Muscle and Neuromuscular Junction

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• Types and structure of muscle cells

• Structural basis of contraction

• Triggering muscle contraction
Skeletal muscle consists of bundles of long, multinucleated cells.

Skeletal muscle is involved most prominently in the movement of limbs but is also responsible for movement of the eyes. It can generate a range of forces from rapid and powerful to slow and delicate. Skeletal muscle is activated by voluntary and reflex signals. The cells of skeletal muscle span the length of entire muscle. So if a muscle is 5 cm, the muscle cells are 5 cm in length. To support the large volume of cytoplasm and all the proteins needed, skeletal muscle cells are multinucleated. The cells evolve from the fusion of many individual cells. Skeletal muscle cells are often referred to as myofibers. Note that the cells are arranged in parallel arrays to generate contraction in one direction.
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Cardiac muscle consists of smaller, interconnected cells. Cardiac muscle cells responsible for pumping blood from the heart. They generate rapid and forceful contractions and are under involuntary control. Cardiac muscle cells are much smaller than skeletal muscle cells and are connected in series to span the length of cardiac muscle. Individual cells are linked and communicate via gap junctions which allows action potentials to pass from one cell to the next. Note that the cells are arranged in parallel arrays to generate contraction in one direction.
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Smooth muscle surrounds most of the internal organs: GI tract, respiratory tract, bladder. It is also surrounds most blood vessels and veins. It provides tone and shape but can also generate slow and powerful contractions to change the size and shape of an organ. Smooth muscle cells are under control of the autonomous nervous system. Smooth muscle is composed of numerous spindled shaped cells. Gap junctions between cells allows coordination of contraction. Note that the smooth muscle cells are arranged in layers that are orthogonal to each other. Smooth muscle often contracts an organ in multiple directions.
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Smooth muscle controls diameter of blood vessels and bronchioles.

To get a better sense of how smooth muscle cells control the shape of an organ, one can look at blood vessels and bronchioles. Here, the smooth muscle cells are arranged circumferentially around the vessels and bronchioles. Contraction of the cells decreases the diameter of the lumen of the vessel to restrict the volume of blood that can flow through the vessel. The cardiovascular system uses smooth muscle to control the distribution of blood to different capillary beds. Smooth muscle cells perform a similar function in the respiratory system. Smooth muscle cells contract to narrow the lumen of the bronchioles. This restricts the amount of air that flows through the bronchiole and is important for preventing access of foreign particles and microorganisms to the deeper aveoli. However, over stimulation or proliferation of smooth muscle cells can lead to pulmonary diseases such as asthma.
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Contraction of all muscle cells driven by myosin and actin filaments.

All three types of muscle cells share some features. All generate movement through contraction. All use force from myosin pulling on actin filaments to generate force for contraction. All use calcium as trigger for contraction. The differences is in the inner architecture of the cells and how myosin is activated to generate contraction.
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Skeletal, cardiac and smooth muscle cells express different types of myosins with different properties.

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>Protein Name</th>
<th>Gene Name</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal Slow-twitch</td>
<td>MHC-β</td>
<td>MYH7</td>
<td>Slow</td>
</tr>
<tr>
<td>Skeletal Type IIa</td>
<td>Myosin IIa</td>
<td>MYH2</td>
<td>Moderately Fast</td>
</tr>
<tr>
<td>Skeletal Type IIx</td>
<td>Myosin IIx/d</td>
<td>MYH1</td>
<td>Fast</td>
</tr>
<tr>
<td>Skeletal Type IIb</td>
<td>Myosin IIb</td>
<td>MYH4</td>
<td>Very Fast</td>
</tr>
<tr>
<td>Cardiac</td>
<td>MHC-α</td>
<td>MYH6</td>
<td>Fast</td>
</tr>
<tr>
<td>Cardiac</td>
<td>MHC-β</td>
<td>MYH7</td>
<td>Slow</td>
</tr>
<tr>
<td>Smooth</td>
<td>Smooth muscle myosin II</td>
<td>MYH11</td>
<td>Very Slow</td>
</tr>
</tbody>
</table>

The different types of muscle express different types of myosin which to a large extend determines the contractile properties of the muscle. There are several different types of skeletal muscle (e.g. slow-twitch and fast-twitch) and each expressed a specific type of myosin. Also note that most non-muscle cells express non-muscle myosin II which helps those cells generate tension and changes in cell shape.
Structure and contraction of skeletal muscle
Skeletal muscle cells contain bundles of myofibrils.

