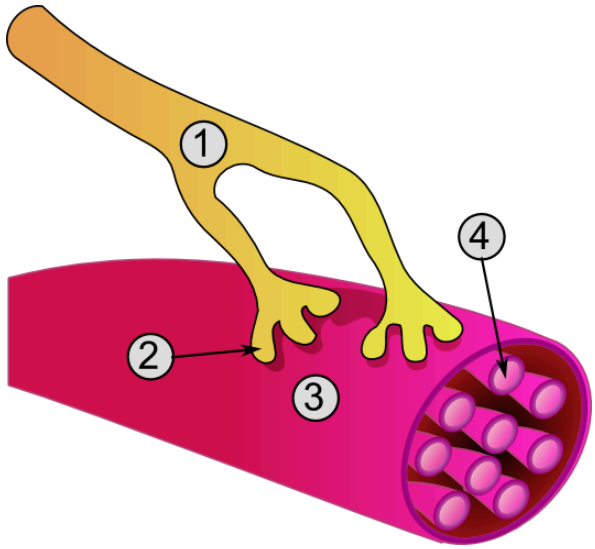
Microscopic Anatomy of Skeletal Muscle Fiber

prerequisites (these will all eventually be their own lessons as well):
you may want to know about the following to make good sense of this:

- basic cellular anatomy
- what a protein is, and what subunits are
- action potentials, graded potentials and electrical communication in the body.
- channel proteins and their different gates

For this lesson, we'll focus on individual skeletal muscle cells. Each skeletal muscle cell is a long cylindrical cell with multiple nuclei. Because of their shape, we call them "fibers". A muscle fiber is the same as saying a muscle cell. They are very large cells, 10-100μm, about 10x average body cell. Some are up to 30cm long. To make such large cells it can take hundreds of embryonic cells to fuse together to make each fiber.

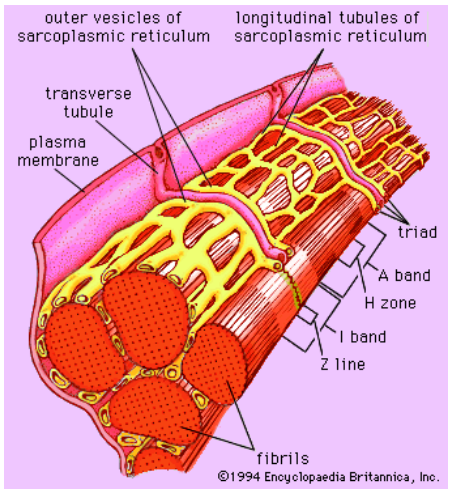
Here is a picture from wikipedia showing some of the special structures of a muscle fiber:



sarcolemma (#3)- the plasma membrane around each fiber.

sarcoplasm - the fiber's cytoplasm filling the cell. beyond what a normal body cell would contain, these fibers also have glycosomes (granules of stored glycogen) and myoglobin (red pigment for storing O_2)

myofibrils (#4) - these rods fill up the fiber. These are what allow the muscle cell, and therefore, the muscle organ to contract and provide locomotion and manipulation. These are the focus of this lesson. These "pipes" are 1-2μm diameter. There are so many that normal organelles are squeezed between them. Each cell can have 100s - 1000s and represent 80% of a cell's volume.

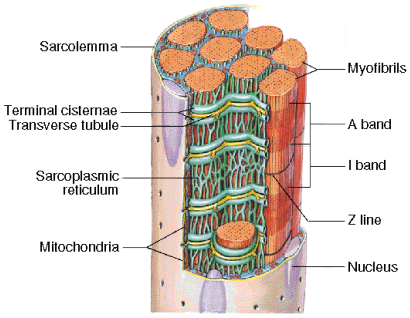


Scarcoplasmic Reticulum (SR)- (yellow tubes in picture) - this is a modified smooth endoplasmic reticulum in muscle fibers. Its job is to release calcium ions for contractions. When your myofibrils get Ca^{2+}, your muscle can contract. Calcium ions mean contraction.

