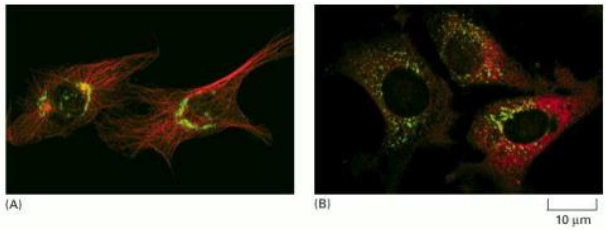
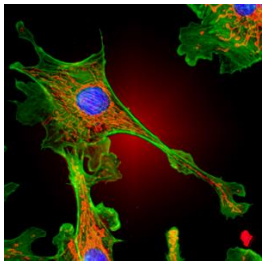


CYTOSKELETON AND CELL MOTILITY



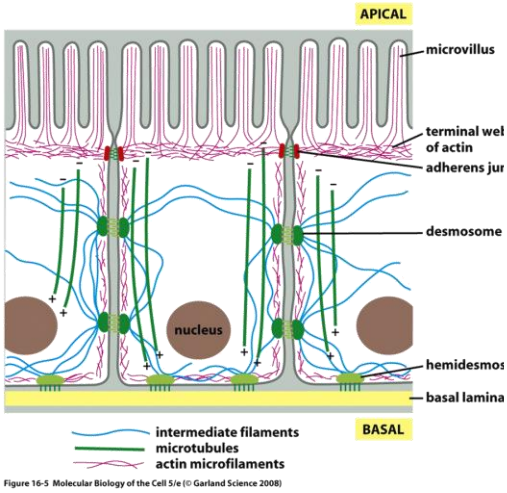
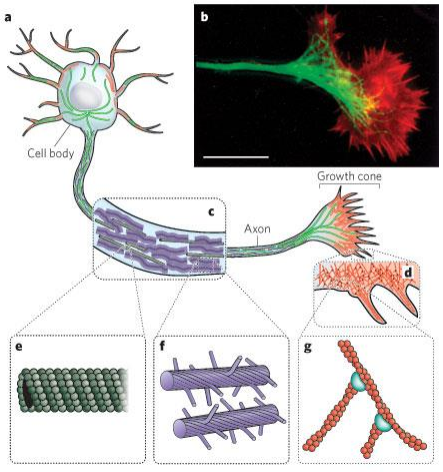


Figure 16-5 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Neurons have elaborate cytoskeletal structures

Cytoskeleton is structural protein that builds up membrane system and cytoplasmic components

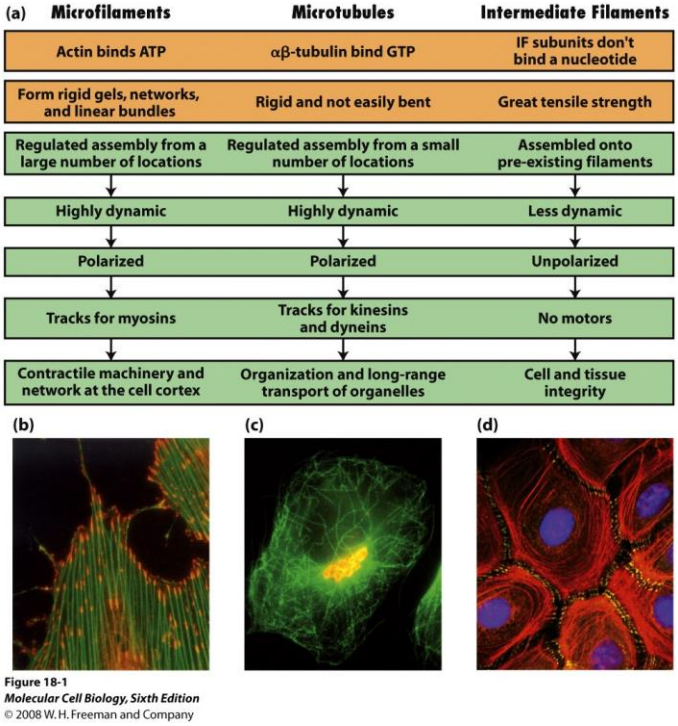
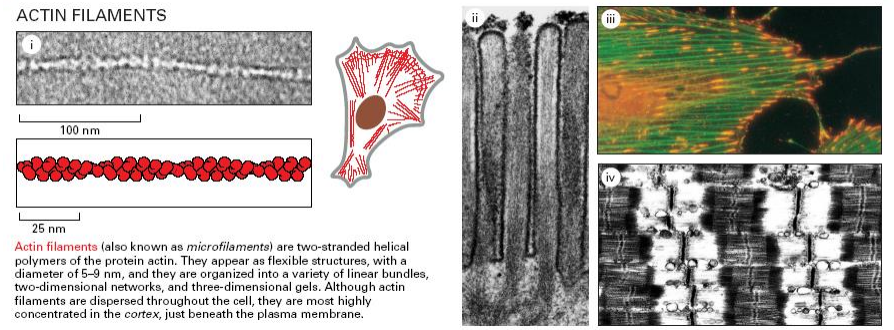
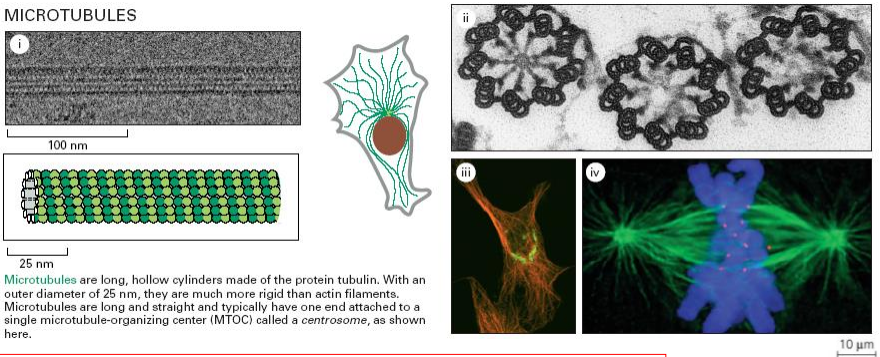


Figure 18-1
Molecular Cell Biology, Sixth Edition



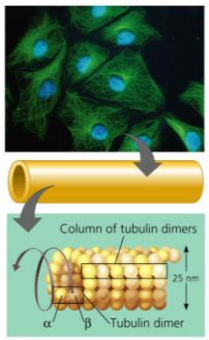
Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

Some functions of actin filaments are:
- to provide mechanical strength to the cell by forming a band under the plasma membrane
- link transmembrane proteins to cytoplasmic proteins
- form contractile ring during cytokinesis in animal cells
- cytoplasmic streaming
- generate locomotion in cells such as white blood cells and amoeba
- Interact with myosin to provide force of muscular contraction



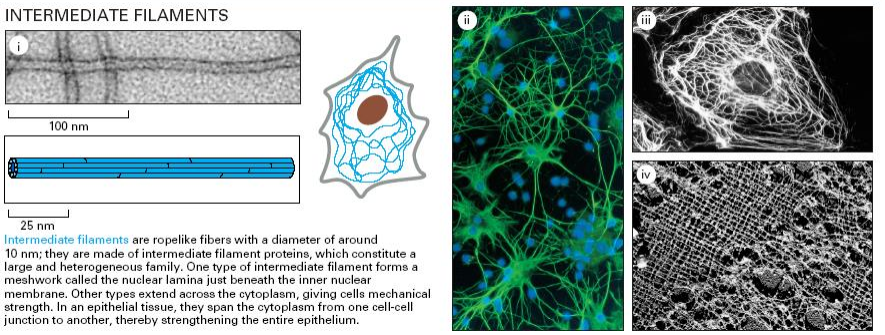
Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

Microtubules participate in a wide variety of cell activities. Most involve motion that is provided by protein "motors" that use ATP. They determine the positions of membrane-enclosed organelles and direct intracellular transport. The migration of chromosomes during mitosis and meiosis takes place on microtubules that make up the spindle fibers.



Other functions:

- **Mechanical – physical + give shape**
- **Neuron: vesicles pathway and cytoplasmic particles**
- **In embryo : conserve elongated shape from axon**
- **conserved internal organization of the cell**

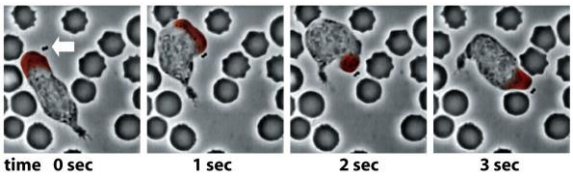


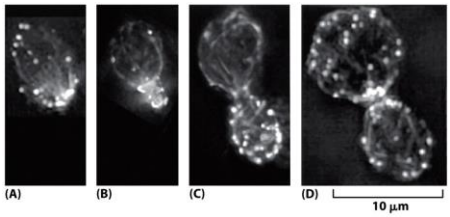
Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.

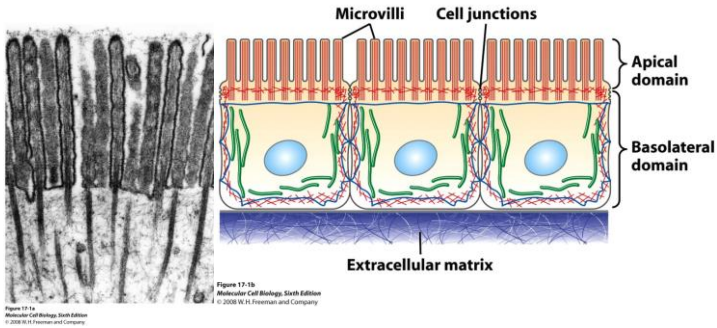
Intermediate filaments provide mechanical strength and resistance to shear stress. There are several types of intermediate filaments, each constructed from one or more proteins characteristic of it.

