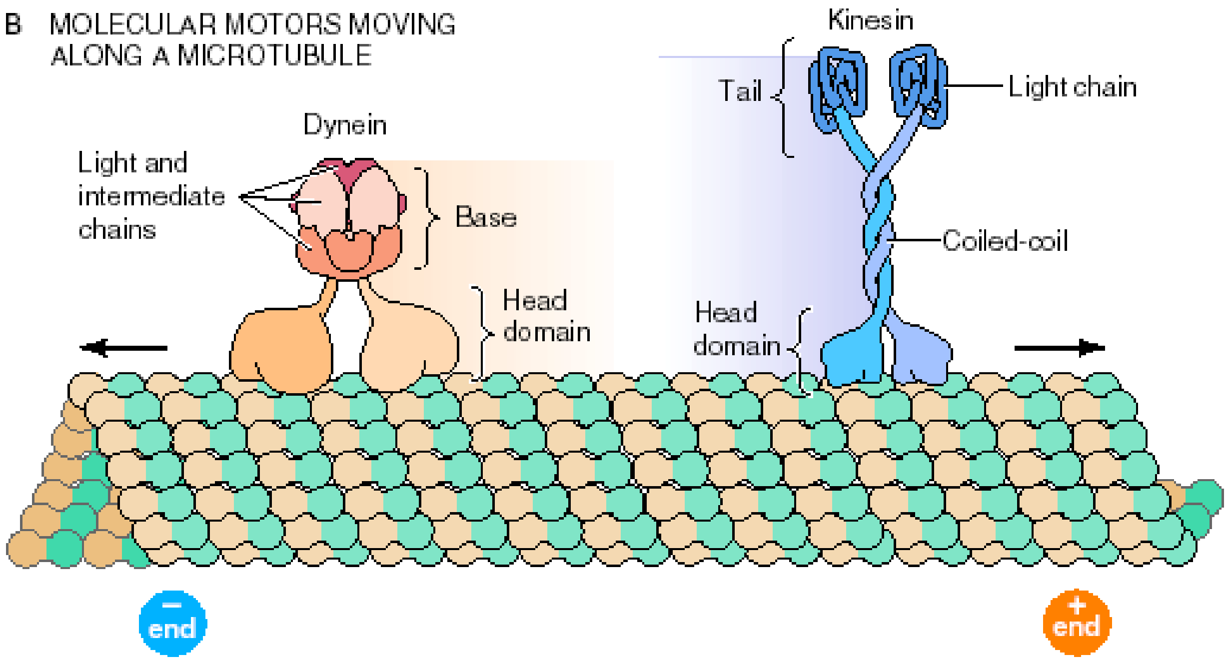
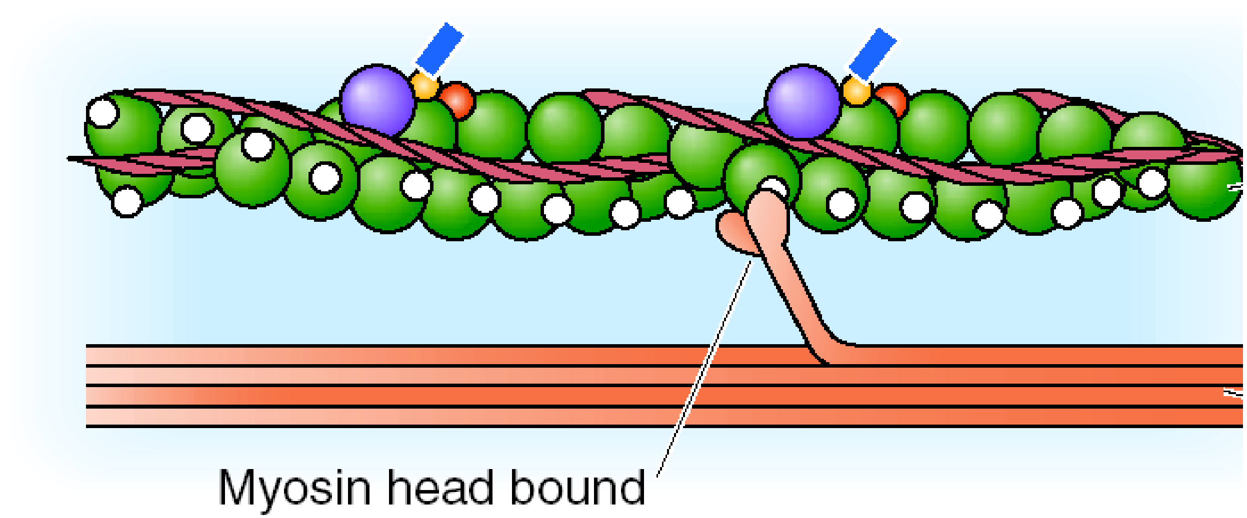
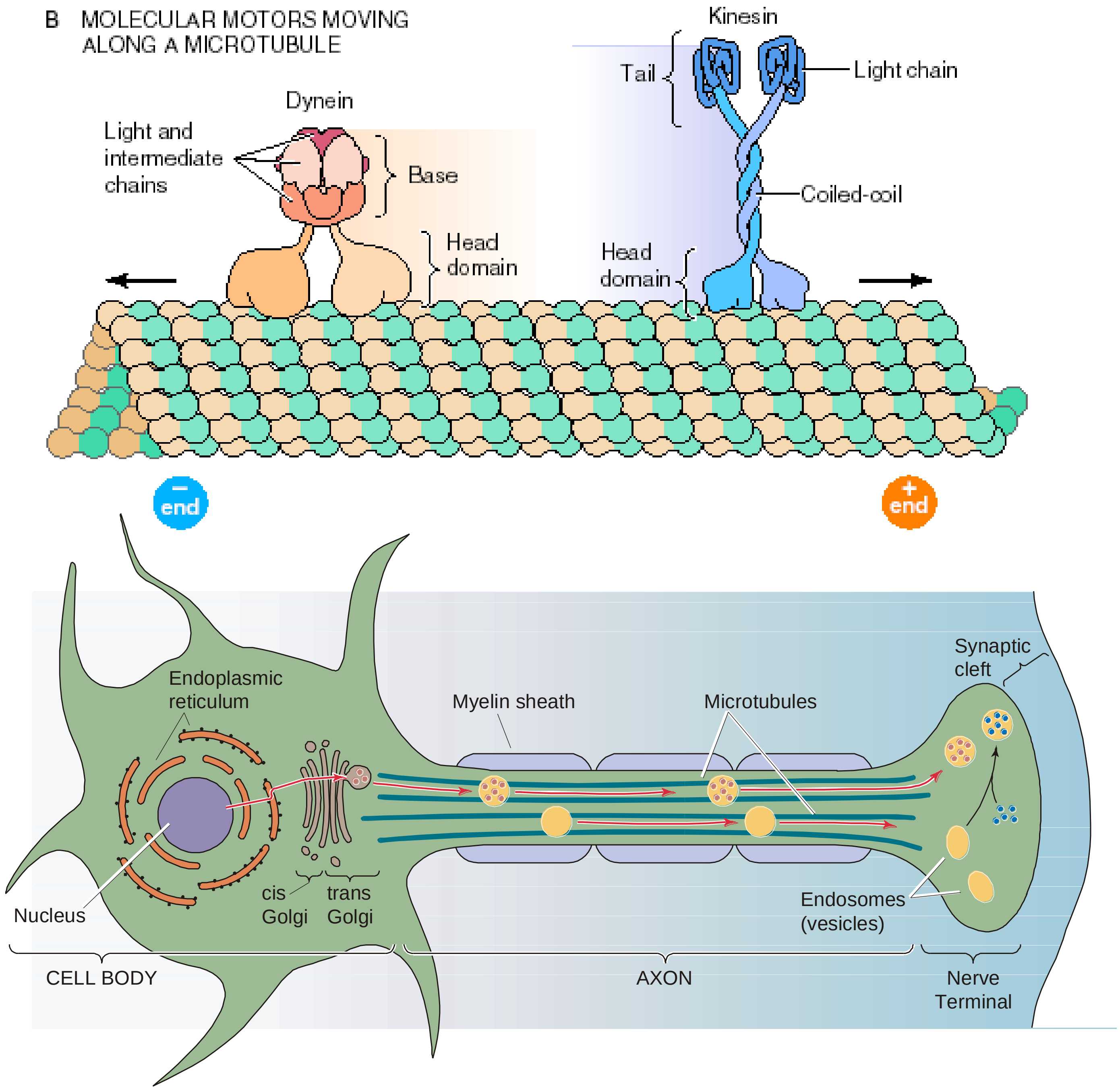
B MOLECULAR MOTORS MOVING ALONG A MICROTUBULE



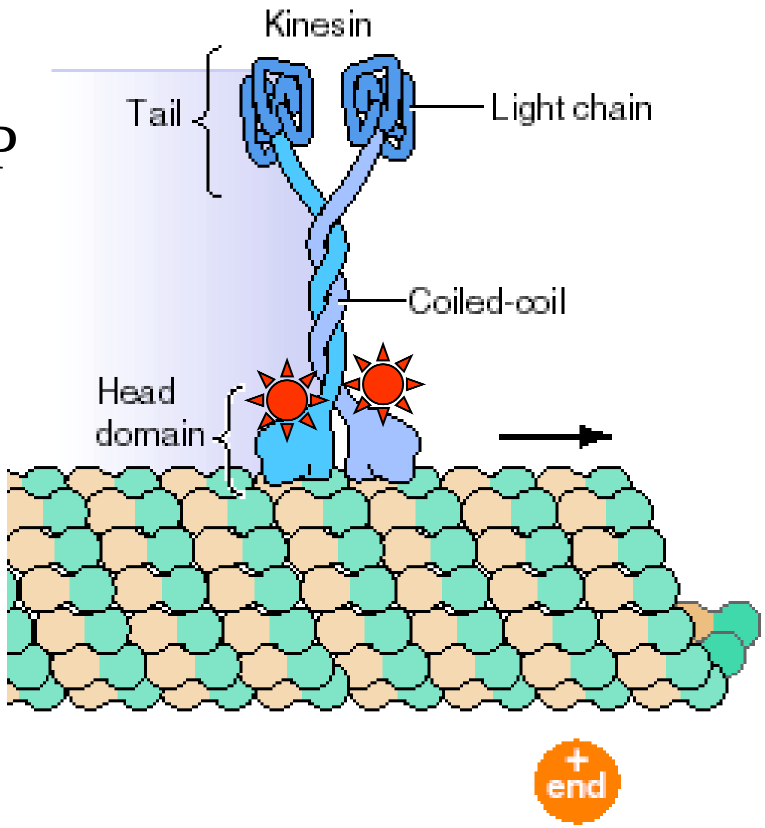
C INTERACTION OF THIN AND THICK FILAMENTS

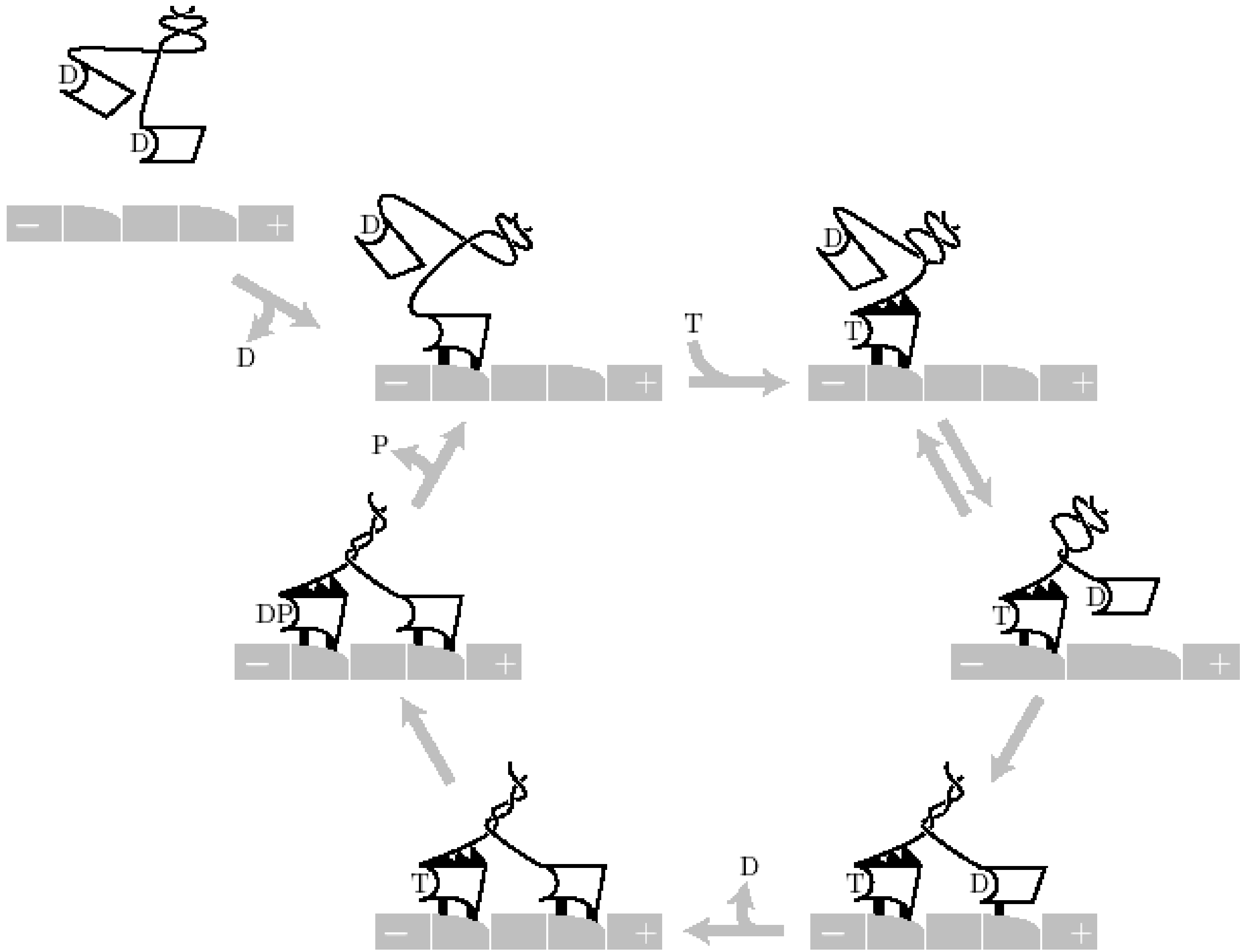


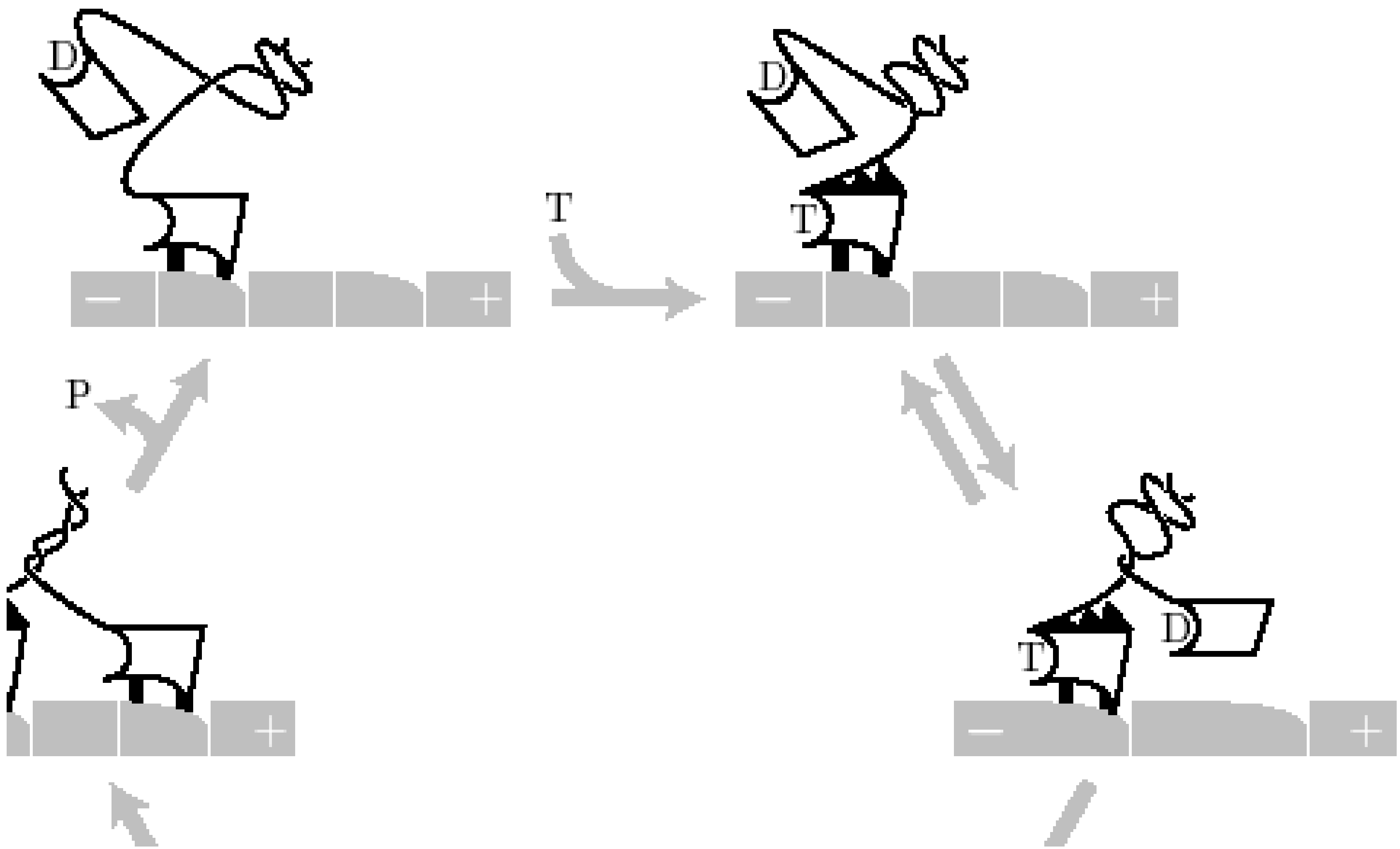


B MOLECULAR MOTORS MOVING ALONG A MICROTUBULE
Dynein
Light and intermediate chains
Base
Head domain
Kinesin
Tail
Light chain
Coiled-coil
Head domain
end
end
Endoplasmic reticulum
Myelin sheath
Microtubules
Synaptic cleft
Nucleus
cis Golgi
trans Golgi
Endosomes (vesicles)
CELL BODY
AXON
Nerve Terminal

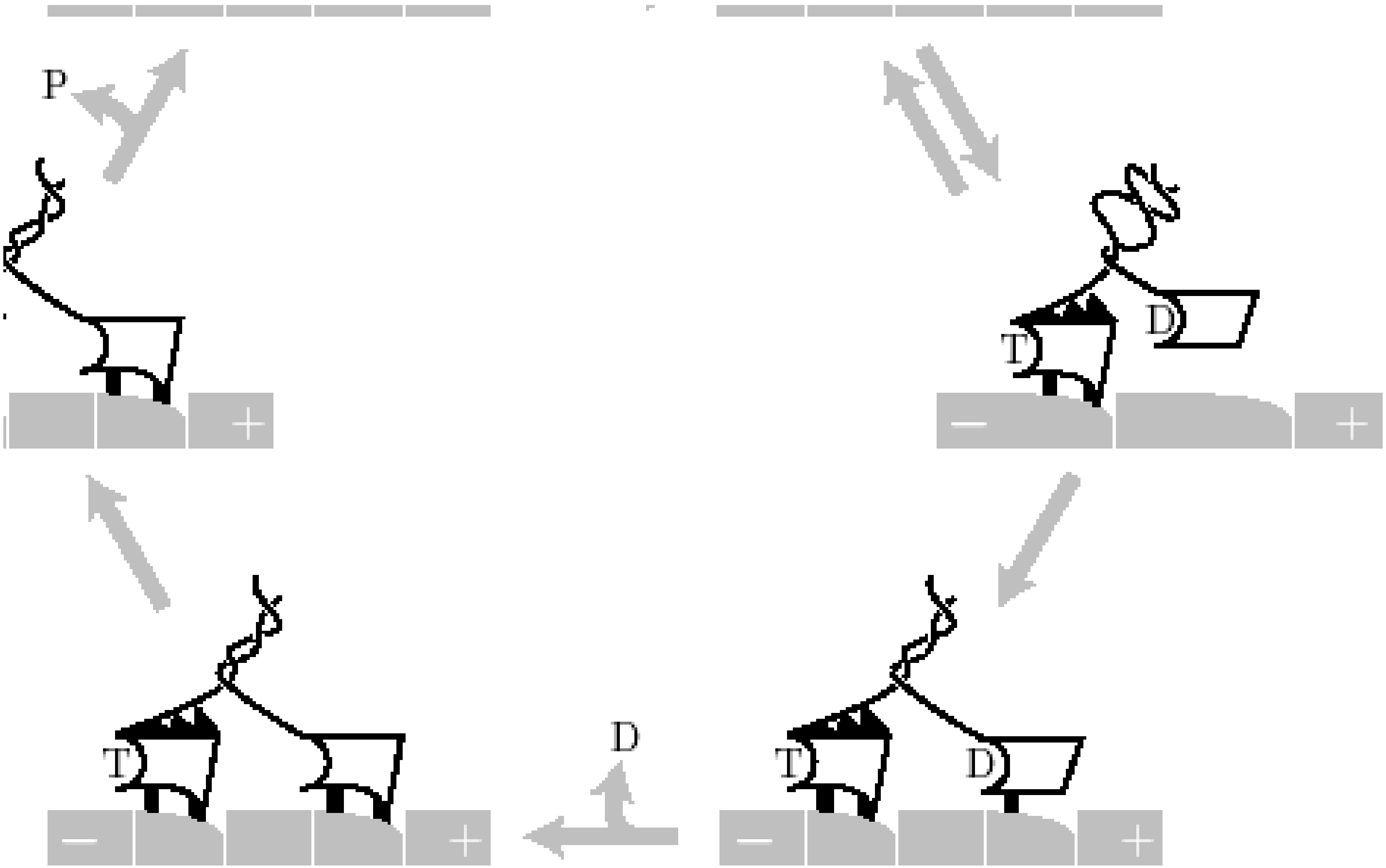
- Double-headed kinesin is a bit short to span one step (about 8 nm)
- Kinesin has enzymatic activity on ATP
  - Binding site for ATP in linker region between head and coil
  - ATP, link is stressed, can now reach full step
  - ATP -> ADP + P_i
  - ADP, link is floppy
  - Coupled with motor activity
- Kinesin without nucleotide binds microtuble strongly
- Microtuble-kinesin-ADP complex is weakly bound due to steric hindrance