T-tubules - the sarcolemma inverts itself to make tubes that wrap around the myofibrils. These are the pink tubes in this second picture. They abut the SR. Its like poking a balloon with a long stick. No matter how long/deep the tube is, the inside (lemma) of the tube is really still the outside of the cell.

Triad - the t-tubule and SR right next to each other. these two organelles work together tightly--so someone gave it a name. The edges of the SR that abut the t-tubes are called "**terminal cisternae**" - the end pots/buckets. The basic function of a triad, which we'll cover more later:

a signal to contract will travel along the sarcolemma, which is the t-tubes;
the t-tubules will tell the SR's terminal cisternae to release Ca^{2+}, which is the SR's job;
the SR releases the Ca^{2+} and the neighboring myofibrils take it up and contract.



Sarcomeres - the structural and functional unit of a muscle fiber. (*this fact is on every A&P test ever created*) sarcomeres are the contractile elements of a muscle.

In a myofibril, you can see areas of light and dark banding. These striations/bandings are what give skeletal muscle the distinctive "striations" that help identify them on lab exams. The sarcomere is one of these dark bands and half of each light band on each side. In this picture you can see two full sarcomeres on the right side.

Sarcomeres are made up of myofilament proteins.

Myofibrils, myofilaments, fibers--these terms can sound similar and confusion. Here's some help remembering these terms:

fiber is a cell
myofibril is that big pipe in a cell (made up of sarcomeres)
myofilaments are proteins (very small) that make up the sarcomere.
therefore, myofilament (proteins) in sarcomeres make myofibrils (big pipes) in the fiber (cell)

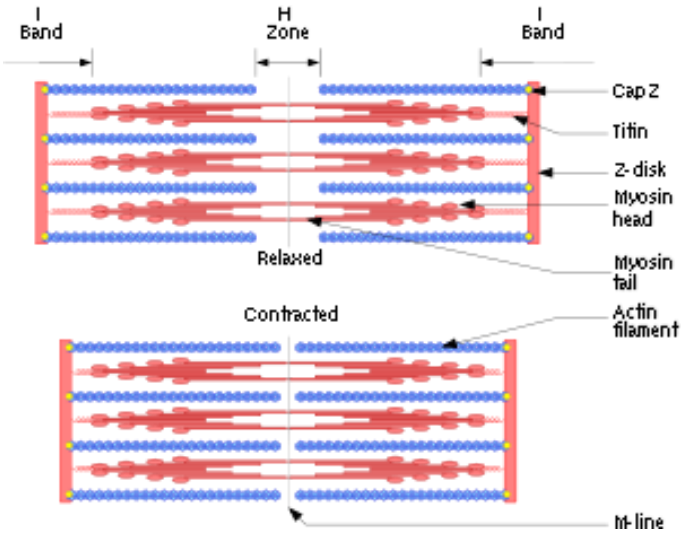
<u>Looking at the Sarcomere's Myofilaments</u>

the sarcomere's striations of dark/light bands all have names and functions we can talk about.

blue horizontal lines: these are thin myofilaments of **actin** proteins. they attach to the vertical pink lines called "Z-discs".

red horizontal lines: these are thick myofilaments of **myosin** proteins. these attach to the central "**M-line**" in black.

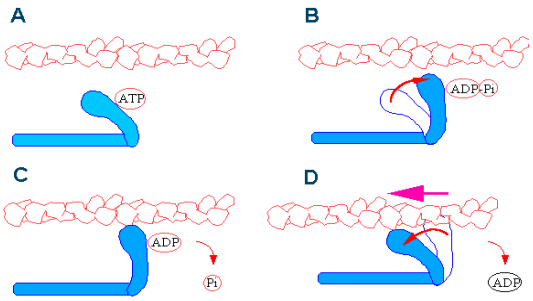
in the middle of the sarcomere is the dark **A-band** of overlapping actin and myosin myofilaments.



