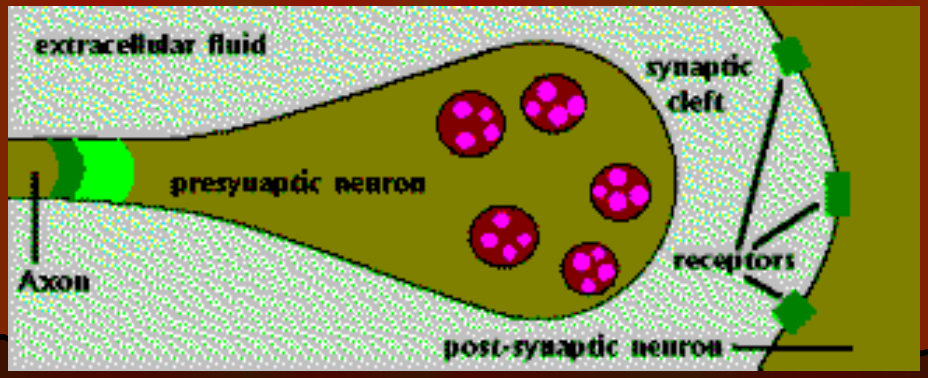
The Neuromuscular Junction
(Neuromuscular Synapse)

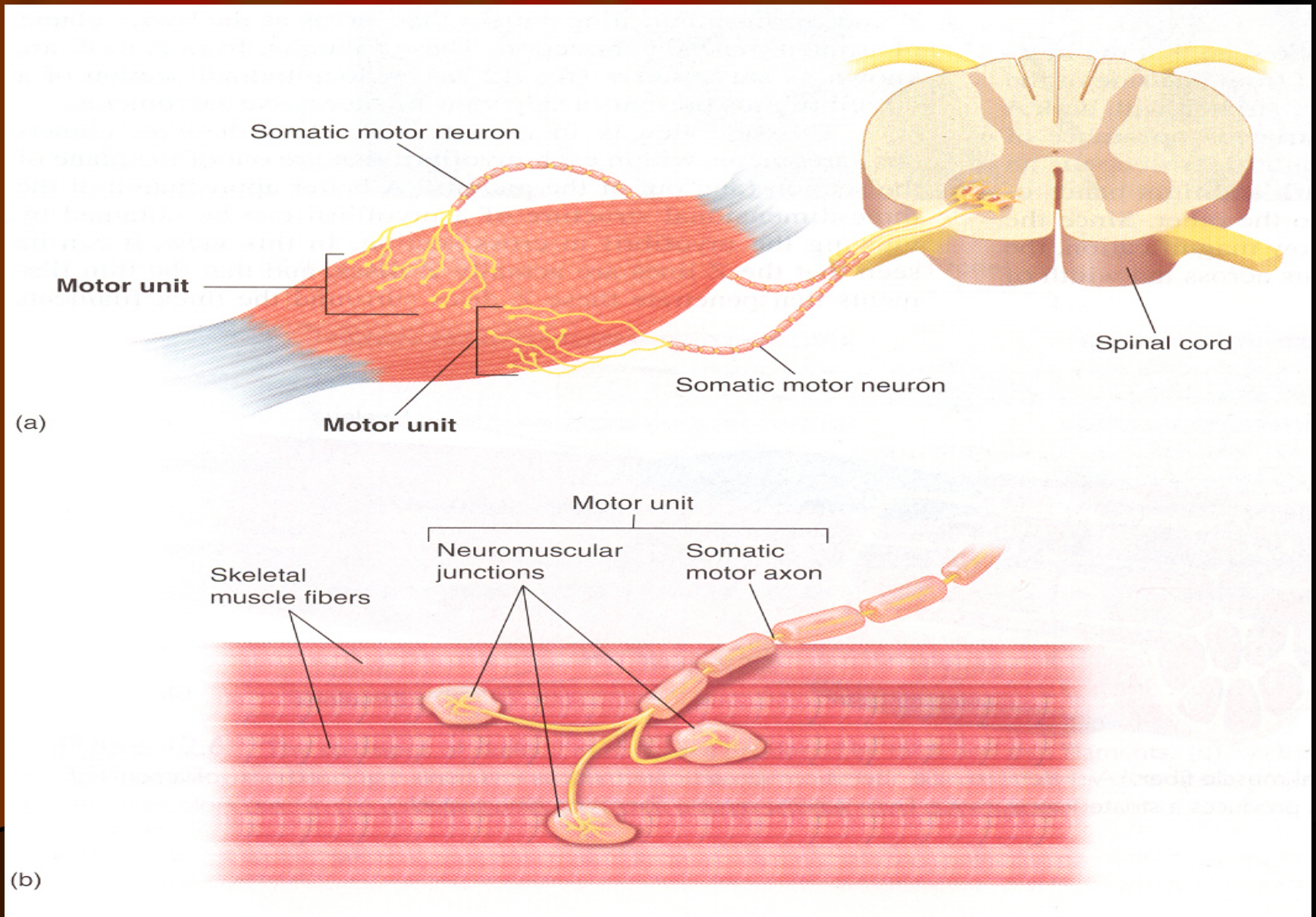
Dr. Taha Sadig Ahmed

Chemical Signals

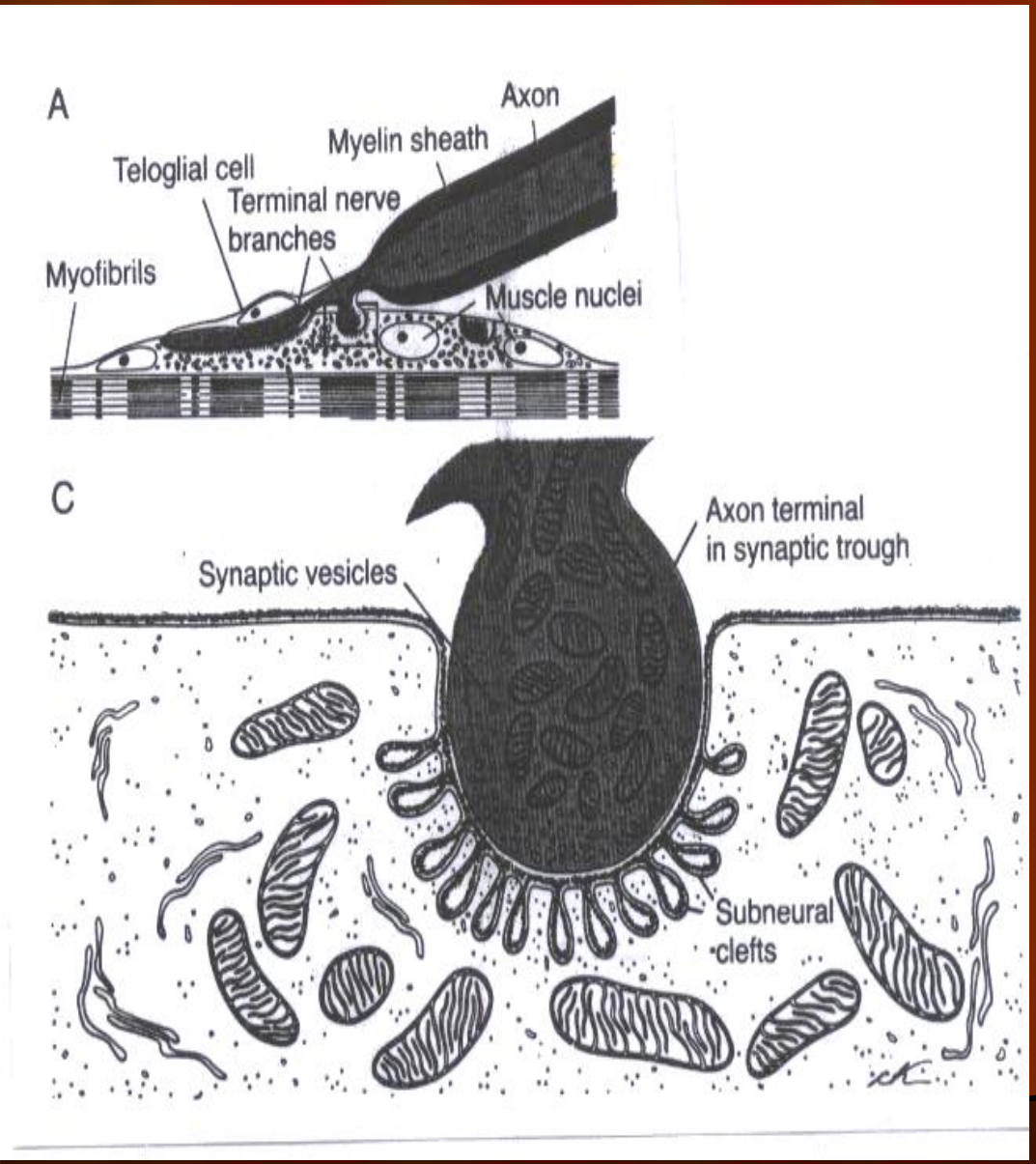
- One neuron will transmit info to another neuron or to a muscle or gland cell by releasing chemicals called neurotransmitters.
- The site of this chemical interplay is known as the synapse.
  - An axon terminal (synaptic knob) will abut another cell, a neuron, muscle fiber, or gland cell.
  - This is the site of transduction – the conversion of an electrical signal into a chemical signal.



Neuromuscular Junction (NMJ)



The Neuromuscular junction consists of



A/ Axon Terminal : contains around 300,000 vesicles which contain the neurotransmitter acetylcholine (Ach).

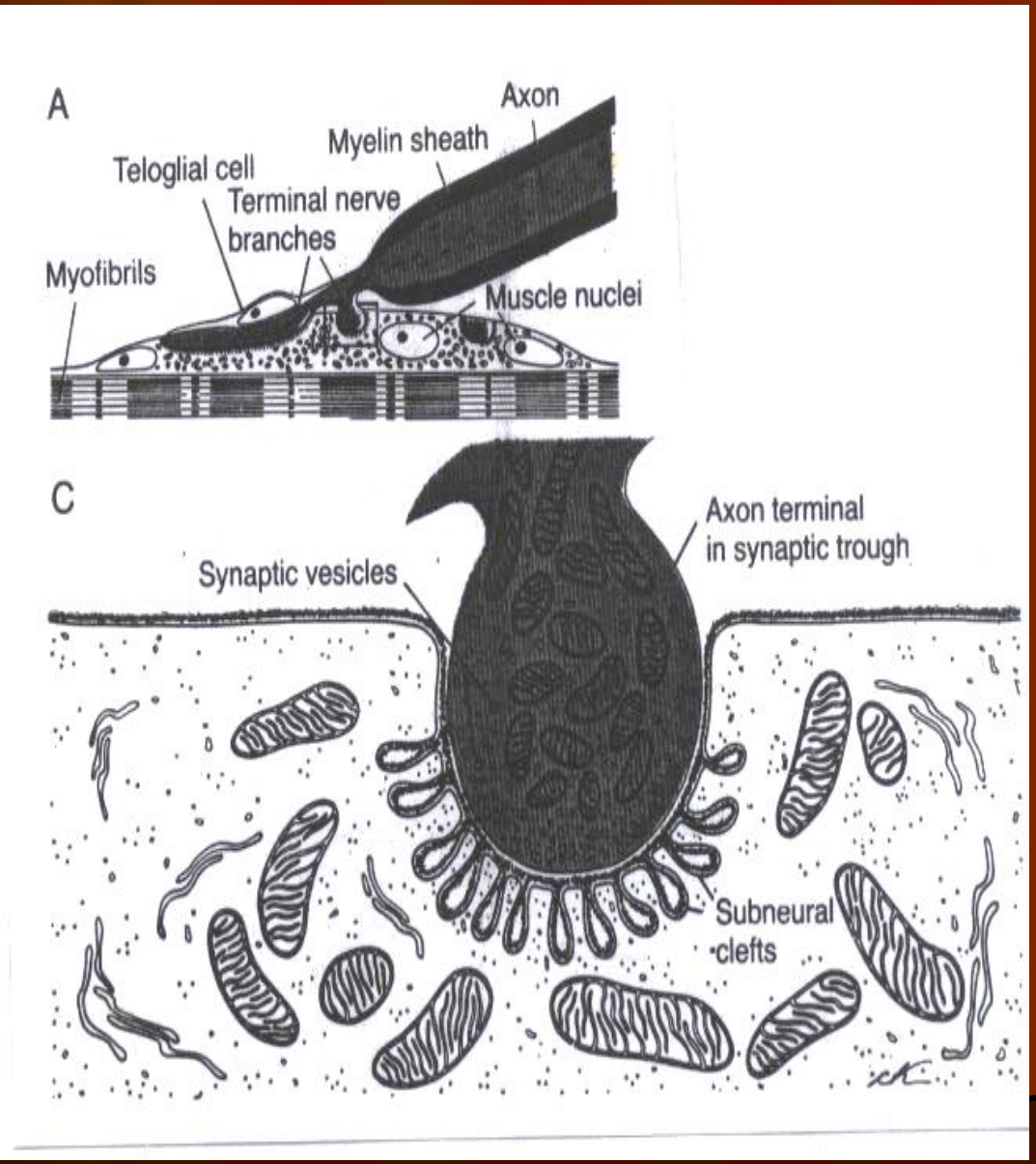
B/ Synaptic Cleft :

20 – 30 nm (nanometer) space between the axon terminal & the muscle cell membrane. It contains the enzyme cholinesterase which can destroy Ach .

C/ Synaptic Gutter (Synaptic Trough)

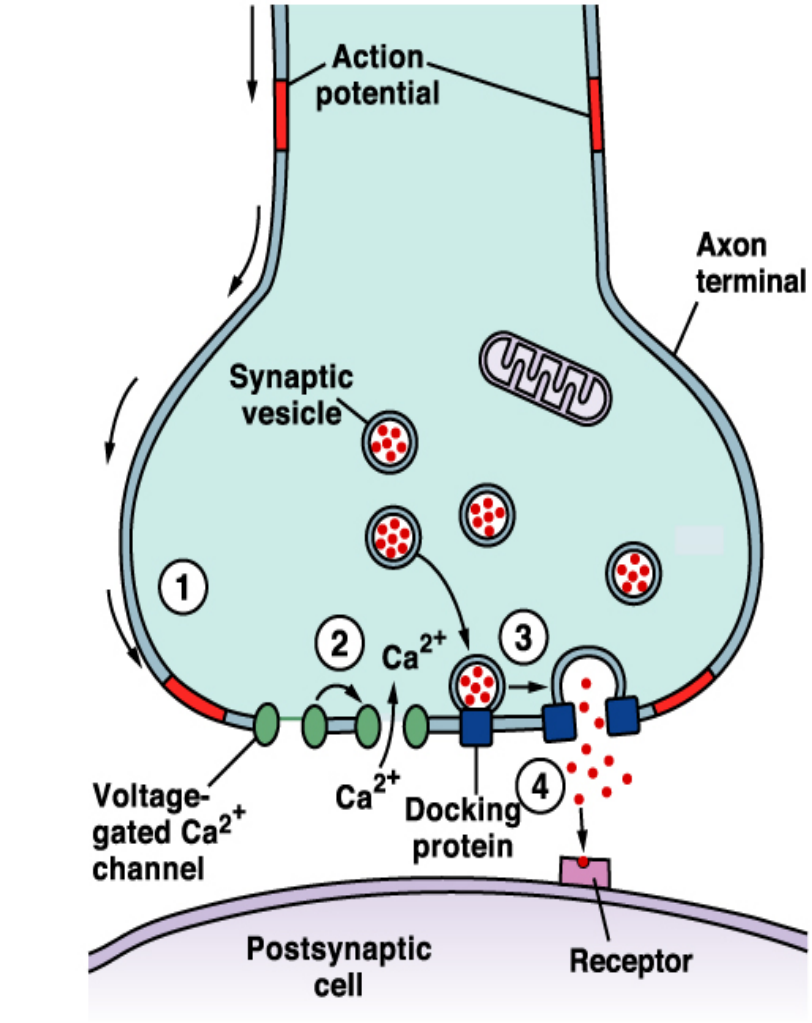
It is the muscle cell membrane which is in contact with the nerve terminal . It has many folds called **Subneural Clefts** , which greatly increase the surface area , allowing for accomodation of large numbers of Ach receptors . Ach receptors are located here .

