

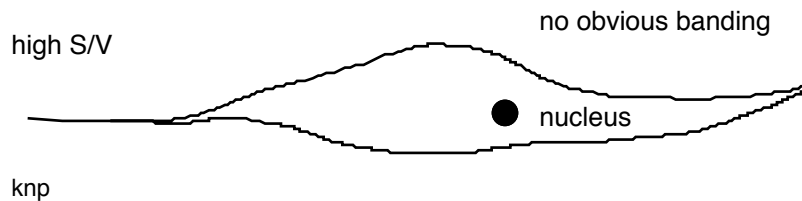
## AN INTRODUCTION TO INVOLUNTARY (ESPECIALLY SMOOTH) MUSCLES<sup>1</sup>

Summary: This section is an introduction to a fascinating and extremely important group of tissue, the smooth muscles. As you will see, their action is quite complex as is their regulation -- which while it bears many similarities to skeletal muscle, is also very different. For those interested in medicine smooth muscle is a far more rewarding tissue to master the function of than skeletal muscle -- smooth muscles are among the most important tissues involved in physiological controls of processes as diverse as airway resistance, blood pressure and flow direction, digestion, excretion, birth, and lactation!

### I. SMOOTH MUSCLE

**A. Introduction:** smooth muscles differ from skeletal muscles in terms of their morphology, biochemistry, and the ways by which their contractions are controlled.

**B. General Morphology:** Individual cells are small and spindle shaped. There is NO extensive SR; NO T-tubules; and no obvious striation or other banding. These cells have very high surface to volume ratios as a consequence of their small elongate spindle (instead of tubular) shape.



### C. Contraction Types and Kinetics:

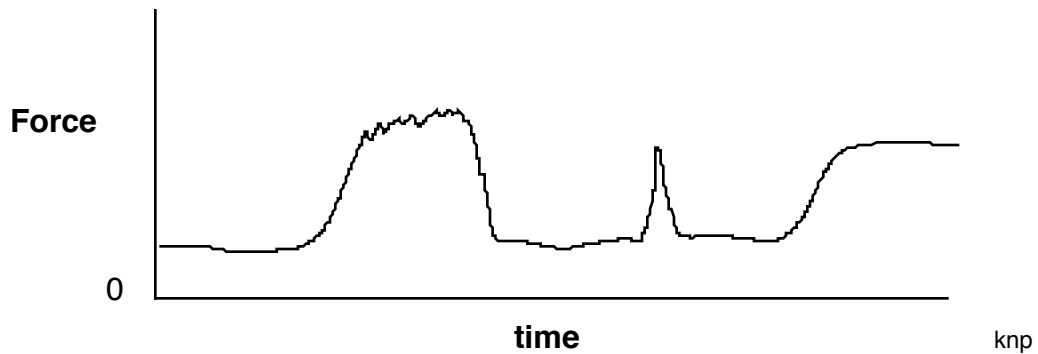
1. Smooth (and cardiac muscles) have some degree of autonomy from the nervous system (unlike skeletal muscles); that is, they have intrinsic activity, we must consider effects on both their force of contraction under a given set of circumstances (e.g., length, recent stretch). This is called **contractility**. Some smooth muscles are also members of functional groups that exhibit a property called **automaticity**. That is, their membrane potentials ( $E_M$ ) change spontaneously with resulting changes in degree of contraction. Thus, we must also consider the effects of extrinsic factors on automaticity. Notice that all of these factors are tied together. For instance, some chemical messenger that increases contractility by changing the internal concentrations of  $Ca^{++}$  or active PKC may in the first case also cause changes in the membrane potential. Likewise, any extrinsic factor that changes automaticity by changing the rates of transmembrane movements of certain ions may also cause changes in the availability of  $Ca^{++}$  (it is one of the ions involved in  $E_M$ ) and therefore change contractility.