At the edges, where there is no longer any of the thick myosin proteins, we call that the light "**I-bands**".

In the middle of the sarcomere is the "**M-line**" surrounded by the "**H-zone**". This "H" stands for "helle", german for bright. Its lighter because there are no thin actin filaments there.

So, the thick myosin proteins are attached to the M-line in the middle. The thin actin proteins are attached to the z-discs on the edges of our sarcomere. How the whole muscle contraction works is this: the thick (red) myosin proteins are going to grab the thin blue actin proteins and pull them inward--contracting the whole sarcomere into a smaller space. You can see this in the bottom of this picture.



In the **sliding filament model of contraction,** the thick myosin protein heads reach up, connect with the thin actin chain forming "**cross bridges**". the thin actin filaments are pulled towards the M-line, which pulls the z-plates together. the cross bridges are cut when the myosin stops holding on to the actin and the contraction comes to an end.

other facts you may want to know:
the m-line is made of a protein called myomesin.
the thick myosin proteins have a core "titin" protein that does connect to the z-discs and anchors everything together.
z-discs are made of alpha-actinin an elastic filament
intermediate filaments of desmin connect Z disc to Z disc

Structurally, the M-line holds neighboring sarcomeres together. The M-line and Z-discs hold myofilaments to sarcolemma. So when the sarcomere contracts, the M-line and Z-discs pull the sarcolemma of the whole fiber/cell together. When all of the cells pull together, the muscle organ contracts.

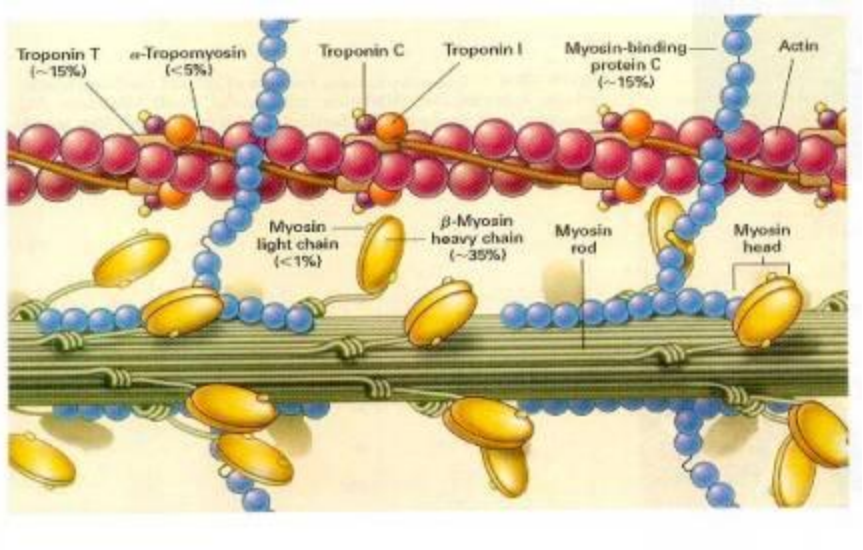
So how does the myosin and actin pull the sarcomere together?

Ultrastructure and Molecular Composition of Myofilaments
The **thick myosin filaments** will provide the power for our muscle contraction. It will hold onto the thin actin filaments and pull them together causing the overall contraction.

Myosin has little heads that point out from a long body. They look a lot like fenugreek bean sprouts: big heads on thin little wispy tails.

These heads contain multiple active sites: ATP binding site, actin binding site, and an ATPase. Lots of these join together as bundles, with their heads sticking out to the side.

In the following picture, myosin is at the bottom--notice its double head sticking out from the myosin rods.



thin actin filaments are made up of two forms of actin:

globular actin (G-actin) has a binding site for myosin to attach to.

fiberous actin (F-actin) has a lot of G-actins attached to them

In this picture you can see the red balls of G-actin that form strings of pearls that run in lines along the F-actin.

