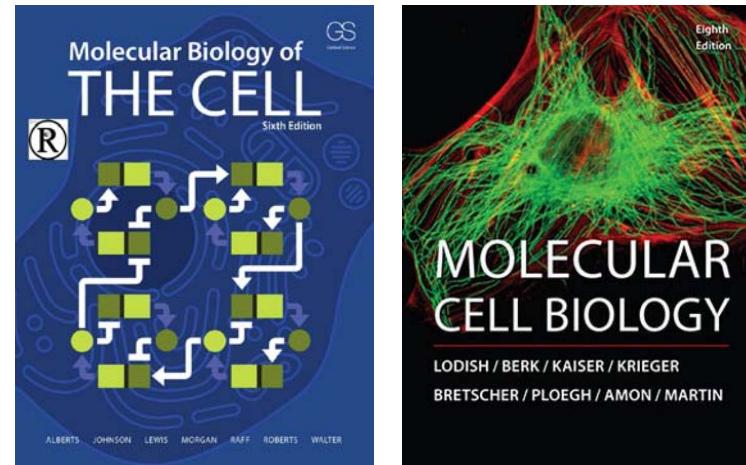
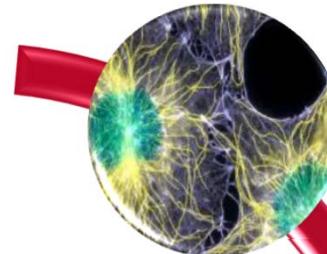
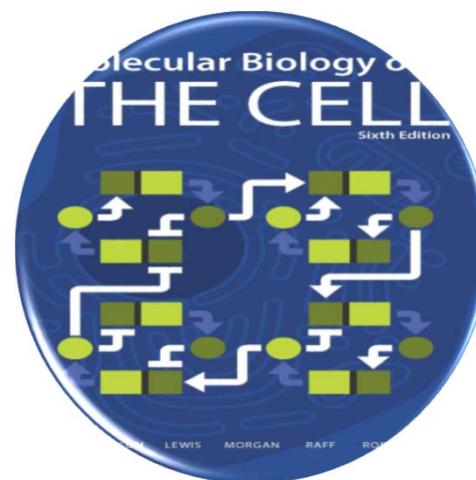

Rehearsal for the final exam

Cell biology

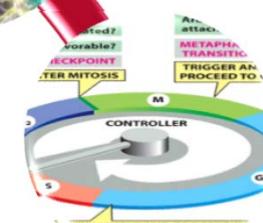


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Where to start?



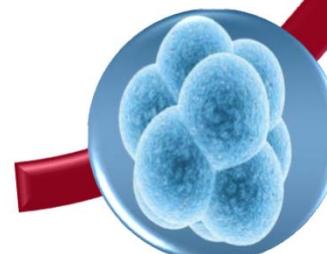
Cell-cell communication,
cytoskeleton & cell
movement



Cell cycle,
apoptosis &
autophagy



Cells in tissues
& ECM



Cancer, stem cells
& neurons

Strategy for straight forward learning

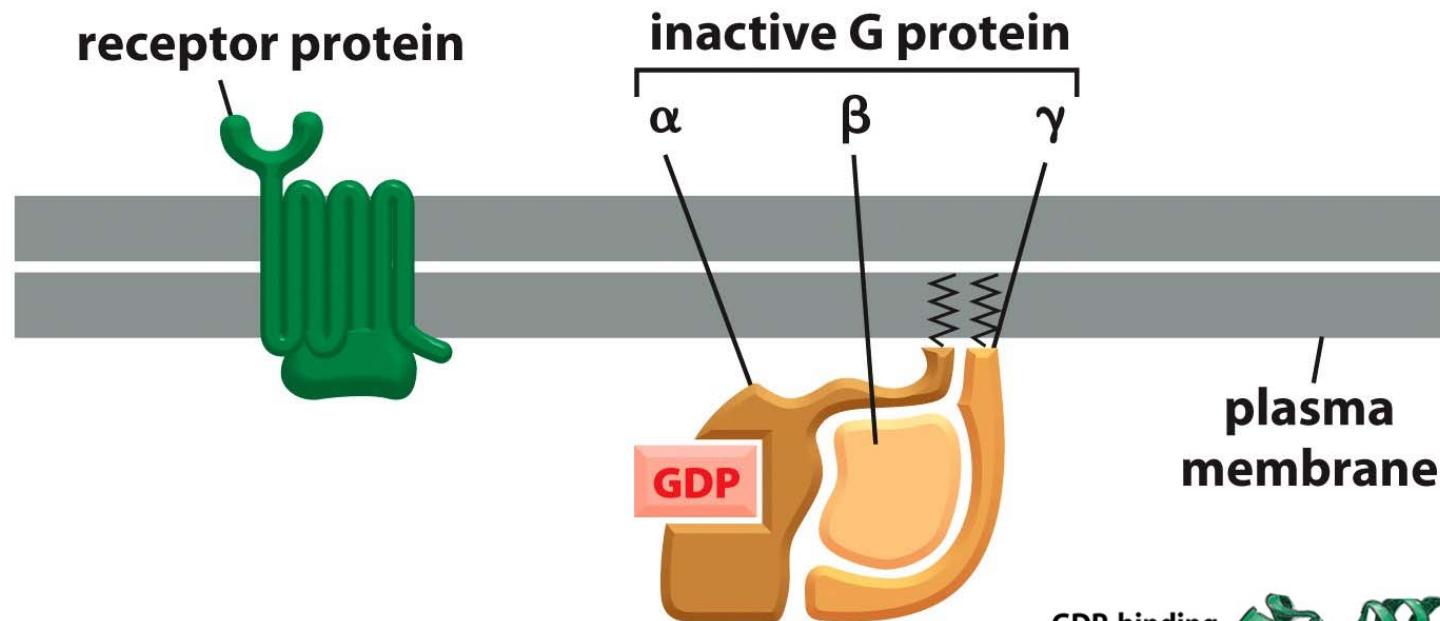
- try to understand the concept first
- principles & mechanisms
- next: key players
- also important: medical relevance
- therapie / analysis
- then: special cases

Examples for straight forward learning

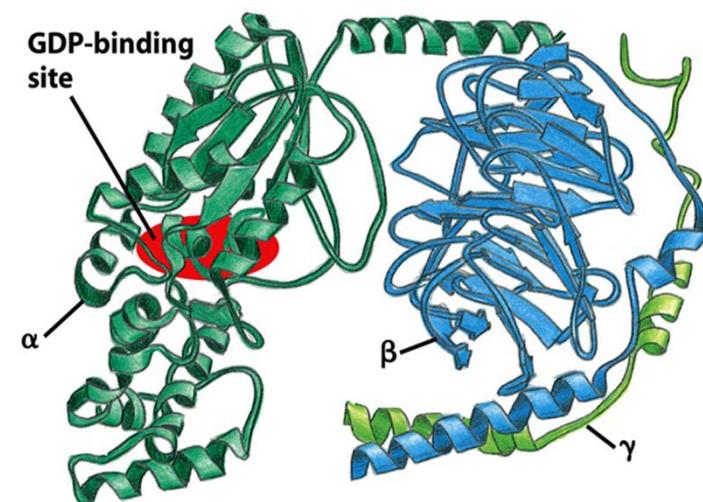
Cell-cell communication

- **Principles**
 - signal perception and transduction
 - reaction cascades
 - modifications phosphorylations: auto, trans etc.
- **different signaling pathways**
- **key players**
 - receptors (G protein coupled receptors) and ligands and G proteins and messengers, signaling molecules etc.
 - primary response, secondary response,
- **Vision - smell**
 - general mechanisms - signaling molecules electrochemical properties

G-protein function is coupled to the receptor function, therefore the name **G-Protein-Coupled Receptor (GPCR)**



- Subunits α and γ are tethered to PM
- Subunit α binds to GTP/GDP and has GTPase activity
- β subunit is bound by α and γ
- β and γ form a functional subunit



2. Activation of a G protein by an activated GPCR

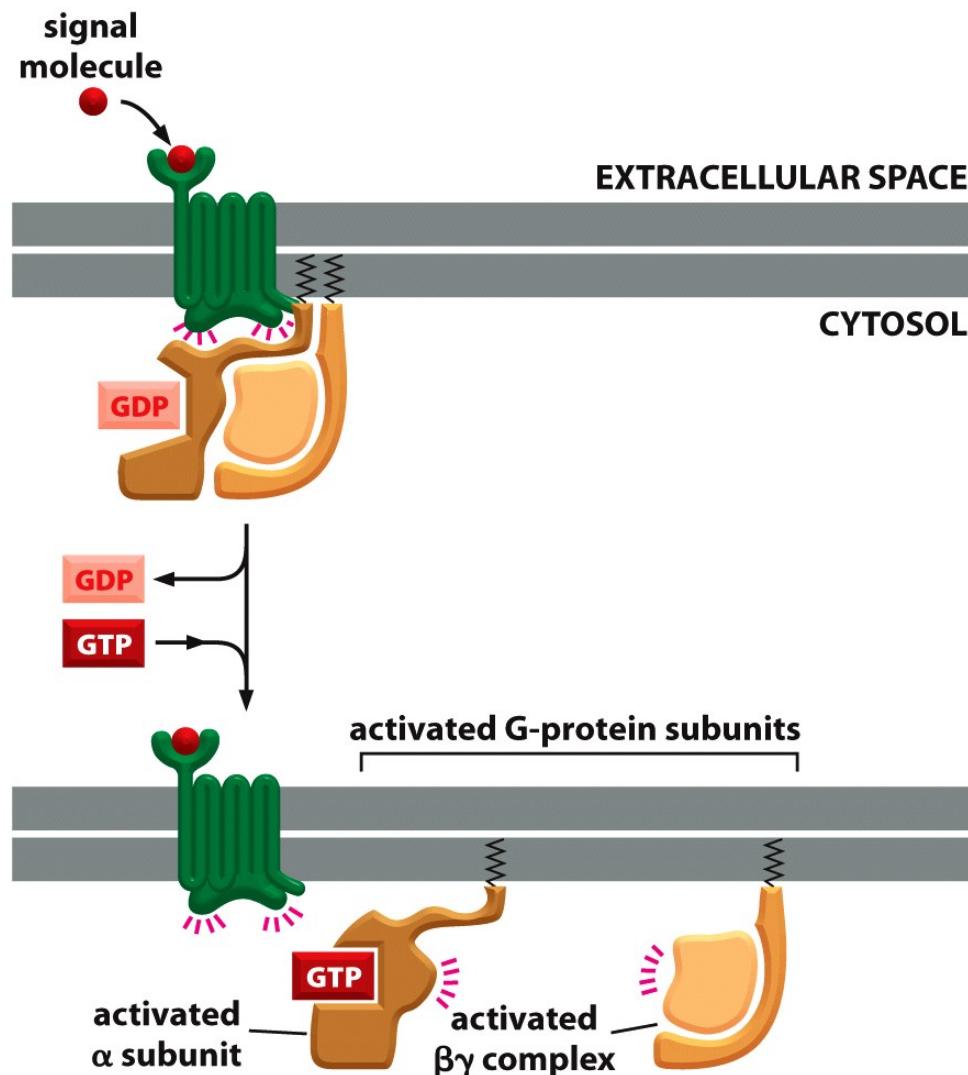


Figure 16-17b Essential Cell Biology 3/e (© Garland Science 2010)

G proteins can be deactivated by GTP hydrolysis

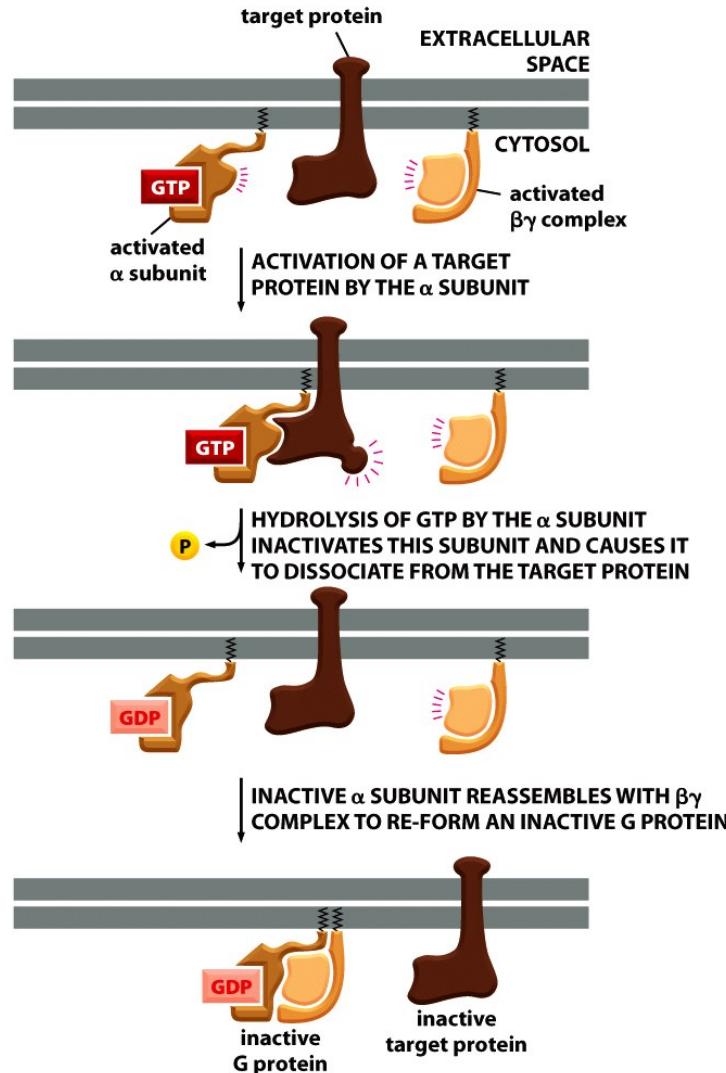
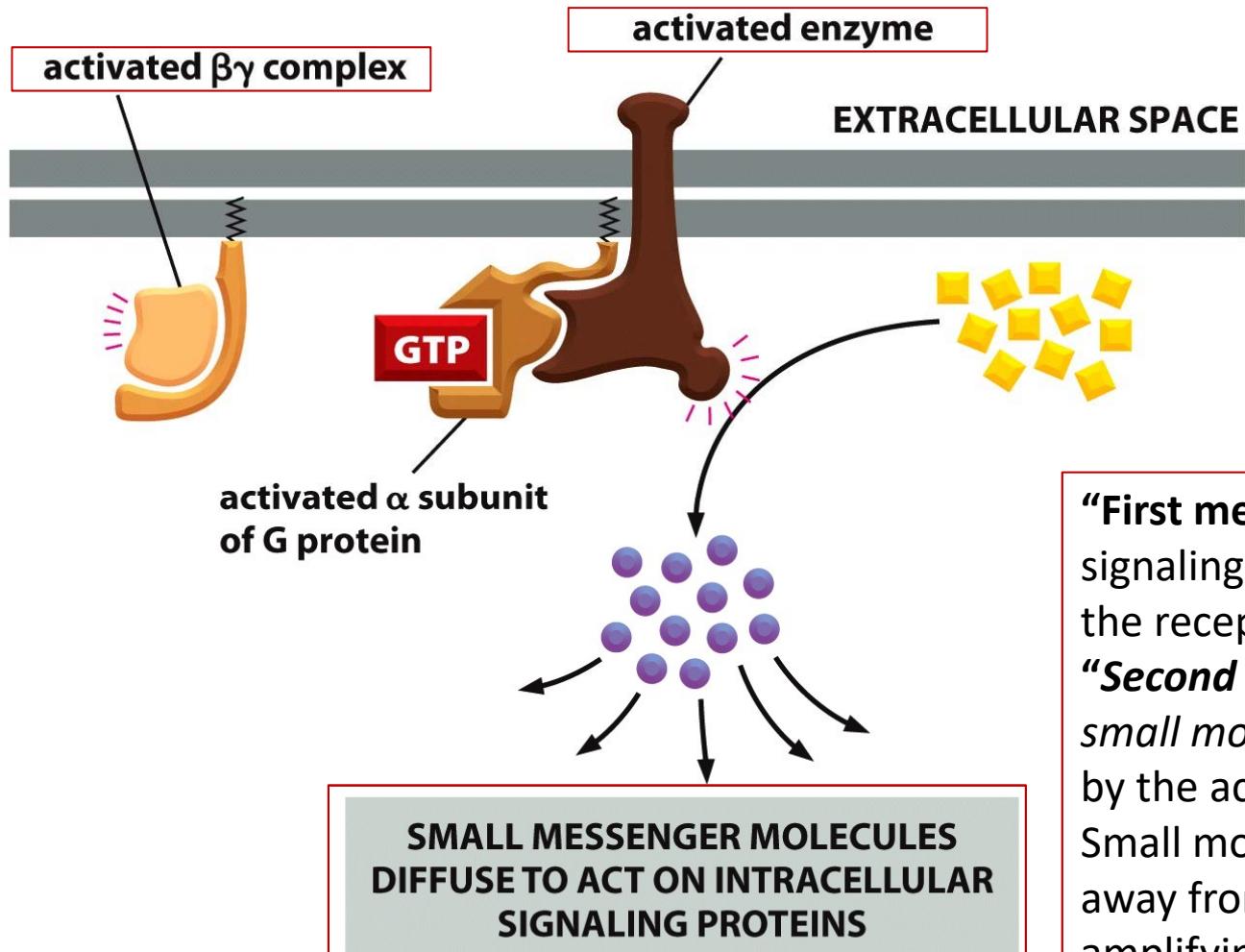


Figure 16-18 Essential Cell Biology 3/e (© Garland Science 2010)

GPCR signaling includes the activation-deactivation cycle of the trimeric G proteins

- Ligand binding activates GPCR, which then acts as a GEF to exchange GDP for GTP and thus activates the G protein
- “Activated trimeric G proteins” dissociate into an active α subunit and an active $\beta\gamma$ complex
- expose functional groups on the activated α subunit and on the $\beta\gamma$ complex.
- Subunit α then acts as GTPase to hydrolyze GTP into GDP, thereby inactivating the G-protein
Alternative inactivation:
RGS(regulator of G protein signaling) acts as α -Subunit-specific GAPs to cause GTP hydrolysis.

How does GPCR signaling activates small messenger molecules?



"First messengers":
signaling molecules that activate
the receptor

"Second messengers":
small molecules that are produced
by the activated enzymes
Small molecules rapidly diffuse
away from their source, thereby
amplifying and spreading the
intracellular signal

Figure 16-20 Essential Cell Biology 3/e (© Garland Science 2010)

Small messenger molecules downstream of GPCR signaling (second messengers)

- **cAMP**
- **DAG** (diacylglycerol) and **IP₃** (inositol 1,4,5 triphosphate)
(produced by **phospholipase C-β**)
- **Ca²⁺**
- **cGMP/cAMP**
(to trigger gated ion channels in smell and vision)

How does GPCR trigger Ca^{2+} release and protein kinase C (PKC) activation?

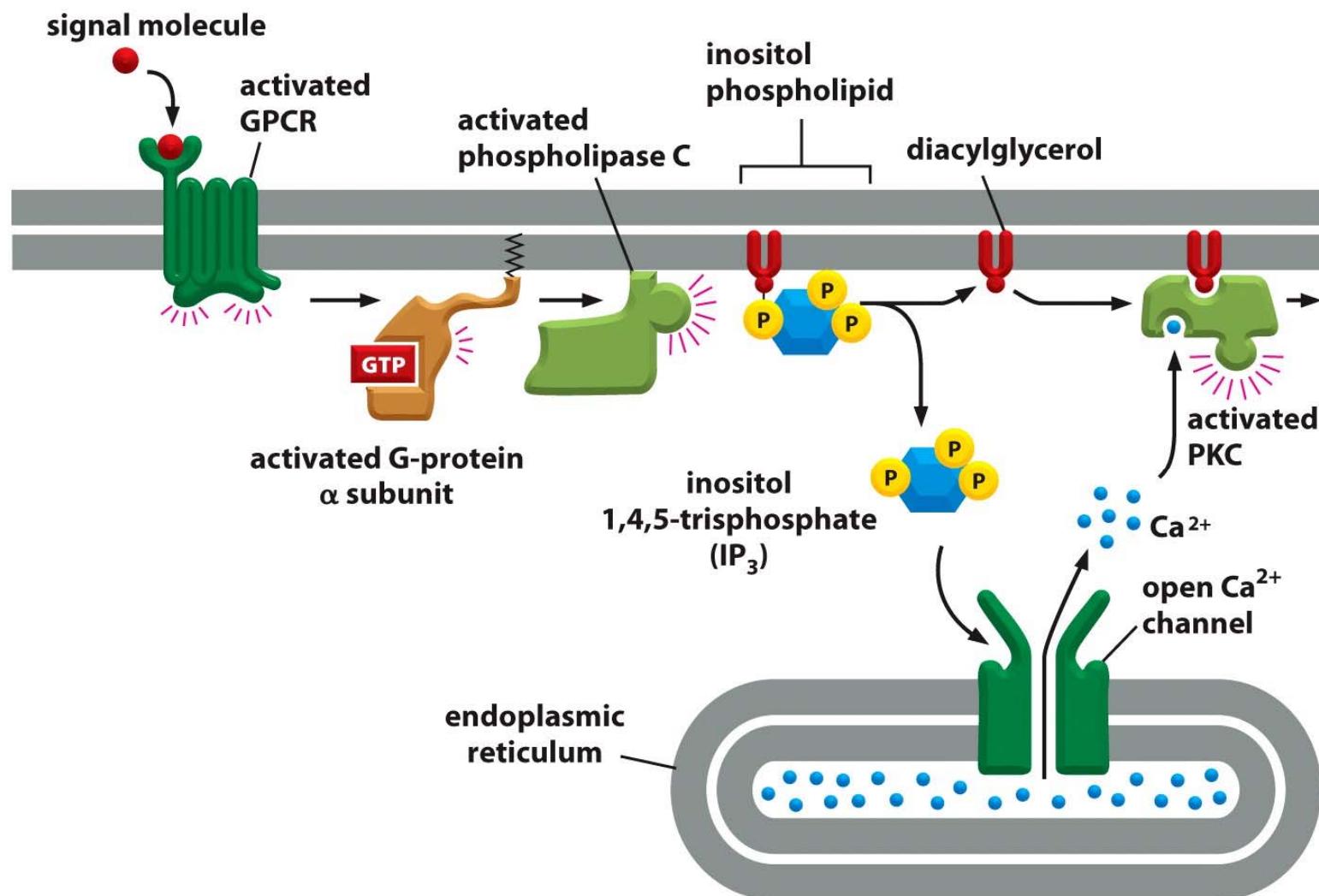
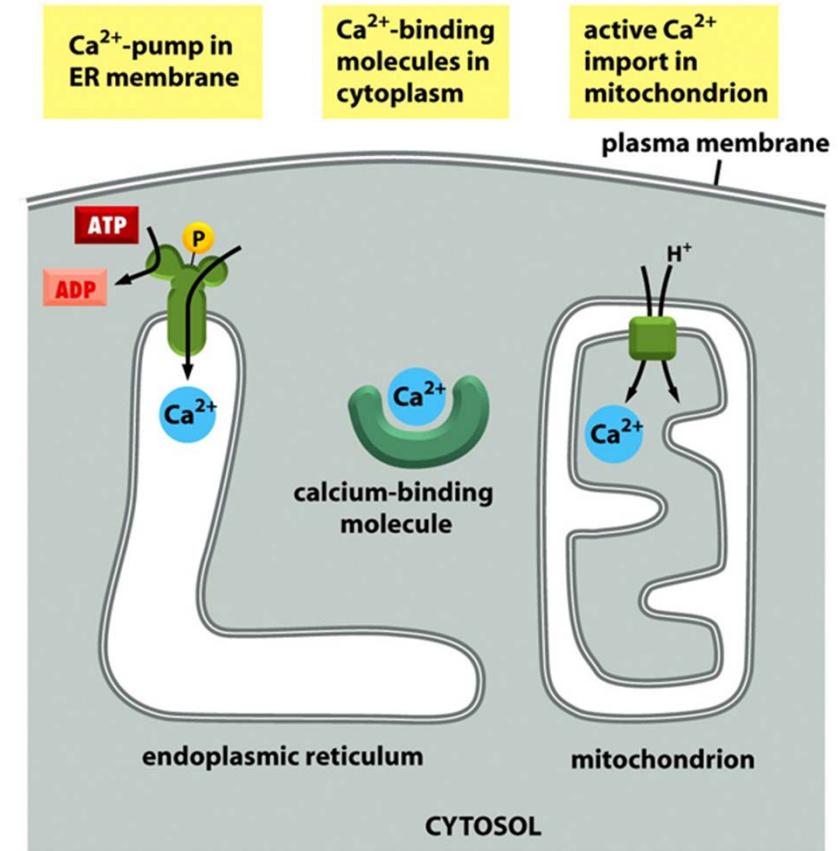
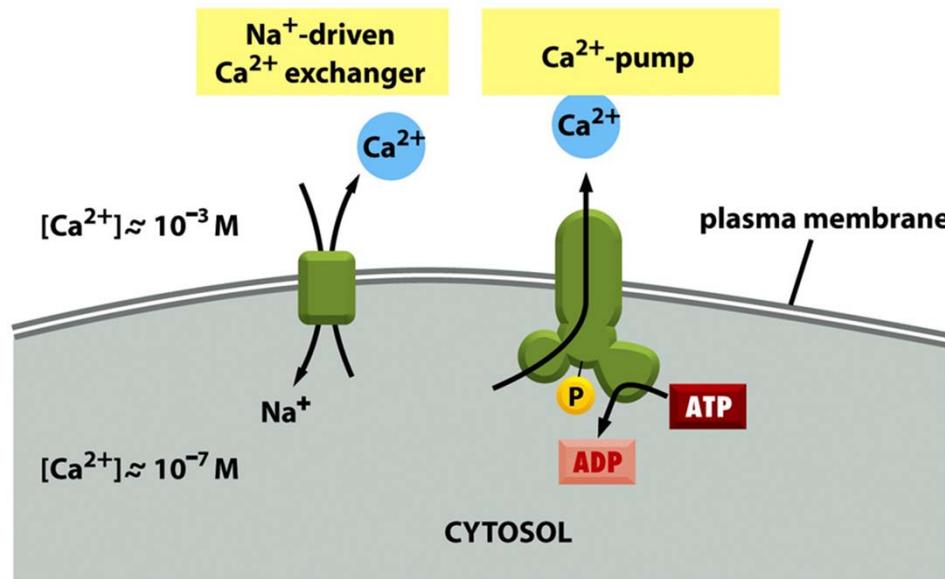


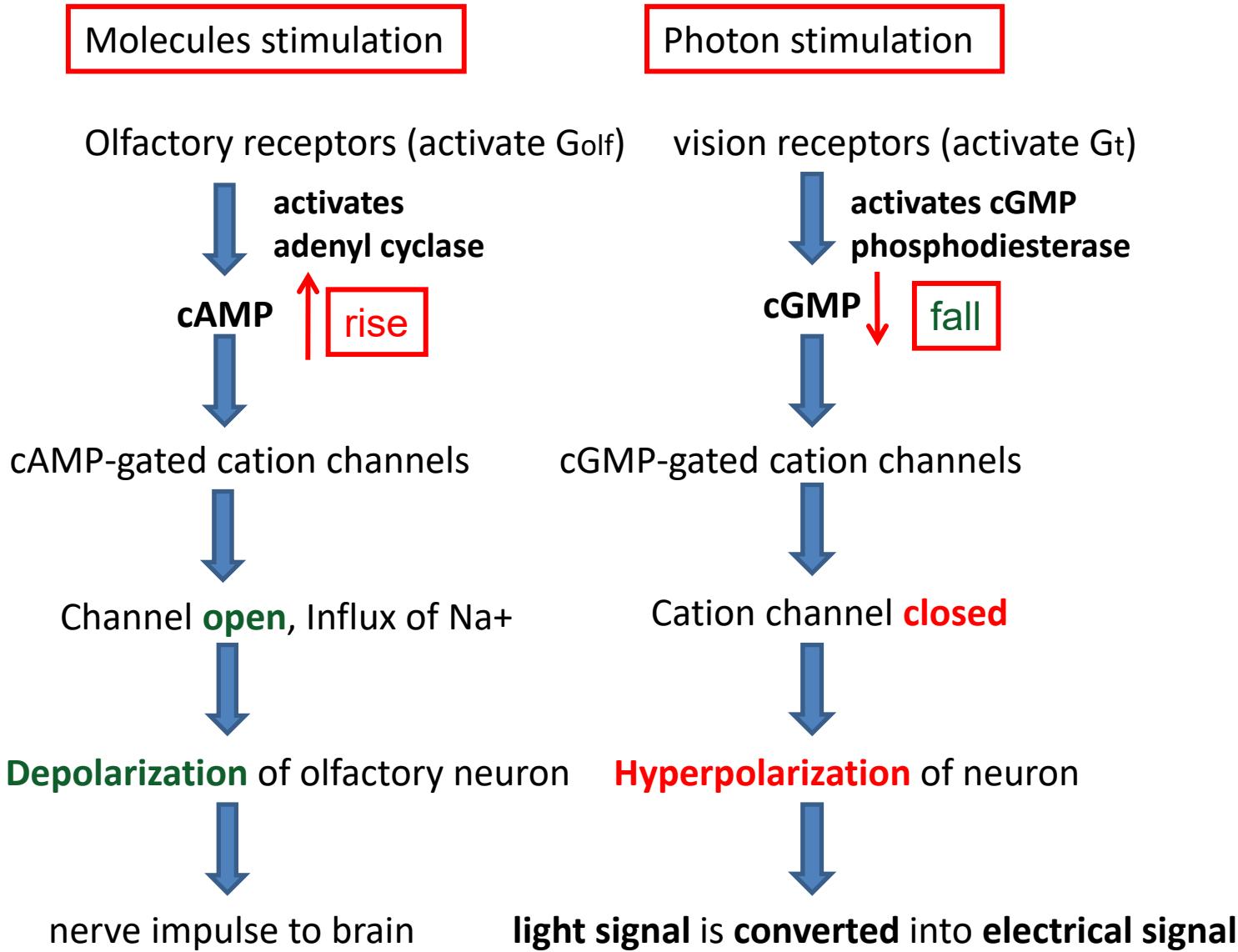
Figure 16-25 Essential Cell Biology 3/e (© Garland Science 2010)

How do cells keep low Ca^{2+} concentrations in the cytosol?

5 different ways to keep cytosolic Ca^{2+} low:

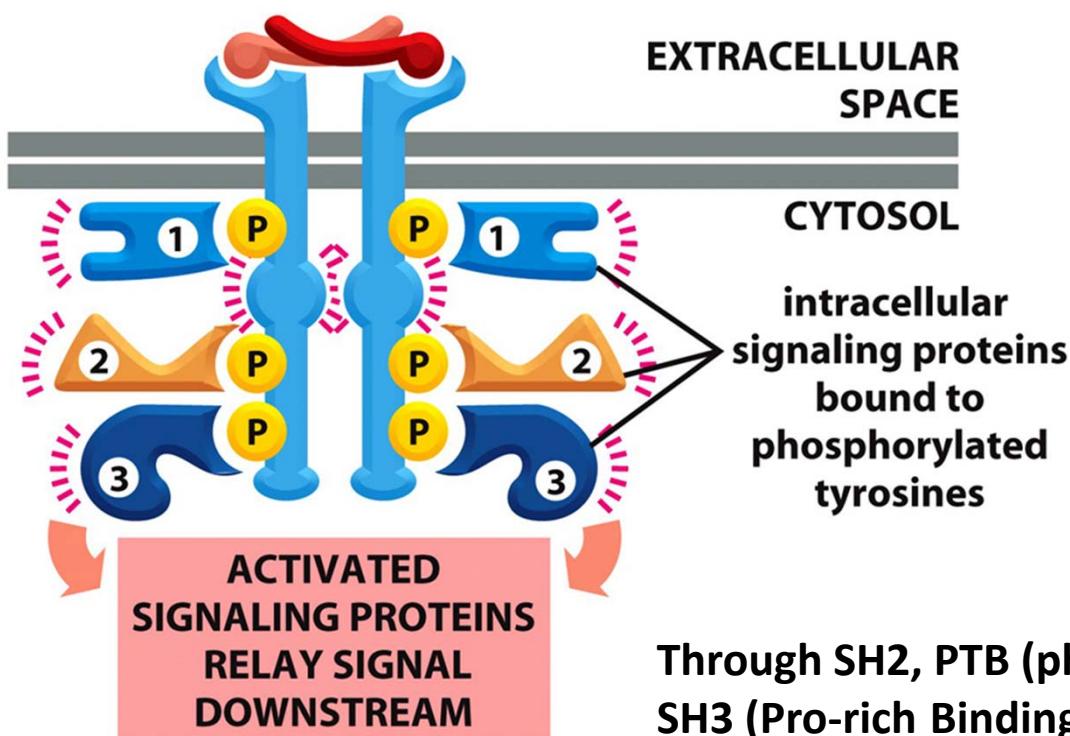


Cyclic-nucleotide-gated ion channels downstream of GPCR in SMELL and VISION



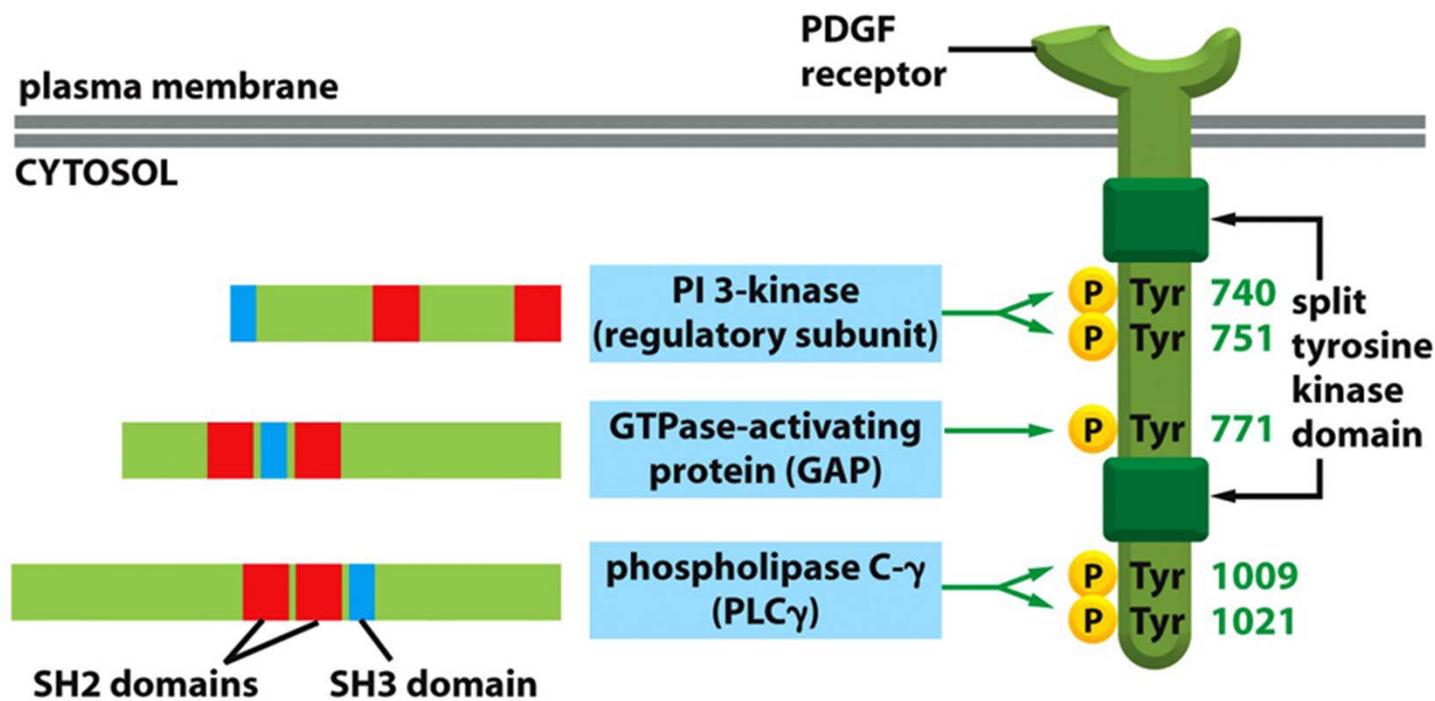
Phosphorylation on RTK has dual roles:

- Activates RTK kinase activity
- Introduce phospho-Tyr that can recruit other protein factors to relay signals



Through SH2, PTB (phospho-Tyr binding) domain, or SH3 (Pro-rich Binding) domains, etc.

