Living organisms properties

Functions

1. Self conservation

- **Nutrition**
- **Respiration**
- **Synthesis**

} **Metabolism**

2. Self regulation

- **Homeostasis**

3. Self reproduction

- **Renewal**
- **Development**
- **Adaptation**

Fundamental mechanisms are constant

Structures

Extraordinary variety

Related to the variety of environments

Ever changing

Living organisms adapt to time changes in the environment

Extreme complexity

At different levels:

…

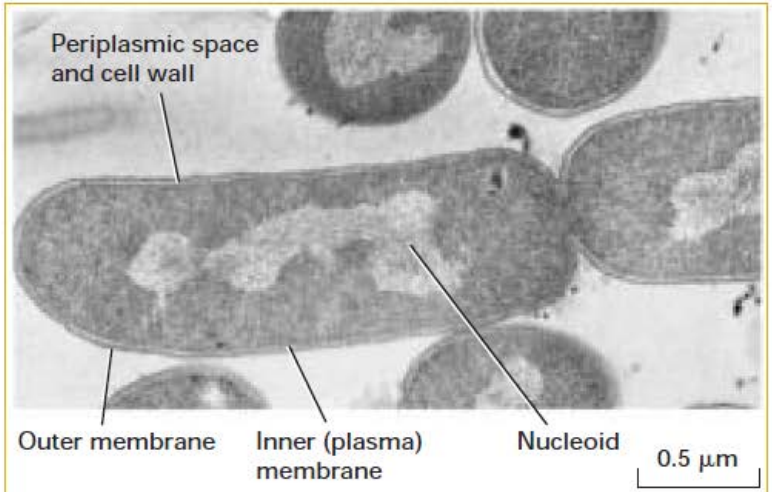
Molecules
Macromolecules
Organelles
Cells
Tissues
Organs

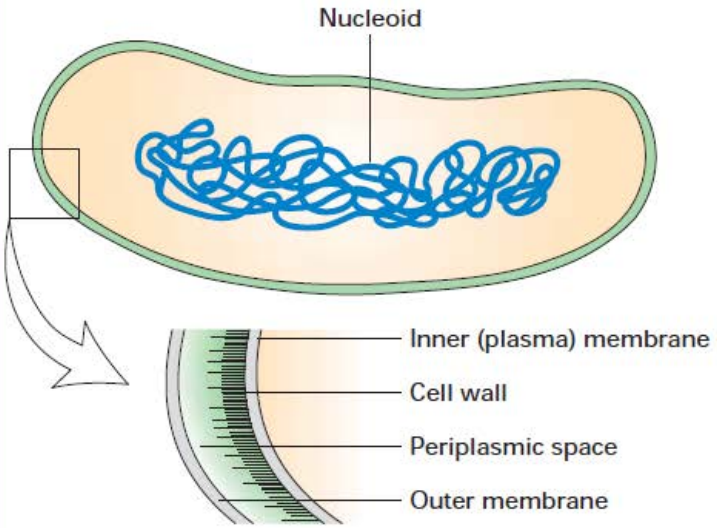
…

Enormous individual variety

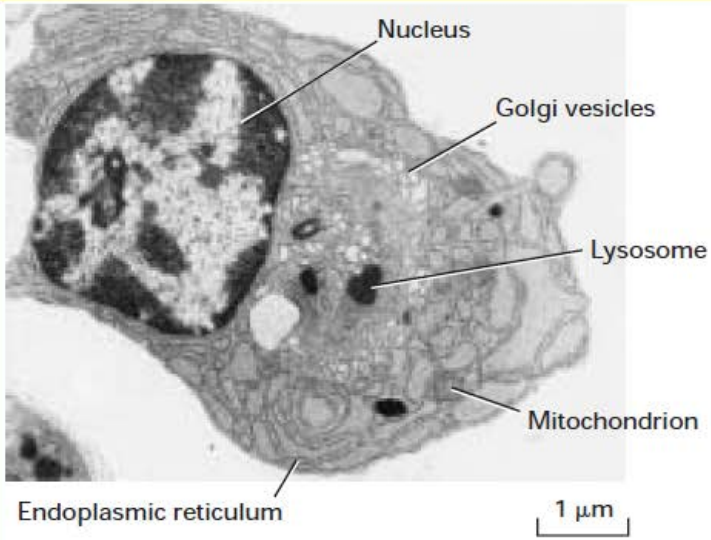
Prokaryotes vs eukaryotes

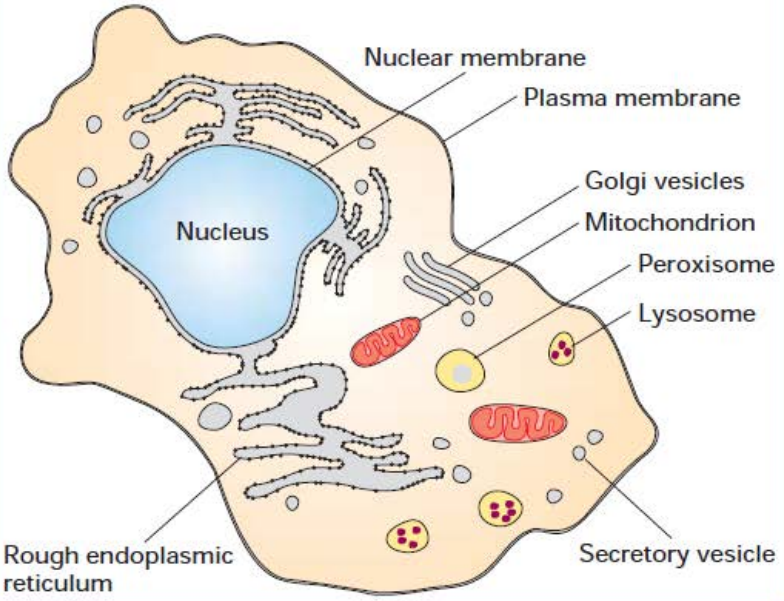
(a) Prokaryotic cell





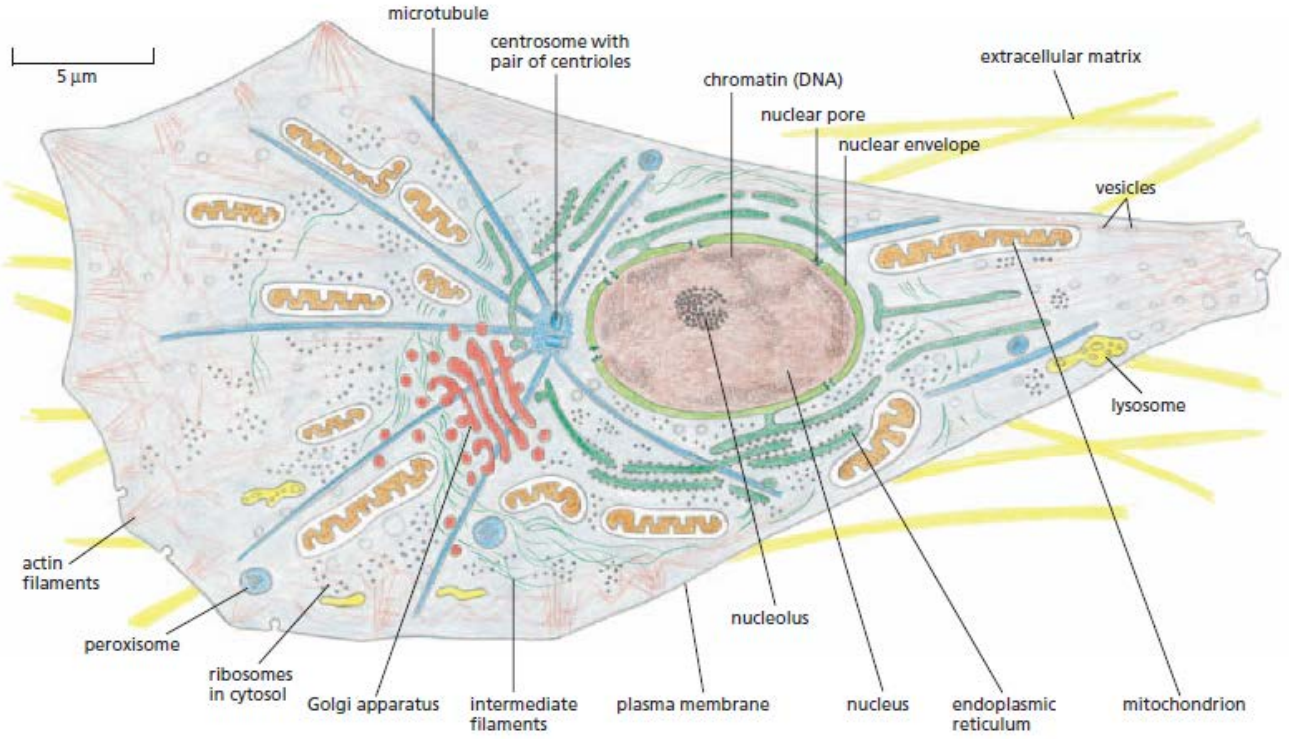
(b) Eukaryotic cell





Advantages of cell compartimentalization

1) The cell is able to retain specific molecules and carry out certain reactions in an orderly manner
2) Organelles membranes allow exchange of materials selectively
3) Specific functions are performed in specific areas of the cytoplasm
4) Incompatible processes can go on simultaneously inside the same cell (but different organelles)



Cell biology and medicine

All diseases are disturbances at the cellular level (Rudolph Virchow, 1858)

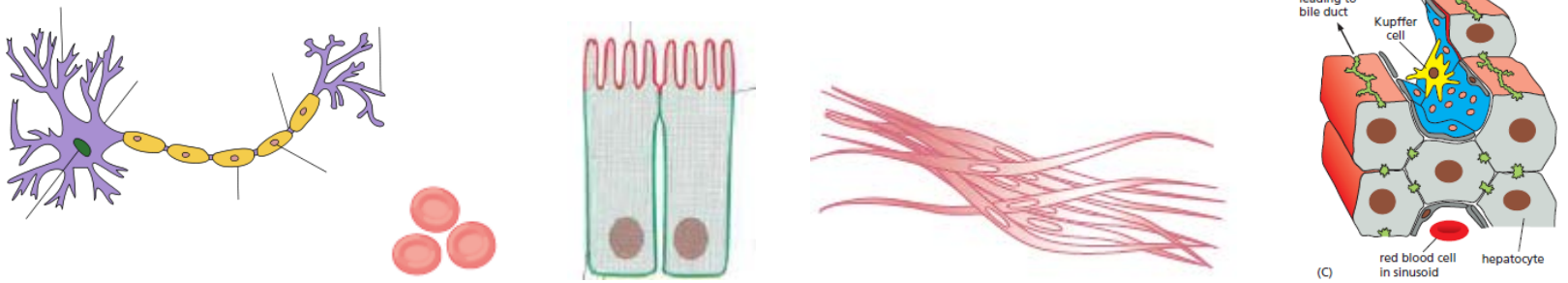
To treat disease, we must understand its cause. To understand the cause of a disease, we must understand the alterations that occur at the level of individual cells.

Update (XXI century)

… and molecules

Rudolph Carl Virchow (13 October 1821 – 5 September 1902) was a German doctor, anthropologist, pathologist, prehistorian, biologist and politician

Cell biology and medicine



Different cells types → Different functions

COMMON GROUND

The molecular biology of the cell

(which is mostly universal for all cells)

Savill J. **Science, medicine, and the future: Role of molecular cell biology in understanding disease.** *BMJ 1997;314:203*

THE CELL MEMBRANE

1. GENERAL CHARACTERISTICS
2. MORPHOLOGICAL OBSERVATION
3. CHEMICAL COMPOSITION
4. MOLECULAR ORGANIZATION
5. BIOGENESIS

GENERAL CHARACTERISTICS

The plasma membrane:

- Is a continuous sheet that spreads over the entire cell surface.
- Has a structure that is similar to that of inner cell membranes. Therefore it is called *unit membrane*, formed by lipids and proteins joined mostly by noncovalent interactions
- Performs very diverse functions, depending on the cell type. These can be summarized as : 1. Delimits the cell volume
  2.Substance exchange
  3. Information exchange.
- Presents a spatial, temporal and functional continuity.
- The spatial and functional organization is heterogeneous.

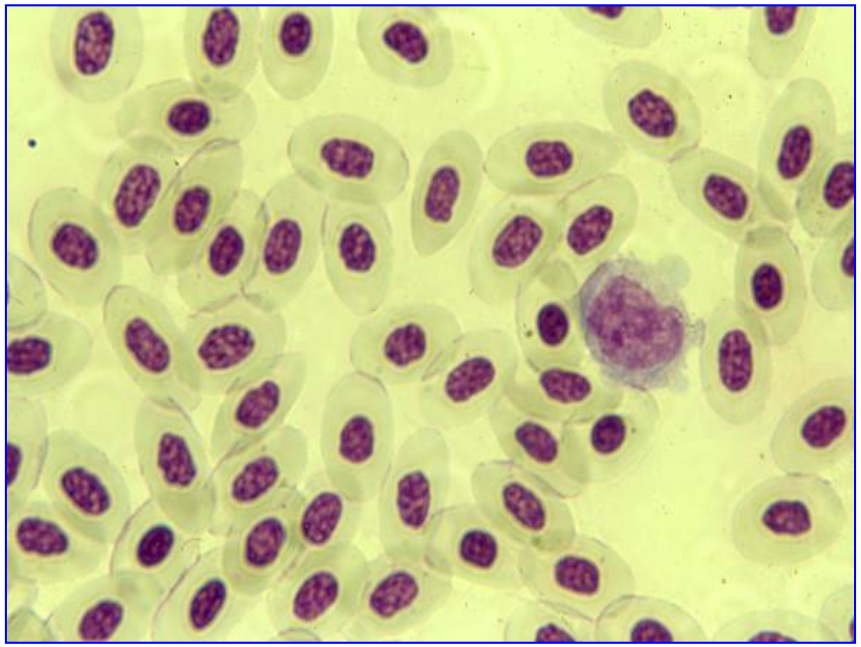
MORPHOLOGY

Optical microscopy

The cell membrane can not be seen with the optical microscope

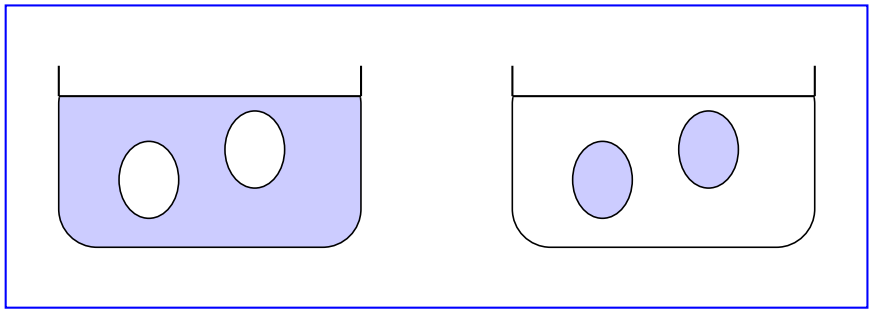
However:

1. An intuitive opinion existed that the cell should have a limiting membranous structure.



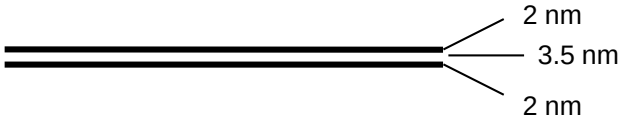
2. Some easy experiments backed this concept.

If a dye was added in the external medium the cell did not stain. If the dye is injected into the cell it does not exit.

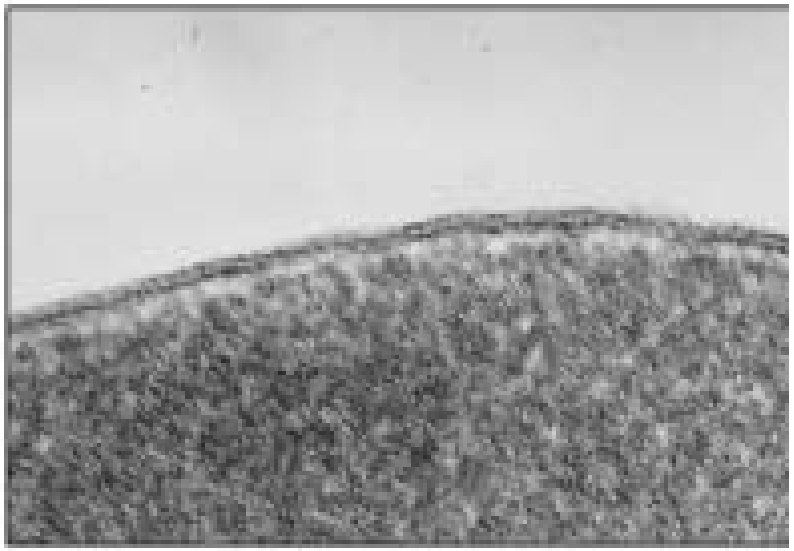


MORPHOLOGY

Electron Microscopy

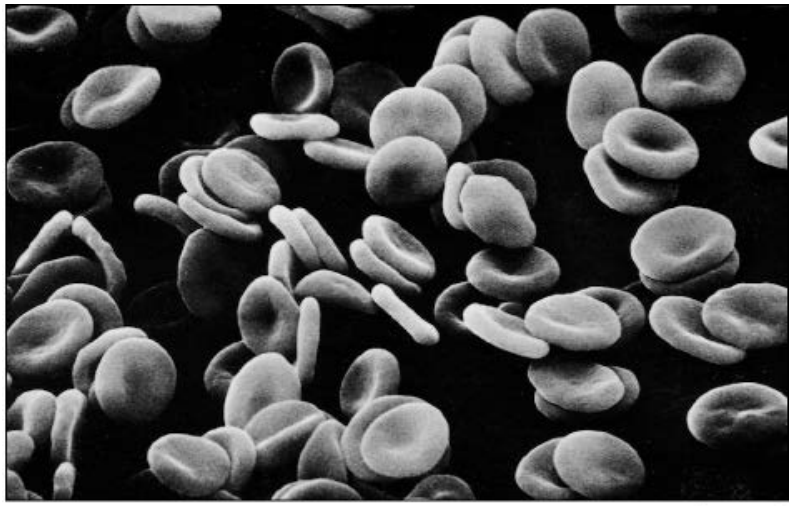


T.E.M.



Trilaminar structure:
Two dark bands (osmiophilic) separated by a clear band (osmiophobic)

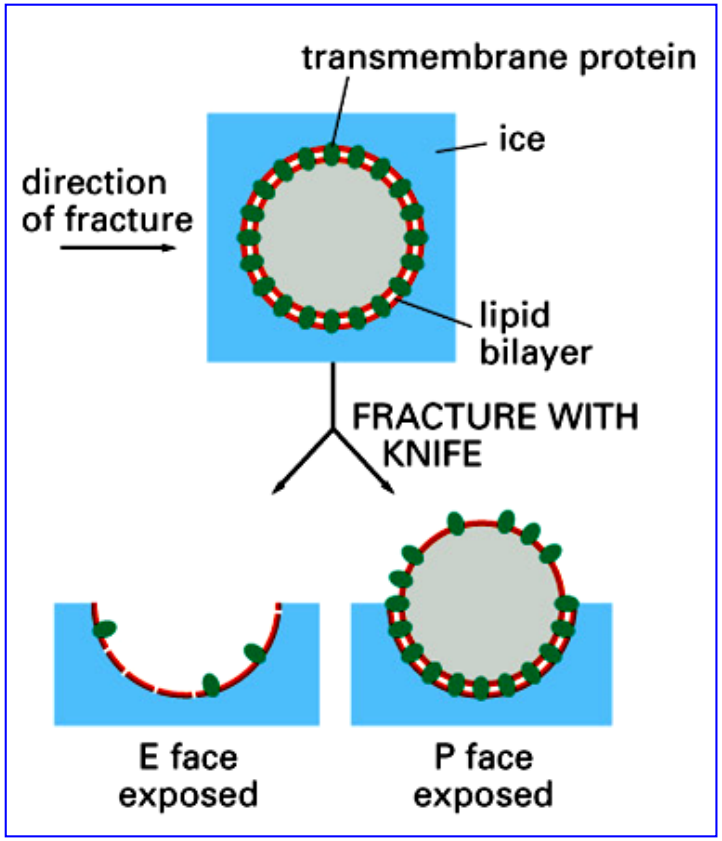
S.E.M.

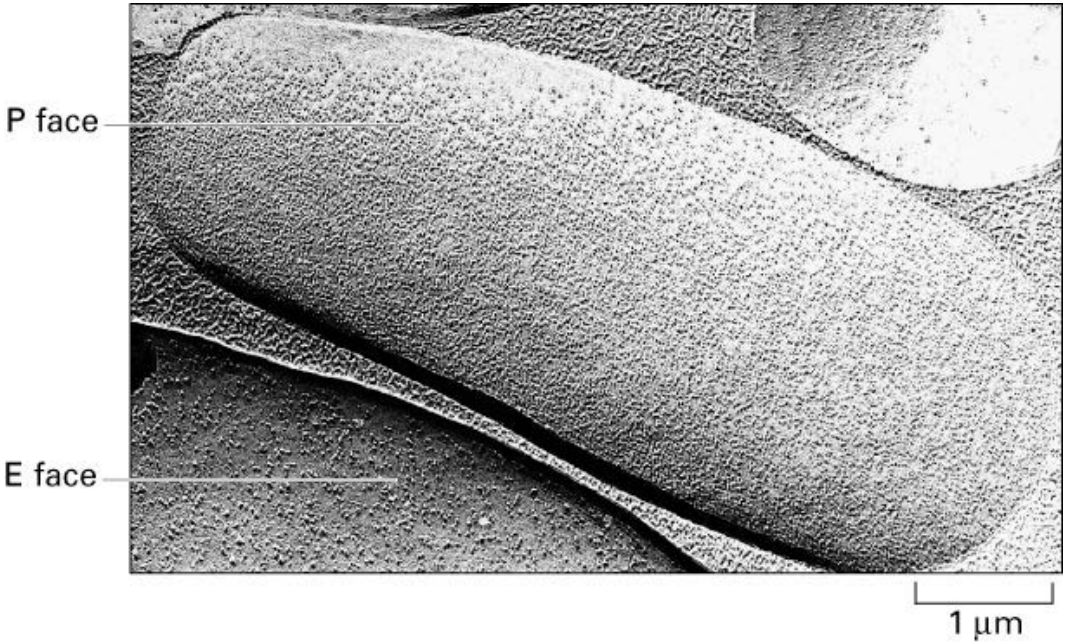


MORPHOLOGY

Electron Microscopy

Freeze-fracture T.E.M.

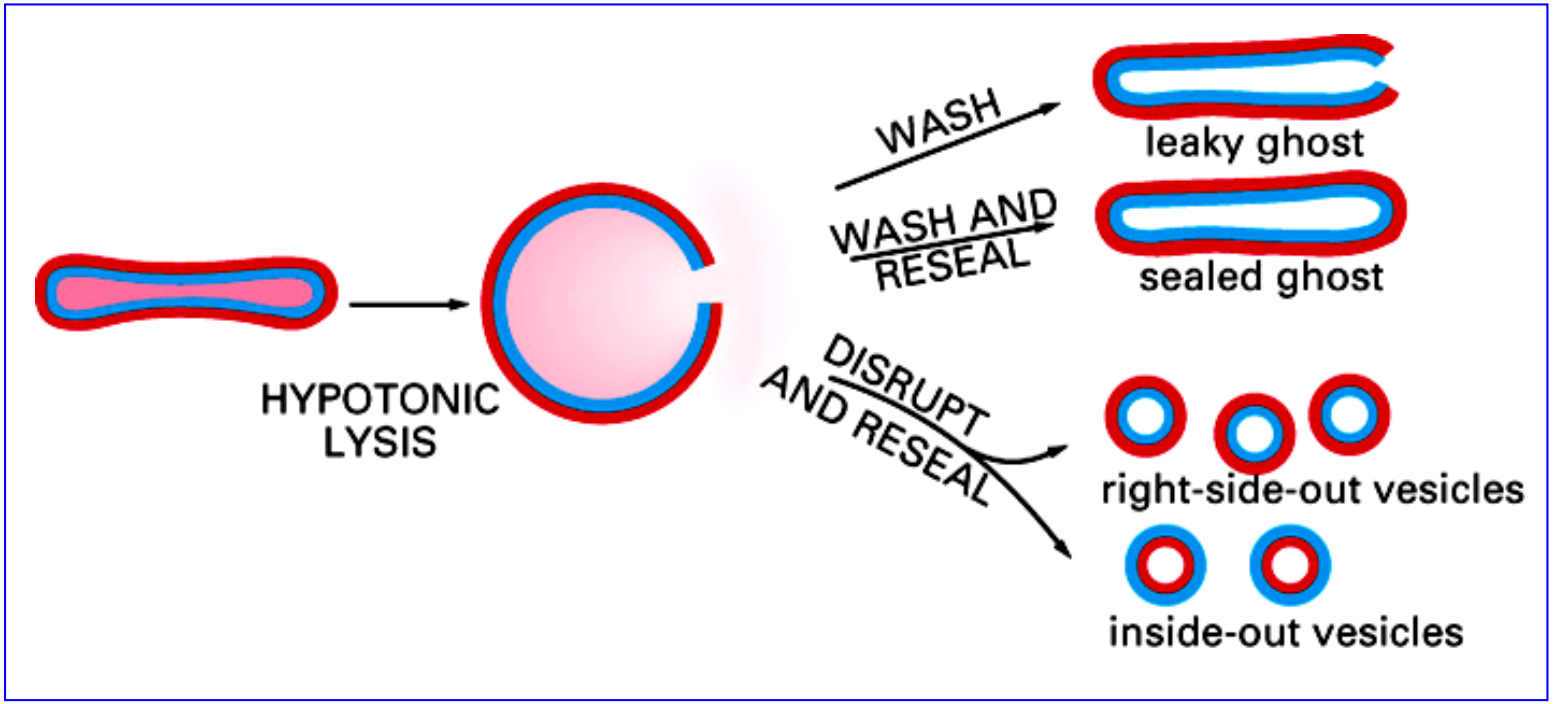




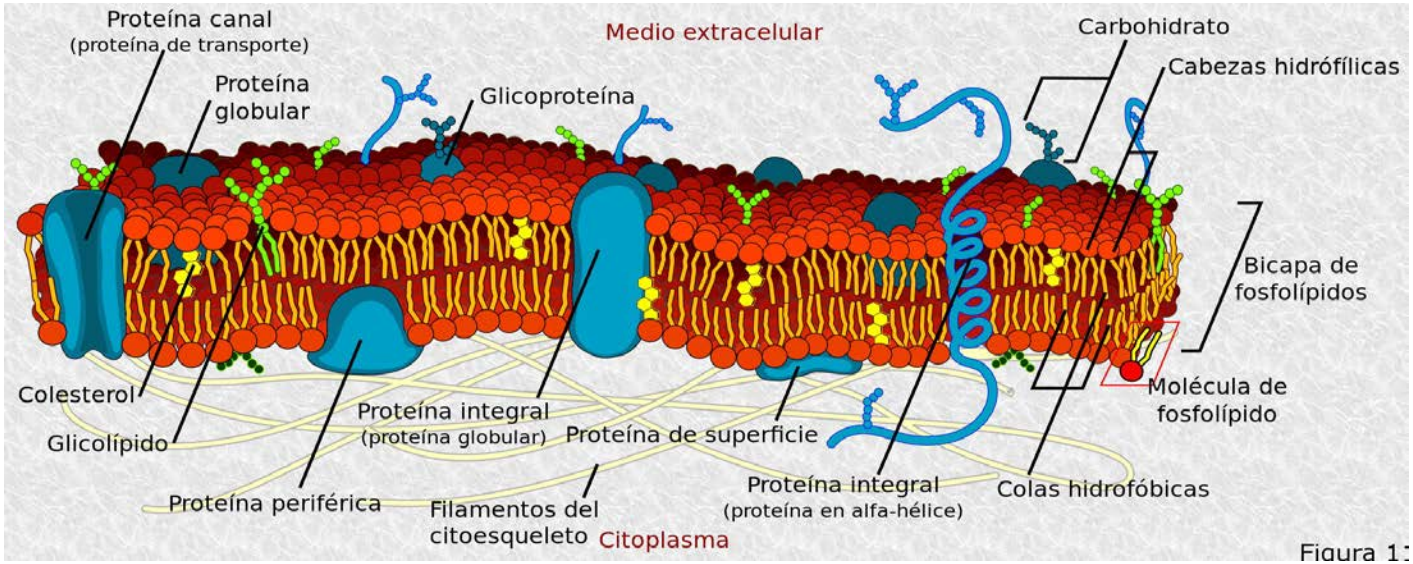
Two hemimembranes (exoplasmic and protoplasmic). Globular particles in the inner space (5-8 nm).

CHEMICAL COMPOSITION

Red Blood Cell Membrane



CHEMICAL COMPOSITION



LIPIDS:

- 50% of membrane mass
- Amphipathics (head-tail)
- Types:
    1. Phospholipids
    2. Cholesterol
    3. Glucolipids (gangliosides)

PROTEINS:

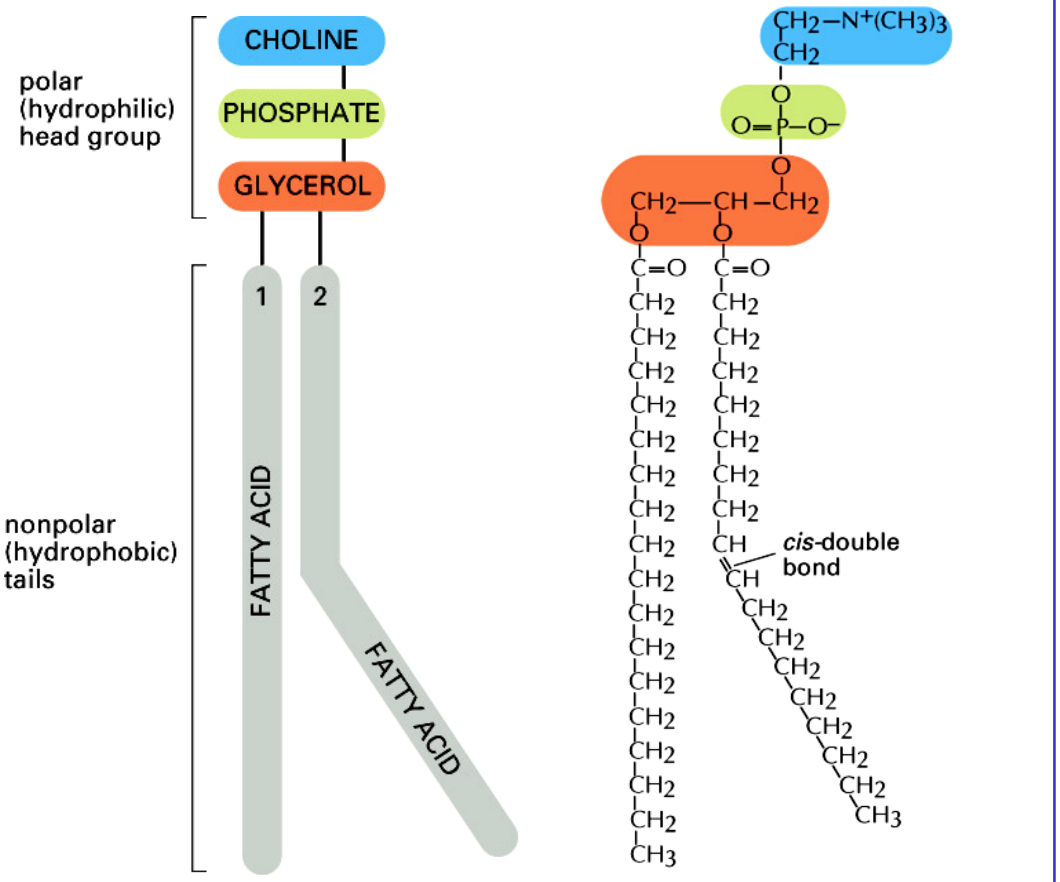
- 50% of membrane mass and very diverse

GLUCIDS:

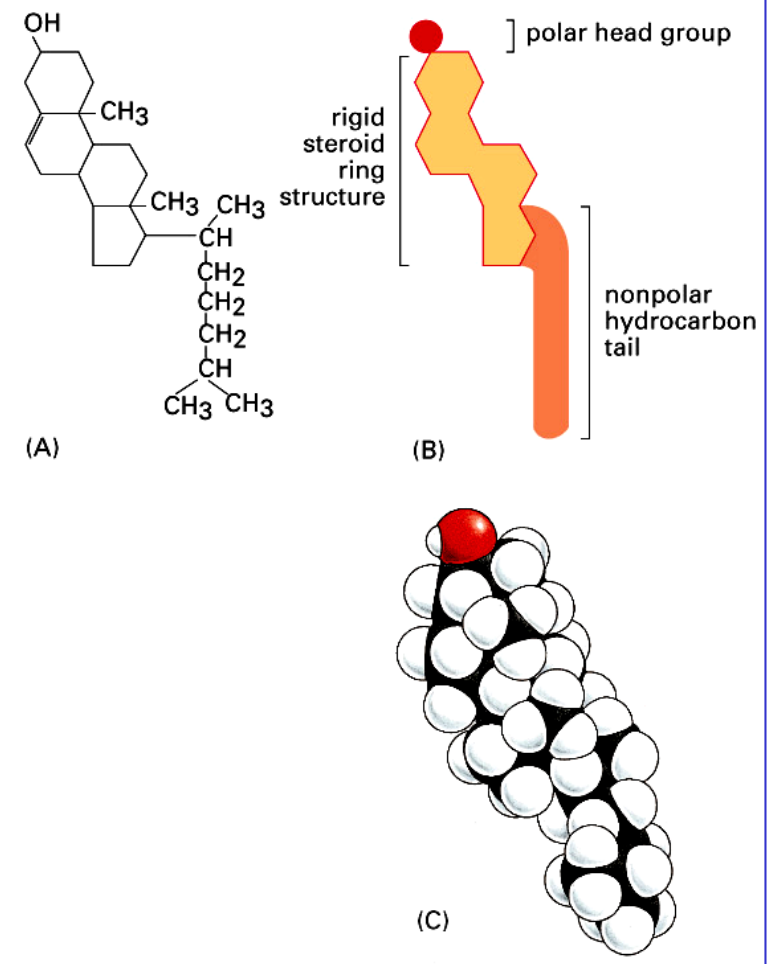
- Always in the outer side linked to lipids or proteins

CHEMICAL COMPOSITION

LIPIDS



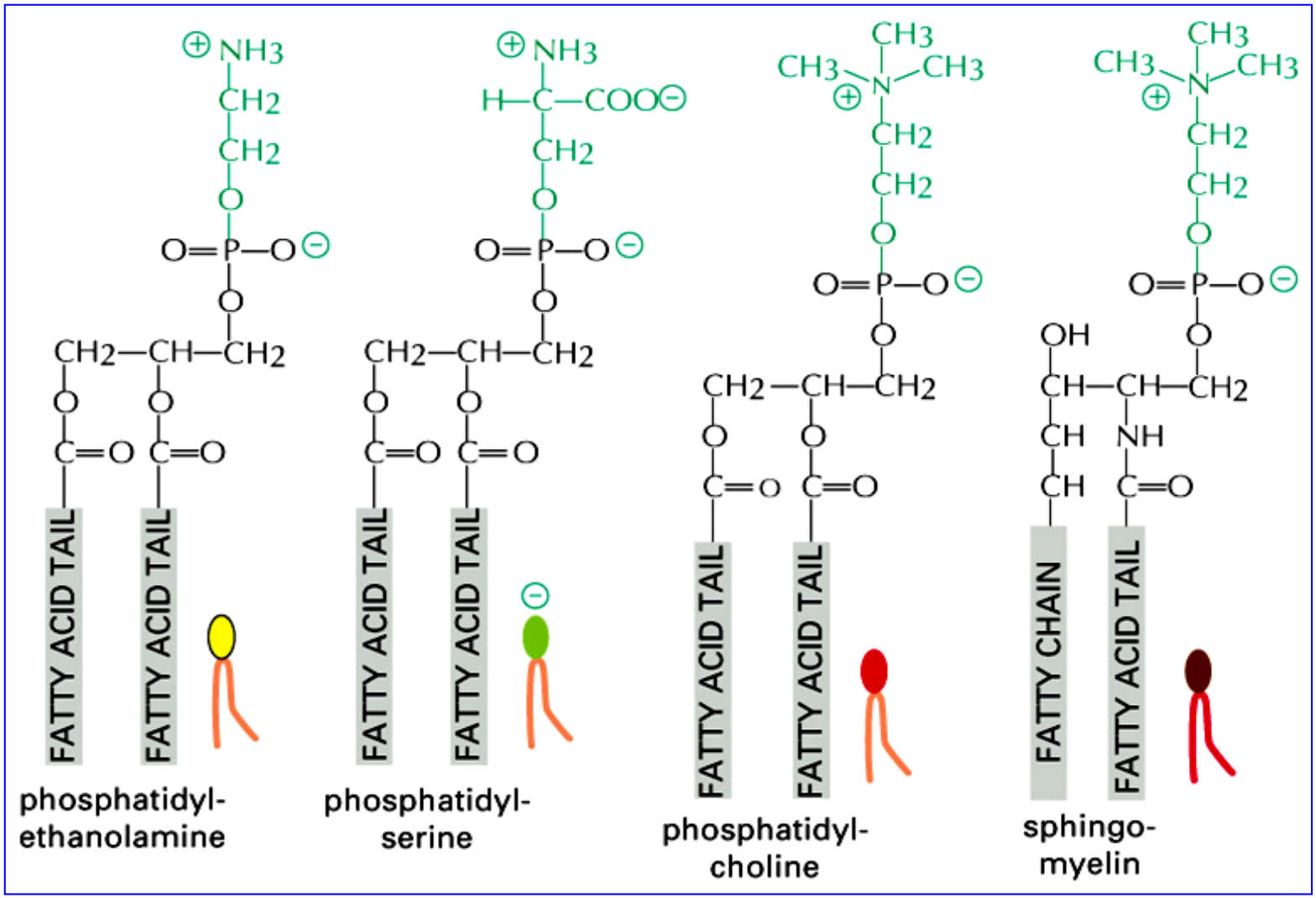
Phospholipids



Cholesterol

CHEMICAL COMPOSITION

PHOSPHOLIPIDS

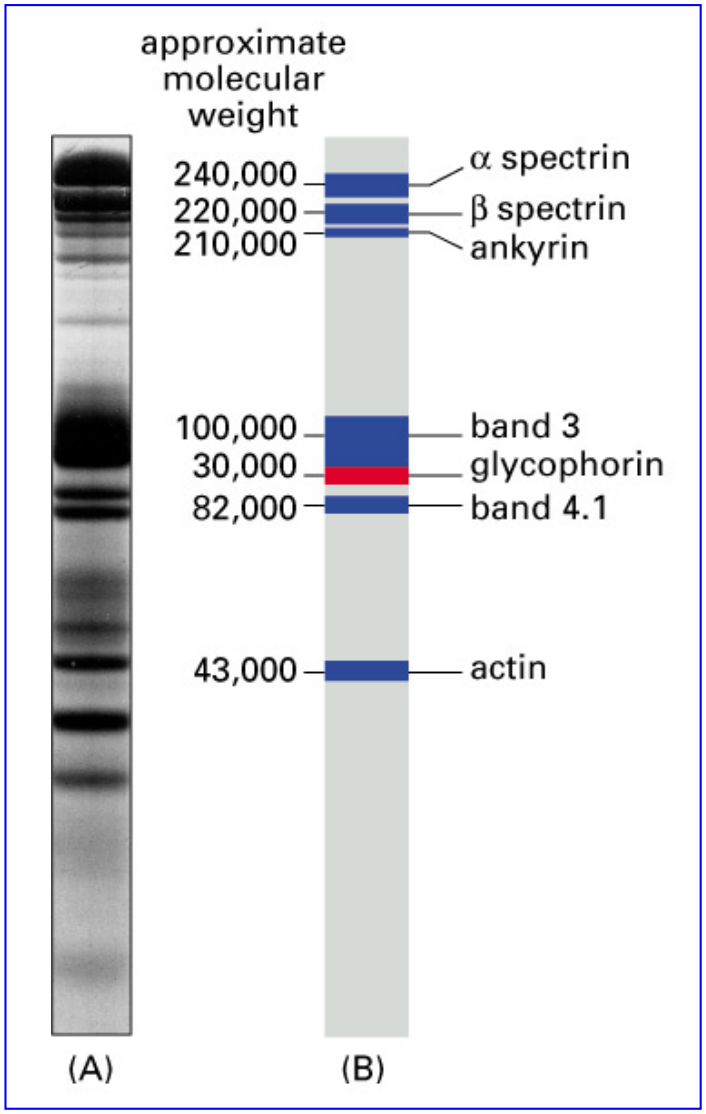


CHEMICAL COMPOSITION

PROTEINS

Integral: strongly joined to the membrane

Peripheral: can be separated easily



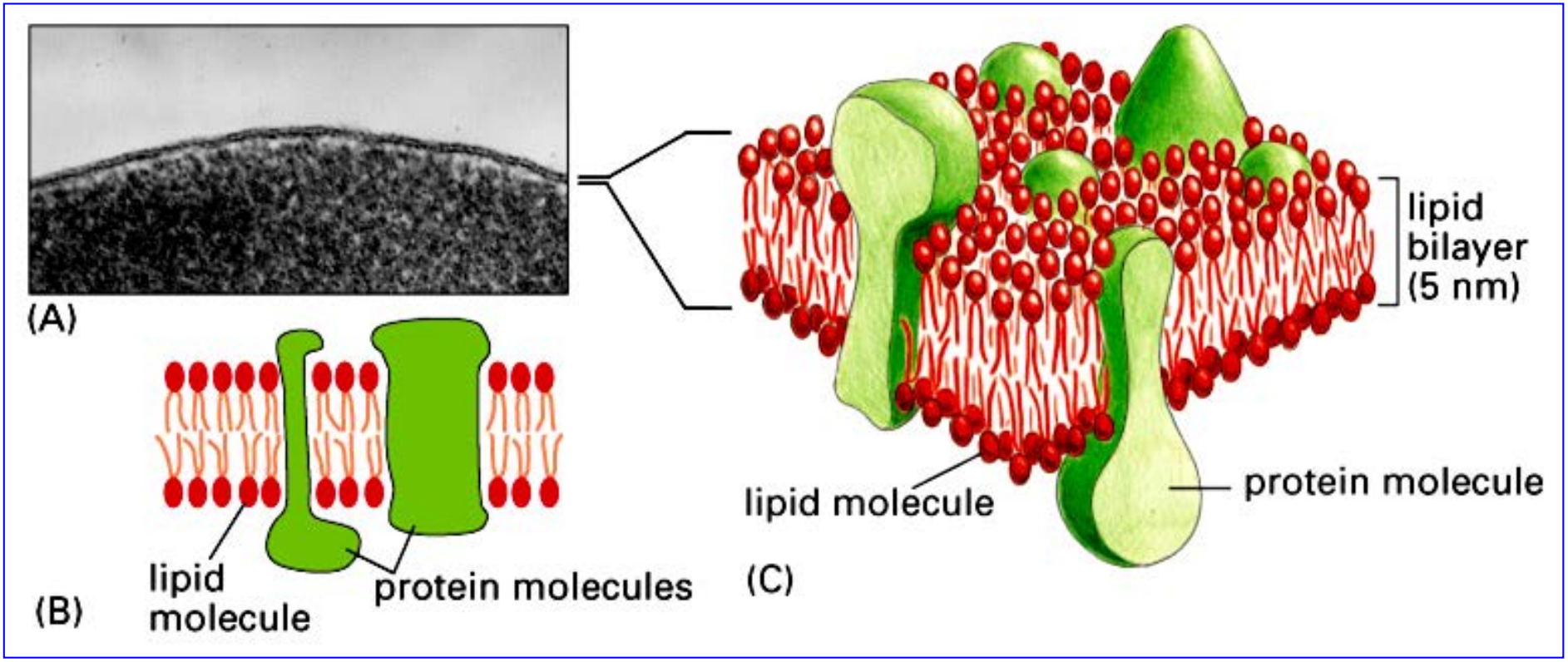
Electrophoretic analysis of human erythrocyte proteins.

MOLECULAR ORGANIZATION

- Lipids
  - Asymmetry
  - Movements
  - Factors that affect fluidity
- Proteins
  - Types
  - Movements
- Sugars

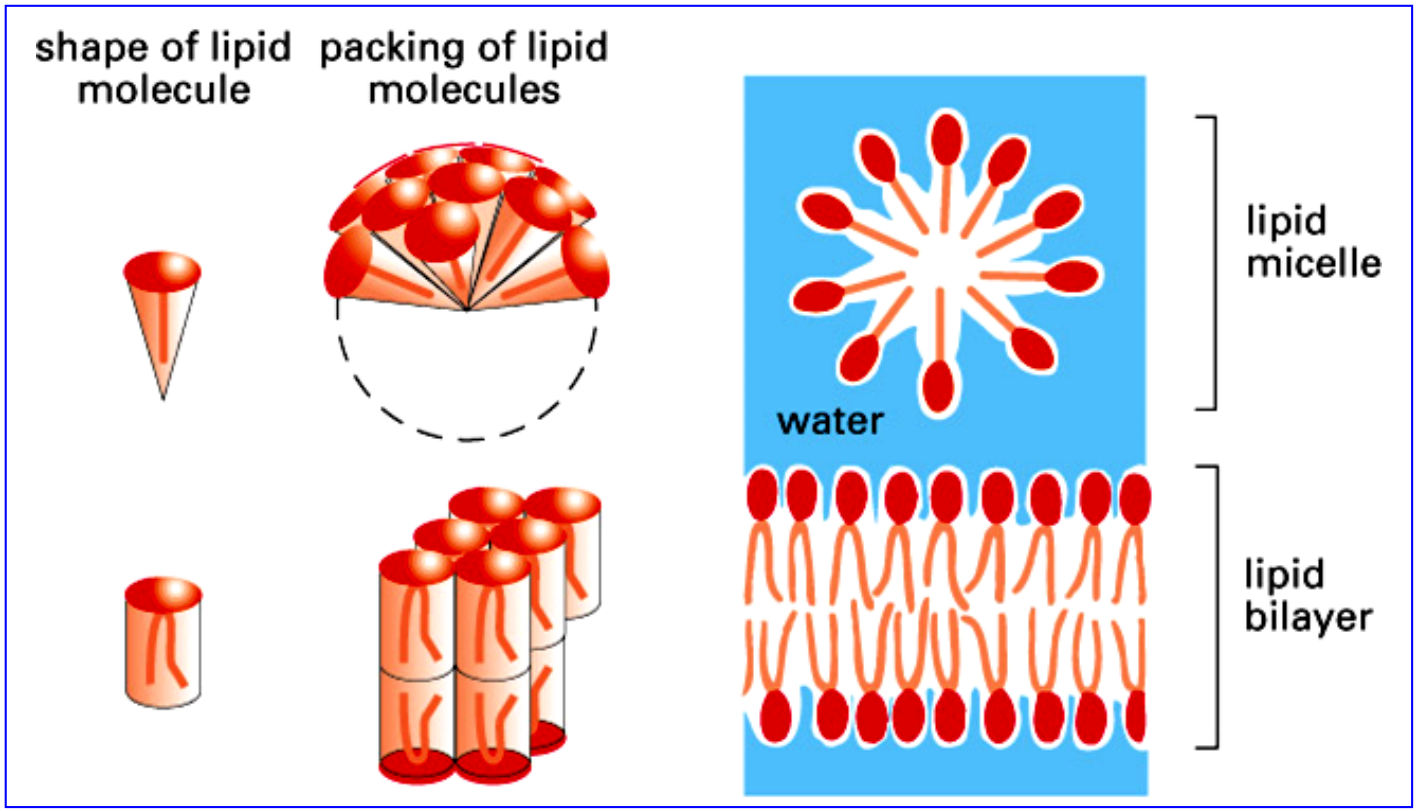
MOLECULAR ORGANIZATION

Fluid mosaic model (Singer and Nicholson, 1972)



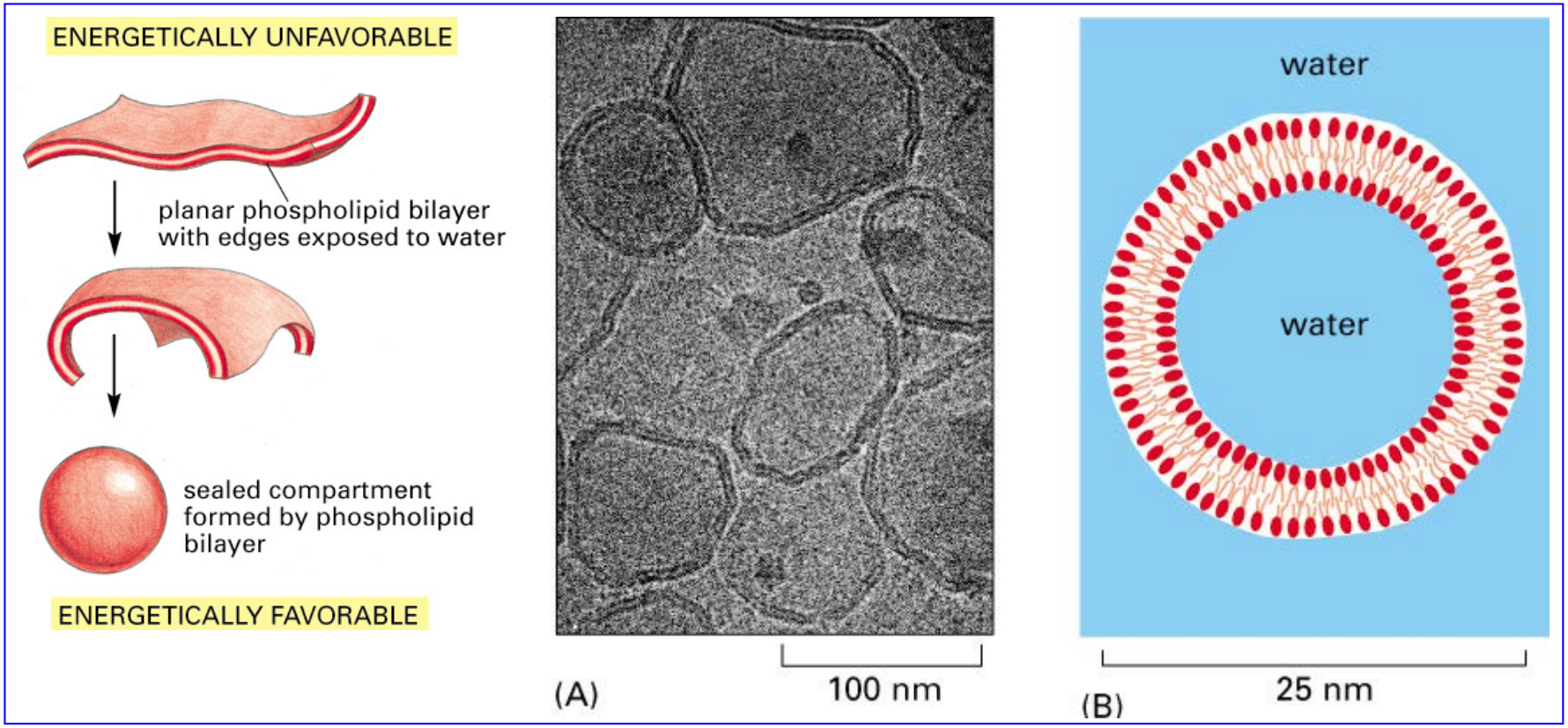
MOLECULAR ORGANIZATION

The lipids of the membrane are amphipathic molecules: they have a polar *head* and nonpolar *tails*.
In contact with water they tend to form micelles or bilayers.



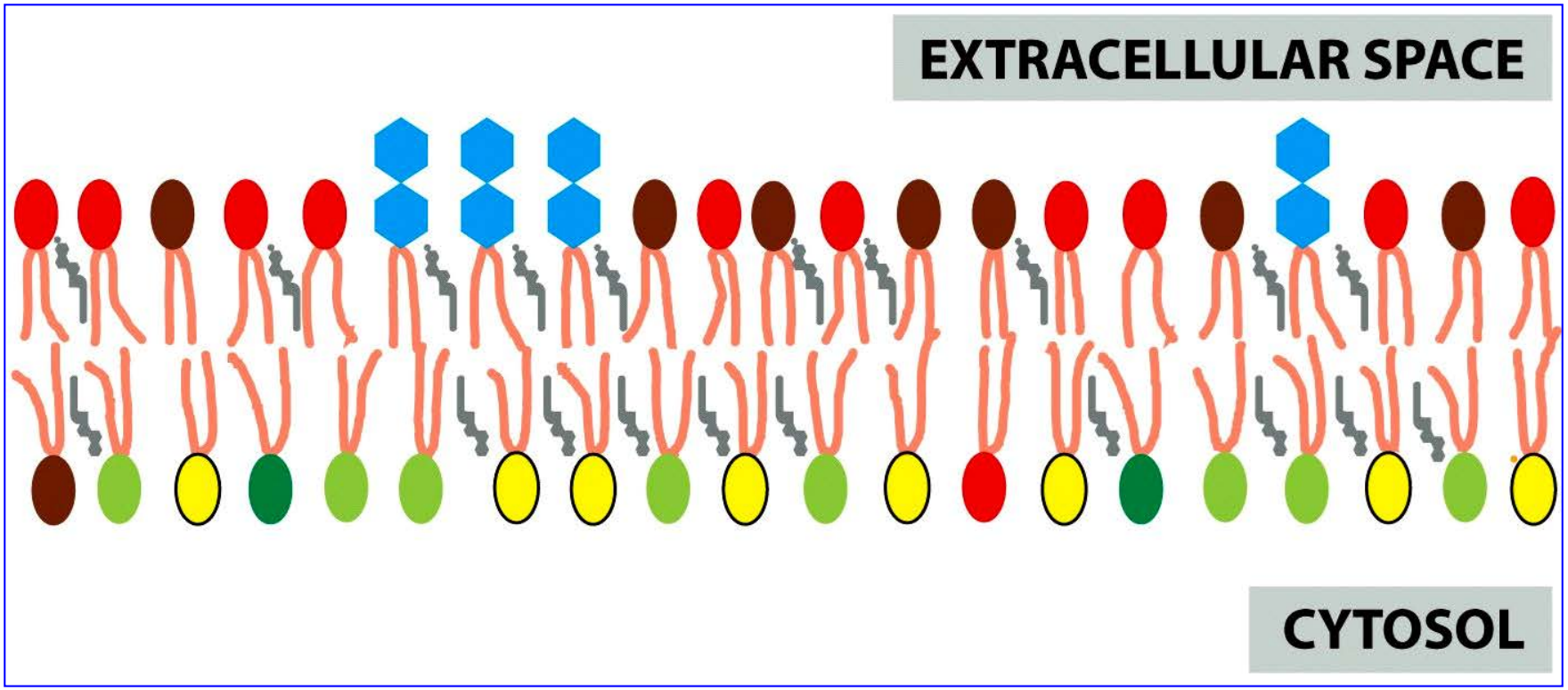
MOLECULAR ORGANIZATION

The arrangement of lipids in a bilayer is more energetically favorable.