2. Smooth muscles have two general modes of contraction: **TWITCH** and **TONUS**.

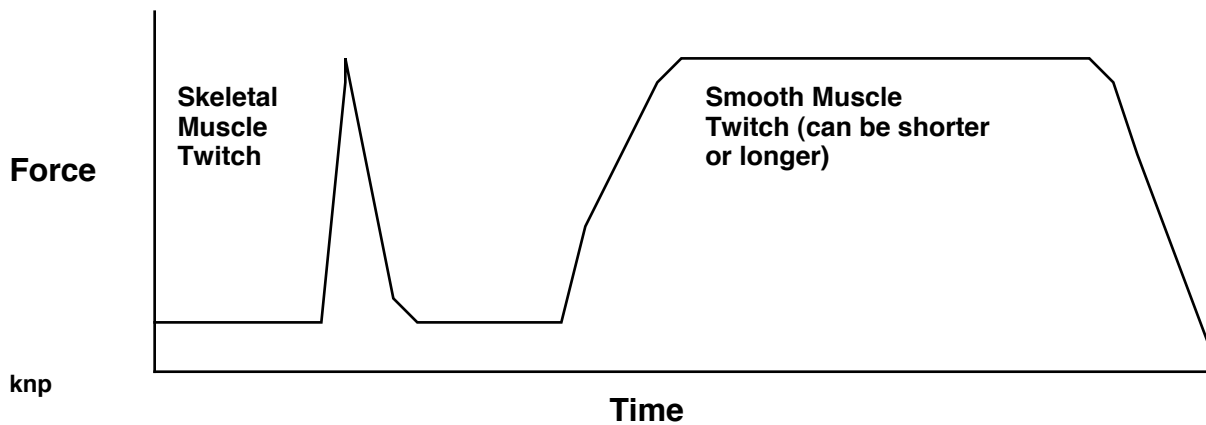
a. **Twitch** contractions in smooth muscles are the shorter duration of the two types, as in skeletal muscles. The figure below shows two tonic contractions with a single twitch in between:

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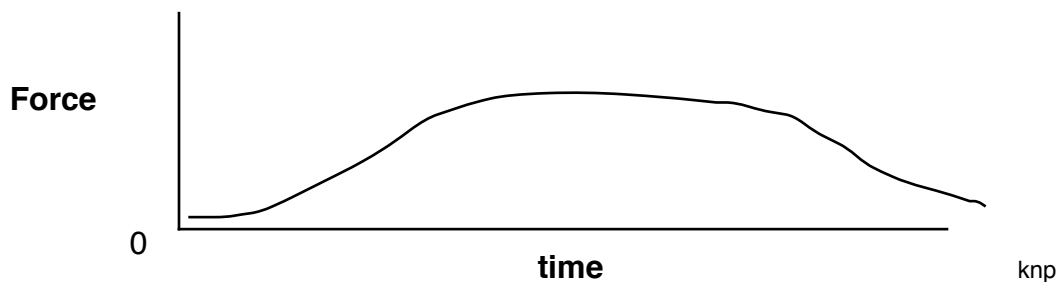
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However, unlike skeletal muscles, twitch contractions can be physiologically important in smooth muscle. And they tend to be of much, much longer duration than skeletal muscle twitches as the graph below shows. They also tend to have a slower onset and relaxation than skeletal muscle contractions:



b. TONUS is a long-term state of contraction that can be modified by various means.



(i) It is quite different from catch-type muscles in that although force remains high for a long period, there is constant cross-bridge cycling. However, the cycling is at a low rate (relative to the rate seen in skeletal muscle).

(ii) In tonus, the **force can either go up or down**.

(iii) Some tonic contractions are **produced by** a series of action potentials, much like tetany in skeletal muscles whereas others are related to long-term changes in membrane potential or to actions by specific hormones or other substances that may or may not alter the membrane

potential (pharmaco-mechanical coupling). These will be discussed in the next two sections of these notes.

### 3. Kinetics:

(a) smooth muscle contraction does not need to be as fast as skeletal muscle contraction; this is another reason why extensive internal membranes are not required. On the other hand,

(b) smooth muscles often need to remain in a contracted state for a long period of time without fatigue or excessively high-energy consumption.

! Both (a) and (b) relate largely to differences in the types of myosin found in smooth muscles -- cross-bridge cycling rates are very different. In addition, smooth muscle myosins are regulated in ways not normally seen in skeletal muscles (see below).

(c) And they may **need to work over a very wide range of operational lengths and these may change over time** (for example, think of uterine muscles during pregnancy). Unlike skeletal muscle, smooth muscles are capable of undergoing considerable reorganization while they contract or in response to long-term loads and stretch. What was once a non-optimal length to produce a certain amount of force can, as a result of re-organization, yield larger forces. Thus, **visco-elastic flows** in response to long-term mechanical stresses can be quite important in smooth muscles.

? Explain why visco-elastic flow should be important in smooth muscle tissues such as the uterus or gut. Why for instance, should the uterus need to be capable of delivery of considerable force over a wide range of lengths? How is this different from skeletal muscle?  
? Why do contractions of smooth muscles not need to be as fast as skeletal muscles?

### D. Excitation and Electrophysiology:

1. General Sources of Control: because of their roles as physiological, not behavioral regulators, smooth muscle needs to be sensitive to more than just neural inputs -- cells often communicate with each other via a range of different chemical signals and smooth muscles are hooked into this physiological network. Thus, they are operated **extrinsically** via both **neural and humoral** inputs. However, smooth muscles also have **intrinsic activity** -- with no external input they can still undergo changes in contractile state.