The PDGF (platelet-derived growth factor)-receptor



Several important signal pathways downstream of RTK

- **Ras** pathway
- **Rho** pathway
- **PI3K** pathway

PI3K pathway promotes cell growth and survival

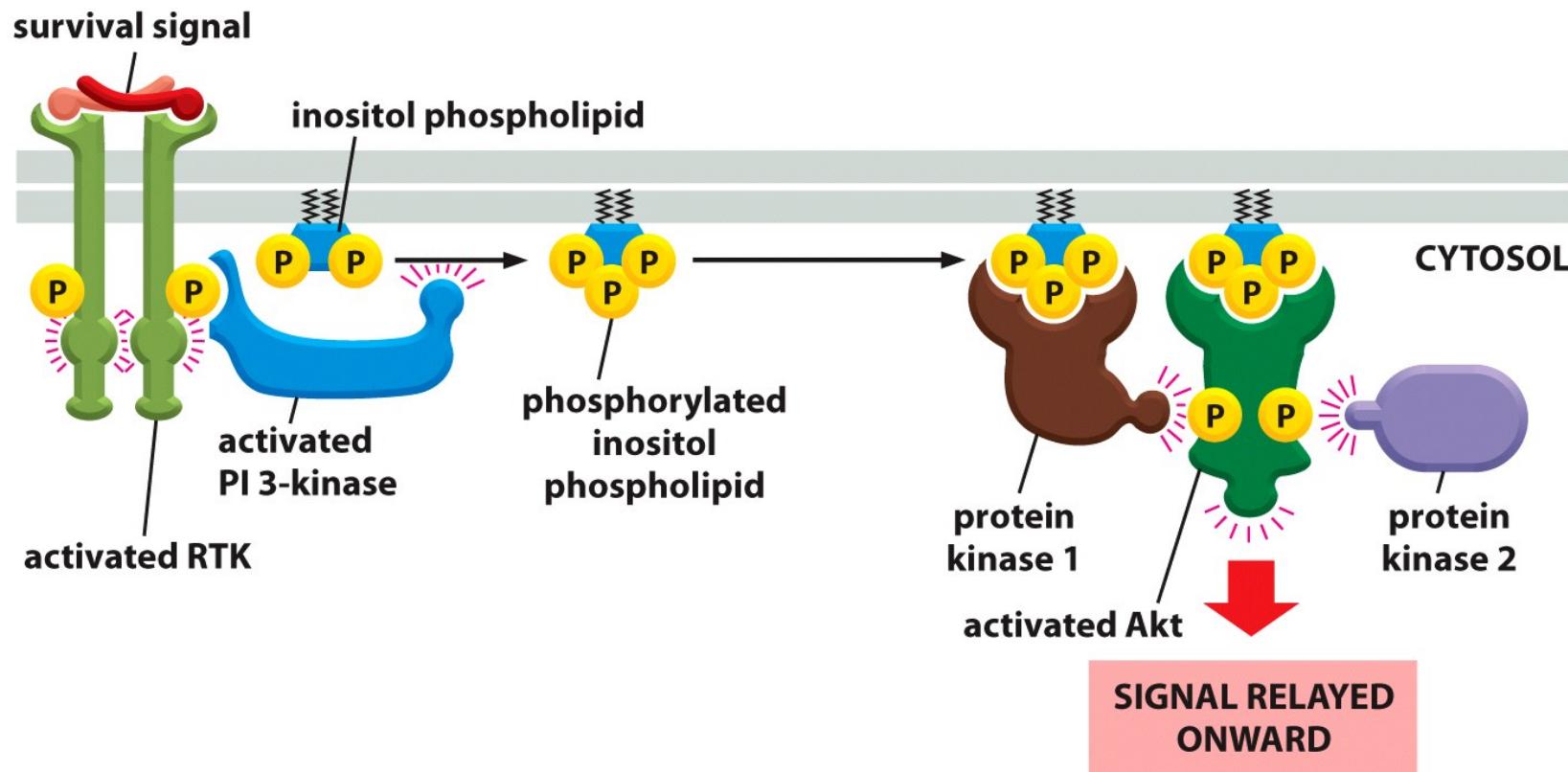
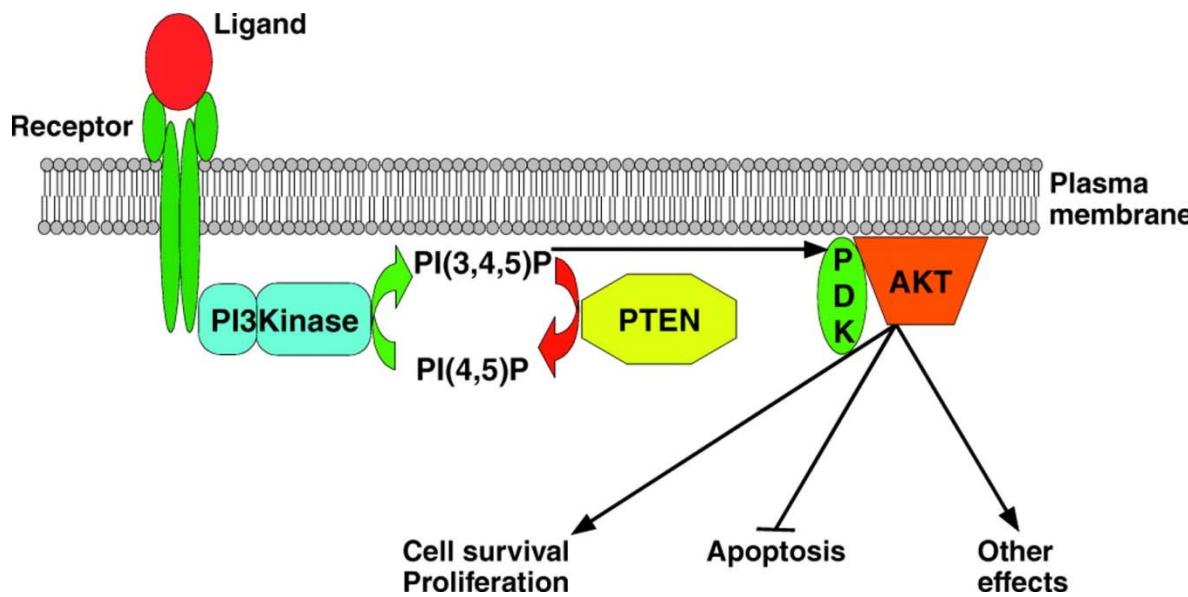


Figure 16-33 Essential Cell Biology 3/e (© Garland Science 2010)

PI3K and PTEN in controlling PIP3

- PTEN: Phosphatase and tensin homolog



PI3K hyperactivation and PTEN loss of function frequently occur in human cancers

Major types of non-receptor tyrosine kinase in cytosol

- **JAK:** the largest family, mediates cytokine signaling
- **Scr family:** cytosolic tyrosine kinase (Src--- sarcoma virus), proto-oncogene, controls cytoskeleton assembly, growth and proliferation.
- **Focal adhesion kinase:** mediate integrin signaling to cytoskeleton during cell adhesion.

JAK-STAT

- JAK-Janus kinase--- cytosolic tyrosine kinase;
- STAT-Signal transducers and activators of transcription
--- transcription factors

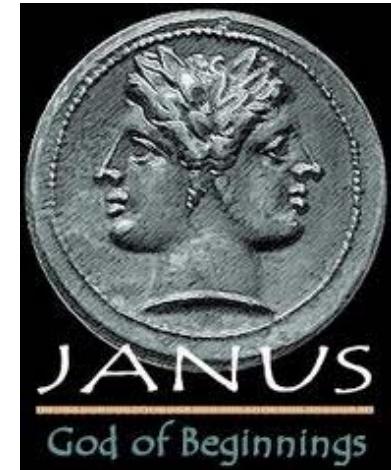


Table 15–6 Some Extracellular Signal Proteins That Act Through Cytokine Receptors and the JAK–STAT Signaling Pathway

SIGNAL PROTEIN	RECEPTOR-ASSOCIATED JAKs	STATS ACTIVATED	SOME RESPONSES
γ-interferon	JAK1 and JAK2	STAT1	activates macrophages
α-interferon	Tyk2 and JAK2	STAT1 and STAT2	increases cell resistance to viral infection
Erythropoietin	JAK2	STAT5	stimulates production of erythrocytes
Prolactin	JAK1 and JAK2	STAT5	stimulates milk production
Growth hormone	JAK2	STAT1 and STAT5	stimulates growth by inducing IGF1 production
GMCSF	JAK2	STAT5	stimulates production of granulocytes and macrophages

How does prolactin promote milk production?

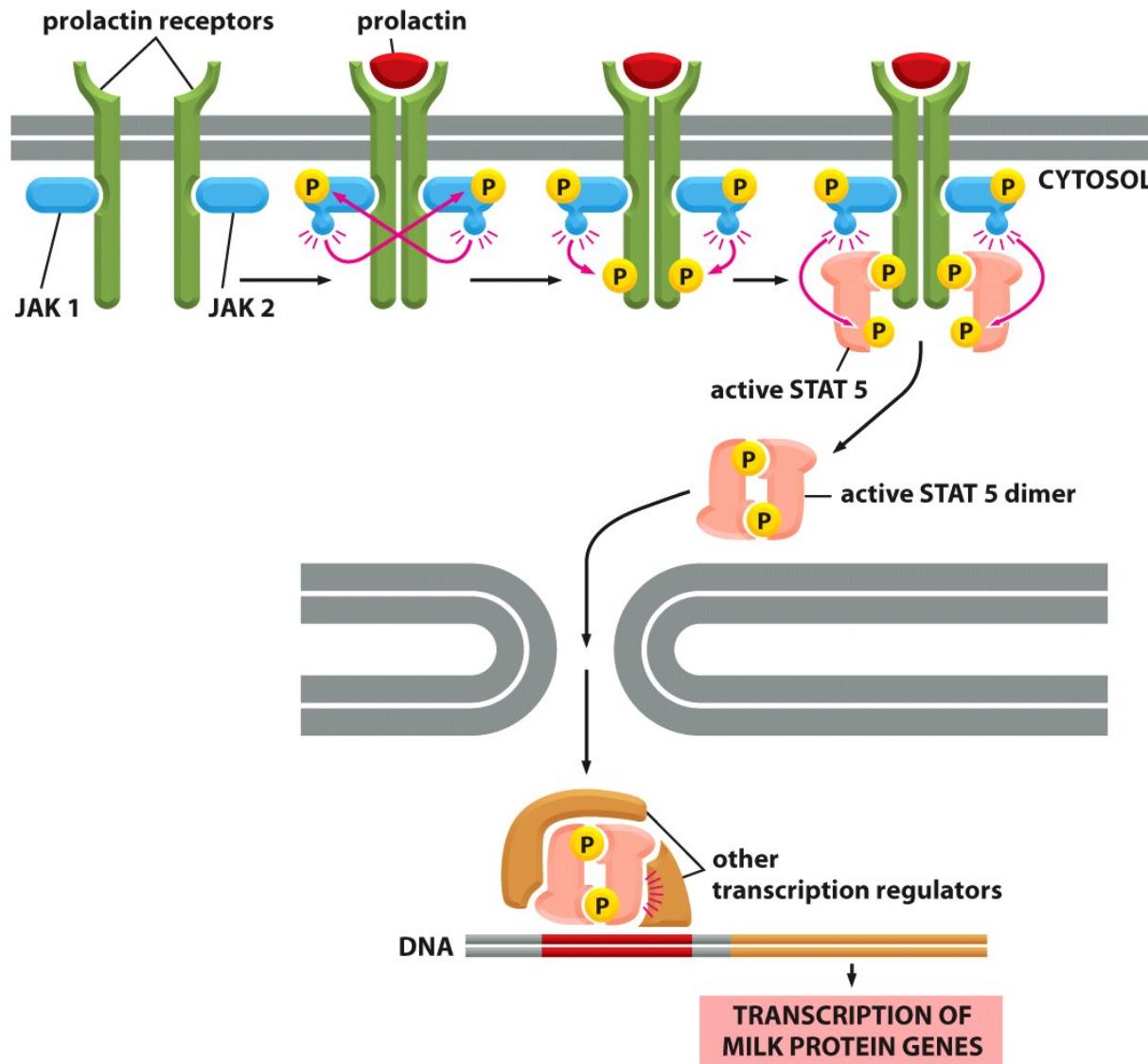


Figure 16-39 Essential Cell Biology 3/e (© Garland Science 2010)

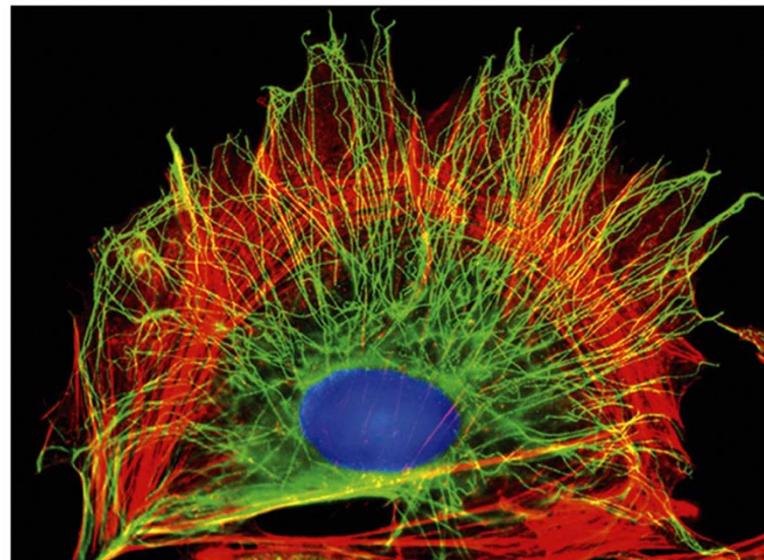
Examples for straight forward learning

Cytoskeleton

- Different structures/type of the cytoskeleton
 - components, actin, tubulin, vimentin
 - in which organisms do they occur?
- Dynamics of the cytoskeleton:
 - assembly (subunits, energy, direction, assistance organization)
 - disassembly (direction)
 - growth / shrinkage and regulation thereof
 - polarity
 - inhibitors/modulators effectors chemicals
- Function
- Higher order structures
 - muscles, cilia flagella, spindle during mitosis
 - interaction / interactors kinesins/myosins directions
- Muscle contraction
- Movement of cells

Types of cytoskeleton system

- Microfilament--- basic unit: **Actin**
- Microtubule---basic unit: **tubulin**
- Intermediate filament-basic unit: **keratin, vimentin, lamin**, etc.

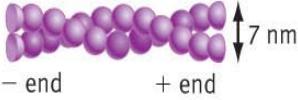
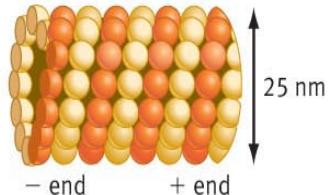


10 μm

A brief summary for the components of the cytoskeleton

SUMMARY TABLE 7.3 Cytoskeletal Filaments

The three types of filaments found in the cytoskeleton are distinguished by their size and structure, and the protein subunit of which they are made.

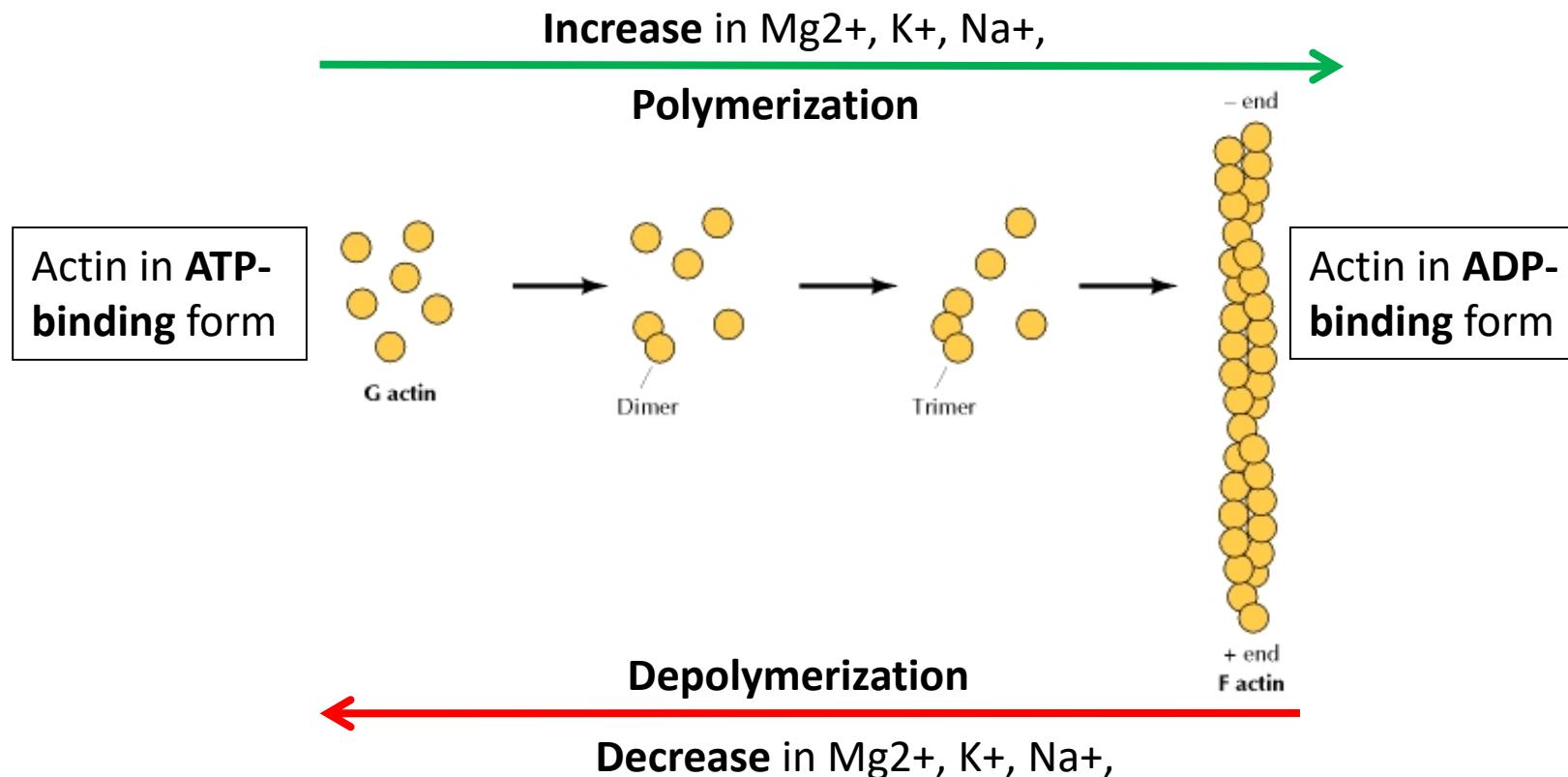
	Structure	Subunits	Functions	
Actin filaments (microfilaments)	Strands in double helix 	Actin 	<ul style="list-style-type: none">• maintain cell shape by resisting tension (pull)• move cells via muscle contraction or cell crawling• divide animal cells in two• move organelles and cytoplasm in plants, fungi, and animals	Semiflexible Motors polarized
Intermediate filaments	Fibers wound into thicker cables 	Keratin or vimentin or lamin or others 	<ul style="list-style-type: none">• maintain cell shape by resisting tension (pull)• anchor nucleus and some other organelles	Flexible No motor unpolarized
Microtubules	Hollow tube 	α - and β -tubulin dimers 	<ul style="list-style-type: none">• maintain cell shape by resisting compression (push)• move cells via flagella or cilia• move chromosomes during cell division• assist formation of cell plate during plant cell division• move organelles• provide tracks for intracellular transport	Stiff rods Motors Polarized

Actin

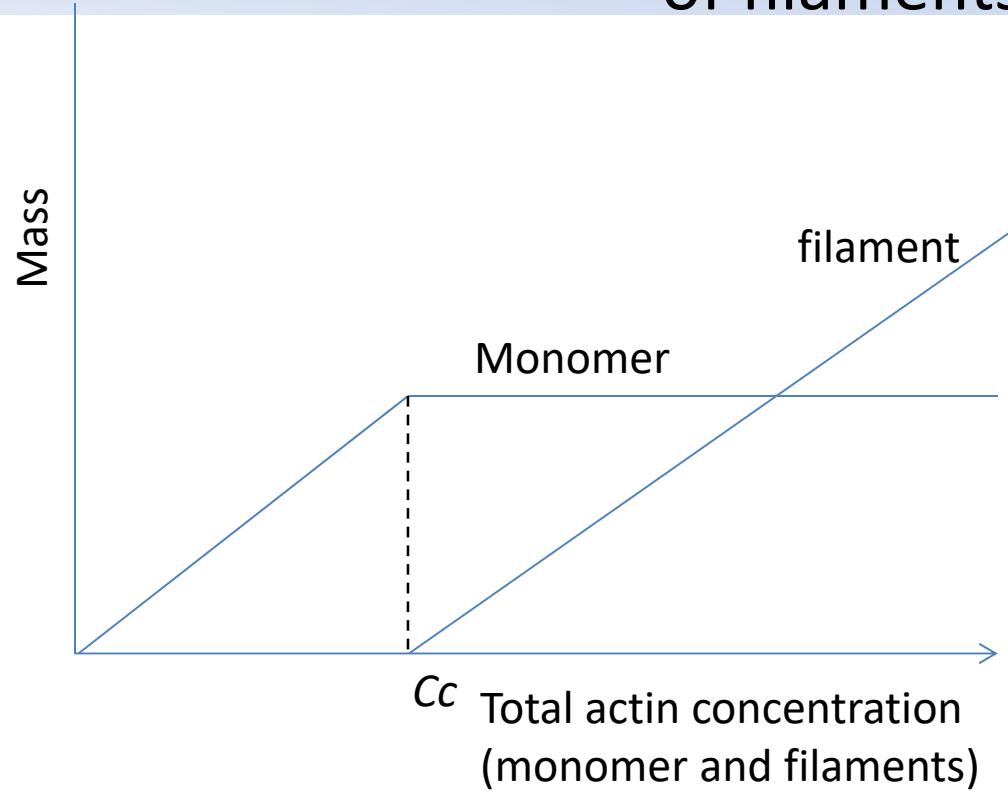
- Highly **conserved** across species,
80% homology between Amoebas and animals
- Most **abundant** protein in cells
(1-5 % cellular protein in non-muscle cells, 10% in muscle cells)
- Exists in **three isoforms** (α -actin, β -actin, γ -actin)
 - α -actin--- contractile structure
 - β -actin--- leading edge and cell cortex
 - γ -actin---stress fibers

G-actin (globular) and F-actin (filamentous)

- G-actin: globular and monomeric actin
- F-actin: filamentous, and linear chain of G-actin



Critical actin concentrations (C_c) for the polymerization of filaments



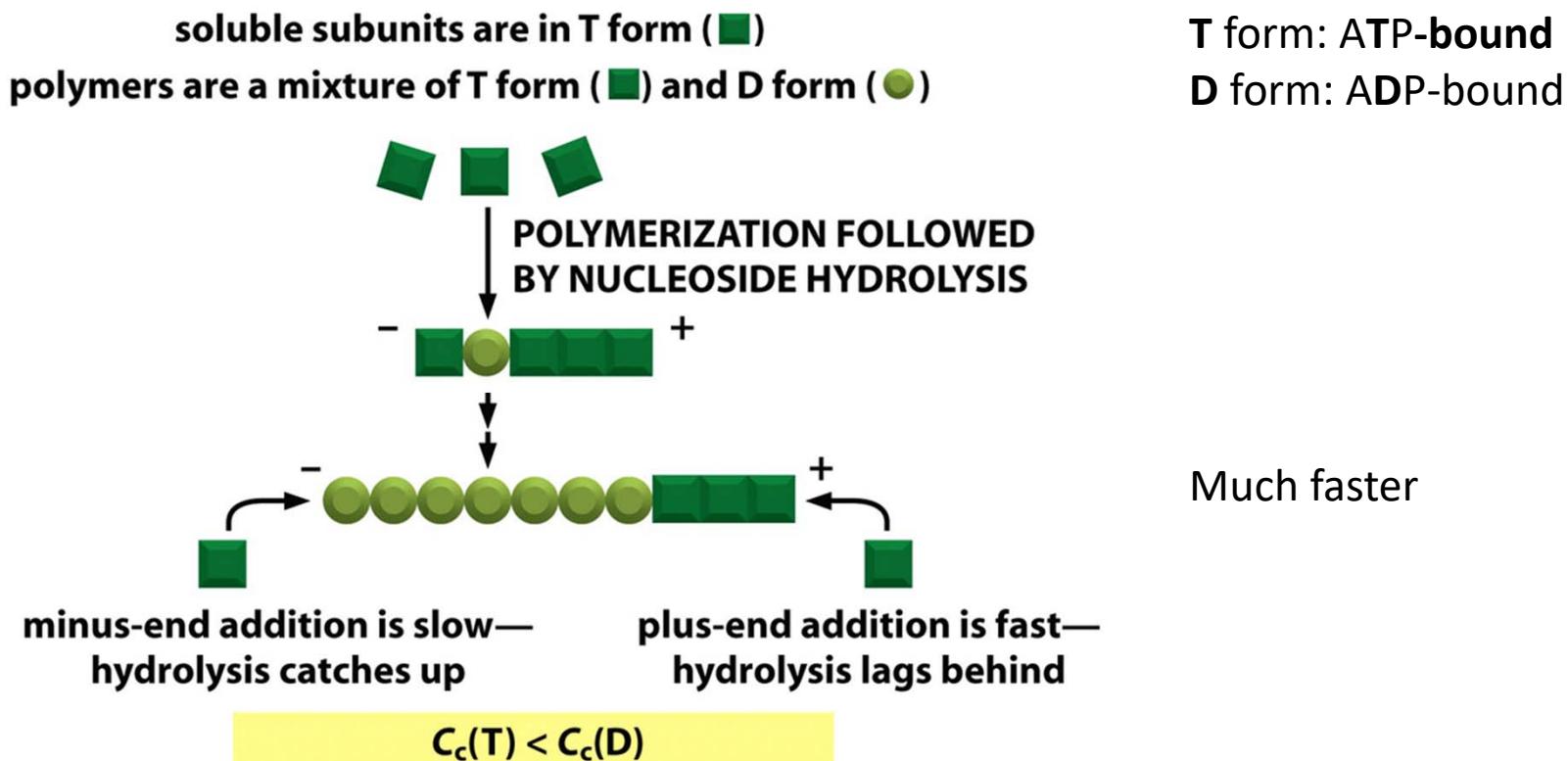
In cells, G-actin levels can be 0.1-0.4mM, C_c is ~0.2 μ M

Definition of C_c :

Concentration of free G-actin at which the assembly onto a filament end is balanced by loss from that end.

Actin treadmilling

- The addition of **ATP-G-actin** at the “+” end with **simultaneous removal** of G-actin at the “-” end of **F-actin**, resulting in a section of filament seemingly “**moving**” across a stratum or the cytosol



Formin mediates straight filament assembly

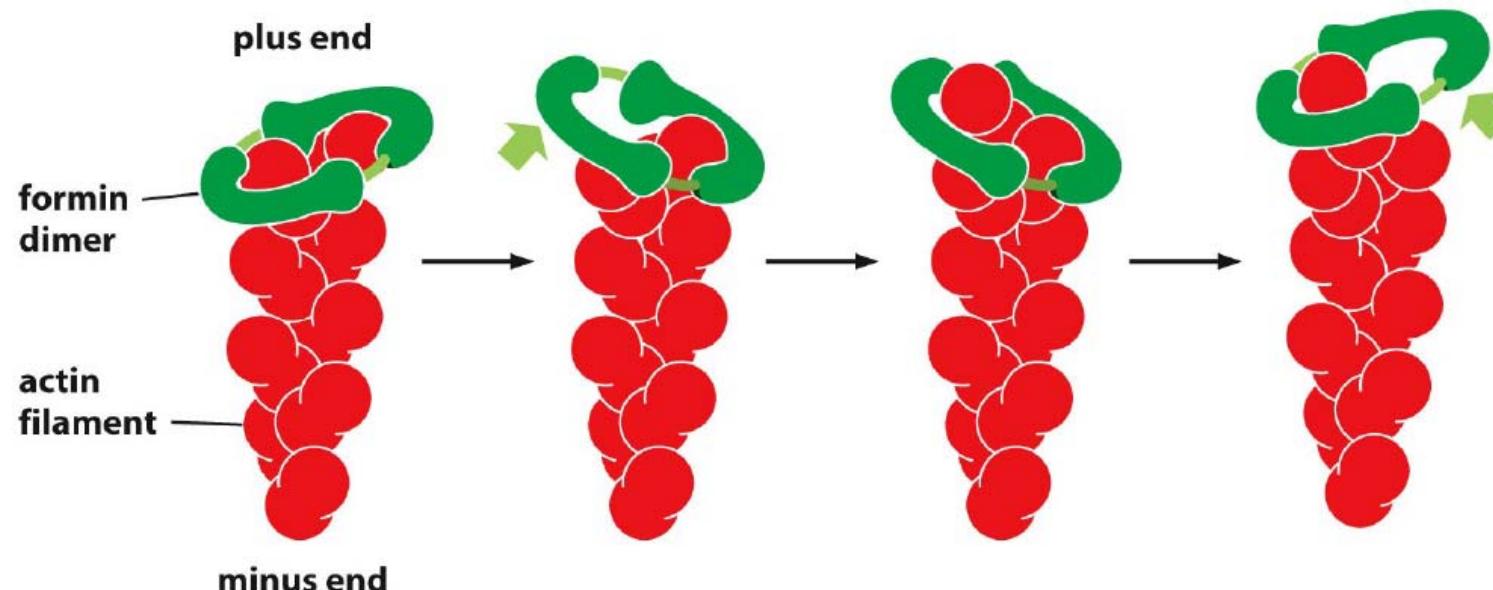
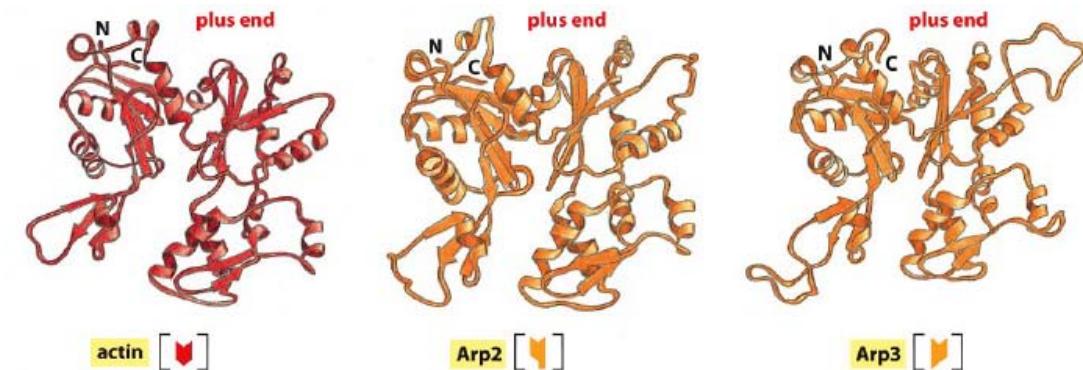


Figure 16-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

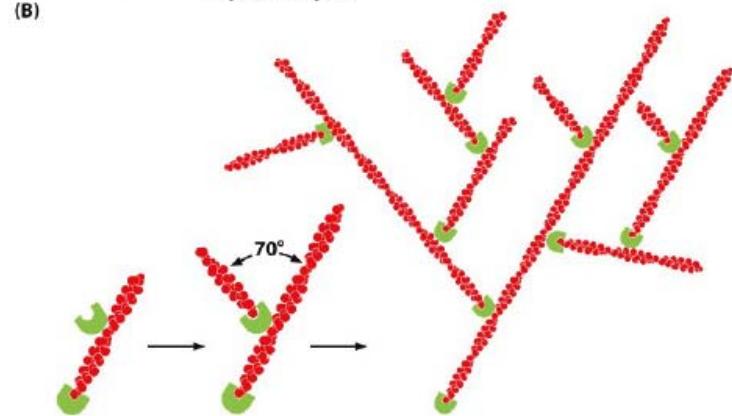
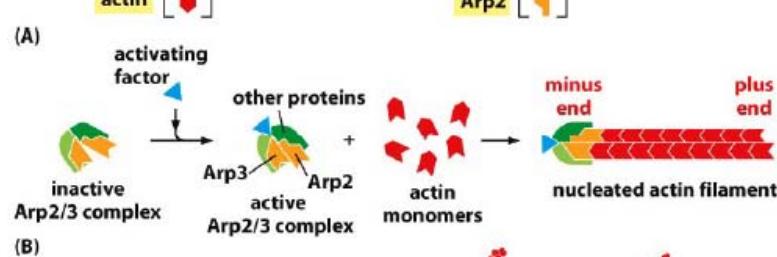
- Formins are **dimeric proteins** that **nucleate the growth of straight, unbranched filaments** that can be cross-linked by other proteins to form parallel bundles.
- **Each formin subunit** has a binding site for **monomeric actin**, and the **formin dimer** appears to nucleate actin filament polymerization by capturing **two monomers**.
- The newly nucleated filament grows and the formin dimer **remains associated with the growing plus end** while **still allowing the addition of new subunits at that end**

Arp2/3 mediates branched filament assembly

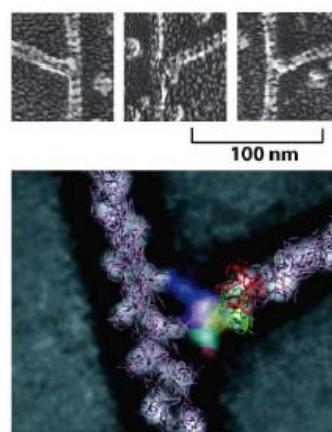
Comparison of structures: actin versus Arp2 and Arp3



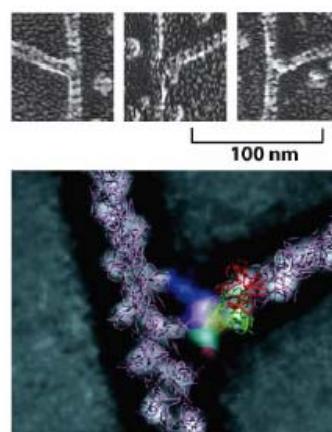
similar at (+) end but **differences** at sides and (-) end **prevent filament formation**



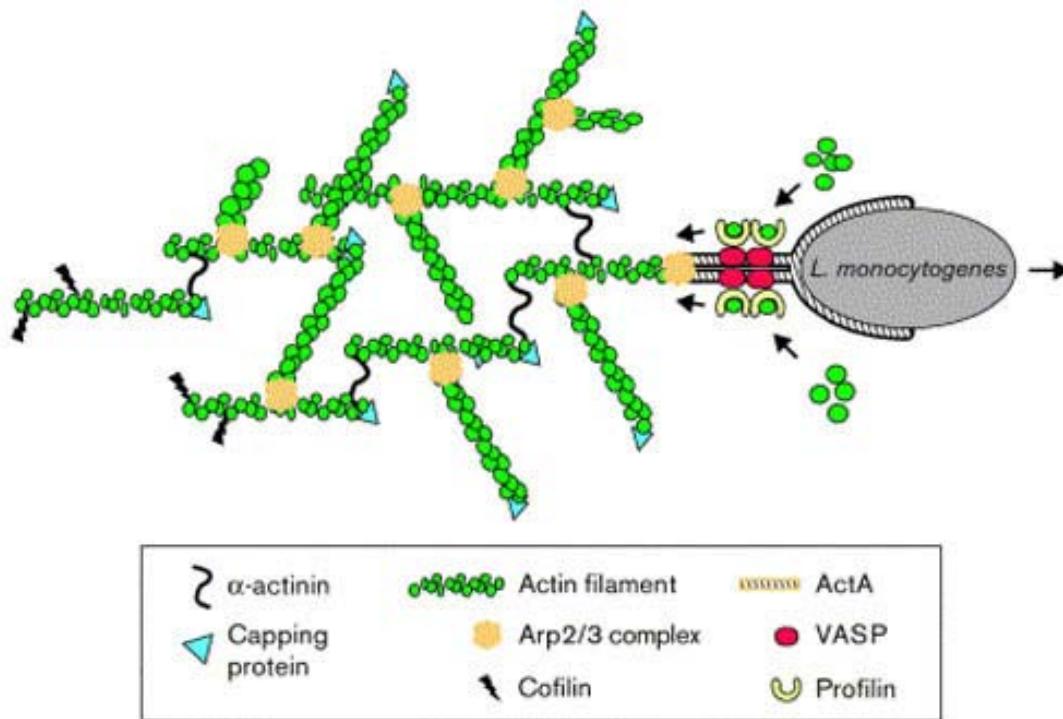
Assembly is most efficient when Arp2/3 is bound to the side of an existing filament:
resulting in branch growth at 70° angles.



Branching: actin (pink) with Arp2/3 complex fitted to the electron density.
Mother filament runs from top to bottom, daughter filament branches to the right



Example 1: How does *Listeria* get around in host cells?



Listeria's cell surface protein ActA functions as a nucleation promoting factor (NPF), which interacts with VASP, Helps to recruit Arp2/3 and enhance ATP-actin assembly.

The recruited Arp2/3 complex nucleates the assembly of actin filaments that generate a substantial force and push the bacterium through the cytoplasm of the cell, at rates of up to 1 $\mu\text{m/sec}$, leaving behind a long actin "comet tail"

Toxins that perturb actin dynamics

- Microfilament depolymerization drugs:

1. **Cytochalasin D**: a fungal alkaloid binds to “+” end of F-actin, blocks addition of subunits.
2. **Latrunculin**: binds to and sequesters G-actin, inhibiting its addition into a filament end.

- Microfilament polymerization drugs:

1. **Jasplakinolide**: enhances nucleation by binding and stabilizing actin dimers and lowering the Cc.
2. **Phalloidin** : binds at the interface between subunits in F-actin, locking adjacent subunits together and preventing actin filaments from depolymerizing.

Actin and myosin perform a lot of functions in non-muscle cells, usually towards the “+” end, only myosin VI moves to the “-”.

