Chromosomes & mitosis & miosis

organelles: functional compartment, many of them are surrounded by lipid membranes
nucleus: a prominent, centrally located organelle containing the genome

Cells divided by MITOSIS and Cytokinesis

Mitosis: chromosome division, chromosomes condense and can be seen as individual entities.
Cytokinesis: cell division

During most of the cell cycle, individual chromosomes cannot be observed, but they become visible during mitosis.
Chromosomes are highly dynamic and flexible, adding to the challenges of study of them.
DNA is packaged into chromosomes, which are much, much shorter than the DNA fiber

2 nm: short region of DNA double helix

The first level of chromatin packaging in eukaryotes is the wrapping of DNA around "**nucleosomes**" to form an 11-nm fiber

11 nm: "beads-on-a-string" from of chromatin

DNA is also packaged into large-scale "loops," which are thought to be made by proteins that can "walk" along DNA like motors.

During interphase, these loops are made by **COHESINS**. During mitosis, substitution of some cohesins by related proteins called **CONDENSINS** lead to further genome compaction.

30 nm (controversial): chromatin fiber of packed nucleosomes
700 nm: chromatin fiber folded into loops
1400 nm: entire mitotic chromosome

REPLICATION ORIGINS - sites along each chromosome where DNA replication initiates
CENTROMERE - specialized site that forms the **KINETOCHORE**, a structure that enables chromosomes to attach to the spindle during mitotic and meiotic division
TELOMERES - unique sequences at chromosome ends enable complete replication of chromosomes; also recruit **SHELTERIN** to protect chromosome ends from fusion/recombination

Chromosomes replicate during S-phase, and divide during mitosis
MITOSIS is the process by which dividing cells partition their chromosomes to produce genetically identical daughters.

interphase:
- G1(gap 1): Cell growth
- S(synthesis): DNA replication
- G2(gap 2): Cell growth

mitosis:
- prophase: crossing-over (genetic exchange)

In most organisms, telomeres associate with the nuclear envelope during early meiotic prophase to facilitate homolog pairing and synapsis.

In some cases this leads to clustering of telomeres, known as the "meiotic bouquet."

prometaphase:

metaphase:

anaphase: "Reductional" division (MI)

telophase: "Equational" division (MII)

cytokinesis: cell division

Homolog pairing is promoted by interactions with the nuclear envelope

Hartwell, Nurse and Hunt:

Cell fusion experiments in the 1970s suggested that a factor in mitotic cells can induce chromosome condensation in an interphase cell. Some soluble factor – designated "MPF" (mitosis-promoting factor) – can cause chromosome condensation when the cytoplasm of mitotic cell and an interphase cell are mixed.

MPF regulates condensins activity to promote chromosome compaction during mitosis. MPF is a serine/threonine kinase (an enzyme that phosphorylates many other proteins on A/T residues)

2 protein subunits work together:

1) A regulatory subunit, Cyclin B, rises and falls in abundance during each cell cycle.

2) The catalytic subunit: cyclin-dependent kinase (Cdk1 does not change in abundance, but its activity rises and falls in response to Cyclin B levels.

Budding yeast: Growth and cell cycle progression are coupled, so the bud size reveals the cell cycle state.

Isolation of temperature-sensitive "conditional" cell division control (cdc) mutations in S. cerevisiae or S. pombe (respectively)

replica plate each plate to 2 new plates, and grow at permissive and restrictive temperatures.

COHESINS and CONDENSINS are evolutionarily related to each other and to other members of the "Structural Maintenance of Chromosomes" (SMC) family.

Unlike histones, these proteins are present in bacteria as well as eukaryotes, and are thus probably the ancestral organizers of DNA genomes.

The **Metaphase-Anaphase transition** is triggered by **destruction of 2 key targets, Cyclin B and securin**, through ubiquitin-mediated proteolysis

The Anaphase-Promoting Complex (APC/C) is a **UBIQUITIN LIGASE** that targets both Cyclin B and securin, leading to their destruction. Securin inhibits a protease called **separase** which cleaves one of the subunits of cohesin to release it from the chromosomes.

Sister chromatid cohesion and separation in mitosis: Some cohesin is released during prophase by a mechanism that reduces its binding to chromatin; at the M–>A transition, a protease called **SEPARASE** cleaves one of the cohesin proteins to release sister chromatid cohesion

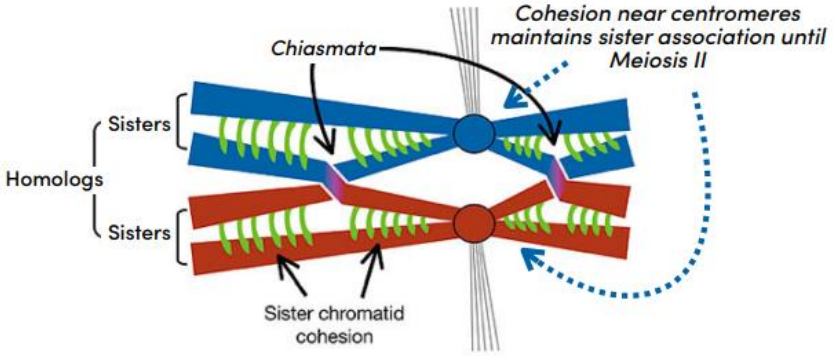
Checkpoints, aka Surveillance Mechanisms, can block the cell cycle at specific points to allow time to complete essential tasks

S-phase entry: DNA damage

M-phase entry: DNA damage
M→A transition: Spindle assembly/ kinetochore attachment defects

Segregation of homologous chromosomes during **MEIOSIS** depends on **pairing, synapsis, and crossover recombination**

Maintenance of cohesion along chromosome arms is necessary to prevent premature homolog separation (which can lead to Meiosis I nondisjunction)



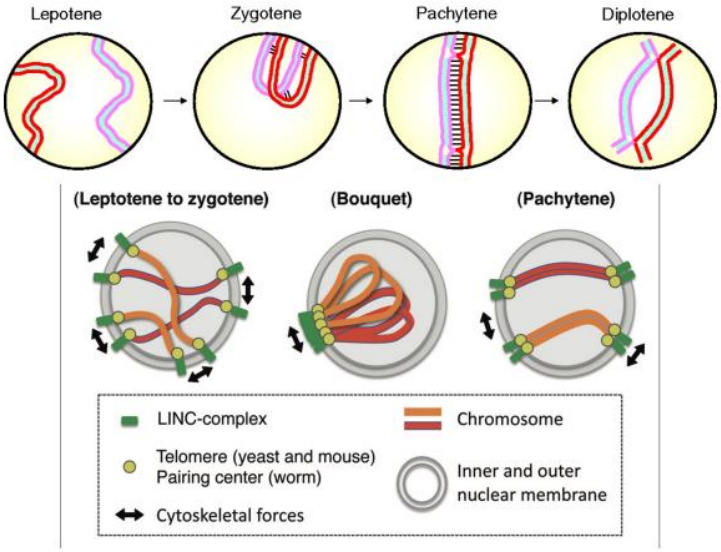
Arm cohesion
(together with crossover formation)
maintains homolog association until
Meiosis I division

Meiotic cohesion is released in 2 steps to allow 2 rounds of chromosome segregation

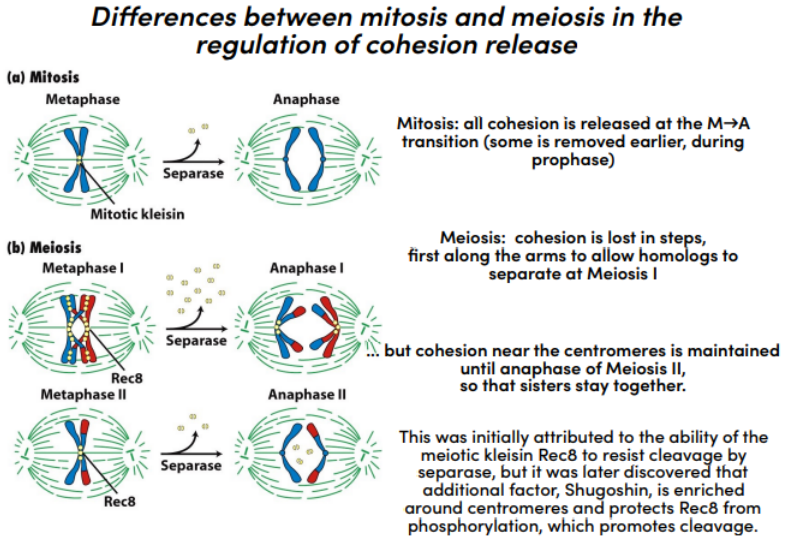
During meiosis, DNA double-strand breaks (DSBs) are deliberately made at semi-random positions along the chromosomes. Crossover recombination is essential to hold homologous chromosomes together so that they can segregate away from each other. At least one DSB, but usually only 1-2 DSBs, is/are repaired as crossovers.

Chromosomes undergo dramatic remodeling at meiotic entry; Double-strand break formation, homolog pairing (stabilized by assembly of a specialized protein structure called the Synaptonemal complex (SC), promoted by interactions with the nuclear envelope)

Homolog pairing is promoted by interactions with the nuclear envelope



Differences between mitosis and meiosis in the regulation of cohesion release



11-1 Cell bio

Viruses are not considered to be “living”, they are not cells (although they can be surrounded by membranes), and they require cellular life forms to replicate/reproduce.

LECA: Last Eukaryotic Common Ancestor
LUCA: Last Universal Common Ancestor
Archaea and eubacteria are both comprised of single-celled organisms, but **archaea and eukaryotes** are more closely related in evolutionary terms

All living cells on earth use similar molecules and biochemical processes, indicating that they arose from a common ancestral cell

Eukaryotic cells are more “compartmentalized” than prokaryotic cells, containing (more) membrane-bound compartments called “organelles”

This distinction is blurrier than it used to be. Advances in microscopy are revealing a surprising degree of compartmentalization in prokaryotes. Also, we are now (re)discovering that not all compartments have membranes around them.

Prokaryotes can also contain membrane-bound compartments. Cells and their organelles are surrounded by membranes, which act as barriers to diffusion of most molecules.

Light microscopy can reveal many features of cells, particularly if they are stained with **probes** to detect specific molecules.

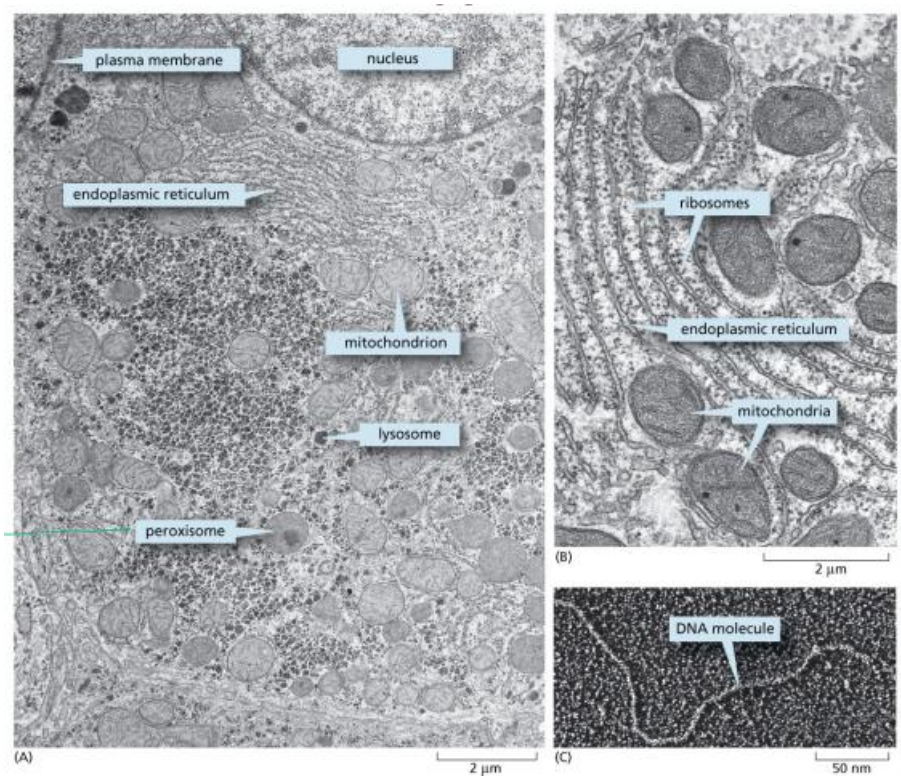
What makes fluorescence useful for biology is our ability to target fluorophores to individual components of the cell in a **highly specific** manner.