The Neuromuscular junction consists of



- **The entire structure of axon terminal , synaptic cleft and synaptic gutter is called " Motor End-Plate " .**
- **Ach is synthesized locally in the cytoplasm of the nerve terminal , from active acetate (acetylcoenzyme A) and choline.**
- **Then it is rapidly absorbed into the synaptic vesicles and stored there.**
- **The synaptic vesicles themselves are made by the Golgi Apparatus in the nerve soma (cell-body).**
- **Then they are carried by Axoplasmic Transport to the nerve terminal , which contains around 300,000 vesicles .**

Acetylcholine (1)



- Ach is synthesized locally in the cytoplasm of the nerve terminal , from active acetate (acetylcoenzyme A) and choline.
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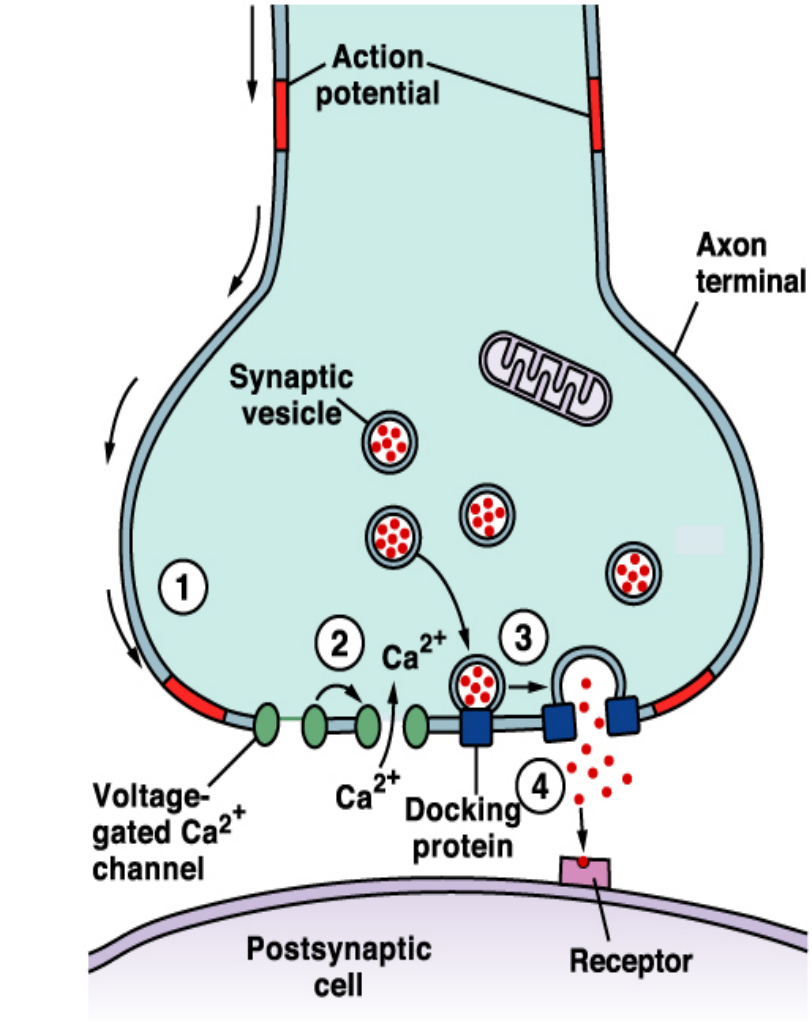
- The synaptic vesicles themselves are made by the Golgi Apparatus in the nerve soma (cell-body).
- Then they are carried by Axoplasmic Transport to the nerve terminal , which contains around 300,000 vesicles .
- Each vesicle is then filled with around 10,000 Ach molecules .

Acetylcholine (2)



- When a nerve impulse reaches the nerve terminal ,
- it opens calcium channels →

  calcium diffuses from the ECF into the axon terminal → Ca++ releases Ach from vesicles by a process of EXOCYTOSIS
- One nerve impulse can release 125 Ach vesicles.
- <u>The quantity of Ach released by one nerve impulse is more than enough to produce one End-Plate Potential .</u>

- Ach combines with its receptors in the subneural clefts. This opens sodium channels → & sodium diffuses into the muscle causing a **local,non-propagated** potential called the " **End-Plate Potential (EPP)",**.
- This EPP triggers a **muscle AP** which spreads down inside the muscle to make it contract .

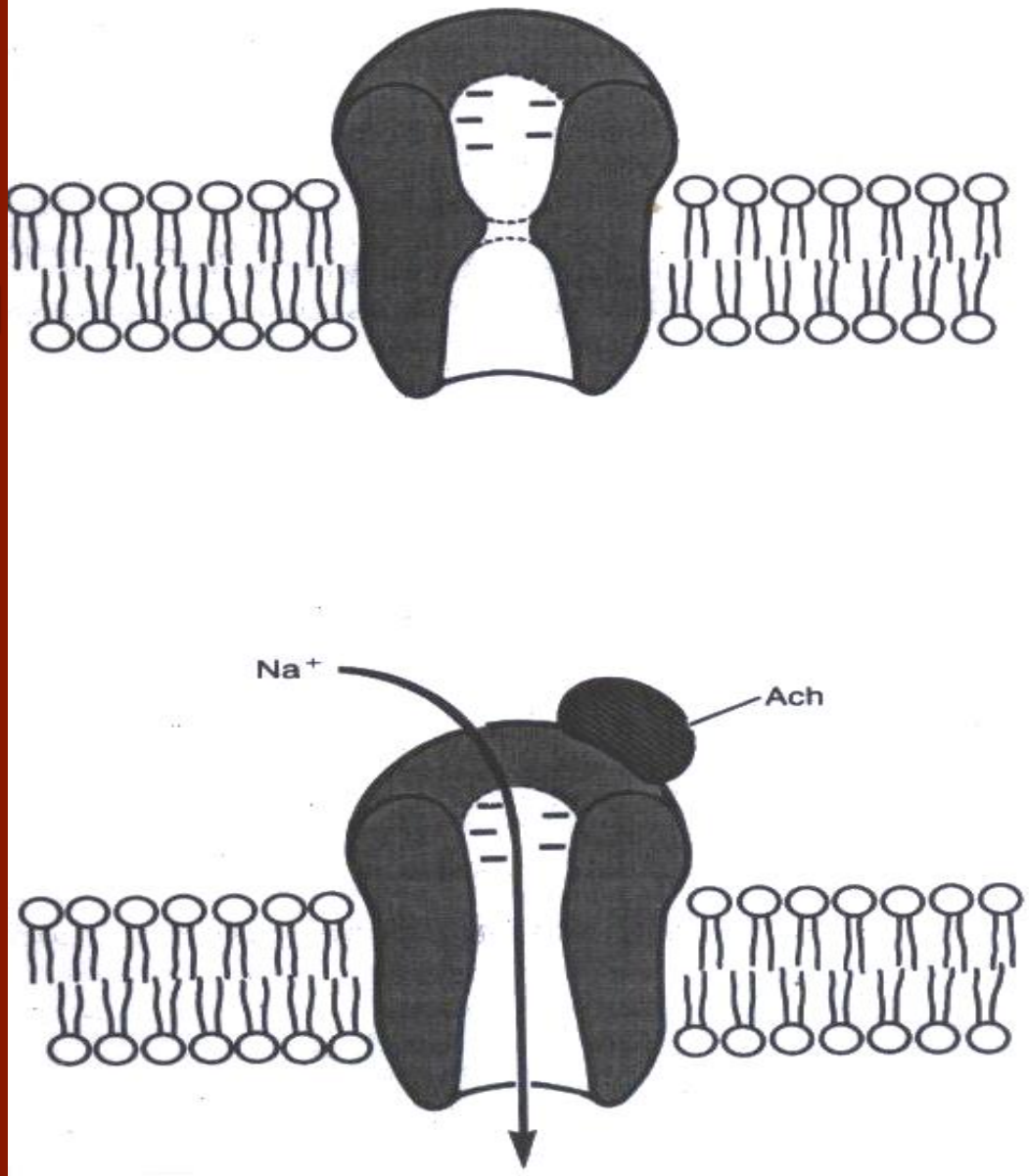
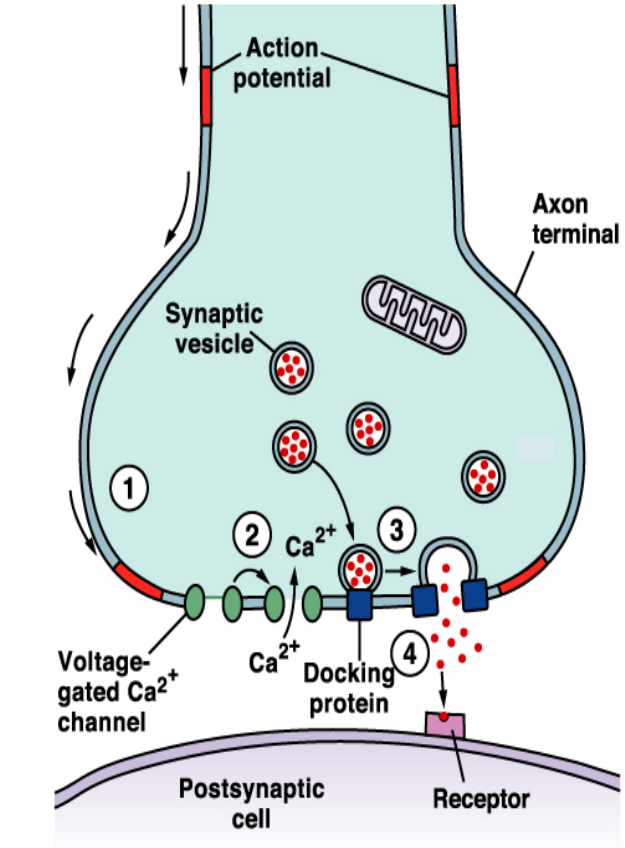
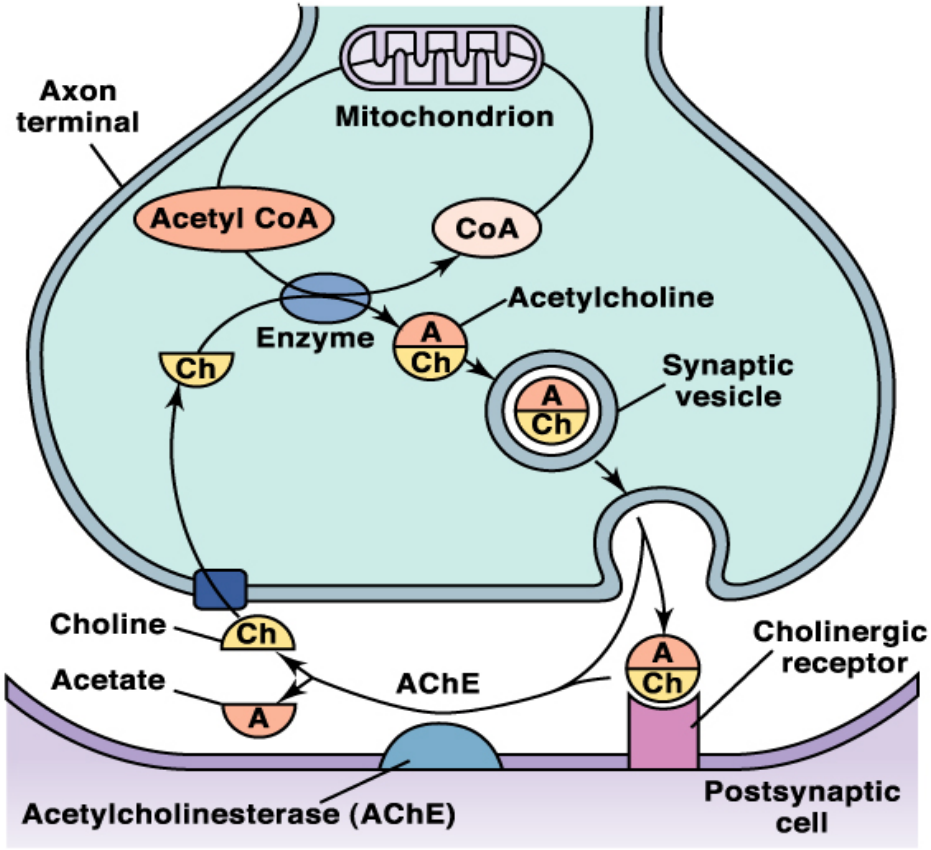


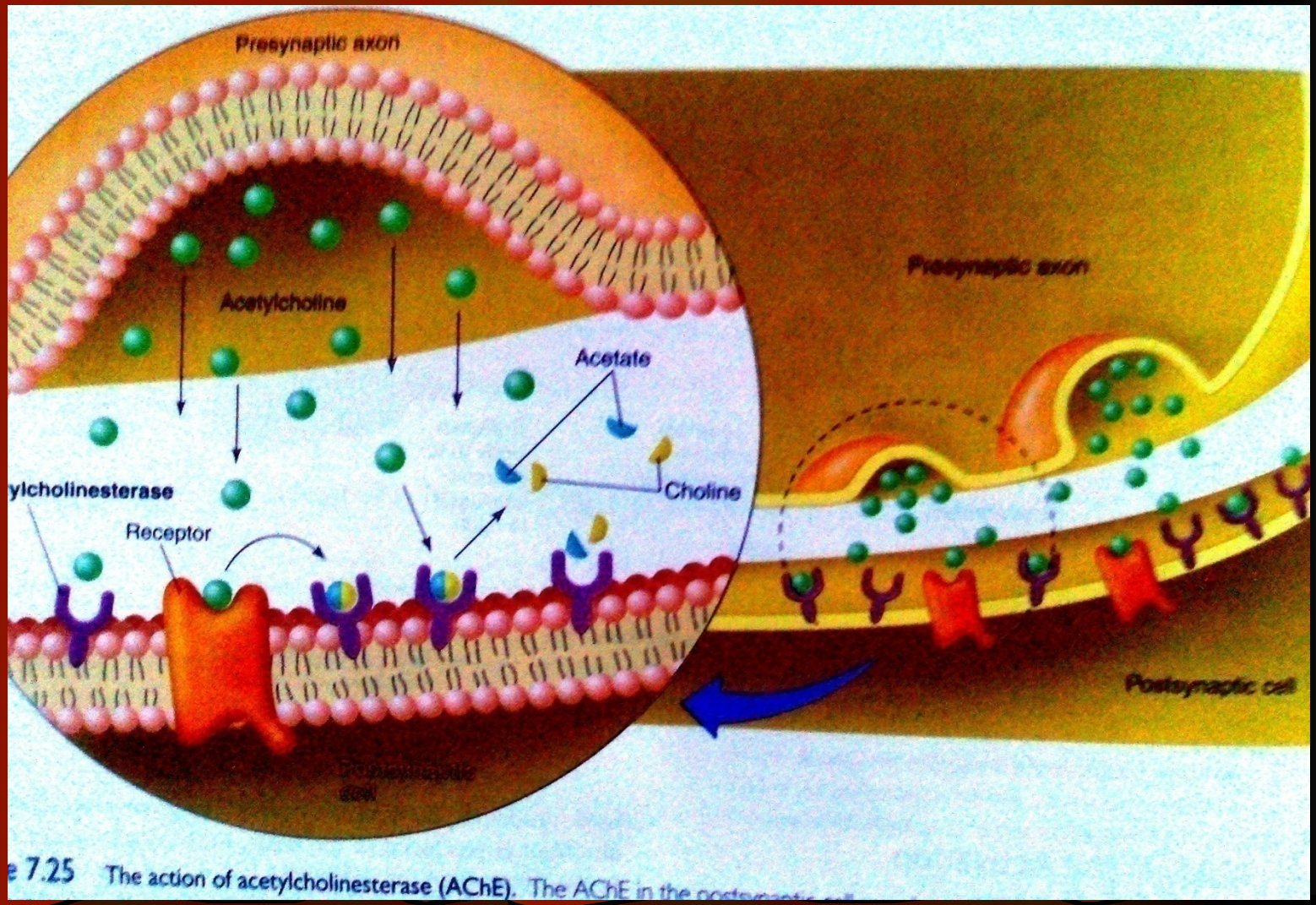
Figure 7–3 The *acetylcholine channel:* Above, while in the closed state. Below, after acetylcholine has become attached and a conformational change has opened the channel, allowing excess sodium to enter the muscle fiber and excite contraction. Note the negative charges at the channel mouth that prevent passage of negative ions.





- After ACh acts on the receptors , it is hydrolyzed by the enzyme Acetylcholinesterase (cholinesterase) into Acetate & Choline . The Choline is actively reabsorbed into the nerve terminal to be used again to form ACh. This whole process of Ach release, action & destruction takes about 5-10 ms .

