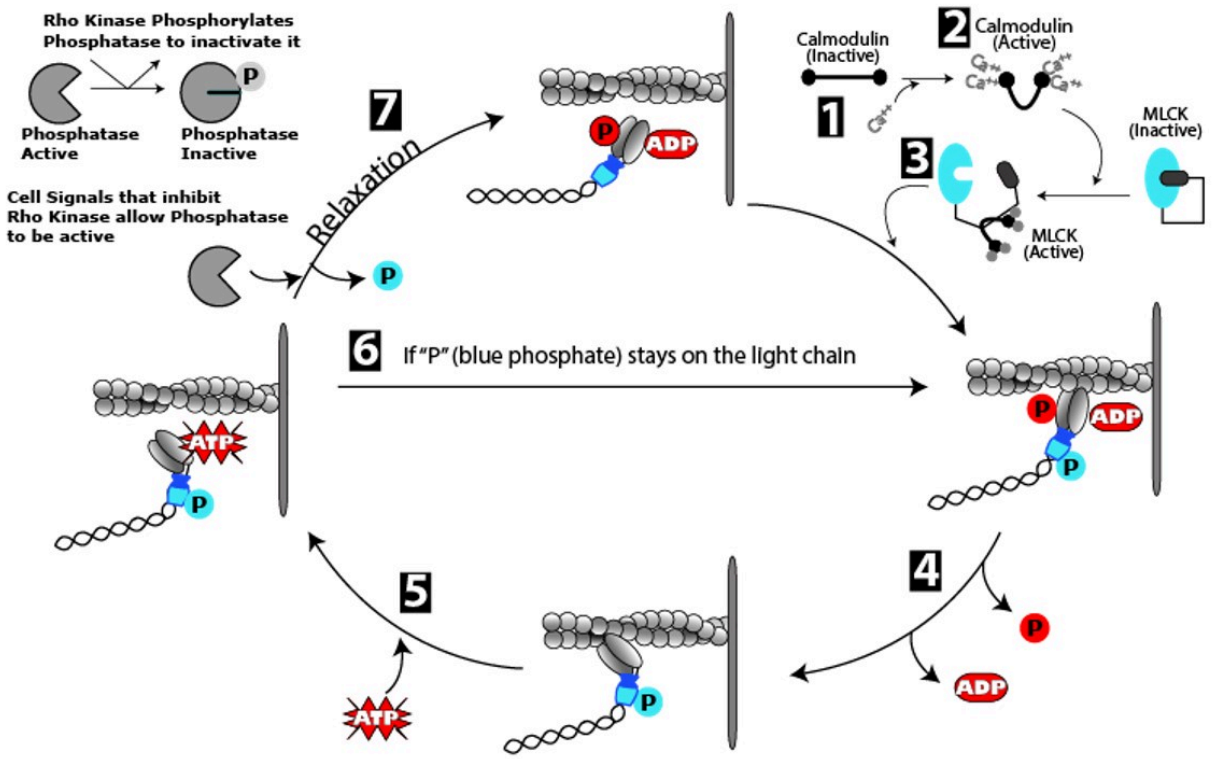
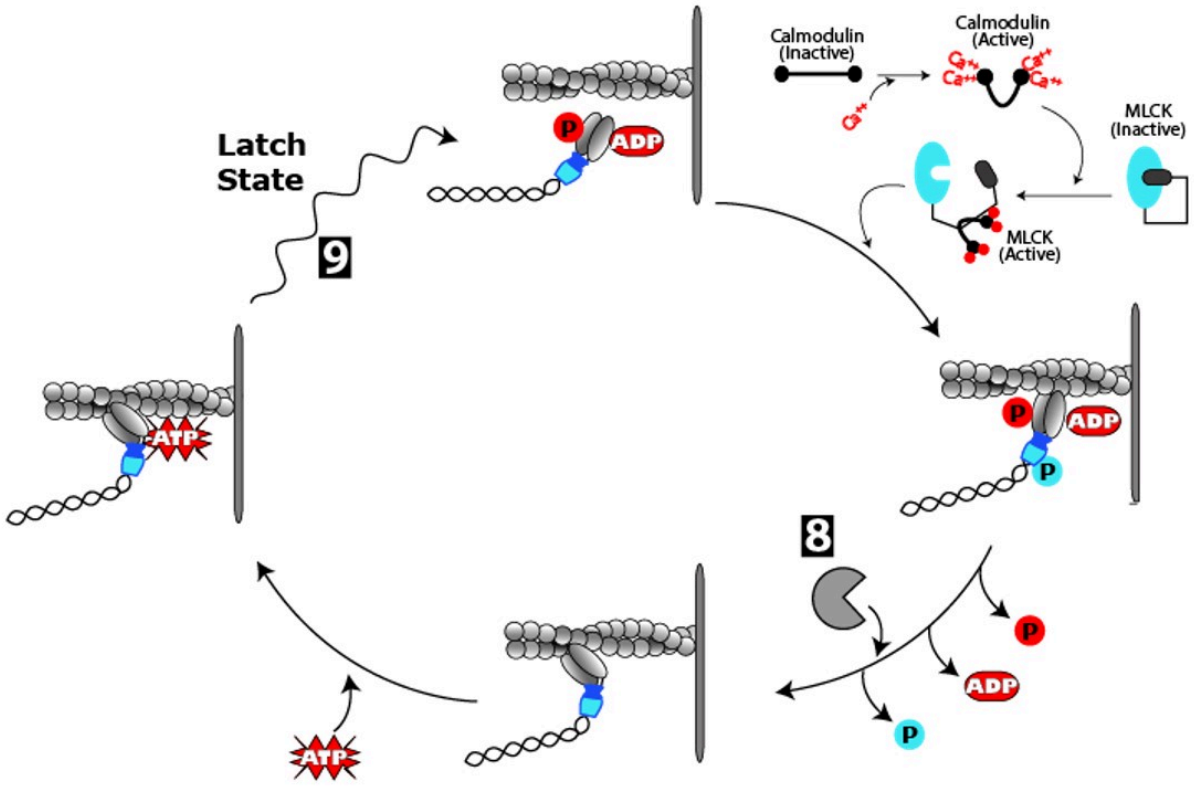
2.4.1

