بسم الله الرحمن الرحيم

اللهم استعملنا ولا تستبدلنا

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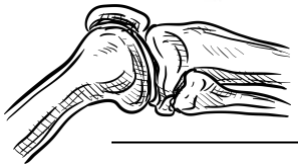
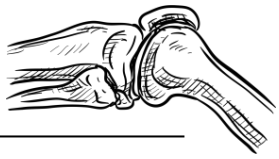


PHYSIOLOGY

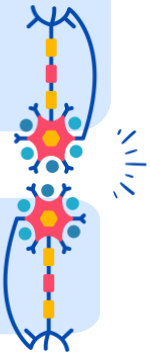


MID | Lecture 2

Neuromuscular Junction (NMJ)

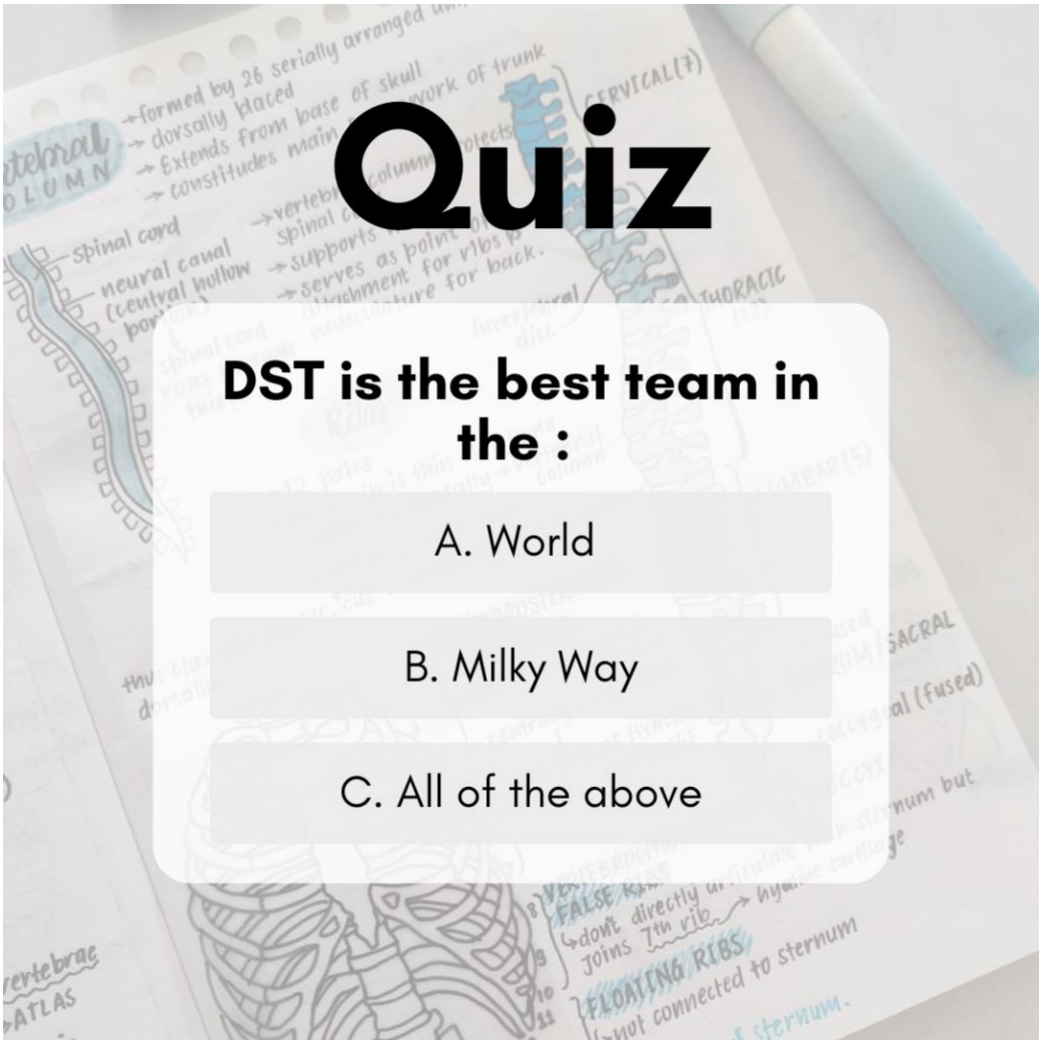
Written by : Sadeel Al-hawawsheh
Leen Mamoon