MOLECULAR ORGANIZATION

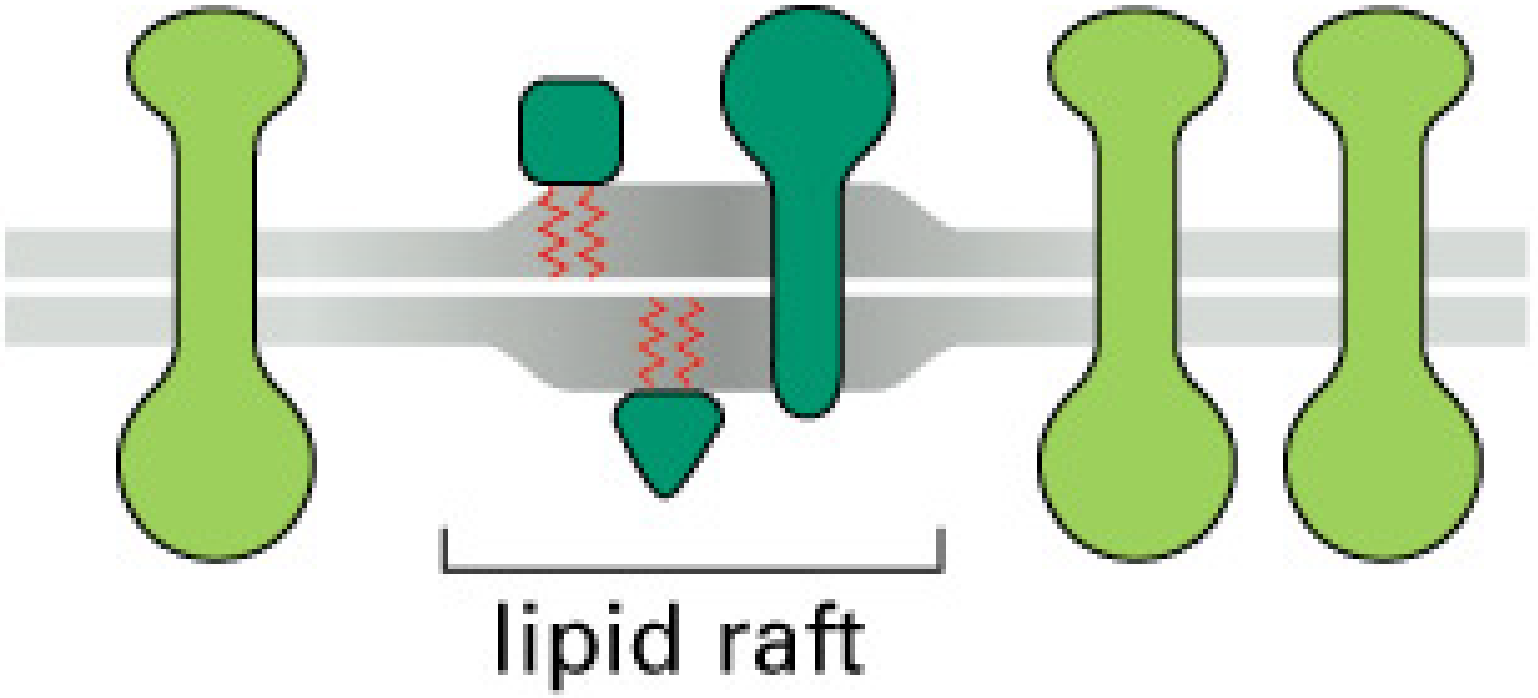
Asymmetry of lipids in the bilayer



- There are different lipids in each layer
- They distribute unevenly (domains) in the same layer
- The thickness of the membrane is heterogeneous

MOLECULAR ORGANIZATION

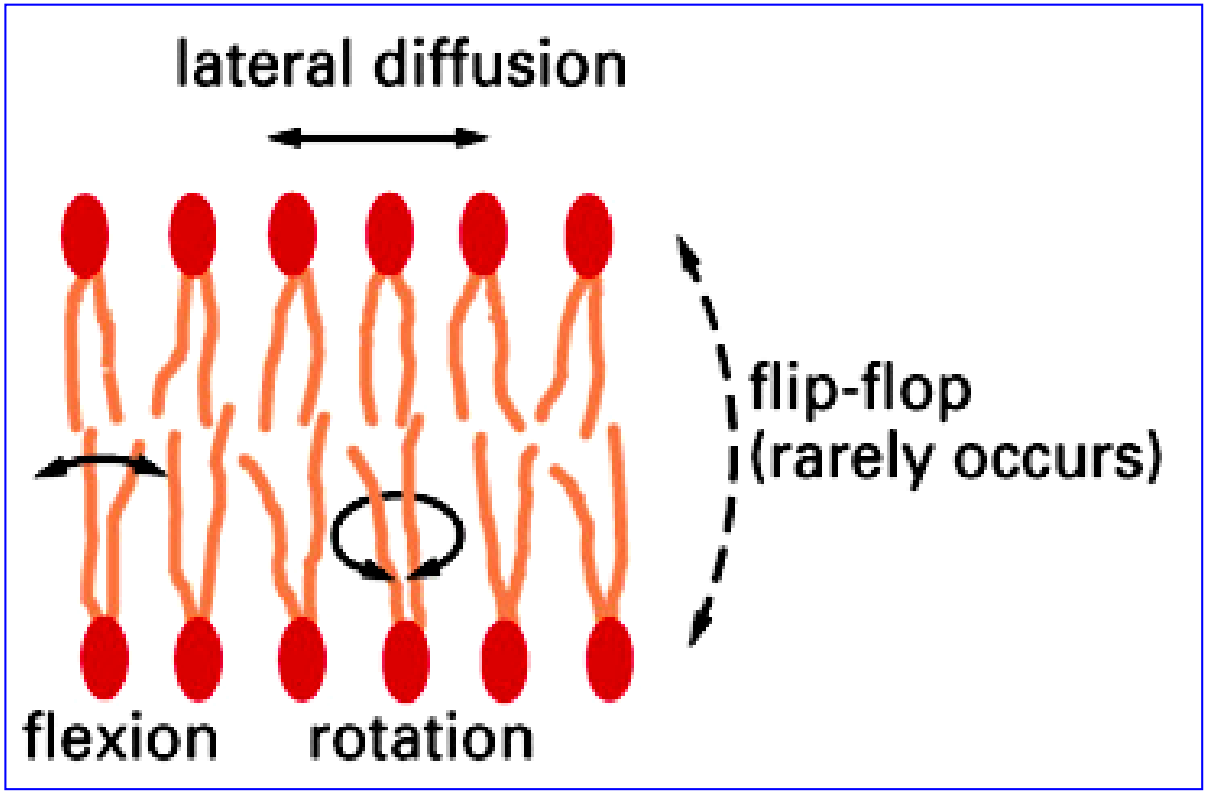
Asymmetry of lipids in the membrane



- Within the same layer they are distributed irregularly (domains).

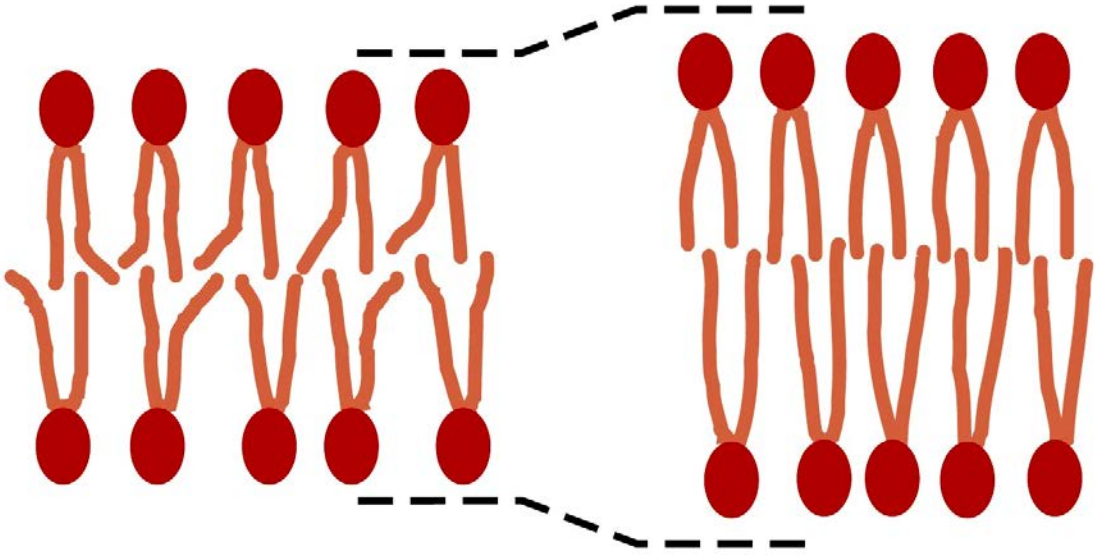
MOLECULAR ORGANIZATION

Lipid movements in the membrane



MOLECULAR ORGANIZATION

Lipid bilayer fluidity

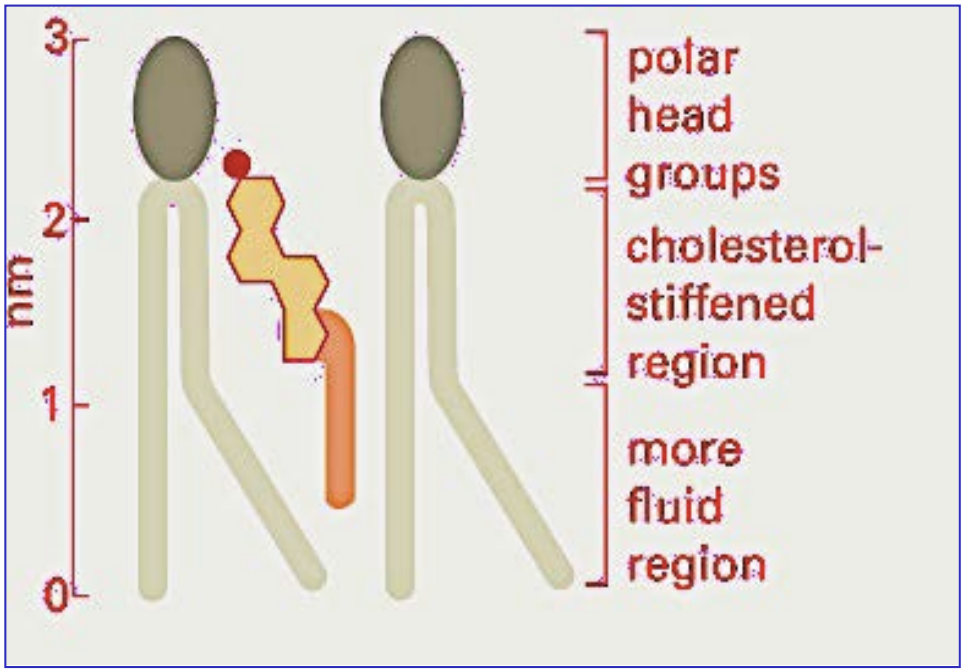


unsaturated hydrocarbon chains with *cis*-double bonds

saturated hydrocarbon chains

MOLECULAR ORGANIZATION

Lipid bilayer fluidity



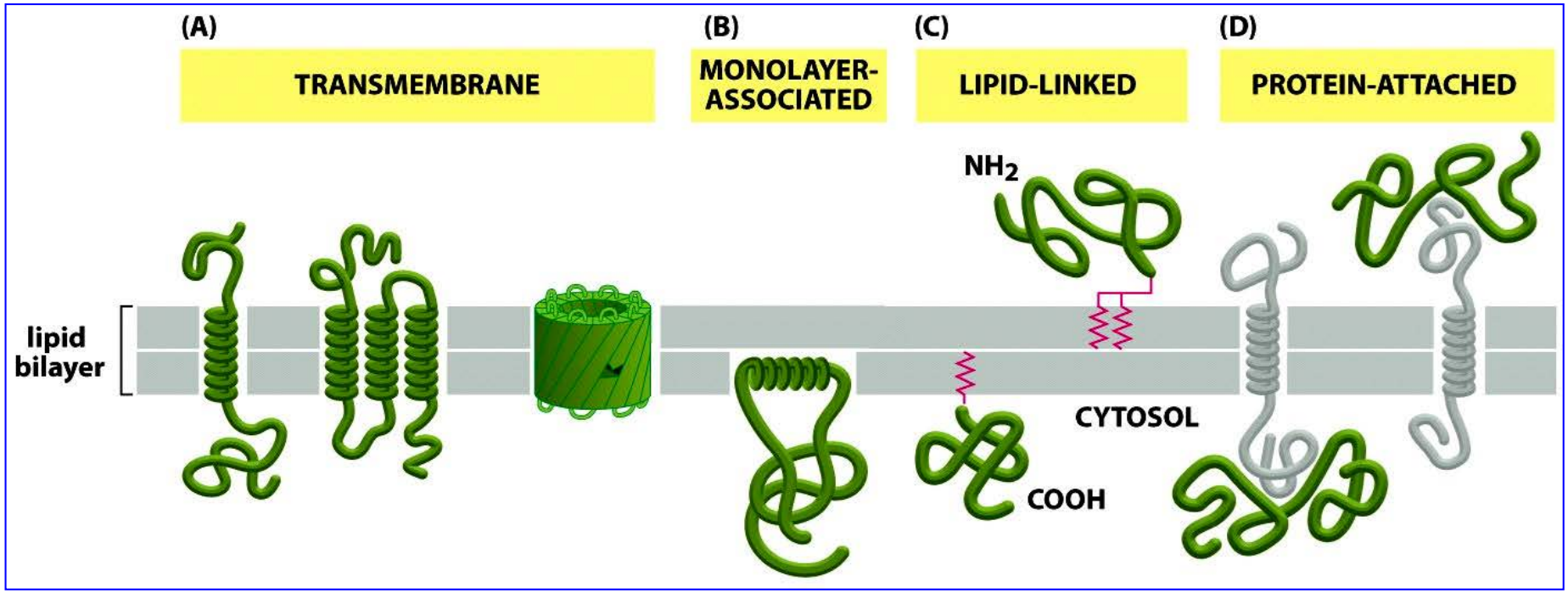
Temperature
(mobility, phase transition)

Lipids properties
(length, unsaturations)

Cholesterol

MOLECULAR ORGANIZATION

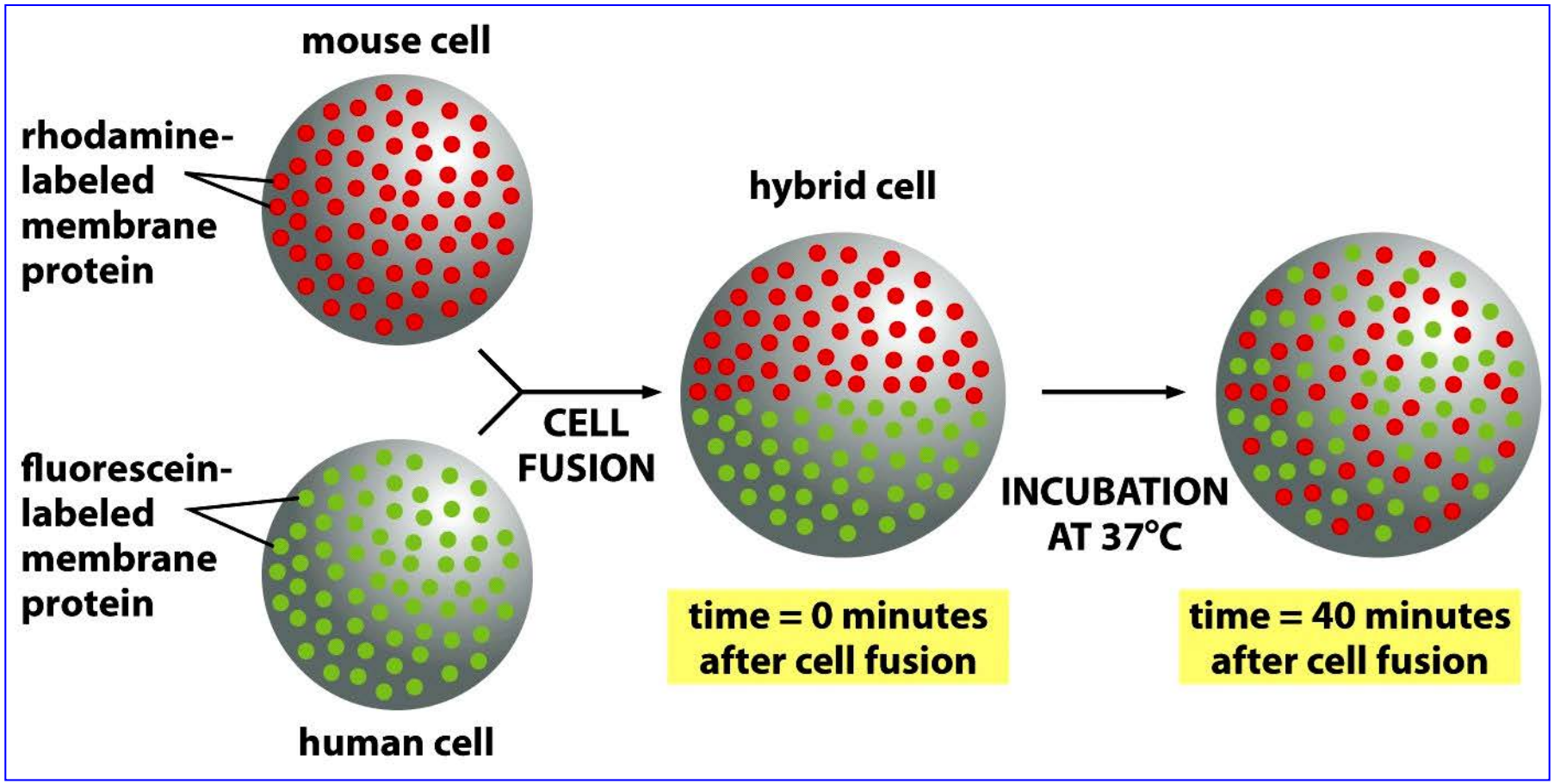
Types of membrane proteins



1. Single pass protein (α helix)
2. Multipass protein (α helices)
3. β barrel (β sheet)
4. Amphiphilic α helix in lipid monolayer
5. Attached by a lipid chain
6. Attached by a GPI anchor
7. Noncovalent interactions
8. Noncovalent interactions

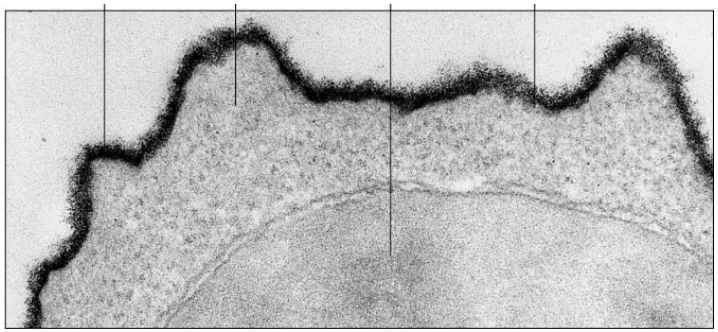
MOLECULAR ORGANIZATION

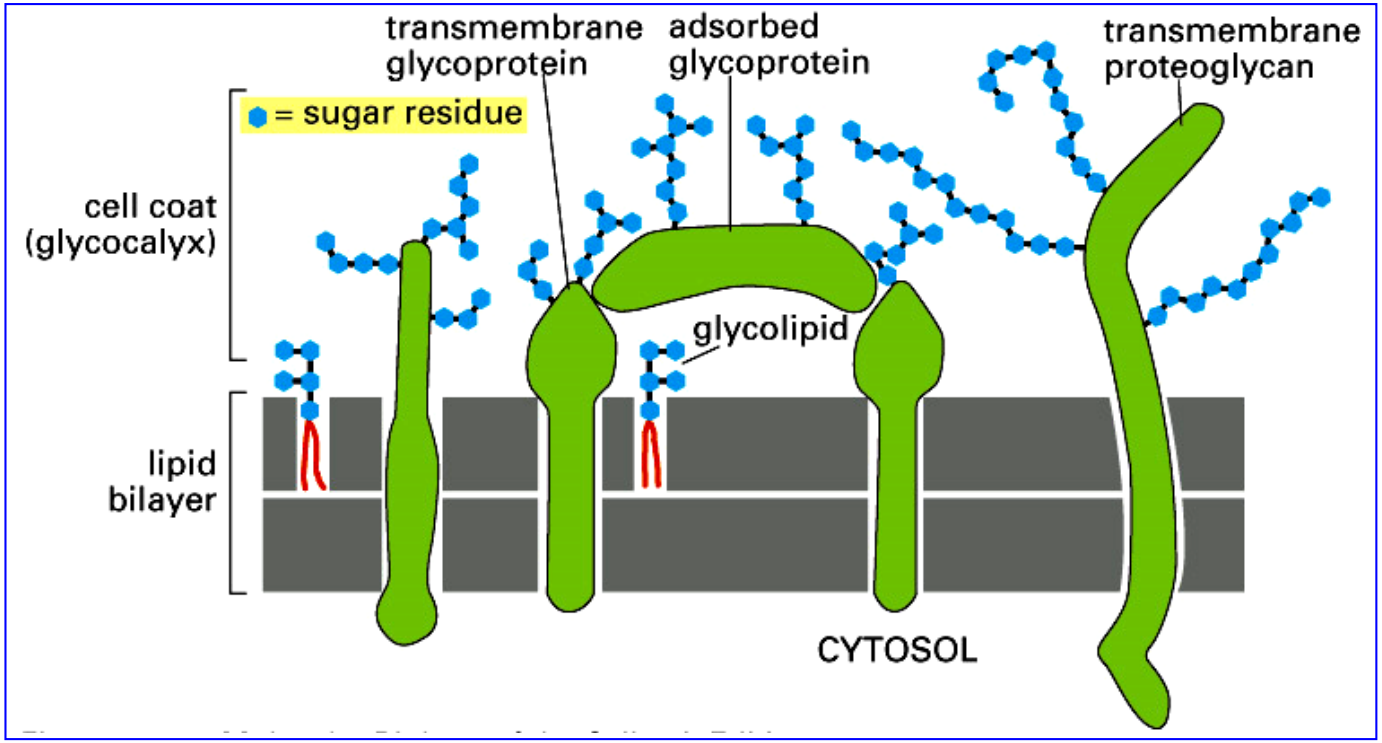
Experiment that demonstrates the fluidity of proteins



MOLECULAR ORGANIZATION

Sugars (Glycocalyx)





BIOGENESIS

The cell membrane renews constantly

Origin of its components:

- The lipids are synthesized by enzymes of the ER membrane .
- The proteins are synthesized at the ribosomes
  - Free ribosomes: internal peripheral proteins
  - R.E.R.: integral and peripheral external proteins.
- The oligosaccharides that will bind the lipids and proteins, in the ER. The terminal glycosylation of lipids and proteins, in the Golgi.

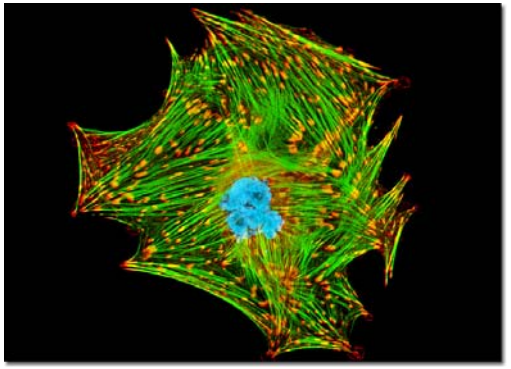
Transport:

- The internal peripheral proteins are sent to the plasma membrane through the cytosol using specific transporters .
- Other components, especially glycoproteins and glycolipids, are preassembled in the ER and Golgi, packed into vesicles and are sent to the cell membrane where they are incorporated.

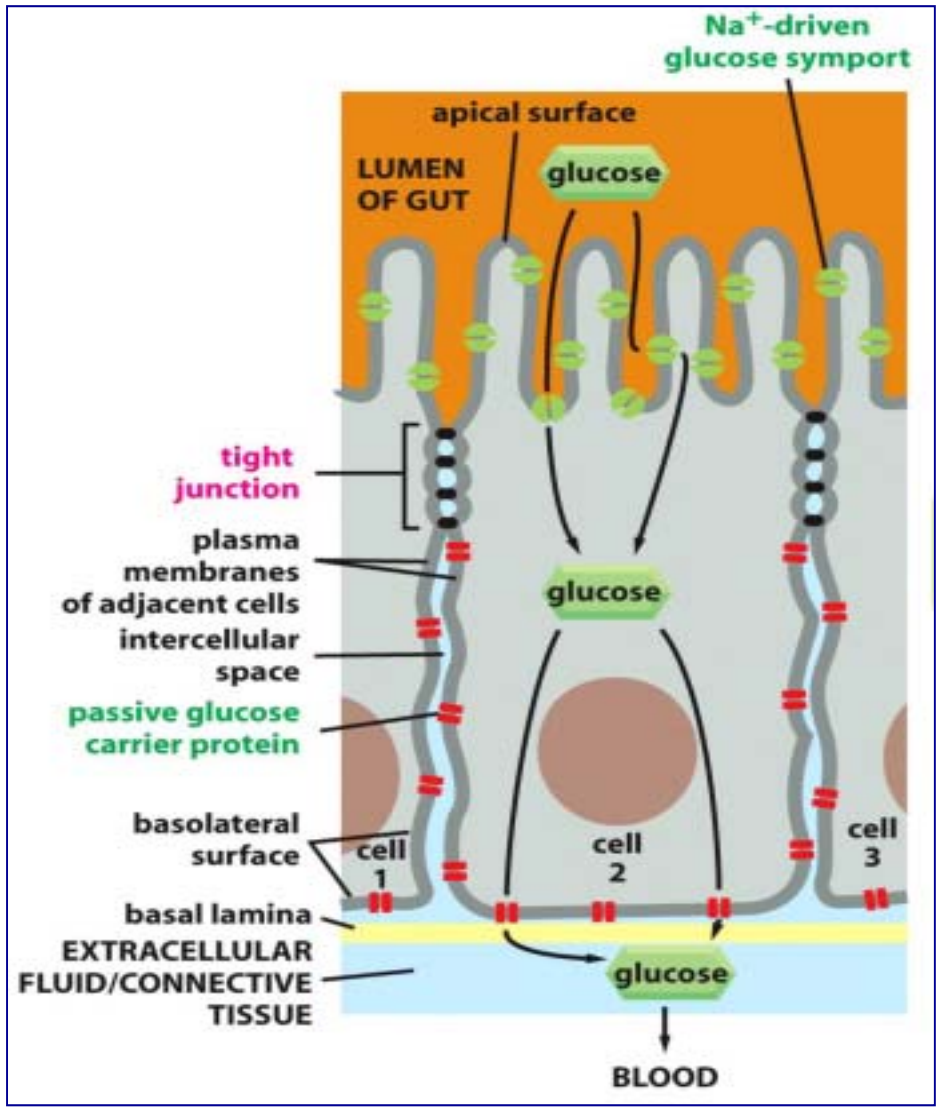
DIFFERENTIATIONS OF THE CELL MEMBRANE

- Heterogeneity of the cell membrane

- Cell junctions
  - Tight junctions
  - Anchoring junctions
  - Gap junctions



HETEROGENEITY OF THE CELL MEMBRANE



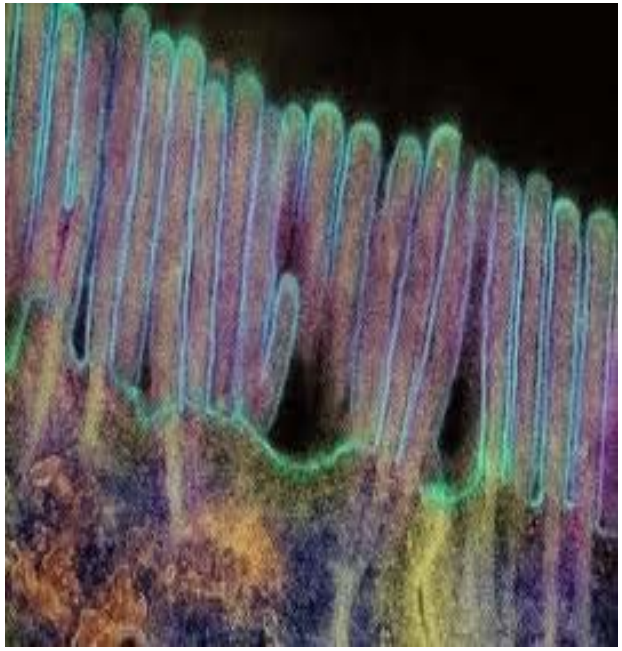
Differentiations

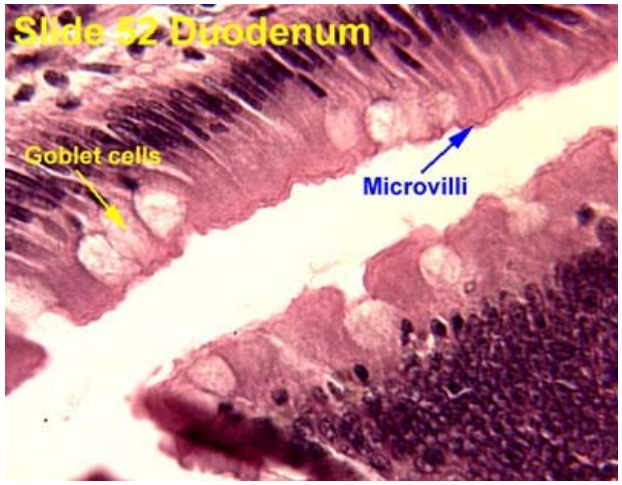
- Microvilli
- Lateral interdigitations
- Basal infoldings
- Cell junctions

Alberts et al. Fig. 19-18

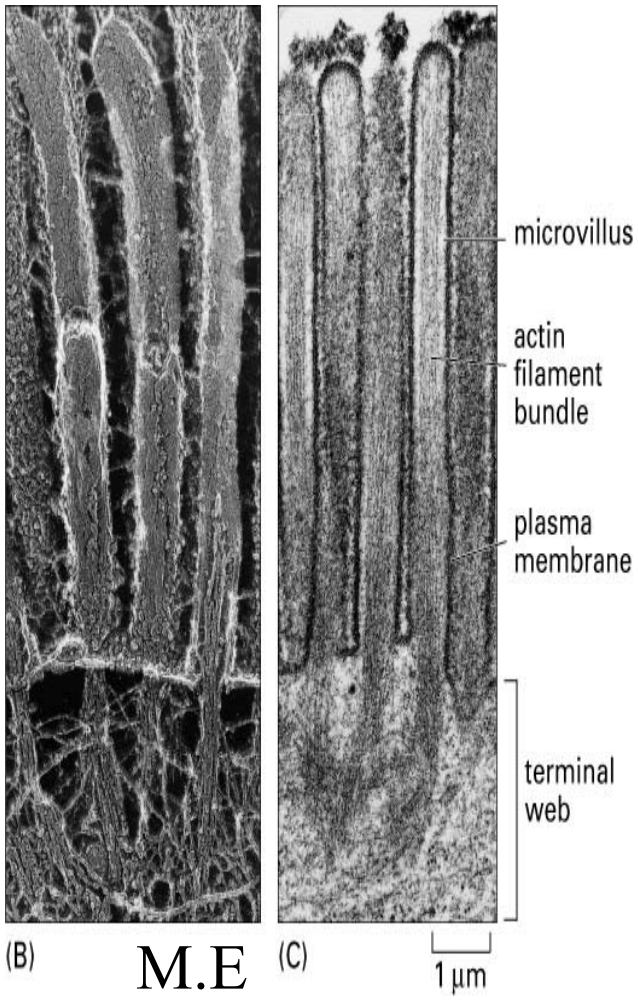
HETEROGENEITY OF THE CELL MEMBRANE

Differentiations: Microvilli





M.O



M.E

Figure 16–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

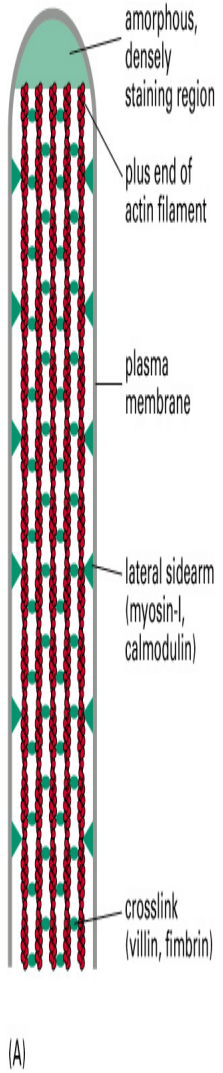
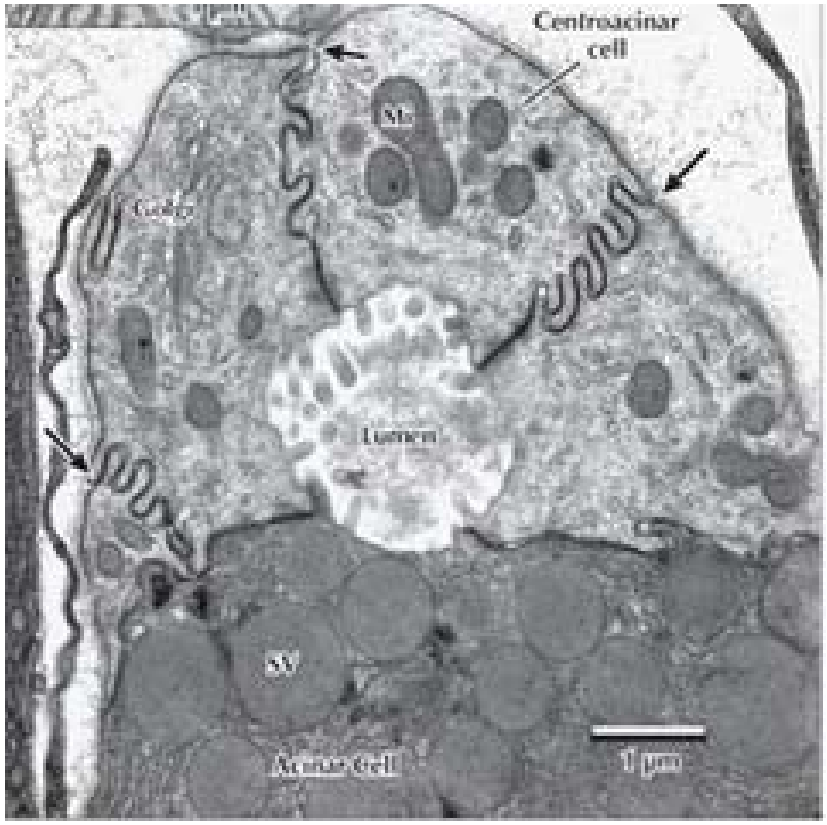
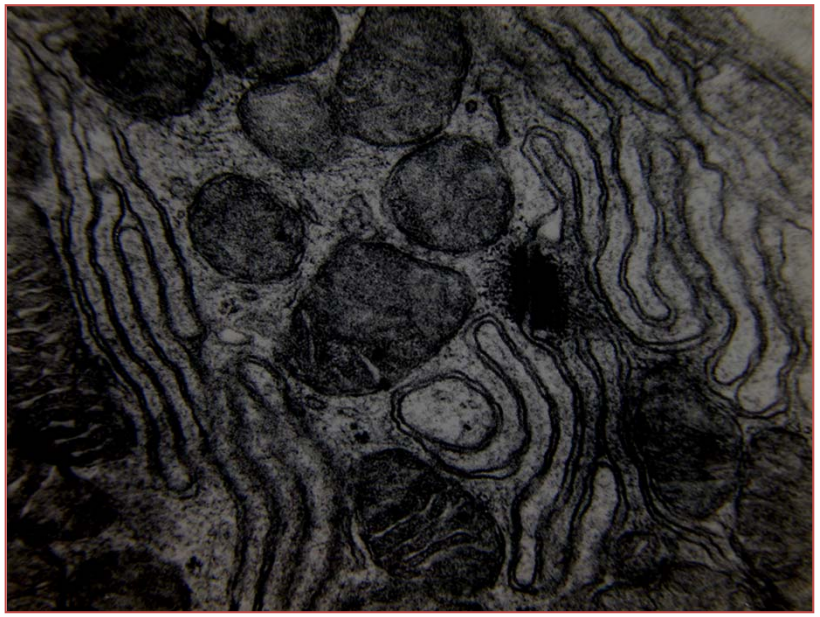


Figure 16–41 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

HETEROGENEITY OF THE CELL MEMBRANE

Differentiations: Lateral interdigitations

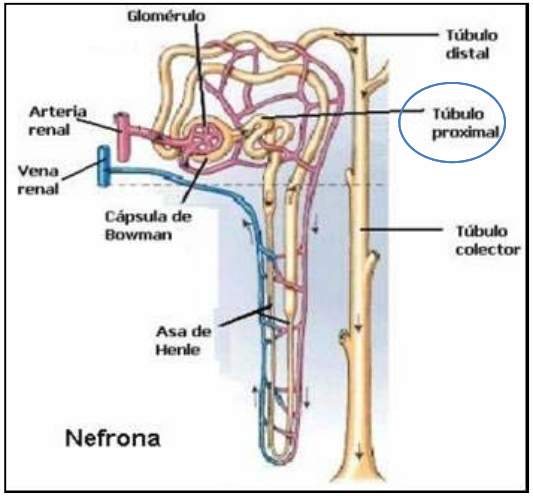


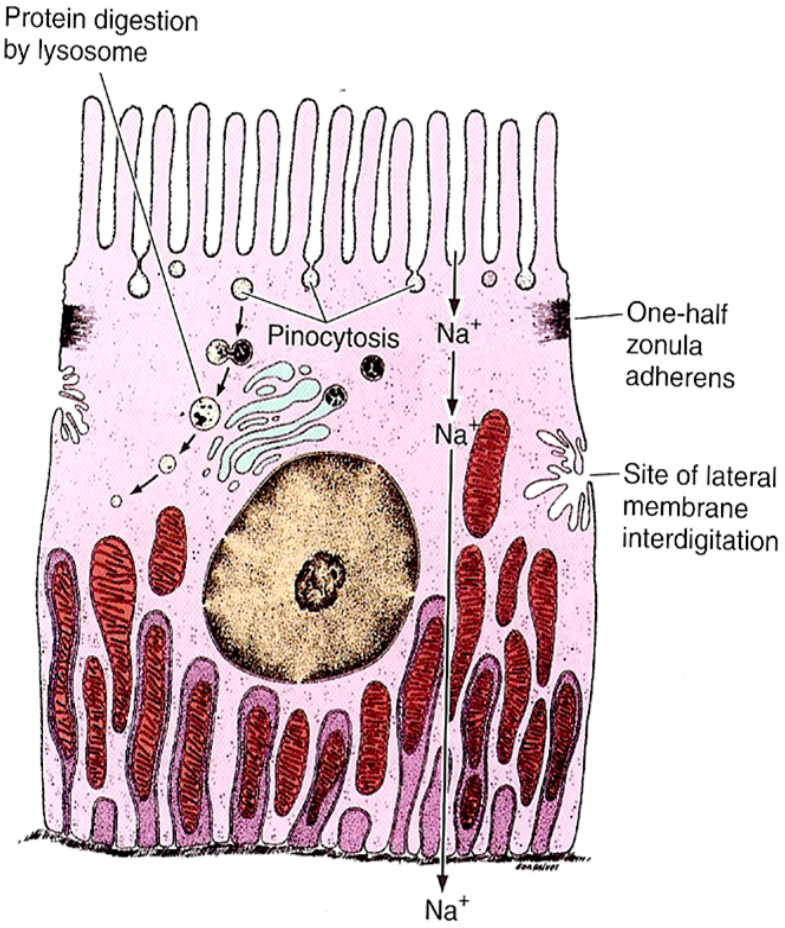


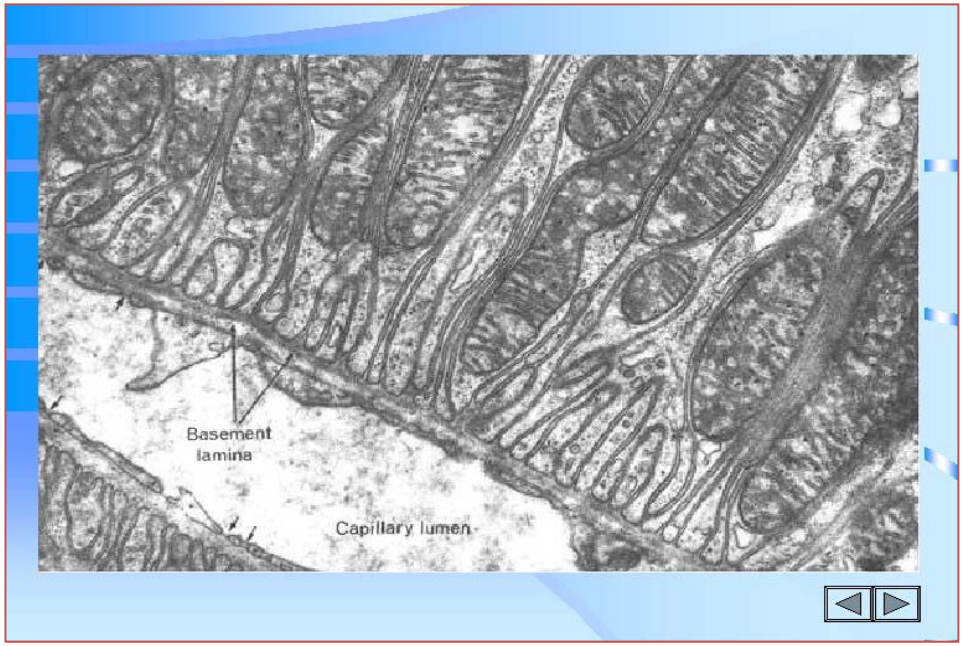
- Mechanical and metabolic function
- Presence of calcium and cytoskeletal participation

HETEROGENEITY OF THE CELL MEMBRANE

Differentiations: Basal infoldings

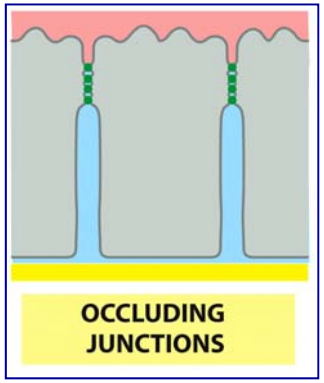


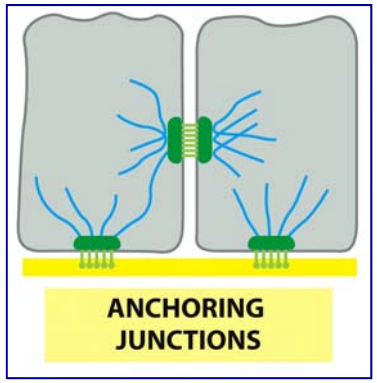


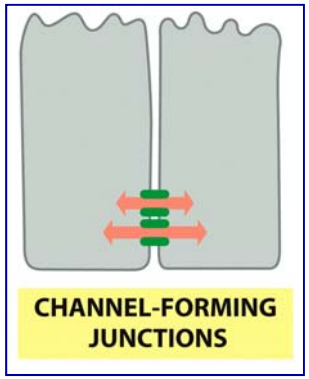


Mitochondria associated

CELL JUNCTIONS:
FUNCTIONAL CLASSIFICATION







Occluding junctions

Tight junctions

Anchoring junctions

- *Actin filament attachment*

  Adherens junctions (cell-cell))

  Focal adhesions (cell-matrix))

- *Intermediate filament attachment*

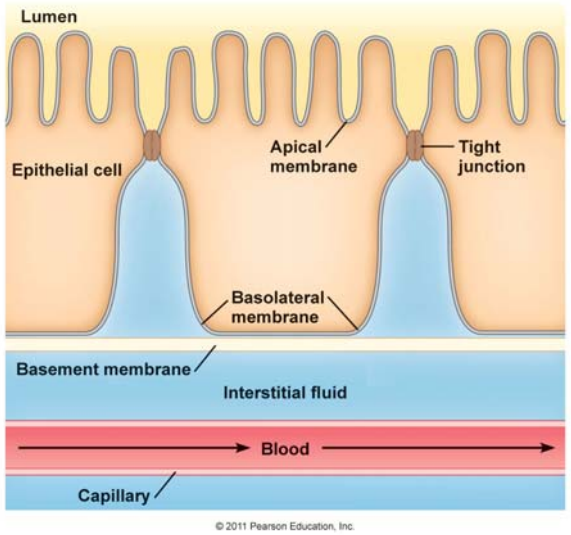
  Desmosomes (cell-cell)

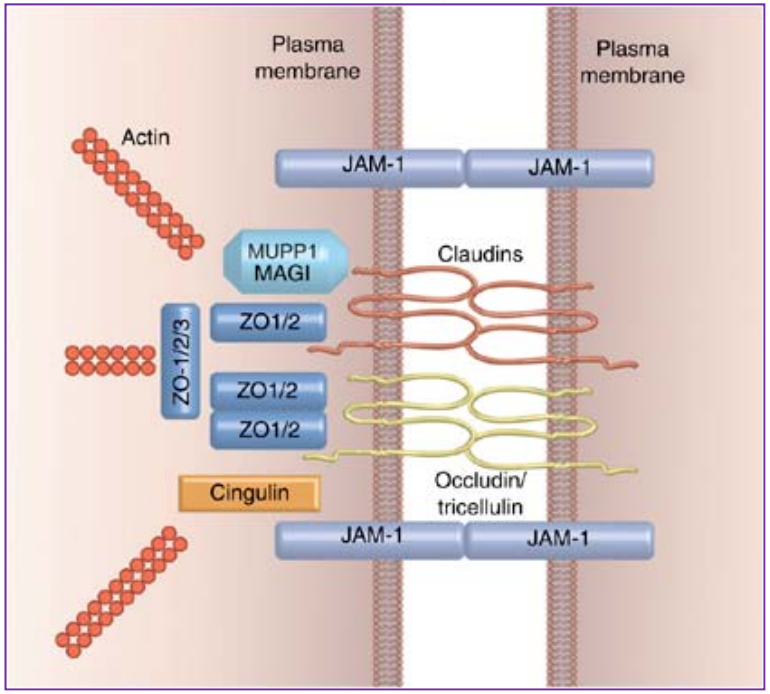
  Hemidesmosomes (cell-matrix))

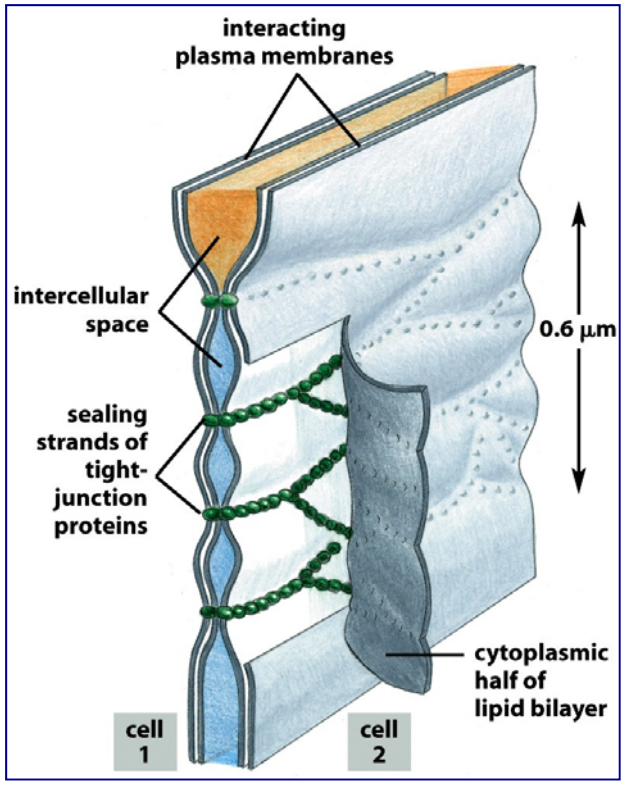
Channel-forming junctions

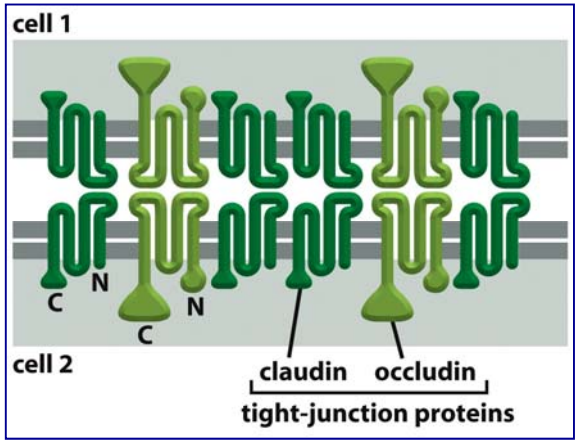
Gap junctions

TIGHT JUNCTIONS



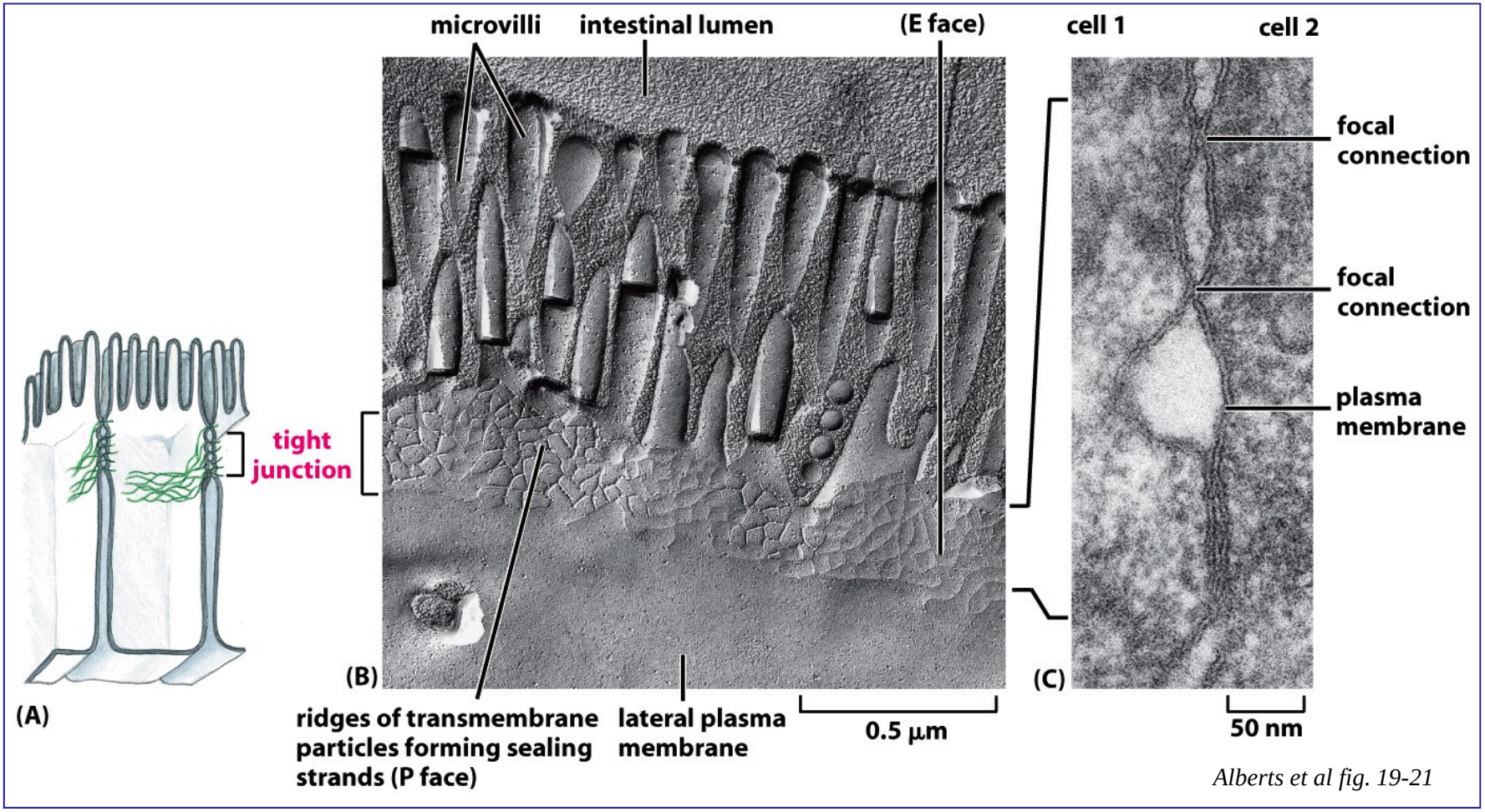






Alberts et al fig. 19-21

TIGHT JUNCTIONS

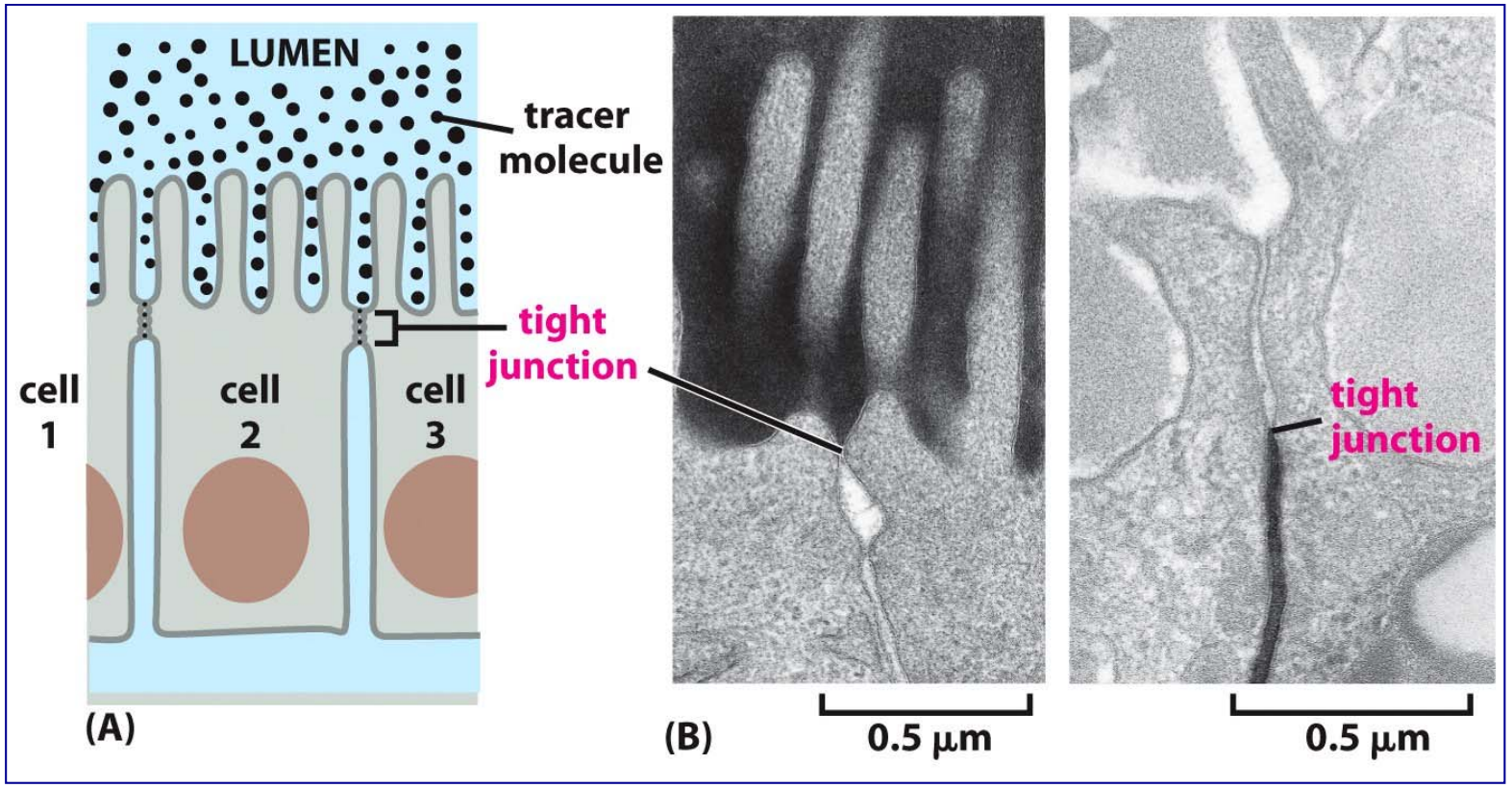


Alberts et al fig. 19-21

Forms a reticular belt that surrounds the cell at the topmost part.

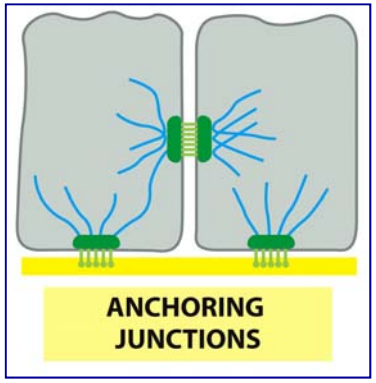
Establishes a strong mechanical junction between adjacent epithelial cells

TIGHT JUNCTIONS: Functions

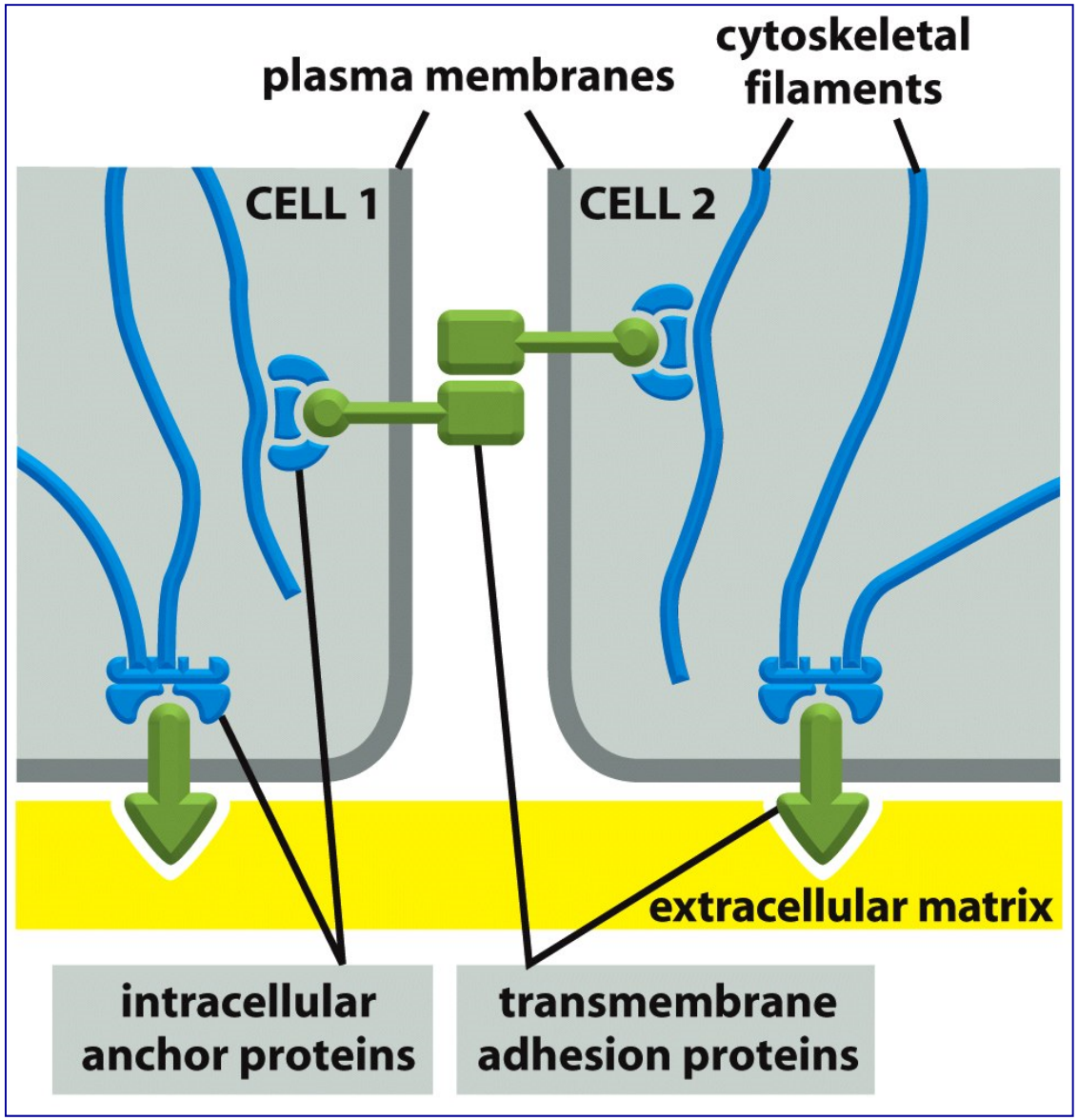


Alberts et al fig. 19-24

- They hold cells together.
- They prevent the diffusion of macromoleculas between the apical and lateral/basal surfaces : Paracellular transport
- Restrict the diffusion of apical and basolateral membrane components.