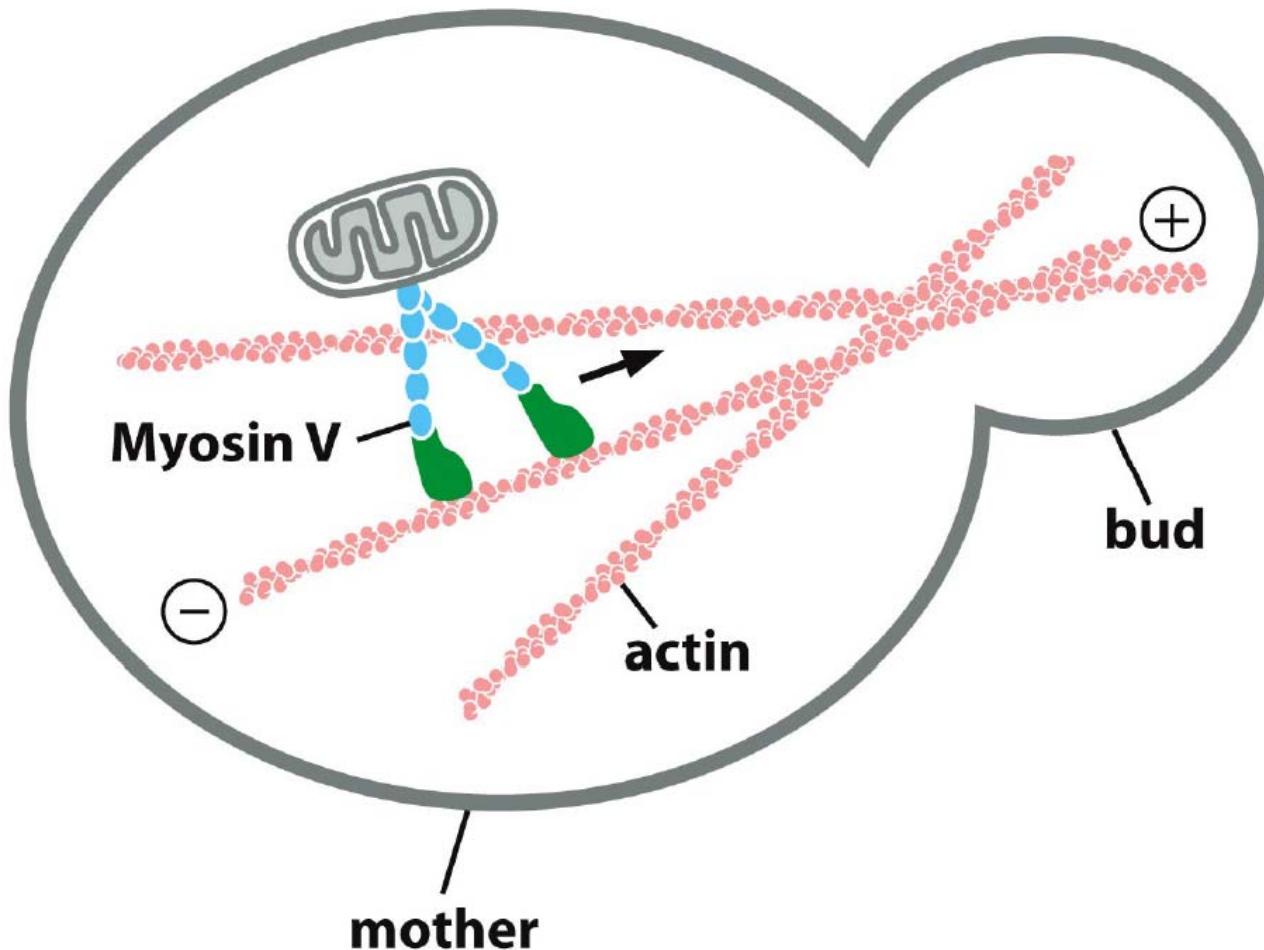
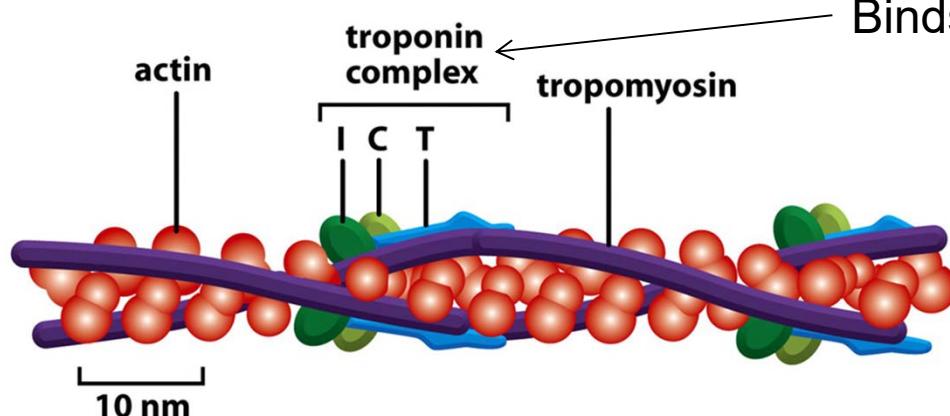
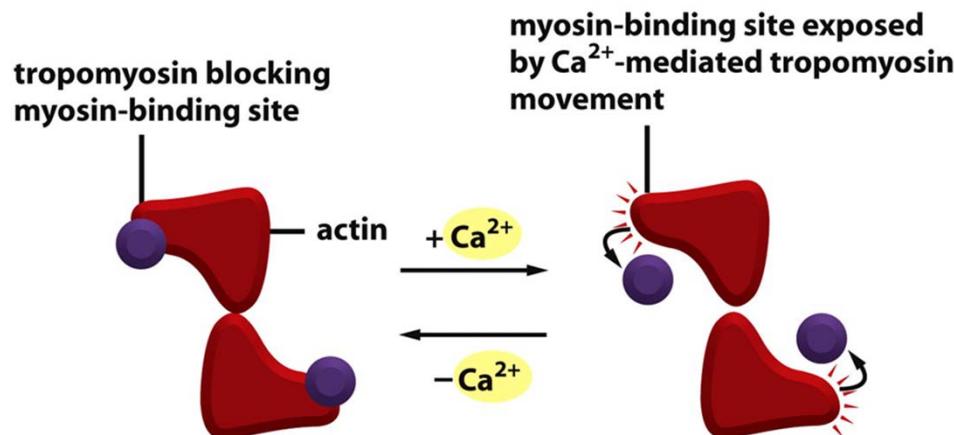


Figure 16-41b Molecular Biology of the Cell 6e (© Garland Science 2015)

2) The control of skeletal muscle contraction by the actin binding proteins troponin and tropomyosin



troponins T, I, and C
(Tropomyosin-binding, Inhibitory, and Ca^{2+} -binding activities)



Binds to 4 Ca^{2+}

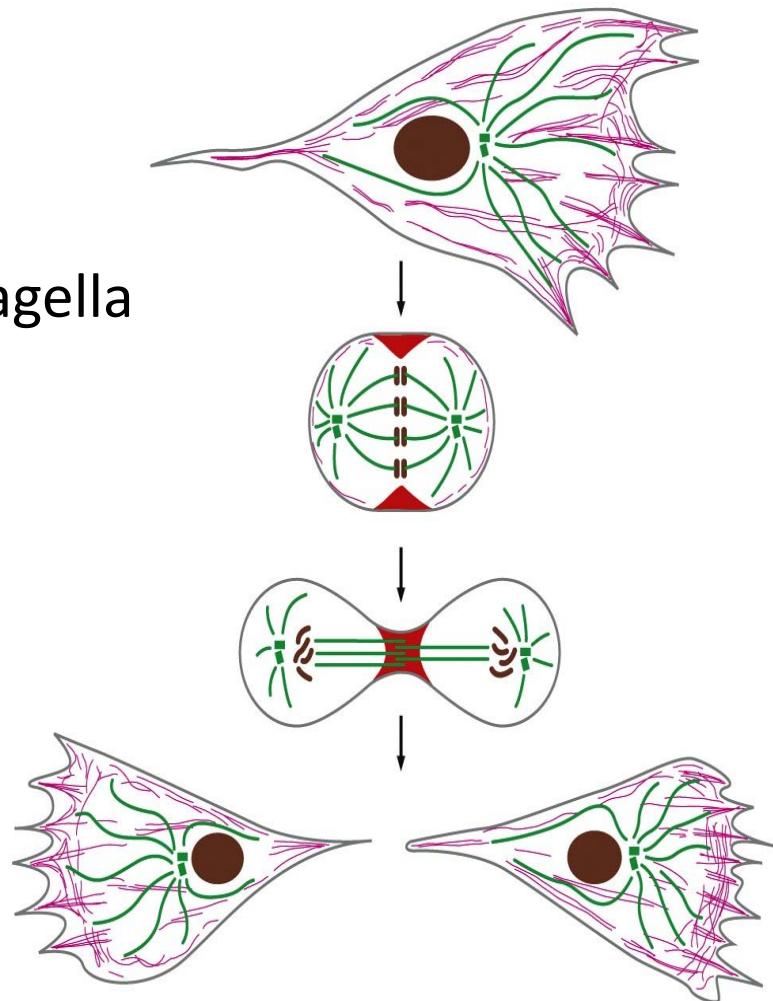
resting muscle:

the troponin I-T complex **pulls the tropomyosin out of its normal binding groove to block binding of myosin heads (no force generating action)**

High Ca_{2+} : troponin C causes troponin I to **release its hold on actin**. This allows the tropomyosin molecules to slip back into their normal position so that the myosin heads can walk along the actin filaments

I. Microtubule structure and organization

- Mitotic spindle
- Structural support in axon
- Structural elements in cilia and flagella
- Centriole
- Basal bodies



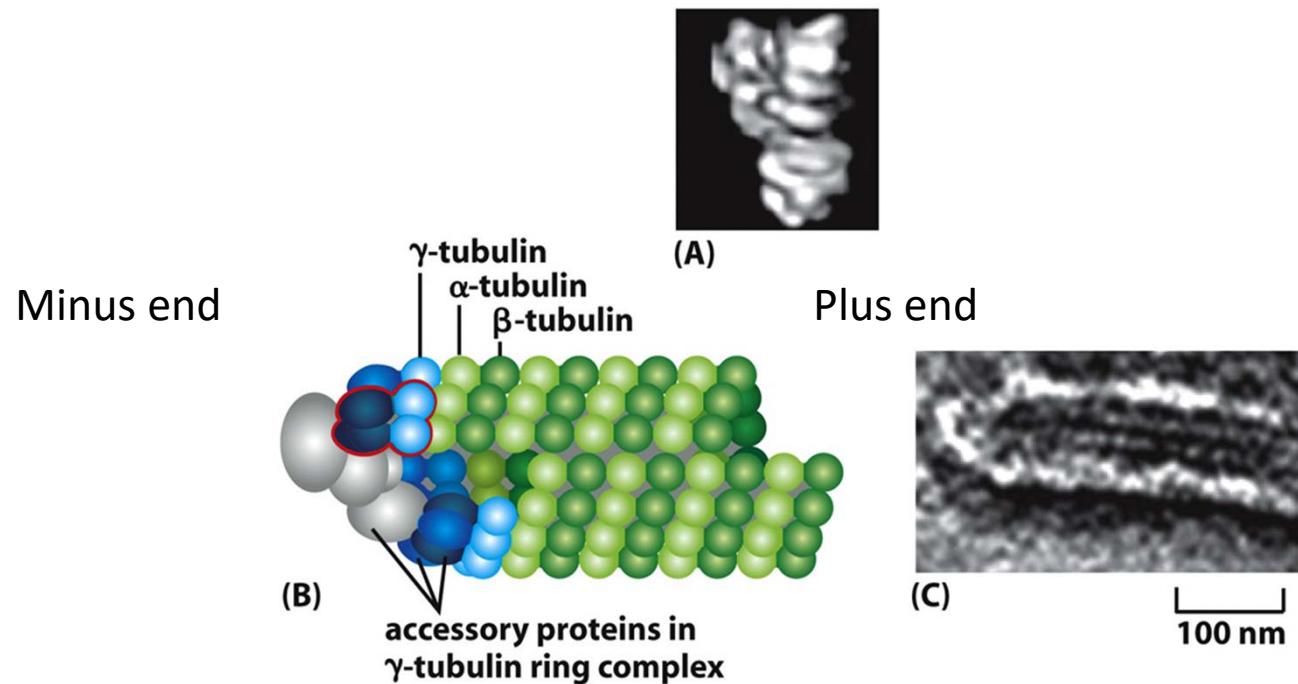
Summary about microtubule proteins

- ♥ Most microtubules are **singlets**, some are in doublet (cilia, flagella), some are in triplet (basal bodies and centrioles)
- ♥ Most have **13 protofilaments**, some have between 11-15.
- ♥ two major types of tubulins, **α -tubulin**, and **β -tubulin**, forming **heterodimer**.
- ♥ All subunits are **oriented in the same way**, the one with **exposed α -tubulin is minus end**, the **one with exposed β -tubulin is plus end**, microtubule has polarity.
- ♥ Each α -tubulin and β -tubulin bind to one molecule of GTP, the **GTP on α -tubulin is never hydrolyzed**, but GTP on β -tubulin is hydrolyzed.
- ♥ **γ -tubulin** is important for microtubule assembly, Microtubule-associated proteins (**MAP**) are important in assembling and dynamics for microbutules.

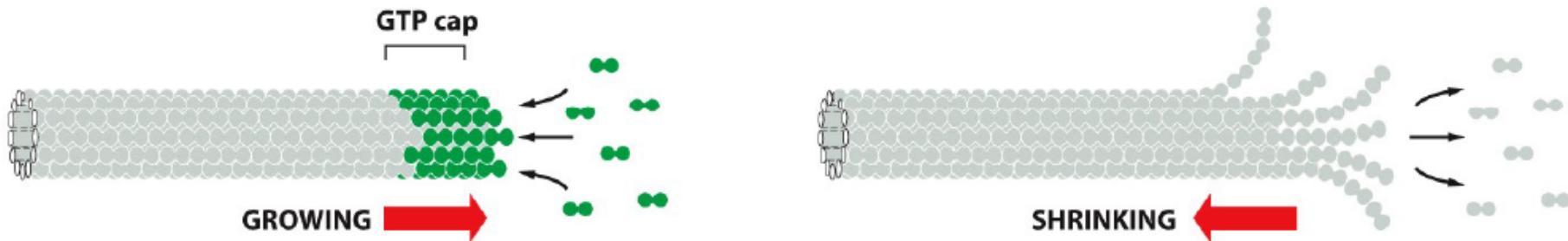
Microtubule assembly

- Very rare spontaneous microtubule assembly
- All microtubules are nucleated from **Microtubule-organizing centers (MTOCs)**, including centrosomes and basal bodies (cilia and flagella)
- Plants use different mechanisms to nucleate microtubules.

γ -tubulin ring complex (γ -TuRC), pericentriolar, is critical to assemble microtubules



Microtubule dynamic instability

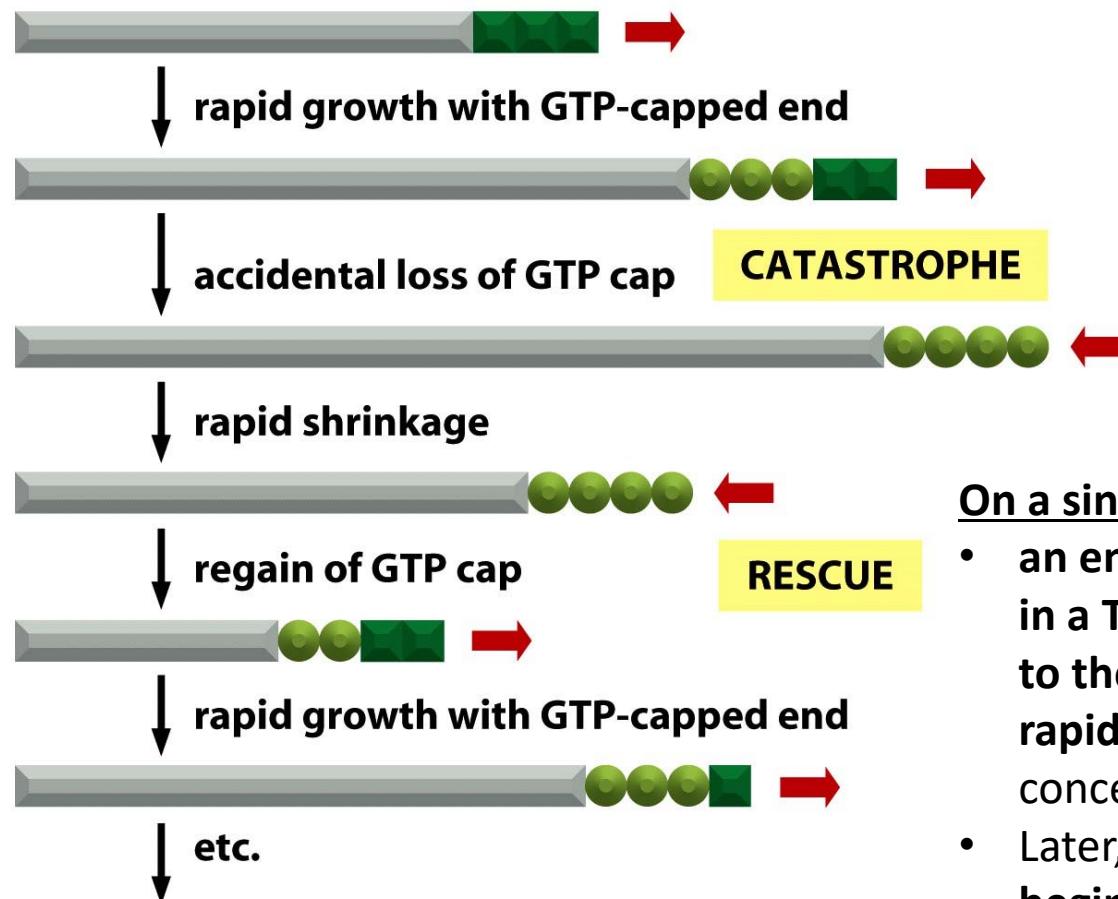


Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called **dynamic instability**.

Panel 16-2 (part 10) Molecular Biology of the Cell 6e (© Garland Science 2015)

- Microtubules **depolymerize** about 100 times faster from an end containing **GDP-tubulin** than from one **containing GTP-tubulin**.
- A **GTP cap** favors growth but if it is lost, then **depolymerization** ensues.

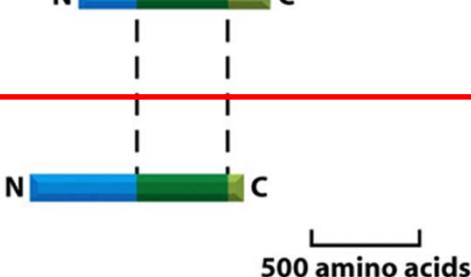
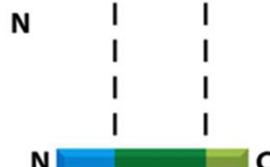
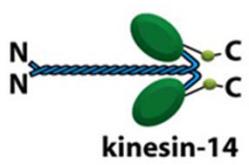
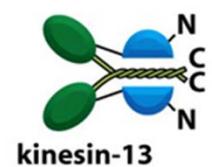
Microtubule dynamic instability



On a single microtubule:

- an end might grow for a certain time in a T form, but then suddenly change to the D form and begin to shrink rapidly, even while the free subunit concentration is held constant.
- Later, it might regain a T-form end and begin to grow again.
- This rapid interconversion between a growing and shrinking state, is called dynamic instability

2. Kinesin family (anterograde)---toward plus end



Diverse roles:

- ♠ organelle transport,
- ♠ mRNA transport,
- ♠ chromosome transport
- ♠ microtubule sliding
- ♠ microtubule depolymerization

Move toward
Minus end

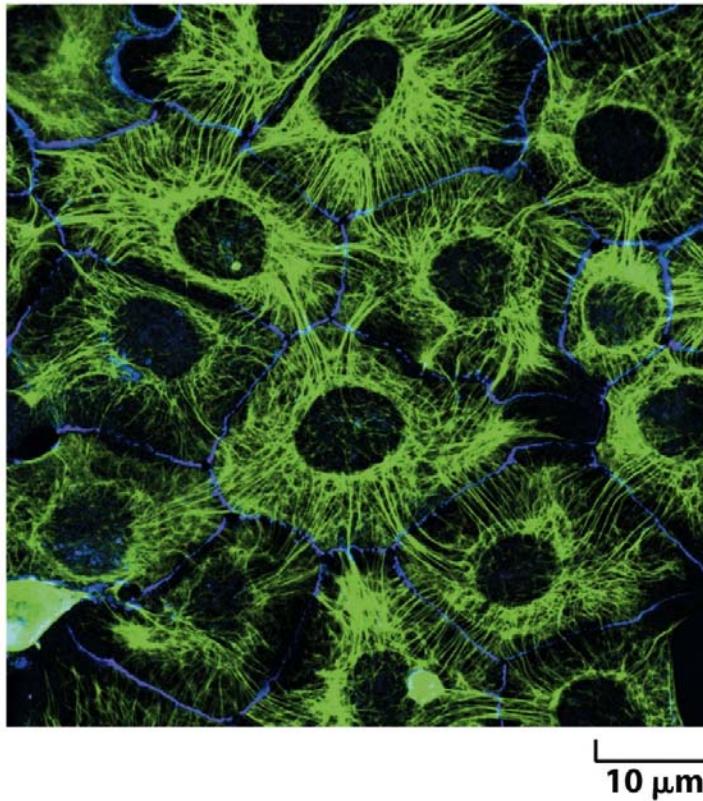
Some drugs to influence tubulin assembly

Table 16–2 Drugs That Affect Actin Filaments and Microtubules

ACTIN-SPECIFIC DRUGS	
Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends
Swinholide	severs filaments
Latrunculin	binds subunits and prevents their polymerization
MICROTUBULE-SPECIFIC DRUGS	
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds subunits and prevents their polymerization
Vinblastine, vincristine	binds subunits and prevents their polymerization
Nocodazole	binds subunits and prevents their polymerization

VI. Intermediate filament

1. No polarity,
2. no motor activity,
3. Tensile and stable
4. hard to be solubilized
5. Not all eukaryotic cells have this, fungi and plants don't have so far.
6. Very heterogeneous
7. Defects in genes for intermediate filaments are associated with ~50 clinical disorders.



Keratin in epithelia

Major types of intermediate filament proteins

Table 16–1 Major Types of Intermediate Filament Proteins in Vertebrate Cells

TYPES OF IF	COMPONENT POLYPEPTIDES	LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial	type I keratins (acidic)	epithelial cells and their derivatives (e.g., hair and nails)
	type II keratins (basic)	
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

Intermediate filaments are crosslinked and bundled into strong arrays

- ♥ Through lateral contacts
- ♥ Through proteins such as filaggrin- keratin filaments, plectin—crosslinks intermediate filaments.
- ♥ Mutation in plectin results in serious human disease characterized by epidermolysis bullosa, muscular dystrophy and neurodegeneration.
- ♥ Keratins are further crosslinked by disulfide bonds.

Examples for straight forward learning

Cell cycle and mitosis

- Phases of the cell cycle
 - what happens at each stage
 - checkpoints, transitions, regulators
 - control of the cell cycle
- Mitosis: all stages (name & description, what happens?)
 - formation of the spindle
 - molecular mechanism of spindle function
(interactors, directions, pulling/pushing etc.)
- Regulators of the cell cycle:
 - which cyclins, which Cdks, which complex at which step; regulators etc
 - mechanism ensuring only a single round of DNA replication
 - substances, mitogens
- Relation mitosis/cytokinesis, growth & development
 - special cases: cleavage growth (oocytes), multinucleation

The 4 major events in cell cycle: What we see (M) and what is happening (G₁, S & G₂)

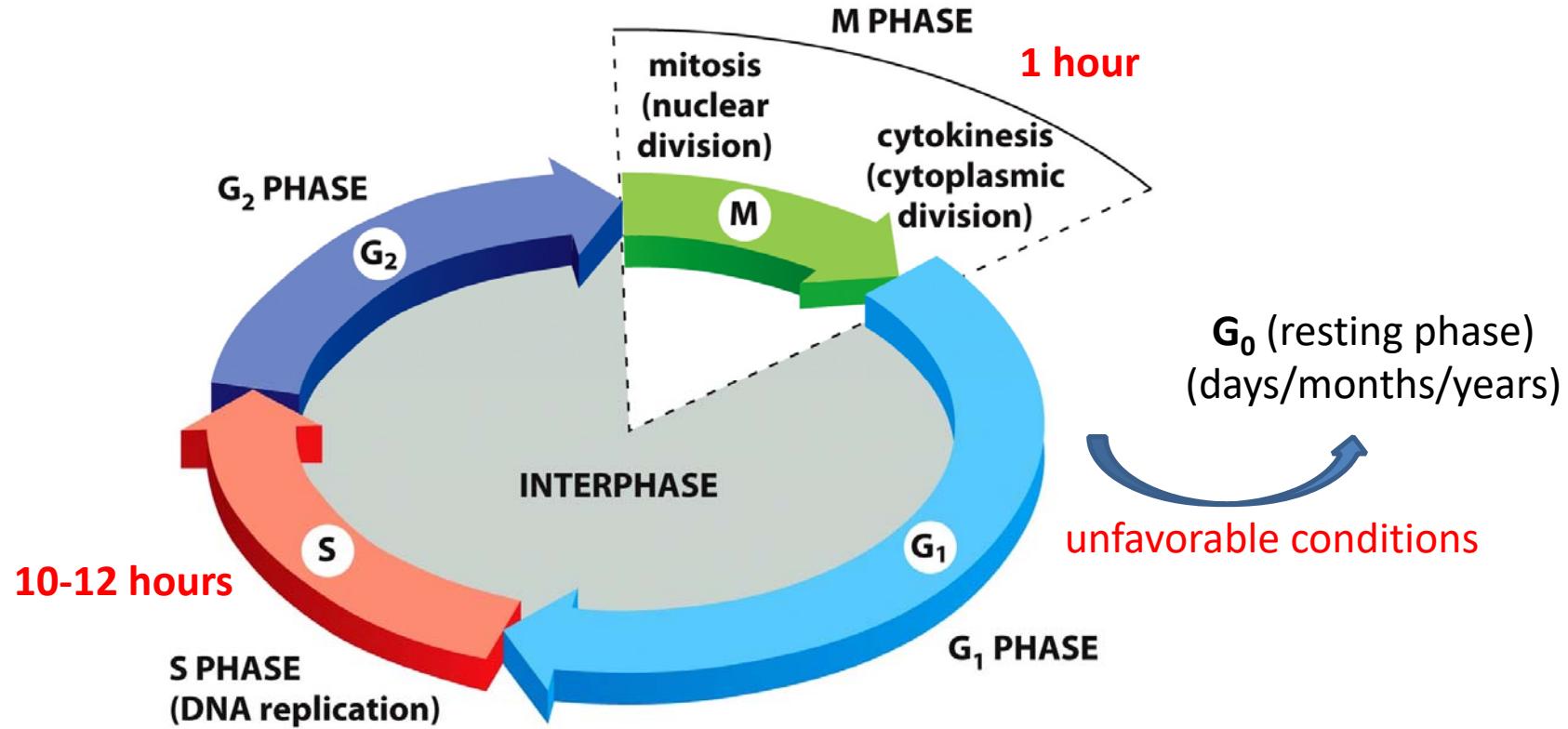
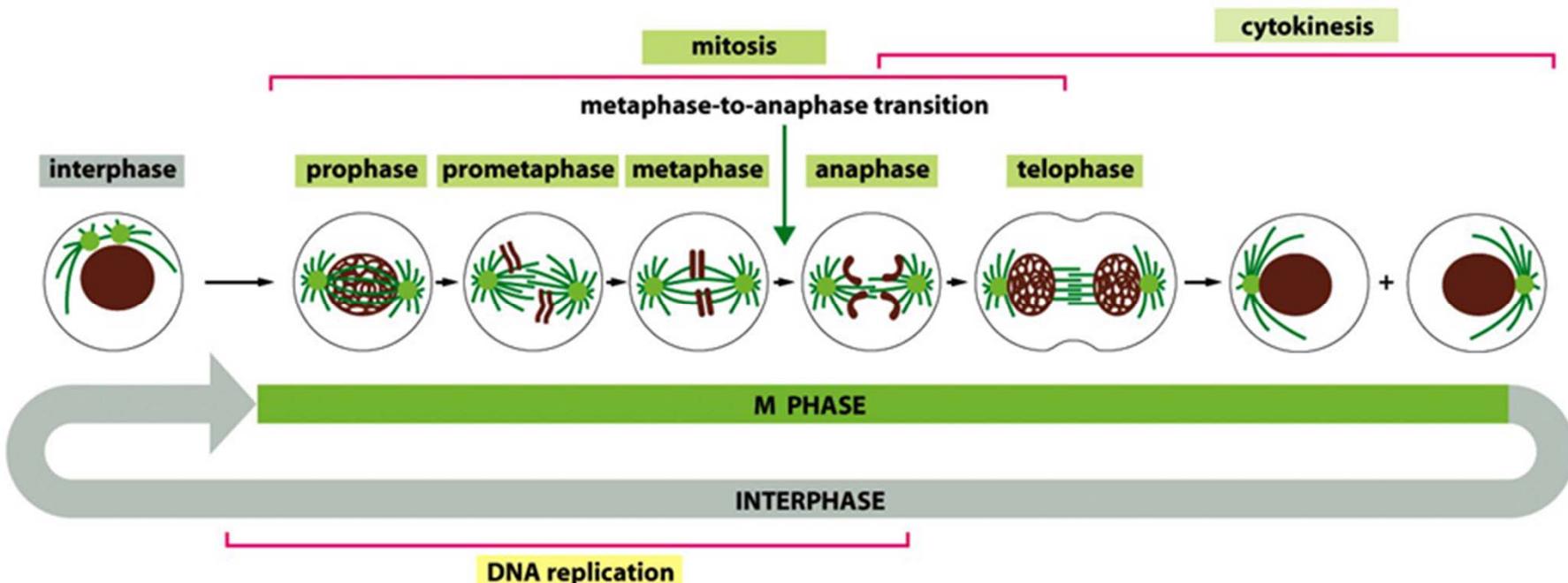


Figure 18-2 Essential Cell Biology 3/e (© Garland Science 2010)

Definitions:

- **M phase:** nuclear division (mitosis) and cell division
- **Interphase:** time between M phases
- **G_{1/2} phases:** gap phases (checking internal state and external environment)
- **S phase:** synthesis

M-phase can be further divided into: mitosis (5 stages) and cytokinesis



End of S phase: DNA molecules in each pair of **duplicated chromosomes are held tightly**

Early in mitosis (prophase): The 2 DNA molecules are condensed into pairs of rigid, compact rods (sister chromatids), which remain linked by sister-chromatid cohesion. **The nuclear envelope disassembles** and the **sister-chromatid pairs become attached to the mitotic spindle**, a giant bipolar array of microtubules.

Metaphase: sister chromatids **are attached to opposite poles of the spindle and, align at the spindle equator**

Anaphase: Destruction of sister-chromatid cohesion **separates the sister chromatids**, which **are pulled to opposite poles** of the spindle.

Telophase: The **spindle disassembles**, segregated chromosomes are **packaged into separate nuclei**

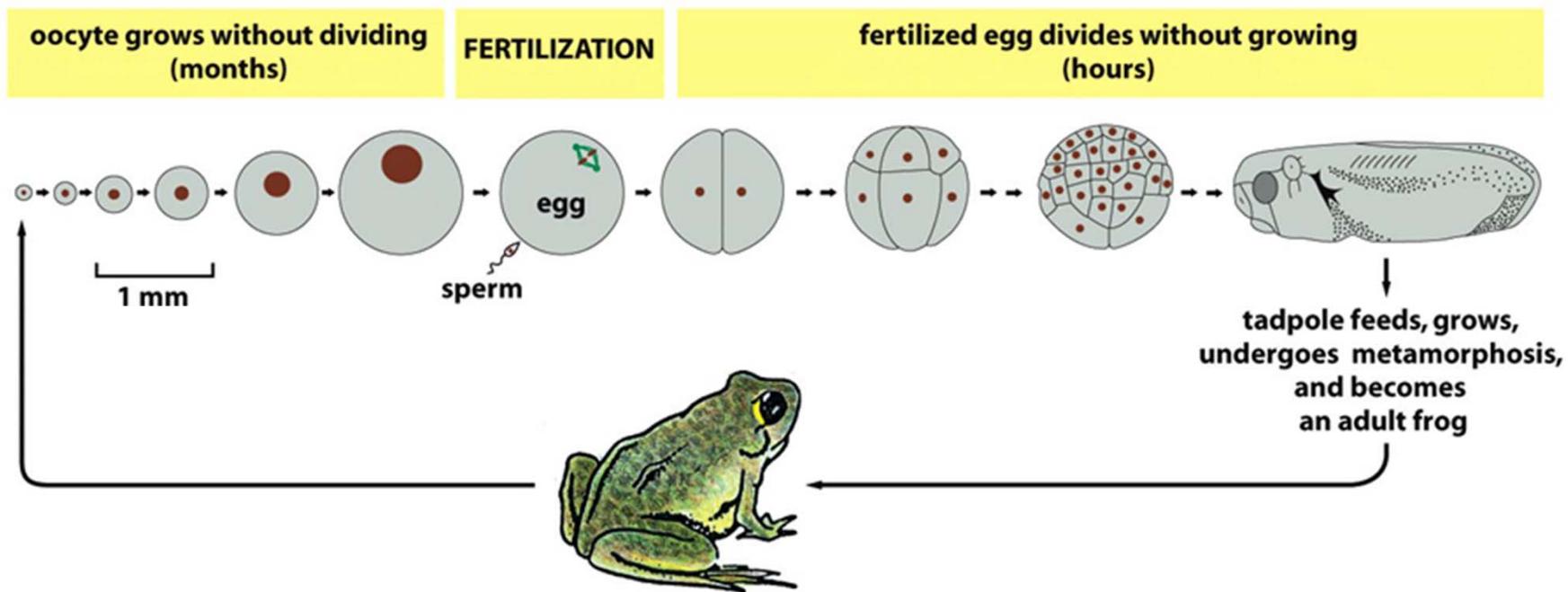
Cytokinesis: **cell division so that each daughter cell inherits one of the two nuclei**

Different Cell cycle time (doubling time) for some eukaryotic cells

Fertilized xenopus oocytes	30 min
Yeast cell	1.5-3 hours
Mammalian intestine epithelial cells	~12 hours
Mammalian fibroblasts	20 hours
Human liver cell	~ 1 year
Tobacco BY2 suspension cultured cells	22 hours

2). Xenopus oocytes

- *Xenopus* oocytes have rich source of cell division proteins
- Fertilized eggs are exceptionally large and divide rapidly (S and M phases, with very short or no G1 or G2 phases)



No new gene transcription in the fertilized egg:

- all of the required mRNAs and proteins are already packed into the very large egg

No cell growth in these early embryonic division cycles (*cleavage divisions*)

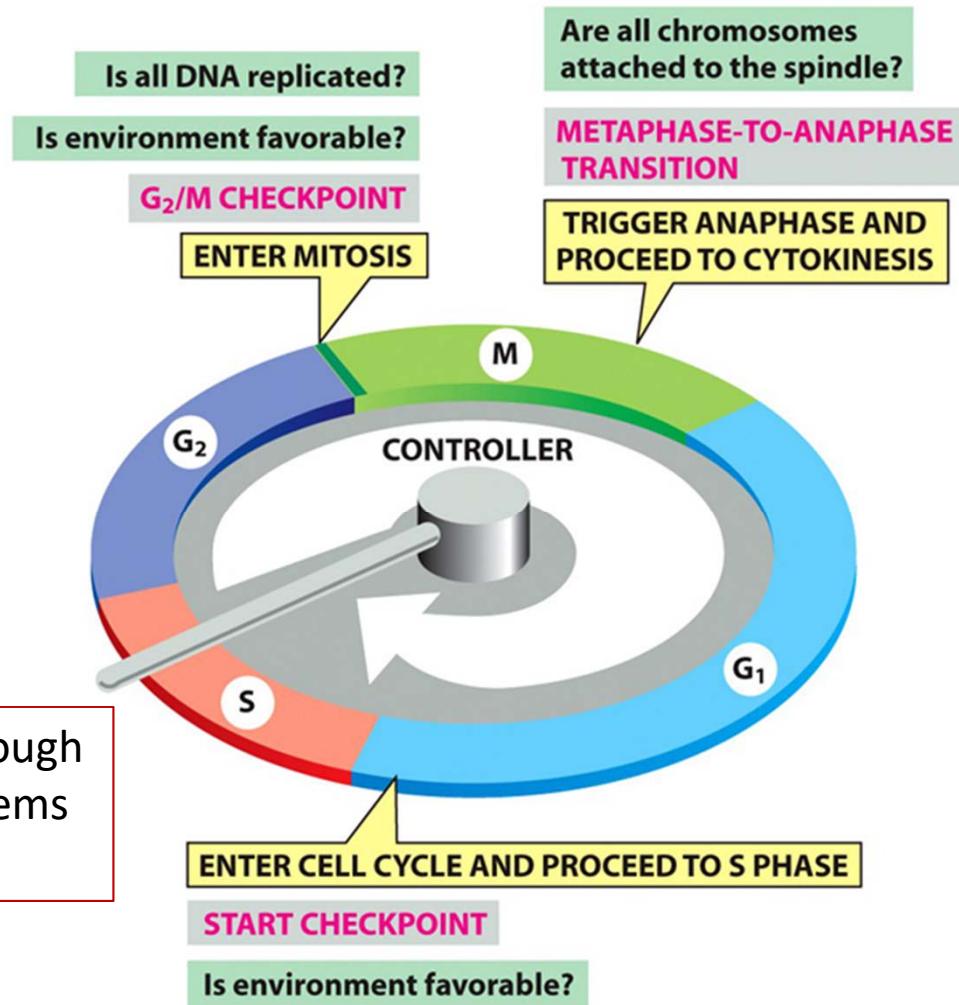
- all cells divide synchronously, growing smaller and smaller with each division

II. The cell cycle control system

The cell cycle control has three major checkpoints:

- **G₁/S-phase transition checkpoint (START)**
- **G₂/M-phase transition checkpoint**
- **Metaphase-to-anaphase transition checkpoint**

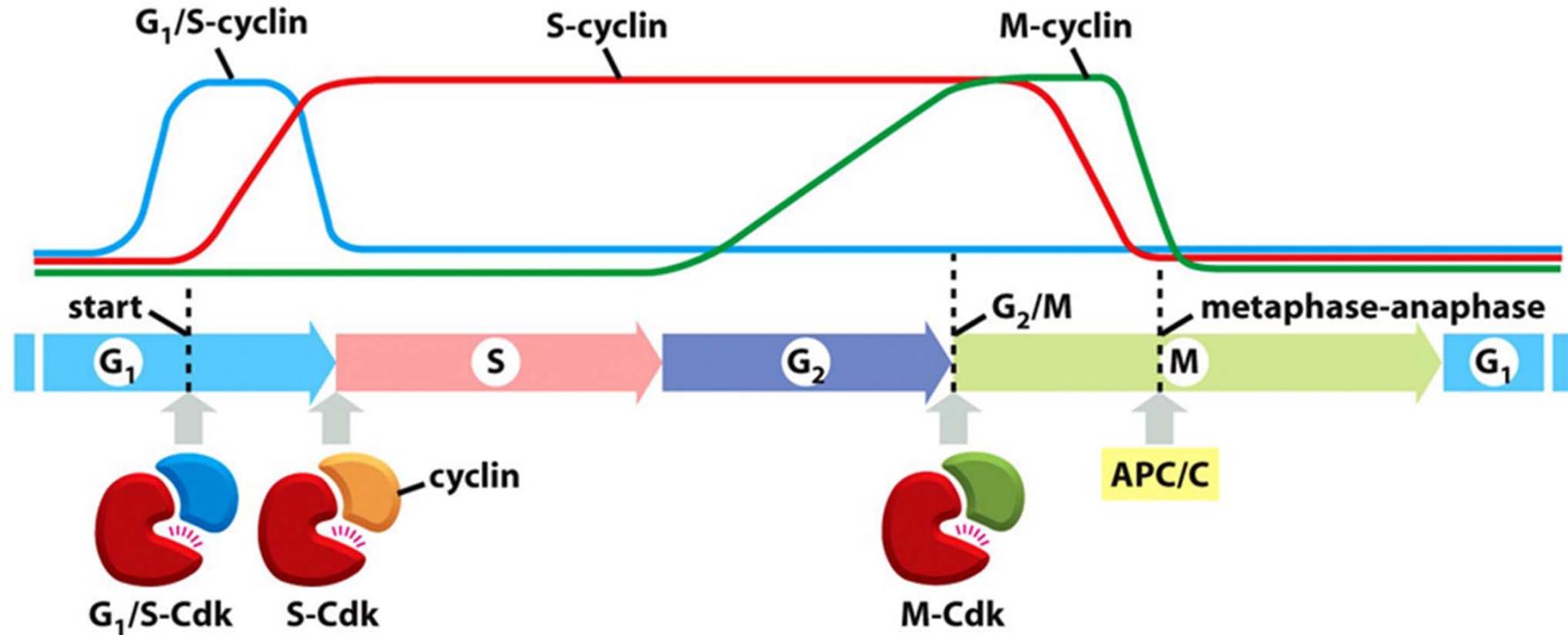
The control system blocks progression through each of these transitions if it detects problems inside or outside the cell.