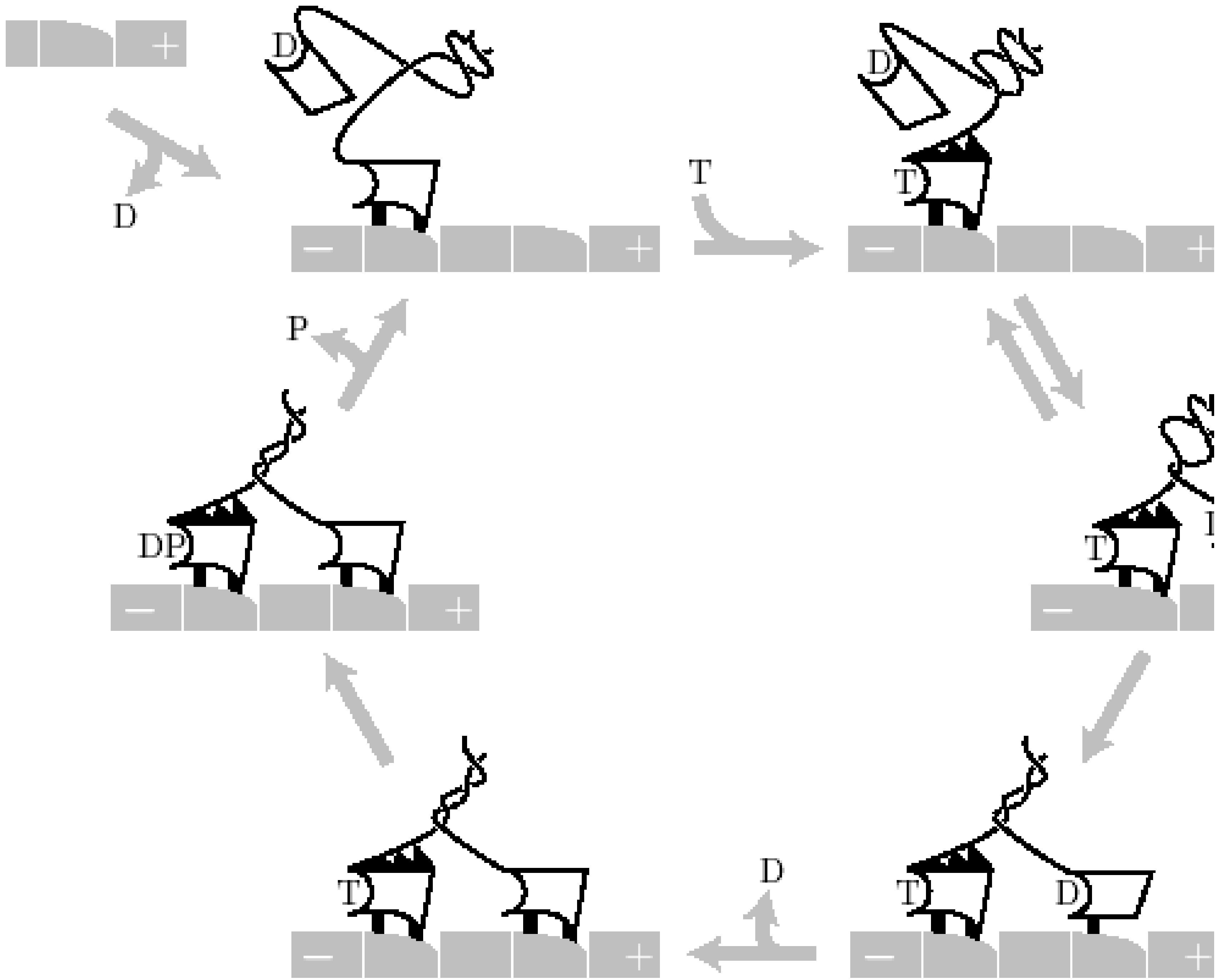


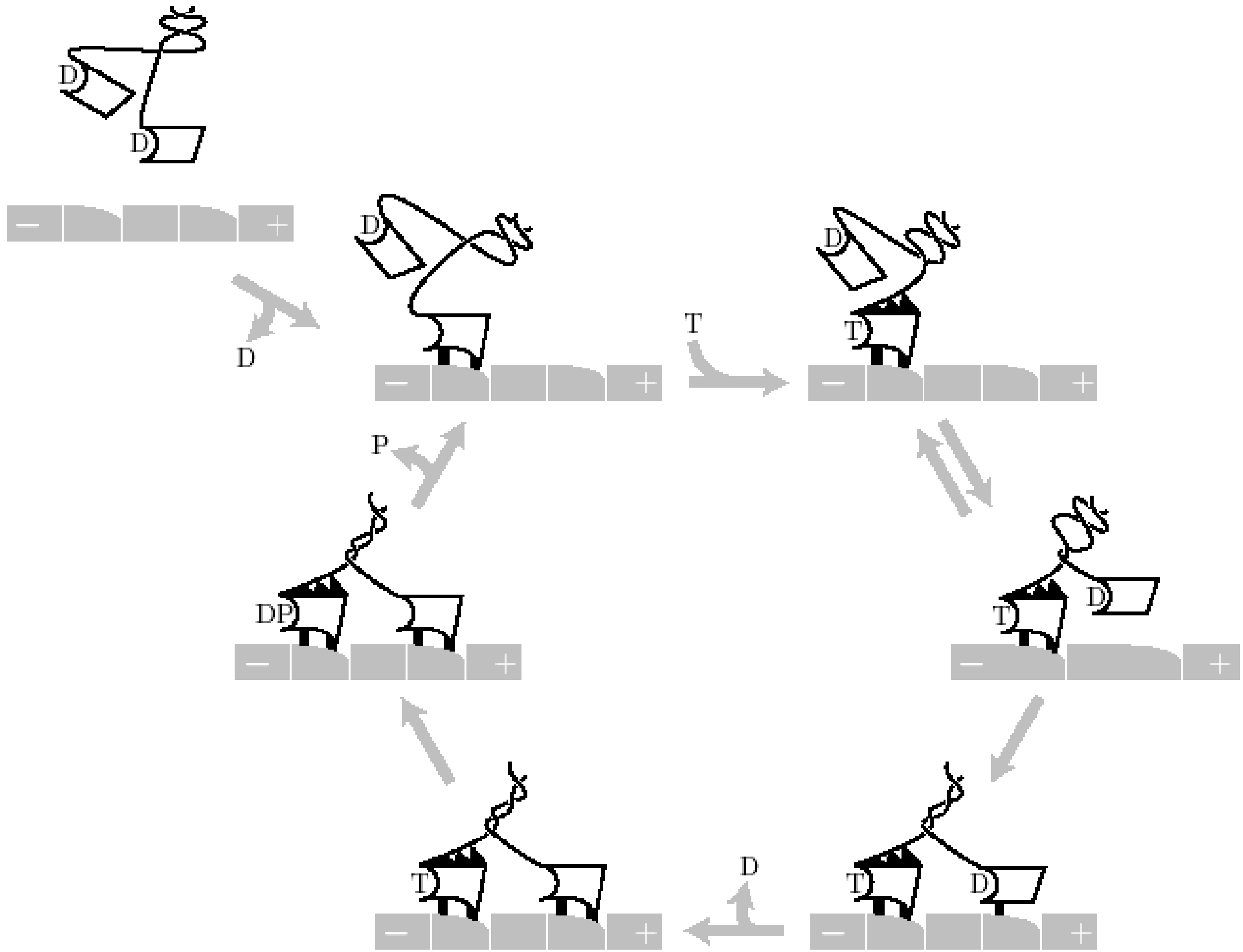

D
D
D
T
D
T
D
T
D
T
D
D
T
P
DP



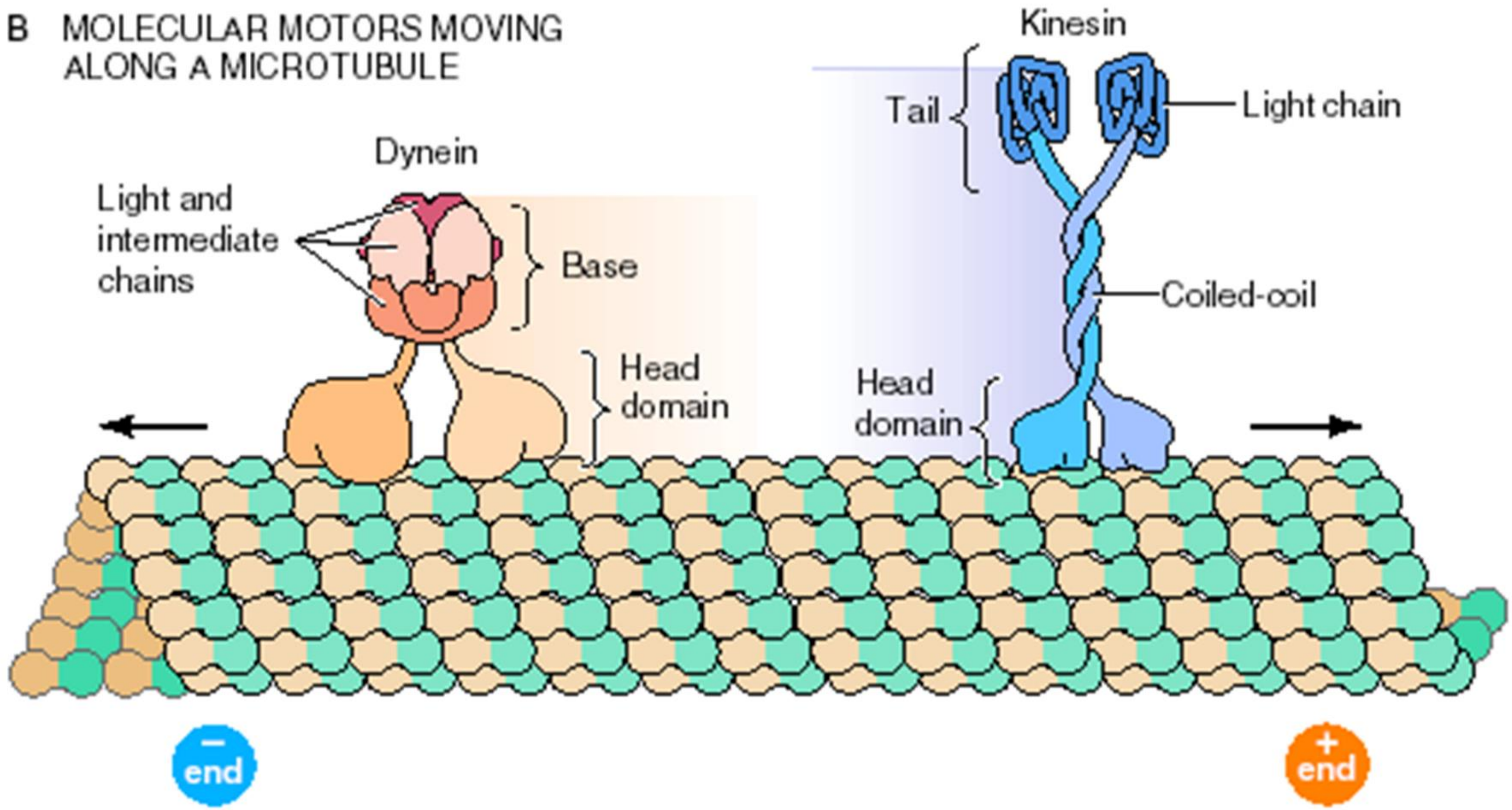
D
T
T
D
P
T
D


P
T
D
T
D
T
D
D

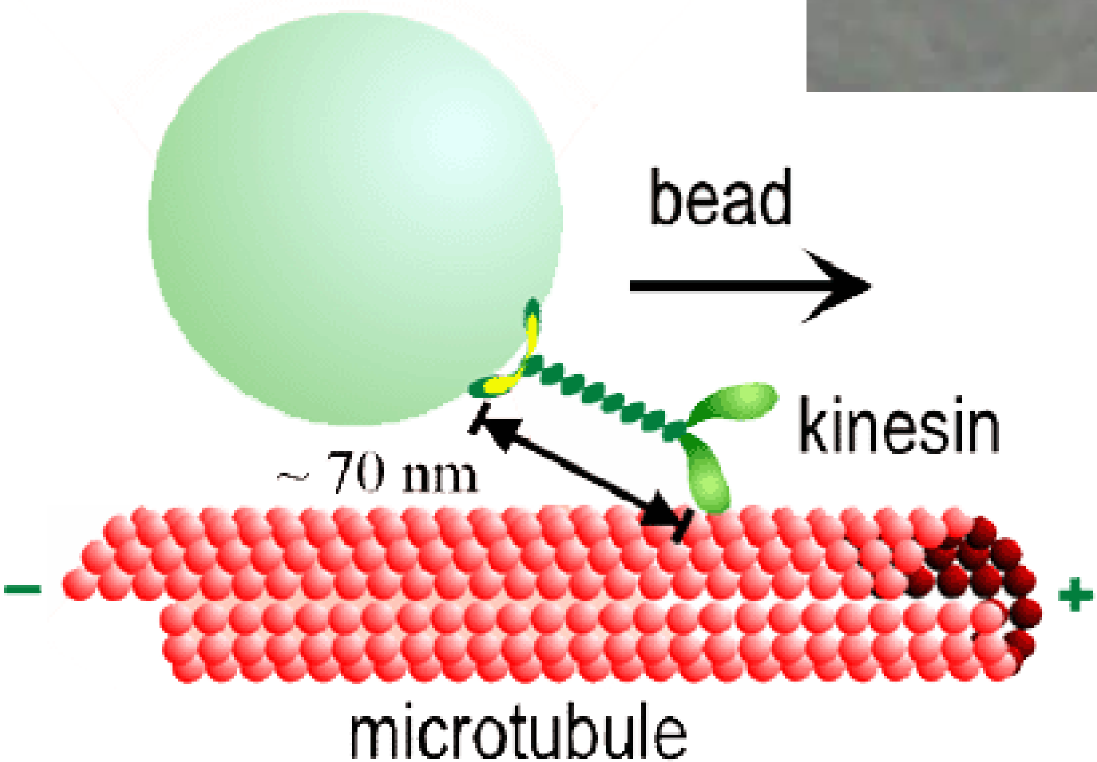

D
D
T
T
D
P
DP
T
T
D
D
T



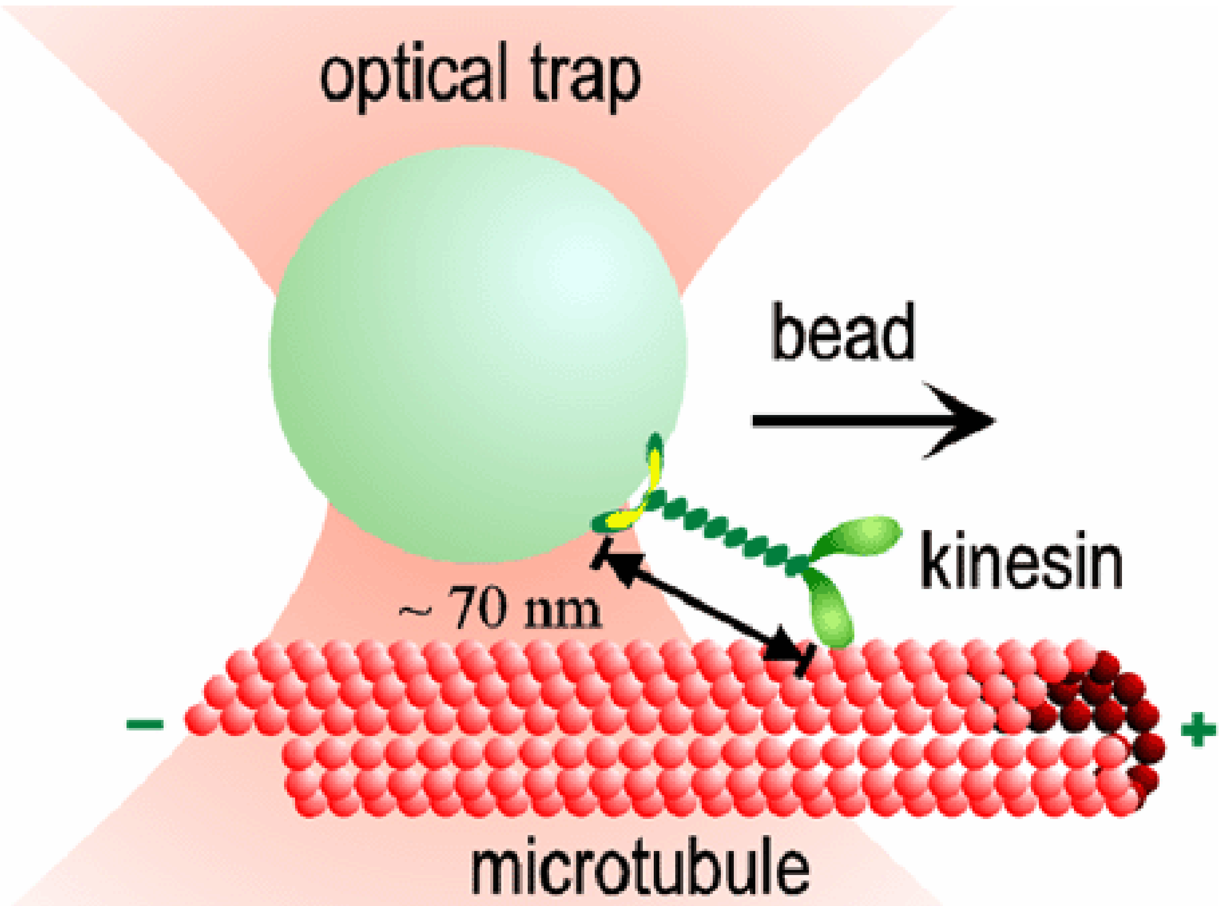
D
D
D
T
T
T
D
P
DP
D
T
D
T

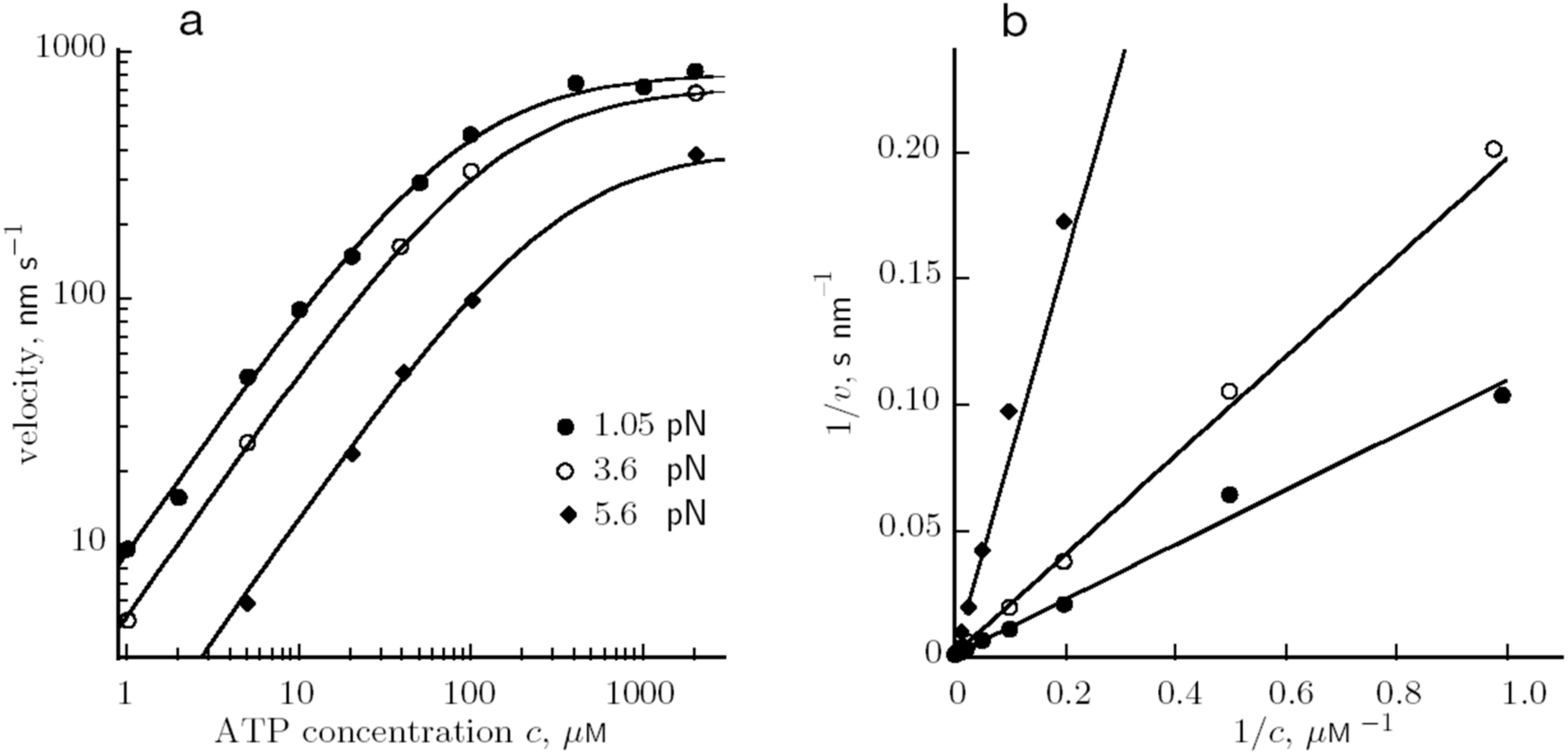


$$M \bullet K + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} M \bullet K \bullet ATP \xrightarrow{k_2} M \bullet K + ADP + \text{movement}$$


bead
kinesin
~ 70 nm
−
+
microtubule

- Bead is pulled into focal point of illumination
- Displacement from focal point produces a restoring force on bead