Reviewed by : Salwa Alawi



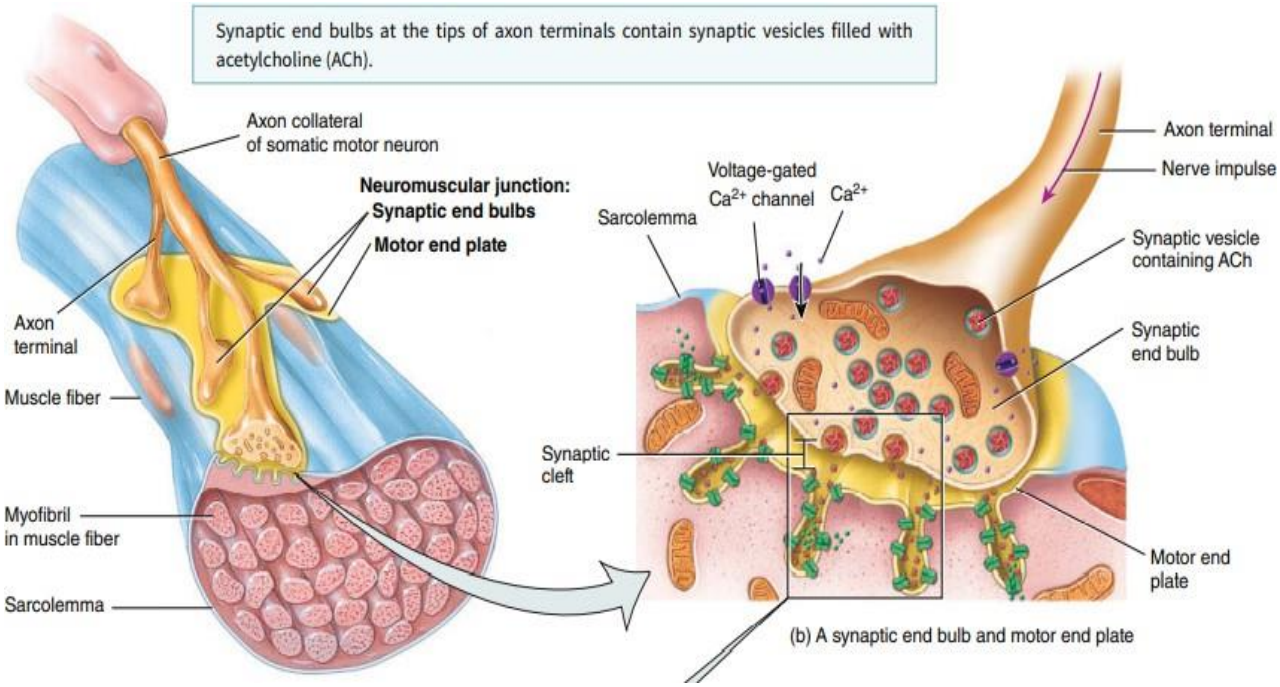
رمضان مبارك

Choose the correct answer to test yourself in the previous lecture



اللهمّ إنّي اسألك فهم النبيّين، وحفظ المرسلين والملائكة المقربين، اللهمّ اجعل ألسنتنا عامرة بذكرك، وقلوبنا بخشيتك، وأسرارنا بطاعتك، إنك على كل شيء قدير.

Neuromuscular junction (NMJ)



Skeletal muscle fibres are innervated by large , myelinated nerve fibers that originate from large motoneurons in the anterior horns of the spinal cord.

Each nerve fiber, after entering the muscle belly, normally branches and stimulates from three to several hundred skeletal muscle fibers. Each nerve ending makes a junction, called the neuromuscular junction, with the muscle fiber near its midpoint.

The action potential initiated in the muscle fiber by the nerve signal travels in both directions toward the muscle fiber ends.

A nerve fiber which extends from the axon terminals , can either connect to another neuron , a muscle fiber , or a gland. In this system , our focus is on the connection between a nerve and a muscle fiber.

The **neuromuscular junction** refers to the point where the nerve ending meets the muscle fiber , facilitating the transmission of stimuli from the neuron to the muscle fiber. At the end , this transmission results in either muscle contraction or relaxation , depending on the stimulus.

Structure of Muscle Fibers and Innervation

A nerve cell branches extensively to ensure that it reaches all parts of the muscle. A muscle is composed of multiple bundled muscle fibers , with each muscle fiber consisting of bundles of myofibrils.

For proper innervation , the nerve must reach the smallest functional unit of the muscle - the myofibril. The nerve cell must induce indentations into the muscle fiber to ensure efficient transmission of action potentials to all myofibrils within the muscle fiber.

Action Potential Propagation

An action potential travels along the nerve cell and reaches the nerve ending. The **synapse** is the area where the **pre-synaptic** cell (nerve ending) interacts with the **post-synaptic** cell (muscle fiber).

Voltage-gated Calcium channels

On the inside surface of the neural membrane are linear dense bars.

To each side of each dense bar are protein particles that penetrate the neural membrane; these are voltage-gated calcium channels.

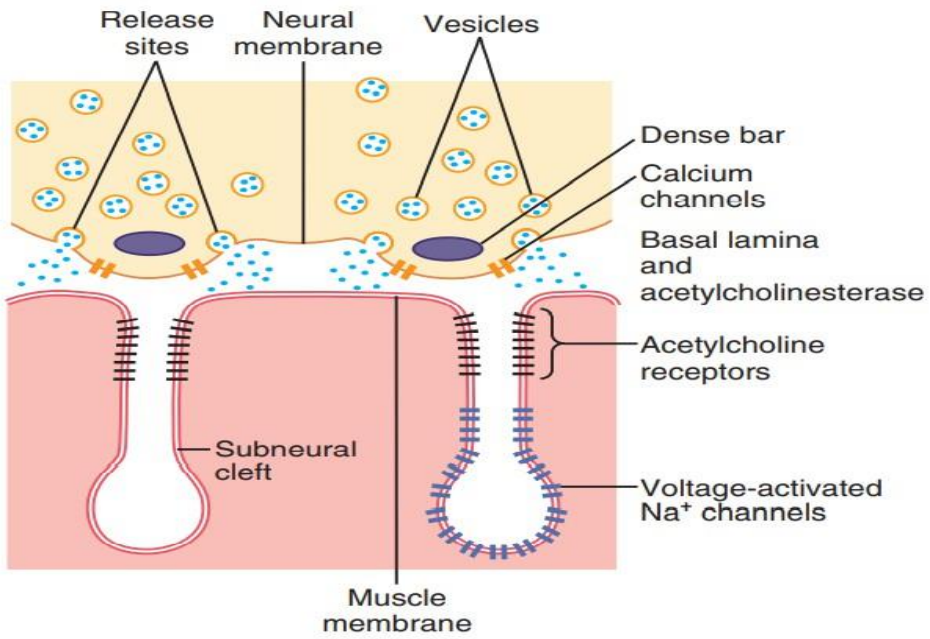
When an action potential spreads over the terminal, these channels open and allow calcium ions to diffuse from the synaptic space to the interior of the nerve terminal.