Destruction of Ach

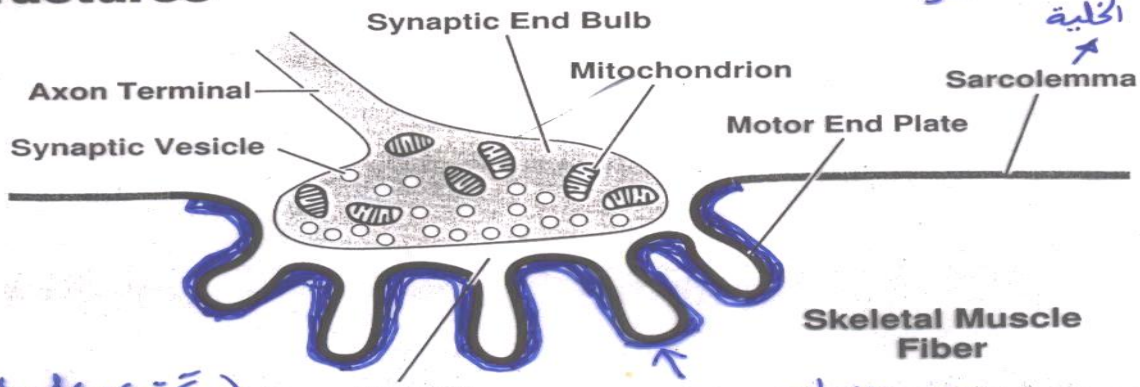


e 7.25 The action of acetylcholinesterase (AChE). The AChE in the

NEUROMUSCULAR JUNCTION

هذه المنطقة يتولد فيها ال A.P الذى هو يطيع قانون ALL-OR-NONE وغير متدرج و ينتشر فى كل أنحاء الخلية

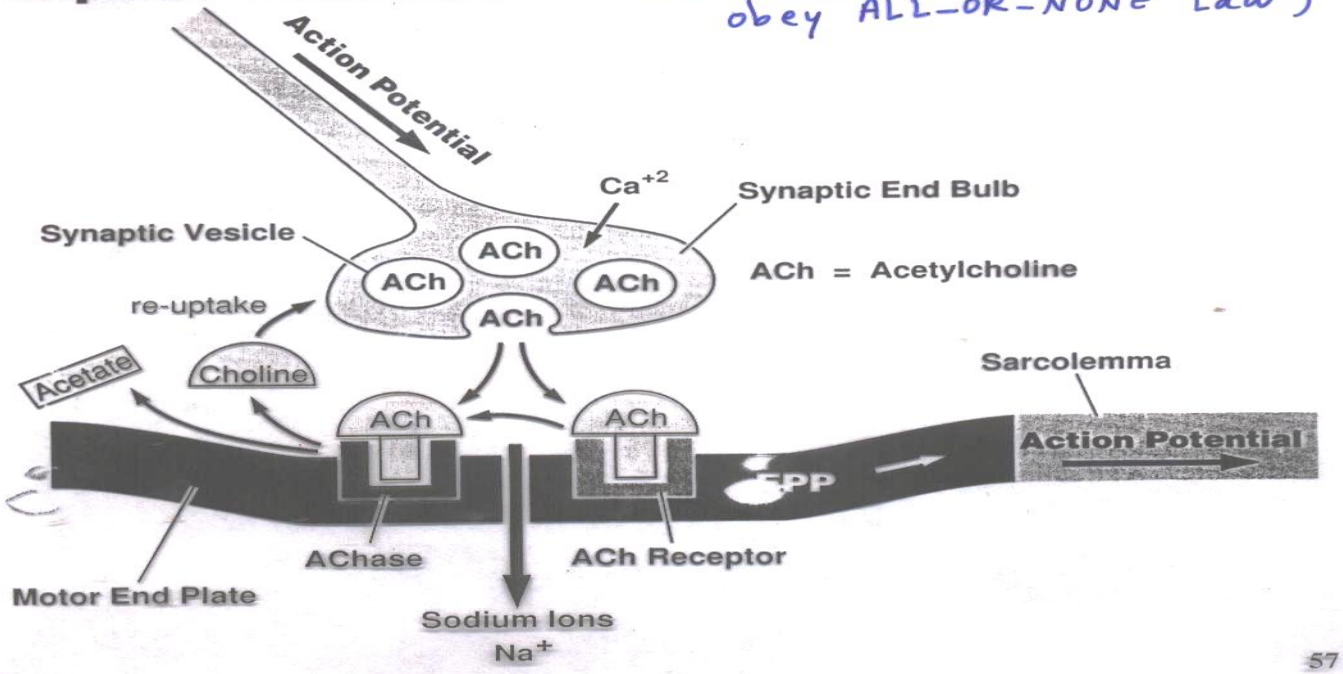
Structures



(تحتوى على ال Cholinesterase)

هذه المنطقة يتولد فيها ال END-PLATE POTENTIAL (Graded, does not spread, can be summated, does not obey ALL-OR-NONE Law)

Impulse Transmission



Drugs that act on the neuromuscular junction

1-Drugs that act on muscle fiber by Ach like action:-

METHACHOLINE- CARBACOL- NICOTINE

they act for minutes or hours—as they do not destructed by Ach estrase enzyme .

2-Drugs that block transmission at neuromuscular junction:-

_CURARE & CURARIFORM like_drugs.

act by competitive inhibition to Ach at its receptors & can not cause Depolarization.

- **3-Drugs that stimulate transmission at neuromuscular junction by inactivation of Ach estrase enzyme:-**
- **A-Neostigmine ,prostigmine and physostigmine:-** inactivates Ach estrase enzyme temporarly
- **b- di-isopropyl – florophosphate(nerve gas poison)** inactivates Ach estrase enzyme for days & weeks -------death because of respiratory muscle spasm

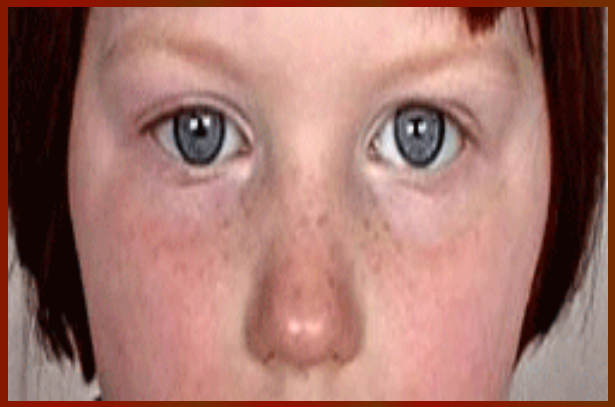
Myasthenia Gravis

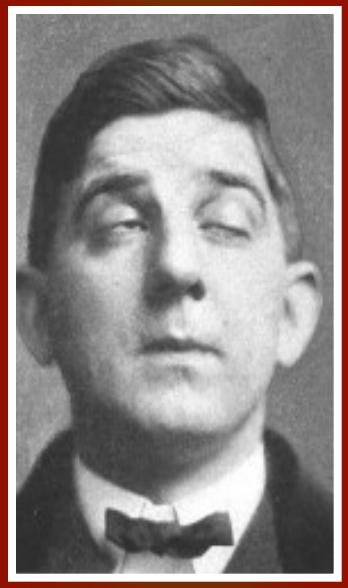
- Auto-immune disease
- Antibodies against Ach receptors destroy many of the receptors → decreasing the EPP , or even preventing its formation → weakness or paralysis of muscles (depending on the severity of the disease) .
- → patient may die because of paralysis of respiratory muscles.
- Treatment : Anti-cholinestersae drugs . These drugs inactivate the cholinesterase enzyme (which destroys Ach) and thereby allow relatively large amounts of Ach to accumulate and act on the remaining healthy receptors → good EPP is formed → muscle contraction .

-An autoimmune disorder

-- body form antibodies against Ach receptors. Patients have 20% of number of Ach receptors .

--the EPPs are too small to trigger action potentials & the muscles can not contract.



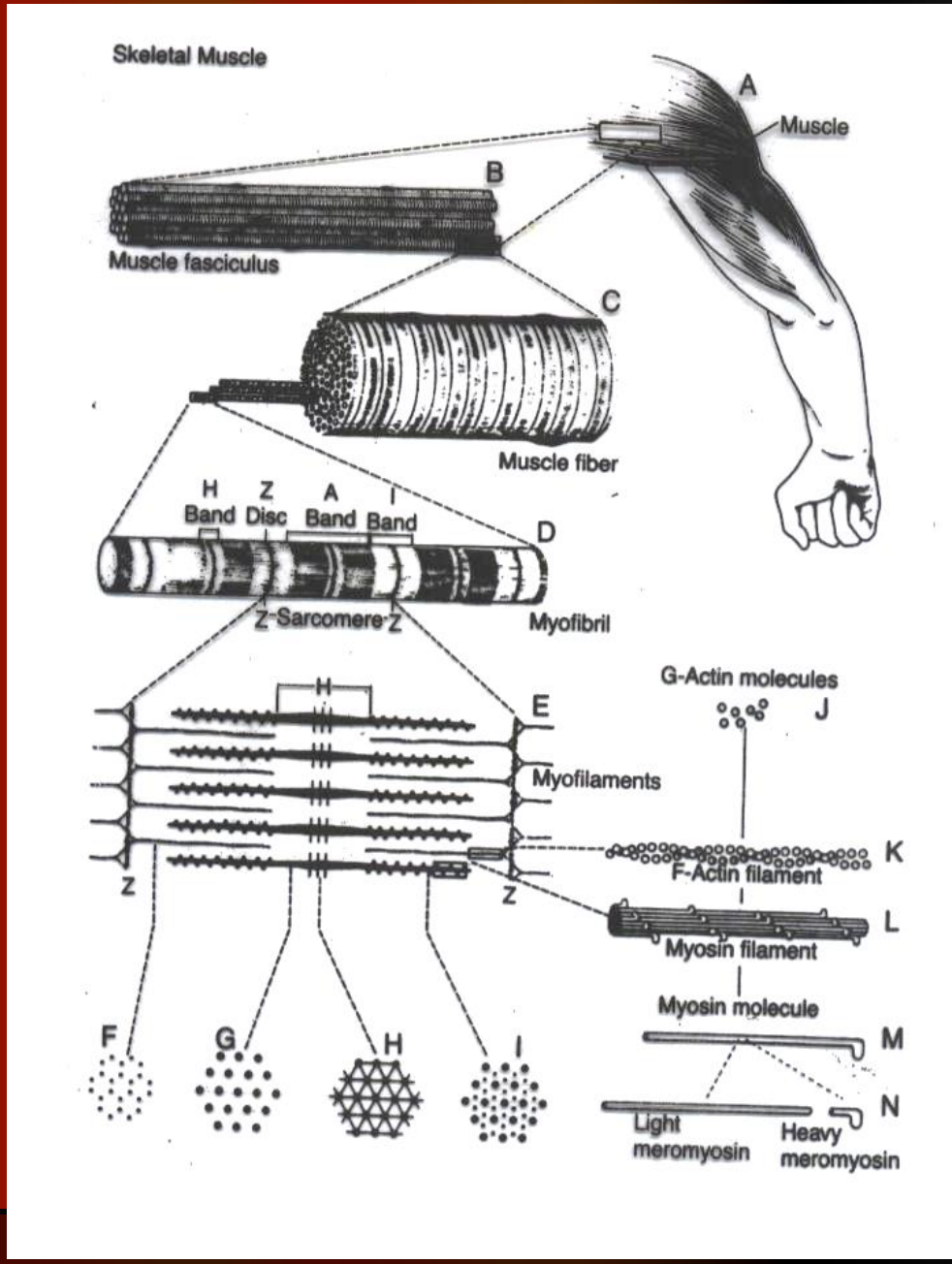


Physiology of Skeletal Muscle Contraction

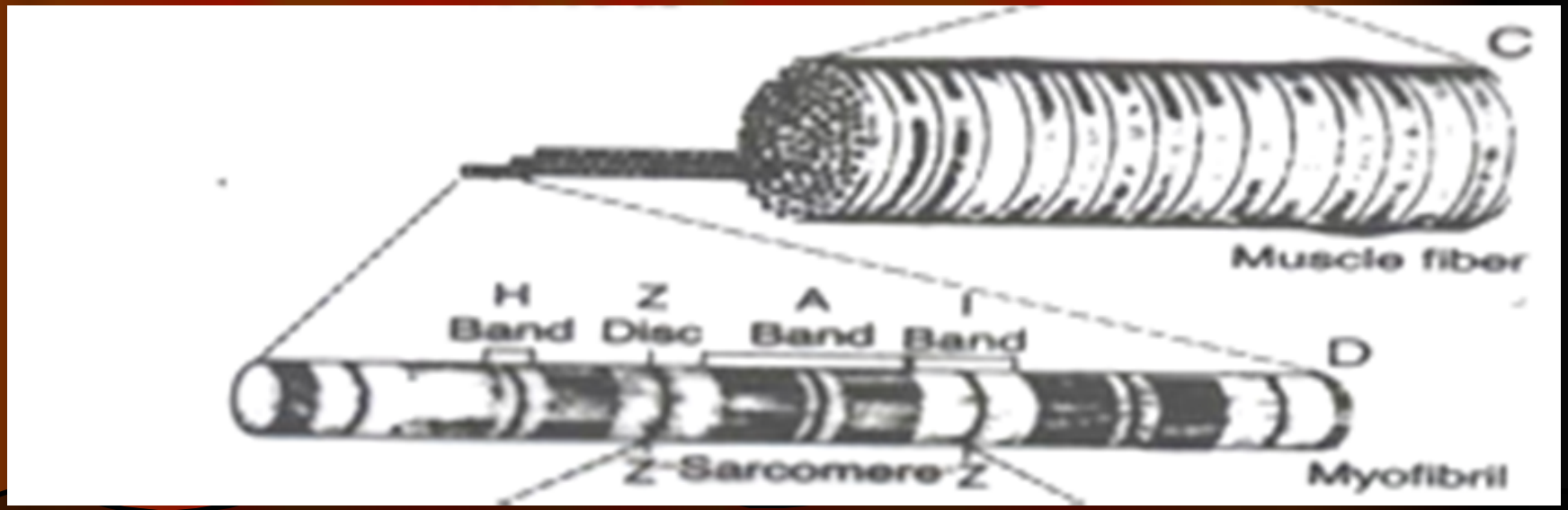
The Muscle Action Potential (AP)

- Muscle RMP = -90 mV (same as in nerves) .
- Duration of AP = 1-5 ms (longer duration than nerve AP , which is usually about 1 ms) .
- Conduction Velocity = 3-5 m/s (slower than big nerves) .

- Each muscle cell (fiber) is covered by a cell-membrane called Sarcolemma.
- Each cell contains between a few hundreds to a few thousands Myofibrils.



- Each Myofibril contains Actin filaments (thin) & Myosin (thick) filaments .
- Each myofibril is striated: consisting of dark bands (called A-bands) and light (I-bands).



Sarcoplasm=

matrix inside muscle fiber in which myofilaments susbended

Sarcoplasmic reticulum=

it is endoplasmic reticulum inside sarcoplasm full of Ca.

