**What is the nerve-muscle relationship?**

**What is the structure and function of the neural muscular junction?**

**What is the significance of the sliding filament theory?**

**What steps occur in a skeletal muscle contraction cycle?**

**What is the structure and function of a motor unit?**

# The Nerve-Muscle Relationship

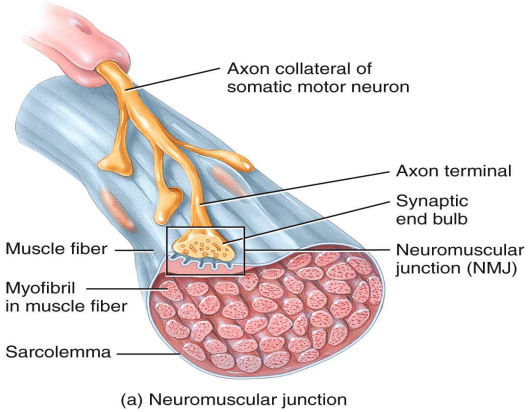
- To preform a voluntary skeletal muscle contraction, an action potential must be initiated for a specific muscle mapped on the motor strip (i.e. precentral gyrus).
- There are two neurons between the motor strip and the skeletal muscle, an upper motor neuron and lower motor neuron.
- If this nerve connection to the muscle is cut then the muscle is paralyzed
- **Denervation atrophy** – occurs when a somatic motor nerve to a skeletal muscle is cut. The muscle is paralyzed and over time muscle atrophy occurs (reduced sarcoplasm volume because myofibils break apart and they are not replaced)
  - This loss of sacroplasm volume (loss of contractile proteins) results in less strength
  - What is disuse atrophy?
  - How may this happen?

# The Nerve-Muscle Relationship

- Low motor neurons are the second segment to the skeletal muscle. The LMN may be either a spinal nerve or a cranial nerve. Both are considered LMN (see handout)
  - LMN's nerve cells' "somas" are in the brain-stem (if cranial nerves) or in spinal cord (if spinal nerve)
  - The terminal knobs of LMN's nerves synapse ("connect to") skeletal muscles
  - A single axon may form many branches at the distal end of the axon – each branch "synapse" with individual muscle fibers (terminal knobs) // all the muscle fibers controlled by this one pathway is call a motor unit
  - Each muscle fiber is only innervated by a single motor unit
  - What are upper motor neurons?

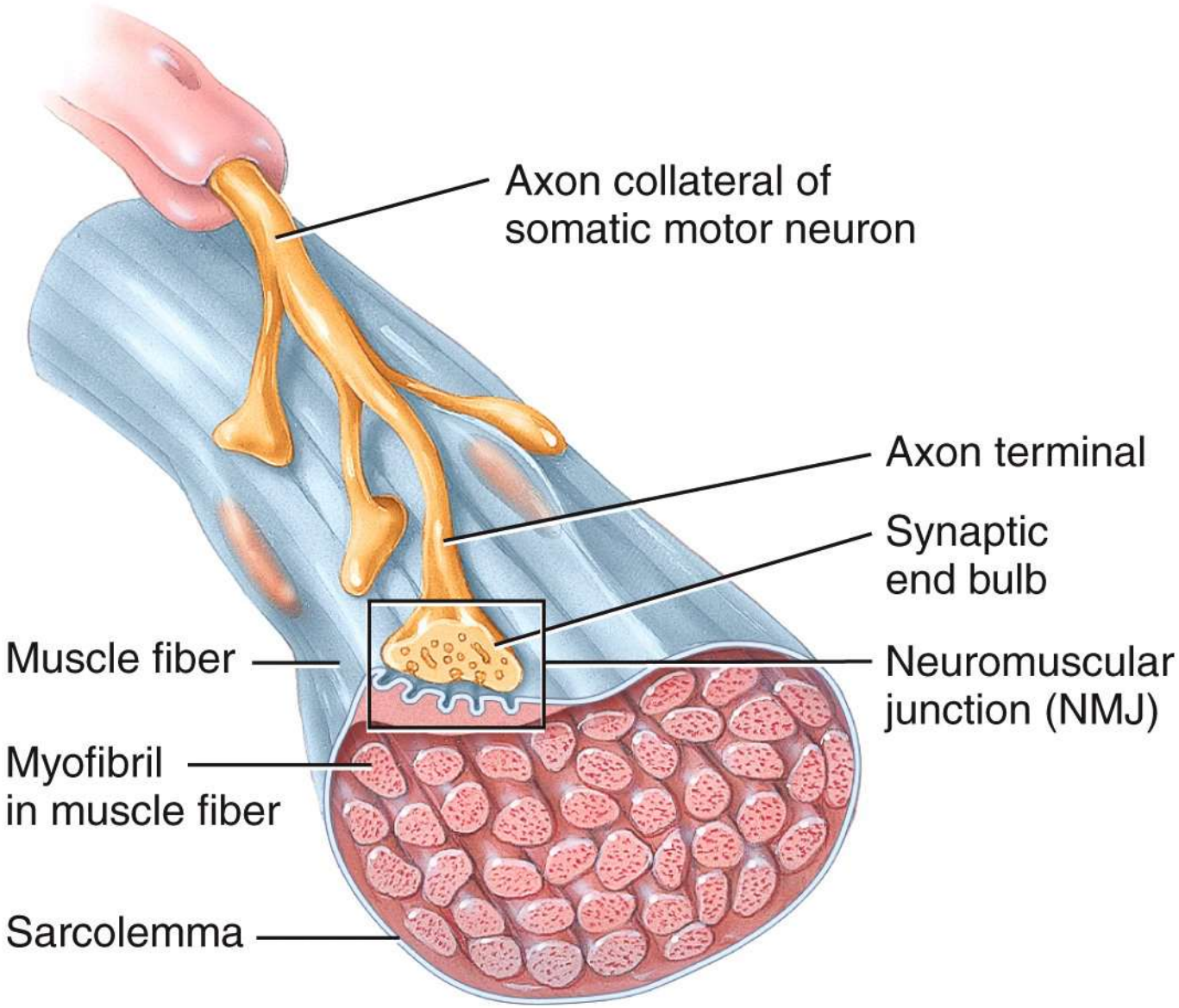
# The Neuromuscular Junction

- Synapse = the location where the terminal end of a nerve (the terminal knob) reaches the target tissue
  - three components of a synapse
    - pre-synaptic membrane
    - synaptic cleft
    - post-synaptic membrane



(a) Neuromuscular junction

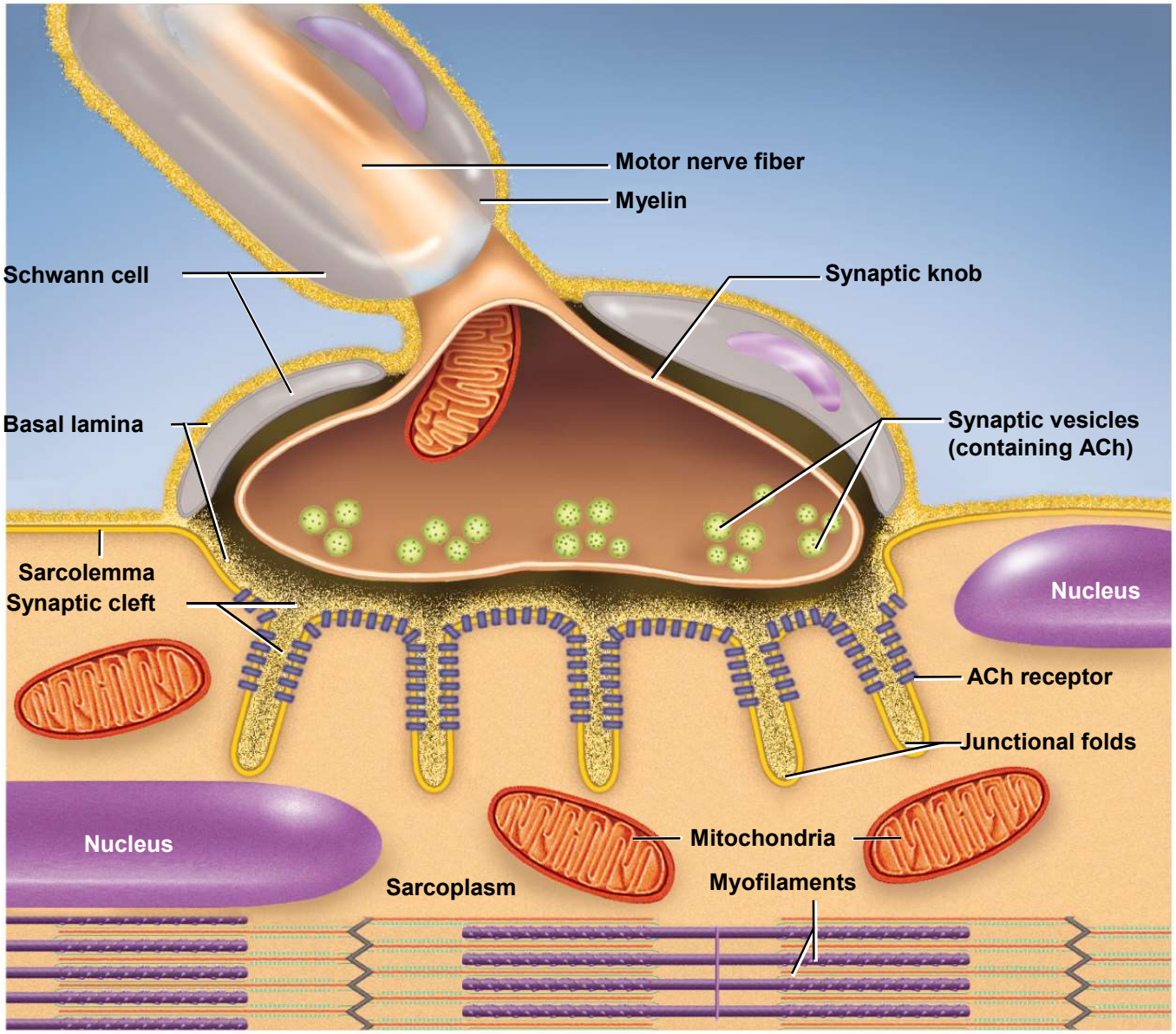
- Neuromuscular junction (NMJ) describes a special type of synapse
- This occurs when a spinal nerve "targets" a muscle fiber