The nerve terminal contains axon terminals, which resemble end plates or buds on the muscle fiber.

As the action potential travels along the nerve cell , voltage-gated **sodium** channels facilitate depolarization, while voltage-gated **potassium** channels manage repolarization.

Upon reaching the nerve ending , voltage-gated **calcium** channels at the presynaptic terminal open due to changes in membrane potential.

This results in an influx of calcium ions (Ca^{2+}) into the nerve ending , further depolarizing it.

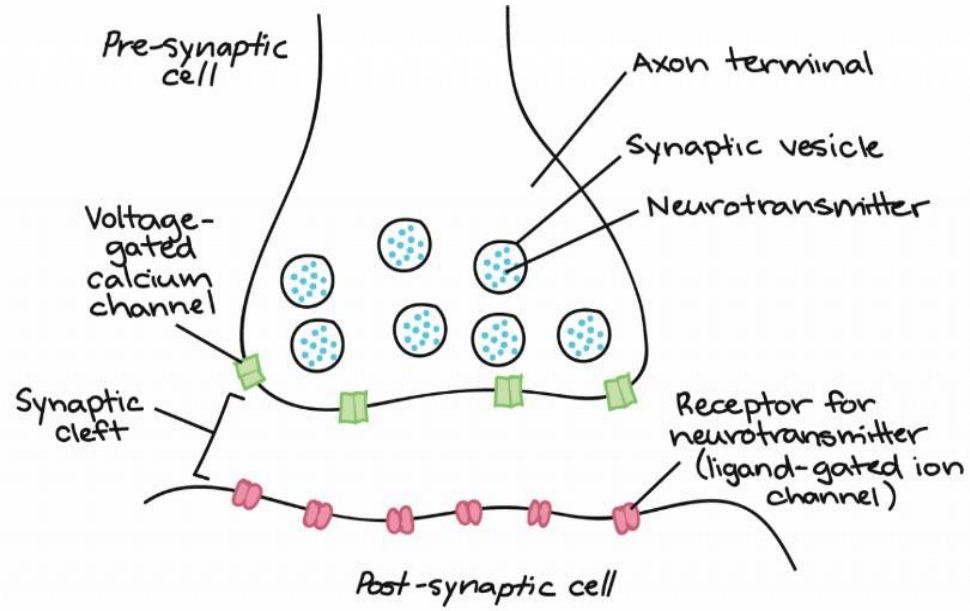


Acetylcholine

In the axon terminal are many mitochondria that supply ATP, the energy source that is used for synthesis of an excitatory transmitter, acetylcholine.

The acetylcholine in turn excites the muscle fiber membrane.

Acetylcholine is synthesized in the cytoplasm of the terminal, but it is absorbed rapidly into many small synaptic vesicles which are normally in the terminals of a single end plate.



Release of Acetylcholine

The calcium ions are believed to activate Ca2+-calmodulin dependent protein kinase, which, in turn, phosphorylates **synapsin** proteins that anchor the acetylcholine vesicles to the cytoskeleton of the presynaptic terminal.

This process frees the acetylcholine vesicles from the cytoskeleton and allows them to move to the active zone of the presynaptic neural membrane adjacent to the dense bars.

The vesicles then dock at the release sites, fuse with the neural membrane, and empty their acetylcholine into the **synaptic space** by the process of exocytosis.

Acetylcholine-gated channels

Acetylcholine receptors in the muscle fiber membrane are acetylcholine-gated ion channels.

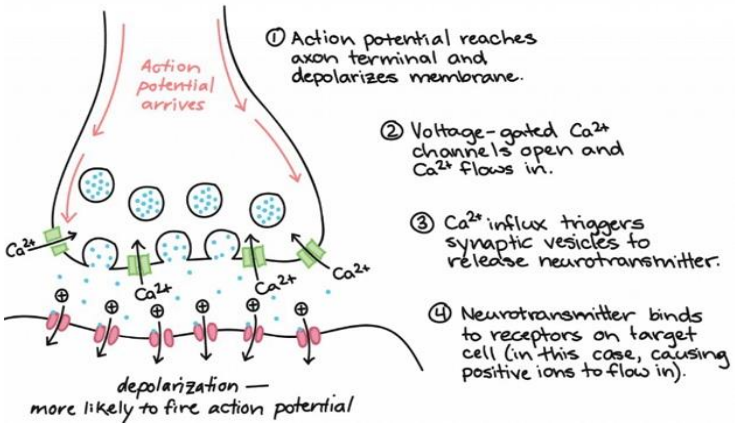
They are located almost entirely near the mouths of the sub-neural clefts lying immediately below the dense bar areas, where the acetylcholine is emptied into the synaptic space.

The released acetylcholine diffuses across the synaptic cleft and binds to **ligand-gated sodium channels** on the muscle fiber membrane (postsynaptic cell).

Two acetylcholine molecules bind to the two **alpha** subunits of the sodium channel, causing it to open.

Sodium ions (Na^+) enter the muscle fiber, leading to depolarization and the generation of an action potential in the muscle.

- Acetylcholine does not enter the muscle fiber but instead activates sodium channels, allowing sodium ions to enter and initiate depolarization.



The ligand-gated sodium channels are only located at the neuromuscular junction (NMJ), specifically at the synapse where the nerve meets the muscle fiber. These channels are NOT distributed along the entire length of the muscle fiber.