The limit of resolution of “conventional” light microscopy is ~200 nanometers (about 1/2 the wavelength of visible light). The **resolution of electron microscopy** can be orders of magnitude greater (10s of picometers).

A number of techniques have enabled fluorescence microscopy to achieve “super-resolution” - beyond the traditional diffraction limit:

STORM super-resolution image

Transmission electron microscopy reveals cellular “ultrastructure” [亚显微结构]



Scanning electron microscopy

Because all organisms are related, we can learn about fundamental cell biological processes by studying appropriate model organisms?

Why there are model organisms for research in biological processes?

Good model organisms:

Can go through their entire life/reproductive cycle under conditions easily controlled in a lab

Require only inexpensive, easily reproduced growth media and food

Are “clonal” - genetically homogeneous

Typically have short life cycles (hours-weeks)

Are amenable to genetic and genomic (as well as cell biological) analysis

Are studied by a large community of researchers (large body of knowledge and resources available)

All organisms on earth are related to each other

Some proteins are “highly conserved” (do not change in sequence rapidly during evolution) while some evolve much more quickly.
The degree of conservation of a protein is not very predictable, but depends on its function (e.g., whether it is an enzyme or a structural protein) and the number of proteins it interacts with.

11-2 Cytoskeleton

3 types of filamentous polymers form the cytoskeleton of animal cells
10nm intermediate filaments: stable, resilient, “rope-like”, less dynamic
25nm microtubules: dynamic, tubular, hollow, intracellular trafficking, chromosome division, and cilia/flagella
7-9nm actin filaments: dynamic, helical structural, cell shape, movement, membrane, trafficking, cytokinesis, and muscle contraction.

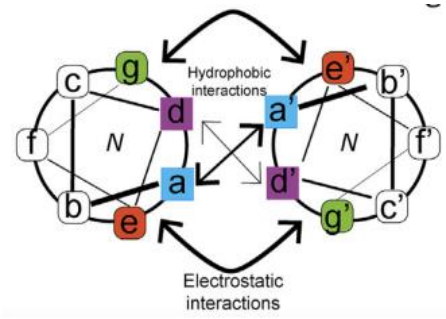
The cytoskeleton provides cells with **mechanical resilience**, allows cell to **organize and move** their internal components, and enables cell motility.

Intermediate filaments

Intermediate filaments form a strong, durable network in the nucleus and cytoplasm
desmosome[桥粒] connecting two cells: bundles of intermediate filaments

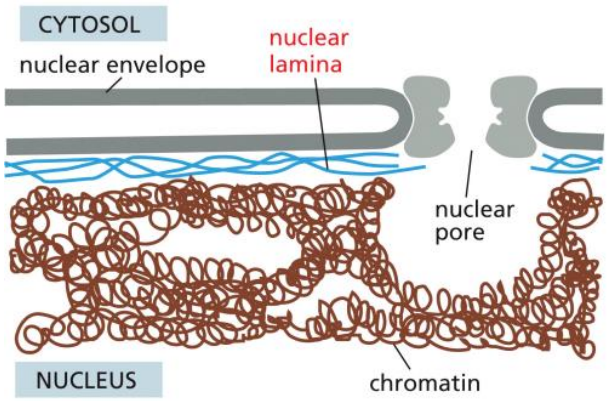
Intermediate filaments are formed by **α-helical proteins (3.6 AA/turn)** that wrap around each other to form **“coiled-coils”,** can be parallel or antiparallel.

Small **hydrophobic (nonpolar) residues** (Leucine, Isoleucine, Valine on one face promote coiled-coil interactions.



Intermediate filaments are formed by α-helical proteins that wrap around each other to form “coiled-coils”, forming strong, durable network in the nucleus and cytoplasm.

The nuclear lamina (made of lamin proteins) is one type of intermediate filament network



Cells use chemical energy to control assembly & disassembly of many structures - including the nuclear lamina in animal cells. **Phosphorylation** of lamin proteins promotes the disassembly of the lamina during mitosis, while **dephosphorylation** promotes assembly after mitosis

Mutations in lamins and associated proteins have been linked to a number of human diseases known as "laminopathies." These include forms of muscular dystrophy and a rare premature aging syndrome known as Hutchison-Gilford Progreia Syndrome (HGPS)

Microtubules

hollow tubes made of **tubulin**
Microtubule organization changes dramatically over the course of a cell division cycle
interphase: astral array
mitosis: bipolar spindle
During the eukaryotic cell cycle, microtubules and chromosomes are **dynamically remodeled** to execute mitosis.

Microtubules are rigid, hollow tubes.

Microtubules are assembled from two closely related tubulin proteins, α- and β-tubulin
α-tubulin: a dead GTPase
β-tubulin: an active GTPase
*polymerization does **not require GTP hydrolysis** (still polymerize with non-hydrolysable GTP analog (e.g. GMPCPP))*

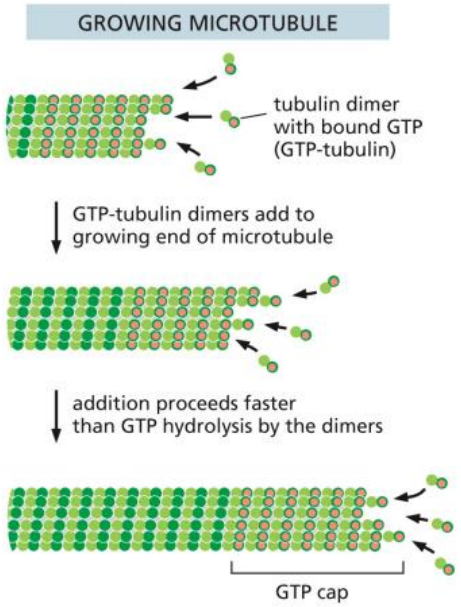
Microtubules usually grow out from an organizing center known as a centrosome, spindle pole, basal body, or microtubule organizing center (MTOC).
The position, number, and activity of the MTOC(s) is regulated by the cell cycle and type of cell.

A microtubule nucleator complex (the gamma tubulin ring complex, or ɣ-TURC) is localized within cells by microtubule organizing centers (MTOCs).
In many nondividing cells, microtubules form a radial array that directs trafficking between the MTOC and the plasma membrane.

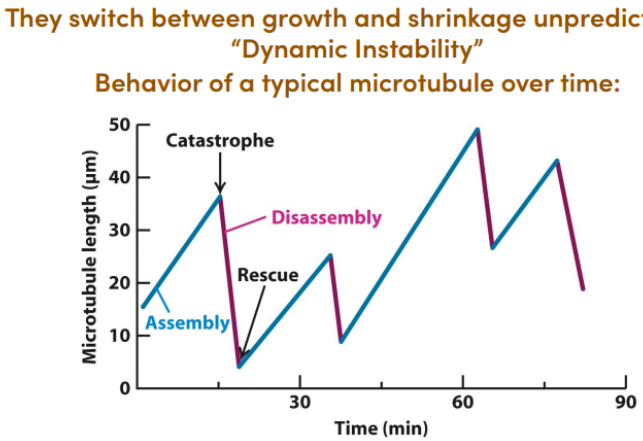
MTOCs often, but not always, have CENTRIOLES at their cores. Centrioles have their own division cycle, which regulates the number of MTOCs. The nucleating activity (γ-TURC) is localized within the pericentriolar material (PCM)[中心周围物质]

Microtubules constantly grow and shrink， they switch between growth and shrinkage unpredictably: "Dynamic Instability", driven by GTP hydrolysis (energy!)

GTP hydrolysis controls the dynamic instability of microtubule
Tubulin dimers carrying GTP (red) bind more **tightly** to one another than do tubulin dimers carrying **GDP** (dark green). The rapidly growing plus ends of microtubules, capped by newly added GTP-tubulin, therefore tend to keep growing.



Microtubule dynamics change dramatically between interphase and mitosis



Both MTOC behavior and the rates of microtubule growth & shrinkage are regulated by the cell cycle:

Microtubule associated proteins (MAPs) proteins "tune" the intrinsic properties of microtubules.

STABILIZING

lattice-binding → stabilizing;

end-binding → promote growth & attach to targets

DESTABILIZING

Catastrophe-promoting factor: Stathmin/Op18; Kin Internal (KinI)

Severing factors: Katanin

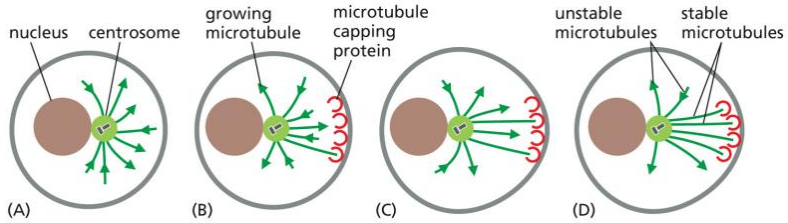
These regulators are modulated by **cell cycle kinases** to assemble/disassemble the mitotic spindle and interphase astral arrays

Microtubule dynamics can also be modified by drugs

Microtubule-specific Drugs	Action
Taxol	Binds and stabilizes microtubules
Colchicine, colcemid	Binds tubulin dimers and prevents their polymerization
Vinblastine, vincristine	Binds tubulin dimers and prevents their polymerization

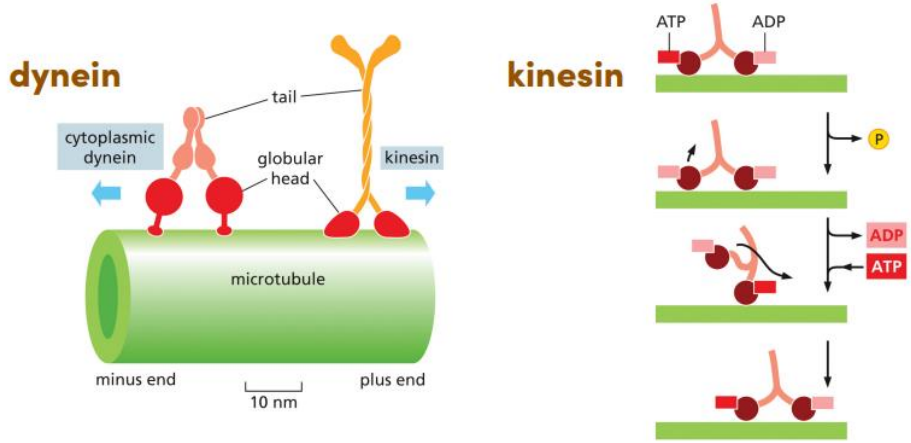
Taxol is used as a chemotherapeutic agent because it preferentially affects dividing cells

Microtubules can be stabilized by attachment to **capping proteins**



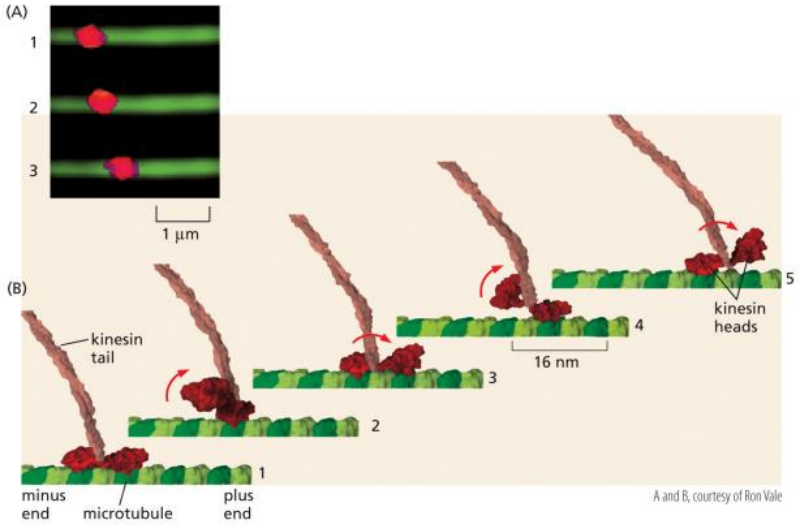
Capping proteins are present within the cell cortex and at kinetochores (where microtubules attach during mitosis)

Microtubule motors - **dynein and kinesin** - take advantage of the polarized organization of microtubules to "walk" towards the + or – end



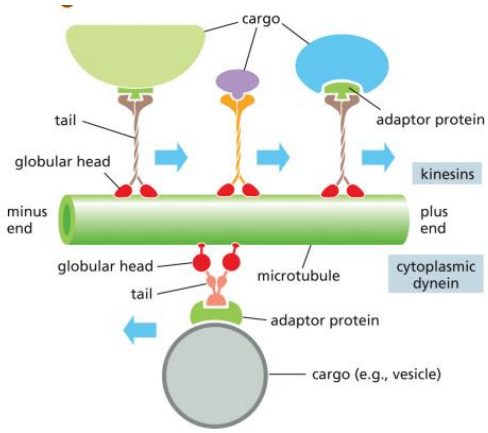
Cytoskeletal motor proteins directly harness chemical energy (usually in the form of **ATP**) to generate force and movement

A single molecule of kinesin moves along a microtubule



Motor proteins are often studied in vitro by immobilizing them **on glass slides** and visualizing how they move microtubules

Motor proteins transport a wide variety of cargo along microtubules



Dynein "walks" towards **the minus end**, while most kinesins are "*plus-end-directed*" microtubule motors.