(a) Neuromuscular junction

# The Neuromuscular Junction

*(You need to be able to draw and label this illustration)*



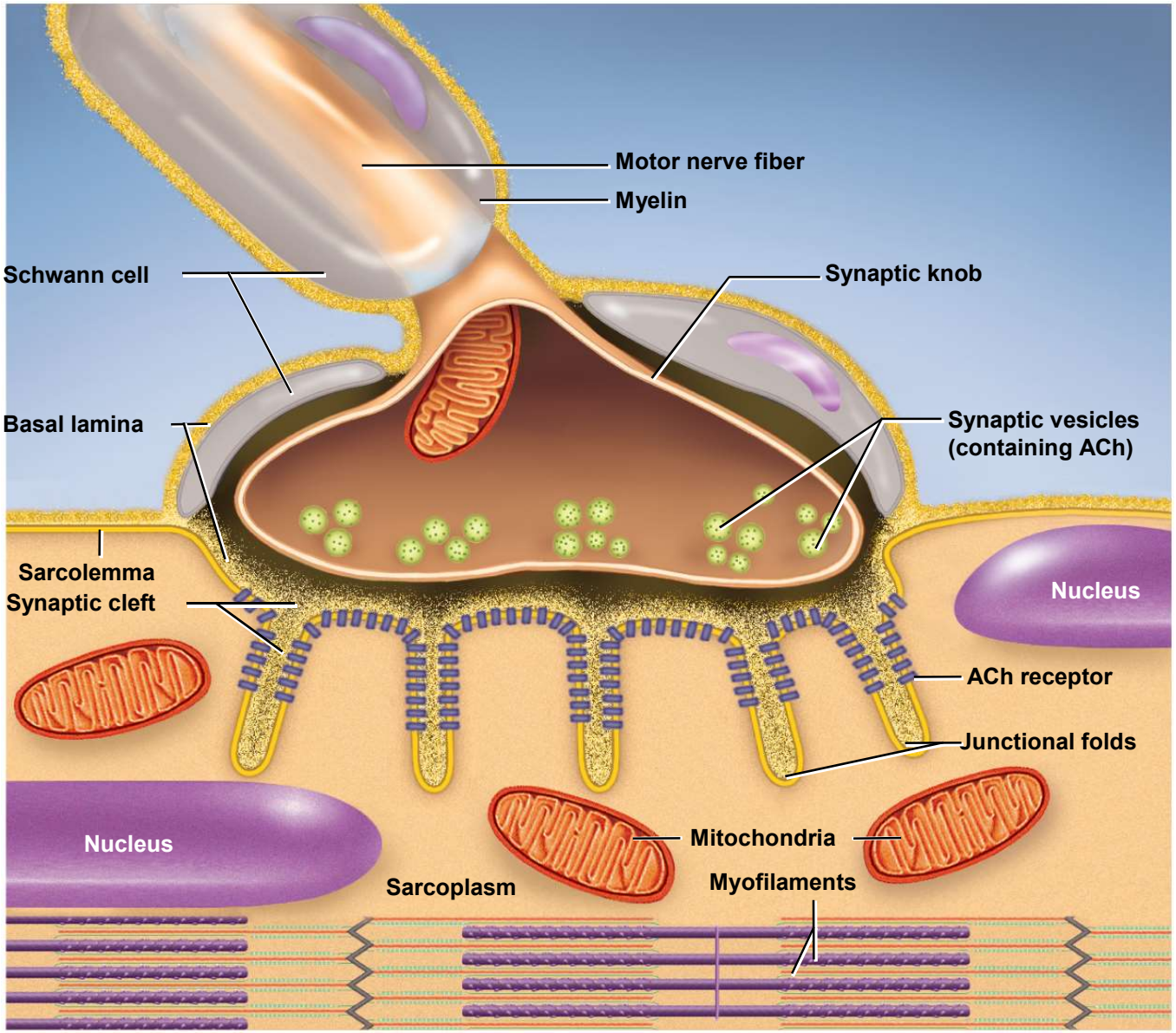
# Components of Neuromuscular Junction

- Synaptic knob - swollen end of terminal end of the nerve fiber
  - contains synaptic vesicles filled with acetylcholine (ACh)
  - synaptic vesicles undergo exocytosis releasing ACh into synaptic cleft
- Synaptic cleft - tiny gap between synaptic knobs and muscle sarcolemma
- Schwann cell - envelops & isolates all of the NMJ from surrounding tissue fluid

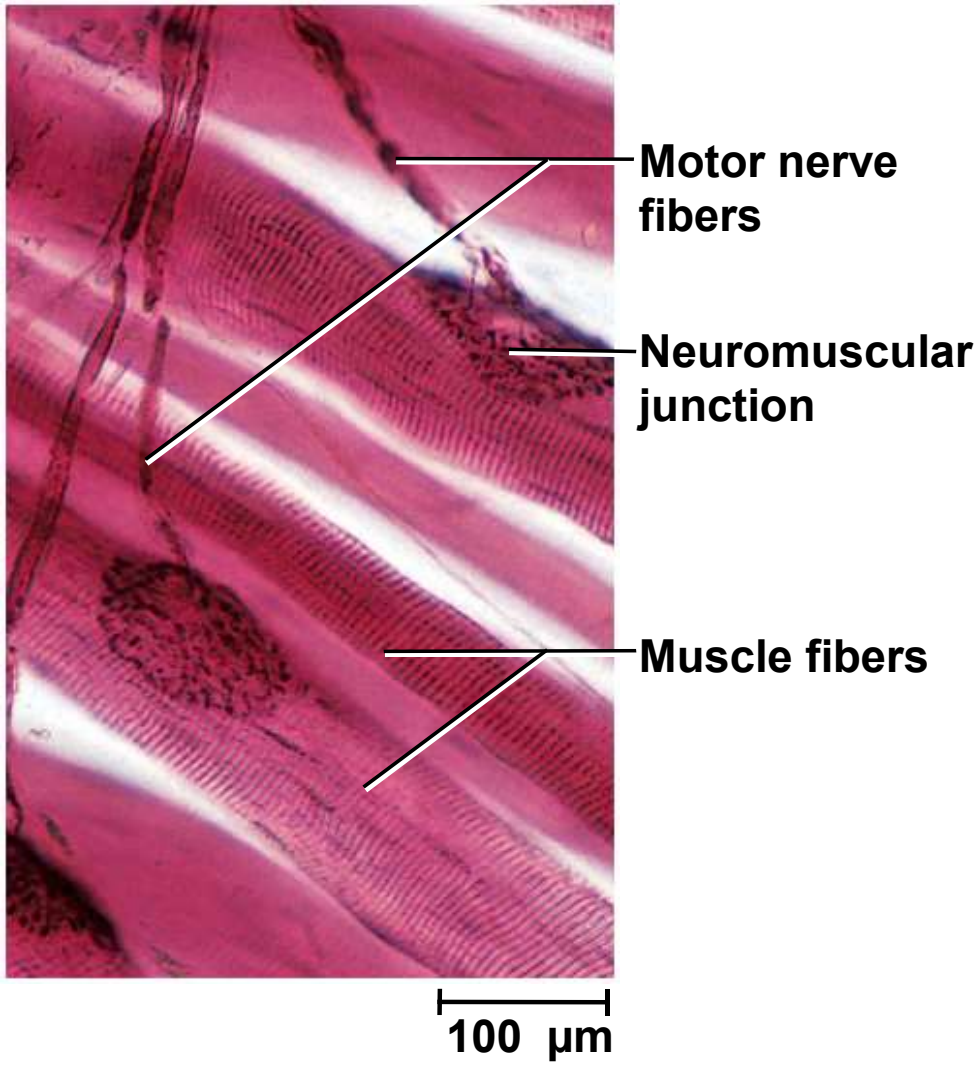
# Components of Neuromuscular Junction

- ACh receptors – 50 million protein receptors are built into muscle cell plasma membrane at synaptic jucntion
  - junctional folds of sarcolemma beneath synaptic knob // increases surface area holding ACh receptors
    - lack of receptors leads to paralysis in disease like myasthenia gravis
- Basal lamina - thin layer of collagen and glycoprotein separates Schwann cell and entire muscle cell from surrounding tissues
  - The enzyme acetylcholinesterase (AchE) is within the synaptic cleft. This enzyme degrades ACh in a sequence of events to stop muscle contraction and allows the muscle to relax.

# The Neuromuscular Junction



# Neuromuscular Junction



# What does it mean to be an excitable cell?

- Muscle and neurons are the only excitable cells. These are the only cells in the body able to move an "action potential" (i.e. current) across their plasma membranes

- As the current moves across the plasma membrane, the current is used to open transmembrane "gates" in the plasma membrane which initiate "down stream events". These are voltage regulated gates (Note: other gates maybe regulated by ligands or mechanical forces)

- Electrophysiology is the study of the electrical activity of cells

- Voltage = a separation of charge across the plasma membrane /// an electrical potential = separation of charge /// in cells it occurs across the plasma membrane with negative charge inside cell and positive charge outside the cell. All cells have a voltage difference across their plasma membrane, called the resting membrane potential.

- Current is the movement of charge. This is like the flow of electrons in a wire. In nerve and muscle cells the resting membrane potential voltage is reversed at a specific point, so the positive charge is now inside the cell and the negative charge is outside the cell. Then, at the point of voltage reversal, the voltage reversal moves from its origin across the plasma membrane. This is the action potential (the current flow).

- All living cells maintain a "resting membrane potential" which is about -90mV. The resting membrane potential is dependent upon the sodium-potassium ATP-ase pump.

- Action Potentials = Current = movement of electrical charge across surface of plasma membrane /// once an action potential is created the action potential will "flow" in one direction across the surface of the membrane

# Excitable Cells

- An excitable cell if not stimulated maintains a resting membrane potential
  - more anions (negative ions) right next to the inside of the plasma membrane than on the outside // highly negatively charge cytoplasmic proteins contribute to this phenomena
  - in the inter cellular fluid, there are anions such as proteins, nucleic acids, and phosphates that cannot penetrate the plasma membrane /// these anions help to make the inside of the plasma membrane negatively charged by comparison to its outer surface
  - there are excess sodium ions ($Na^+$) in the extracellular fluid (ECF)
  - there are excess potassium ions ($K^+$) in the intracellular fluid (ICF)
  - The net effect of all these ions is to create a voltage difference across the plasma membrane

# Electrically Excitable Cells

- **Muscle and nerve cells in a resting membrane potential state may be stimulated to generate an action potential**
  - quick up-and-down voltage shift from the negative RMP to a positive value, and back to the negative value again.
  - seen in an **active stimulated cell**
  - an action potential at one point on a plasma membrane causes another one to happen immediately in front of it
  - this then triggers another one a little farther along and so forth (appropriates a wave of negativity moving across the plasma membrane) – propagates the action potential across surface
- **RMP (resting membrane potential)** is a stable voltage potential seen in all cells but only in muscle or nerve cell may a RMP be changed into an action potential

# What steps occur to change a resting potential into an action potential in excitable cells?

- Sodium ion gates open in the plasma membrane (these are voltage regulated gates!) Remember, sodium concentration is higher outside of the cell!

- Sodium ion diffuses instantly down their concentration gradient into the cell

- These cations override the negative charges in the cytosol

- This causes "depolarization" - inside of the plasma membrane becomes briefly positive

- Now, the $Na^+$ gates close and $K^+$ gates open. Remember, potassium ion concention is higher inside the cell.

- Now $K^+$ to rush out of cell – making interior once again more negative (i.e. repolarize cytosol to restore negative state) /// repelled by the positive sodium charge and partly because of its concentration gradient

- The sodium-potasium-ATPase pump moves ions (Na and K) across the membrane to readjust the concentration of sodium and potassium across the plasma membrane and restore the resting membrane potential