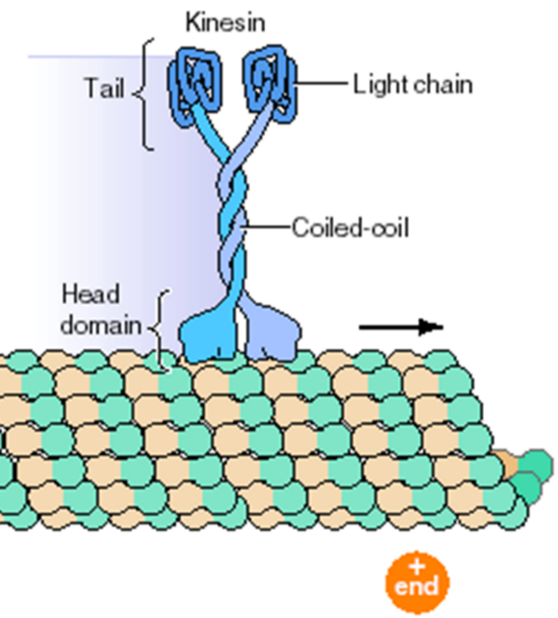


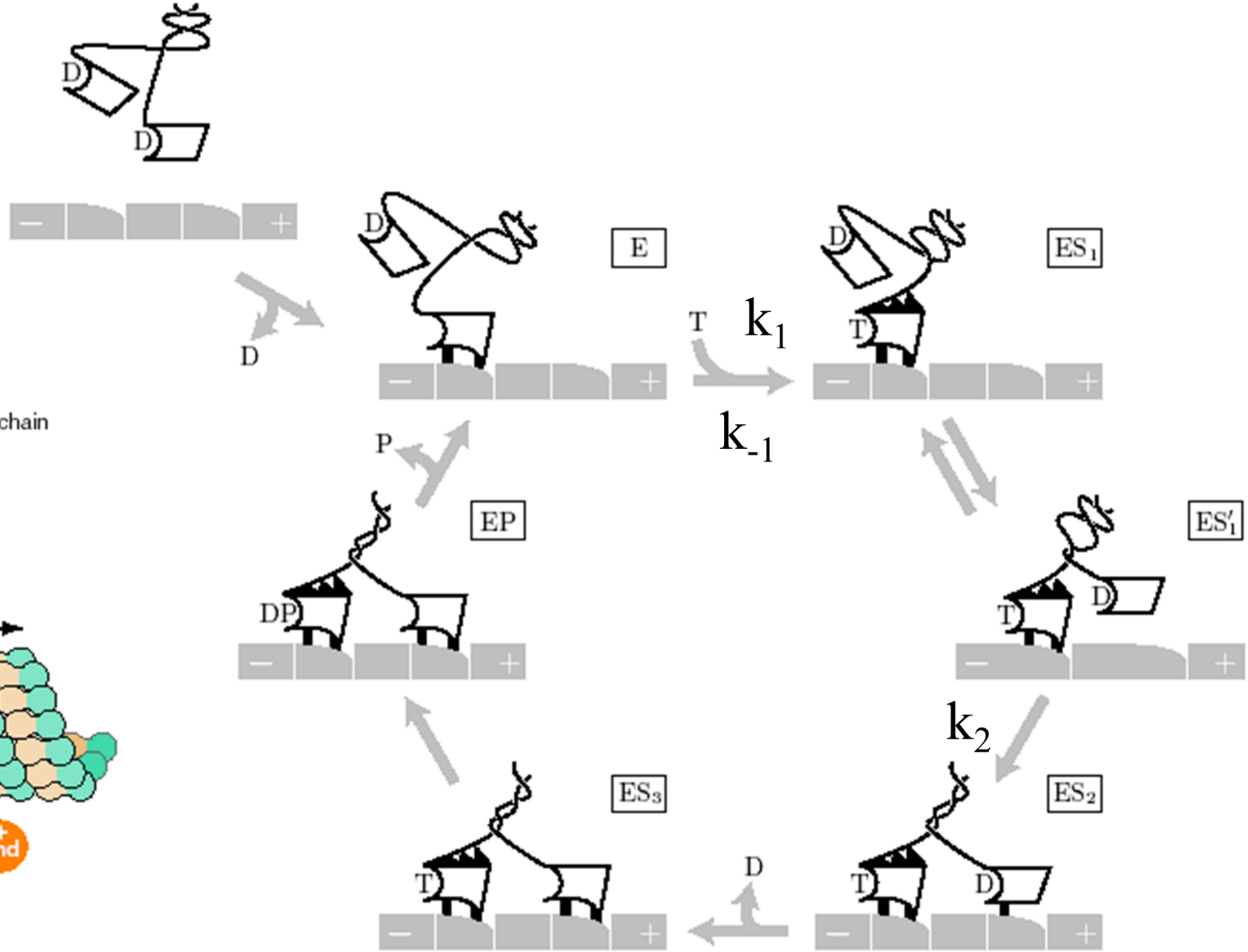


load, pN	v_{max}, nm/s	K_M, μM
1.05	813±28	88±7
3.6	715±19	140±6
5.6	404±32	312±49

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

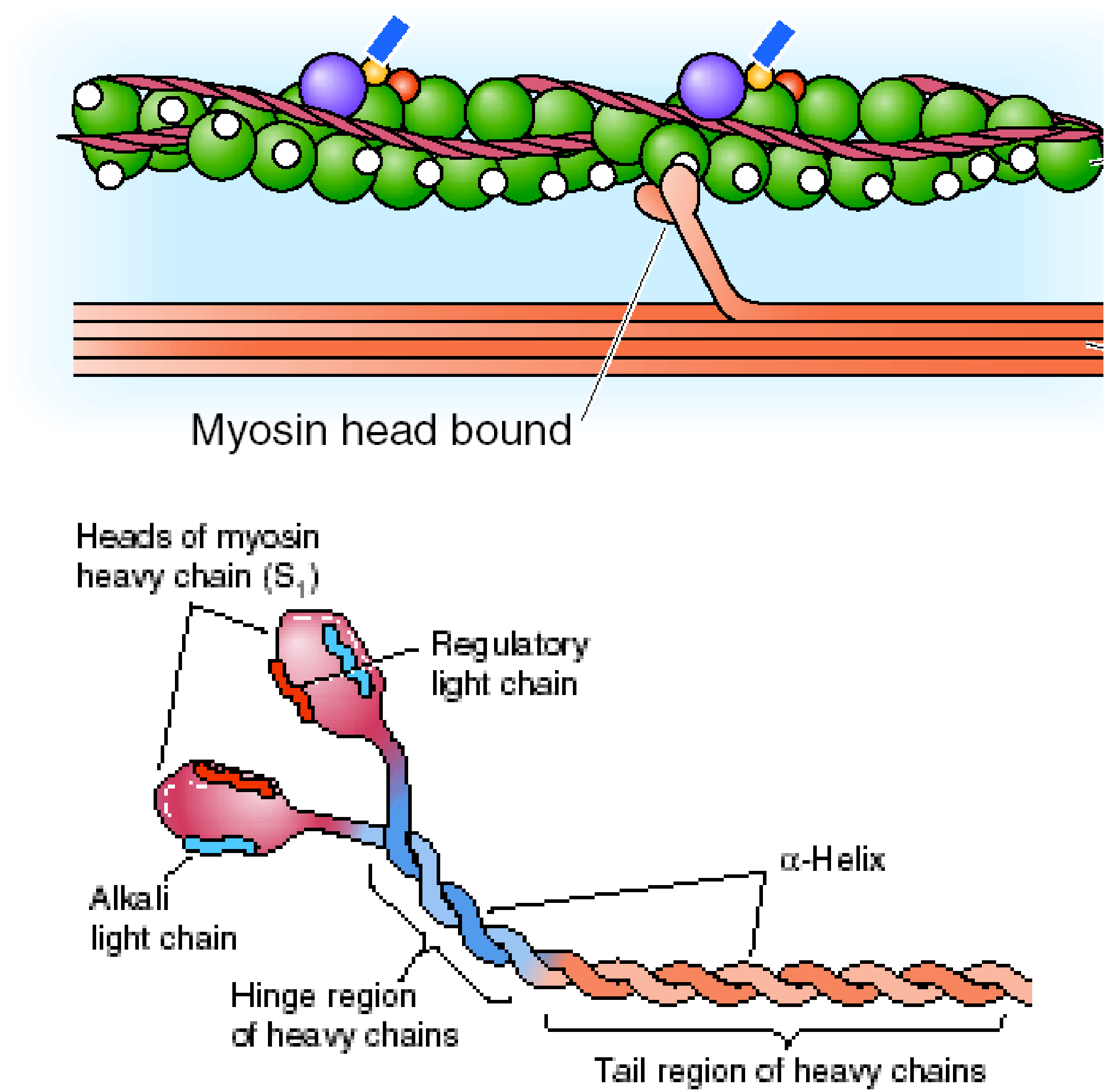
$$M \bullet K + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M \bullet K \bullet ATP \xrightarrow{k_2} M \bullet K + ADP + \text{movement}$$

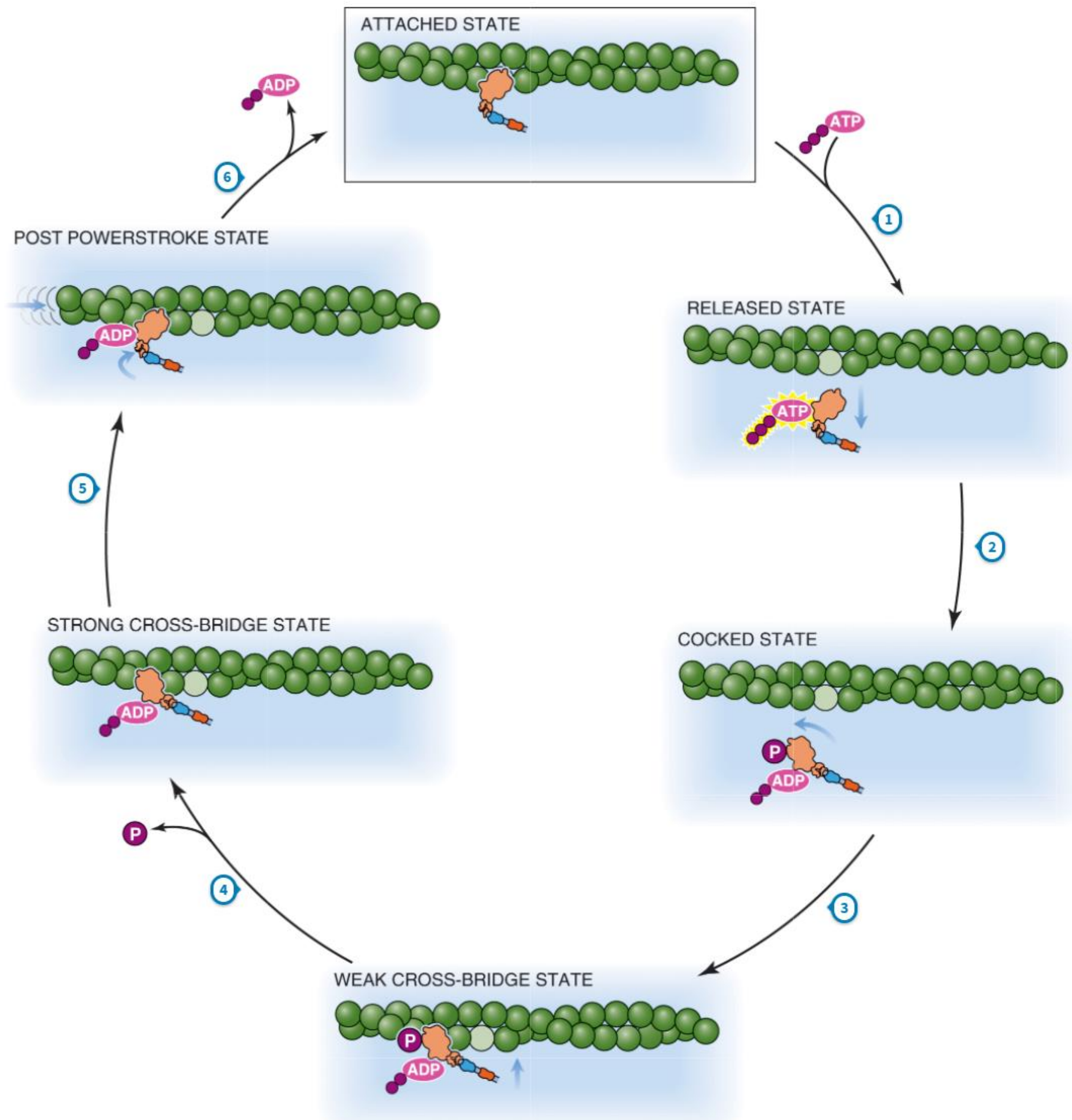


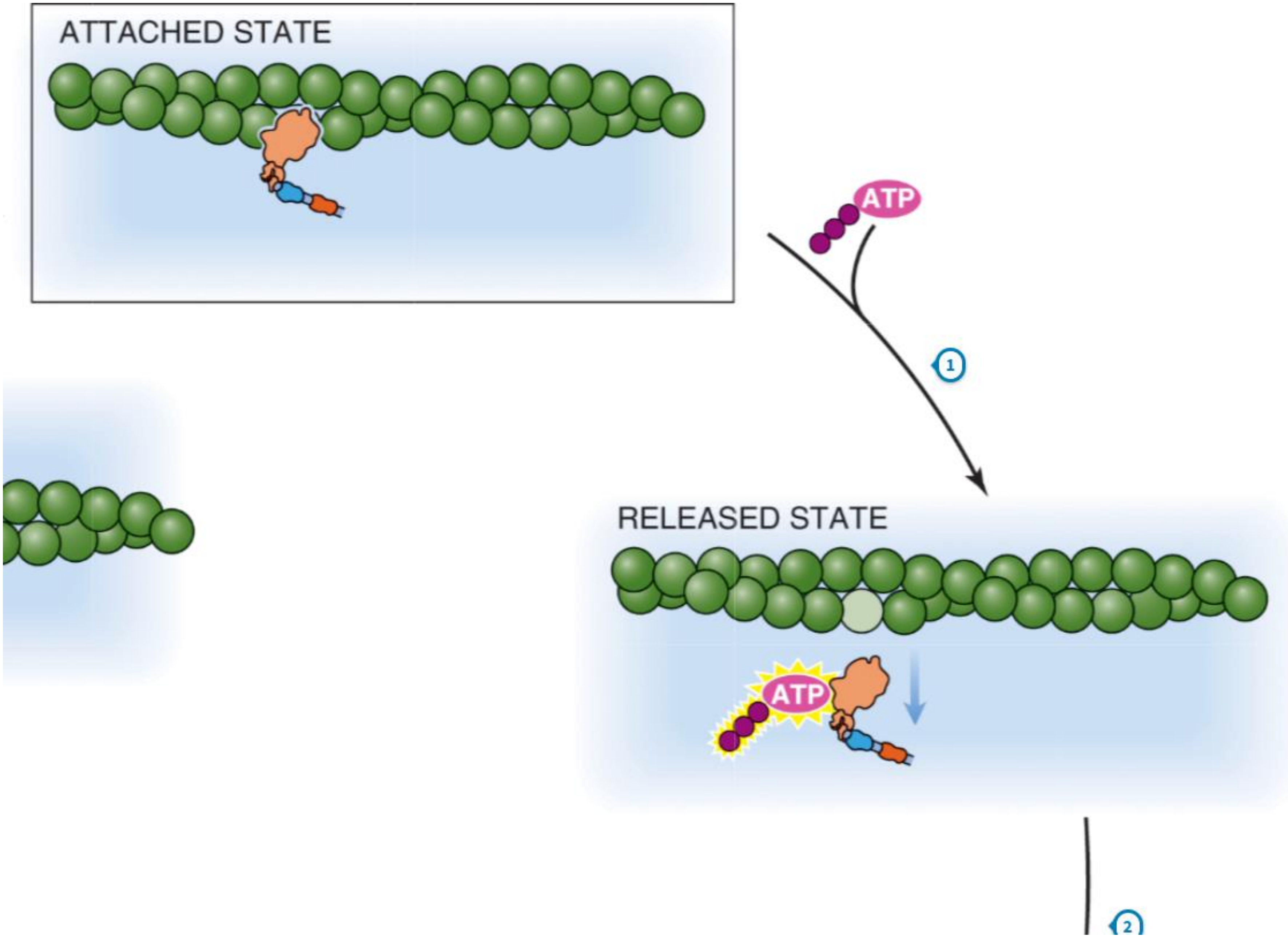


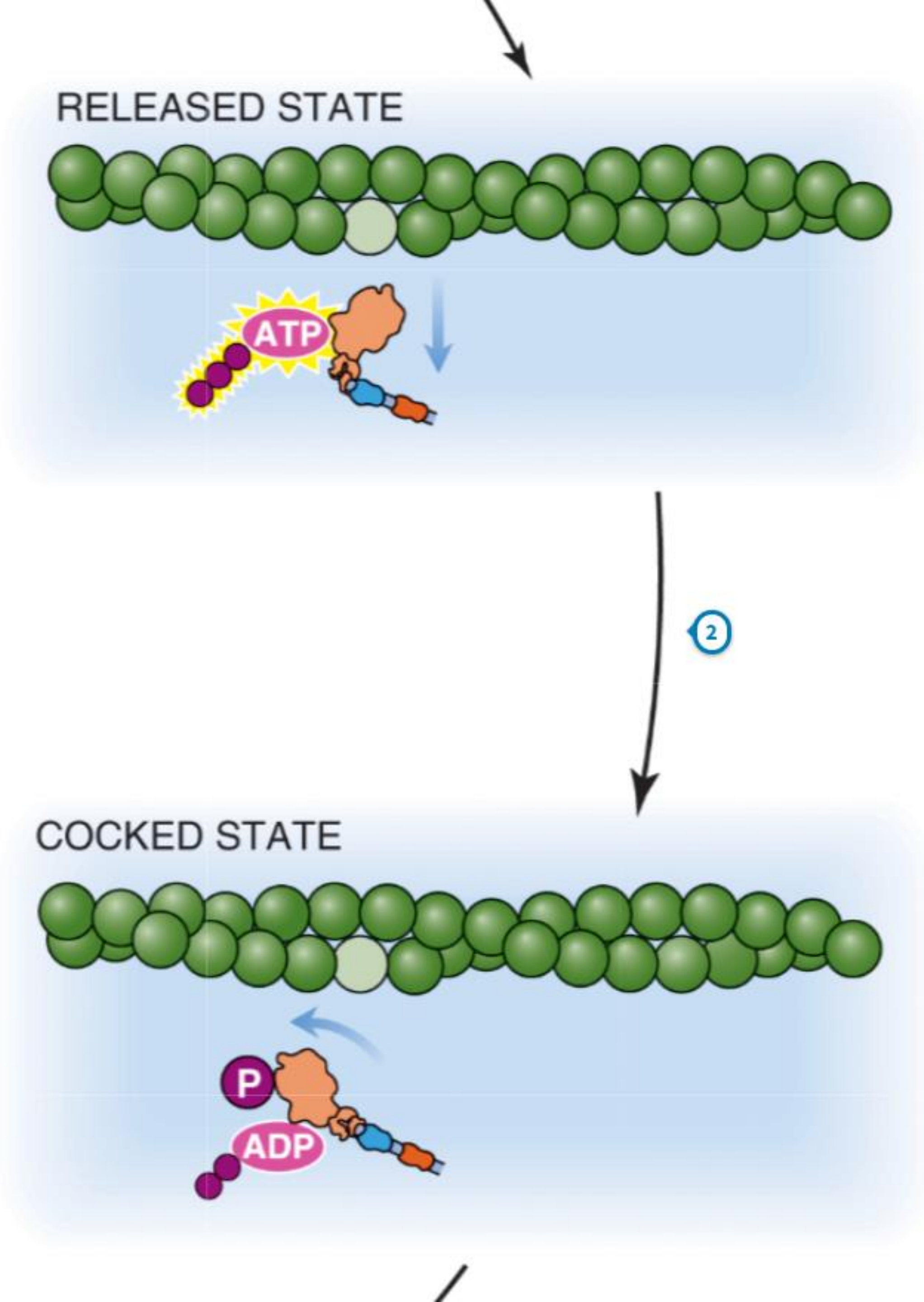
$$M \bullet K + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} M \bullet K \bullet ATP \xrightarrow{k_2} M \bullet K + ADP + \text{movement}$$

