Myosins: A Superfamily of Actin-Dependent Molecular Motors

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Molecular Motors

Myosins —interact with actin filaments

Kinesins —interact with microtubules
Dyneins —interact with microtubules

Bacterial flagellar motor —rotary motor imbeded in cell membrane

DNA/RNA polymerases —interact with nucleic acid polymers

Translational Elongation Factor G —the motor of the ribosomes—interacts with nucleic acid polymers

F_1-ATPase —proton motive rotary motor imbeded in mitochondia matrix

Traditionally, when you think of myosins, this is what you envision

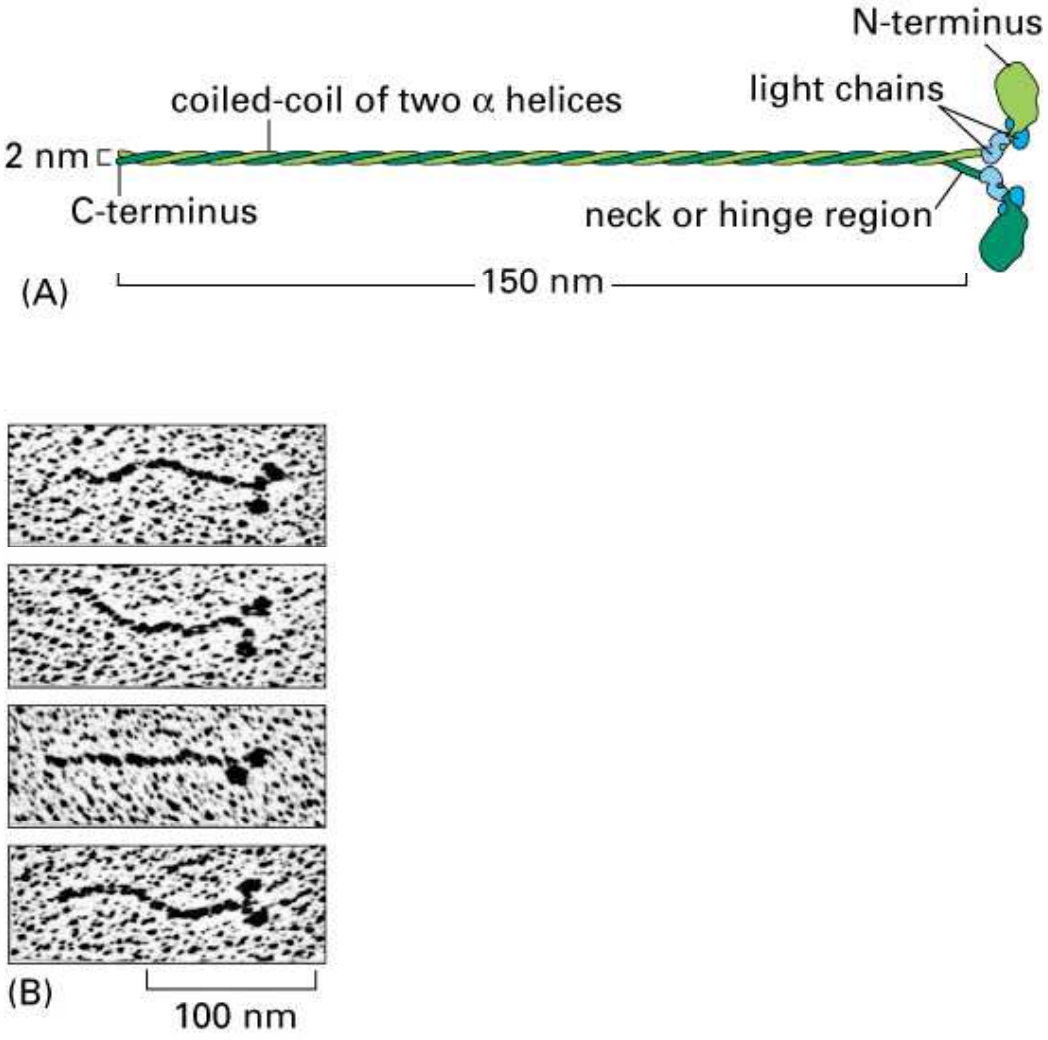


Figure 16–51. Molecular Biology of the Cell, 4th Edition.

These individual molecules polymerize to form a thick filament

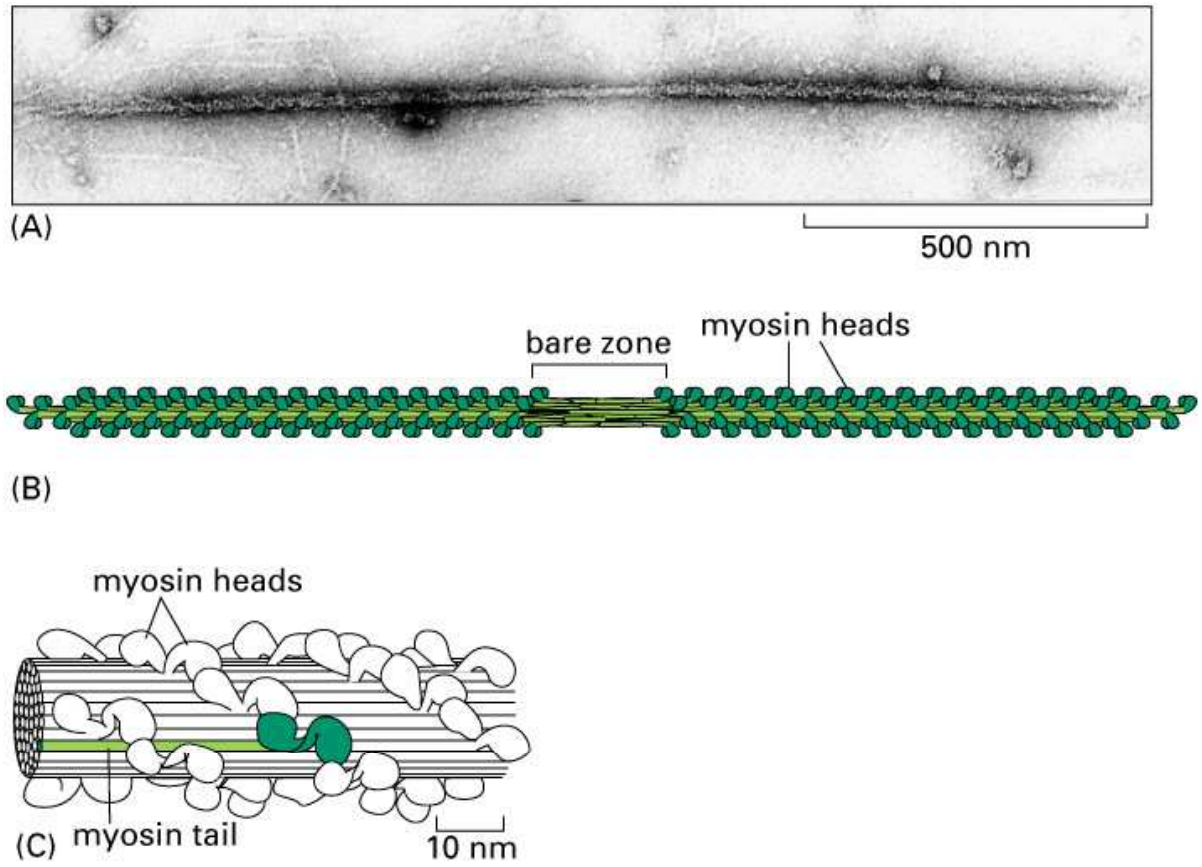
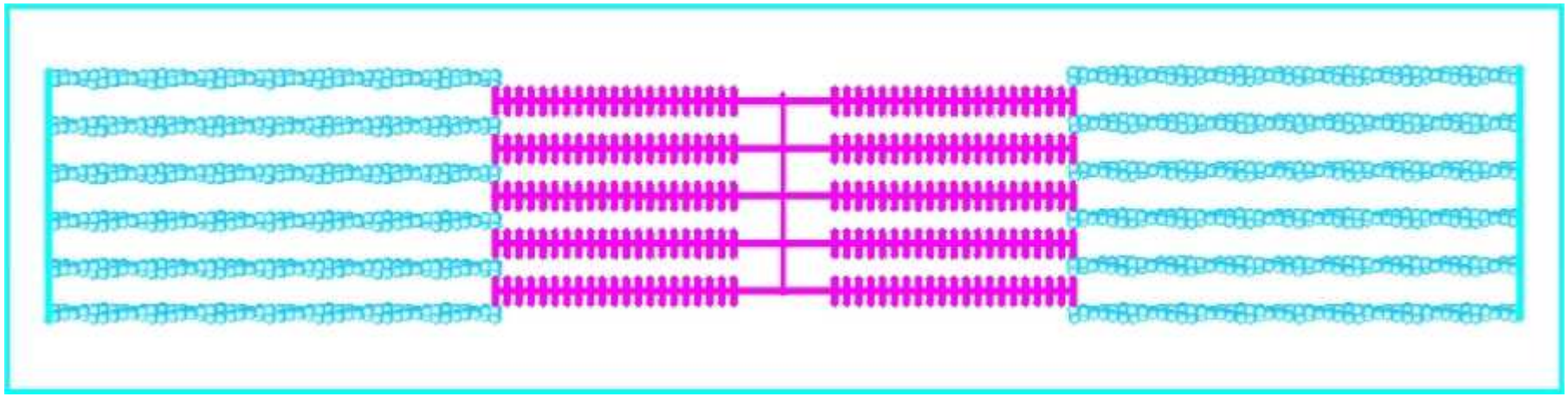


Figure 16–52. Molecular Biology of the Cell, 4th Edition.

Myosin II Powers the Muscle Contraction

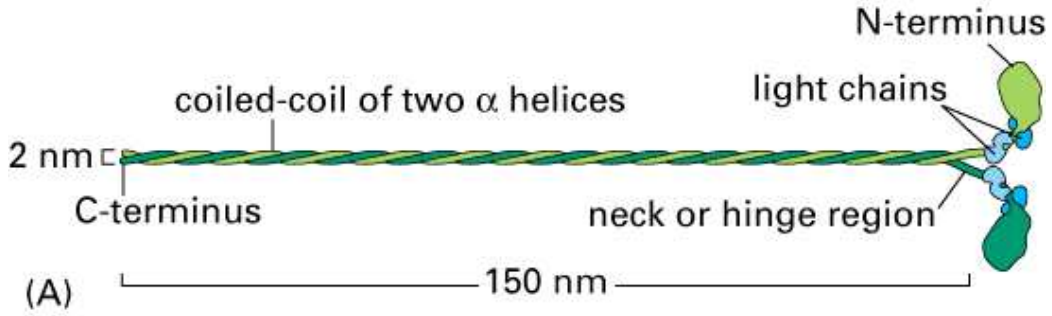
I-band
Actin filaments

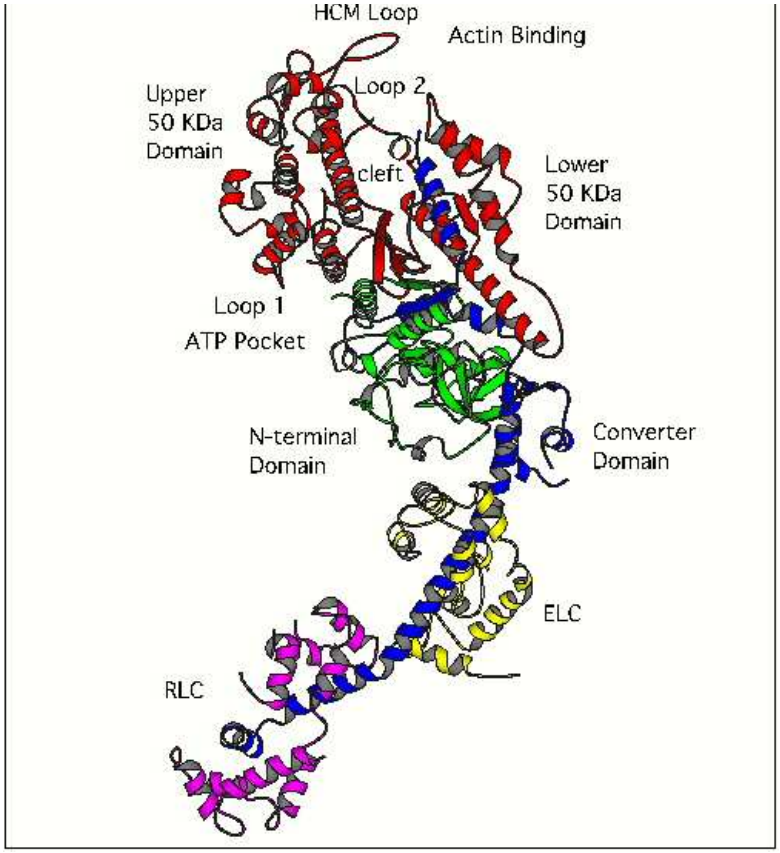
A-band
Myosin filaments



Z-line

Crystal Structure of the Head of Rabbit Skeletal Muscle Myosin



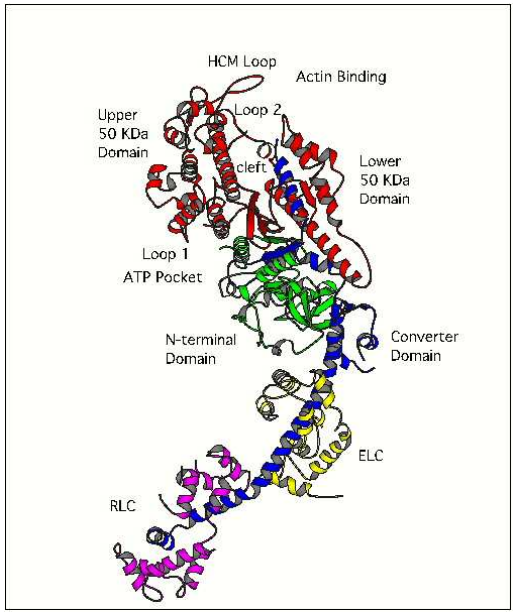


The motor domain of kinesin has structural homology to the core motor region of myosin. Both have homologies to that of the G-proteins. In other words, all three classes of proteins have a switch I, a switch II and a P-loop.

Light Chains Bind to IQ Motifs in the Neck Region of the Heavy Chain

IQXXXRGXXXR

I	Isoleucine
Q	Glutamine
R	Arginine
G	Glycine
X	Others

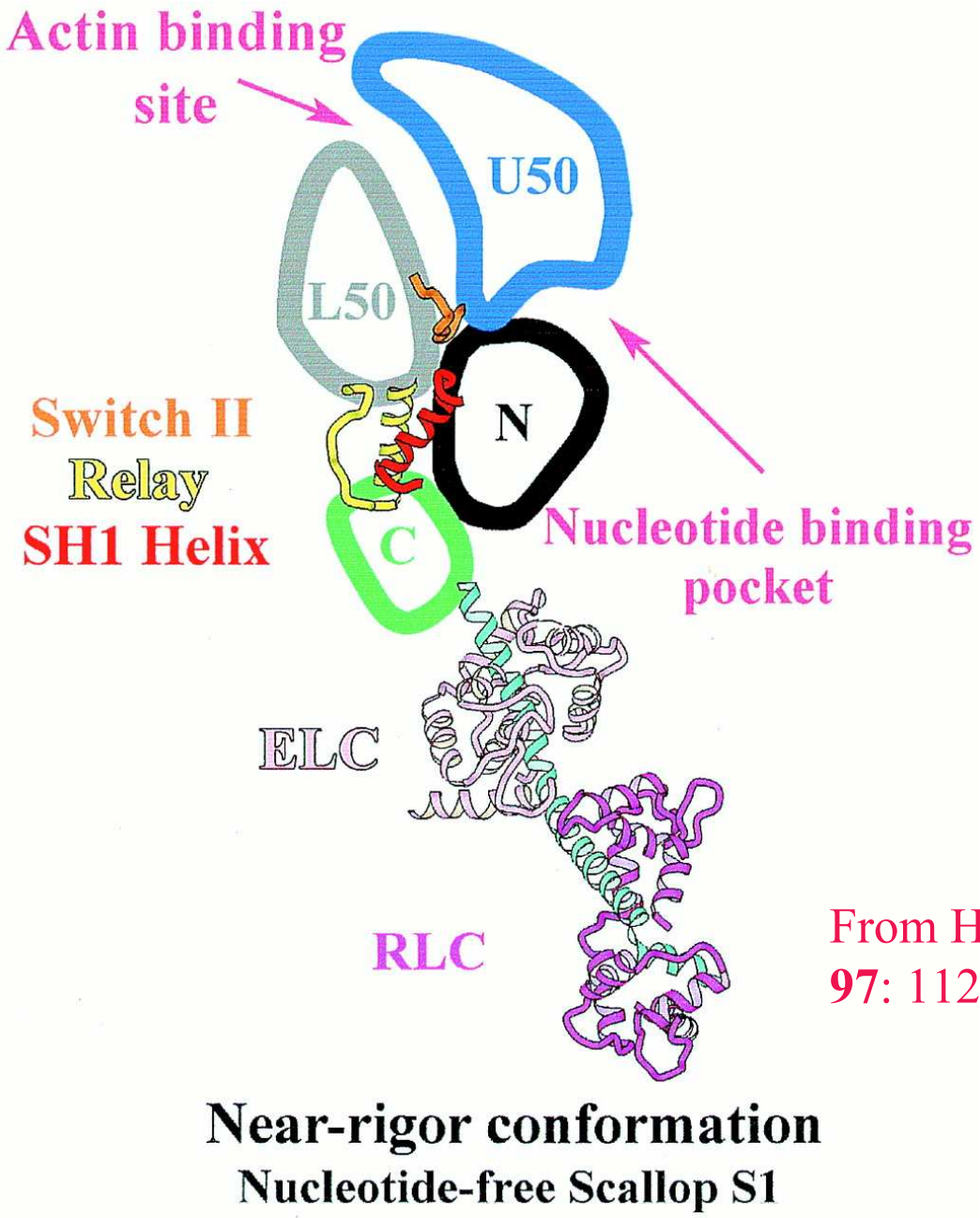


Light chains may be identical to those found in myosin II, calmodulin, or low molecular weight calmodulin-like proteins

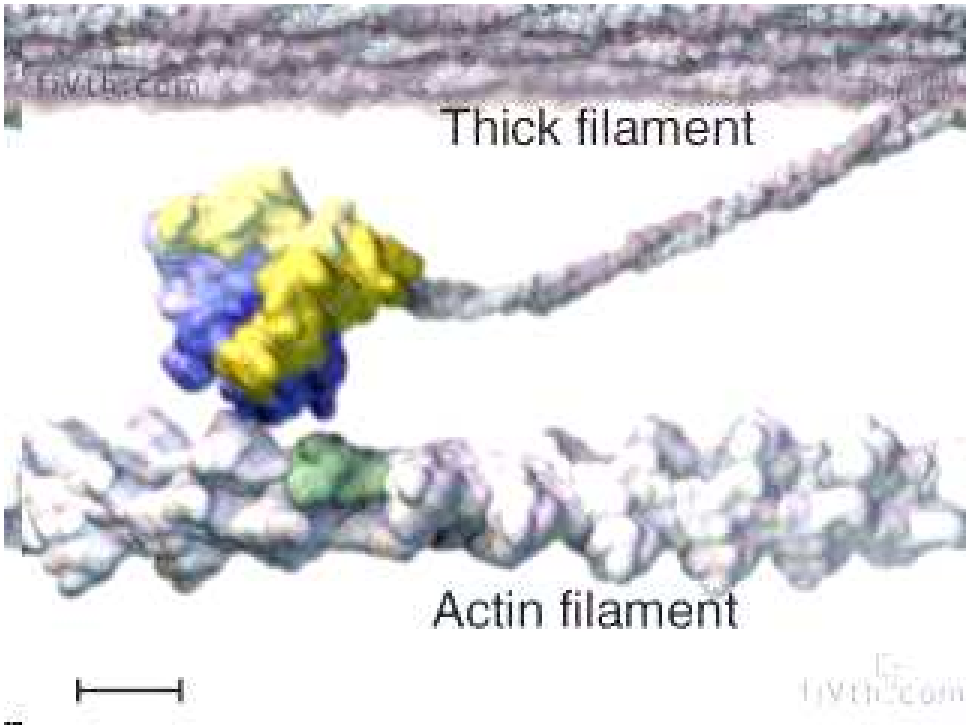
In myosin II there are 23 amino acids separating the two IQ motifs

In unconventional myosin the separation varies between 22 and 26 amino acids

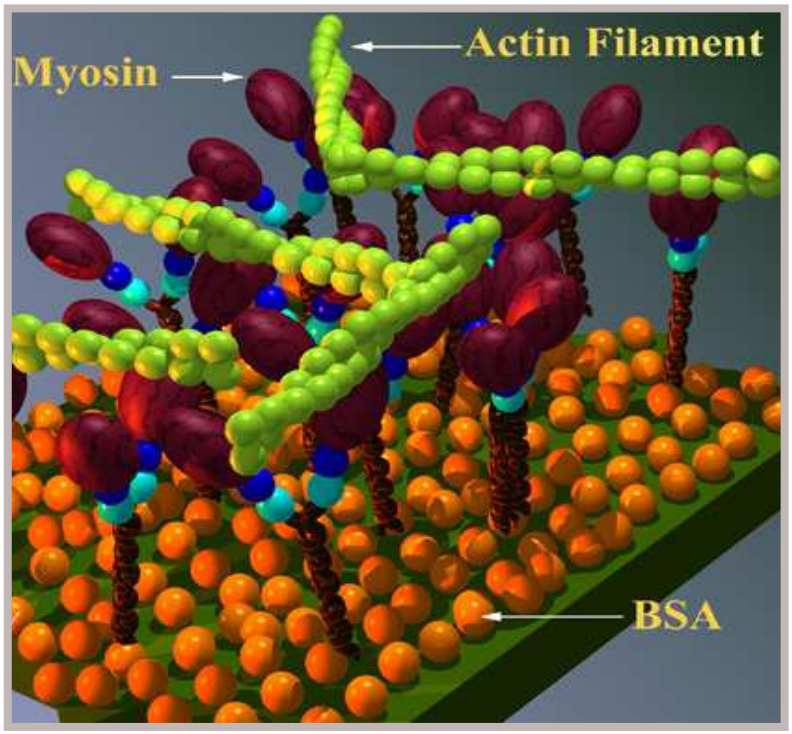
Light chains may be involved in phosphorylation-dependent regulation in some myosin II molecules and in calcium-dependent regulation in other myosins



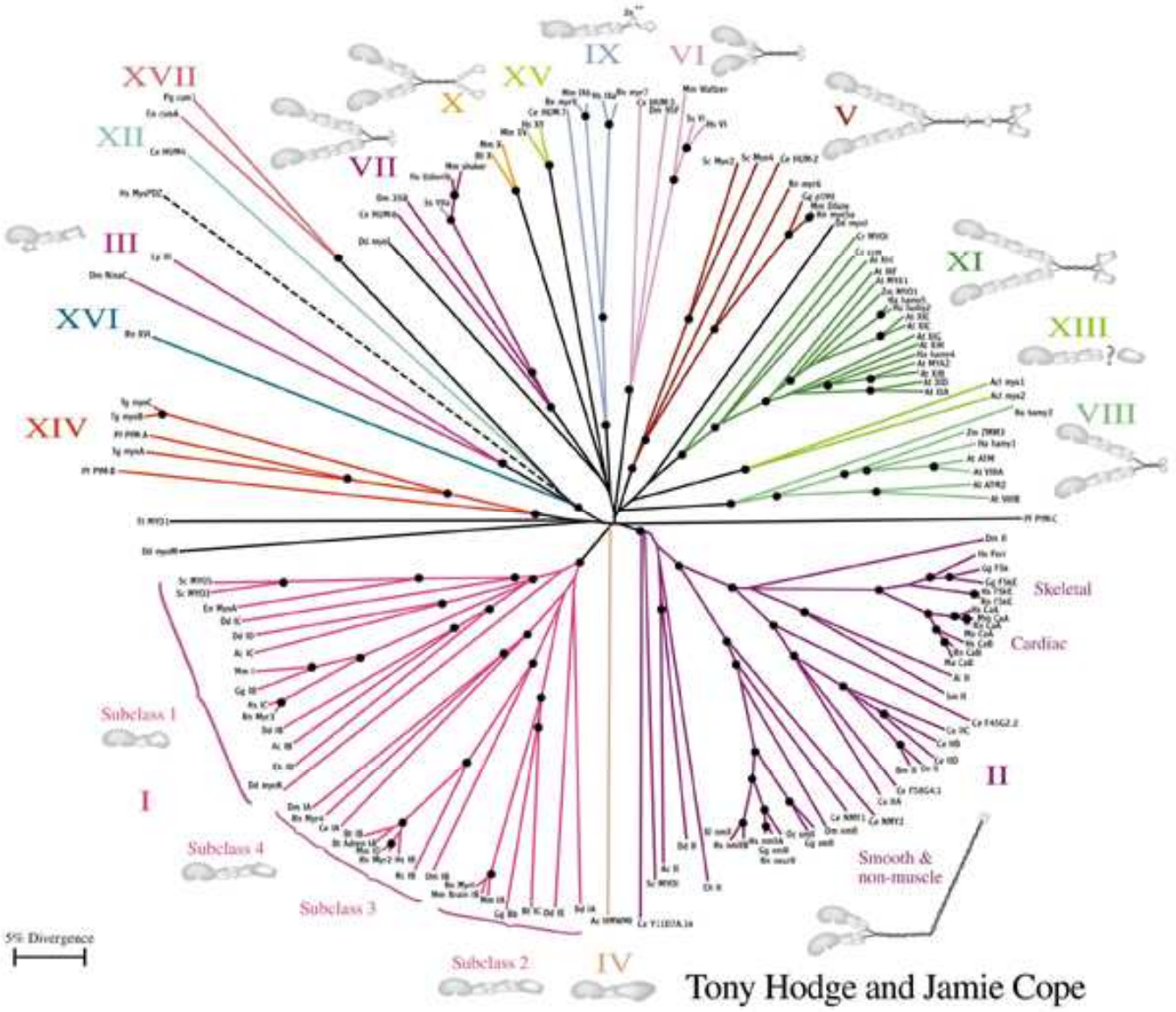
From Houdusse et al. *PNAS* **97**: 11238, 2000