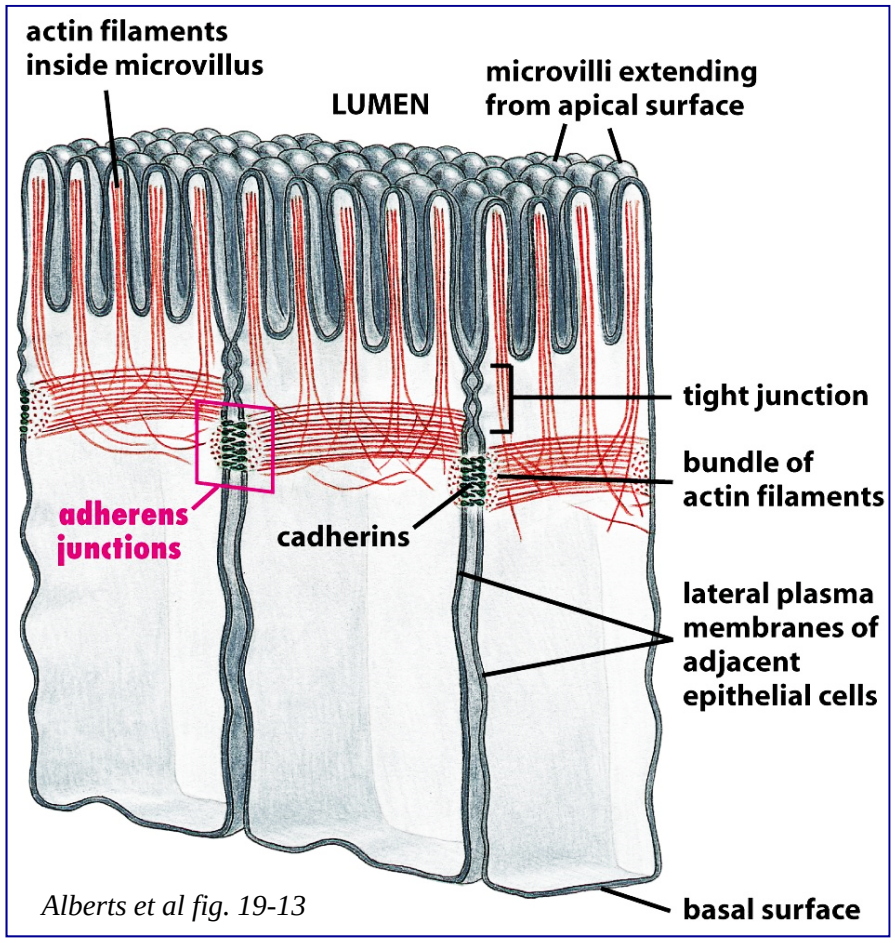


ANCHORING JUNCTIONS

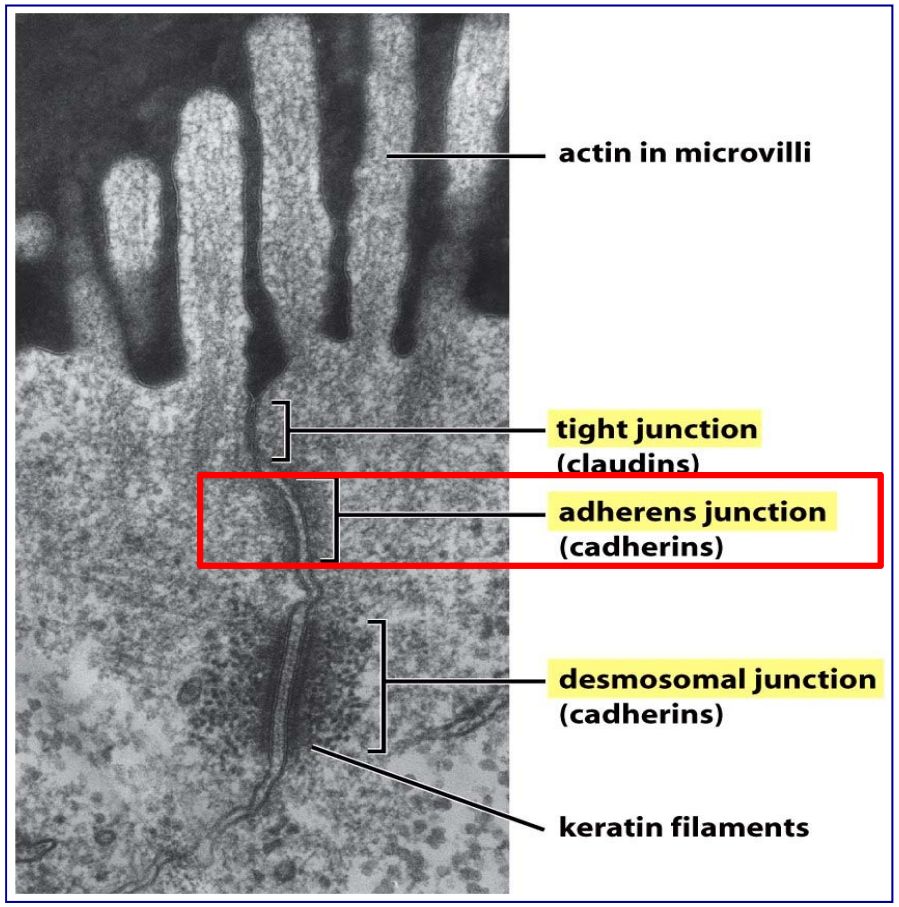


Alberts et al fig. 19-3

Adherens junctions

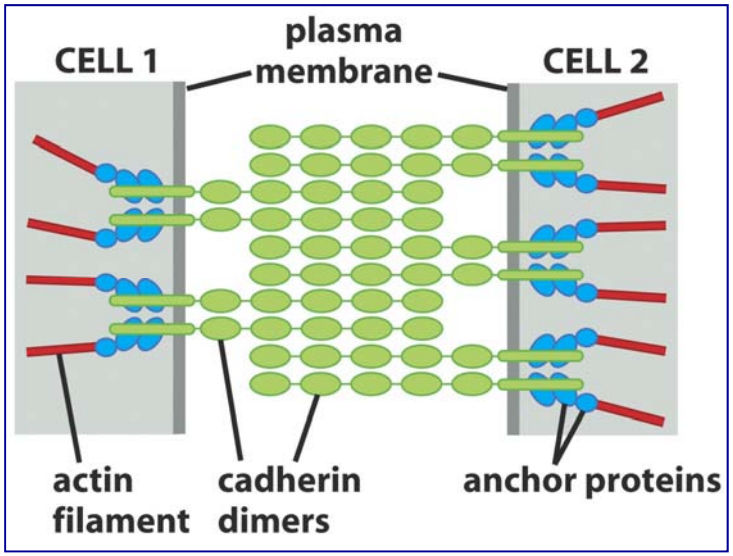


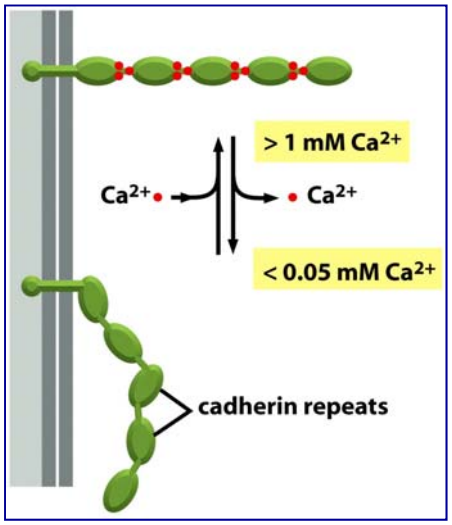
Alberts et al fig. 19-13

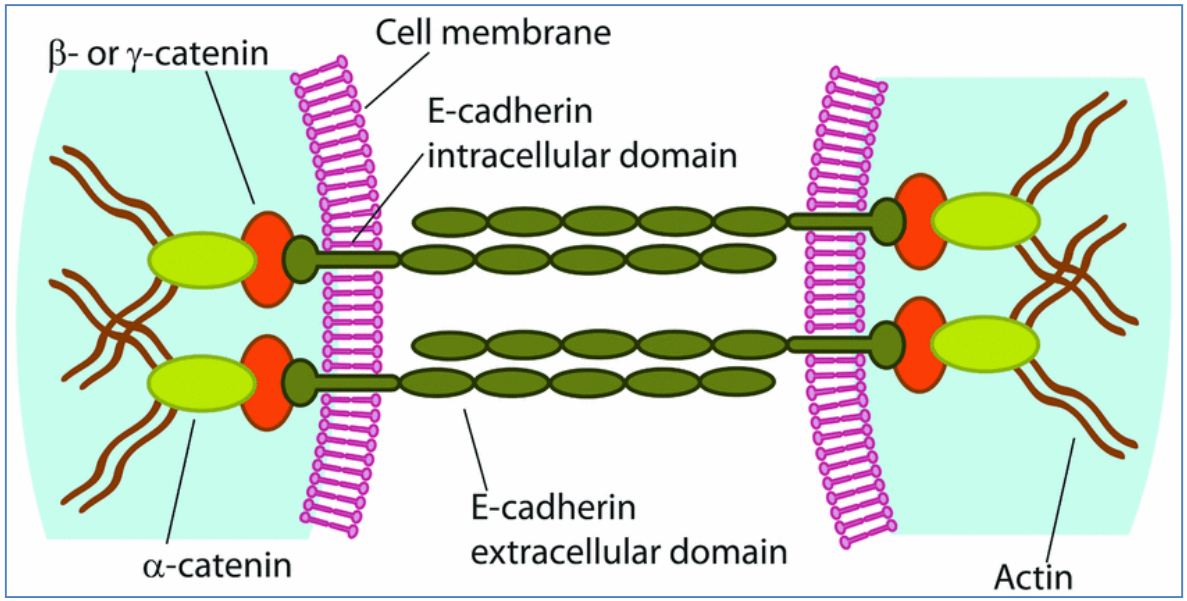


An adherens junction is a cell junction whose cytoplasmic face is linked to the actin cytoskeleton.

Adherens junctions







Transmembrane adhesion proteins

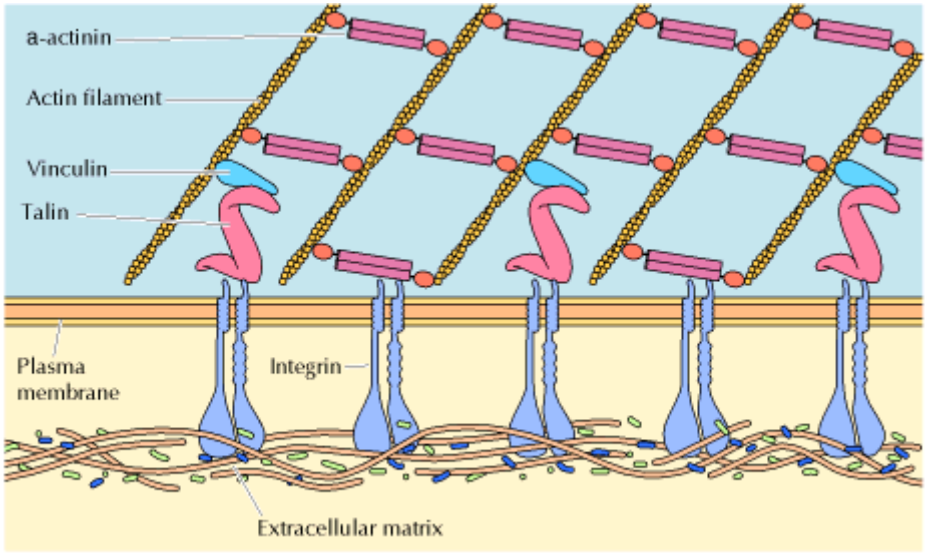
Cadherins are the principal molecules of intercellular adhesion.

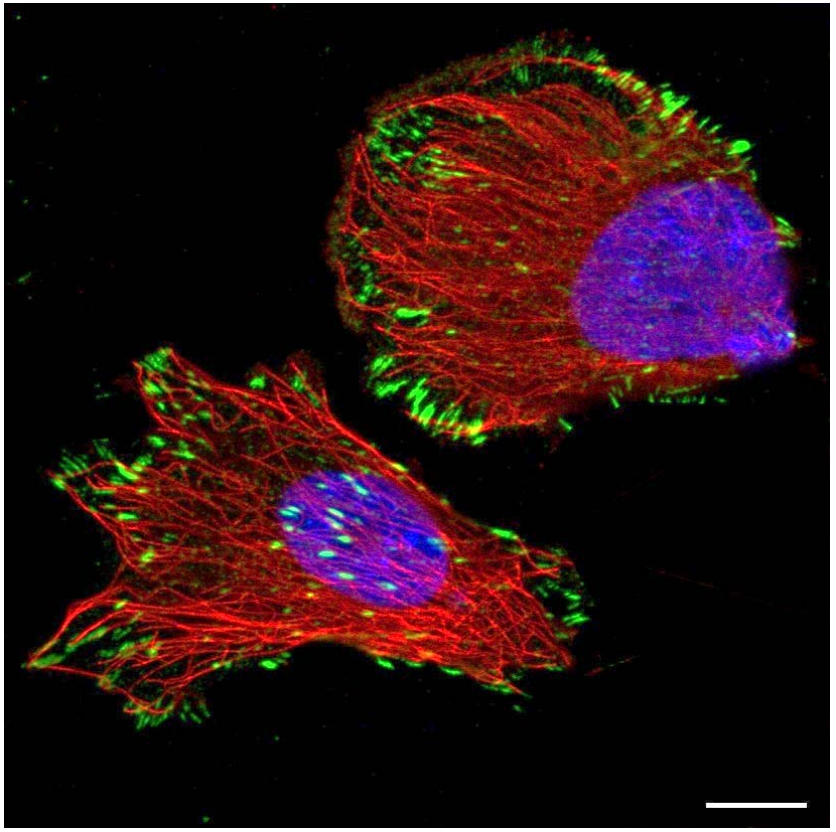
They form homodimers and depend on Ca++ to perform their adhesion function.

Intracellular anchor proteins

Catenin, α-actinin….

Focal adhesions

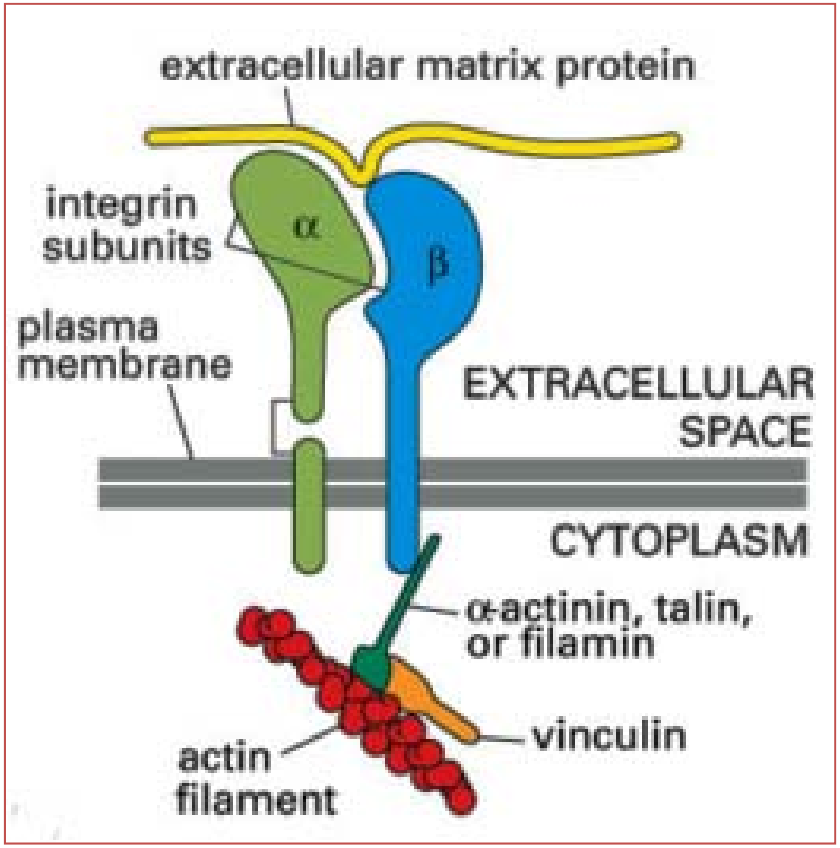




Green: focal adhesions
Red: Actin filaments
Blue: nucleus

- Focal adhesions attach the cells to the substratum or to extracellular matrix
- They are involved in cell motility and migration

Focal adhesions

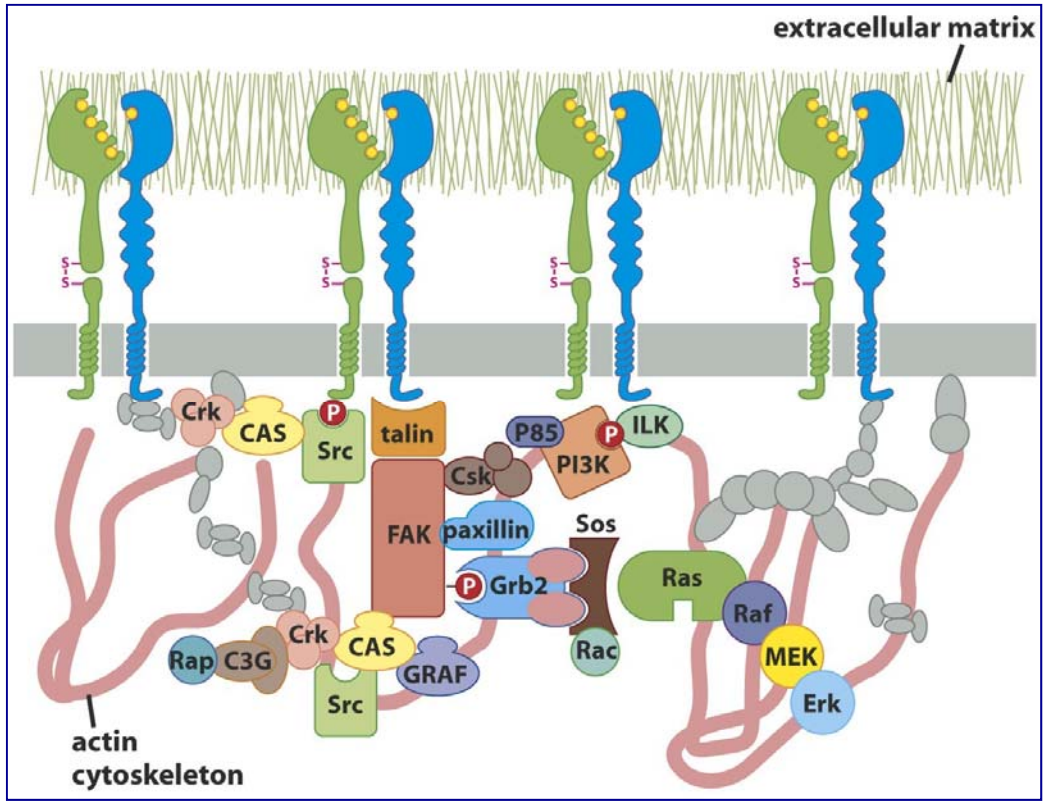


Integrins are the principal adhesion molecules between the cell and the extracellular matrix.

There are many varieties of integrins and they form heterodimers.

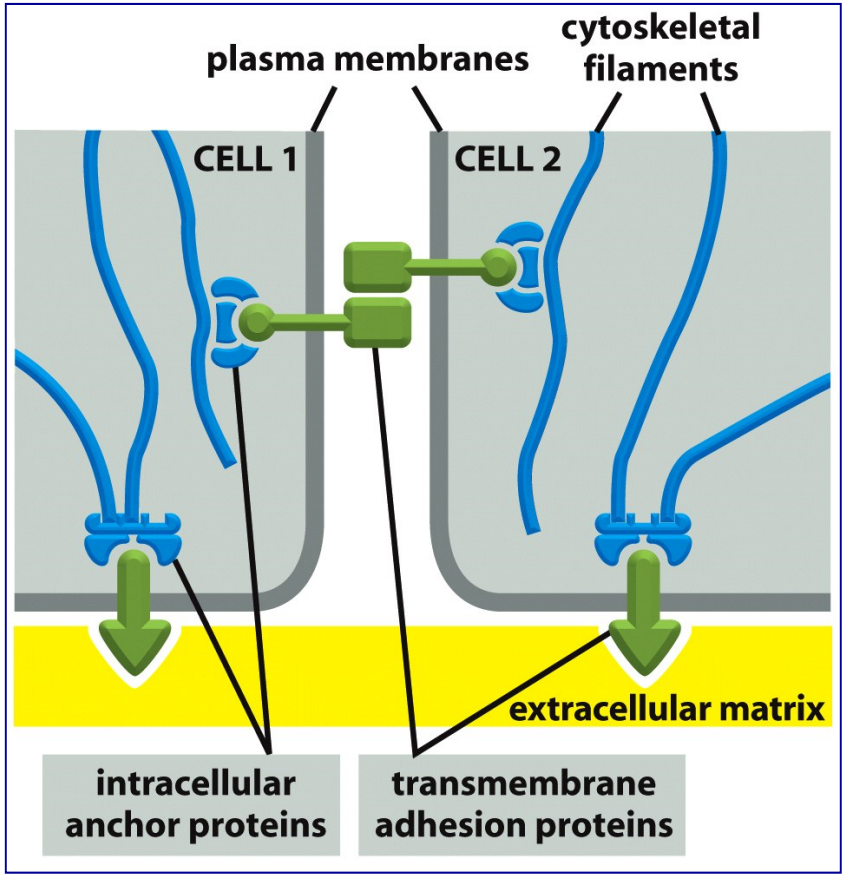
ANCHORING JUNCTIONS

Focal adhesions

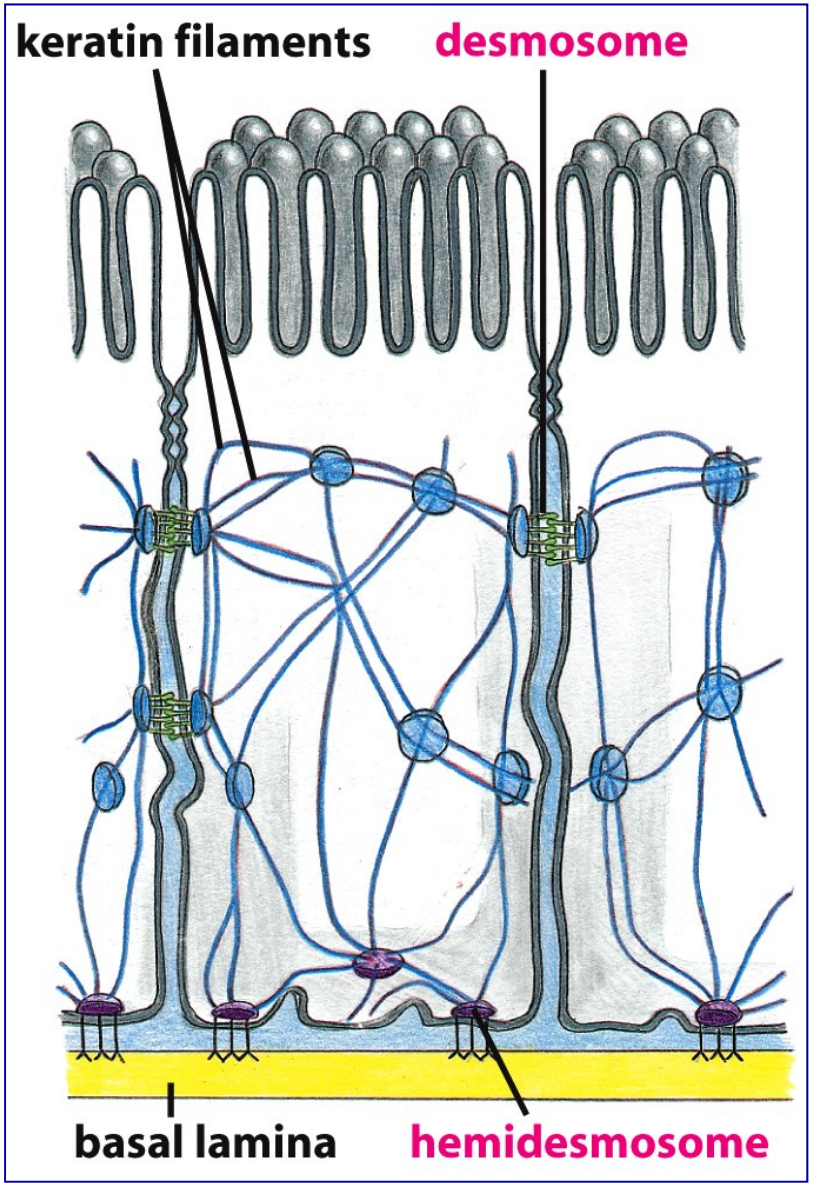


Integrins are also implicated in complex mechanisms of signal transduction.

Desmosomes and hemidesmosomes

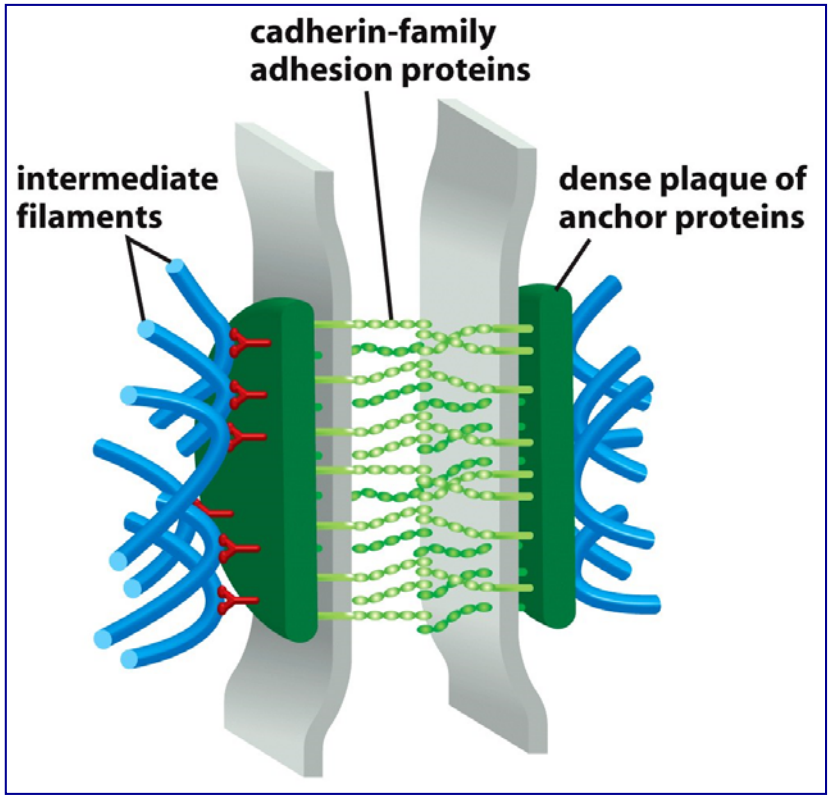


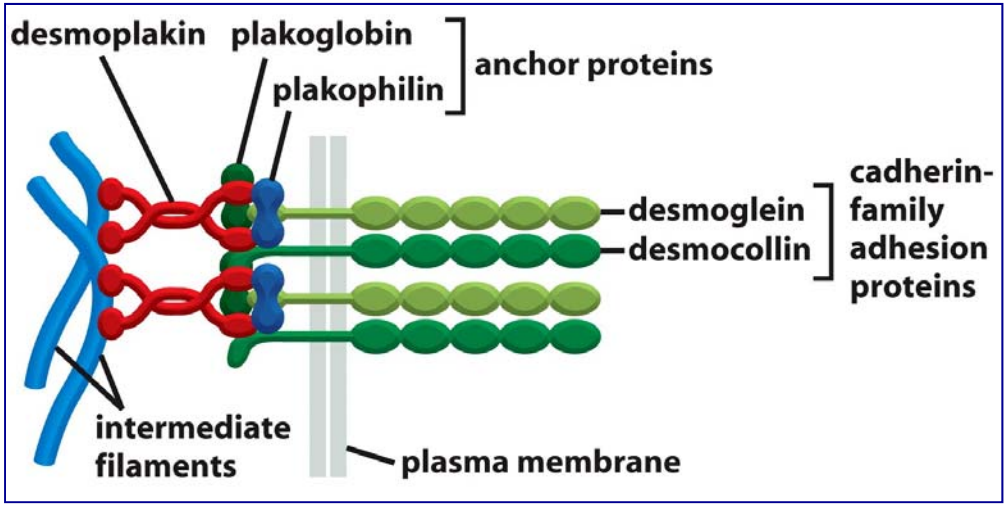
Alberts et al fig. 19-3



Alberts et al fig. 19-17

Molecular organization of desmosomes

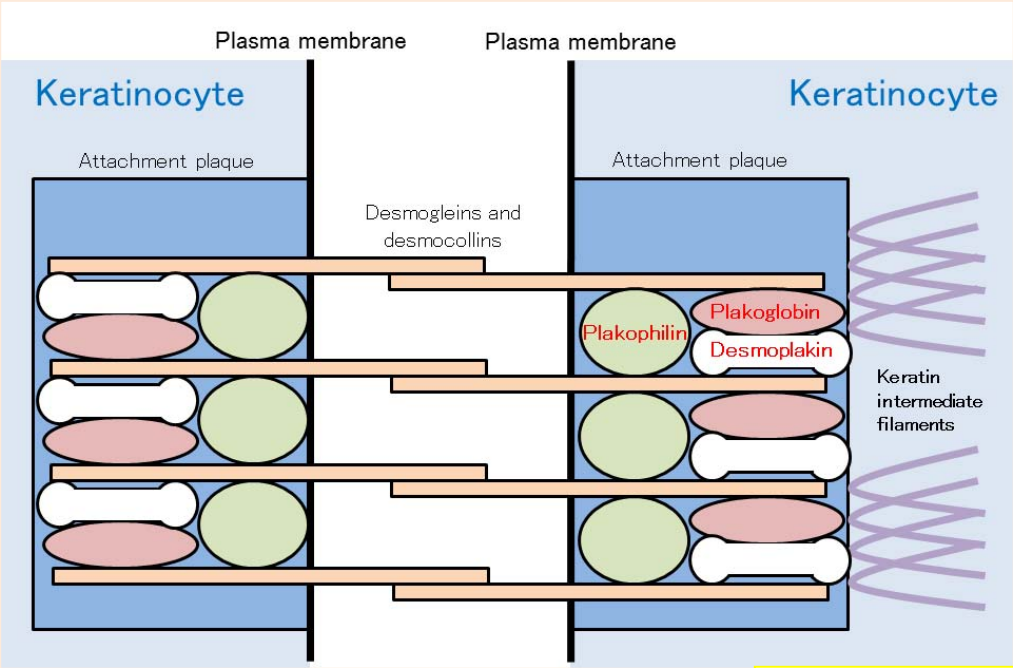




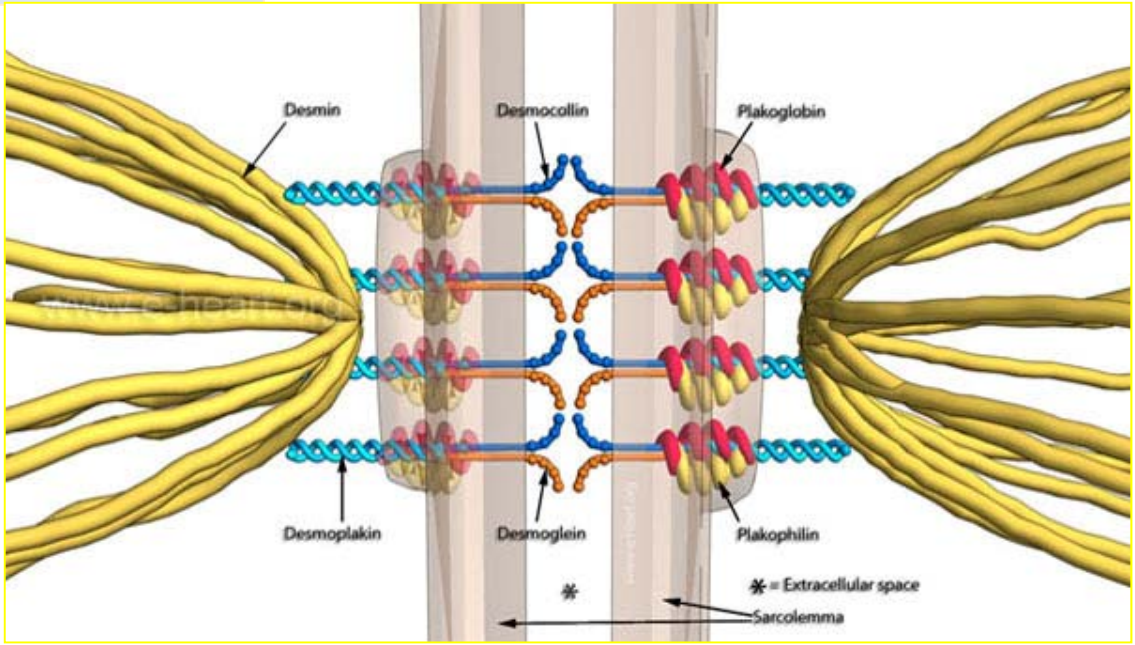
Alberts et al fig. 19-16

Anchoring function between cells

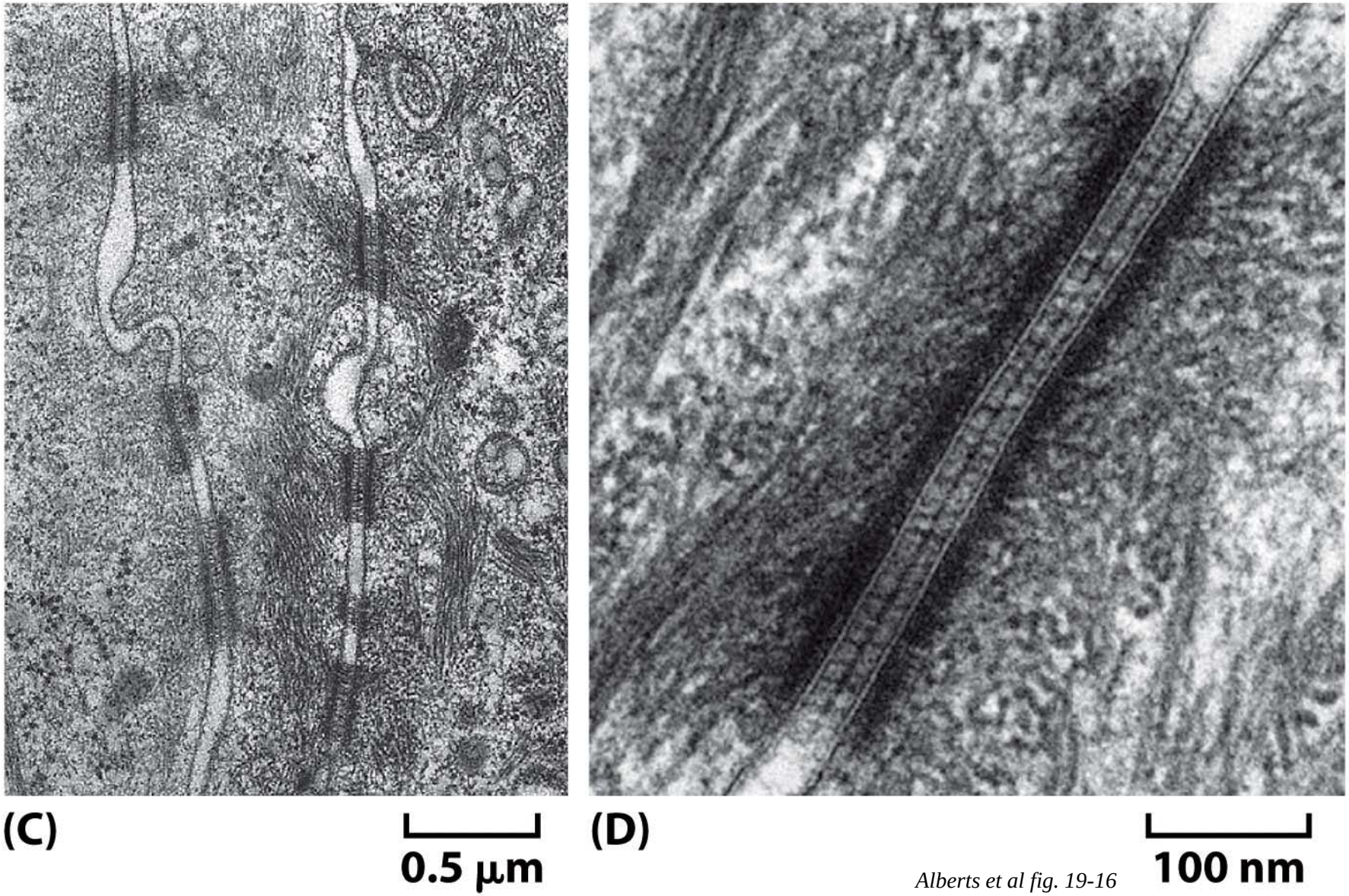
ANCHORING JUNCTIONS



Molecular organization of desmosomes

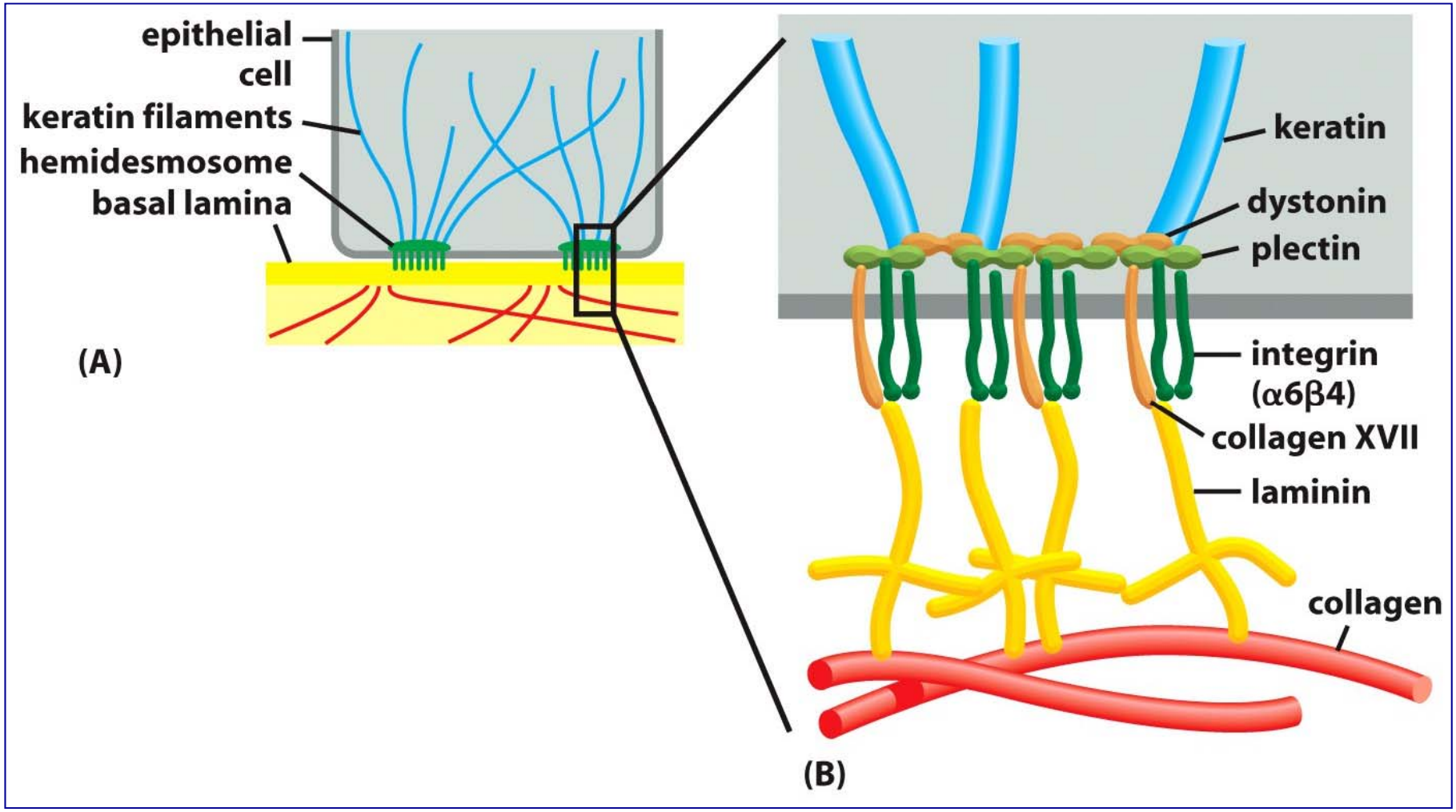


Desmosomes



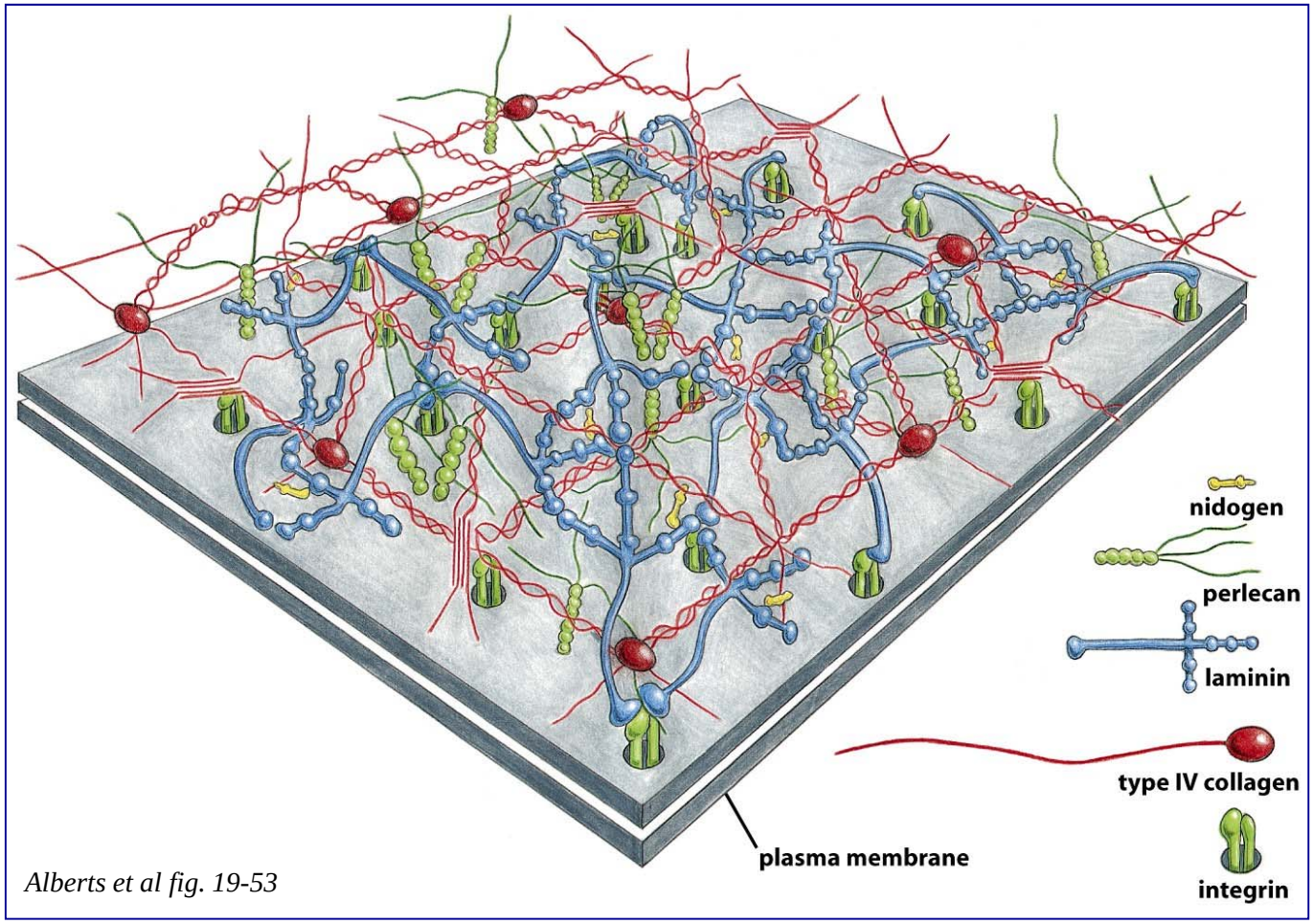
Alberts et al fig. 19-16

Hemidesmosomas. Molecular organization



Alberts et al fig. 19-56

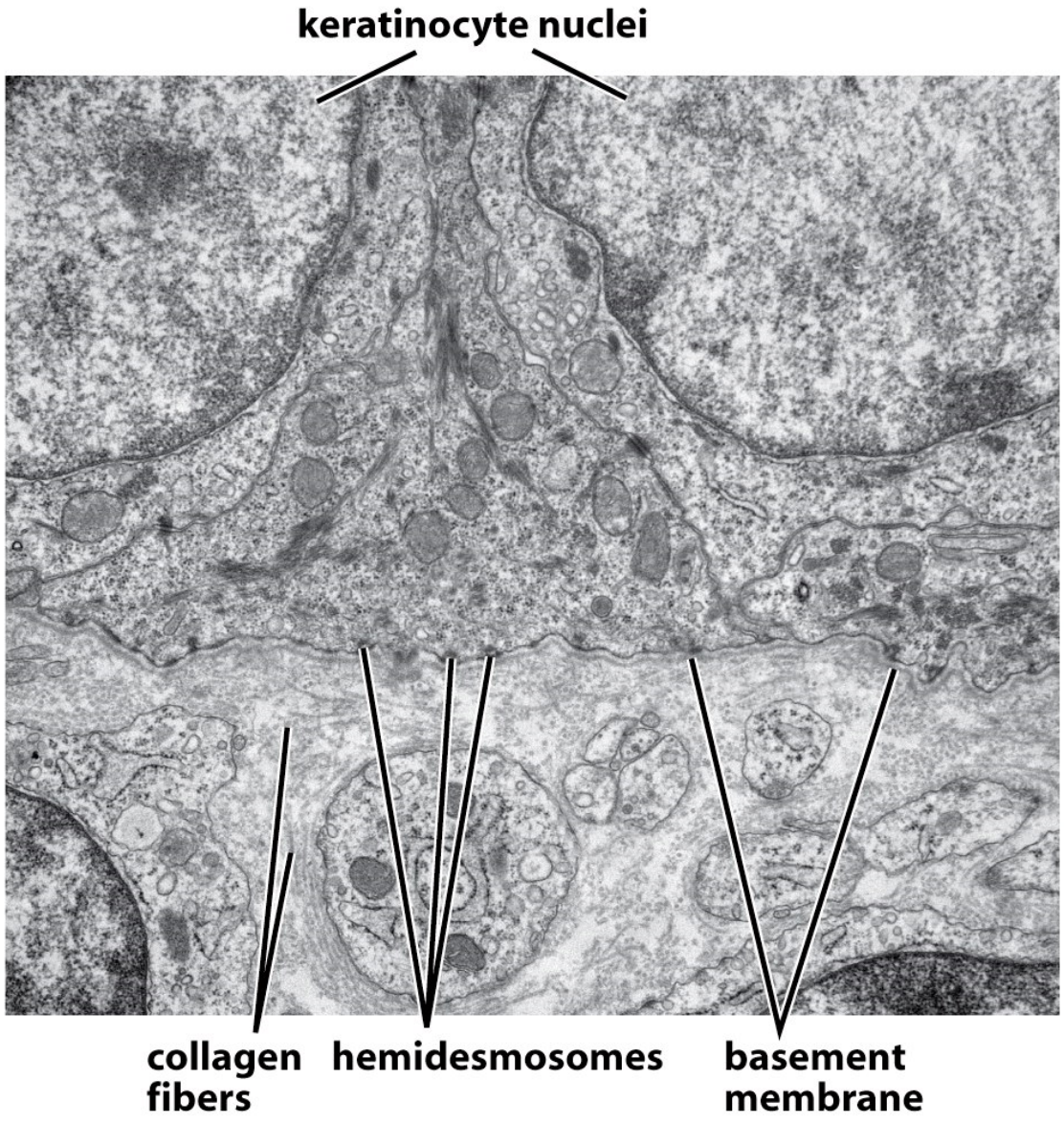
Molecular organization of hemidesmosomes



Alberts et al fig. 19-53

Integrins are connected to a complex web of filaments of the extracellular matrix.

Hemidesmosomes



GAP JUNCTION

Gap junctions create gaps that connect animal cells.

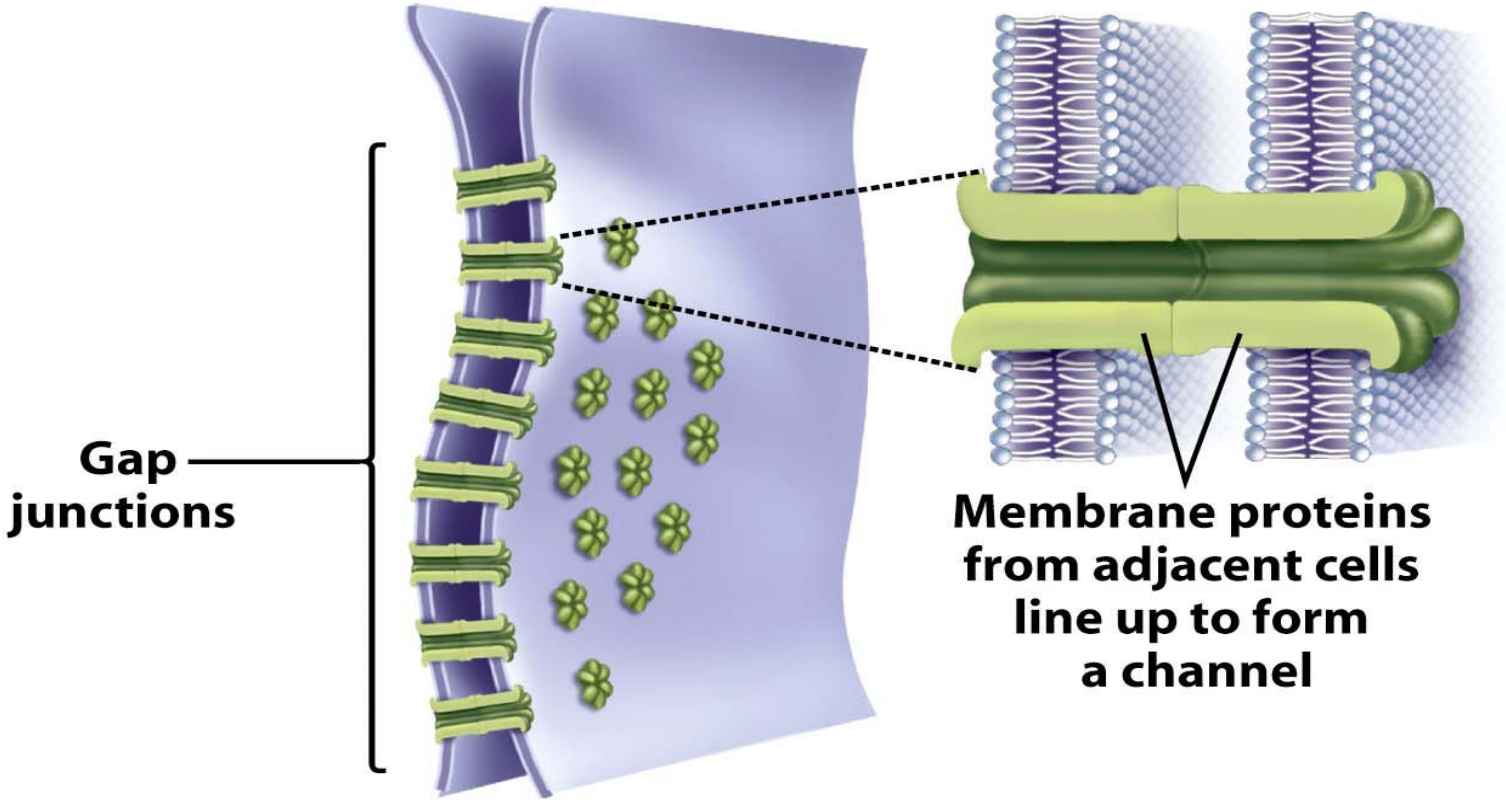
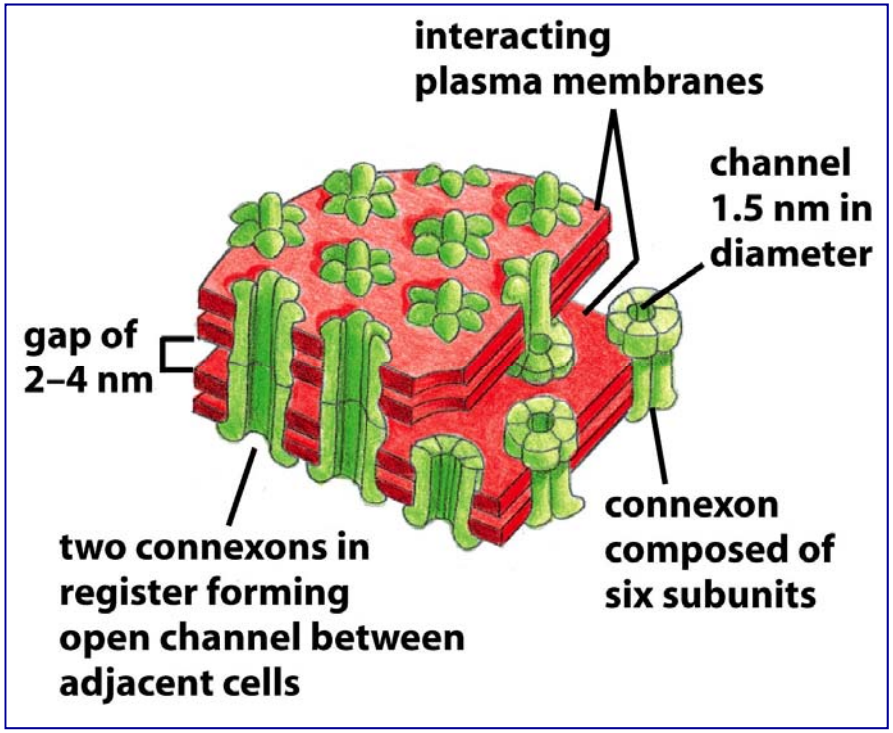
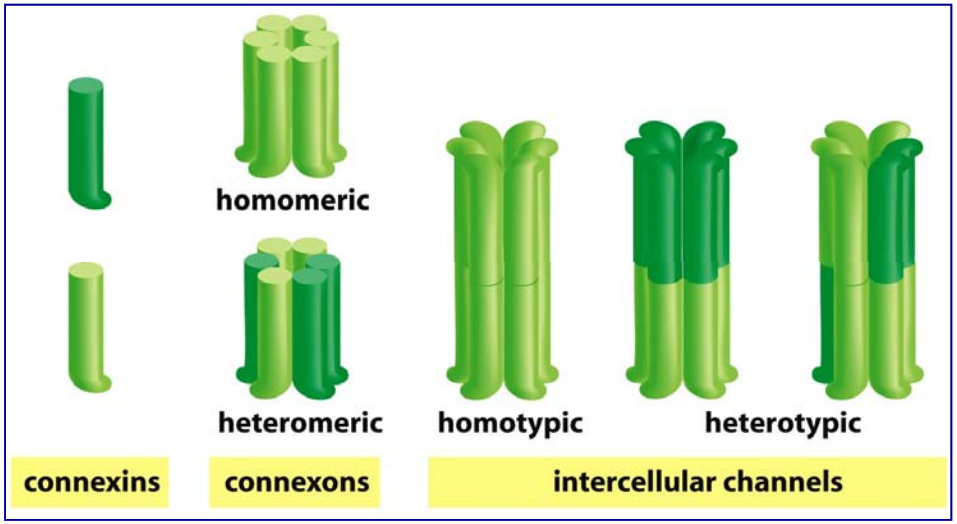


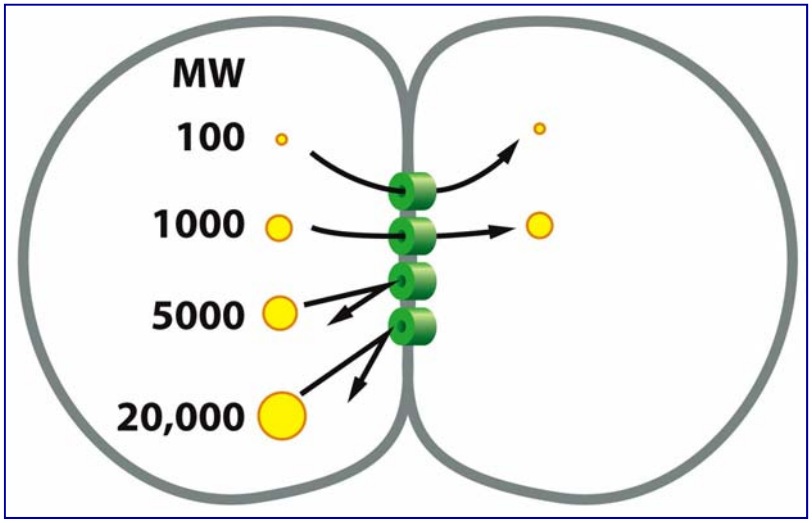
Figure 8-13b part 2 Biological Science, 2/e