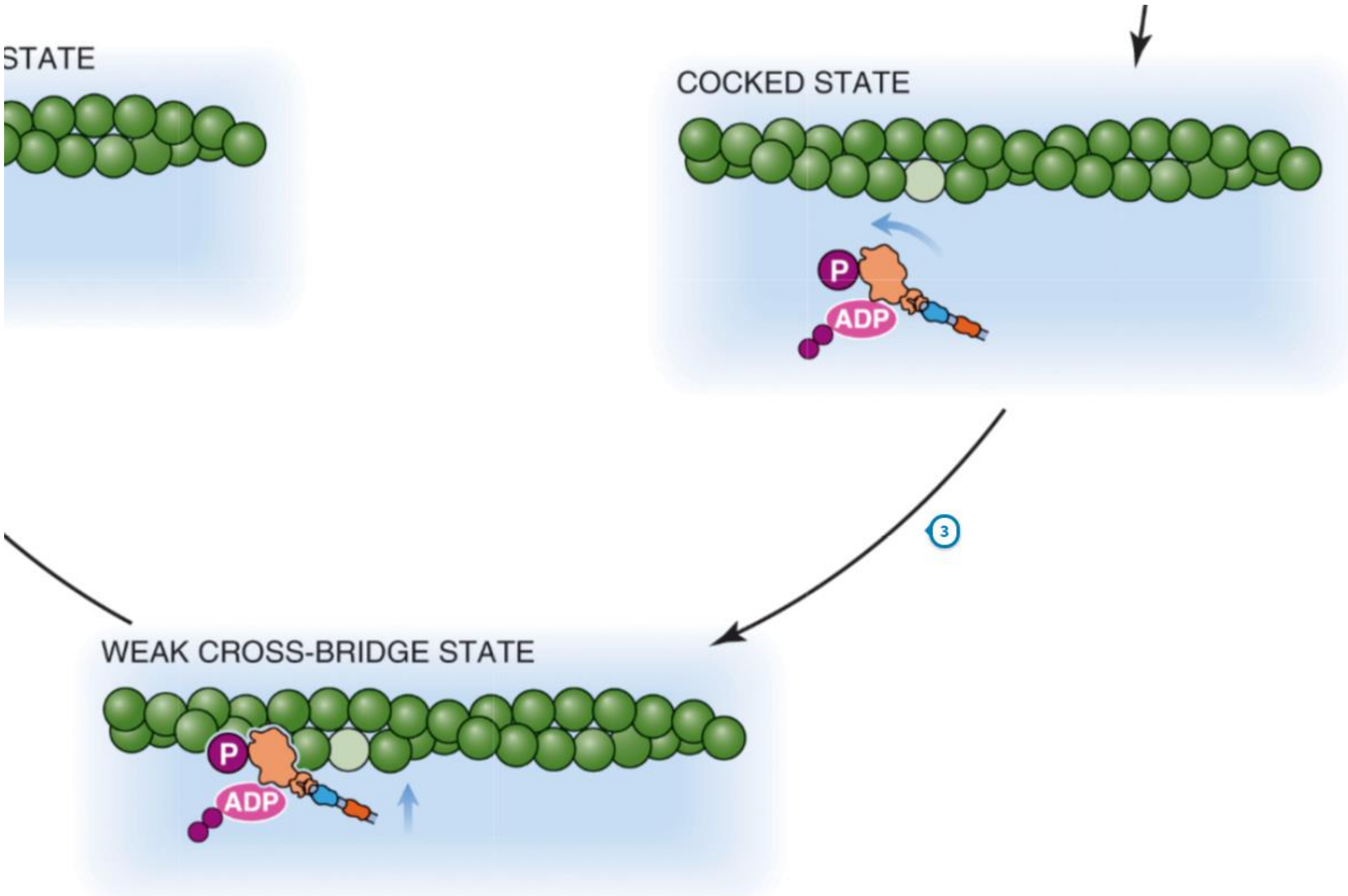
C INTERACTION OF THIN AND THICK FILAMENTS

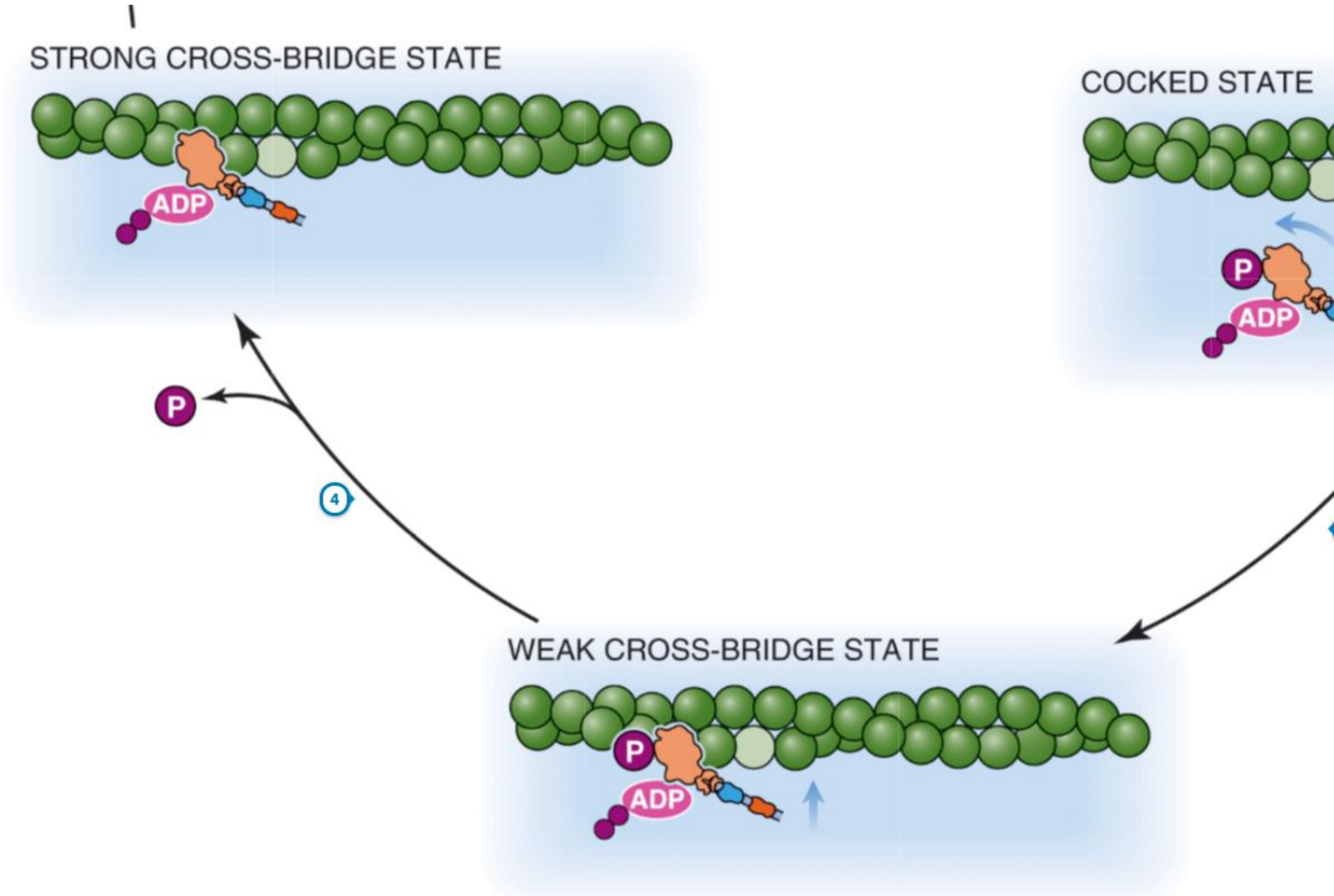



ATTACHED STATE
ATP
1
RELEASED STATE
ATP
2
COCKED STATE
P
ADP
3
WEAK CROSS-BRIDGE STATE
P
ADP
4
P
STRONG CROSS-BRIDGE STATE
ADP
5
POST POWERSTROKE STATE
ADP
6
ADP