Along with the actin we have the toponin-tropomyosin complex that stops contractions from occurring.

tropomyosin (brown rod) is a rod that covers binding sites of the G-actin while muscle is at rest. As long as the binding sites are covered, the myosin heads can't reach up and grab on.

troponin (purple/orange/lightbrown) is made up from 3 protein subunits:

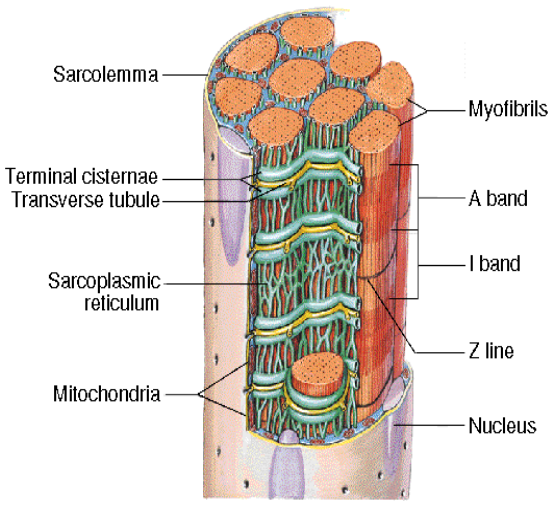
1 subunit binds to actin (purple)

1 subunit to tropomyosin (lightbrown)

the third subunit (purple) is ready for Ca^{2+} (yellow) to come and activate the whole protein.

The Triad: Sarcoplasmic Reticulum and T-tubules

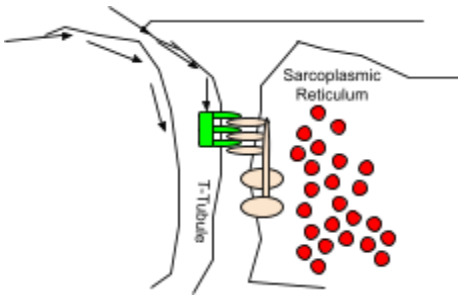
Lets back up and look at the triad again. The triad is a t-tubule (yellow) abutting two SR-terminal cisternae (thick green), one on either side. The action potential signal to contract will travel along the sarcolemma (the cell wall) down the t-tubule which will activate the SR to release Calcium thus activating muscle.



Sarcoplasmic Reticulum (SR) (green)- as I said before, this is an elaborate smooth ER. We care about this because it regulates the Ca ion "go signal" that will help initiate contractions. The SR is a sleeve of interconnecting tubules wrapped around each myofibril (the big pipes in the fiber). There are lots of

mitochondria and glycogen granules near the SR to help provide power for our contractions.

The **T-tubules** come down like a tube to wrap around each myofibril (the big pipes) from the sarcolemma (that outer wall of the cell) at the A-band/I-band junctions.



So, so far we have the t-tubules coming close to the SR. the t-tubules as extensions of the sarcolemma can carry the action potential we started with a nerve signal. the SR has the calcium (seen here as red dots) we need to start muscle contractions, but how do they communicate?

The Triad relationship comes from a double zipper of internal proteins. The t-tubule has voltage-gated channel proteins that stick out towards the SR. The SR has mechanically-gated calcium channels called “foot proteins” that zipper-into the t-tubule proteins. A voltage change comes down (arrows) the sarcolemma, trips the voltage gates on the green channels. That causes the green channels to physically deform. The interlocked foot proteins (tan) are then deformed which opens up the nearby Calcium channel. Once thats open, all the calcium ions flood out of the SR into the muscle myofibrils.

Starting the whole process

Skeletal muscles are widely regarded as voluntary muscles. So, to start this process, we need to have a nerve stimulate our muscle fiber to initiate this entire process. Nerve axons get close to the muscle fiber’s receptor pit at the neuromuscular junction.