Vesicles move along microtubules within cells.

The microtubule array is usually tethered to the nucleus, and gives cells a (polar) coordinate system.

In neurons, microtubules extend from the cell body along axons and dendrites

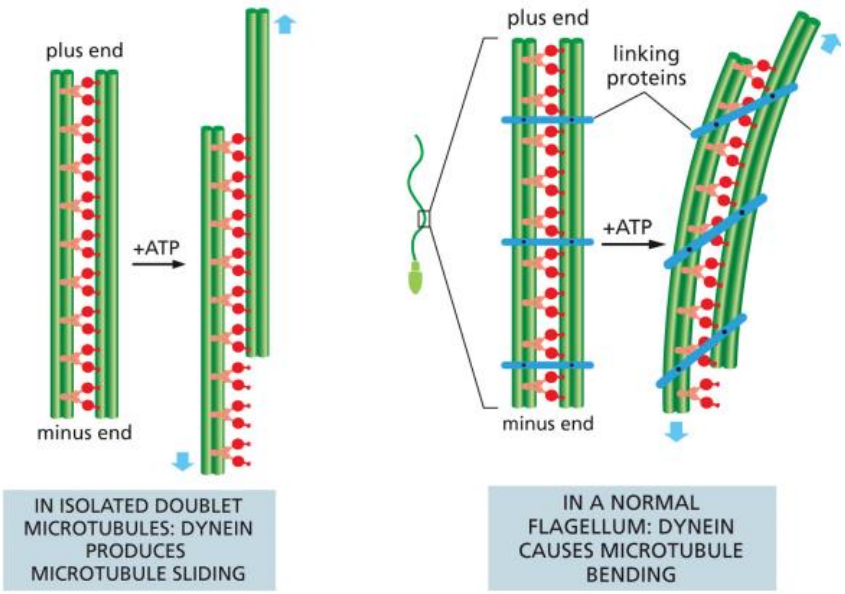
They act as "railroad tracks" for the transport of organelles, vesicles, and macromolecules

Microtubules act as "tracks" for the transport of organelles, vesicles, and macromolecules

Cilia and flagella contain stable microtubules moved by dynein

Microtubules in a cilium or flagellum are arranged in a "9 + 2" array

The movement of **dynein** causes a flagellum to bend



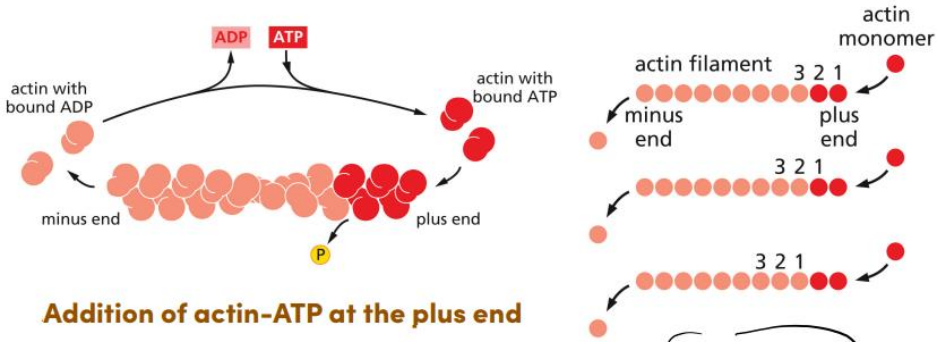
Actin filaments

Actin filaments are required for cell shape and rigidity, cell movement, cytokinesis, cell polarity, muscle contraction.

Actin filaments allow animal cells to adopt **a variety of shapes and perform diverse functions.**

Actin **monomers (a.k.a. "G-actin")** assemble into polar filaments with a diameter of **7 nm**.
Actin is an ATPase, it can incorporate into filaments when bound to ATP, and dissociates more readily when bound to ADP.

Addition of actin-ATP at the plus end, treadmilling.



Actin and tubulin form **helical** polymers: A helix forms naturally when a **chiral/asymmetrical** unit (like a protein) can bind to itself
The accidental filamentous polymer: Sickle-cell anemia is caused by a single amino acid change in hemoglobin, which results in helical filaments (14 protofilaments)

The ability of tubulin and actin to form dynamic filaments that cells can control makes them very useful for a wide variety of function.

Actin polymers (like microtubules) have POLARITY = nonequivalence of two ends. Actin decorated with myosin S1 (proteolytic fragment) **"barbed" appearance** is due to **myosin binding.**

-allows cell to generate asymmetric structures and shapes

-basis of directional motility

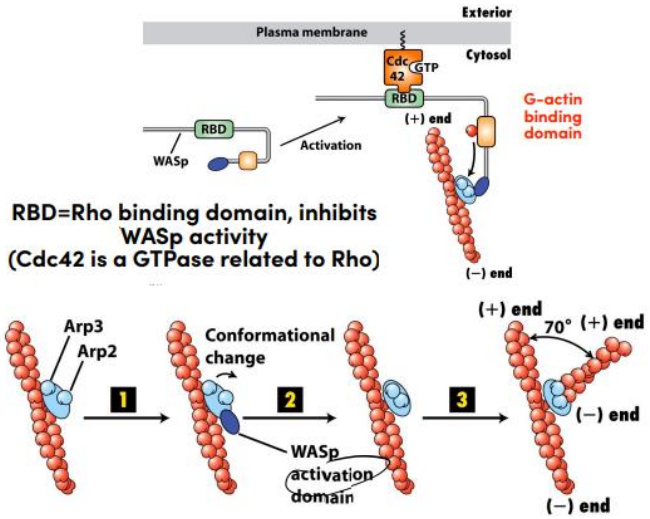
-motor proteins recognize polymer asymmetry, providing directionality for force production, moving cargo.

Assembly is strongly favored at the + (barbed) ends, this implies that the critical concentration (Cc) is nonequivalent at the 2 ends.

Web of actin filaments allow animal cells to migrate, regulated formation and retraction of actin filaments allow animal cells to crawl

WASp (Wiskott-Aldrich Syndrome protein) is required for efficient actin polymerization nucleated by Arp 2/3.

RBD, Rho binding domain, inhibits WASp activity.



Actin filaments are quickly capped after nucleation, by Capping Protein.

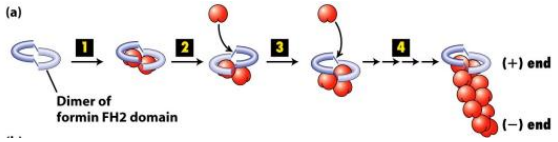
Profilin-actin[肌动蛋白结合蛋白]

This keeps polymer growth focused in the region of nucleation near the membrane, and results in short filaments that are able to do work (longer ones would be too bendy)

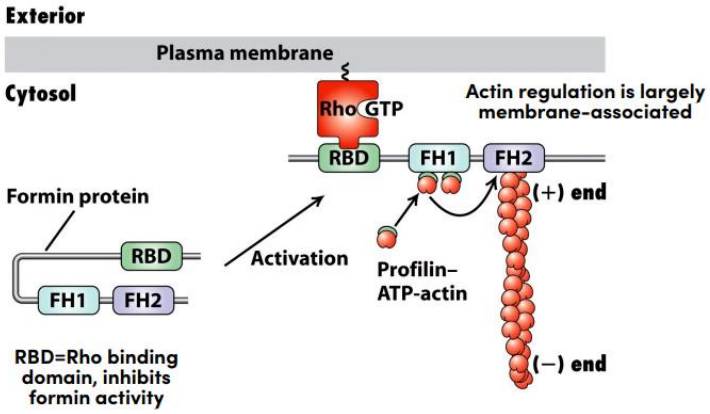
Arp2/3 + WASp nucleate branched actin filaments that can push against membranes

Formin proteins nucleate unbranched actin filaments:

Surprisingly (at first): Formin is associated with the + end of growing filaments



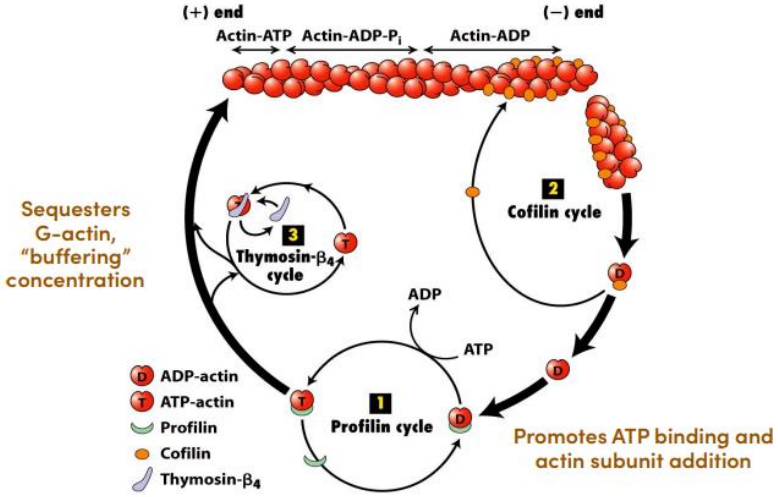
Formins nucleate **long, straight actin filaments**, the activity of formin is regulated by a small GTPase, Rho, at the plasma membrane.



Activation of different Rho-family GTPases triggers different forms of actin polymerization

Extracellular signals can also modify actin dynamics by regulating other actin-associated proteins. e.g. PKC

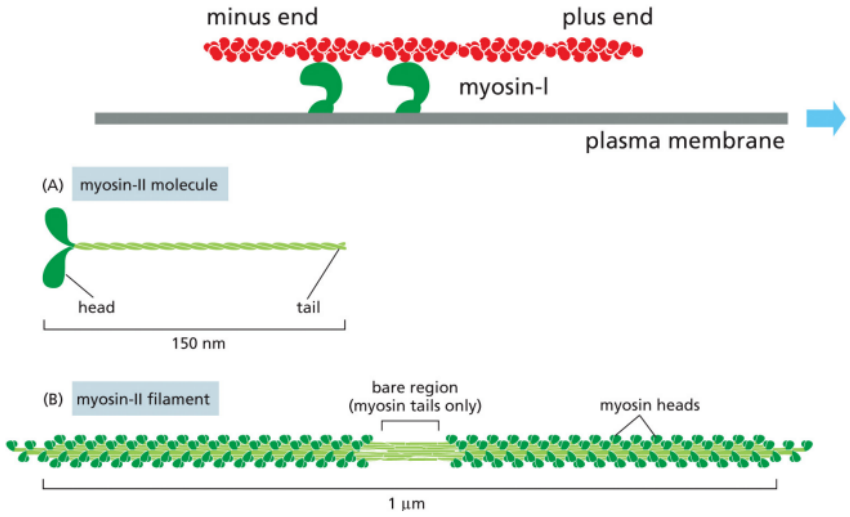
Although actin is present at a high concentration within cells, its effective concentration is controlled by a **monomer-binding proteins**, including **thymosin and profilin,** In cells, actin filaments are nucleated by nucleation factors



Pathogens often modify the actin cytoskeleton from the outside to anchor themselves or promote uptake into cells, bacteria can modify the actin cytoskeleton from outside the cell to promote their own entry.
How Listeria moves: ActA on the surface behaves like WASp; Listeria and many other bacteria use host cell actin to move within and between cells.