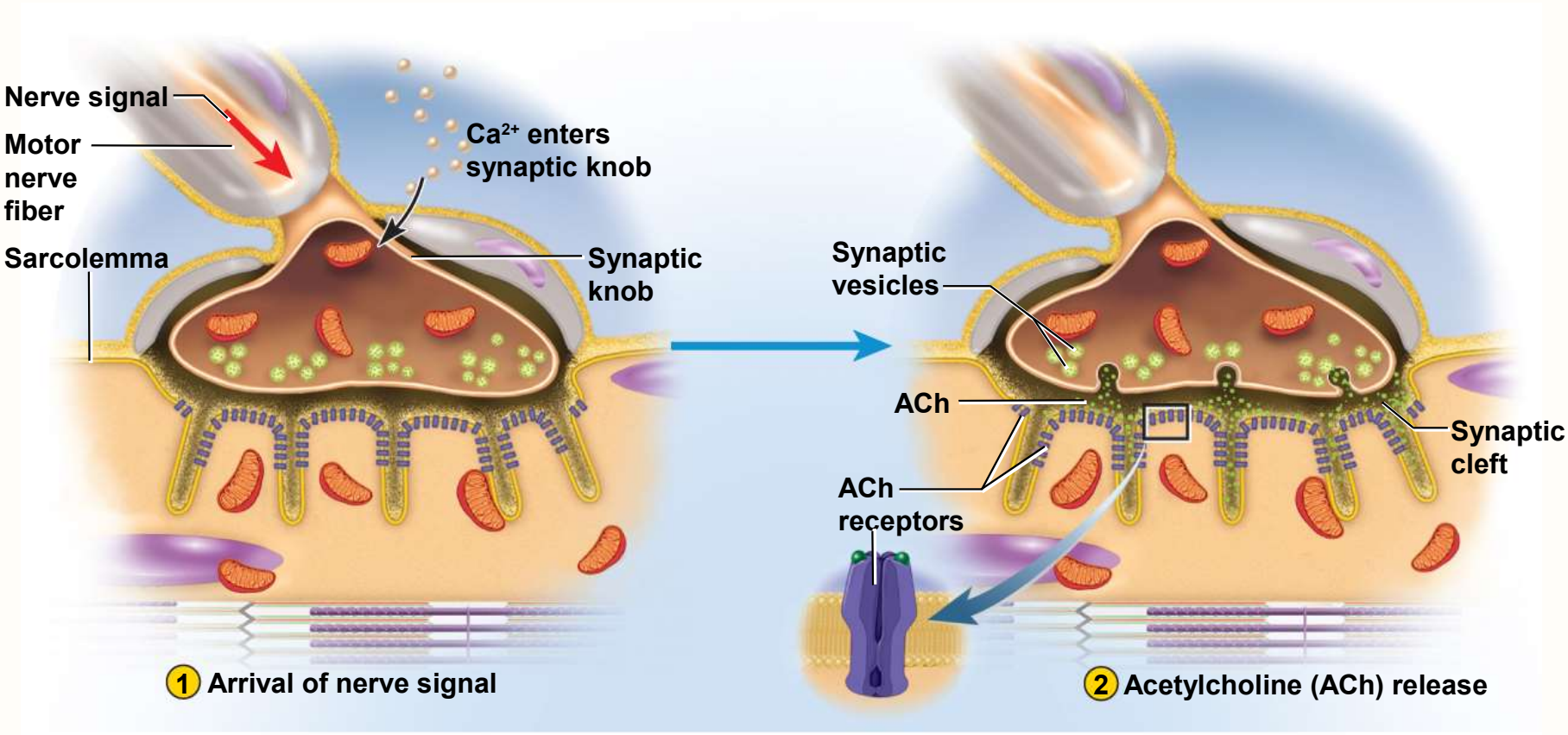
# The Skeletal Muscle's Sliding Filament Theory

- In the early 1950s, a hypothesis to explain skeletal muscle contractions was to think of proteins folding like an accordion
- With the discovery of the electron microscope, scientist could “see” the thin and thick proteins inside the skeletal muscle.
- These proteins did not shorten during contraction (no accordion like action)
- Therefore, the original hypothesis was wrong!
- A new hypothesis suggested the muscle fibers shortened by having the proteins sliding across each other.
- This hypothesis was proven to be “true” and is now called the sliding filament theory.

# Sliding Filament Theory's Contraction & Relaxation

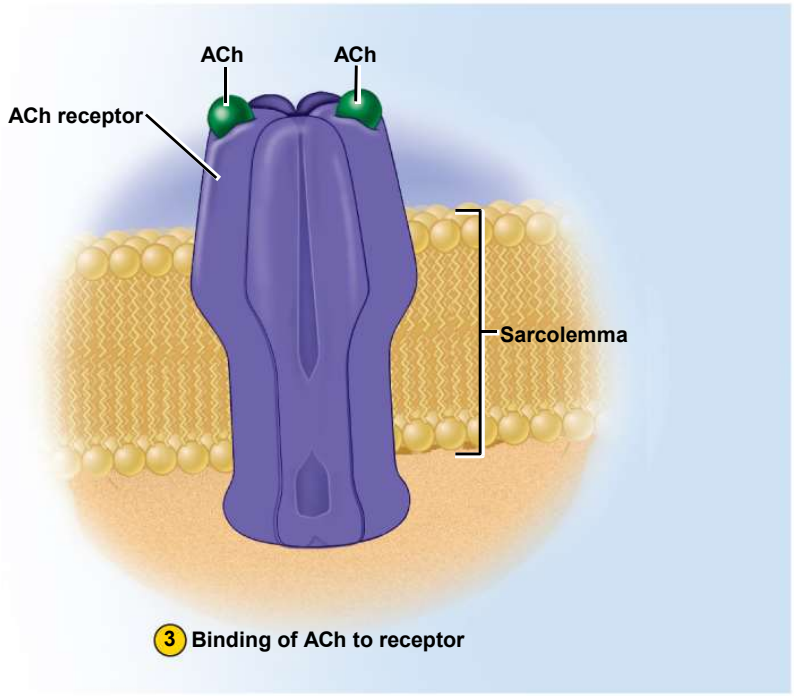
- **Four phases of a skeletal muscle contraction cycle**
  - **Excitation >** the process in which nerve action potentials lead to a muscle action potentials
  - **Excitation-contraction coupling >** moves the muscle membrane's (sarcolemma) action potential to the membrane of the sarcoplasmic reticulumactivation where voltage regulated gates open to release calcium // calcium release allows the protein (the myofilaments), to interact causing a power stroke
  - **Contraction (the power stroke) >** step in which the muscle fiber develops tension and the contractile proteins “slide” over each other
  - **Relaxation >** after tension is created, events occur to allow a muscle fiber to lose tension and return to its resting length

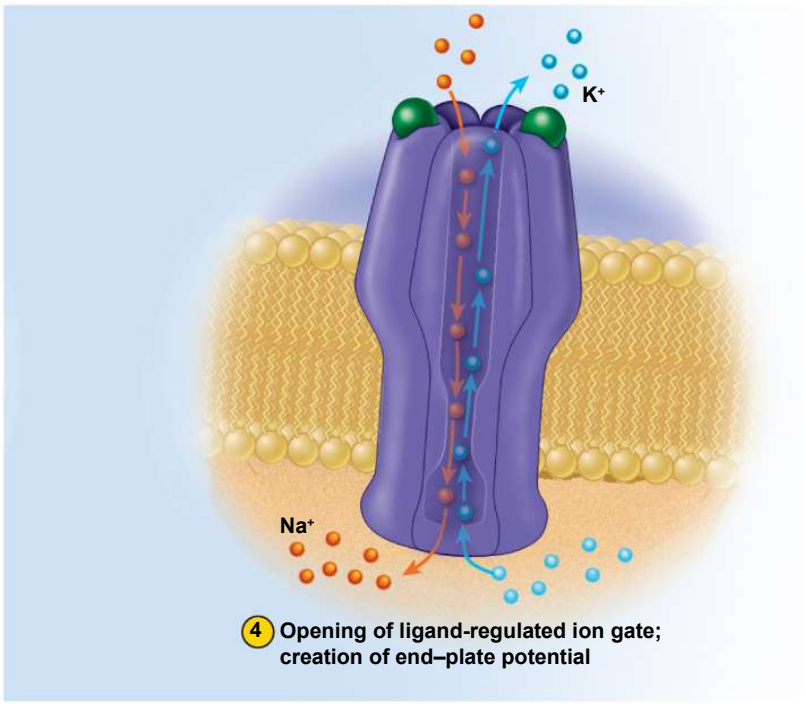
# Start of Excitation (steps 1 and 2)




- nerve signal at end of axon opens voltage regulated calcium gate channels at distal end of synaptic knob
- calcium stimulates exocytosis of ACh from synaptic vesicles
- ACh released into synaptic cleft

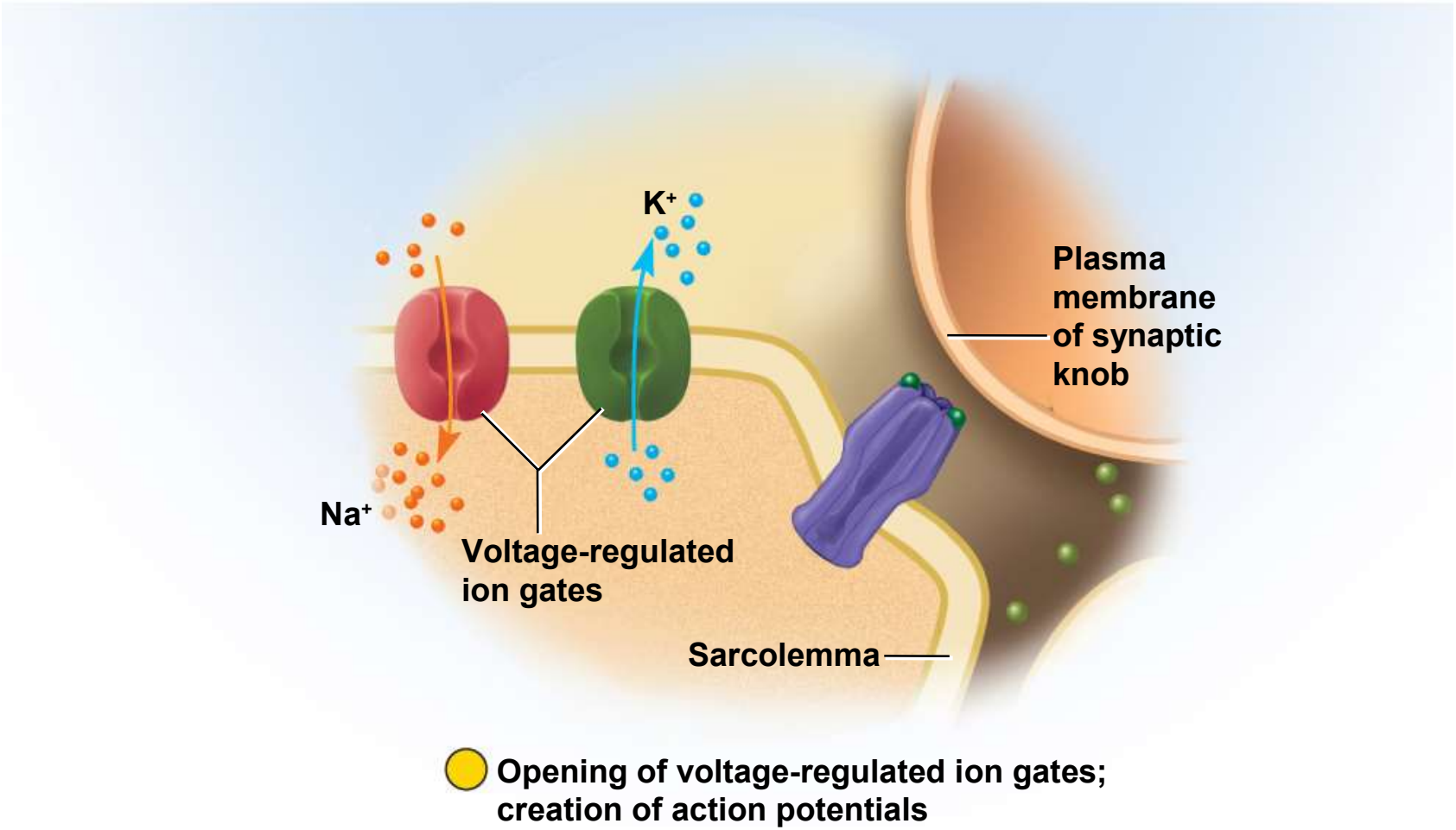
# Excitation (steps 3 and 4)