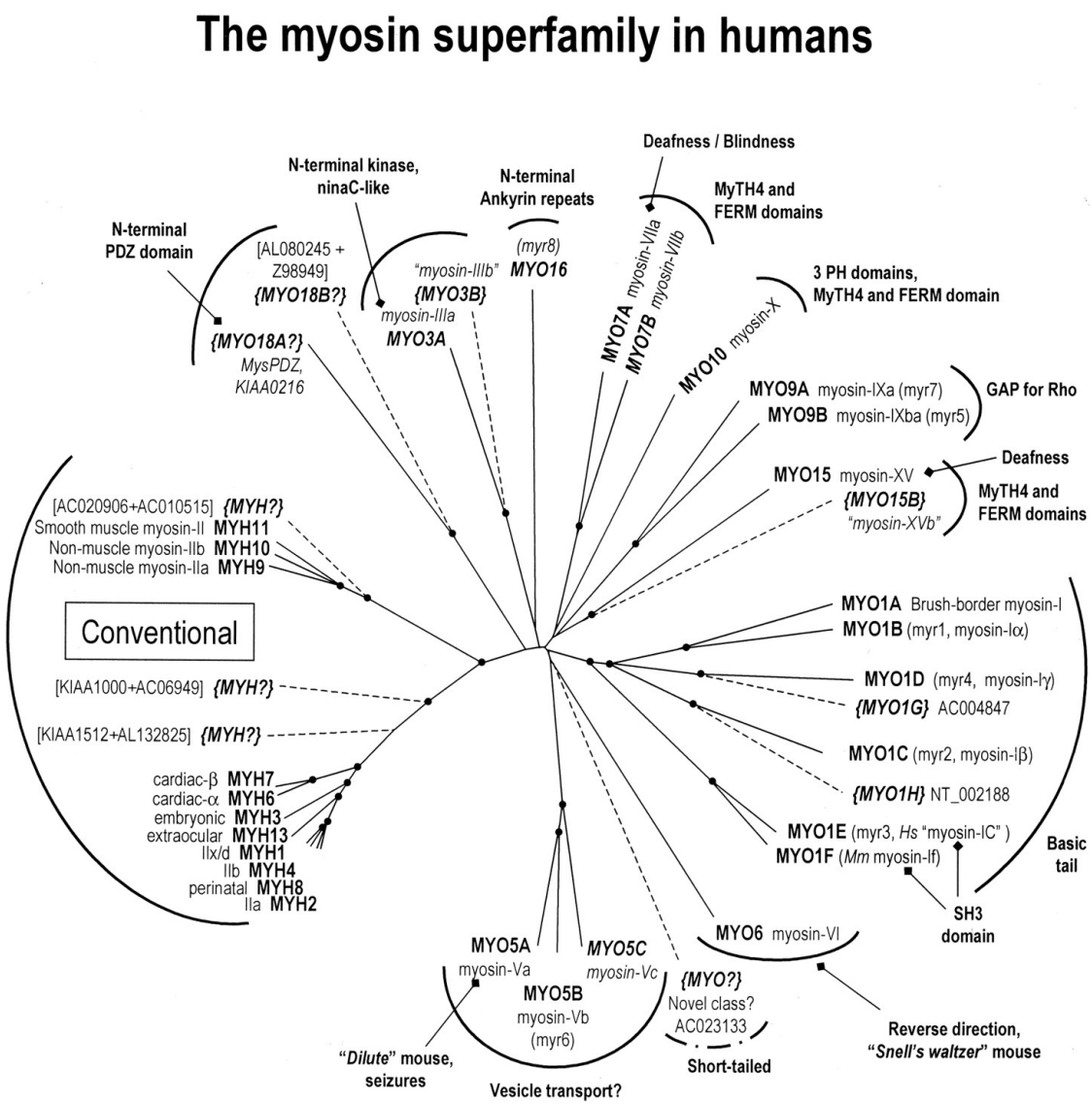

Thick filament
Actin filament

In Vitro Motility Assay with Rabbit Skeletal Muscle Actin



Diversity of the Myosin Motor Family





Yeast have only 5 myosins:
2 Myosin I genes
1 Myosin II gene
2 Myosin V genes

Humans have

39 myosins

From 12 classes

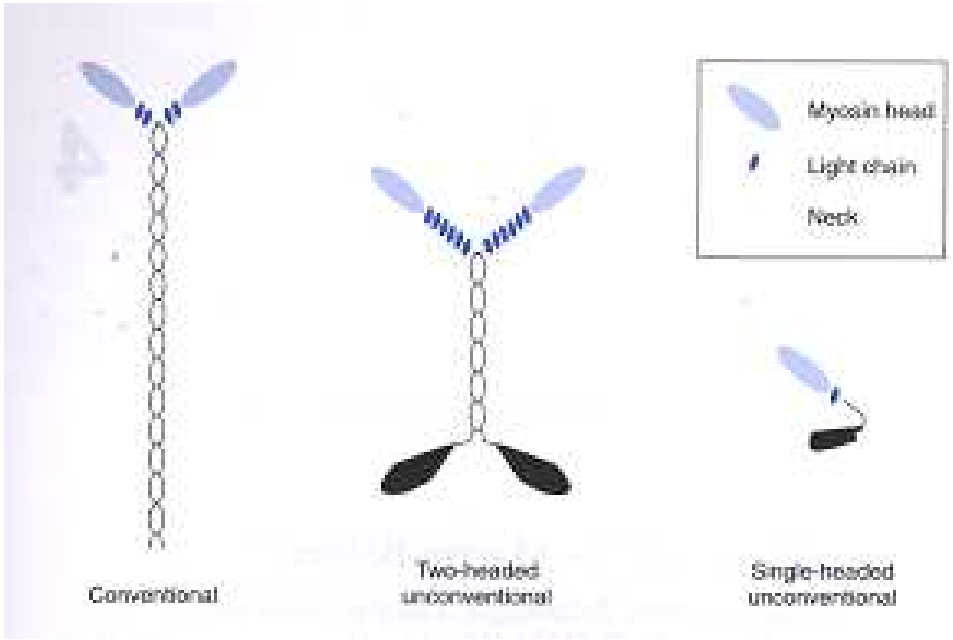
From Berg et al. *Mol. Biol. Cell* **12**: 780, 2001

Most Myosins Have a Head, a Neck and a Tail

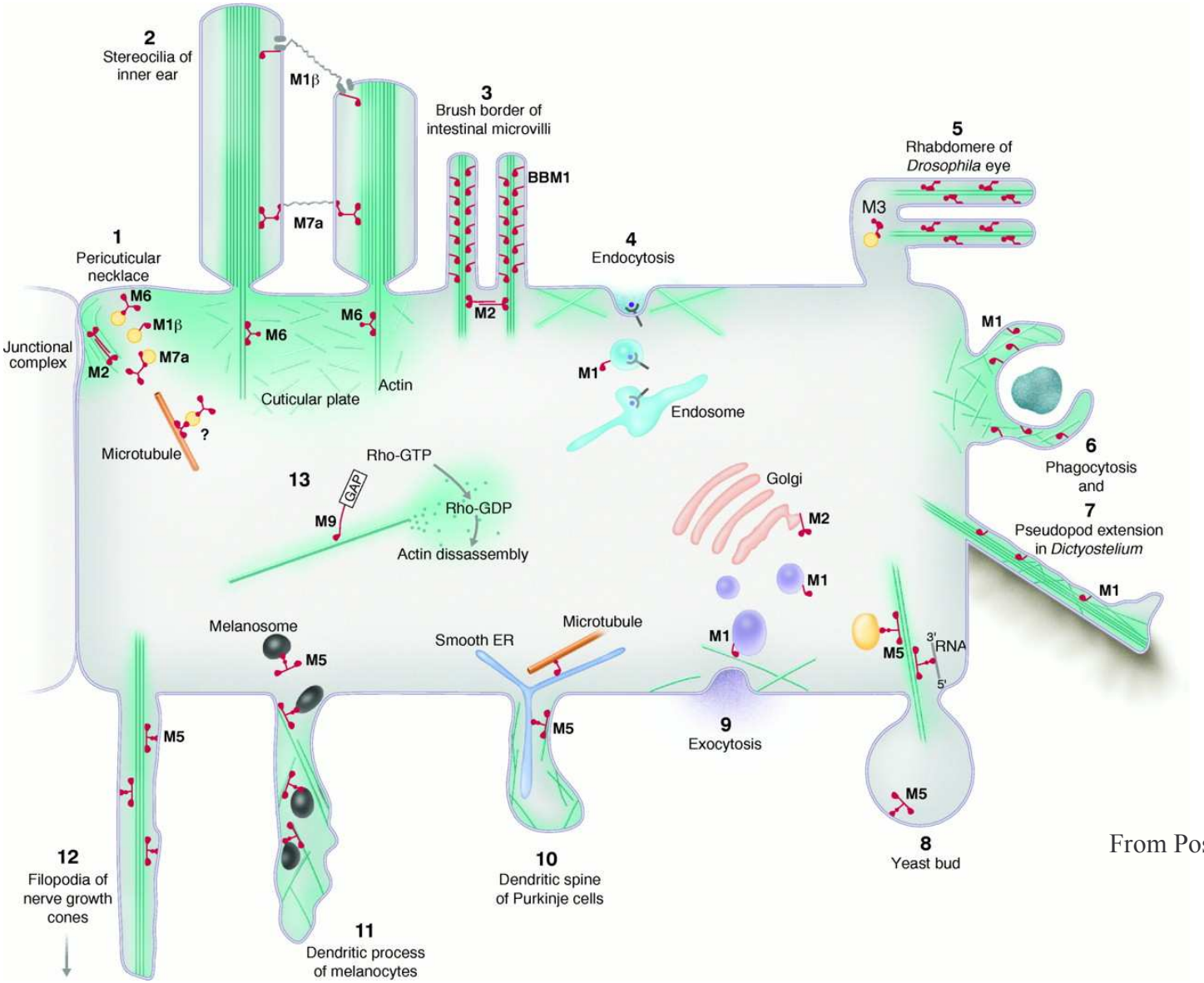
Head, conserved motor domain

Neck, light chain binding domain

Tail, may contain functional domains or phosphorylation sites. Responsible for targeting or anchoring the molecule within the cell



Possible Myosin Functions in the Cell



From Post et al.

Myosins clearly perform a diverse set of task within cells.

Some of these functions are common to most cells such as cell division, maintenance of cortical tension, adhesion, Golgi transport.

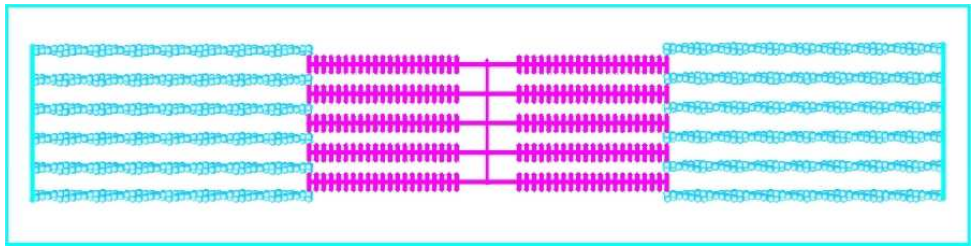
Other functions may occur only in specialized cells such as maintenance of the structural integrity of stereocelia in the inner ear or in retinal cells

Myosin XIV may be highly specialized to be involved in the infectious pathway of parasitic cells such as *Plasmodium falciparum.* It is only found in a few such organisms.

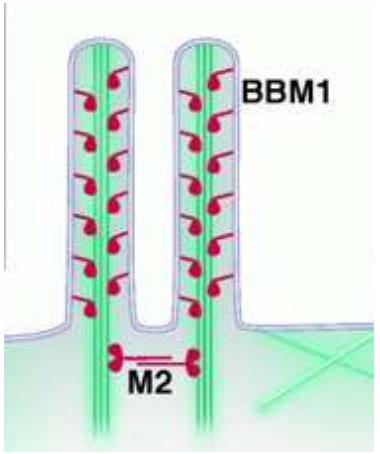
To accomplish this diversity, myosins have undergone evolution of both their molecular structure (primarily the neck and tail) as well as their kinetic pathways to give rise to molecules capable of carrying out many different functions.

Three very different myosin functions

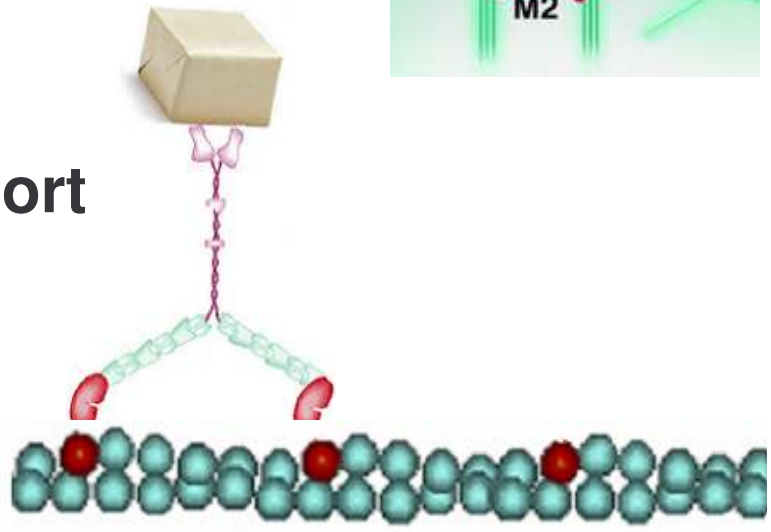
Myosin II in muscle contraction



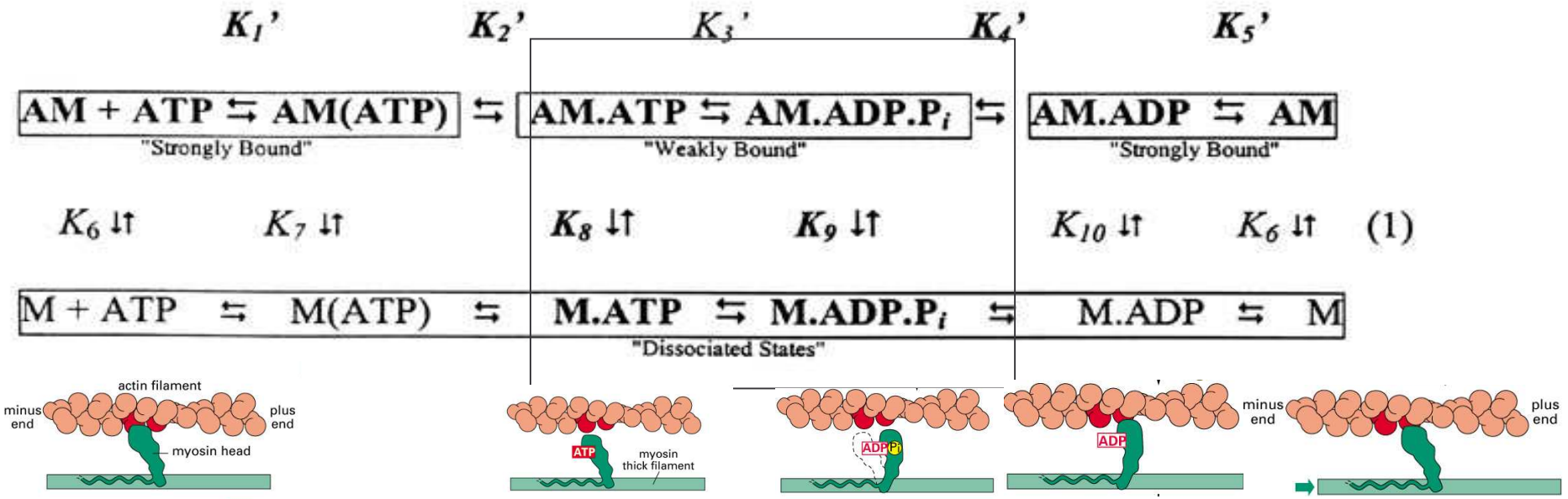
Myosin I in microvilli



Myosin V in cargo transport



Kinetic Evolution of Different Myosins Allow for Different Tasks

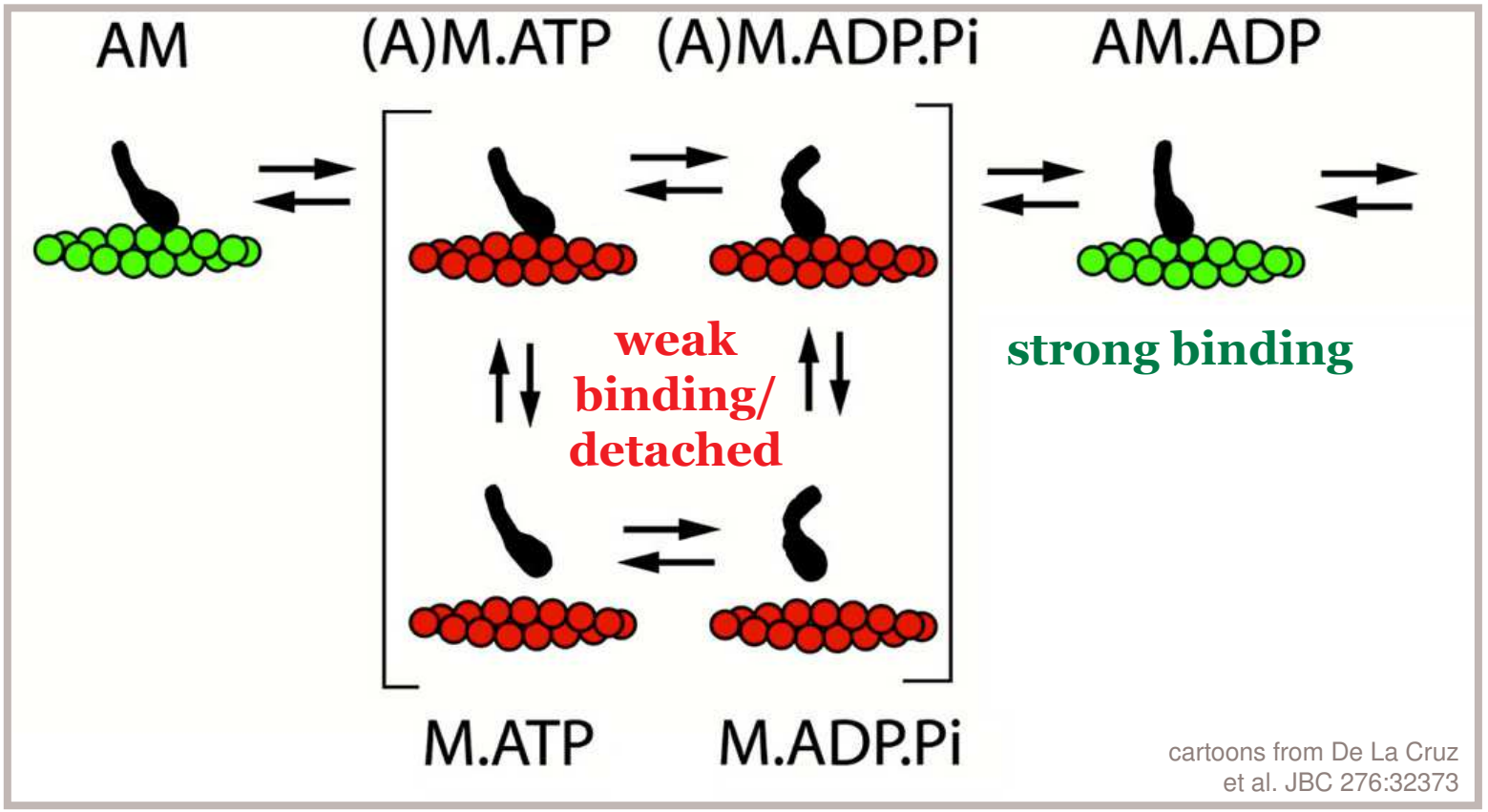


Rapid Equilibrium
Mostly Detached

Attached

Duty ratio is the percentage of time myosin spends strongly bound to actin

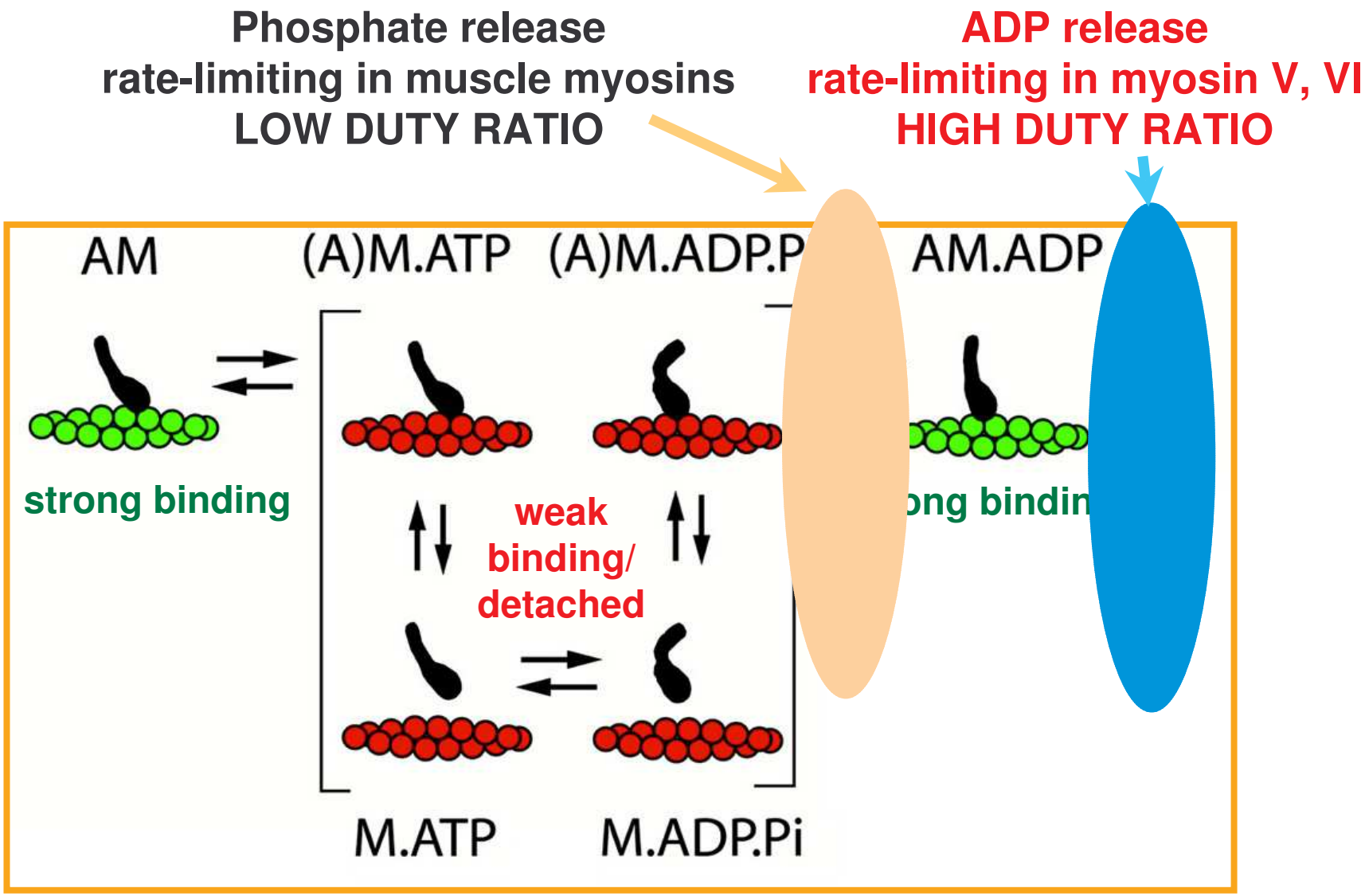
Actomyosin working cycle



Duty ratio: fraction of cycle time spent in strongly actin-bound states

Processivity: ability to take multiple steps on actin without detachment

Using this same basic kinetic cycle, nature has evolved myosins capable of carrying out a plethora of functions by varying the magnitude of certain key rate constants



