What is the Nerve Muscle Relationship?

What is the Structure and Function of the Neural Muscular Junction?

What Are the Steps in a Skeletal Muscle Contraction Cycle?

What is the Significance of a Skeletal Muscle's Motor Unit?

The Nerve-Muscle Relationship

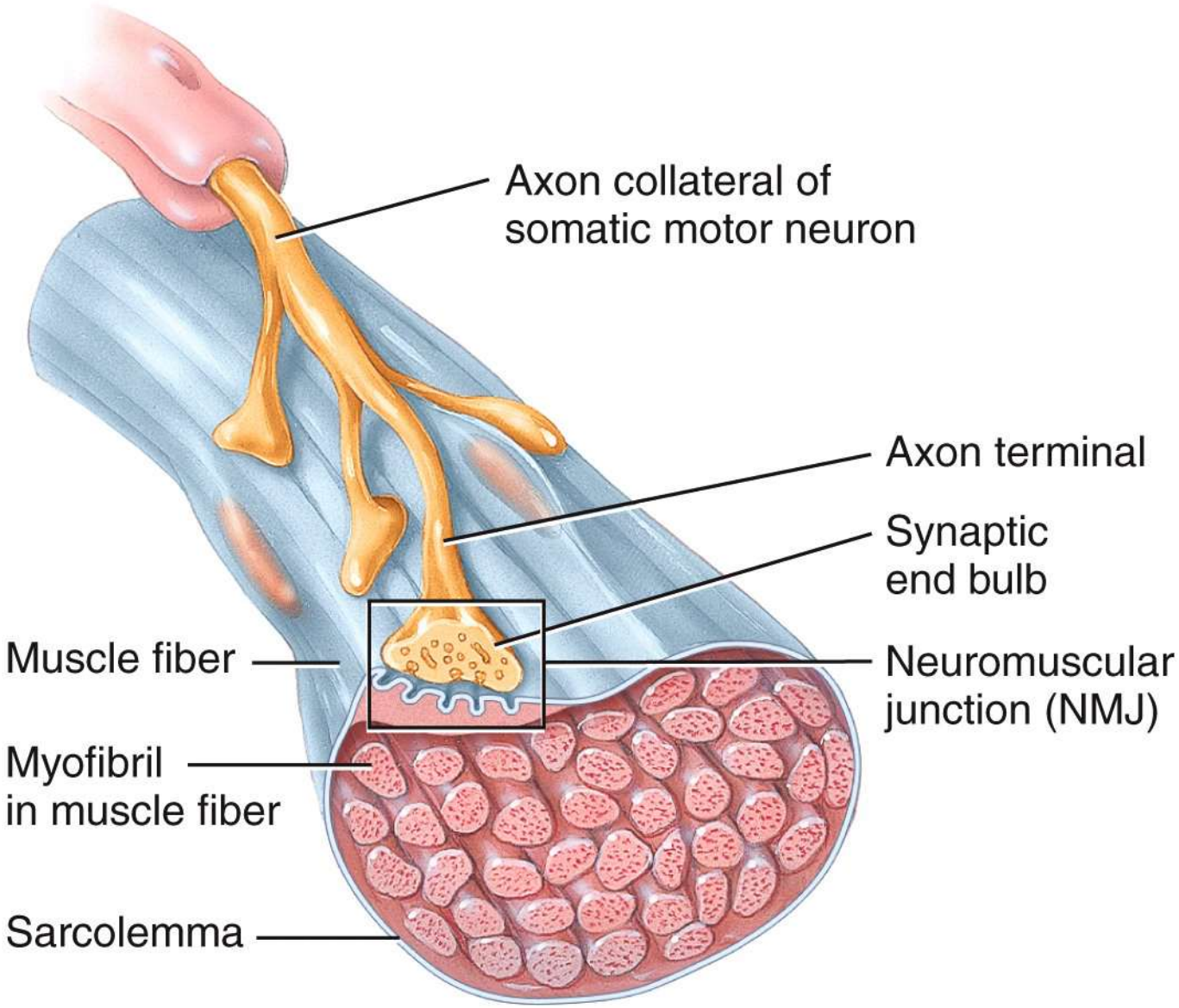
- Skeletal muscles require nerve stimulation to contract
  - if nerve connection to the muscle is cut then the muscle is paralyzed
  - **denervation atrophy** – cut somatic nerve to skeletal muscle /// muscle paralyzed and atrophy (reduced sarcoplasm volume because myofibils break down and are not replaced)
    - experience a loss of sacroplasm – loss of contractile proteins /// result in less strength
  - What is disuse atrophy? When may this happen?

The Nerve-Muscle Relationship

- Skeletal fibers are innervated by either somatic motor neurons or cranial nerves
  - LMN's nerve cells' "somas" are in the brainstem or spinal cord
  - LMN's nerve's axon "connect to" skeletal muscles
  - Single axon form branches – each branch "synapse" with individual muscle fibers (terminal knobs)
  - each muscle fiber is innervated by only one motor neuron which may have several "terminal knobs"

The Neuromuscular Junction

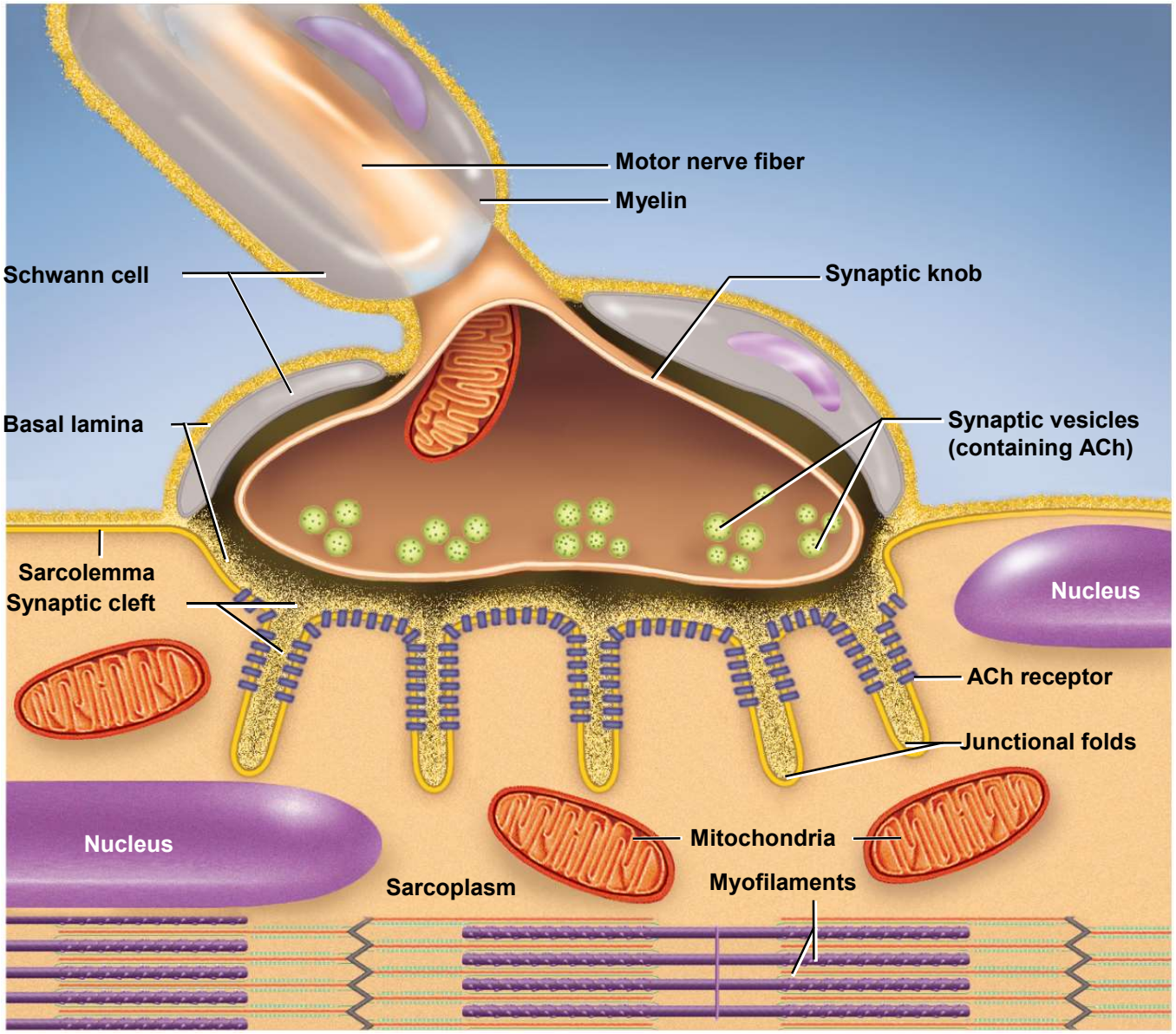
- Synapse = the location where the terminal end of a nerve (the terminal knob) reaches its target tissue
  - three components of a synapse = pre-synaptic membrane / synaptic cleft / post-synaptic membrane
- Neuromuscular junction (NMJ) = special type of synapse // when target cell is a muscle fiber
  - each terminal branch of the nerve fiber's axon forms a separate NMJ synaptic junctjion with a single muscle fiber



(a) Neuromuscular junction

The Neuromuscular Junction

(You should be able to draw and label this graphic!)