Major players of the cell cycle control system: **Cyclins and cyclin-dependent kinases (Cdks)**

- **Cyclins:**
 - named cyclins because they undergo a cycle of **synthesis & degradation** during each cell cycle
 - **activate cyclin-dependent kinases (Cdks)**
 - different cyclins oscillate in cell cycle and bind to or control the activity of different Cdks; it decides cdk substrates specificity and activates Cdk.
- **Cyclin-dependent kinases (Cdk):**
 - Protein kinasees, phosphorylate a subset of substrates to control cell cycle progression at specific checkpoints.
 - **level of Cdks is constant throughout the cell cycle**

Cyclin-Cdk complexes of the cell-cycle control system



G₁/S-cyclins activate Cdk's in late G₁ trigger progression through the Start transition
(Commitment to cell-cycle entry).

S-cyclins activate Cdk's soon after Start and trigger DNA replication and early mitotic events.

M-cyclins activate Cdk's to trigger entry into mitosis at the G₂/M transition.

APC/C, initiates the metaphase-to anaphase transition

Major cyclins and Cdks of vertebrates and budding yeast

Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

CYCLIN-CDK COMPLEX	VERTEBRATES CYCLIN	CDK PARTNER	BUDDING YEAST CYCLIN	CDK PARTNER
G ₁ -Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G ₁ /S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).

** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

A fourth class of cyclin, cyclin G1, helps to govern the activities of the G1/S cyclins.

Metaphase-to-anaphase transition is controlled by degradation of the S/M-cyclins

- Progression through the **G₁/S** and **G₂/M transitions** is driven by **activation of cyclin–Cdk complexes**.

but

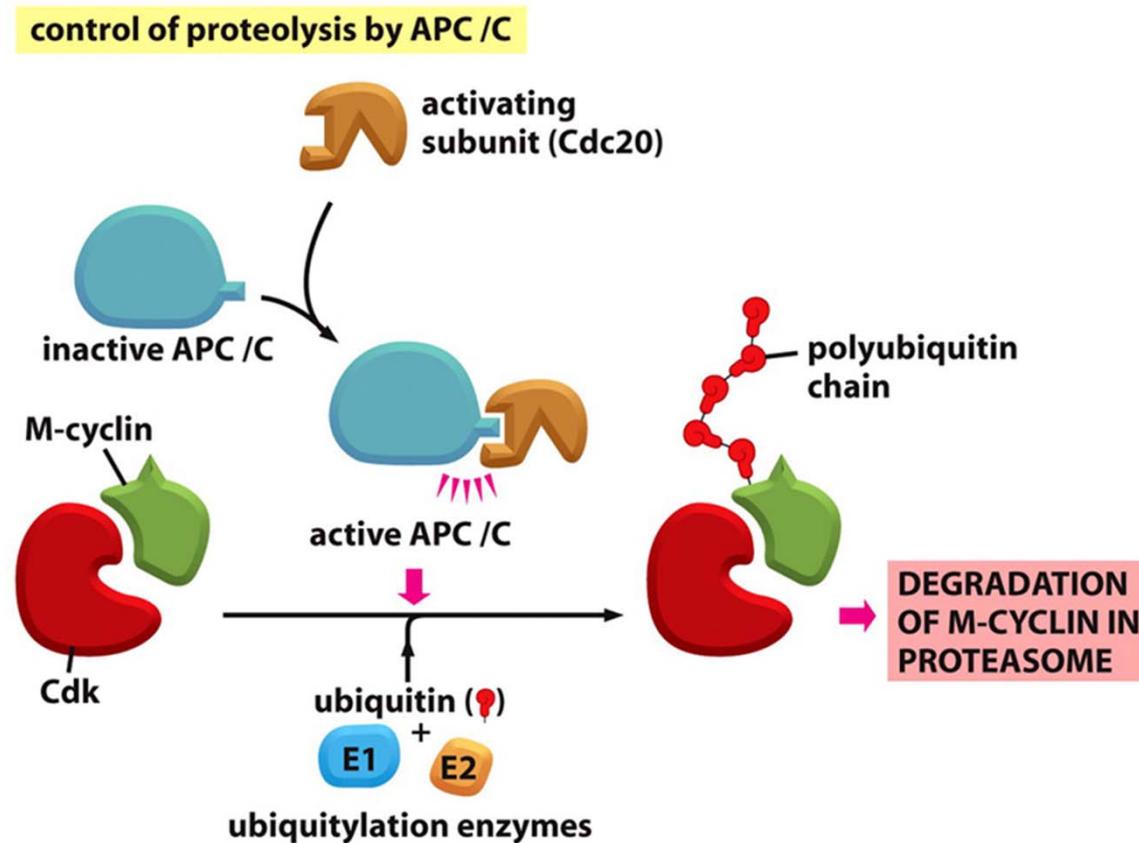
- Progression through the **metaphase-to-anaphase transition** is triggered by **protein destruction**.

One Key player needed:

The anaphase-promoting-complex or cyclosome (APC/C), this complex is a multi-protein **ubiquitin ligase**...

Reminder: protein degradation mediated by ubiquitination

APC/C is an E3 ubiquitin ligase that marks the S/M-cyclins for degradation by the 26S proteasome



... but protein degradation can also be used to trigger the activation of cyclin-Cdk complexes! Guess how?

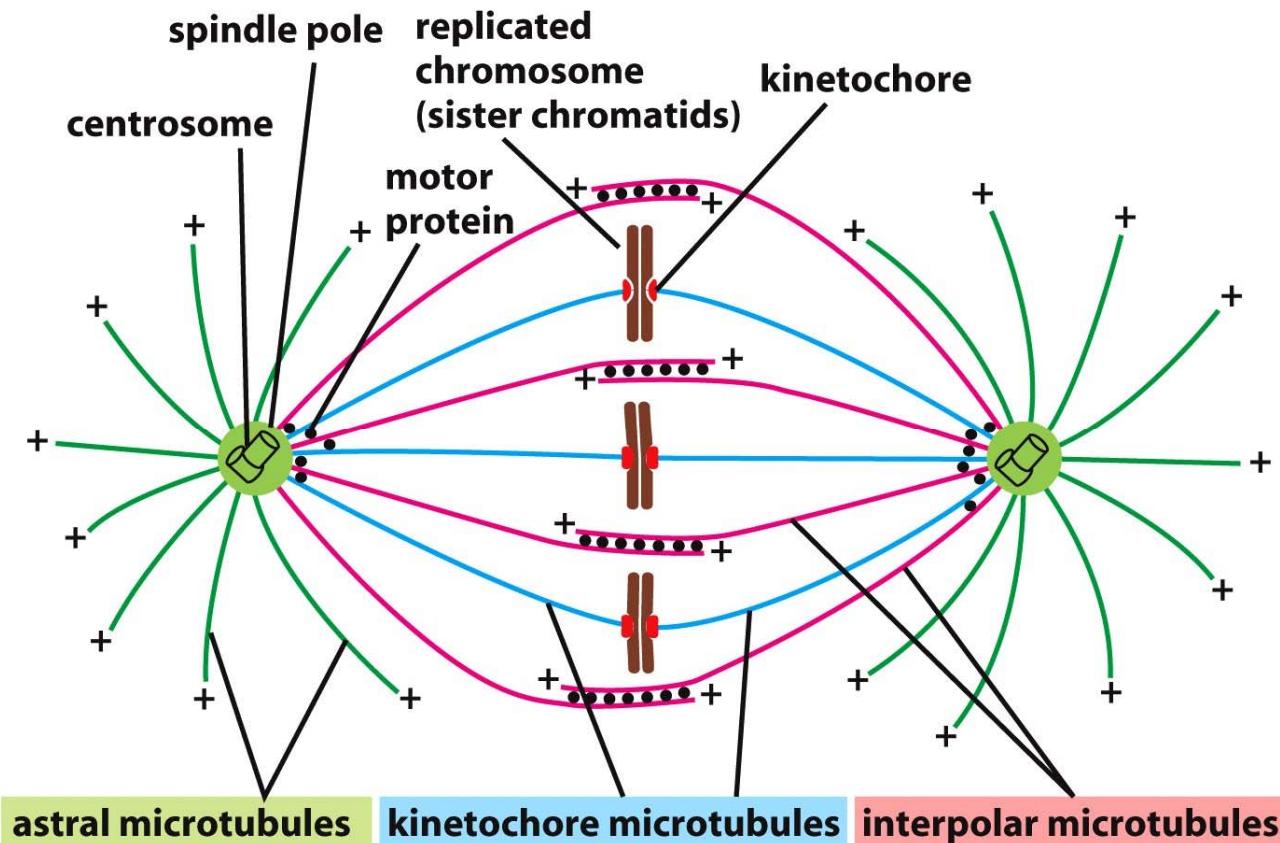
Answer: temporal separation of DNA priming for replication and the start of the replication

- **Pre-replication complex** (pre-RC) - “prime and licensing” activated by APC/C in late M and early G1 when APC/C activity is high.
- **Pre-initiation complex** – DNA unwinding, replication activated by S-Cdk in late G1 when APC/C activity is low, pre-RC is partially dismantled and cannot assemble again.

Levels of S-Cdks and M-Cdks remain high until after late mitosis, when APC/C regains its activity and targets the cyclins for degradation, and starts the next round of pre-RC formation.

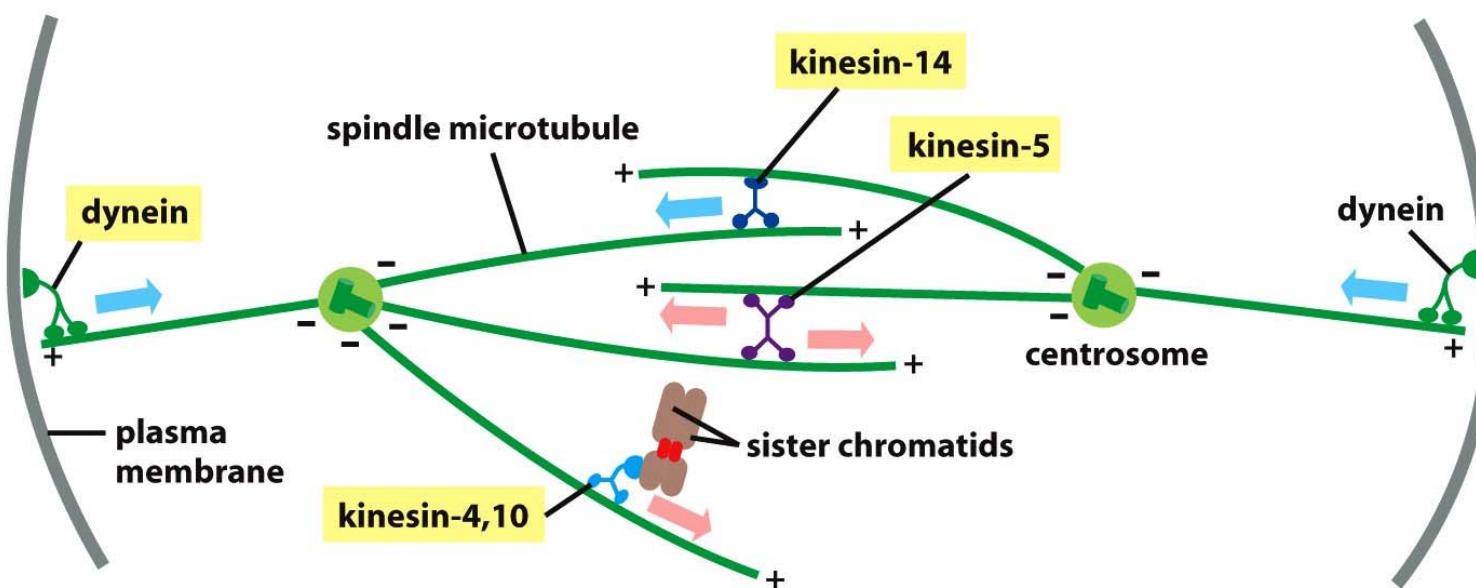
M-Cdk also triggers formation of mitotic spindle

three classes of microtubules:



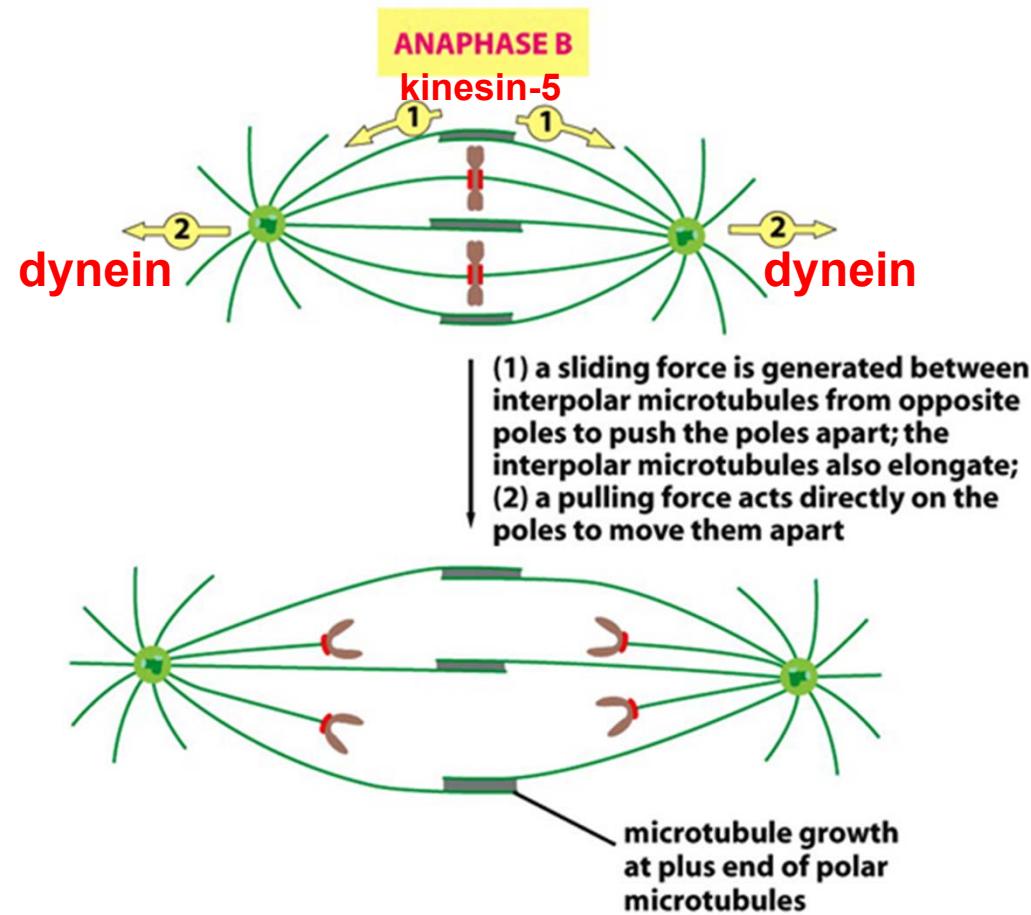
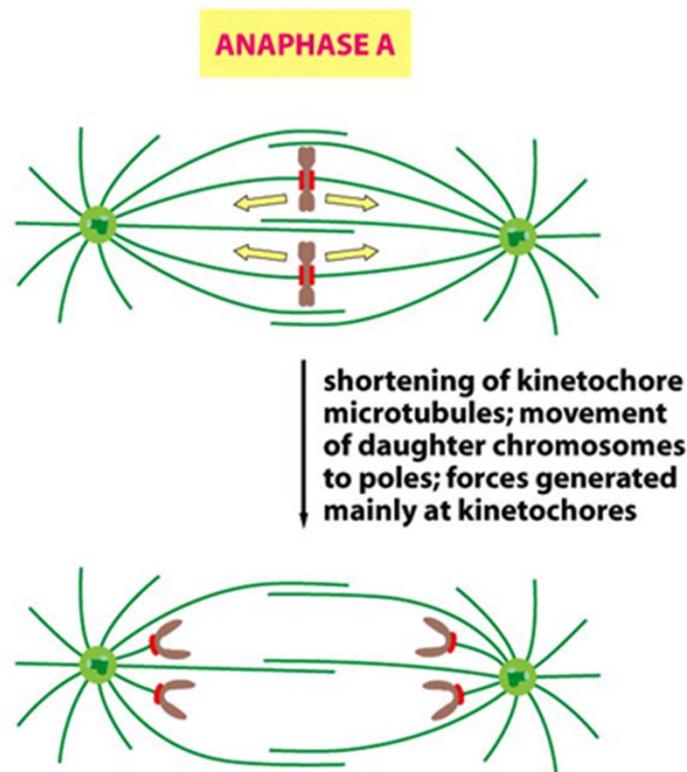
Microtubule-dependent motor proteins govern spindle assembly and function

- Kinesin-5: push poles apart
- Kinesin-14: pull poles together
- Kinesins-4,-10: chromokinesins, push the attached chromosomes away from the pole
- Dynein: pull spindle poles away from each other



Chromosomes segregate in anaphase in two stages

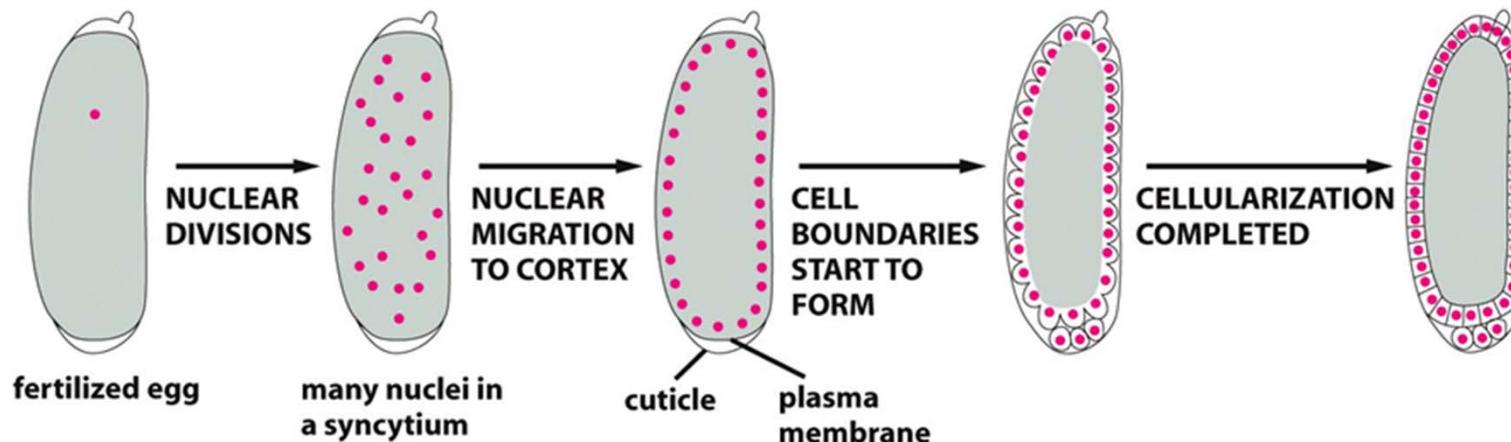
- **Anaphase A:** initial stage, shortening of the kinetochore microtubules
- **Anaphase B:** later stage, separation of the spindle poles
(motor proteins: kinesin-5 & dynein).



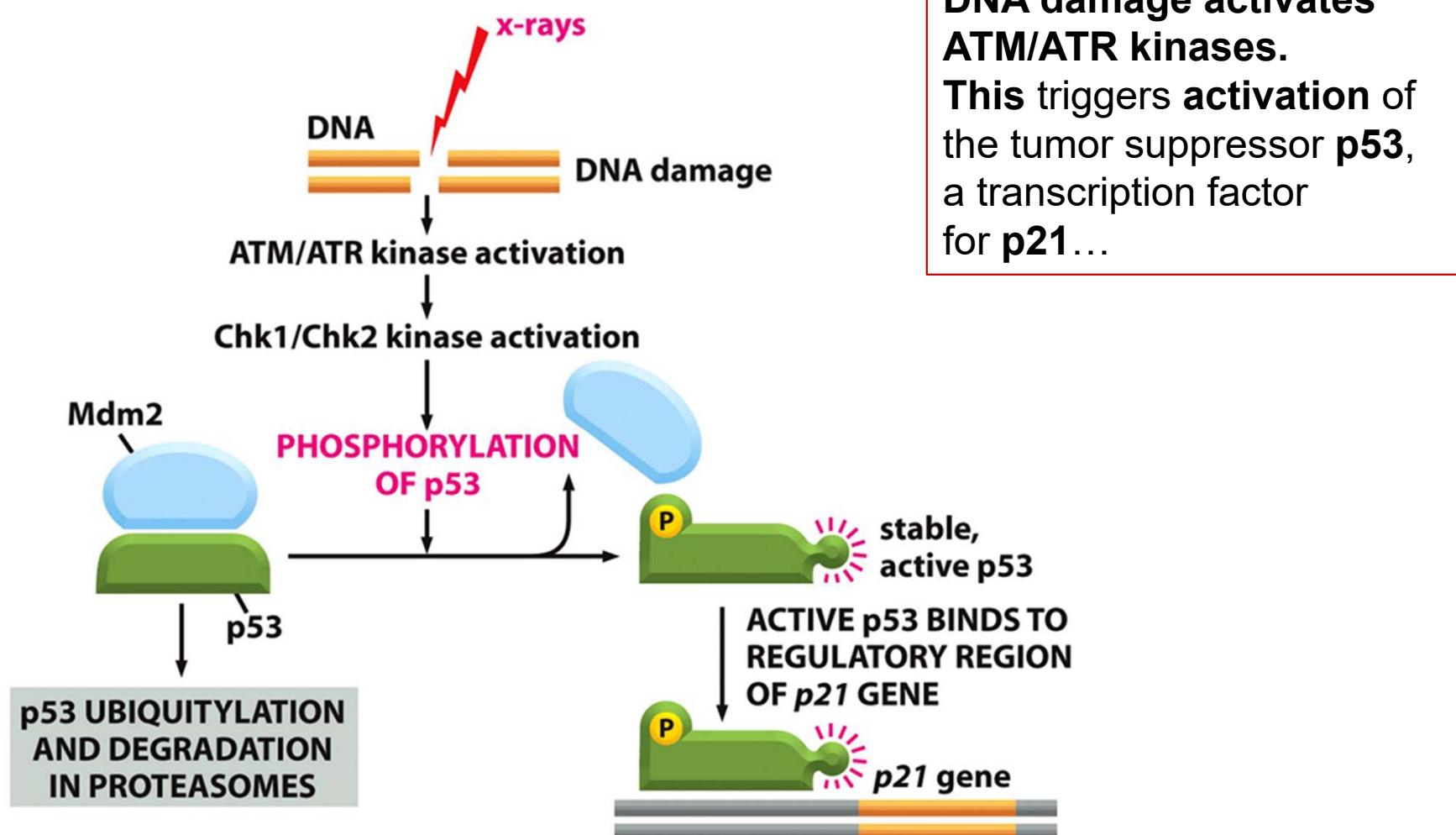
Mitosis without cytokinesis

- *Drosophila* embryo
- Megakaryocytes (blood platelets)
- Hepatocytes
- Heart muscle cell

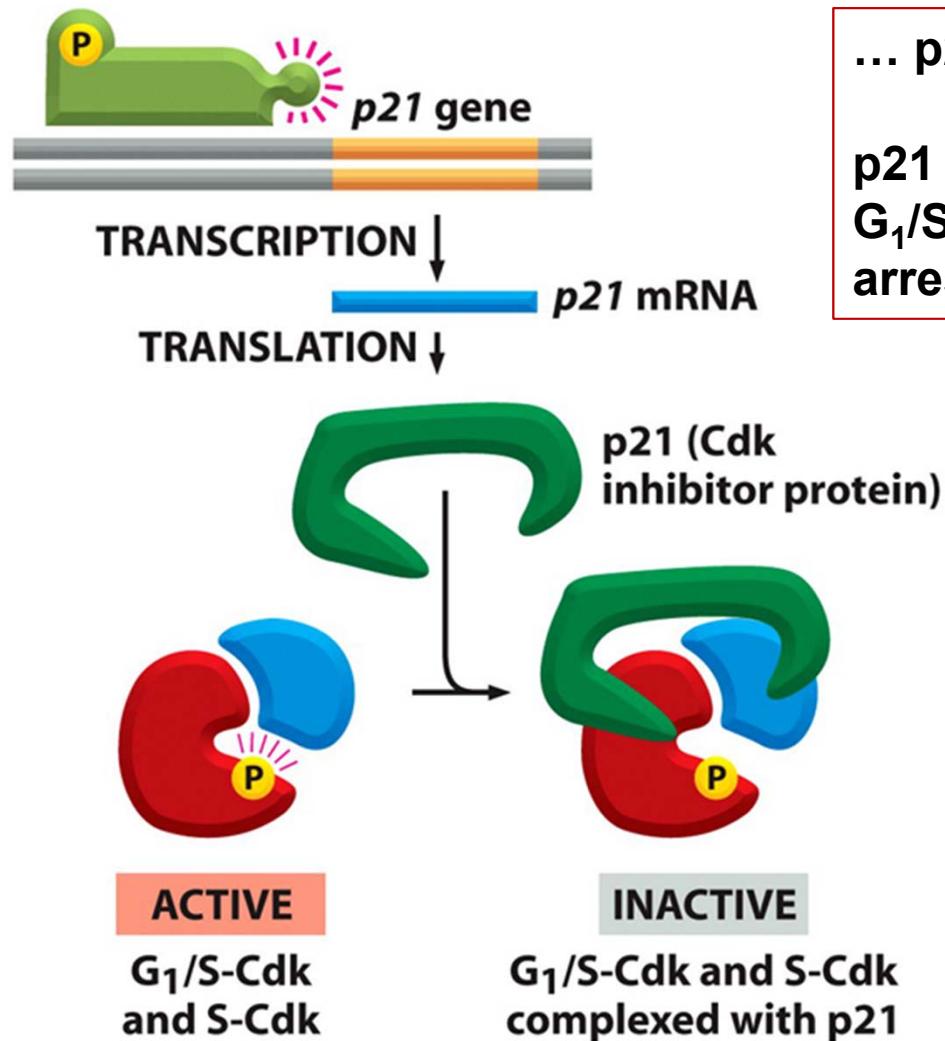
syncytium and cellularization for *Drosophila* embryo



2. DNA damage response triggers cell cycle arrest: activation of the tumor suppressor p53



p21 triggers the arrest of the cell cycle: both at G₁/S and G₂/M transition.

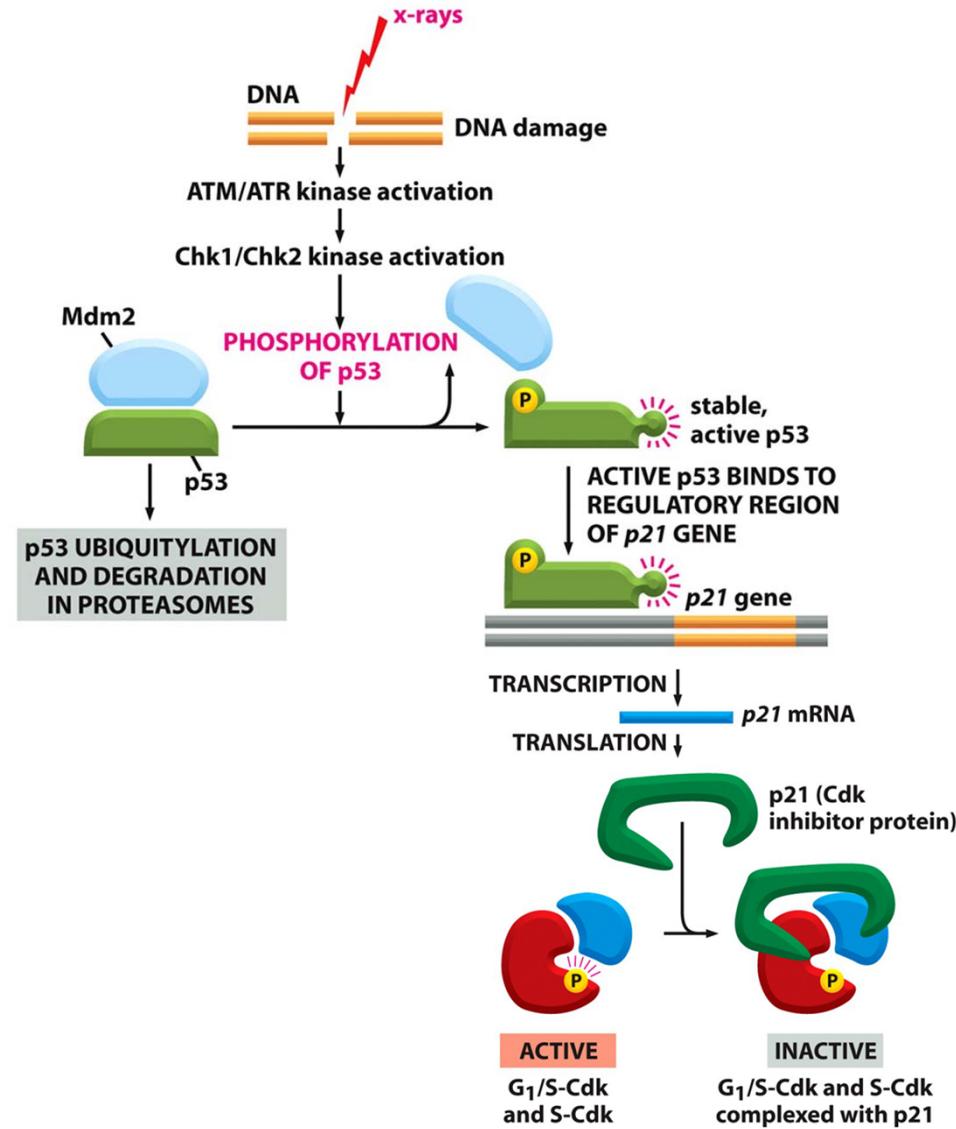


... p21 is a Cdk inhibitor protein!

p21 triggers inactivation of
G₁/S-Cdk and S-Cdk and thus
arrests the cell cycle

Lecture 15:

DNA damage response triggers cell cycle arrest: activation of tumor suppressor p53



- **DNA damage activates ATM/ATR kinases.**
- **This triggers activation of the tumor suppressor p53, a transcription factor for p21...**

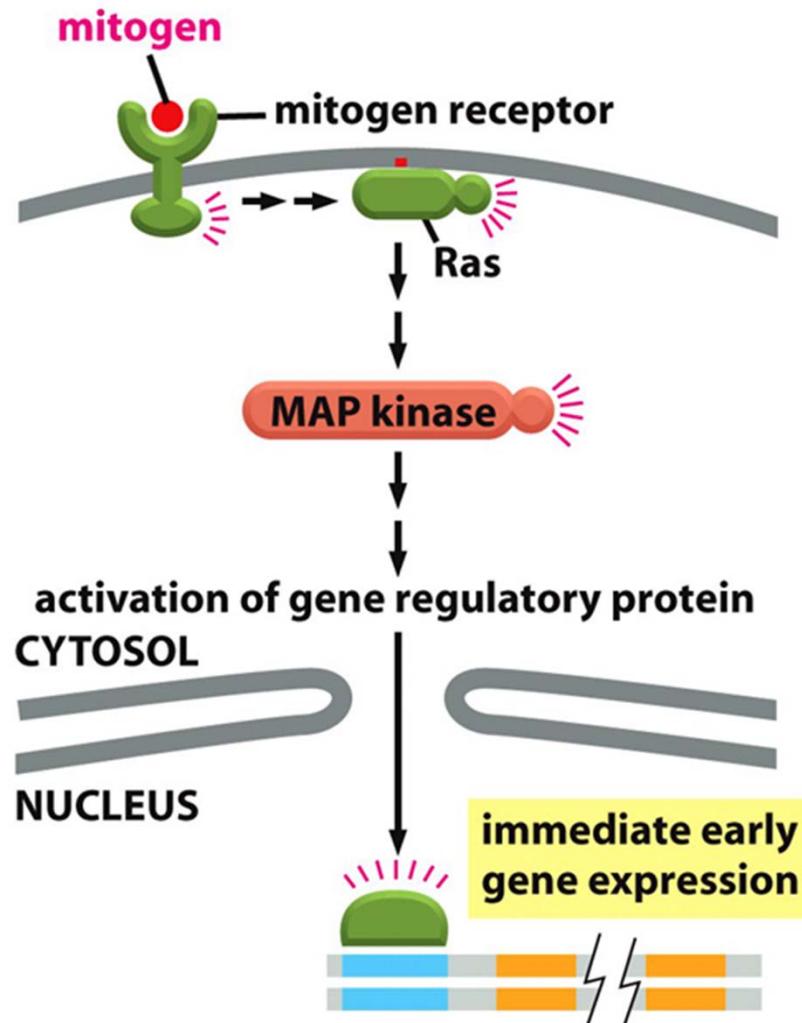
- **p21 is a Cdk inhibitor protein!**
- **p21 triggers inactivation of G₁/S-Cdk and S-Cdk and thus arrests the cell cycle**

Defects in DNA damage response have severe consequences

- **ATM mutation:** Ataxia-telangiectasia (**Louis–Bar syndrome**)
 - serious genetic disease
 - hypersensitive to sunlight
 - few live beyond 20s
 - neurodegenerated,
 - Highly susceptibility to cancer
- **p53 mutation/loss:**
 - cancer,
 - 50% of human cancer have p53 loss or mutation

The signaling pathway controlling cell cycle progression

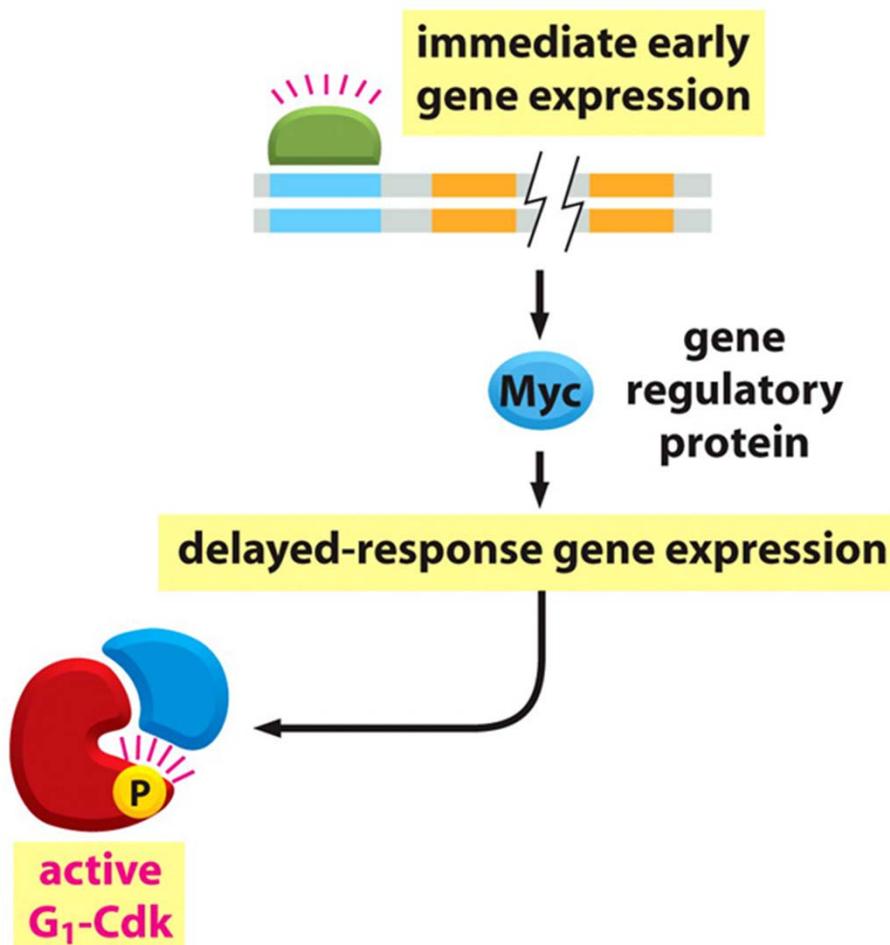
Mitogens control the rate of cell division by acting in the G1 phase of the cell cycle



Signaling pathway:

- **mitogen** activates receptor
- receptor activates **Ras**
- **Ras** activates the mitogen-activated protein (MAP) kinase cascade
- The **MAP kinase cascade** activates transcription factors and triggers immediate early gene expression
- one of these genes is the gene regulatory protein (transcription factor) **Myc...**

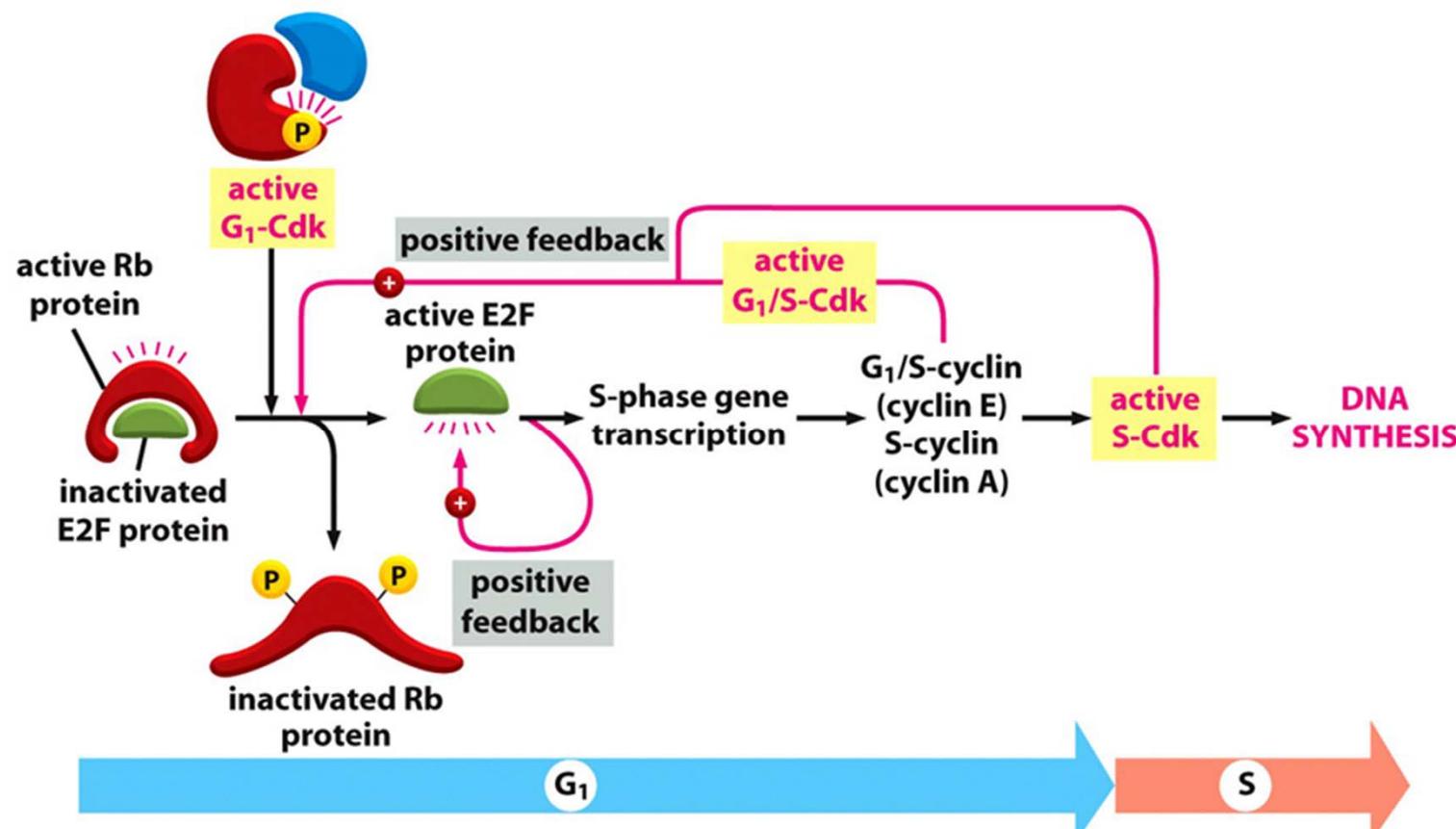
MAP transcriptionally activates c-Myc



Signaling pathway:

- the gene regulatory protein (transcription factor) Myc promotes expression of **G₁ cyclins (D cyclins)**
- **G₁ cyclins (D cyclins)** activate **G₁-Cdk** and trigger cell cycle entry
- but G₁-Cdk activates also other transcription factors e.g. the E2F proteins.....