Keratins are found in epithelial cells, hair and nails
Nuclear lamins form a meshwork that stabilizes the inner nuclear membrane
Neurofilaments strengthen the long axons of neurons
Vimentins provide mechanical strength to muscle and other cells

- Cytoskeletal filaments are dynamic and adaptable
- Cytoskeleton can form stable structures

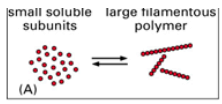


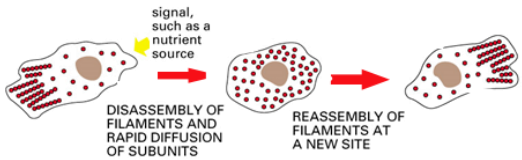




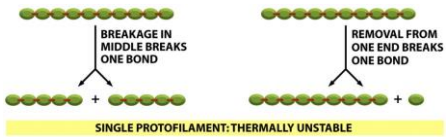
- **Monomer-polimer → filamen :**

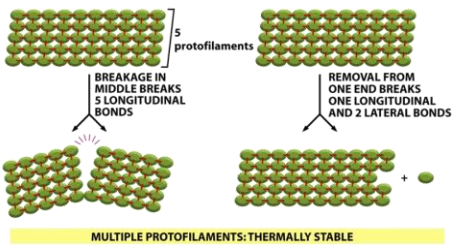
1. Microtubule : Tubulin
2. Filament : Actin
3. Intermediate Filamen : "fibrous"protein

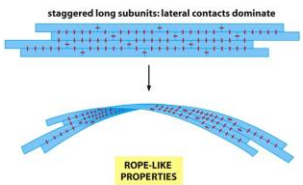




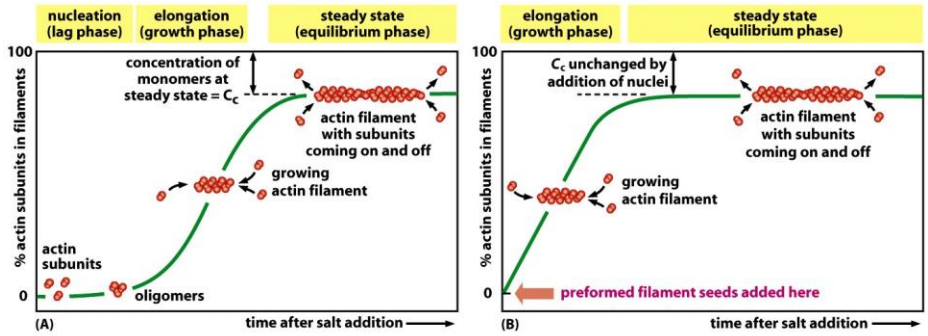
Multiple protofilaments
-resist thermal breakage while leaving the filaments ends as dynamic structure → addition and loss of subunits can occur rapidly
- IF tolerate streching, bending, strong





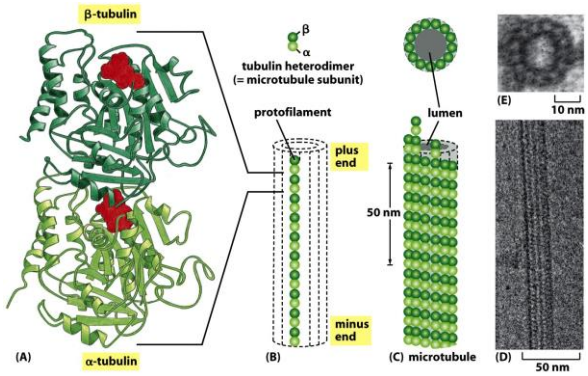


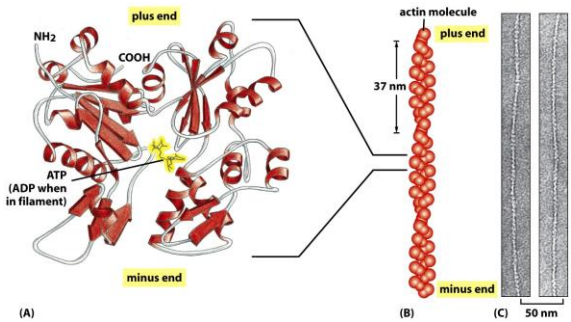
Cytoskeletal polymer formation



critical concentration/Cc=

- The concentration of free subunits in solution.
- rate constant for subunit loss divided by the rate constant for subunit addition that is, Cc = koff / kon.





- Two distinct ends
  - Plus end
    - Fast growing end
  - Negative end
- Different growing rates

polymer dynamics: 3 cases

•linear polymers

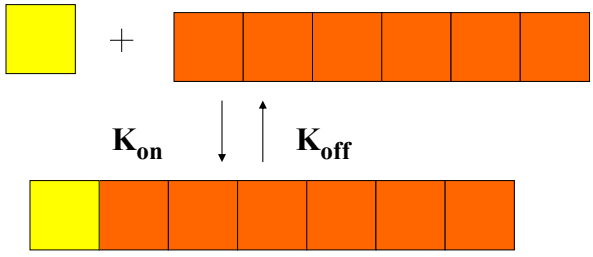
• polar polymers: asymmetric subunits
undergo conformational change during assembly

actin and microtubules

• complex polymers: non-equilibrium
➔ subunit nucleotide hydrolysis (energy input)

linear Polymer

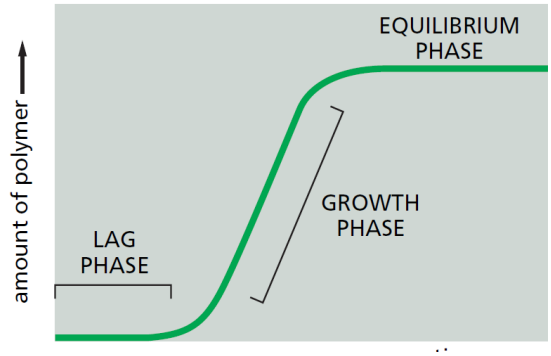
Assembles/disassembles by addition/loss of subunits at ends
Rates = K_{on} and K_{off}



K_{on} depends on concentration of subunit, units of $M^{-1}sec^{-1}$
K_{off} does not (unimolecular), units of sec^{-1}

Time course of polymerization

1) lag due to kinetic barrier to nucleation
2) growth
3) equilibrium

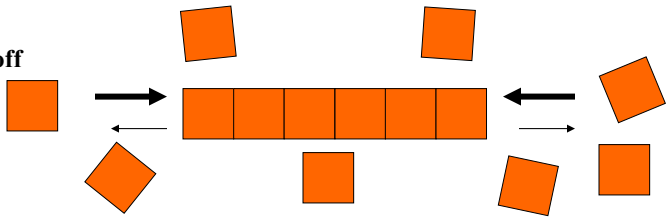


rate of subunit addition = rate of loss

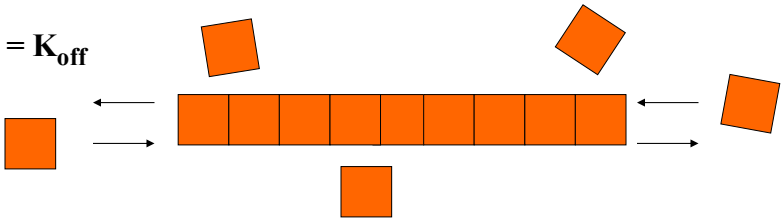
polymer grows, subunit concentration drops until $K_{on}[C] = K_{off}$, when [C] = critical concentration Cc

($M^{-1}sec^{-1}[M] = sec^{-1}$)

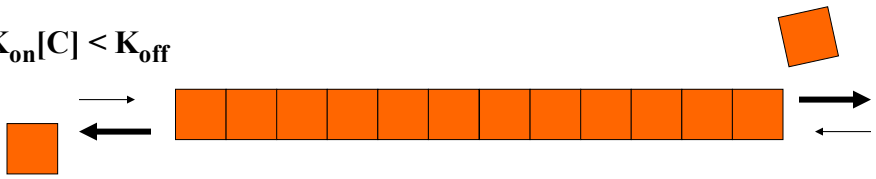
$K_{on}[C] > K_{off}$



$K_{on}[C] = K_{off}$



$K_{on}[C] < K_{off}$



Critical Concentration

- **Concentration of free subunits at which rate of subunit addition $K_{on}C$= rate of loss (Koff)**

- **Above Cc ➔ net growth, below Cc ➔ net shrinkage**

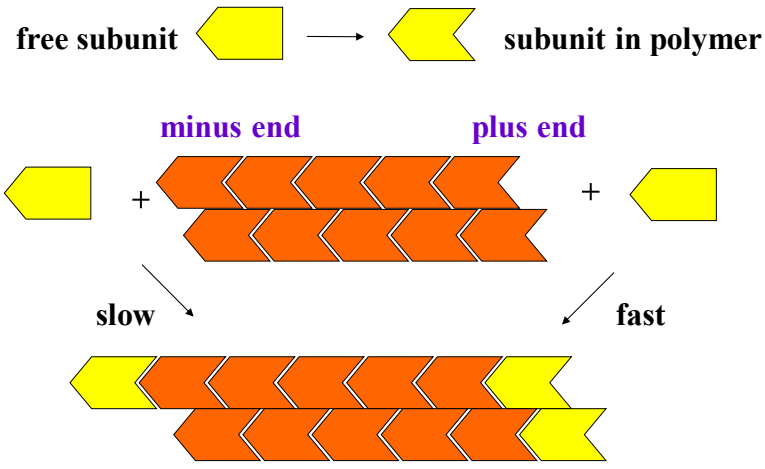
- **Equilibrium constant K_{eq} determined by change in free energy between free subunits and polymer**

$$k_{on}\, C = k_{off}$$

$$C_c = \frac{k_{off}}{k_{on}} = \frac{1}{K}$$

Polar Polymer

Two ends polymerize and depolymerize at different rates
BECAUSE
subunit conformation changes as it incorporates into the polymer



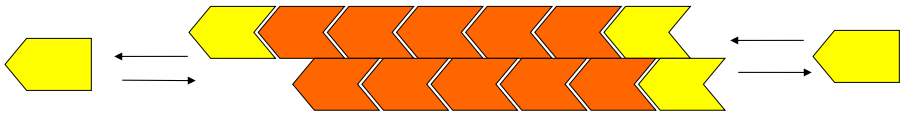
Plus and minus ends:

- Different K_{on} and K_{off}
- But!