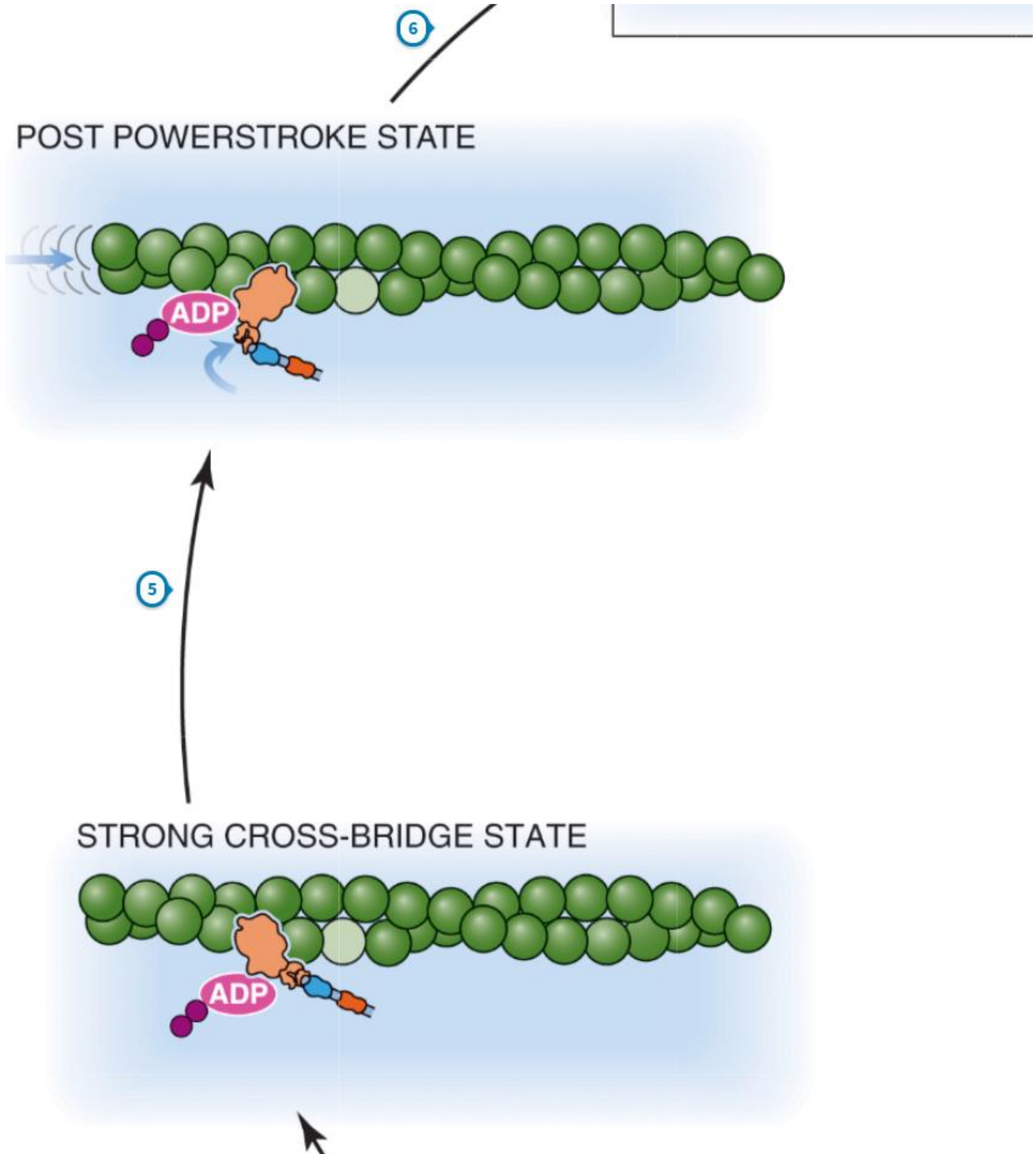

ATTACHED STATE
ATP
1
RELEASED STATE
ATP
2

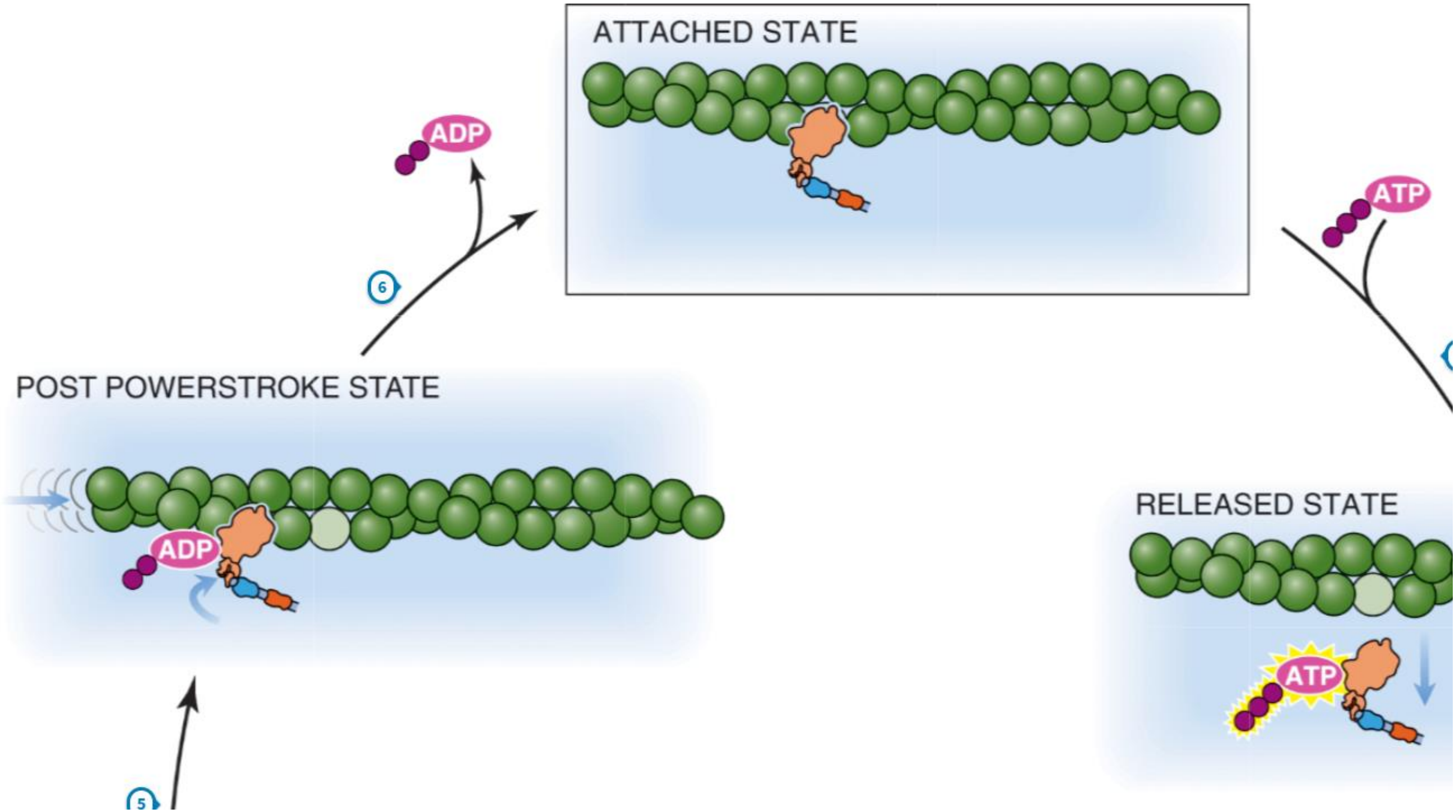

RELEASED STATE
ATP
2
COCKED STATE
P
ADP

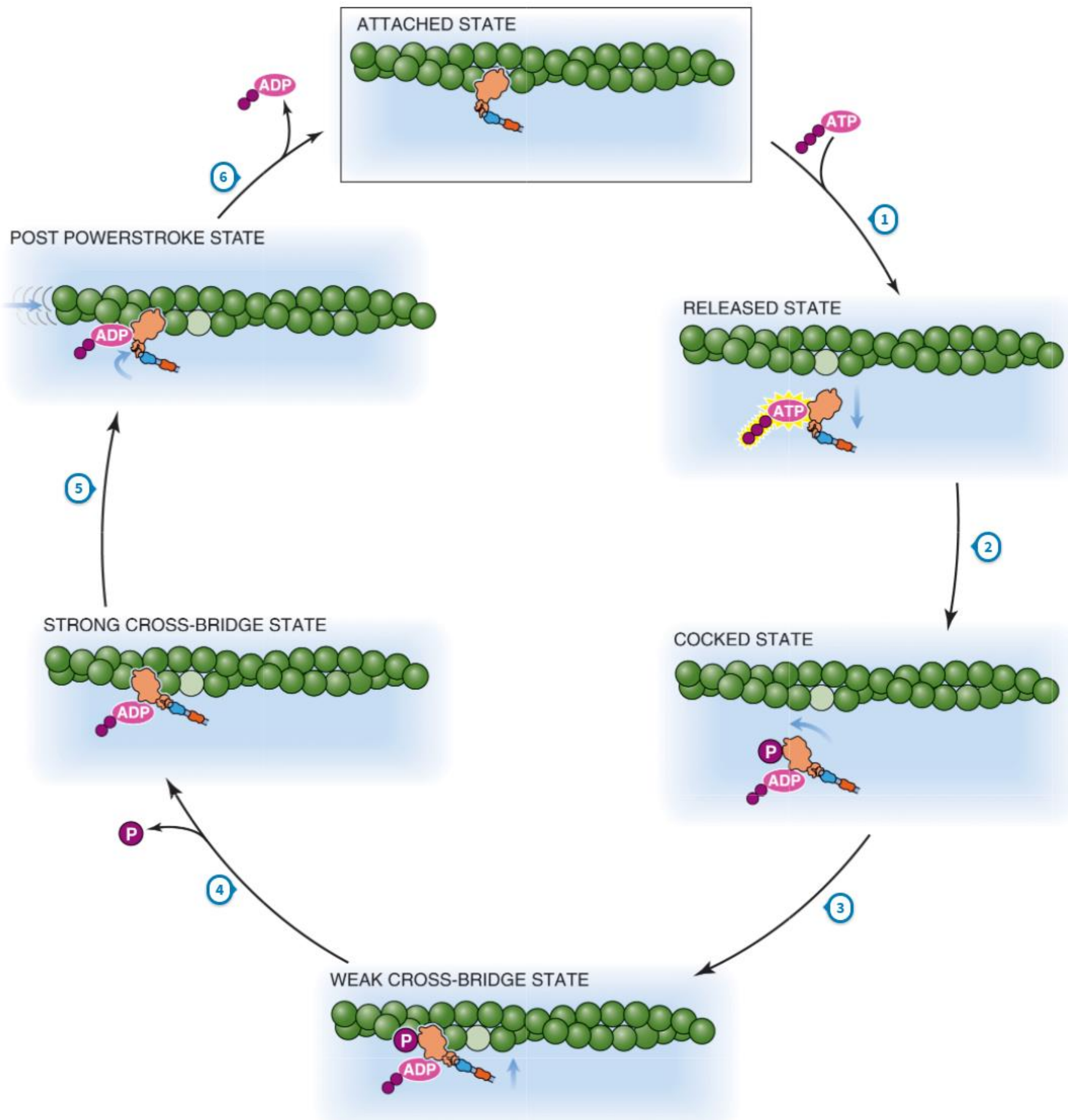

STATE
COCKED STATE
P
ADP
3
WEAK CROSS-BRIDGE STATE
P
ADP

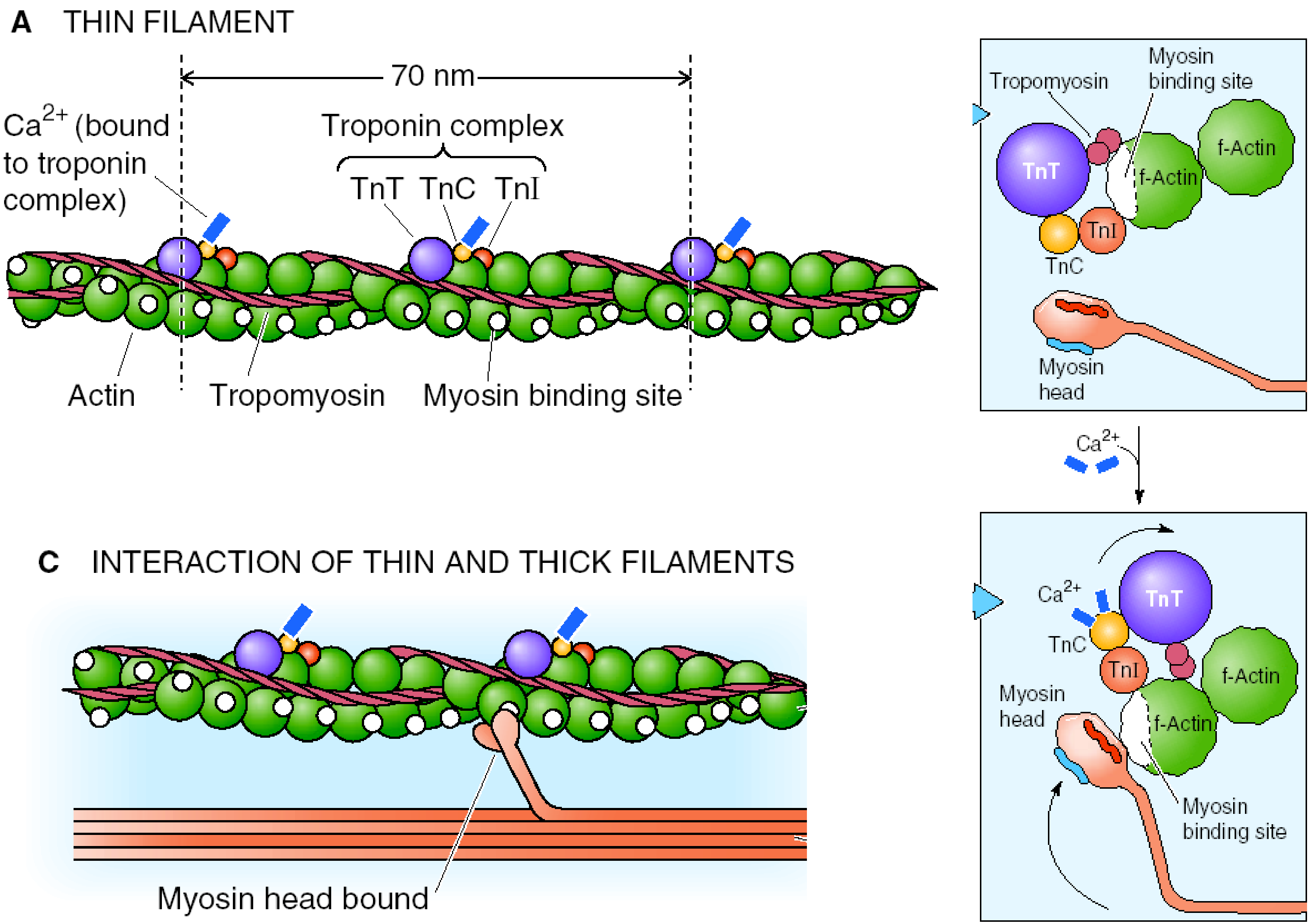


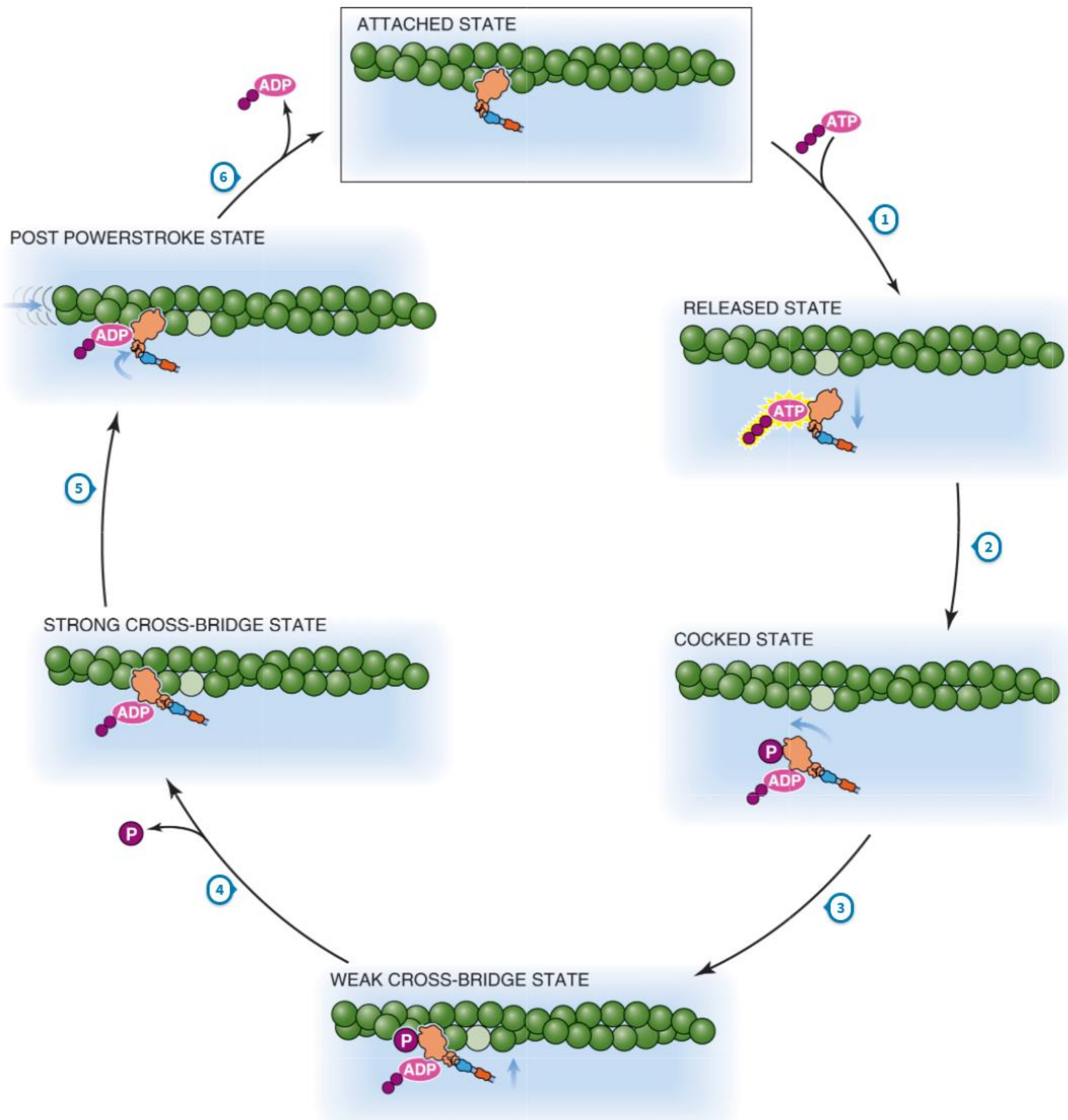
STRONG CROSS-BRIDGE STATE
ADP
COCKED STATE
P
ADP
P
4
WEAK CROSS-BRIDGE STATE
P
ADP


6
POST POWERSTROKE STATE
ADP
5
STRONG CROSS-BRIDGE STATE
ADP


ATTACHED STATE
ADP
6
ATP
POST POWERSTROKE STATE
ADP
RELEASED STATE
ATP
5


ATTACHED STATE
ADP
ATP
1
6
POST POWERSTROKE STATE
ADP
RELEASED STATE
ATP
2
5
STRONG CROSS-BRIDGE STATE
ADP
COCKED STATE
P
ADP
P
4
3
WEAK CROSS-BRIDGE STATE
P
ADP

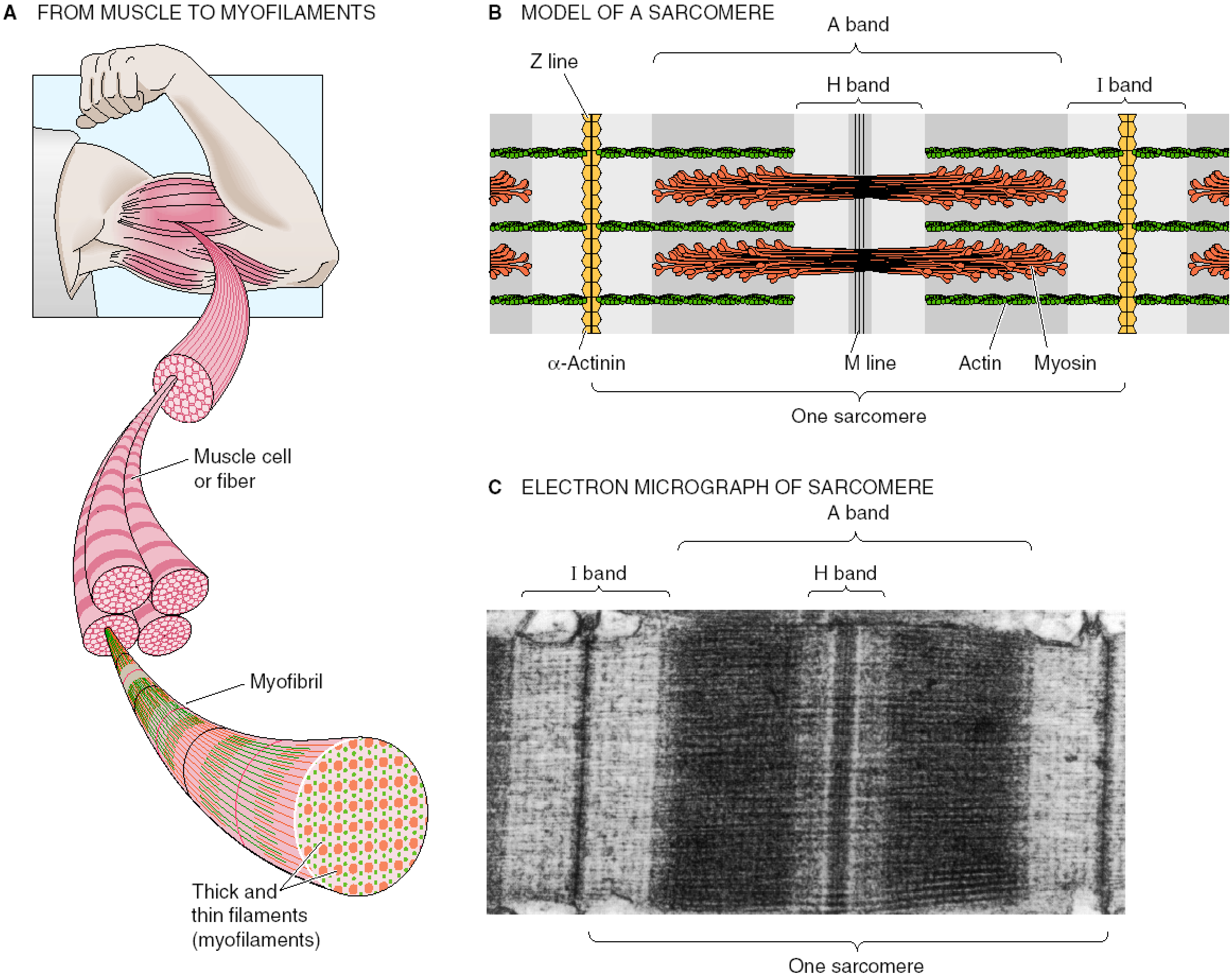

A THIN FILAMENT
70 nm
Ca2+ (bound to troponin complex)
Troponin complex
TnT TnC TnI
Actin
Tropomyosin
Myosin binding site
Tropomyosin
Myosin binding site
TnT
f-Actin
f-Actin
TnI
TnC
Myosin head
Ca2+
C INTERACTION OF THIN AND THICK FILAMENTS
Myosin head bound
Ca2+
TnT
TnC
TnI
f-Actin
Myosin head
f-Actin
Myosin binding site


ATTACHED STATE
ATP
1
RELEASED STATE
ATP
2
COCKED STATE
P
ADP
3
WEAK CROSS-BRIDGE STATE
P
ADP
4
P
STRONG CROSS-BRIDGE STATE
ADP
5
POST POWERSTROKE STATE
ADP
6
ADP

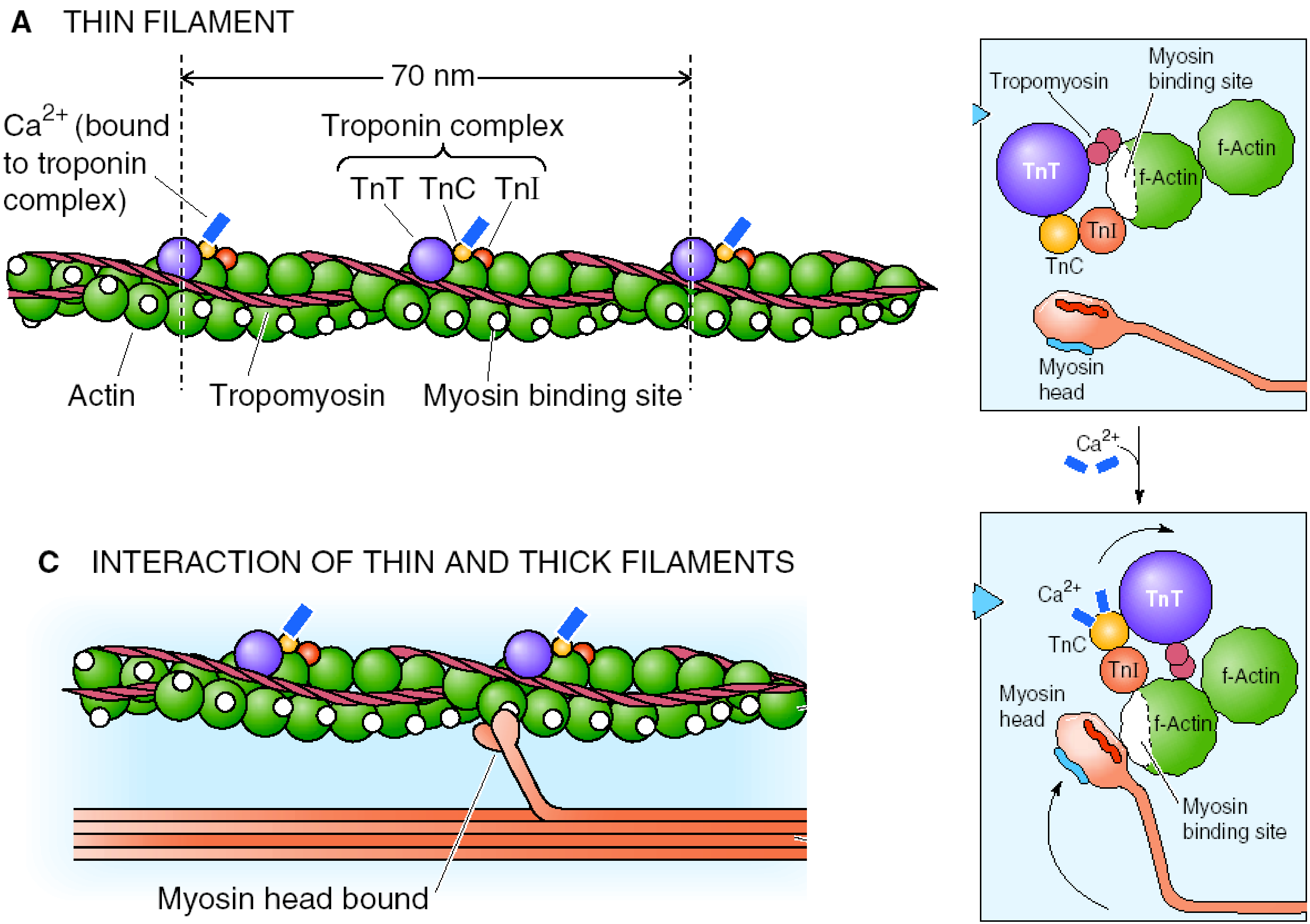
Muscle physiology

- 3 general types
  - skeletal muscle - voluntary movement, the one thought of as muscle
  - cardiac muscle - highly coordinated, moderate control
  - smooth muscle - slower-responding, tone of viscera, other tissues

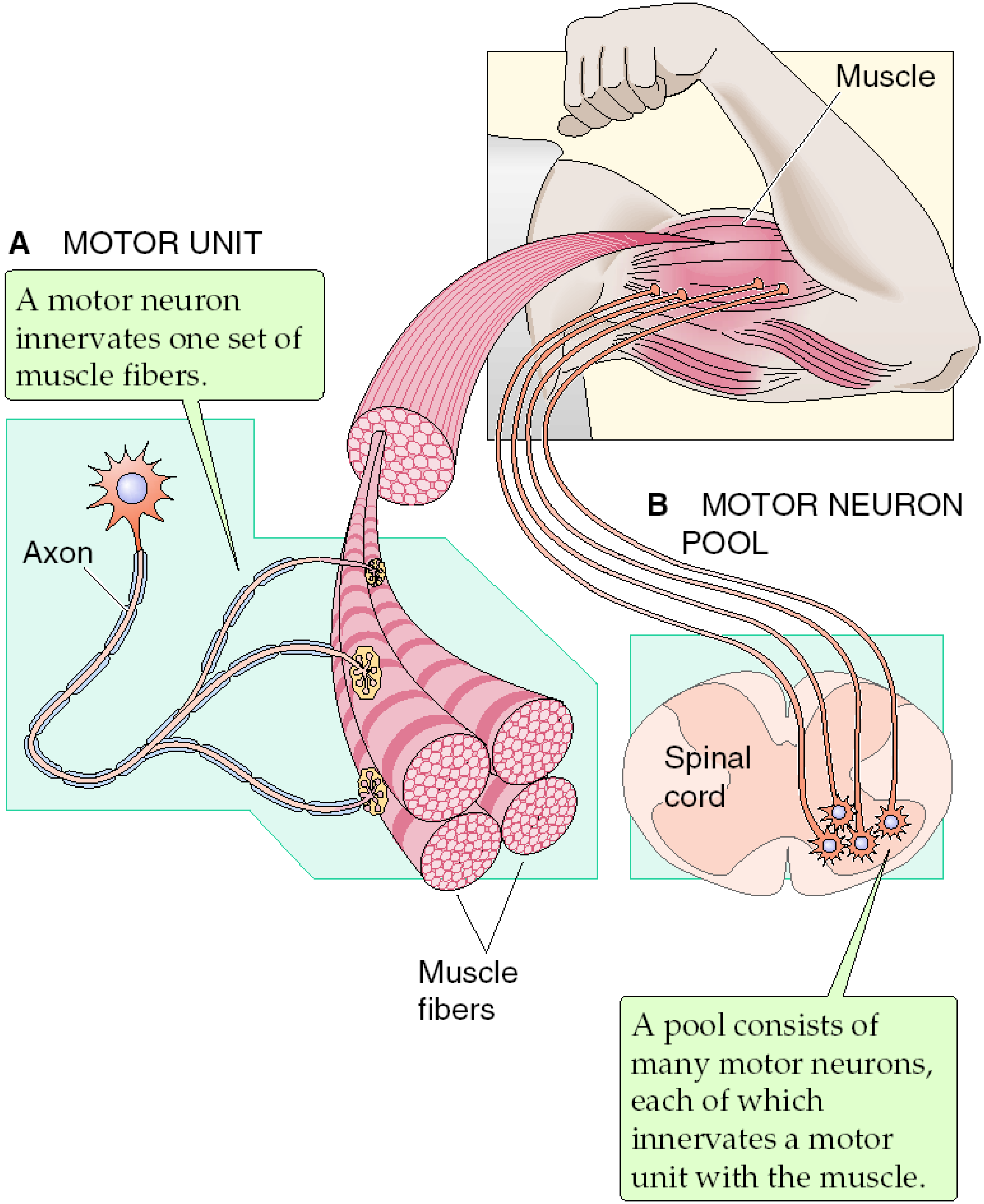
- *striated*: highly organized actin/myosin interactions - skeletal and cardiac muscle



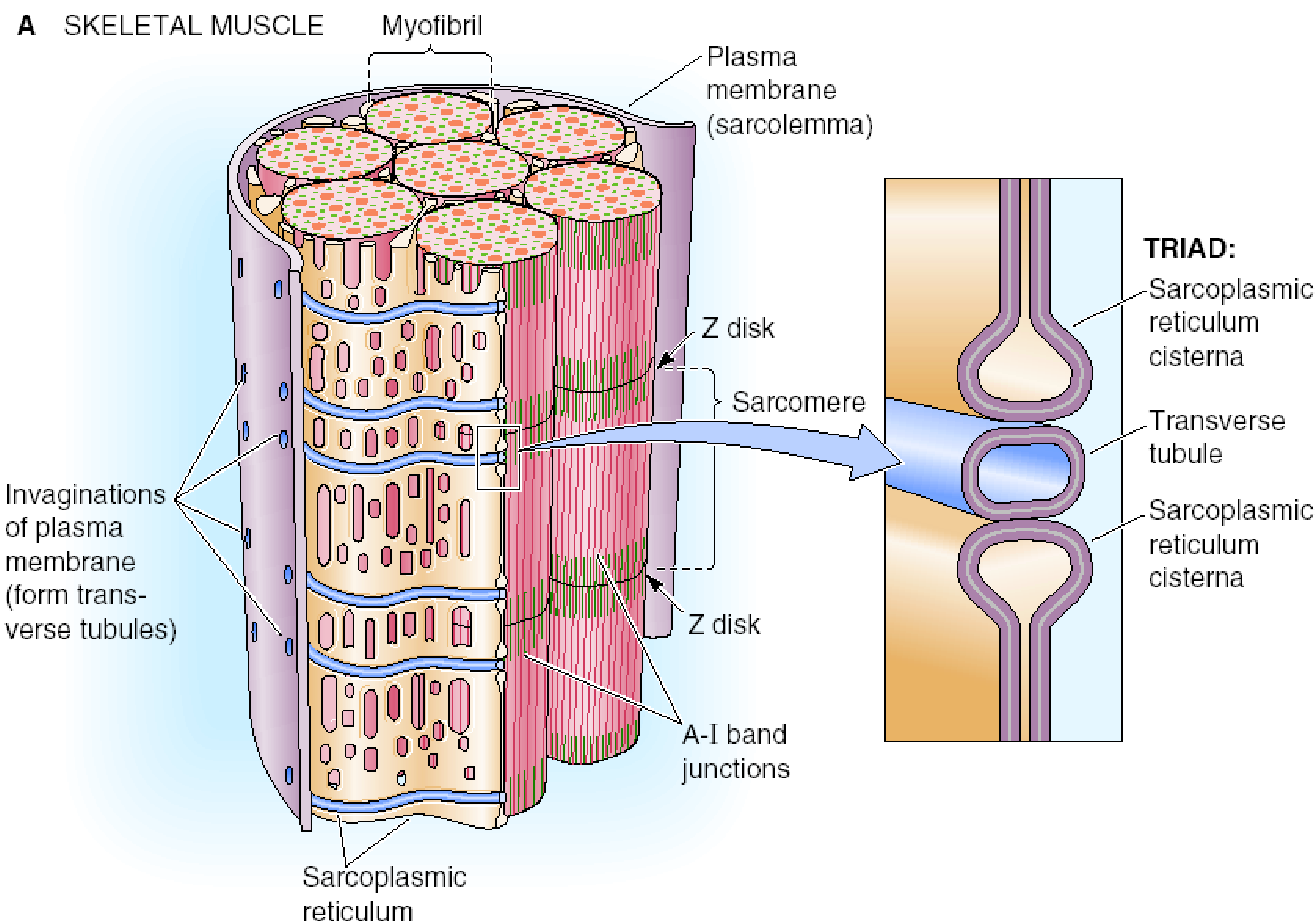
A FROM MUSCLE TO MYOFILAMENTS
Muscle cell
or fiber
Myofibril
Thick and
thin filaments
(myofilaments)
B MODEL OF A SARCOMERE
A band
Z line
H band
I band
α-Actinin
M line
Actin
Myosin
One sarcomere
C ELECTRON MICROGRAPH OF SARCOMERE
A band
I band
H band
One sarcomere