At the NMJ, the nerve ending makes contact with the muscle fiber at a central location rather than along its entire surface. This ensures that when an action potential is triggered, it spreads in both directions along the muscle fiber.

Once the initial depolarization occurs at the NMJ due to the opening of ligand-gated sodium channels, the rest of the muscle fiber relies on voltage-gated sodium channels to propagate the action potential.

Acetylcholine esterase

The acetylcholine, once released into the synaptic space, continues to activate the acetylcholine receptors as long as the acetylcholine persists in the space. However, it is removed rapidly by two means:

(1) Most of the acetylcholine is destroyed by the enzyme **acetylcholinesterase**, which is in the synaptic space. It destroys acetylcholine a few milliseconds after it has been released from the synaptic vesicles.

(2) a small amount of acetylcholine diffuses out of the synaptic space and is then no longer available to act on the muscle fiber membrane.

The short time that the acetylcholine remains in the synaptic space—a few milliseconds at most—normally is sufficient to excite the muscle fiber. Then the rapid removal of the acetylcholine prevents continued muscle re-excitation.

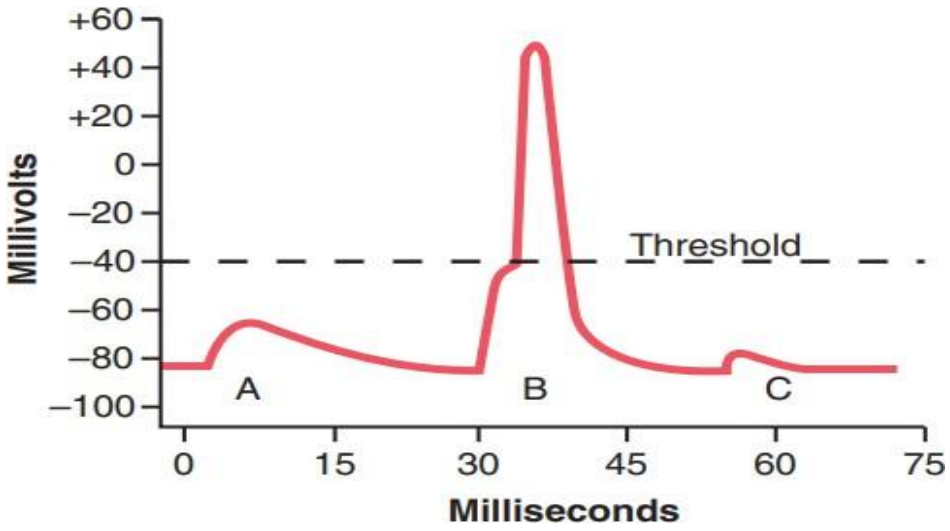
(3) Reuptake into the Presynaptic Cell. Some sources suggest that acetylcholine or its breakdown products (particularly choline) can be taken back up by the presynaptic neuron. This

reuptake mechanism allows the nerve cell to store and recycle choline for the synthesis of new acetylcholine molecules, ensuring availability for the next action potential.

End plate potential

The principal effect of opening the acetylcholine-gated channels is to allow large numbers of sodium ions to pour to the inside of the fiber, carrying with them large numbers of positive charges.

This action creates a local positive potential change inside the muscle fiber membrane, called the end plate potential. A sudden increase in nerve membrane potential of more than 20 to 30 millivolts is normally sufficient to initiate more and more sodium channel opening, thus initiating an action potential at the muscle fiber membrane.



Skeletal muscle action potential

Initiation and conduction of action potentials in nerve fibers applies equally to skeletal muscle fibers, except for quantitative differences. Some of the quantitative aspects of muscle potentials are as follows:

1. Resting membrane potential is about −80 to −90 millivolts in skeletal fibers—the same as in large myelinated nerve fibers. However, the value may reaches -70 in other nerve fibres.

2. Duration of action potential is 1 to 5 milliseconds in skeletal muscle—about five times as long as in large myelinated nerves.

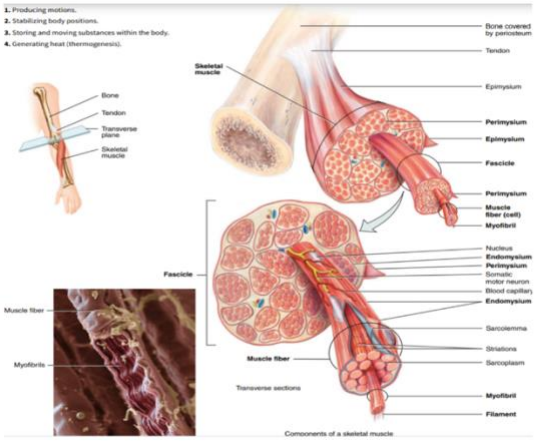
3. Velocity of conduction is 3 to 5 m/sec—about 1/13 the velocity of conduction in the large myelinated nerve fibers that excite skeletal muscle

(The duration of the action potential in a muscle fiber is longer than that in a nerve cell → The conduction velocity in a nerve cell is greater than that in a muscle cell).

Skeletal muscles

The bone is connected to the tendon, which, in turn, is attached to the muscle. A muscle is made up of muscle fibers that are grouped together in **fascicles**.

Each muscle fiber consists of myofibrils that are bundled together.If we take a single myofibril and observe it in a longitudinal view, we can see its structure, which includes various components crucial for skeletal muscle function, such as the **sarcomere** (to be discussed) and other elements.