Actin cross-linking proteins link actin filaments to each other, and to other cell structures.
Actin crosslinking proteins can create **stiff bundles of actin filaments**.

Myosins are a family of motors that can move along actin filaments



Muscle cells express a form of myosin II that forms filaments.

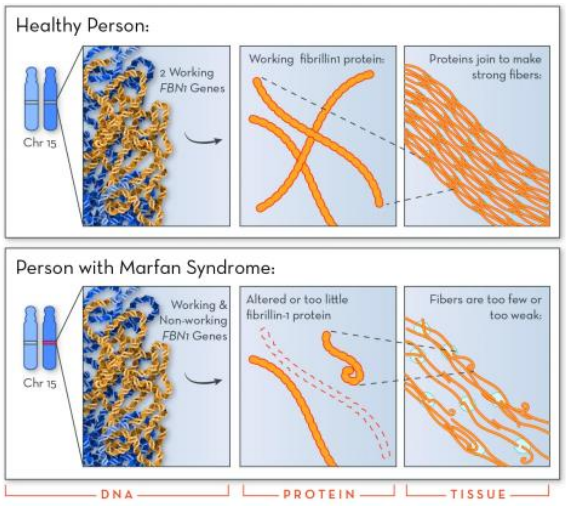
TABLE 17–2 DRUGS THAT AFFECT FILAMENTS	
Actin-specific Drugs	**Action**
Phalloidin	Binds and stabilizes filaments
Cytochalasin	Caps filament plus ends, preventing polymerization there
Latrunculin	Binds actin monomers and prevents their polymerization

Actin in fixed cells is often visualized with **fluorescent phalloidin**

Genetically dominant disorders caused by mutations that **disrupt polymers**

Osteogenesis imperfecta (OI), brittle bone disease: caused by mutations in collagen proteins.
Amelogenesis imperfecta (AI), defects in tooth enamel: Has both dominant and recessive forms affecting different proteins involved in enamel production.

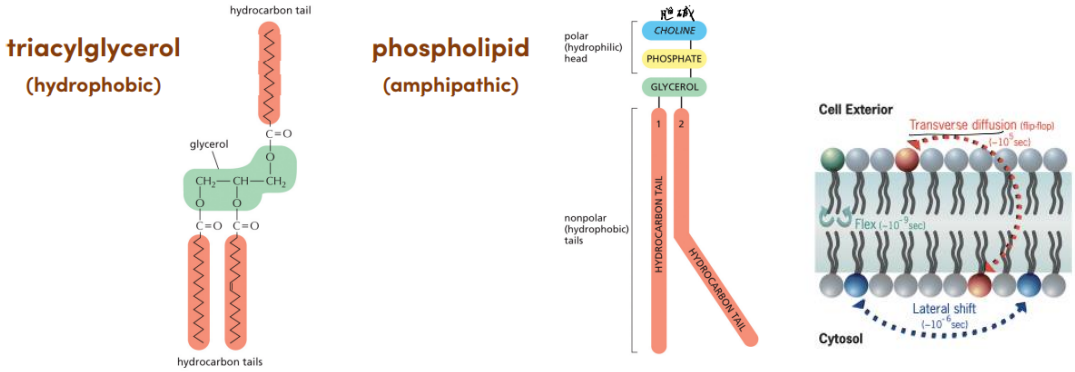
Marfan syndrome: mutations in fibrillin (FBN1)



Hypertrophic cardiomyopathy: caused by mutations in genes that encode muscle proteins

12-1 Membrane and labeling

Lipid membranes enable the concentration & compartmentalization of biochemical processes within cells and organelles
The plasma membrane is involved in **cell communication, import and export of molecules, and cell growth and motility.**
Cell membranes are phospholipid bilayers, along with many associated proteins.
Fatty acids are the building blocks for membranes They are stored within cells as **triacylglycerols** (fats)
Lipids are synthesized from triacylglycerols (fats), which are stored as oily droplets.



Membranes also incorporate sterols and glycolipids. These molecules are also amphipathic.

Unsaturated tails (double bonds) kink lipid structure, limiting the tight packing of the hydrocarbon tails and thus increase the mobility (fluidity) of the lipids within the membrane. Membranes in cells/organisms at higher temperatures (e.g. animals) tend to **have more saturated hydrocarbon tails.**

Conversely, **cholesterol** can fill the gaps created by unsaturated bond angles, making membranes **less fluid and stiffer**, Cholesterol is an essential component of animal membranes (plants do not have it).

Lipid molecules can rapidly diffuse within one layer (a.k.a. “leaflet”) of the membrane, but there is **a larger energy barrier** to flipping to the opposite side.

Membranes are fluid - lipid molecules move rapidly within the layer that they’re in, but rarely flip spontaneously
“Hydrophobic interactions” are not driven by an affinity of hydrophobic surfaces for each other, but rather because the presence of hydrophobic molecules reduces entropy by constraining the network of hydrogen bonds in water. When hydrophobic molecules come together this entropic cost is minimized.

The hydrophobic nature of the lipid tail drives many aspects of lipid structure and dynamics.

Newly synthesized phospholipids are added to the cytosolic side of the ER membrane and then redistributed "scrambles," which transfer them from one half of the lipid bilayer to the other,

Biosynthetic enzymes (not shown) bound to the cytosolic face of the ER membrane produce new phospholipids from free fatty acids and insert them into the cytosolic monolayer. Transporters called scramblases then randomly transfer phospholipid molecules from one monolayer to the other, allowing the membrane to grow as a bilayer in which the two leaflets continuously equilibrate in size and lipid composition.

"Flippases" reestablish asymmetric distribution of phospholipids characteristic of animal cell membranes

When membranes leave the ER and are incorporated in the Golgi, they encounter a different set of transporters called flippases, which selectively remove phosphatidylserine (light green) and phosphatidylethanolamine (yellow) from the noncytosolic monolayer and flip them to the cytosolic side.

Proteins can associate with the surface of membranes, or have segments that go through membranes. Transmembrane domains are usually **α-helices**, which are typically 20-25 amino acids long and easy to predict computationally.

The amino acid side chains of transmembrane helices are hydrophobic.

Some proteins form transmembrane *pores* comprised of bundles of amphipathic helices, with a hydrophobic exterior and a hydrophilic interior. They allow water or other molecules to pass through.

Proteins can also be modified to associate with membranes by addition of lipophilic molecules.

Proteins can also be modified to associate with membranes by addition of lipophilic molecules, usually at the C-terminus. A "CaaX" motif at the C-terminus of lamin A (and other proteins) is recognized and modified by enzymes that add **hydrophobic farnesyl or geranylgeranyl groups.** Farnesyl group promotes association with inner nuclear membrane, and is later cleaved.

The plasma membrane is reinforced by an underlying "cell cortex"

The mobility of plasma membrane proteins can be restricted in several ways:

Proteins can be tethered

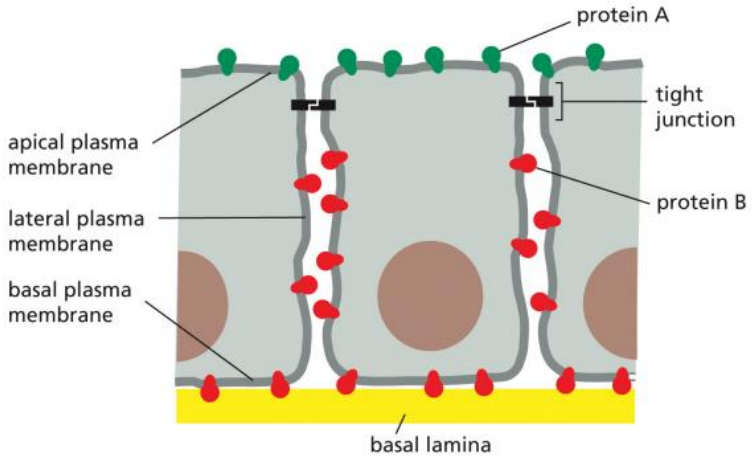
(A) to the cell cortex (cytoskeletal proteins and crosslinkers) inside the cell,

(B) to extracellular matrix molecules outside the cell, or

(C) to proteins on the surface of another cell.

(D) Diffusion barriers such as tight junctions (shown as black bars) can restrict proteins to a particular membrane domain.

Membrane proteins are restricted to particular domains of the plasma membrane of epithelial cells.



Tight junctions create are barriers that create distinct "apical" and "basolateral" domains. Proteins can diffuse laterally within a domain but cannot cross this barrier.
The basal lamina (yellow) is a mat of extracellular matrix that supports epithelial sheets

Proteins within cell membranes also move by diffusion: Visualization of the mobility of membrane-associated proteins was first visualized in the 1970s by labeling specific membrane proteins in a mouse cell and human cells using different antibodies. When these cells were induced to fuse, the proteins intermixed.

Immunofluorescence - lighting up cell components with antibodies:
Injection of an "antigen" (usually a purified protein or protein fragment) into a mouse, guinea pig, rabbit, etc. leads to production of antibodies that bind tightly to that molecule. This takes several weeks and is fairly expensive, but it produces one of the most valuable experimental tools for cell biology.
Fluorescence is a property of some dye molecules. They absorb light at one wavelength, undergo an internal energy conversion, then emit light of lower energy (longer wavelength)
FLUORESCENCE microscopy is a key tool for modern cell biology It enables researchers to **visualize any specific molecule** that they can tag with a fluorescent dye
However, exciting the dye may also lead to an irreversible (permanent) change in its structure that renders it non-fluorescent, this is called "photobleaching"

"Indirect immunofluorescence" uses two layers of antibodies
Immunofluorescence enables us to label cellular proteins in a highly specific manner.
However, labeling of *internal* components usually requires **fixation and permeabilization**, and is thus **incompatible with live cells**.

Green Fluorescent Protein (GFP)
The dynamics of membrane proteins can be studied using **FRAP (Fluorescence Recovery After Photobleaching).**

Photoactivatable GFP enabled asynchronous[非同步] detection of molecules within a sample, which is how **PALM s**uper-resolution works.
Photoconversion of fluorescent proteins has enabled new alternatives to FRAP for measuring cell dynamics, STORM is the same concept as PALM, except with different dyes

Intracellular Compartments and Protein Transport

In eukaryotic cells, **internal membranes** create enclosed compartments that spatially and biochemical segregate different metabolic processes.