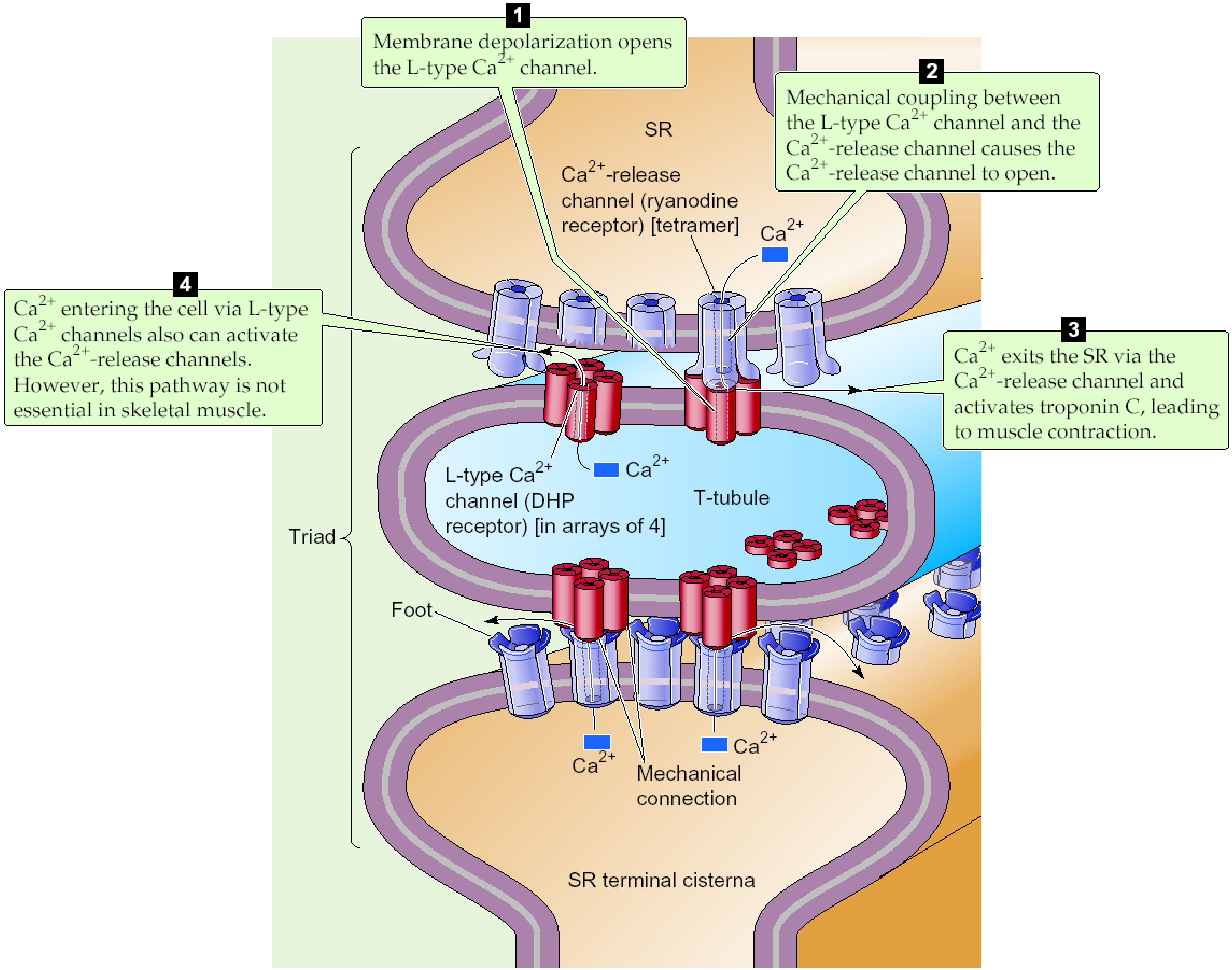


A THIN FILAMENT
70 nm
Ca2+ (bound to troponin complex)
Troponin complex
TnT TnC TnI
Actin
Tropomyosin
Myosin binding site
Tropomyosin
Myosin binding site
TnT
f-Actin
f-Actin
TnI
TnC
Myosin head
Ca2+
Ca2+
TnT
TnC
TnI
f-Actin
Myosin head
f-Actin
Myosin binding site
C INTERACTION OF THIN AND THICK FILAMENTS
Myosin head bound



Muscle
A MOTOR UNIT
A motor neuron innervates one set of muscle fibers.
Axon
B MOTOR NEURON POOL
Spinal cord
Muscle fibers
A pool consists of many motor neurons, each of which innervates a motor unit with the muscle.

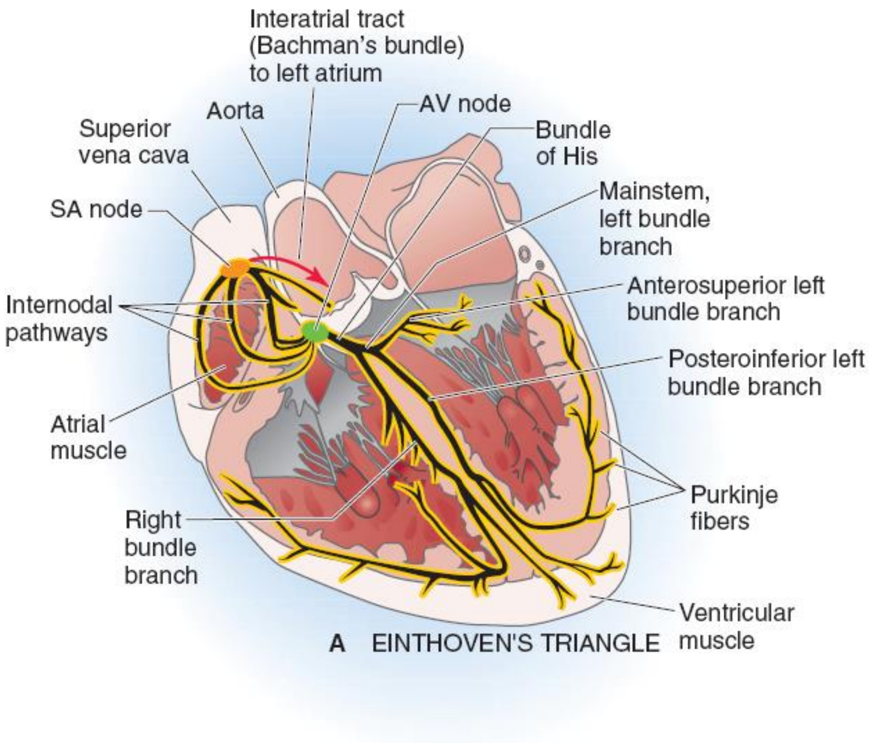

A SKELETAL MUSCLE
Myofibril
Plasma membrane (sarcolemma)
Z disk
Sarcomere
Z disk
A-I band junctions
Sarcoplasmic reticulum
Invaginations of plasma membrane (form transverse tubules)
TRIAD:
Sarcoplasmic reticulum cisterna
Transverse tubule
Sarcoplasmic reticulum cisterna

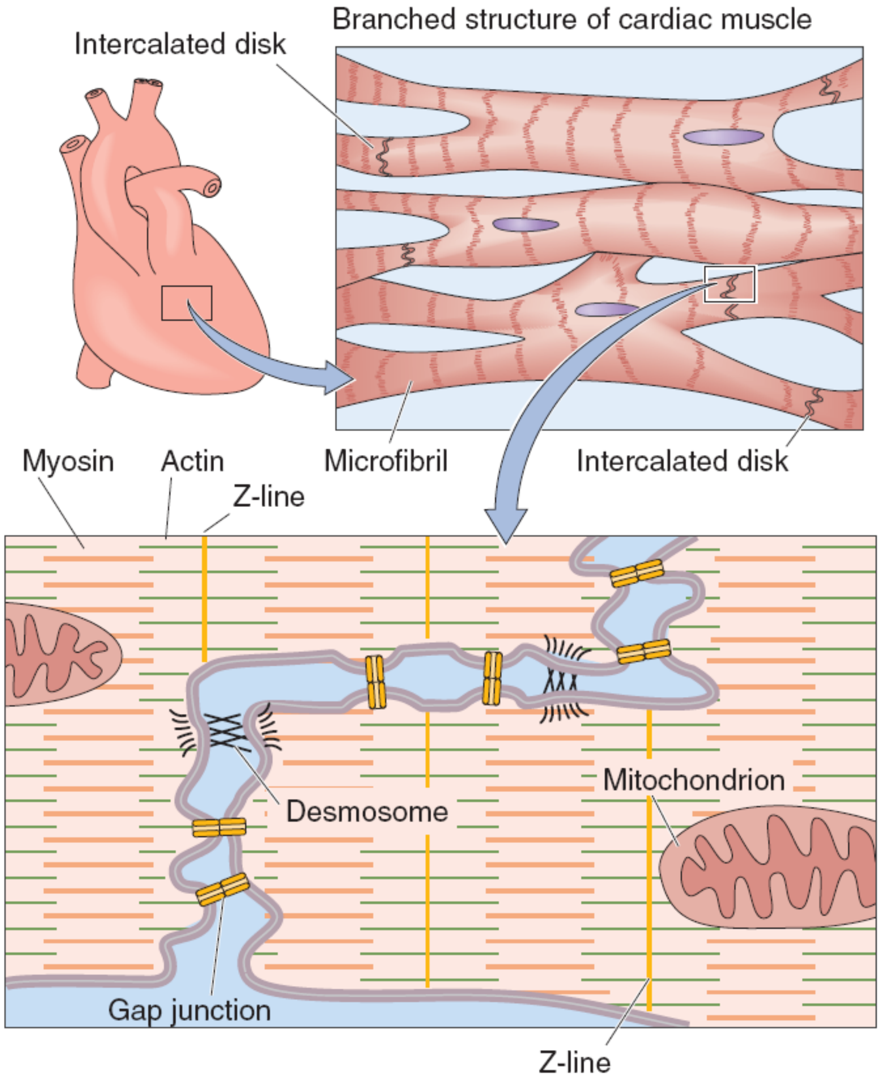


1
Membrane depolarization opens the L-type Ca^{2+} channel.
2
Mechanical coupling between the L-type Ca^{2+} channel and the Ca^{2+}-release channel causes the Ca^{2+}-release channel to open.
3
Ca^{2+} exits the SR via the Ca^{2+}-release channel and activates troponin C, leading to muscle contraction.
4
Ca^{2+} entering the cell via L-type Ca^{2+} channels also can activate the Ca^{2+}-release channels. However, this pathway is not essential in skeletal muscle.
SR
Ca^{2+}-release channel (ryanodine receptor) [tetramer]
Ca^{2+}
L-type Ca^{2+} channel (DHP receptor) [in arrays of 4]
Ca^{2+}
T-tubule
Triad
Foot
Ca^{2+}
Ca^{2+}
Mechanical connection
SR terminal cisterna

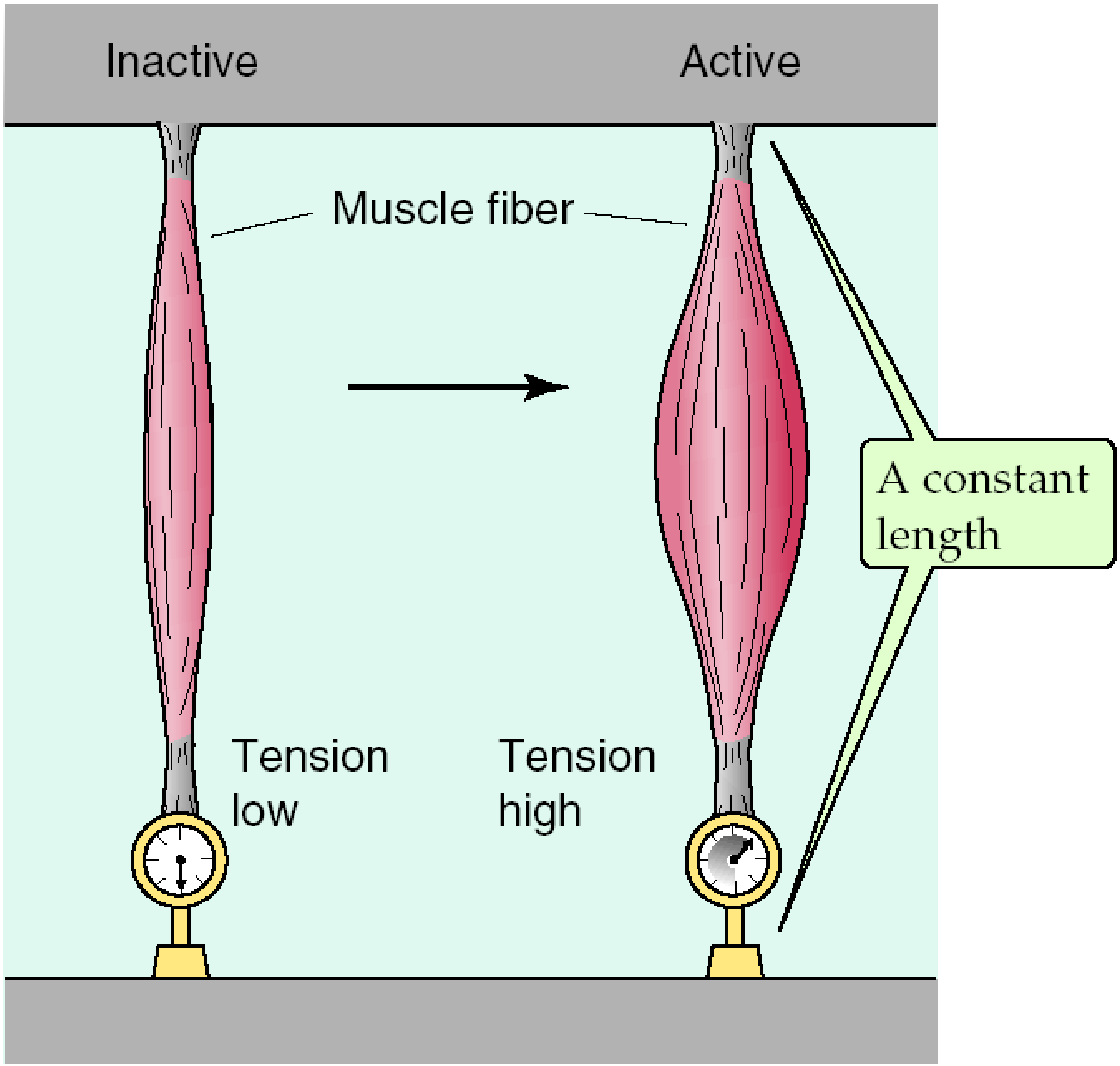
Cardiac muscle

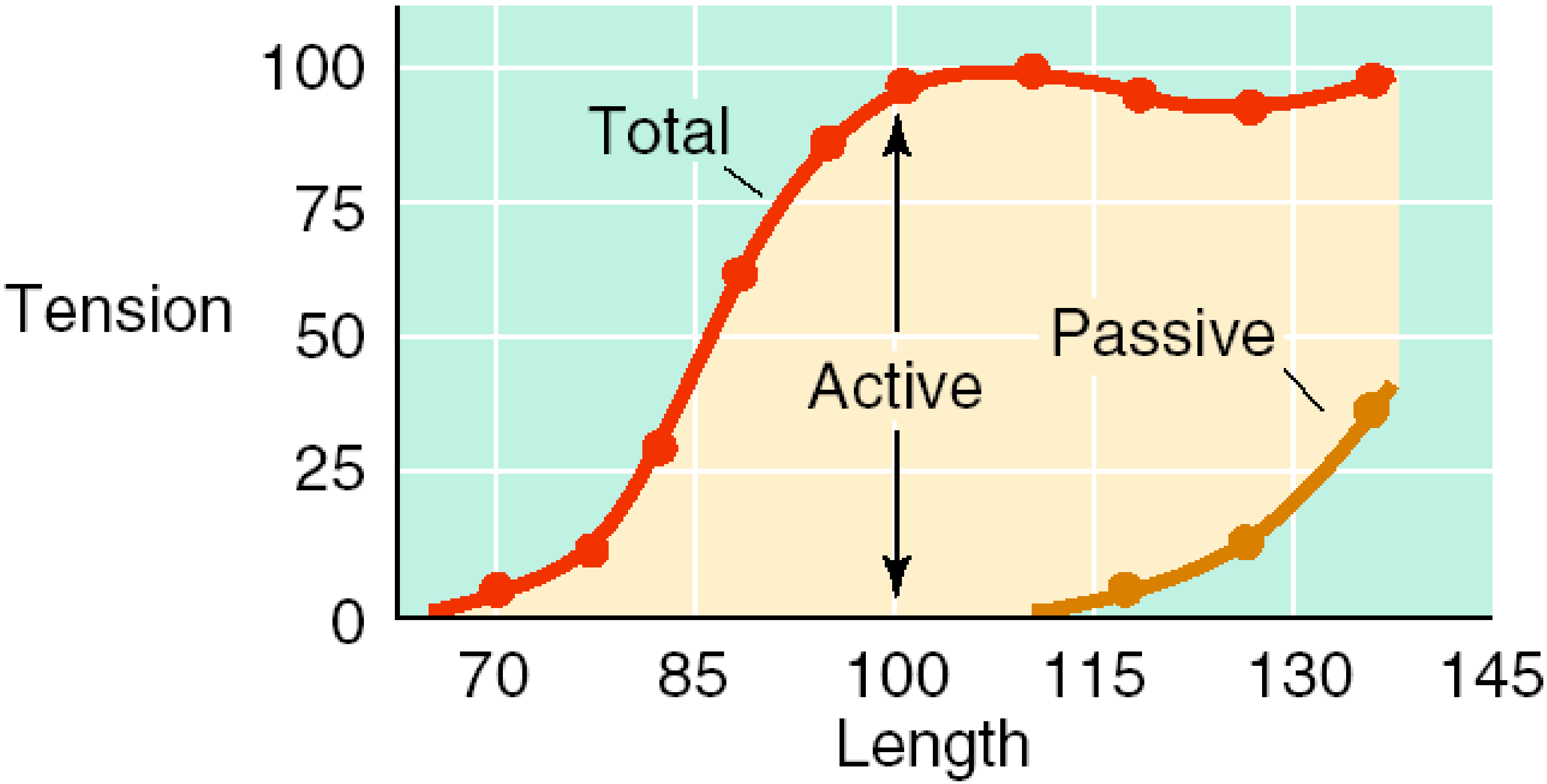
- variant of skeletal muscle
- modified action potential
- pacemaker activity