GAP JUNCTION



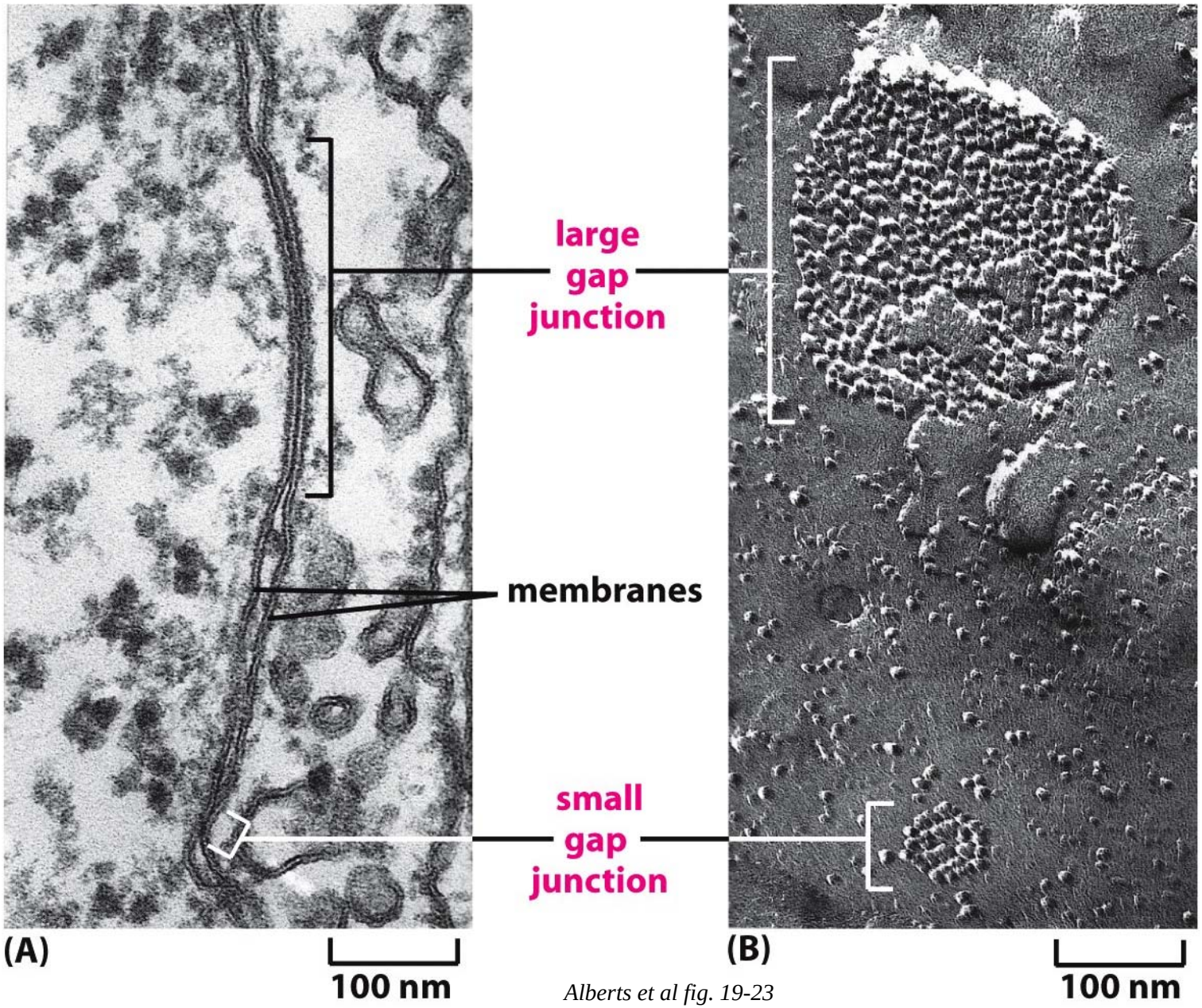


- The gap junctions are unstable
- Function: electric coupling and metabolic cooperation

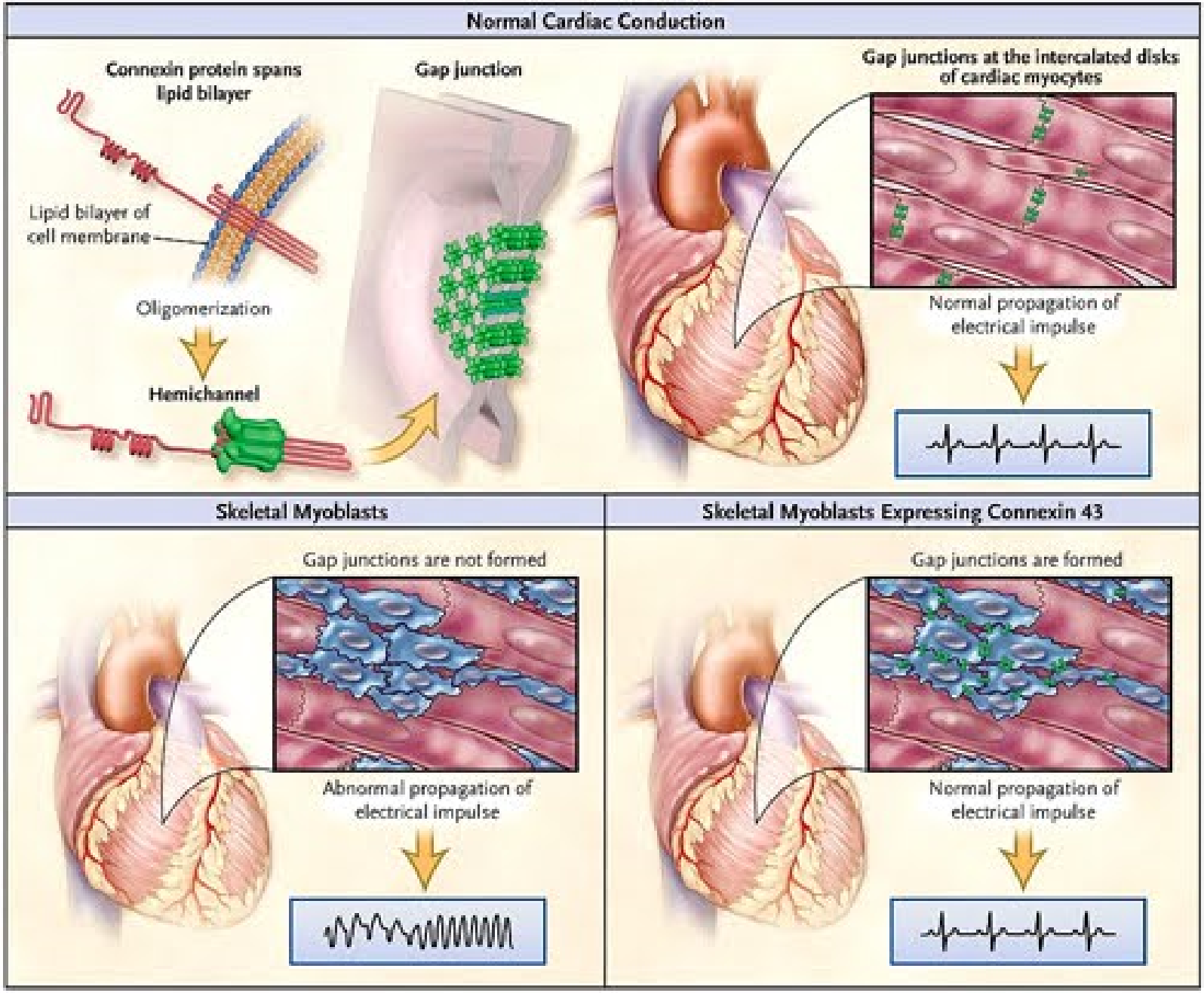


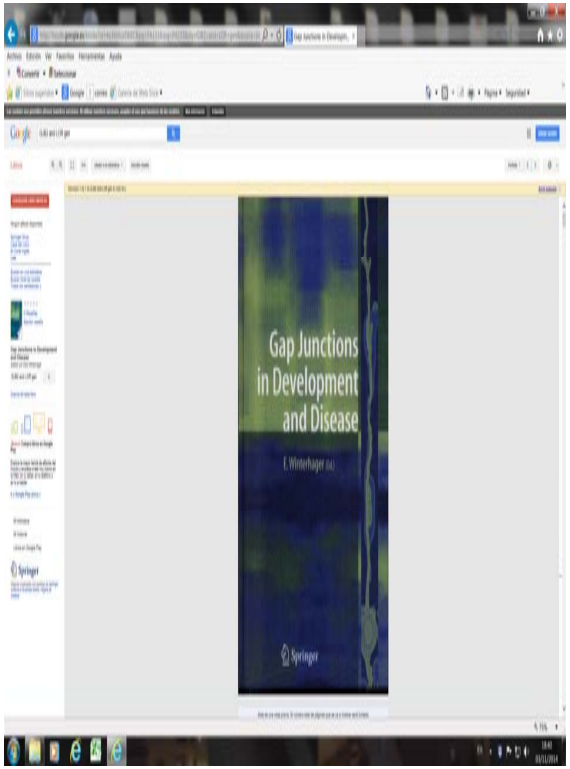
Alberts et al fig. 19-24,25

GAP JUNCTION



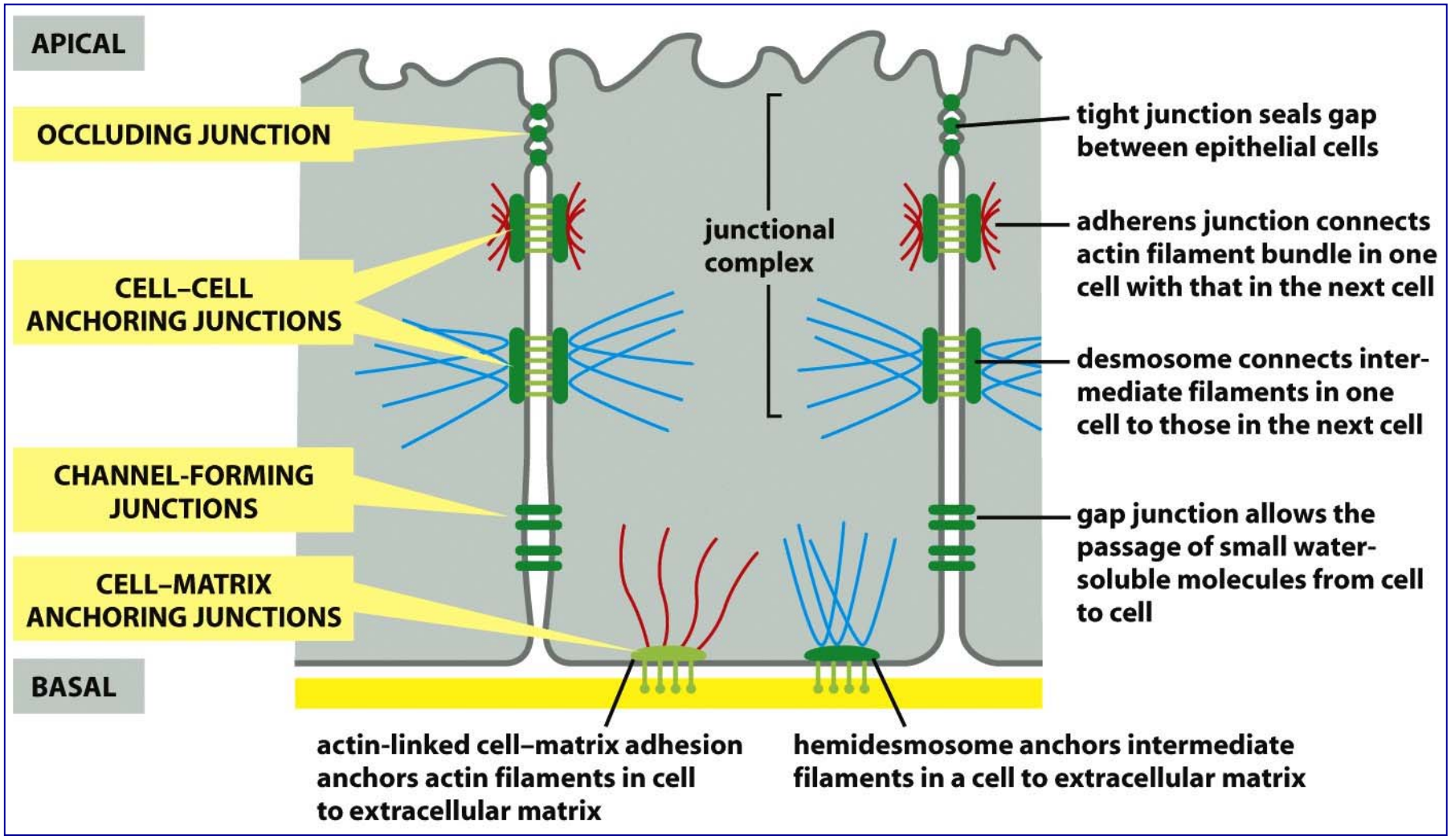
Alberts et al fig. 19-23


Normal Cardiac Conduction
Connexin protein spans lipid bilayer
Lipid bilayer of cell membrane
Oligomerization
Hemichannel
Gap junction
Gap junctions at the intercalated disks of cardiac myocytes
Normal propagation of electrical impulse
Skeletal Myoblasts
Gap junctions are not formed
Abnormal propagation of electrical impulse
Skeletal Myoblasts Expressing Connexin 43
Gap junctions are formed
Normal propagation of electrical impulse


Gap Junctions
in Development
and Disease
Springer

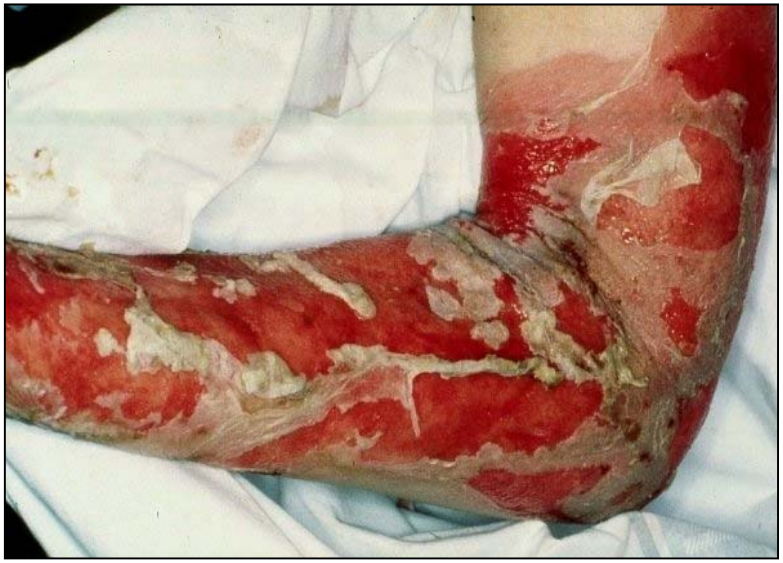

Gap Junctions
MOLECULAR BASIS OF CELL COMMUNICATION
IN HEALTH AND DISEASE
Camillo Peracchia

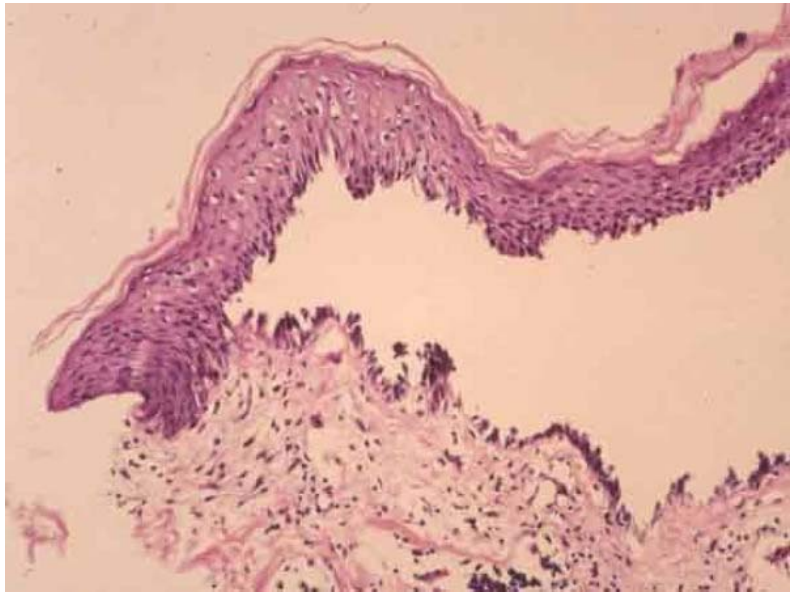
CELL JUNCTIONS IN EPITHELIA

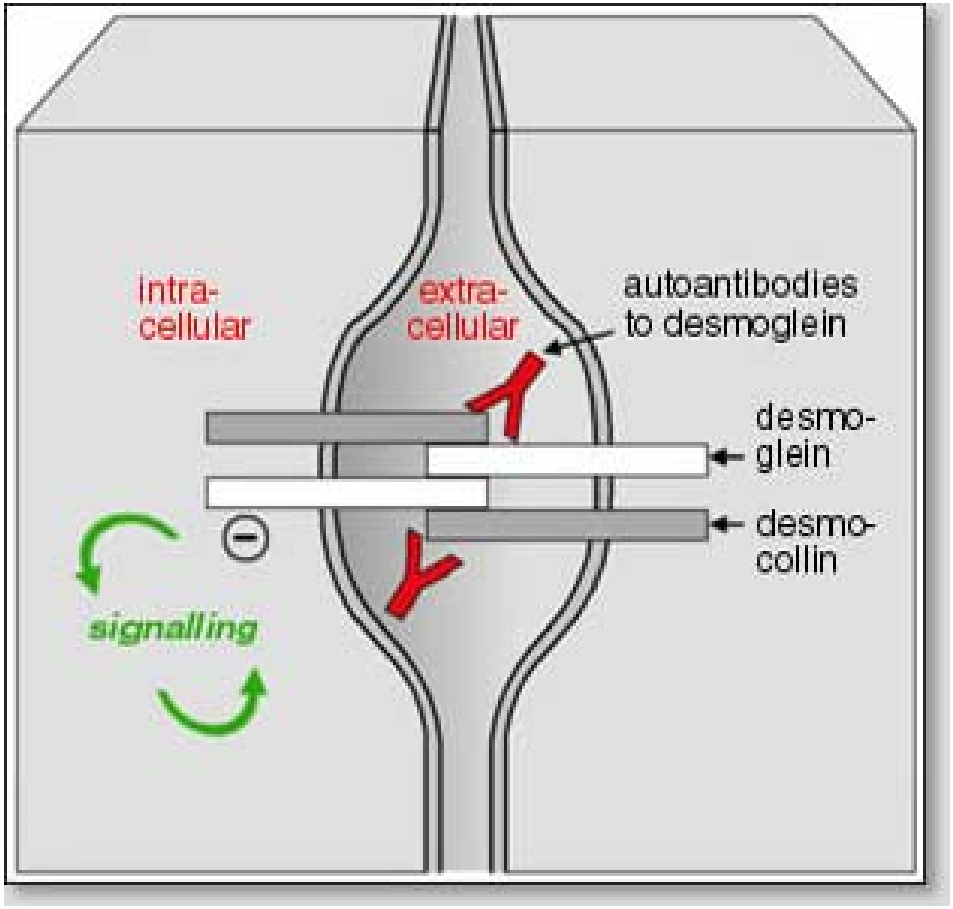


Alberts et al fig. 19-2

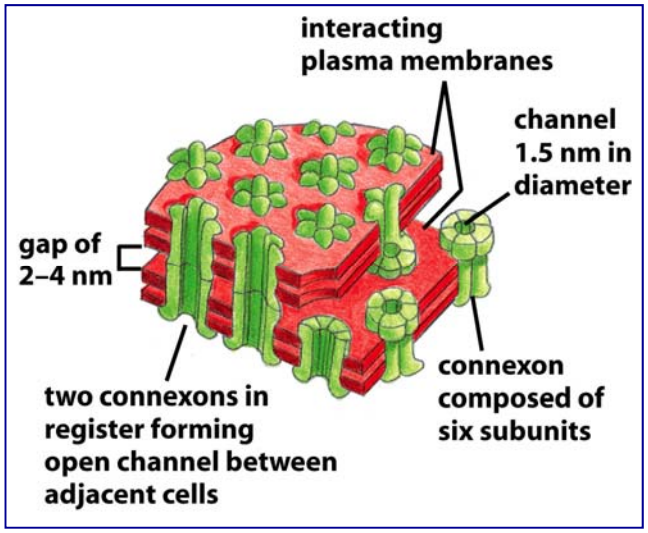
Desmosomes: Pemphigus

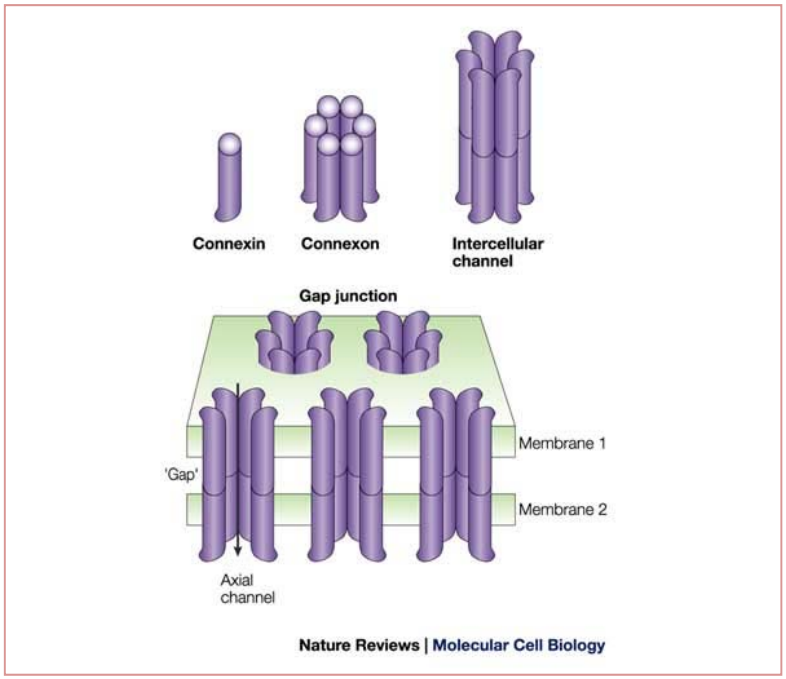


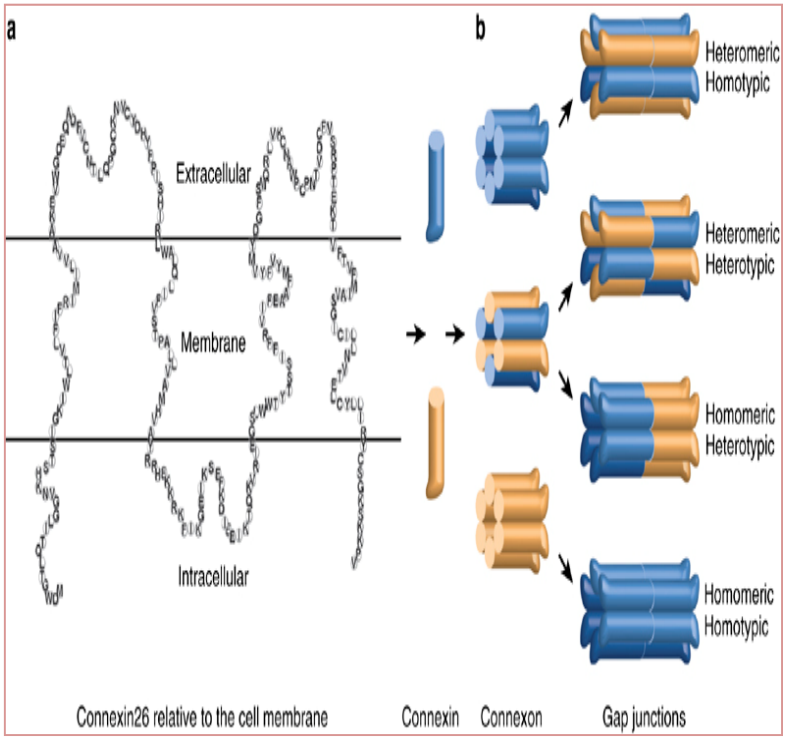


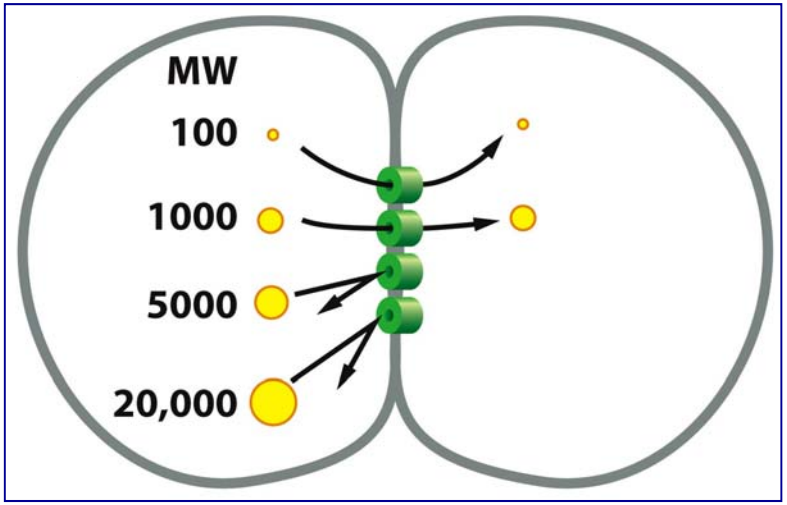


GAP JUNCTION









CELL MEMBRANE, INTERACTION WITH THE ENVIRONMENT AND ADHESION MOLECULES

1. Introduction
2. Extracellular matrix
3. Adhesion molecules
    - Generalities
    - Cadherins
    - Selectins
    - Integrins
    - IGSF-CAM

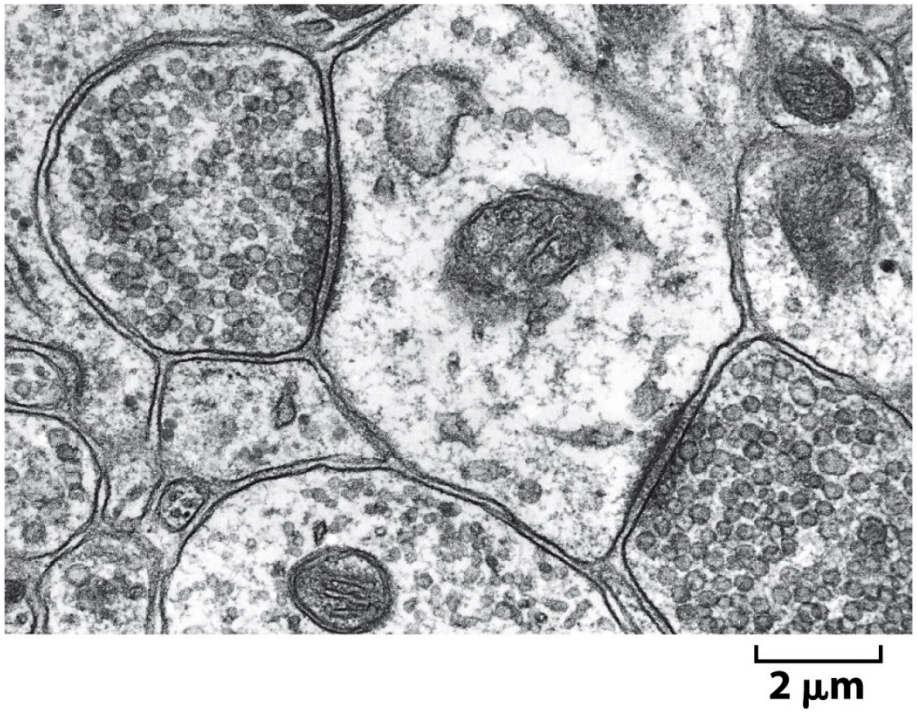


Figura 19-22a *Biología molecular de la célula*, quinta edición (© Garland Science 2008 y Ediciones Omega 2010)

1. INTRODUCTION

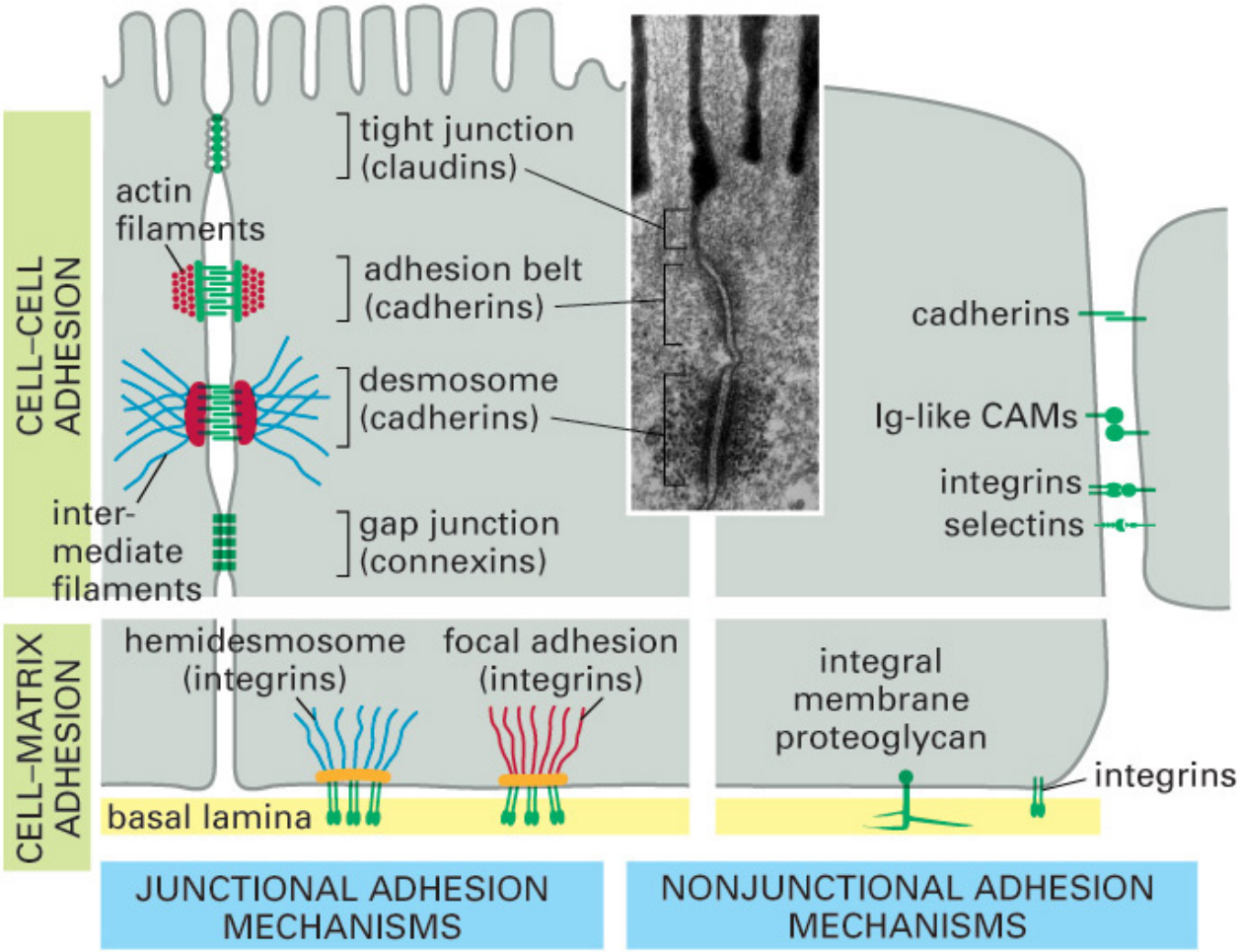
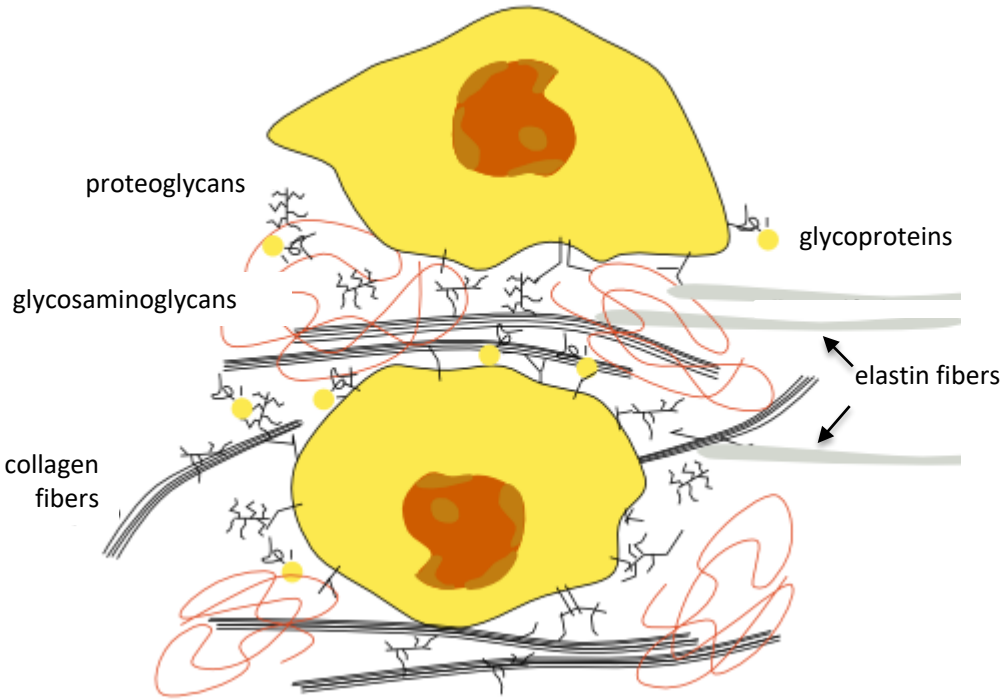


Figure 19-32 *Molecular Biology of the Cell, 4th edition* (© Garland Science 2002)

2. EXTRACELLULAR MATRIX

Set of extracellular substances that give cohesion and strength to the tissues

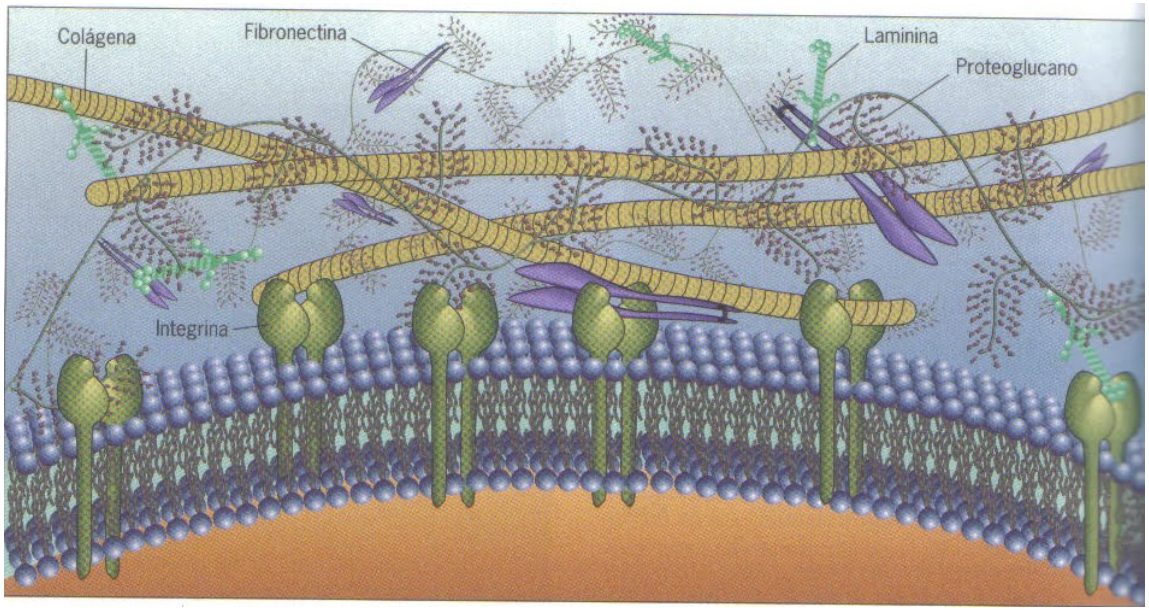


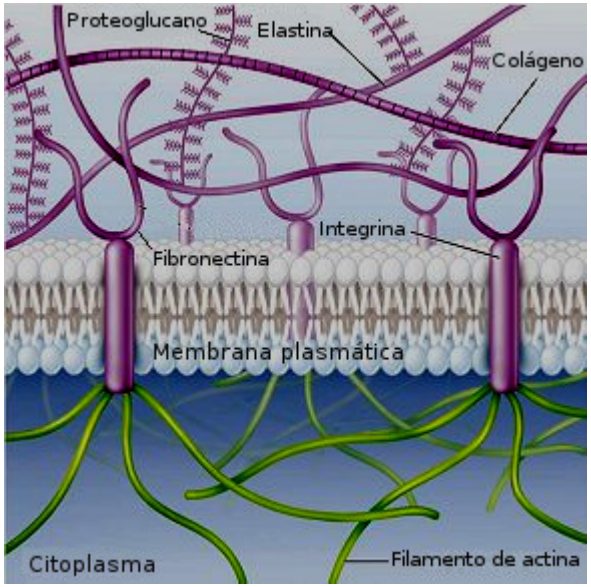
<u>Fibers</u>

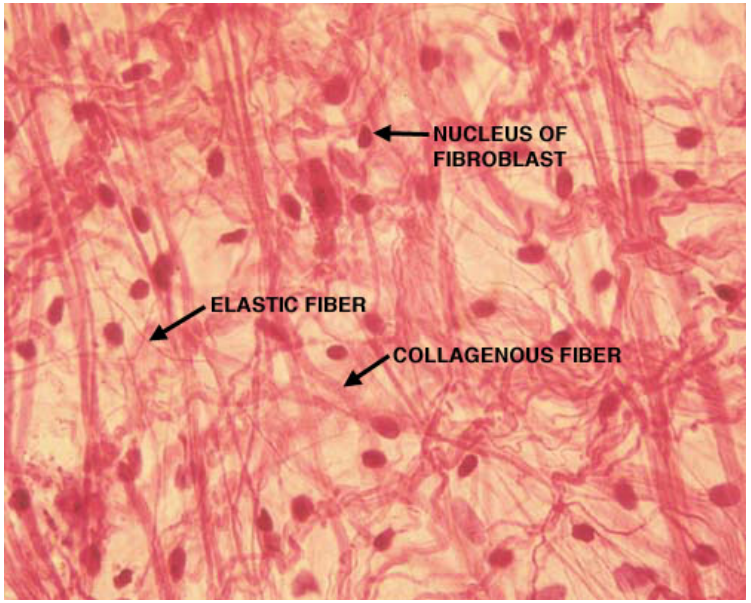
- **Collagenous**
- **Reticular fibers**
- **Elastic fibers**

<u>Ground substance</u>

- **Glycosaminoglycans**
- **Proteoglycans**
- **Adhesive glycoproteins**
- **Water, salts ...**


Colágena
Fibronectina
Laminina
Proteoglucano
Integrina


Proteoglucano
Elastina
Colágeno
Integrina
Fibronectina
Membrana plasmática
Citoplasma
Filamento de actina

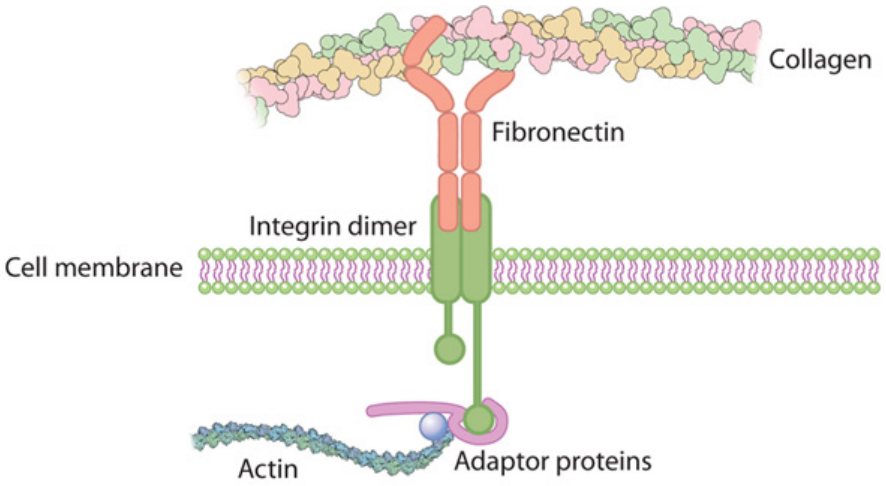

NUCLEUS OF FIBROBLAST
ELASTIC FIBER
COLLAGENOUS FIBER

Adhesive glycoproteins

Fibronectin
Laminin
……

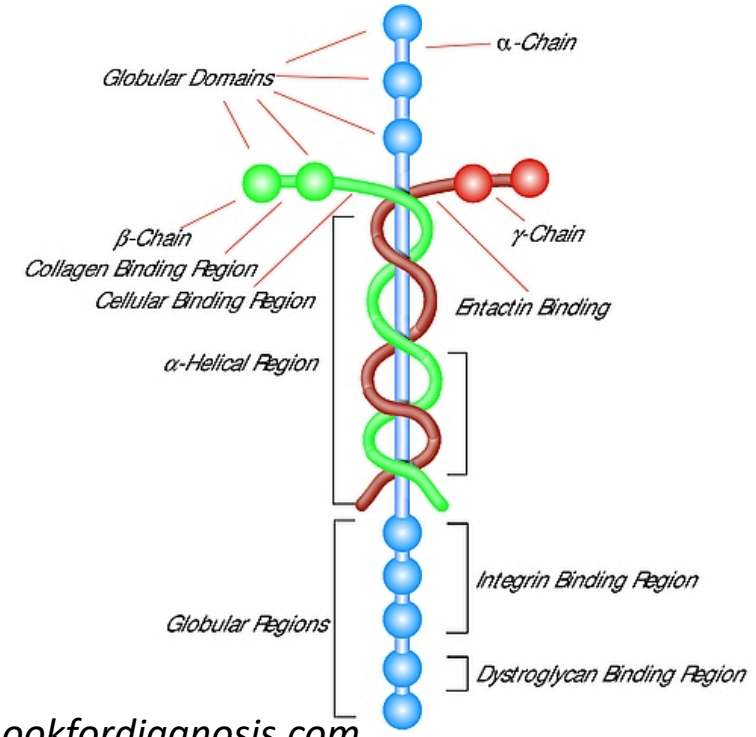
Integrins

Fibronectin



Nature.com

Laminin



Lookfordiagnosis.com

Membrane or basal lamina

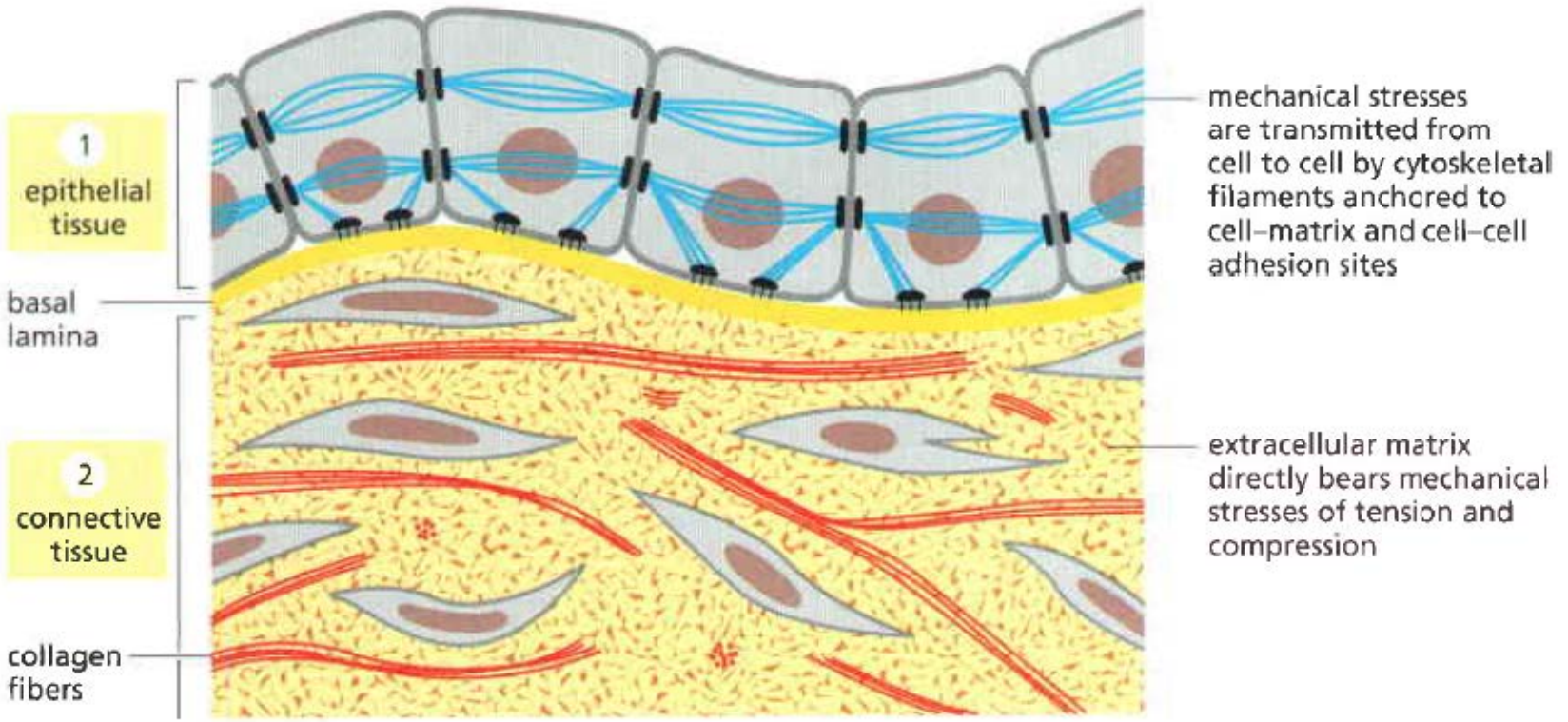
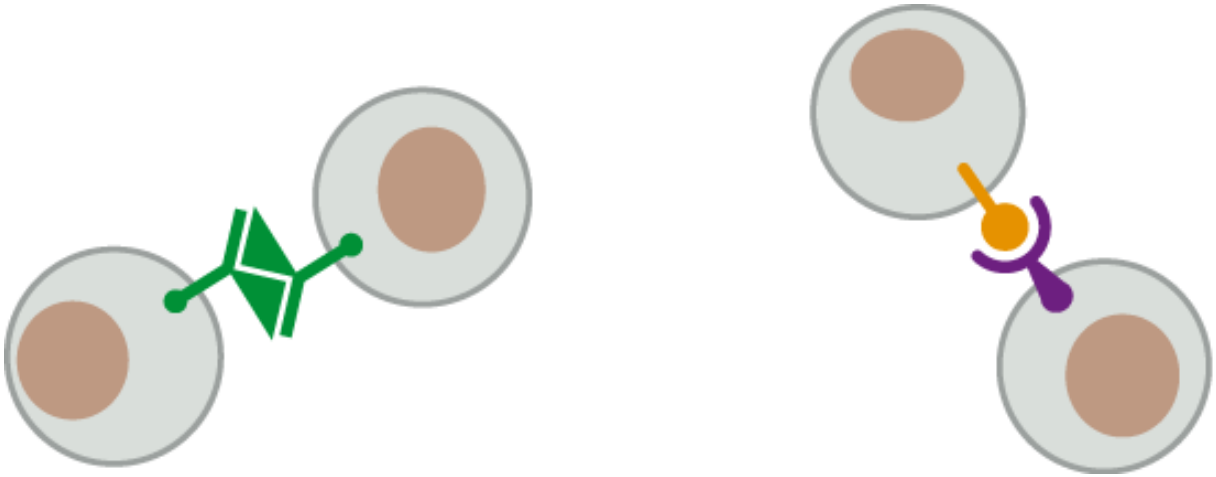


Figure 19-1 *Molecular Biology of the Cell, 5th edition* (© Garland Science 2008)

3. ADHESION MOLECULES (CAMs)

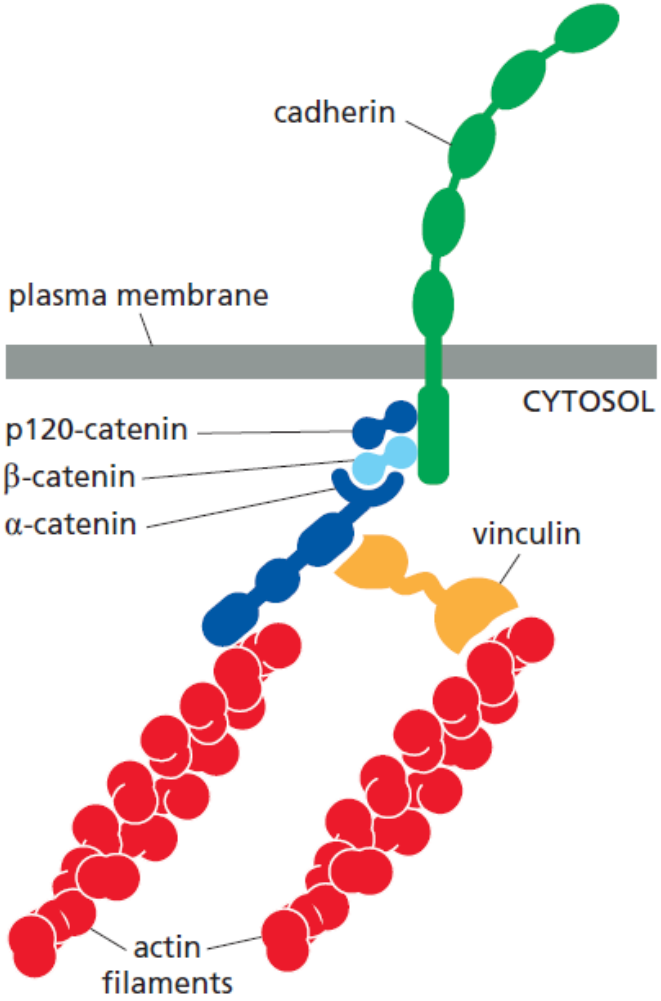
- Protein conformational status
- Type of interaction: cell-cell or cell-matrix
- Divalent ions involvement: Ca++, Mg++, none
- Type of union: homophilic or heterophilic
- Stability of the union: stable or transient



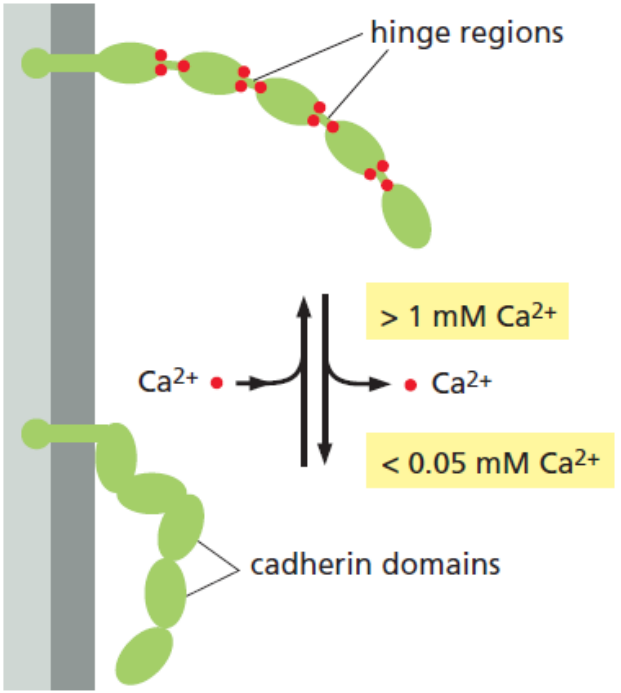
HOMOPHILIC HETEROPHILIC

Figure 19-8 *Molecular Biology of the Cell, 5th edition* (© Garland Science 2008)

3.1 CADHERINS. Structure



- Single pass transmembrane proteins
- Length: 700-750 aminoacids
- 5-6 domains in the extracelular región, similar to Ig



- Interaction with neighbour cell: homophilic

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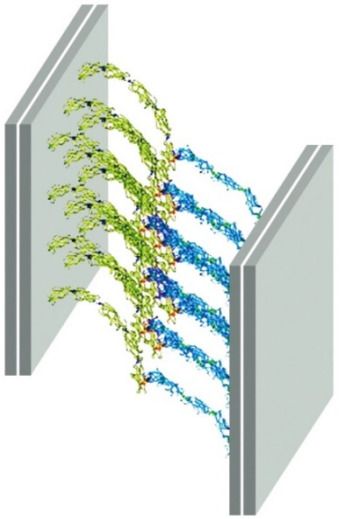
CADHERINS. Features



Molecular Biology of the Cell, 5th edition (© Garland Science 2008)

- Form a superfamily. Types:

  Classic cadherins

  - Cadherin E, in many ephitelia
  - Cadherin N, in muscle, nervous and cristalline cells
  - Cadherin P, in placenta and epidermis

  Non-classic cadherins

  - Cadherins in desmosomes (desmoglein, desmocollin
  - Protocadherins in the brain (more tan 50 different types)
  - Cadherin T (no adhesion role)

- Beyond the adhesión role, many are signal transductors
- They are expressed (at least 1) in all the vertebrate cells
- They are the main cell-cell adhesion molecules during embryonary development

CADHERINS. Pathology

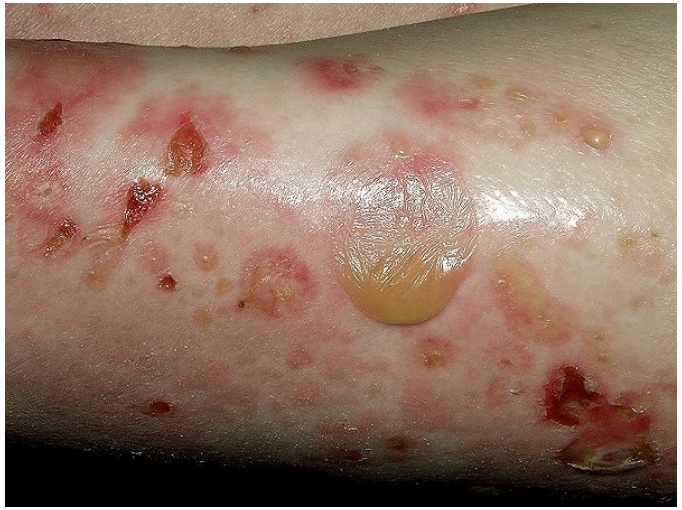
Pemphigus vulgaris

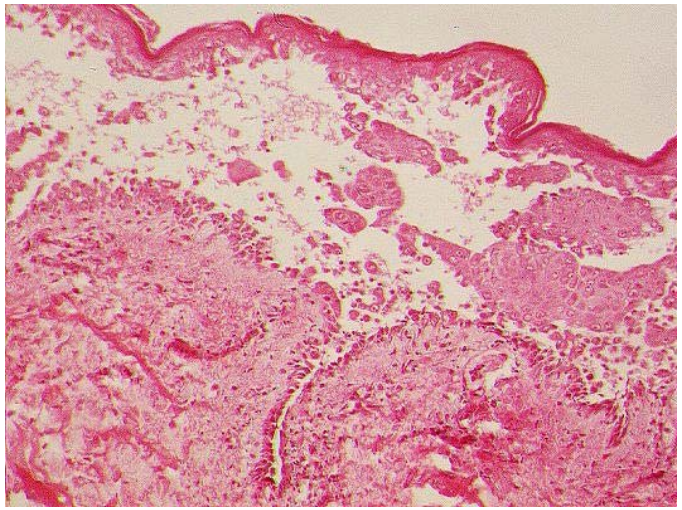
- Unknown etiology.
  - Autoimmune disease.
  Autoantibodies: anti-desmoglein 3, anti-desmoglein 1 (Skin and mucous membranes)

- Destruction of desmosomes (keratinocytes)

- Main Feature: Typical blisters.

- Symptomatic treatment: corticosteroids, immunosuppressants, analgesics and anti-infectives when necessary.





http://entornomedico.blogspot.com.es/2009/04/dermatologia-penfigo-vulgar.html

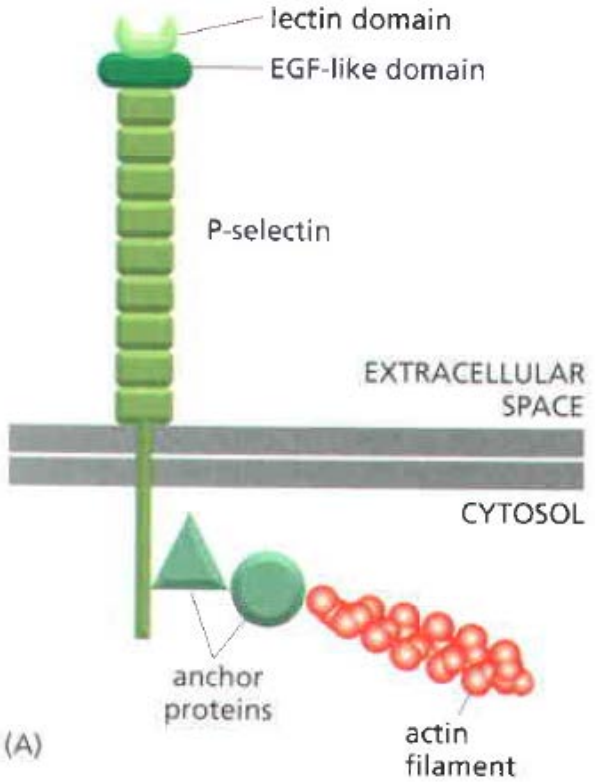
3.2 SELECTINS. Structure.