Duty ratio: fraction of cycle time spent in strongly actin-bound states

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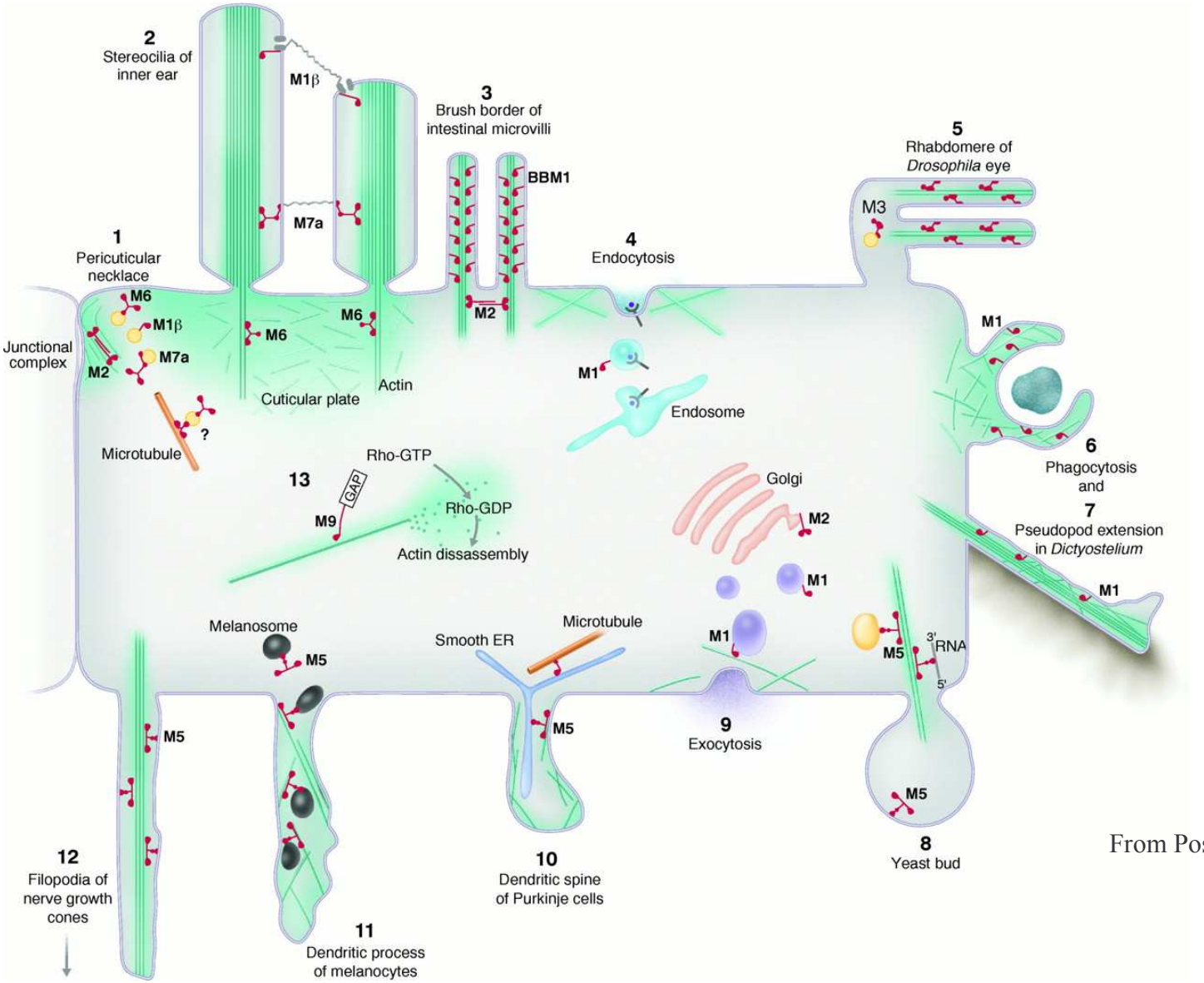
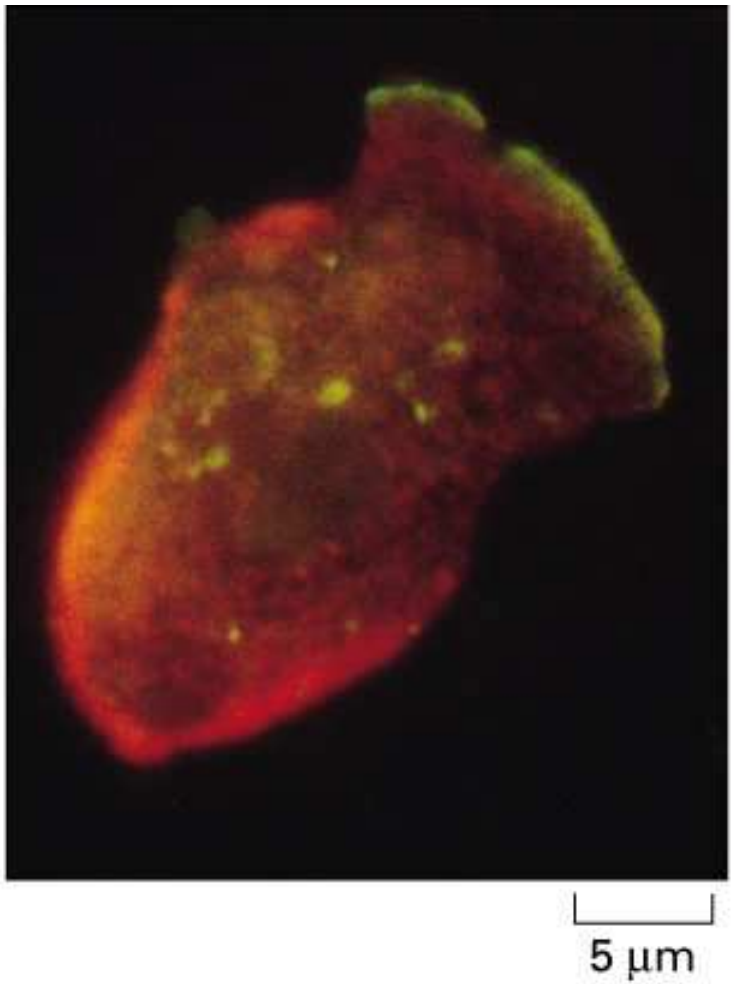
What determines localization in cells



From Post et al.

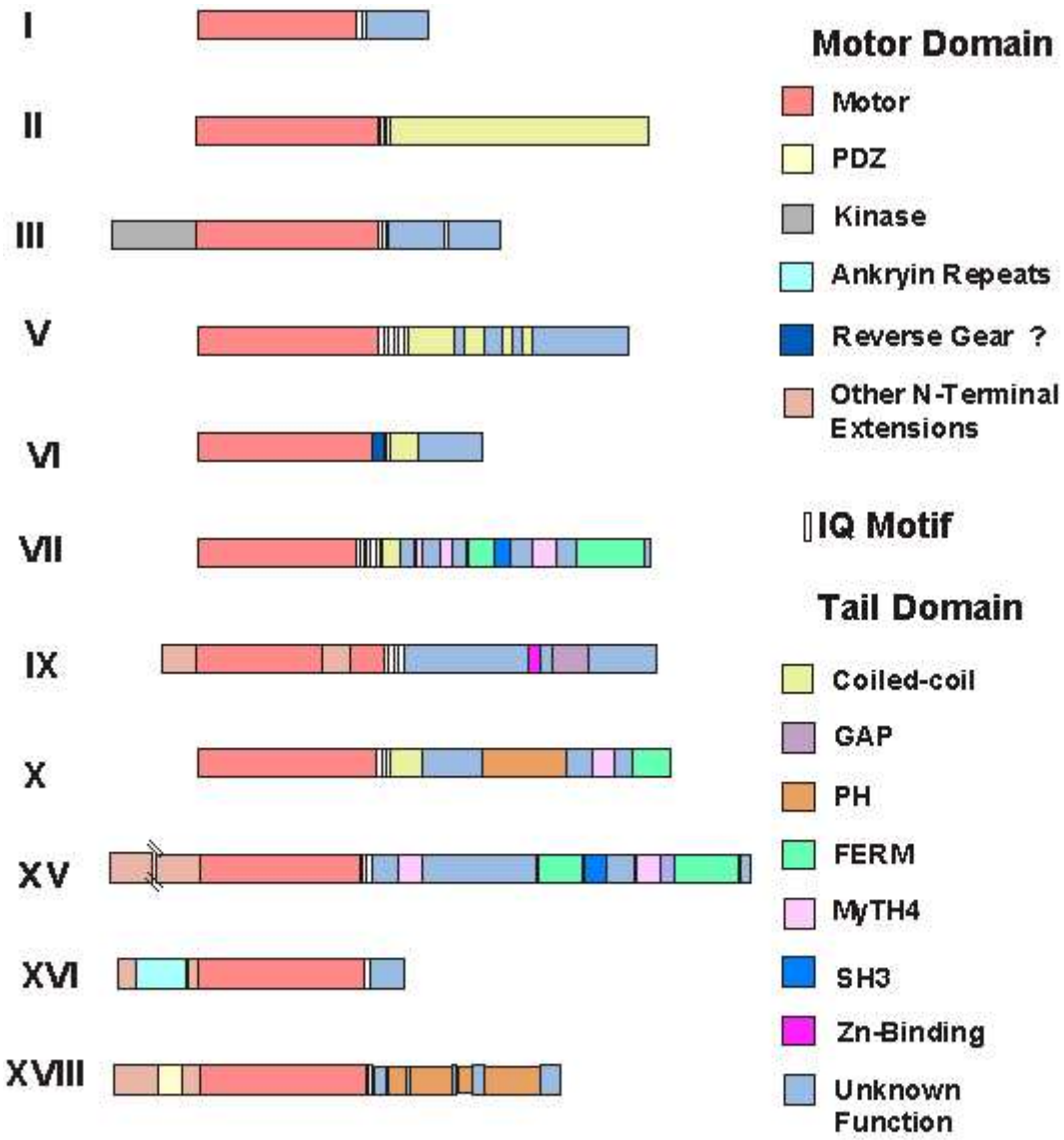
Migrating *Dictyostelium discoideum* cell



Myosin I

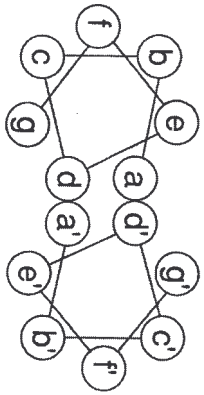
Myosin II

Figure 16–93. Molecular Biology of the Cell, 4th Edition.


I
II
III
V
VI
VII
IX
X
XV
XVI
XVIII
Motor Domain
Motor
PDZ
Kinase
Ankryin Repeats
Reverse Gear ?
Other N-Terminal Extensions
IQ Motif
Tail Domain
Coiled-coil
GAP
PH
FERM
MyTH4
SH3
Zn-Binding
Unknown Function
200 amino acids

Coiled-coil Forming Sequences Are a Means to Dimerize Myosin Heavy Chains

Arrangement of
Amino
Acids in a Coiled-coil



Myosin II class molecules have the longest coiled-coil sequences.They self association via their tails to form filaments.

Other myosin classes appear to use the coiled-c to make dimers, but do not self associate to forr filaments. Often their coiled-coilRegions are much shorter

Myosin V

Amino acids in the “a” and “d” positions are usually hydrophobic

What do you want to know about a motor once you've discovered it?

- What is its function in cells?
- What is its subunit composition?
- Where is it located in cells/tissues?
- What are the receptors for this localization?
- What regulates the binding to the receptors?
- What are the enzymatic parameters of the motor?
- How is the enzymatic activity regulated?
- What is the directionality of the motor?
- What is the molecular mechanism of the motor?
- Determine the 3D structure of the motor and/or motor-receptor complex.

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- Determine the 3D structure of the motor and/or motor-receptor complex.

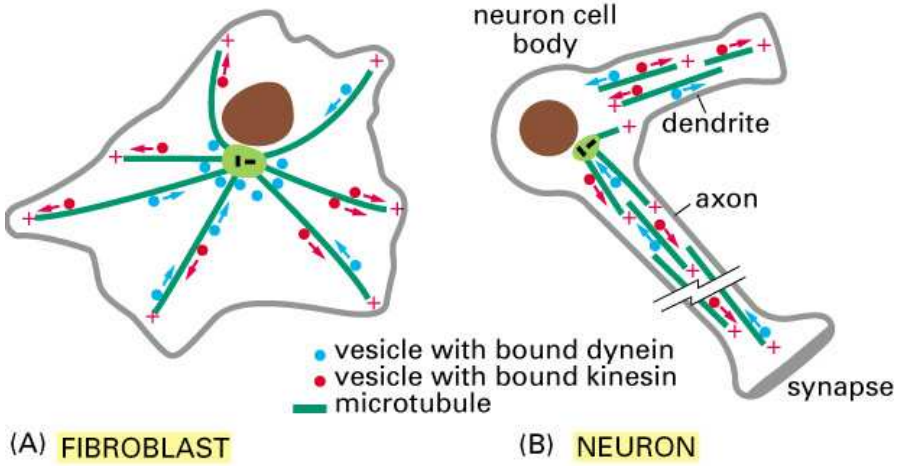
Motor proteins move unidirectionally on their tracks

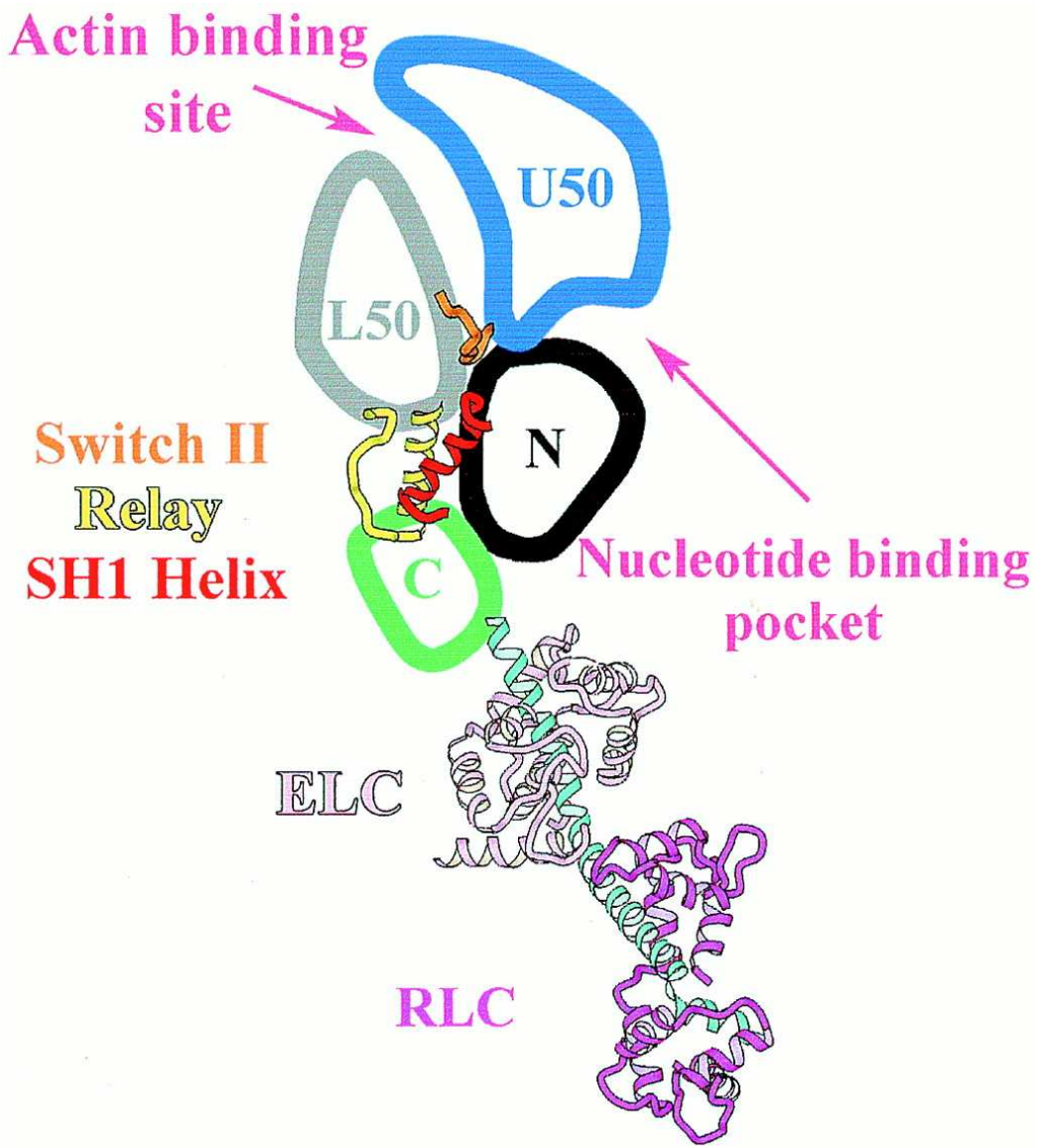
In general, myosins move toward the + end of actin filaments. Actin filaments are typically oriented with the + end towards the plasma membrane.

Myosin VI moves in the opposite direction, i.e. towards the – end

Some kinesin isoforms move in the + (antereograde) direction and others move in the – (retrograde) direction.

Dynein moves in the – direction.





Near-rigor conformation

Nucleotide-free Scallop S1

Myosin II

Humans have 14 myosin II genes of which 10 are found in skeletal or cardiac muscle tissue. Some of these fast isoforms and some are slow giving rise to different contractile speeds of muscle. Some are developmentally regulated.

There is one smooth muscle specific myosin genes and three "nonmuscle" myosin II genes. These are closely related in terms of structure and regulation. Different cells may have various combinations of the three nonmuscle isoforms.

Studies demonstrate that the nonmuscle isoforms have differing functions within the cell. They are often located in different regions and have slightly different kinetic properties.

Functions of non muscle myosin II

Cytokinesis
Cell-surface and cell-cell adhesion
Retraction of trailing cell body in motility
Neurite retraction
Maintenance of cortical tension

Regulation of nonmuscle myosin II occurs via phosphorylation of the "regulatory" light chain by myosin light chain kinase or by terminal kinases of various signal transduction pathways such as rho kinase.

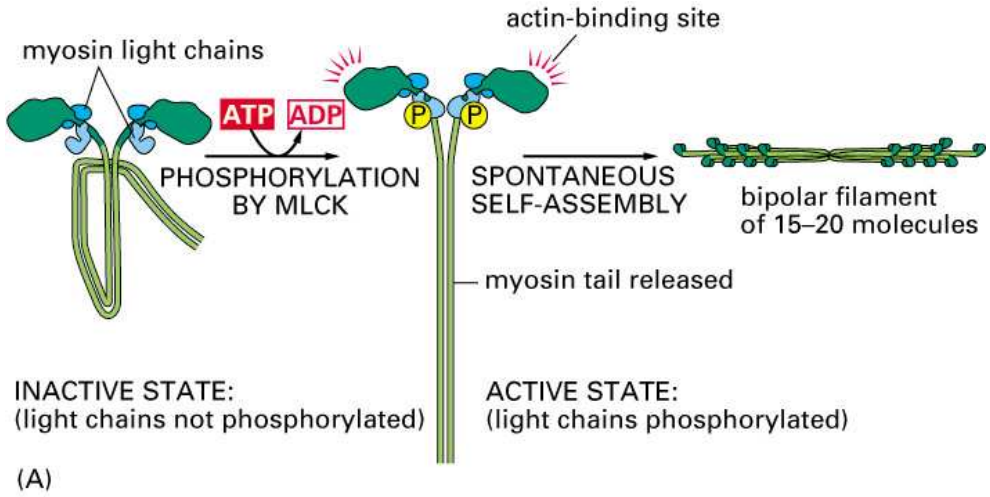
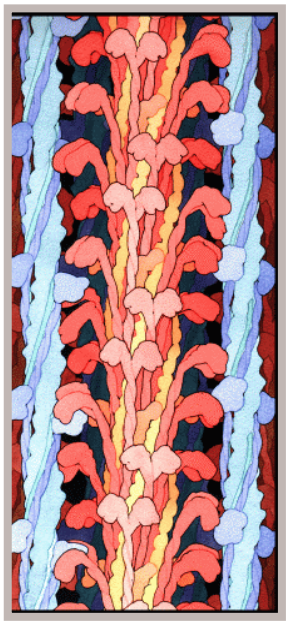
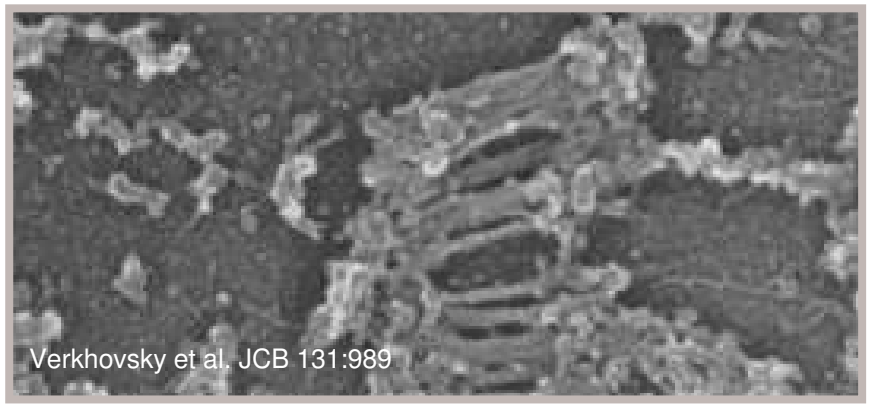
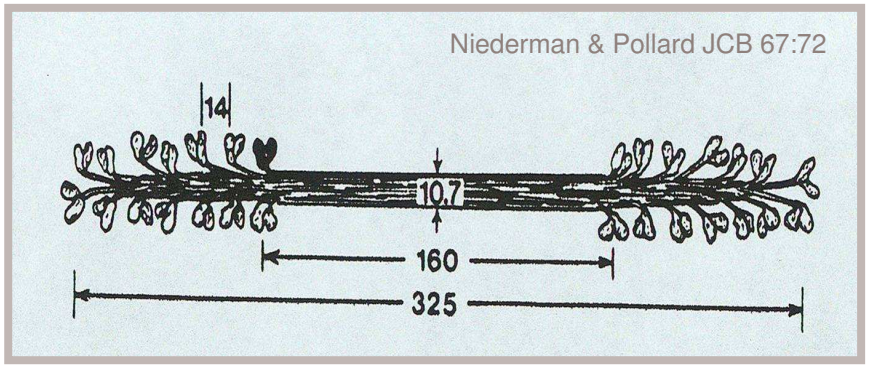


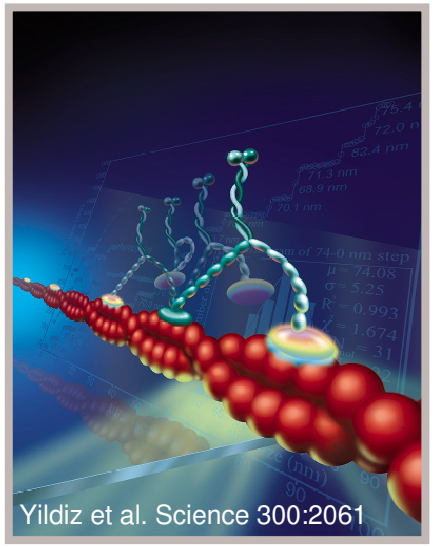
Figure 16–67 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Non-muscle myosin II works in minifilaments









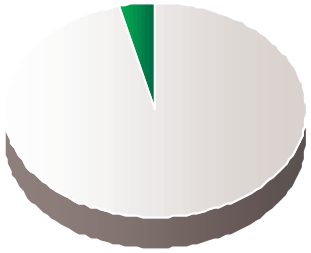
non-muscle myosin II

>1000 heads **56 heads** **2 heads**

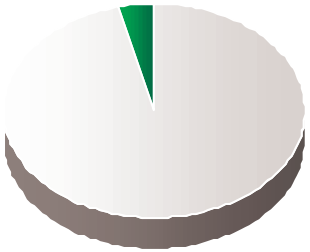
Duty ratio

muscle myosin II

skeletal muscle



smooth muscle



vesicle motors

myosin V

myosin VI

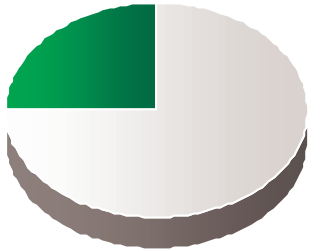
weakly bound to actin/detached

strongly bound to actin

non-muscle myosin II

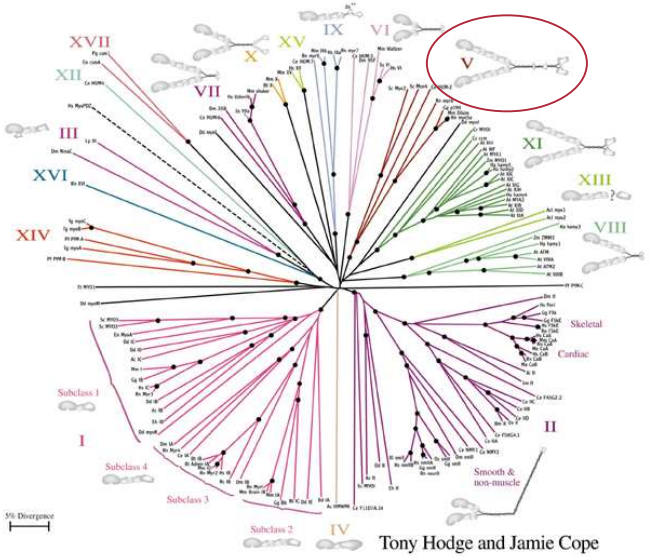
NMIIA

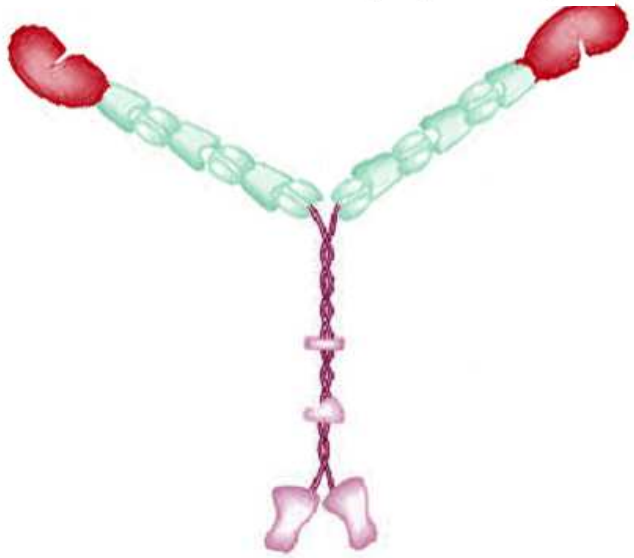
NMIIB



NMIIC

Myosin V





May be the only universally expressed myosin

Implicated in cargo transport in melanocytes and in neurons and in mRNA transportation in yeast