K_{off}/K_{on} ratio or C_c must be the same for both ends:

>The same interactions are broken when a subunit dissociates from either end

>The final state of the subunit is identical



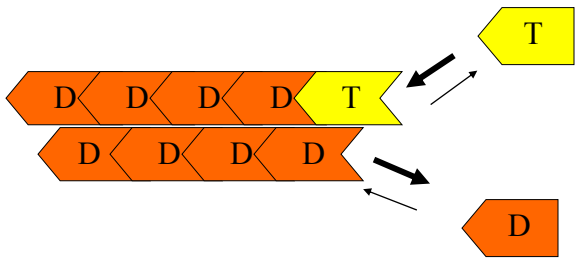
If the plus end grows 3 times faster it must also shrink 3 times faster.
Above Cc both ends grow, below Cc, both shrink

Complex Polymer (non-equilibrium): microtubules, actin filaments

Due to nucleotide hydrolysis upon assembly of subunit into polymer:

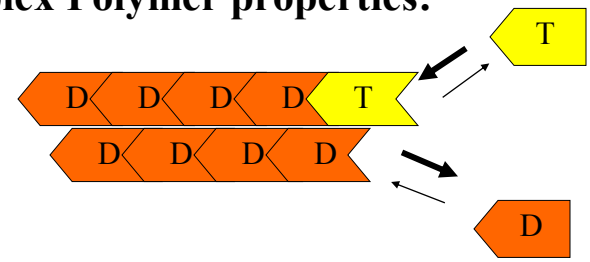
D= nucleotide diphosphate

T= nucleotide triphosphate



Nucleotide hydrolysis reduces binding affinity

Complex Polymer properties:



➲ **Internal subunits have different dynamic properties than the ends**

T form binds, D form dissociates

$K^{T}_{on} >> K^{D}_{on}$ $K^{D}_{off} >> K^{T}_{off}$

Cc = "steady state" concentration:

$K^{T}_{on}[C] = K^{D}_{off}$

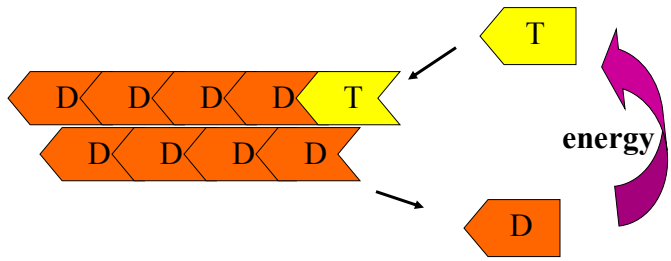
$Cc = K^{D}_{off} / K^{T}_{on}$

Steady State Dynamics

No longer true equilibrium, rather steady state because ATP or GTP subunits must be replenished

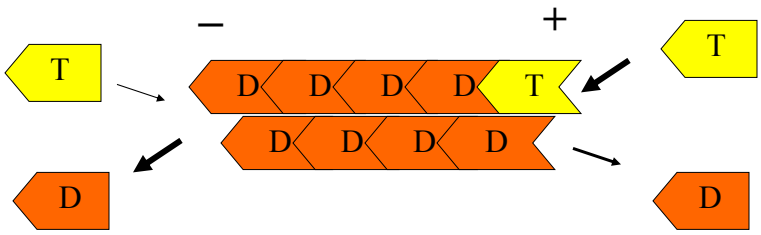
D= diphosphate

T= triphosphate



Consequences for polymer dynamics

Treadmilling (actin and microtubules)



- **Two different reactions at each end of the polymer**
- **Critical concentration different**

  Cc(- end) > Cc(+ end)

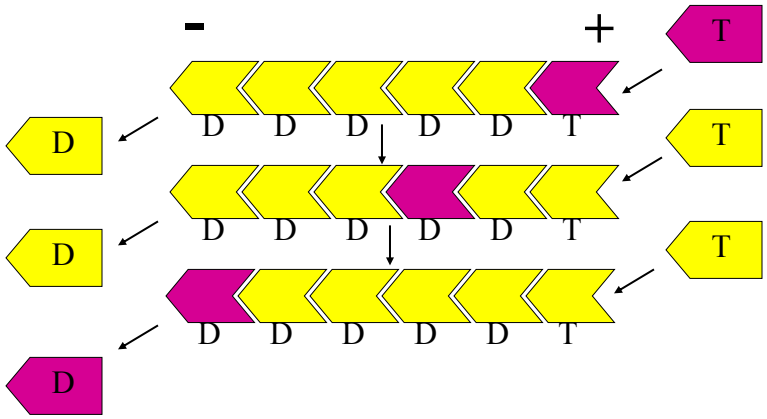
Treadmilling

Both ends exposed:
Steady state occurs at concentration between Cc(- end) and Cc(+ end)

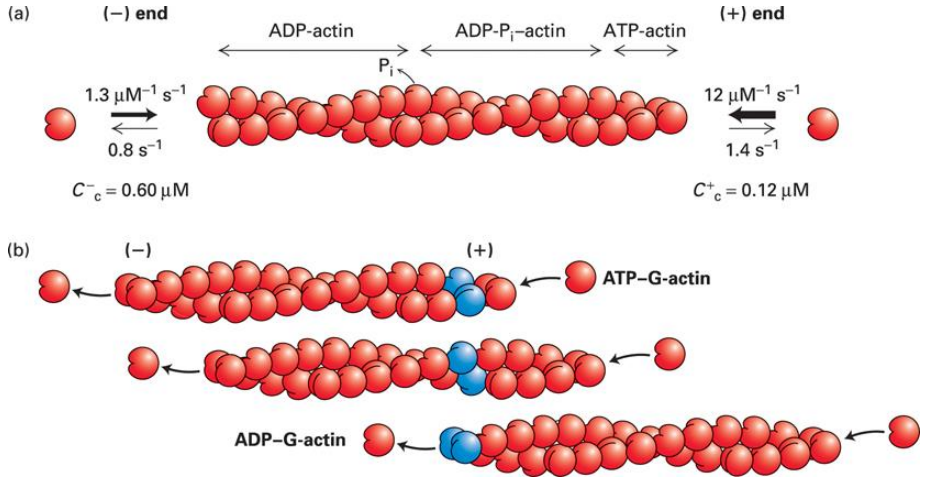
➲ **net assembly at the plus end**
➲ **net disassembly at the minus end**

subunits "flux" through the polymer

Treadmilling



Actin treadmilling.

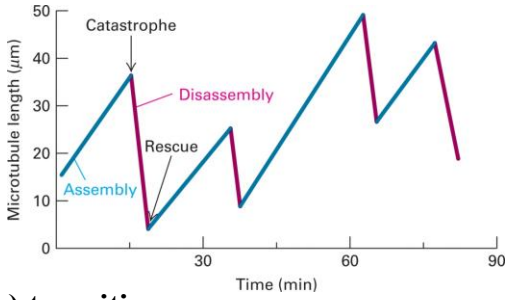


Dynamic instability (microtubules):

- **subunit addition is faster than nucleotide hydrolysis**
- **cap of GTP-tubulin on polymer ends**