T- tubules:-

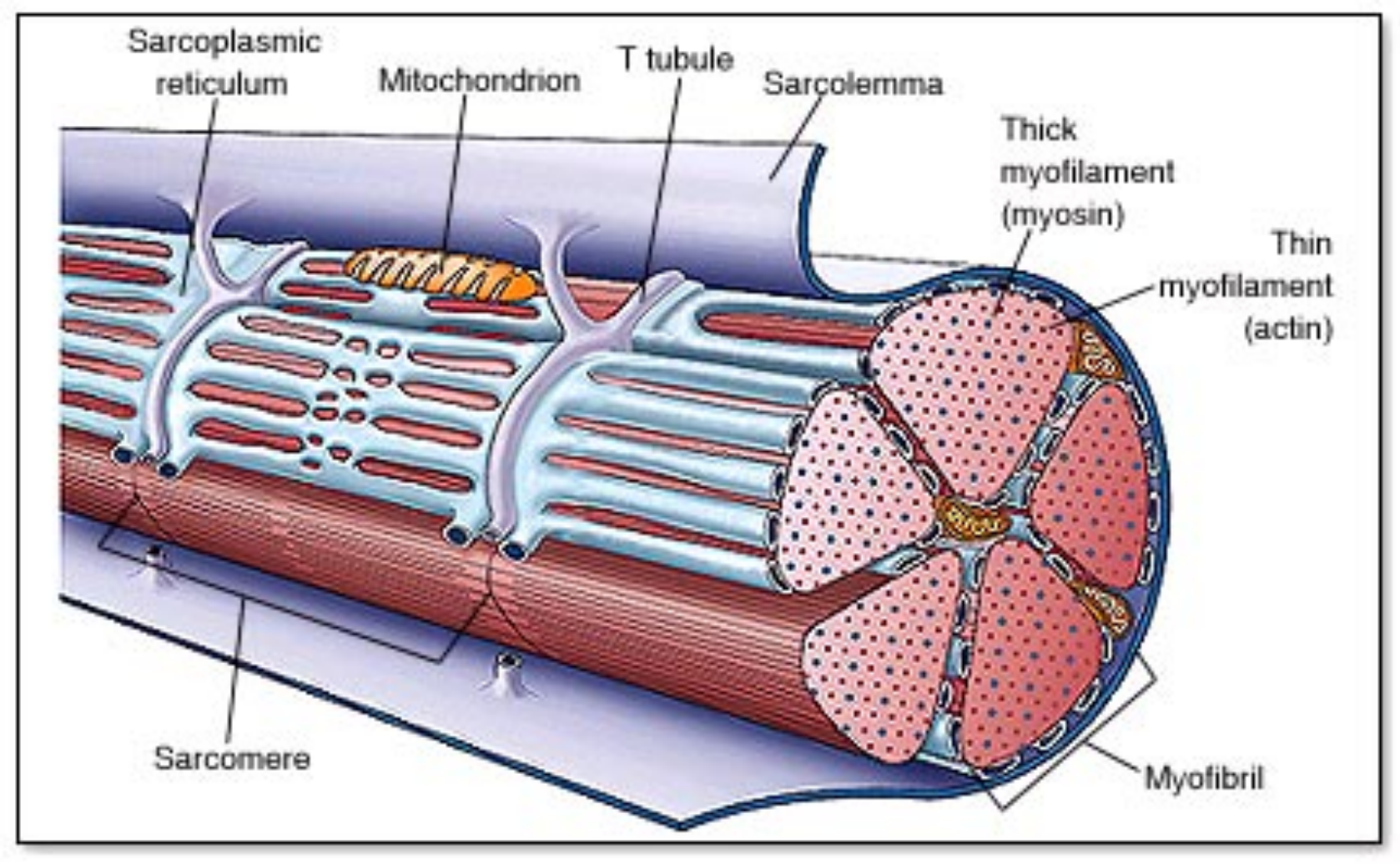
extend from one side of muscle to other (function?).

Sarcomere=

contractile unit of muscle, it is the zone between two Z lines (discs)=2 micrometer in length in resting state.

Z discs (lines) = lines extend all way across myofibrils

The functional unit of a myofibril is the **Sarcomere**

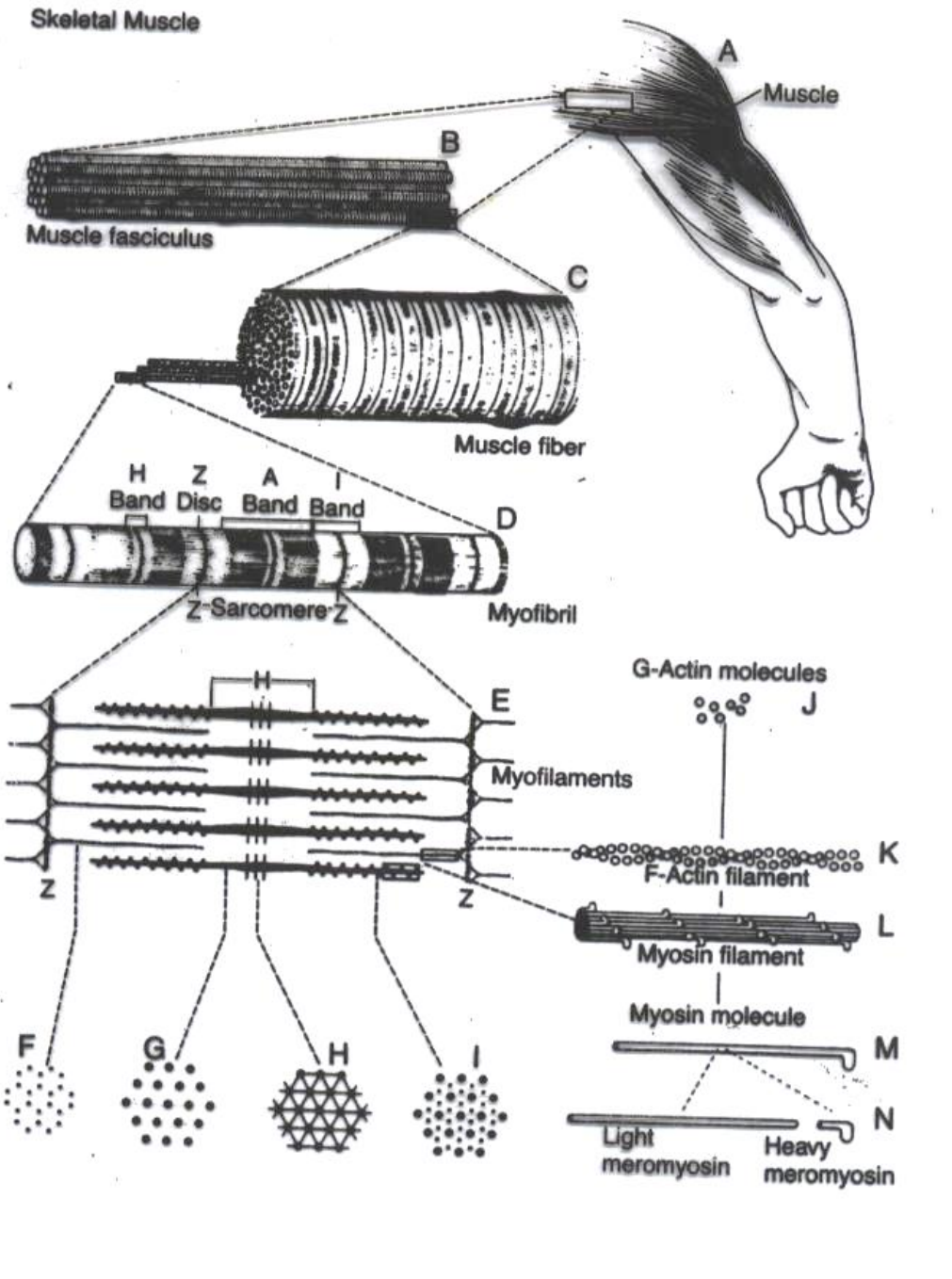


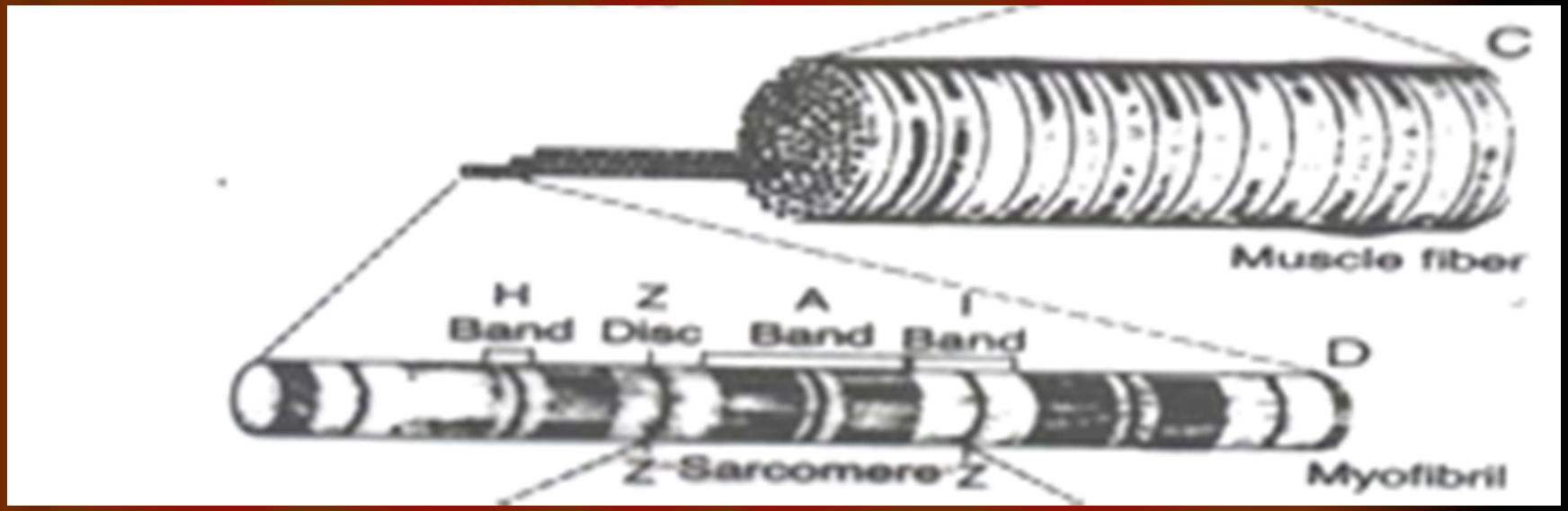
Inside each sarcomere there are 3 bands:-