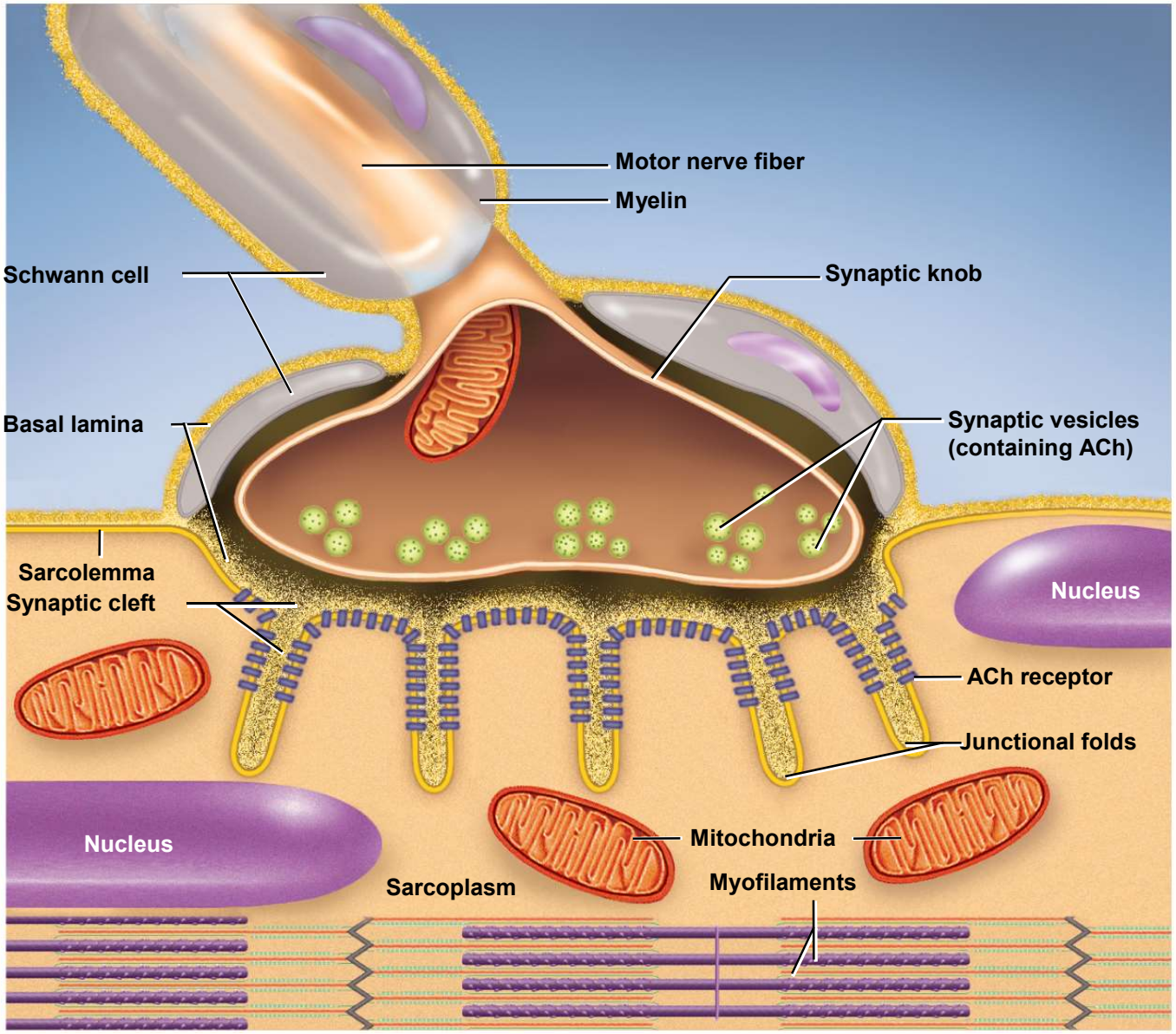
Components of Neuromuscular Junction

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

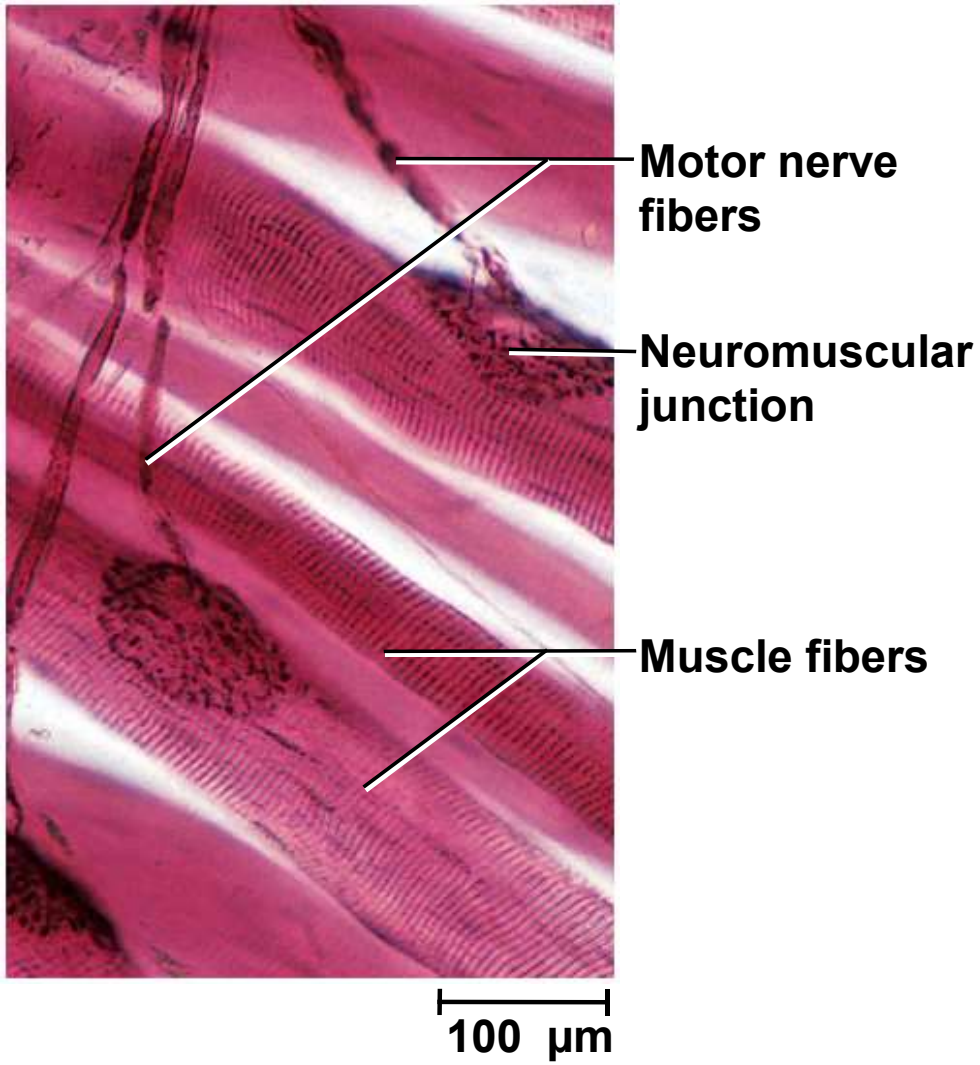
Components of Neuromuscular Junction

- ACh receptors – 50 million protein receptors incorporated into muscle cell plasma membrane
  - junctional folds of sarcolemma beneath synaptic knob // increases surface area holding ACh receptors
    - lack of receptors leads to paralysis in disease myasthenia gravis
- Basal lamina - thin layer of collagen and glycoprotein separates Schwann cell and entire muscle cell from surrounding tissues
  - contains acetylcholinesterase (AChE) that breaks down ACh after contraction causing relaxation

The Neuromuscular Junction



Neuromuscular Junction - LM



What Does it Mean to be an Electrically Excitable Cell?

- Only muscle fibers and neurons are excitable cells, able to conduct current across their plasma membranes
  - muscle and neuron cells may open regulated channels in their plasma membranes in response to stimulation // these channels (ie regulated gates) are controlled by voltage, ligands, or mechanical forces

- Electrophysiology - the study of the electrical activity of cells
- Voltage = a separation of charge /// an electrical potential = separation of charge /// in cells it occurs across the plasma membrane
- Resting membrane potential // about -90mV // maintained by sodium-potassium ATP-ase pump /// all cells have RMP
- Action Potential = Current = movement of electrical charge across surface of plasma membrane /// see reversal of charge that moves across membrane /// flows in one direction across surface

Electrically Excitable Cells

- A cell that is not stimulated = cell with a resting membrane potential
  - there are more anions (negative ions) on the inside of the plasma membrane than on the outside
  - the plasma membrane is electrically polarized (charged)
  - there are excess sodium ions (Na^+) in the extracellular fluid (ECF)
  - there are excess potassium ions (K^+) in the intracellular fluid (ICF)
  - in the ICF, there are anions such as proteins, nucleic acids, and phosphates that cannot penetrate the plasma membrane
  - these anions make the inside of the plasma membrane negatively charged by comparison to its outer surface

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) – propigates 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 generate an action potential

Events Across the Plasma Membrane Associated with Action Potentials in Muscle or Nerve Cells

- Sodium ion gates open in the plasma membrane (these are voltage regulated gates!)
- Sodium ion diffuses instantly down its 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
- This allows 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
- Now sodium-potasium-ATPase pump moves ions (Na and K) to “fine tunes” these ion concentrations so voltage across membrane is restored to the cells resting membrane potential

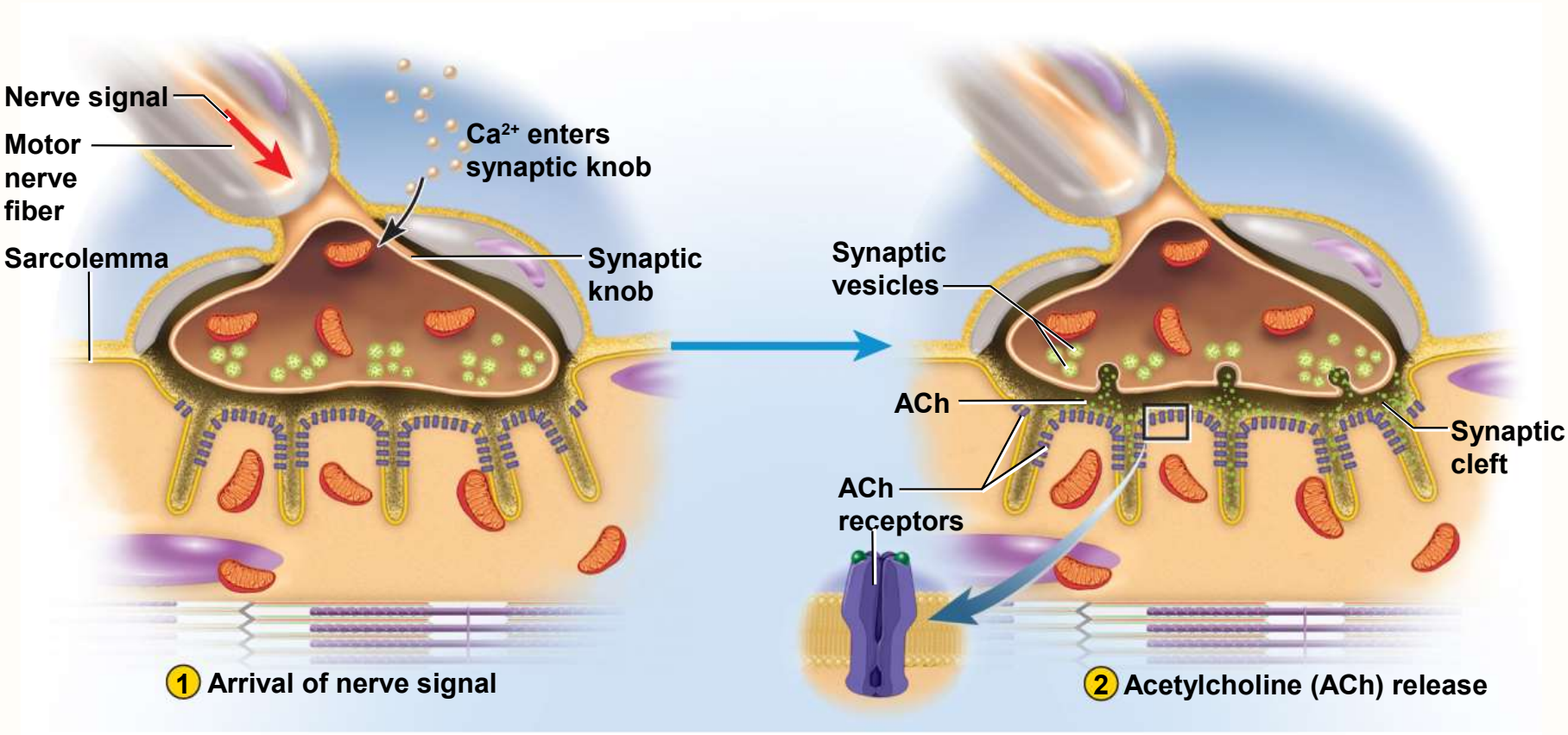
The Sliding Filament Theory of Skeletal Muscle

- In the early 1950s, one hypothesis to explain skeletal muscle function was to think of proteins folding like an accordion
- With the discovery of the electron microscope, scientist could “see” the thin and thick proteins in skeletal muscles.
- These proteins did not shorten during contraction (no accordion like action)
- Original hypothesis was wrong and
- New hypothesis formulated suggesting muscle fiber shortened by proteins slideing across each other. This hypothesis was proven to be correct and is called the sliding filament theory.