K_{Doff}>>K_{Toff}: GTP cap favors growth

GTP Cap present:
Growth
GTP Cap lost:
rapid disassembly



- **stochastic (unpredictable) transitions**

- **frequency correlates with tubulin concentration**

Dynamic Instability

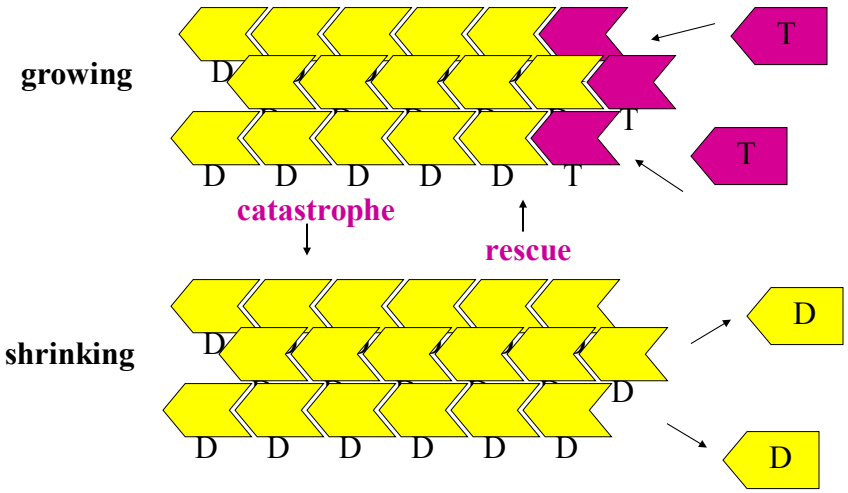
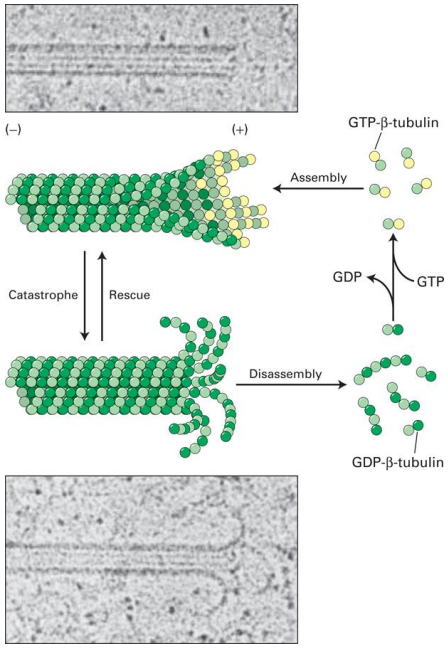
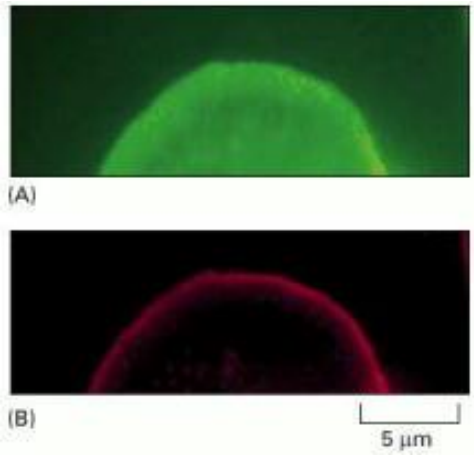


Figure 18.11 **Dynamic instability depends on the presence or absence of a GTP-β-tubulin cap.**



Actin Filaments



- The tip of the leading edge of a cell nucleates actin filaments.
- Actin filament nucleation most frequently occurs at the plasma membrane → highest density of actin filament is at the cell cortex

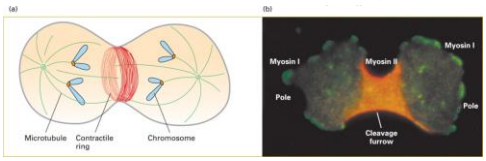
Actin Filaments

Actin function:

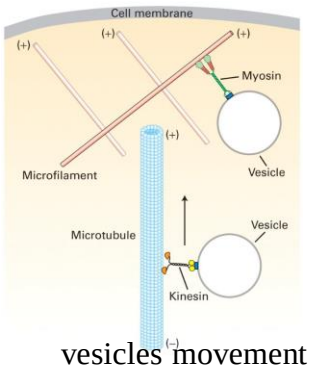
Cell movement

Cell shape

Cytoplasmic contractile

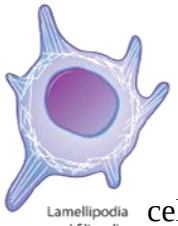


"Contractile bundles"



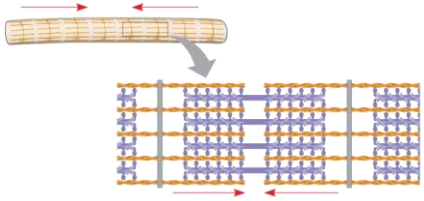
vesicles movement (in actin filaments)

Microvilli



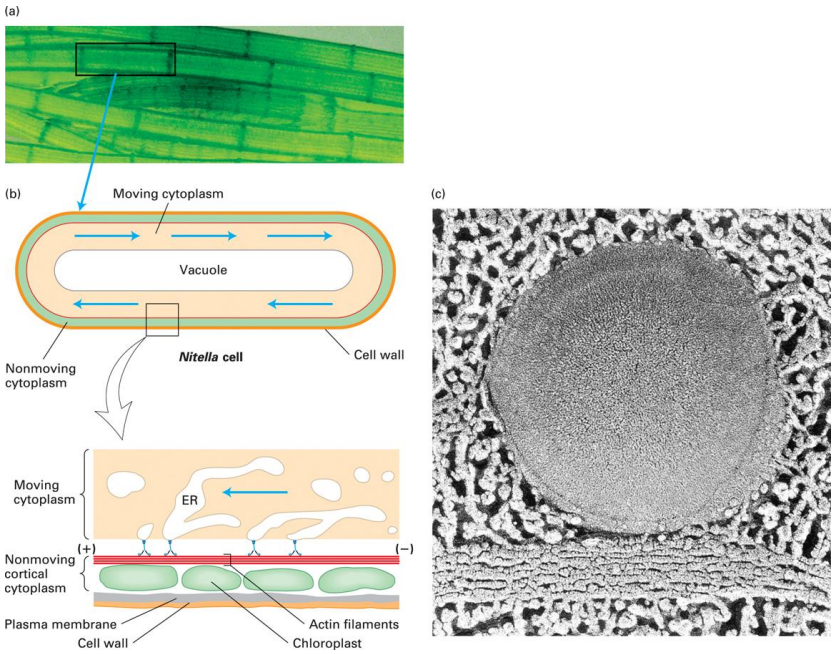
Lamellipodia and filipodia

cell migration



"Thick and thin filaments" (e.g.muscle contraction)

Cytoplasmic streaming in cylindrical giant algae.



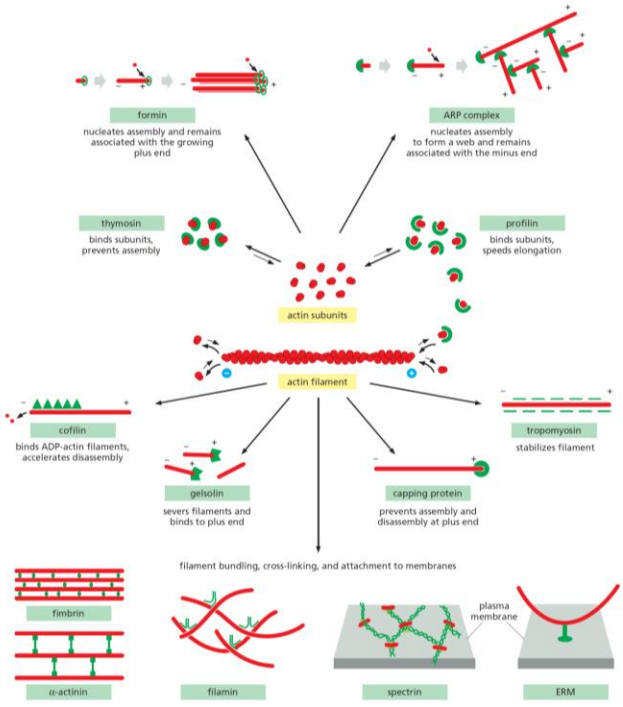
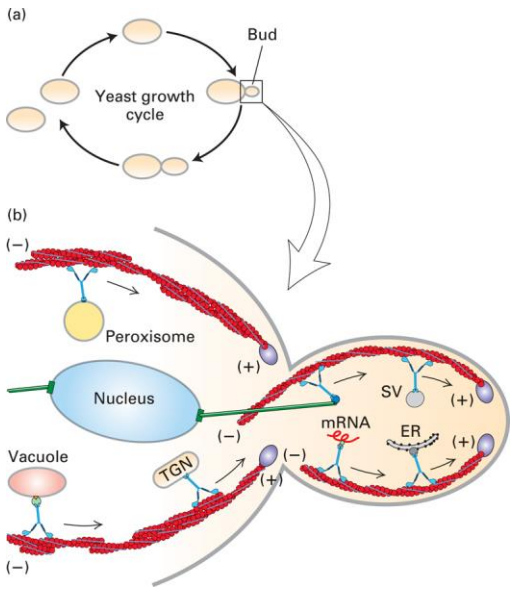
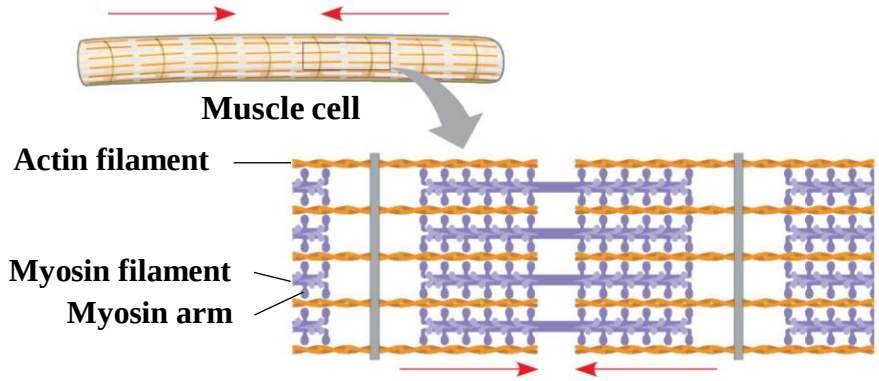