2. **Contractile Units**: smooth muscle cells are usually linked:

(a) to each other by **electrical synapses**. And they are

(b) linked to neurons by chemical synapses but they use different receptors and transmitters in addition to ACH. Review the notes on the autonomic nervous system for some depth in this matter, but for the moment simply recall that:

(i) The controlling nervous system for most smooth muscle is the **AUTONOMIC NERVOUS SYSTEM** (as vs. the Somatic Nervous System for skeletal muscles).

(ii) The role of the autonomic system is to **MODULATE** the activity of the smooth muscles, as stated before, most of them have some degree of **endogenous activity**

(c) When referring to a smooth muscle tissue, the pattern of electrical linkage between cells and also between exogenous neural controls is important because it determines certain differences in function and control. The two general patterns are referred to as **motor unit types**:

(i) **Single Unit Smooth Muscles:** where **all cells are electrically coupled to each other**, as a result they form a **functional syncytium** and **respond as one unit**. Ex: much of the digestive system.

(ii) **Multi-Unit Smooth Muscles:** where **individual cells are small groups of cells are isolated from other such groups**; each group (unit) is controlled endogenously and via external nervous and endocrine inputs. Ex.: vascular smooth muscle, especially in the arterioles.

### 3. Electrophysiology -- Smooth Muscle Membrane potentials ( $E_m$ )

a. **Smooth muscles can conduct APs**, but they are of lower amplitude than in nerves and skeletal muscle.

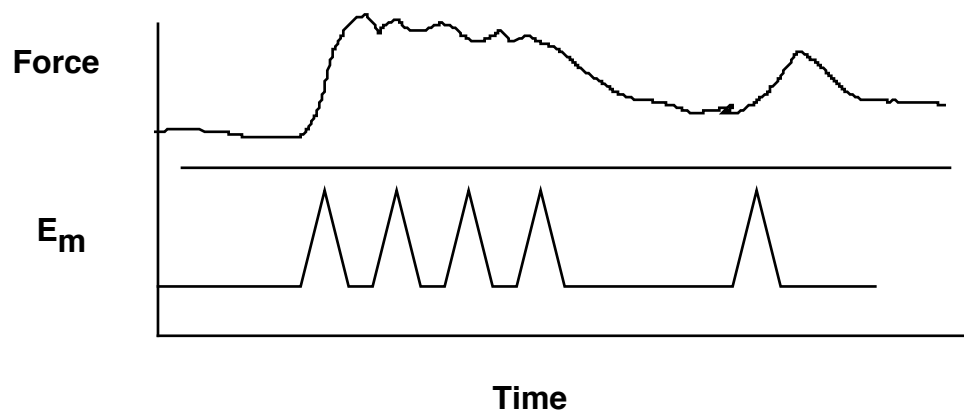
b. **The upswing of these APs is due primarily to  $Ca^{++}$  entry** (and some  $Na^+$ ) through what is termed the  **$Ca^{++}/Na^+$  slow channel**. These channels can be blocked by several different **PHARMACOLOGICAL ANTAGONISTS**, in particular:

- i. **high concentration of either  $Mg^{++}$  or  $Mn^{++}$** ; or
- ii. **verapamil**.

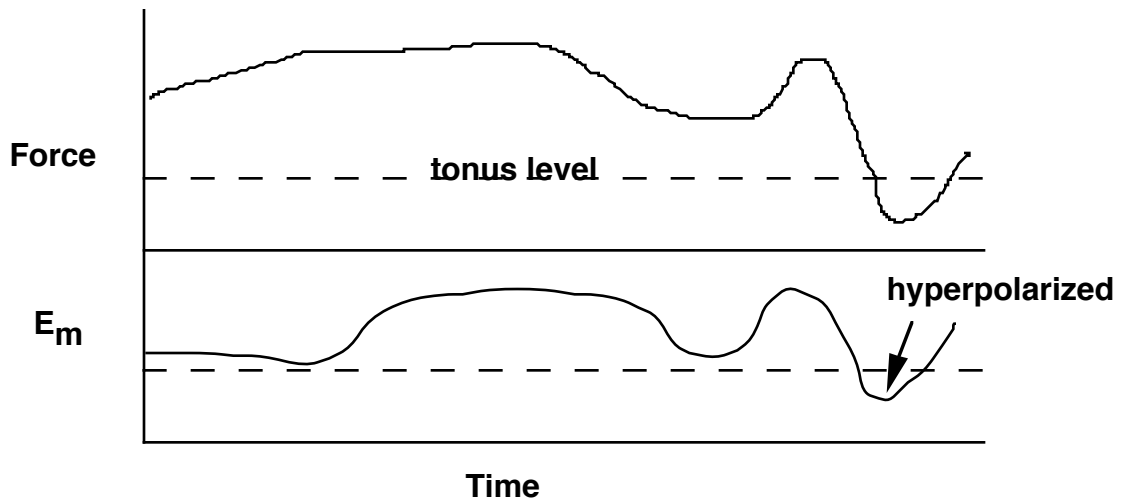
c. The  **$Ca^{++}$  that enters during these spikes (also called  $Ca^{++}$  spikes) is used to control the events of EC-coupling**.