Moves processively along actin filaments, taking 36 nm steps

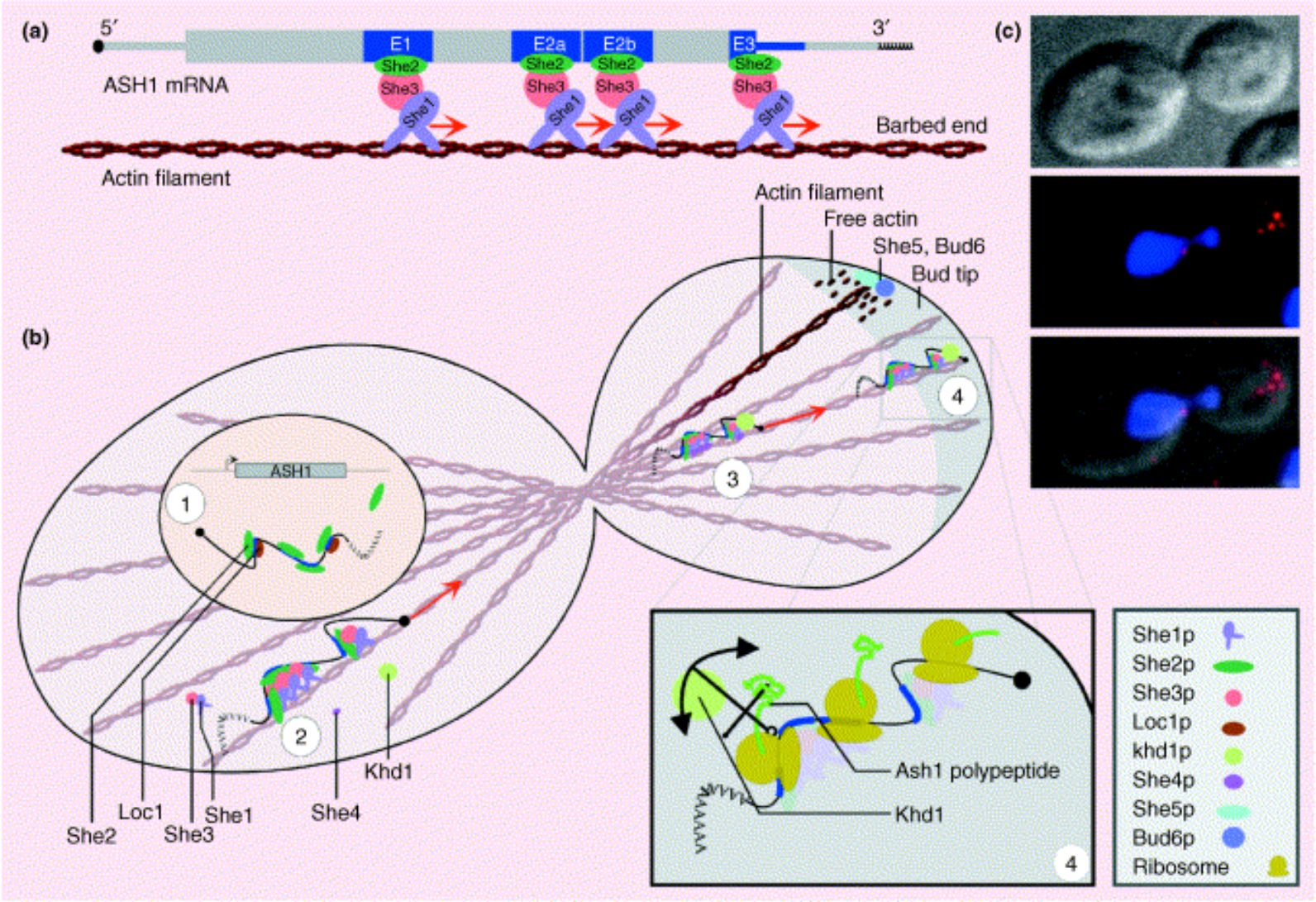
Human disease –Griscelli's disease

***Dilute* mice have myosin V mutations and severe defects in melanosome transport. Also neurological defects**

Expresses well in baculovirus/Sf9 system

Abundant enough to purify from tissue

Myosin V (she1p) is involved in mRNA localization during asymetric cell division in yeast



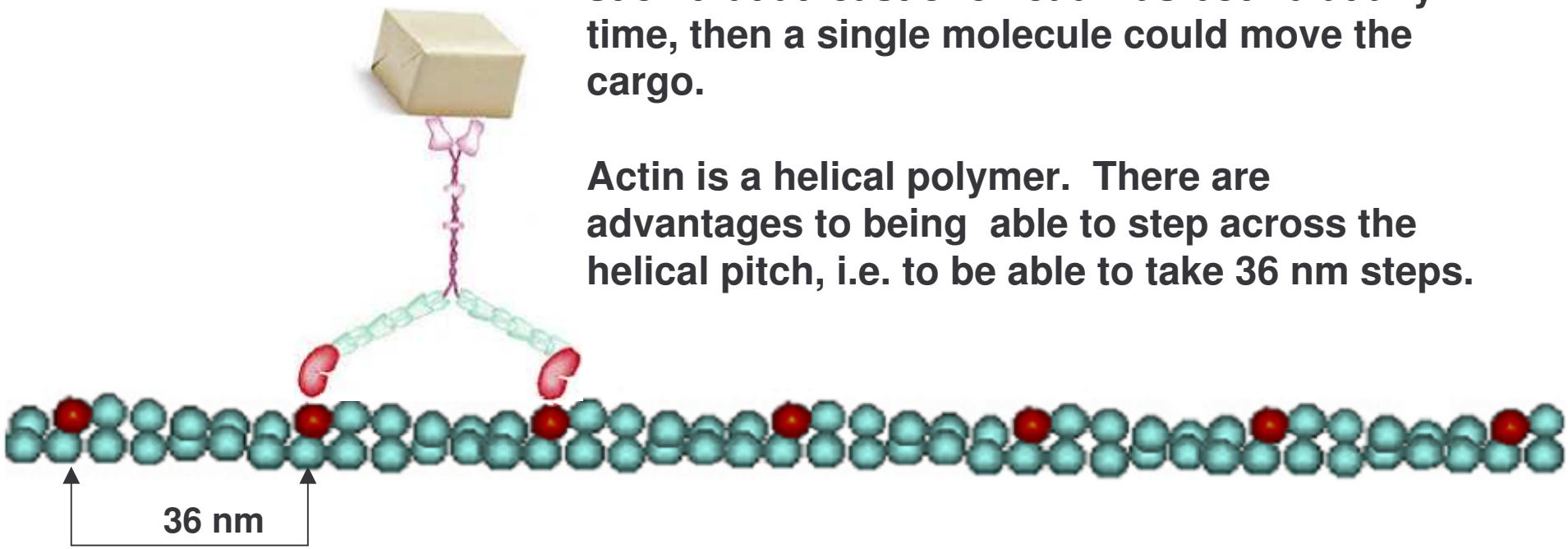
Current Opinion in Microbiology

Myosin V As a Cargo Motor

If myosin V had kinetics like myosin II, a large number of molecules would need to be present on the surface.

If the heads of myosin V worked cooperatively, such that at least one head was bound at any time, then a single molecule could move the cargo.

Actin is a helical polymer. There are advantages to being able to step across the helical pitch, i.e. to be able to take 36 nm steps.



Myosin V: Strides = helical repeat → linear walk

Evidence that Myosin V is a Processive Motor Capable of Moving in a Linear Fashion on Actin

Kinetics suggest that it should be strongly bound to actin for most of its kinetic cycle

Is able to move actin filaments at very low density in the in vitro motility assay

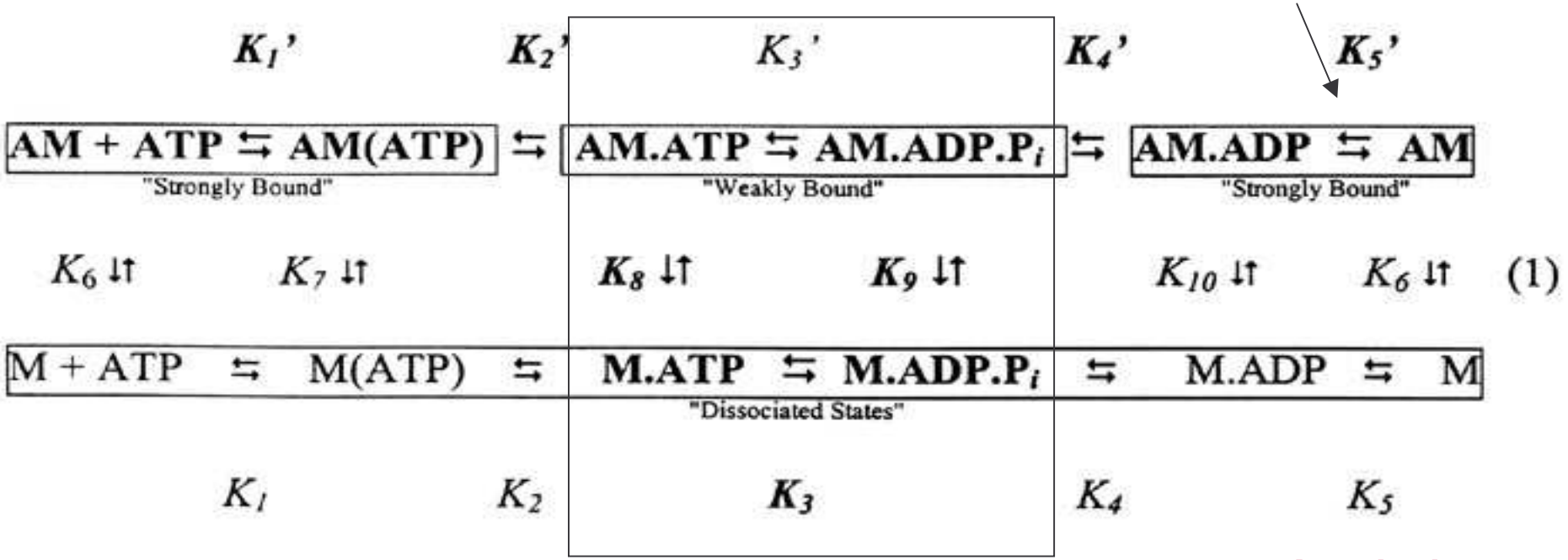
Has multiple steps per diffusional encounter in the optical trap. Steps are 36 nm apart.

Can bind to myosin via two heads even in the presence of ATP at dilutions required for electron microscopy. The two heads are bound 36 nm apart

Single fluorescently labeled myosin V molecules can move on actin filaments

Kinetic Evolution of Different Myosins Allow for Different Tasks

Rate limiting step
For myosin V. Means
that steady products
bind actin strongly.
Well adapted for a



Attached

Rapid Equilibrium
Mostly Detached

Myosin V is a high duty ratio motor

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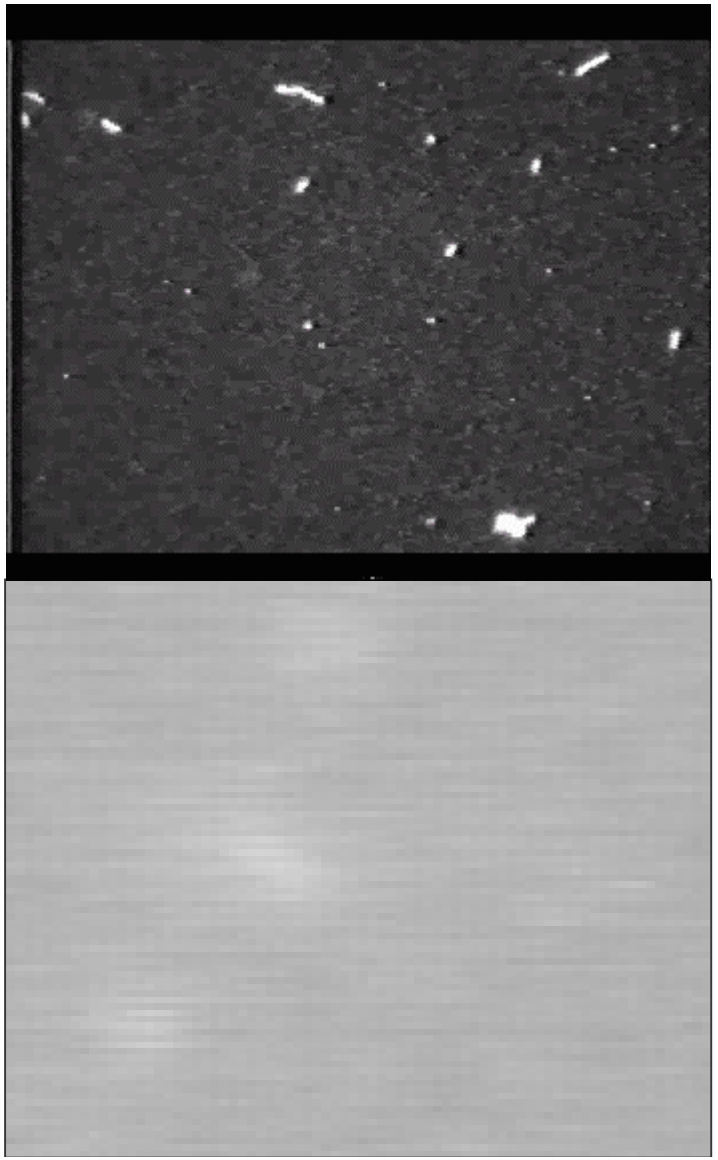
Myosin V Has a High Duty Cycle

- Myosin II requires a high surface density



- Myosin V moves actin filaments at a very low density in the in vitro motility assay
- In some instances the actin filaments appear to be moving about a single point

Evidence that Myosin V is a Processive Motor Capable of Moving in a Linear Fashion on Actin

Kinetics suggest that it should be strongly bound to actin for most of its kinetic cycle

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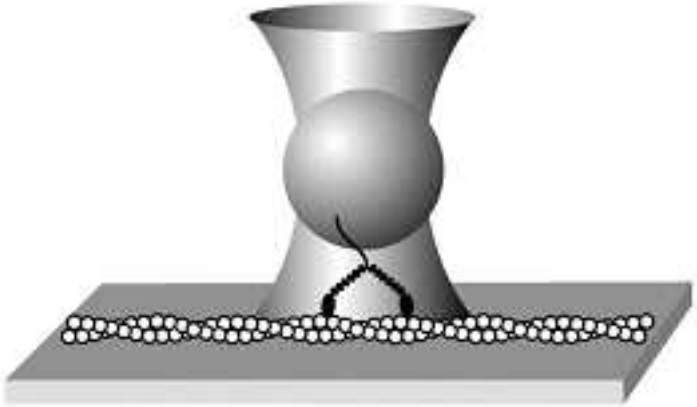
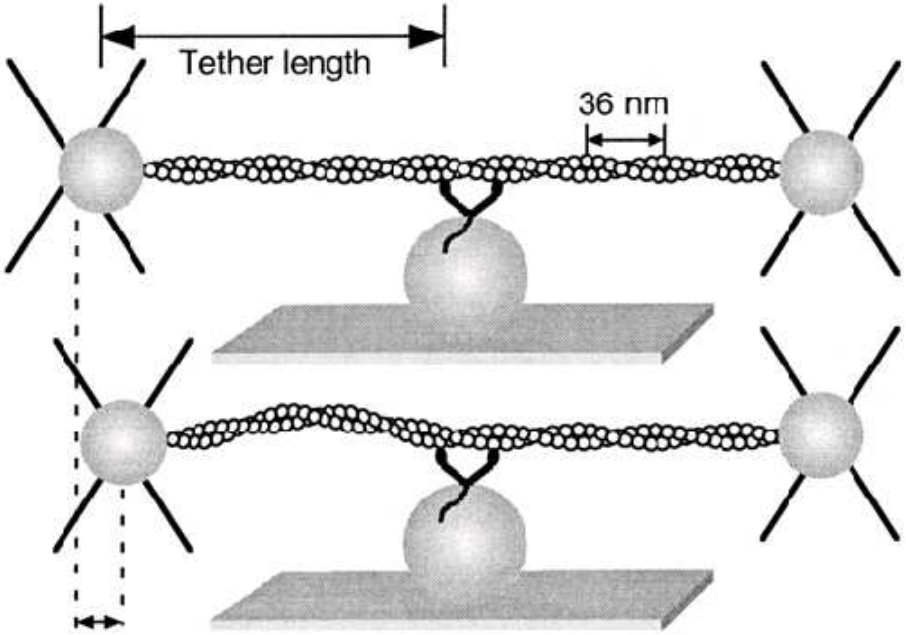
Single fluorescently labeled myosin V molecules can move on actin filaments

Two types of Optical traps

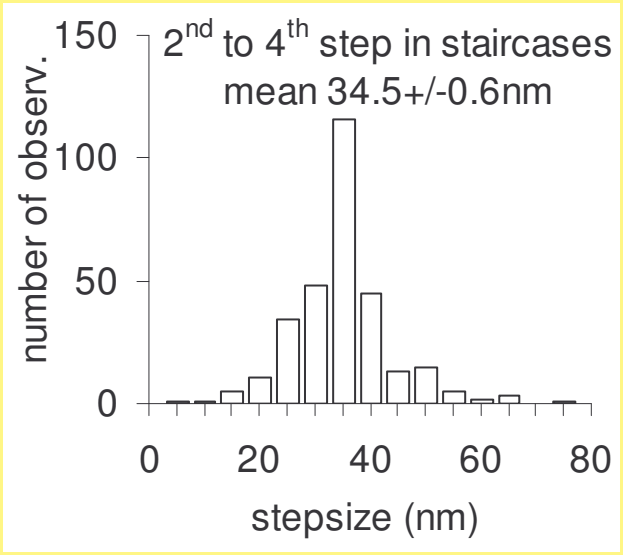


Single Bead Trap



Three Bead Trap

Amplitude of “single events” indicates working stroke is **smaller than** stepsize



Evidence that Myosin V is a Processive Motor Capable of Moving in a Linear Fashion on Actin

Kinetics suggest that it should be strongly bound to actin for most of its kinetic cycle

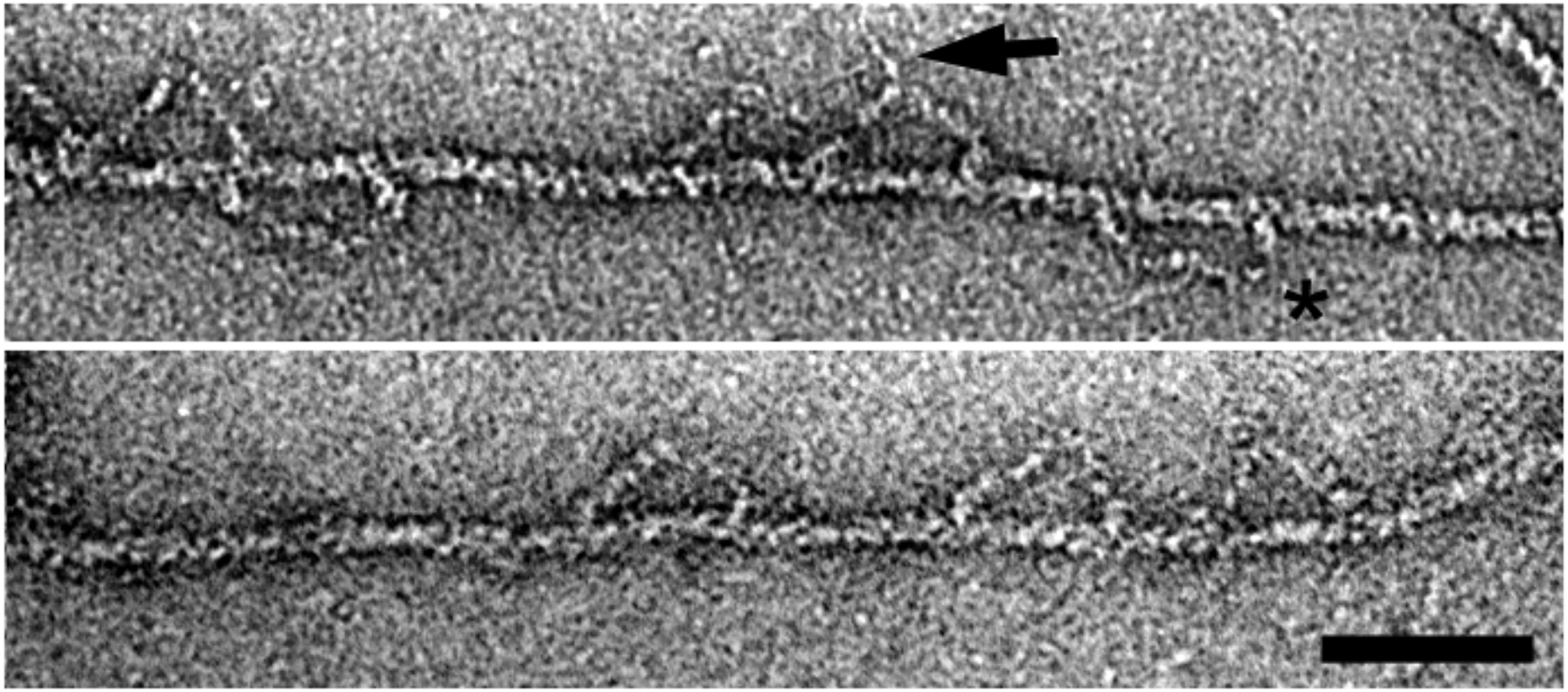
Is able to move actin filaments at very low density in the in vitro motility assay

Has multiple steps per diffusional encounter in the optical trap. Steps are 36 nm apart.