Skeletal Muscle Contraction & Relaxation

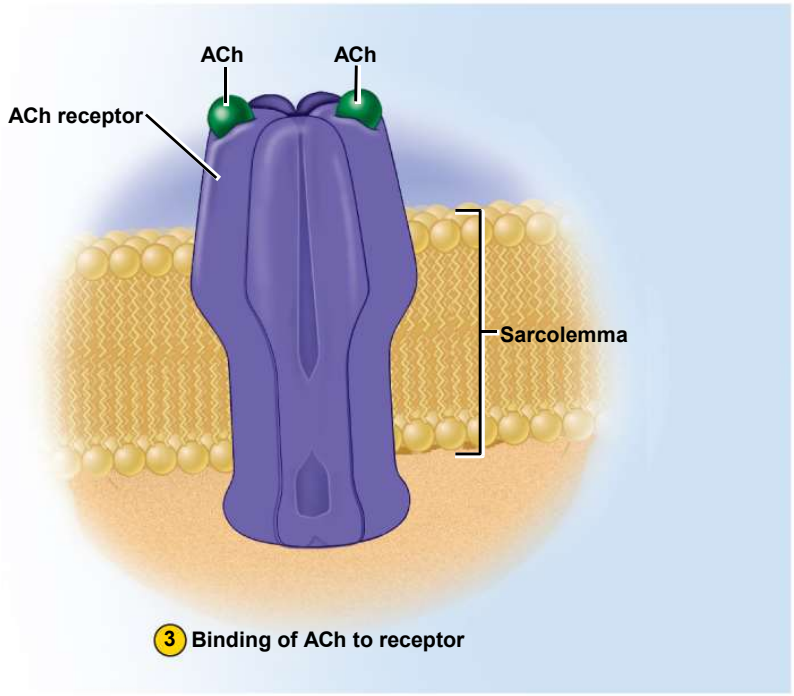
- **Four phases of a skeletal muscle contraction cycle**
  - **excitation**
    - the process in which nerve action potentials lead to a muscle action potentials
  - **excitation-contraction coupling**
    - events that link the muscle action potential on the sarcolemma to activation of the myofilaments, thereby preparing them to contract
  - **contraction (the contraction cycle)**
    - step in which the muscle fiber develops tension and contractile proteins “slide” over each other
  - **relaxation**
    - after tension is created, events that allow a muscle fiber to return to its resting length

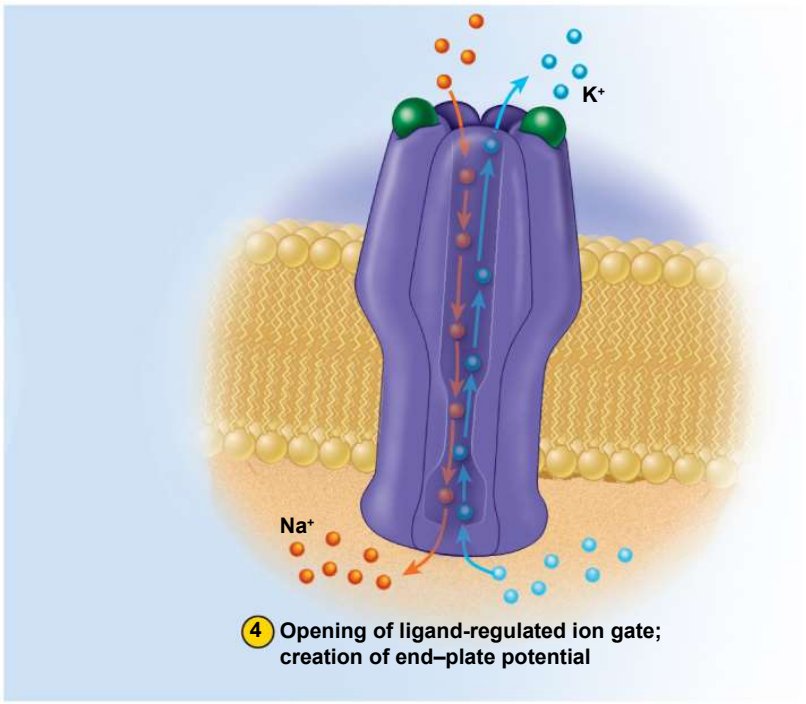
Start of Excitation (steps 1 and 2)



- nerve signal opens voltage regulated calcium gated channels in synaptic knob
- calcium stimulates exocytosis of ACh from synaptic vesicles
- ACh released into synaptic cleft

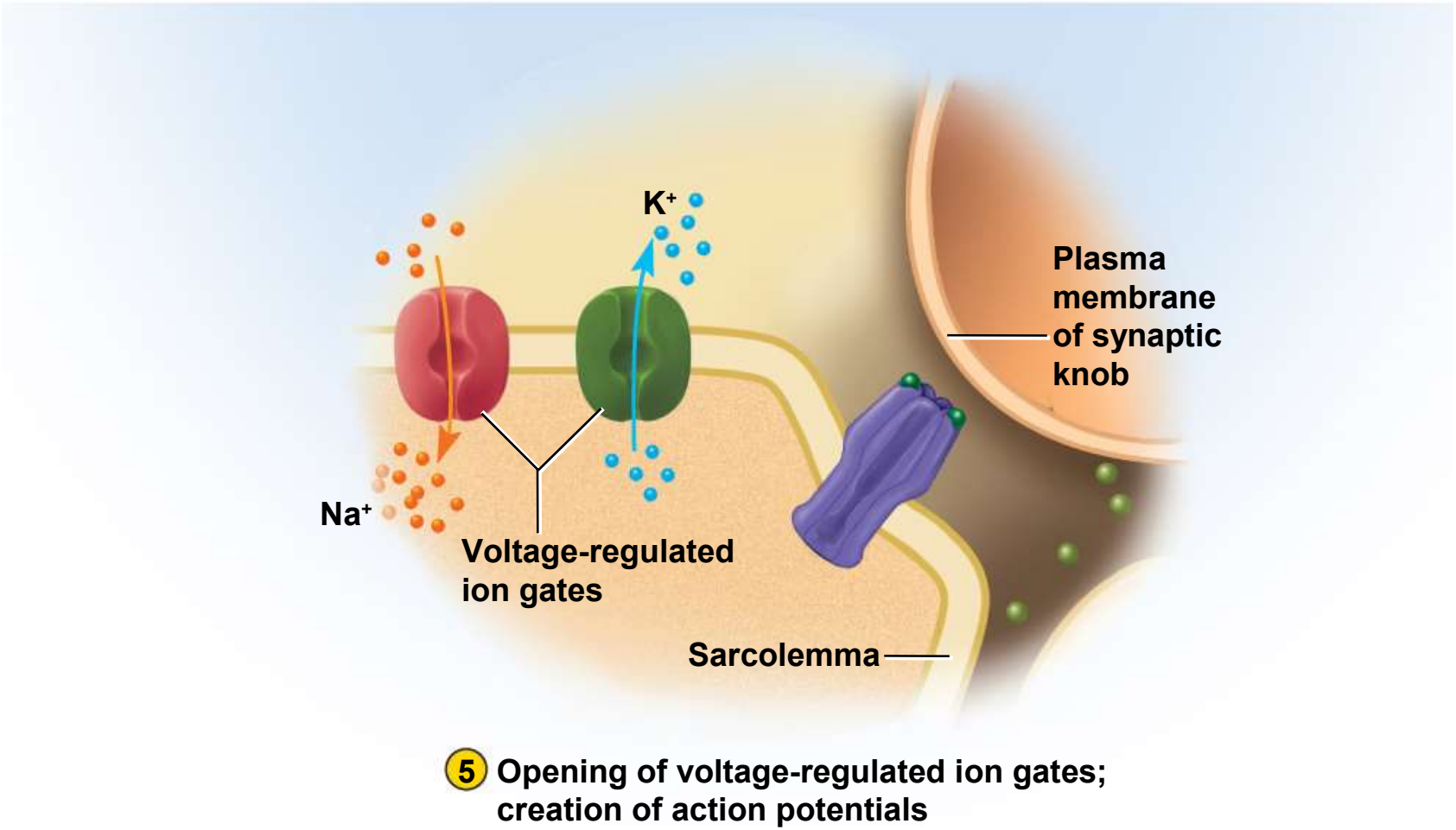
Excitation (steps 3 and 4)