Rb-E2F pathway in cell cycle control



Signaling pathway:

- **G₁-Cdk** activates **E2F proteins**, which activate transcription of **S-phase proteins** like **G₁/S-cyclin & S-cyclin**, resulting in further activation of **G₁-Cdk & S-Cdk** and thus further inactivation (phosphorylation) of the Rb protein (**positive feedback loop**) and the cell cycle starts....

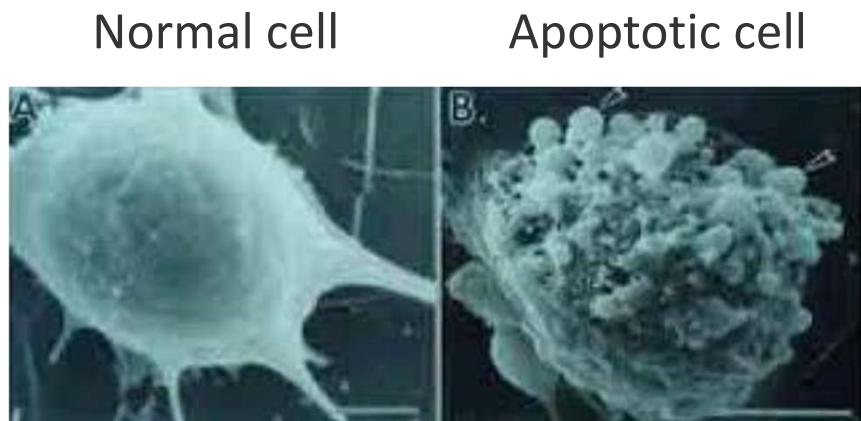
Examples for straight forward learning

Apoptosis

- What is apoptosis?
- How does it differ from necrosis?
- When does apoptosis occur?
- What are the key players:
 - different caspases have different functions
 - regulators/modulators?
- How is apoptosis triggered:
 - internally (which molecules / compartments and proteins are involved?)
 - externally (which components (receptors ligands) are involved?)
- What fail-safe mechanisms prevents “accidental” start of apoptosis?
 - (proteins/molecules/mechanism)
- Medical conditions related to apoptosis?

What is apoptosis?

During apoptosis, cells undergo morphological changes:



Characteristics of apoptosis :

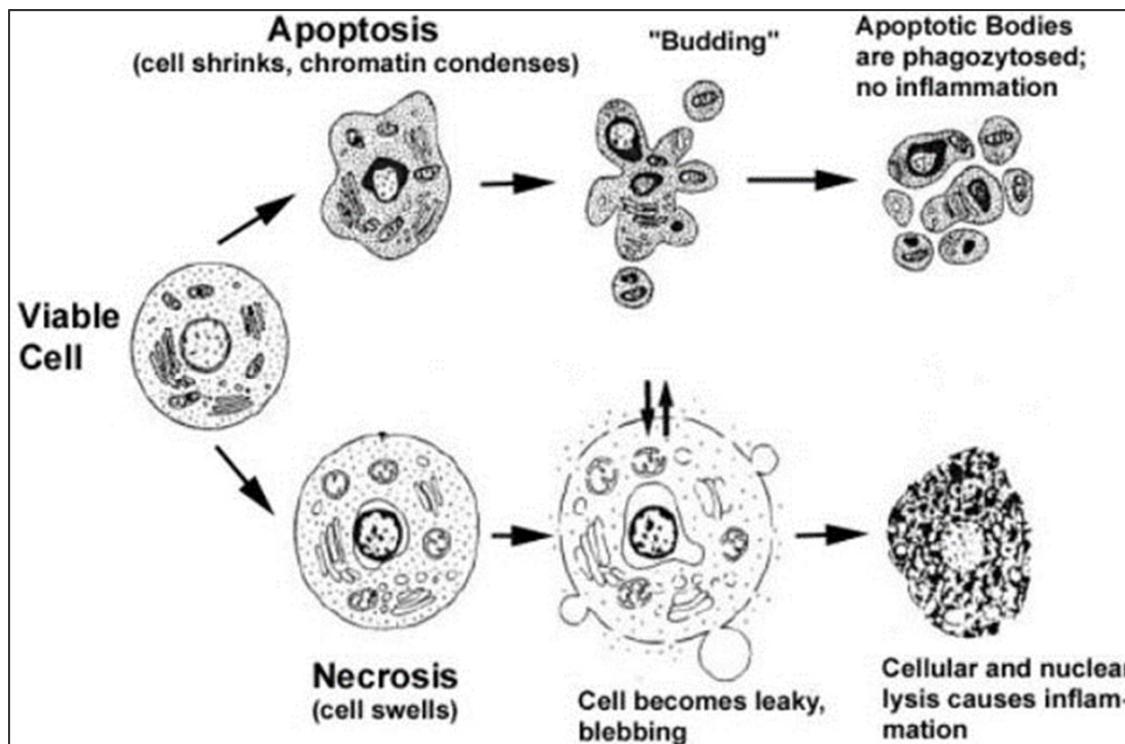
1. Cell shrinkage and chromatin condensation
2. PS flipping to outside
3. DNA fragmentation
4. Nuclear membrane disruption
5. Cytoskeleton collapses
6. Cell surface blebs---apoptotic bodies

Different types of death: apoptosis versus necrosis

Apoptosis: cells die clean and tidy

(eaten and digested by neighboring cells or macrophages)

Necrosis: cells swell and burst, content spillage, can cause inflammation,
necrosis is usually due to acute insults



Apoptosis depends on an intracellular proteolytic cascade

Proteolysis is catalyzed by caspases:

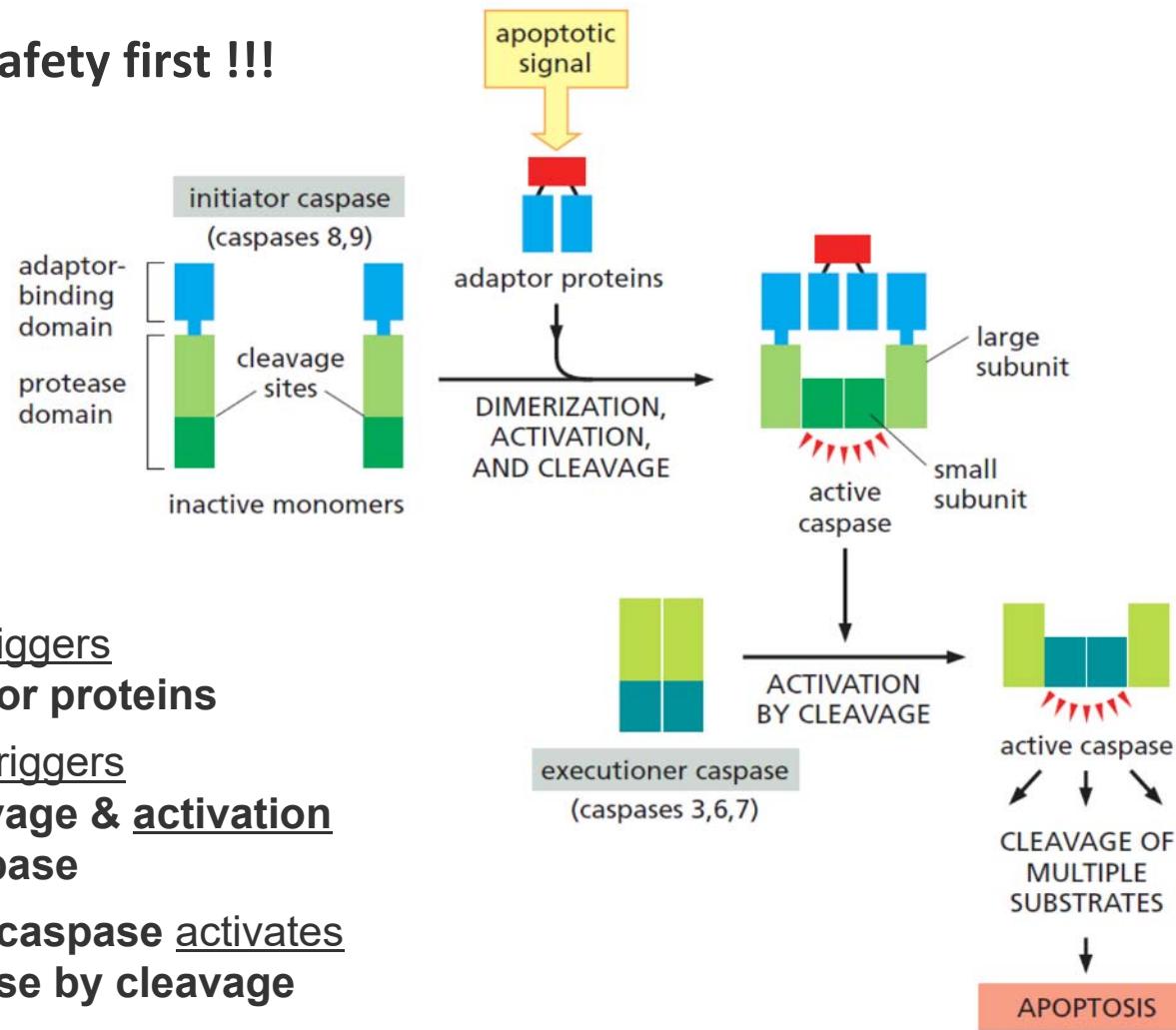
- Caspases have a cysteine in their active site and cleave their target proteins at specific aspartic acids (→ caspase)
- Caspases are zymogens (synthesized as inactive precursors), →**procaspase**



- Two types of caspases:
 - initiator caspases
 - executioner caspases
- Apoptosis is triggered by a cascading reaction of initiator and executioner caspases

Signal-mediated cascading activation of apoptosis

Death is dangerous: safety first !!!

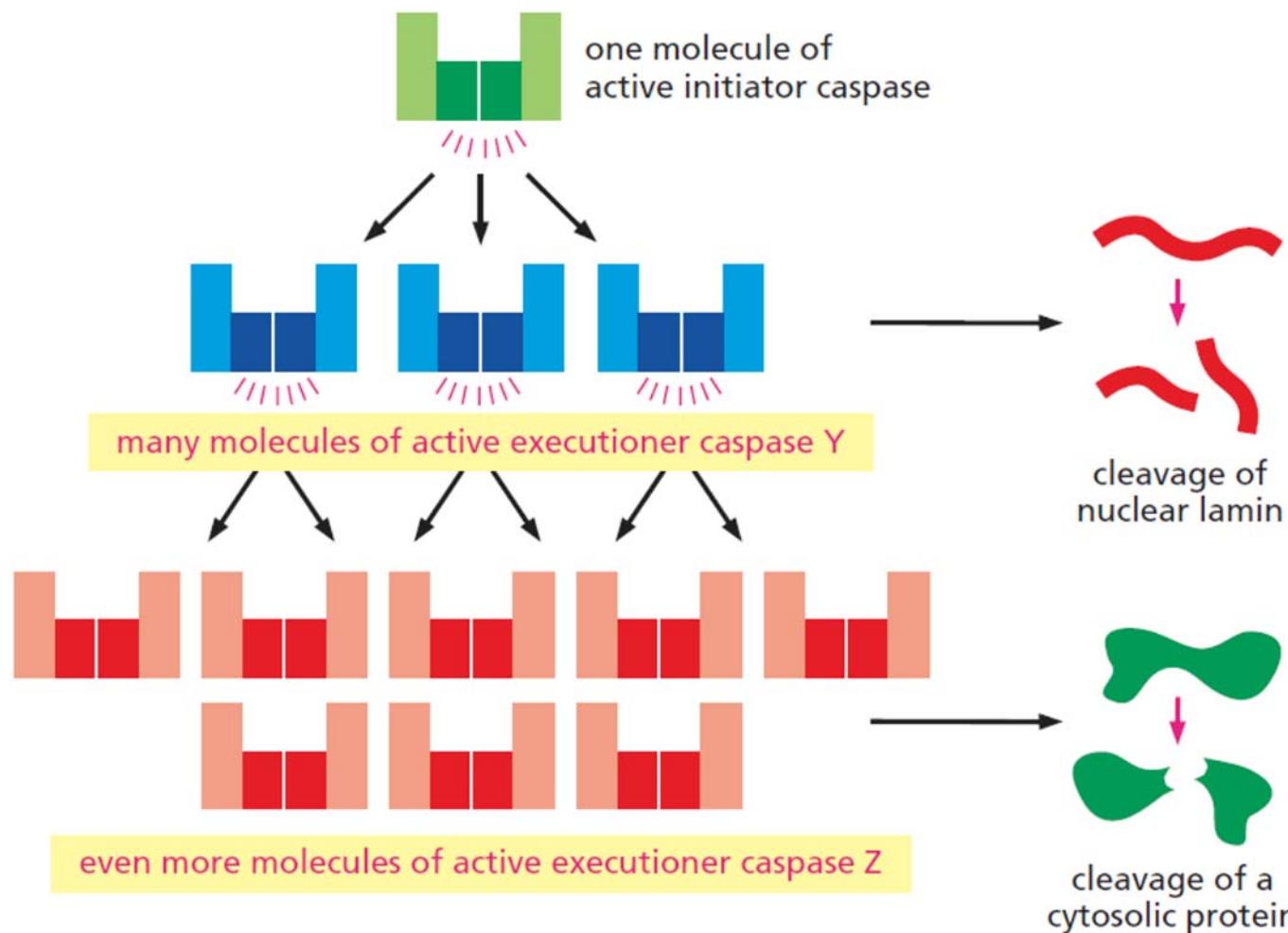


Activation cascade:

- 1) Apoptotic signal triggers assembly of adaptor proteins
- 2) Adaptor complex triggers dimerization, cleavage & activation of the initiator caspase
- 3) Activated initiator caspase activates executioner caspase by cleavage
- 4) Activated executioner caspase cleaves multiple substrates, resulting in **cell death**.

The amplifying caspase cascade: the point-of-no-return

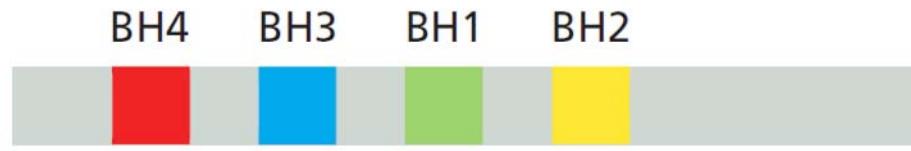
A single **initiator caspase** activates many molecules of **executioner caspases**



The intrinsic pathway of apoptosis is regulated by Bcl2 proteins

- Bcl2 () protein family

anti-apoptotic
Bcl2 family protein
(e.g., Bcl2, BclX_L)



pro-apoptotic
effector Bcl2 family
protein
(e.g., Bax, Bak)

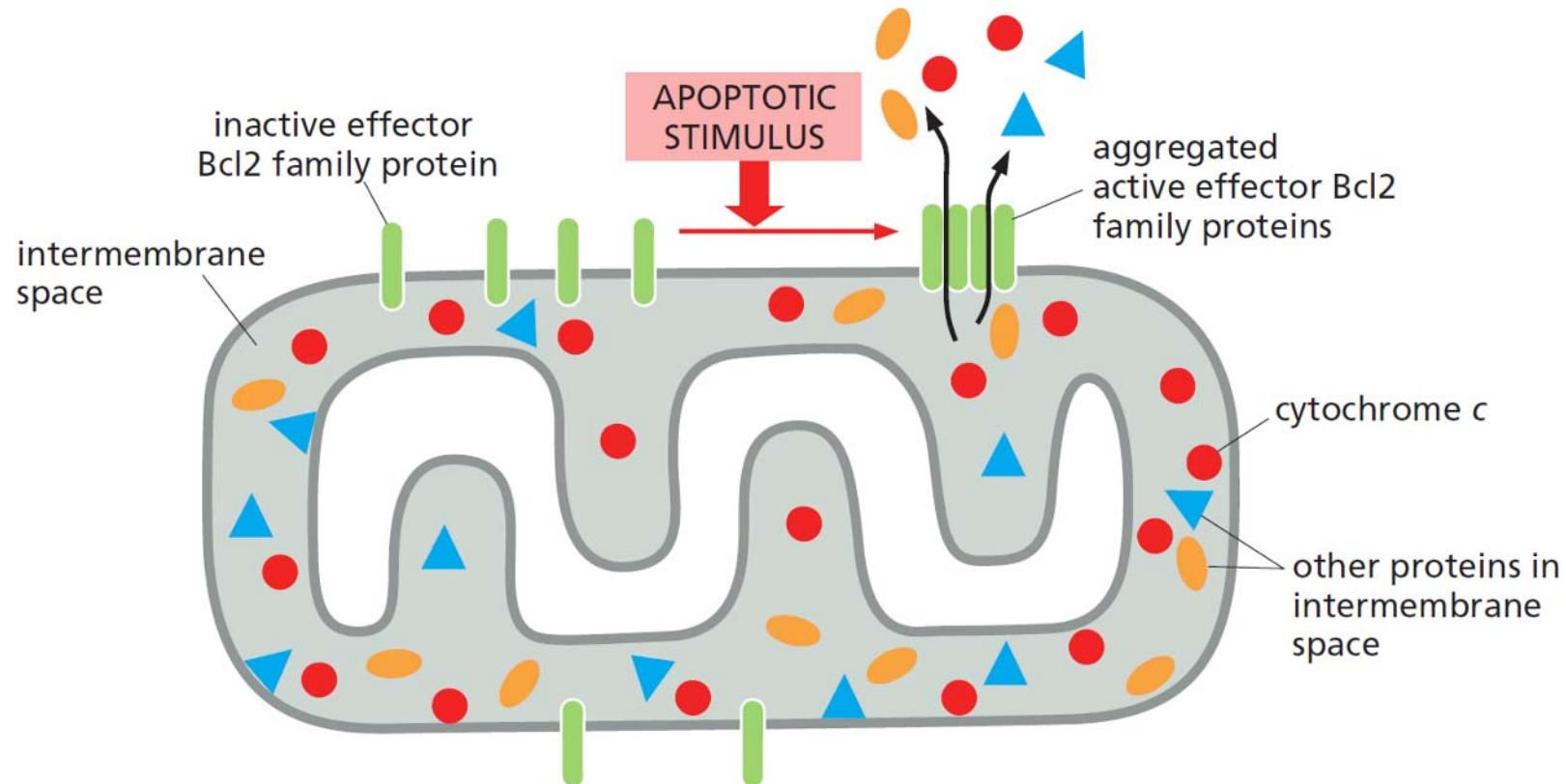


pro-apoptotic
BH3-only protein
(e.g., Bad, Bim,
Bid, Puma, Noxa)



How are cytochrome c and IMS proteins released?

the pro-apoptotic effectors Bax and Bak aggregate in the outer membrane of mitochondria to release cytochrome c and IMS proteins

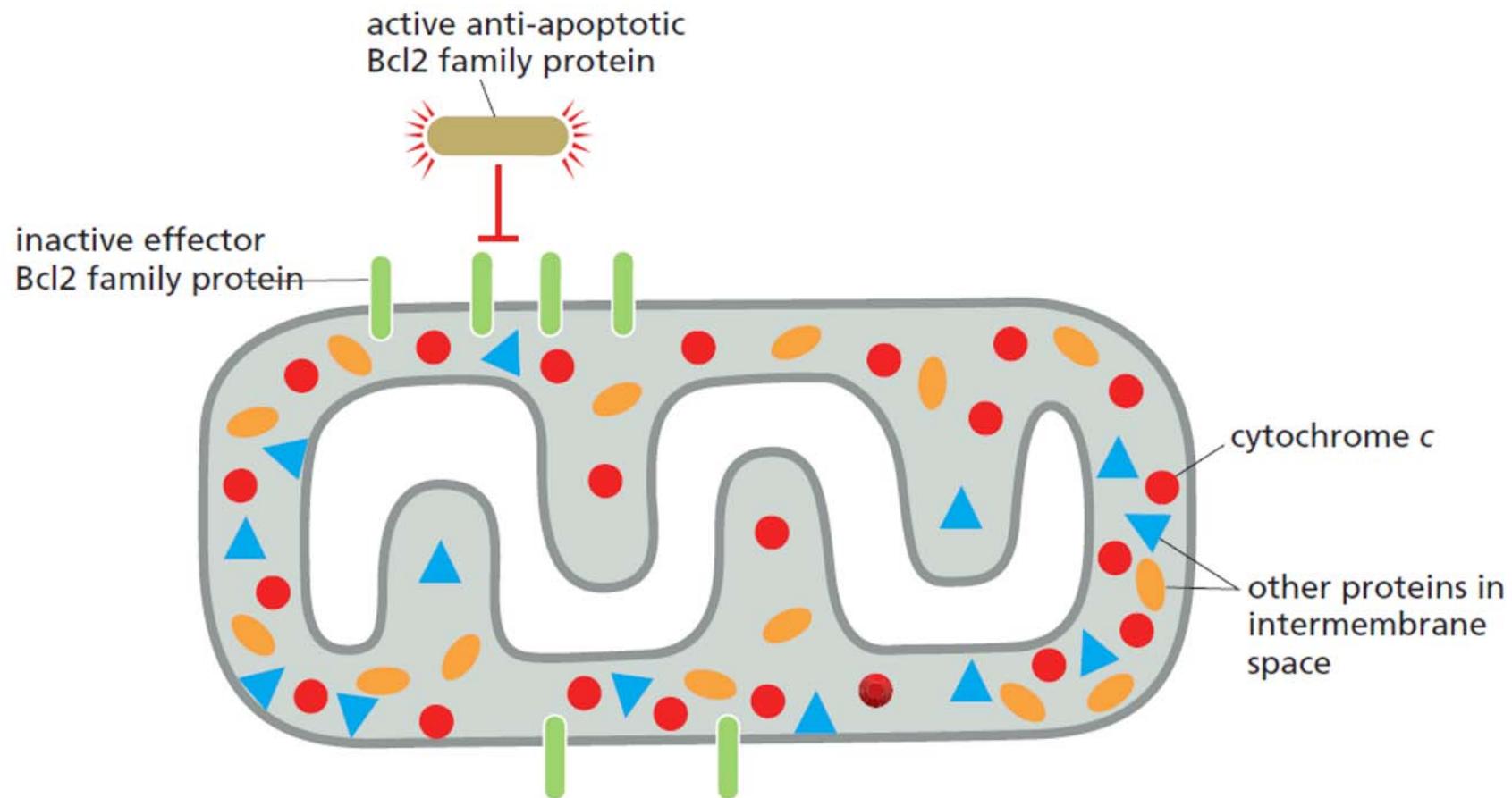


Absence of signal: Bak is mainly in the outer membran, whilst Bax is cytosolic.
Upon signaling: Bax relocates to the outer membrane and interacts with BAK
to trigger the efflux from the IMS

Anti-apoptotic Bcl-2 binds and inhibits Bax and Bak aggregation

active anti-apoptotic Bcl2 family proteins prevent aggregation of receptors at the outer mitochondrial membrane

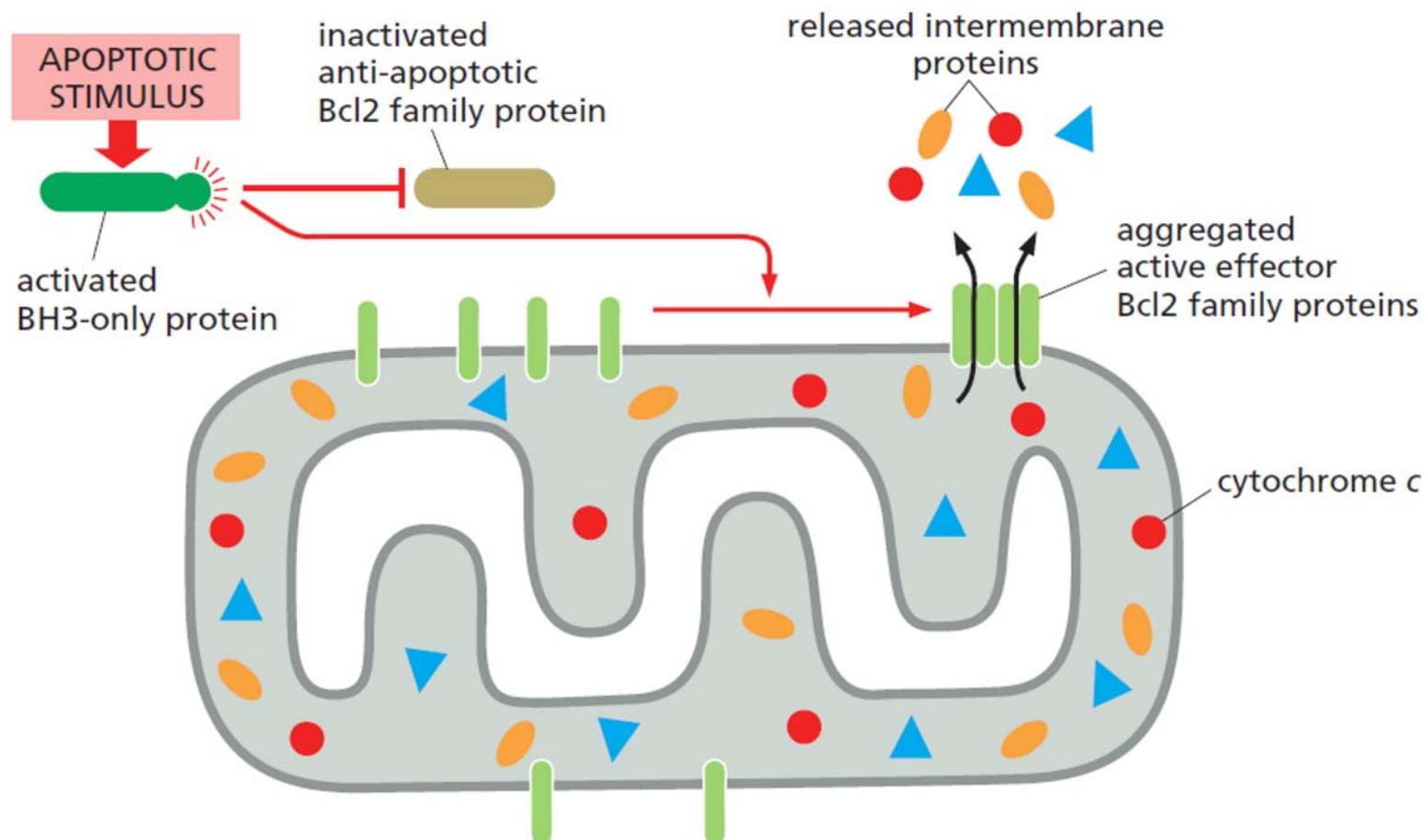
(A) INACTIVE INTRINSIC PATHWAY



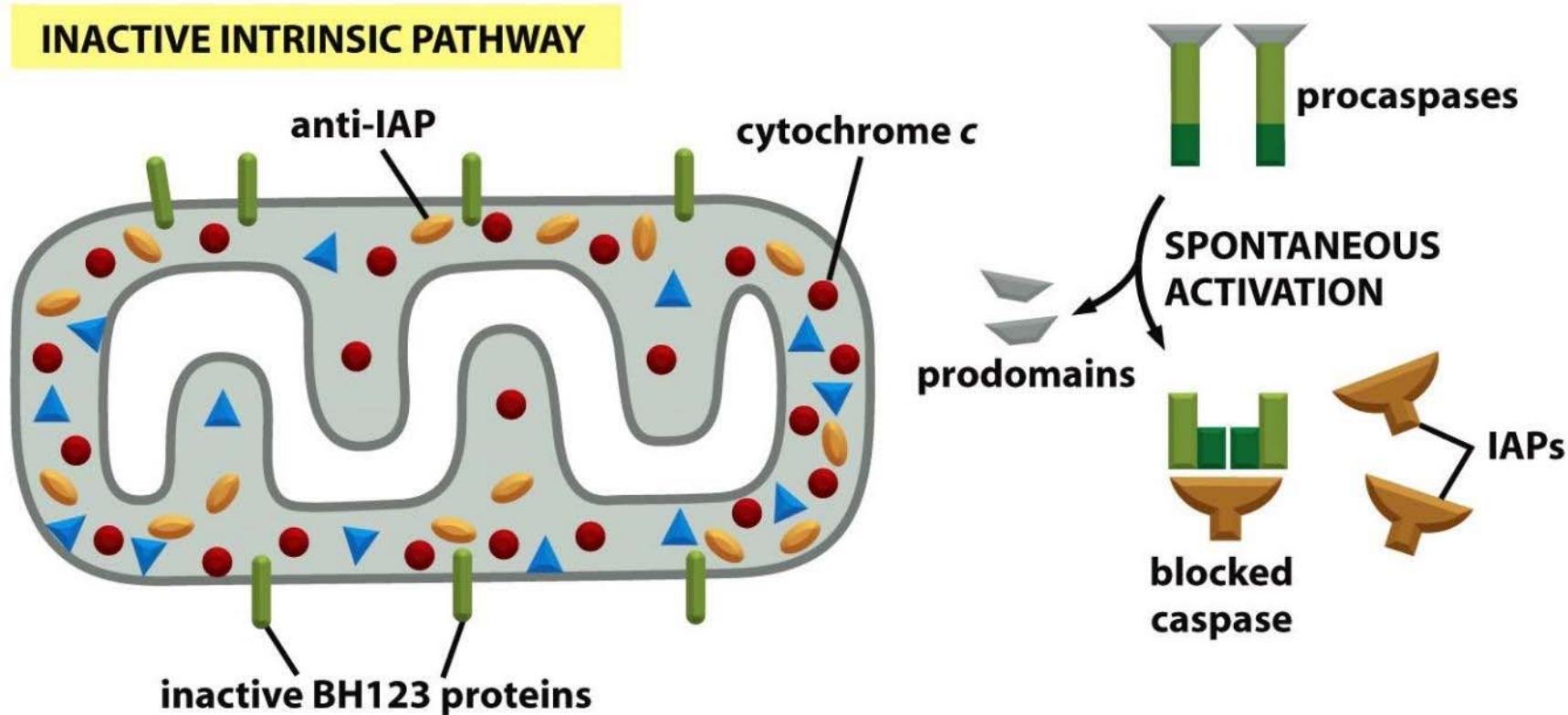
BH3- only proteins inhibit anti-apoptotic Bcl2 family proteins

Inactivation of anti-apoptotic Bcl2 proteins by BH3-only proteins allow for Bak/Bax aggregation and thus for apoptosis