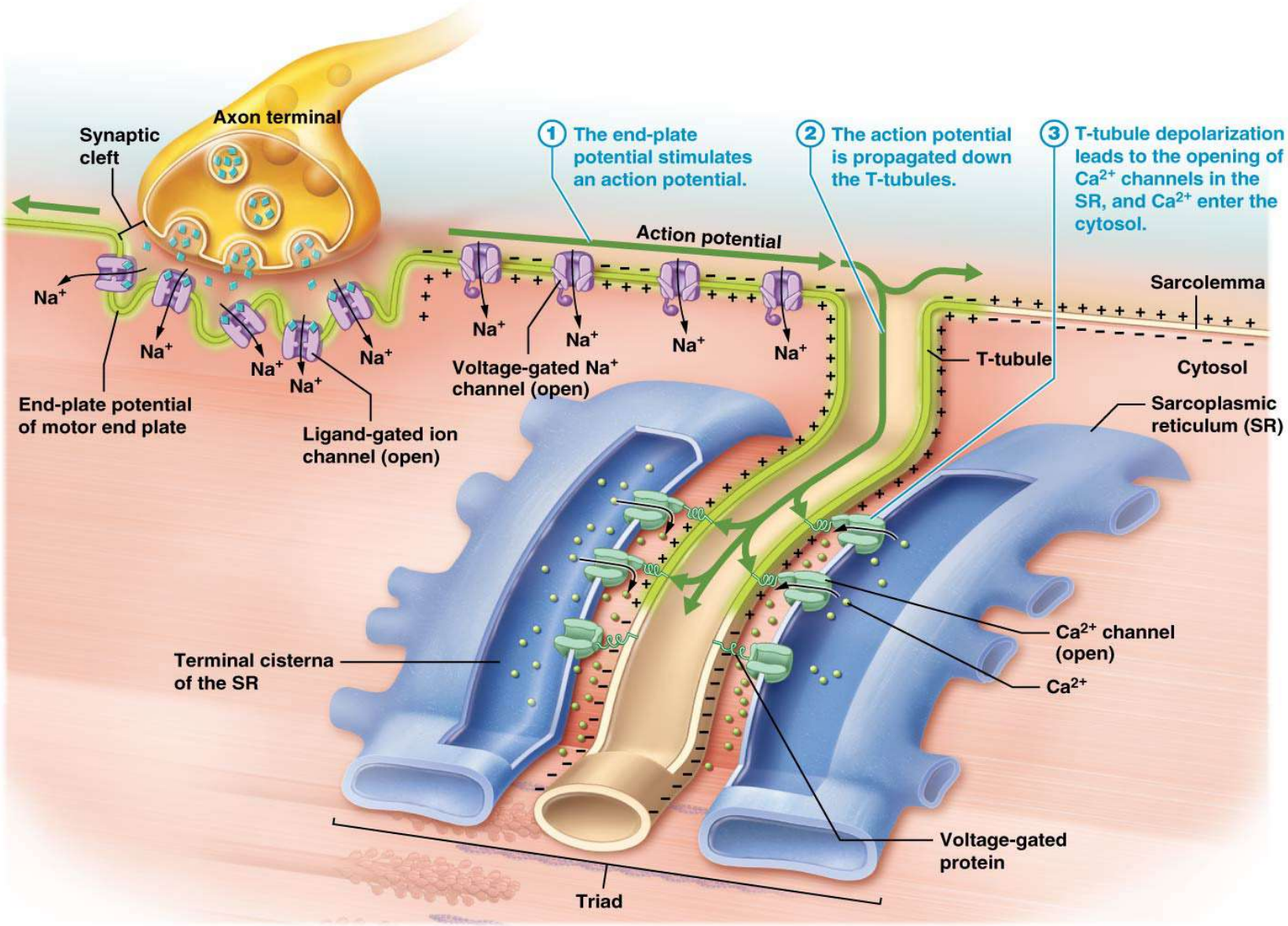

- two ACh molecules bind to each receptor protein on post synaptic sarcoplasm, this opens ligand regulated $Na^+$ and $K^+$ channels. (i.e. Ach is the ligand)
- $Na^+$ first ion to move through channel and enters interior of cell - shifting the RMP /// goes from -90mV to +75mV - this depolarizes sarcoplasm
- then $K^+$ exits the cell and RMP returns to -90mV
- quick voltage shift is called the **end-plate potential (EPP)** (this is a type of action potential)

# Completion of Excitation (step 5)

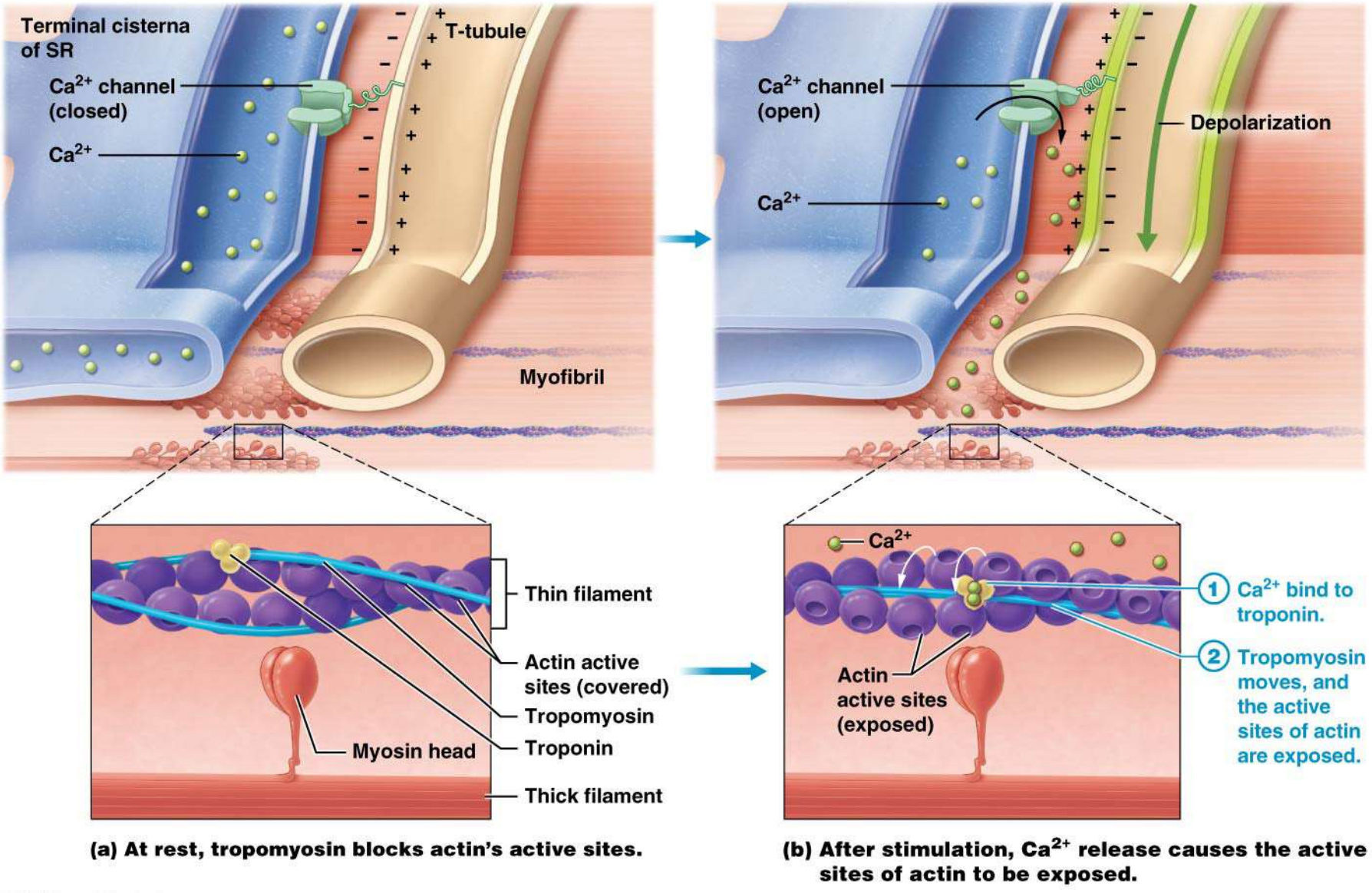



Voltage change caused by ligand (within end-plate region) creates an action potential that spreads to nearby voltage regulated Na and K gated channels just outside end-plate. Now the AP opens first the Na voltage regulated gate follow by the K voltage regulated gate opening. This produces a second action potential just outside of the neuromuscular junction. This "action potential" then spreads over the entire muscle surface.

# Excitation-contraction coupling: events at the sarcolemma and sarcoplasmic reticulum.



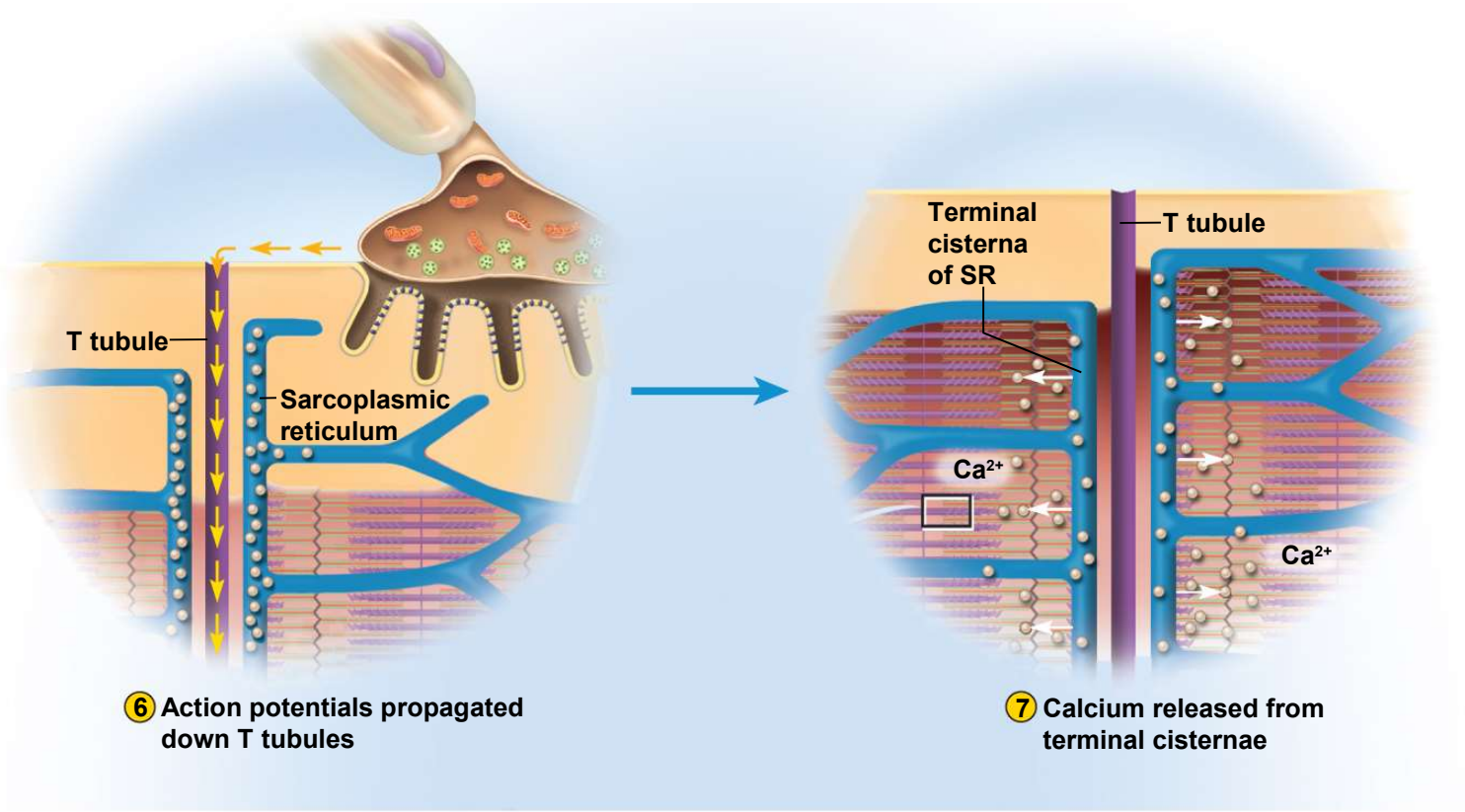
# Excitation-contraction coupling: preparation for contraction (regulatory events at the myofibril)




(a) At rest, tropomyosin blocks actin's active sites.