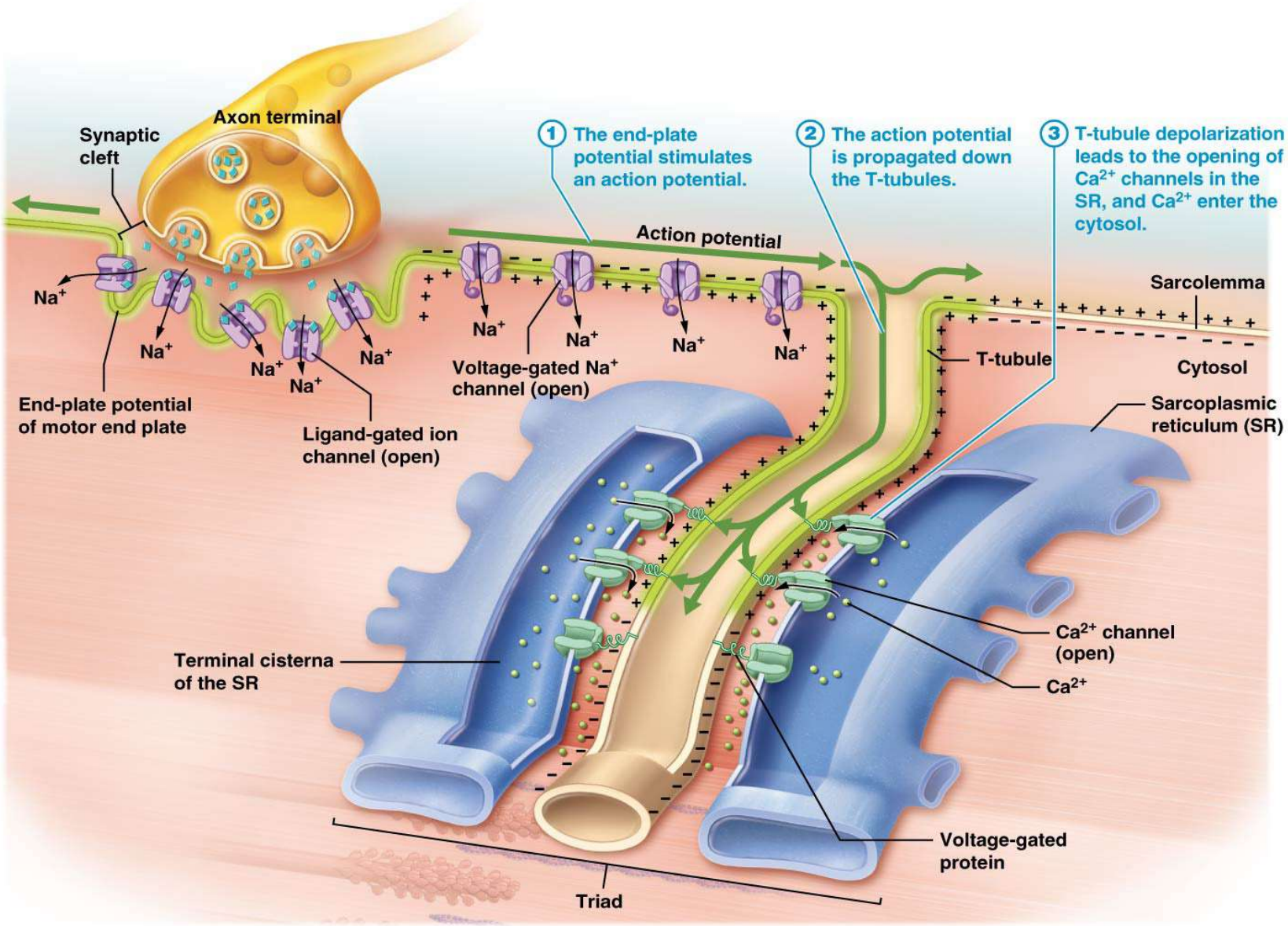
- two ACh molecules bind to each receptor protein, this opens ligand regulated Na^+ and K^+ channels. (i.e. Ach is the ligand)
- Na^+ first ion to move and enters interior of cell - shifting RMP /// goes from -90mV to +75mV - this depolarizes sarcoplasm
- then K^+ exits and RMP returns to -90mV
- quick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

Completion of Excitation (step 5)

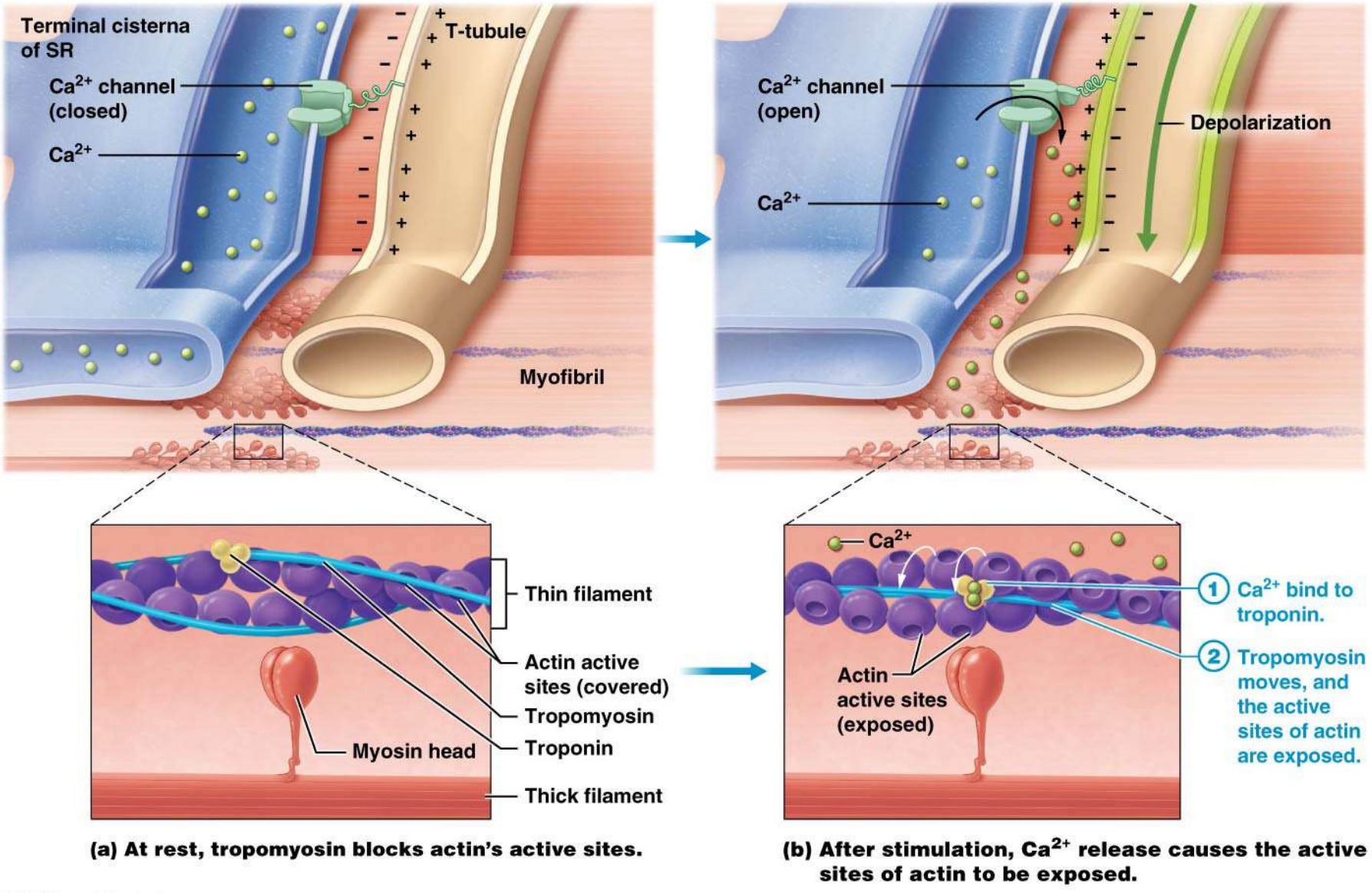


Voltage change caused by ligand (within end-plate region) spreads to nearby voltage regulated gated Na and K channels outside end-plate which then produce an action potential just outside of the neuromuscular junction. This “action potential” then spreads over entire muscle surface.

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



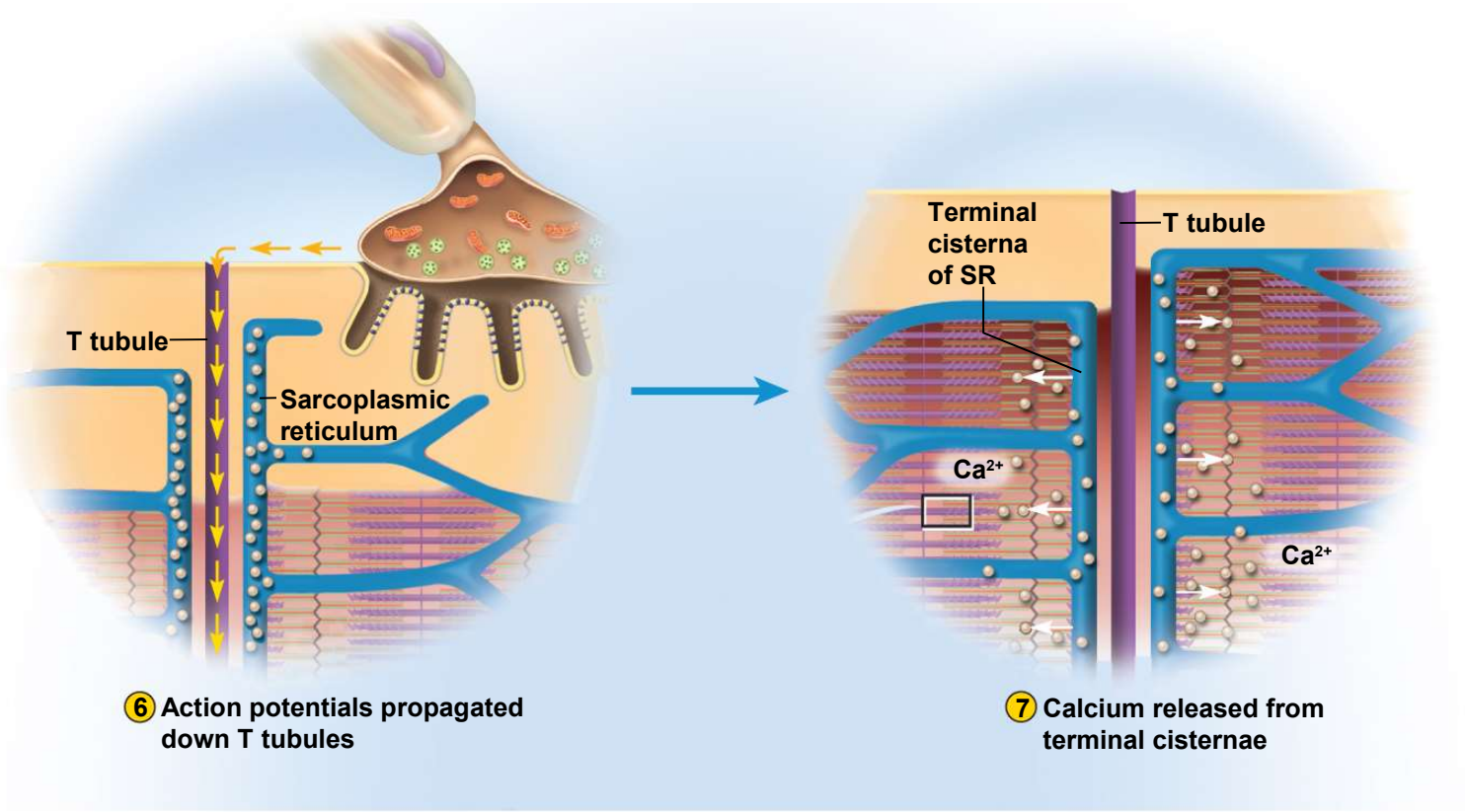
Excitation-contraction coupling: preparation for contraction (regulatory events at the myofibril)