d. The  **$Ca^{++}$  is removed by membrane-bound  $Ca^{++}/ATPase$**  that pumps the  $Ca^{++}$  back out of the cell. Several other mechanisms also exist to control the  $[Ca^{++}]$  in smooth muscles, we will not learn these. When  $Ca^{++}$  enters, it will serve as a trigger for contraction as in other muscle types. However, as you will see below, the exact mechanism is different.

The diagram below shows several schematic spike potentials and the resulting contraction. Note that the first four produce a tetanus-like contraction (tonus) and the last a twitch. Although it is not obvious from this graph, the time course of all of these events is much greater than in skeletal muscle, particularly the duration of the contraction. Further, the onset and offset of the contraction is much slower than in skeletal muscle:

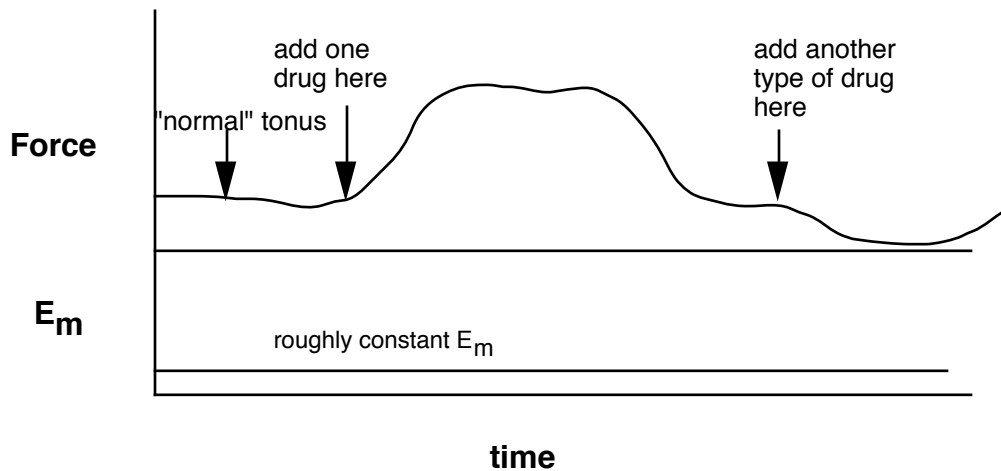


e. The  **$E_m$  may slowly change as a result of changes in the electrogenic pump's rate of discharge of ions**. This may or may not result in changes in tension of the muscle:



knp

f. **PHARMACO-MECHANICAL COUPLING:** certain hormones and drugs will produce a **contraction in the absence of any change in  $E_m$** . To a certain degree this is true of xanthines (such as caffeine and theobromine), which increase the levels of cyclic AMP, often without markedly affecting the  $E_m$ . It is also true of a number of other substances. **These substances all may cause changes in internal  $[Ca^{++}]$  and/or changes in the activity of protein kinase C (PKC), a major controller of contraction in smooth muscles (see later sections):**



knp

**Summary:** Thus, the force of contraction in smooth muscles is regulated by:

1.  $Ca^{++}$  entry during changes in  $E_m$ .
2. Endocrine effects
3. Myosin type
4. Stretch on the muscle
5. Various drugs

? How does the list above differ from what you would expect for skeletal muscles?

Do you think the system that smooth muscles use is less "advanced" and more "primitive" than that used in skeletal muscle? Why?

### E. Biochemistry and EC Coupling in Smooth Muscle

**Note:** The last word is most likely not in on this complicated topic – this is the fourth time I have re-written this section since about 1990. I will try to label the parts that seem to be best understood and also let you know which ideas are more tentatively accepted or unclearly demonstrated.

1. General: Regulation of contraction in smooth muscle is quite different and much more complicated than what occurs in skeletal muscle. This is because regulation is achieved via a number of different pathways

a. **membrane potential -  $Ca^{++}$  pathway** as in skeletal muscle this is the main control on contraction. But the details are totally (or at least mostly) different. We will concentrate on how  $Ca^{++}$  regulates myosin. There is no troponin. Moreover,  $Ca^{++}$  regulation is not the only pathway. There are a number of others, perhaps most notably the:

b. **protein kinase pathway** whose operation does not necessarily involve changes in membrane potential.