Muscle cells span length of muscle and are multinucleated. Contraction of the cells will shorten muscle. Each cell is filled with myofibrils that span the length of the muscle cell. Myofibrils are bundles of actin and myosin filaments that form the contractile apparatus.
Skeletal muscle cell cytoplasm is filled with myofibrils and mitochondria.

A cross section of a skeletal muscle cell reveals a cytoplasm that is filled with myofibrils (parallel arrays of actin and myosin filaments). Mitochondria are abundant in some skeletal muscle cells to provide ATP via oxidative phosphorylation. The nucleus of skeletal muscle cells is pushed to the periphery to accommodate the myofibrils.
Actin and myosin filaments are precisely arranged in sarcomeres.

A cross section of myofibril reveals precise arrangement of actin and myosin filaments in a myofibril. 6 actin filaments arranged radially around one myosin filament. Motors in one myosin filament contact 6 different actin filaments.
Myofibrils are longitudinally organized into sarcomeres.

Histologically, skeletal muscle appears in longitudinal sections to contain a series of light and dark bands. This pattern gives skeletal muscle its other name: striated muscle. The light and dark bands reflect the presence or absence of actin and myosin filaments. The light bands are regions where there are only actin filaments and the dark bands contain both actin and myosin filaments. This repeated pattern can be divided into a structural and functional unit called the sarcomere. By EM, sarcomeres are defined by two dark bands called Z-disks. Sarcomeres are arranged in series and run the entire length of the muscle cell.
Sarcomeres contain bundles of actin and myosin filaments.

This image shows the arrangement of actin and myosin filaments in a sarcomere. Actin filaments extend from Z-line or Z-disc toward the center of the sarcomere. The plus ends of the actin filaments are stabilized in the Z-discs and the minus ends are located toward the middle of the sarcomere. Actin filaments end before reaching the center of the sarcomere. Myosin filaments span the central region of the sarcomere and overlap part of the actin filaments. Each sarcomere is about 2.4 μm in length.
Sliding of actin and myosin filaments contracts sarcomeres.

The myosin filaments are bipolar in that motors on one side of the filament walk toward one Z-disc and motors on the other side of the filament walk toward the opposite Z-disc. When activated, myosin tries to walk to opposite Z-disks, pulling on actin filaments which shortens distance between Z-disks. Each sarcomere shortens by about 0.4 µm during muscle contraction. This distance is tiny, but when 80,000 sarcomeres in series contract 0.4 µm, the total length of contraction is 3.2 cm.
Actin and myosin filaments arranged in sarcomeres in cardiac muscle cells.

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Smooth muscle cells lack sarcomeres.

In contrast to skeletal and cardiac muscle cells, smooth muscle cells lack sarcomeres. Instead, the actin and myosin filaments in smooth muscle cells are arranged in more directions. Actin filaments are anchored at the cell membrane and in the cytosol at structures called dense bodies. The plus ends of the filaments are anchored at the dense filaments. Dense bodies are the functional equivalent of Z-discs.
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Actin filaments anchored at plasma membrane and dense bodies in cytoplasm.

Bipolar myosin filaments interdigitate between actin filaments. Activation of myosin pulls on the cell membrane to shrink the cell.
Triggering muscle contraction
Skeletal muscle cells innervated by motor neurons at neuromuscular junctions.

Muscle cells innervated by motor neurons that arise from spinal cord. Activation of motor neuron triggers contraction of muscle cell.
Muscle cell innervated by single neuron at motor end plate.