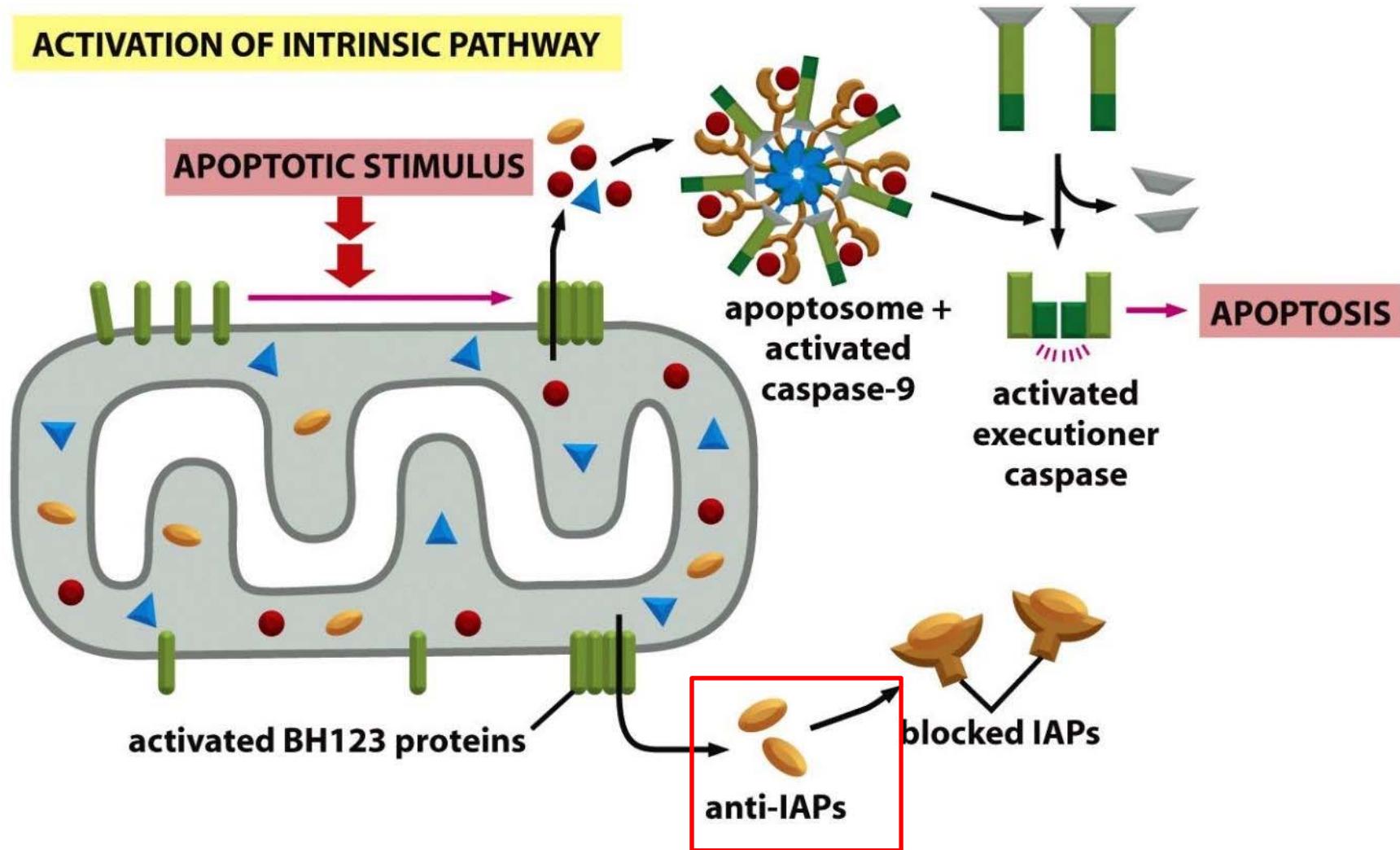
(B) ACTIVATION OF INTRINSIC PATHWAY



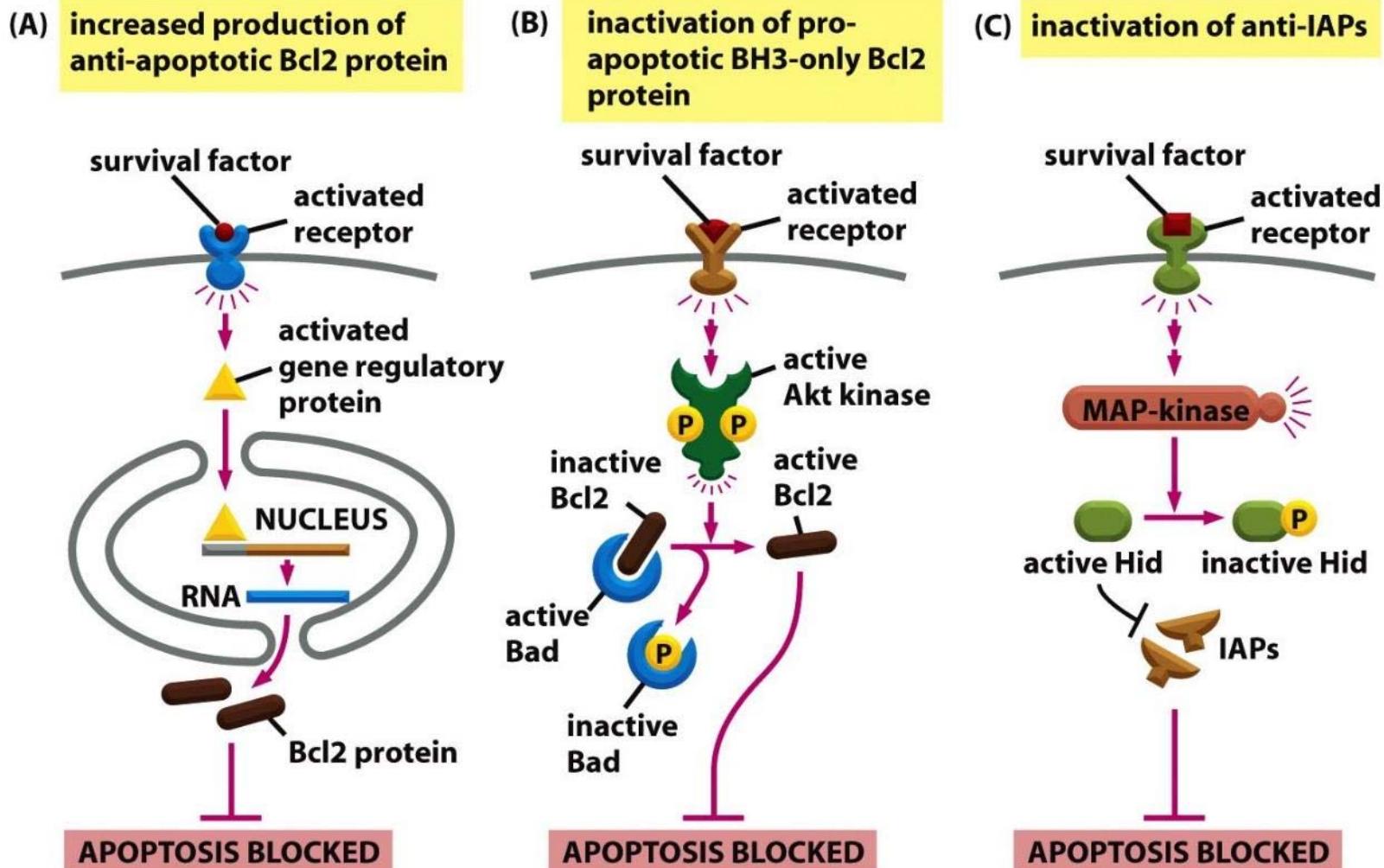
IAPs (inhibitors of apoptosis) inhibit caspases



ACTIVATION OF INTRINSIC PATHWAY



Three ways for survival factors to inhibit apoptosis



Examples for straight forward learning

Autophagy

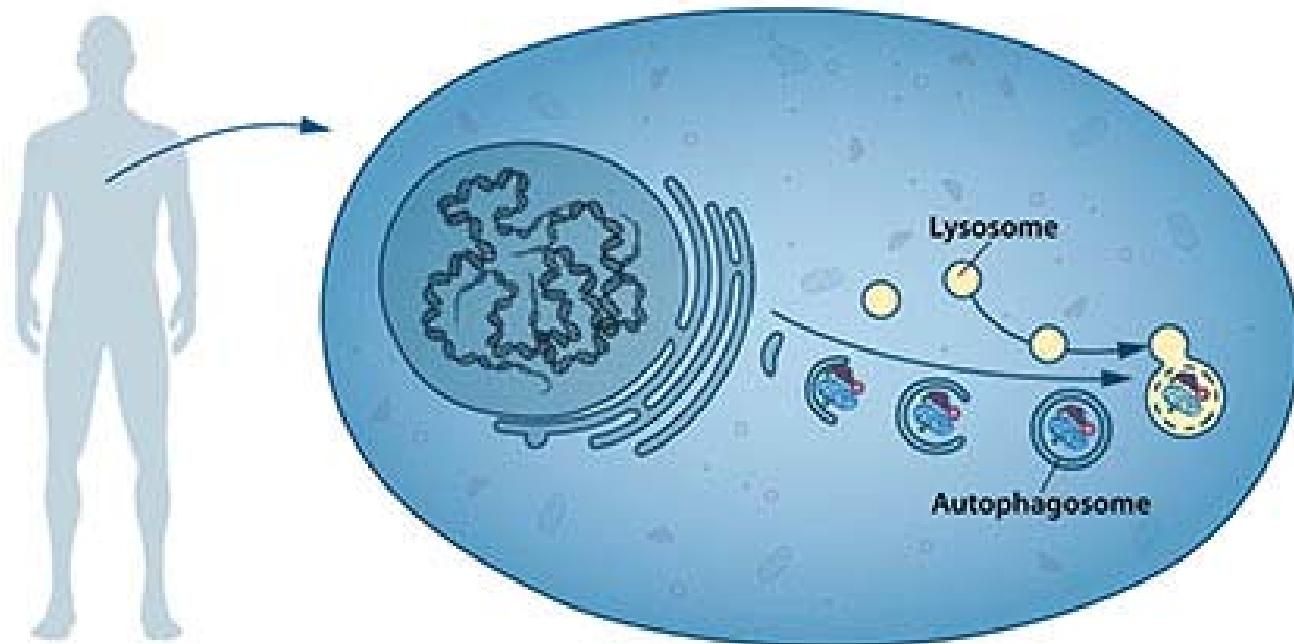
- What is autophagy and when does it occur
- key players

Autophagy concept in 1963

- Autophagy occur at a low basal level in normal condition
- It can be induced by stress such as starvation
- It may have roles in pathogenesis or disease
- It occurs in a wide range of cells including amoeba, tetrahymena, insect, frogs , etc.

Autophagy remained a mystery for ~ 30 years...

What they knew was:



To study autophagy was difficult at that time:

- autophagosomes are transient and only exist for about 20-30 minutes before fusing with lysosome... until

In early 1990's, Yoshinori Ohsumi



- Made seminar discoveries about autophagy using yeast model system
- He was awarded 2016 Nobel prize in Physiology/Medicine
- He **identified multiple key genes involved in autophagy** and revealed the molecular mechanism for autophagy initiation, formation, regulation, **and link to human diseases, etc.**



1945 - Japan

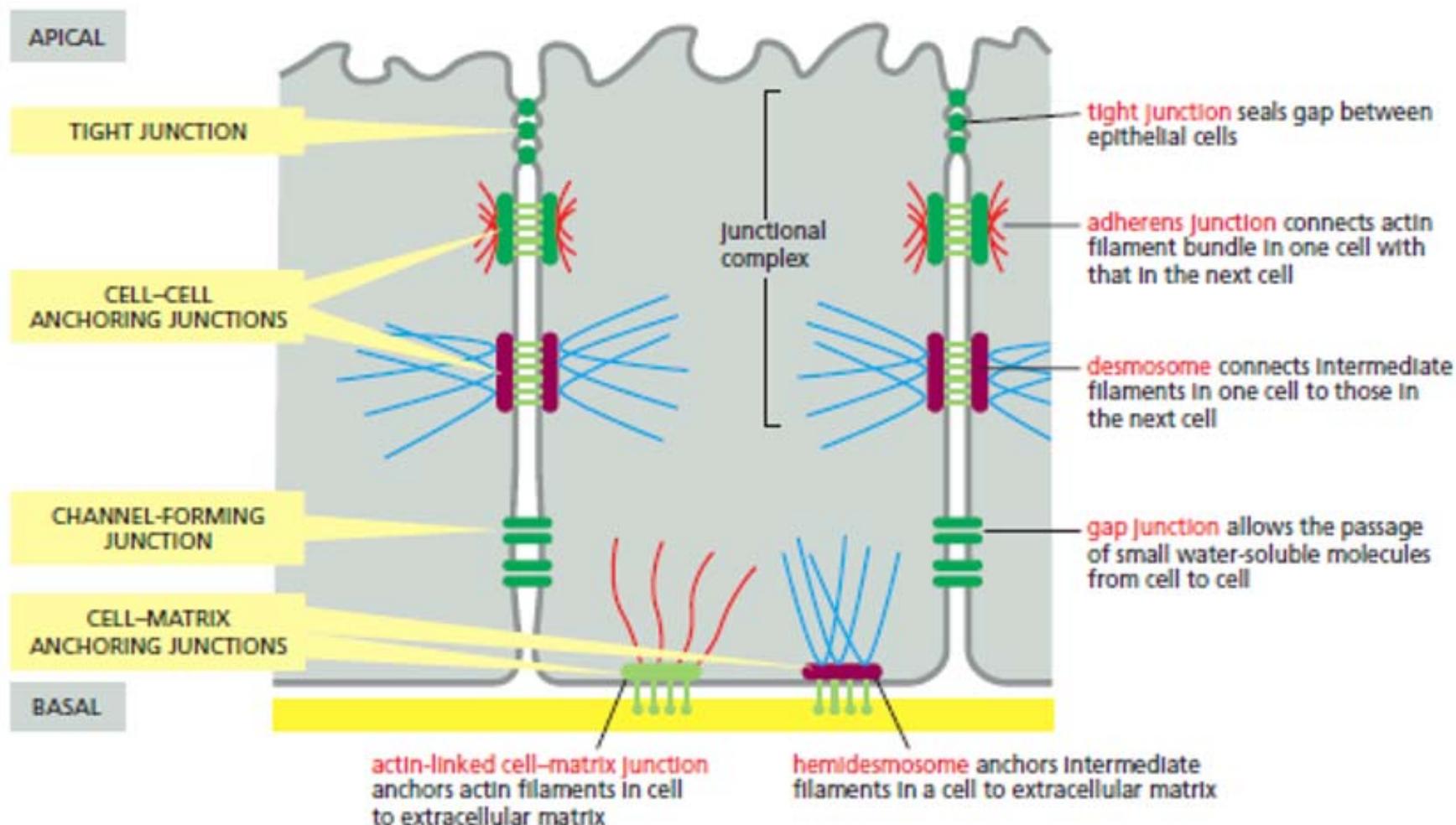
Examples for straight forward learning

Junctions

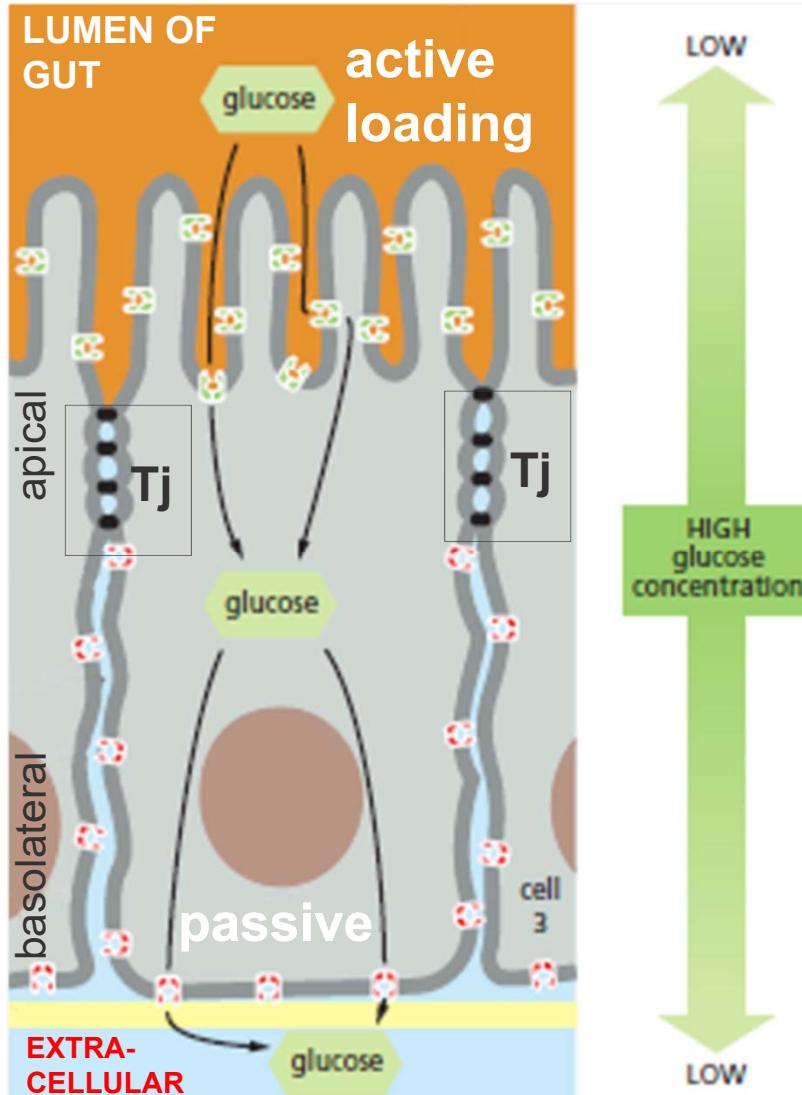
- Types of junctions?
 - differences vertebrates invertebrates
- Locations
- Functions
- Key player & components
 - differences
- Medical conditions?

Where to start?

... and then reveal more details, step by step...



Tight junctions maintain cell polarity: fencing function



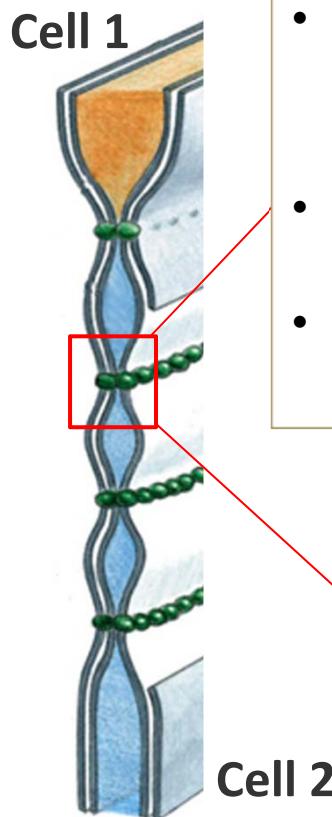
This segregation permits a vectorial transfer of nutrients across the epithelium from the gut lumen to the blood.

Function of tight junctions:

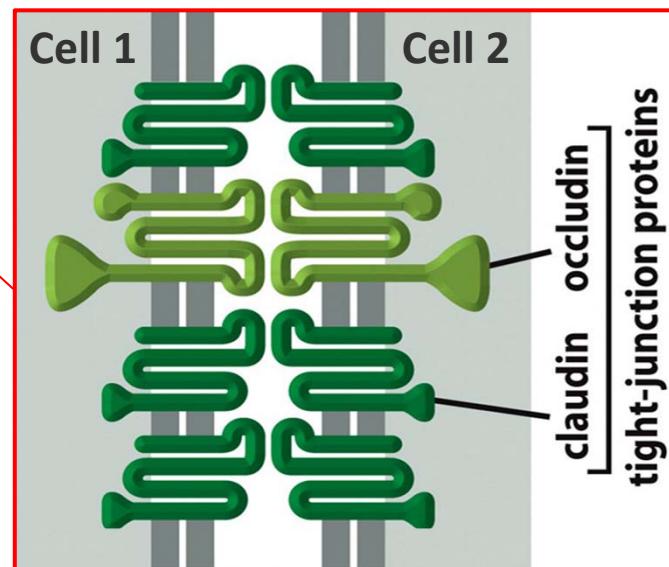
1. They **seal** the intercellular space to form a barrier
2. Serve as “**fences**” to **separate domains** within the PM
3. Tjs **Maintain cellular polarity**
4. Tjs are **selective for ions**, but do not allow macromolecules to pass.

Molecular organization of tight junctions

Three key proteins form tight junctions: claudin, occludin & tricellulin

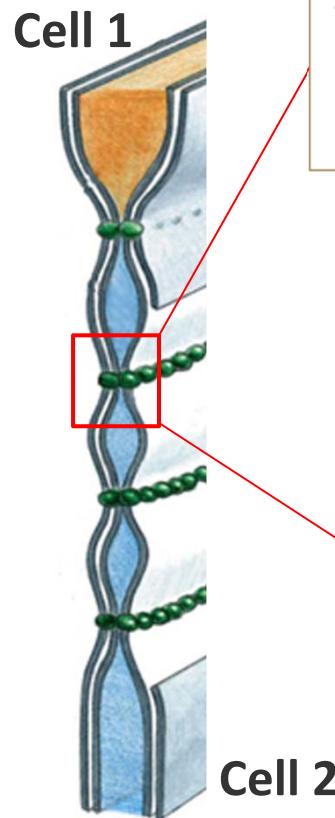


- **Claudin:**
 - **Loss** causes dehydration and death in mice
 - **Overexpression** causes tight junction formation in fibroblasts
- **Occludin:**
 - Detailed function unknown
- **Tricellulin:**
 - Required to seal membrane

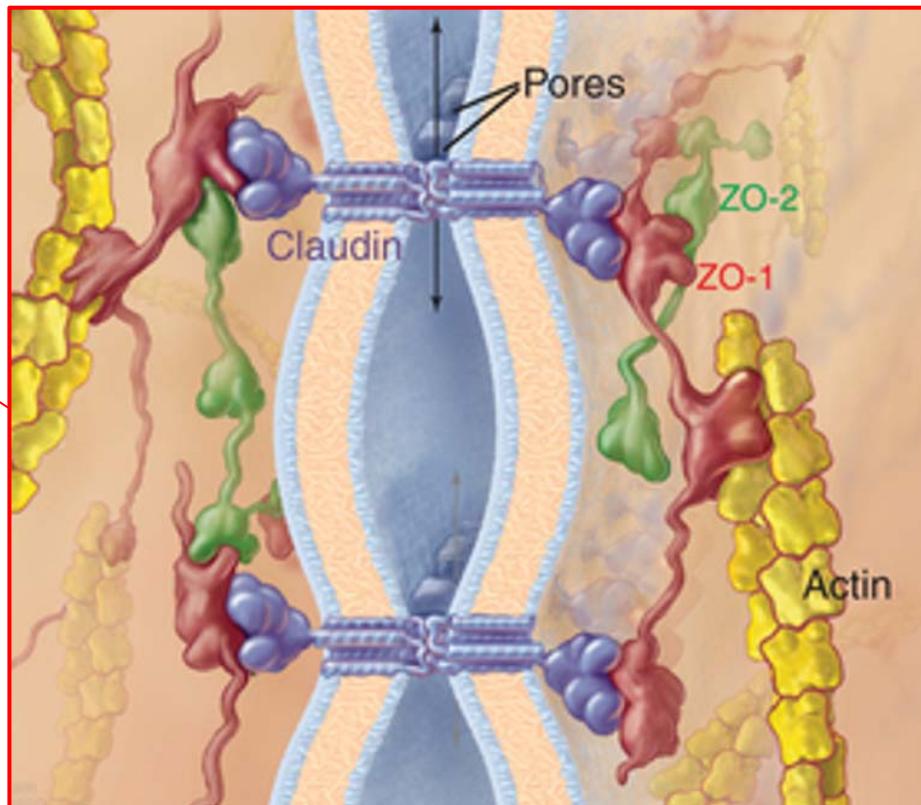


Molecular organization of tight junctions

Scaffold proteins (**ZO/Tjp**) link claudins to the actin-cytoskeleton

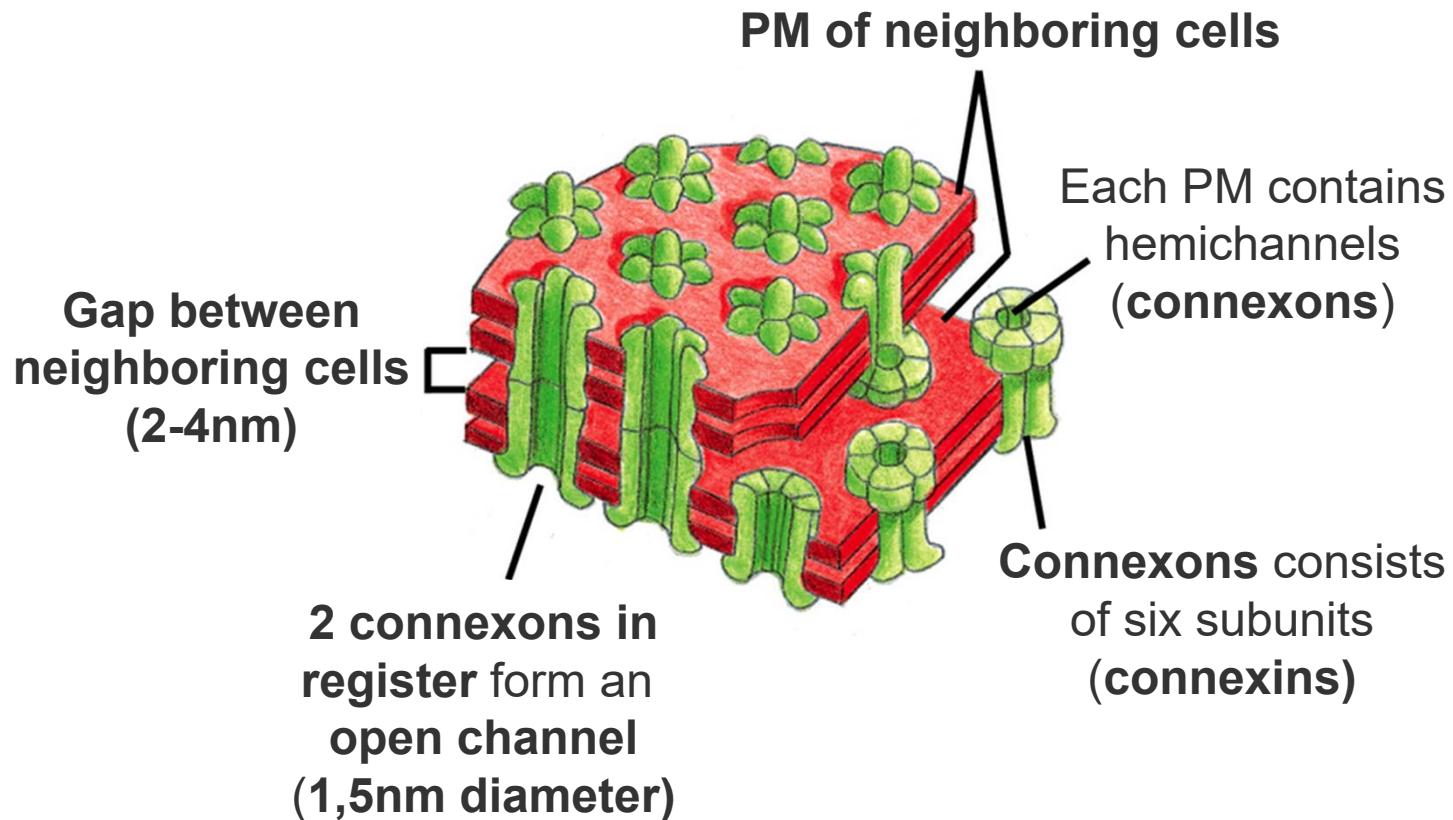


- **ZO** (zonula occludens) proteins (**ZO-1/-2**)
also called **Tjp** (tight junction proteins)
are anchor sites for tight-junctional strands



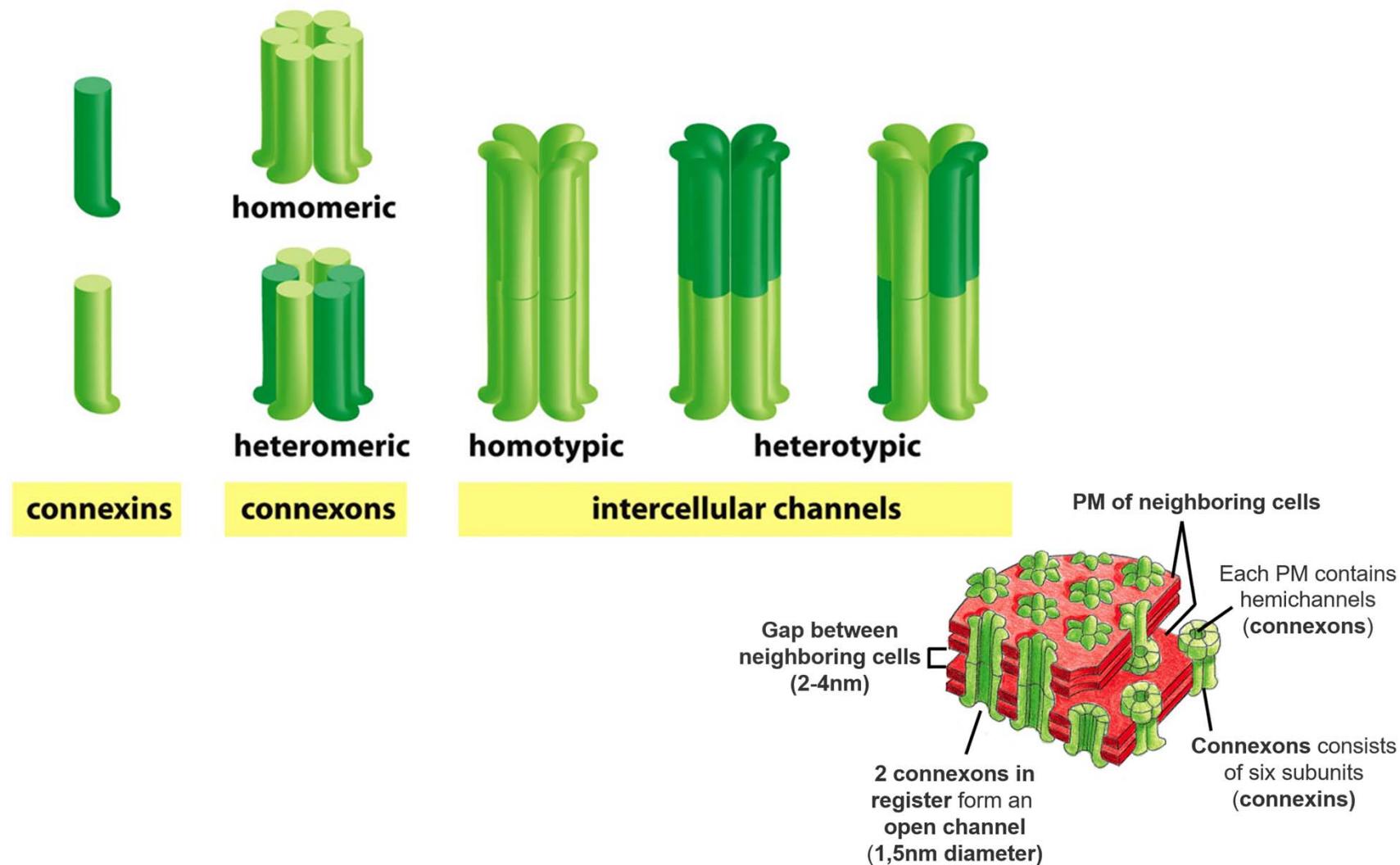
Structure of Gap junctions

Six connexins form one connexon and two connexons build one channel



Structure of Gap junctions

Connexons can be homomeric or heteromeric



Examples for straight forward learning

ECM

- What is it, where does it come from?
- Different types of ECM and their individual composition
 - connective tissue
 - basal lamina
- Components (Major key components)
 - properties & differences and compositions

III. The basal lamina

What is the basal lamina?



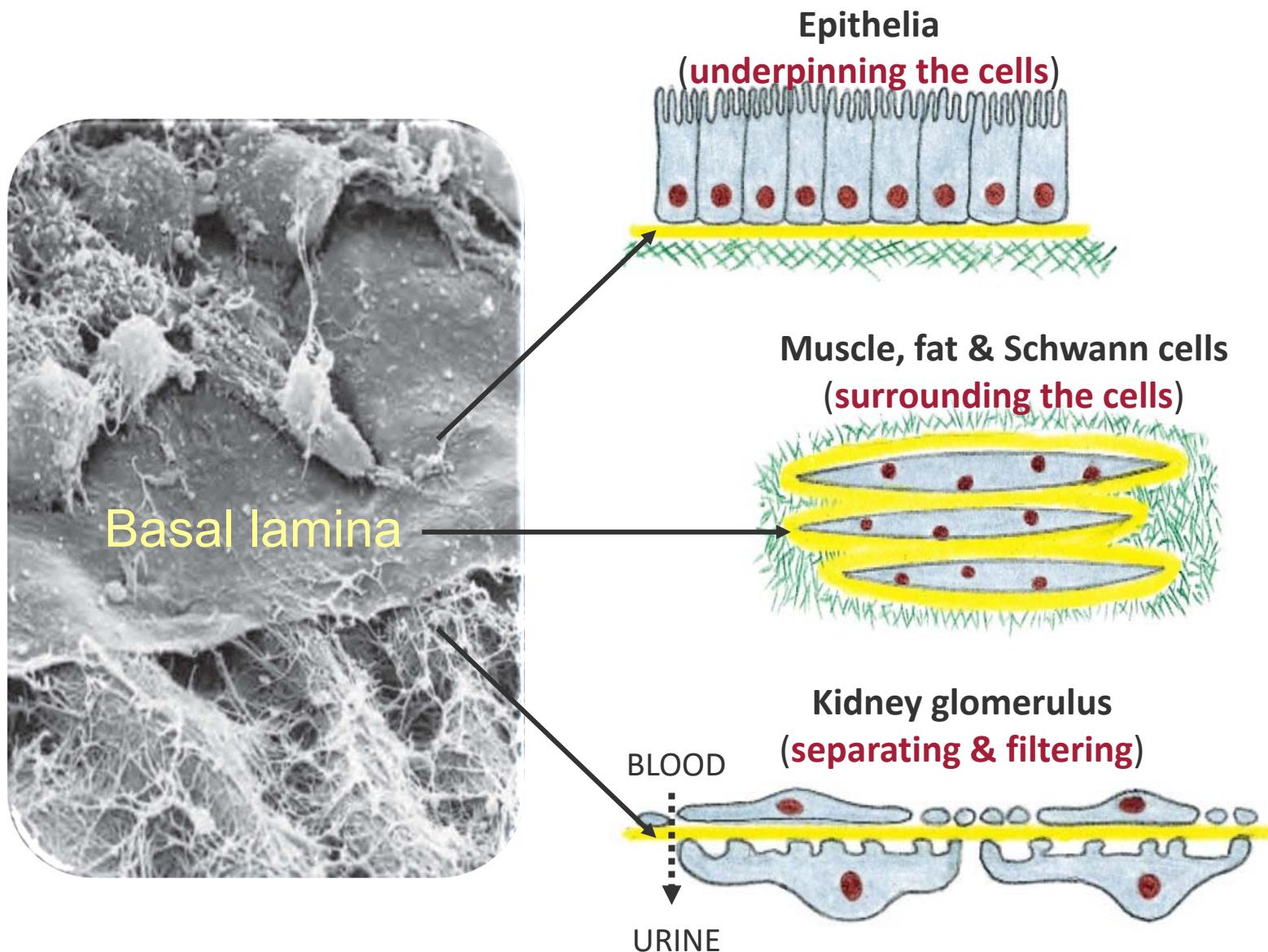
Basal lamina is a structure
of the ECM
(extracellular matrix)

Produced by both:
epithelia & the stroma,

40-120 nm thick

Plays important mechanical role
(strength of the epidermis)

The basal lamina comes in different configurations:



The ECM consists of proteoglycans & fibrous proteins

ECM: substances & organization:

Proteoglycans

- proteins with polysaccharides (**GAGs**)

Fibrous proteins

- proteins with short oligosaccharide side chains

The **proteoglycans** form a hydrated **gel-like matrix**, in which the **fibrous proteins** are **embedded**

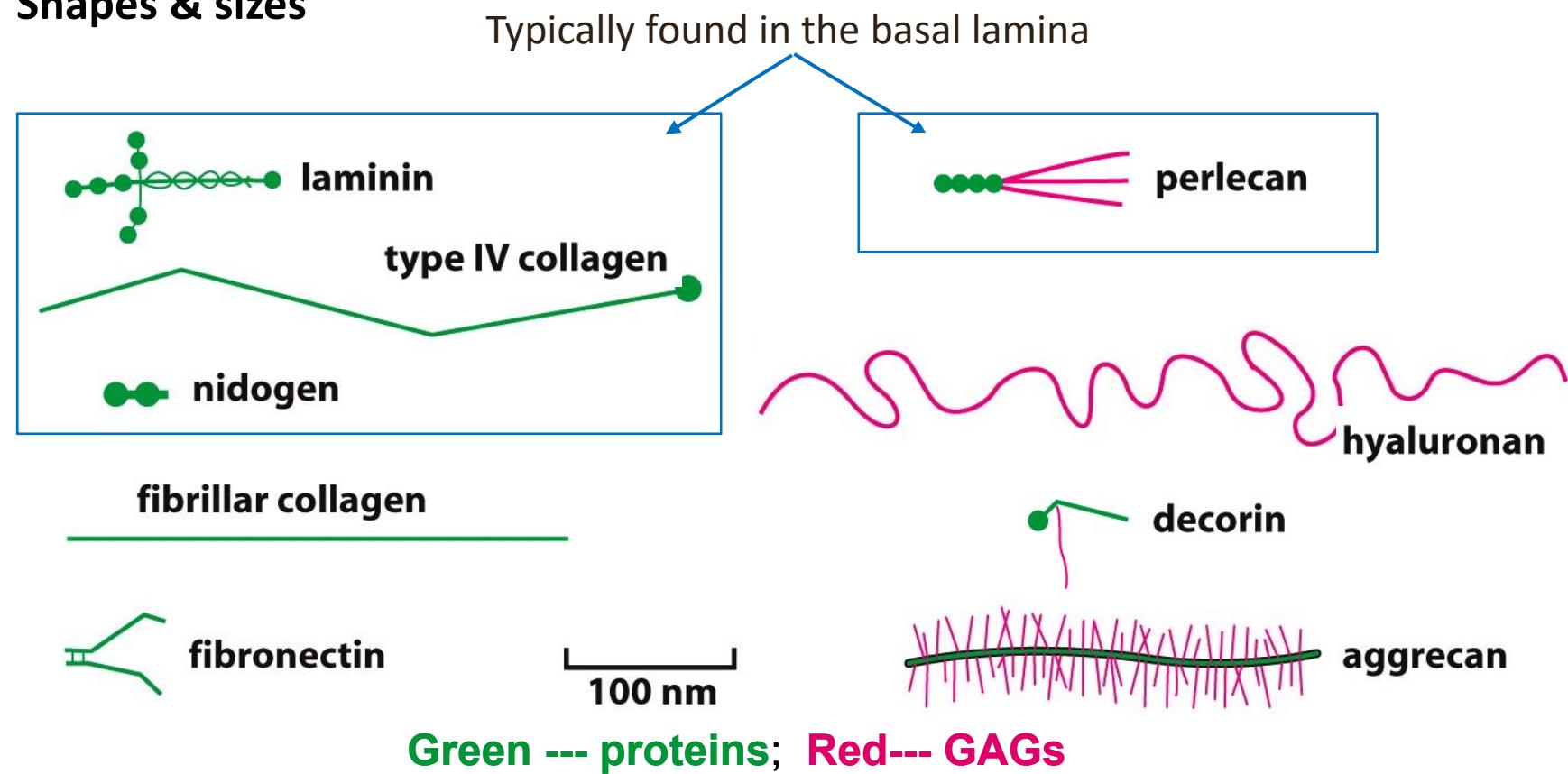
ECM consistency is the result of the composition