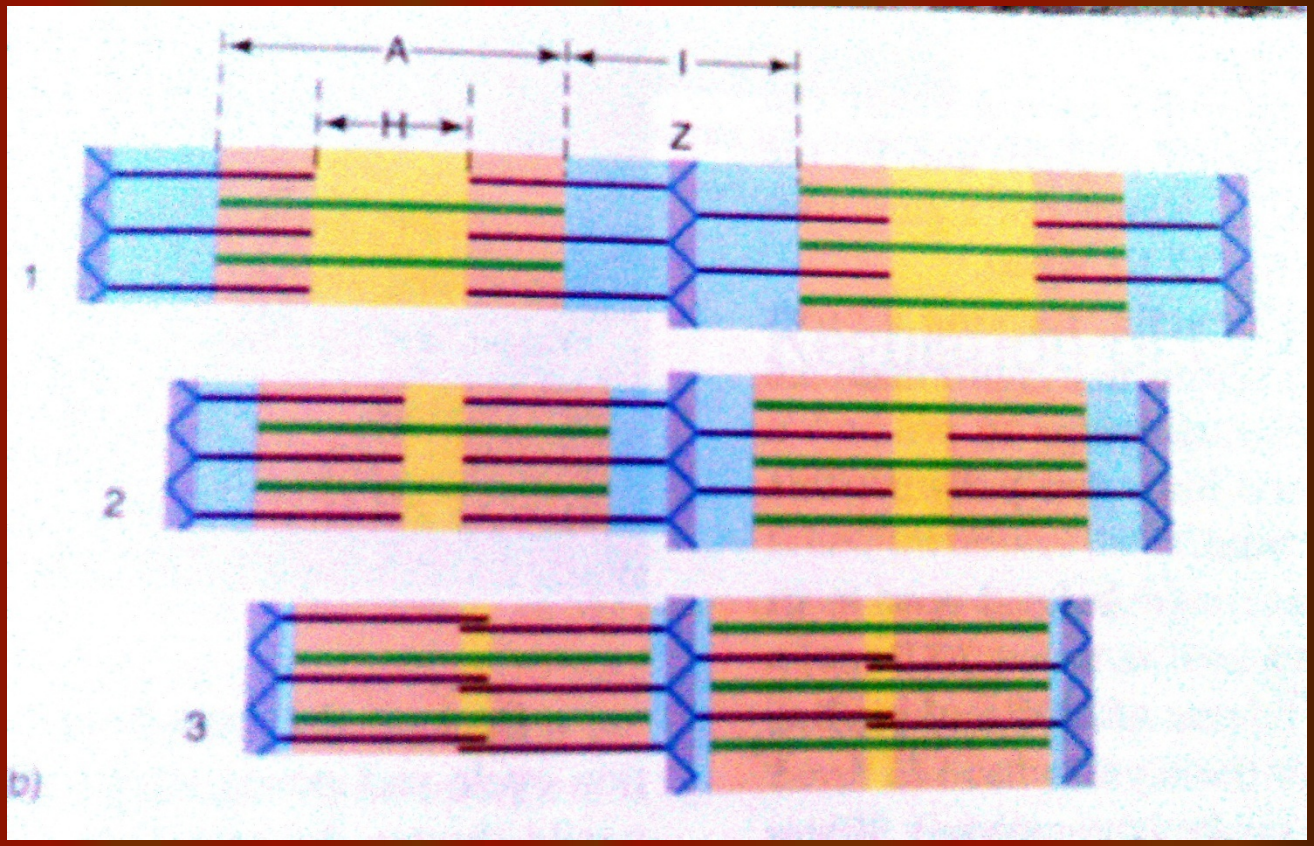
I band = of actin only-

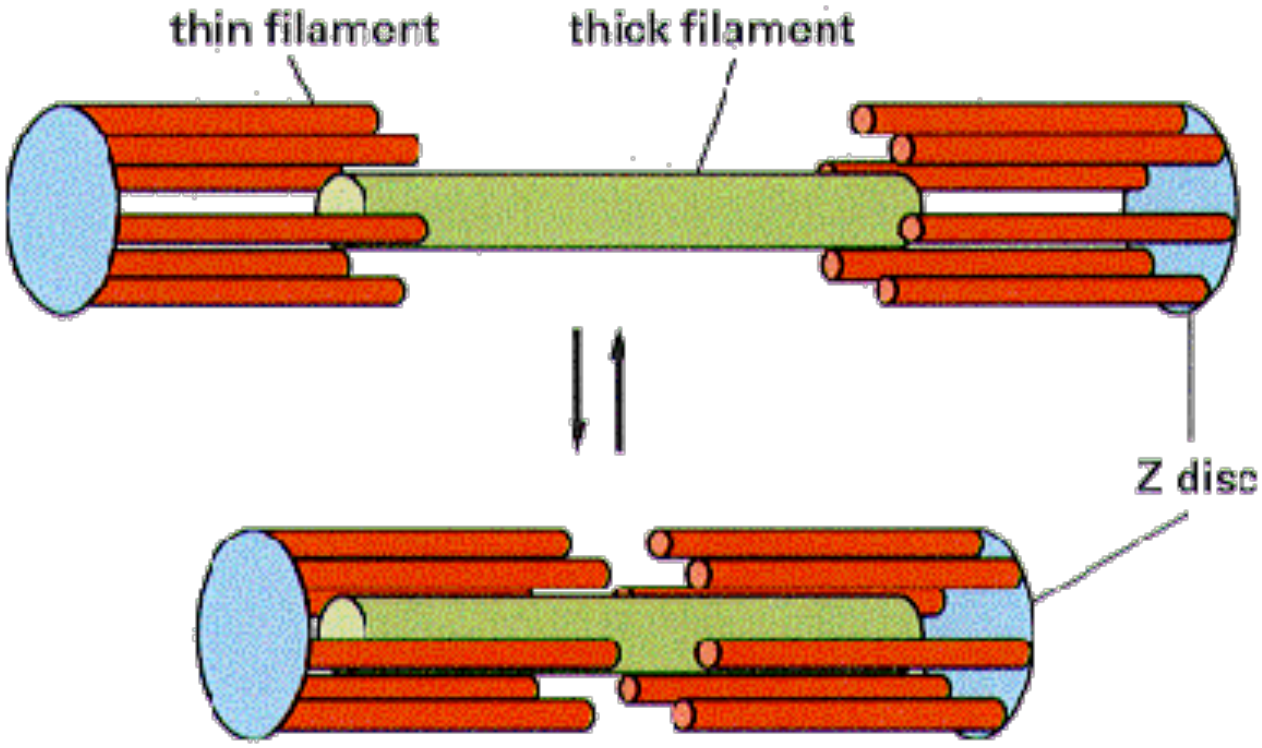
- H band= of myosin only

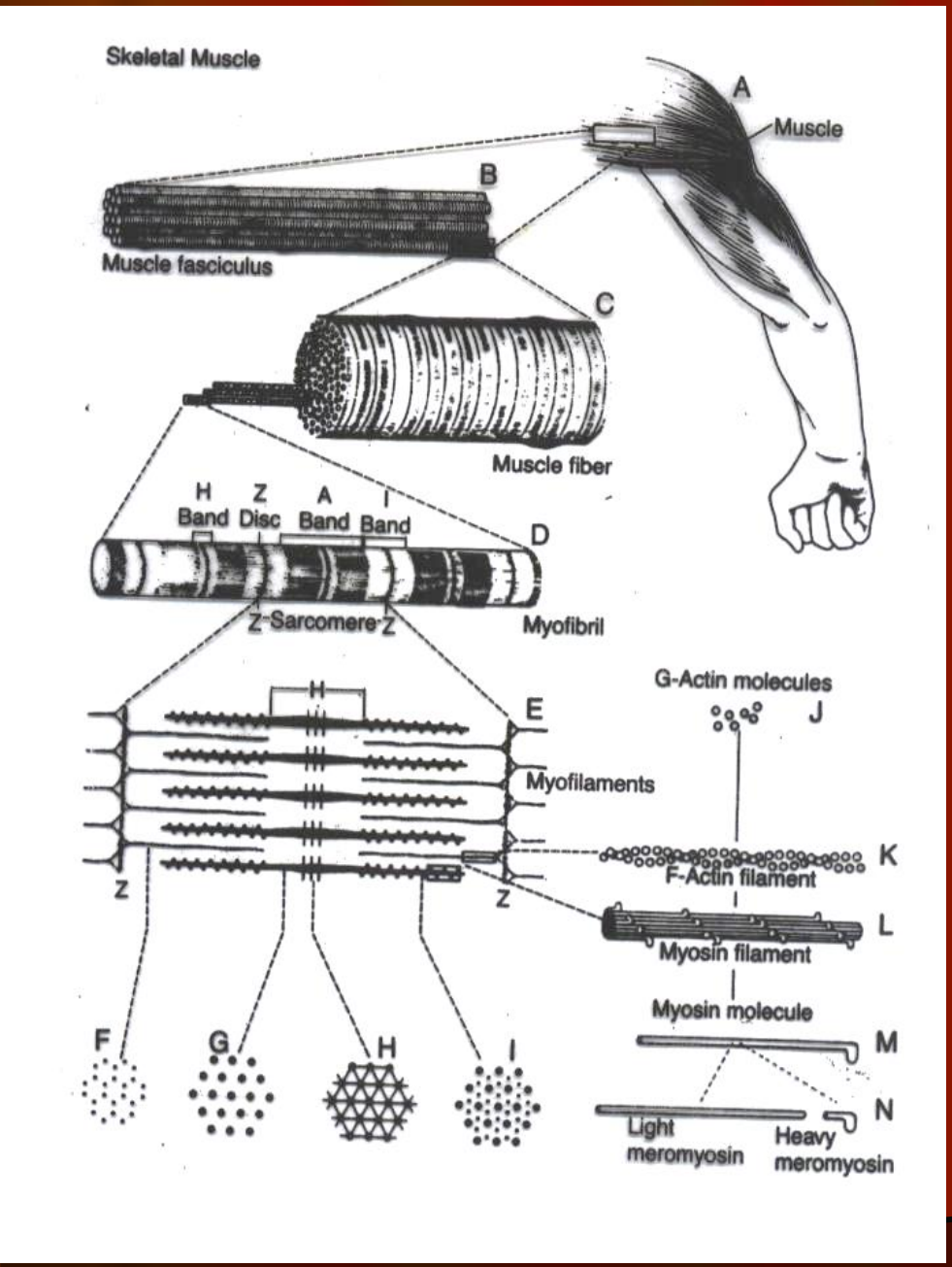
- A band= formed of actin & myosin filaments


Skeletal Muscle
A
Muscle
B
Muscle fasciculus
C
Muscle fiber
H Band
Z Disc
A Band
I Band
D
Z Sarcomere Z
Myofibril
H
E
Myofilaments
Z
Z
G-Actin molecules
J
K
F-Actin filament
L
Myosin filament
Myosin molecule
M
N
Light meromyosin
Heavy meromyosin
F
G
H
I


C
Muscle fiber
H
Band
Z
Disc
A
Band
I
Band
D
Z
Sarcomere
Z
Myofibril

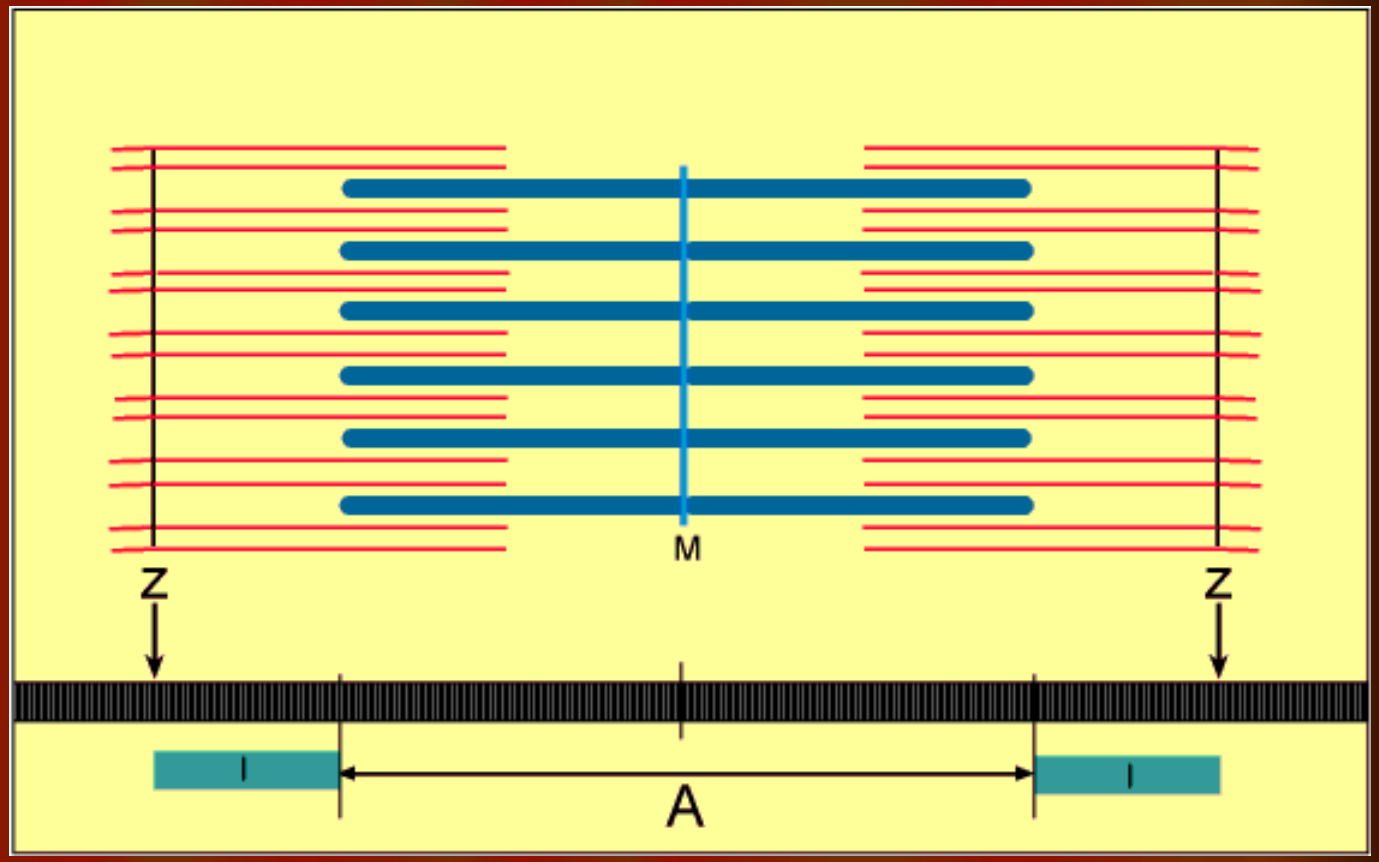

A
I
H
Z
1
2
3
b)


thin filament
thick filament
Z disc



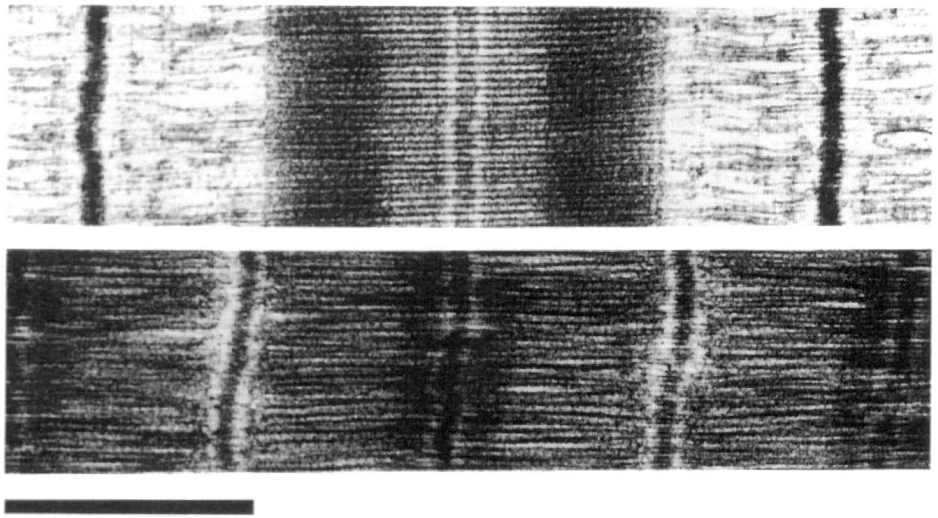
- When contraction takes place Actin & Myosin slide upon each other , & the distance between two z-discs decreases : This is called

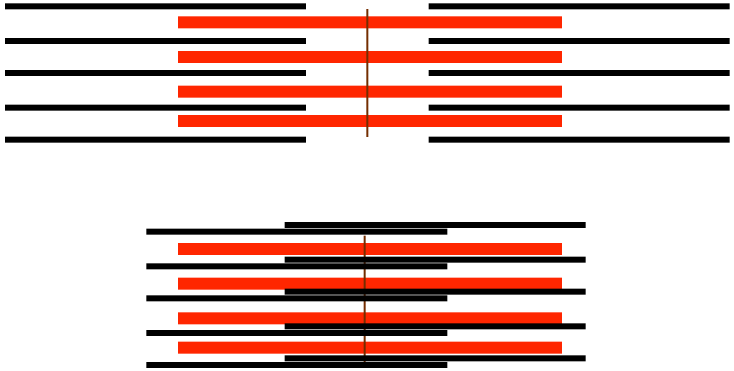
Sliding Filament Mechanism



From: http://www.3dotstudio.com/zz.html

EM Evidence for Sliding Filaments





Muscle Contraction

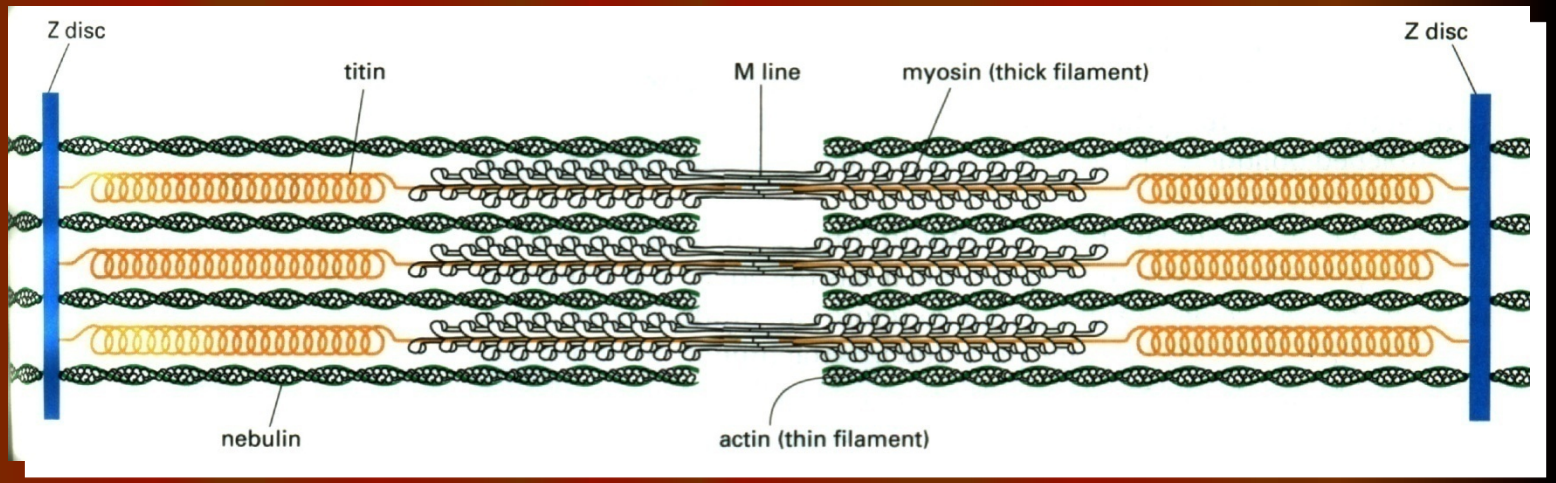
muscle proteins :

a. Thick filament: **Myosin**

b. Thin filament :

1. **Actin**
2. **Troponin** →
3. **Tropomyosin** →

Sarcomere filamentous proteins



From: Alberts et al.. 1994 Molecular Biology of the Cell.

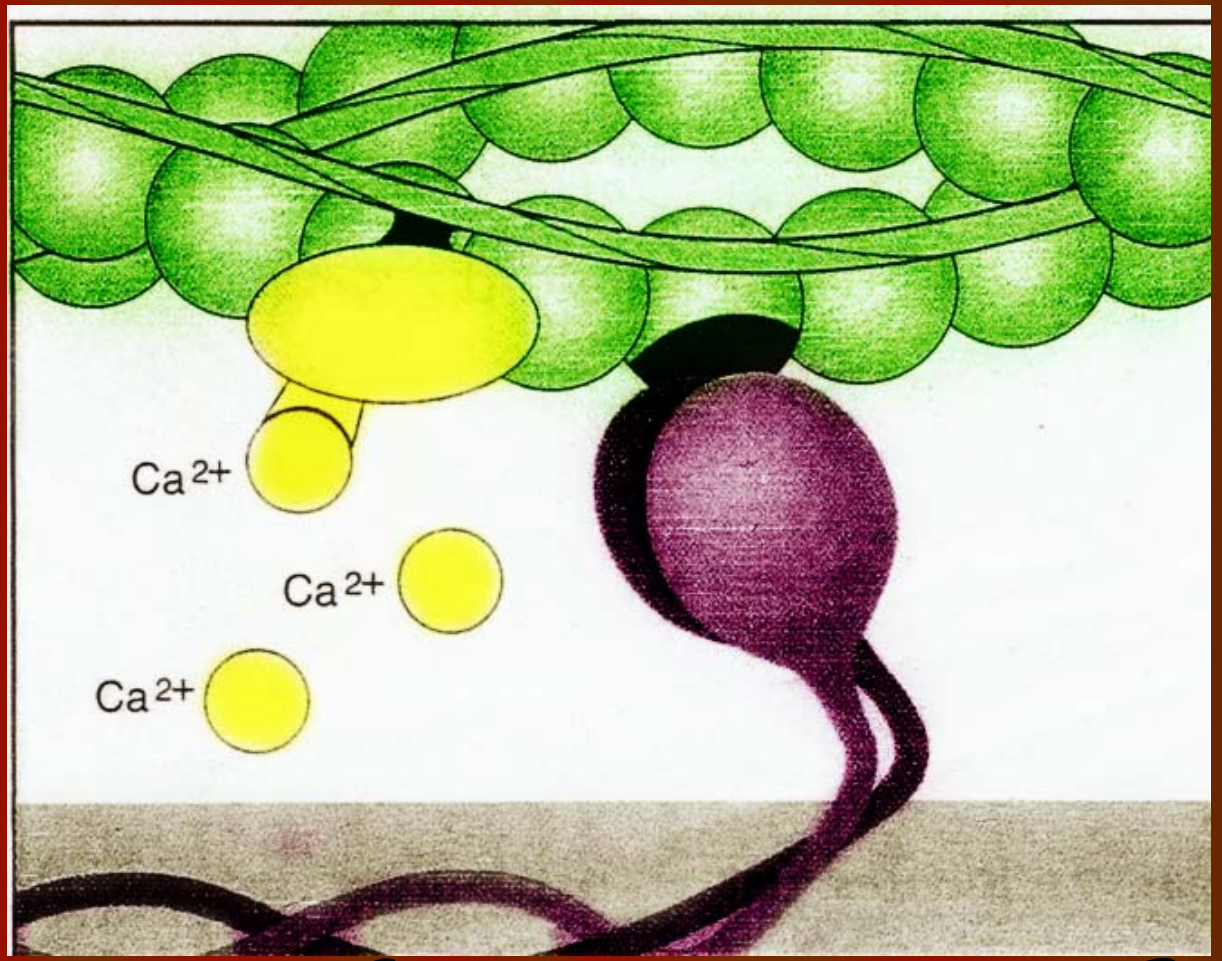
Thick filament:

Myosin filament

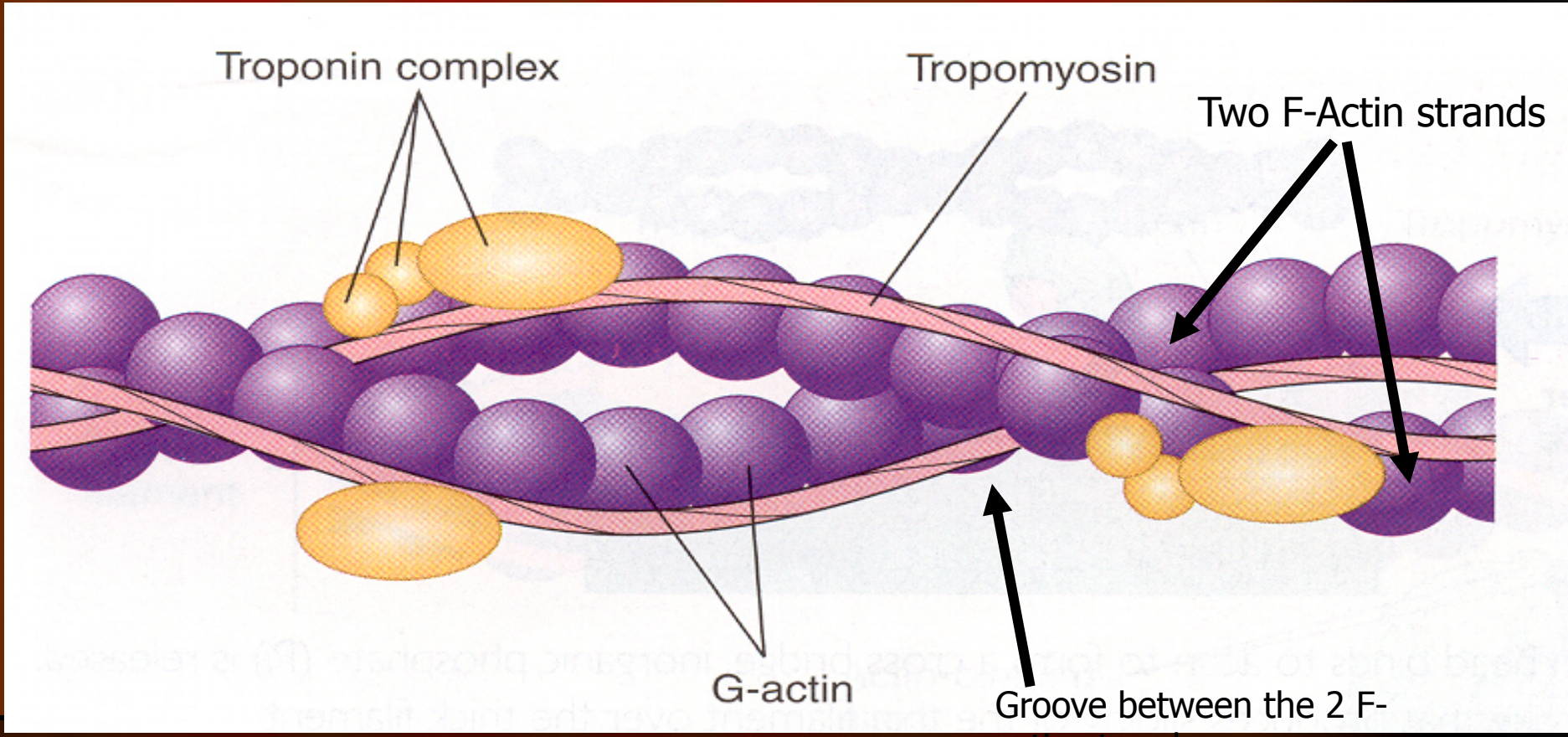
it has head + tail

cross bridges (?)

- Head has ATP site -?


Ca 2+
Ca 2+
Ca 2+

Thin filament



MOLECULAR MECHANISM OF MUSCLE CONTRACTION

Excitation –contraction coupling

Events of muscle contraction: ***

- Acetylcholine released by motor nerve »»»»»» EPP »»»»»» depolarization of CM (muscle AP) »»»»»»
- Spread of AP into T tubule »»»»»»release of Ca from sarcoplasmic reticulum into the cytoplasm
- »»»»»» Ca combines with troponin »»»»»» troponin pull tropomyosin sideway »»»»»» exposing the active site on actin »»»»»» myosin heads with ATP on them, attached to actin active site
- »»»»»» myosin cross bridges bend pulling actin toward center of sarcomere (Power stroke) using energy of ATP»»»»»»ADP & P released »»»»»» Linkage between actin & myosin broken as new ATP binds to myosin cross bridge >>> ATP hydrolyzed and cross bridge go back to its original conformation.

Events of muscle contraction:

- When a new ATP occupies the vacant site on the myosin head, this triggers detachment of myosin from actin

- The free myosin swings back to its original position, & attached to another actin, & the cycle repeat its self

Events of muscle relaxation:

- When ca is pumped back into sarcoplasmic reticulum
- »»»»»» ca detached from troponin »»»»»» tropomyosin return to its original position
- »»»»»» covering active sit on actin »»»»»» prevent formation of cross bridge »»»»»» relaxation

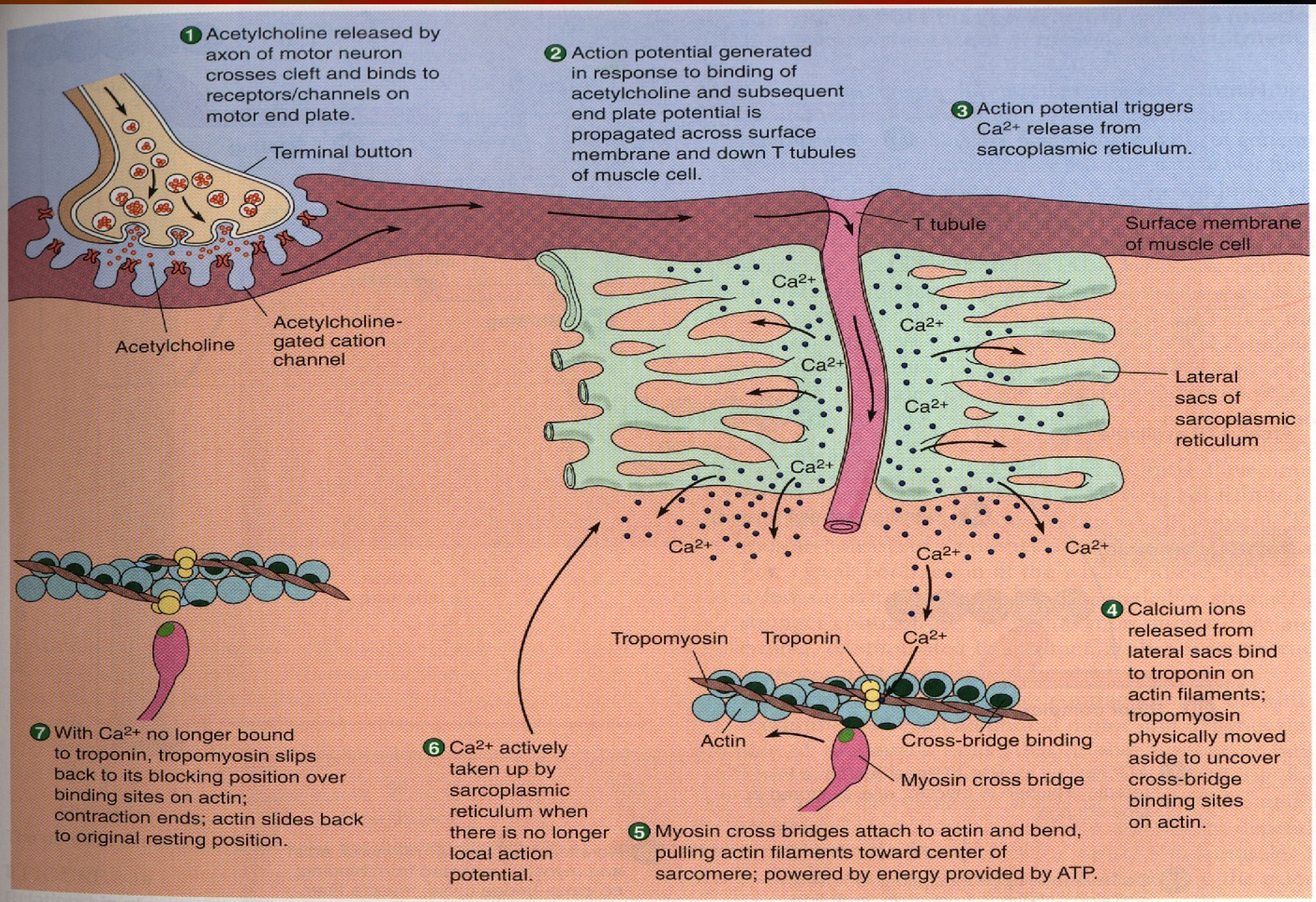


FIGURE 8-12

Calcium release in excitation-contraction coupling

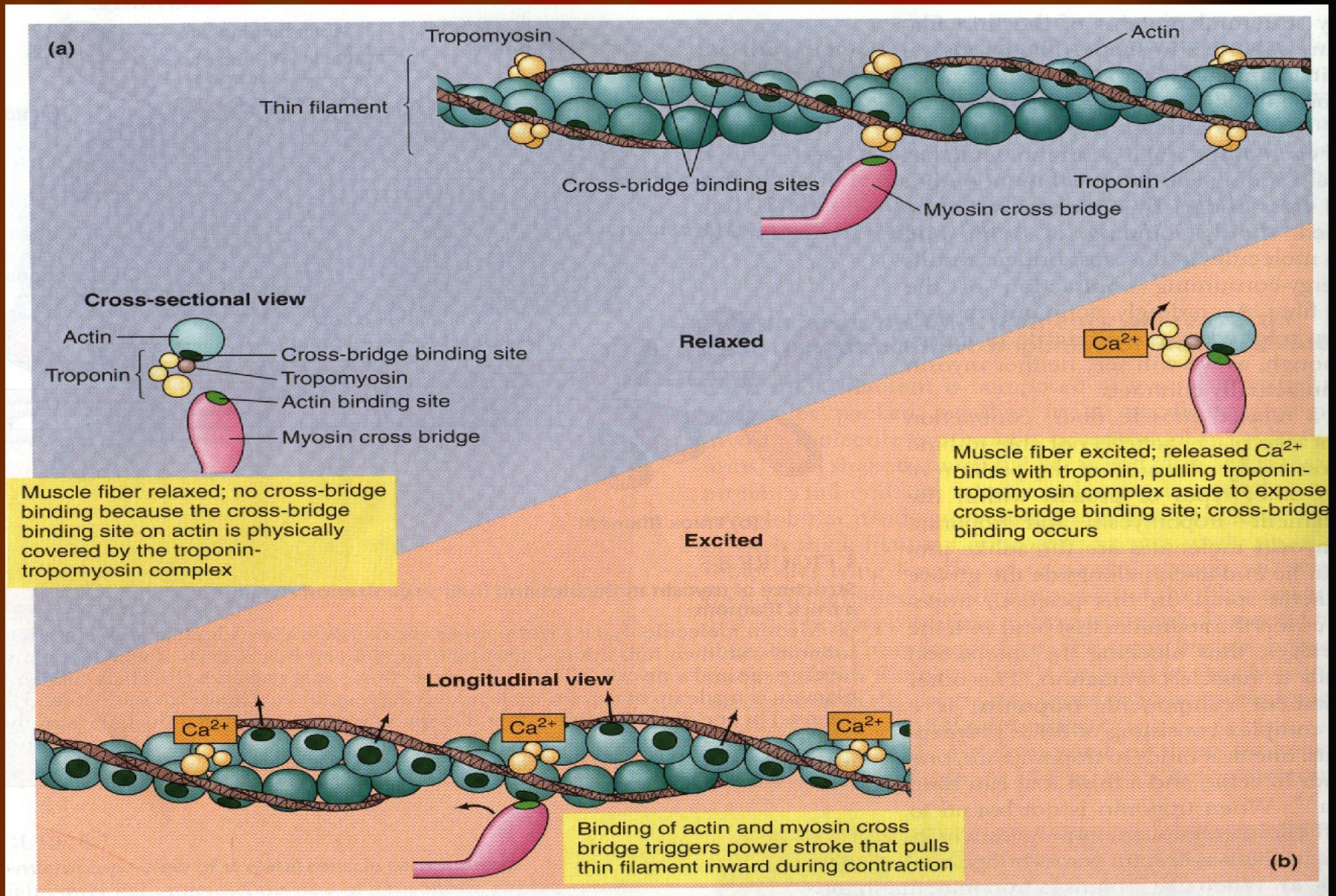
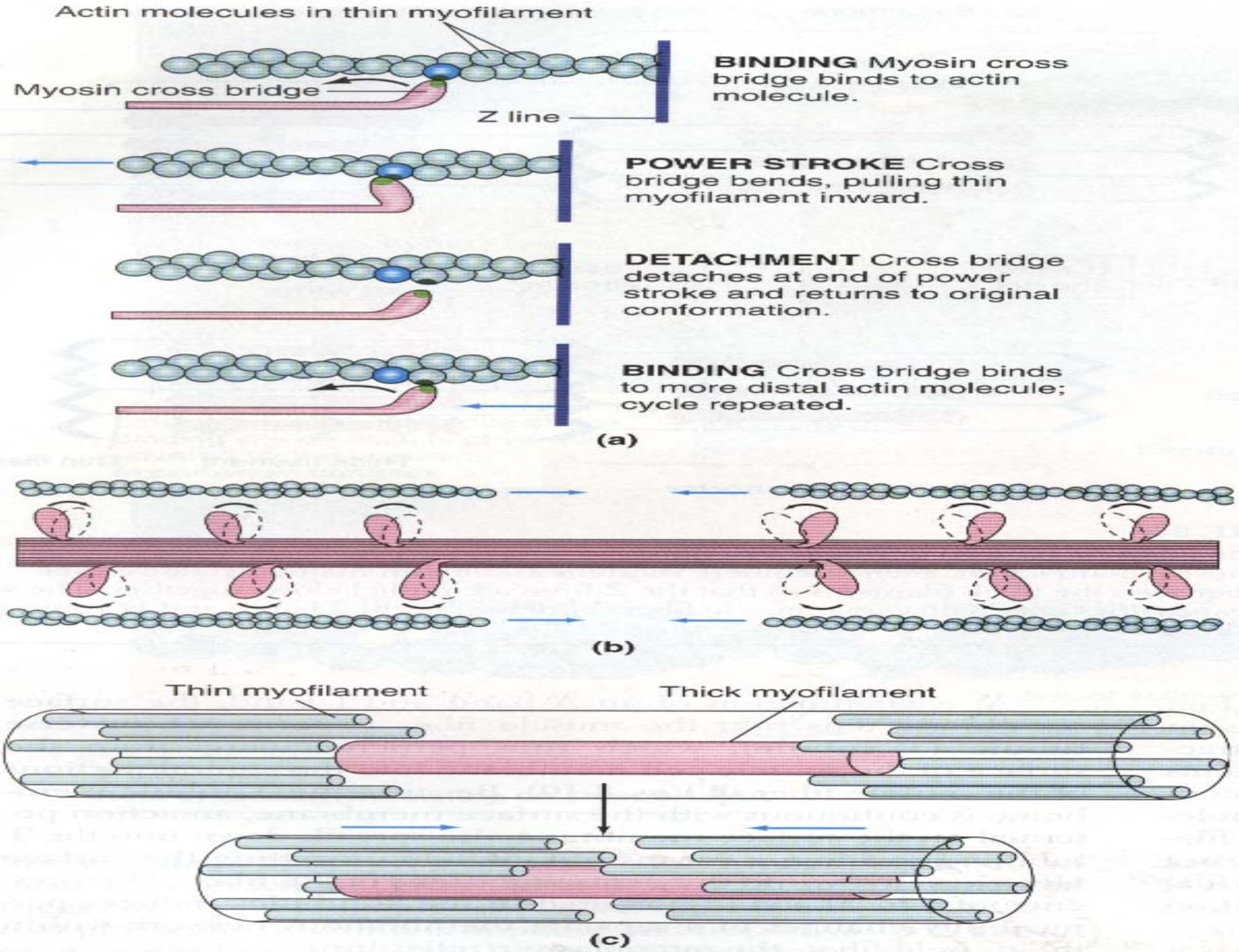
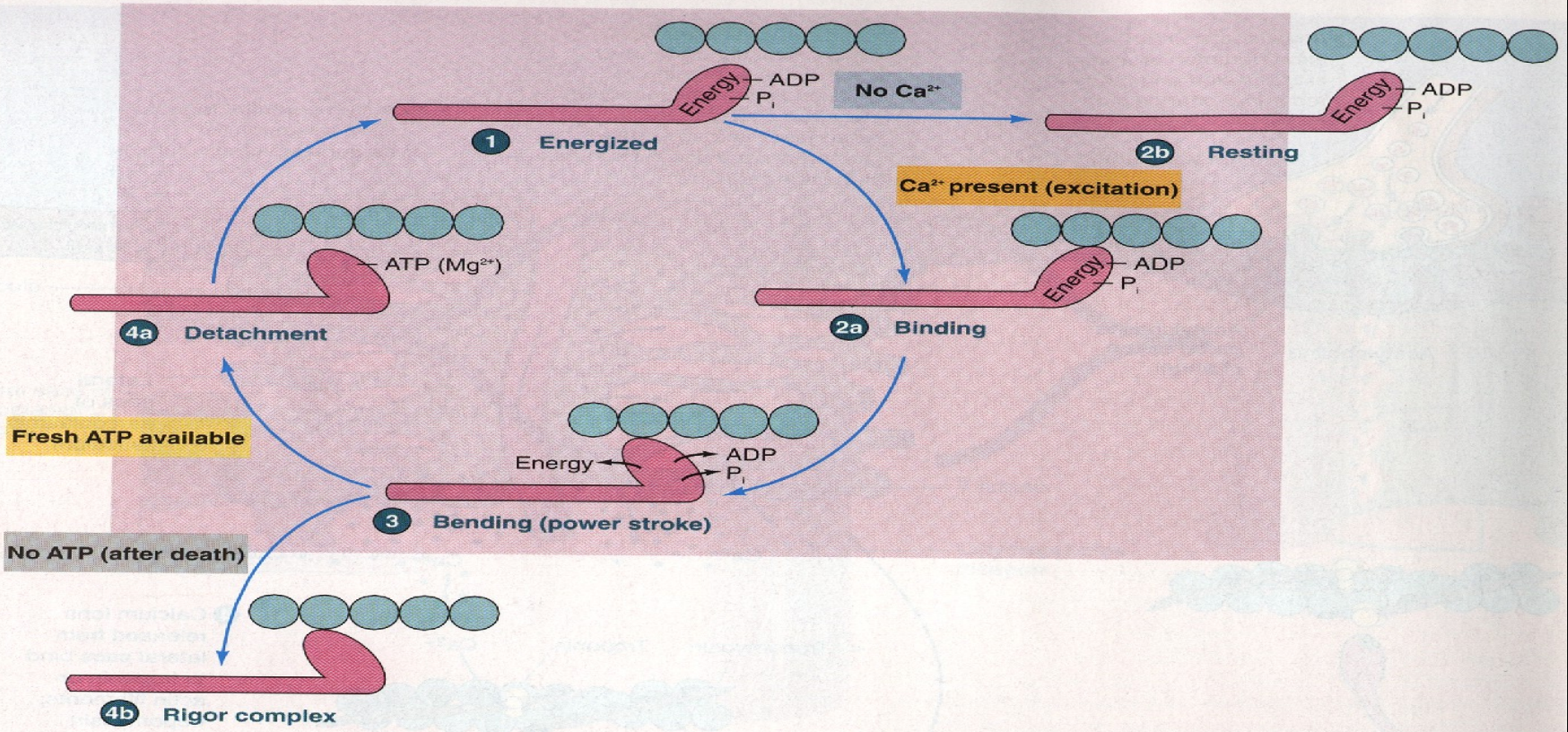