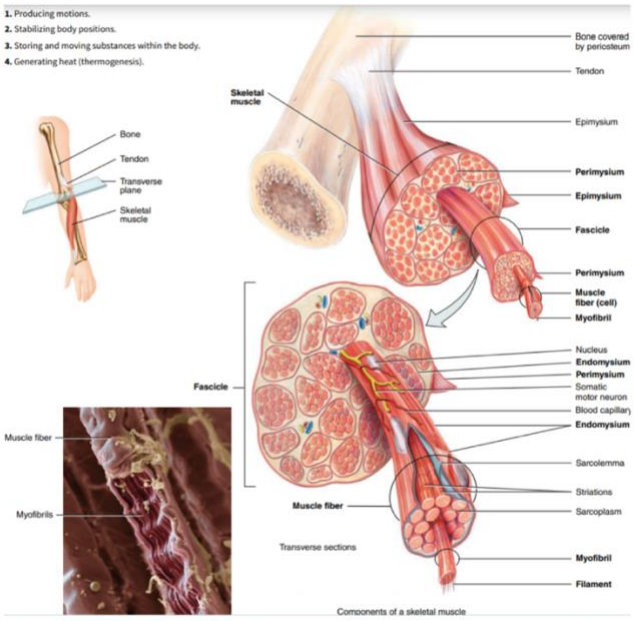
Skeletal muscle

Physiological anatomy of skeletal muscles

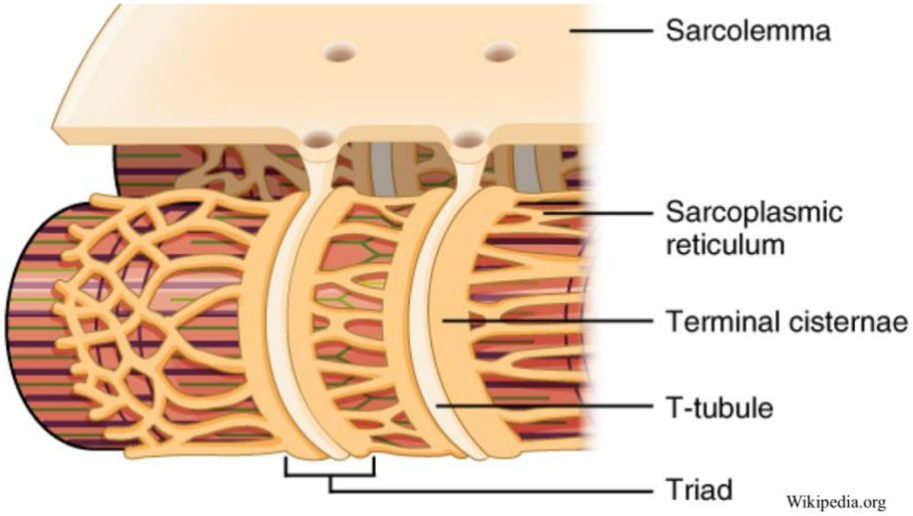
- In 98%of muscle fibers in skeletal muscles, each fiber innervated by one nerve ending, located near the middle of the fiber.

- Each muscle fiber contains several hundred to several thousand **myofibrils.**

- Each myofibrils is composed of thousands of **myosin** and **actin filaments**.



Skeletal muscle



- ✓ Muscle fibers contain multiple myofibrils, which are covered by a specialized plasma membrane known as the **sarcolemma**

- ✓ The **sarcoplasm** (refers to the cytoplasm within the muscle fiber) contains large quantities of potassium, magnesium, and phosphate, plus multiple protein enzymes. Also present are tremendous numbers of mitochondria that lie parallel to the myofibrils.

- ✓ The muscle fiber also contains a modified endoplasmic reticulum called **The sarcoplasmic reticulum** has a special organization that is extremely important in regulating calcium storage, release, and reuptake and therefore muscle contraction.

  Calcium is the most important ion in the contraction process.

T tubules

The sarcolemma has indentations that allow the action potential to reach all the myofibrils composing the muscle fiber upon arrival. These indentations are called T-tubules (transverse tubules).

The skeletal muscle fiber is so large that action potentials spreading along its surface membrane cause almost no current flow deep within the fiber.

Maximum muscle contraction, however, requires the current to penetrate deeply into the muscle fiber to the vicinity of the separate myofibrils.

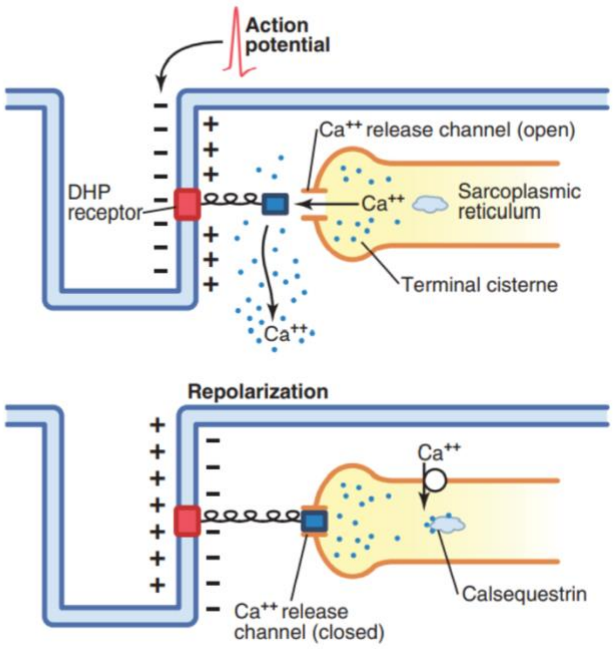
This penetration is achieved by transmission of action potentials along transverse tubules (T tubules) that penetrate all the way through the muscle fiber from one side of the fiber to the other.