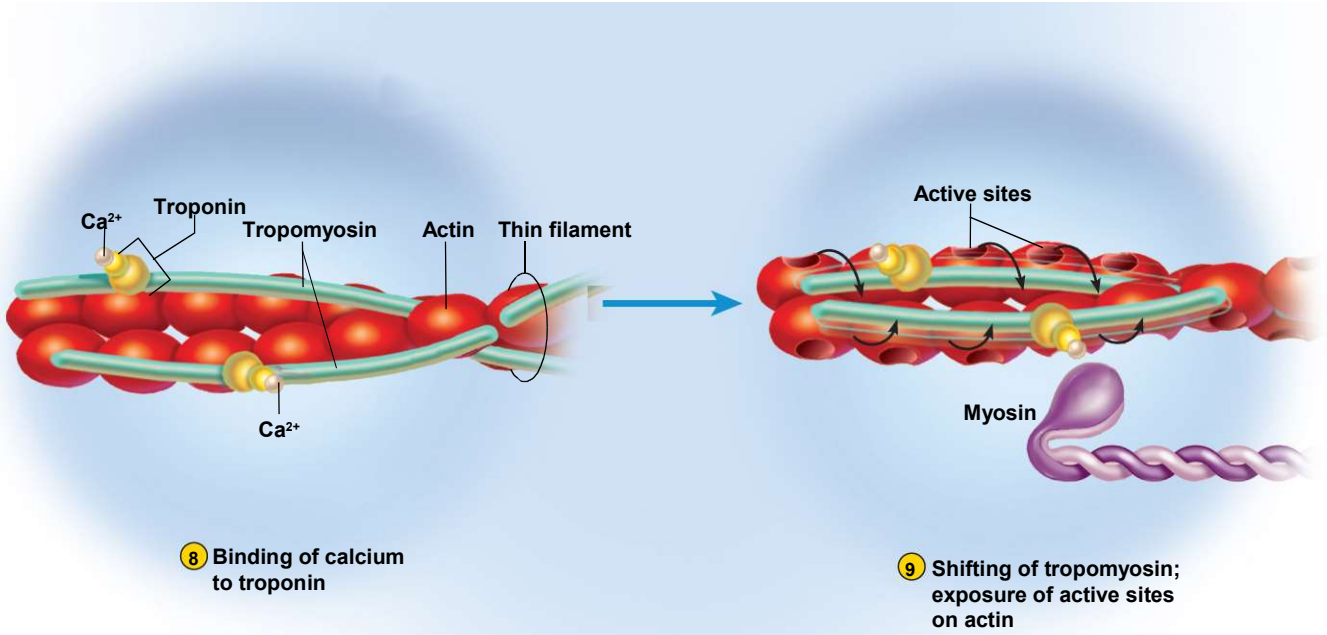
(b) After stimulation, $Ca^{2+}$ release causes the active sites of actin to be exposed.

# Excitation-Contraction Coupling
## (steps 6 and 7)




- action potential (AP) spreads from sarcoplasm into T tubules
- AP flows from T tubules to sarcoplasmic reticulum
- AP opens voltage regulated gated calcium ion channels in SR
- $Ca^{+2}$ diffuse into the cytosol

# Excitation-Contraction Coupling
## (steps 8 and 9)



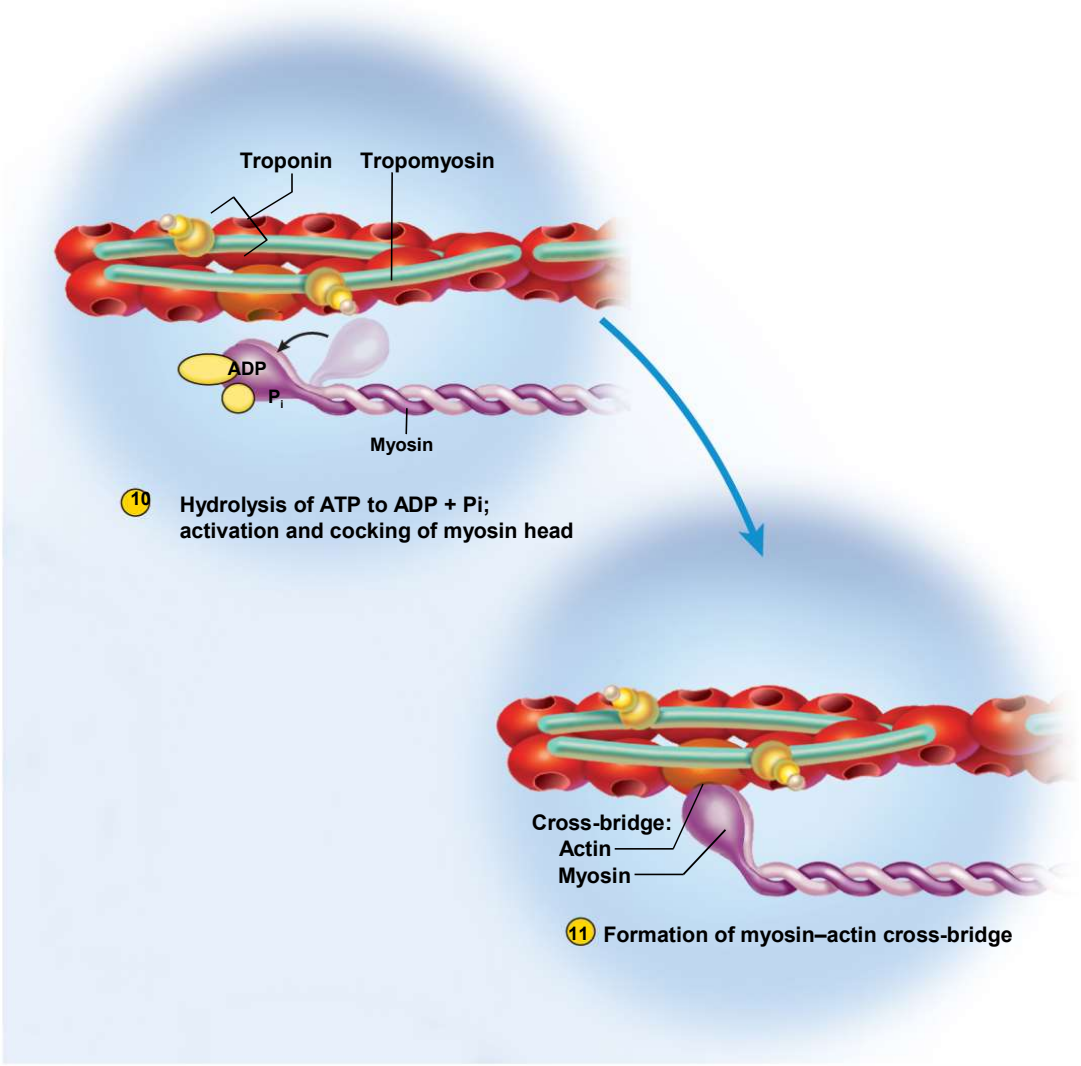

- calcium binds to troponin on the thin filaments // causes troponin-tropomyosin complex changes shape, moves to exposes myosin head binding site on actin

- ATP was used earlier to cock the myosin heads (load them with energy) – when this occurs ATP becomes ADP and ADP stays attached to the cocked myosin head /// when at rest all myosin heads are pre-loaded with energy // ready for contraction!

- If the myosin head are able to dock to the myosin head binding site, then the myosin head releases its energy and pulls on the actin // moving the two actin fibers towards the center of the sarcomere

- Now there is a cross bridge between thick and thin filaments // the cross bridge may only be broken if a new ATP binds to the myosin head. // at the same time this pops the “old” ADP off the myosin head

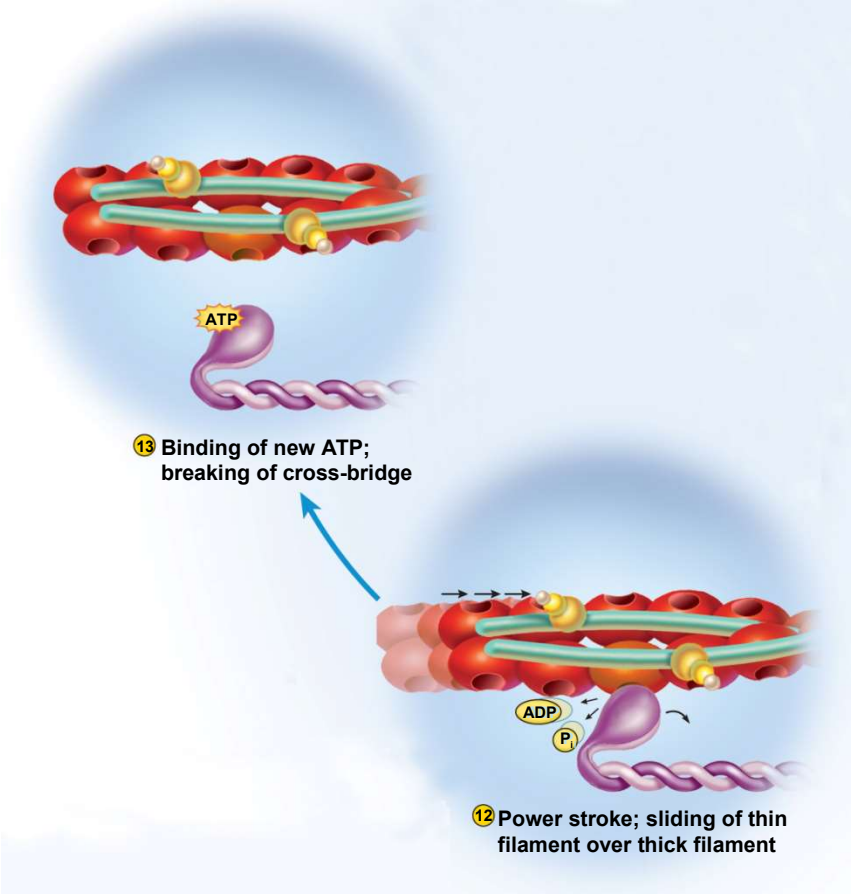
# Contraction
## (steps 10 and 11)




- ATP binds to myosin head // myosin-ATPases removes third phosphate from ATP to make ADP and releases energy /// used to "cock myosin head" (i.e. extend the head)
- This reaction occurs independent and before the actin – troponin – tropomyocin event
- Myosin head is now activated /// /// note: ADP + $P_i$ remain attached to head
- Cocked head of myosin binds to exposed myosin head receptor on actin molecle to form a myosin – actin – cross-bridge
- With the formation of crossbridge the energy in the extended myosin head is released /// now myosin head flexes back to its non-extended position
- *At this point the ADP + P is released from the myosin head*
- *This is the "Power Stroke"*