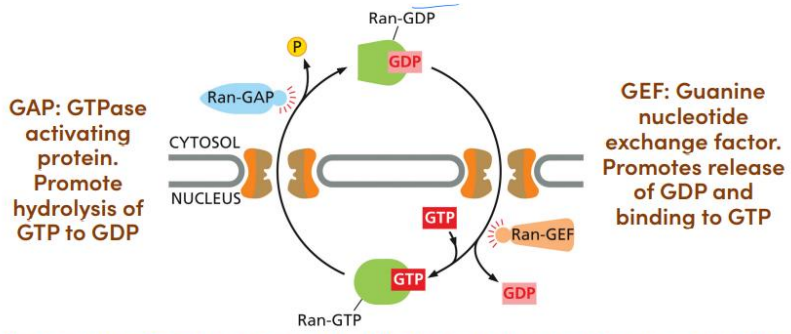
Proteins are targeted to the plasma membrane and to membrane-bound organelles by 3 distinct mechanisms:

-Transport through nuclear pores
-Transport across membranes
-Transport by vesicles

Proteins contain signal sequences, or signal peptides, that determine their ultimate location within the cell… sort of like a ZIP code
Some of these can be located anywhere within a protein sequence; some are always at the N- or C-terminus

Transport through nuclear pores

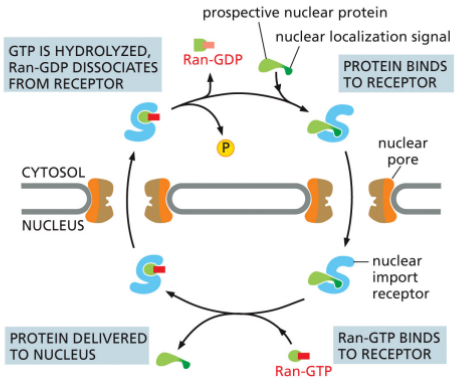
Proteins are carried into and out of the nucleus by binding to nuclear import receptors (importins) and nuclear export receptors (exportins), collectively known as "karyopherins"[核转运蛋白]. These special transport proteins can pass through the nuclear pores and carry other proteins with them.



Ran binds to GTP and hydrolyzes it to GDP. The concentration of Ran bound to GTP is higher in the nucleus than in the cytoplasm, due to the presence of a RanGAP protein on the cytoplasmic surface of the nuclear pore, and Ran-GEF in the nucleus

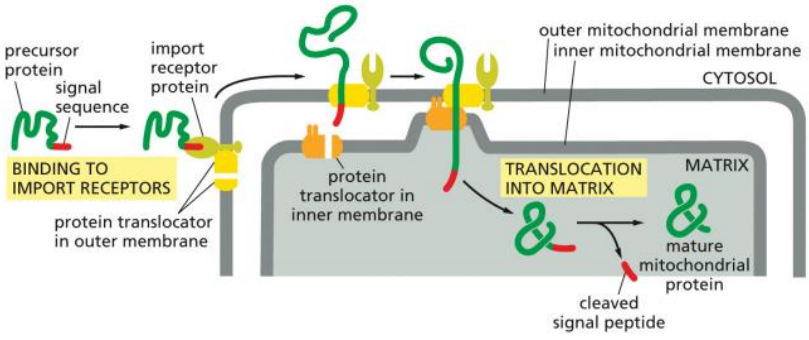
Nuclear import receptors bind to nuclear localization signals (NLSs) in the presence of RanGDP (in the cytoplasm) and release them in the presence of RanGTP (in the nucleus).
Nuclear export receptors do the opposite: they bind to nuclear export signals (NESs) in the presence of high Ran-GTP and ferry proteins into the cytoplasm, where they release them.

Nuclear import receptors bind to nuclear localization signals (NLSs) in the presence of RanGDP (in the cytoplasm) and release them in the presence of RanGTP (in the nucleus)



Nuclear export receptors do the opposite: they bind to nuclear export signals (NESs) in the presence of high Ran-GTP and ferry proteins into the cytoplasm, where they release them.

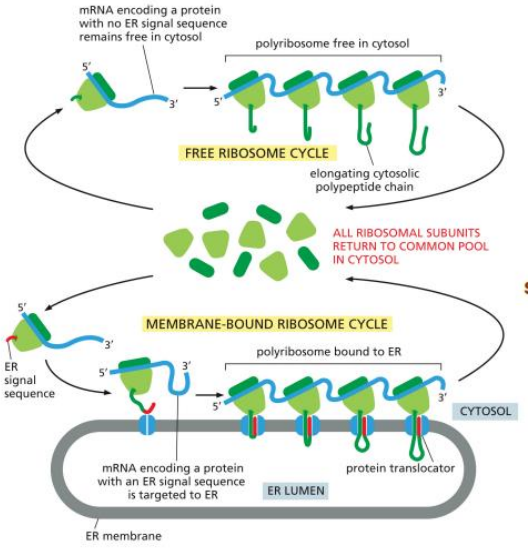
Mitochondrial proteins are transported across membranes, proteins are imported into chloroplasts by a similar mechanism



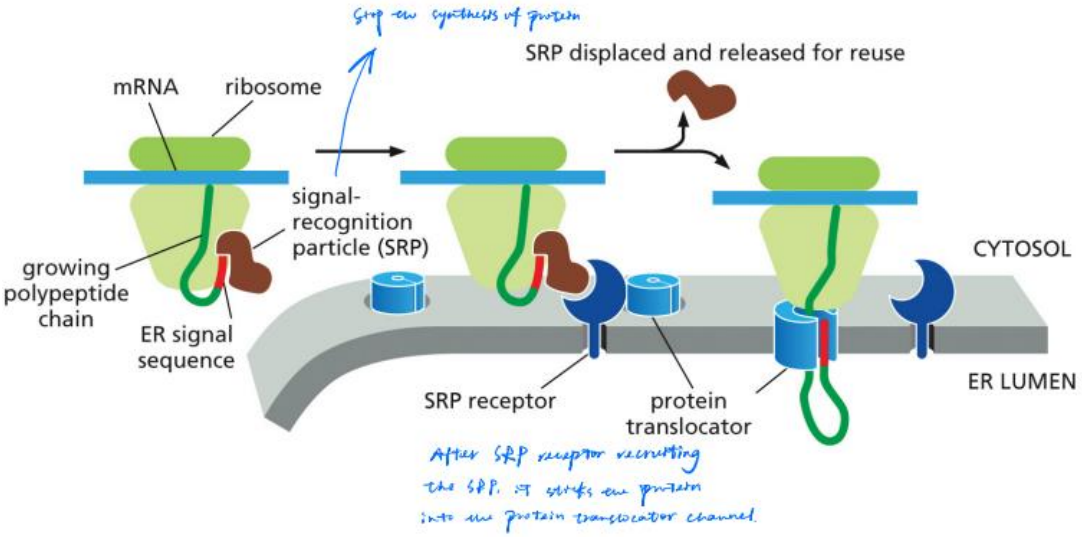
The endoplasmic reticulum is the most extensive membrane network in eukaryotic cells.

Transmembrane membrane proteins and secreted proteins are synthesized by ribosomes on the **cytoplasmic face** of the ER

Ribosomal subunits are located in the cytosol. When a ribosome starts to synthesize a protein that has an ER signal, they become tethered to the ER by binding of a ***single recognition protein (SRP)*** *that binds to a receptor (SRP receptor) on the surface of the ER*

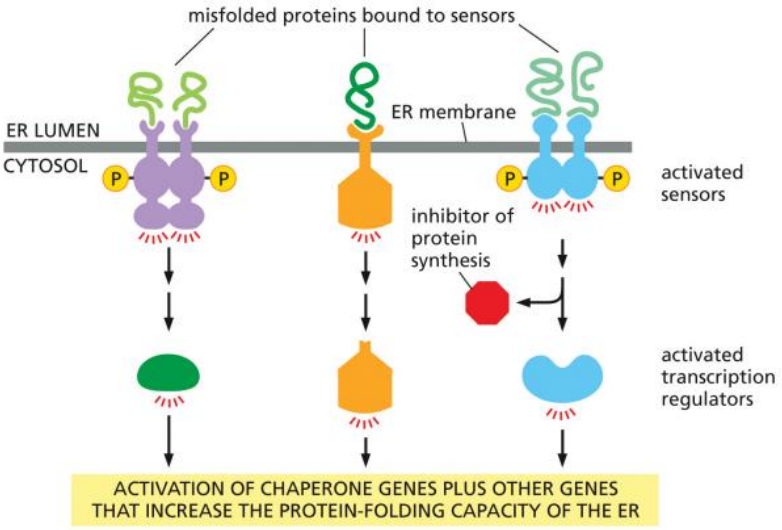


An ER signal sequence and an SRP direct a ribosome to the ER membrane



Transmembrane proteins are inserted into the ER membrane

Accumulation of misfolded proteins in the ER lumen triggers an **unfolded protein response (UPR)**.



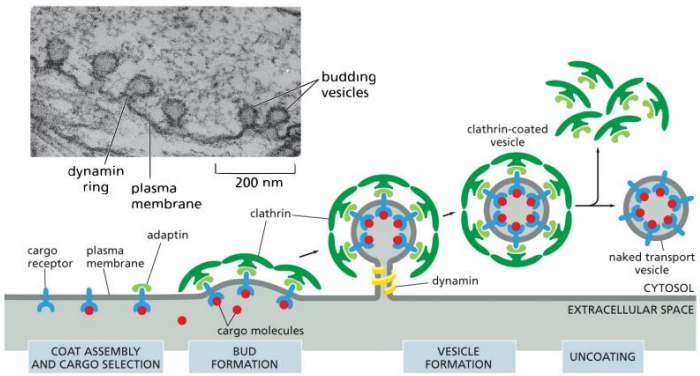
Budding and fusion of membrane vesicles enables transport of soluble proteins and membrane into, out of, and around the cell, all of this requires energy (ATP & GTP).

Vesicular transport carries proteins from the ER to their eventual destination in **other organelles or the cell surface**

Clathrin protein on the internal (cytoplasmic) surface of a membrane interacts with receptors that span[跨] the membrane. These interactions concentrate receptors into a “coated pit” that buds off to form a “coated vesicle”.

Clathrin molecules form basketlike cages that help shape membranes into vesicles

Clathrin-coated vesicles transport selected cargo molecules



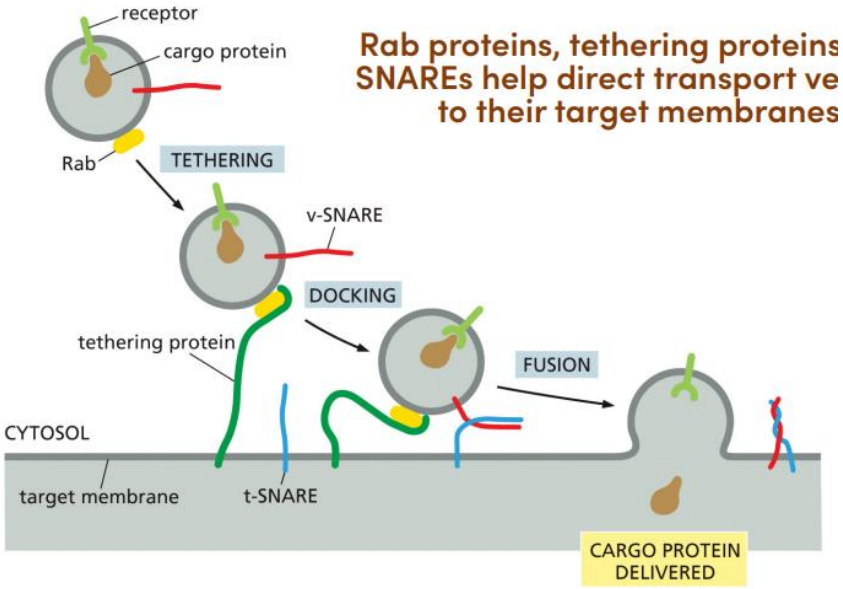
Viruses can enter cells via receptor-mediated endocytosis

COPI and COPII coat vesicles that emerge from the Golgi and ER, respectively

Type of Coated Vesicle	Coat Proteins	Origin	Destination
Clathrin-coated	clathrin + adaptin 1	Golgi apparatus	lysosome (via endosomes)
Clathrin-coated	clathrin + adaptin 2	plasma membrane	endosomes
COPII-coated	COPII proteins	ER	Golgi cisterna
COPI-coated	COPI proteins	Golgi cisterna	ER

The Golgi apparatus consists of a stack of flattened sacs called cisternae. cis=near ER; trans=export sites

Rab proteins, tethering proteins, and SNAREs help direct transport vesicles to their target membranes.