The T tubule action potentials cause release of calcium ions inside the muscle fiber in the immediate vicinity of the myofibrils, and these calcium ions then cause contraction. This overall process is called excitation-contraction coupling.

Also, where the T tubules originate from the cell membrane, they are open to the exterior of the muscle fiber. Therefore, they communicate with the extracellular fluid surrounding the muscle fiber and contain extracellular fluid in their lumens.

The sarcoplasmic reticulum

Calcium release from SR

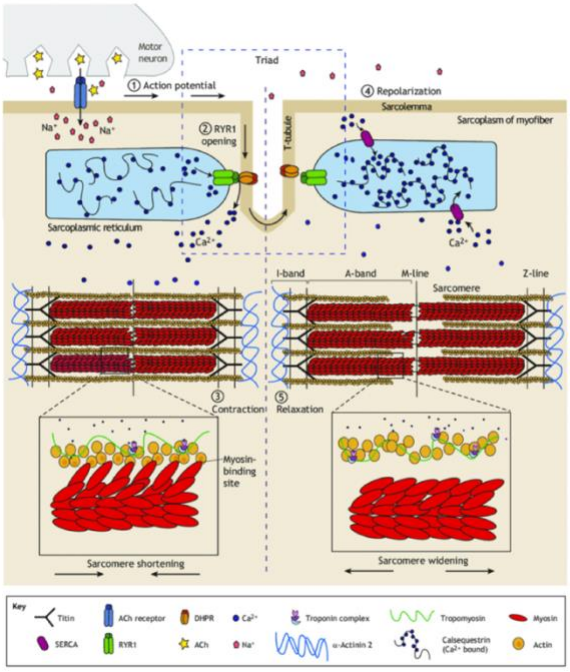


- The action potential of the T tubule causes current flow in to sarcoplasmic reticular cisternae where they abut the T tubule.
- As the action potential reaches the T tubule, the voltage change is sensed by dihydropyridine receptors (DHP) that are linked to calcium release channels, also called ryanodine receptor channels (RYR), in the adjacent sarcoplasmic reticular cisternae. (بكونوا مثل المسننات)
- Activation of dihydropyridine receptors triggers the opening of the calcium release channels in the cisternae, as well as in their attached longitudinal tubules. These channels remain open for few milliseconds, releasing calcium ions into the sarcoplasm surrounding the myofibrils and causing contraction.

Sarcoplasmic reticulum is composed of two major parts:

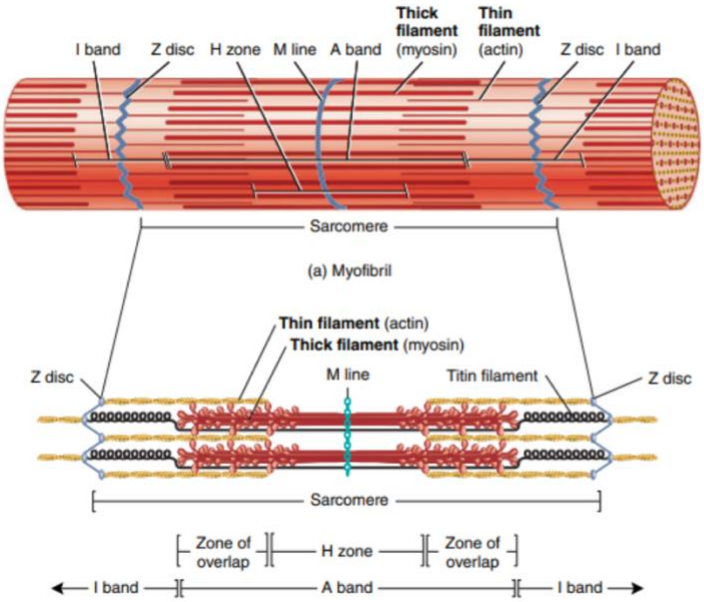
- ✓ large chambers called **terminal cisternae** that abut the T tubules.
- ✓ **long longitudinal tubules** that surround all surfaces of the actual contracting Myofibrils functions primarily as a calcium storage site.

Calcium release from SR



Fusto et al. The use of models to understand core myopathies, 2019.

- ✓ During repolarization (muscle relaxation), Ca^{2+} is actively pumped back into the sarcoplasmic reticulum (SR),This calcium reuptake reduces the Ca^{2+} concentration in the sarcoplasm, leading to muscle relaxation.



Sarcomere

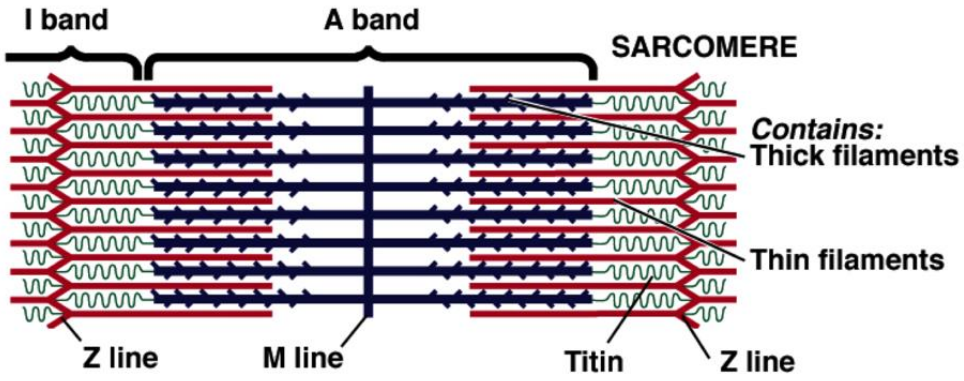
- The sarcomere is the basic contractile unit of the myofibril.
- Myofibrils are composed of multiple sarcomeres arranged in a repeating pattern along their length.