Single pass transmembrane proteins of superfamily lectins:

- Monomeric
- Ca ++ dependent
- Lectin domain: specific oligosaccharide binds to the cell that interacts with
- Heterophilic union
- Transient adhesion

Types:

- Selectin L (leukocytes)
- Selectin P (platellets and endotelial cells)
- Selectin E, (endotelial cells)



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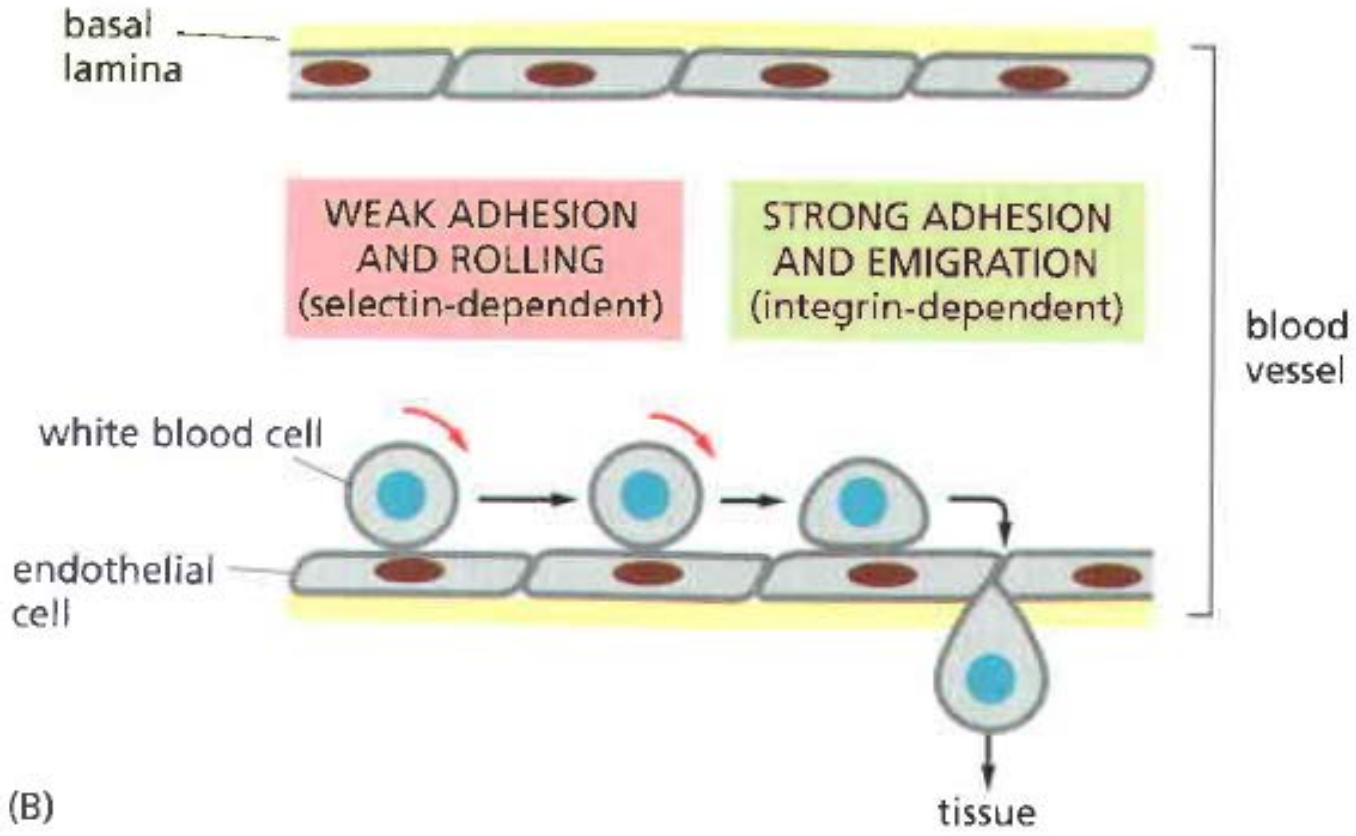
SELECTINS. Function

Role in extravasation: binding of leukocytes to endothelial cells lining the vessel wall



Weak and transient adhesion. Final union by integrins.

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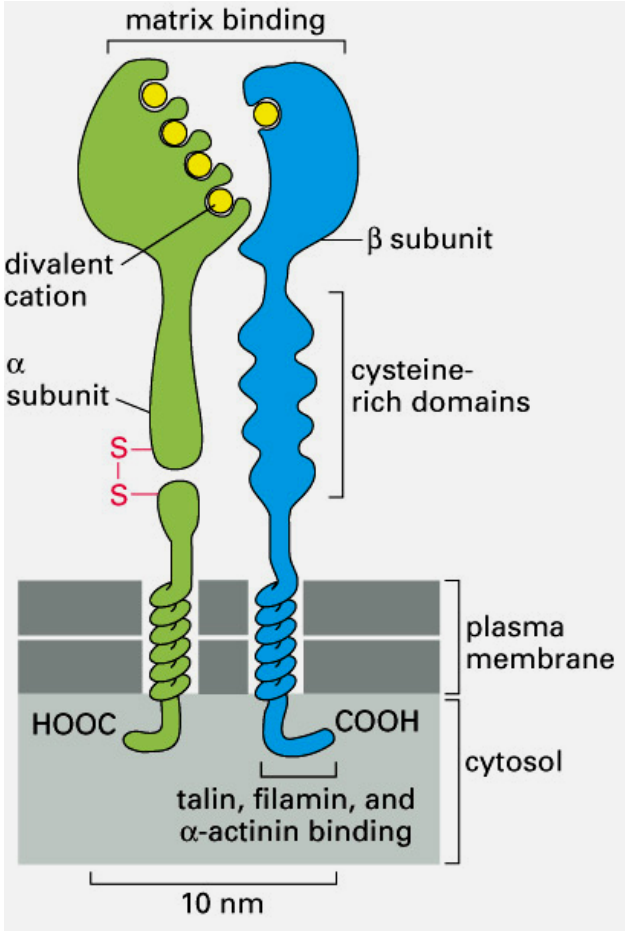
3.3 INTEGRINS. Strcture and features.

- Transmembrane proteins
- Heterodimer: subunit α + β subunit (non-covalent binding)
- Extracellular part: domains binding divalent ions (Ca ++ or Mg ++ depending on the integrin)
- Binding to diverse ligands (heterophile and promiscuous)
- STABLE: hemidesmosome (cell-matrix)
- TRANSIENT (low affinity, high number): leukocyte extravasation (cell-cell), focal unions (cell-matrix)



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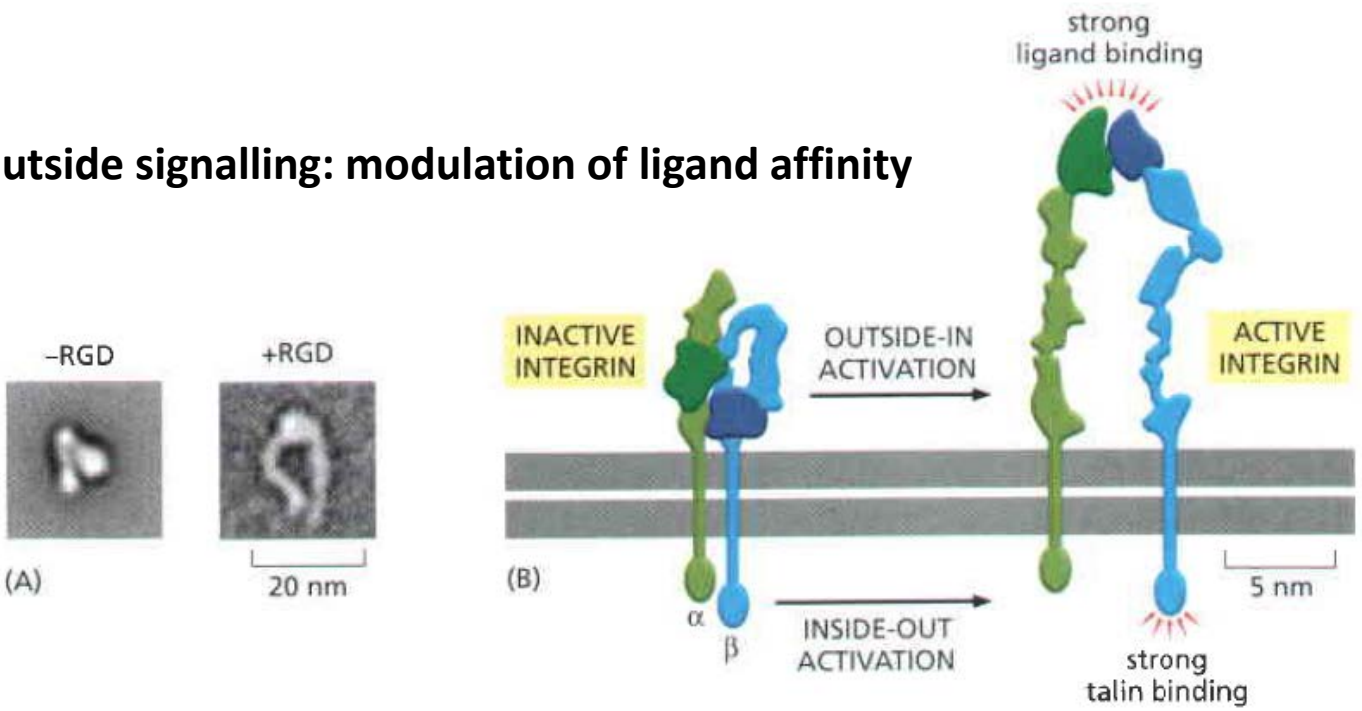
Main cell-matrix adhesion molecules

INTEGRINS

Heterophilic and promiscuous union:

- Heterodimers composed of at least 9 types of subunits β and 24 subunits α (diversity)
- In different cell types, the same integrin may bind to different ligands and vice versa.

Inside-outside signalling: modulation of ligand affinity



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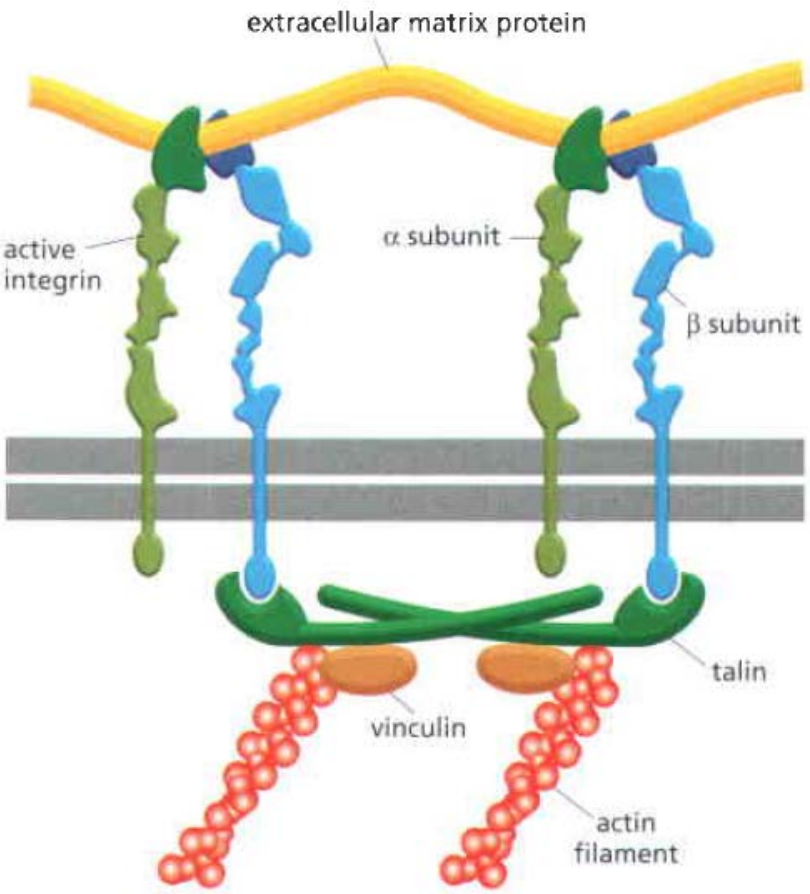
INTEGRINS. FUNCTIONS

- Most integrins bind to actin by binding the β subunit to anchoring proteins as talin, α actinin and filamin (focal joints)
- Connect cell cytoskeleton filaments with the matrix: shape, orientation and cell movement
- Integrin α 6 β 4 in hemidesmosome: connects to intermediate filaments (epithelia)
- Signal transduction: CNS development (growing axons)
- Leukocyte extravasation.



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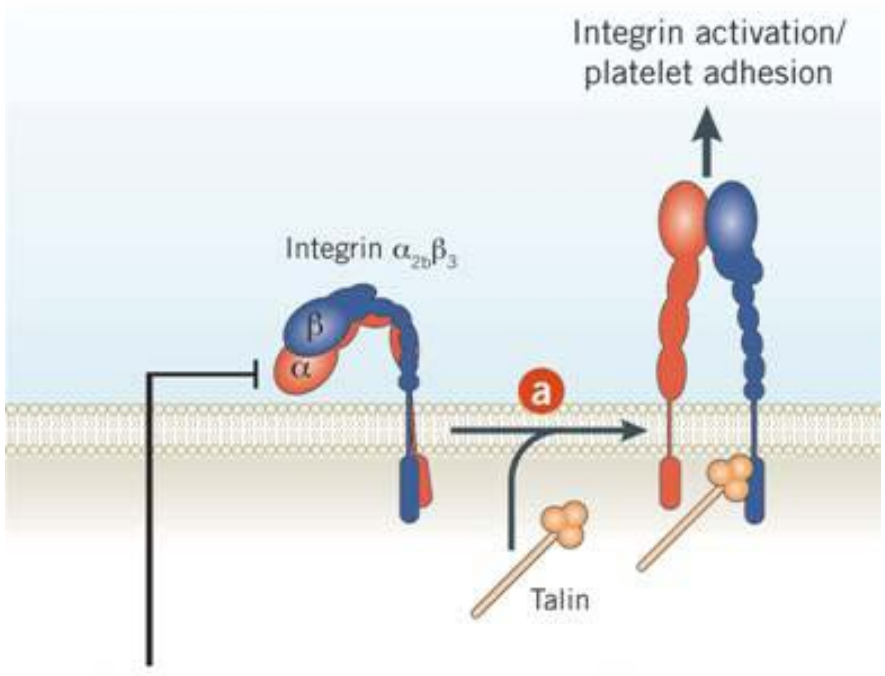
INTEGRINS. Related problems.

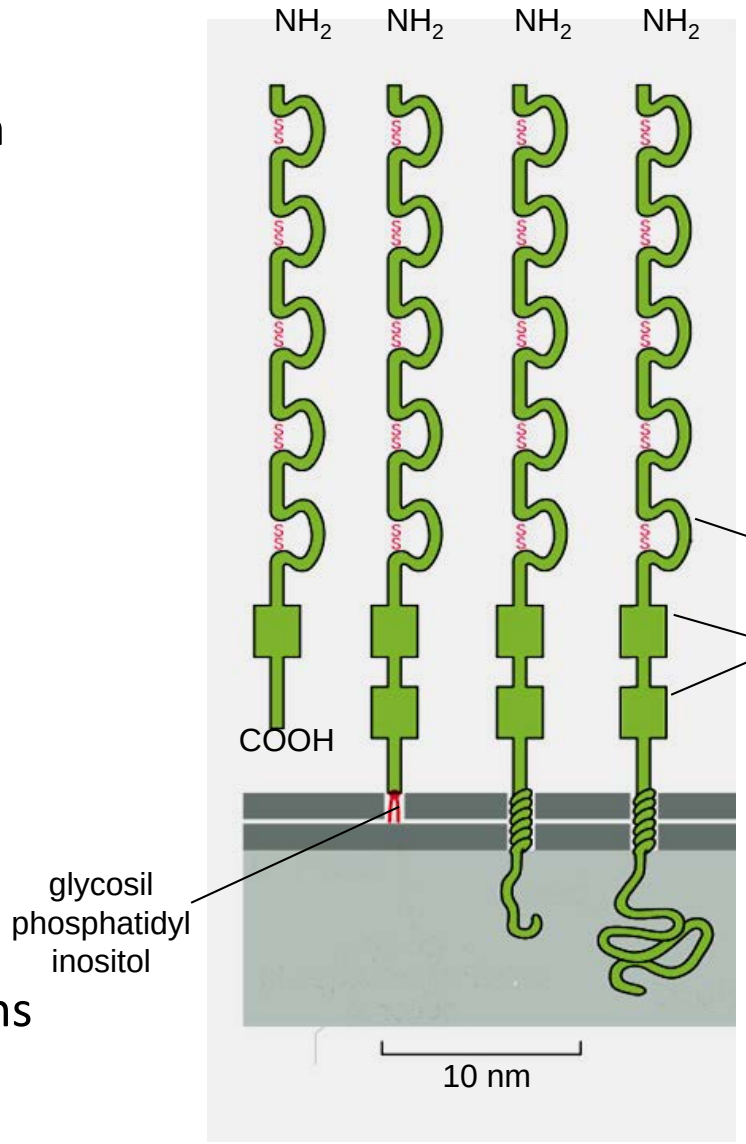
- If β1 integrin is not synthesized, the embryo dies at implantation.
- If integrin α 7 (which is the companion of β 1 in the muscle) is not synthesized, the embryo is viable but has muscular dystrophy.
- Glanzmann's disease. β3 genetic deficiency: blood clotting problems and frequent bleeding



www.biocentury.com

3.4 IgSF-CAM PROTEINS

- Belong to the immunoglobulin superfamily (Ig)
- Transmembrane proteins
- Monomerics
- One or more Ig-like domains
- Ca++ independent
- Cell-cell interaction
- Homophilic/heterophilic unions



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IgSF-CAM PROTEINS

Homophilic unions (CNS)

N-CAM (*neural cell adhesion molecules*)

At least, 20 different forms. 5 extracelular domains homologues to those in Ig.

Less adhesion than cadherins in the same cells (because sialic acid link).

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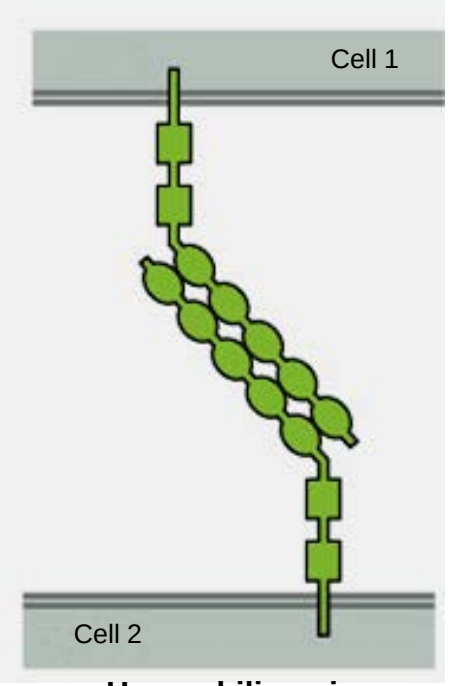
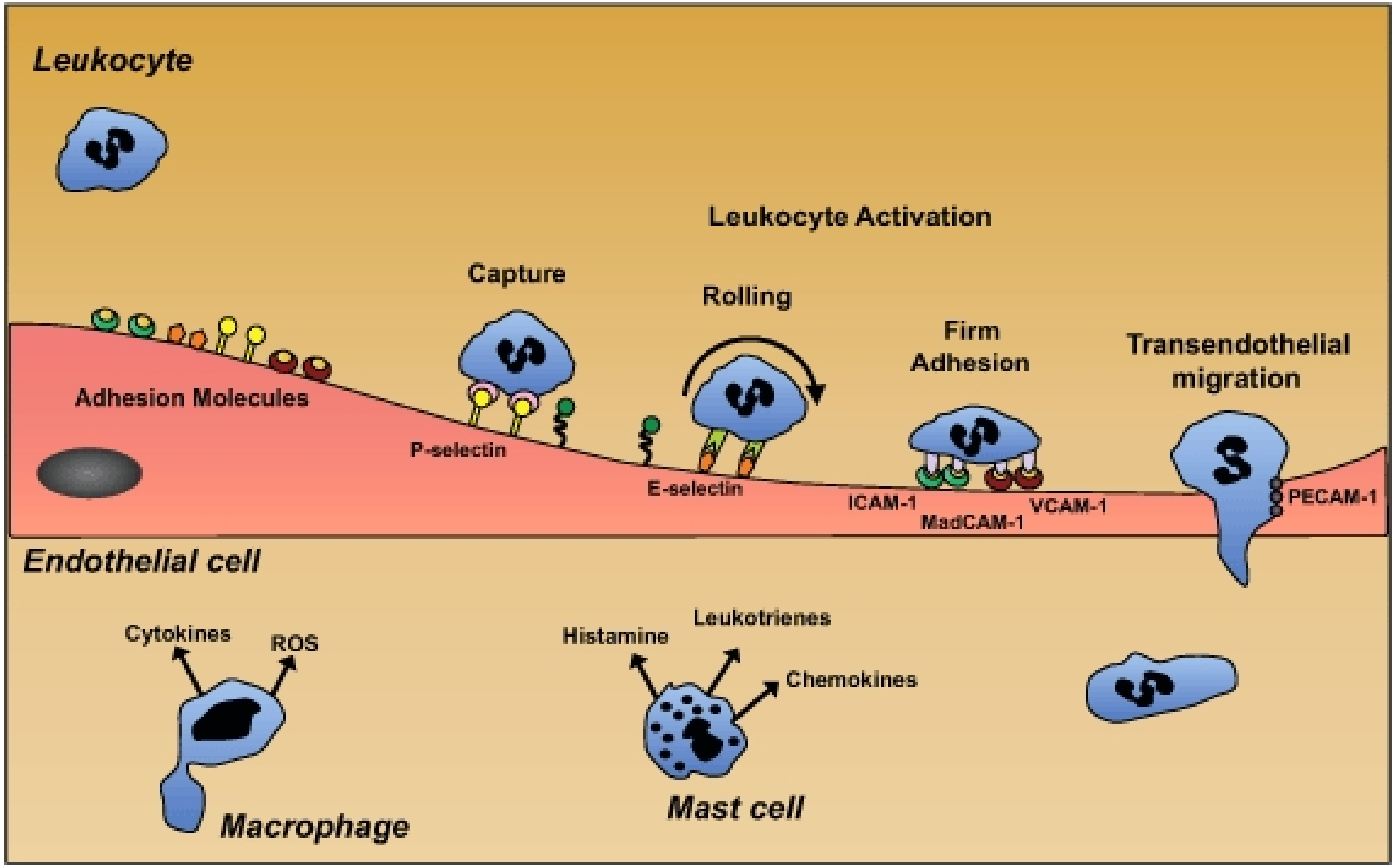


Homophilic union

Heterophilic unions (endothelia)

- I-CAM (*intercellular adhesion molecules*) and other
- They bind to leukocytes integrins and selectins


Leukocyte
Leukocyte Activation
Capture
Rolling
Firm Adhesion
Transendothelial migration
Adhesion Molecules
P-selectin
E-selectin
ICAM-1
VCAM-1
MadCAM-1
PECAM-1
Endothelial cell
Cytokines
ROS
Macrophage
Histamine
Leukotrienes
Chemokines
Mast cell

	Conformation	Interaction	Divalent ions	Union	Stability
Cadherins	Dimeric	C-C	Ca2+	Homophilic	Stable
Selectins	Monomeric	C-C	Ca2+	Heterophilic	Transient
Integrins	Dimeric	C-M	Ca2+ o Mg2+	Heterophilic	Stable/ Transient
	Dimeric	C-C	Ca2+ o Mg2+	Heterophilic	Transient
IGSF-CAM	Ig-like	C-C	Not needed	Homophilic/ Heterophilic	Transient

FUNCTIONS OF THE CELL MEMBRANE

Information exchange

- Signaling cells
- Target cells
- Classes of signaling cells
- Classes of cell surface receptors
- Classes of intracellular receptors
- Complexity of information exchange

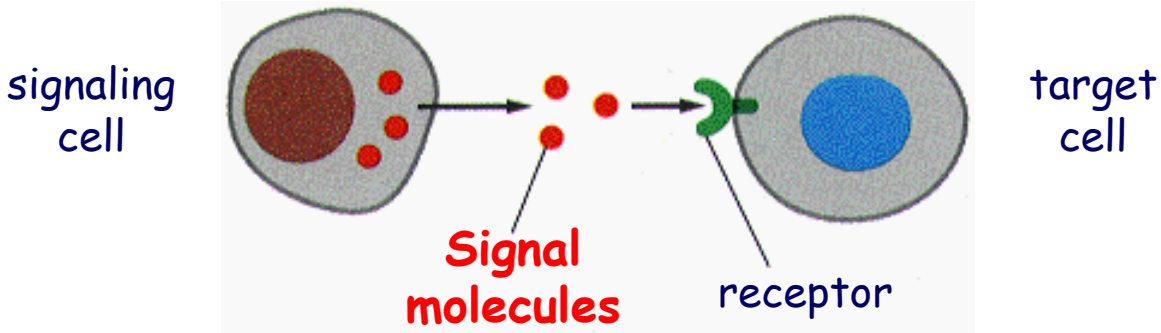
Substance exchange

- Transport through the lipid phase
- Transport through the protein phase
- Cytosis
  - Exocytosis
  - Endocytosis
  - Transcytosis

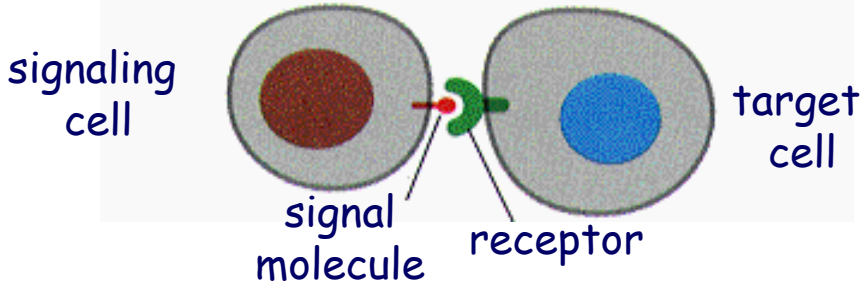
INFORMATION EXCHANGE

Signaling cells

Signaling by secreted molecules



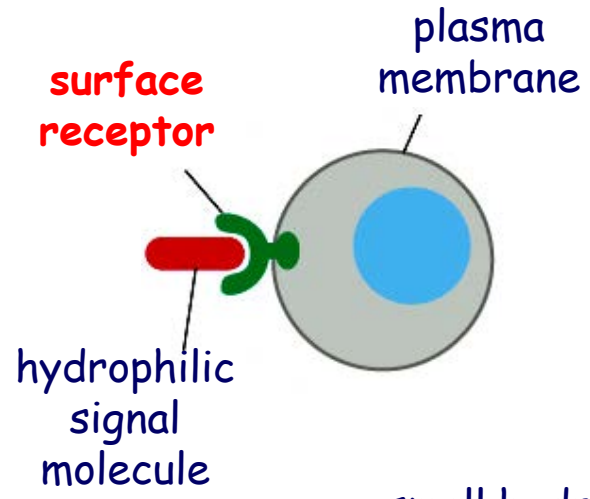
Contact-dependent signaling



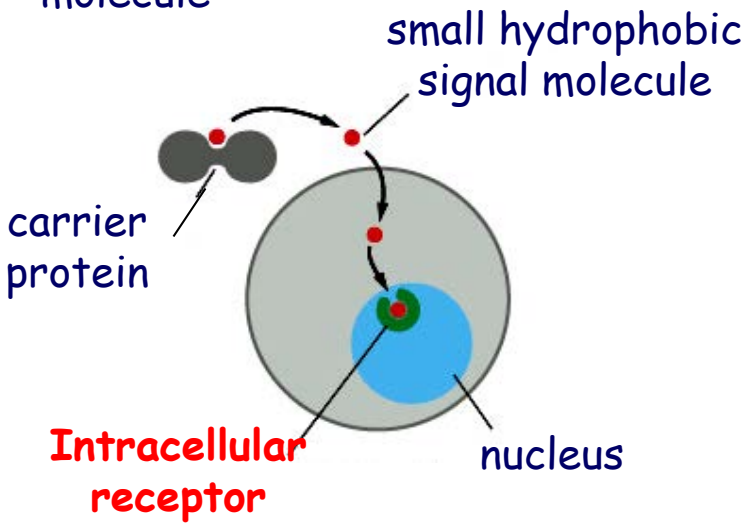
INFORMATION EXCHANGE

Target cells

Cell-surface receptors

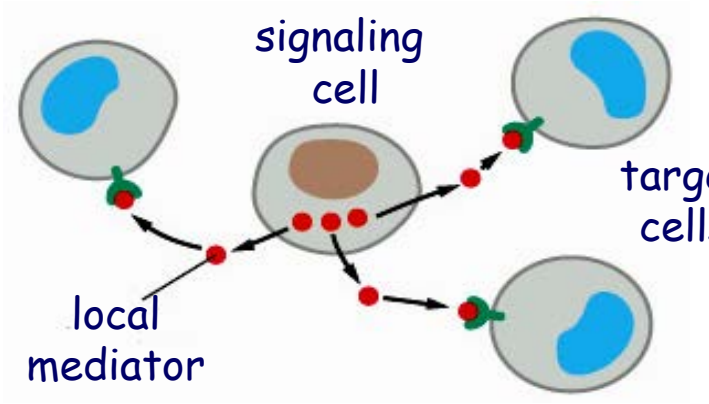


Intracellular receptors

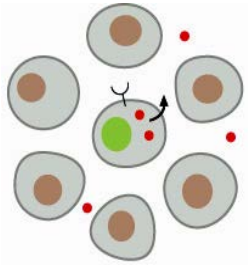


Classes of signaling cells

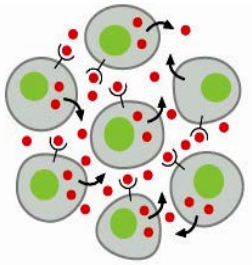
PARACRINE



AUTOCRINE

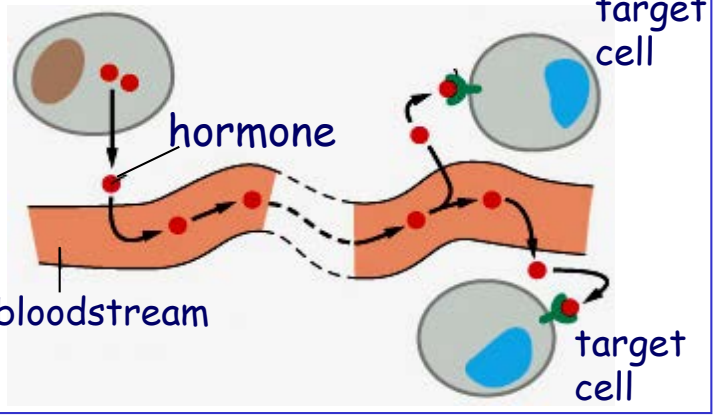


A single signaling cell receives a weak autocrine signal

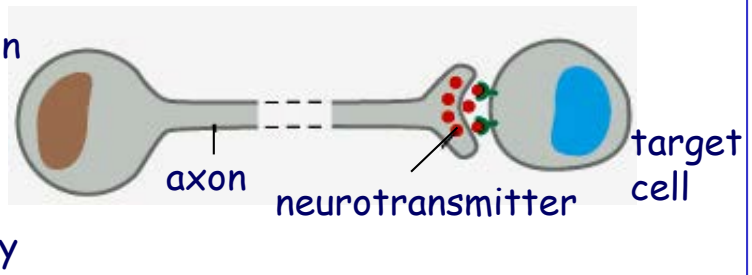


A group of the same signaling cells receive a strong autocrine signal

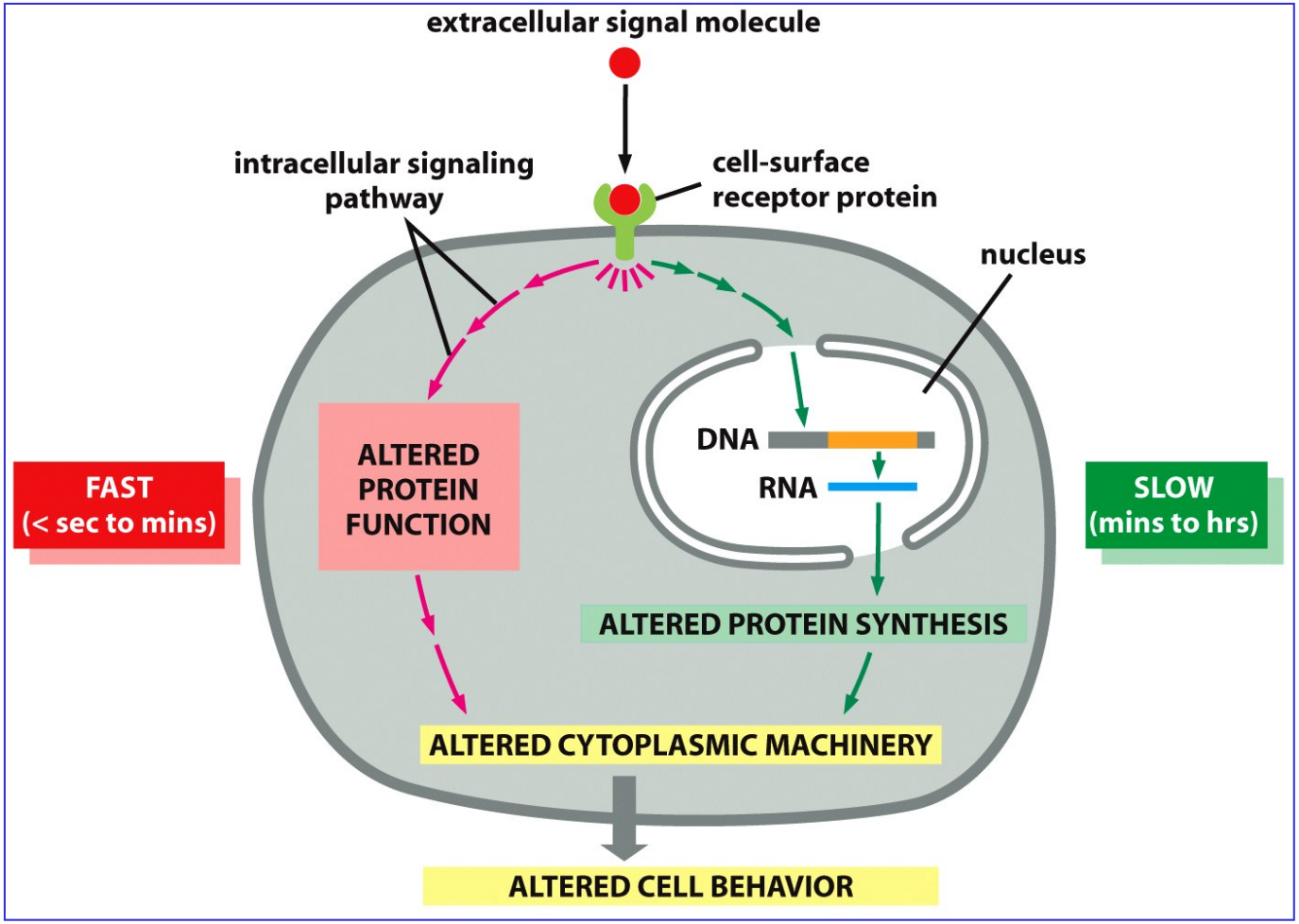
ENDOCRINE



SYNAPTIC SIGNALING



The extracellular signals can work fast or slowly

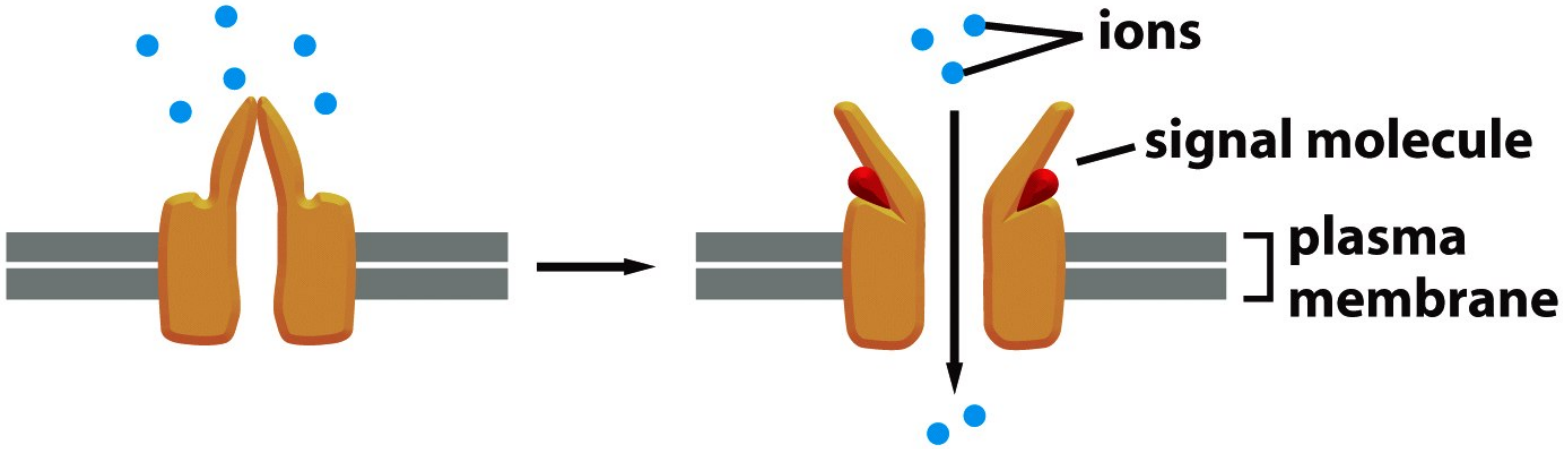


- The speed of response to an extracellular signal depends not only the mechanism of signal distribution, but also the mechanism of response in the target cell

Cell-surface receptors

There are three main classes:

1.- **ION-CHANNEL-COUPLED RECEPTORS**

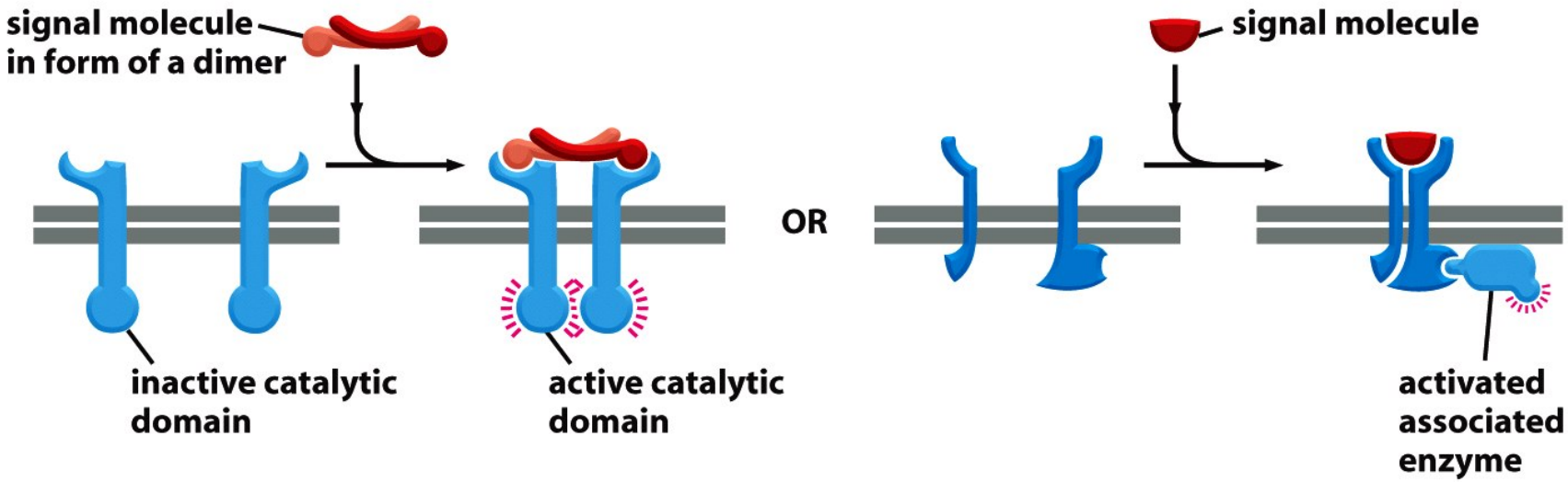


Is mediated by a small number of neurotransmitters that open or close transiently an ionic channel formed by a protein to which they are attached. Briefly alter the ionic permeability

Cell-surface receptors

There are three main classes:

2.- ENZYME-COUPLED RECEPTORS



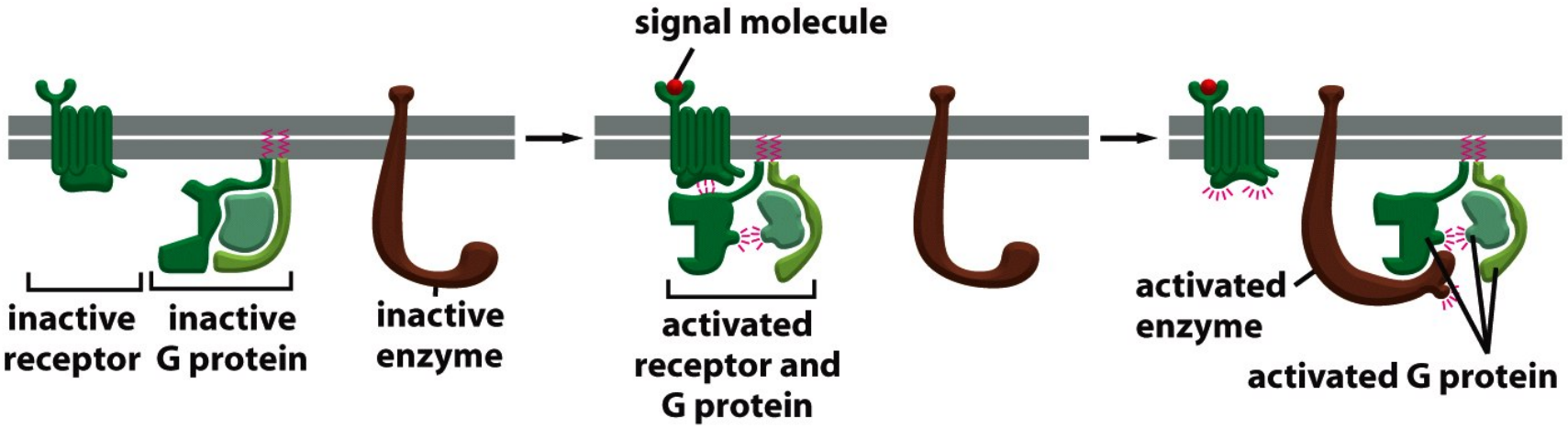
Is a transmembrane receptor, where the binding of an extracellular ligand causes enzymatic activity on the intracellular side.

Examples :
Receptor tyrosine kinase, as different growth factor receptors.
Serine/threonine-specific protein kinase,as in bone morphogenetic protein.

Cell-surface receptors

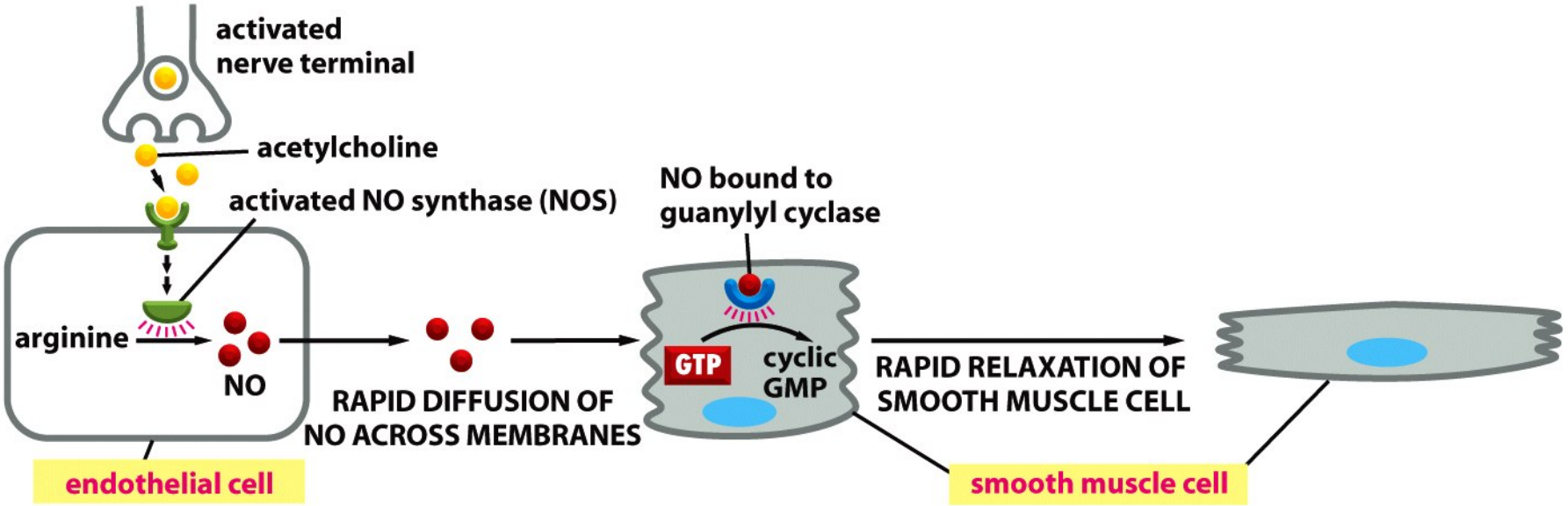
There are three main classes:

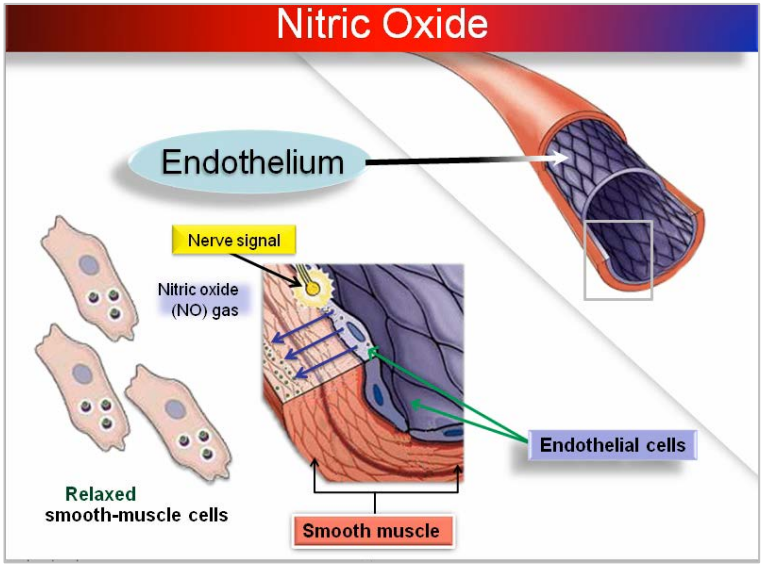
3.- **G-PROTEIN-COUPLED RECEPTORS**

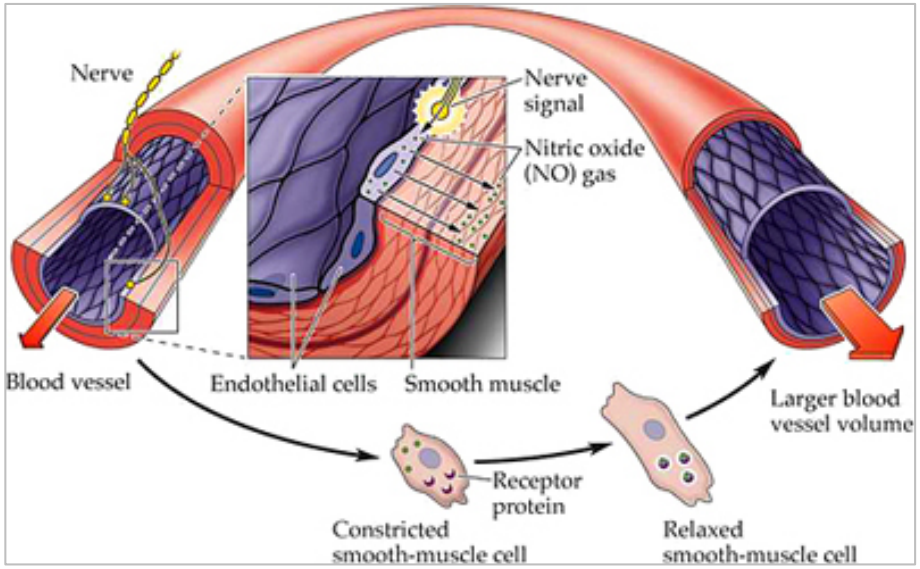


G protein-coupled receptors (GPCRs), constitute a large protein family of receptors that sense molecules outside the cell and activate inside signal transduction pathways and, ultimately, cellular responses

Intracellular receptors: *Cytoplasmic enzymes*

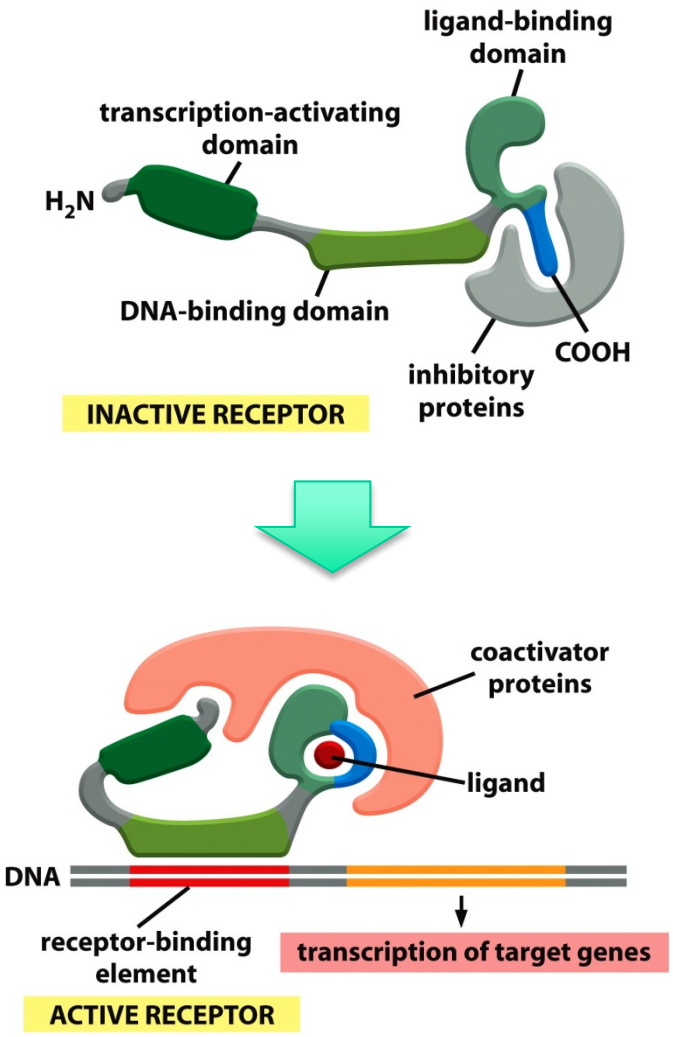


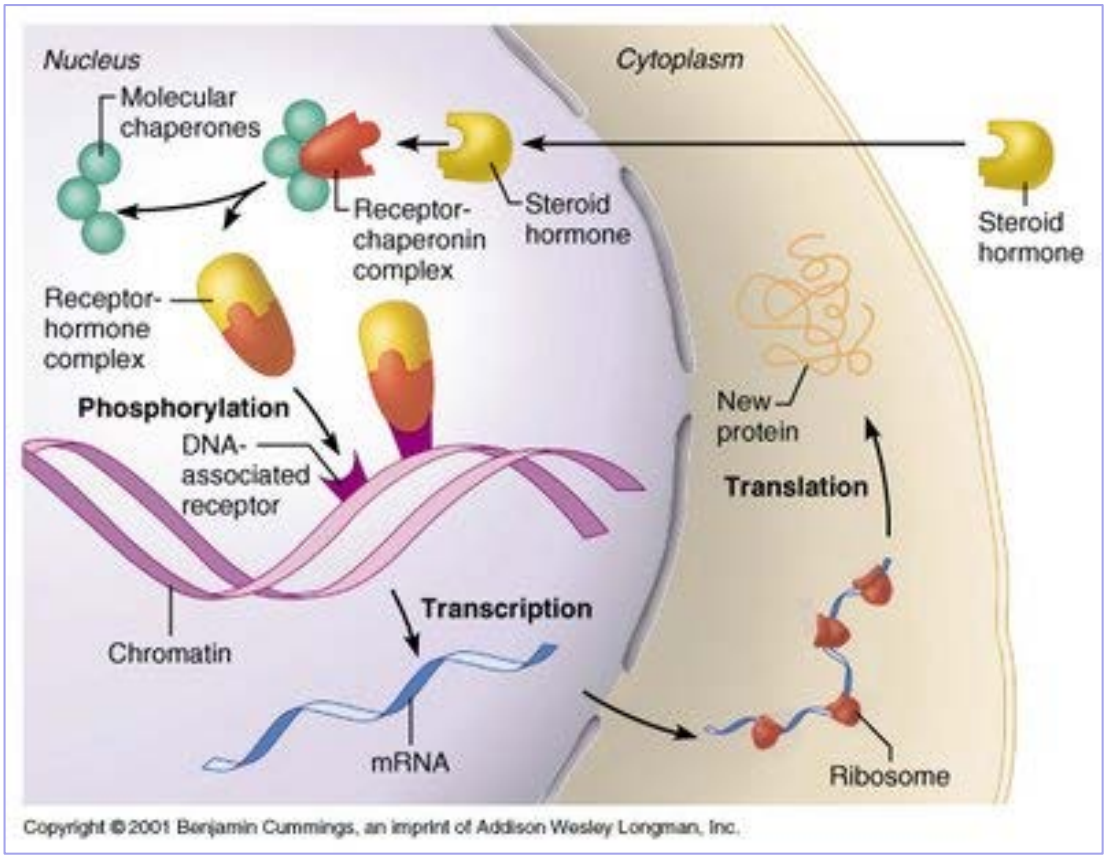




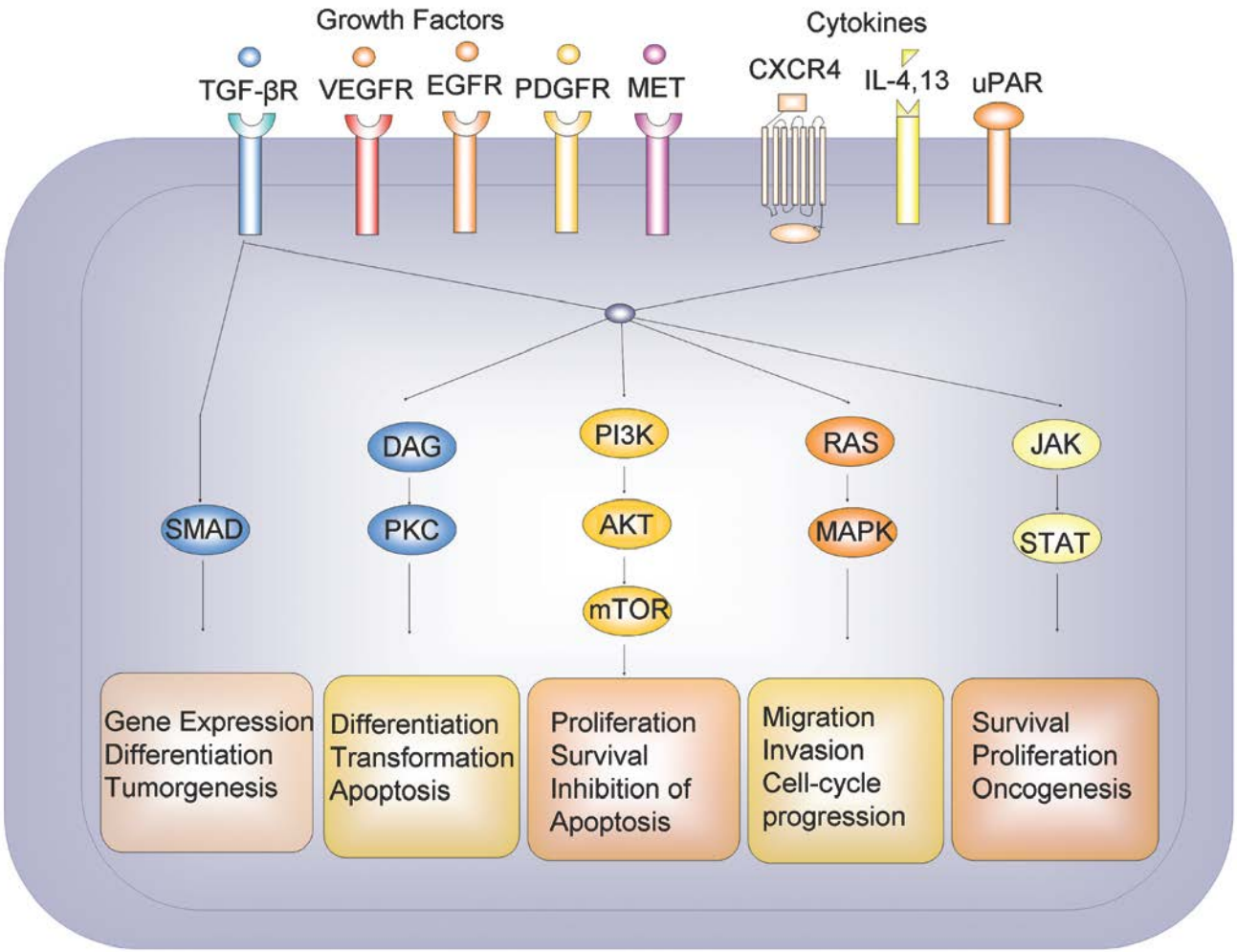
Intracellular receptors

Nuclear receptor



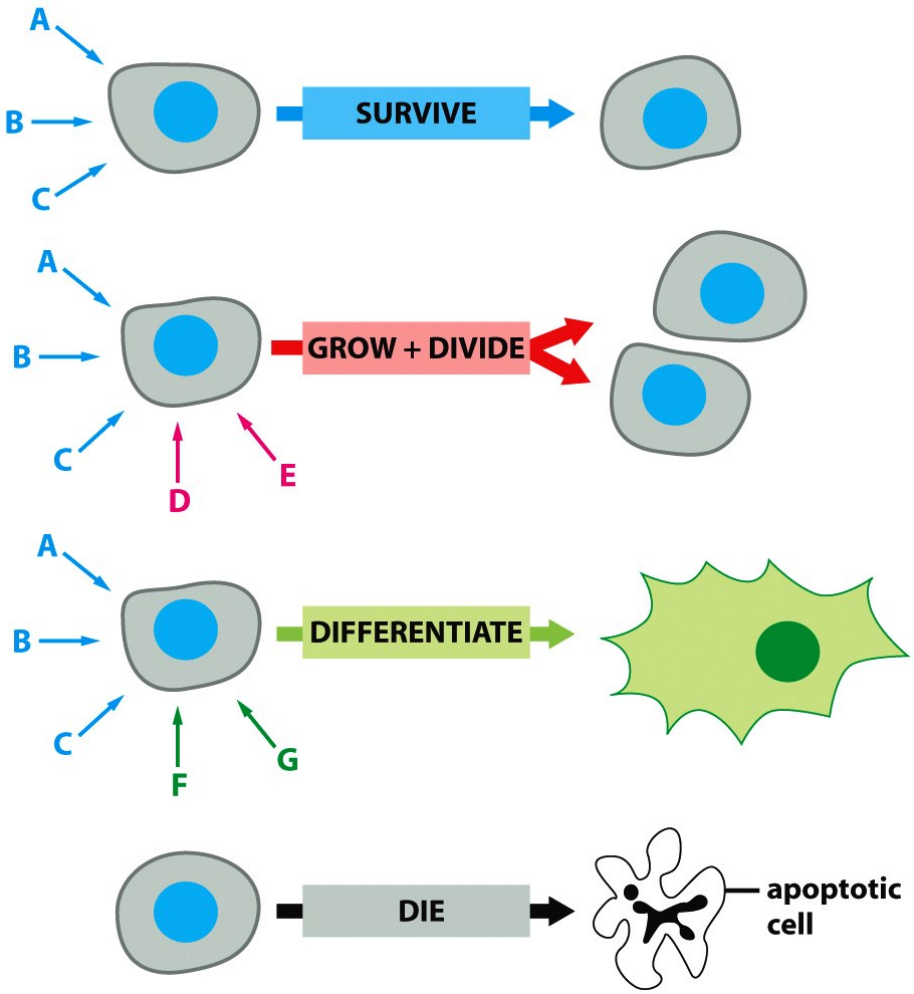


Complexity of information exchange



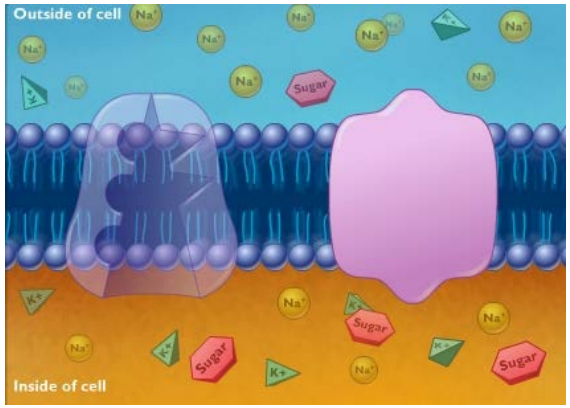
- A signal molecule may activate a signaling pathway leading to various effector proteins, thus modifying cell behavior
- In a particular signaling pathway may be involved very different types of proteins, from the receptor to the effector molecule activated