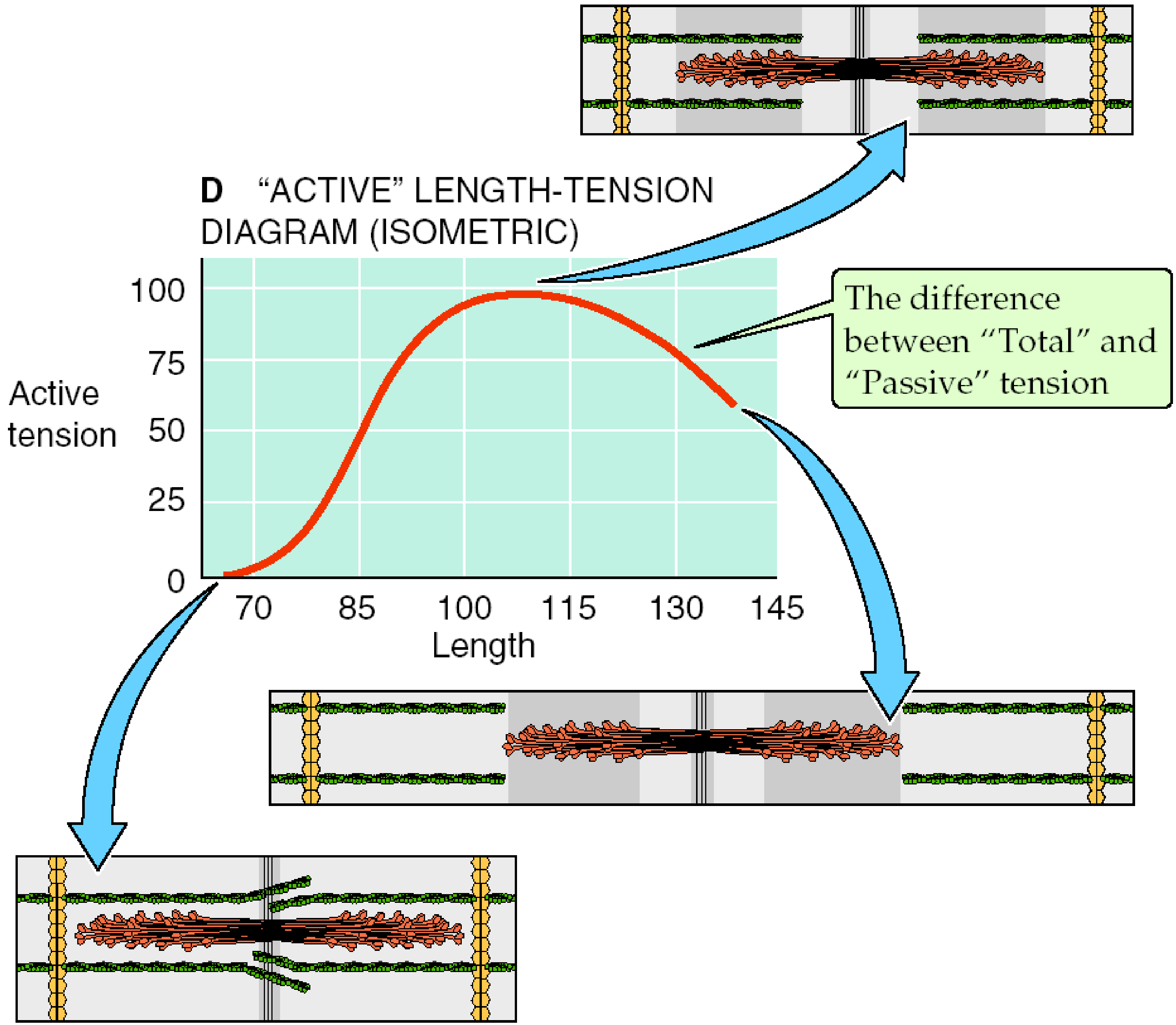


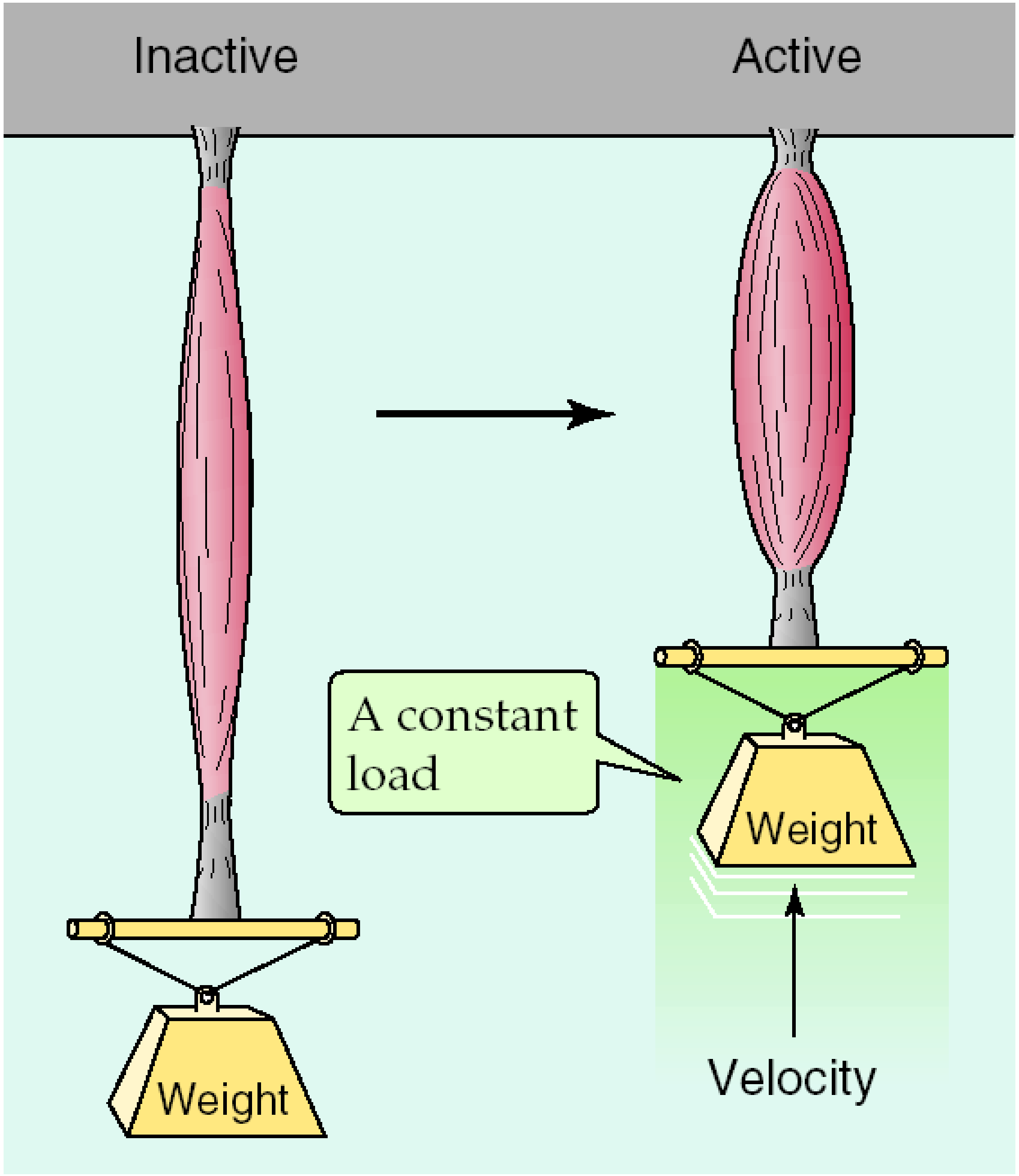
A ISOMETRIC


Inactive
Active
Muscle fiber
A constant length
Tension low
Tension constant high

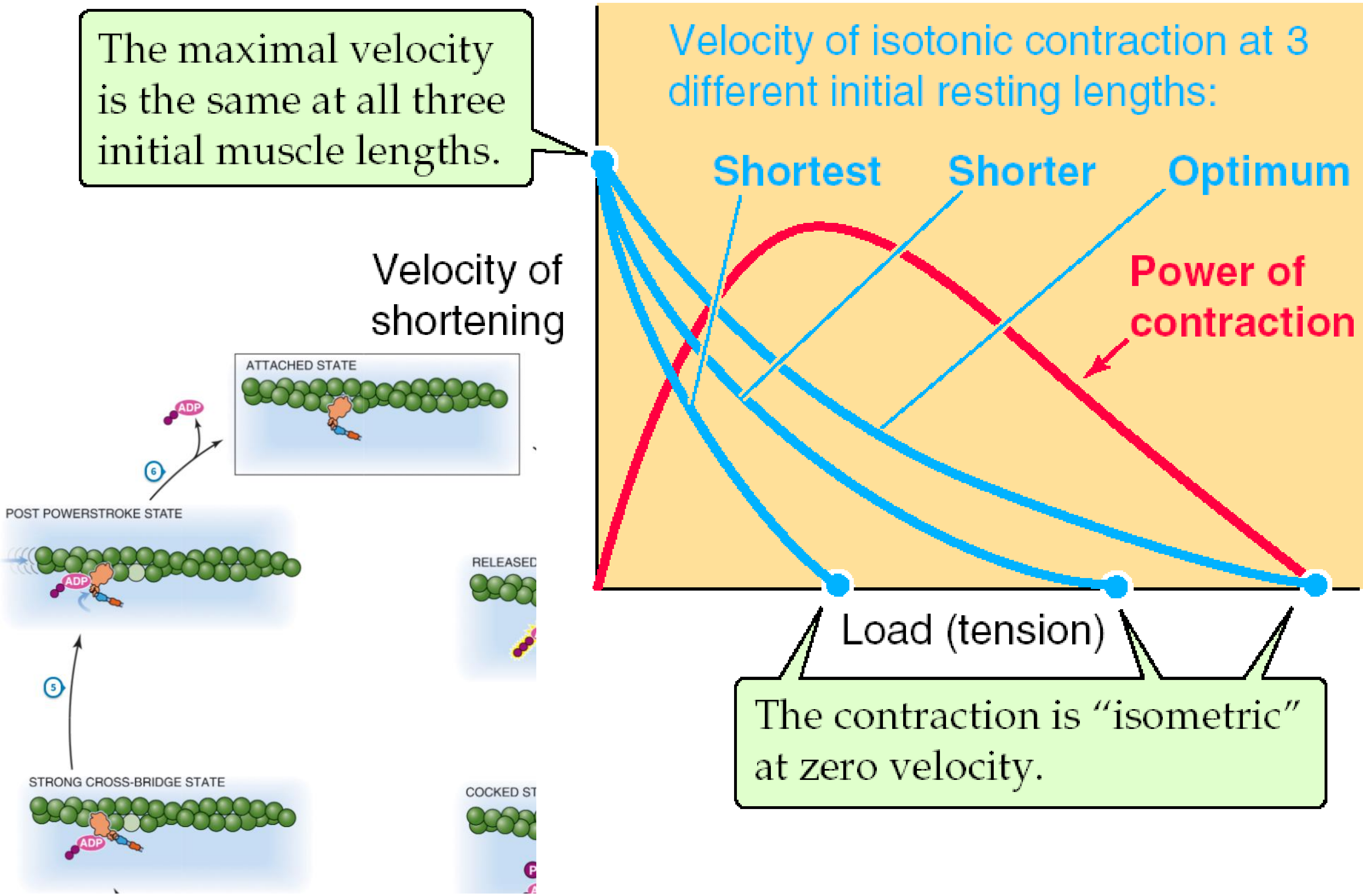


Tension
100
75
50
25
0
Total
Active
Passive
70
85
100
115
130
145
Length


D “ACTIVE” LENGTH-TENSION
DIAGRAM (ISOMETRIC)
Active
tension
100
75
50
25
0
70
85
100
115
130
145
Length
The difference between “Total” and “Passive” tension

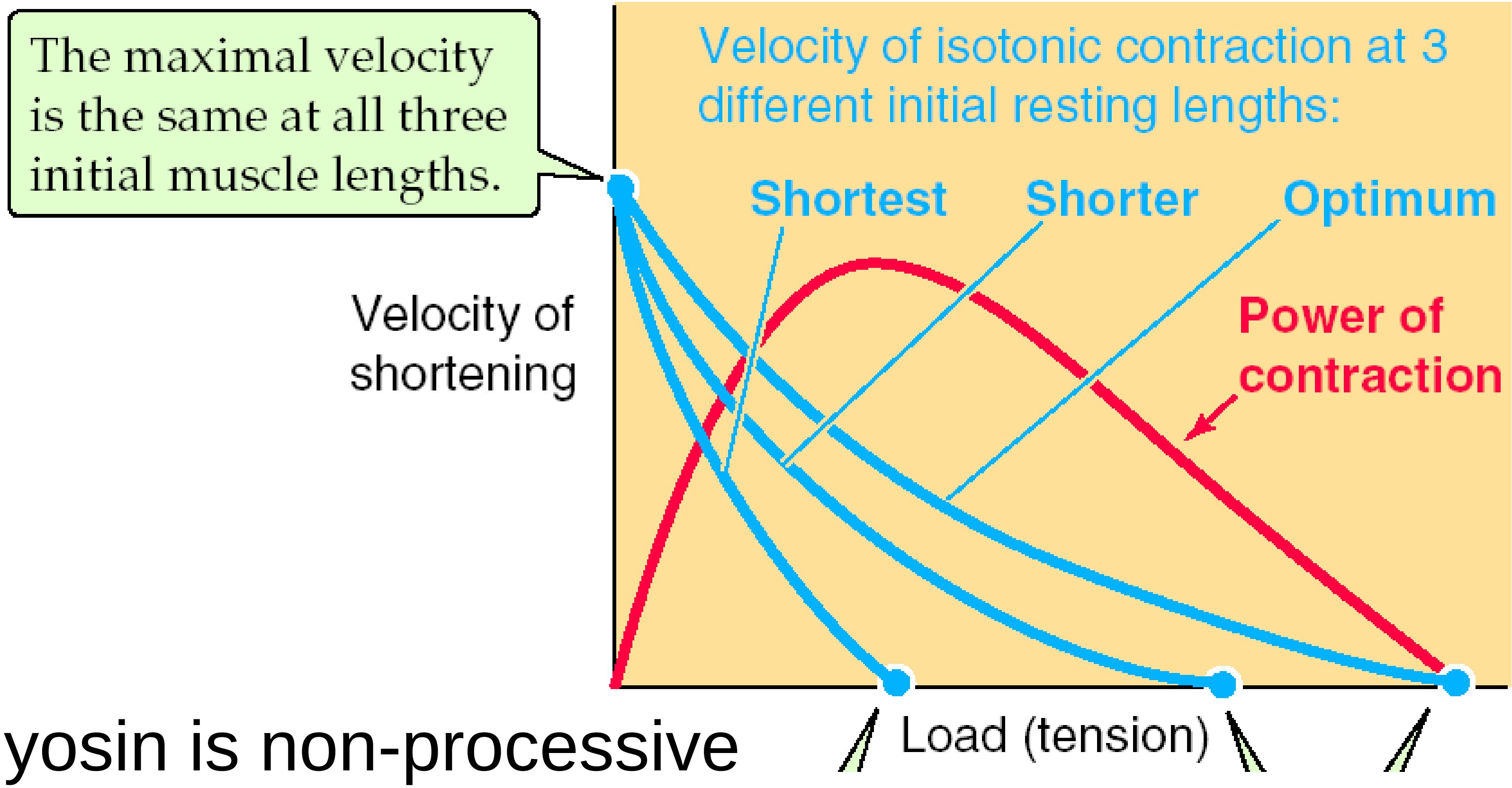


isotonic contraction



Huxley (1957) applied crossbridge theory to explain the force-velocity curve

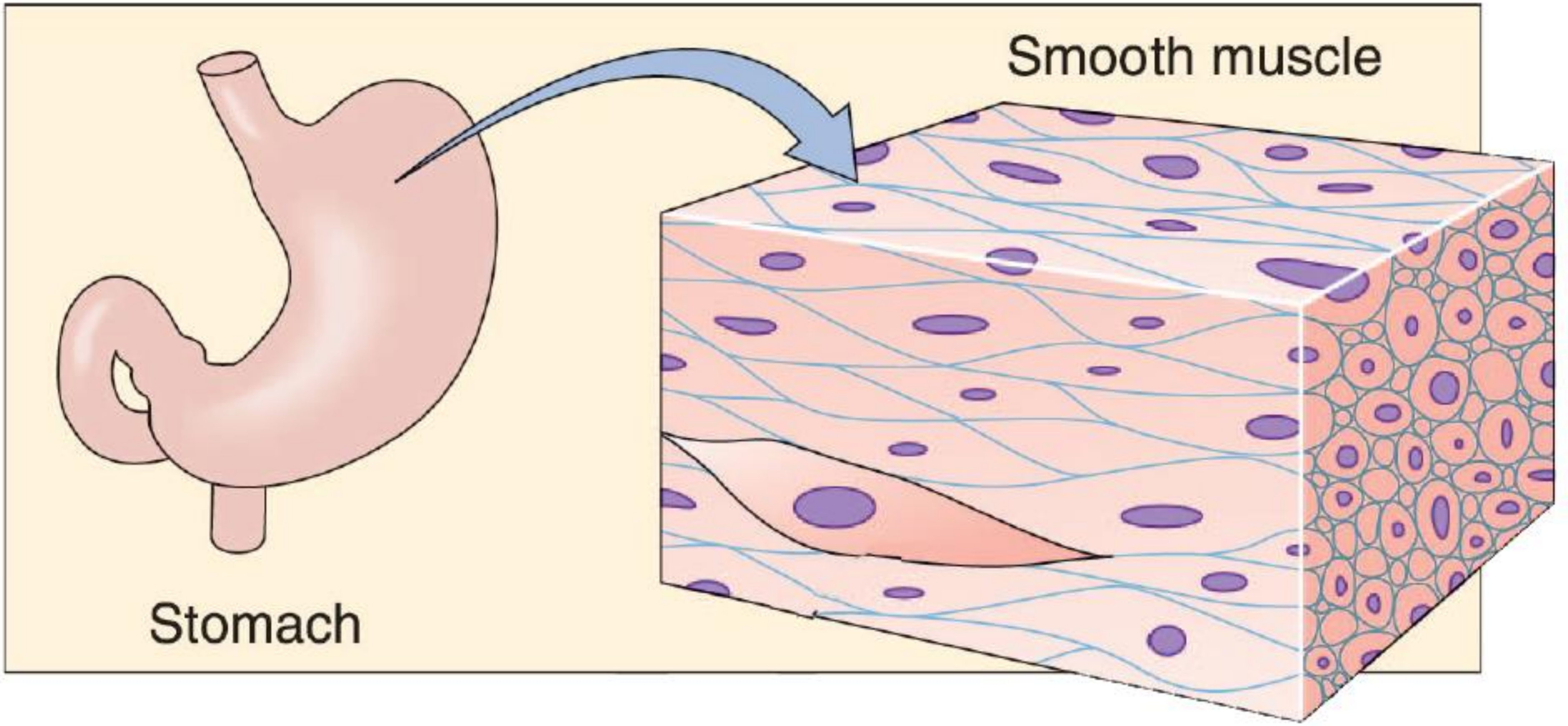
- high load, more interactions involved in maintaining length of muscle
- low load, all actin-myosin interactions lead to motion, max velocity
- lots of curve fitting, assumptions on quantitative relations.

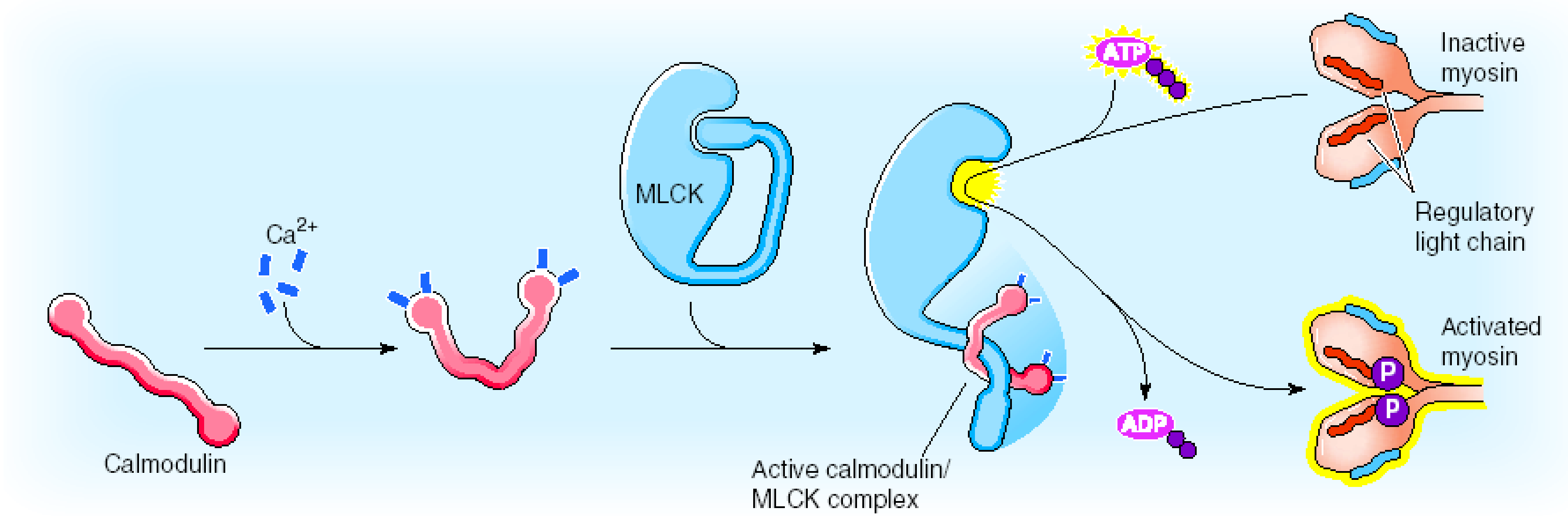


- Myosin is non-processive
  - muscle can contract quickly, 10 μm/sec unloaded
  - 10 nm per step * 20 ATP/sec = 200 nm/sec ???
  - 2 % duty cycle, so could reach contractile speed