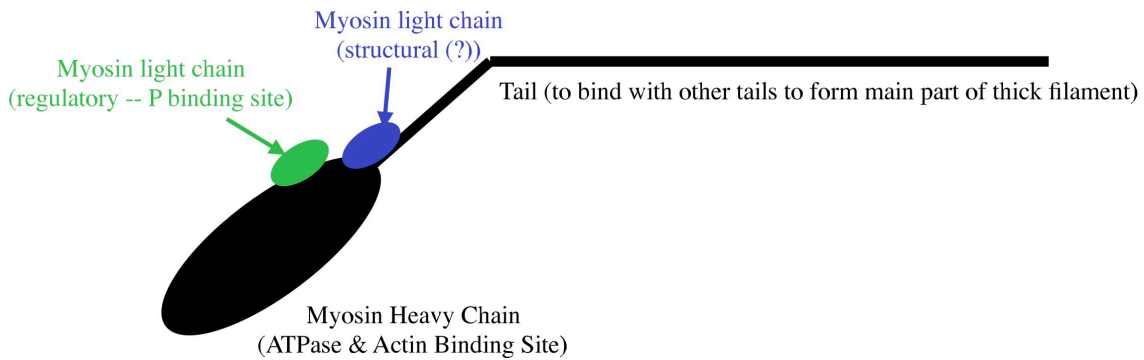
These notes will serve as an introduction to the regulation of smooth muscle contraction. Since the  $Ca^{++}$  regulation is most important, we will focus on it and I will hope you learn about the other pathways in some other course.

#### 2. $Ca^{++}$ Control of Contraction:

a. As in skeletal muscle,  $Ca^{++}$  enters the cell via  $Ca^{++}$  gates in the plasma membrane. The opening of the gates is voltage dependent (thus, these are termed voltage-dependent gates). As with skeletal muscles, the exit of  $Ca^{++}$  from the cytosol is via a  $Ca^{++}$  ATPase whose maximum rate is not as great as the rate for entry via the gates. For our purposes, the main difference between smooth and skeletal muscles is that these gates and pumps are in the plasma membrane, not the cisternae of the SR.

b. Once the  $Ca^{++}$  is inside the cell, it can promote cross-bridging. The best documented mechanism and probably the most important is through the regulation of the ability of myosin to form crossbridges. We will concentrate on this and we will term it **thick filament or myosin light chain regulation of contraction**. It also appears likely (but there is some dispute) that  $Ca^{++}$  may also have an effect on thin filaments that helps regulate contraction – we will briefly discuss thin filament regulation at the end of these notes.

c. **Calcium-controlled regulation of myosin**: When we considered skeletal muscles earlier we saw that myosin molecules have a regulatory light chain attached to their heads. We saw that in skeletal muscles, these chains are not an important regulatory site. However, they are extremely important in smooth muscle and somewhat important in cardiac muscle:

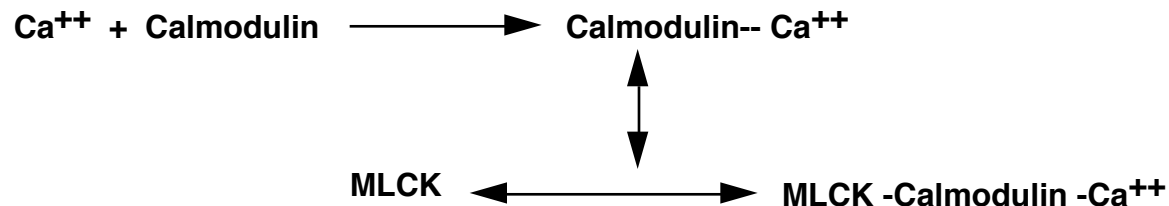


1.  $\text{Ca}^{++}$ -dependent MLCK regulation of the regulatory myosin light

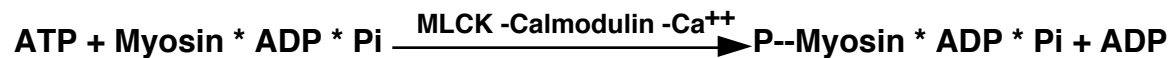
chain:

(a)  $\text{Ca}^{++}$  regulation of the myosin light chain is indirect  $\text{Ca}^{++}$  must first bind to **calmodulin**. This binding is reversible and therefore concentration and affinity-dependent.

(b)  $\text{Ca}^{++}$  - calmodulin can interact with a type of protein kinase called **myosin light chain kinase (MLCK)**.