Figure 17.38 **Cargo movement by myosin Vs in budding yeast.**



http://bcs.whfreeman.com/lodish7e/#800911__816642__

Actin/Myosin Fibers: muscle contraction



(a) Myosin motors in muscle cell contraction

Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes

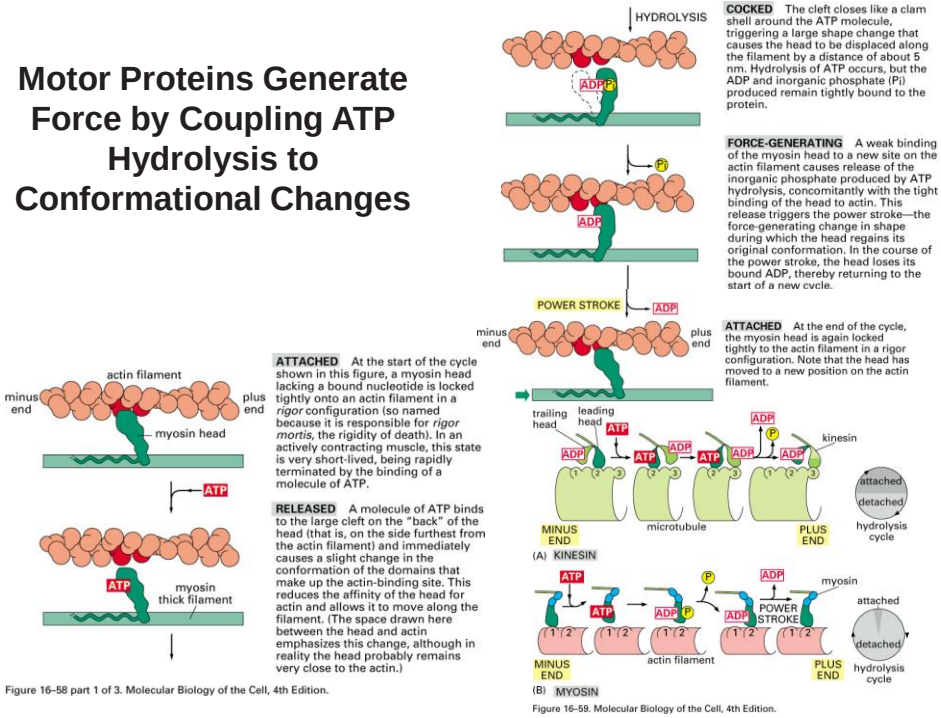


Figure 16–58 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

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

Motor proteins transition/cycle between different conformations:
one step is driven by the hydrolysis of ATP,
thereby making the cycle essentially irreversible and movement unidirectional

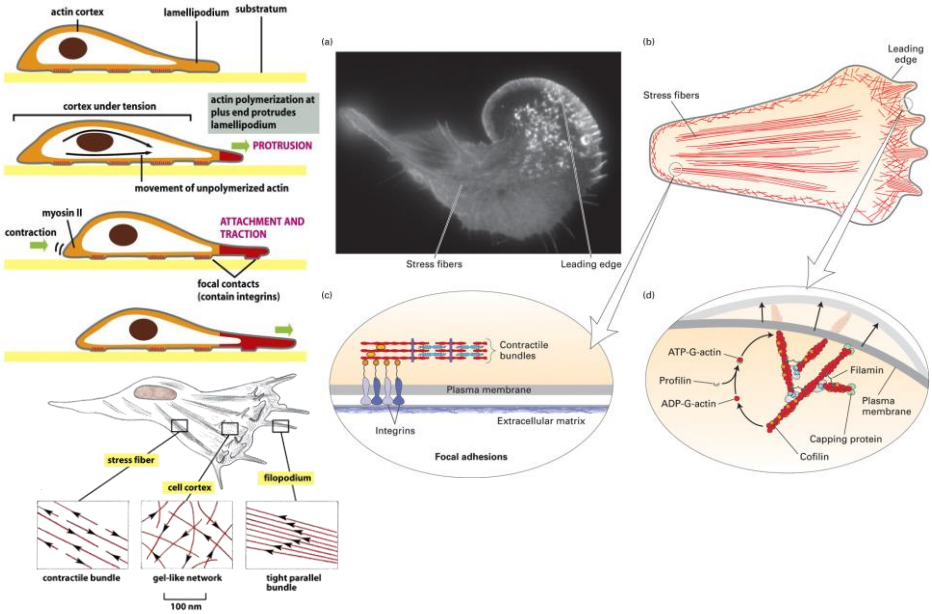
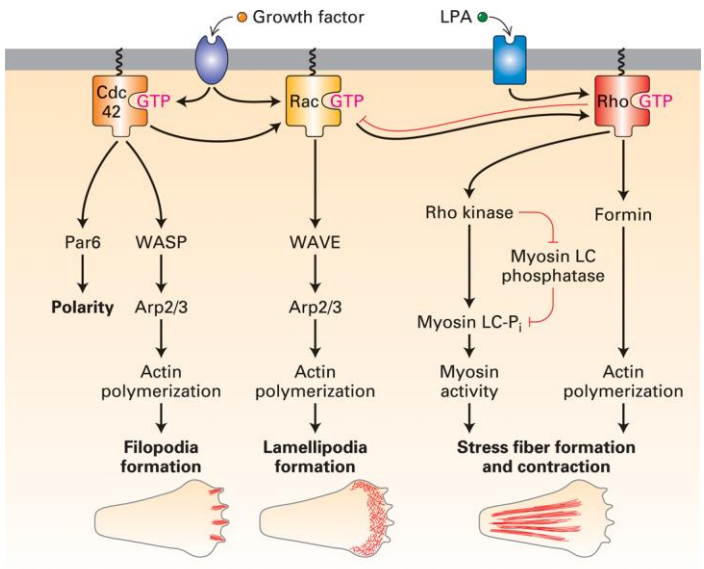
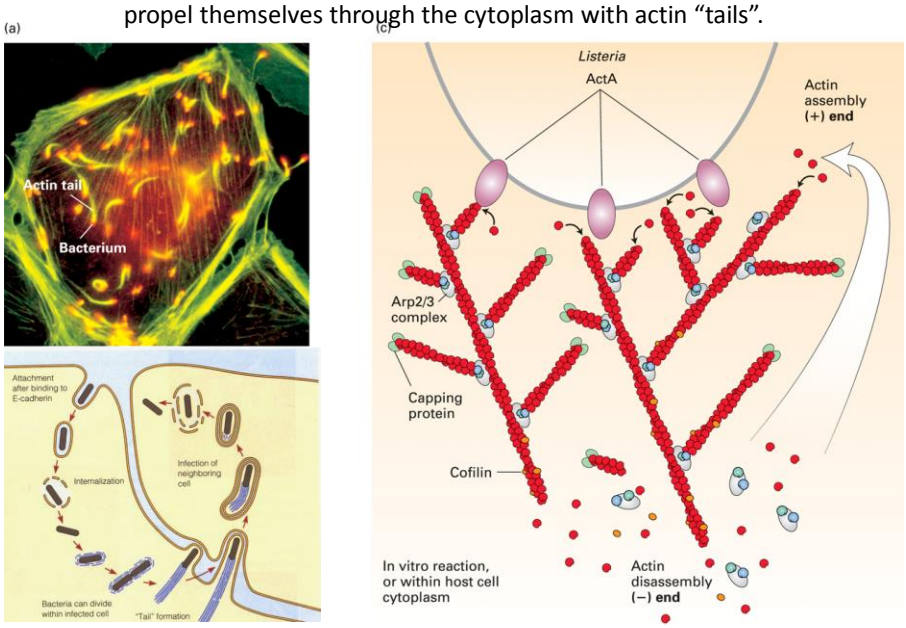


Figure 17.44 **Summary of signal-induced changes in the actin cytoskeleton.**

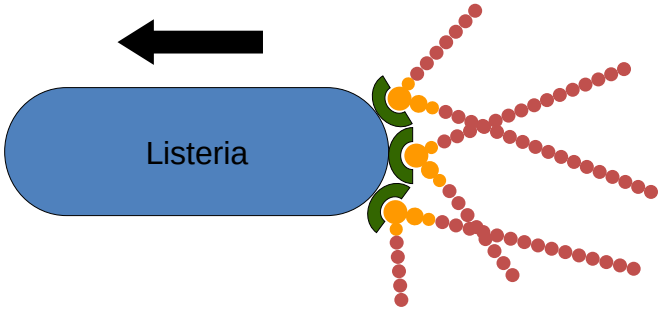


Some intracellular pathogens such as the bacteria *Listeria* and *Shigella* and the vaccinia virus usurp the host cell's mechanism of assembling actin networks and propel themselves through the cytoplasm with actin "tails".