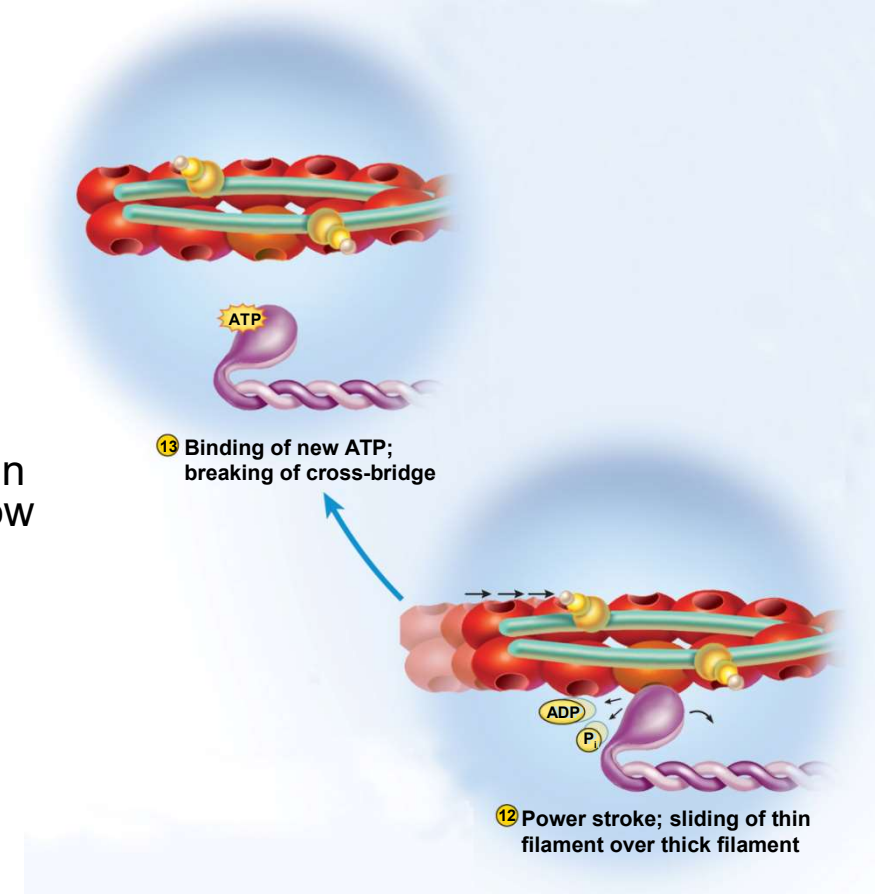
# Contraction = Power Stroke (steps 12 and 13)

- **The Power Stroke**
  - When the “precocked myosin head” is allowed to bind to the actin receptor for myosin – the stored energy in the cocked myosin head is released
  - For this to occur – regulatory proteins must move out of the way to expose the myosin binding site
  - After the “cross bridge” between the myosin and actin is formed – the energy released from the cocked myosin molecule pulls the thin filament over thick filament // the Z disc move closer together
  - Myosin head can not release the actin cross bridge until new ATP molecule binds to myosin // this “breaks the bridge”



# Contraction (steps 12 and 13)

- After power stroke the actin-myosin-cross bridge can not be “broken”
- To break the cross bridge “new” ATP must bind to the myosin head
  - ATP allows myosin to release actin
  - At same time it immediately “recocks” myosin head and it is again loaded with energy // now the power stroke is repeated
  - each head performs 5 power strokes per second
  - each stroke utilizes one  molecule of ATP
  - as one bridge is broken many more are formed which maintains tension in muscle



# Power Stroke and the Sliding Filament

- Lets Review:

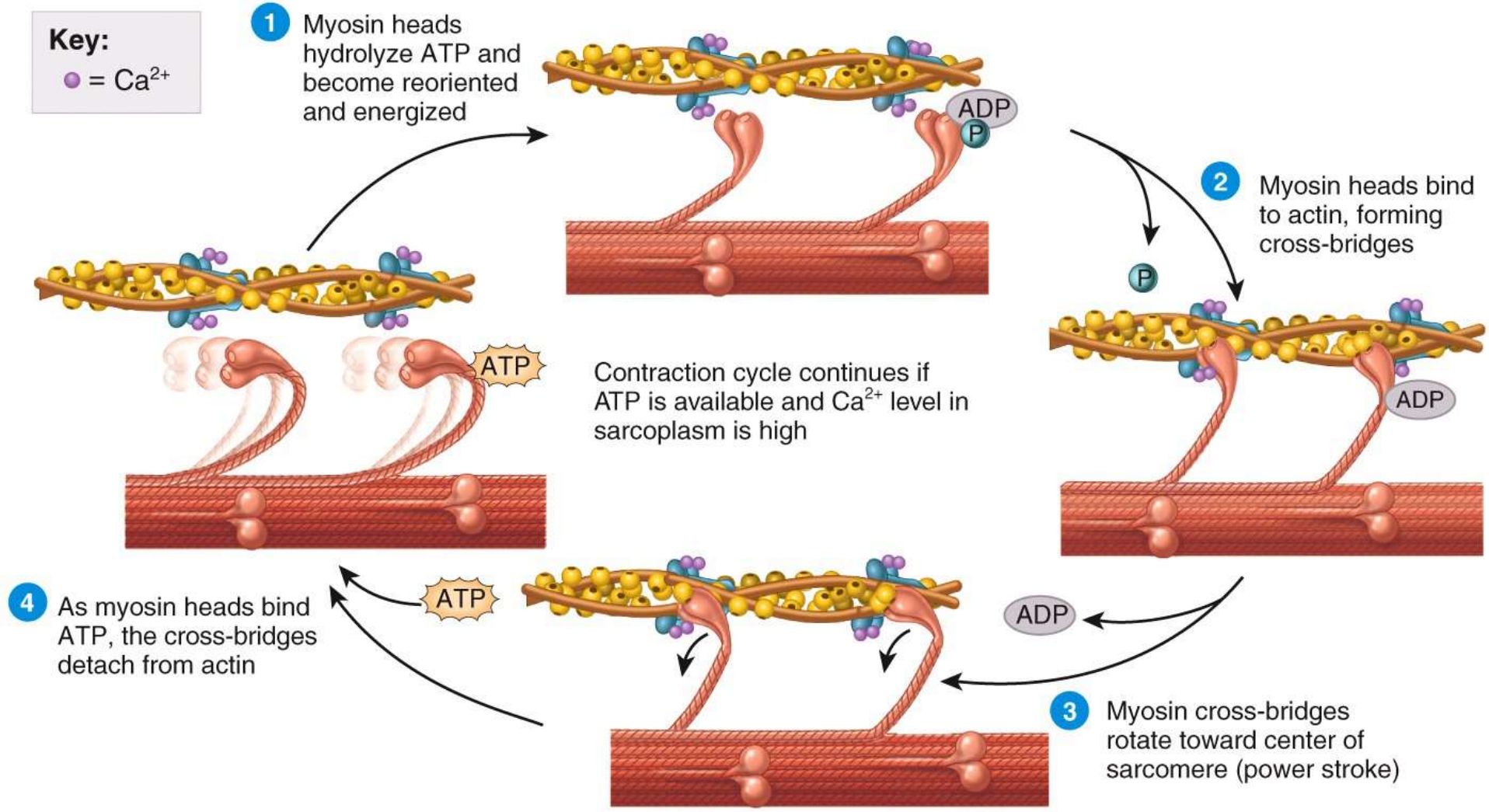
- To start, lets assume that there are myosin head not attached to actin and the myosin head has not been “loaded with energy” (ATP is used to cock or energize myosin head)

- 1 - ATP docks on myosin head and hydrolysis of ATP to ADP // this will cock myosin head (head is loaded with energy)

- 2 – Formation of myosin-actin cross bridge (myosin head binds to receptor on actin molecule) – after the cross bridge forms then an enzyme will allows the energy of the myosin head to be released, next....

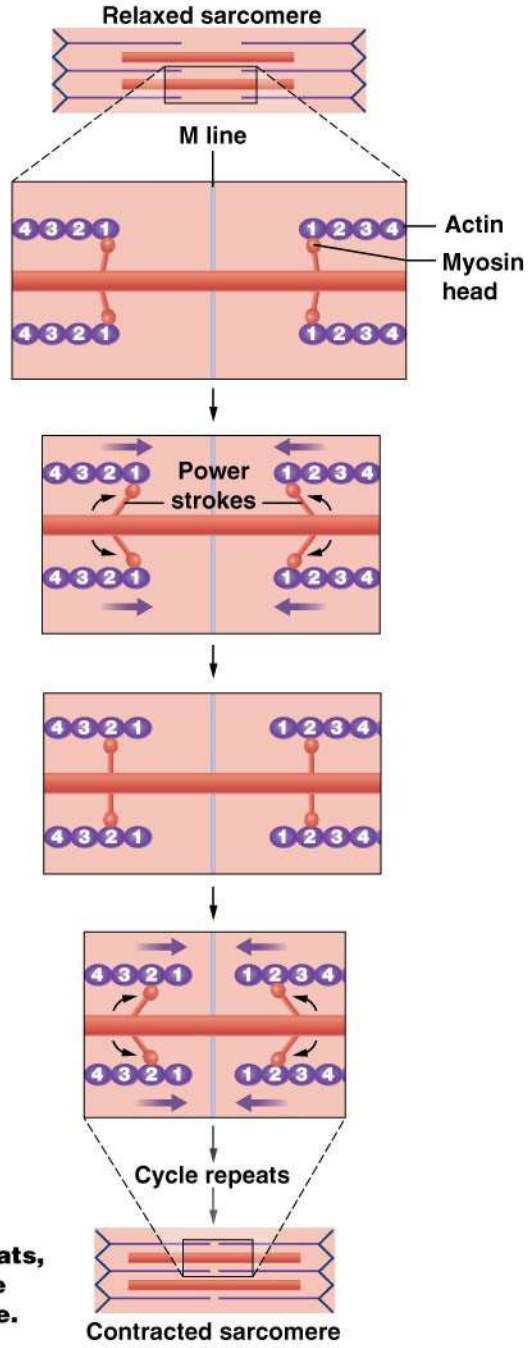
- 3- Power stroke, the myosin head pivots and now thin filament slides over thick filament //  note: the cross bridge is still in attacked!

- 4 – Binding of another ATP to myosin head will break cross bridge and cock the myosin head with energy again  ///  if no ATP is available then the cross brindge may not be broken


Key:
= $Ca^{2+}$
1 Myosin heads hydrolyze ATP and become reoriented and energized
ADP
P
2 Myosin heads bind to actin, forming cross-bridges
P
ADP
ATP
Contraction cycle continues if ATP is available and $Ca^{2+}$ level in sarcoplasm is high
4 As myosin heads bind ATP, the cross-bridges detach from actin
ATP
ADP
3 Myosin cross-bridges rotate toward center of sarcomere (power stroke)