FIGURE 8-7
Role of calcium in turning on cross bridges


Actin molecules in thin myofilament
Myosin cross bridge
Z line
BINDING Myosin cross bridge binds to actin molecule.
POWER STROKE Cross bridge bends, pulling thin myofilament inward.
DETACHMENT Cross bridge detaches at end of power stroke and returns to original conformation.
BINDING Cross bridge binds to more distal actin molecule; cycle repeated.
(a)
(b)
Thin myofilament
Thick myofilament
(c)



1 ATP split by myosin ATPase; ADP and P_i remain attached to myosin; energy stored in cross bridge.

...or...

2a Ca^{2+} released upon excitation; removes inhibitory influence from actin, enabling it to bind with cross bridge.

2b No excitation; no Ca^{2+} released; actin and myosin prevented from binding; no cross-bridge cycle; muscle fiber remains at rest.

3 Power stroke of cross bridge triggered upon contact between myosin and actin; ADP and P_i released.

...or...

4a Linkage between actin and myosin broken as fresh molecule of ATP binds to myosin cross bridge; cross bridge assumes original conformation; ATP hydrolyzed (cycle starts again at step 1).

4b If no fresh ATP available (after death), actin and myosin remain bound in rigor complex.

FIGURE 8-13
Cross-bridge cycle

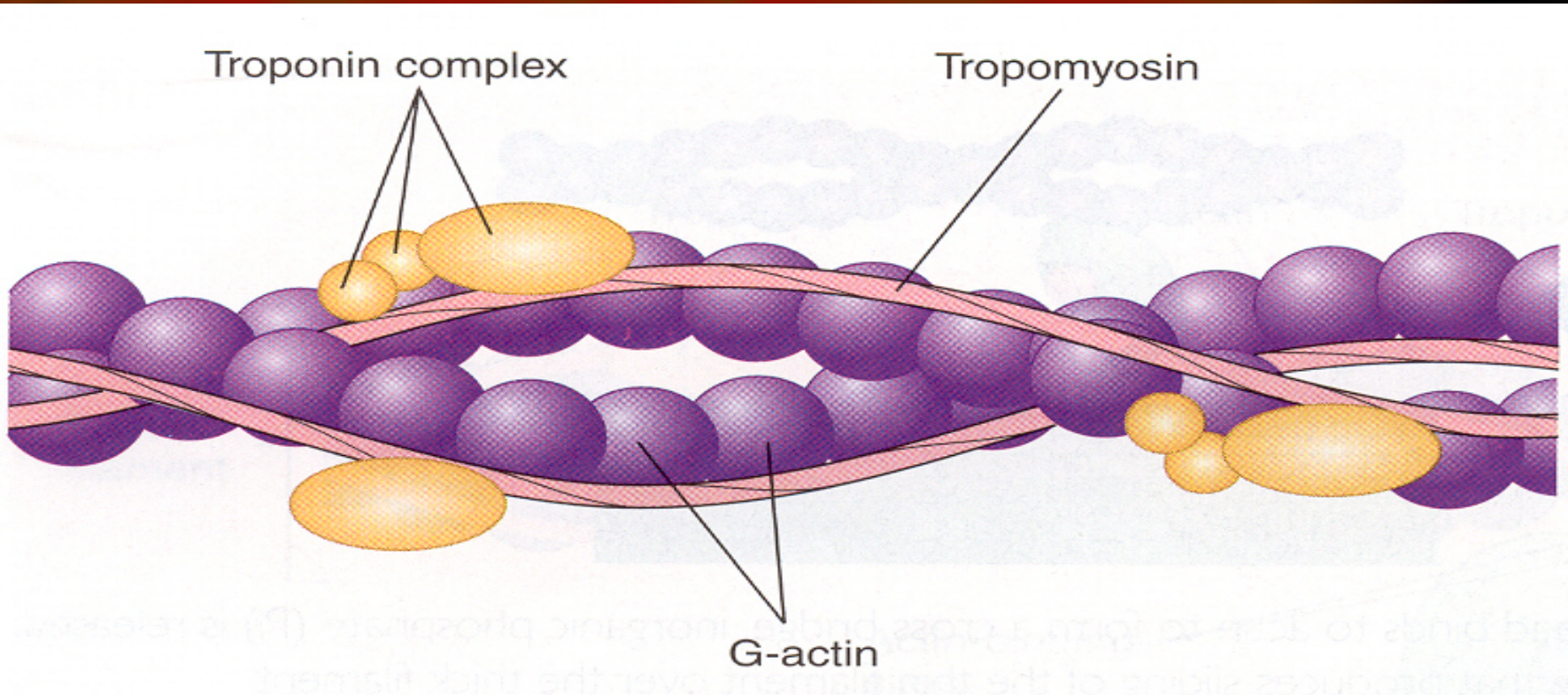
> Each G-Actin molecule has a binding site for Myosin head (called actin active sites)

> These active sites are covered and hidden from the Myosin head by the inhibitory protein Tropomyosin

> When Troponin is activated by Ca++ it will move the Tropomyosin away from these sites and expose them for Myosin.

> then myosin immediately gets attached to them .

> when the myosin head attaches to actin it forms a " cross-bridge"



The diagram of Guyton

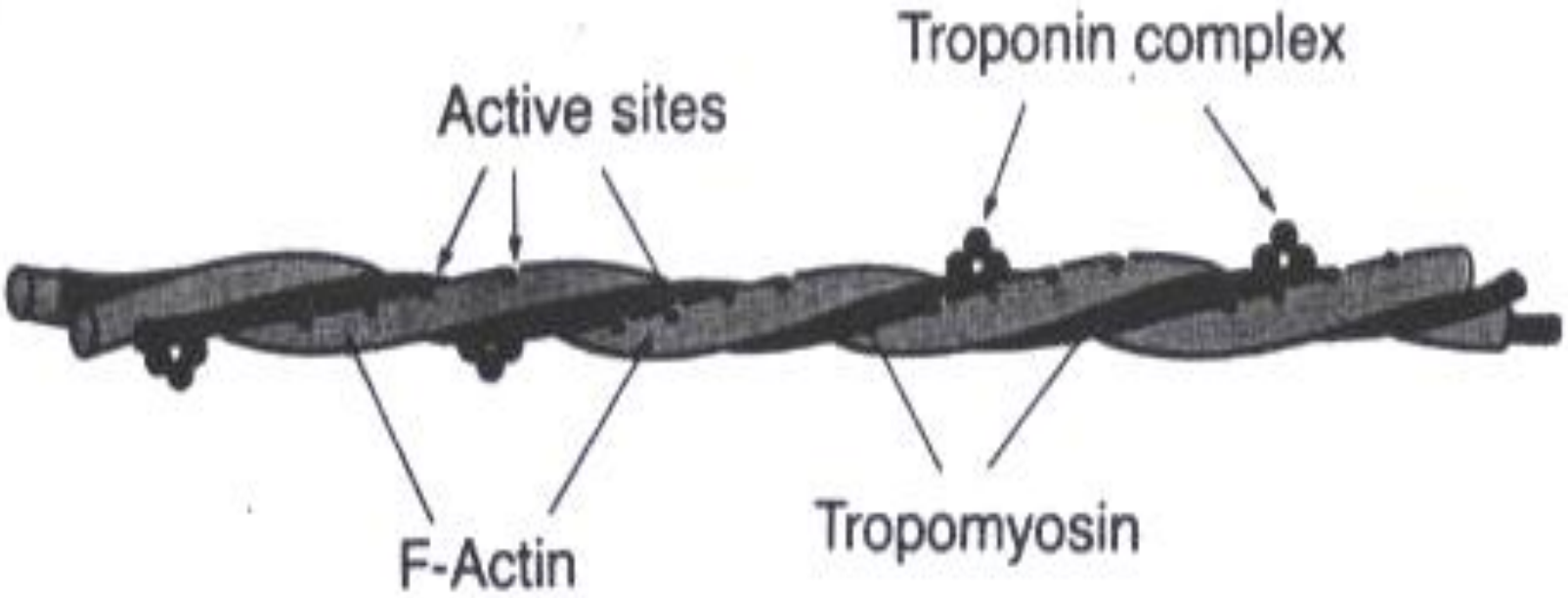


Figure 6–6 The actin filament, composed of two helical strands of F-actin and tropomyosin molecules that fit loosely in the grooves between the actin strands. Attached to one end of each tropomyosin molecule is a troponin complex that initiates contraction.

Myosin (1)

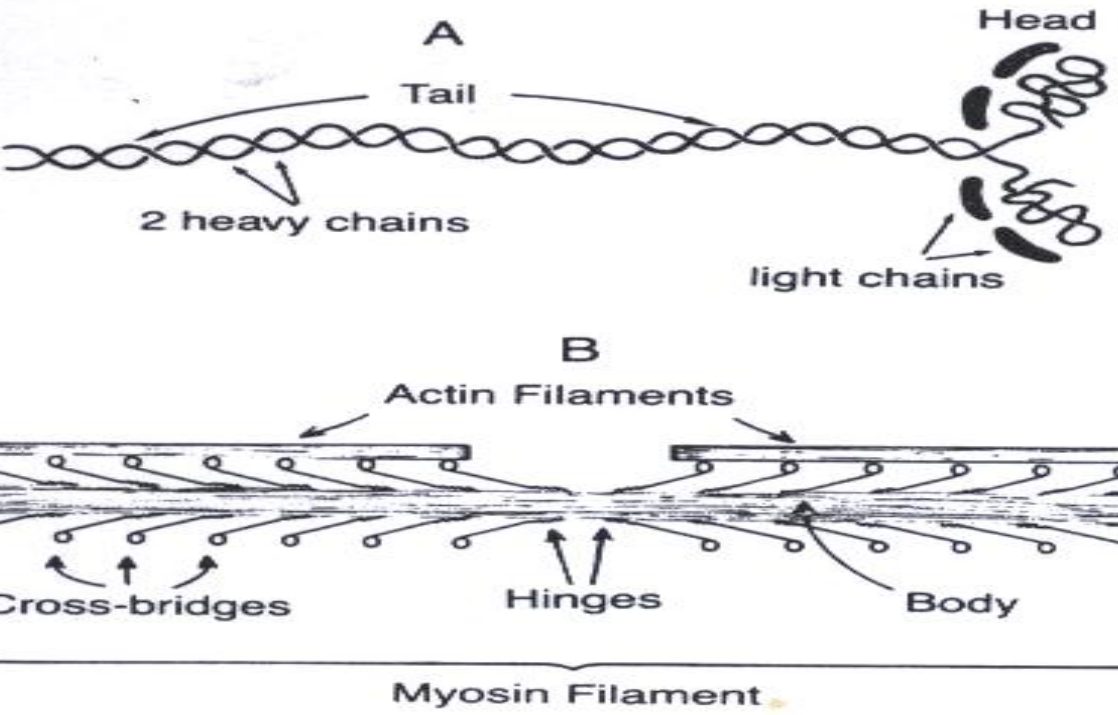


Figure 6–5 *A,* The myosin molecule. *B,* Combination of many myosin molecules to form a myosin filament. Also shown are the cross-bridges and the interaction between the heads of the cross-bridges and adjacent actin filaments.

- Each Myosin molecule has (1) Head (2) Hinge (joint) and (3) Tail ; and each myosin head contains an ATP binding site as well as ATP-ase enzyme .