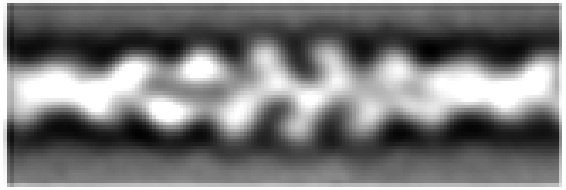
Can bind to actin via two heads even in the presence of ATP at dilutions required for electron microscopy. The two heads are bound 36 nm apart

Single fluorescently labeled myosin V molecules can move on actin filaments

Structure of myosins bound to actin in the presence of ATP

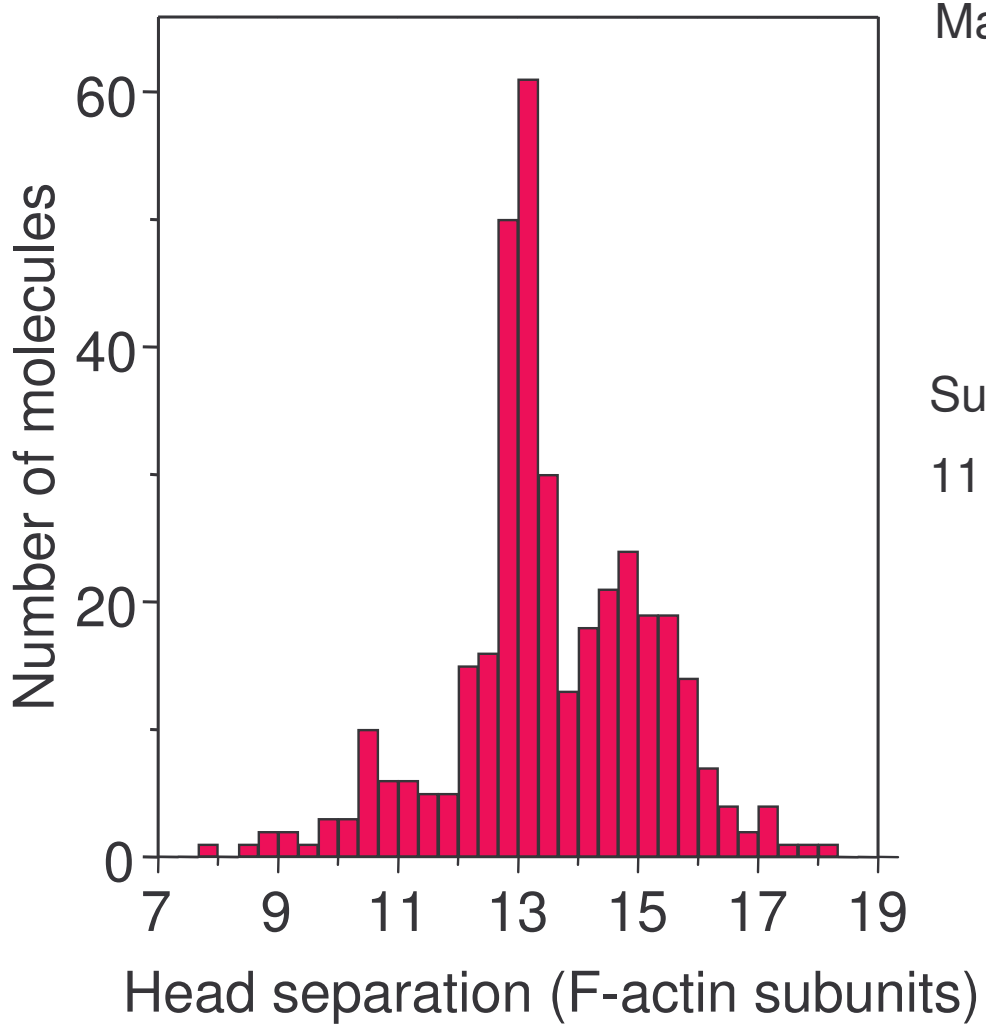


- Polar structure: like telemark skiing stance
- Telemarks on one filament all point one way
- Correlate with actin polarity



In collaboration with Peter Knight and John Trinick

Myosin V Motor Separation on Actin in ATP



Major peak 13 subunits apart

= helical repeat for 13/6

Subsidiary peaks:

11 & 15 subunits apart

Evidence that Myosin V is a Processive Motor Capable of Moving in a Linear Fashion on Actin

Kinetics suggest that it should be strongly bound to actin for most of its kinetic cycle

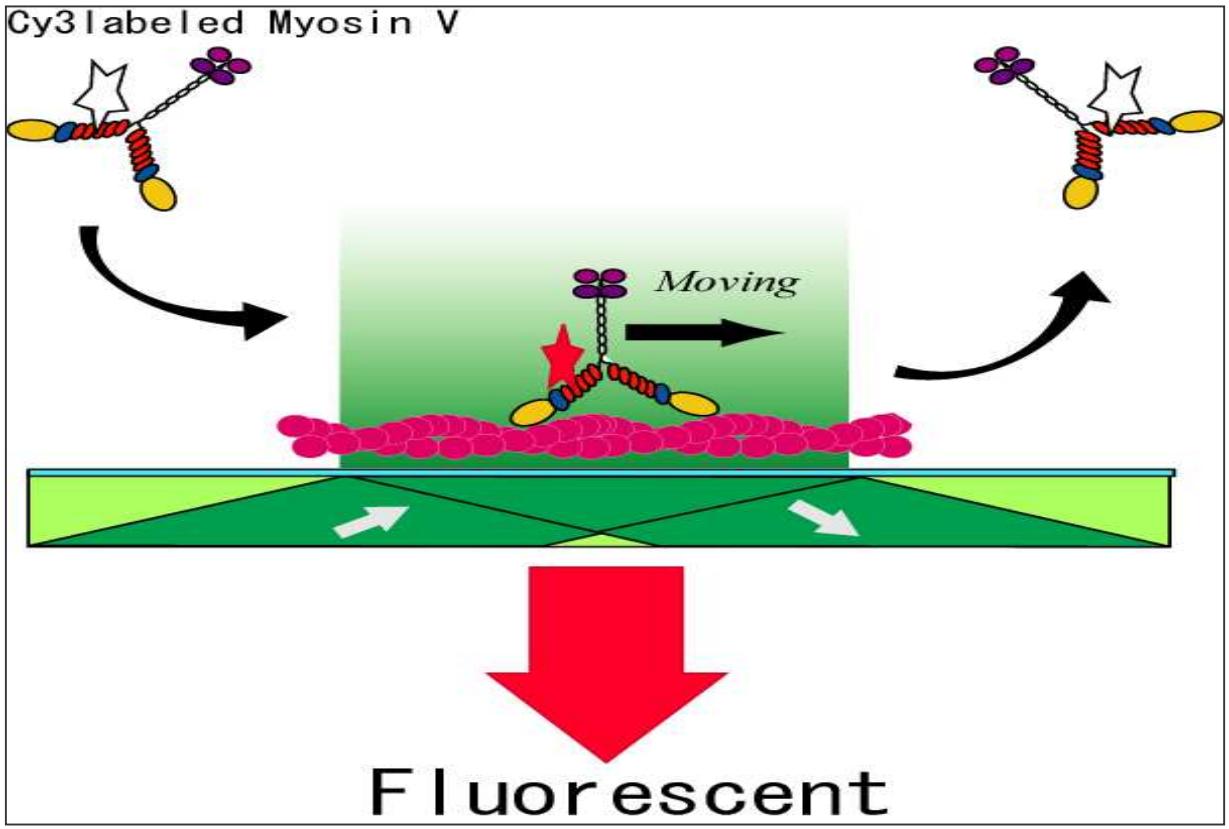
Is able to move actin filaments at very low density in the in vitro motility assay

Has multiple steps per diffusional encounter in the optical trap. Steps are 36 nm apart.

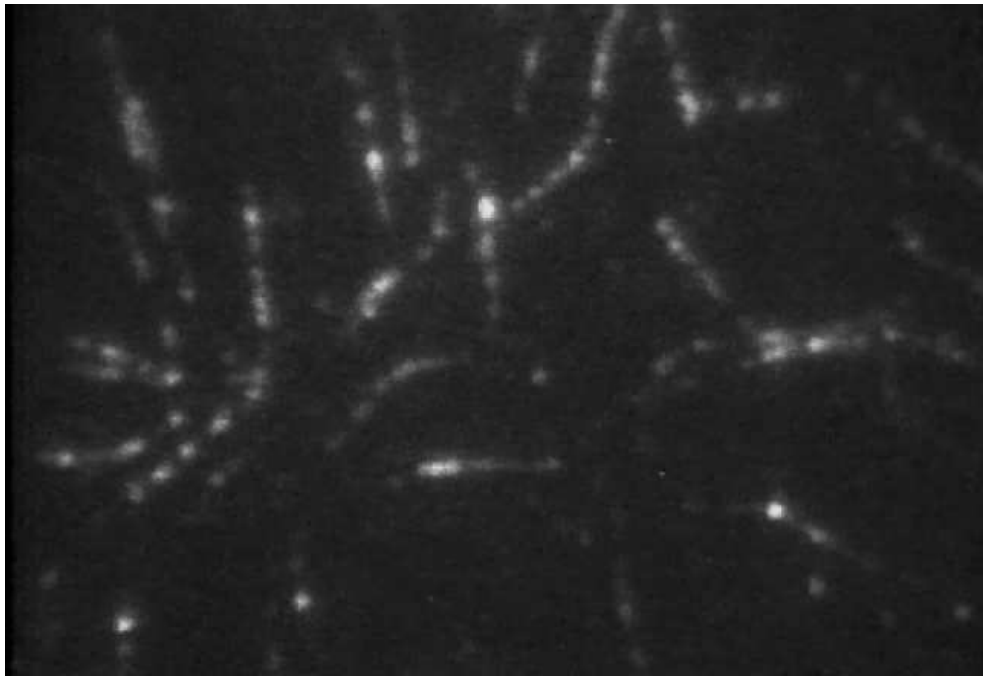
Can bind to myosin via two heads even in the presence of ATP at dilutions required for electron microscopy. The two heads are bound 36 nm apart

Single fluorescently labeled myosin V molecules can move on actin filaments

Single myosin V molecules can be observed using TIRF Microscopy



Movement of Single Molecules of Myosin V on Actin Visualized by Total Internal Reflectance Microscope



Lesson for today: Make use of the unique properties of a particular myosin to understand how myosins in general work

In Search of the Holy Grail of Muscle Research

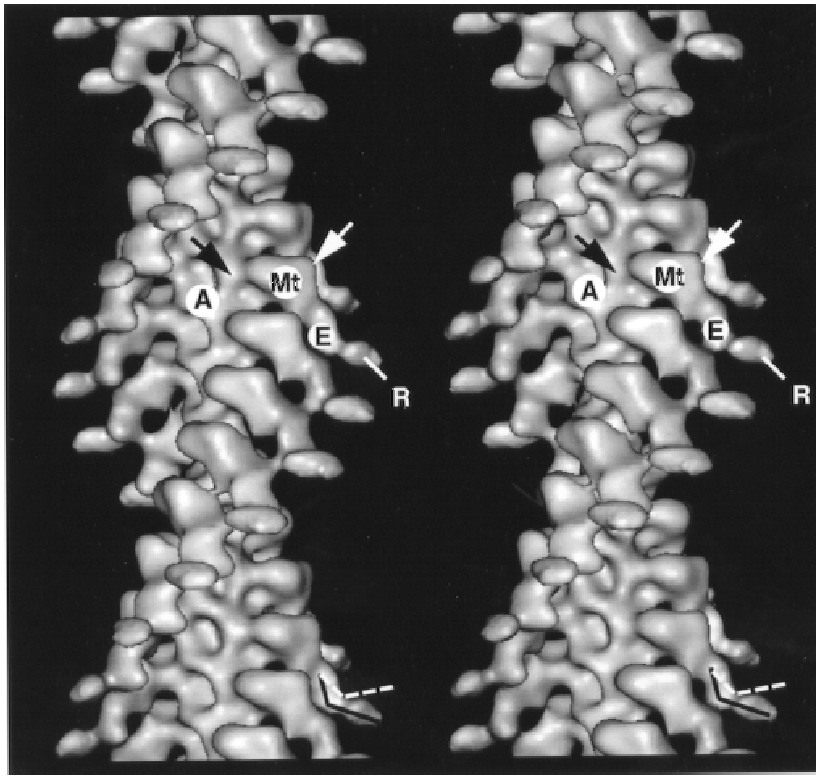
Which is, of course, the direct visualization of the myosin powerstroke in action

To do this we collaborated with John Trinick, Stan Burgess and Peter Knight of the University of Leeds

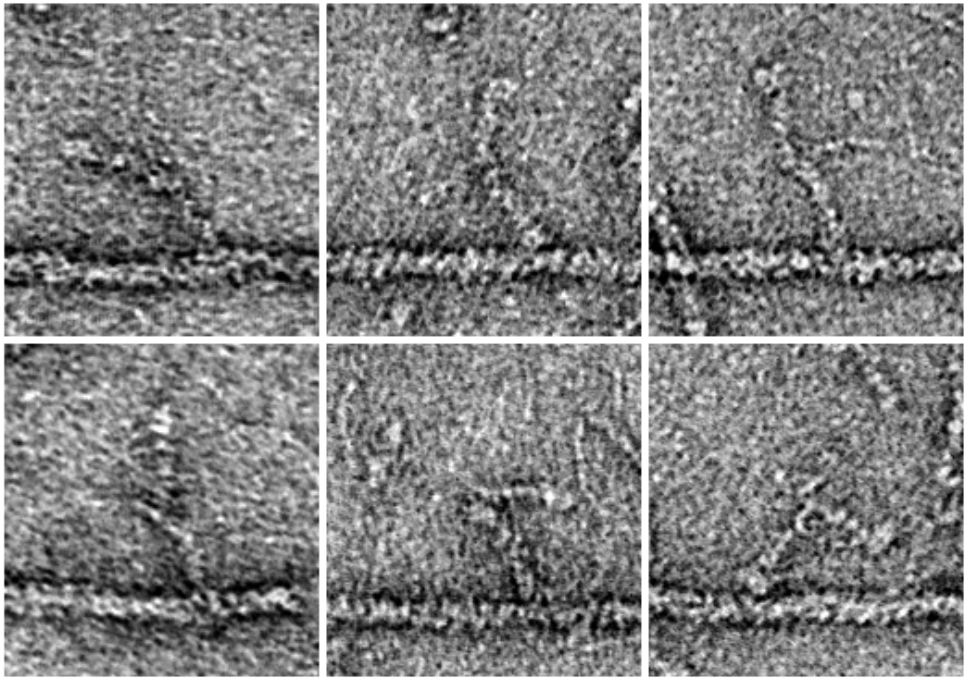
In the absence of nucleotide or in the presence of ADP the binding of myosin II to actin is very tight (Kd= nanamolar range. The problem is that myosin with ATP bound has a Kd of around 20 μM.

To do EM on the isolated proteins they must be diluted down to less than 1μM which means that virtually all of the myosin II rapidly dissociates.

Myosin V has a submicrolar Kd for actin in the presence of ATP, suggesting that it may work for this experiment.



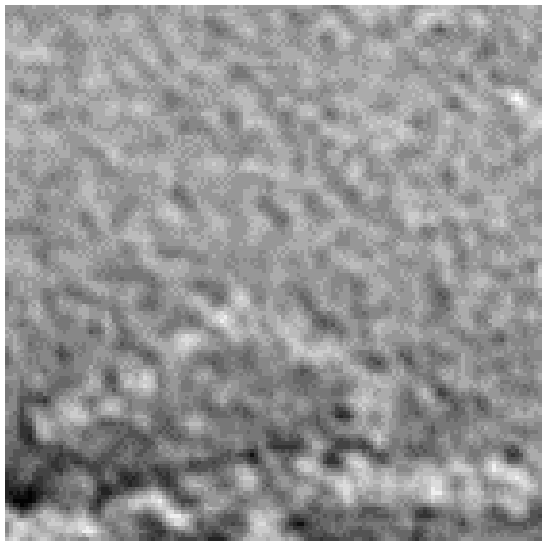
Singly- attached Molecules on Actin in ATP



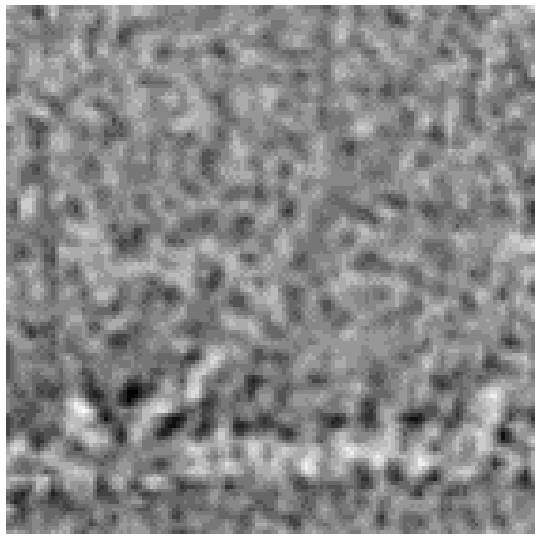
Variety of angles of attachment: some beyond 90°

Seeing the start of the working stroke for the first time!

Motion of detached heads



Attached head pre-power stroke

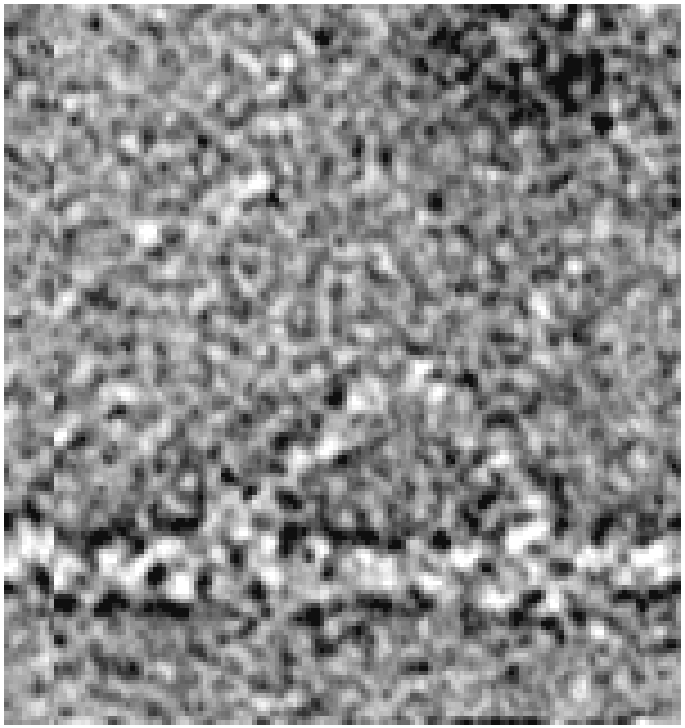


Attached head post-power stroke

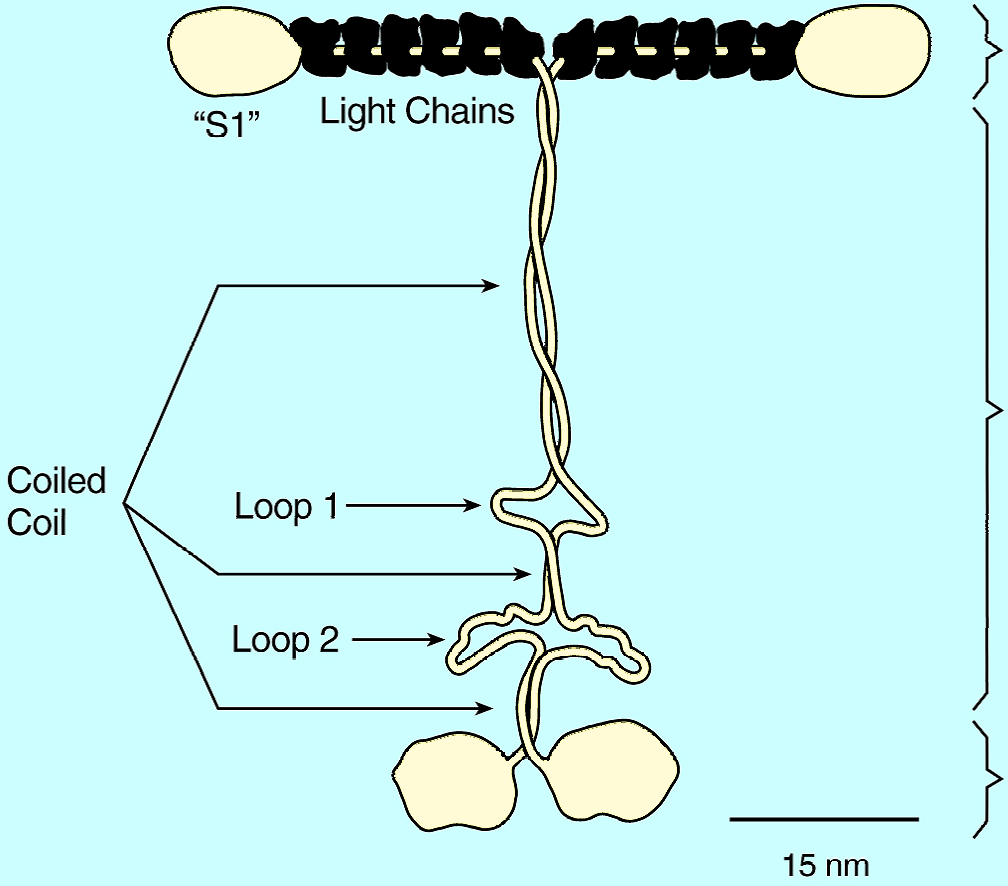
Detached head:
- **cannot reach next attachment site**

Detached head:
- **can reach next site**
- **cannot reach previous site**

Animated Walking Cycle



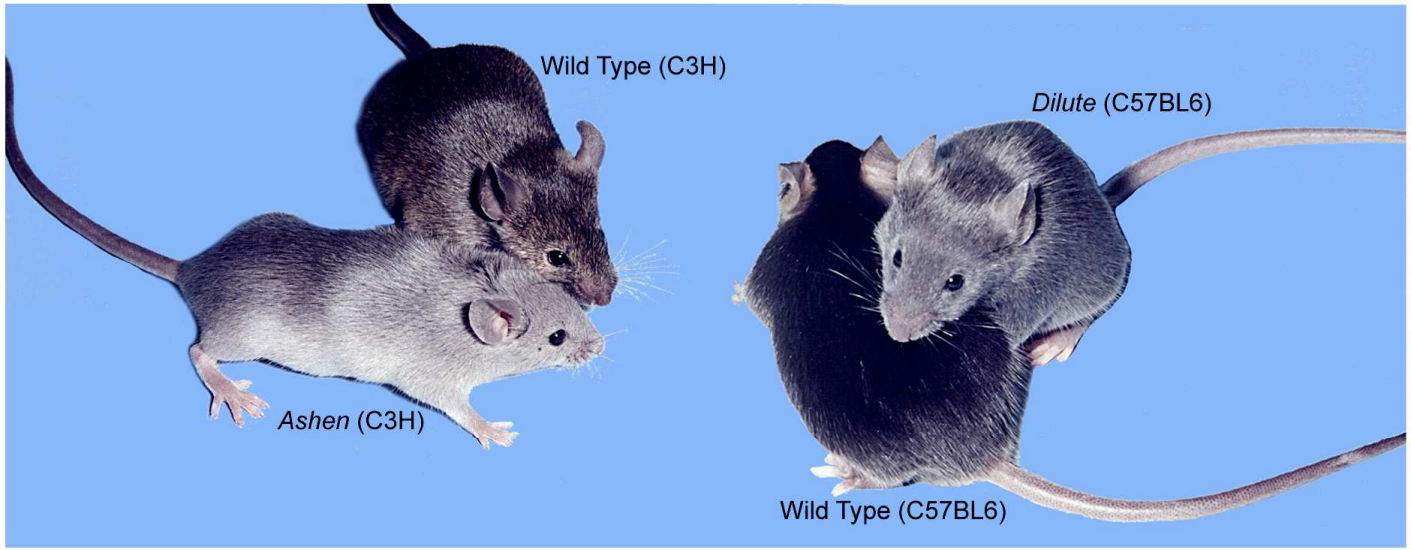
MYOSIN Va *(DILUTE)*

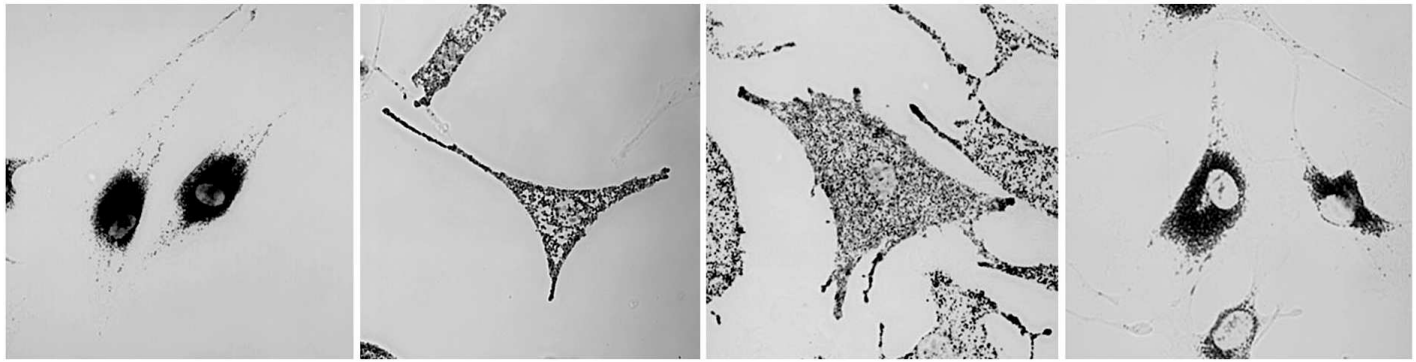


Domain	Function
Head/Neck (~100 kDa)	Processive Motor
Stalk (~ 55 kDa)	Dimerization
Globular Tail (~ 45 kDa)	Vesicle Binding Kinesin Interaction

Cheney et. al., *Cell* (1993)
Mehta et. al., *Nature* (1999)
Huang et. al., *Nature* (1999)