Motor neurons live in brain/spinal cord and have their axons reach out to each muscle fiber. Near the ends, axons divide profusely as they enter each muscle. Each ending branches to single muscle fibers that approach the neuromuscular junctions. They don’t physically connect, but instead leave a gap known as the synaptic cleft. This process is very similar if not identical to neuron-neuron communication. Briefly, synaptic vesicles in the presynaptic neuron release acetylcholine (ACh). on the postsynaptic side, the muscle fiber’s sarcolemma has junctional folds for ACh receptors. These receptors are ligand-gated channels that can initiate a graded potential in that area. ACh falls off the receptors. Some is disposed of by floating away, some by being reabsorbed by the pre-synaptic neuron, but most of it is ripped apart by acetylcholinesterase (AChE) that removes the acetyl from the choline.

If there is ever a shortage of ACh receptors (AChR), then any given amount of ACh will provide less of a response in the post-synaptic muscle. This leads to apparent muscle weakness known as myasthenia gravis (asthen=weakness, gravi=heavy). Its an autoimmune disease caused by ACh receptor antibodies being found by the immune system.

Generation of Action Potential across sarcolemma

The sarcolemma, the cell membrane, has potential across it making the inside slightly negative.

1) ACh receptors open up local ligand-gated ion channels for K^+ and Na^+ causing **local depolarization** generation of end-plate potential. more Na^+ diffuses than K^+ causing potential changes
2) local depolarization of end plate potential spreads out to adjacent areas opening voltage-gated

Na^+ channels. Na^+ enters, threshold reached, **action potential** generated which continues to open voltage-gated channels for Na^+.

reminder: Action potential is unstoppable. AP's result in muscle fiber contraction

3) **repolarization** - Na-channels close, voltage-gated K^+ open restoring negative potential inside. this is refractory period. a time when that area of the fiber can't be stimulated. Repolarization restores electric potential not ionic balance!! For that we need Na/K pumps. Hundreds of action potentials can occur before ionic imbalance

excitation-contraction (EC) coupling

1) ACh-receptor is activated bringing about an action potential (AP) along the sarcolemma
2) AP down into T-tubule, activating voltage-sensitive tubule protein which changes shape this opens Ca^{2+} release channels in terminal cisternae of SR. Ca^{2+} floods out into myofibrils.
3) Ca^{2+} in myofibrils binds to troponin. Troponin changes shape, twisting tropomyosin, exposing binding/active sites on actin filaments
4) myosin binds to actin forming cross bridges. sarcomeres, and therefore the whole muscle, contracts due to cross-bridge cycling
    4a) energized myosin attaches to actin active sites, this is the cross bridge
    4b) the power stroke that pulls the sarcomere together: ADP-P_i released causing myosin head to pivot and bend, which slides actin towards M-line
    4c) cross-bridge detachment - ATP attaches breaking cross-bridge link
    4d) cocking-head - ATP hydrolized to ADP-P_i, myosin head returns to high-energy "cocked" position
    4e) cycle starts anew
5) EC coupling is over now

This process repeats many times, walking actin towards M-line. At any given time, only half of the myosins are ever pulling at the same time. others are searching for next binding site.

Two breakdowns that can occur during this process:
hydroxyapatite crystals: calcium needs to be kept low otherwise inorganic phosphate would make hydroxyapatite crystals, hard salts of bone matrix. Any muscle cells that were calcified like this would would die.

rigor mortis (death rigor) - because cross-bridge detachment is ATP driven, when ATP is no longer available after death, there isn't any way for cross bridges to detach themselves. The muscles stay in a permanent contraction. Well, not completely permanent--after some time the proteins themselves will start to fall apart as apart of rotting and the contraction will be released.

Here's something neat!!
Muscle Contraction shows a good review of gated channels:

1) **voltage-gated** Ca channels in the presynaptic nerve cause the release of ACh
2) ACh receptors are **ligand-gated** Na/K channels to
3) local depolarization **voltage gated** channels make AP
4) AP travels along the T-tubules which have their own voltage gated channels bound up with SR

foot proteins
5) SR foot proteins are **mechanically gated** Ca^{2+} channels.