Smooth Muscle Contraction

Perhaps what makes smooth muscle so unique (both multiunit and single unit) is its activity patterns during contraction. Smooth muscle cells can exhibit rhythmic activity similar to skeletal muscle where separate contractions are easily delineated or they can exhibit a single long duration contraction (ie. Sphincters). Smooth muscle can remain in the tetanic state indefinitely without fatiguing. Rhythmic contractions are termed **phasic** while sustained contractions are termed **tonic**. Whether phasic or tonic, smooth muscle contraction begins with phosphorylation of the myosin light chain. For this reason, smooth muscle is sometimes called "heavy chain regulated". In skeletal muscle, it is troponin and tropomyosin that regulates when the myosin head can bind to actin. Therefore, in skeletal muscle we call it "thin chain regulated". Smooth muscle lacks troponin, so calcium activation for contraction is not a matter of moving tropomyosin. However, smooth muscle still requires a rise in intracellular Ca^{2+} to initiate contraction. Smooth muscle also lacks T-tubules which makes the rise in intracellular Ca^{2+} also unique.

We will outline the common steps of smooth muscle contraction. Please use the full page sized picture below while reading the following mechanisms. The numbers in the text match numbers on the picture as an attempt to help you visualize these steps.





Images by BYU-I JS F17

1 The smooth muscle cell experiences an increase in intracellular Ca^{2+} . This can come from the sarcoplasmic reticulum or from extracellular stores in response to an action potential, or a chemical signal (like a hormone) that does not require an action potential. If the signal is an action potential, we see voltage gated calcium channels open to let extra cellular calcium in. If the signal is a chemical ligand, it is common to see an IP3 second messenger system that can release intracellular calcium from the SR. There are also examples of chemical ligands stimulating membrane bound messenger systems that result in the opening of extra cellular ligand gated calcium channels. Thus, in smooth muscle the calcium used to initiate contraction can come from intracellular or extracellular stores. In contrast, skeletal muscle only uses intracellular stores of calcium for contraction. The regulation that will be discussed below has similarities to skeletal muscle but only when skeletal muscle is fighting off fatigue and trying to conserved ATP.

2 The newly introduced Ca^{2+} ions bind to an intracellular protein called calmodulin which has four Ca^{2+} binding sites

3 The newly formed Ca^{2+} /calmodulin complex activates another enzyme called myosin light chain kinase by exposing a previously hidden active site. MLCK acts to phosphorylate the regulatory light chain on the myosin molecule (represented as the “blue” phosphate in the figure). This phosphorylation increases the binding affinity of myosin to actin. In addition, this step requires another molecule of ATP that is different than the molecule of ATP required to “cock” the myosin head (which is represented as red in the figure).

4 Once phosphorylated by Myosin Light Chain Kinase (MLCK), the myosin head can attach to actin. The release of the phosphate (red) and ADP (also red) allow the myosin head to bind actin tightly and complete a conformational change towards a lower energy state for the myosin protein. Notice in the picture how the myosin head flexes forward. You can imagine how this would pull on the actin filament and it often referred to as the “power stroke”. We have to be careful though. The myosin head is not using “power” or energy to complete the powerstroke, rather it is returning to a

lower energy state once the ADP and P have dissociated from the protein. It will require another ATP to come and attach so that the high energy state of the myosin can be obtained again ("re-cocked").

5 An ATP comes in and attaches to the myosin head. This attachment causes a change in the myosin protein that results in the dissociation of the myosin from the actin active site. However, the higher energy state ("cocking") of the myosin head is not acquired at this time. This will happen after ATP is hydrolyzed to ADP and P. In step 5 we have only used ATP to dissociate the myosin from the actin active site.