Complexity of information exchange

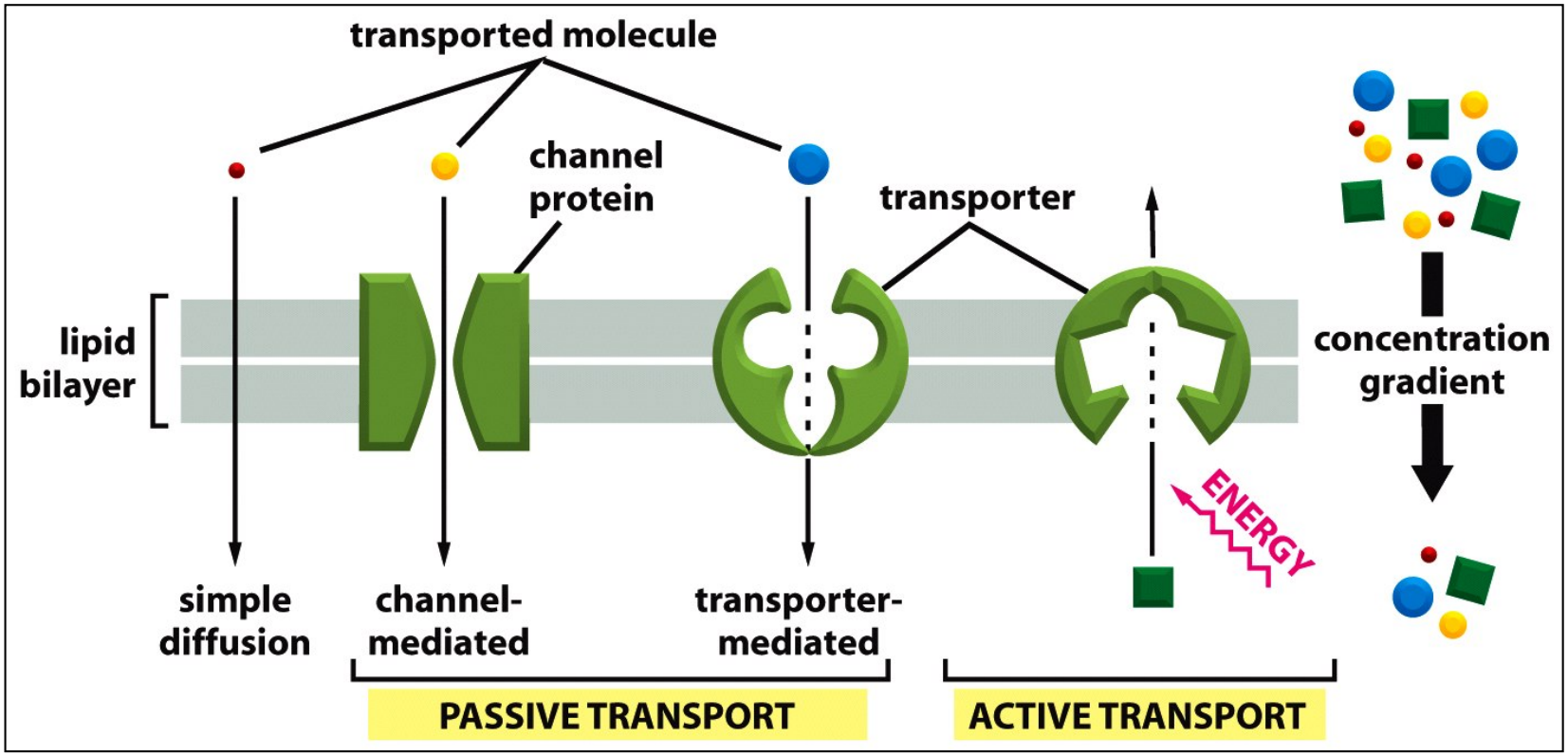


Signal molecules act in combination and regulate cell behavior

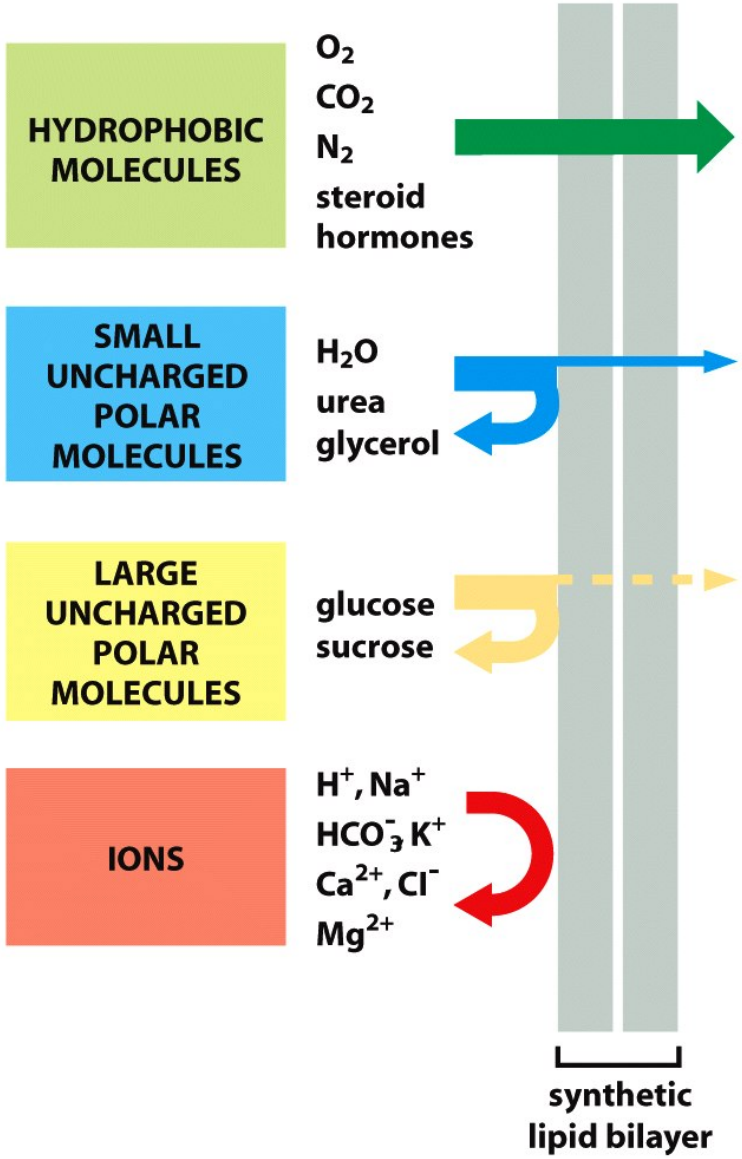
SUBSTANCE EXCHANGE



- Permeability
- Cytosis



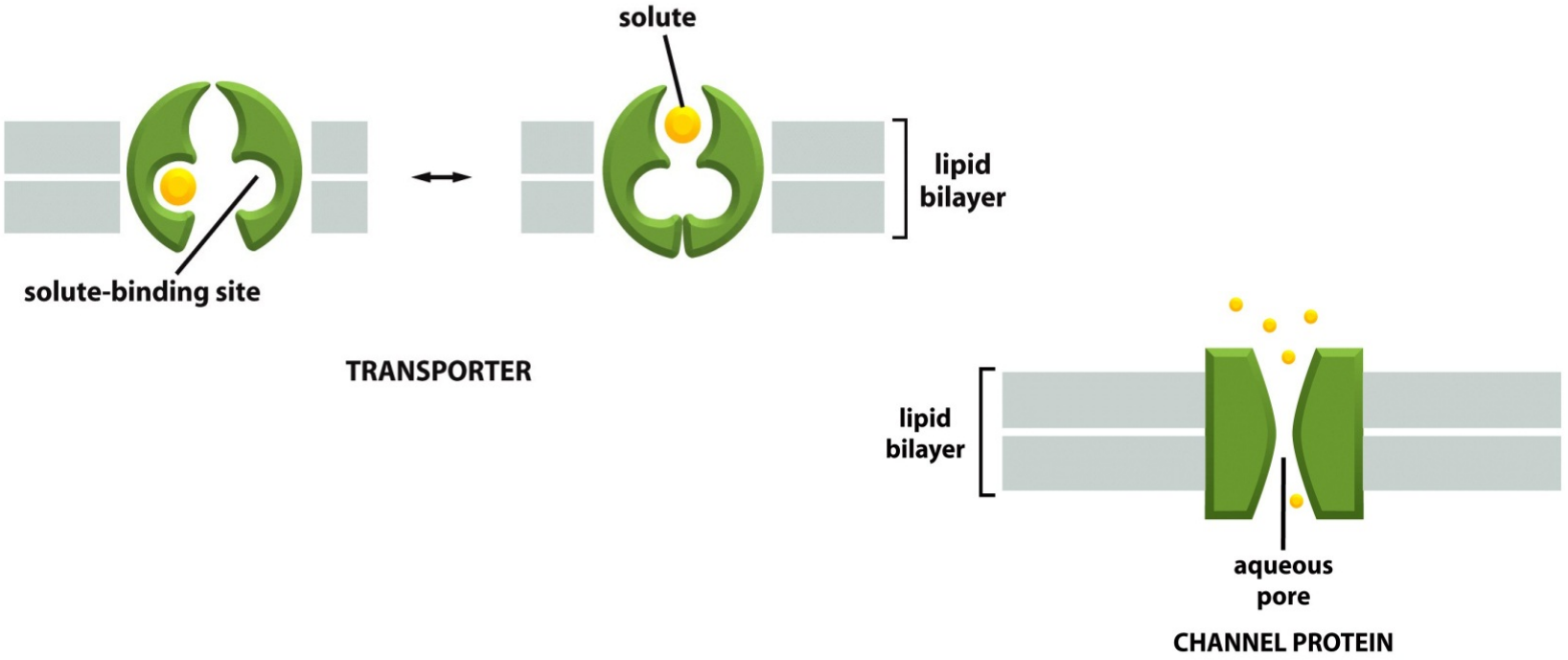
Transport through the lipid phase



Transport through the protein phase

There are two major classes of membrane transport proteins:

- Transporters (permeases or carriers): bind the solute, change conformation and transfer the bound solute across the membrane.
- Channels: form hydrophilic pores that allow solutes to cross the membrane

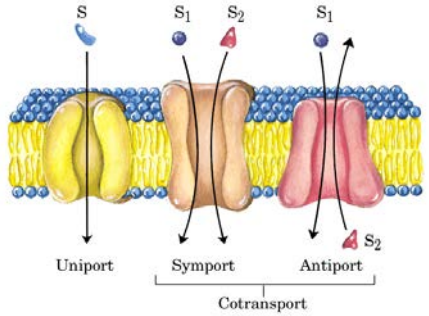


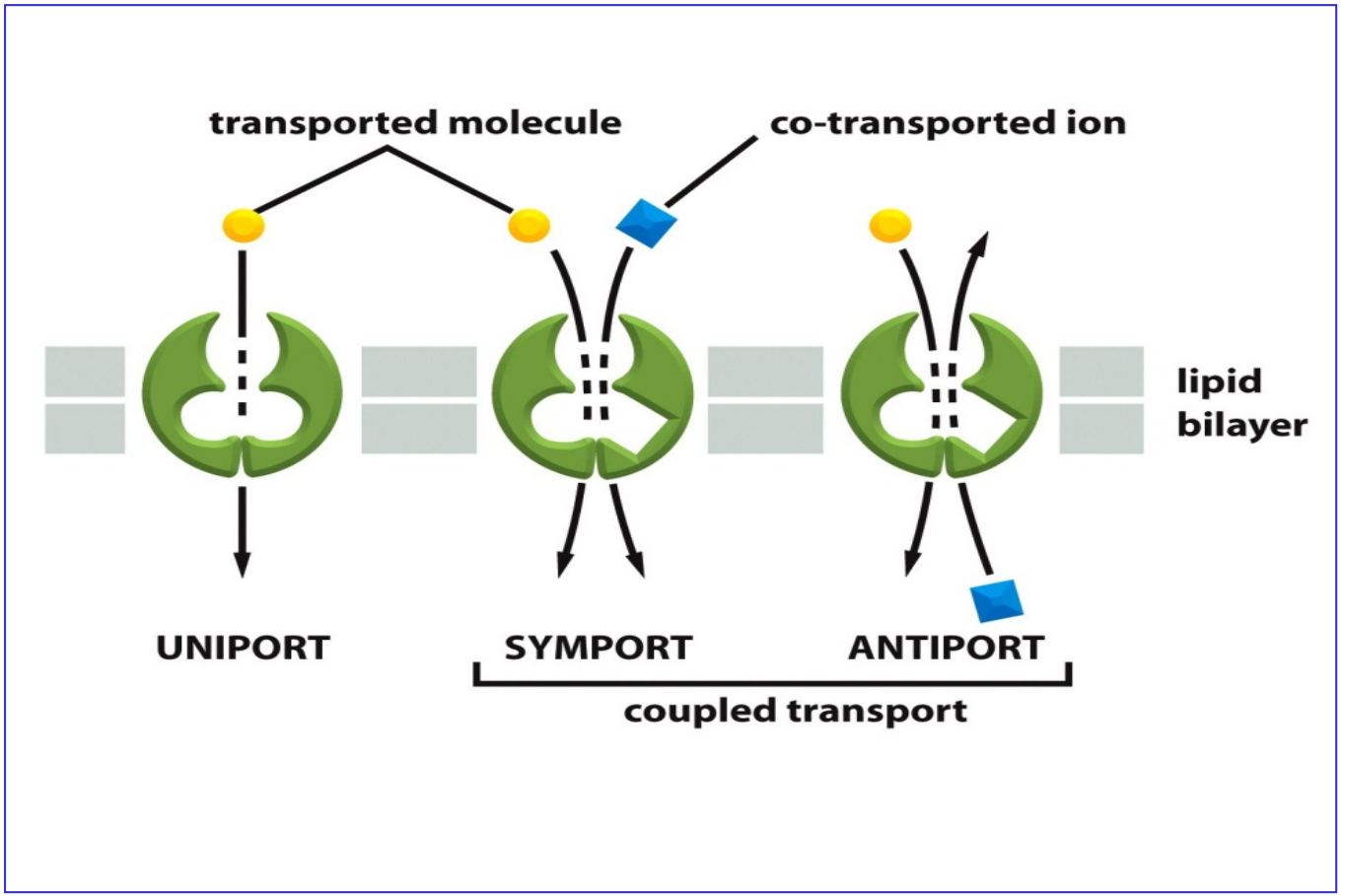
All channels and many transporters allow ***passive transport*** , also called ***facilitated diffusion***.

Transport through the protein phase

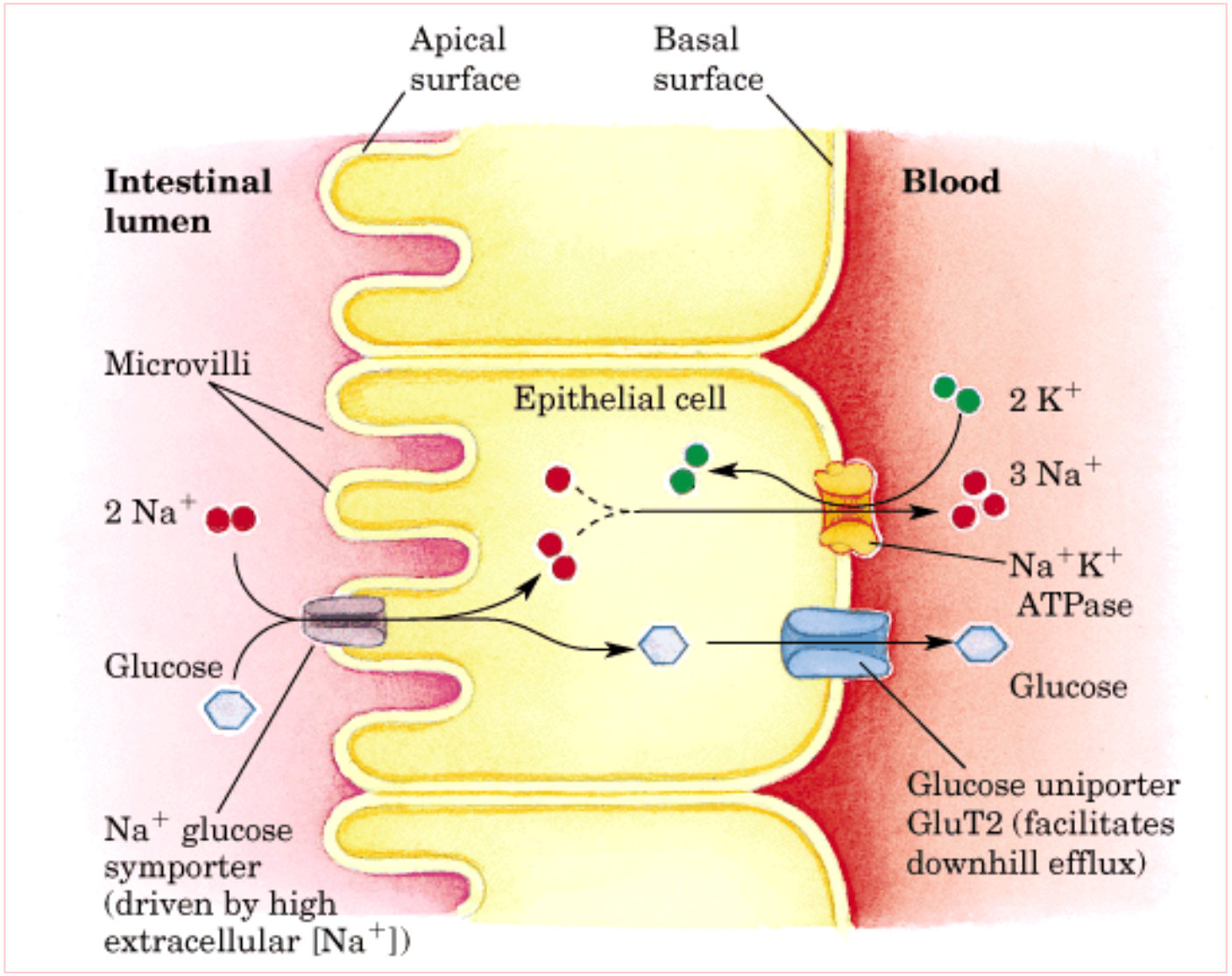
Transport systems:

- Uniport
- Co-transporters
  - Symport
  - Antiport

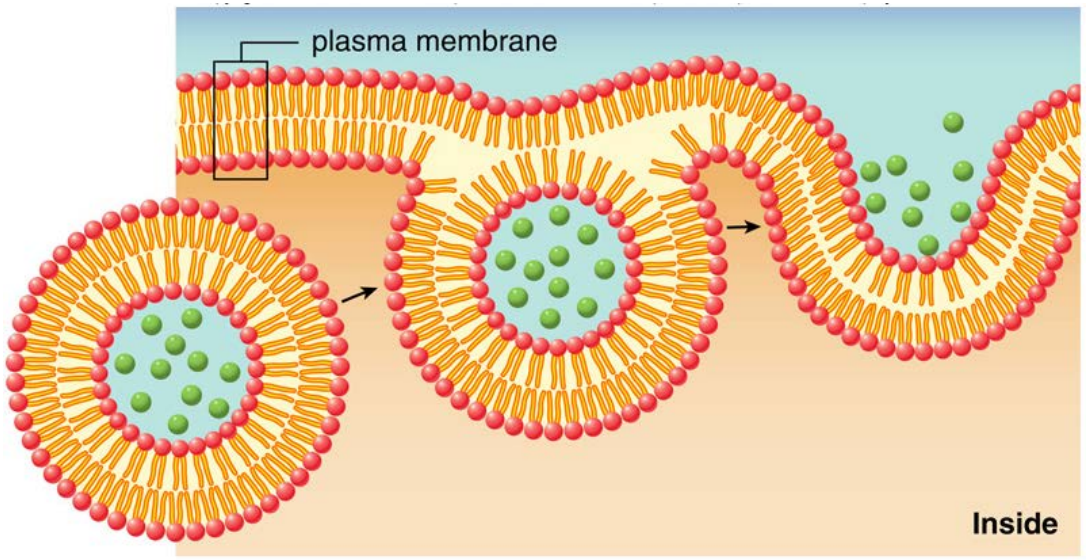




Transport through the protein phase



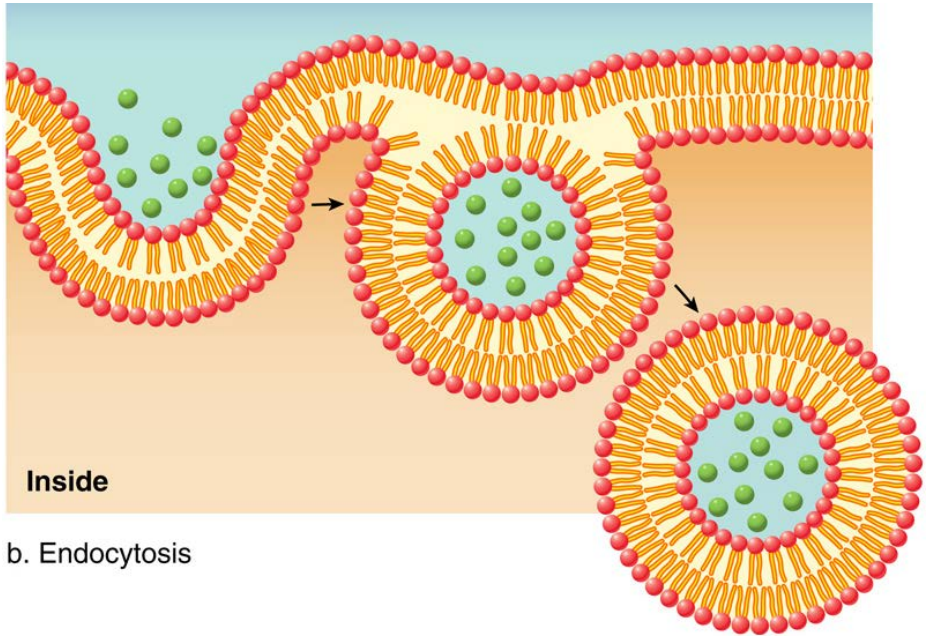
CYTOSIS



a. Exocytosis

Exocytosis is the durable, energy-consuming process by which a cell directs the contents of secretory vesicles out of the cell membrane and into the extracellular space.

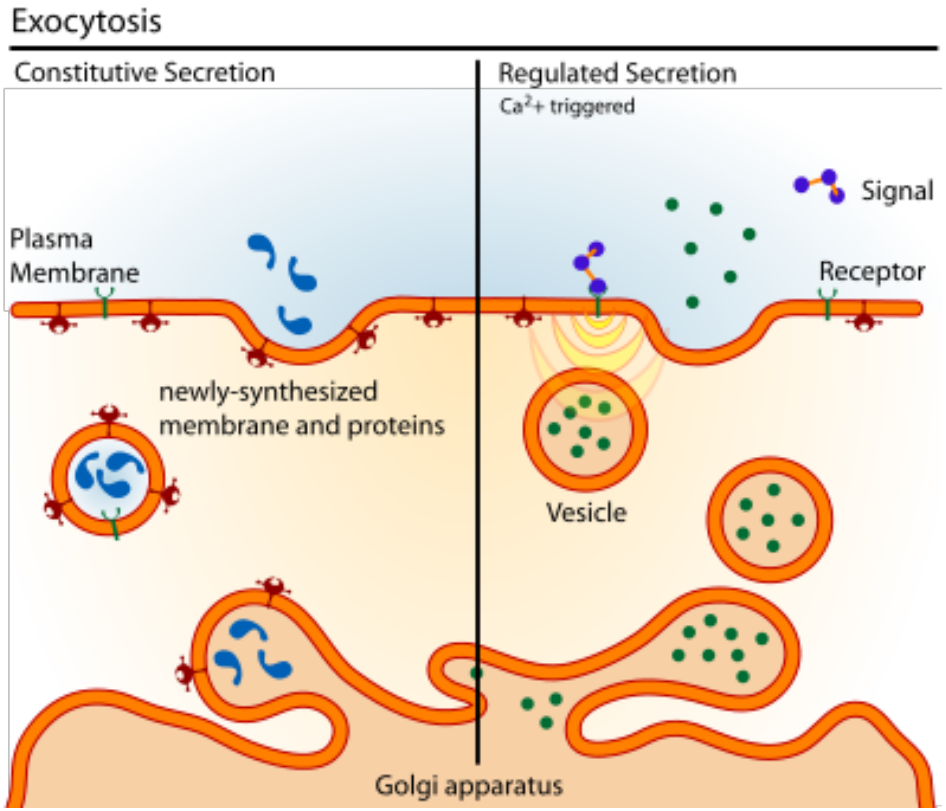
Endocytosis is an energy-using process by which cells absorb molecules (such as proteins) by engulfing them. It is used by all cells of the body because most substances important to them are large polar molecules that cannot pass through the hydrophobic plasma or cell membrane.



b. Endocytosis

CYTOSIS

Exocytosis



Constitutive: operates continuously.

Function: secretion of products to the extracellular matrix renewal of the cell membrane

Regulated (induced): only in response to a signal.

Found in cells specialized in secreting products rapidly on demand

CYTOSIS: Exocytosis

Constitutive

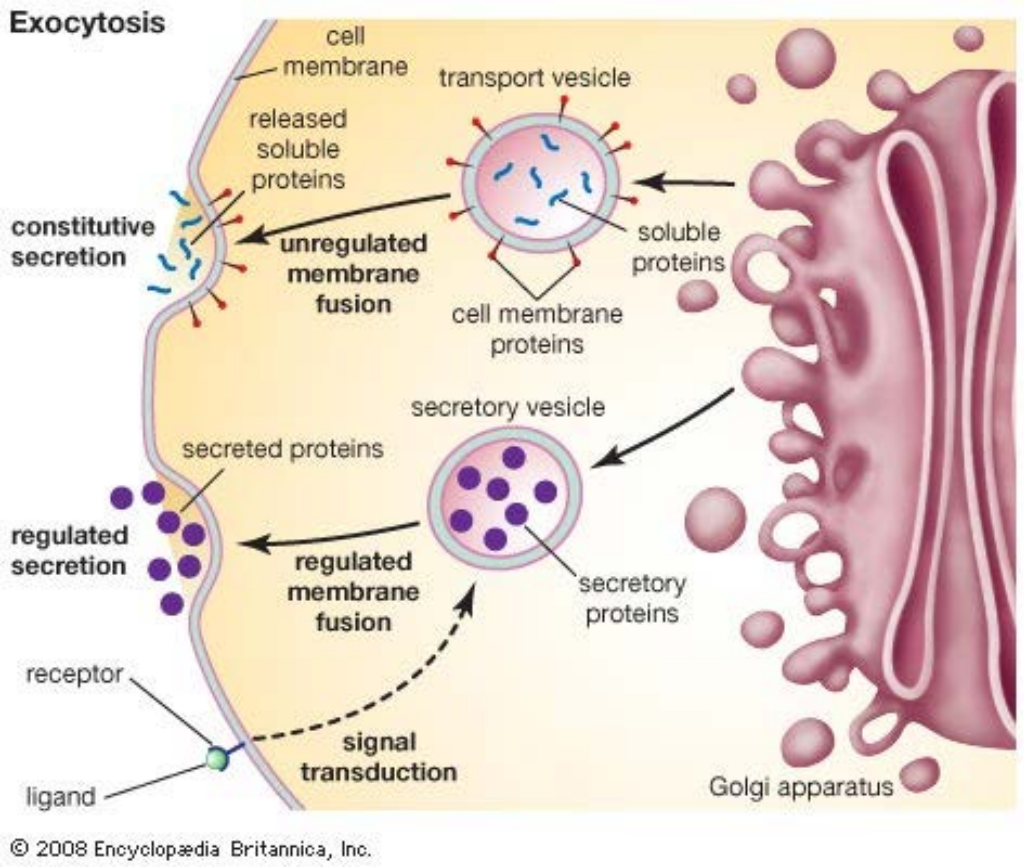
In all eukaryotic cells there is a continuous flow of vesicles that leave the Golgi apparatus and go towards the cell membrane.

Regulated

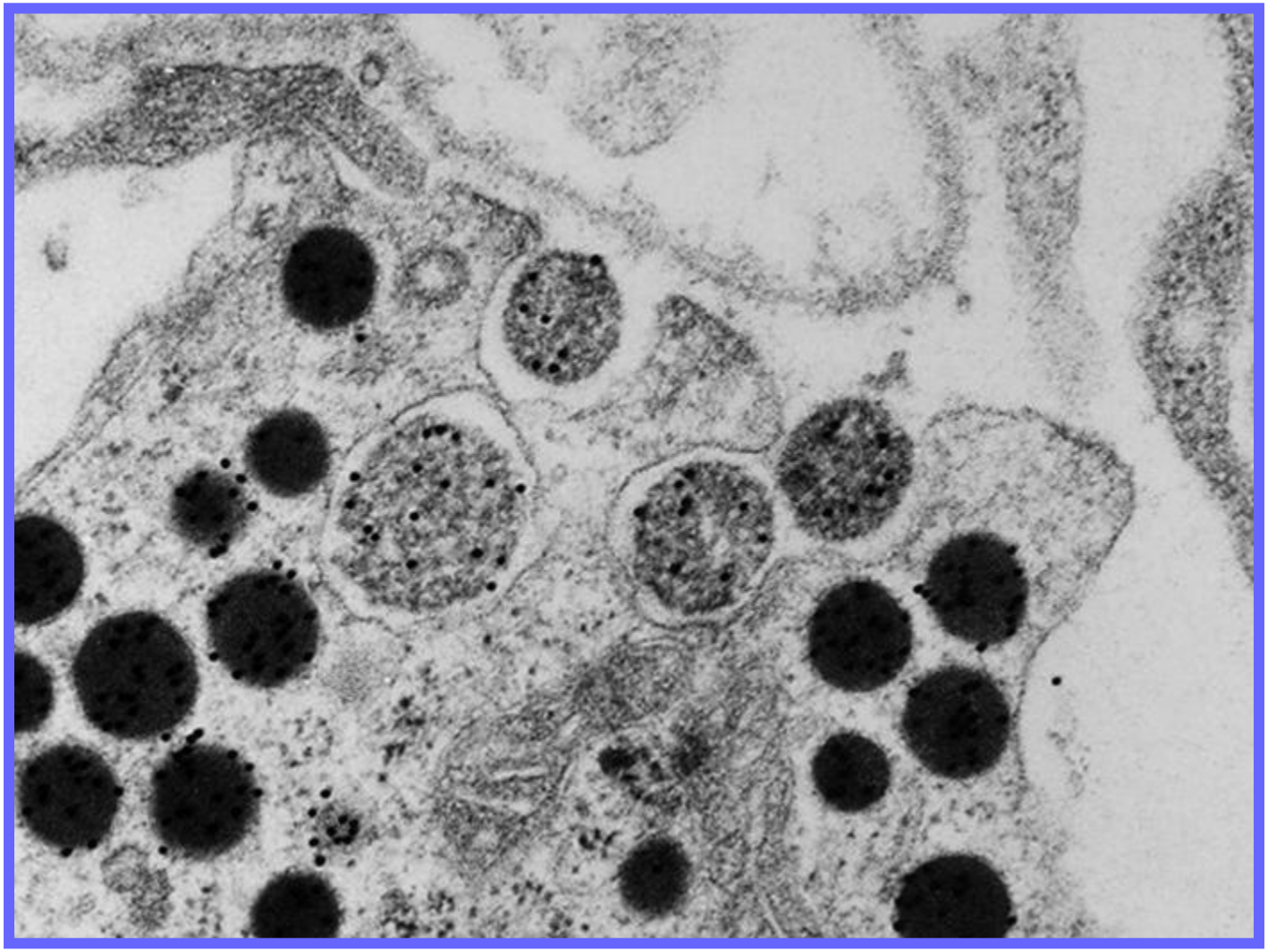
The secretory cells produce large amounts of a product that are stored in secretory vesicles.

These vesicles are formed in the Golgi apparatus and accumulate near the cell membrane.

When the cells receive an external stimulus (signal) the vesicles fuse with the cell membrane and discharge their content into the extracellular matrix.

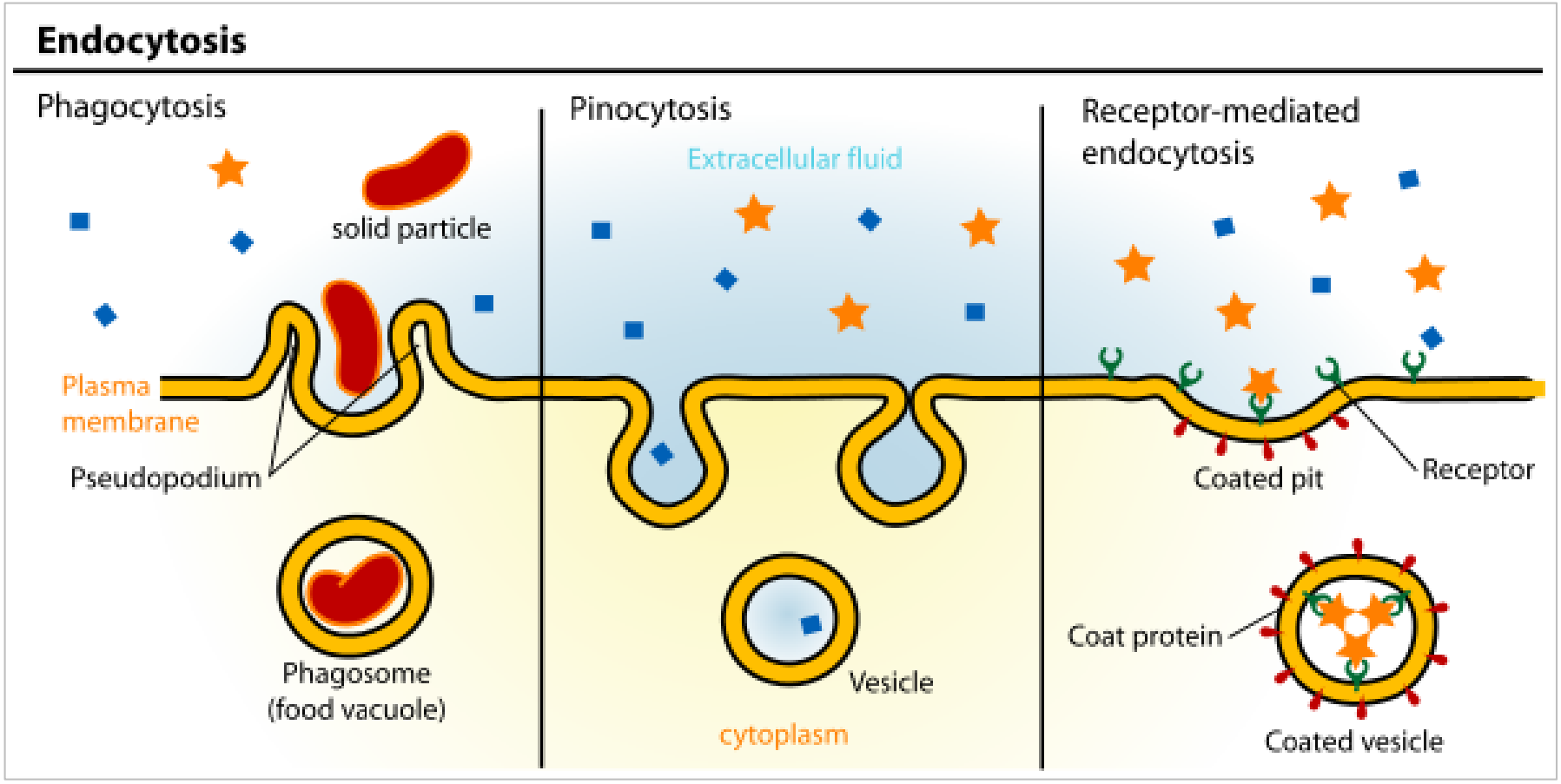


Exocitosis

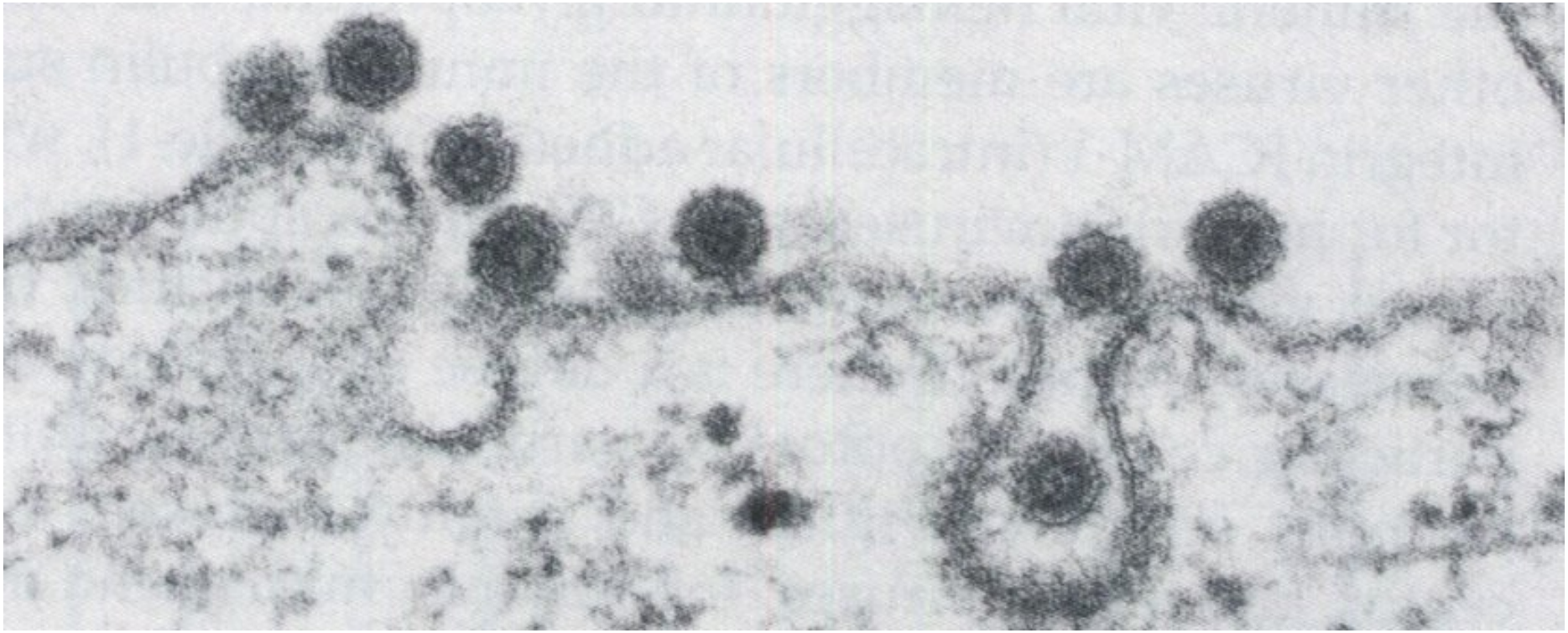


CYTOSIS

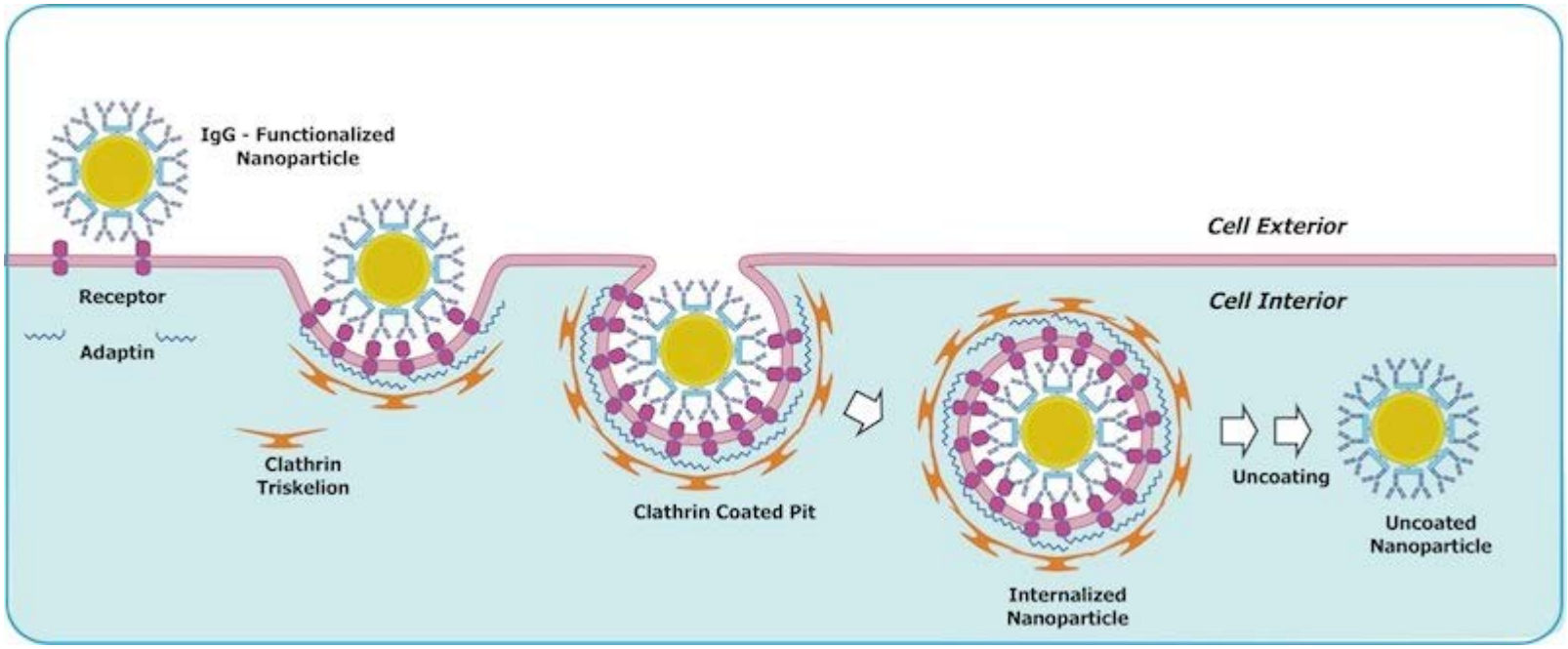
Endocytosis: ingestion of substances by vesicles

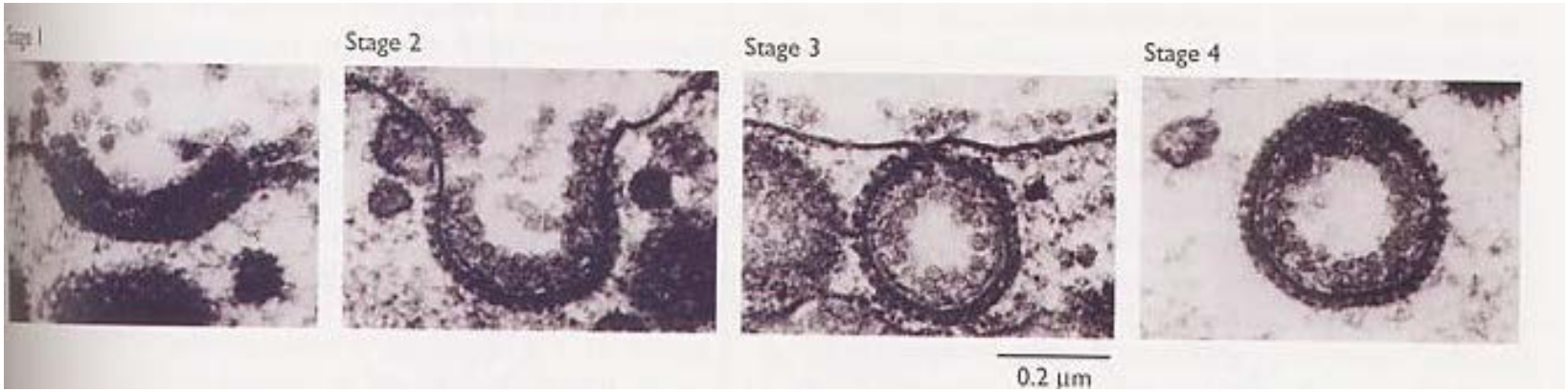


CYTOSIS: Endocytosis

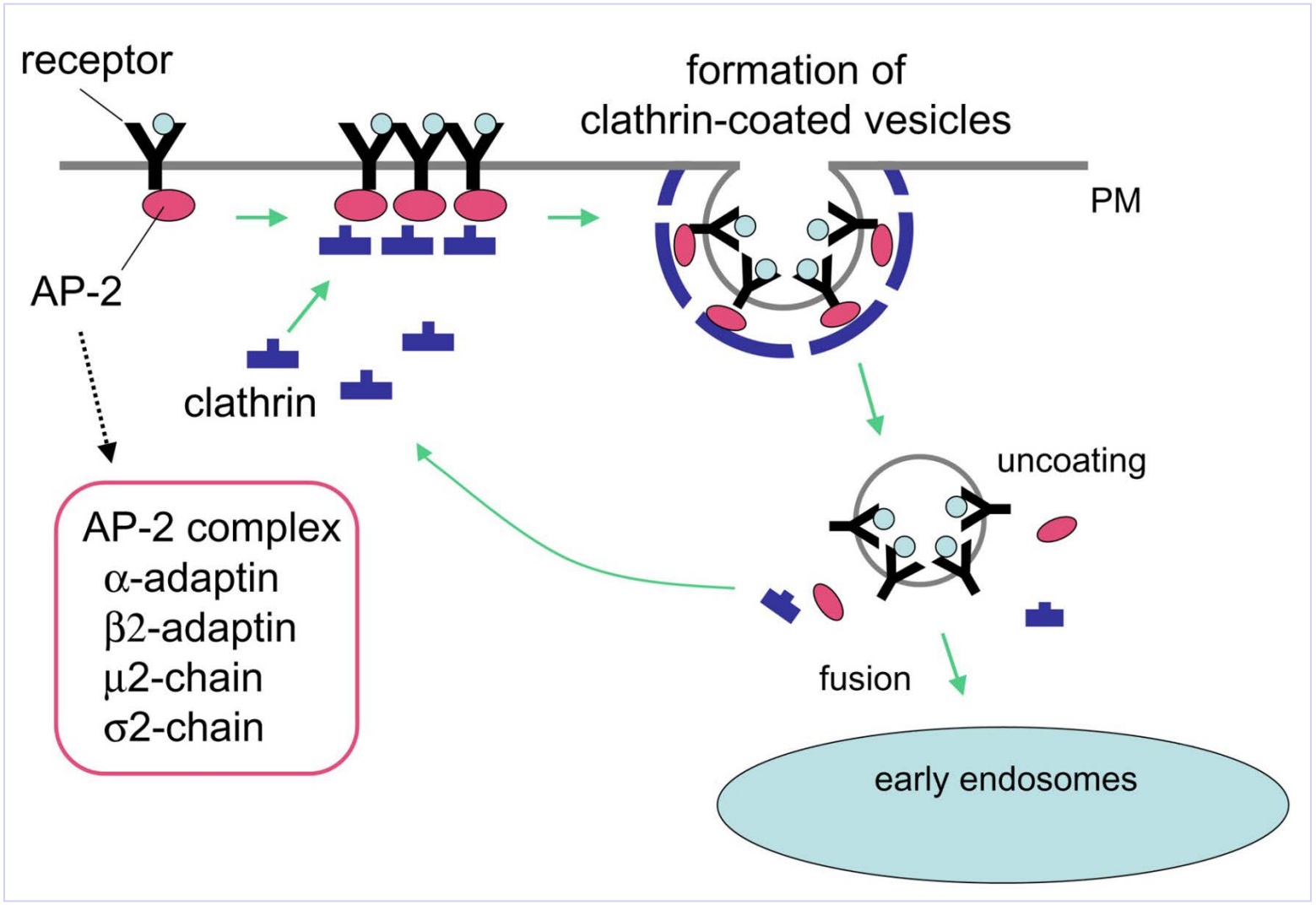


CYTOSIS


IgG - Functionalized Nanoparticle
Cell Exterior
Receptor
Cell Interior
Adaptin
Clathrin Triskelion
Clathrin Coated Pit
Uncoating
Uncoated Nanoparticle
Internalized Nanoparticle

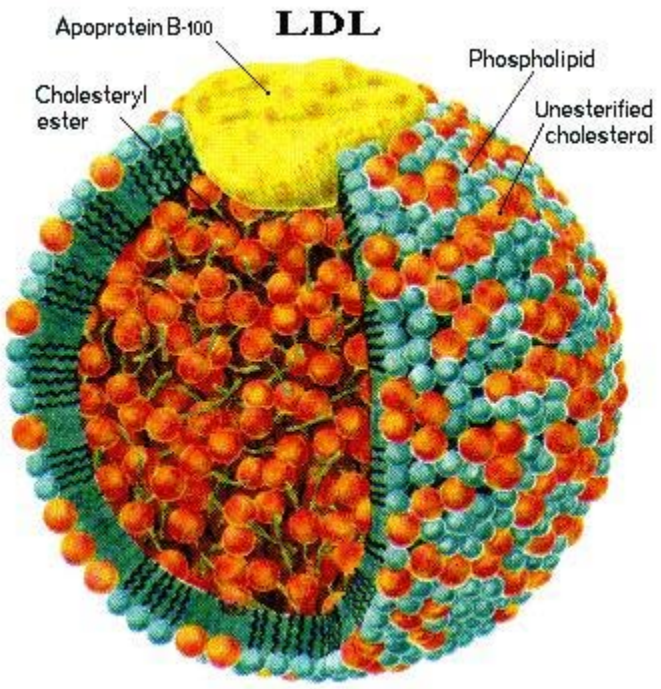

Stage 2
Stage 3
Stage 4
0.2 µm

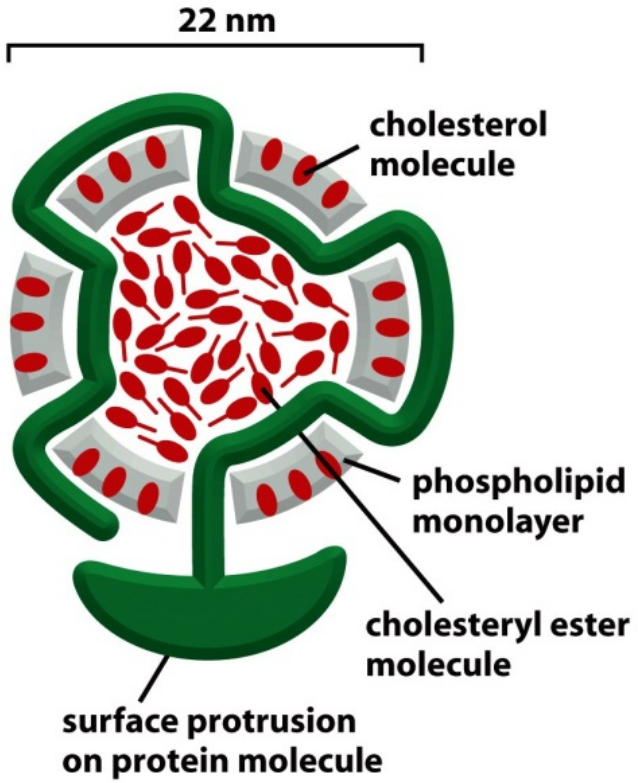
Receptor-mediated endocytosis



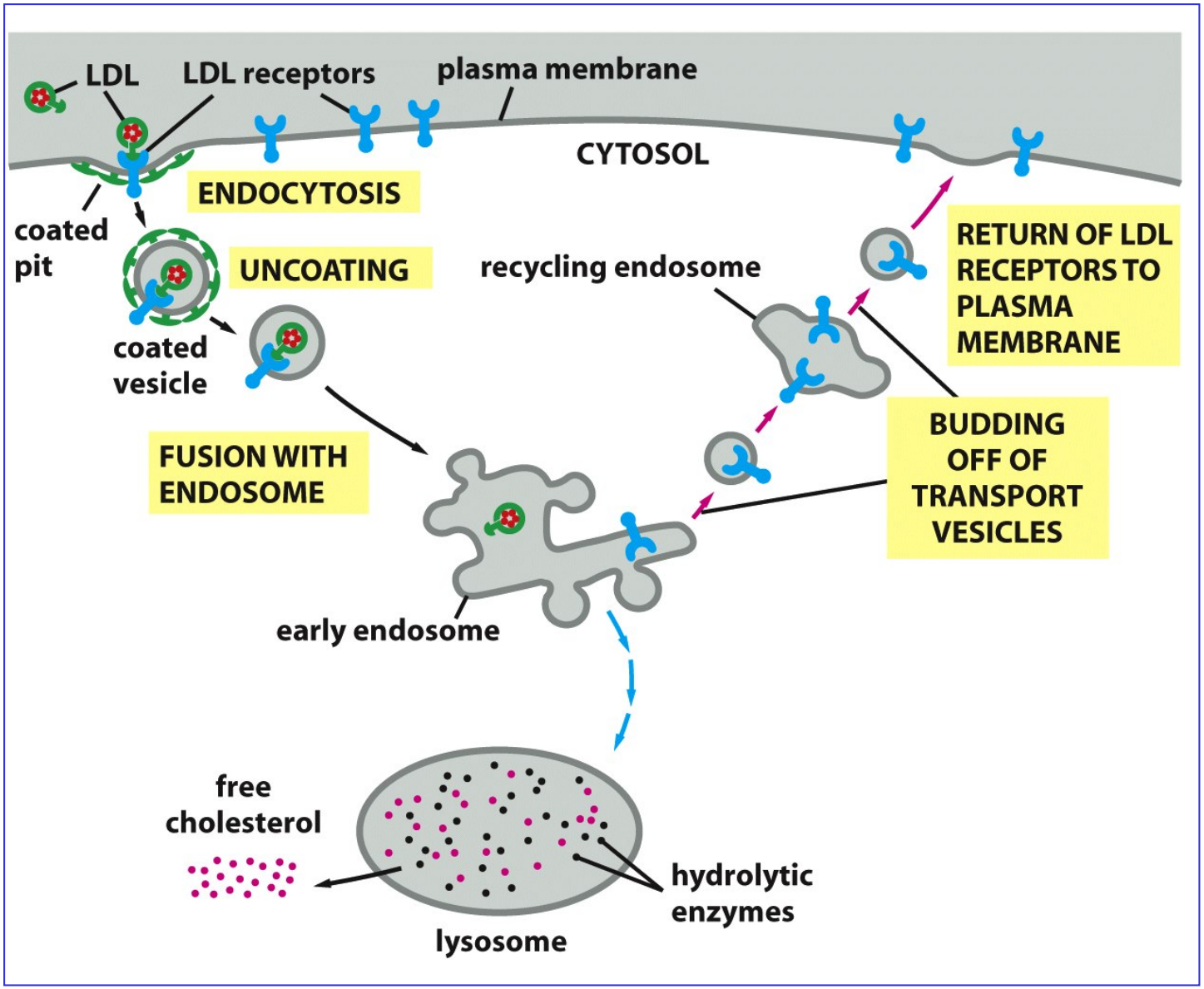
COAT ASSEMBLY AND CARGO SELECTION | BUD FORMATION | VESICLE FORMATION | UNCOATING

