

Lectures 16 & 17: Muscle Structure and Mechanisms of Contraction

The contraction of muscle is complex, starting with an electrical stimulus (an action potential arriving from a motor neuron) and proceeding through a series of biochemical steps to produce mechanical force. How is it that a motor impulse causes a muscle to physically shorten?

1. Gross Structure

A skeletal muscle is generally anchored at each end by a tendon attached to a bone. By contracting, the muscle changes the relationship of the two bones to which it attaches, to cause movement. Cardiac and smooth muscle contract in a similar fashion, but the force they produce is used to push substances through the lumen of a tube (vein, gut, etc).

(Overhead: Eckert Fig 10-1).

Vertebrate skeletal muscle has a highly organized, hierarchical structure. ***This rigid physical organization is necessary because the function of muscle is to produce force & motion.*** If muscle had a random or amorphous structure (like secretory tissue, for example), then the subunits of a muscle would exert their forces in random directions, rather than summing the forces in a single direction.

A muscle is composed of many muscle fibers or ***myofibers***, which are long thin cells that lay parallel to one another. Myofibers are multinucleate cells, with each myofiber arising by the fusion of many embryonic ***myoblast*** cells. Each myofiber is composed of many ***myofibrils***, which also run in parallel. A myofibril is composed of many ***sarcomeres*** laid end to end.

2. Molecular Structure

(Overheads: Eckert Figs 10-1, 10-2, 10-3)

Sarcomeres are the basic contractile units of muscle. Because their structure is highly organized structure at the molecular level, individual sarcomeres can be seen under a light microscope as light and dark bands or striations, which is why vertebrate skeletal muscle is also called ***striated muscle***.

A sarcomere is composed of two types of ***protein myofilaments***, which lie parallel to each other:

Actin (thin filaments)

Myosin (thick filaments)

Striations are alternating regions in which:

Solely actin filaments occur (***I band***)

Myosin filaments occur either alone or overlapping with actin (***A band***)

Solely myosin filaments are found (***H zone*** - a subset of the A band)

Actin filaments are anchored to ***Z-disks***, which are fixed points against which the protein filaments pull during contraction.

(Overhead: Eckert Fig 10-5)

Actin filaments are double-helical structures, with two long filaments of ***F-actin*** (F = filament) wound around each other. The F-actin is composed of many smaller units, each a globular actin molecule, ***G-actin***. A long protein molecule, ***tropomyosin***, lies in the groove between the two F-actin filaments. Every 40 nm along the actin filament lies a troponin, which is made of several complex globular proteins.

(Overhead: Eckert Fig 10-5)

Myosin filaments are made of many individual myosin molecules. Each myosin molecule has a head and a tail. Myosin molecules spontaneously organize themselves into chains, with the heads projecting out at regular intervals.

(Overhead Eckert Fig 10-7 & 10-4)

3. Cross Bridges and Mechanics of Contraction

Contraction is due to myofilaments sliding past each other. This ***sliding filament*** motion causes each sarcomere to shorten slightly (about 0.3 μm). Because many sarcomeres lie end to end in a myofibril, the total length shortens substantially when all sarcomeres contract in unison.

(Overhead: Eckert Fig 10-8A)

Myosin heads form ***cross bridges*** between myosin and actin, by attaching to specific binding sites on G-actin molecules. By:

- Binding to actin
- pivoting
- detaching
- and binding to actin again,

myosin heads ratchet the actin filament along to shorten the sarcomere.

This mechanism requires a coordinated sequence of binding, movement and rebinding. Obviously, if the myosin head did not release at the correct time, they would eventually prevent actin filament from sliding past. What controls the binding and unbinding of myofilaments?

Control of cross bridge formation and breakage depends on ATP and Ca^{2+} ions.

Role of ATP in contraction

ATP is necessary for unbinding of myosin head to break the cross bridge.

Actin and myosin spontaneously bind to form a stable actomyosin complex. If myosin is bound to ATP, then the actomyosin-ATP complex that forms is not stable, allowing actin and myosin to uncouple.

1. Actin + Myosin \rightarrow Actomyosin (Stable - forms spontaneously and stays bound)
2. Actin + Myosin-ATP \rightarrow Actomyosin-ATP (unstable, forms only briefly)

\rightarrow Actin + Myosin + ADP + Pi

(Overhead: Eckert Fig 10-10) (shows that actual sequence is slightly more complex)

Rigor mortis - after death, all available ATP is quickly used up, so muscles lock in position as actin & myosin are permanently bound.

While the above sequence occurs, ATP binding to myosin is also involved in ***pivoting of myosin head***:

- There are 4 binding sites on the myosin head - M1, M2, M3 & M4.
- M1 is the first to bind to actin, because of the physical arrangement of M1 to M4
- M2 forms a more stable bond with actin than M1, so bond shifts from M1 \rightarrow M2
- M3 forms a more stable bond with actin than M2, so bond shifts from M2 \rightarrow M3
- M4 forms a more stable bond with actin than M3, so bond shifts from M3 \rightarrow M4

- M1 to M4 are arranged in a linear sequence on the myosin head, so this binding sequence causes the head to pivot.

- Pivoting head pulls actin filament past myosin filament.