6 The intrinsic ATPase activity of the myosin head will cleave ATP. This enzyme activity is able to engage only after the myosin head dissociates from actin. The hydrolyzing of ATP to ADP and P will "cock" or move the myosin head to a high energy conformation. As long as the light chain remains phosphorylated (blue) then the myosin head is ready to attach to another actin active site and perform another cycle starting at step 4.

7 If Myosin Light Chain Phosphatase (MLCP) is available, then the MLCK phosphate (blue) is removed and the myosin head is unable to attach to another actin active site. The myosin head will remain in the high energy "cocked" position and the muscle fiber would be in a relaxed state (myosin unattached to actin and not pulling on actin). If steps 1-3 are initiated again, then the cross bridge cycling will start again.

** Notice in step 7 that MLCP (the "pac-man" icon) is inactivated by an intracellular enzyme called Rho kinase. If Rho kinase is available and active, it will put a phosphate (gray) on the MLCP to inactivate it. However, if Rho Kinase is not available or it is inactivated, then MLCP will be active to do its job of removing the MLCK phosphate (blue). The current hypothesis is that Rho kinase is inactivated by sustained levels of intracellular calcium. This inactivation is most likely the result of other, yet, unidentified enzymes.*

8 The ratio of MLCP to MLCK is important. If MLCP is high and MLCK is relatively low or inactive, then fewer phosphorylated myosin heads will be reaching step 7 and the extra MLCPs will find opportunity to remove MLCK phosphate ("blue") not just in step 7 but also in step 4 or rather step 8 for this explanation. If we see MLCP removing the MLCK phosphate (blue) from the myosin light chain while myosin is bound to actin, then ATP will attach but not be able to cause an easy dissociation of myosin from actin. The myosin molecule will enter a state called the latch state. The latch state is the result of a slowing of the rate of dissociation of myosin from actin. This means that force can still be maintained, but without additional expenditure of energy. Removing the phosphate while myosin is still attached causes a slowing of the cross bridge cycle. In the latch state it has been observed that only 20 to 30% of myosin heads are phosphorylated, thus, most of the heads are unphosphorylated but bound.

9 Normally, when ATP binds the myosin head, a protein change occurs that allows immediate dissociation from the actin active site. Also, once dissociated, the intrinsic ATPase activity of the myosin head is engaged to hydrolyze the ATP into ADP and P. Once again, this all results in the restoration of the high energy state for the myosin head. In the "Latch State", the absence of the MLCK phosphate from the myosin head interferes with ATPs ability to trigger the myosin head dissociation from actin active sites. The dissociation will eventually occur, but it takes a much longer time (this extra time is represented by the wavy line in the figure) and this is called the "Latch State"

Summarizing Phasic vs Tonic Contraction and Ca^{2+}

Smooth muscle draws on two sources of Ca^{2+} , one from extracellular stores and another from the intracellular stores found in the sarcoplasmic reticulum (SR). Similar to skeletal muscle, Ca^{2+} release from the SR is controlled by the ryanodine receptor but it is not voltage dependent nor is it linked to the DHP channel. Instead, the release of Ca^{2+} from the SR occurs through the ryanodine channel or is mediated by a G-protein second messenger cascade involving IP3. Various hormones and neurotransmitters (ie., norepinephrine, angiotensin II, vasopressin) can bind to receptors that

utilize the IP3 signaling cascade. Once generated, IP3 will bind to the SR in a way that calcium is released. Calcium in the myoplasm can bind to RyR receptors on the SR and activate them to release more calcium. This is called calcium induced calcium release. The rapid increase in intracellular Ca^{2+} results in the activation of MLCK (calmodulin MLCK complex).

Rho-kinase (ROK), which phosphorylates MLCP and inactivates it, is activated or inactivated by other second messenger systems started at the membrane surface by various chemical ligands. It has been shown that in situations where intracellular calcium is maintained for long periods, Rho-kinase activity decreases. The combination of chemical signals, depolarization events and duration of intracellular calcium increase results in changes to the MLCK / MLCP ratios. For example, in response to a rise in myoplasmic calcium concentrations, MLCK activity will increase and is relatively high compared to MLCP. This will cause a spike in muscle tension as the cross bridge cycling increases and a phasic contraction will ensue. However, if the Ca^{2+} concentrations are sustained a tonic contraction will ensue (extracellular source). This sustained calcium influx tends to diminish MLCK sensitivity (poorly understood mechanisms). In addition there are a series of chemical ligands that attach membrane bound receptors an initiate signals to inhibit intracellular Rho Kinase. The decrease activity of Rho Kinase results in an increase activity of MLCP. These events will result in a relatively high level of MLCP compared to MLCK in response to the sustained calcium influx. This will result in more dominance for step 8 and the latch state will occur (Tonic contraction).

Hopefully you can see how smooth muscle is regulated by a complicated combination of action potentials, chemical ligand stimulation and calcium influx duration. Just how each smooth muscle tissue uses these variables to regulate functional contraction is an area of active research and there is much we still don't know.

[Click Here] to watch a video that goes through the concepts your read about above.