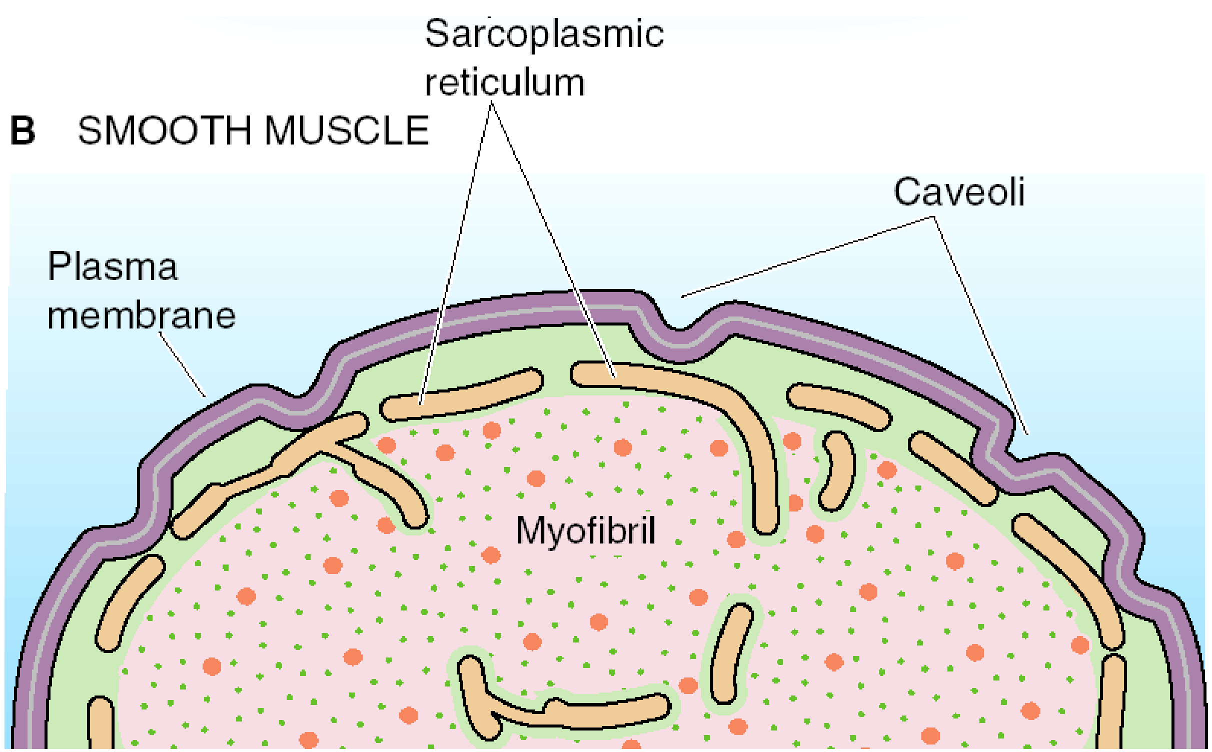
Smooth muscle

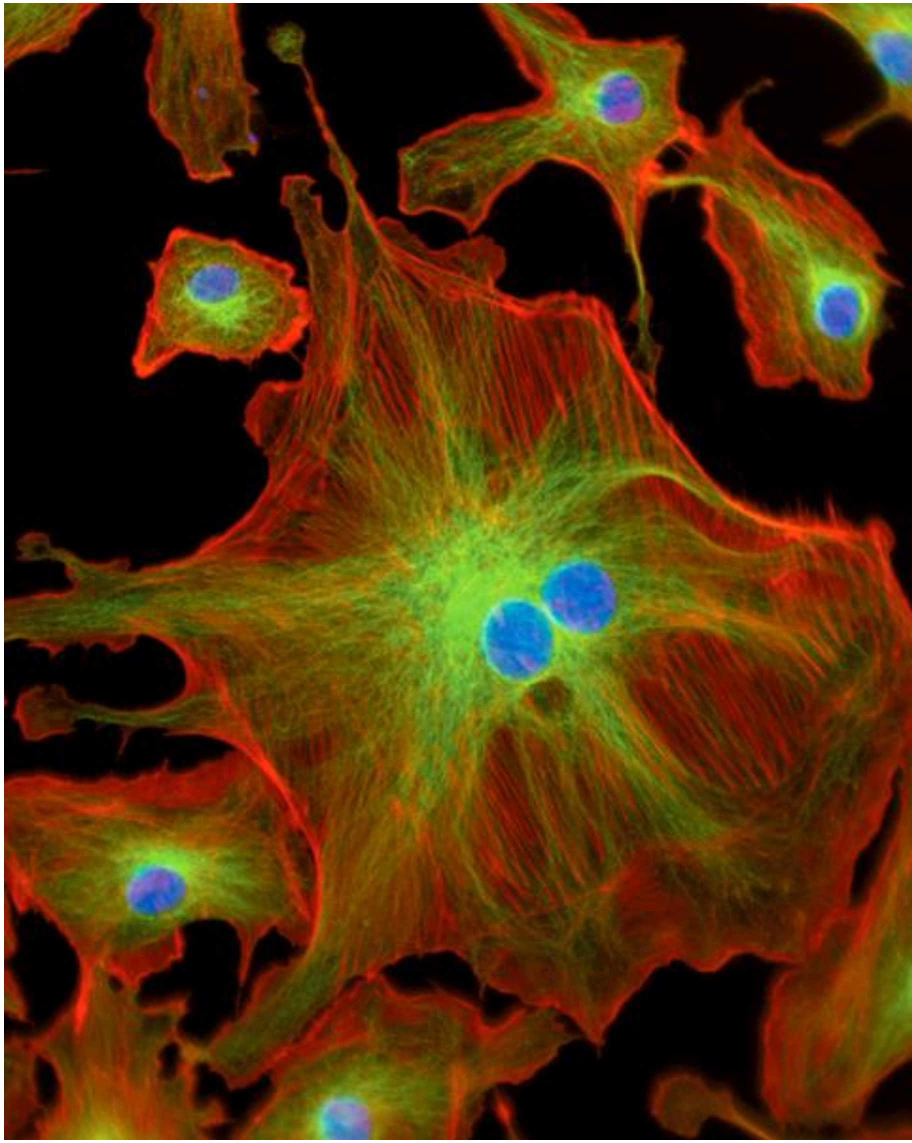
- less organized actin/myosin structure than striated muscle
- slower response

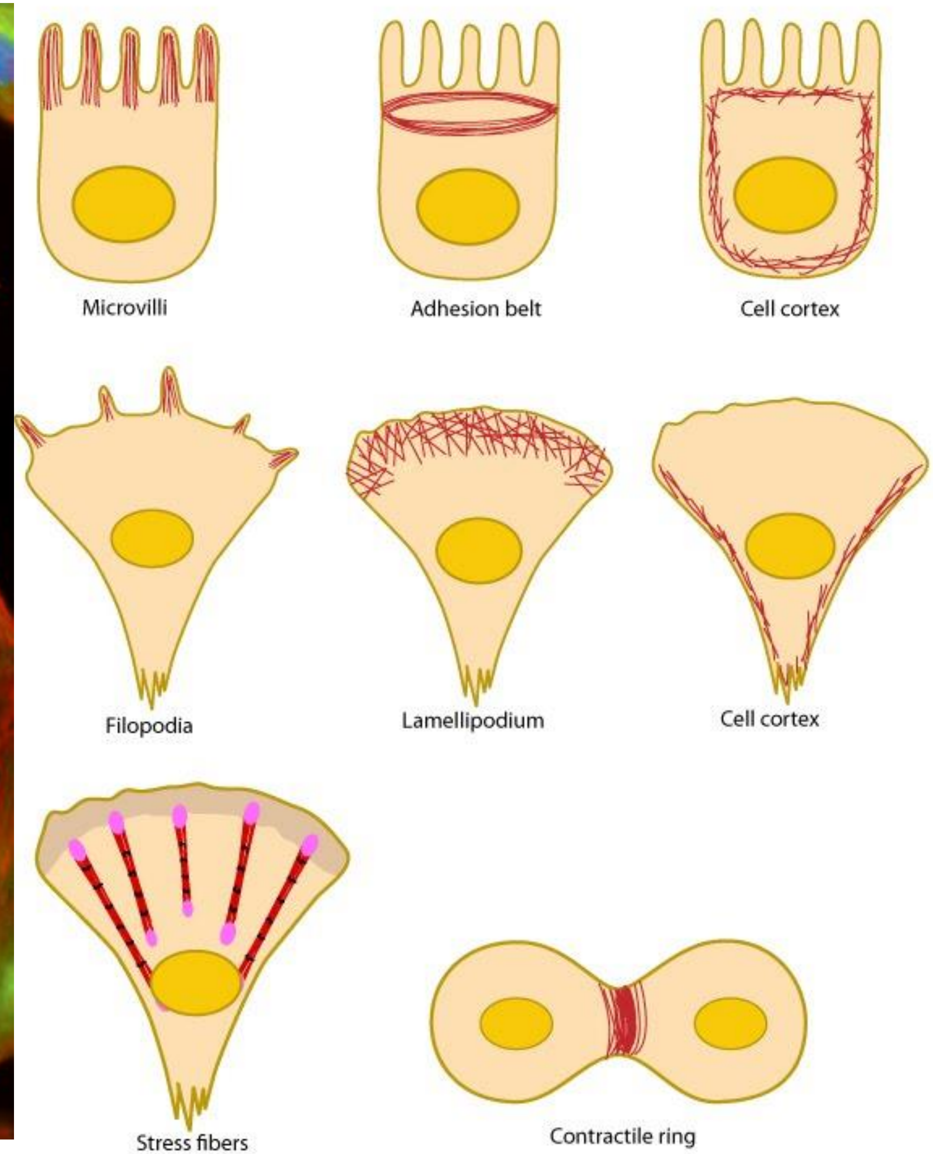


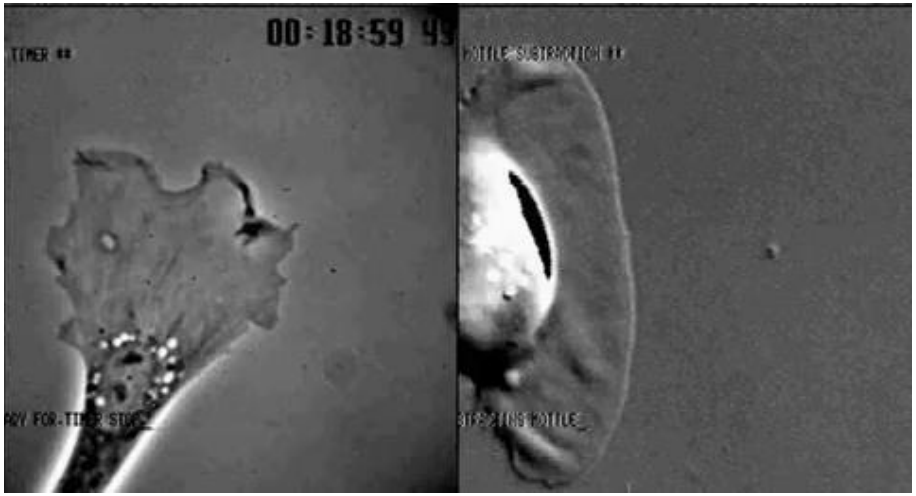


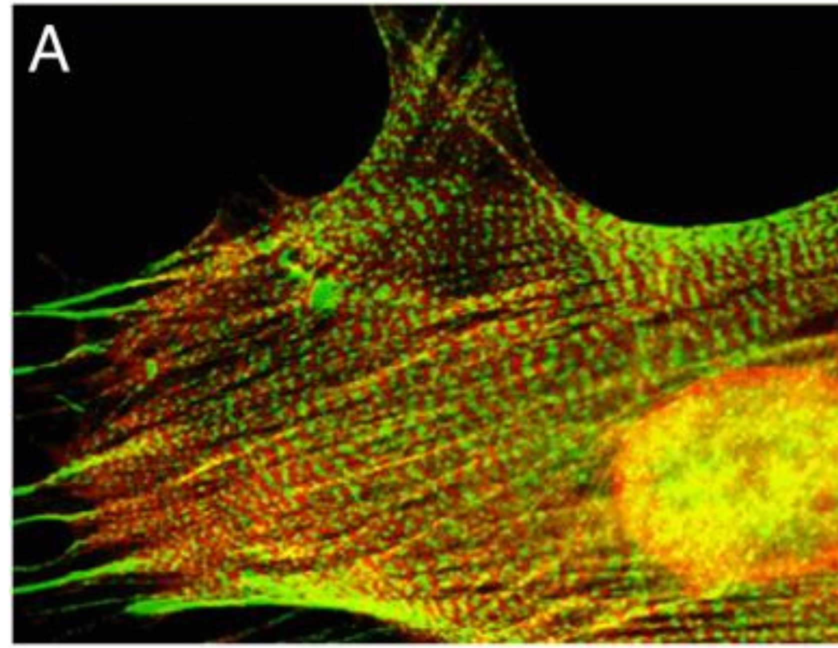
B SMOOTH MUSCLE

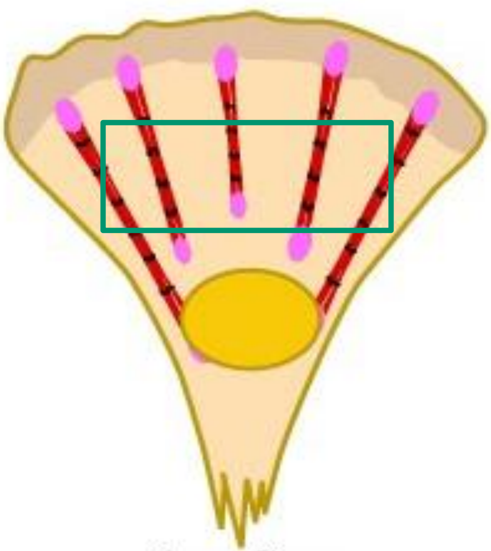




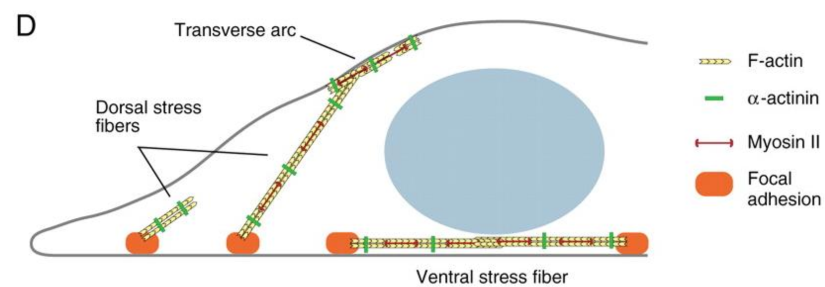

Microvilli
Adhesion belt
Cell cortex
Filopodia
Lamellipodium
Cell cortex
Stress fibers
Contractile ring



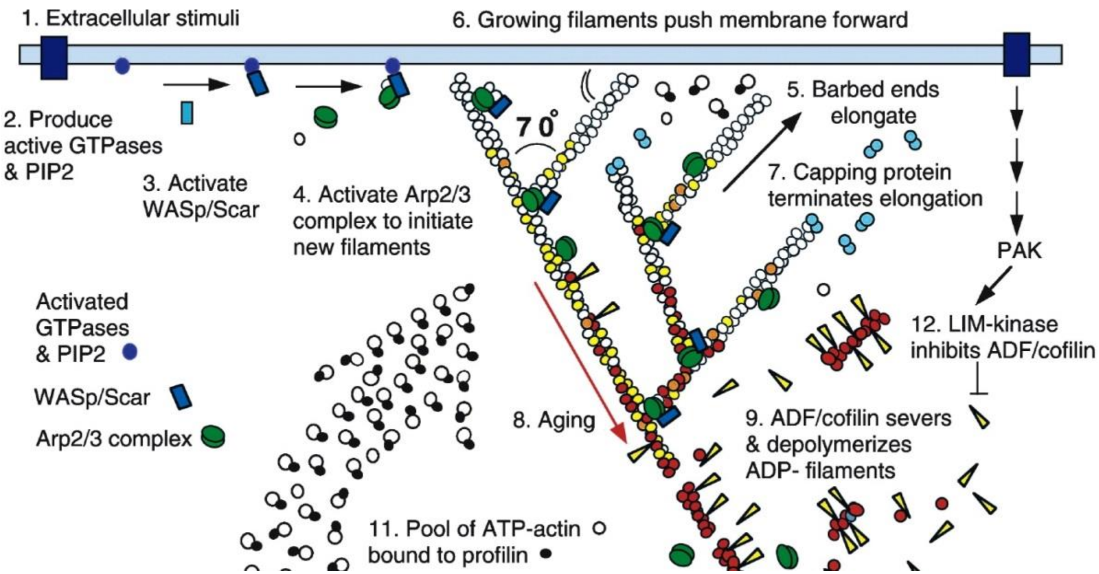


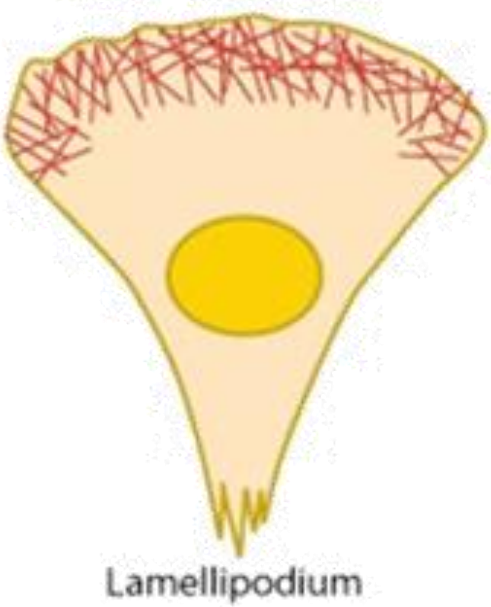


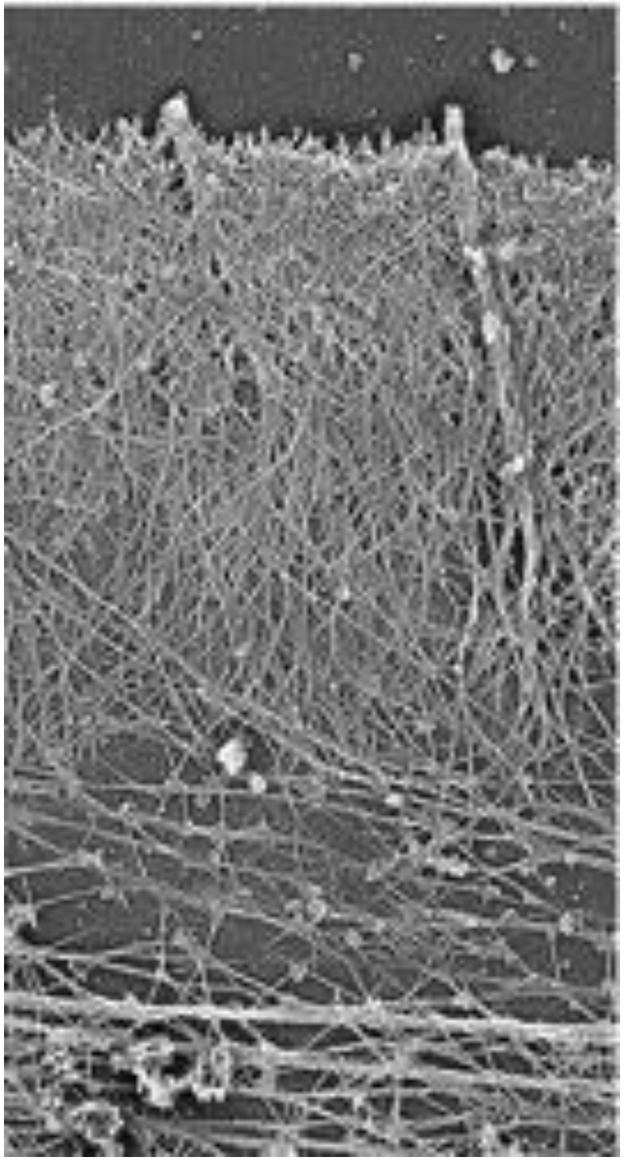
Stress fibers

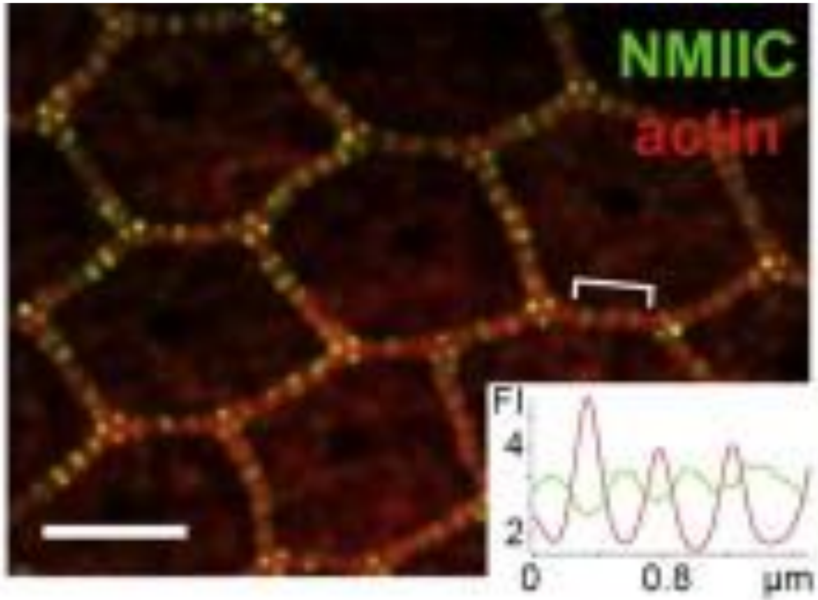


Pellegrin & Mellor, JCS, 120:3491 (2007)

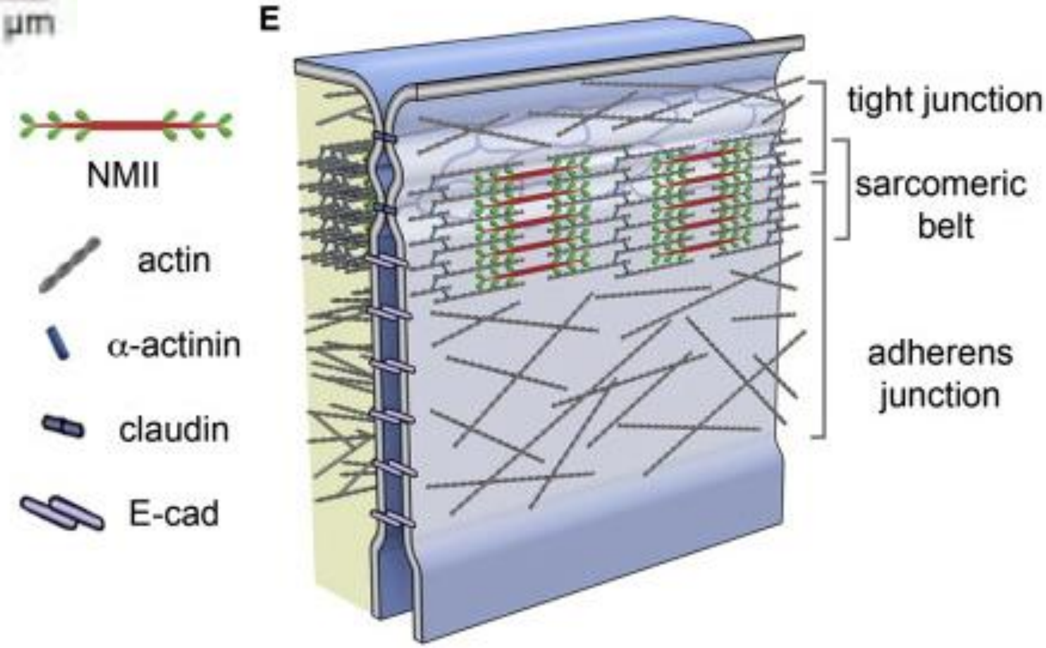

1. Extracellular stimuli
6. Growing filaments push membrane forward
2. Produce active GTPases & PIP2
3. Activate WASp/Scar
4. Activate Arp2/3 complex to initiate new filaments
70°
5. Barbed ends elongate
7. Capping protein terminates elongation
PAK
12. LIM-kinase inhibits ADF/cofilin
Activated GTPases & PIP2
WASp/Scar
Arp2/3 complex
8. Aging
9. ADF/cofilin severs & depolymerizes ADP- filaments
11. Pool of ATP-actin bound to profilin


Lamellipodium





Adhesion belt



Ebrahim *et al*., *Current Biology*, 23:731 (2013)