Composition of the basal lamina

The basal lamina consists of 2 main classes of secreted macromolecules:

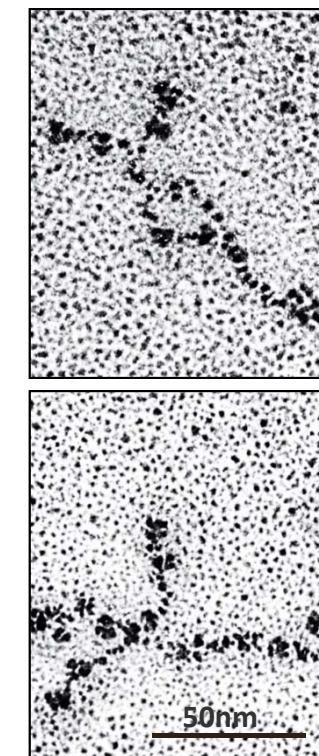
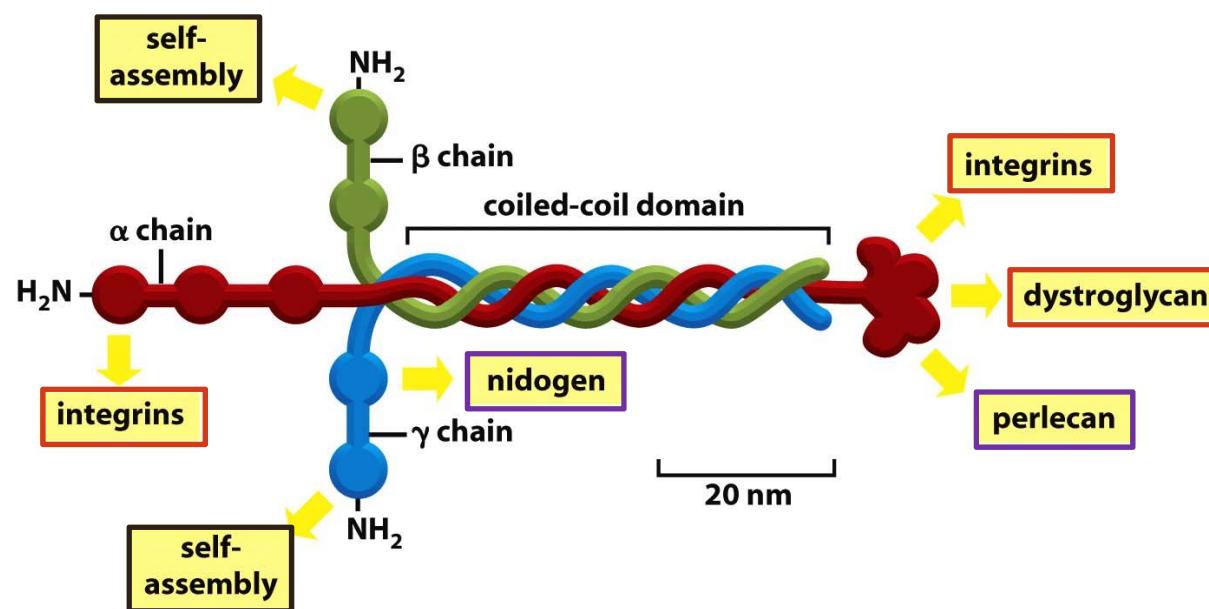
- Fibrous proteins (with short oligosaccharide side chains)
- Proteoglycans (proteins with polysaccharides, glycosaminoglycans, **GAGs**)

Shapes & sizes



Composition of the basal lamina: laminin

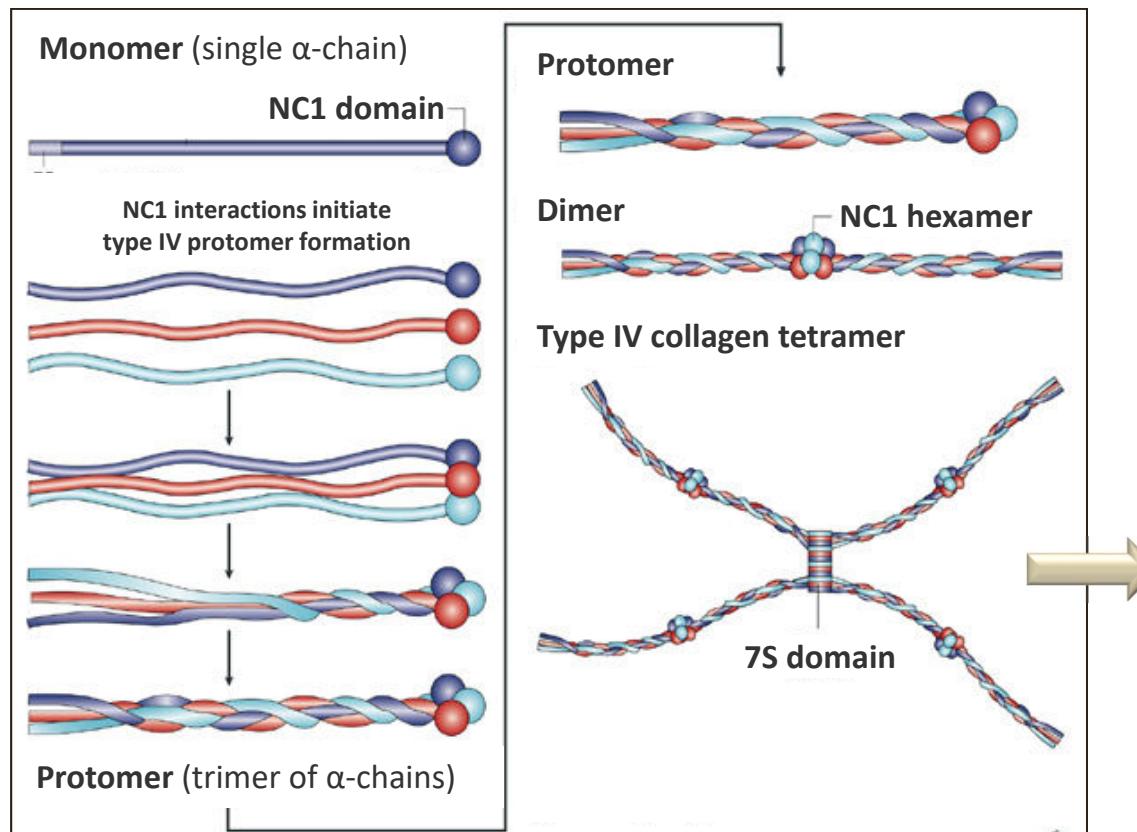
- Laminin is the primary organizer for the basal lamina structure
- Large heterotrimeric complex, consisting of α -, β -, γ -chains (about 3000 amino acids each subunit), which are held together by disulfide bonds.
- Can self-assemble via their head domains into a network *in vitro*



Composition of the basal lamina: type IV collagen

Second essential component in the basal lamina

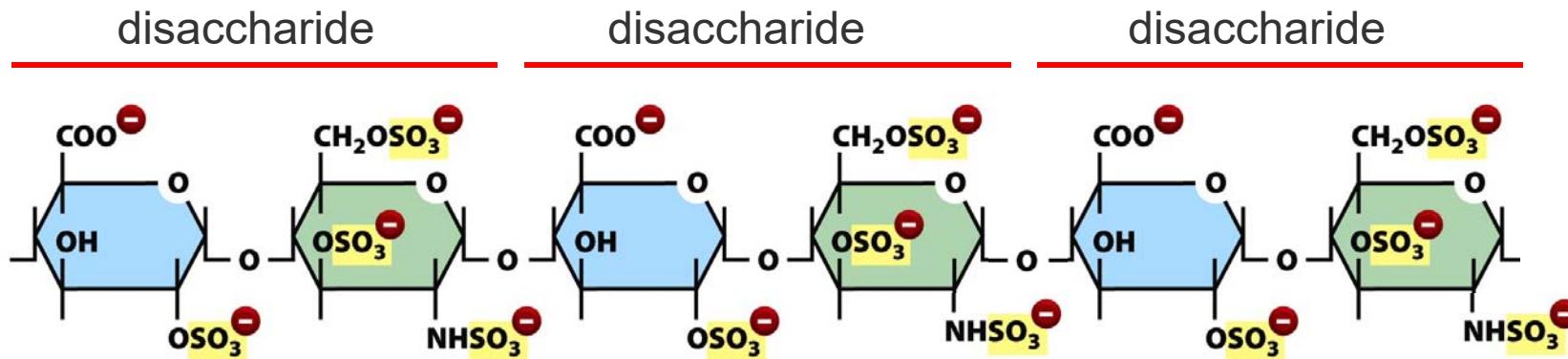
- Three separate chains twist together to form rope-like superhelix, with multiple bends.
- Interact with other basal lamina proteins via their terminal domains.



Glycosaminoglycans (GAGs)

What are GAGs?

- GAGs are **unbranched** chains of **repeating** disaccharides (up to 200) **Hyaluronan** as special case contains **up to 25,000** disaccharide repeats
- high density of **negative charges** along the chain due to carboxyl (COO^-) and sulfate groups (SO_3^-)

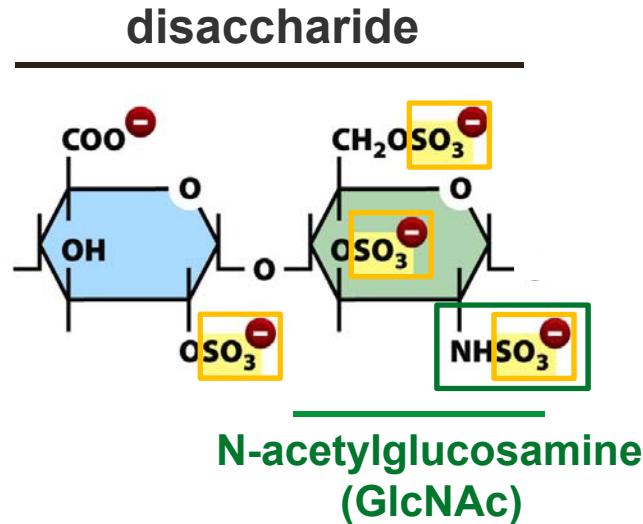


GAGs are the most anionic molecules that are produced by cells

Glycosaminoglycans (GAGs)

Why are they called glycosaminoglycans?

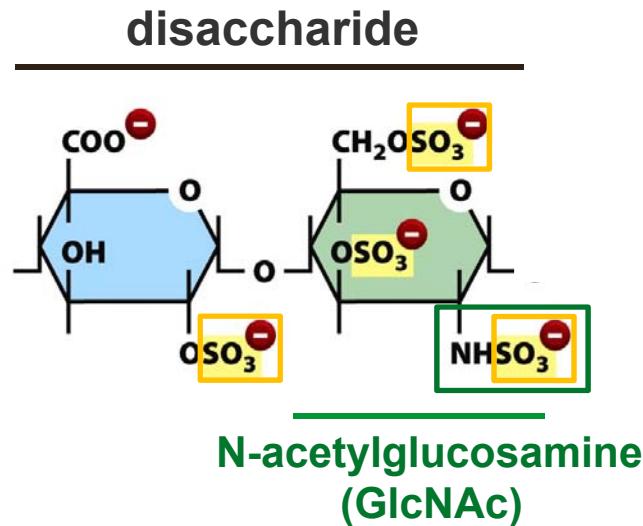
- One of the two sugars is always an **amino** sugar, frequently **sulfated**:
N-acetylglucosamine (GlcNAc) (based on **glucose**) or
N-acetylgalactosamine (GalNAc) (based on **galactose**)



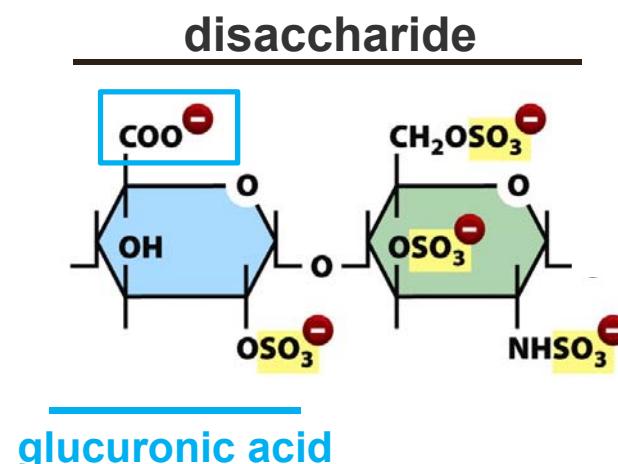
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N-acetylgalactosamine (GalNAc) (based on **galactose**)



- The second sugar is a **uronic acid** (oxidation \rightarrow carboxyl group, COO^-). Here, **glucuronic acid** (based on glucose), also **iduronic acid** (based on **idose**)

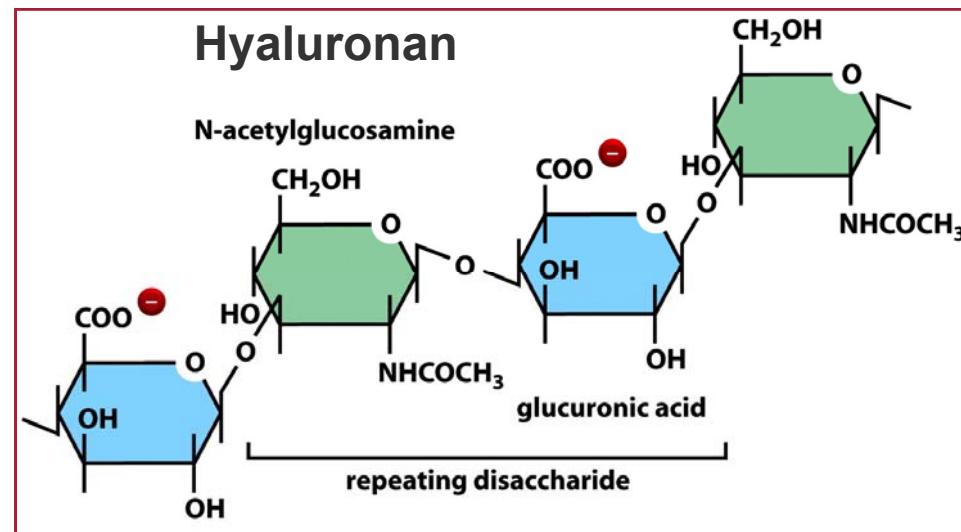


Glycosaminoglycans (GAGs)

Four different groups of GAGs are classified according to:

- the type of sugar
- type of linkage between the sugars
- number and location of sulfate groups

1. Hyaluronan
2. Chondroitin sulfate
3. Heparin sulfate
4. Keratan sulfate

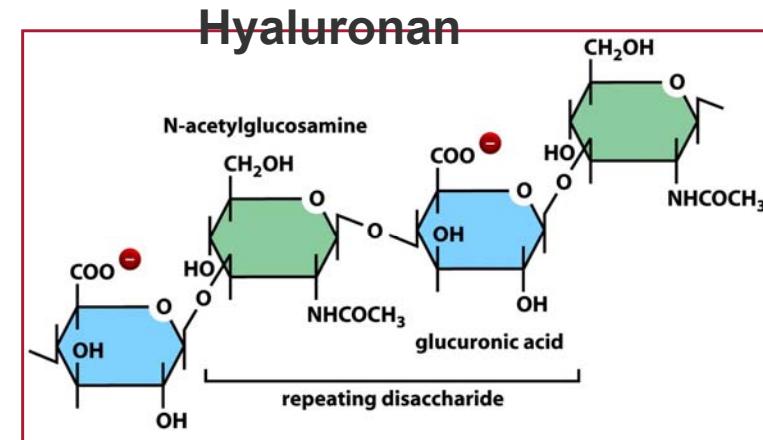


Glycosaminoglycans (GAGs)

Hyaluronan:

...actually, it is not a typical GAG:

- doesn't contain sulfated sugars
- only GlcNAc & glucuronic acid dimers
- not linked to proteins at all
- not secreted by cells, it
- is synthesized by enzymes **on the cell surface**, instead



Major functions:

- **guides cell migration** during tissue morphogenesis and repair
- it occupies a large volume compared to its mass:
it **provides** the space and it **fills** the **space** up
- it is degraded by **hyaluronidase**

Proteoglycans: almost limitless heterogeneity

Core proteins:

- diverse group of core proteins
- shared feature: the “link” domain

GAG side chains:

- diverse in composition and combination of sugars
- variable in modifications (sulfatation)
- a single core protein can carry a
highly variable number of different types of GAG side chains
 - sometimes more than 100 GAGs on a core protein (e.g. aggrecan)
 - sometimes only 1-10 (e.g. decorin)

Assembly of polymeric complexes:

- GAGs & proteoglycans can associate to form **polymeric complexes**:
 - aggrecan and hyaluronan form aggregates in cartilage matrix
the **size of a bacterium!**
 - GAGs & proteoglycans can associate with **fibrous proteins** like **collagen** and with **protein meshworks (basal lamina)** to extremely complex structures

General functions of proteoglycans

- Form gels (matrices) of varying pore size and charge density
- Serve as a selective sieves to regulate traffic of molecules and cells
- Regulate signaling through **binding** e.g. FGF (fibroblast growth factor), trTGF β (transforming growth factor) and chemical attractants (chemokines)
This allows control of growth and movement of cells
- Regulate activities of other proteins in the matrix e.g. proteolytic enzymes
- Some cell surface bound proteoglycan such as **syndecans** acts as co-receptors for growth factors to participate in cell signaling

Proteoglycans are not simple glycoproteins

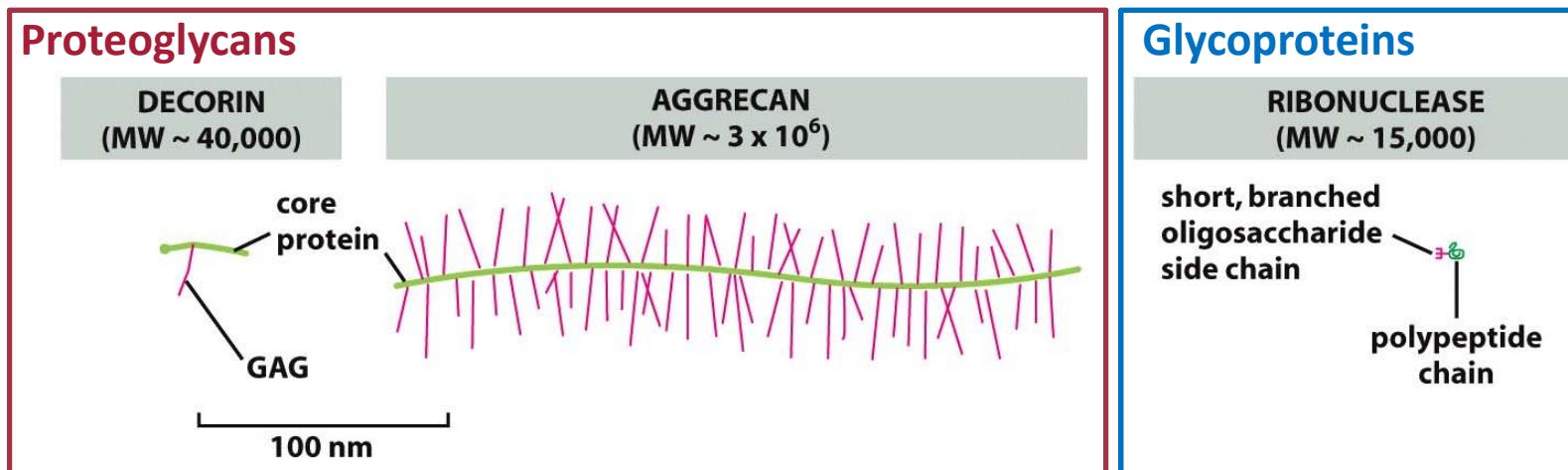
Comparison between proteoglycan and glycoproteins:

Glycoproteins:

- sugar content usually only (1-60%) but usually only few percent in many short & branched sugar chains
- usually low molecular weight proteins (only few hundred kDa)

Proteoglycans:

- **sugar content up to 95%**
- contain at least one GAG - a long (up to 25,000), unbranched sugar chains
- usually very high in molecular weight, up to 3000 kDa.



Degradation of extracellular matrix

Two different classes of proteases:

1. **Matrix metalloprotease (MMP), Ca^{2+} or Zn^{2+} dependent)**
2. **Serine protease**

Three ways to activate & regulate these proteases:

1. **Local activation:**

The protease is transported as an **inactive precursor**.

It's activation occurs upon transport at it's destination.

Example:

The **protease plasmin**, which helps to break down blood clots in the capillaries, is **secreted as an inactive precursor, called plasminogen**.

Activation occurs by the plasmin-activating proteases in the blood vessel

Degradation of extracellular matrix

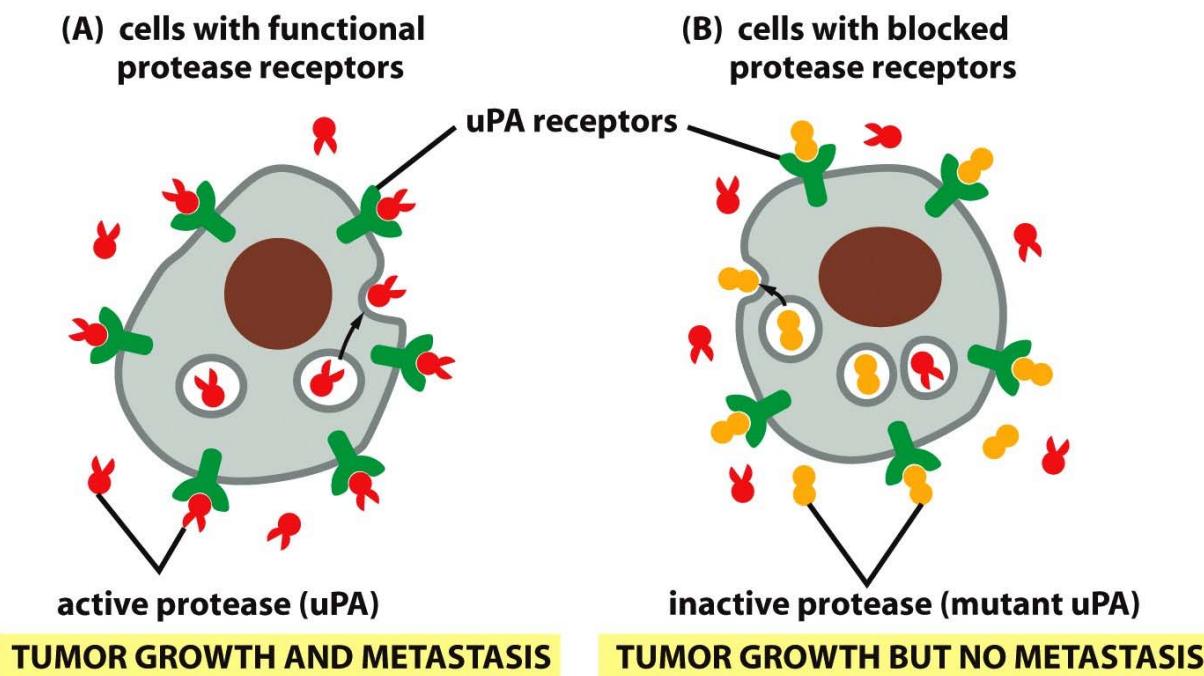
Three ways to activate & regulate these proteases

2. Confined to membrane surface:

Proteases are bound at the cell surface and are only active in the vicinity of the cell, to clear a pathway during cell migration

(Membrane bound matrix metalloproteases (MMPs))

Urokinase-type plasminogen activator)



Degradation of extracellular matrix

Three ways to activate & regulate these proteases

3. Secretion of inhibitors

- Tissue inhibitors of metalloproteases (TIMPs)
- Serine protease inhibitors--- serpins

Examples for straight forward learning

Cancer

- Definitions
- Cancer-critical genes : protooncogenes, tumor suppressors
 - function, activation, regulation
- Features of cancer cells:
 - morphology / genome / behavior
- Master regulators and their mechanisms:
 - Src, Ras, Rb, p53, p21
- Treatment
- Metastasis
 - principles, steps,

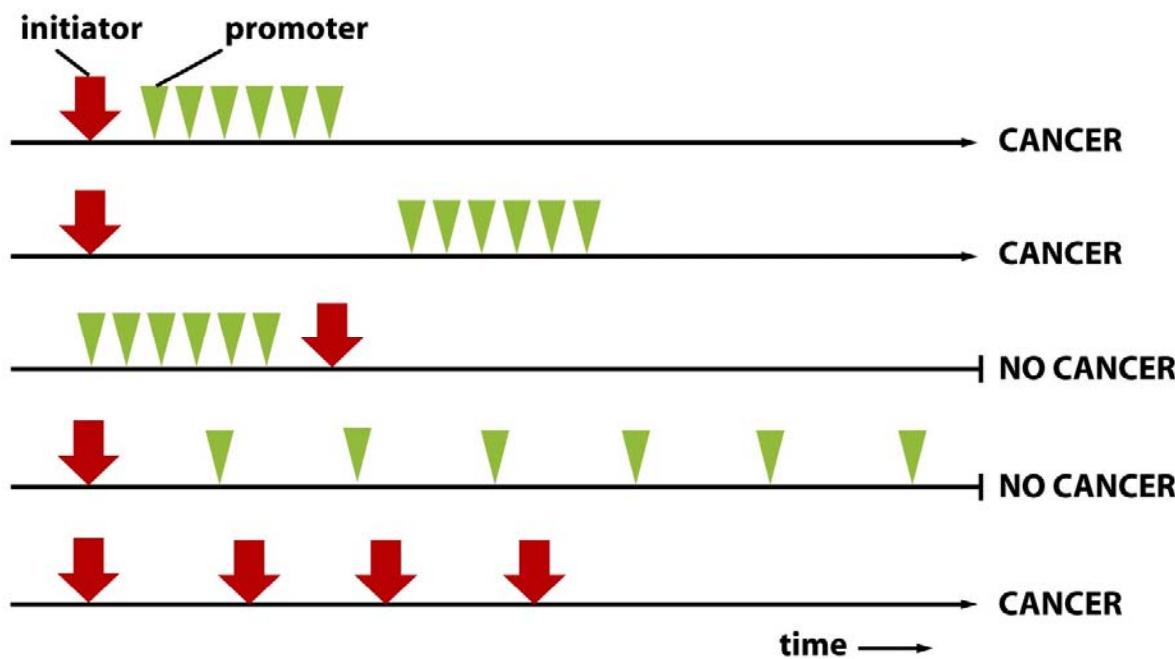
1. Life style - minimize your personal cancer risk



Cancer onset: Tumor initiator (mutagenic) & tumor promoter (non-mutagenic)

Differences between tumor initiator (mutagenic) and tumor promoter (non-mutagenic)

- Tumor initiators causes mutations but do not trigger immediate tumor growth
 - they set the stage for greatly increased incidence of cancer
- Tumor promotores do not cause mutations but stimulate/trigger proliferation (targeting the cell cycle or, in its easiest case even simple wounding reactions)



More specific cancer drug development

Drug targets in cancer treatment

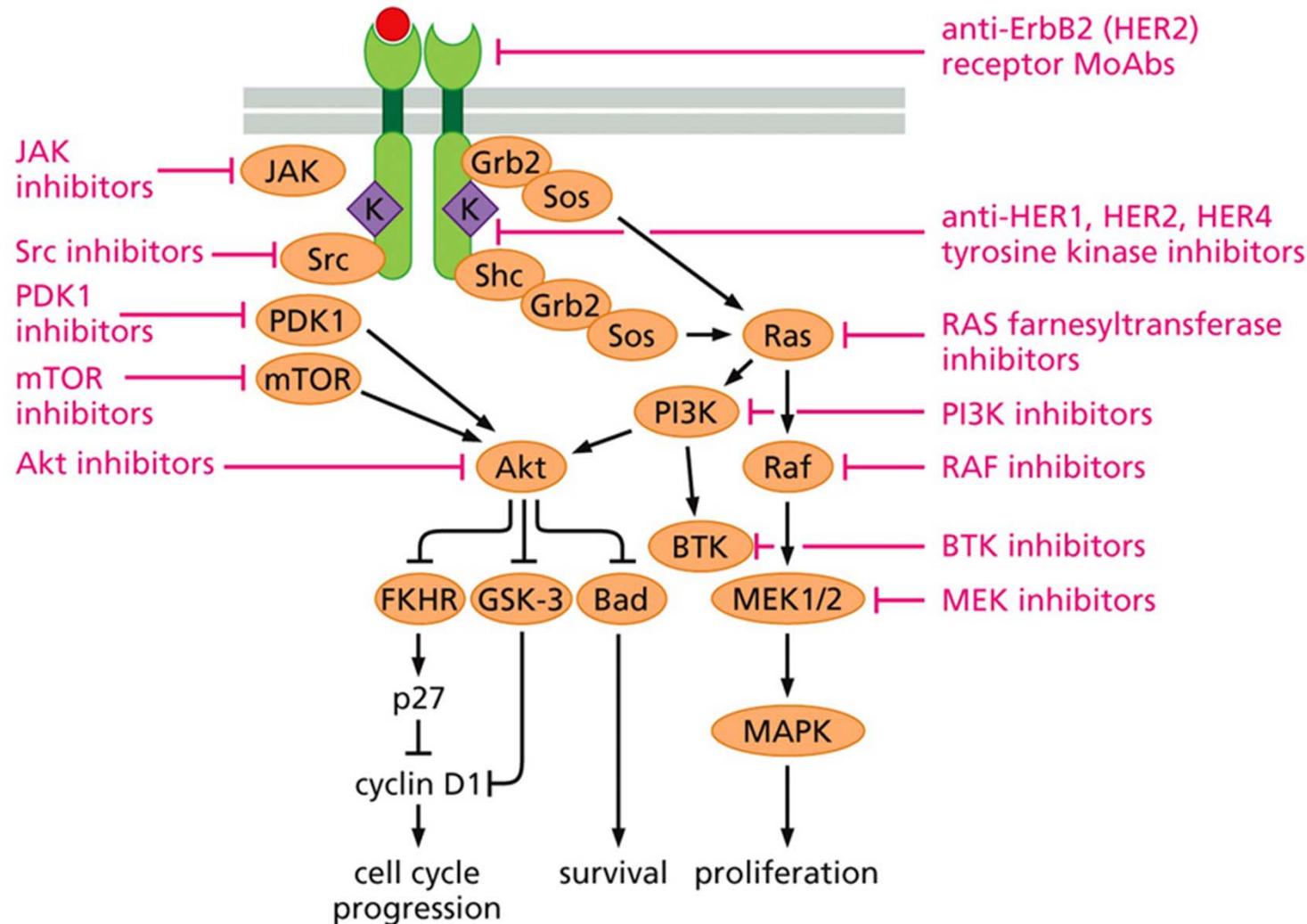
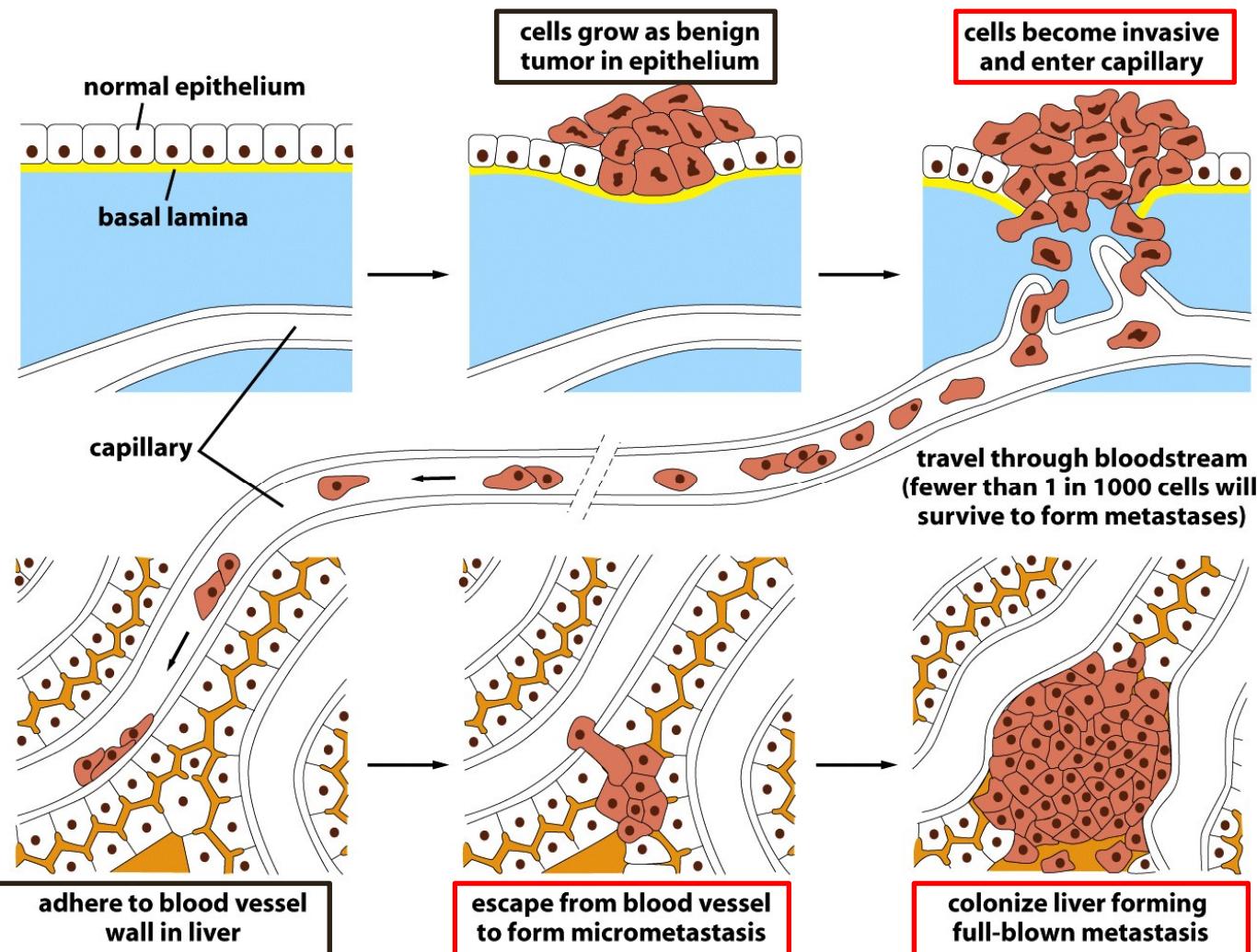


Figure 16.9 The Biology of Cancer (© Garland Science 2014)

Metastasis: The fatal step

Metastasis is a multi-step procedure



Angiogenesis: new formation of blood vessels

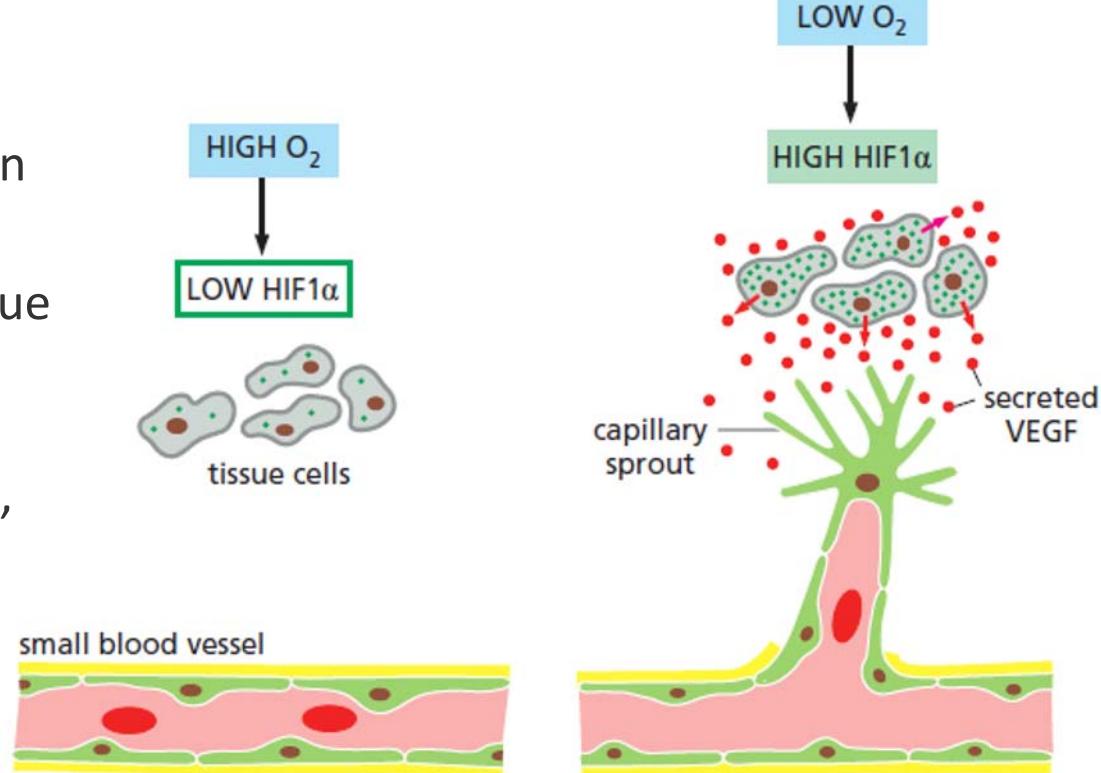
Cells sense the amount of available oxygen and nutrients.
If levels are too low, they call for supply...