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

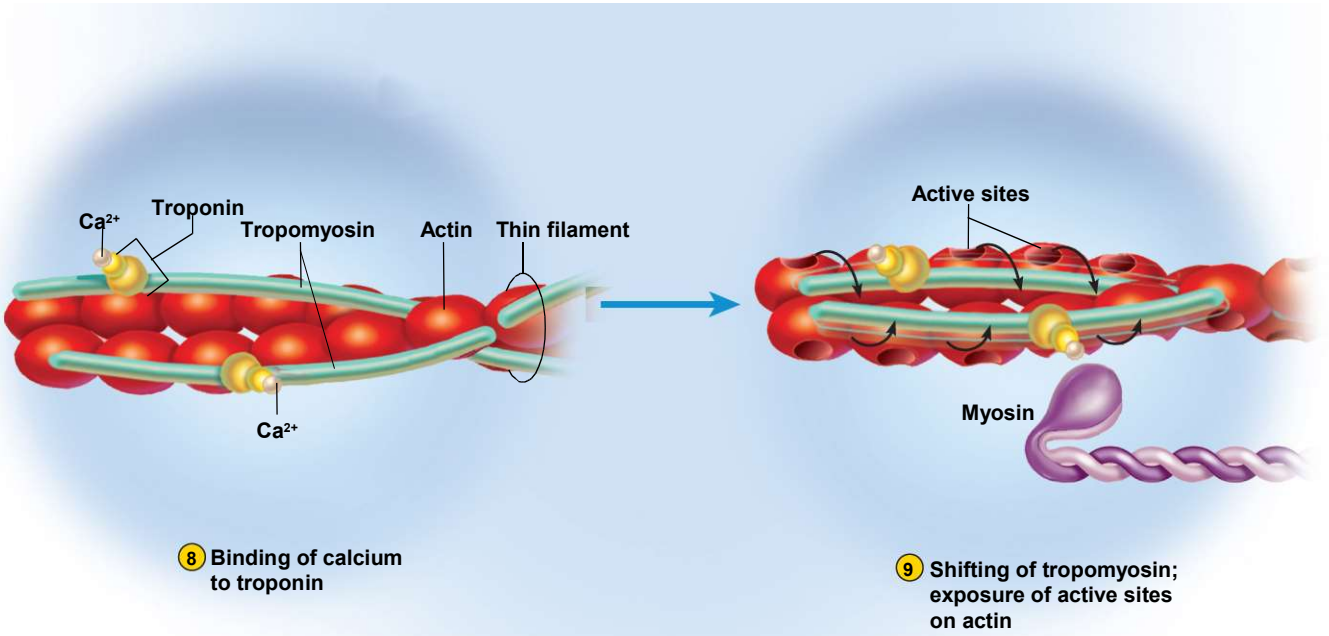
(b) After stimulation, Ca^{2+} release causes the active sites of actin to be exposed.

Excitation-Contraction Coupling
(steps 6 and 7)



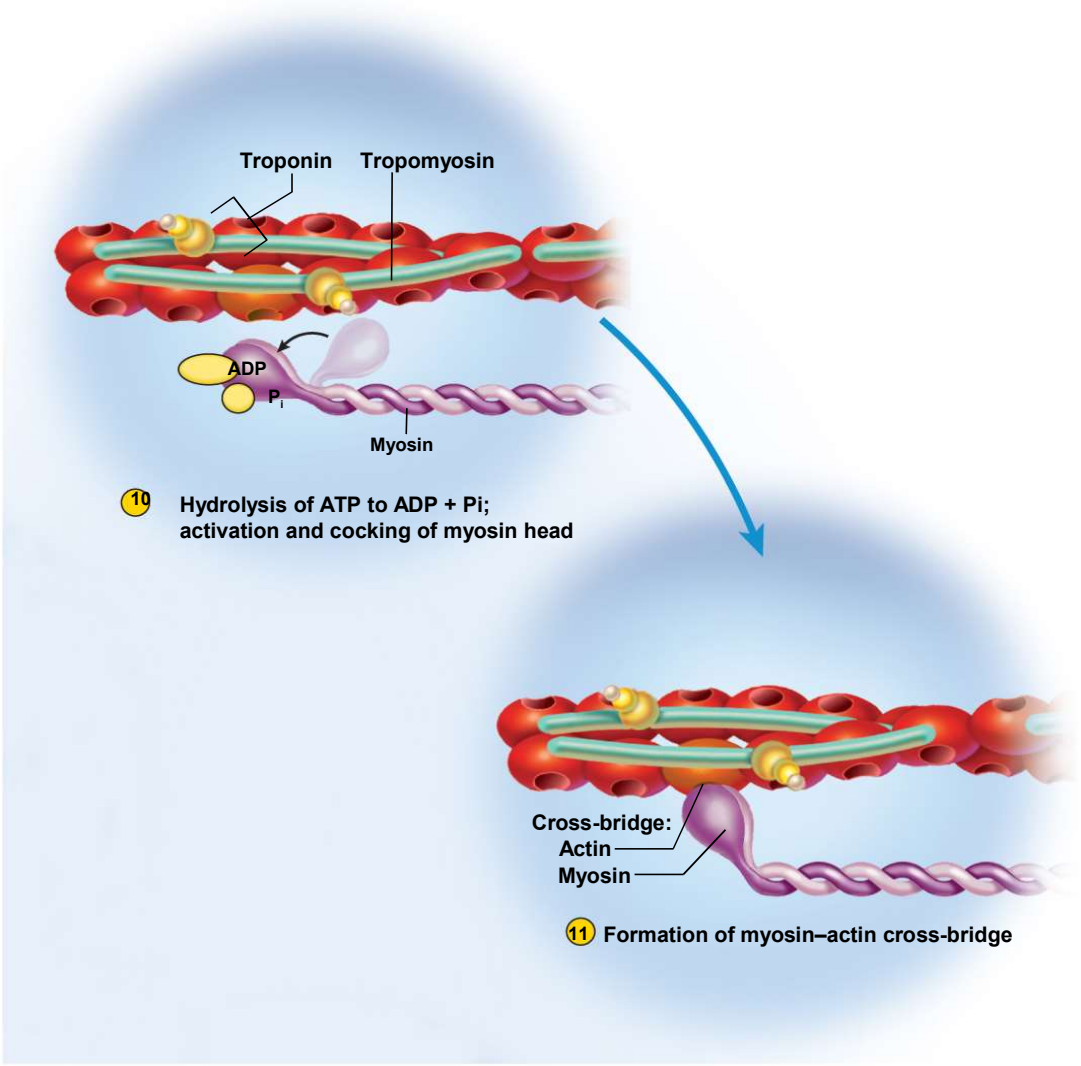
- action potential (AP) spreads from sarcoplasm into T tubules
- AP flows from T tubules to sarcoplasmic reticulum
- AP opens voltage regulated gated calcium ion channels in SR
- Ca^{+2} diffuse into the cytosol

Excitation-Contraction Coupling
(steps 8 and 9)



- calcium binds to troponin in thin filaments
- troponin-tropomyosin complex changes shape and exposes active sites on actin
- this is the site where the “energized myosin head” must bind to in order for a muscle to shorten

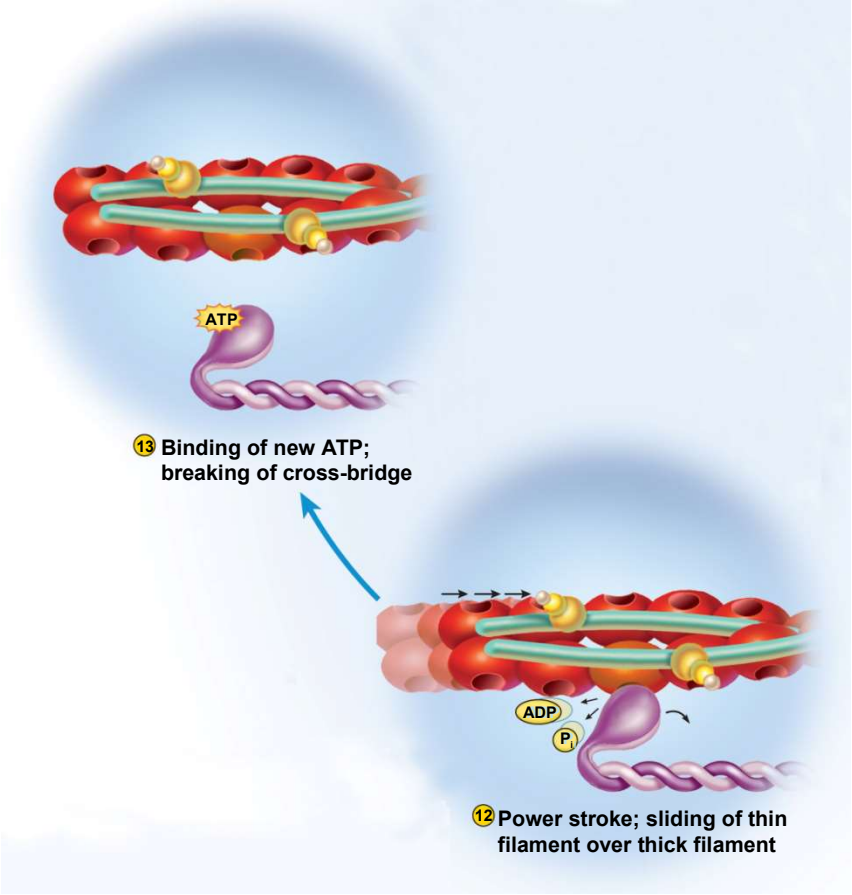
Contraction
(steps 10 and 11)



- Myosin ATPase enzyme in myosin head hydrolyzes ATP molecule
- This reaction occurs independent of the actin – troponin – tropomyocin event
- Myosin head is activated = the head "cocks" to extends head /// ADP + P_i remain attached to head
- Head of myosin binds to actin active site forming a myosin – actin - cross-bridge
- *Now ADP + P released from the myosin head*
- *Now the "Power Stroke" will occur!*