Following vesicle docking, SNARE proteins can catalyze the fusion of the vesicle and target membranes

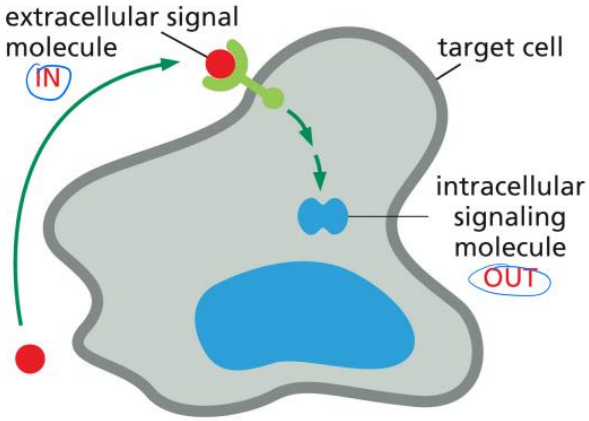
Pathogens often modify the actin cytoskeleton from the outside to anchor themselves or promote uptake into cells. Bacteria are too large to be taken up by receptor-mediated endocytosis, but they

can modify the actin cytoskeleton from outside the cell to promote their entry into cells through other processes

Our understanding of the **secretory pathway** was greatly expanded using **live-cell microscopy** and **GFP to tag newly-synthesized proteins**

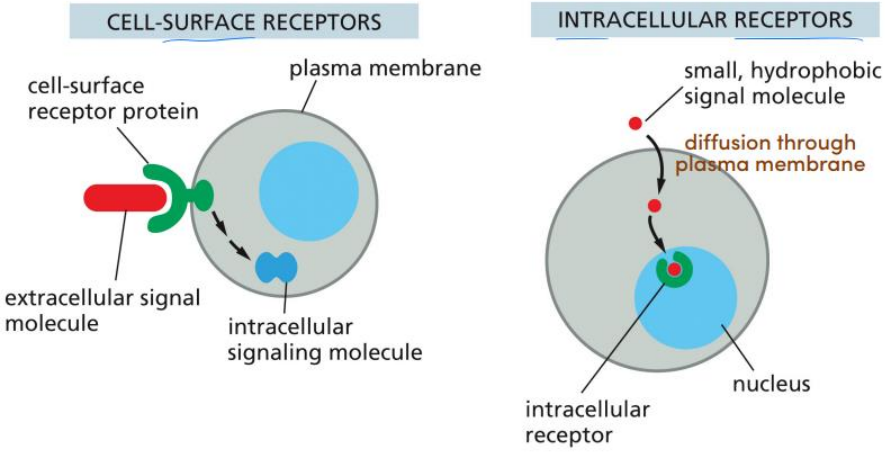
Cell Signaling

Cells convert ("transduce") extracellular signals into intracellular signals



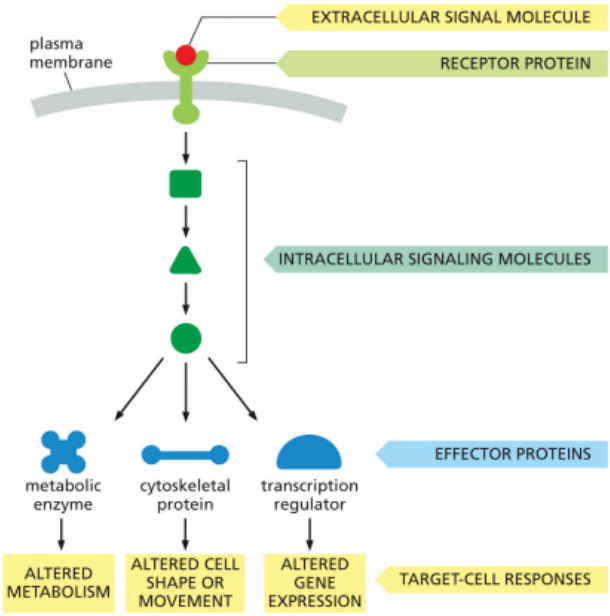
Animal cells use extracellular signal molecules to communicate with one another in various ways:
Endocrine: Extracellular signals can travel through the bloodstream,
Paracrine: be secreted by nearby cells,
Neuronal: be released at synapses,
Contact-dependent: or be tethered to the surface of adjacent cells

Signal molecules bind either to cell-surface receptors or to intracellular receptors



Some **small, hydrophobic hormones** bind to intracellular receptors that act as transcriptional regulators: steroid hormones, thyroid hormone.

Extracellular signals activate intracellular signaling pathways to change the behavior of the target cell

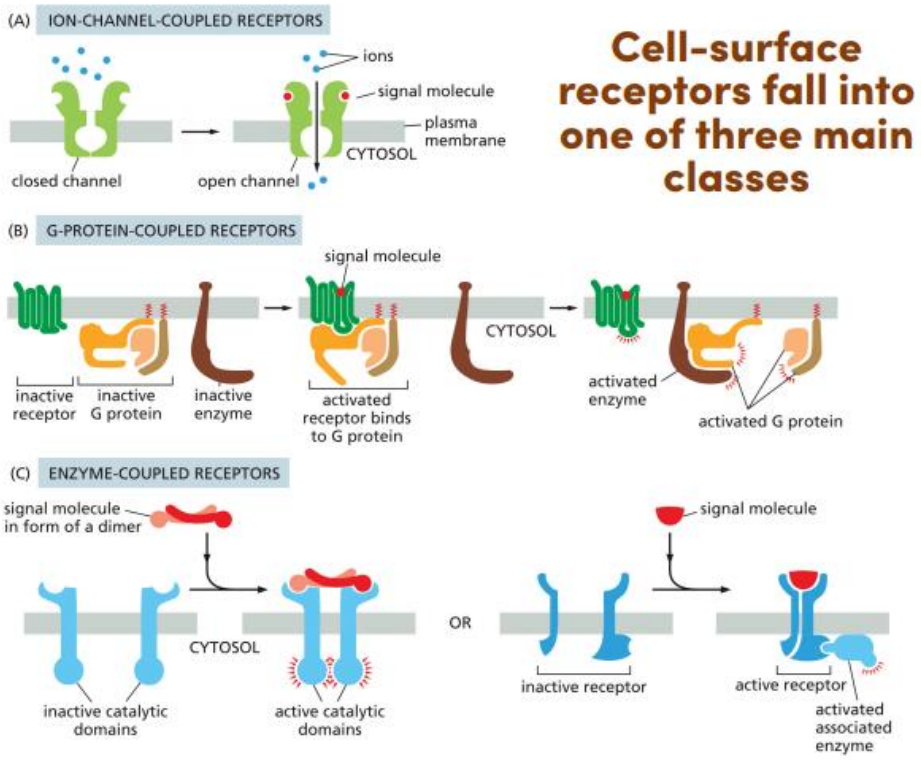


Cell-surface receptors fall into one of three main classes:

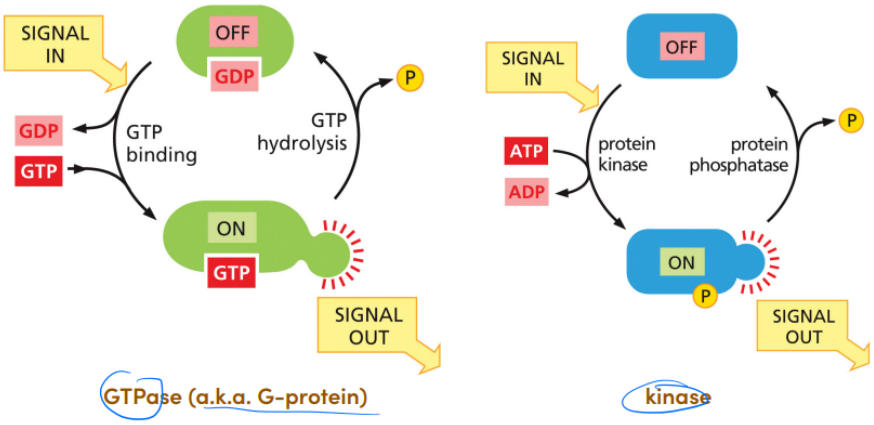
Ion-channel-coupled receptors

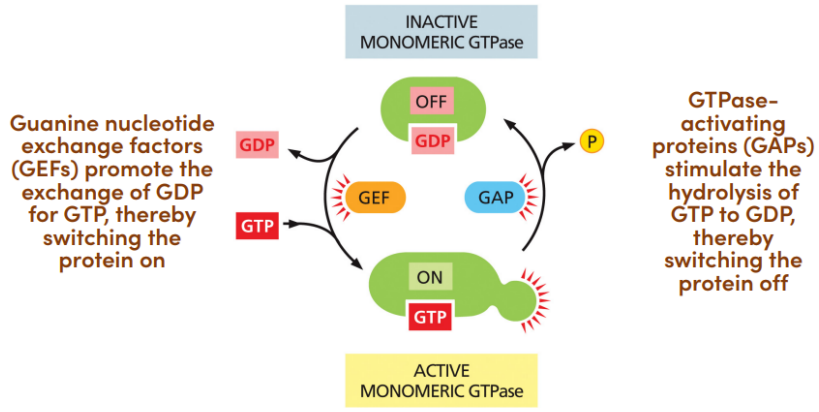
G-protein-coupled receptors

Enzyme-coupled receptors

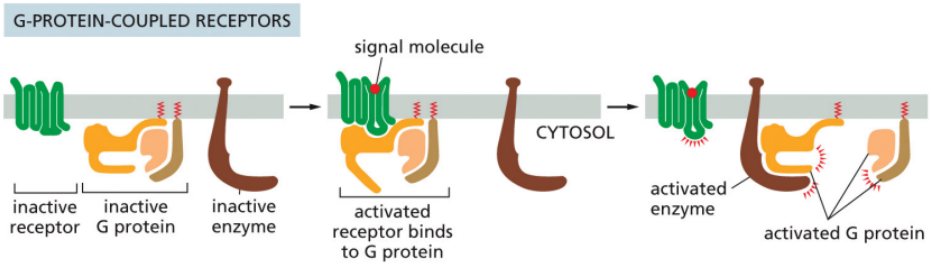


Many intracellular signaling proteins act as molecular switches: **GTPase, kinase**



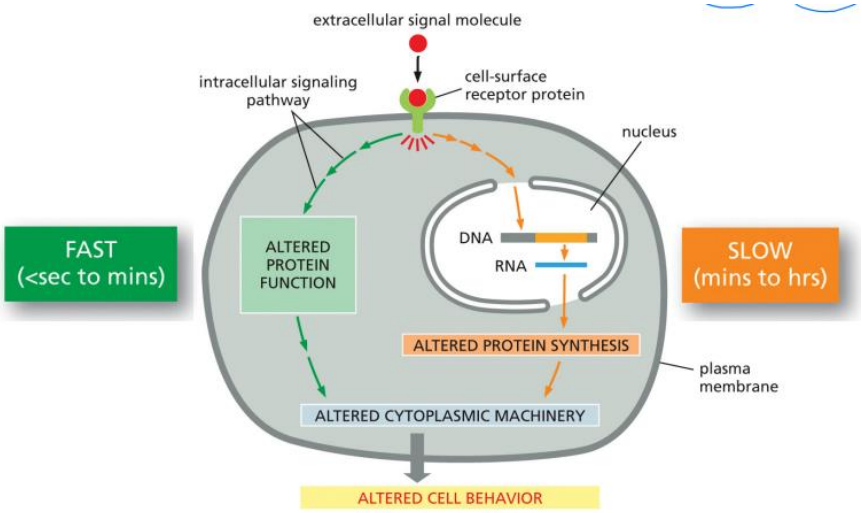


G-protein coupled receptors (GPCRs) interact with more complex, heterotrimeric GTPases



Binding of a signal (a.k.a. ligand) to a GPCR activates the associated G-protein, which can activate one or more targets