The axon of motor neurons will often branch to allow a single motor neuron to synapse with multiple skeletal muscle cells. However, each muscle cell is innervated by only one neuron.
Motor neurons release acetylcholine from synaptic vesicles to trigger muscle contraction.

Motor neurons contain high concentration of synaptic vesicles filled with acetylcholine which is the neurotransmitter that triggers contraction of skeletal muscle cells. At the neuromuscular junction, the cell membrane of the skeletal muscle is arranged into a fold. This increases the surface area of cell membrane to accommodate more acetylcholine receptors and voltage gated sodium channels. Basal lamina in folds contains acetycholinesterase that degrades acetylcholine.
Acetylcholine binds and opens ion channels in muscle plasma membrane.

Acetylcholine is bound by ligand–gated ion channels in the cell membrane of the skeletal muscle cells. When bound to acetylcholine the ion channels open allowing primarily sodium to enter the cell. The influx of sodium depolarizes membrane and leads to the opening of voltage–gated ion channels. The opening of voltage–gated ion channels initiate an action potential along muscle cell plasma membrane.
Motor neurons release excess acetylcholine to depolarize membranes beyond threshold.

One unique feature of the neuromuscular junction is that the amount of acetylcholine released and the number of ligand-gated ion channels in the membrane of most muscle cells are sufficient to depolarize the membrane to a level far beyond what is needed to trigger an action potential. The difference between the depolarization caused by acetylcholine and the threshold level needed to trigger an action potential is called the safety factor.
Safety factor ensures action potentials are triggered after repeated rounds of stimulation.

The reason for the safety factor is that skeletal muscle cells are often repeatedly stimulated by a motor neuron at high frequency. Each subsequent synaptic event releases less acetylcholine, but due to the excess of acetylcholine and the large number of receptors in the skeletal muscle cell membrane, an action potential is still triggered.
Increase in intracellular calcium triggers muscle contraction.

Action potentials lead to increases cytosolic calcium that trigger contraction of skeletal muscle.
Calcium causes shift in tropomyosin to expose myosin-binding site on actin filaments.

Actin filaments wrapped by filament called tropomyosin. Each tropomyosin covers 7 actin monomers and adjacent tropomyosins are linked to cover an entire filament. The Position of tropomyosin on actin occludes myosin binding site and prevents myosin from binding to actin. So, even though the myosin is active, it can't bind actin to generate force and contraction. Calcium causes tropomyosin to shift, exposing myosin-binding site. Troponin complex is calcium sensing protein along actin filaments. It binds calcium induces shift in tropomyosin. When calcium levels fall, the tropomyosin shifts back to occlude the myosin-binding site and knock myosin off the filament.
How does activation at surface of muscle cell trigger contraction in center of cell?

Skeletal muscle cells are innervated at the cell membrane but each cell can be hundreds of microns in diameter. How does a signal at the cell surface reach the myofibrils in the center of the cell to generate contraction?
Plasma membrane of muscle cells penetrates into center of cell as T-tubules.
Action potential at NMJ passes through T-tubules triggering calcium release throughout muscle cell.

T-tubules run adjacent to the sarcoplasmic reticulum of the skeletal muscle. The sarcoplasmic reticulum is the equivalent of the ER and is the major reservoir of calcium. Action potentials that start on the surface of the cell will proceed down the T-tubules. The action potential in the T-tubules will trigger the adjacent sarcoplasmic reticulum to release calcium into the cytosol.
Calcium channels in T-tubules mechanically open calcium channels in ER.

Plasma membrane in T-tubules and ER (sarcoplasmic reticulum in muscle cells) run together throughout cell in triads. The close distance allows physical interaction between proteins in both membranes. Voltage-gated calcium channels, dihydropyridine (DHP) receptors, in T-tubules open during action potential. DHP receptors are linked directly to calcium–releasing channels, ryanodine receptor, in the sarcoplasmic reticulum. Opening of DHP receptors causes the ryanodine receptors to open, releasing calcium into the cytoplasm.
Cardiac muscle consists of smaller, interconnected cells.

