin in the regulation of contraction in smooth muscle.* Adv Exp Med Biol 644, 110-123