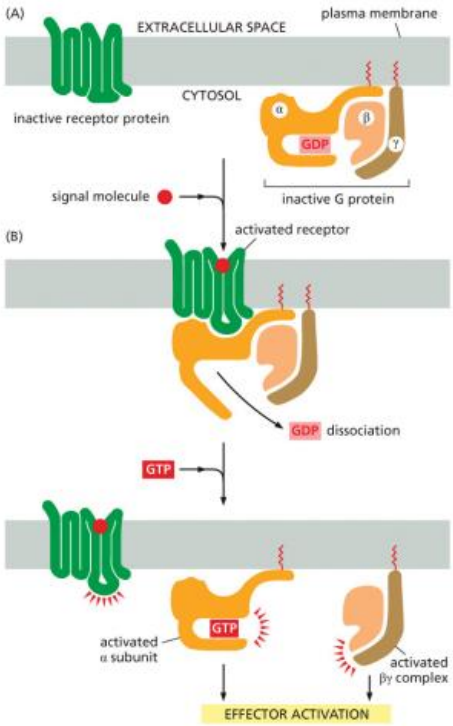
Cellular responses to extracellular signals can be fast or slow



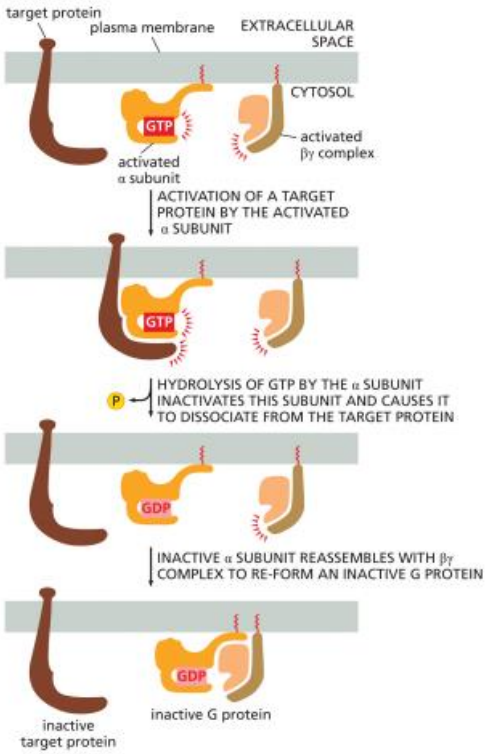
An activated GPCR activates a G protein by inducing the GTPase (the α subunit) to release GDP and bind to GTP.

In other words, the receptor acts as a GEF for a G-protein.

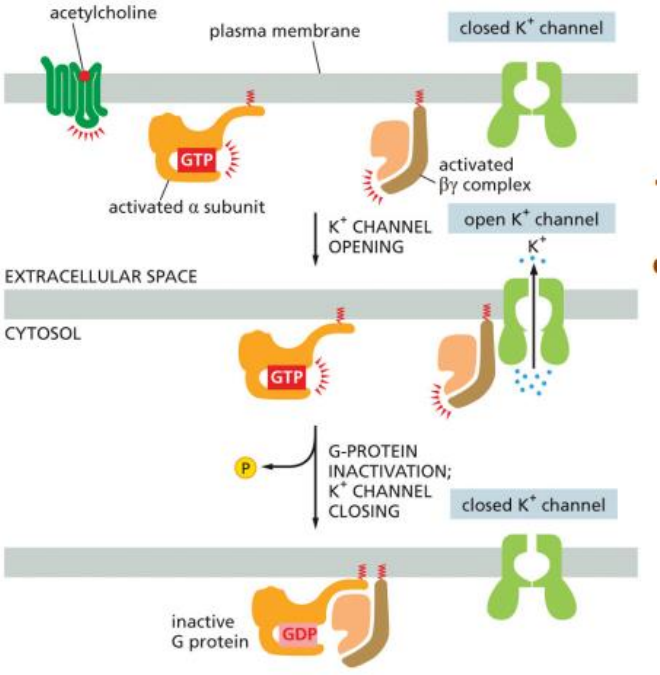
When the α subunit binds to GTP it releases the other two subunits (β and ɣ), thereby activating them as well.



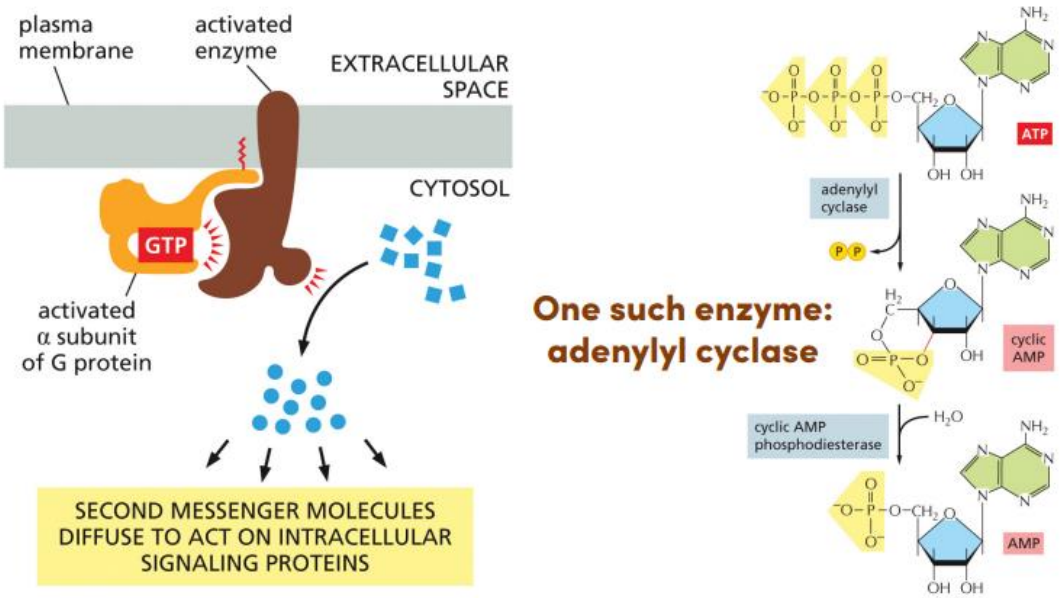
The activated α subunit will then bind to and activate other proteins, until it hydrolyzes GTP and thereby turns itself off



The activated βγ complex can also activate downstream targets, such as (in this example) a K+ channel in the plasma membrane.



Some enzymes activated by G proteins increase the concentrations of small intracellular signaling molecules, a.k.a. “second messengers”



Visualization of a rise in cAMP in a neuron receiving an extracellular signal (serotonin)

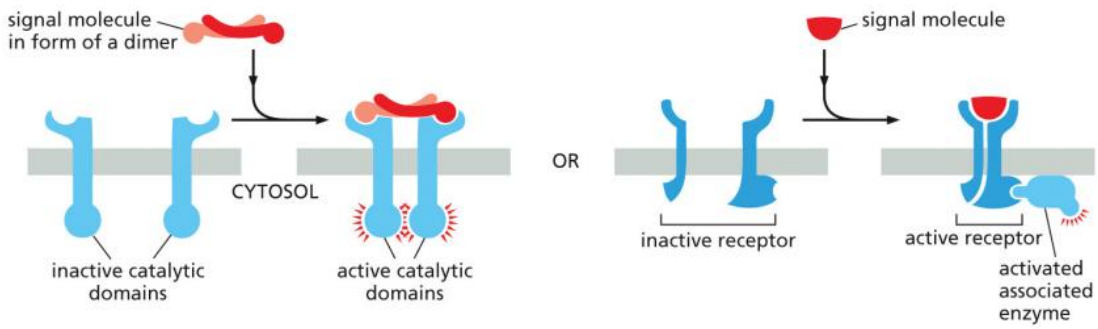
cAMP can trigger a wide range of responses, depending on the identity of the target cell. In skeletal muscle cells, cAMP activates a kinase, Protein Kinase A, which phosphorylates targets to trigger glycogen[糖原] breakdown.

Protein Kinase A can also go into the nucleus and phosphorylate transcription factors to activate gene expression

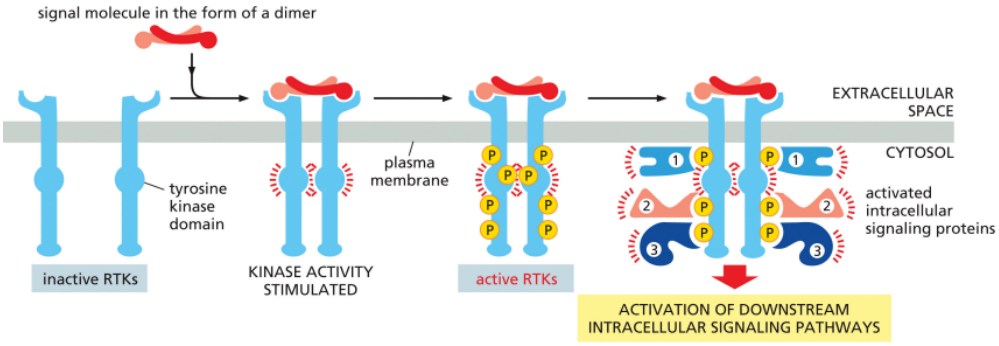
Second messengers can massively **amplify** a signal, resulting in highly sensitive signaling.

For example, photoreceptor cells in the retina express a GPCR called rhodopsin. A conformational change in rhodopsin triggered by a single photon can be detected by the brain due to signal amplification.

Enzyme-coupled receptors



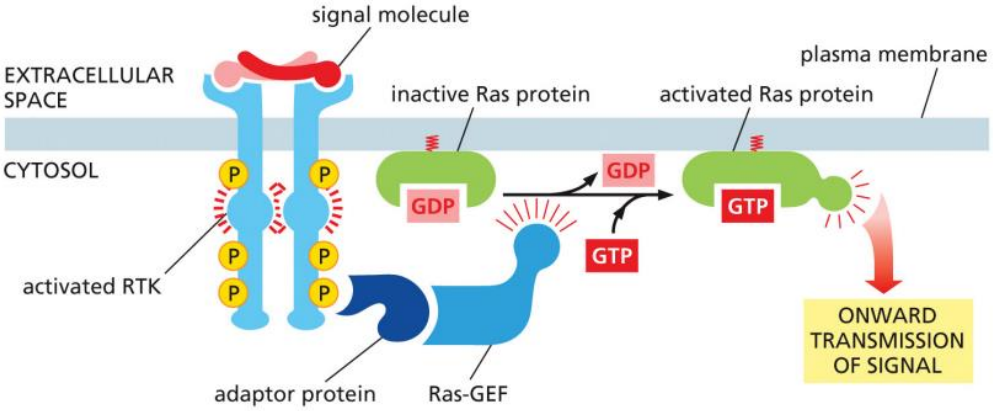
Receptor tyrosine kinases (RTKs) are activated by signal binding



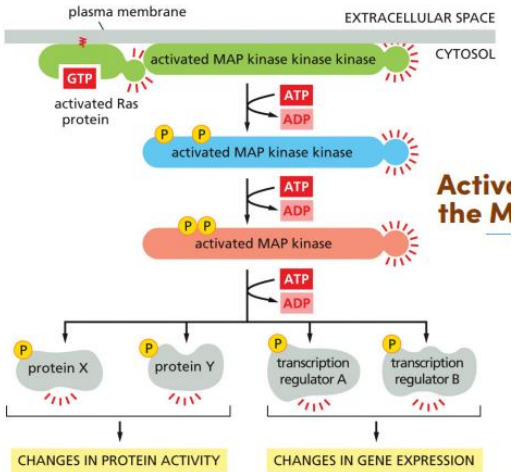
Activation of their kinase activity leads to the assembly of an intracellular signaling complex

Most RTKs activate the monomeric GTPase Ras

Ras is one of the most frequently mutated genes in cancer. Constitutive (signal-independent) activation of Ras leads to cell proliferation and loss of growth control



Activated Ras stimulates the MAP kinase pathway



Both GPCRs and RTKs can activate multiple intracellular signaling pathways

The same signal molecule can induce different responses in different target cells

Animal cells integrate responses to combinations of signals to achieve different outcomes

"Scaffolds" are (usually) non-enzymatic proteins that physically bring together components in a signaling network, making it more efficient and/or more specific

Scaffold proteins organize cellular information flow, expression of different scaffolds under different conditions or in different cell types can prime cells to respond to signals in specific ways.