Receptor-mediated endocytosis

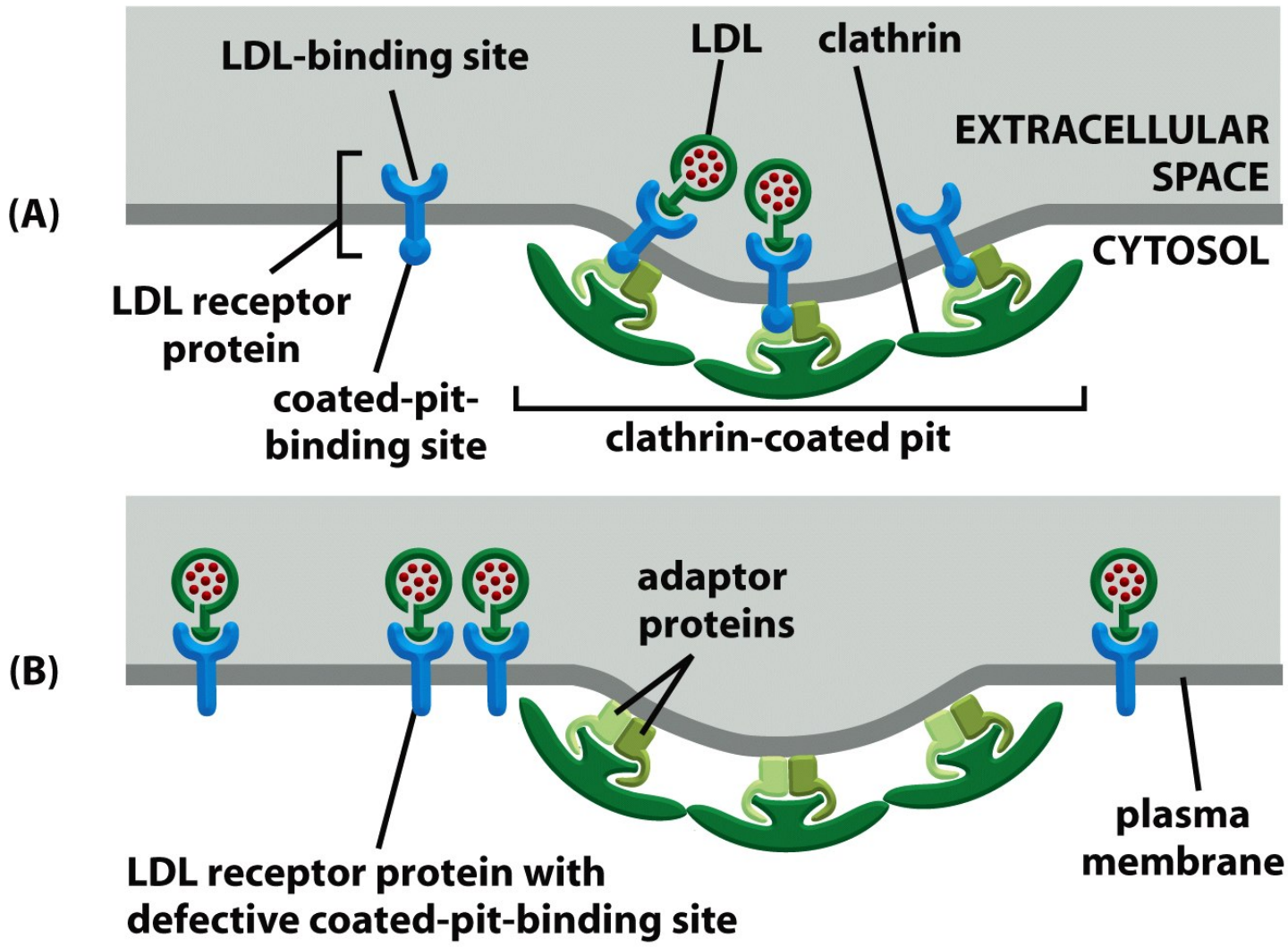




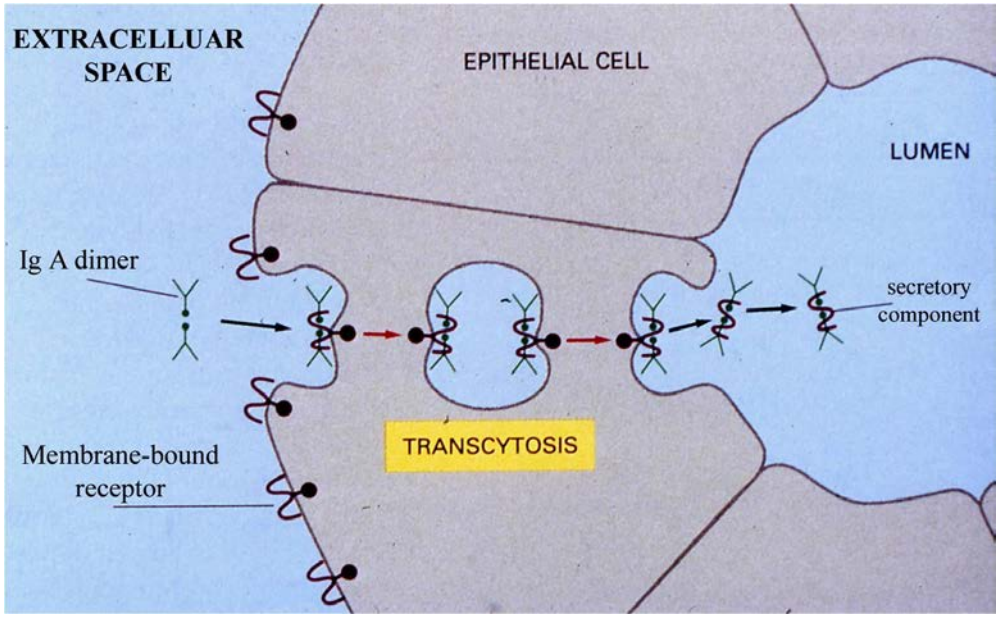
Receptor-mediated endocytosis

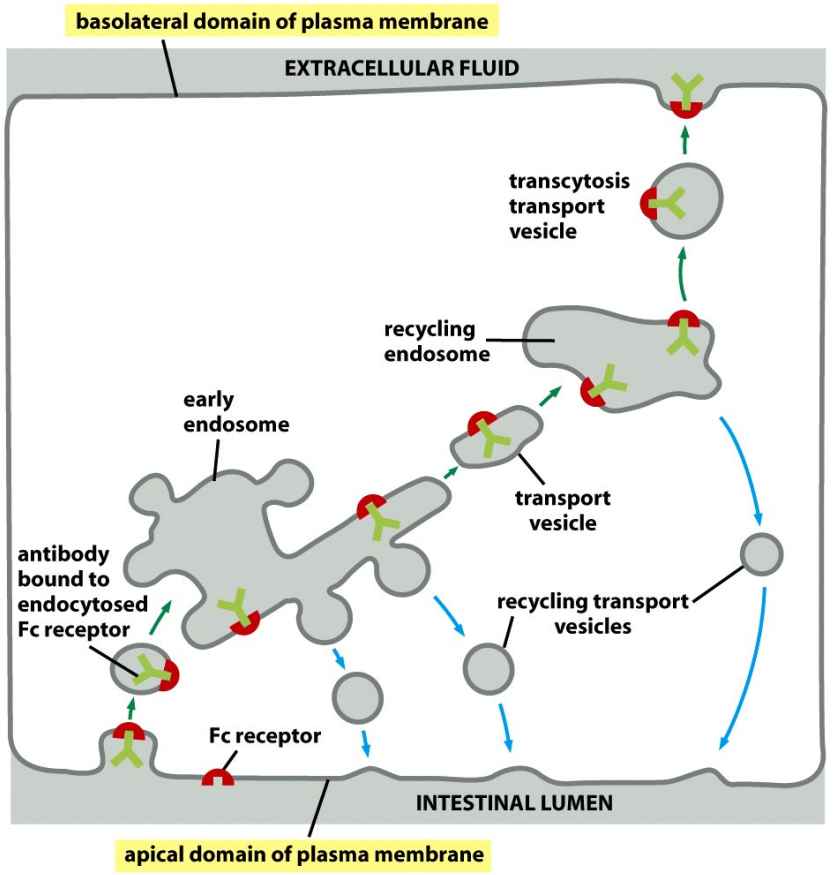


Receptor-mediated endocytosis



CITOSIS: transcytosis

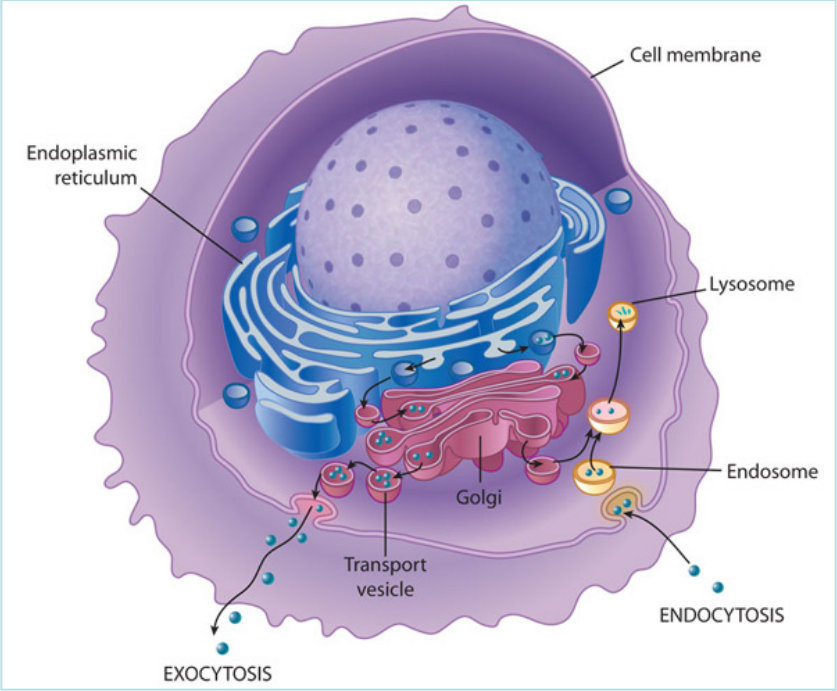


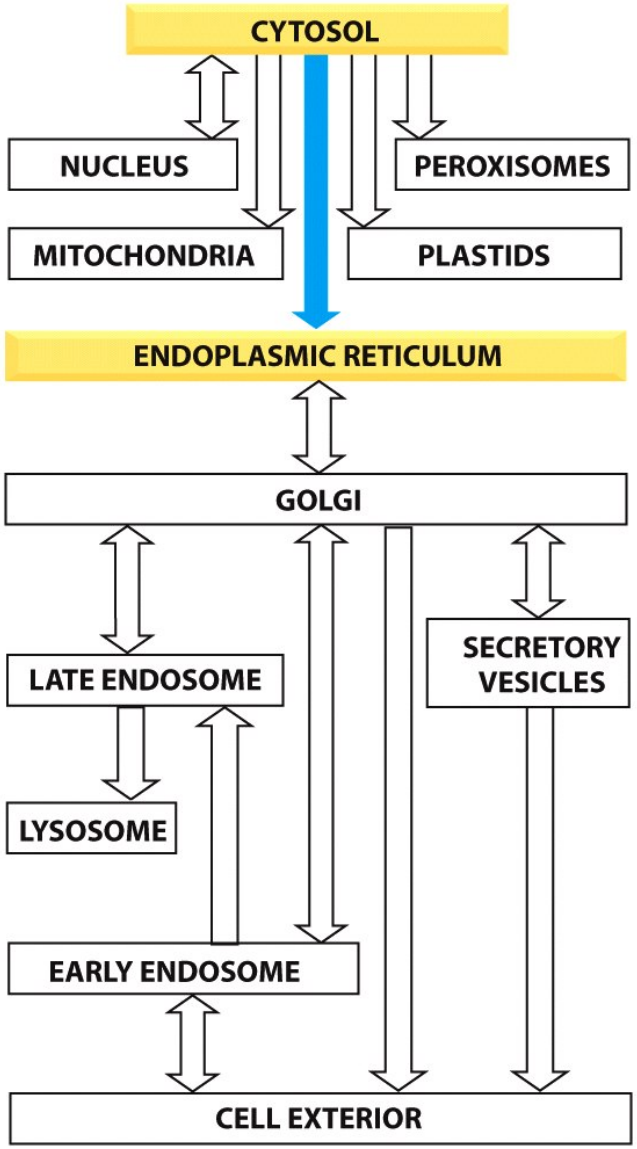


ENDOMEMBRANE CELL SYSTEM:

ENDOPLASMIC RETICULUM

1. General characteristics
2. Functions:
   - Protein synthesis
   - Glycosylations
   - Lipid synthesis
   - Detoxification
   - Ca^{2+} storage
   - Intracellular transport
   - Product storage
3. Biogenesis





Molecular Biology of the Cell (6ª ed. Fig 12-31)

ENDOPLASMIC RETICULUM: STRUCTURE

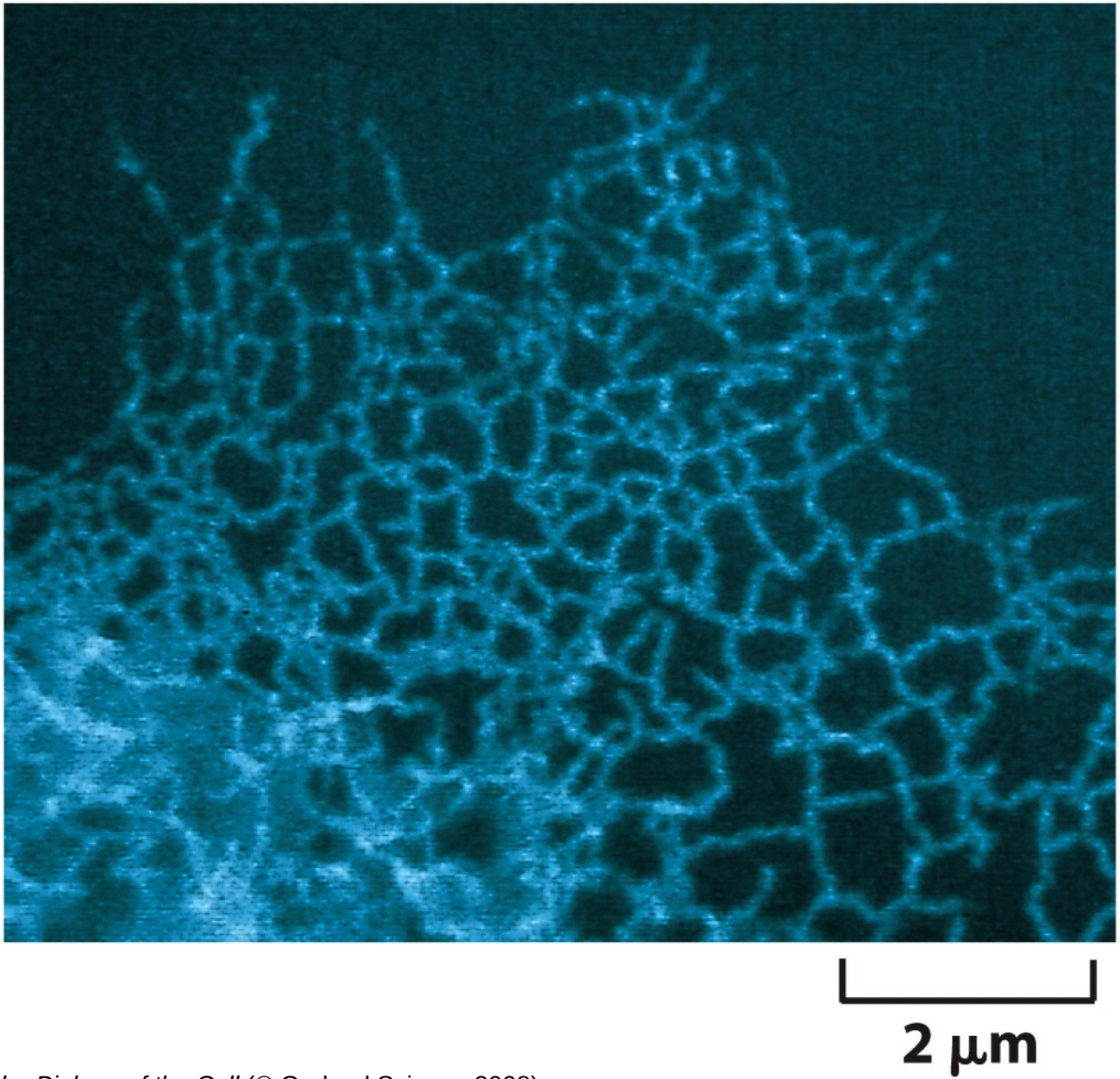
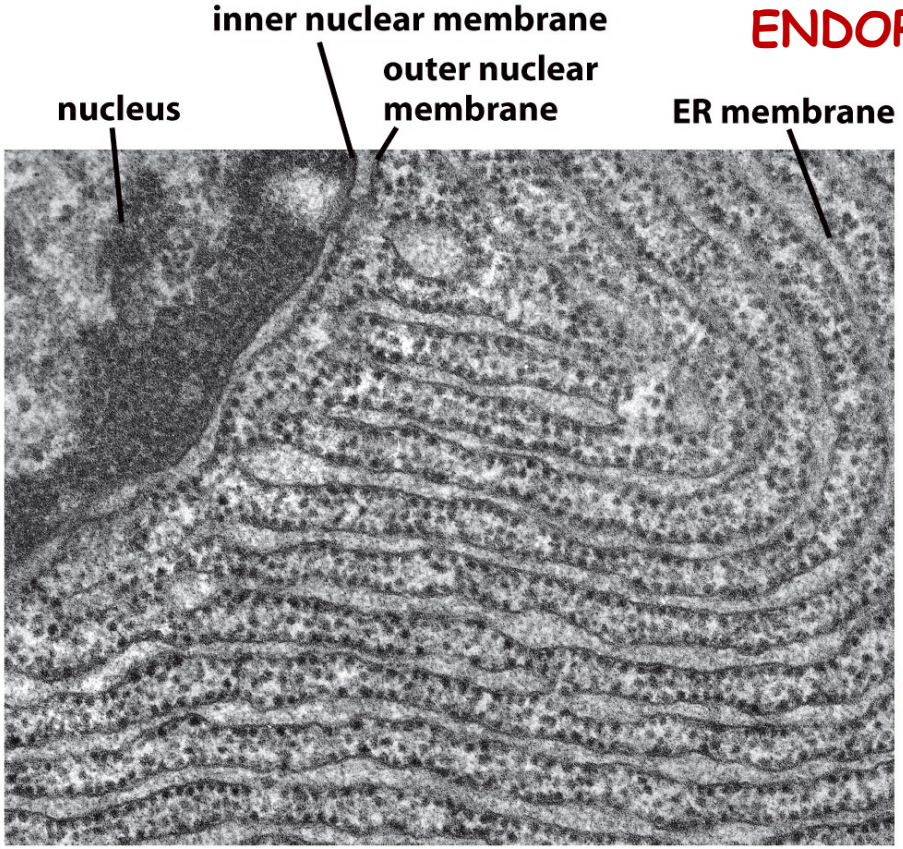


Figure 12-34a *Molecular Biology of the Cell* (© Garland Science 2008)

ENDOPLASMIC RETICULUM: STRUCTURE



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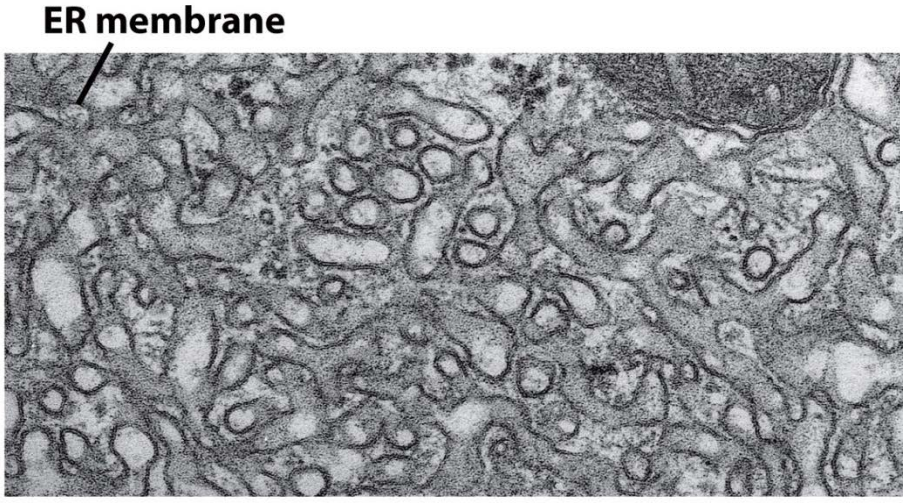


SER

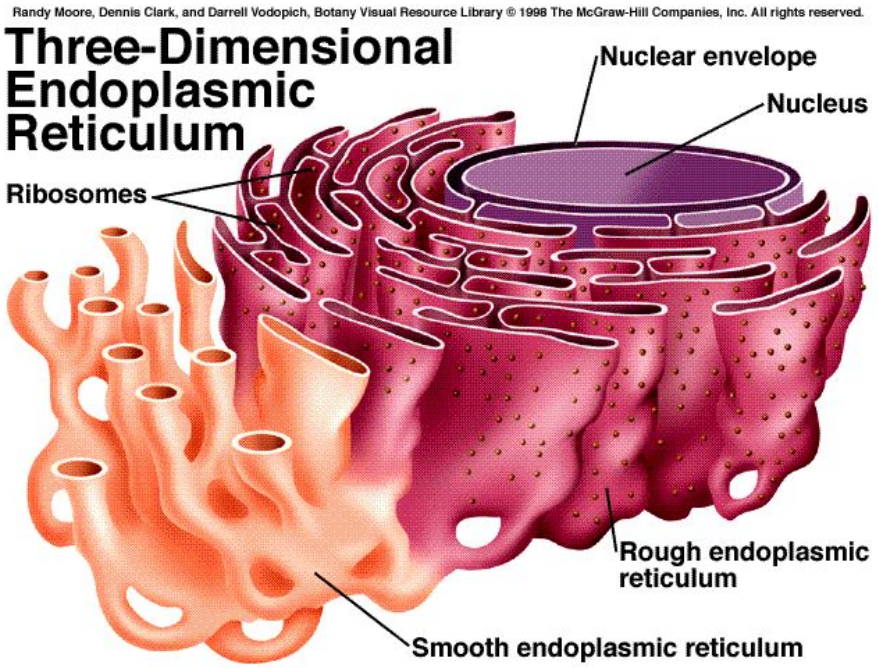
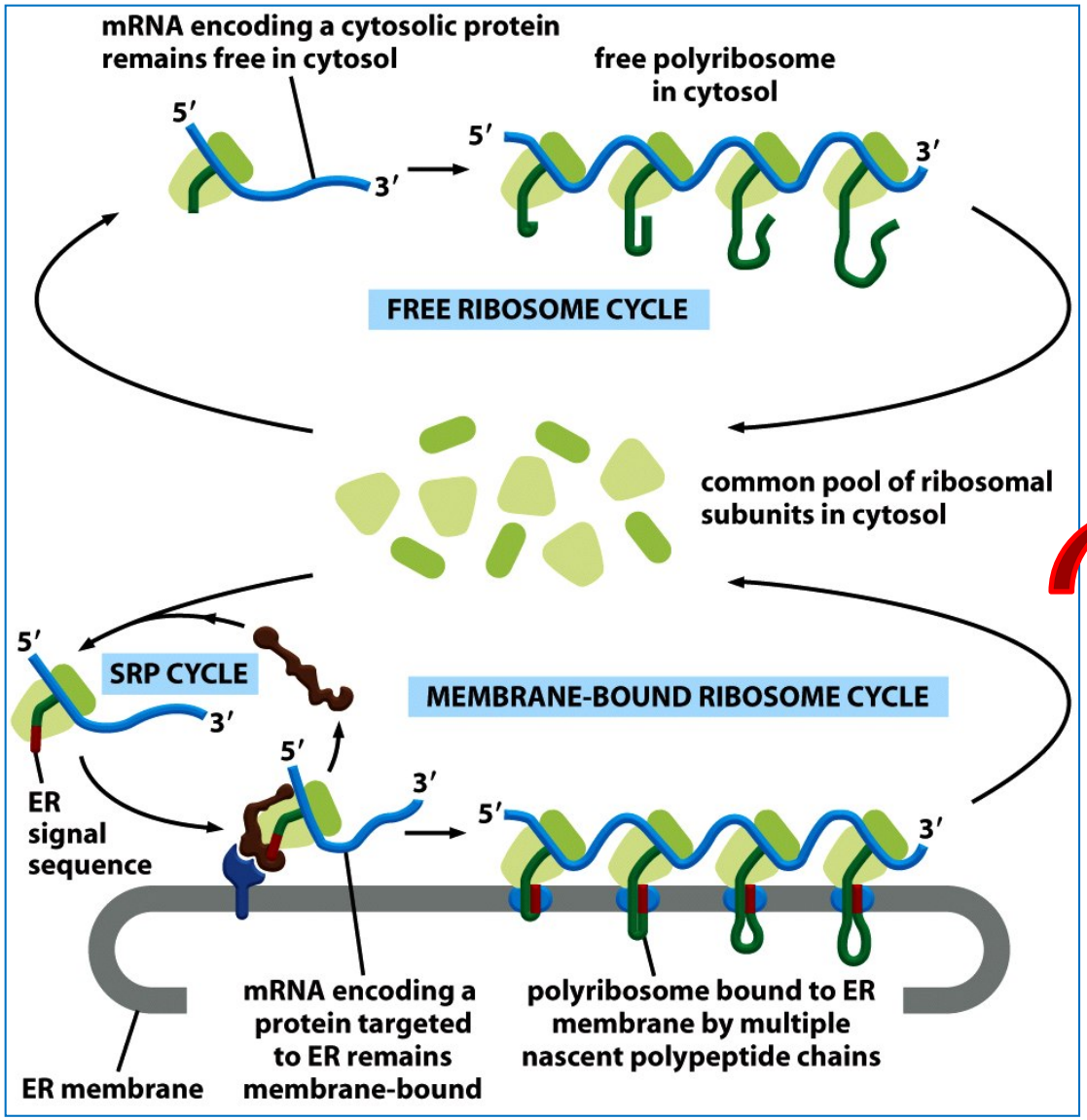


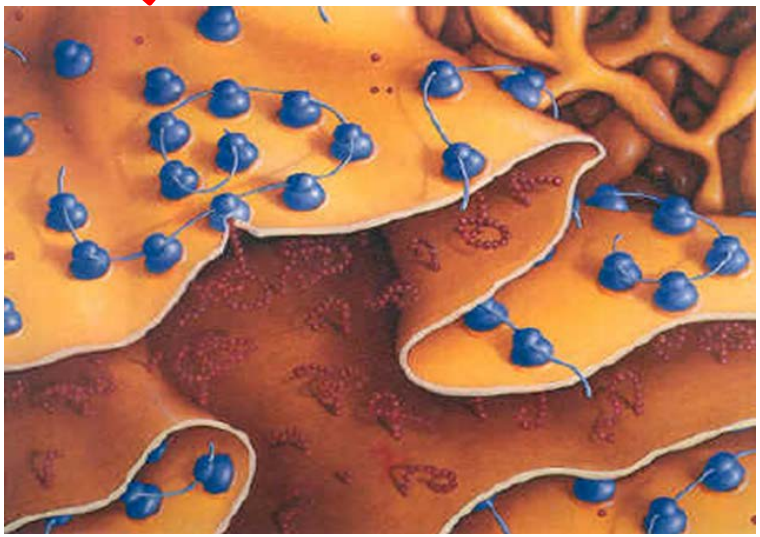
Figure 12-36c *Molecular Biology of the Cell* (© Garland Science 2008)

Functions:

- Protein synthesis
- Glycosylations
- Lipid synthesis
- Detoxification
- Ca^{2+} storage
- Intracellular transport
- Product storage

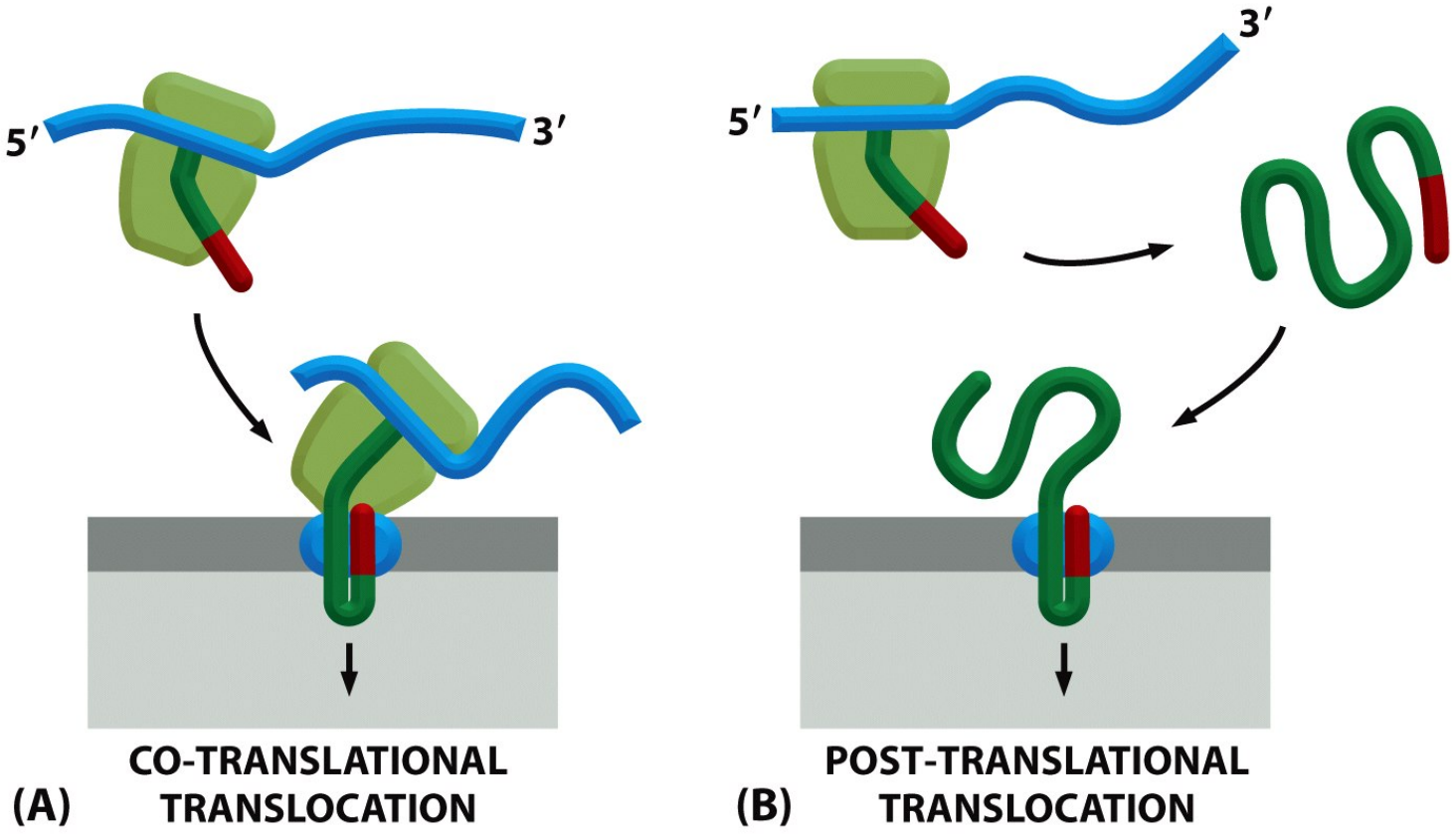
FUNCTIONS: PROTEIN SYNTHESIS





Molecular Biology of the Cell (6ª ed. Fig 12-38)

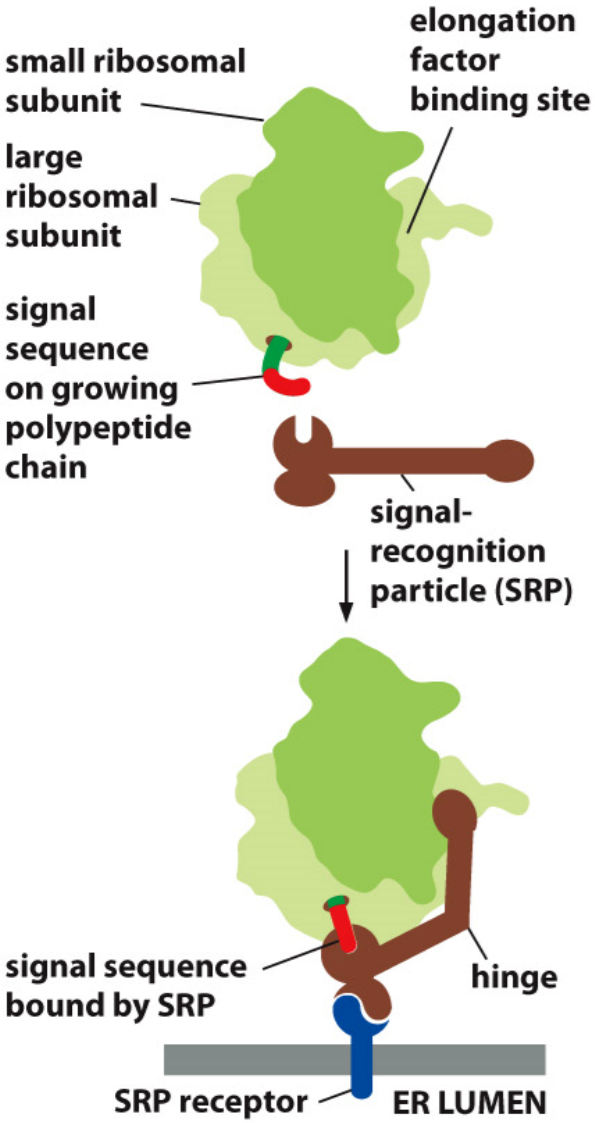
FUNCTIONS: PROTEIN SYNTHESIS



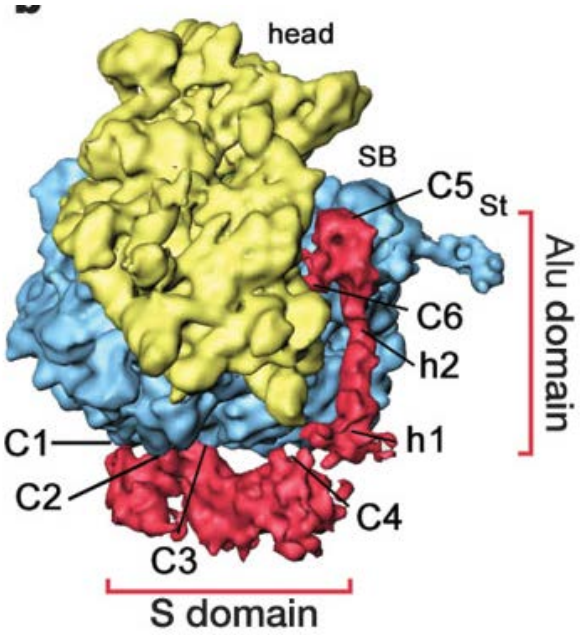
Protein synthesis in the ER

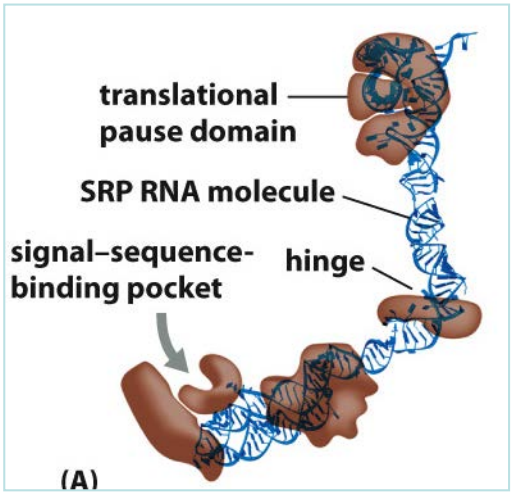
Molecular Biology of the Cell (6ª ed. Fig 12-32)

FUNCTIONS: PROTEIN SYNTHESIS



SRP





Molecular Biology of the Cell (6ª ed. Fig 12-36)

FUNCTIONS: PROTEIN SYNTHESIS

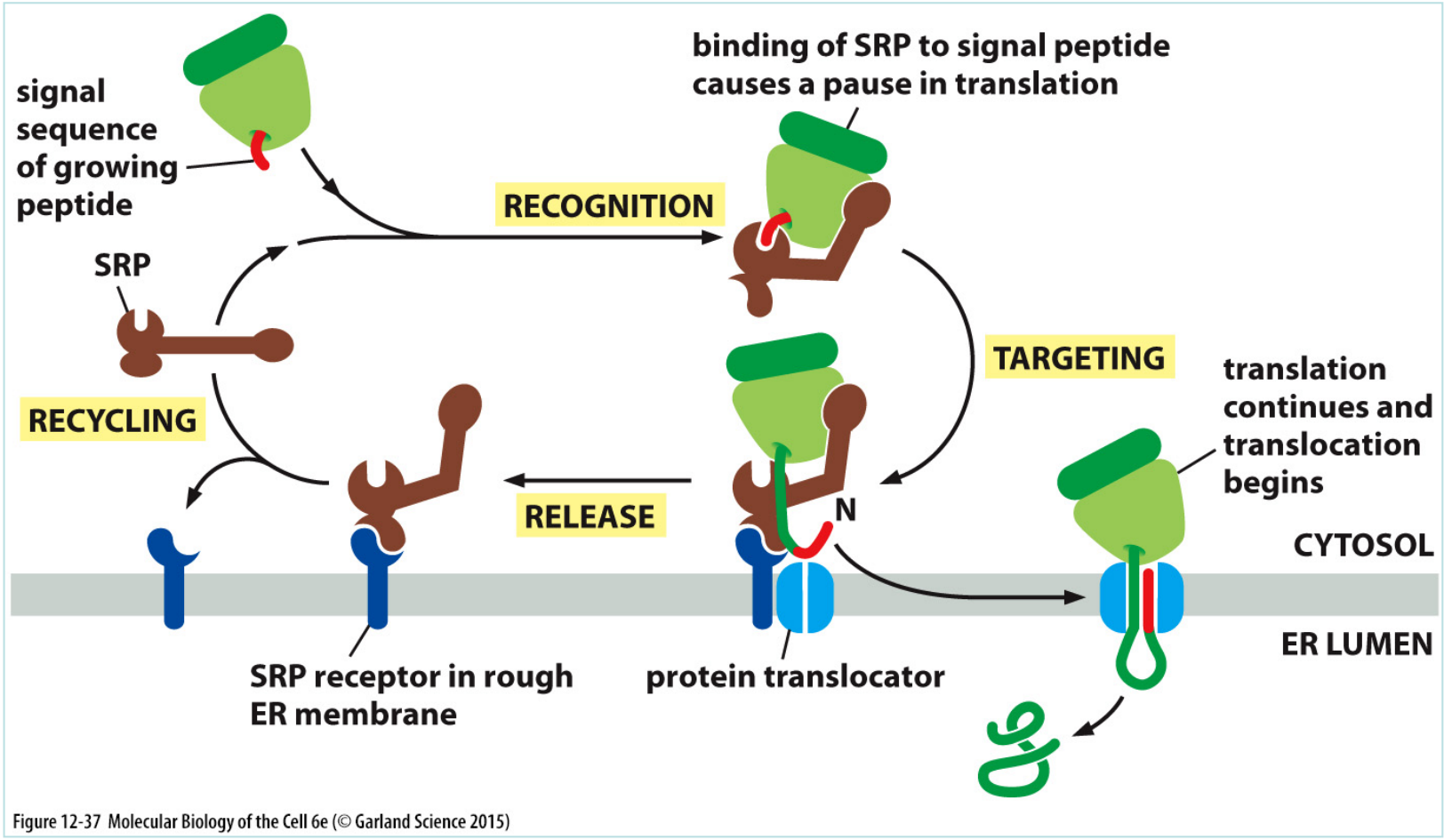


Figure 12-37 Molecular Biology of the Cell 6e (© Garland Science 2015)

FUNCTIONS: PROTEIN SYNTHESIS

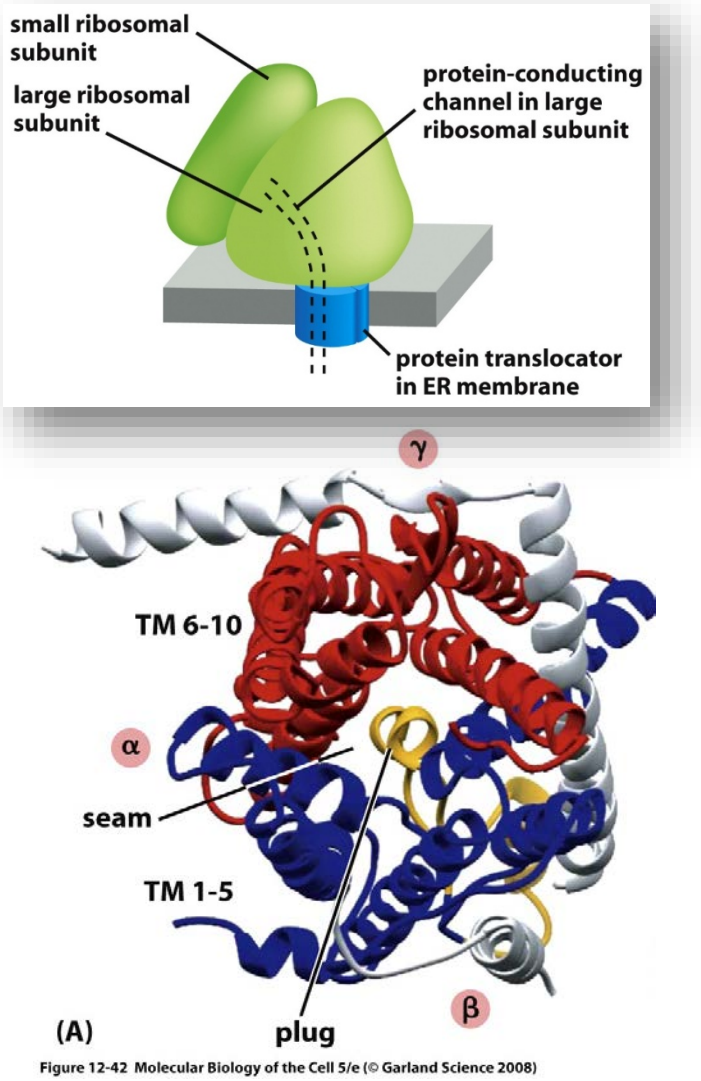
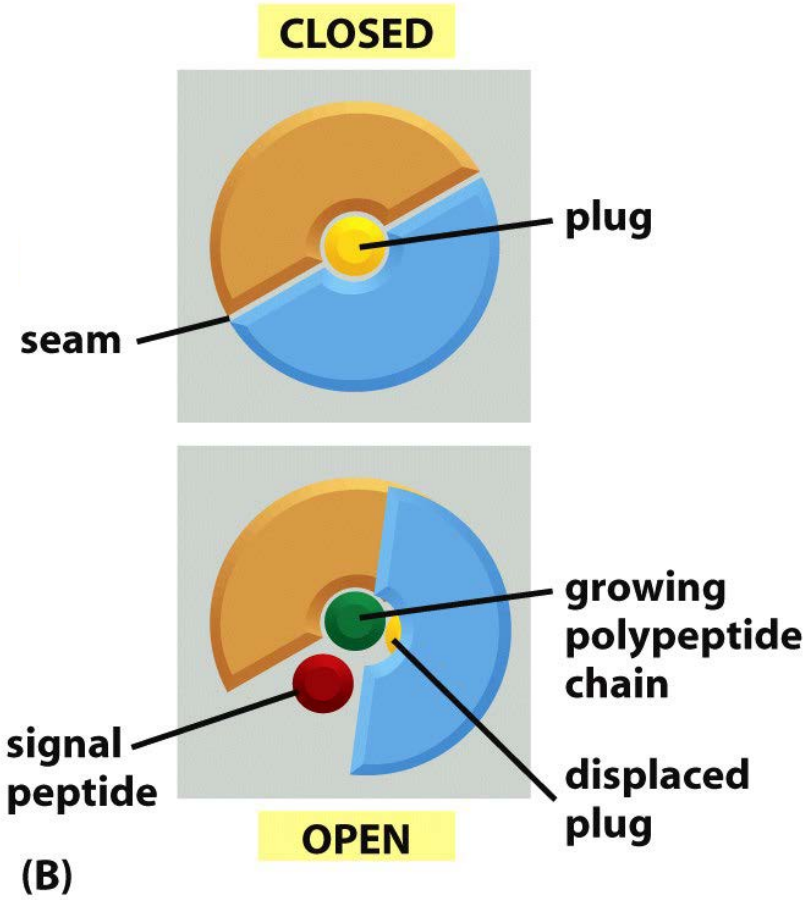


Figure 12-42 Molecular Biology of the Cell 5/e (© Garland Science 2008)



Molecular Biology of the Cell (6ª ed. Fig 12-39)

Conformational transitions of **Sec61** during co-translational protein translocation and membrane insertion.

FUNCTIONS: PROTEIN SYNTHESIS

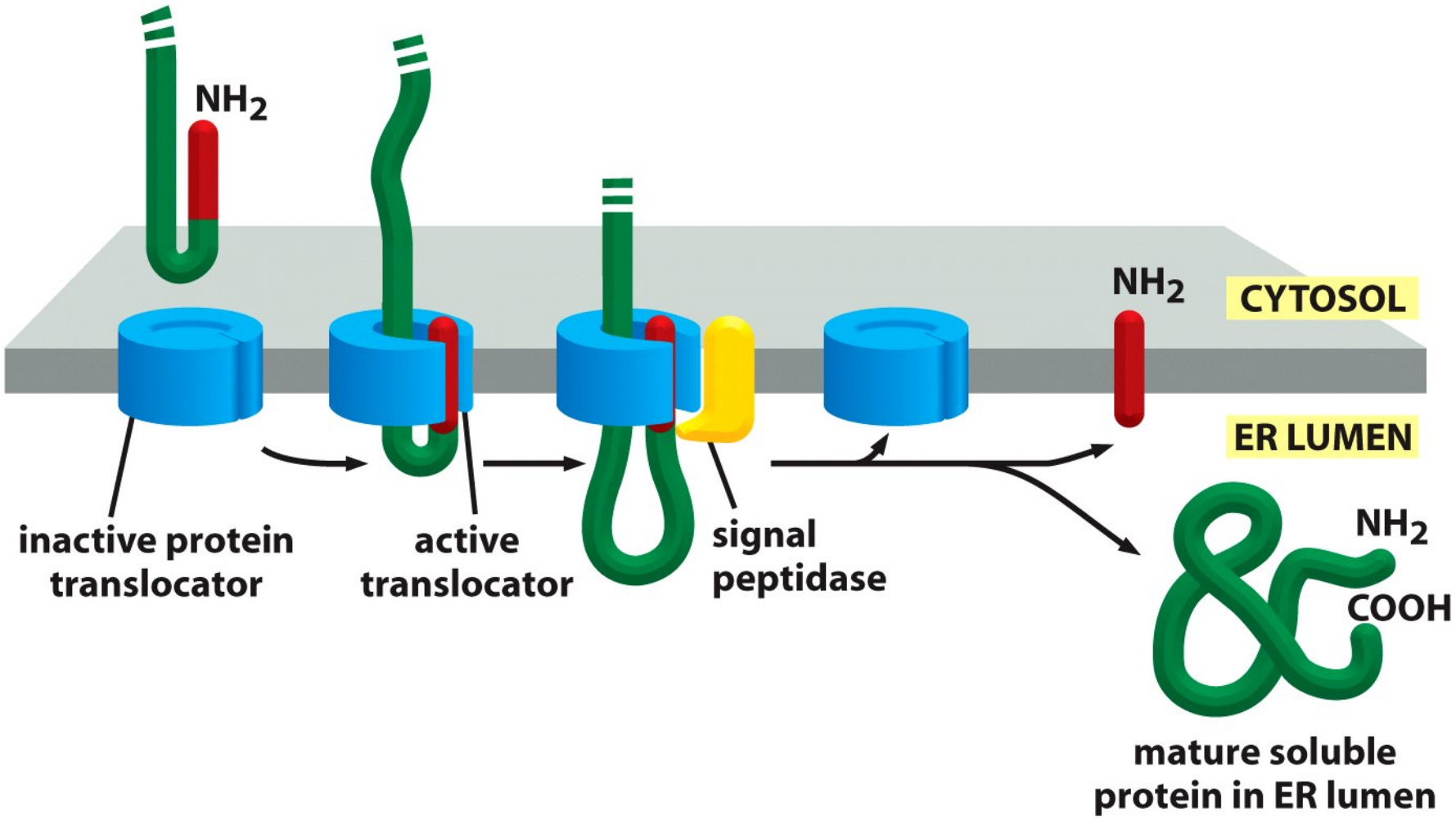


Figure 12-45 *Molecular Biology of the Cell* (© Garland Science 2008)

FUNCTIONS: PROTEIN SYNTHESIS

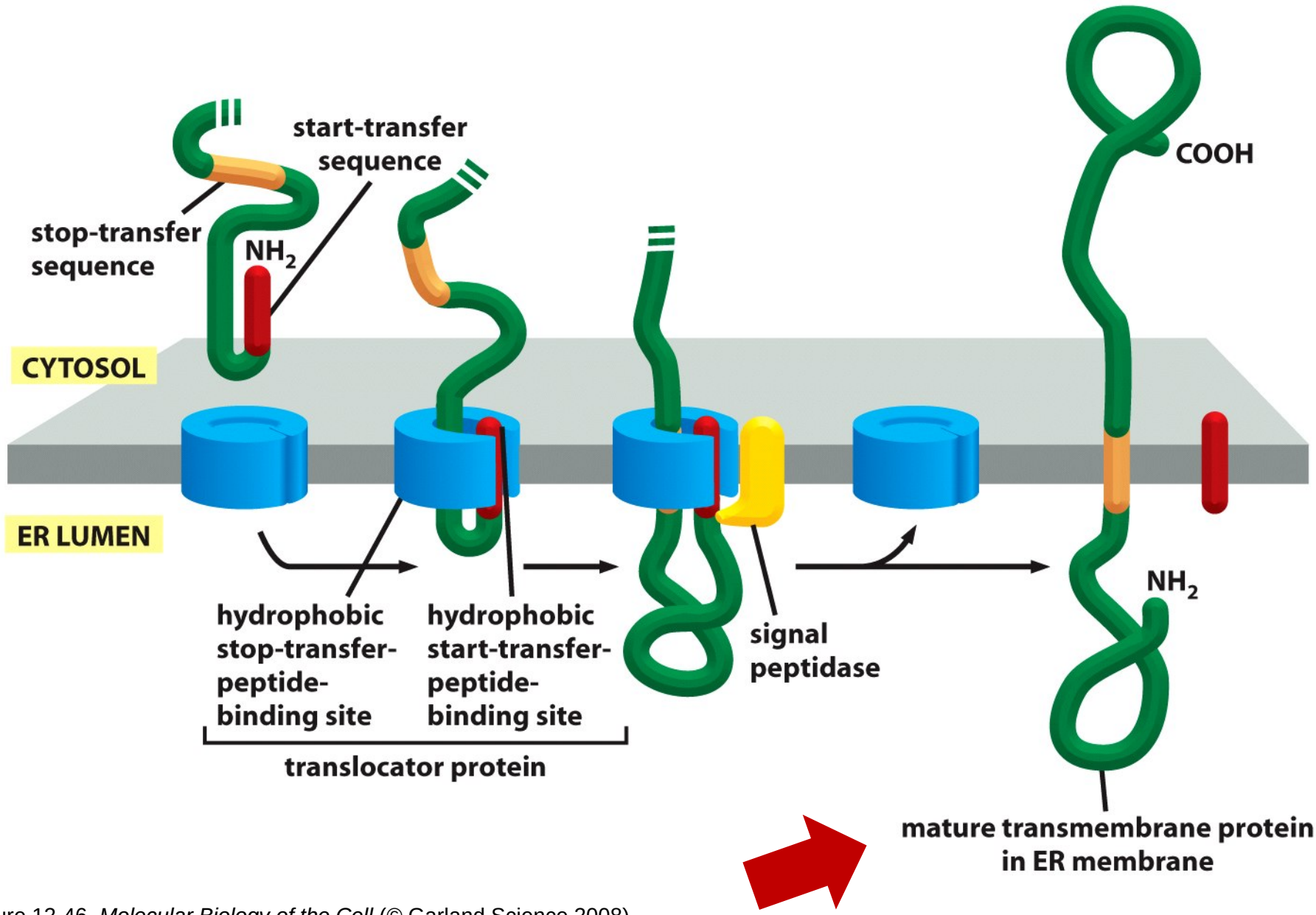


Figure 12-46 *Molecular Biology of the Cell* (© Garland Science 2008)

FUNCTIONS: PROTEIN SYNTHESIS

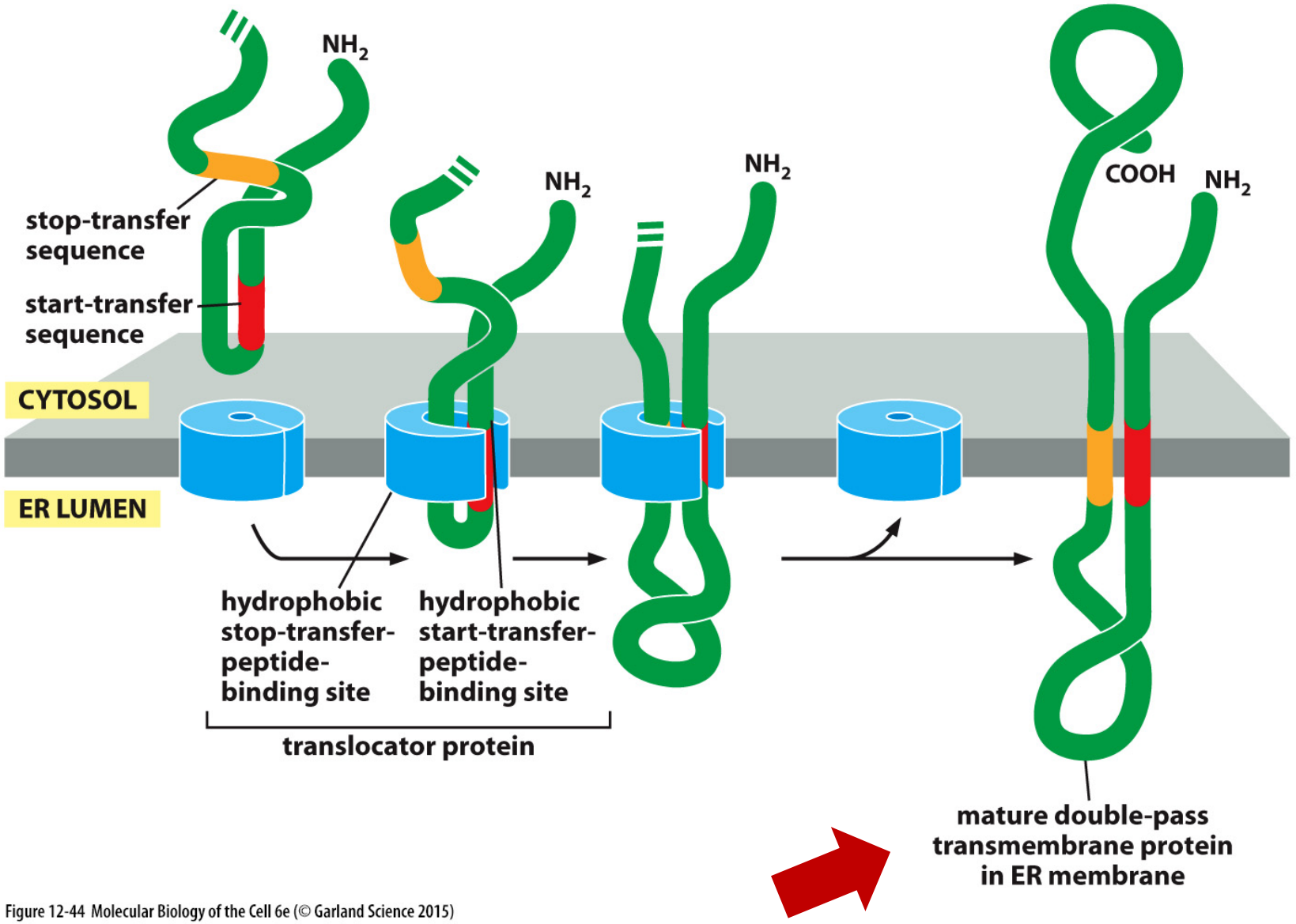


Figure 12-44 Molecular Biology of the Cell 6e (© Garland Science 2015)

FUNCTIONS: PROTEIN SYNTHESIS

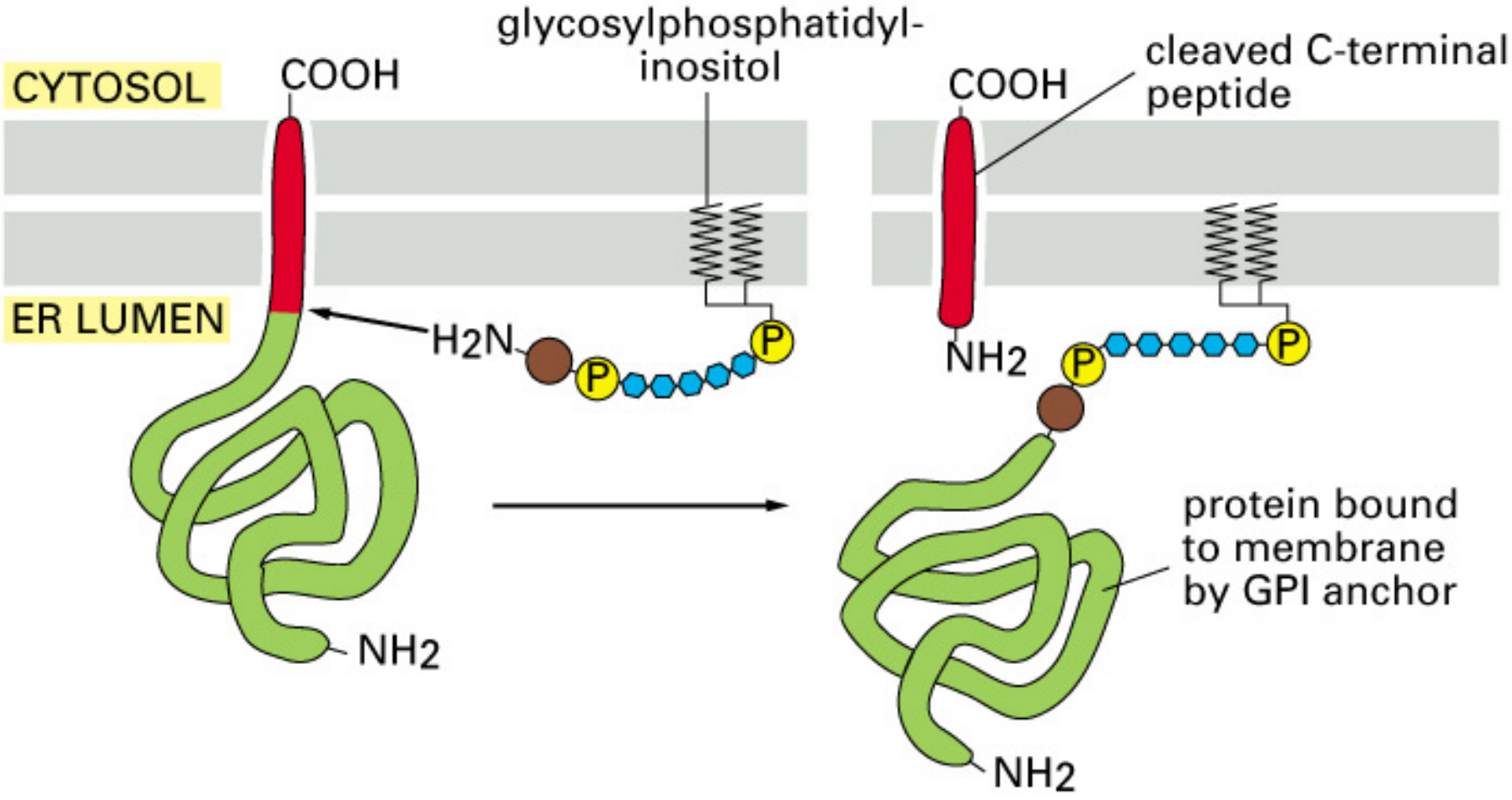
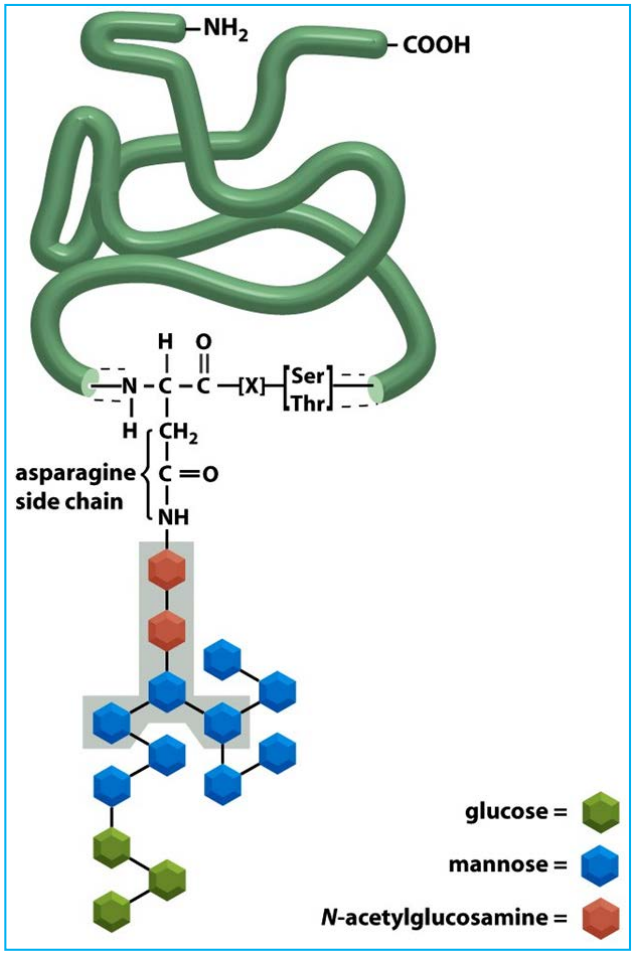
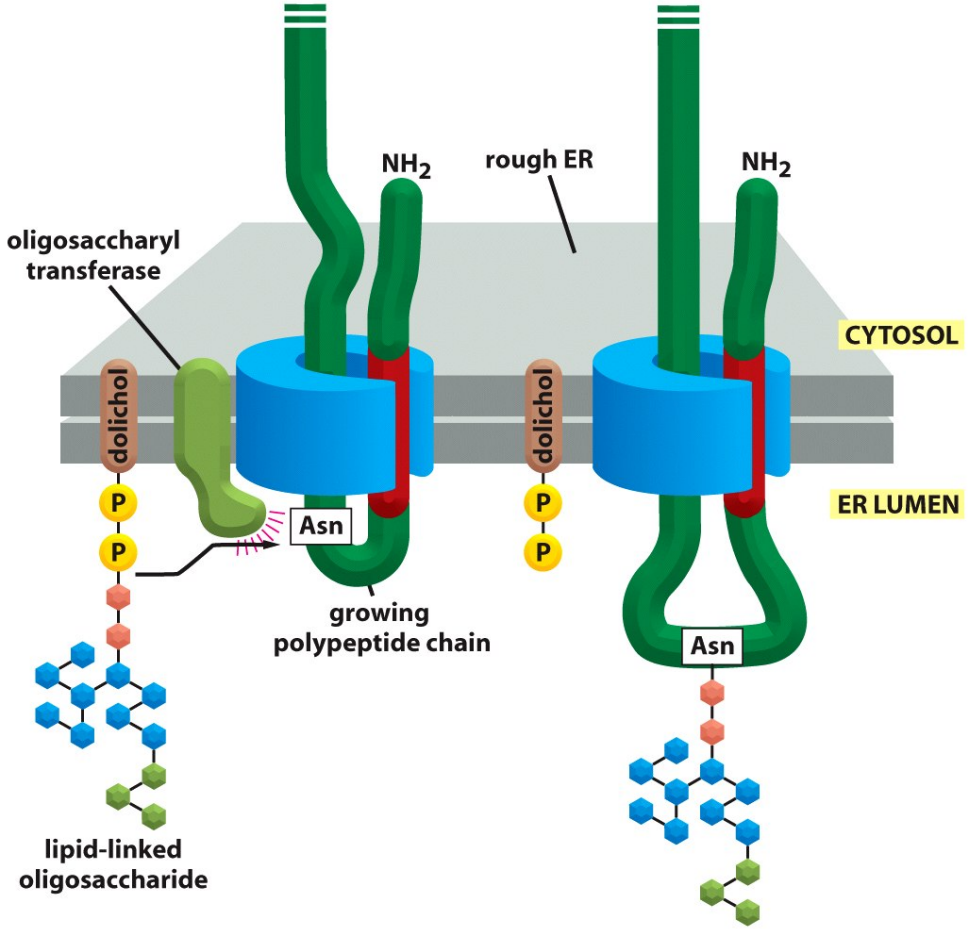


Figure 12–57. Molecular Biology of the Cell, 4th Edition.