The key players:

- VEGF (vascular endothelial growth factor) protein
- HIF1 α (hypoxia-inducible factor 1 α) transcription factor for VEGF

Mechanism:

- low oxygen increases intra-cellular levels of HIF1 α
- HIF1 α stimulates transcription of *Vegf*, raising VEGF levels
- VEGF is secreted into the tissue and reaches endothelial cells
- VEGF induces production of proteases in endothelial cells, allowing them to digest the basal lamina and to sprout towards the tumor



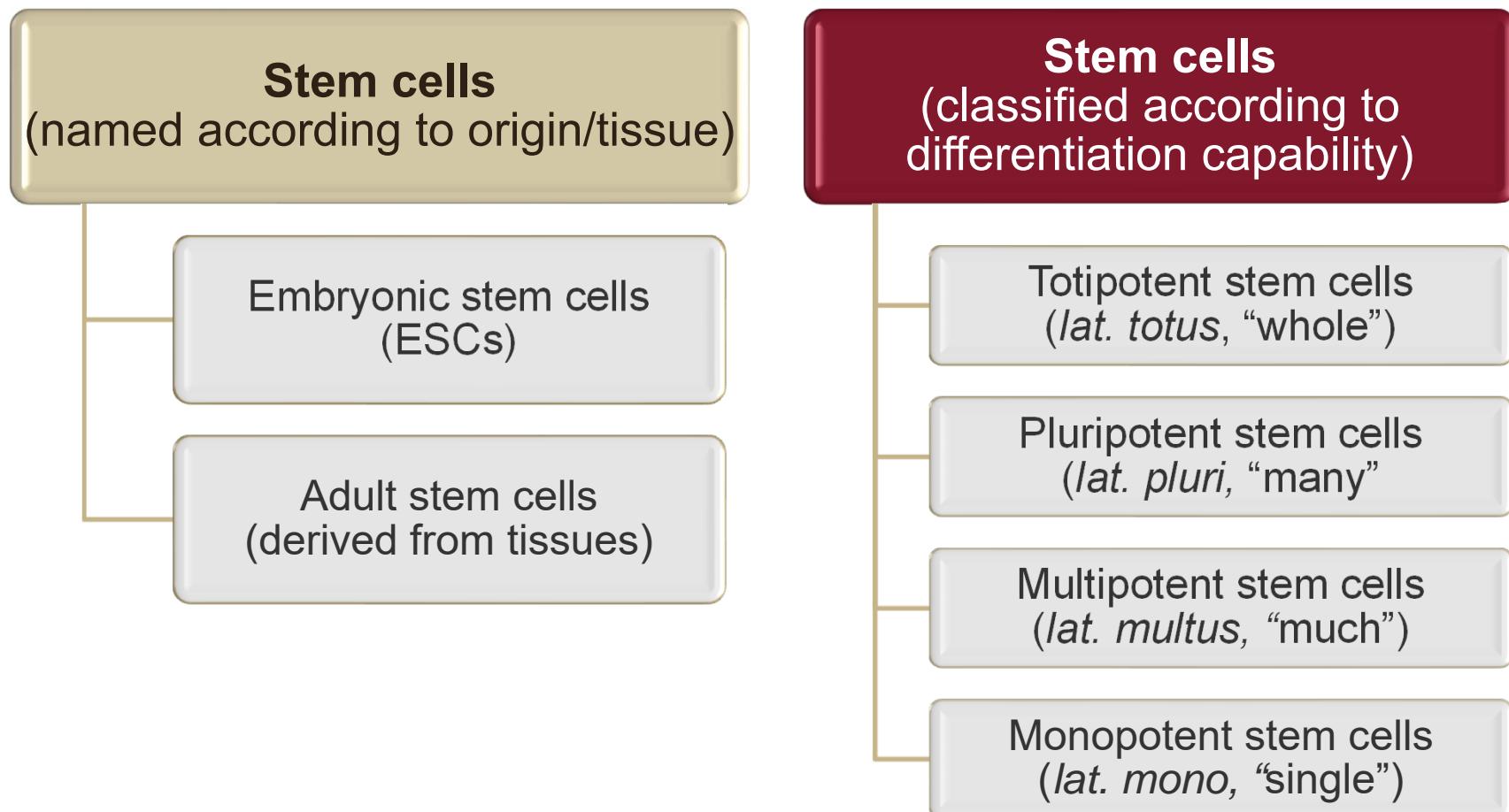
Examples for straight forward learning

Stem cells

- What are stem cells
 - definition
 - occurrence/location
 - totipotent, pluripotent, multipotent, monopotent (representatives)
- early embryogenesis
 - stages

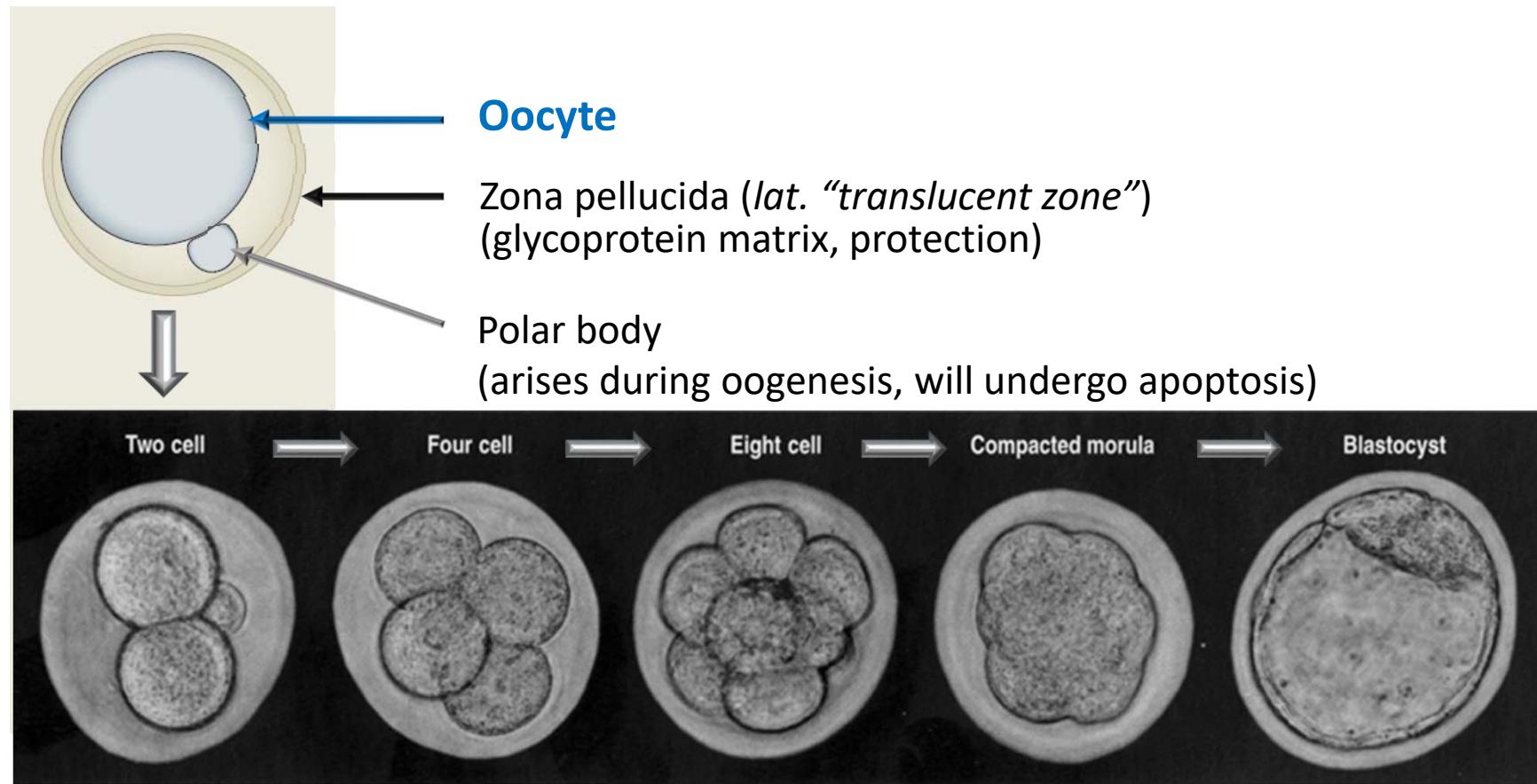
I. Stem cells: Different types of stem cells

Stem cells can be named after their location/fate or according to their differentiation capability



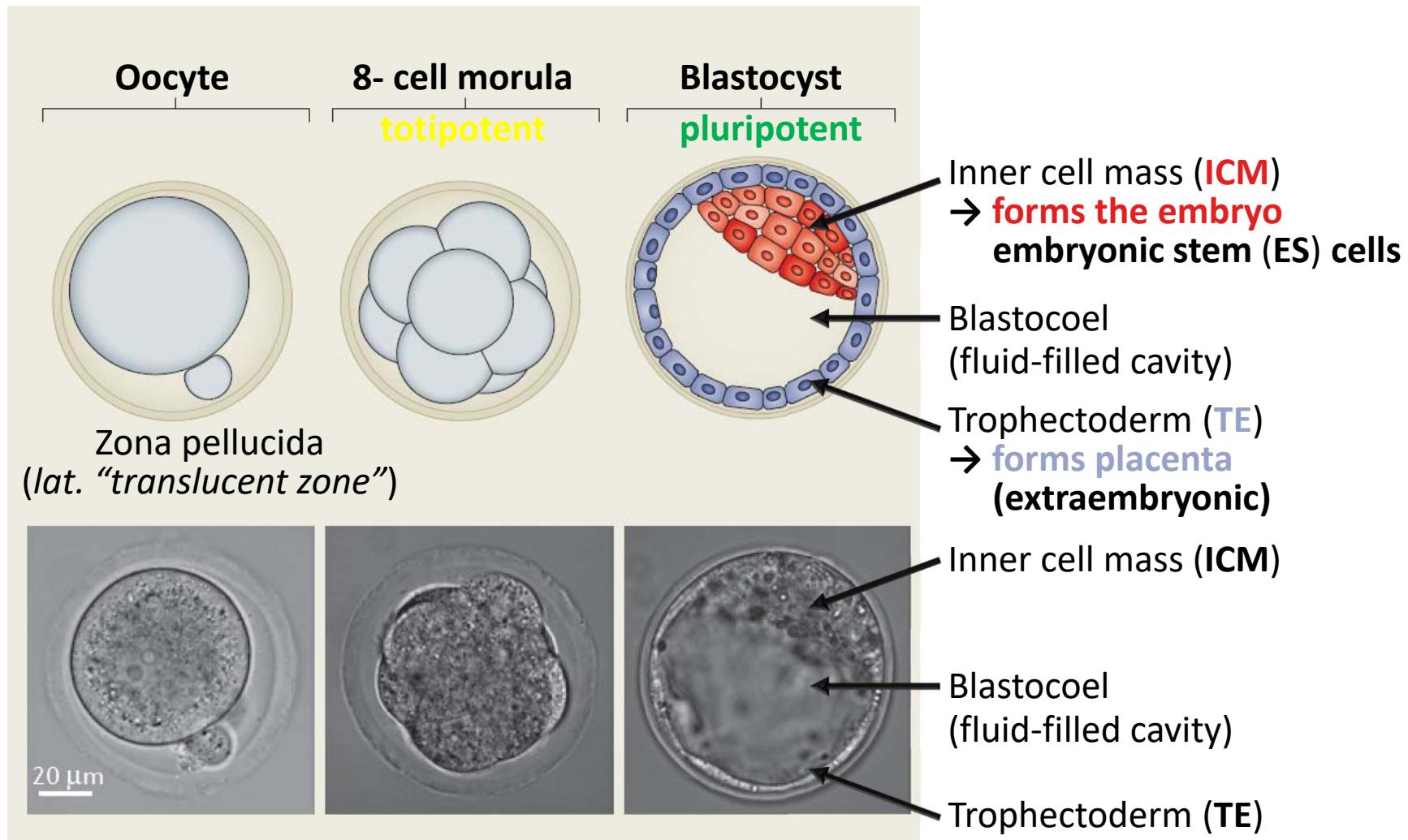
Embryogenesis: it starts with the fertilized oocyte...

The first 5 days: from the 2-cell stage to the 64 cell stage, the blastocyst



The blastocyst

The first 5 days: from the 2-cell stage to the 64 cell stage, the blastocyst

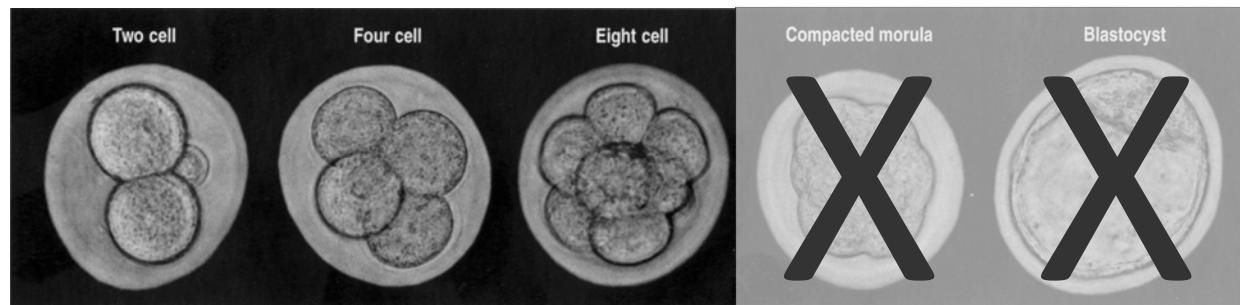


Totipotent cells

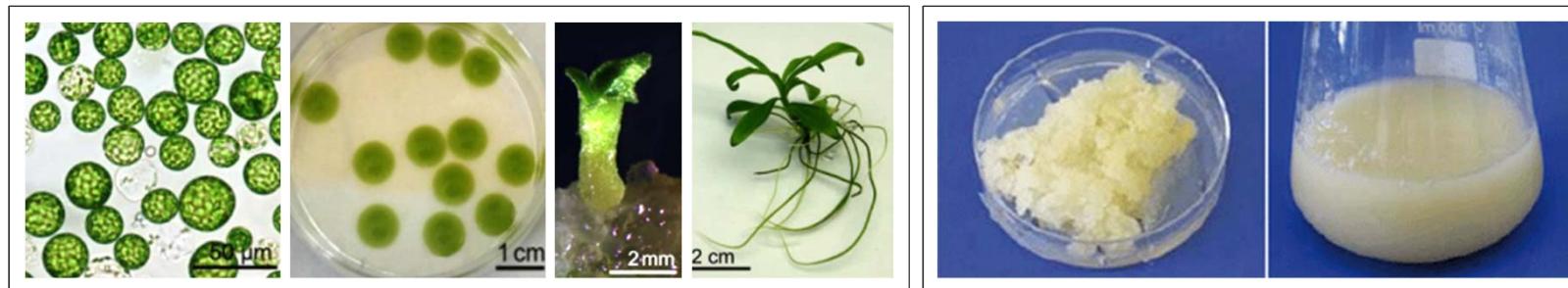
A totipotent cell can differentiate into a whole organism:

Examples:

- fertilized egg
- each cell during the development from the zygote **up to the 8-cell stage**



- **Many cells in a plant are totipotent** and a whole plant can be regenerated from a single cell of e.g. a leaf; suspension cultured cells have eternal growth



Other stem cells: pluri-, multi- & mono-potent stem cells

Pluripotent cells can differentiate into a wide variety of cells, but can not differentiate to form the whole organism

Examples:

- embryonic stem cells
- bone marrow stromal stem cells
- nerve stem cells
- embryonic germ

Multi- and **monopotent** stem cells can differentiate into a few types of cells or only in a special type of cells, respectively

Examples:

- neuroglial cells
- nerve stem cells
- satellite cells
- epithelial stem cells
- hematopoietic stem cells

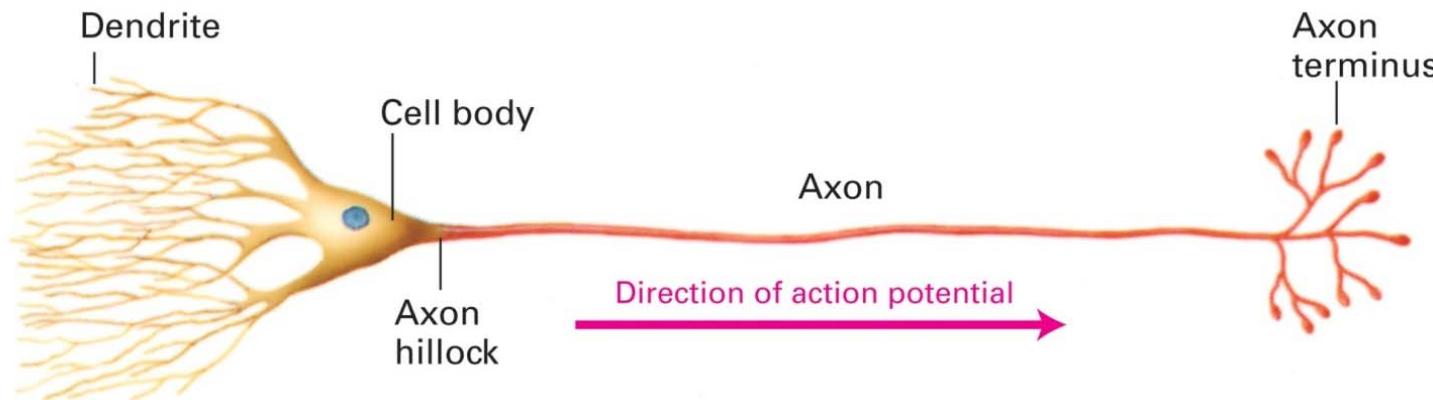
Examples for straight forward learning

Neurons

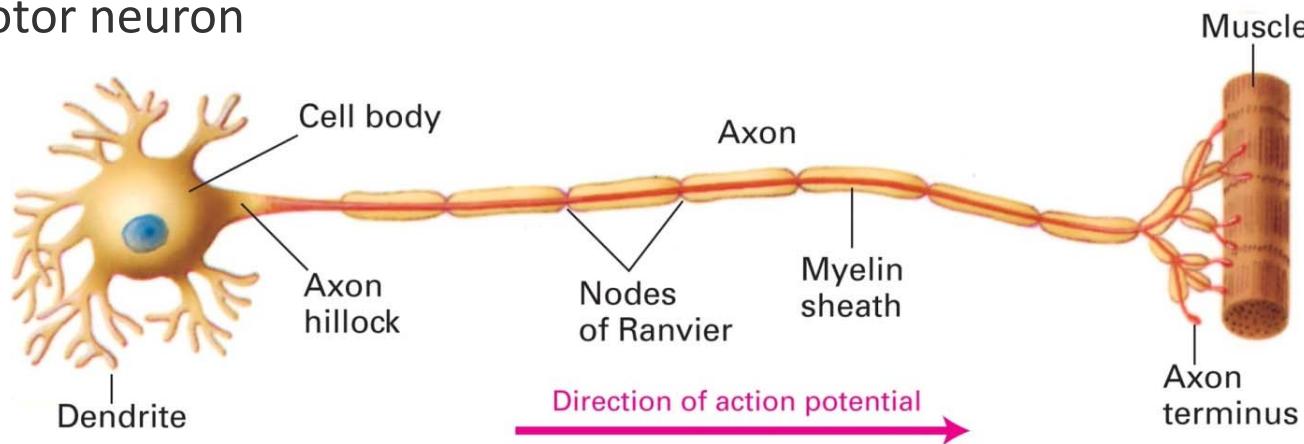
- Morphology
- Function / signaling /rest potential & action potential
- Synapses:
 - signaling mechanism (start and stop)
 - components
 - mechanism
- Cellular context
 - interacting cells
- All time favourite:
 - muscle contraction - the full scheme starting with the nerve!

Two types of neurons: multipolar interneuron and motor neuron

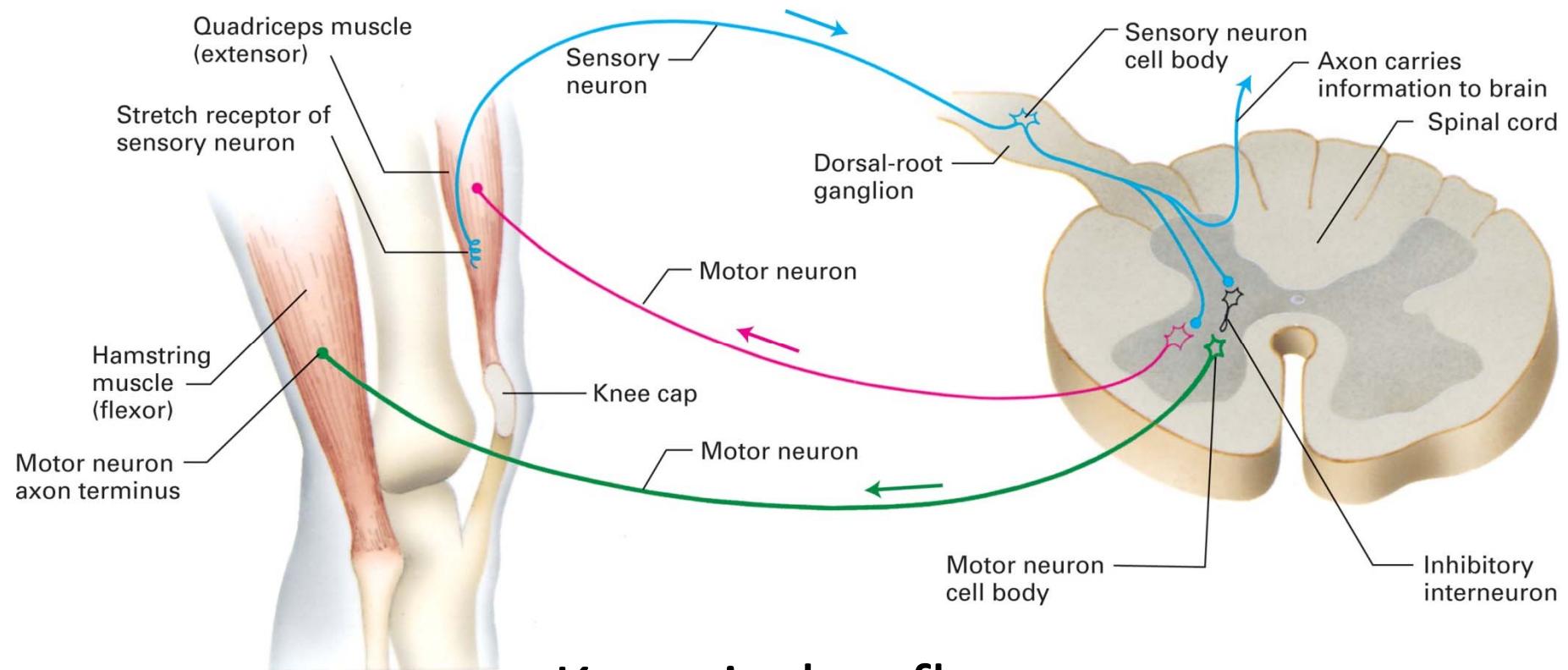
Multipolar interneuron



Motor neuron



Multiple neurons in signaling circuits



Knee-jerk reflex

Sensory neurons

Effector neurons

Interneurons: the largest group

Glia cells

Glia cells form myelin sheaths and support neurons

Glia cells can be divided into three categories:

1. **Oligodendrocytes:**

- make sheaths for the **central nervous system**

2. **Schwann cells:**

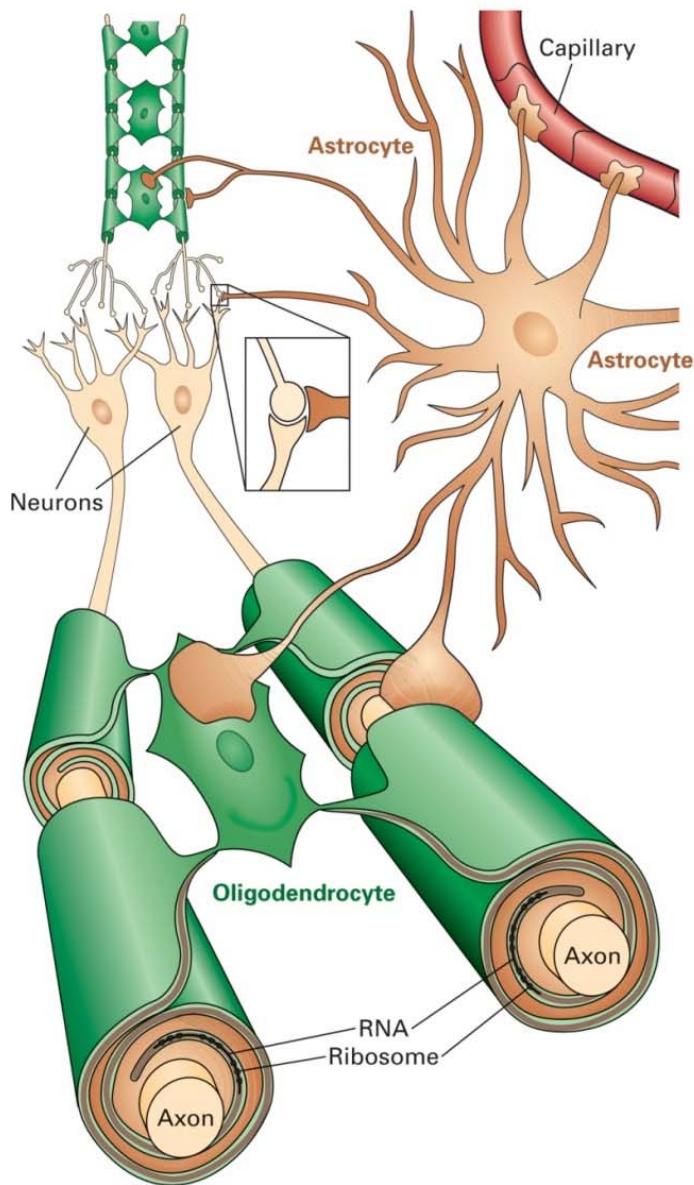
- make sheaths for the **peripheral nervous system**

3. **Astrocytes:**

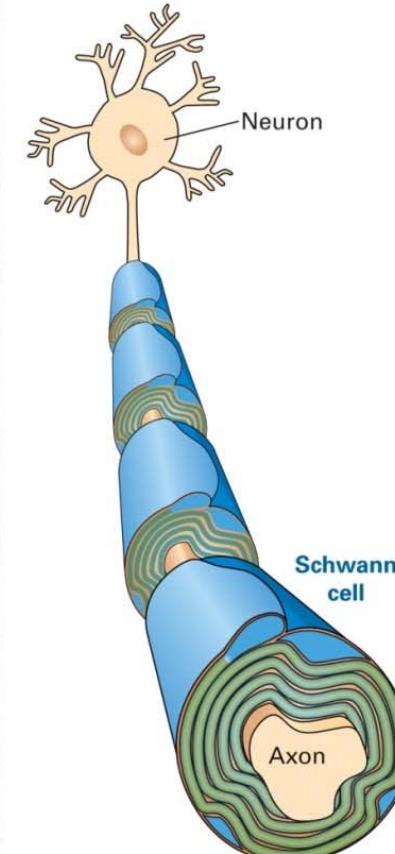
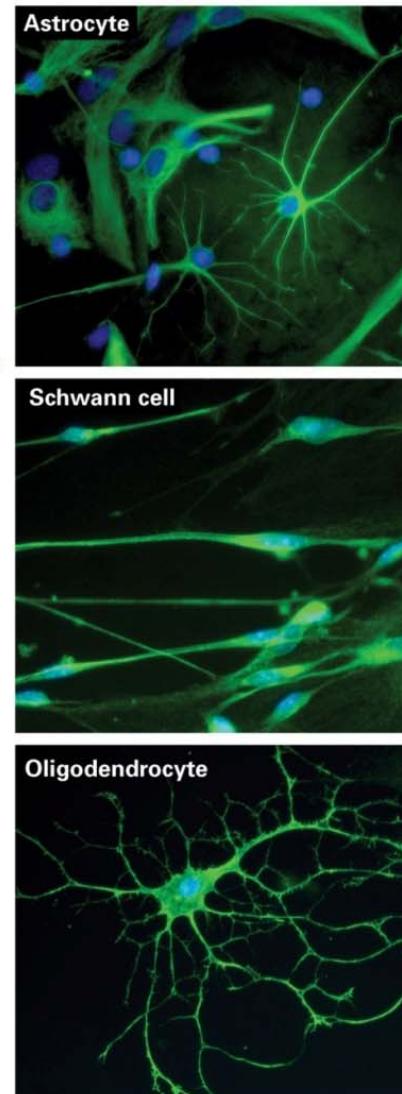
- star-like shape,
- provide growth factors and other signals to neurons
- receive signals from neurons and induce synapse formation between neurons.

The three types of glia cells

(a) Central nervous system glia

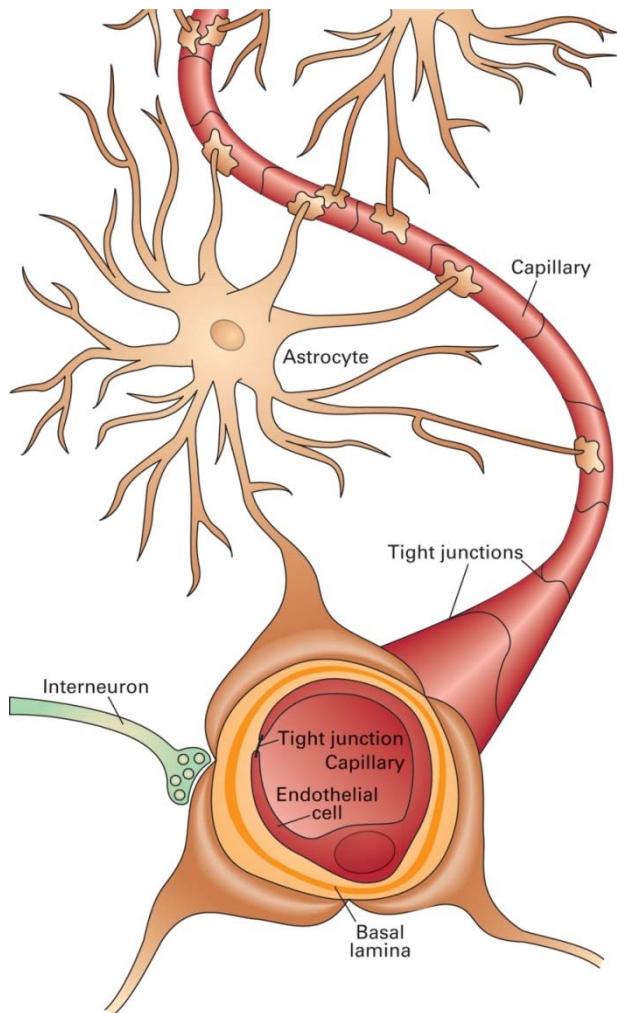


(b) Peripheral nervous system glia



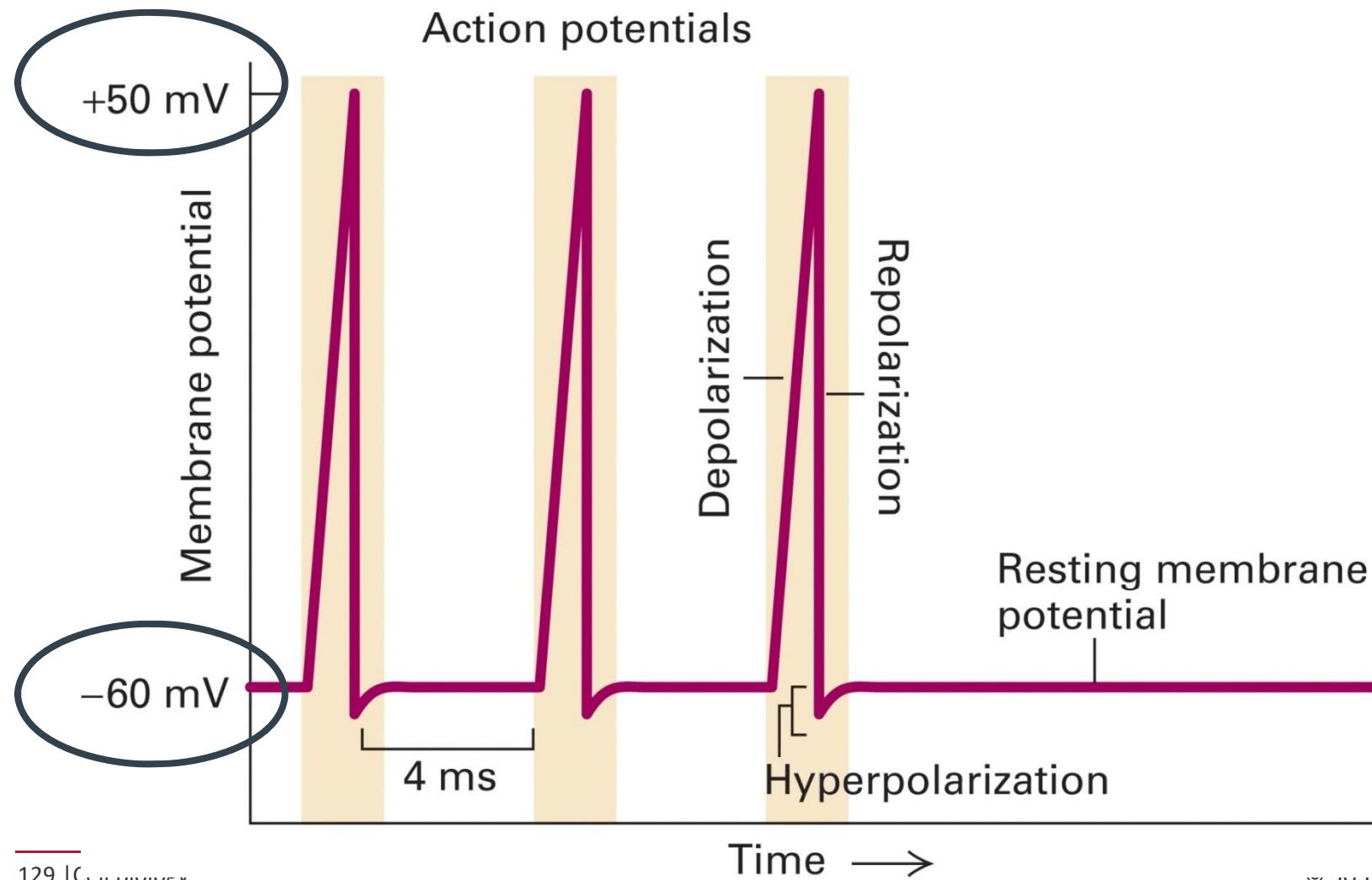
Astrocytes

Astrocytes interact with endothelial cells at the blood-brain barrier

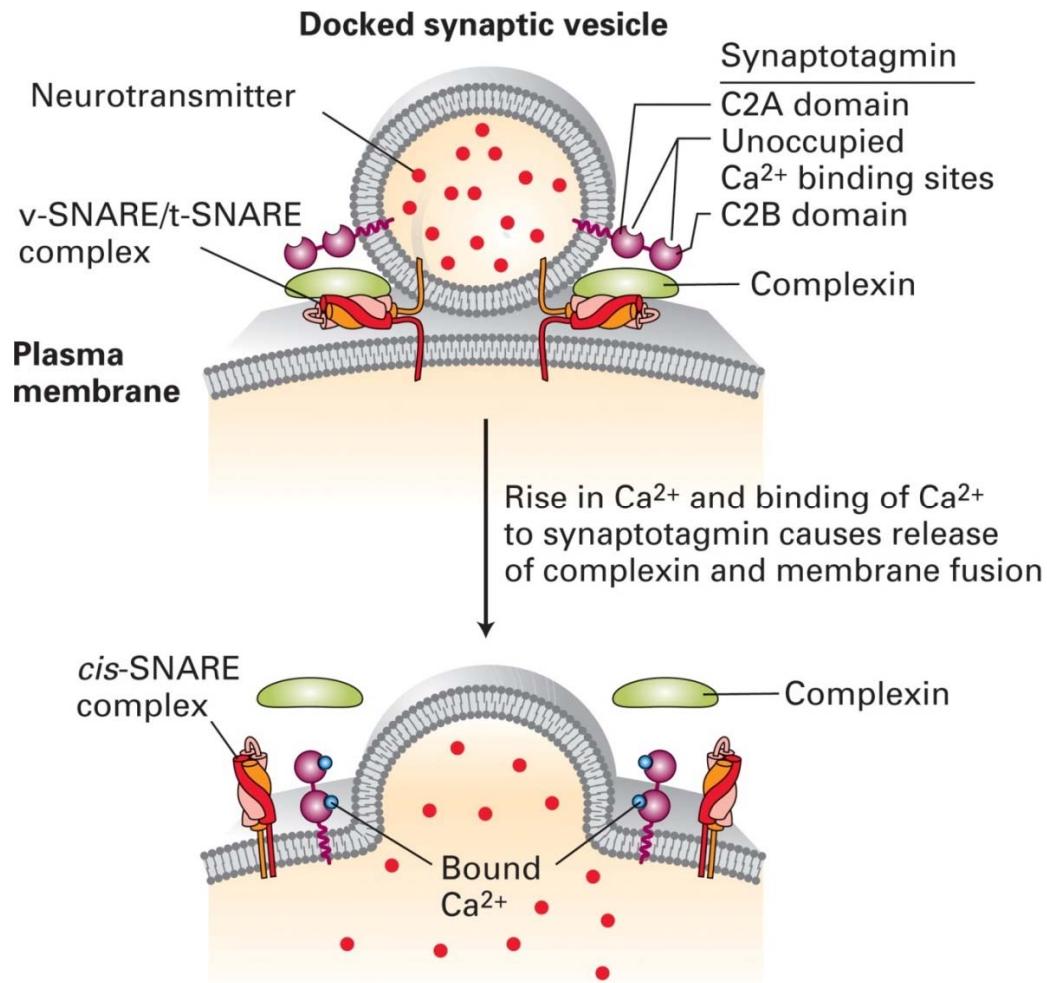


- Approximately 1/3 of the brain mass and about 1/2 of the total brain cells are astrocytes.
- Produce ECM proteins.
- Joined together by gap junctions

Nerve resting potential and action potential



Fusion of synaptic vesicles with the plasma membrane



Signaling at synapses is terminated by degradation or reuptake of neurotransmitters

Neuromuscular transmission