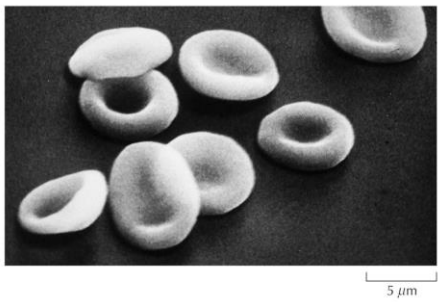
Stealing the machinery

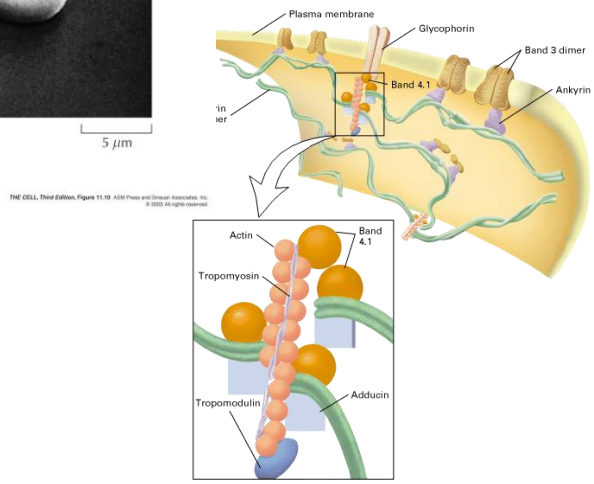
- Listeria has on its surface the protein **ActA**
- **ActA** *recruits* **Arp2/3** from the cytoplasm and *activates* it (basically substituting for WASP)
- Promotes actin filament nucleation and growth



Biconcave Red Blood Cells

the structural basis for the cortical cytoskeleton in erythrocytes-spectrin





Actin specific drugs

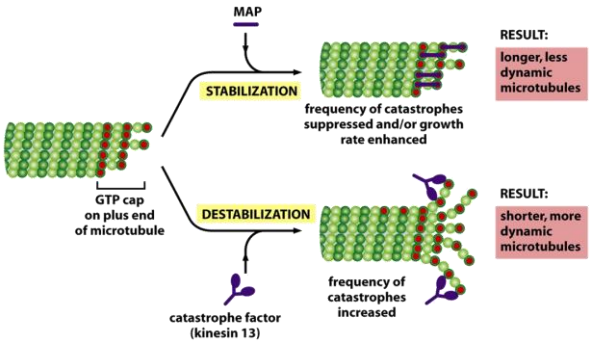
- **Phallotoxin (phalloidin)**
  - an actin filament stabilizer
  - the poison in some mushroom genera
  - It kills by *stabilizing* actin filaments (inhibiting disassembly)
  - Immediate cause of death is liver failure
- **Cytochalasin**
  - an actin filament de-stabilizer
  - also derived from mushrooms
- **Swinholide**
  - Severs filaments
- **Latrunculin**
  - Binds subunits and prevents their polymerization

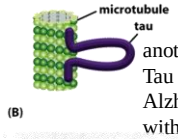
Death Cup mushroom

MICROTUBULE

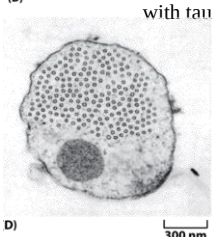
Structure and composition :

- Rod shape (tubule) exist almost in all eukaryote cells
- Function in mitosis and cell movement →cilia dan flagella
- Consist of proto -filaments → paralel along the axis of tubules.
- Protofilament consists of 2 kind of tubulin molecules :
  α dan β tubulin



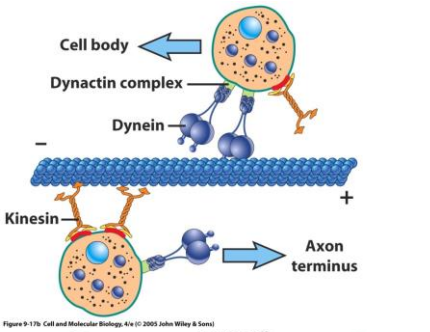


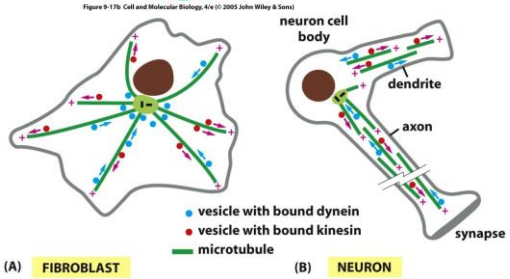
another type of MAP: Tau
Alzheimer – problem with tau protein

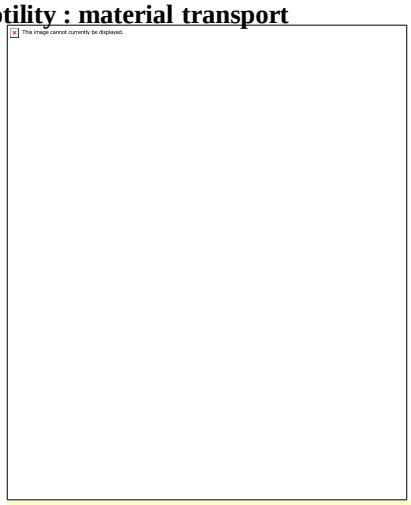


Microtubule play a role in intracellular motility : material transport

Protein motor : kynesin, dynein

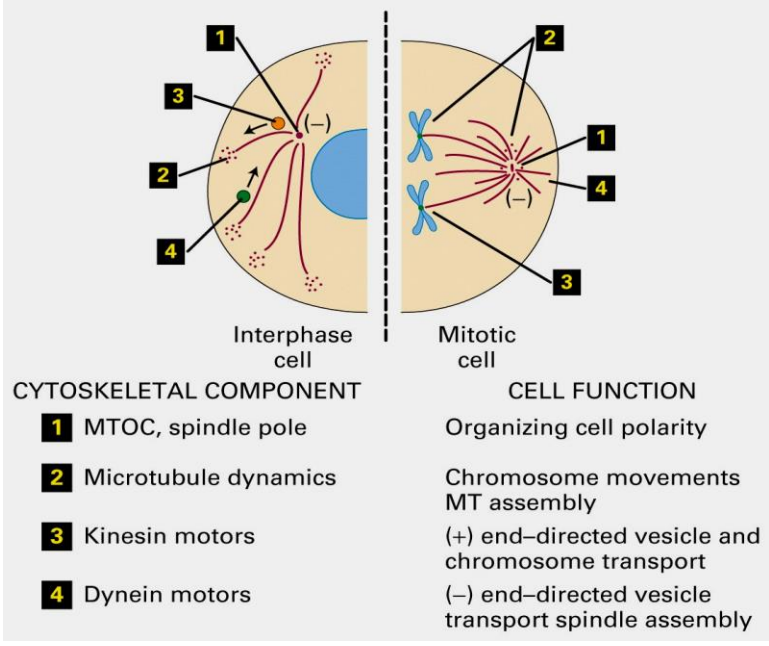






kynesin & dynein → vesicle movement from cell to synaptic terminal & organelles transport

dynein → cilia and flagella movement



Organization of MT around the MTOC and spindle poles

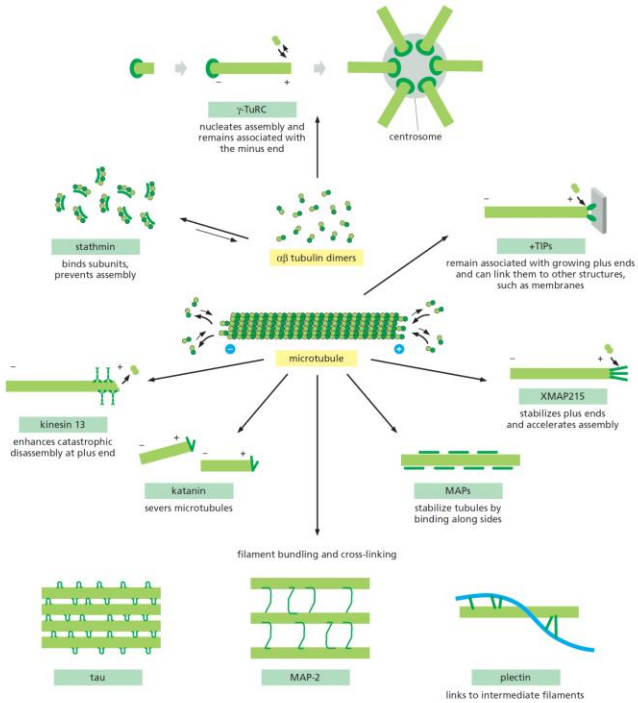
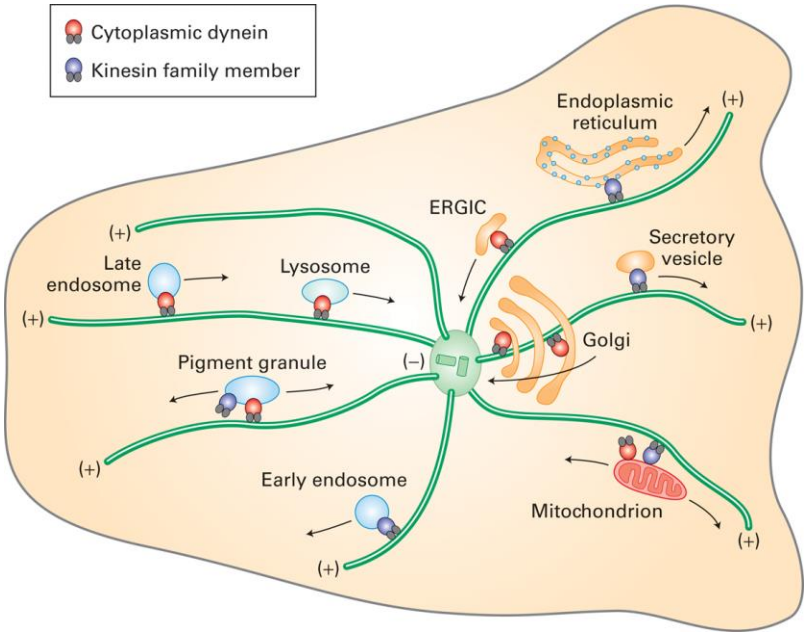
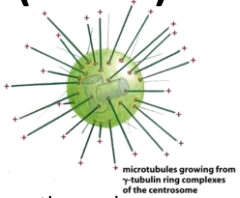


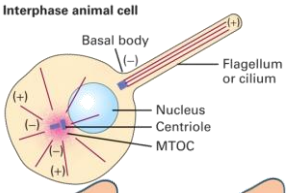
Figure 18.27 **Organelle transport by microtubule motors.**