(c) **Activated MLCK can phosphorylate a site on the regulatory light chain**; the phosphate is transferred from ATP. Note that this site is quite separate from the myosin ATPase. The reaction is:



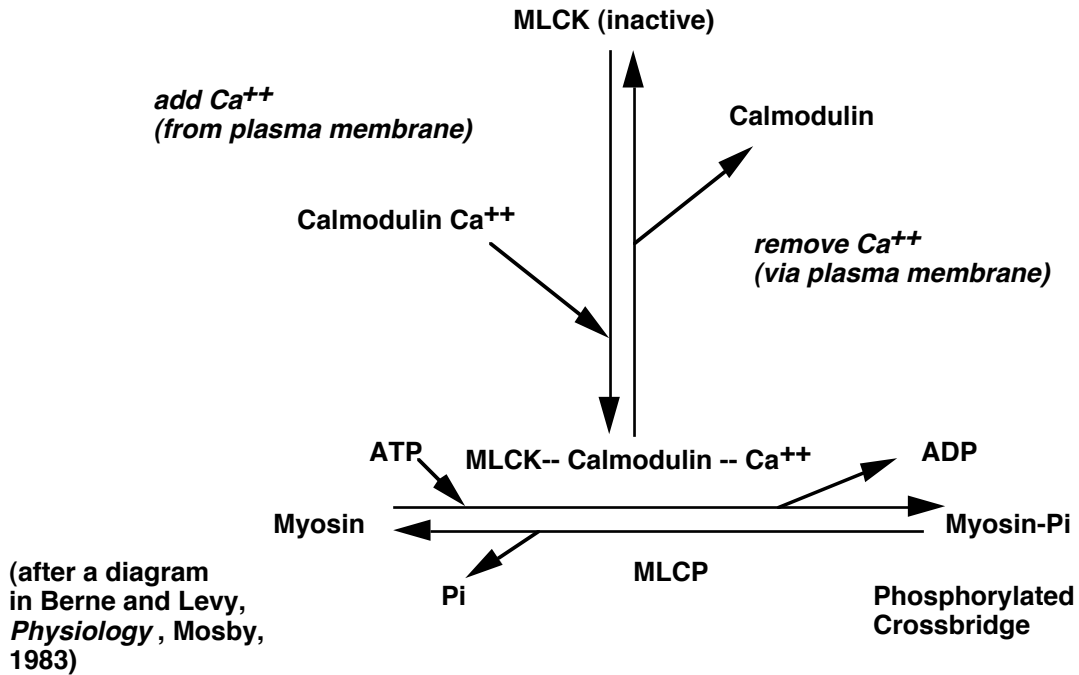
(d) When the regulatory light chain is phosphorylated, its affinity for actin is greatly increased and crossbridging can begin. Crossbridging can continue as long as the binding site for myosin on actin is available and the light chain is phosphorylated.

(e) Thus, when  $[\text{Ca}^{++}]$  is low, the activity of MLCK is low -- it is unable to phosphorylate the myosin light chain. However, when  $[\text{Ca}^{++}]$  increases sufficiently, the  $\text{Ca}^{++}$  binds reversibly to calmodulin (as with the caldesmon process outlined below):

(f) Light chain phosphorylation is reversed by **MYOSIN LIGHT CHAIN PHOSPHATASE (MLCP)** which is also known as **PHOSPHATASE**. This enzyme removes  $\text{P}_i$  from the myosin light chain.



This will be the predominant reaction when  $[Ca^{++}]$  decreases and calmodulin dissociates from MLCK -- as a result there is no additional phosphorylation of myosin and as the MLCP dephosphorylates myosin, relaxation occurs. The overall myosin light chain MLCP-site process:

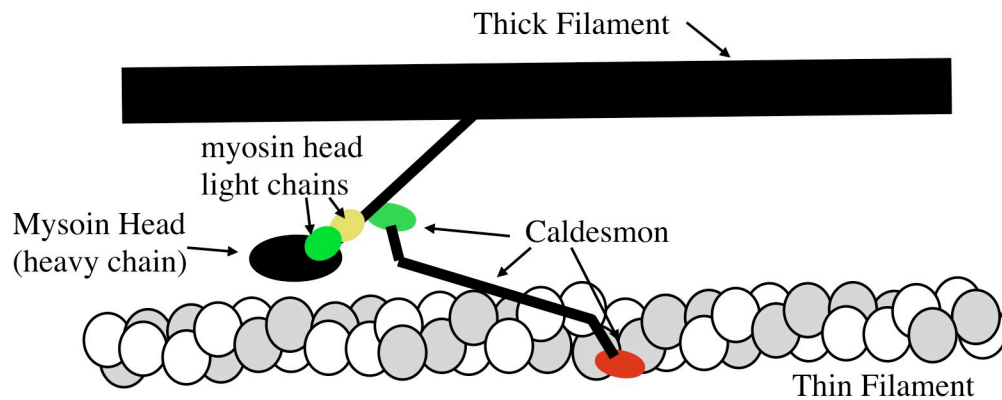


(g) Just to make things more complicated, MLCP is also regulated – one type of regulation is inhibition by a  $Ca^{++}$  activated pathway (why does this make sense) but there are apparently other independent regulatory paths!