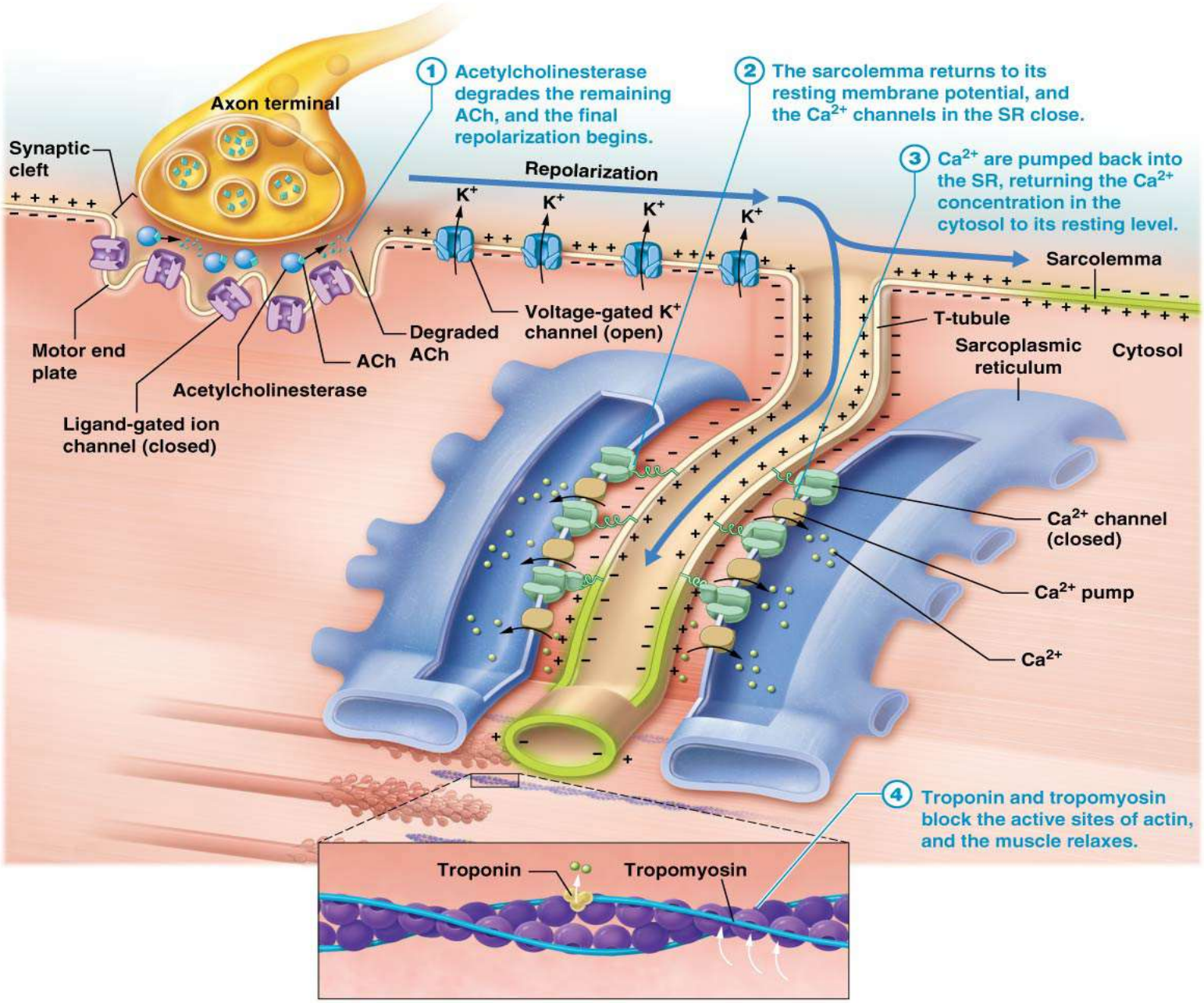
## The Contraction Phase

**A cross bridge cycle brings the thin filaments closer to the center**

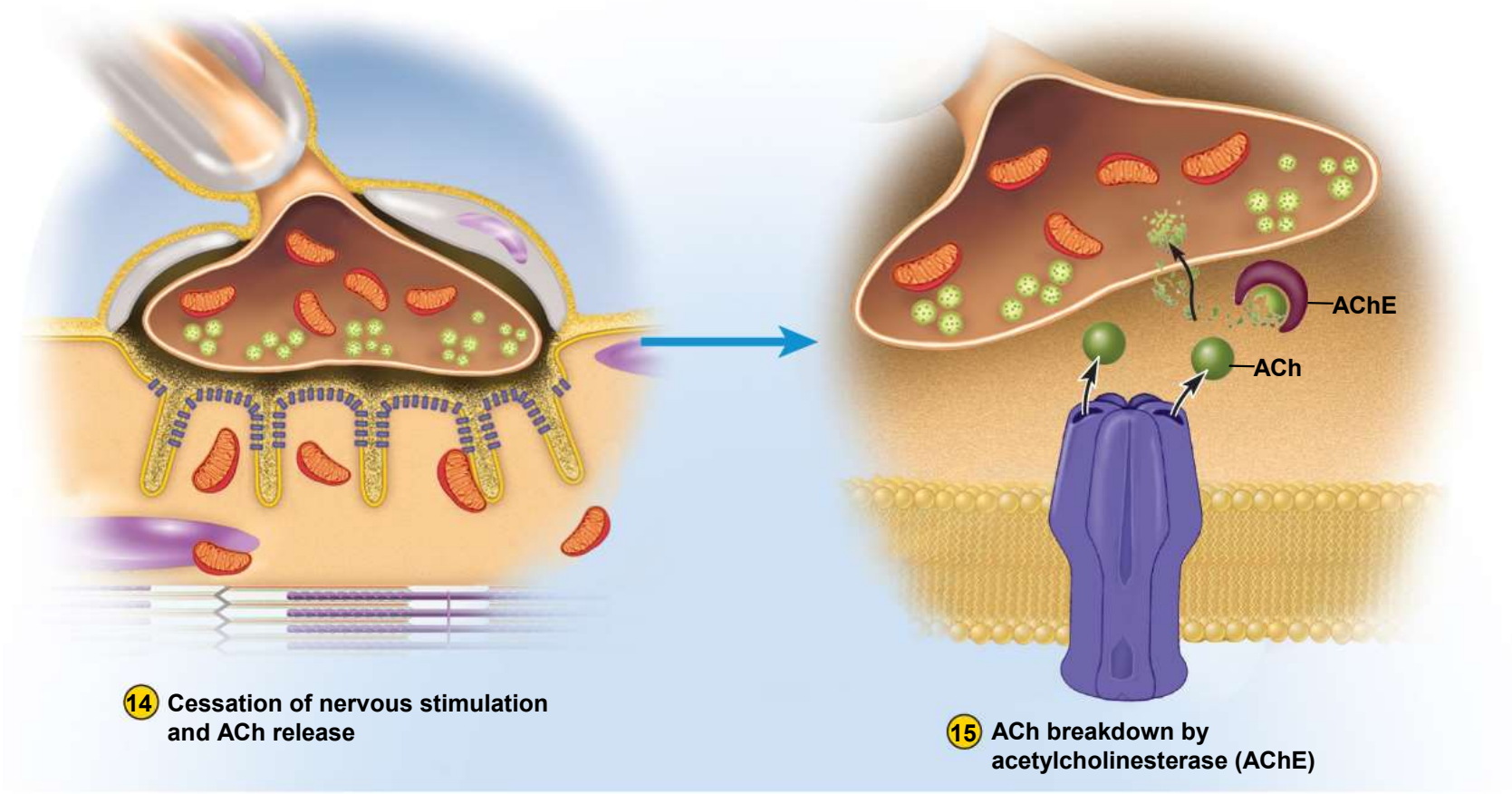



(b) Crossbridge cycle repeats, pulling actin toward the center of the sarcomere.

# Relaxation Phase
## the process of muscle relaxation.



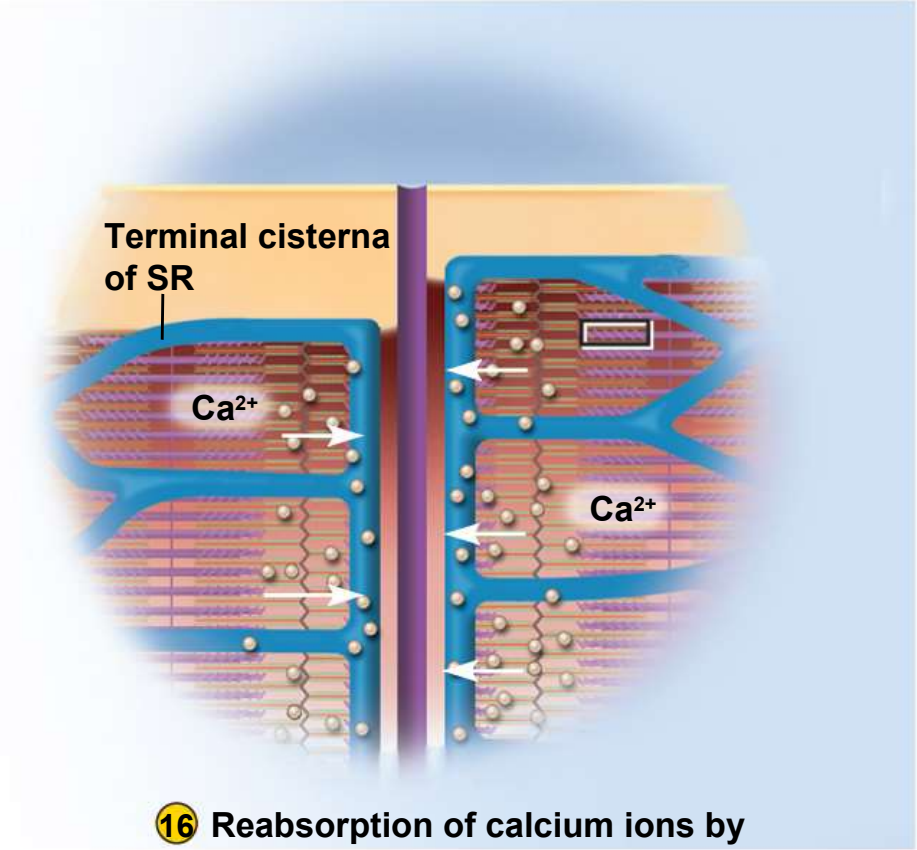
# Relaxation (steps 14 and 15)




- If you stop new nerve action potentials then this will close voltage regulated calcium channel at synaptic knob. Now this stops exocytosis of any more ACh into synaptic cleft
- ACh-Esterase breaks down any Ach already in synaptic cleft // fragments reabsorbed into synaptic knob
- This prevents excitation by ACh and all the "downstream" events are reversed

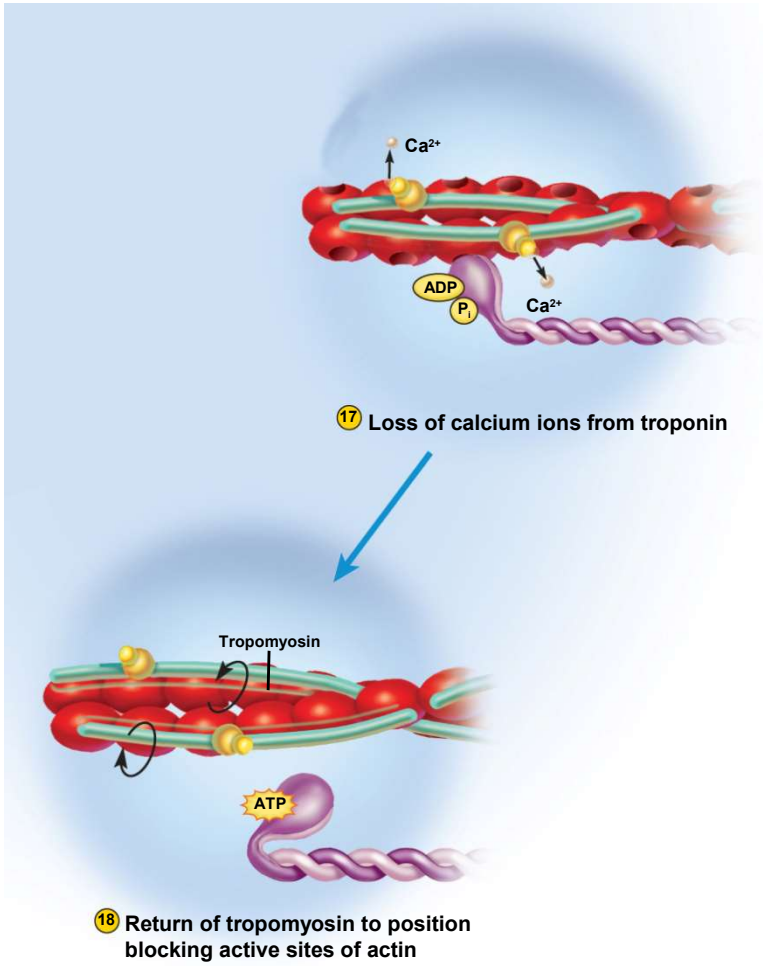
# Relaxation (step 16)

- $Ca^{+2}$ is pumped back into SR by active transport. // Why is this is active transport?
- $Ca^{+2}$ binds to calsequestrin while in storage in SR
- <u>Muscle funtion requires a lot of ATP because ATP is required for both muscle contraction and muscle relaxation.</u>