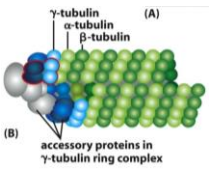


Microtubule-Organizing center (MTOC)

- **the place where enucleation of tubulin is happened**
  - **tubulin molecule start to organize and elongate**
  - **centrosome, basal body**
- **Centrosome**
  - **Only in animal cells**
  - **Consists of 2 centrioles & peri-centriolar material,** located near the nucleus
- **basal body**
  - **Microtubules from cilia and flagella start from basal body**
- **Polymerization in MTOC**
  - is started with the arrangement of γ-tubulin in the nucleation center and then polymerization will continue with the arrangement of α and β tubulin







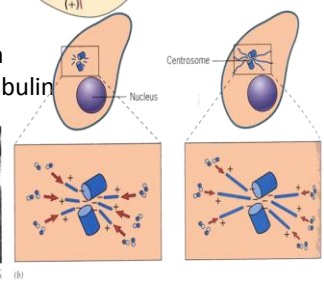
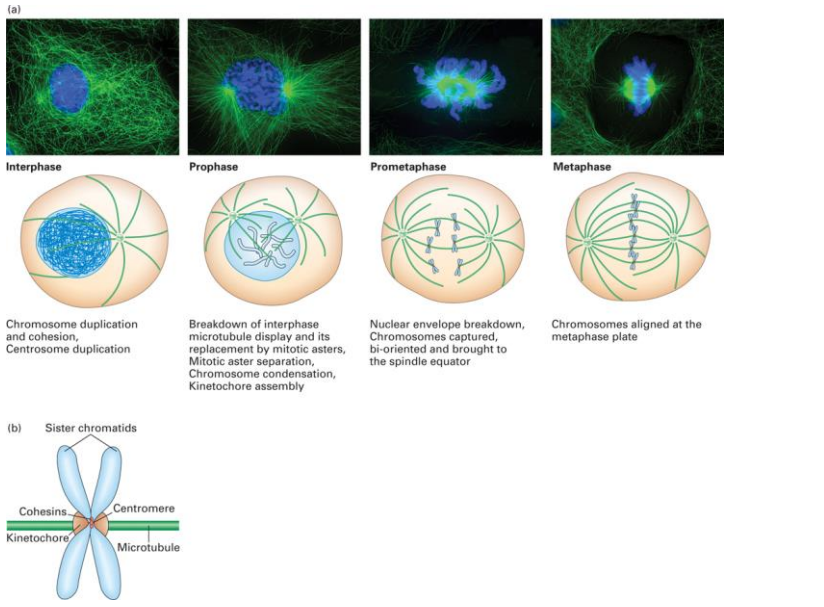
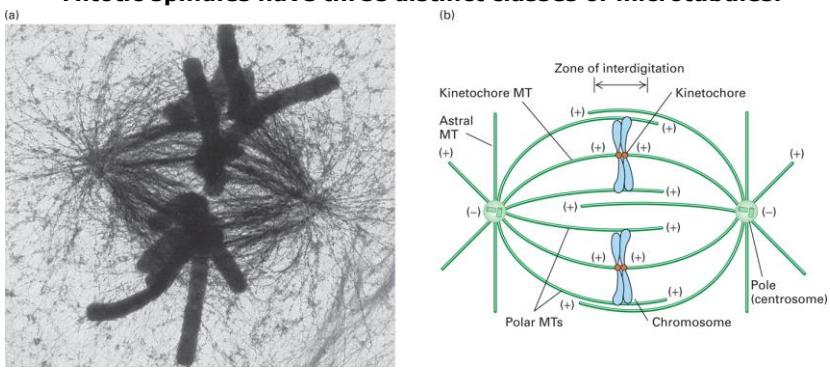


Figure 18.36 **The stages of mitosis.**



Mitotic spindles have three distinct classes of microtubules.



The structure of a mammalian kinetochore

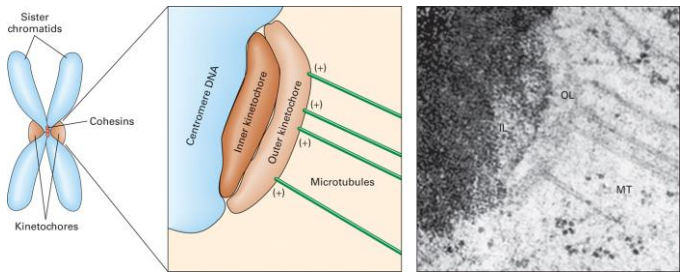


Figure 18.40 **Chromosome capture and congression in prometaphase.**

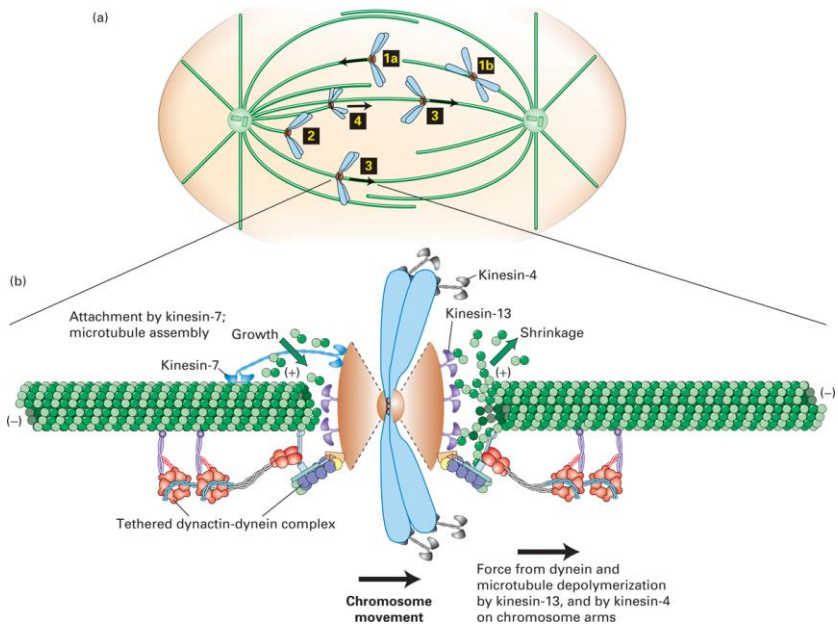
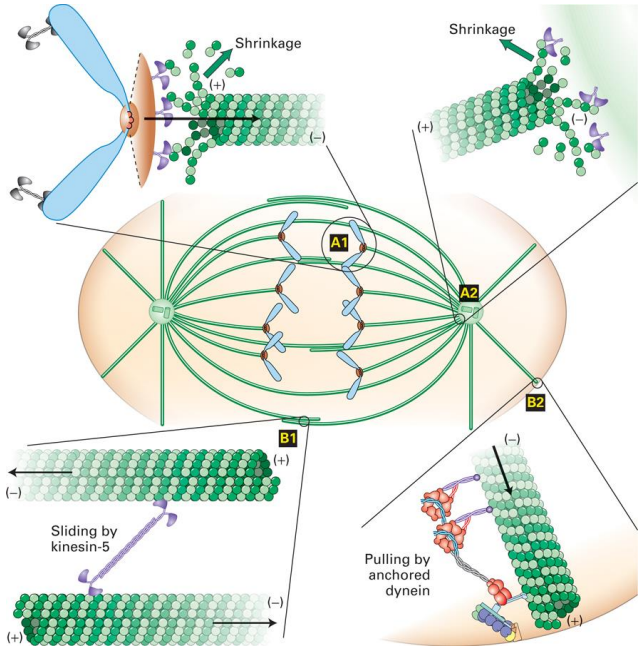
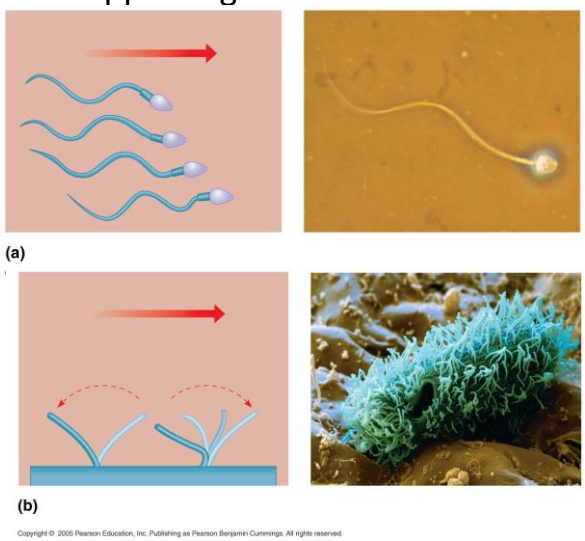


Figure 18.42 **Chromosome movement and spindle pole separation in anaphase.**



Cilia and Flagella

- Microtubules control the beating of cilia and flagella, locomotor appendages of some cells