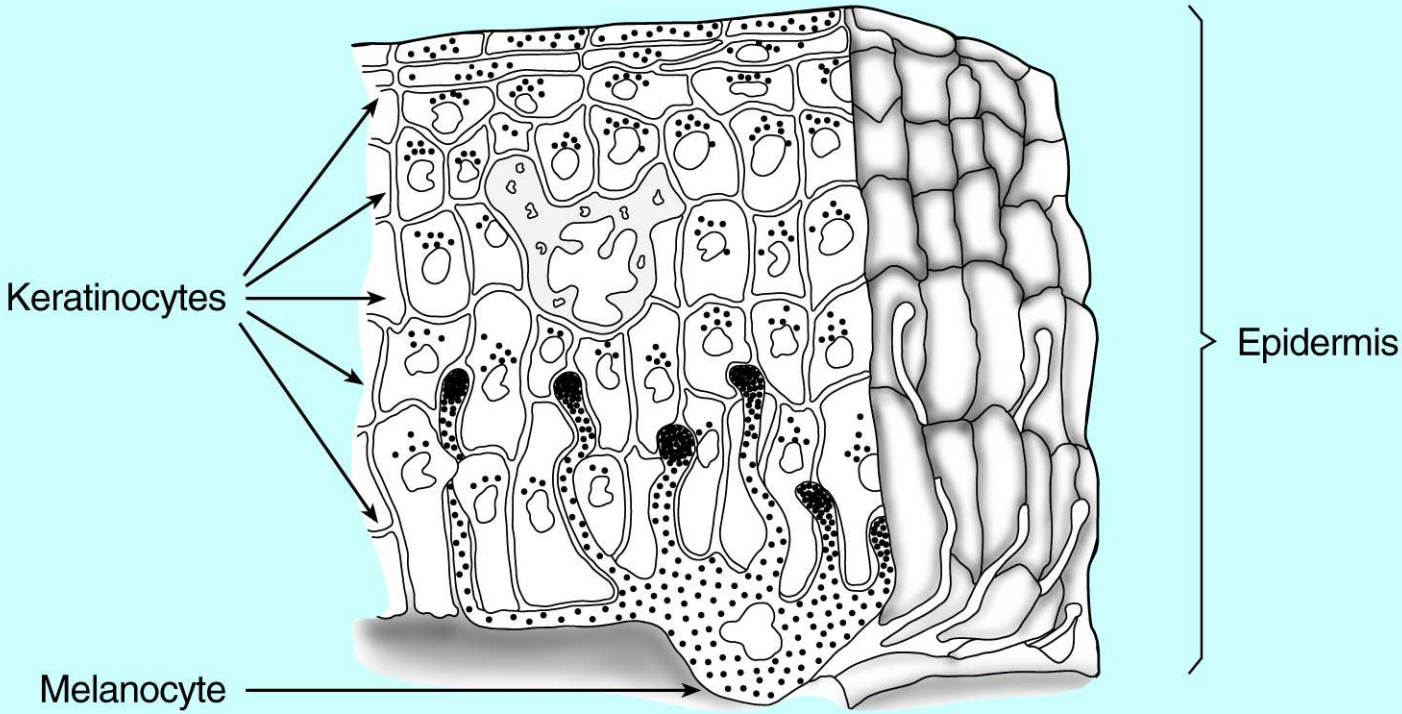
Using Mouse Coat Color Mutants to Investigate Organelle Transport and Distribution

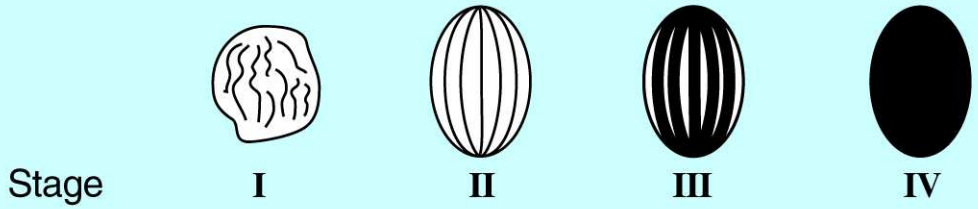




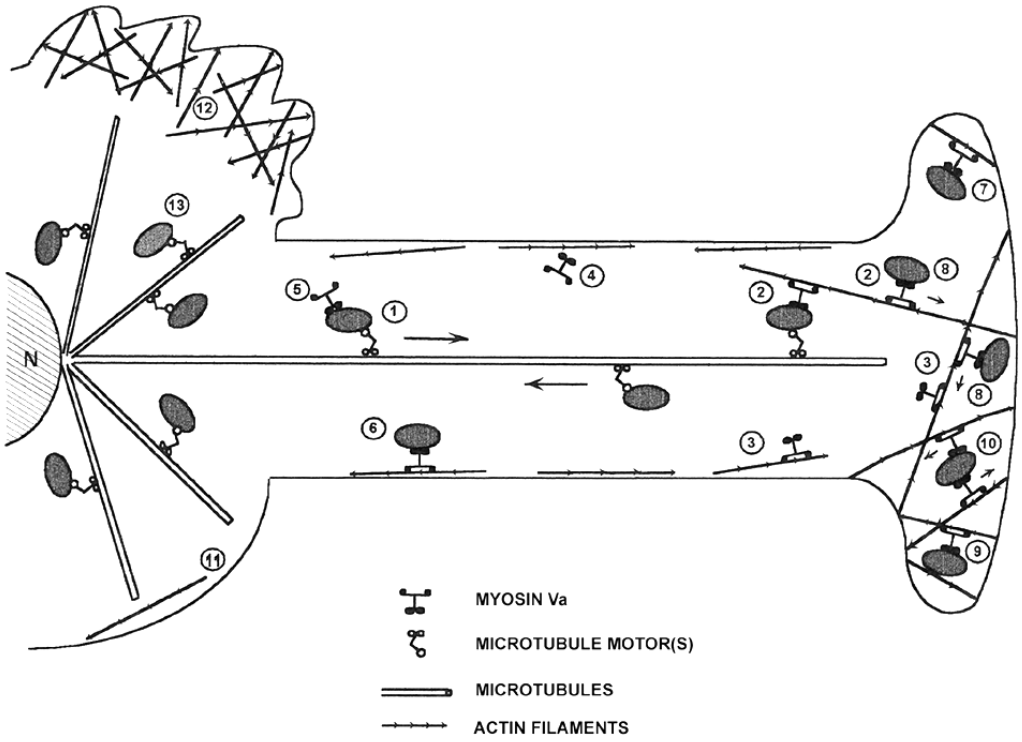
Ashen (C3H) melanocytes | Wild Type (C3H) melanocytes | Wild type (C57BL6) melanocytes | *Dilute* (C57BL6) melanocytes

MAMMALIAN PIGMENTATION





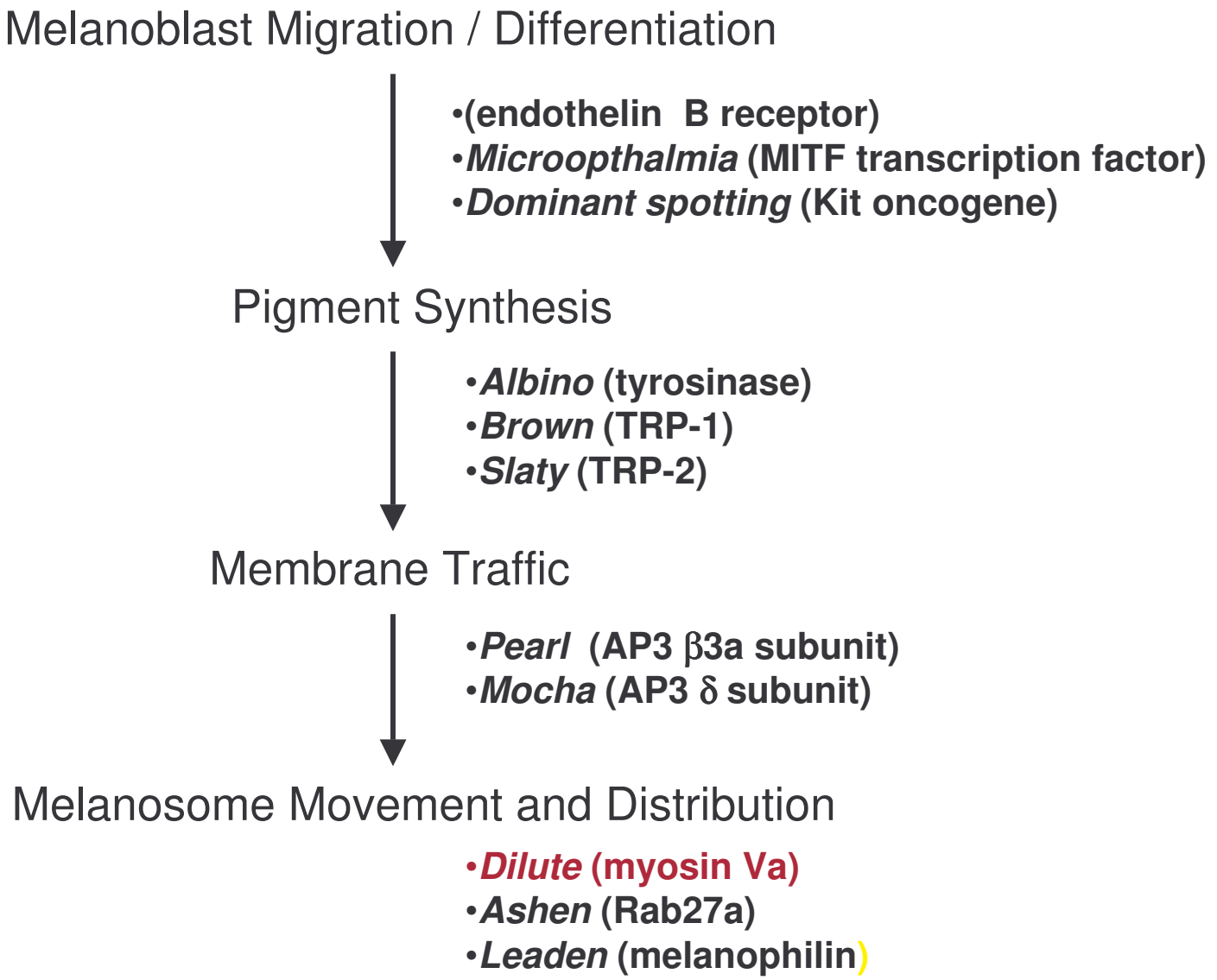
Polarized Secretion
Melanosome = Secretory Lysosome

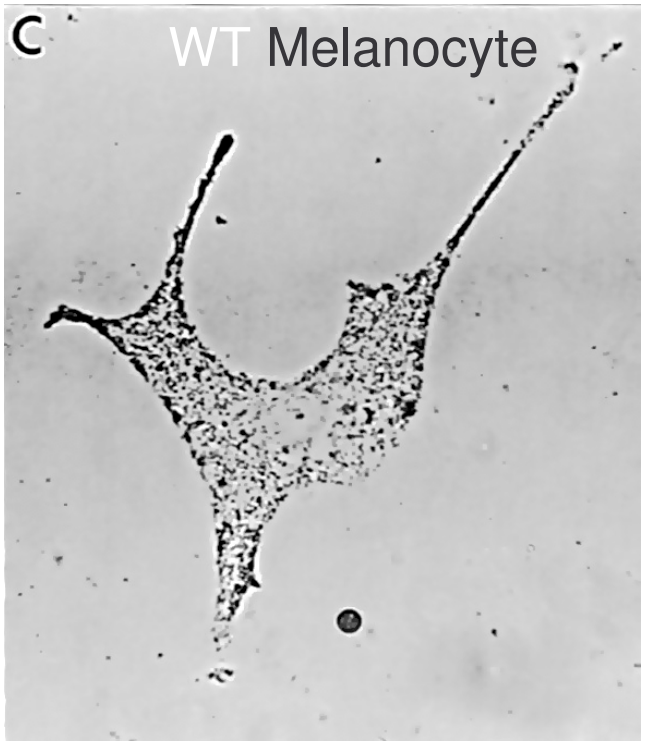


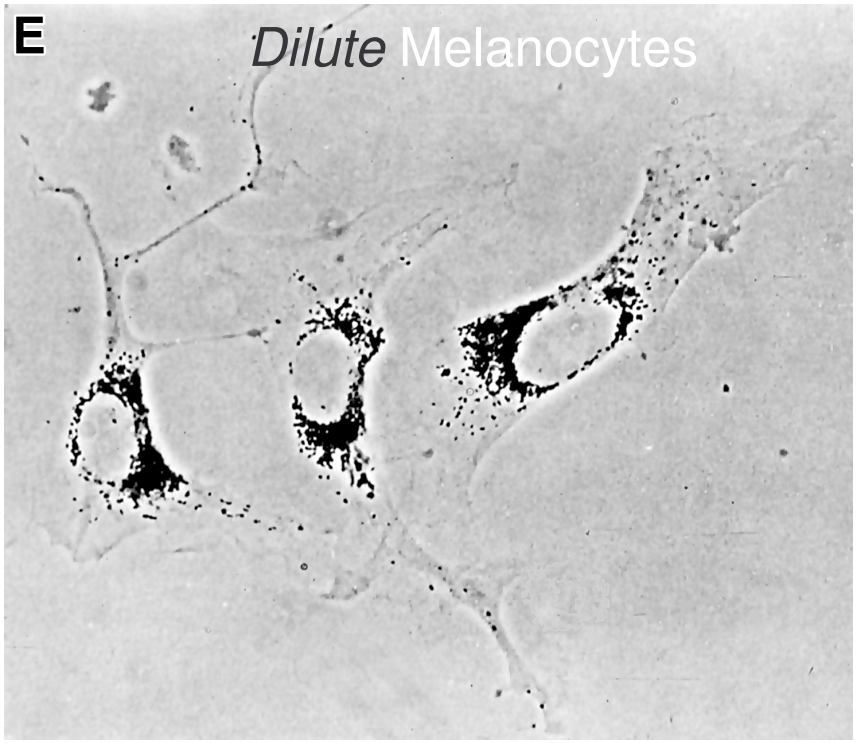
Wu, Bowers, Rao, Wei, and Hammer. 1998. JCB. Vol.143. pp1899-1918

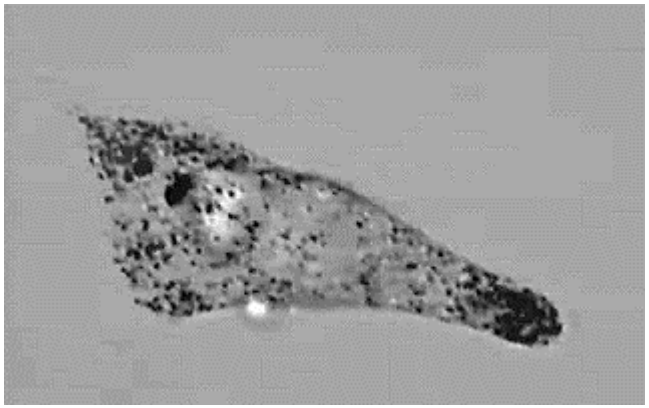
The myosin Va-dependent interaction of melanosomes with F-actin in the periphery prevents a fraction of melanosomes delivered there by centrifugal microtubule-dependent movements from being returned to the cell center by centripetal microtubule-dependent movements, thereby causing their distal accumulation.

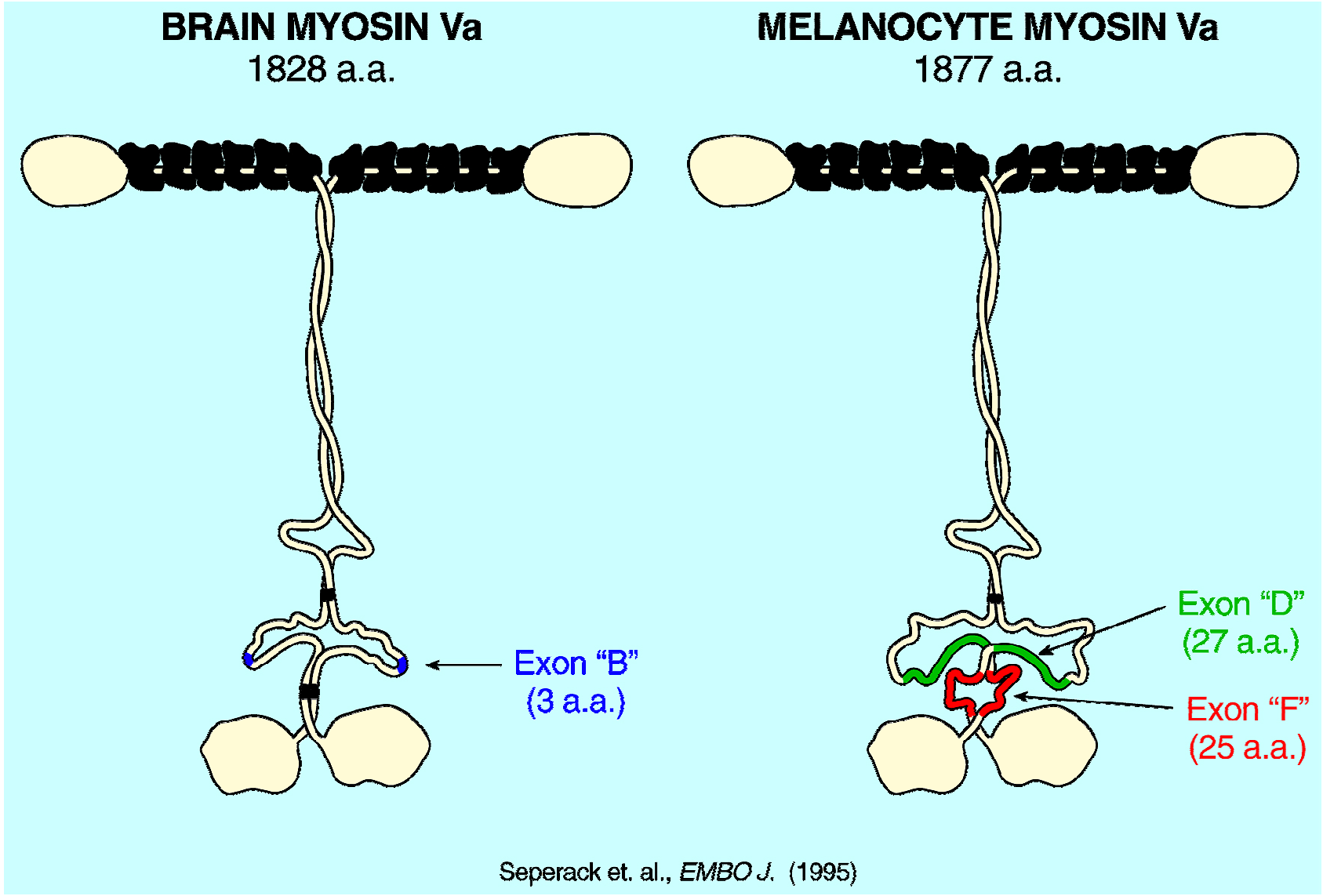
Mouse Coat Color Mutants



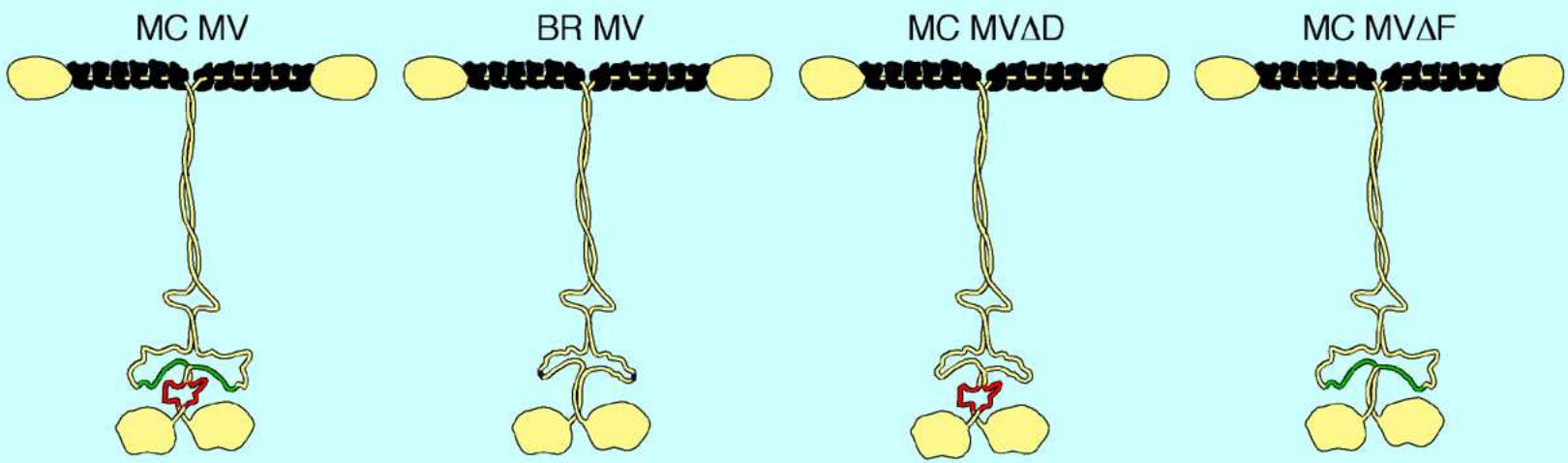

C
WT Melanocyte


E
Dilute Melanocytes




BRAIN MYOSIN Va
1828 a.a.
MELANOCYTE MYOSIN Va
1877 a.a.
Exon "B"
(3 a.a.)
Exon "D"
(27 a.a.)
Exon "F"
(25 a.a.)
Seperack et. al., EMBO J. (1995)

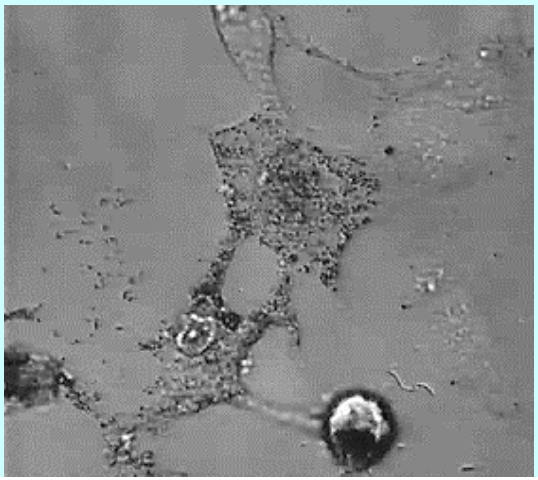
RESCUE



	MC MV	BR MV	MC MVΔD	MC MVΔF
Rescue:	YES	NO	YES	NO
Colocalization:	YES	NO	YES	NO

DOMINANT NEGATIVE

	MC ST	BR ST	MC STΔD	MC STΔF	MC STK	GTD
Dominant Negative:	YES	NO	YES	NO	NO	NO
Colocalization:	YES	NO	YES	NO	NO	NO



Exon B
Exon D
Exon F

Mouse Coat Color Mutants

Melanoblast Migration / Differentiation

(endothelin B receptor)
- ***Microopthalmia*** **(MITF transcription factor)**
- ***Dominant spotting*** **(Kit oncogene)**

Pigment Synthesis

- ***Albino*** **(tyrosinase)**
- ***Brown*** **(TRP-1)**
- ***Slaty*** **(TRP-2)**

Membrane Traffic

- ***Pearl*** **(AP3 β3a subunit)**
- ***Mocha*** **(AP3 δ subunit)**

Melanosome Movement and Distribution

- ***Dilute*** **(myosin Va)**
- ***Ashen*** **(Rab27a)**
- ***Leaden*** **(melanophilin)**

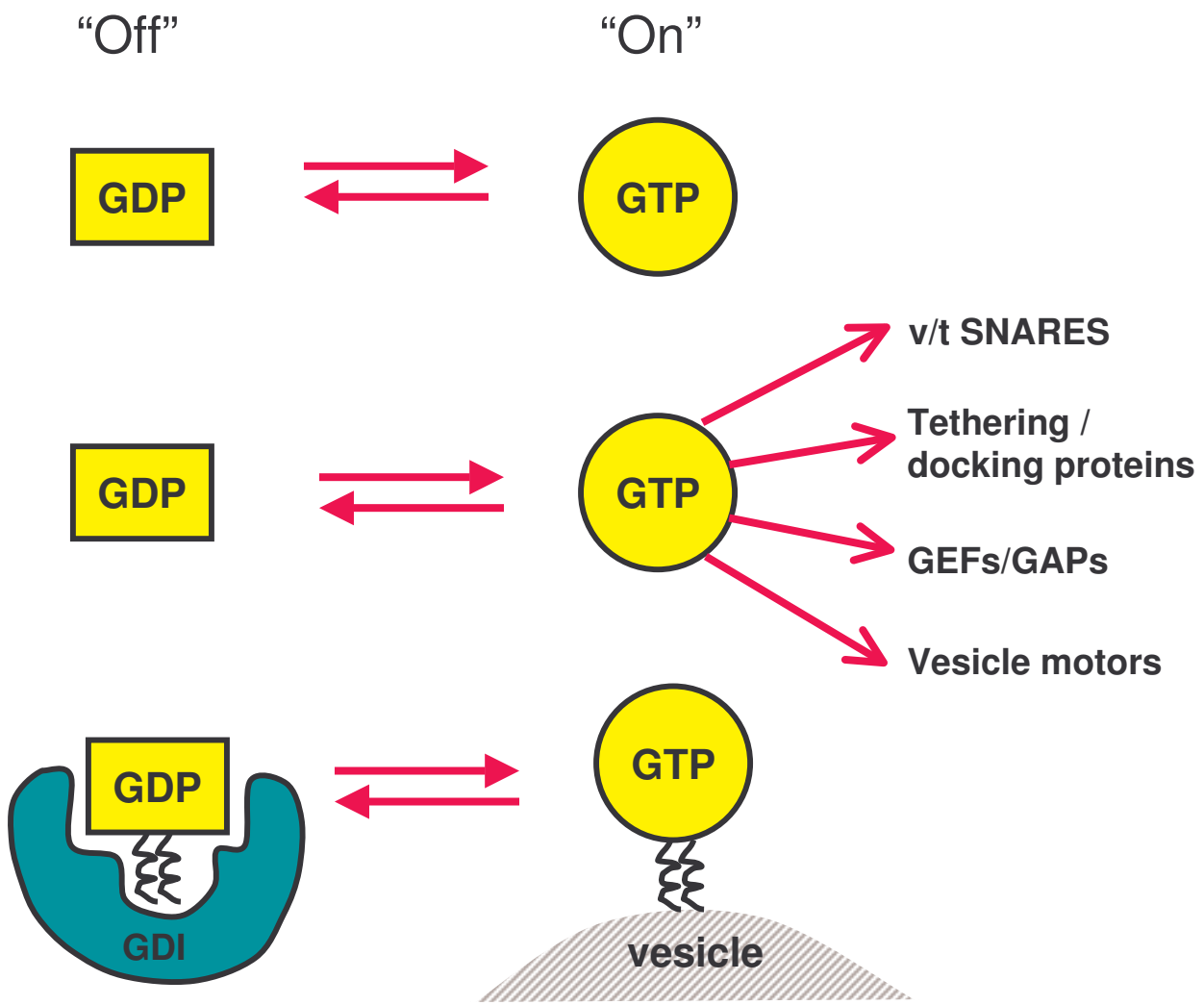
Rab GTPases

Reside on the surface of organelles/vesicles in the endocytic and secretory pathways.