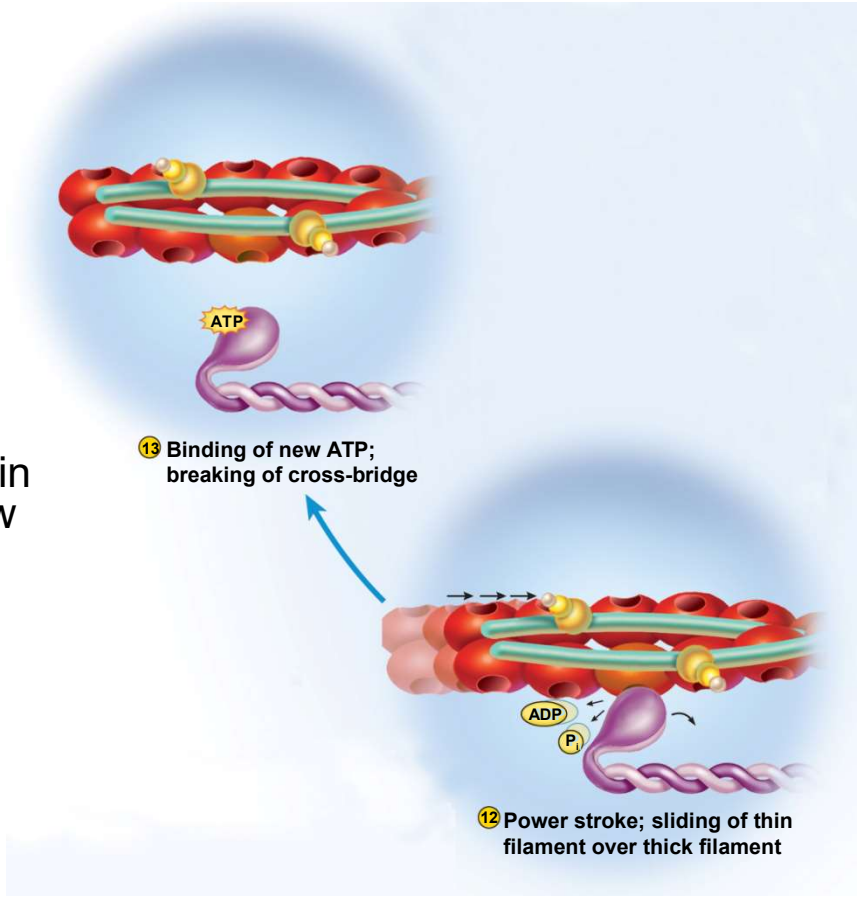
Contraction = Power Stroke (steps 12 and 13)

- **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”



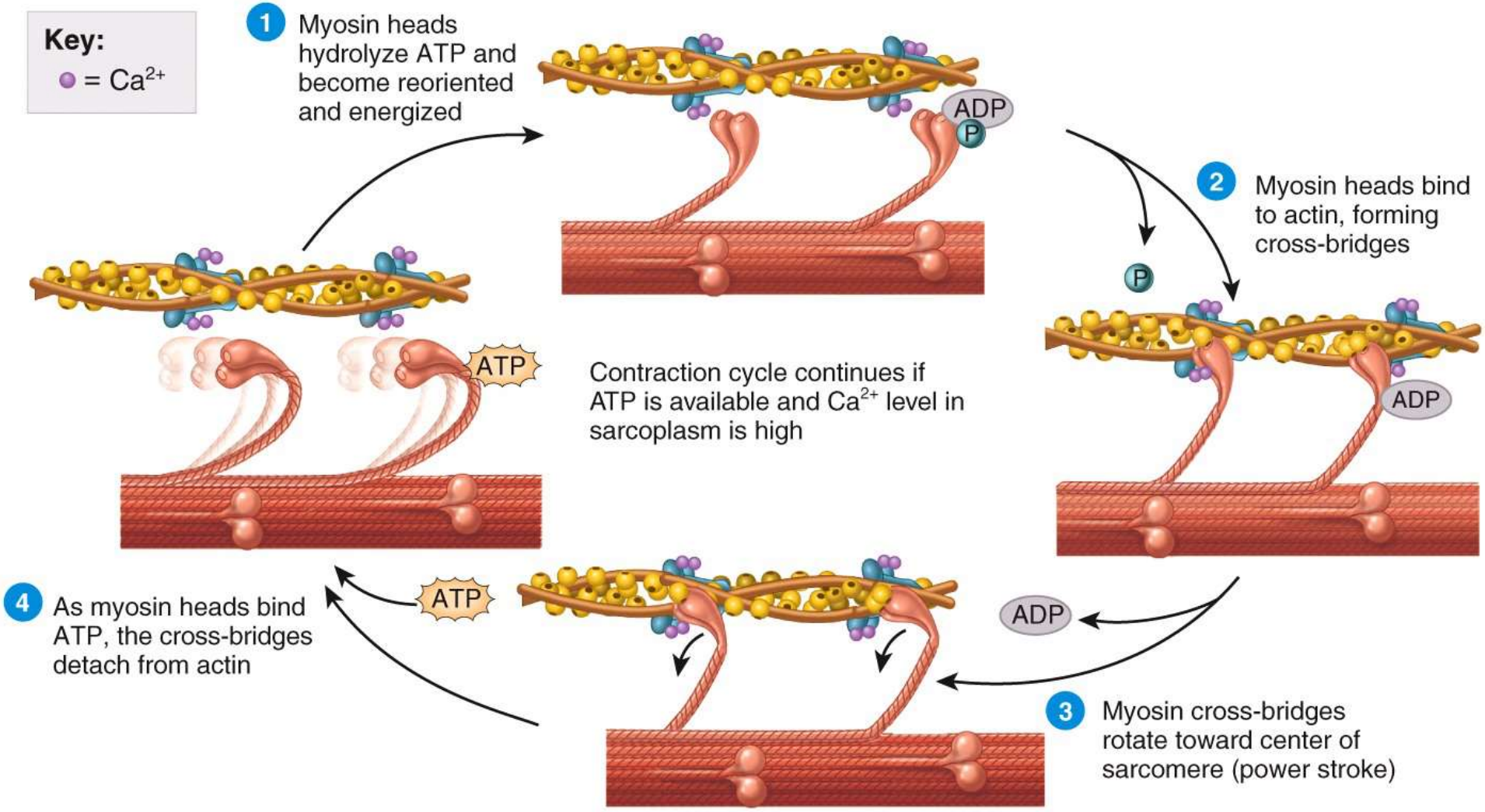
Contraction (steps 12 and 13)

- 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 immeadiately “recocks” myosin head and it is again loaded with energy // now the power stroke maybe 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