Myosin (2)

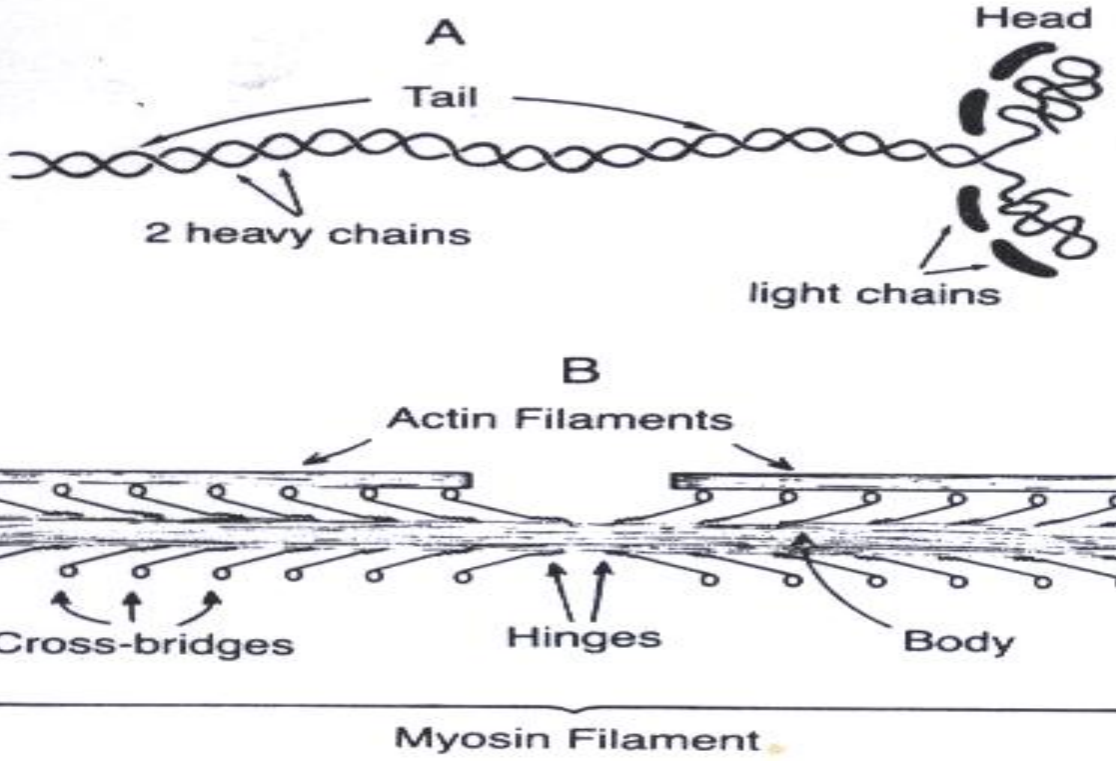


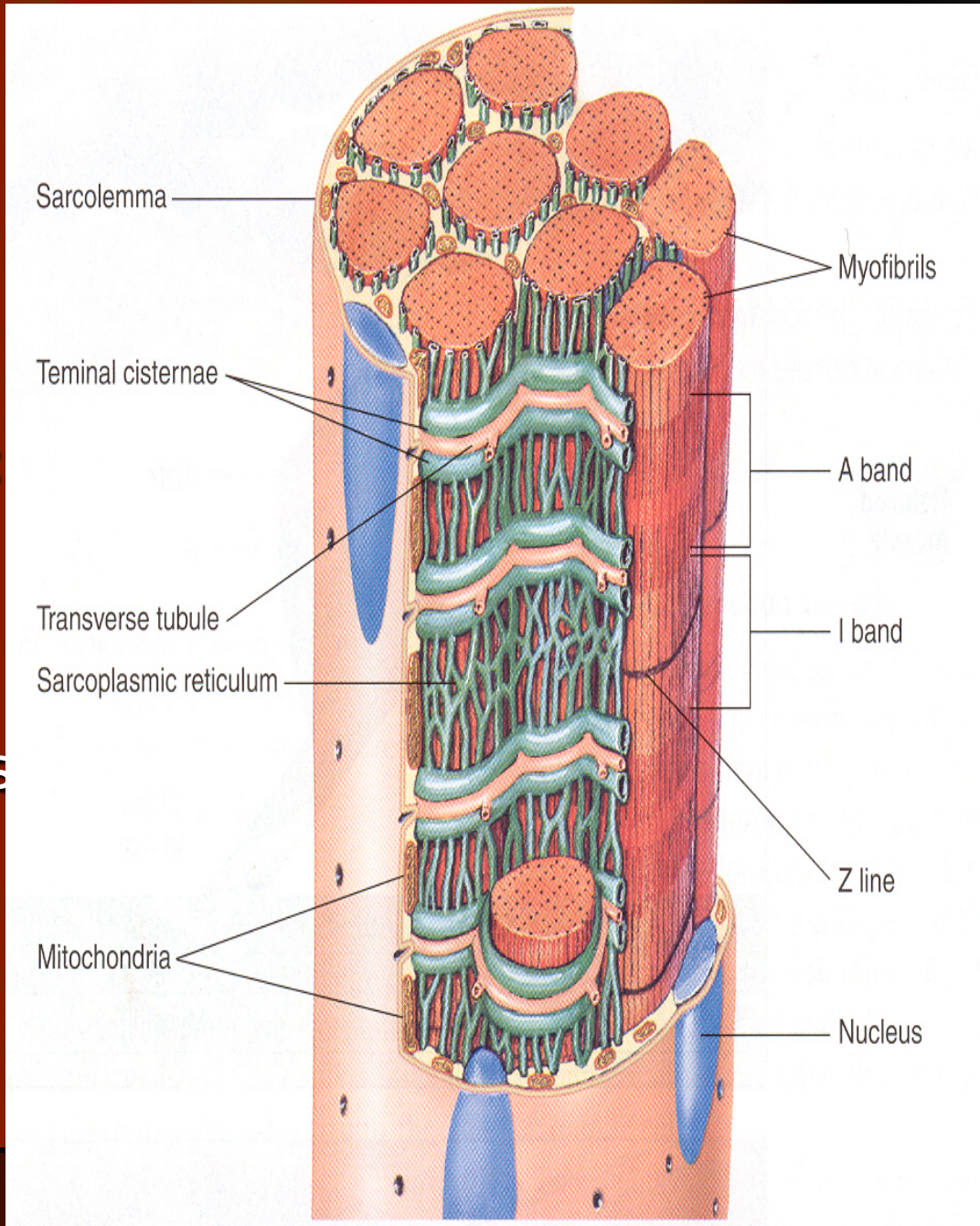
Figure 6–5 *A,* The myosin molecule. *B,* Combination of many myosin molecules to form a myosin filament. Also shown are the cross-bridges and the interaction between the heads of the cross-bridges and adjacent actin filaments.

- Each 200 myosin molecules aggregate to form a myosin filament , from the sides of which project myosin heads in all directions .

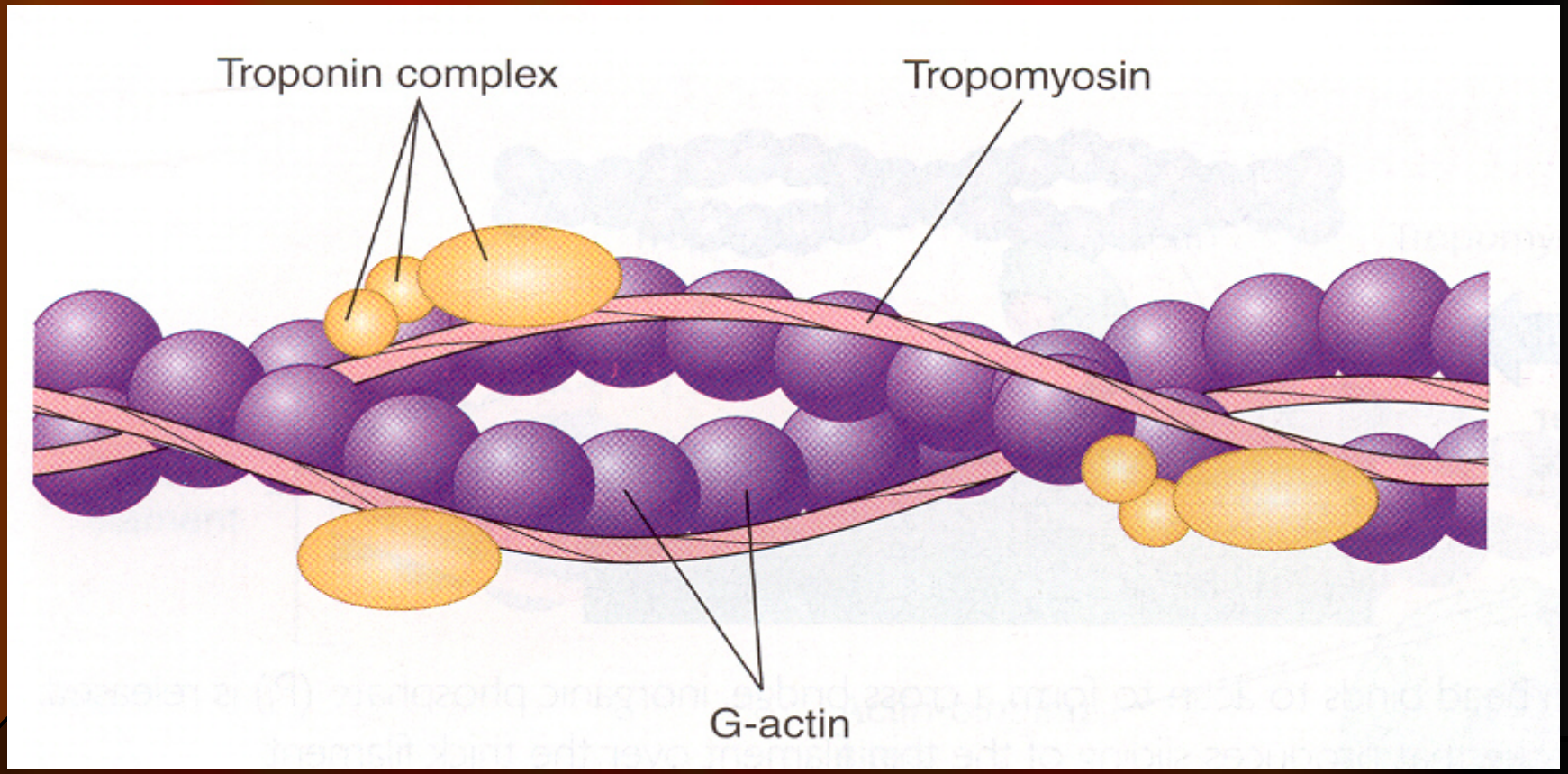
- The EPP at the motor end-plate triggers a muscle AP
- The muscle AP spreads down inside the muscle through the Transverse Tubules (T-tubules)

  to reach the Sarcoplasmic Reticulum (SR) .
- In the SR the muscle AP opens calcium channels

  (in the walls of the SR) → calcium passively flows out (by concentration gradient) of the SR into muscle cytoplasm→ Ca++ combines with Troponin



➢The activated troponin pulls the inhibitory protein tropomyosin away from the myosin binding sites on actin
→ and once these sites on Actin are exposed (uncovered)
→ myosin heads quickly bind to them



This binding activates the enzyme ATPase in the Myosin Head → it breaks down ATP releasing energy → which is used in the "Power Stroke " to move the myosin head

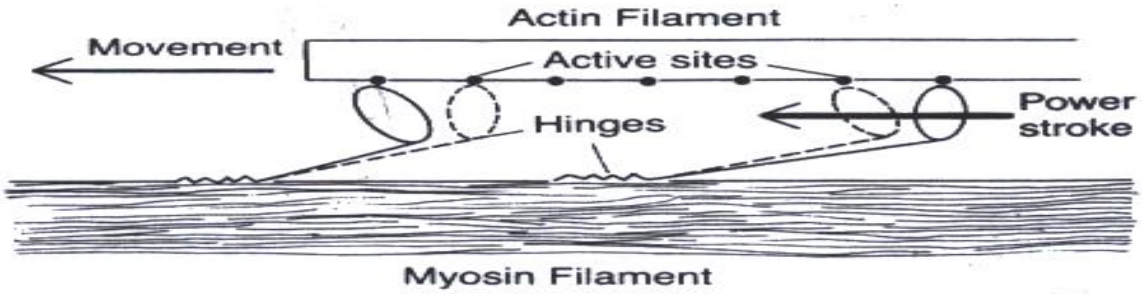


Figure 6–7 The "walk-along" mechanism for contraction of the muscle.

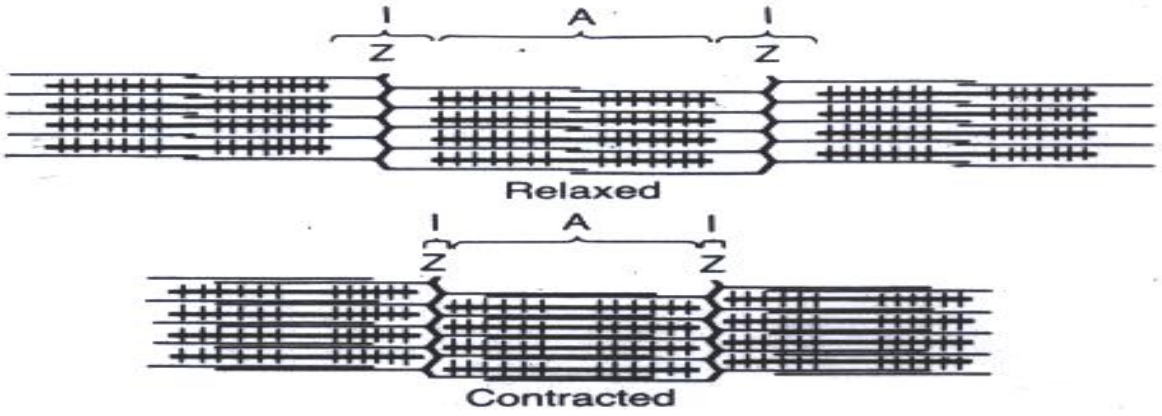


Figure 6–4 The relaxed and contracted states of a myofibril, showing sliding of the actin filaments (black) into the spaces between the myosin filaments (red).

The “ power stroke ” means tilting of the cross-bridge head (myosin head) and dragging (pulling) of actin filament

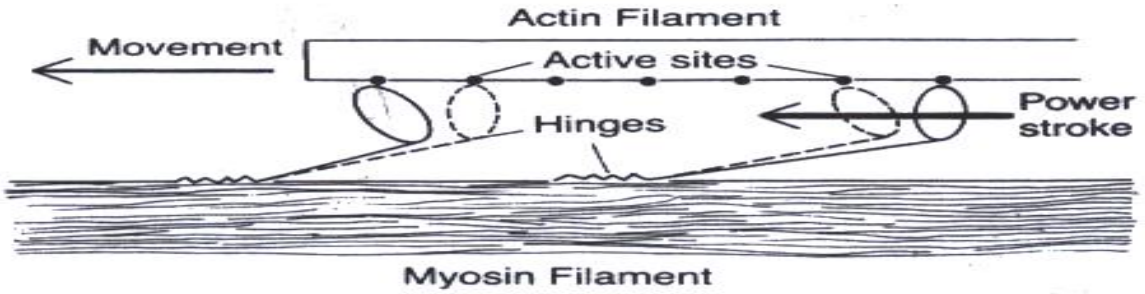


Figure 6–7 The “walk-along” mechanism for contraction of the muscle.

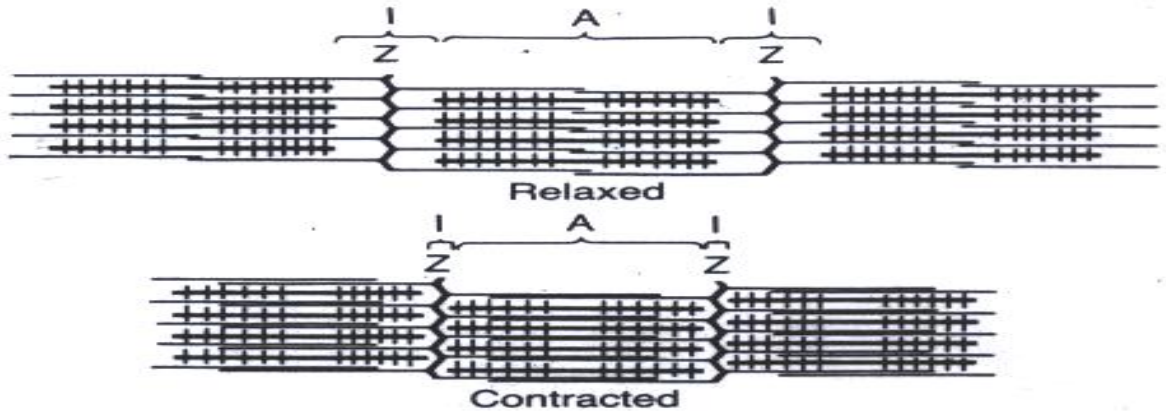


Figure 6–4 The relaxed and contracted states of a myofibril, showing sliding of the actin filaments (black) into the spaces between the myosin filaments (red).

Summary (1)

(1) Muscle AP spreads through T-tubules
(2) it reaches the sarcoplasmic reticulum where → opens its Ca++ channels → calcium diffuses out of the sarcoplasmic reticulum into the cytoplasm → increased Ca++ concentration in the myofibrillar fluid .
(3) Ca++ combines with Troponin , activating it
(4) Troponin pulls away Tropomyosin
(5) This uncovers the active sites in Actin for Myosin
(6) Myosin combines with these sites
(7) This causes cleavage (breakdown) of ATP and release of energy
(8) This released energy is used to produce Power Stroke
(9) Myosin and Actin slide upon each other → contraction
(10) A new ATP comes and combines with the Myosin head → this causes detachment (separation)of Myosin from Actin .

- Therefore , on order to release the head of Myosin from Actin , a new ATP is needed to come and combine with the head of Myosin .
- Q: What is Rigor Mortis ?
- Q: ATP is needed for 3 things : what are they ?
- ATP is needed for 3 things :
- (1) Power stroke .
- (2) Detachment of myosin from actin active sites
- (3) Pumping C++ back into the Sarcoplasmic reticulum .
- Q: Is muscle relaxation a passive or active process ?
- A : it is active ; Why ? Because it needs ATP .

- Q: What happens to A-band and I-band during contraction ?
- Q: Ca++ is needed in nerve & muscle : when and where ?
- A : In nerve → needed for exocytosis (& release of Ach)
- In Muscle → needed for contraction .

- Thanks