Play critical roles in the targeting and fusion of these vesicles with their appropriate acceptor membrane.

Participate in the formation and /or function of SNARE complexes.

Rab Cycles


"Off"
"On"
GDP
GTP
GDP
GTP
v/t SNARES
Tethering /
docking proteins
GEFs/GAPs
Vesicle motors
GDP
GDI
GTP
vesicle

Does myosin Va directly interact with Rab27a?

Can look at this using myosin V affinity columns?

The answer is…

No… There must be some intermediate protein that interacts with both myosin Va and Rab27a

Mouse Coat Color Mutants

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- ***Mocha*** **(AP3 δ subunit)**

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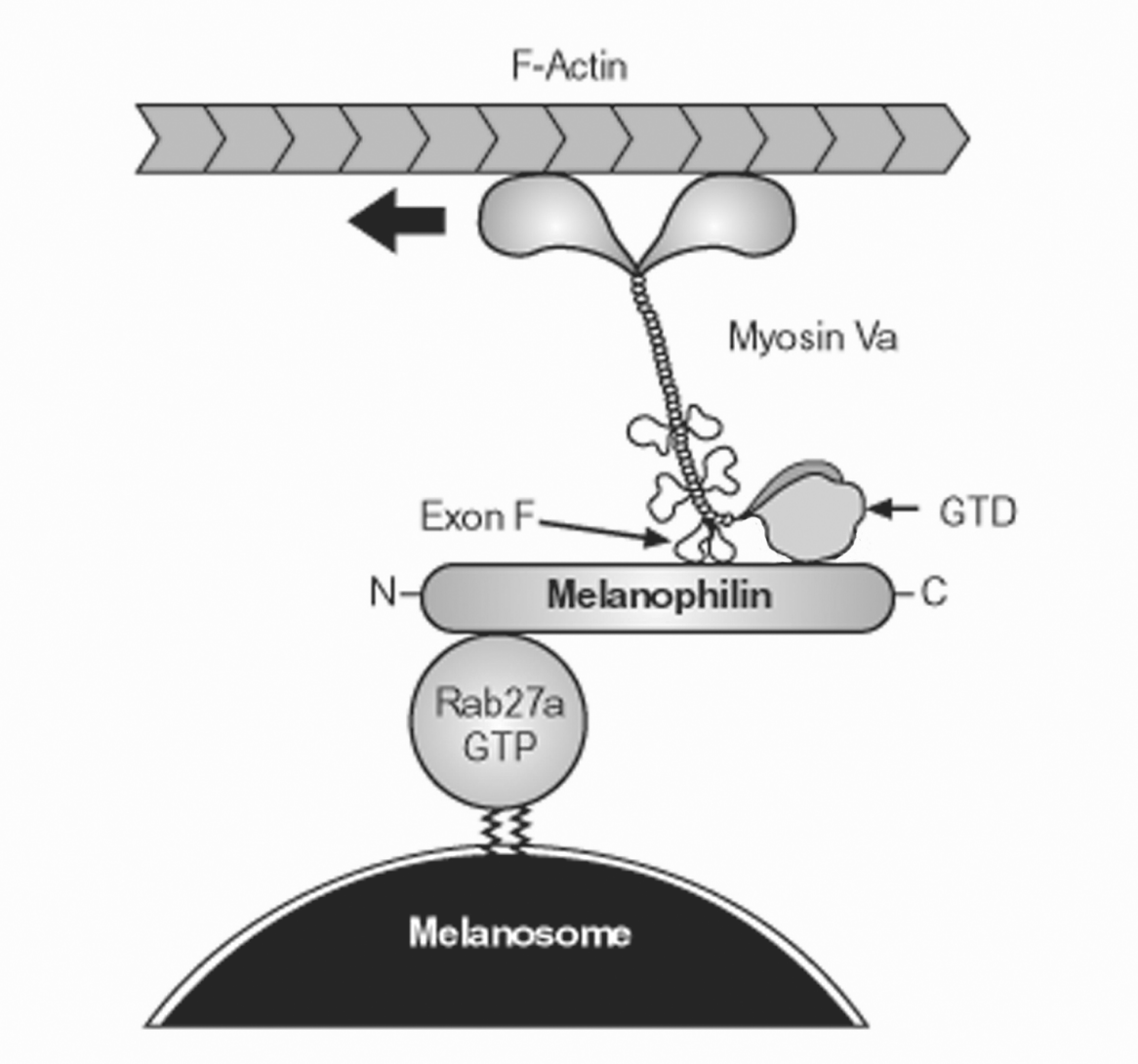
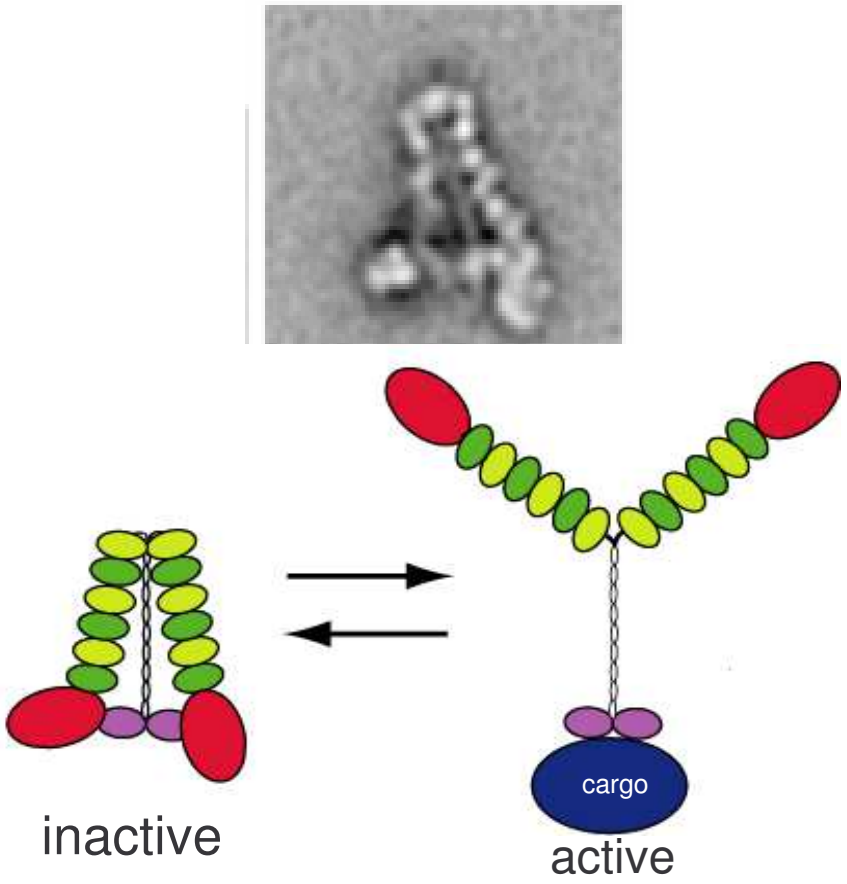
- ***Dilute*** **(myosin Va)**
- ***Ashen*** **(Rab27a)**
- ***Leaden*** **(melanophilin)**

Organization of the melanosome receptor for myosin Va

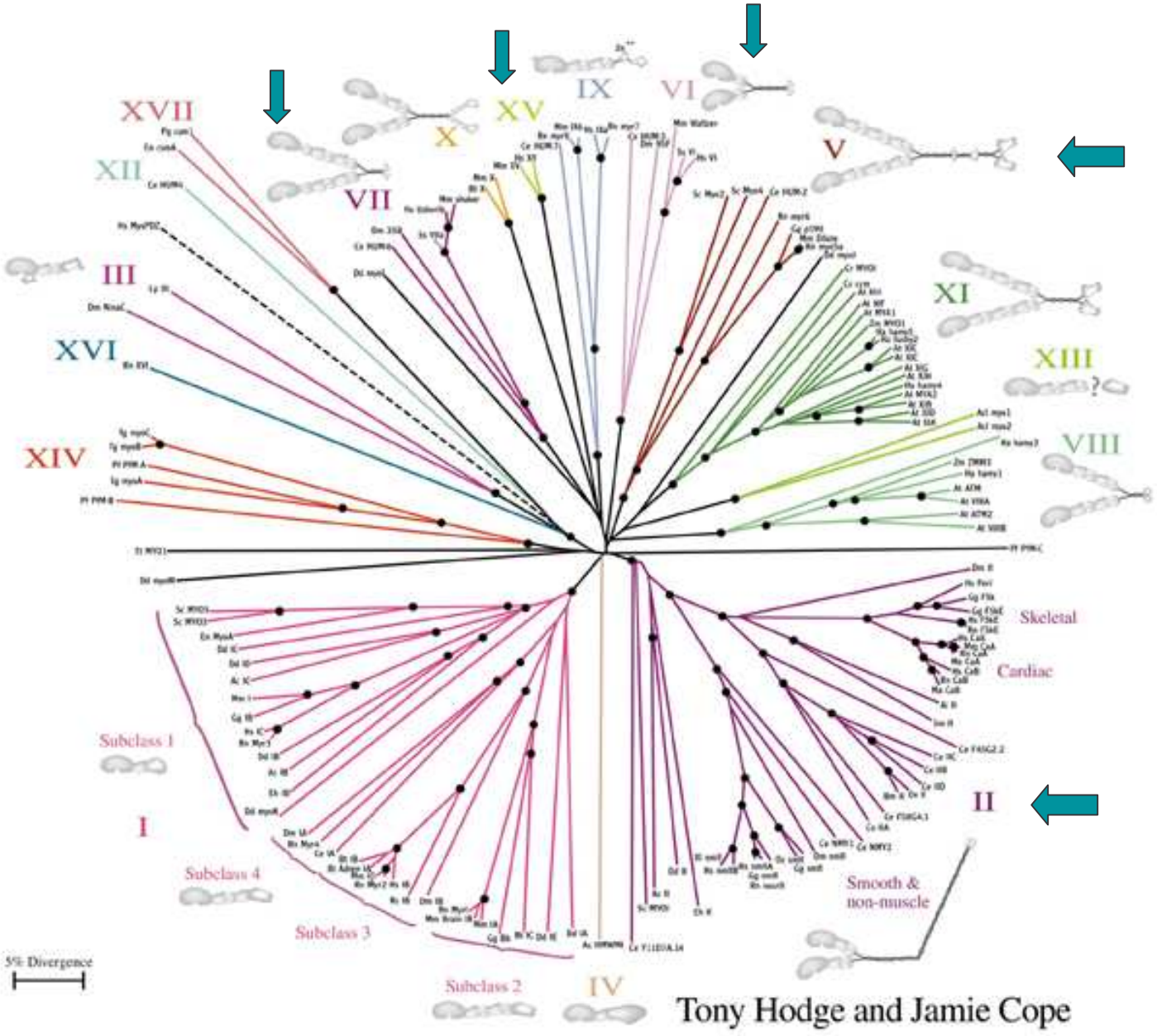


Regulation of myosin V

In the absence of calcium myosin V forms a folded, inactive complex in which the globular tail domain interacts with the heads. In vitro, we can activate the enzymatic activity of myosin V by adding calcium, but it is likely that in cells myosin V is activated by binding of cargo.



Myosins Linked to Human Diseases



Myosins involved in familial deafness

Myosin IA	**DFNA48**
Myosin IIA	**DFNA17**
Myosin III	**DFNB30**
Myosin VI	**DFNA22; Snell's Waltzer mouse**
Myosin VIIA	**Usher's IB Syndrome,DFNB1, Shaker1 Mouse**
Myosin XV	**DFNB3, Shaker2 mouse**

Interestingly, the Snell's Waltzer mouse is deaf and has circling behavior, but is relatively normal in other respects. It reproduces, looks normal and has no other obvious defects. Myosin VI, however is present in all cell types and has been implicated in endocytosis and Golgi transport. Its functions in these processes must not be essential.

Differential Localizations of Myosins in the Inner Ear

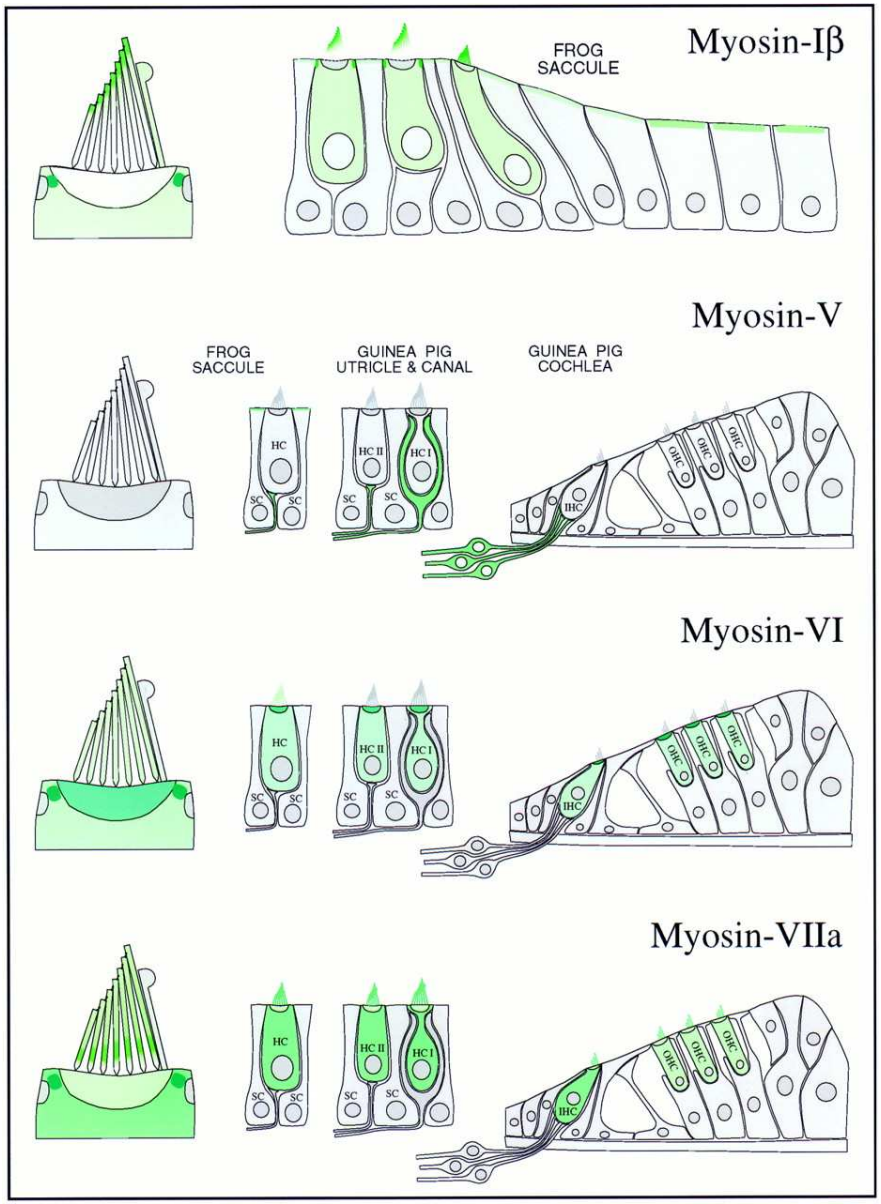


Figure from Hasson et al. J.Cell Biol. **137**: 1287, 1997

Myosin Iβ may be involved in adaptation, by moving the insertion point of the tip link which connects one stereocelia to another. The stereocelia contain a core of actin filaments.

Myosin V is only involved in neurotrans-mission, not in sensory perception, itself.

Myosin VI mutations are associated with deafness in mice and humans . It is found in the region ina the cuticular plate region which lies beneath the stereocelia and in the cell body. Mutations in myoVI result in degeneration of stereocelia.

Myosin VII mutations are associated with deafness and, in humans, also with blindness. It is located near the base of the stereocelia and in the cell body.

Nonmuscle Myosin IIA is associated with human deafness. It is located in the terminal web region

Myosin XV is associated with deafness in humans and mice. It is located at the tip of the stereocelia.

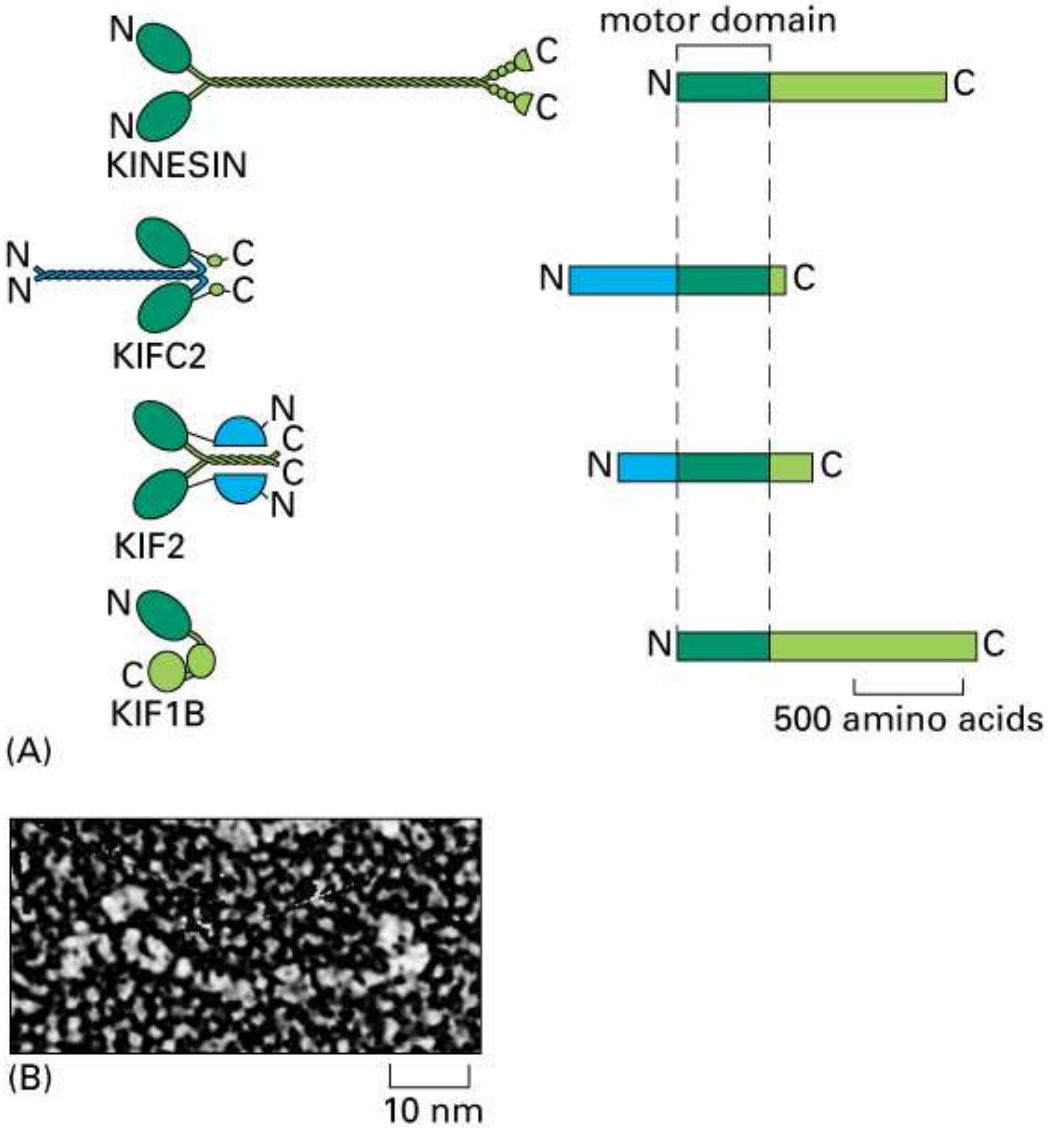
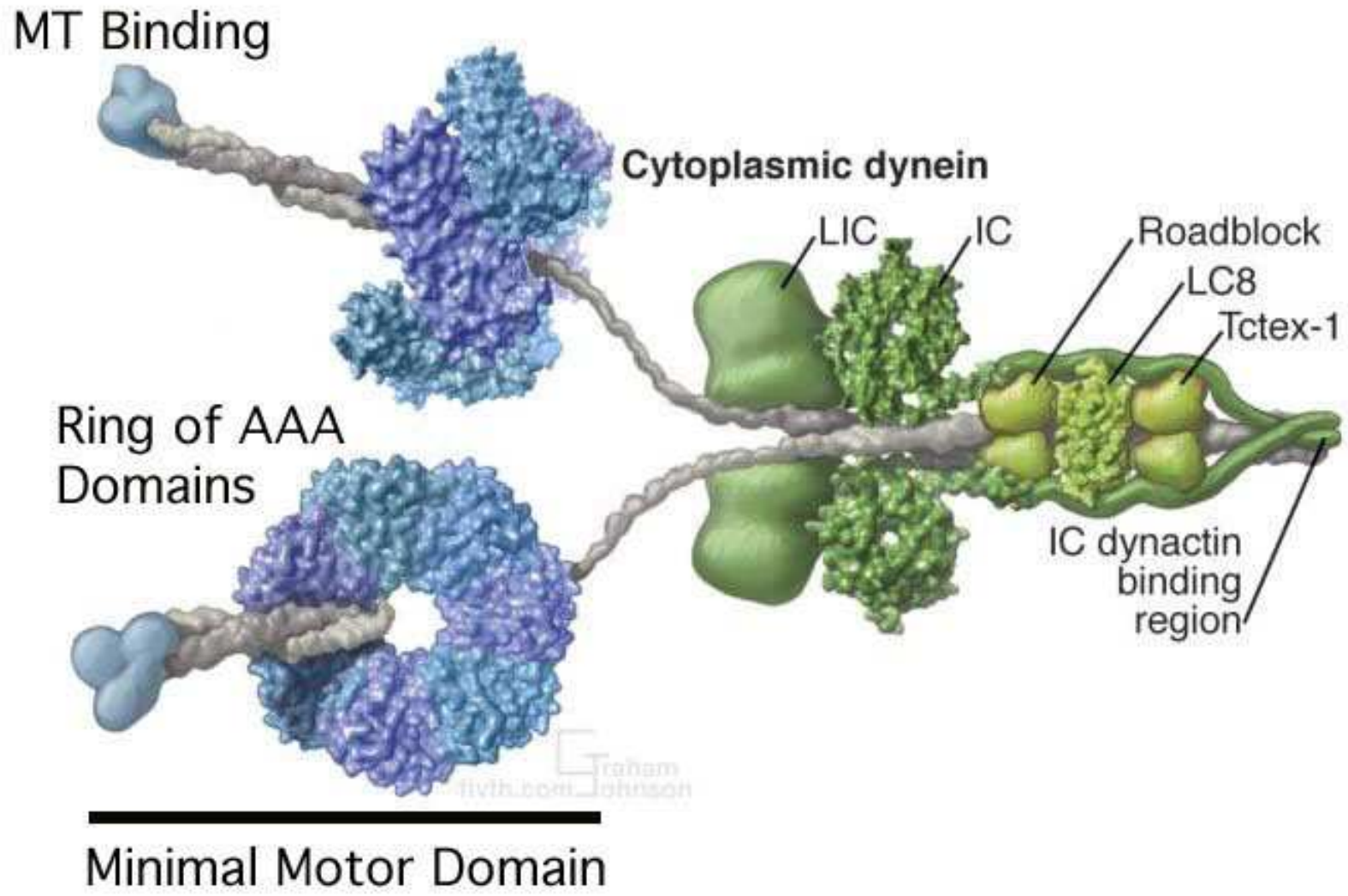


Figure 16–55. Molecular Biology of the Cell, 4th Edition.