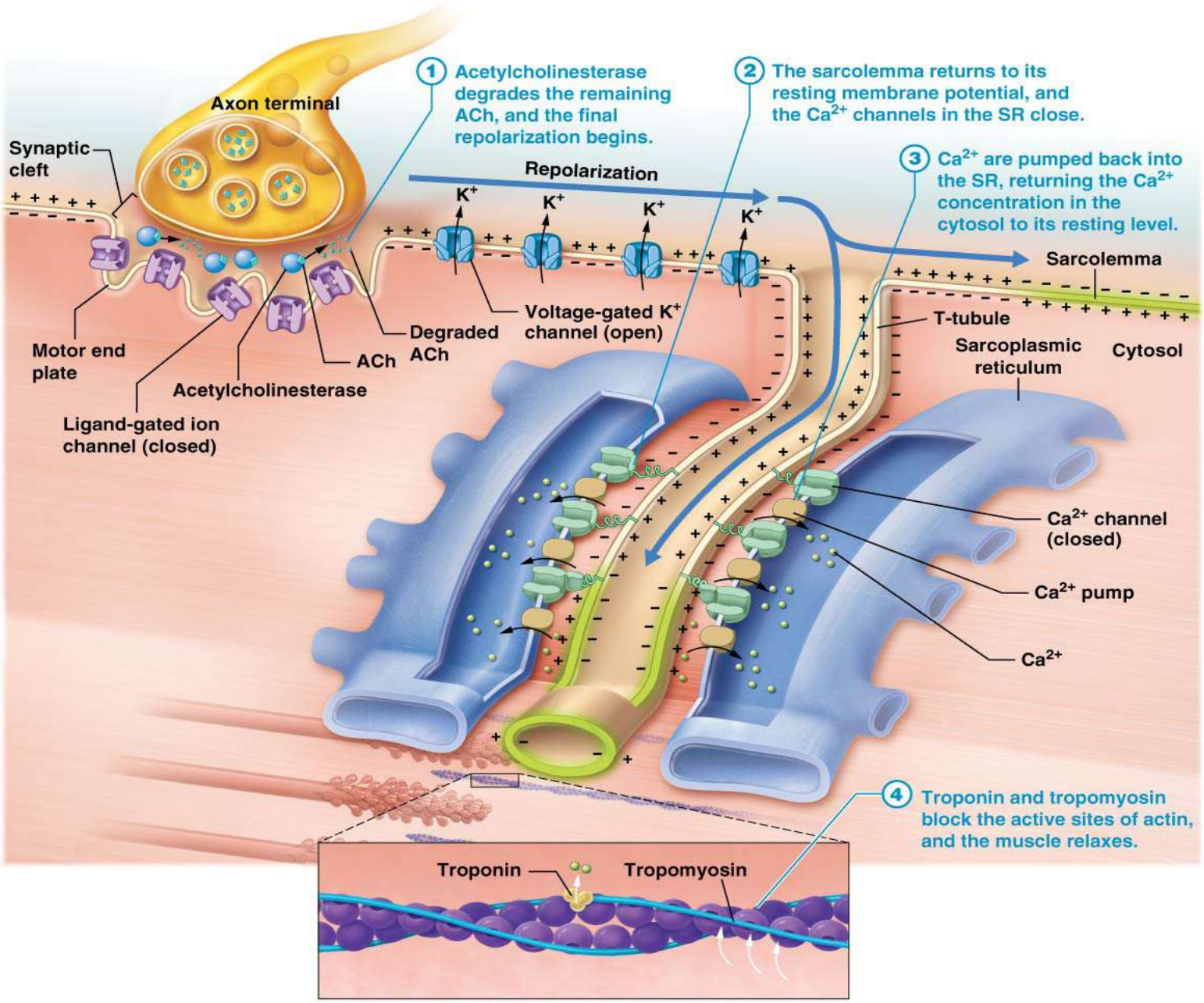


Review of Contraction Cycle

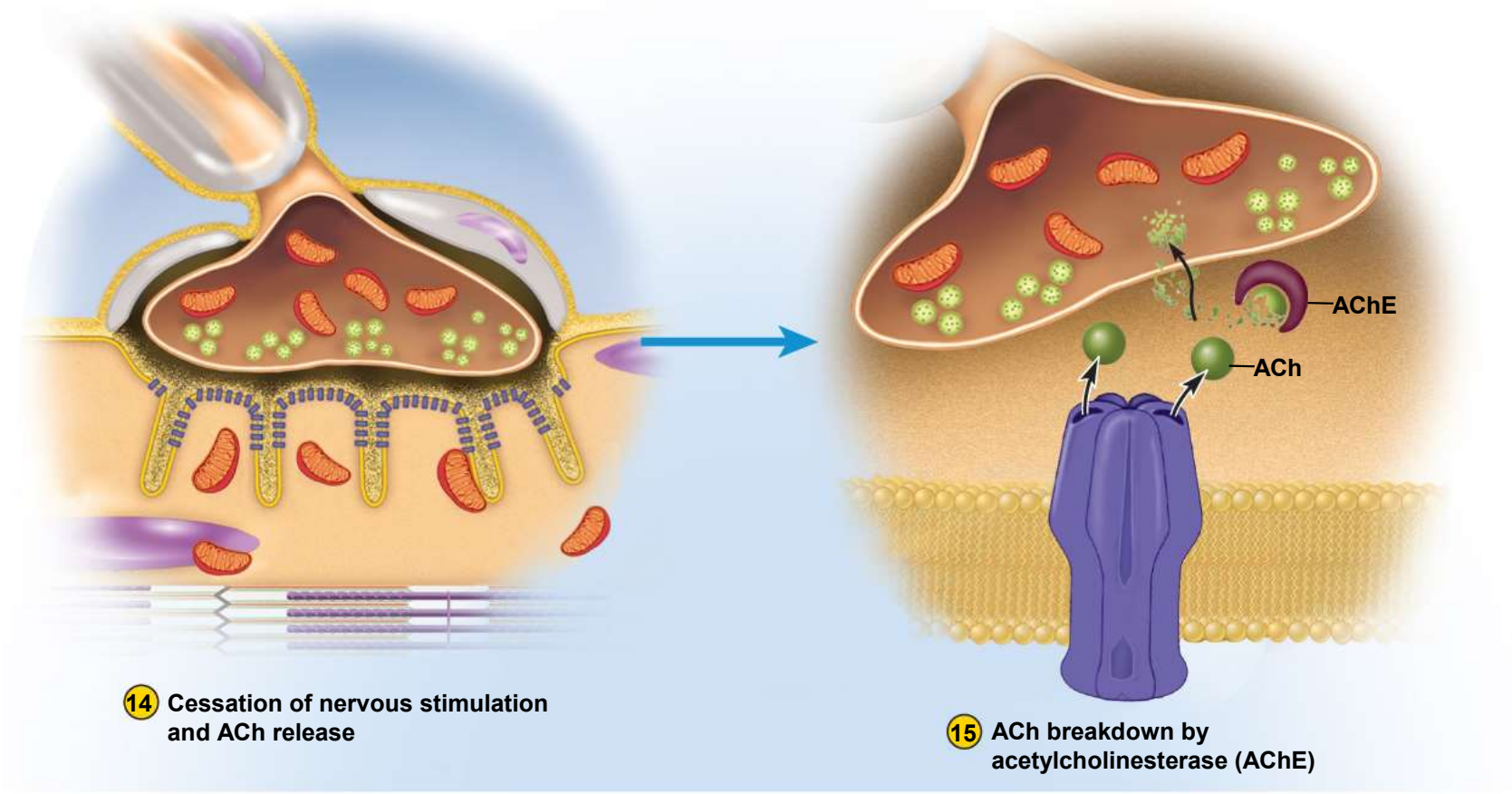
- Contraction cycle = repeating sequence of events
- Results = myosin and actin sliding across each other
- Brings Z-disc closer together (muscle shortening)
- Four Steps in contraction cycle:
  - 1 - ATP hydrolysis (myosin head energized)
  - 2 - Attachment of myosin to actin (forms cross-bridge)
  - 3 - “The Power Stroke” (rotation of myosin molecule)
  - 4 - New ATP molecule binds to myosin /// now myosin cross-bridge to actin broken (ends contraction cycle)


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)

Relaxation phase: the process of muscle relaxation.



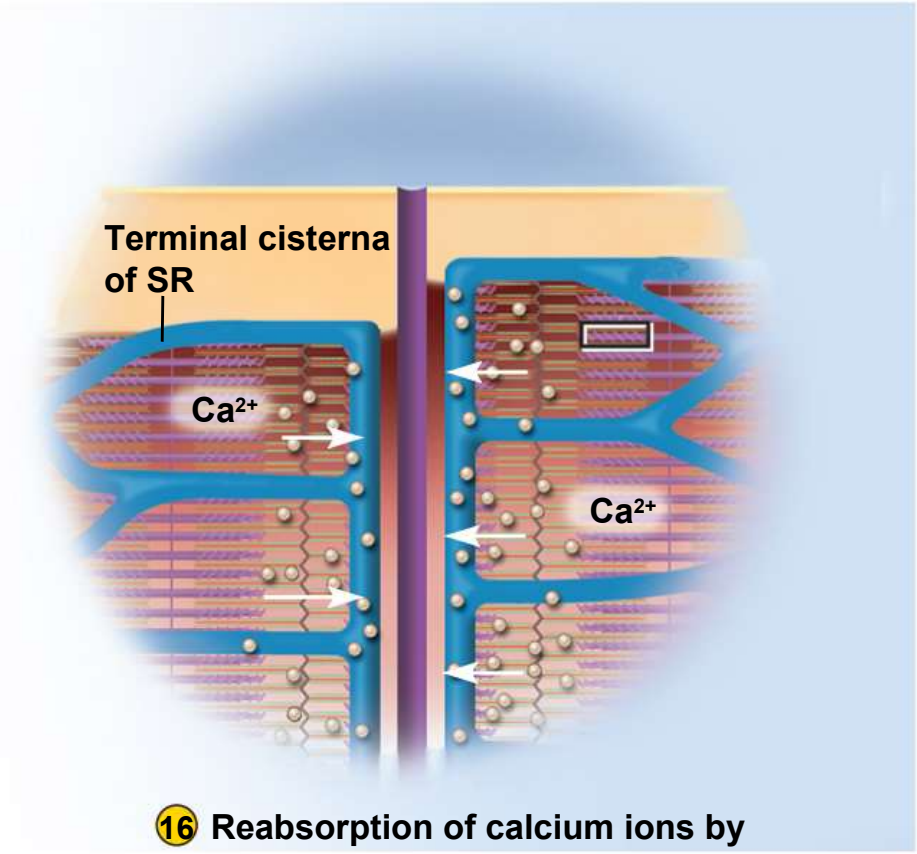
Relaxation (steps 14 and 15)



- Stopping nerve stimulation will stop ACh release
- ACh-Esterase breaks down Ach // fragments reabsorbed into synaptic knob
- This stops stimulation by ACh // now all “downstream” events are reversed

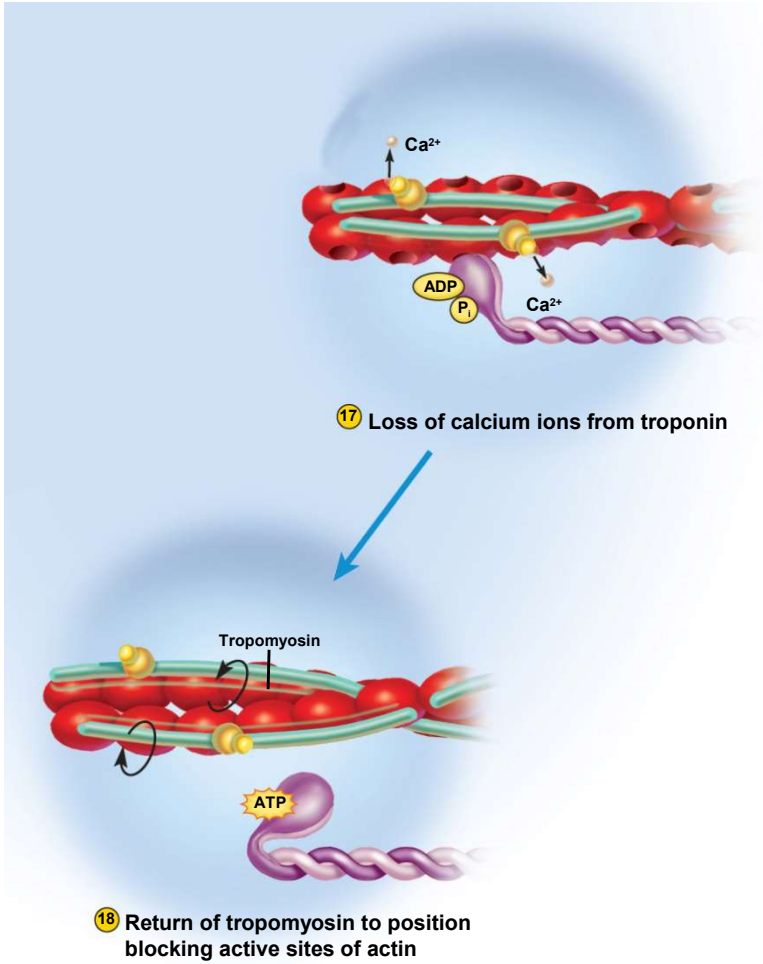
Relaxation (step 16)

- Ca^{+2} pumped back into SR by active transport. // Why is this is active transport?
- Ca^{+2} binds to calsequestrin while in storage in SR
- <u>ATP is needed for both</u>
  - <u>muscle relaxation</u>
  - <u>as well as muscle contraction.</u>



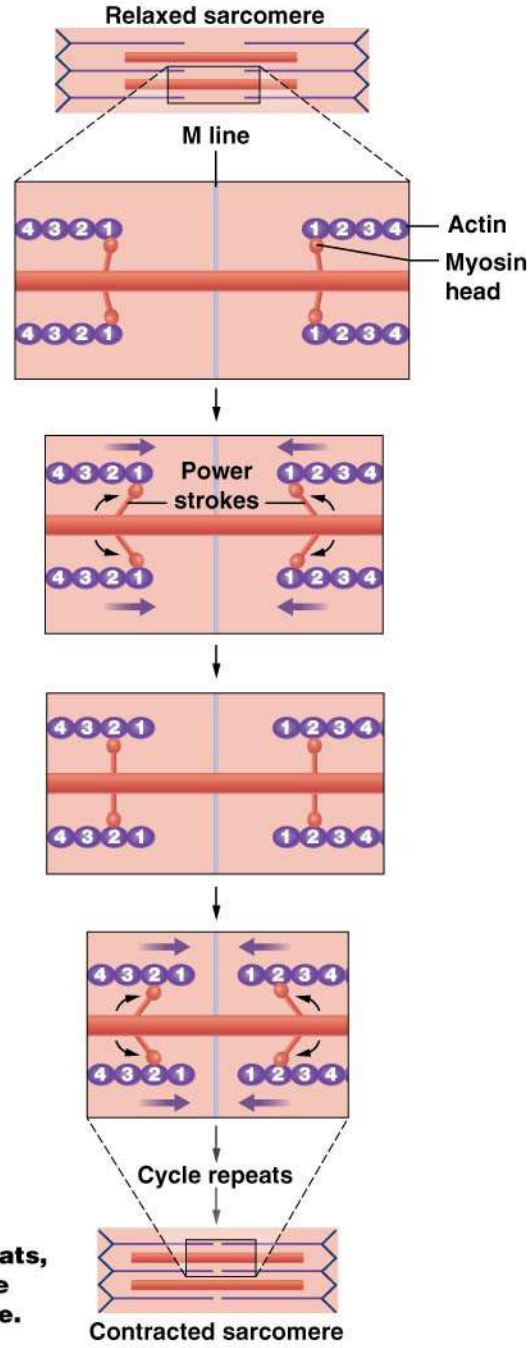
Relaxation (steps 17 and 18)