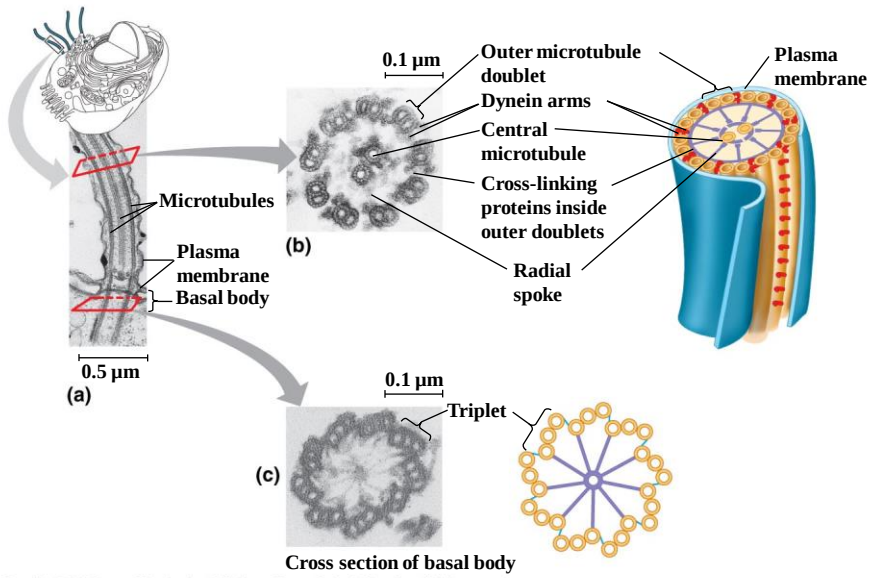


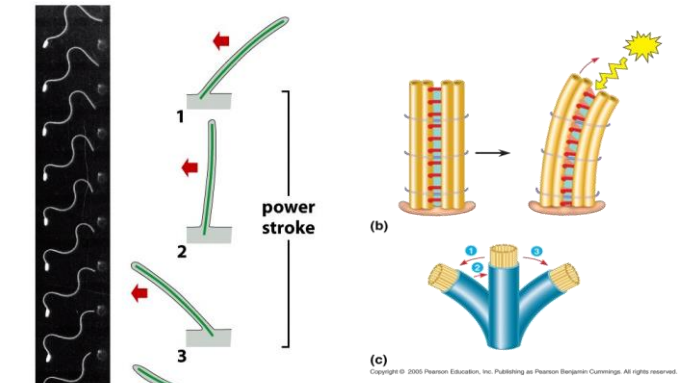
flagella

cilia

Cilia and Flagella

- Cilia and flagella share a common ultrastructure:
  - **Flagella is longer than cilia**
  - **Consists of axonem (center) that surrounded by 9 double microtubules**
  - **MTOC : basal body →** A basal body that anchors the cilium or flagellum
  - A motor protein called dynein, which drives the bending movements of a cilium or flagellum

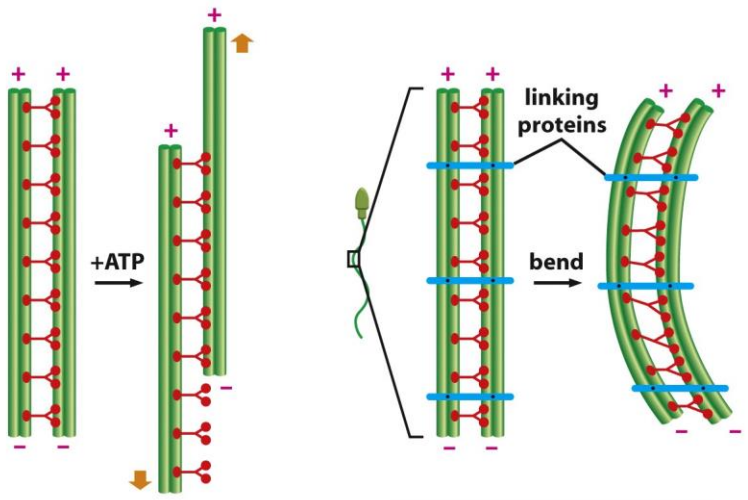




The contrasting motions of flagella and cilia.

(A) The wave-like motion of the flagellum of a sperm cell from a tunicate.

(B) The beat of a cilium, which resembles the breast stroke in swimming. A fast power stroke *(red* arrows), in which fluid is driven over the surface of the cell, is followed by a slow recovery stroke.



(A) IN ISOLATED DOUBLET MICROTUBULES: DYNEIN PRODUCES MICROTUBULE SLIDING

(B) IN NORMAL FLAGELLUM: DYNEIN CAUSES MICROTUBULE BENDING

Substances that interfere microtubule

- **NOKODAZOL → inhibit polymerization → substance binds to tubulin →inhibit + end addition**
- **COLCHICINE → de-polymerization**
  - from the Autumn Crocus (a lavender)
  - causes disassembly of microtubules

- **VINBLASTIN & VINCRISTIN → de-polymerization of microtubules**
- **TAXOL → increase microtubule's stability → as anticancer drug**

Coordination and cooperation between cytoskeletal elements

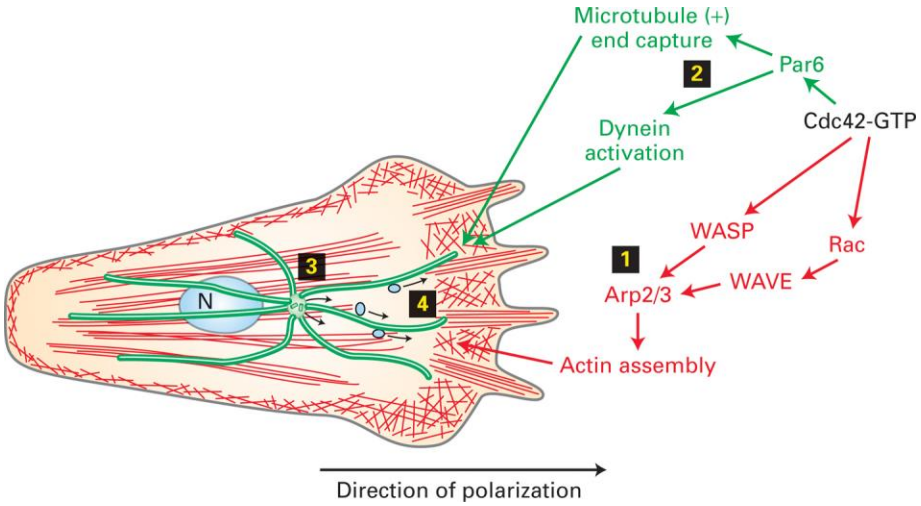
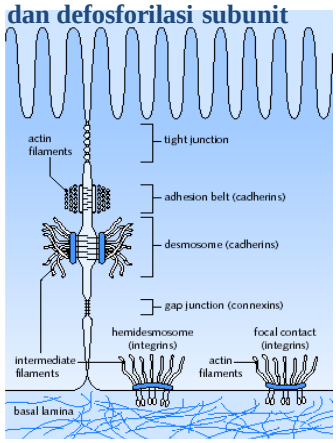


Figure 18.51 **Independent Cdc42 regulation of microfilaments and microtubules to polarize a migrating cell.**

Intermediate filament

Resistant to pressure, e.g in cornified skin (including human skin)→ IF , skin is waterproof, resistant against bacteria or chemical substances

Assembly and disassembly → because phosphorilasi dan defosforilasi subunit



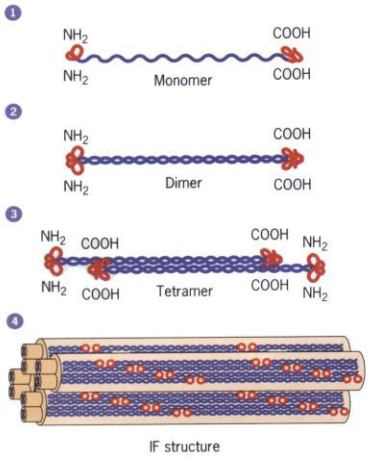


Figure 9.41 A model of intermediate filament assembly and architecture. Each monomer has a pair of globular terminal domains separated by a long α-helical region (step 1). Pairs of monomers associate in parallel orientation with their ends aligned to form dimers (step 2). Depending on the type of intermediate filament, the dimers may be composed of identical monomers (homodimers) or nonidentical monomers (heterodimers). Dimers in turn associate in an antiparallel, staggered fashion to form tetramers (step 3), which are thought to be the basic subunit in the assembly of intermediate filaments. The organization of the tetrameric subunits within the filament is shown in step 4.

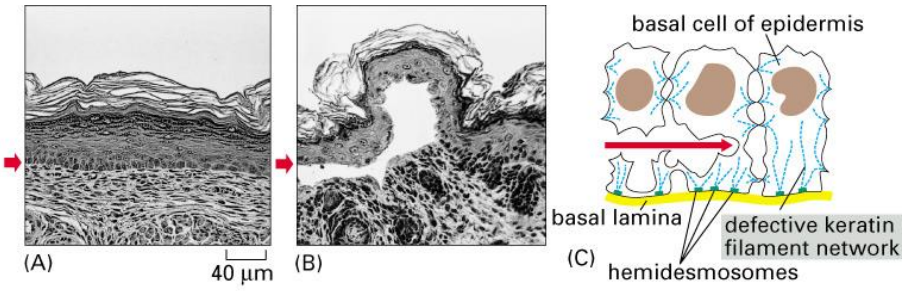


Figure 16–19. Molecular Biology of the Cell, 4th Edition.

Mutant of keratin gene causes peel of/ wound in the skin (Epidermolysis bullosa simplex)

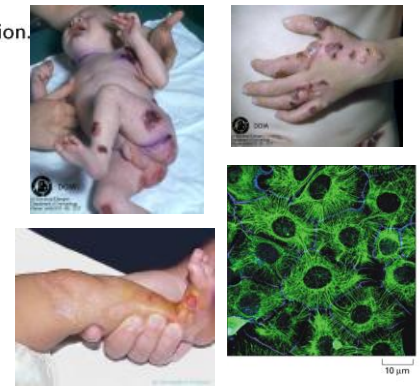


Figure 16–18. Molecular Biology of the Cell, 4th Edition.