Structure of the Sarcomere:

The Z-disk (Z-line) marks the boundary of each sarcomere,It has a zigzag appearance and serves as an anchor for thin filaments (actin).

The region between two Z-disks forms one sarcomere, which contains the essential contractile proteins responsible for muscle contraction, Within each sarcomere, thin filaments (actin) and thick filaments (myosin) interact to generate contraction.

The sliding of these filaments past one another (rather than shortening) is the primary mechanism of muscle contraction,When contraction occurs within individual sarcomeres, it leads to the overall contraction of the muscle.



University of Jordan

The sarcomere consists of specific regions based on the arrangement of actin (thin) and myosin (thick) filaments:

1. I-band:

• Contains only thin filaments (actin).

• Appears as a light region under a microscope.

• Spans across two adjacent sarcomeres, and is divided by the Z-disk.

2. A-band:

• Contains the entire length of the thick filaments (myosin).

• Includes both overlapping and non-overlapping regions of actin and myosin.

• Appears dark under a microscope.

3. H-zone:

•The central region of the A-band, where only myosin is present (no actin overlap).

• Becomes narrower during contraction as actin filaments slides inward.

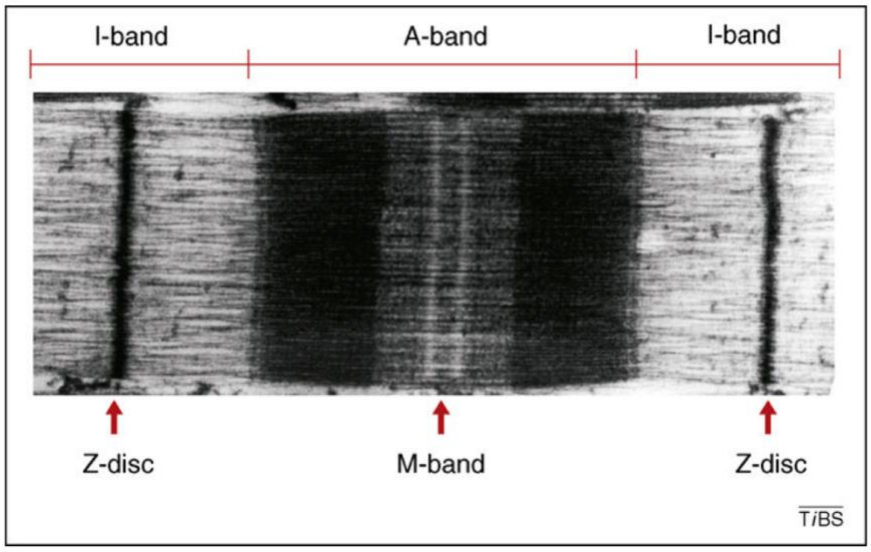
4. M-line:

• A protein-rich region in the center of the H-zone, serving as an attachment site for myosin filaments.

5. Z-disk (Z-line):

• Defines the boundary of each sarcomere.

• Anchors the thin filaments (actin) and connects adjacent sarcomeres.



Types of Sarcomere Proteins:

1. Structural Proteins → Maintain sarcomere organization.

•The side-by-side relationship between the myosin and actin filaments is maintained by a large number of filamentous molecules of a protein called **titin**.

•Springy titin molecules act as a framework that holds the myosin and actin filaments horizontally in place so that the contractile machinery of the sarcomere will work.

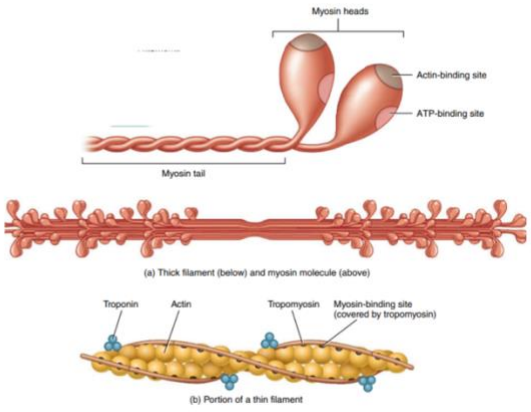
2. Regulatory Proteins → Control contraction

• Tropomyosin → Blocks myosin-binding sites on actin during rest

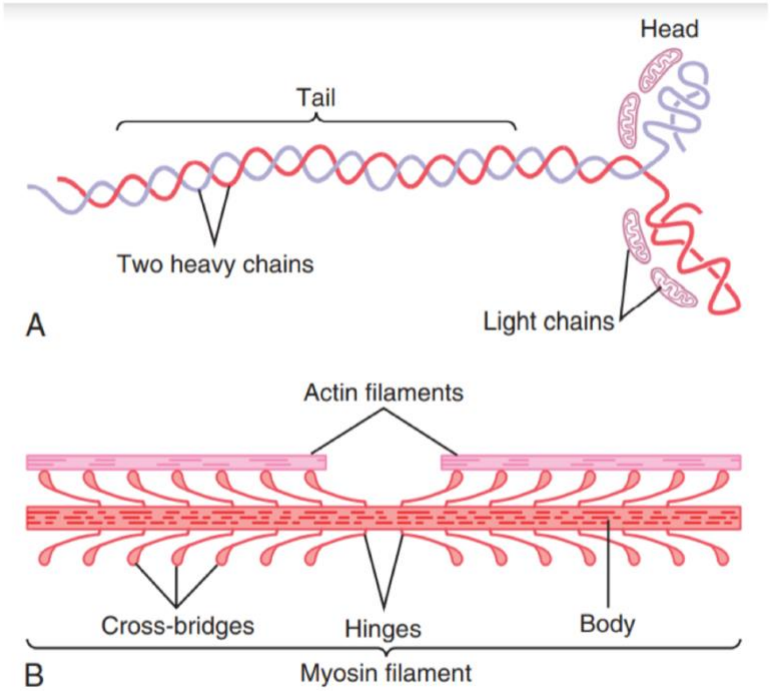
• Troponin → Binds Ca^{2+}, triggering contraction

3. Contractile Proteins → Generate force.

Thick and thin filaments



1.Myosin filament



• The protruding arms and heads together are called **cross-bridges.**

•Each cross-bridge is flexible at two points called **hinges**: one where the arm leaves the body of the myosin filament, and the other where the head attaches to the arm.