- Ca^{+2} removed from troponin as calcium is pumped back into SR
- Now tropomyosin once again can block the myosin binding sites
- Muscle fiber ceases to produce or maintain tension
- Muscle fiber returns to its resting length
  - due to recoil of elastic components & contraction of antagonistic muscles



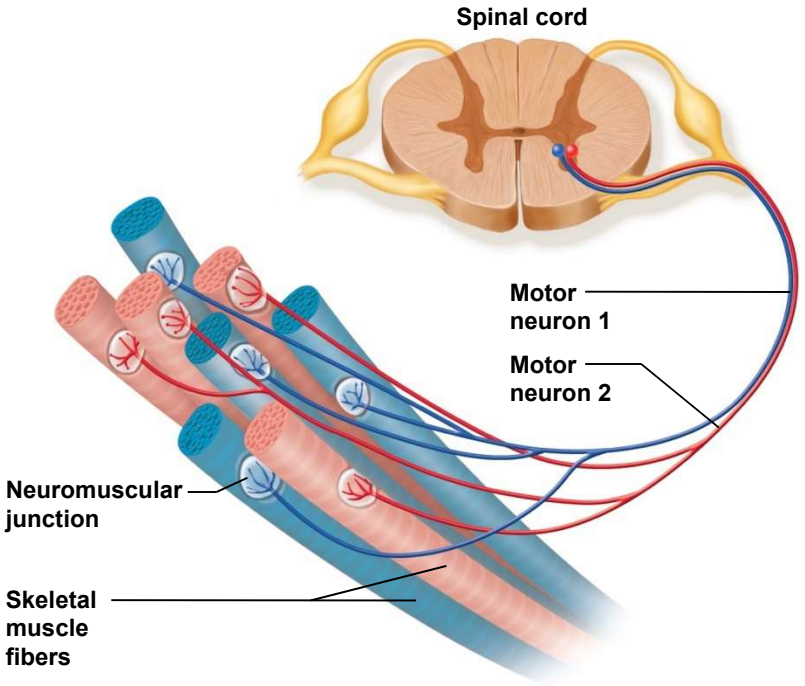
Contraction Phase:

the crossbridge cycle of the sliding-filament mechanism.



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

What is the significance of a motor unit?



Motor unit = one nerve fiber's axon and all the muscle fibers innervated by that onenerve fiber

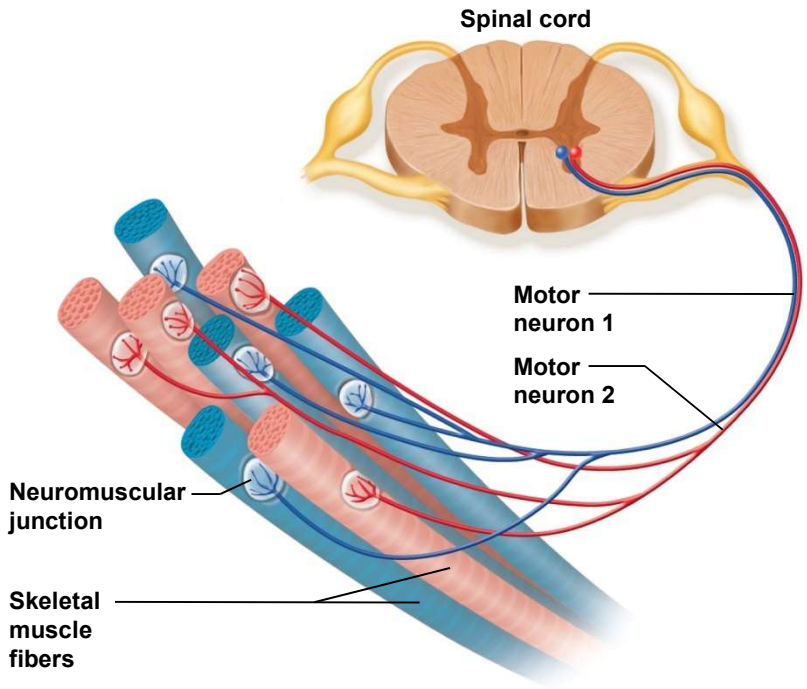
Motor Units

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

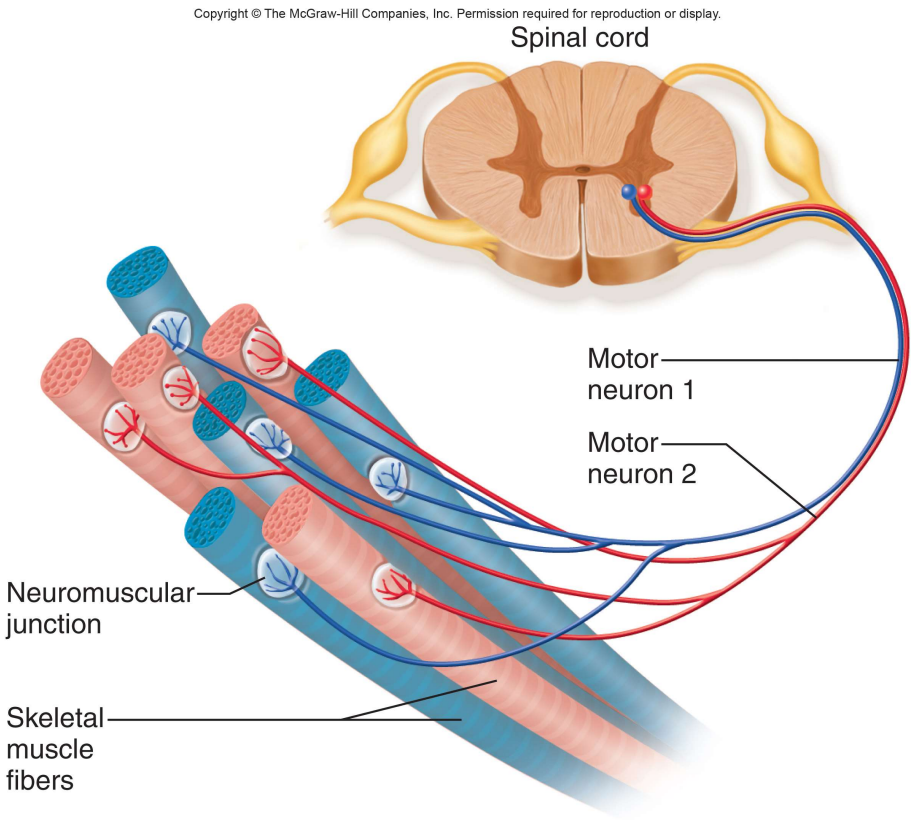
- large motor units – more strength than control // many muscle fibers per motor unit

  – powerful contractions supplied by large motor units

  – gastrocnemius has 1000 muscle fibers per neuron



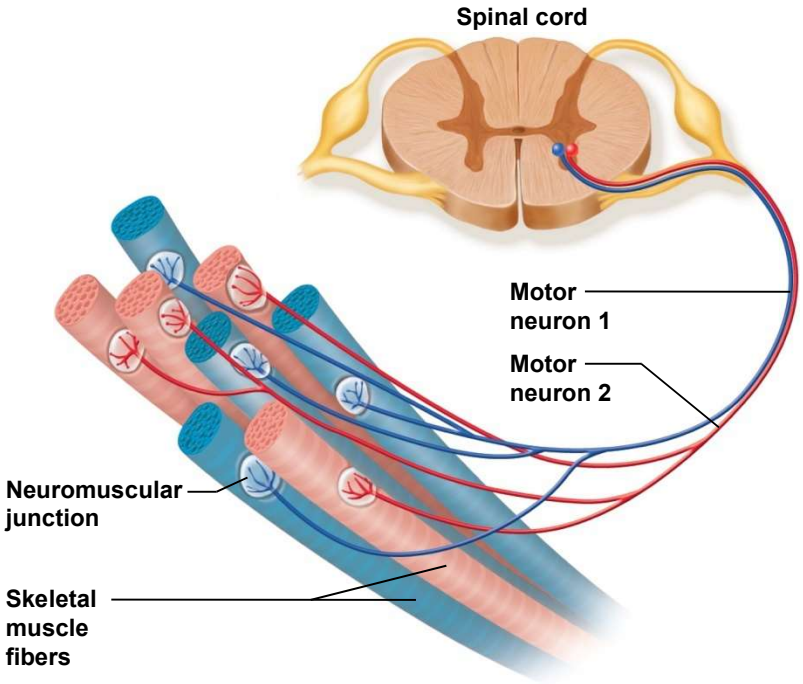
Motor Units



How do we use motor units?

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”



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 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 induced by breakdown of sarcoplasmic reticulum that releases Ca^{+2}

  - Deteriorating sarcolemma allows Ca^{+2} to enter cytosol /// also lack of ATP means no active transport to keep calcium inside SR

  - Ca^{+2} activates myosin-actin cross-bridge – can not break cross bridges /// also no new ATP being prtoduced so can not remove calcium ions from cytosol

  - Gradually, muscle generates more tension as more and more calcium is released from sacroplasm reticulum /// more myosin-actin cross bridges are formed

  - But muscle can not relax // why? /// because after death - new ATP can not be formed /// ATP is required to “break” myosin-actin cross bridge

Rigor Mortis

- Under normal conditions, muscle relaxation requires ATP to break myosin-actin cross bridges
  - ATP only last a few seconds in the cytosol
  - ATP production is not produced after death
  - So following rigor mortis, the muscle organ will gradually lose tension but this now ccurs 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 it from degrading Ach
    - spastic paralysis - a state of continual contraction of the muscles
    - 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 and 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 stimulating the muscles
    - 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
    - botox cosmetic injections for wrinkle removal.