Cytoplasmic Dynein



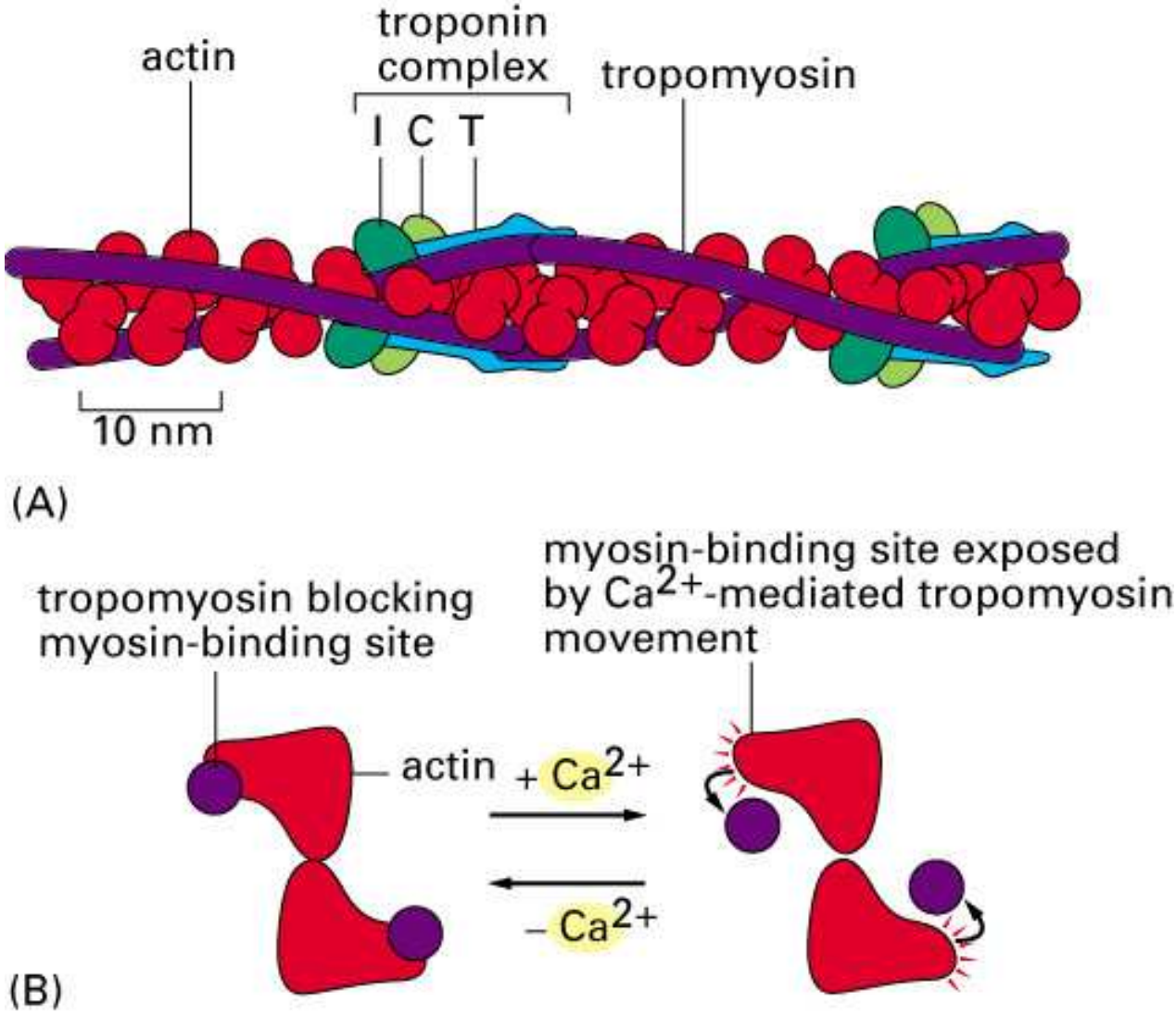
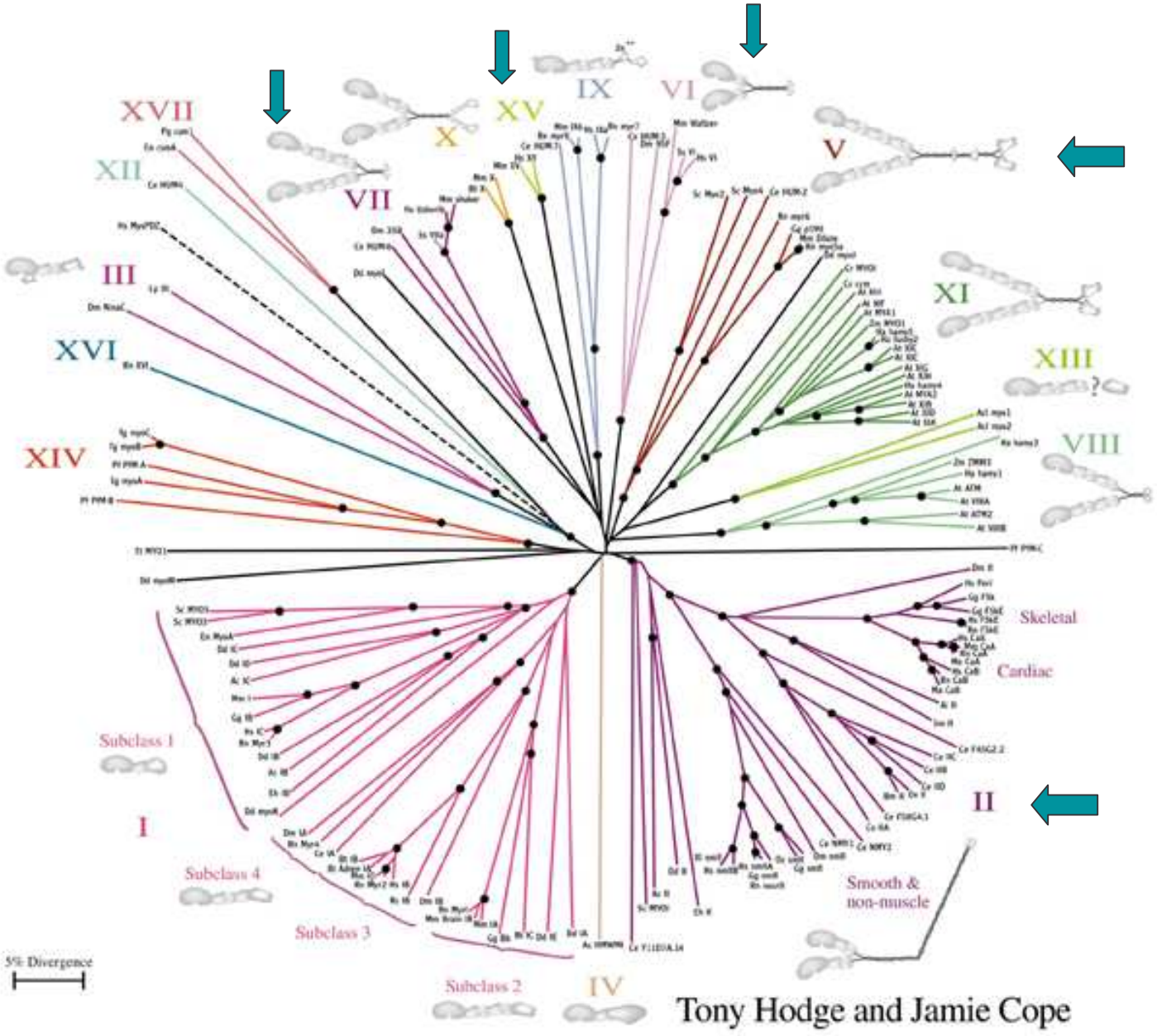
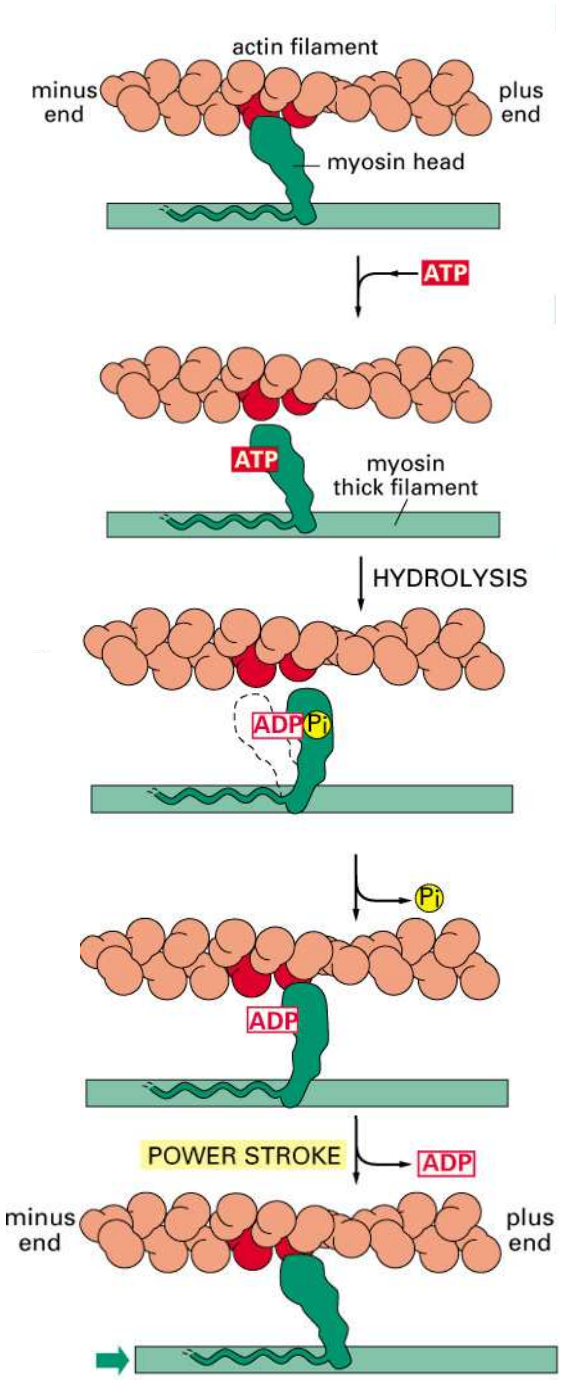


Figure 16–74. Molecular Biology of the Cell, 4th Edition.

Myosins Linked to Human Diseases




actin filament
minus end
plus end
myosin head
ATP
ATP
myosin thick filament
HYDROLYSIS
ADP
Pi
Pi
ADP
POWER STROKE
ADP
minus end
plus end

Tip link
Anchor
Filament 2
Elastic
Filament 1
Tip
Receptor
F_M
F
α
F

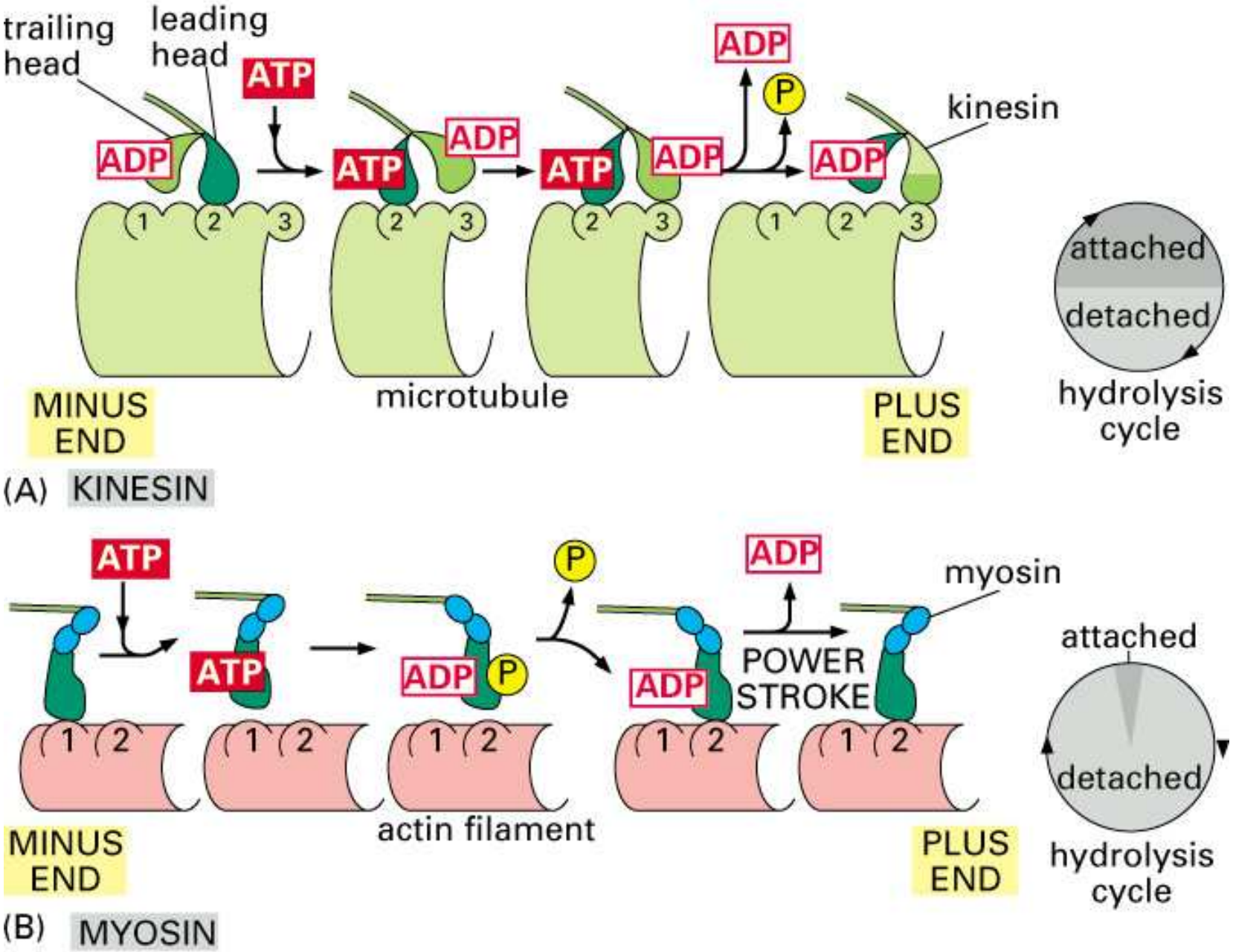


Figure 16–59. Molecular Biology of the Cell, 4th Edition.

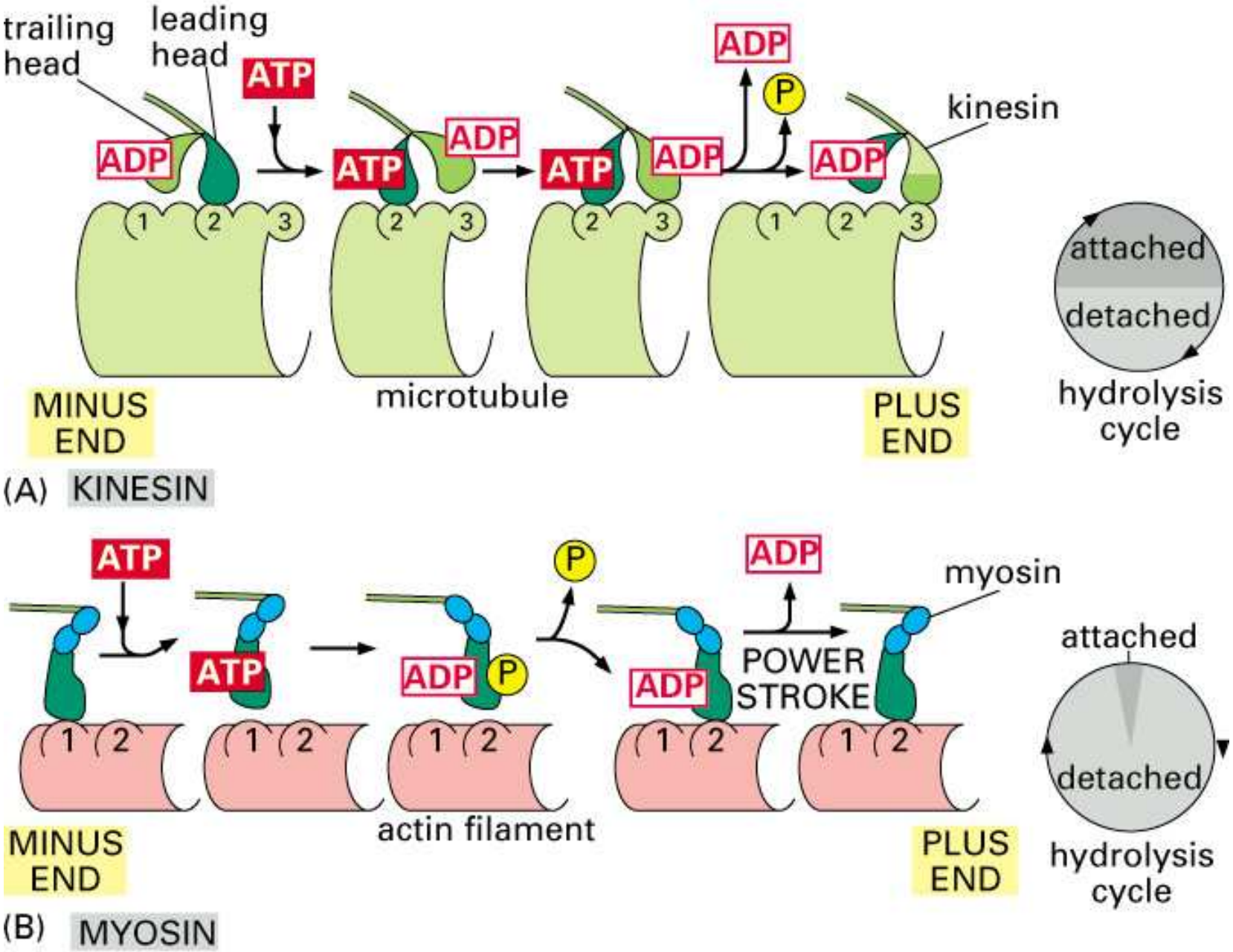
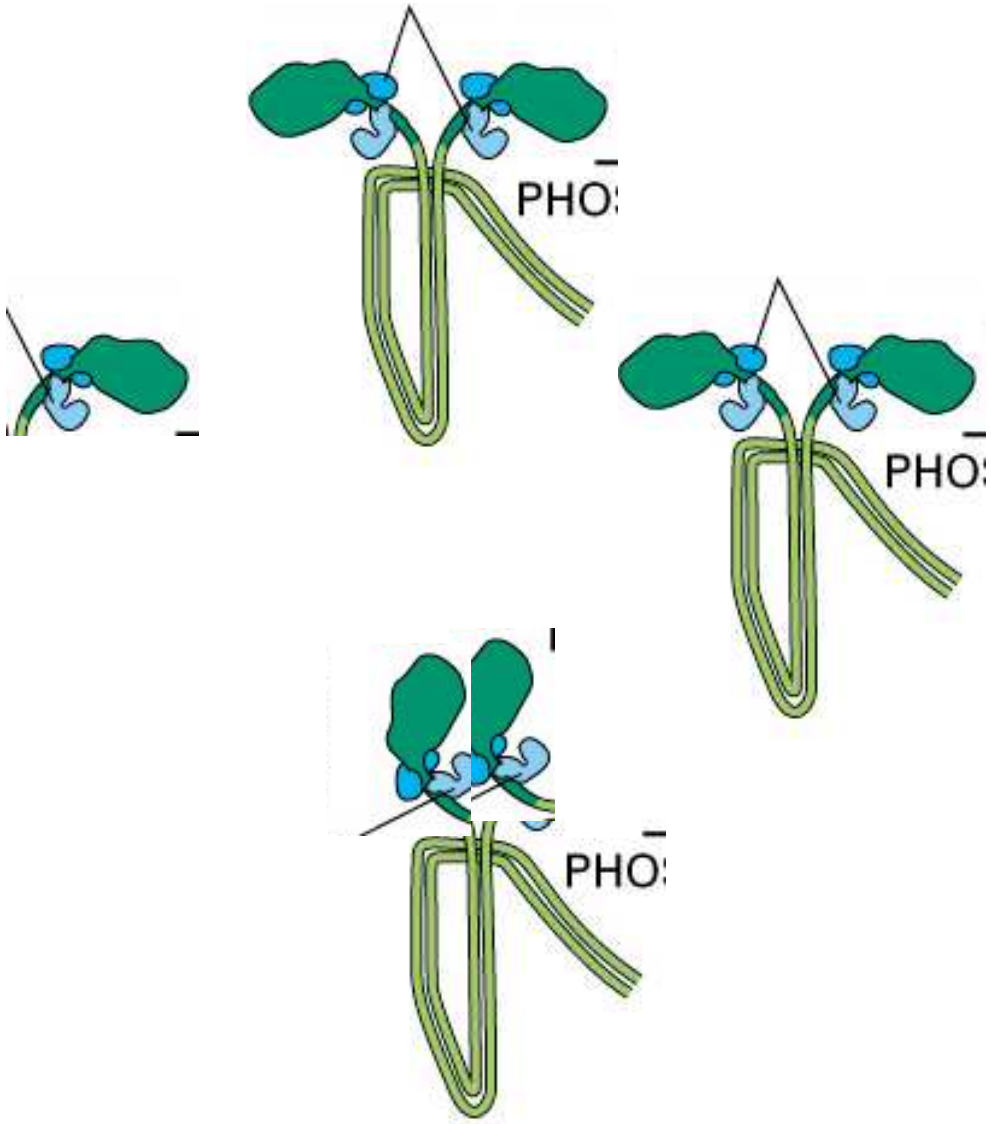


Figure 16–59. Molecular Biology of the Cell, 4th Edition.


PHO
PHO
PHO

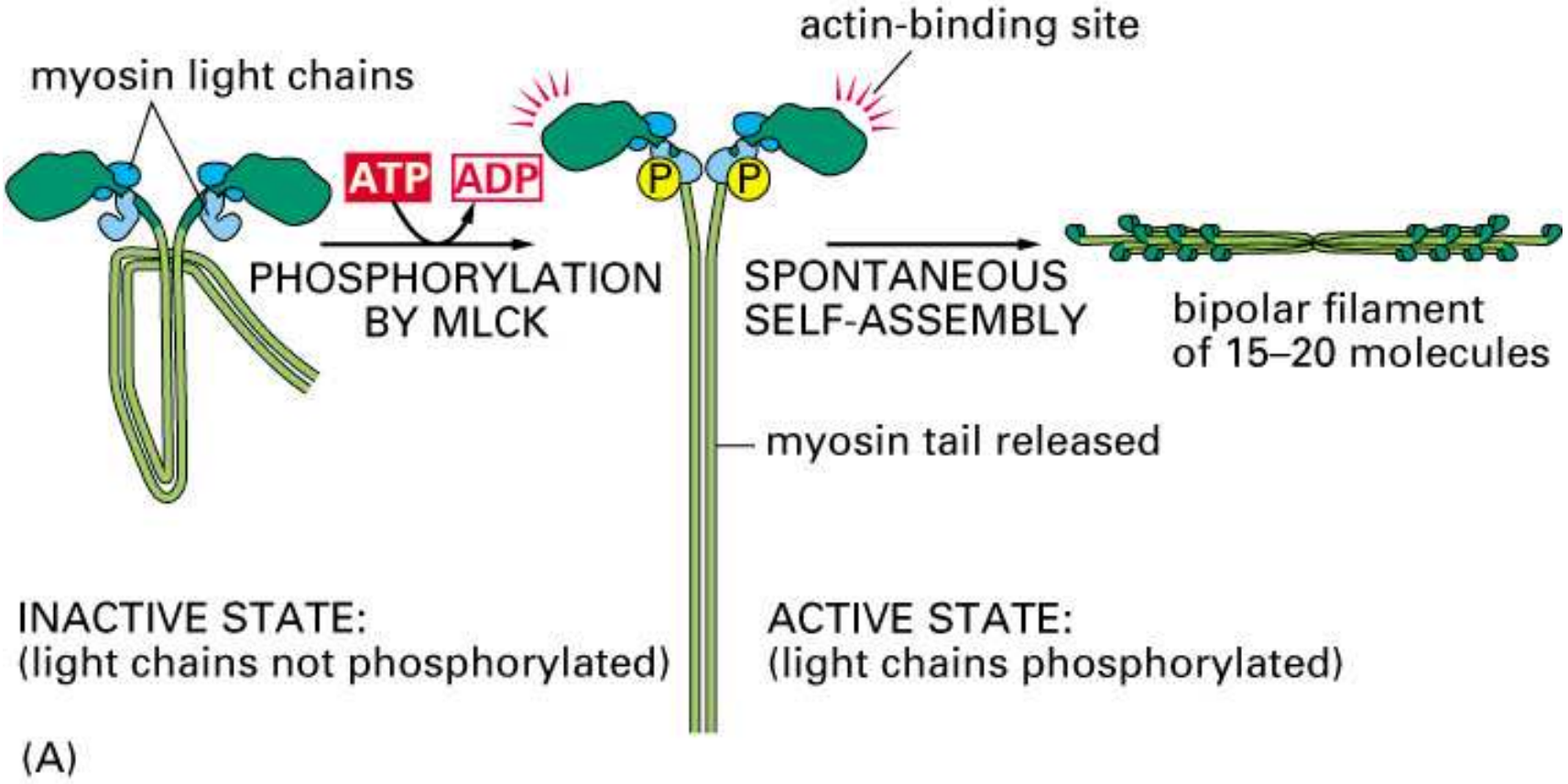


Figure 16–67 part 1 of 2. Molecular Biology of the Cell, 4th Edition.