(Overhead: Eckert Fig 10-11 B & C)

Role of Calcium ions in contraction

Ca^{2+} play an opposite role to that of ATP - *Ca^{2+} ions are necessary for binding of myosin head to actin to form cross bridges.* In particular, Ca^{2+} ions must be present in the myoplasm (cytoplasm of muscle fiber) at greater than 10^{-7} M for contraction.

(Overhead: Eckert Fig 10-15 B)

(Overhead Eckert Fig 10-23)

Steric (shape) inhibition of actin-myosin binding: In absence of Ca^{2+} tropomyosin is physically in the way of the binding site on G-actin molecules, to which myosin head would bind.

- Ca^{2+} ions bind to the troponin complex.
- Ca^{2+} binding alters shape of troponin complex (which are located every 40 nm along tropomyosin molecule)
- Altered shape of troponin- Ca^{2+} complex pulls tropomyosin away from binding site for myosin head
- Removes steric inhibition

(Overhead: Eckert Fig 10-16)

4. Excitation-Contraction Coupling

Obviously, muscles are controlled by nerves, but how does the arrival of an action potential from a motor neuron lead to contraction?

Review of neuromuscular junctions (also see lecture of 18 September):

(Overheads: Eckert Fig 6-13, 6-12A & 6-15A,B,C)

- AP travels down myelinated (fast) motor nerve
- Motor nerve branches, so each neuron innervates a myofiber (or many myofibers) - for fine control (e.g. fingers), few fibers per neuron; for course control (e.g. massive leg muscles), many fibers per neuron.
- AP arrives at ***neuromuscular junction***
- Synaptic vesicles bind to cell membrane of neuron, releasing ***acetylcholine*** (neurotransmitter) into synaptic cleft
- Acetylcholine binds to receptors on muscle cell membrane at ***junctional folds*** that lie at the neuron's endplate.
- Binding of Acetylcholine stimulates depolarization of muscle fiber's cell membrane
- Depolarization causes myofiber to contract (twitch) in all-or-none manner

(Overhead: Eckert Figs. 10-18 & 10-19)

The depolarization of the muscle fiber is due to opening of ion channels in membrane. Resting potential is -90mV (due to ion gradient) During an AP, voltage-gated Na^+

channels open, allowing ions to cross membrane, which briefly depolarizes (actually overshoots to +50 mV).

What occurs within the myofiber when depolarization occurs?

The *simple answer* is that calcium ions are released, disinhibiting actin-myosin binding. This answer makes sense, but there is a *problem* \Rightarrow contraction occurs within two milliseconds of AP across entire cross section of fiber. This is *too fast* for calcium to move from the cell membrane to myofibrils in the center of the fiber, so movement of Ca^{2+} into the cell cannot be the mechanism

Solution \Rightarrow Depolarization moves deep into cell from surface, rather than calcium moving into cell. A *process can move faster than a substance*.

T tubules are extensions of the myofiber's plasma membrane that extend into the cell to contact myofibrils far from the surface.

The lumen of T tubules is extracellular - like surface of a swiss cheese. AP propagates down the T tubules, just as it moves across other parts of cell membrane.

(Overheads: Eckert Figs 10-20 & 10-21)

In vertebrate striated muscle, T tubules run parallel to Z-lines. Next to each T tubule, one on each side, is a *terminal cistern* of the *sarcoplasmic reticulum*. *Triad* \Rightarrow t-tubule and 2 terminal cisterns together.

(Overhead: Eckert Figs 10-21)

(Overhead: Eckert Fig 10-25)

Sarcoplasmic reticulum is a structure of tubes or bags that lie next to myofibrils. Membrane of SR contains *Ca^{2+} pumps*, which are channels in membrane that actively move Ca^{2+} ions out of myoplasm into the SR.

- In the SR, calcium is bound to *calsequestrin* to lock it up. This allows Ca^{2+} to be highly concentrated within SR, without pumps working against huge ion gradient.
- Using ATP for energy, SR calcium pumps keep the Ca^{2+} concentration in myoplasm below 10^{-7} M, preventing contraction.
- When AP travels down T tubule, it causes adjacent terminal cistern of SR to release sequestered Ca^{2+} , initiating contraction.
- Rapid re-uptake of Ca^{2+} by SR ends contraction (repumping begins even while release occurs) by removing Ca^{2+} from troponin.

(Overhead: Eckert Fig 10-23)

Plunger model of Ca^{2+} release

(Overhead: Eckert Figs 10-24 & 10-25)

Why does AP in T tubule cause adjacent SR to release Ca^{2+} ?

- Membrane of T-tubule has embedded ***dihydropyridine receptors***
- Dihydropyridine receptors are ***voltage sensitive*** \Rightarrow change shape as AP wave passes
- Membrane of SR has embedded ***ryanodine receptors*** directly across from dihydropyridine receptor.
- Ryanodine receptors act as plugs for pores in SR membrane
- When Dihydropyridine receptor changes shape, it physically pulls on ryanodine receptor, opening the SR pore and allowing Ca^{2+} to flow out.
- Because Ca^{2+} is much more concentrated inside SR than in myoplasm, it flows out passively.
- Ca^{2+} in myoplasm above 10^{-7} M \Rightarrow disinhibition of actin-myosin crossbridge formation \Rightarrow contraction

There is an excellent summary of mechanisms in contraction - relaxation cycles, step by step, on page 471-472 in Hill et al textbook

(Overhead: Eckert Fig 10-40)