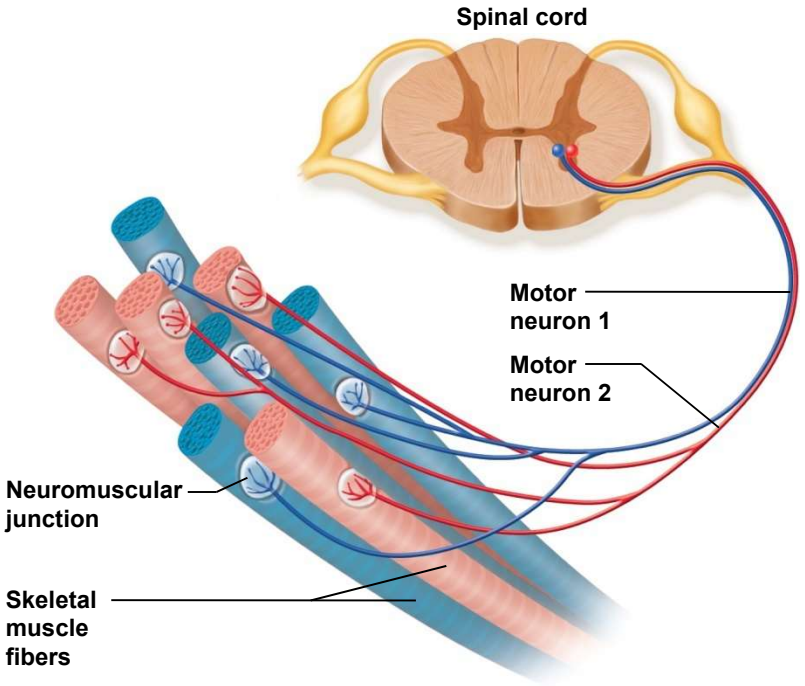


# Relaxation (steps 17 and 18)

- $Ca^{+2}$ removed from troponin as calcium is pumped back into SR
- Now tropomyosin moves back to block the myosin binding sites
- Muscle fiber ceases to produce or maintain tension
- Note: in relaxation more ATP is used to cox myosin heads for future contractions
- Muscle fiber returns to its resting length due to recoil of elastic components & contraction of antagonistic muscles



# What is the significance of a motor unit?




Motor unit = one axon and all the muscle fibers innervated by the terminal knobs of that nerve fiber //  note – a single axon may have one or more terminal knobs

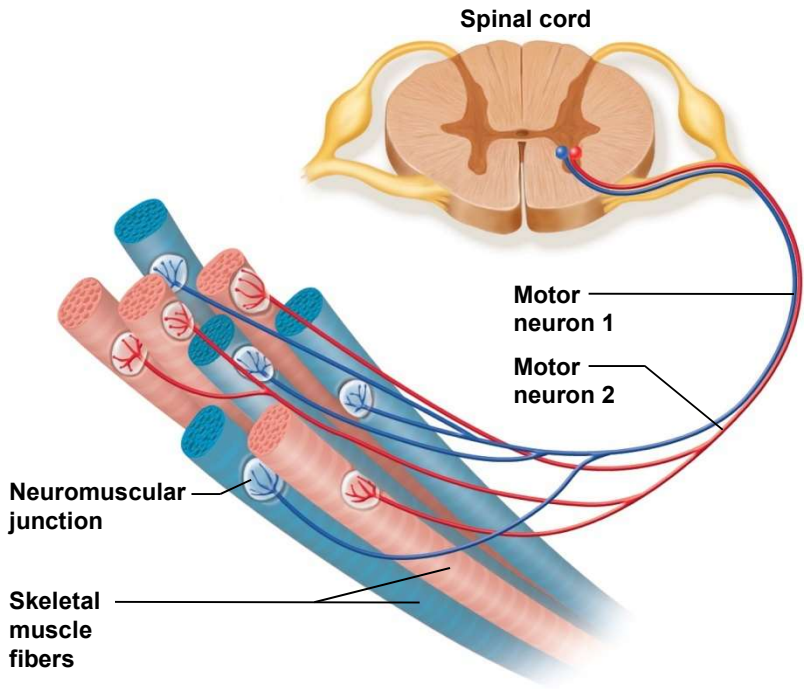
# Motor Units

- small motor units - fine degree of control // 3-6 muscle fibers per neuron // eg eye and hand muscles

- large motor units – more strength than control // many muscle fibers per motor unit

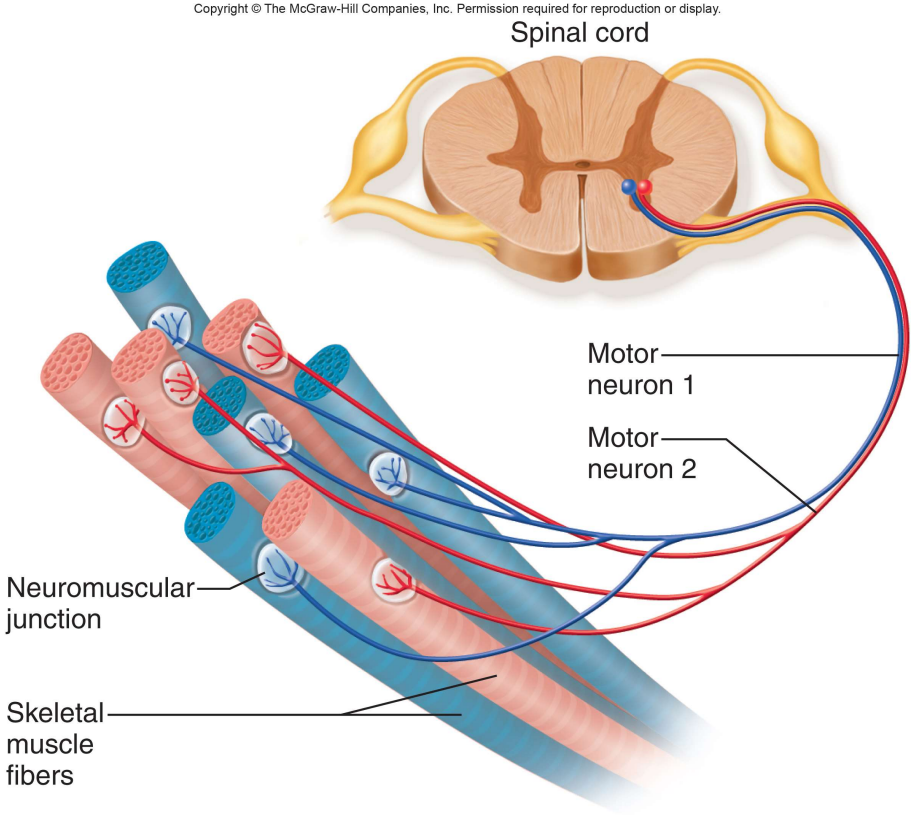
  – powerful contractions supplied by large motor units

  – gastrocnemius has 1000 muscle fibers per neuron



# How do we use motor units?

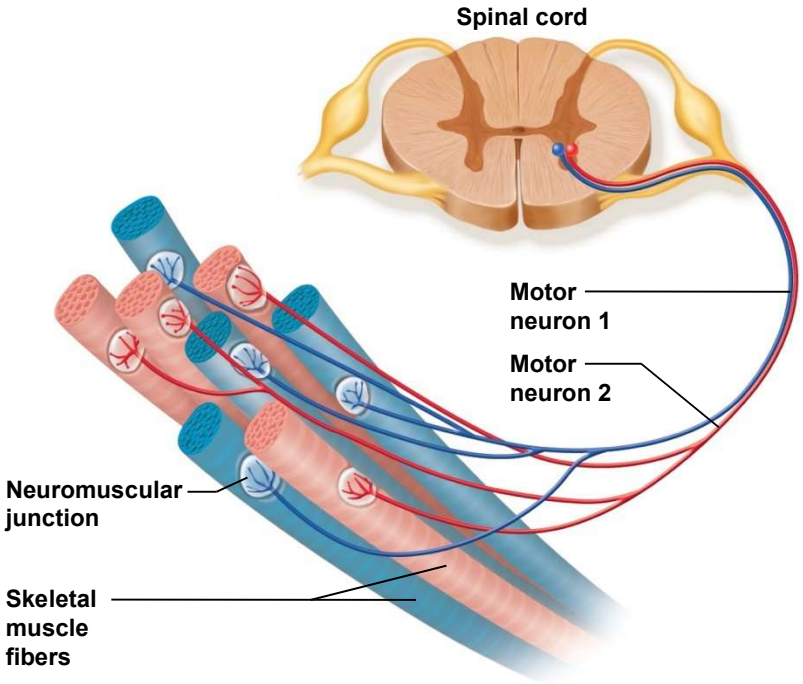





Skeletal muscles are voluntary. This means we “consciously” use our brain to send signals to our muscle fibers to contract. Our brains must signal how many motor units to activate in order to generate the appropriate force (eg. pick up a dime VS pick up a chair)

# Motor Units

- Dispersed throughout the muscle organ
- More MU activated to increase strength of contraction
- Activation of fewer MU produce weak contraction over wide area
- Also provides ability to sustain long-term contraction by “rotating” use of different motor units //  take turns contracting (e.g. postural control)
- Effective contraction usually requires the contraction of several motor units at once to create the force equal to the “load the muscle needs to overcome”



# The Physiology of Rigor Mortis

- Hardening of muscles and stiffening of body that occurs after death

  - This begins 3 to 4 hours after death // tension in tissue peaks after twelve hours /// muscle tension then diminishes over the next 48 to 60 hours

  - At time of death all myosin molecules are “loaded with ATP” and capable of initiating a “power stroke”

  - Rigor mortis starts to develop because there is no ATP being produce that can be usd to keep calcium in the sarcoplasmic reticulum (lack of ATP means no active transport to keep calcium inside SR)

  - Furthermore, deteriorating of the sarcolemma reticulum allows even more $Ca^{+2}$ to enter cytosol

  - Released $Ca^{+2}$ exposes myosin binding site on actin // all myosin heads were pre-loaded with ATP and cocked before death // now contraction cycle occurs and myosin-actin cross-bridges are formed – since the dead cell can not make new ATP // there is no available ATP to break the cross bridges // muscle tension stays in the muscle

  - See next slide

# Rigor Mortis

- Over time, muscle generates even more tension as more calcium escapes from the  sacroplasm reticulumand more myosin-actin cross bridges are formed

- During this phase of rigor mortis, the muscle can not relax.

- Because after death - new ATP can not be formed.  And the ATP formed prior to death only last a few millisecond (ATP is not stored)

- ATP is required to “break” myosin-actin cross bridges.  Under normal conditions, muscle relaxation requires ATP to break myosin-actin cross bridges

- After 48 to 60 hours muscle tension starts to decrease.  This occurs because the myofilaments (i.e. the proteins) are hydrolyzed by lysosomal enzymes

# Neuromuscular Toxins

Some toxins interfere with synaptic function and can result in either spastic paralysis or flaccid paralysis of the muscles

- Spastic paralysis = over stimulated and muscle can not relax
  - some pesticides contain cholinesterase inhibitors
    - bind to acetylcholinesterase and prevent Ach hydrolysis
    - spastic paralysis - a state of continual contraction of the muscles
    - this may cause paralysis of diaphram // possible suffocation
  - tetanus (lockjaw) is a form of spastic paralysis caused by toxin of *Clostridium tetani*
    - glycine in the spinal cord normally stops motor neurons from producing unwanted muscle contractions
    - tetanus toxin blocks glycine release in the spinal cord /// lack of glycine causes over stimulation and spastic paralysis of the muscles

# Neuromuscular Toxins

Some toxins interfere with synaptic function and can result in either spastic paralysis or flaccid paralysis of the muscles

- Flaccid paralysis – a state in which the muscles are limp and cannot contract
  - curare – compete with ACh for receptor sites, without causing an action potential (prevents muscle contraction)
    - plant poison used by South American natives to poison blowgun darts
  - botulism – type of food poisoning caused by a neuromuscular toxin secreted by the bacterium *Clostridium botulinum*
    - blocks release of ACh causing flaccid paralysis
    - use botox cosmetic injections to remove wrinkles in skin