## 2. Thin filaments.

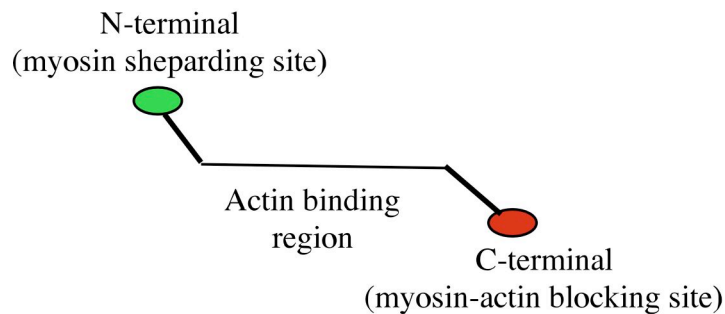
a. As mentioned previously, there is no troponin on thin filaments. The overall structure of thin filaments is as diagramed below:





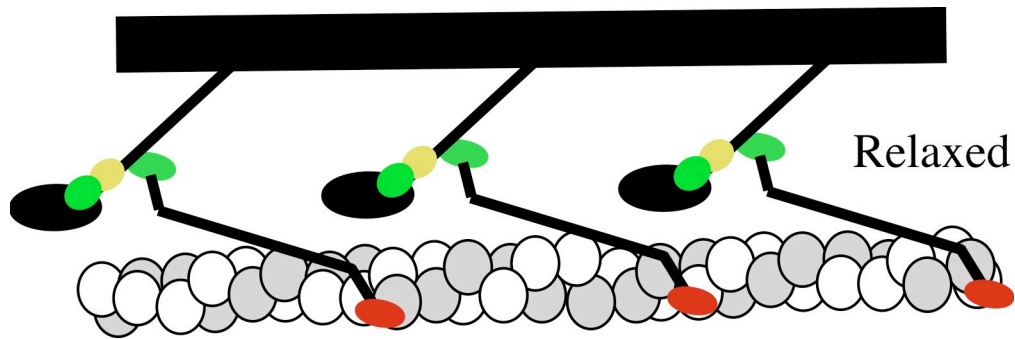
A Model of the role of caldesmon in smooth muscle contraction.  
 From Wang (2001) *Cell Biochem & Biophys.* 35:275-288

Note the presence of a protein that we did not consider in skeletal muscles (and is not found in them) – **caldesmon**. Caldesmon is a curved chain with two binding sites. The N-terminal head has a binding site for myosin and the C-terminal binds with actin in such a way that sometimes it may act to block binding of myosin to actin (but this is apparently not well established yet):



A Model of the role of caldesmon in smooth muscle contraction. From Wang (2001)  
*Cell Biochem & Biophys.* 35:275-288

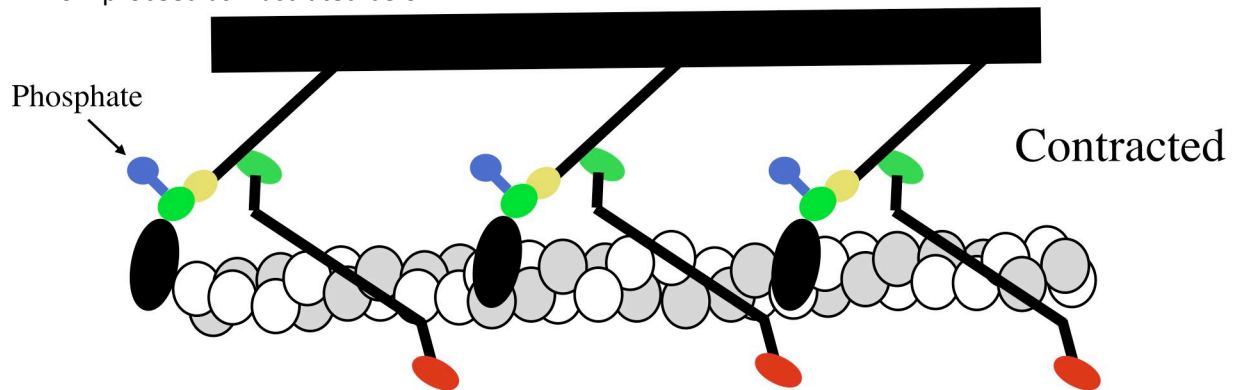
Here is how the whole complex will look in a relaxed muscle. Notice that the caldesmon holds the myosin near the thin filament and also apparently blocks the binding site for myosin on actin. In the situation diagramed here, the myosin light chain is not phosphorylated and the muscle is relaxed.