Cardiac muscle consists of thousands of cells in series and individual innervation of each cell would be nearly impossible given the rate of contraction.
Current spreads through gap junctions between cardiac muscle cells to trigger contraction.

Instead, cardiac muscle cells rely on gap junctions to allow action potentials to proceed from one cell to the next. An action potential in one cell leads to an influx of cation that diffuses through the gap junctions to the neighboring cell. The amount of positive charge is sufficient to open voltage-gated channels in the second cell, leading to an action potential. This process continues down the muscle. Similar to skeletal muscle, the action potential and depolarization of the cell membrane leads to an increase in cytosolic calcium. The increase in calcium causes tropomyosin to shift, allowing myosin to bind and pull on actin filaments to generate contraction.
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Smooth muscle cells can be individually innervated or work in groups via gap junctions.

Smooth muscle cells can be innervated in different ways. Some cells are individually innervated in what are called multiunit smooth muscle. Other smooth muscle cells rely on gap junctions for a stimulatory signal to pass from a single innervated cells. In multiunit smooth muscle, neurotransmitter binds G-protein coupled receptors that initiate a signaling pathway to activate contraction. In unitary smooth muscle, neurotransmitter initiates an action potential.
In smooth muscle cells, calcium triggers enzymatic activation of muscle myosin.

One major difference between smooth muscle and skeletal/cardiac muscle is the mechanism by which contraction is initiated. In smooth muscle, myosin is inactive and is converted to an active form when the smooth muscle cell is stimulated. Stimulation of smooth muscle cells generates an increase in cytosolic calcium, but instead of causing tropomyosin to shift as in skeletal and cardiac muscle, calcium leads to the activation of a kinase called myosin light chain kinase. Calmodulin binds calcium and then converts MLCK into an active form. MLCK phosphorylates myosin light chains which associate with muscle myosin. When the light chains are phosphorylated, the myosin is active and able to generate contraction.
Smooth muscle cells slower to contract but maintain force longer.

One consequence of this mechanism to active myosin is that smooth muscle cells take longer to generate contraction and contractive force increases slowly over time. One benefit is that smooth muscle cells can maintain contraction long after the stimulus is removed. This is due in part to the length of time it takes to dephosphorylate myosin light chain and thereby inactive muscle myosin. Another contributing factor is the length of time it takes muscle myosin to release from actin filaments. Once bound to actin filaments, muscle myosin in smooth muscle cells very slowly releases from actin filaments. After a contractile event, it takes a considerable amount of time for all of the myosin motors to release from actin filaments and allow complete relaxation of the muscle cell. This allows smooth muscle to generate and maintain contraction without consuming a lot of ATP.
Clinical correlation
Myasthenia gravis is an autoimmune disease with antibodies against the acetylcholine receptor.

Myasthenia gravis is an autoimmune disorder in which antibodies are generated against the acetylcholine receptor. The binding of antibodies reduces synaptic transmission at the neuromuscular junction by preventing receptors from binding acetylcholine or causing the cell to endocytose the receptor. The net effect is that there are fewer available receptors to bind acetylcholine.
Loss of available acetylcholine receptors reduces responsiveness of muscle to repeated stimulation.

The decrease in the number of available acetylcholine receptors reduces the level of membrane depolarization during muscle stimulation. The lower level of depolarization reduces the safety factor and consequently, subsequent stimulation events are not able to trigger muscle contraction.
Take home points...

• There are three types of muscle: skeletal, cardiac and smooth.

• Contraction of all muscle mediated by myosin and actin filaments and triggered by increase in cytosolic calcium.

• Skeletal and cardiac muscle contain sarcomeres but smooth muscle does not.

• Motor neurons secrete acetylcholine to trigger contraction of skeletal muscle.

• Cardiac and smooth muscle cells utilize gap junctions to transmit signals and coordinate contraction.