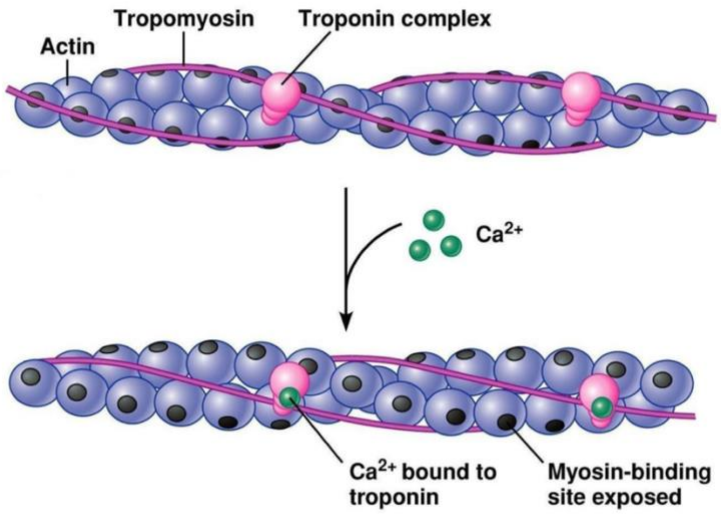
•The hinged arms allow the heads to be either extended far outward from the body of the myosin filament or brought close to the body.

•The hinged heads in turn participate in the actual contraction process.

•Another feature of the myosin head that is essential for muscle contraction is that it functions as an ATPase enzyme.

•This property allows the head to cleave ATP and use the energy derived from the ATP's high-energy phosphate bond to energize the contraction process.

2.Actin Filament



•Actin Filaments Are Composed of Actin, Tropomyosin, and Troponin.

•The backbone of the actin filament is a double-stranded F-actin protein molecule.

•Each strand of the double F-actin helix is composed of polymerized G-actin molecules.

In the relaxation state , The myosin binding site (Actin active site,The dark points) it's covered by protein called **Tropomyosin.**

•These molecules are wrapped spirally around the sides of the F-actin helix.

•In the resting state, the tropomyosin molecules lie on top of the active sites of the actin strands so that attraction cannot occur between the actin and myosin filaments to cause contraction, and they bind to Troponin.

Troponin

•A complex of three loosely bound protein subunits, each of which plays a specific role in controlling muscle contraction:

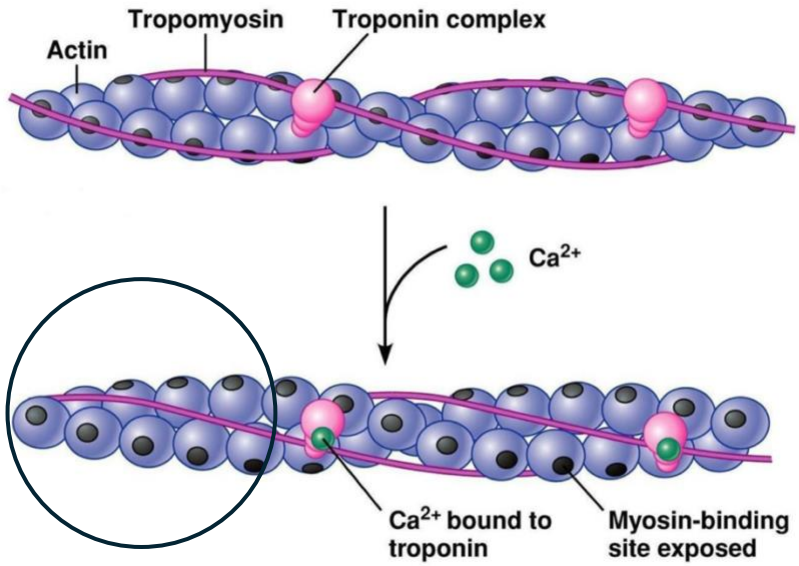
•Troponin I has a strong affinity for actin,troponin T for tropomyosin, and troponin C for calcium ions.

•This complex is believed to attach the tropomyosin to the actin.

Effect of Calcium on Myosin-Actin binding

•The strong affinity of the troponin for calcium ions is believed to initiate the contraction process.

•The affinity of the troponin for calcium ions is stronger than the affinity of the troponin with Tropomyosin, so once the calcium binds to troponin, it will take its place.



Once this region is exposed, the myosin head will bind to it. The active site on the myosin head, known as the active binding site, interacts with the myosin binding site on the actin filament.

•In the presence of large amounts of calcium ions, the inhibitory effect of the troponin-tropomyosin on the actin filaments is itself inhibited. The mechanism of this inhibition is not known.

Additional Resources:

رسالة من الفريق العلمي:

1.1.Junqueira's Basic Histology Book

2. Structure of skeletal muscle fibers

3. Sarcomere contraction

(وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

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