A Model of the role of caldesmon in smooth muscle contraction.  
From Wang (2001) *Cell Biochem & Biophys.* 35:275-288

Not shown in the diagram above is **tropomyosin**. Its function in smooth muscle is not well understood – but it is believed to have a role in strengthening the thin filament (and not in regulation as a blocking agent as in skeletal muscles).

When  $\text{Ca}^{++}$  (or a number of other signals) results in the activation of myosin, there is apparently some other pathway that also involves slight movements of caldesmon that exposes binding sites. Contraction will now proceed as illustrated below.

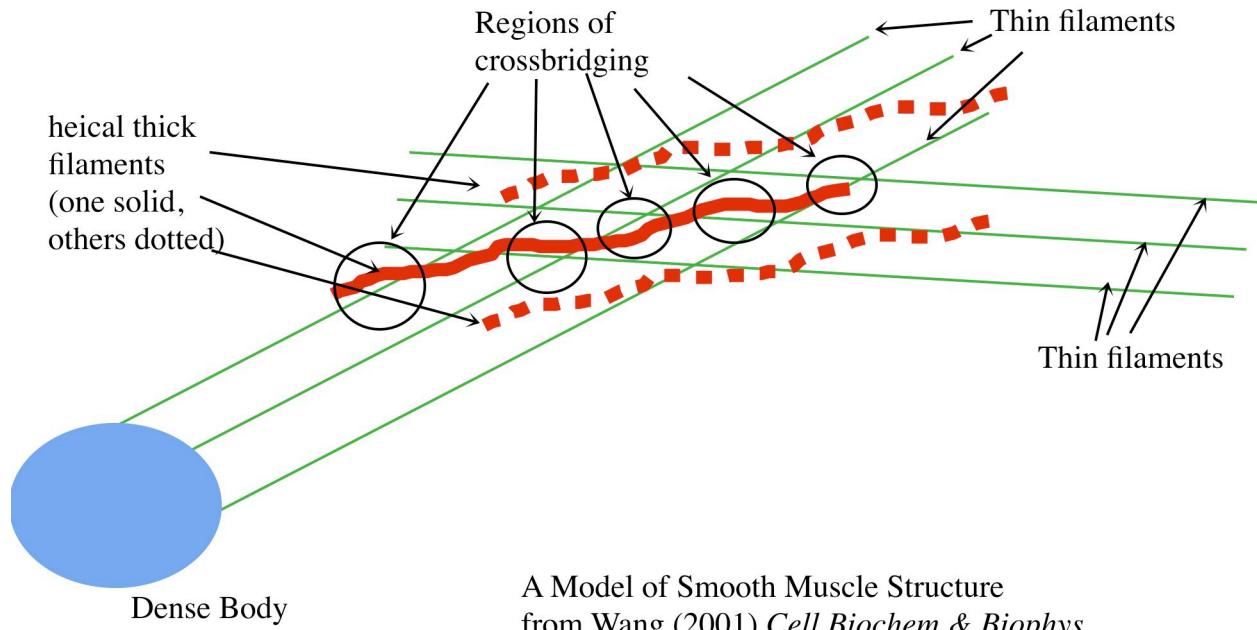


A Model of the role of caldesmon in smooth muscle contraction.  
From Wang (2001) *Cell Biochem & Biophys.* 35:275-288

Contraction in smooth muscle can be further complicated by additional pathways and by what are called latching when a muscle continues to show tension in the absence of high  $\text{Ca}^{++}$  or other signals. A number of ideas have been invoked to account for this property – very slow kinetics of some myosin heads and the crossbridge like effects of caldesmon in relaxed muscles (see above).

Finally, it is of interest to see the geometry of pulling. It is well known that smooth muscles often pull at a number of angles at once. It appears that thick filaments have a somewhat helical structure that

allows crossbridges to occur between the thick filament and thin filaments that are at oblique angles. Note this in the cartoon below – also note the dense body – the anchor points for thin filaments in smooth muscle.



A Model of Smooth Muscle Structure  
from Wang (2001) *Cell Biochem & Biophys.*  
35:275-288

! One final note: Hopefully you have seen that different rates of contraction in different types of smooth muscle can be achieved by the modification of the various protein players and/or membrane potential. The actual picture is far more complex than what has been presented here, but hopefully this will give you a good overview.