Some pathogens also express their own scaffolds that **"rewire" signaling pathways** in infected cells

Compartment not surrounded by membranes

Cellular bodies that arise through **phase separation**

Membraneless organelles

Biomolecular condensates (or just condensates)

Coacervates[凝聚物] (condensates involving multiple molecules, which include most biological condensates)

Phase-separated (liquid) compartments

"Germ granules[胚粒]" are found around the periphery of nuclei in the germline of most, if not all, animals.

*Shortly after fertilization of C. elegans embryos, P-granules become concentrated at the **posterior**….*

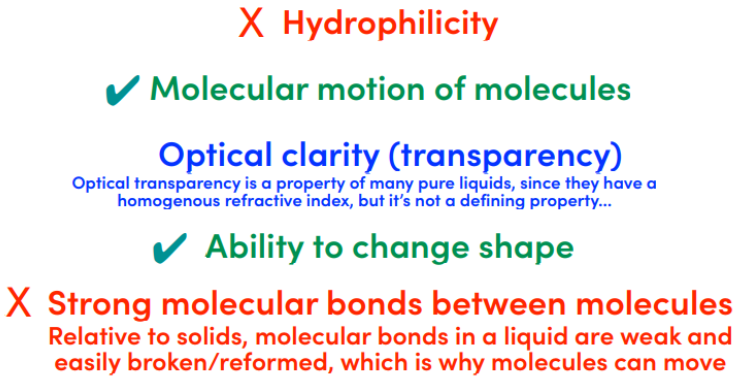
It was long assumed that they migrate there.

However, in vivo imaging revealed that P-granules actually spontaneously form (condense) near the posterior and disappear (dissolve or decondense) near the anterior

P granule localization is not due to cytoplasmic flow.

P-granules also display liquid-like behavior, They undergo rapid shape-changes, fusions, and "dripping" along the nuclear surface. Proteins within them move quickly (can be measured using FRAP) and also undergo constant exchange with the surrounding cytoplasm (the "dilute" phase)

Defining features of a liquid:



Nuclear pores create a size exclusion barrier to passive diffusion between the nucleus and cytoplasm

The interior of the nuclear pore is comprised of “intrinsically disordered” protein domains rich in phenylalanine and glycine (a.k.a. **FG-repeat domains**)

P-granules act as an extension of the nuclear pore
P granules exhibit size exclusion properties, much like nuclear pores

Nuclei contain a number of different “bodies” in which particular types of biochemistry (transcripton, splicing, etc.) are carried out
The largest of these bodies is/are usually one or more “nucleoli”. Nucleoli are sites of ribosome assembly.

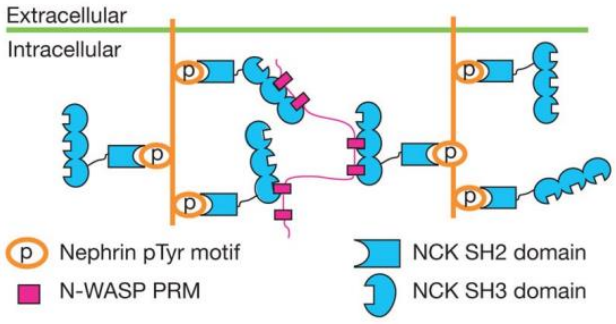
Nucleoli also exhibit liquid-like properties: Manipulating nucleoli into contact with each other in Xenopus oocytes results in fusion
Nucleoli also exhibit liquid-like properties Nucleoli also undergo fission under shear stress

Measurement of intracellular dynamics helps to reveal the properties of biomolecular condensates
Molecules can rapidly join or leave condensates from the surrounding “dilute phase”

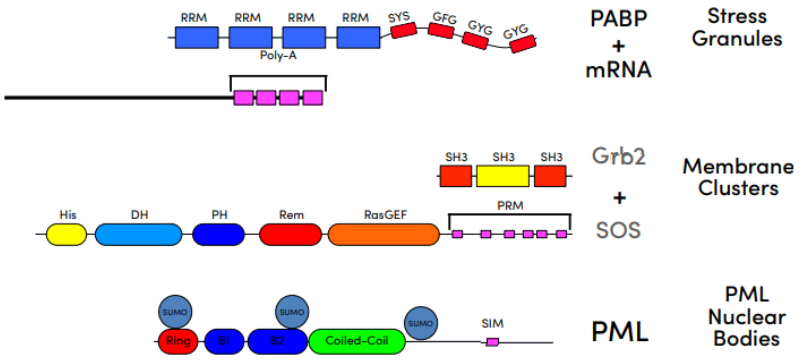
Liquid-liquid demixing/phase separation can concentrate macromolecules within a subcellular compartment, or exclude them

Loss of entropy due to condensation must be offset by enthalpy of binding.

Actin meshwork nucleation in podocytes involves a network of multivalent proteins



A clue to molecular mechanism: Putative phase-separated structures in cells are enriched in multivalent proteins & multivalent ligands:

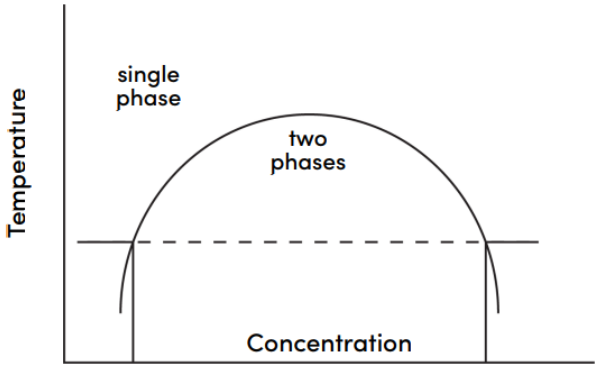


A hypothesis from polymer chemistry:
Multivalent interactions can drive polymerization, which decreases solubility, promoting phase separation. Could these mechanisms underlie formation of phase-separated cellular bodies?

An increase in valency promotes phase separation at lower concentrations.
-Droplets coalesce, increasing in size over time
-Proteins are concentrated in the droplets ~100-fold
-Fluorescence recovers quickly after photobleaching (t1/2 ~ 30s)

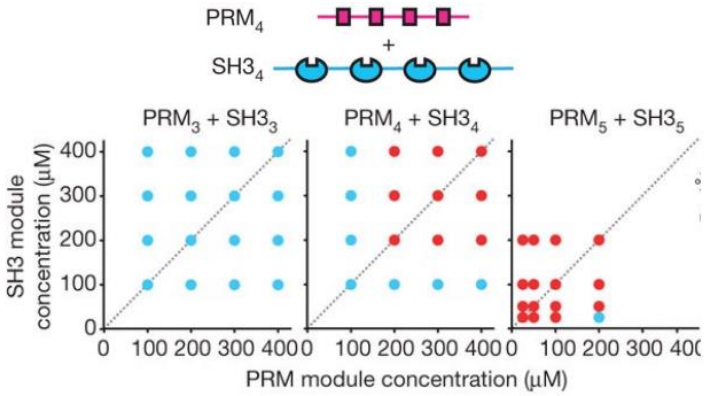
"Liquid-liquid demixing" occurred when solutions contain multivalent proteins

Phase separation can make a mixture look **cloudy or "opalescent."**
This is due to light scattering at the interface between droplets and the surrounding



Lower temperatures and higher concentrations often favor phase separation

At high valencies (#s of interacting domains), the concentration required for phase separation drops dramatically, into a physiologically relevant range.



a) Phase diagrams of multivalent SH3 and PRM proteins. The concentrations are in terms of the modules. The red circles indicate phase separation, and the blue circles indicate no phase separation.

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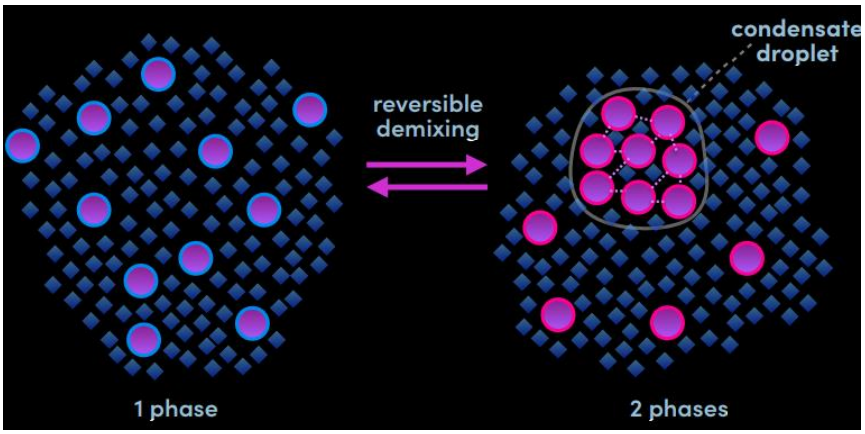
Similar droplet formation was also observed when synthetic multivalent proteins were expressed in cells
"Intrinsic disorder" is a feature of many protein domains, no crystal structure. Computer programs can identify regions of intrinsic disorder
Such regions are often enriched in regulatory sites, such as phosphorylation sites

While formation of condensates is often driven by multivalent proteins (“scaffolds”), they can also recruit lower-valency “clients”

How is the formation of biomolecular condensates regulated in cells?
Protein condensation can by driven by changes in surface properties
- e.g., enhanced protein-protein a!nity and/or reduced solubility

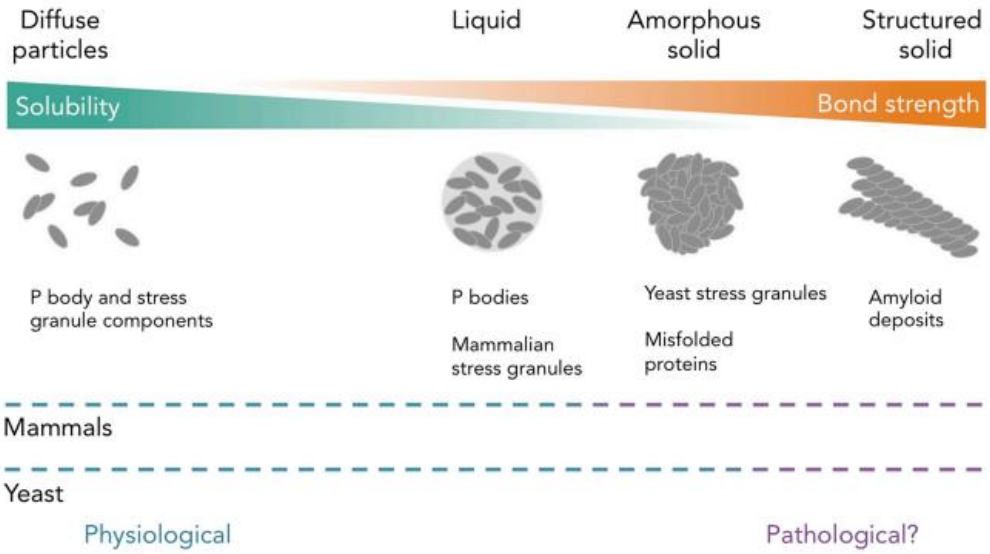


“Active droplets” can assemble around a localized protein modification activity

Phase separation/de-mixing can be driven by:
Low temperature, low or high salt (in vitro)
Multivalent interactions, molecular crowding, and high local concentrations
Nucleic acid binding (which also adds valency to a network of interactions)
Hydrophobic interactions (weak and labile, thus often lead to liquid-like behavior)
Posttranslational modifications that promote protein-protein interactions or reduce solubility of components

How is phase separation useful to cells?
Provides a rapid, readily reversible mechanism to spatially organize sets of cellular components
Concentrates enzymes & substrates to expedite chemistry
Conversely, provides a way to arrest chemistry by separating enzymes from substrates, e.g. to sequester & protect transcripts from translation or degradation during times of stress
May provide a unique biochemical environment to facilitate some reactions
Due to rapid equilibrations within liquid media, phase separation provides a way to orchestrate a signal or biochemical transition across a large compartment
Other functions/roles remain to be discovered

High concentrations of proteins within phase-separated droplets can lead to aberrant sol-gel transitions, which can be irreversible/pathological



This type of behavior may underlie neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)