FUNCTIONS: GLYCOSYLATIONS

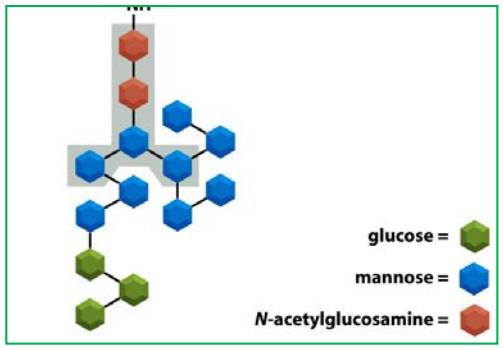




Oligosaccharid N-linked or asparagine-linked

Figure 12-51 *Molecular Biology of the Cell* (© Garland Science 2008)

FUNCTIONS: GLYCOSYLATIONS



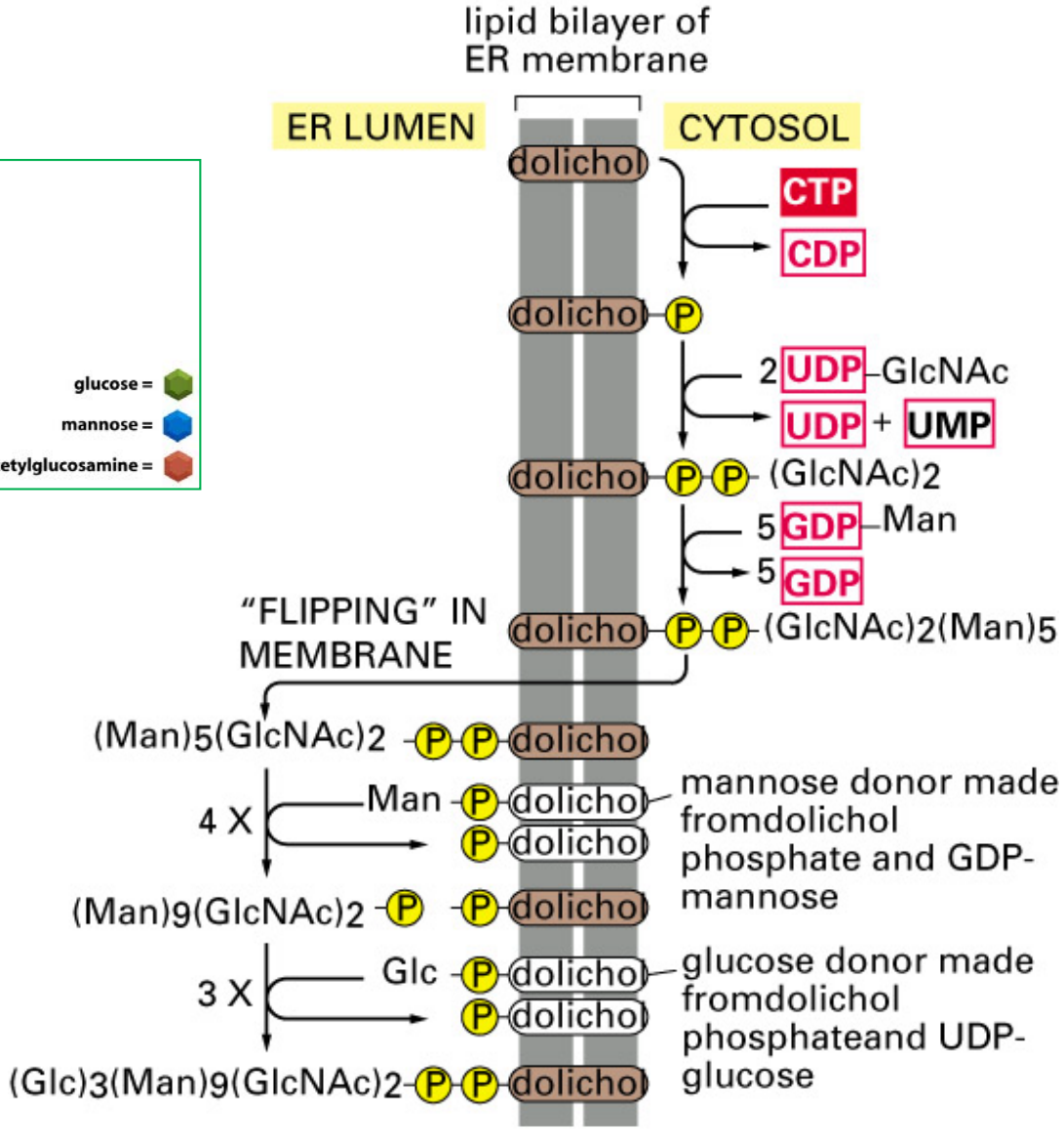


Figure 12–53. Molecular Biology of the Cell, 4th Edition.

FUNCTIONS: GLYCOSYLATIONS

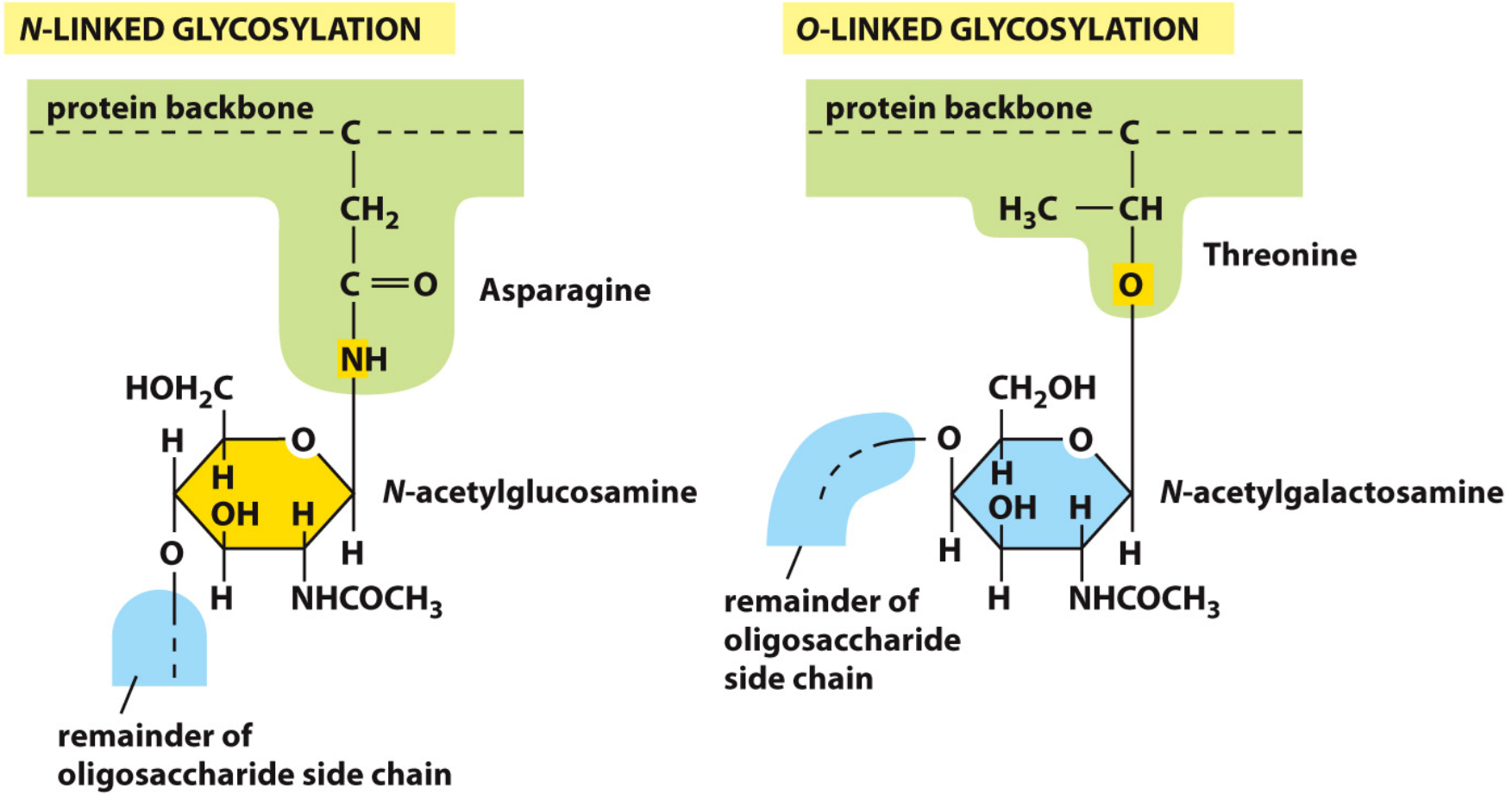


Figure 13-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

FUNCTIONS: LIPID SYNTHESIS

- ER synthesizes nearly all the major classes of lipids, including both phospholipids and cholesterol required for the production of new membranes

- ER also produces ceramide. Ceramide is exported to the Golgi apparatus, where it serves as a precursor for the synthesis of glycosphingolipids and sphingomyelin.

- The ER is the site of production of lipids for most of the cell organelles, including the ER itself, the Golgi apparatus, lysosomes, endosomes, secretory vesicles, and the plasma membrane. The ER membrane also makes most of the lipids for mitochondria and peroxisomal membranes.

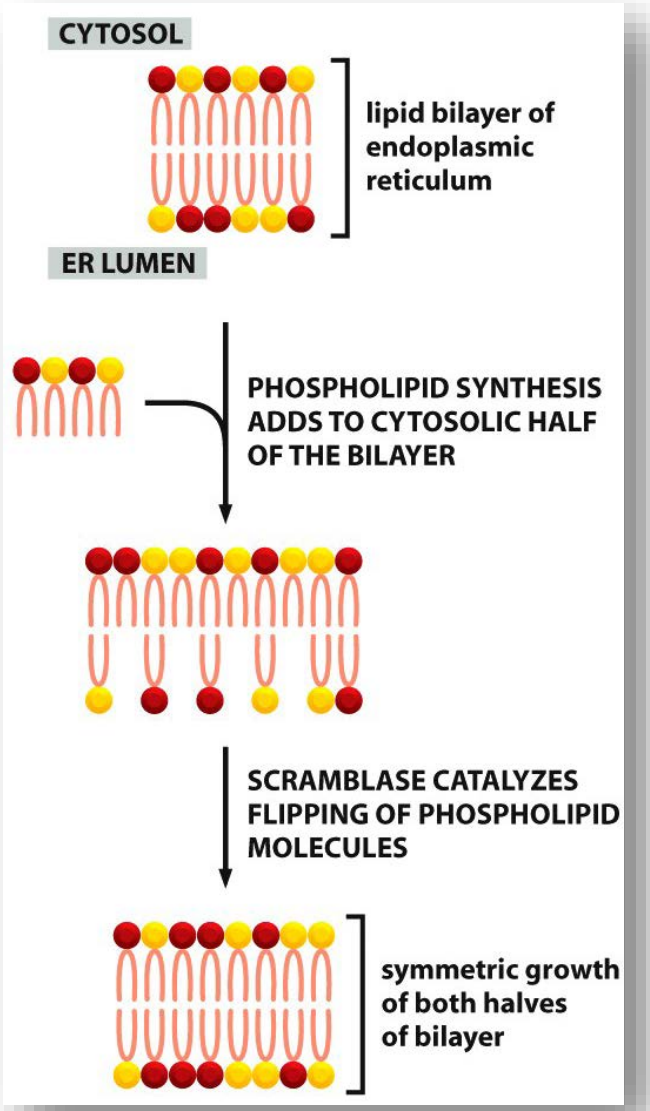
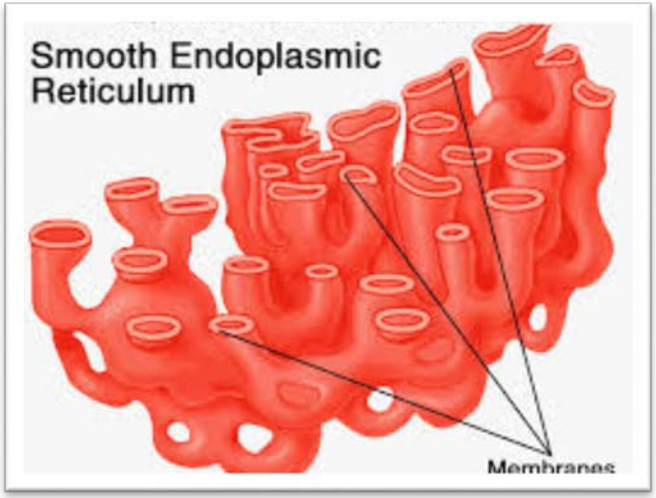
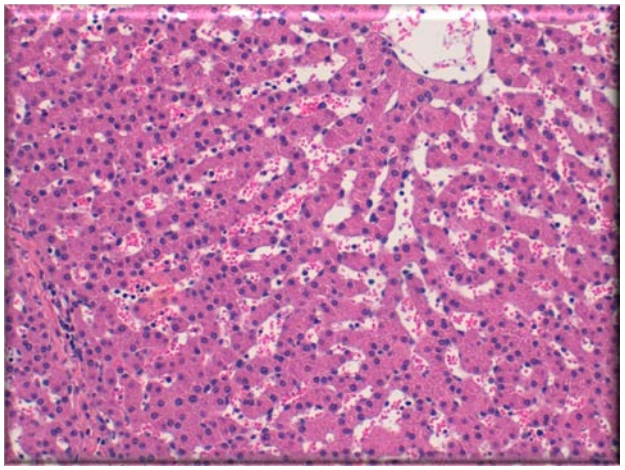


Figure 12-58 *Molecular Biology of the Cell* (© Garland Science 2008)

ENDOPLASMIC RETICULUM : DETOXIFICATION

- In hepatocytes, smooth ER contains enzymes that catalyze a series of reaction to detoxify both lipid-soluble drugs and various harmful compounds produced by metabolism.
- Cytochrome P450 family of enzymes catalyze a series of reactions in which water-insoluble drugs or metabolites that would otherwise accumulate to toxic levels in cell membranes are rendered water-soluble and excreted by the urine.
- The amount of smooth ER can increase notably to perform this detoxification function.

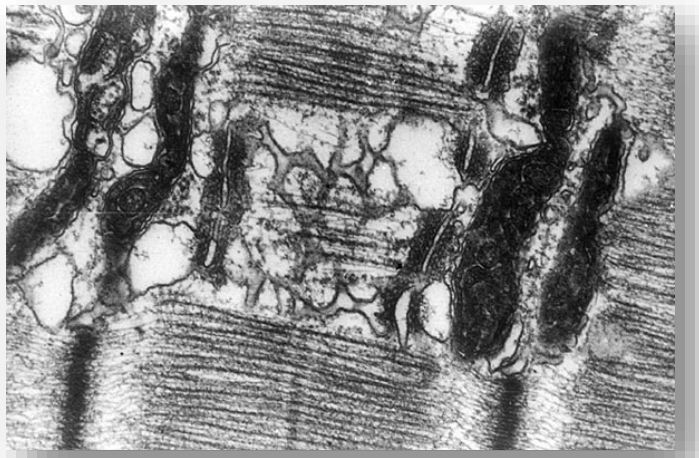




ENDOPLASMIC RETICULUM : Ca^{2+} STORAGE

- In most cells Ca^{2+} is sequestered in the ER. A Ca^{2+} pump transports Ca^{2+} from the cytosol into the ER lumen. A high concentration of Ca^{2+} -binding proteins in the ER facilitates Ca^{2+} storage.
- The release of Ca^{2+} into the cytosol from the ER, and its subsequent reuptake, occurs in many rapid responses to extracellular signals.

- Muscle cells have an abundant, modified smooth ER, called the **sarcoplasmic reticulum**.
- The release and reuptake of Ca^{2+} by the sarcoplasmic reticulum trigger myofibril contraction and relaxation, respectively, during each round of muscle contraction.



ENDOPLASMIC RETICULUM : INTRACELLULAR TRANSPORT

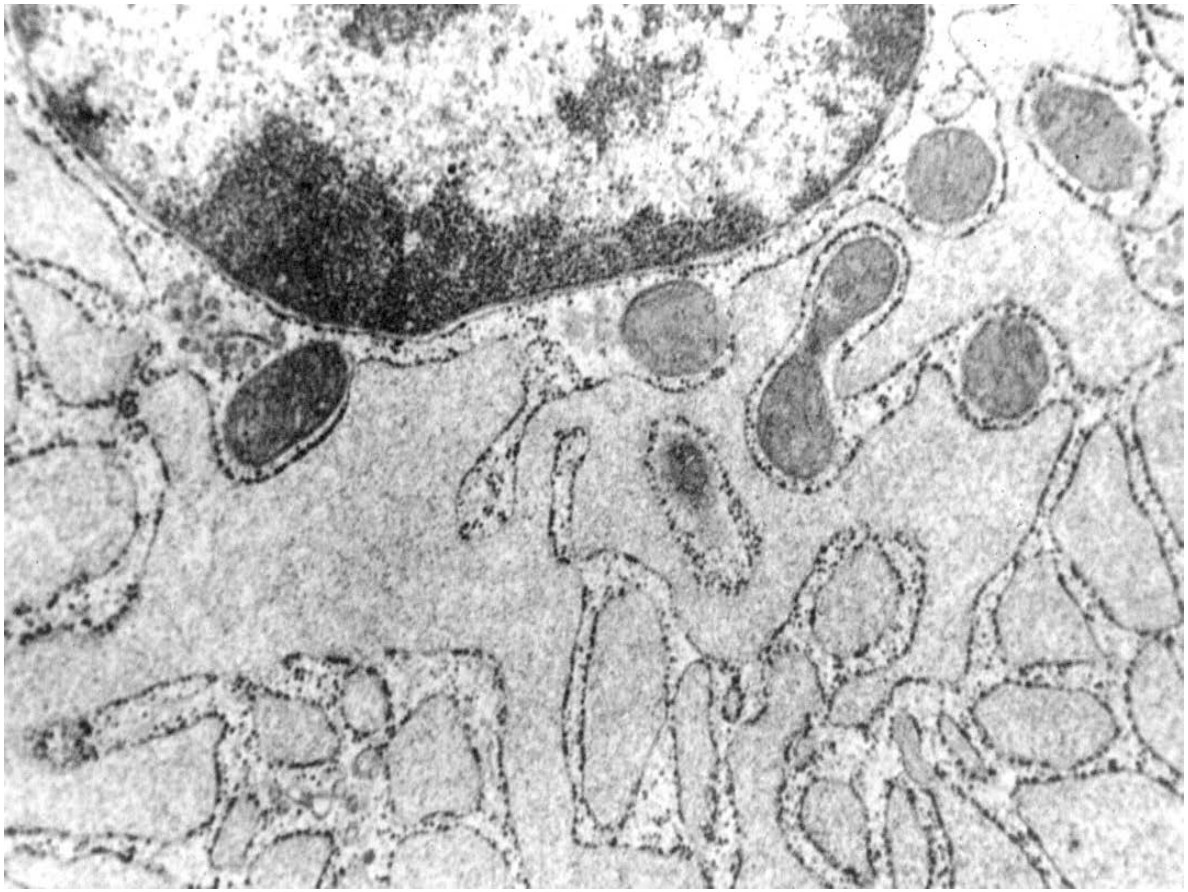
- ER forms a separate compartment within the cell where molecules can be transported to different locations within the cell without contact with the cytosol.

- Membrane proteins and lipids are transported to the transitional reticulum for vesicle formation and transport to the Golgi apparatus.

- ER resident proteins contain a signal ER retention of four amino acids at its C-terminus, which is responsible for retaining the protein in the ER: Lys-Asp-Glu-Leu-COO

ENDOPLASMIC RETICULUM : PRODUCT STORAGE

Products of ER can be stored within the lumen, appearing when viewed with electron microscopy as extensively dilated cisternae.



EXPORT AND DEGRADATION OF MISFOLDED ER PROTEINS

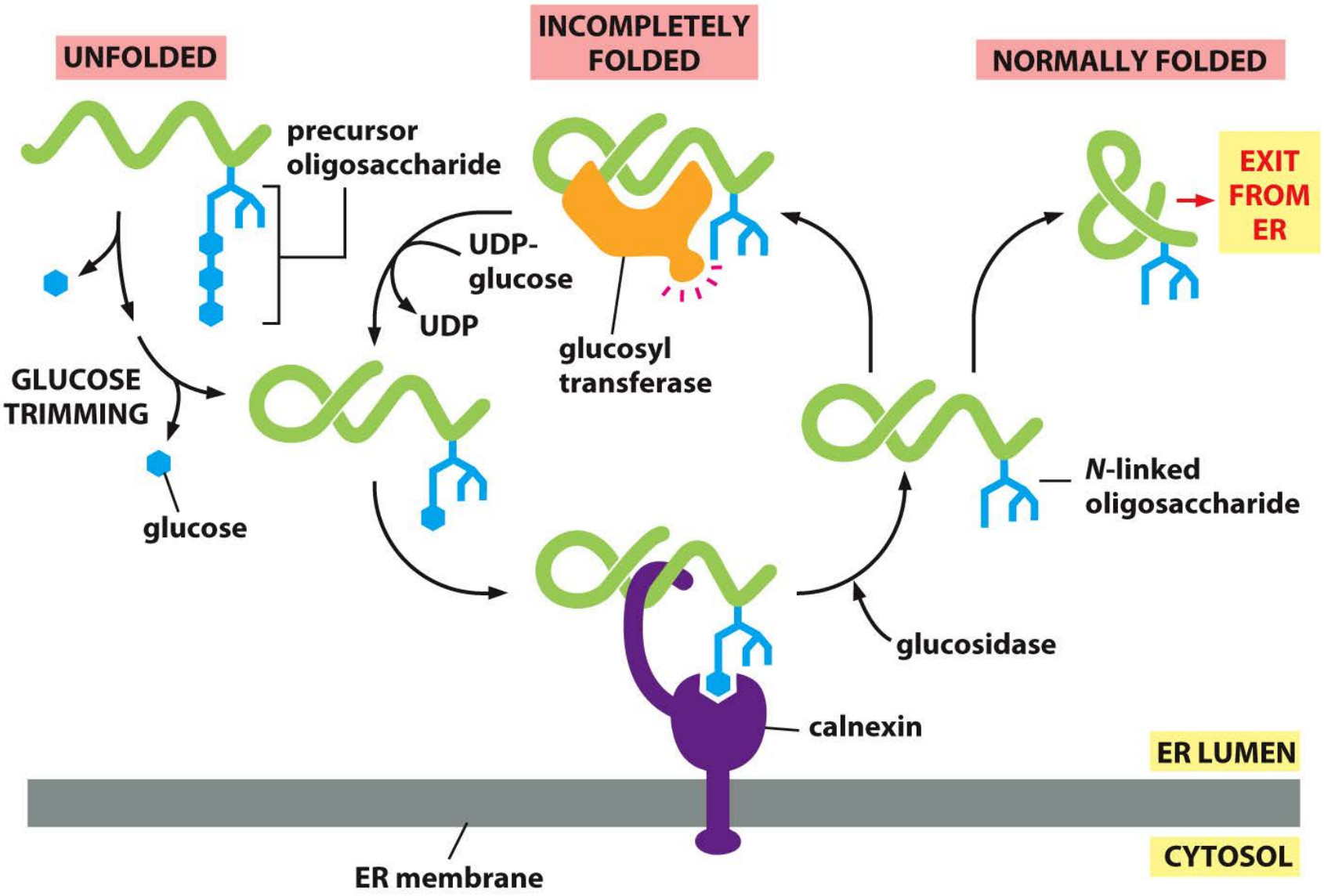


Figure 12-49 Molecular Biology of the Cell 6e (© Garland Science 2015)

EXPORT AND DEGRADATION OF MISFOLDED ER PROTEINS

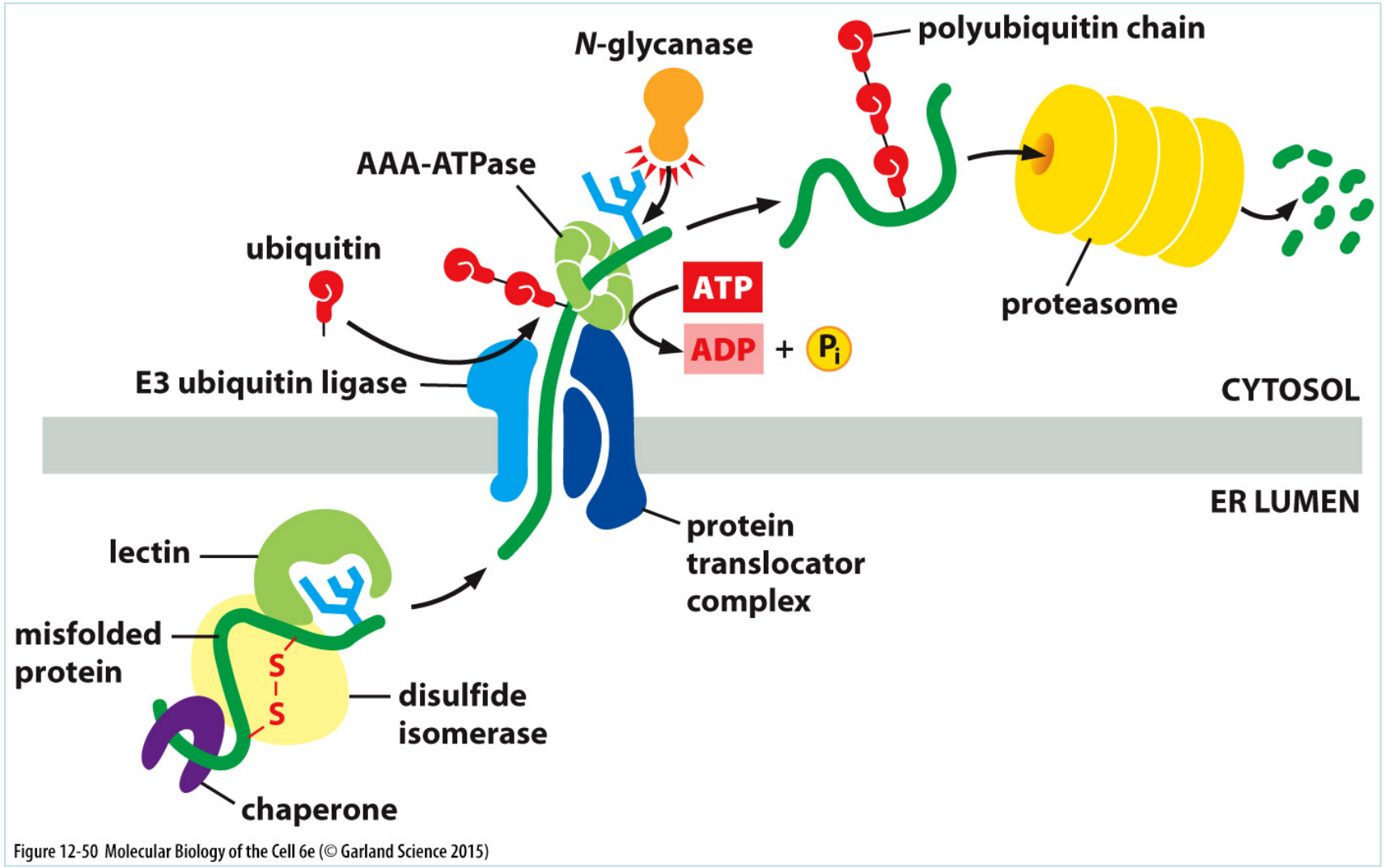
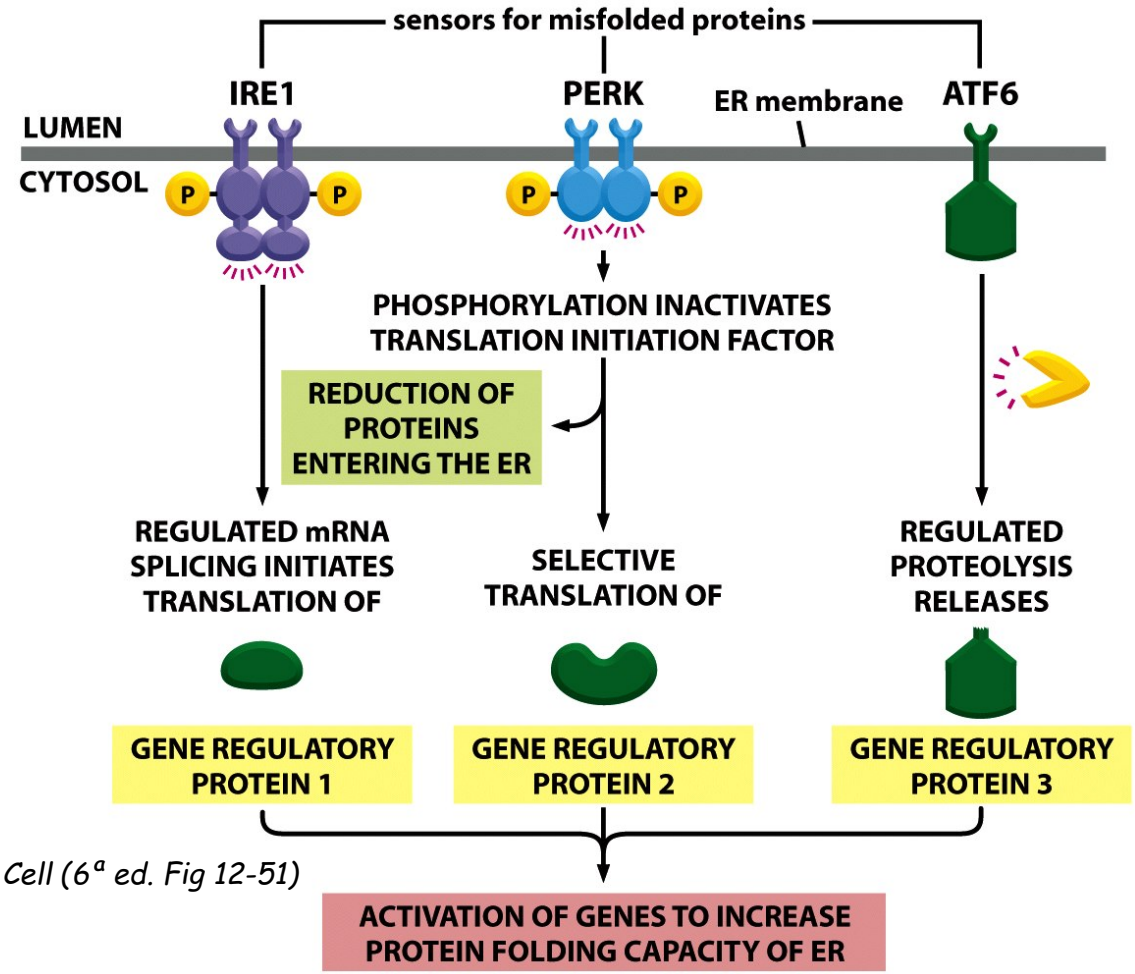


Figure 12-50 Molecular Biology of the Cell 6e (© Garland Science 2015)

SYSTEM "UPR": UNFOLDED PROTEIN RESPONSE



Molecular Biology of the Cell (6ª ed. Fig 12-51)

The UPR has three aims:

- Initially to restore normal function of the cell by halting protein translation.
- Degrading misfolded proteins.
- Activating the signaling pathways that lead to increasing the production of molecular chaperones involved in protein folding.

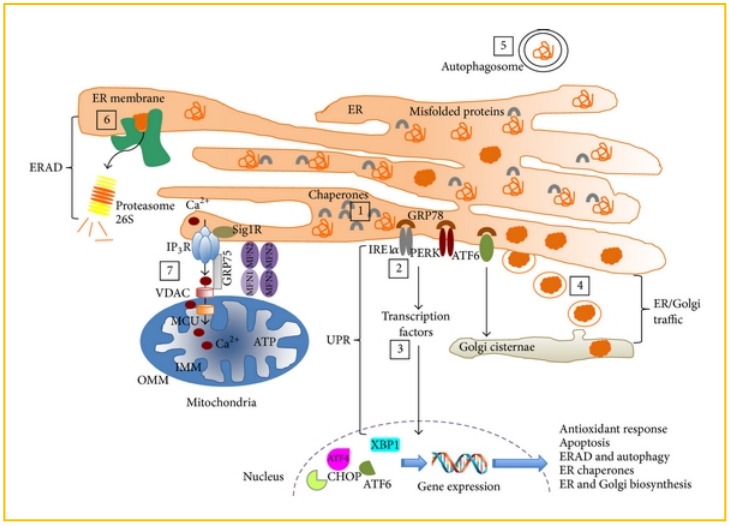
The **unfolded protein response** (UPR) is a cellular stress response related to the endoplasmic reticulum

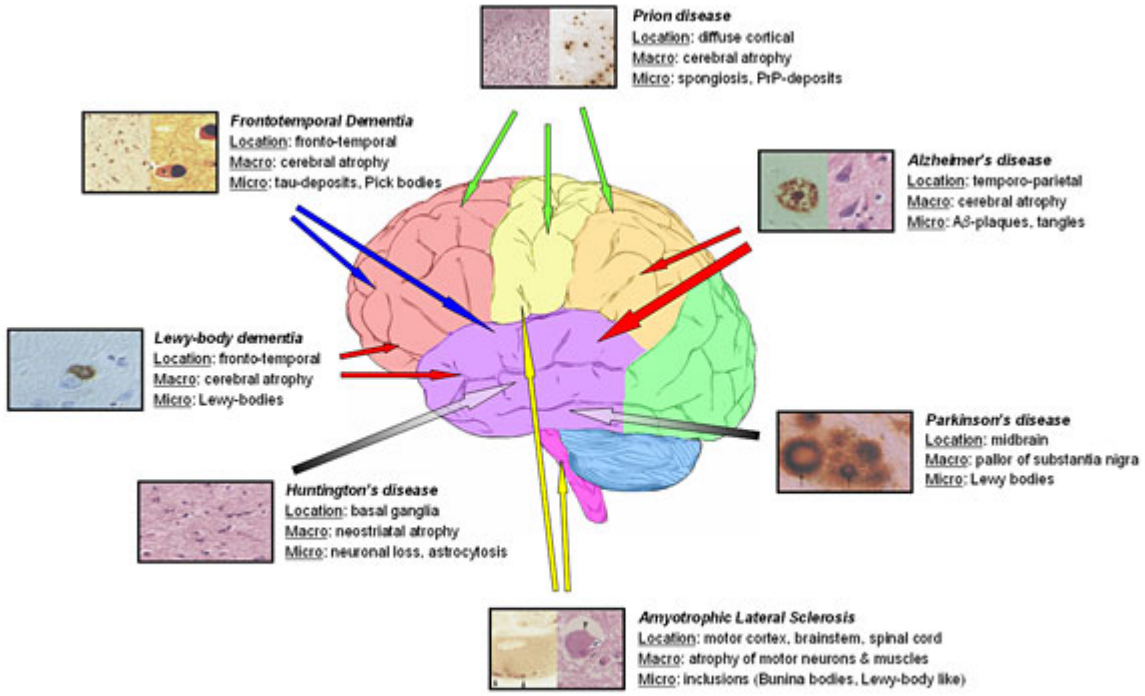
BIOPATHOLOGY

❑ Neurodegenerative diseases

Alzheimer
Parkinson
Huntington

Several neurodegenerative diseases are classified as proteopathies as they are associated with the aggregation of misfolded proteins.





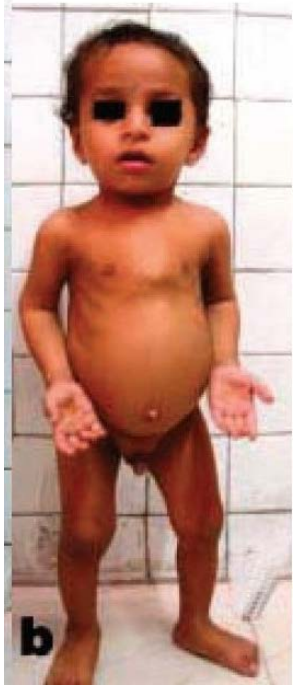
Bertram and Tanzi, *JCI*, 2005

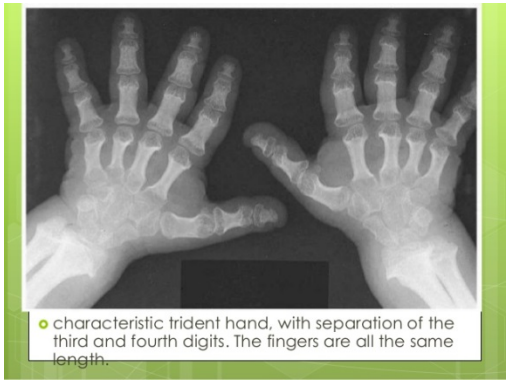
BIOPATHOLOGY

❑ Pseudoachondroplasia

Caused by a mutation in the COMP gene ("cartilage oligomeric matrix protein")

The COMP protein is located in the extracellular matrix surrounding the cells that make up ligaments and tendons, and near cartilage-forming cells (chondrocytes). Chondrocytes play an important role in bone formation (osteogenesis).





Mutations → *Aggregation of misfolded proteins in the RE*

BIOGENESIS

- Membrane lipids of the ER are produced by the ER.
- Most of the membrane proteins that define the ER and perform many of its functions are themselves products of the ER.
- Some completely synthesized proteins are, however, imported into the ER. Proteins that are transported into the ER by a post-translational mechanism are first released into the cytosol, where they bind chaperone proteins to prevent folding.

GOLGI APPARATUS

1- Ultrastructure

2- Functions

- Protein modifications
- Protein secretion
- Membrane renewal
- Lysosome formation

3- Vesicle formation and transport

4- Biogenesis. Organization models

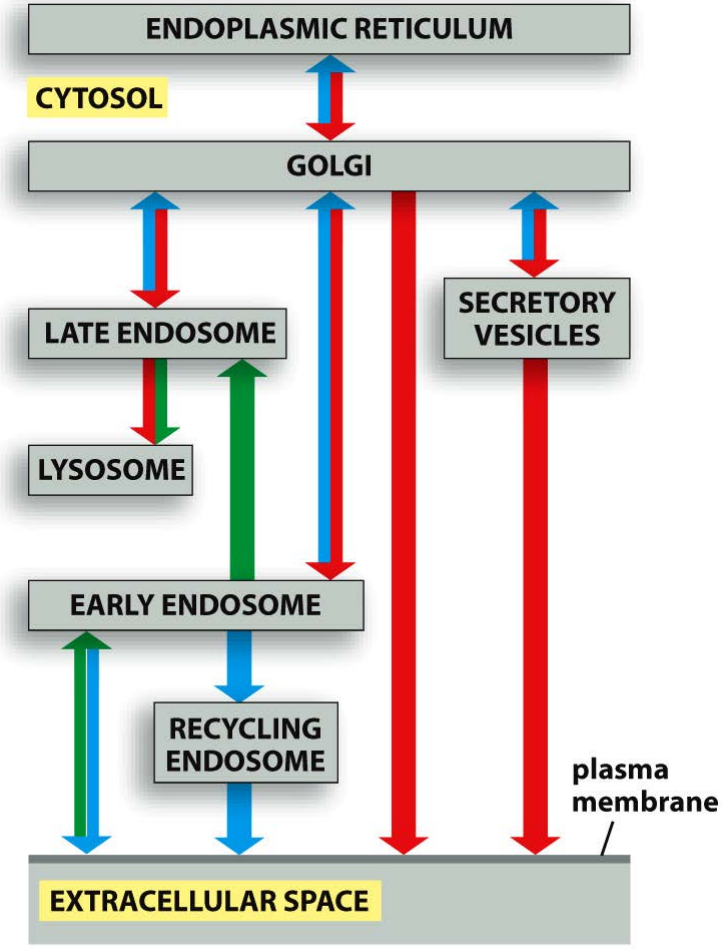
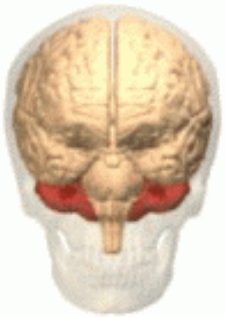
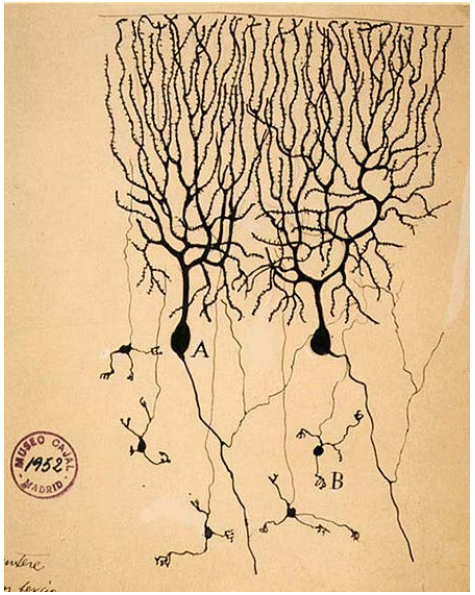


Figure 13-3a Molecular Biology of the Cell 6e (© Garland Science 2015)

GOLGI APPARATUS





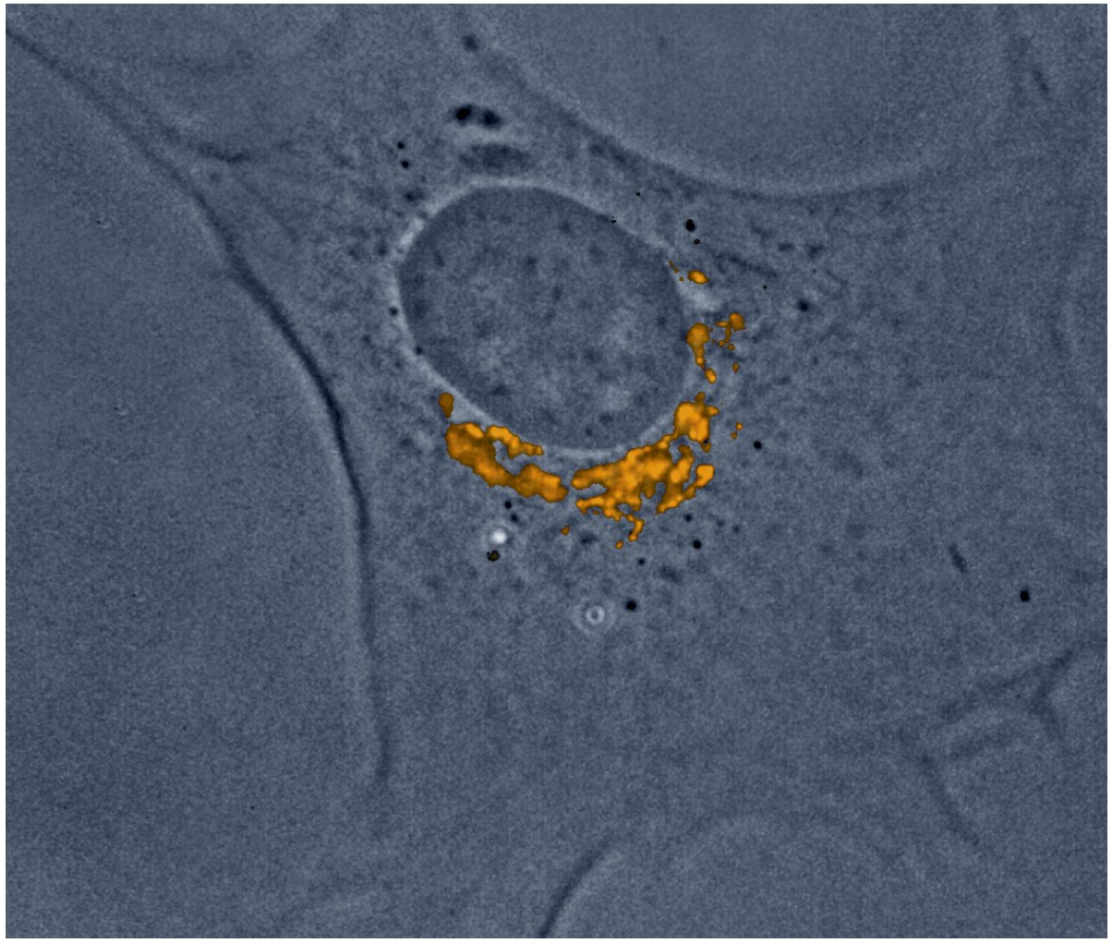
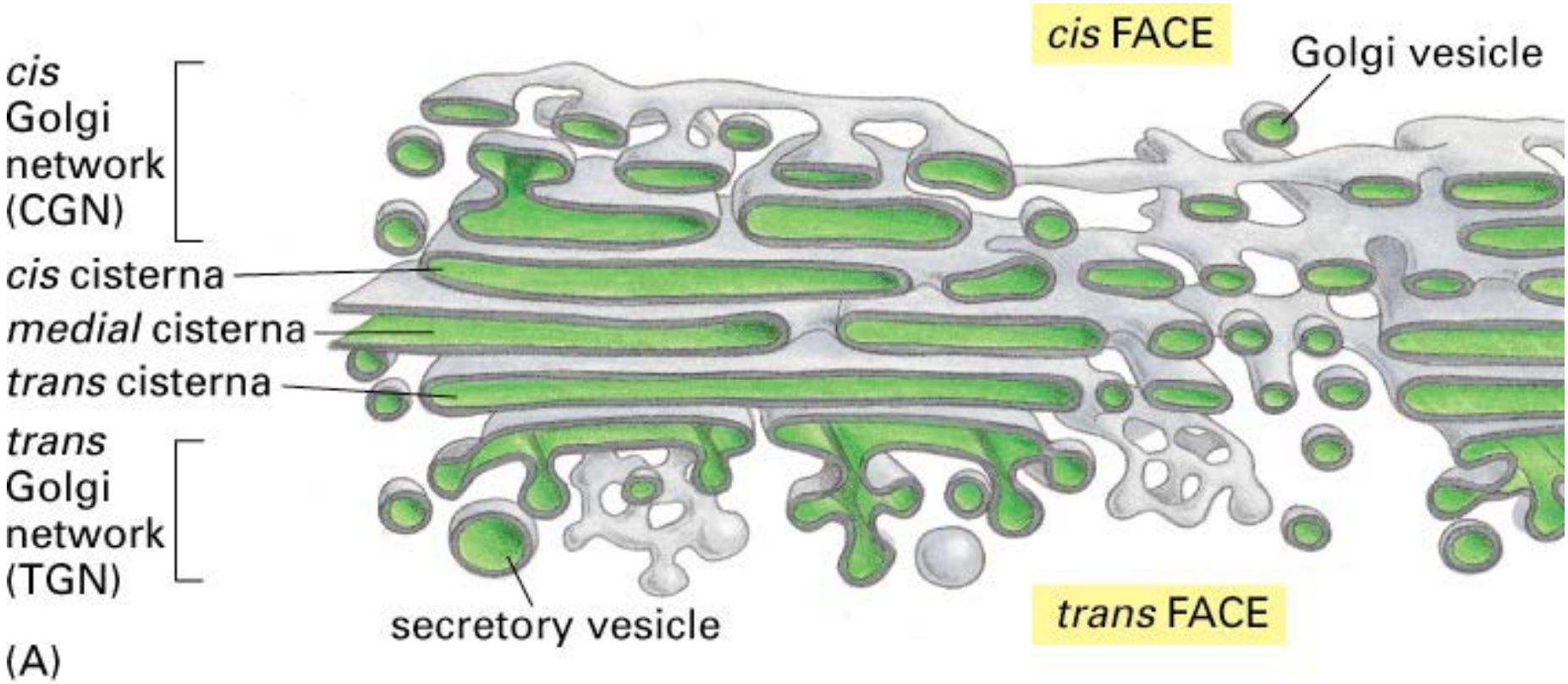


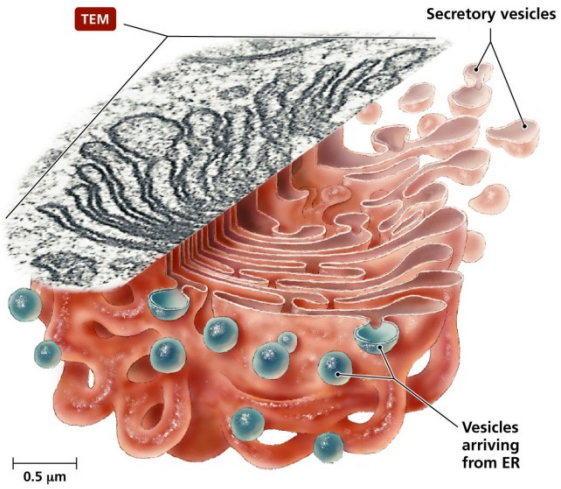
Figure 13-26a *Molecular Biology of the Cell* (© Garland Science 2008)

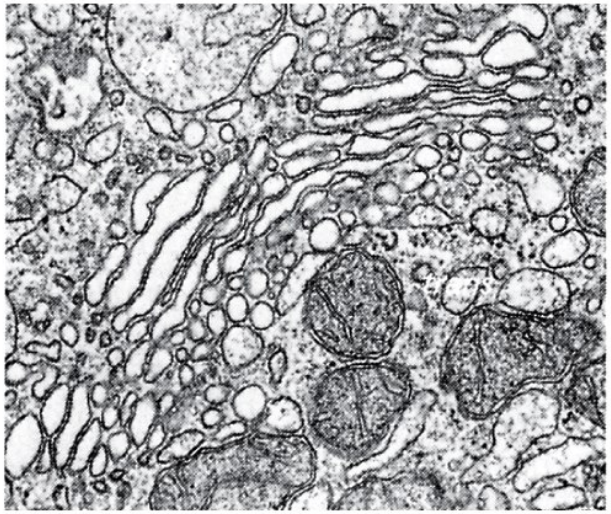
ULTRASTRUCTURE



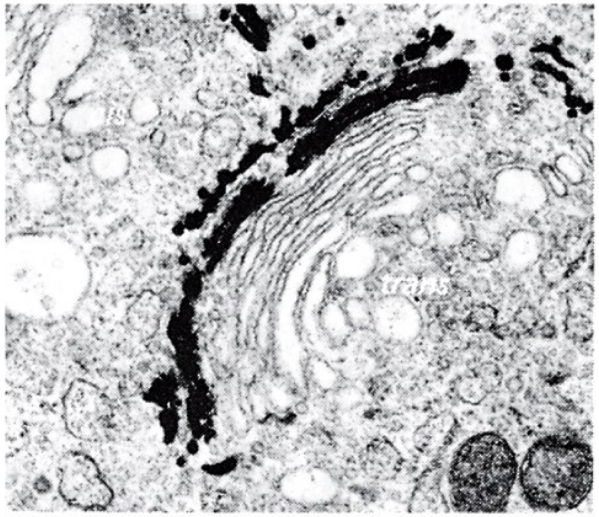
Alberts, sixth ed. Fig. 13-26

ULTRASTRUCTURE

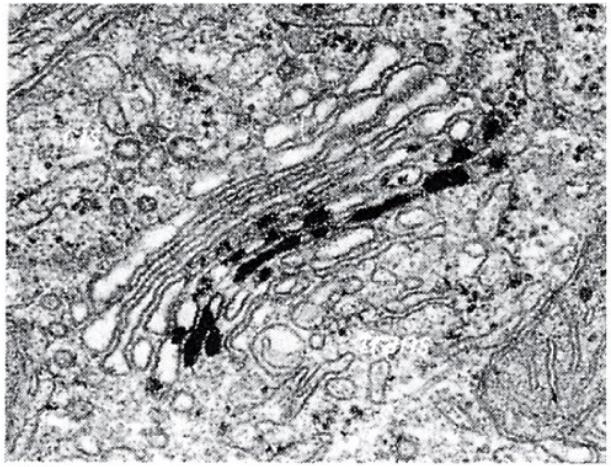




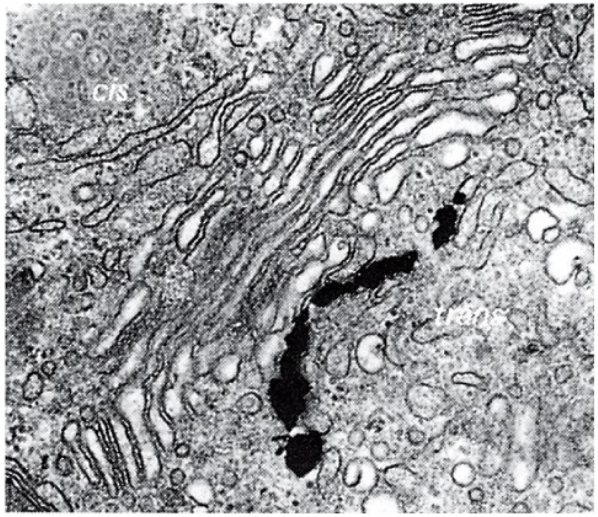
(A) Unstained



(B) Osmium



(C) Nucleoside diphosphatase



(D) Acid phosphatase

FUNCTIONS

Protein modifications

Protein secretion

Membrane renewal

Lysosome formation

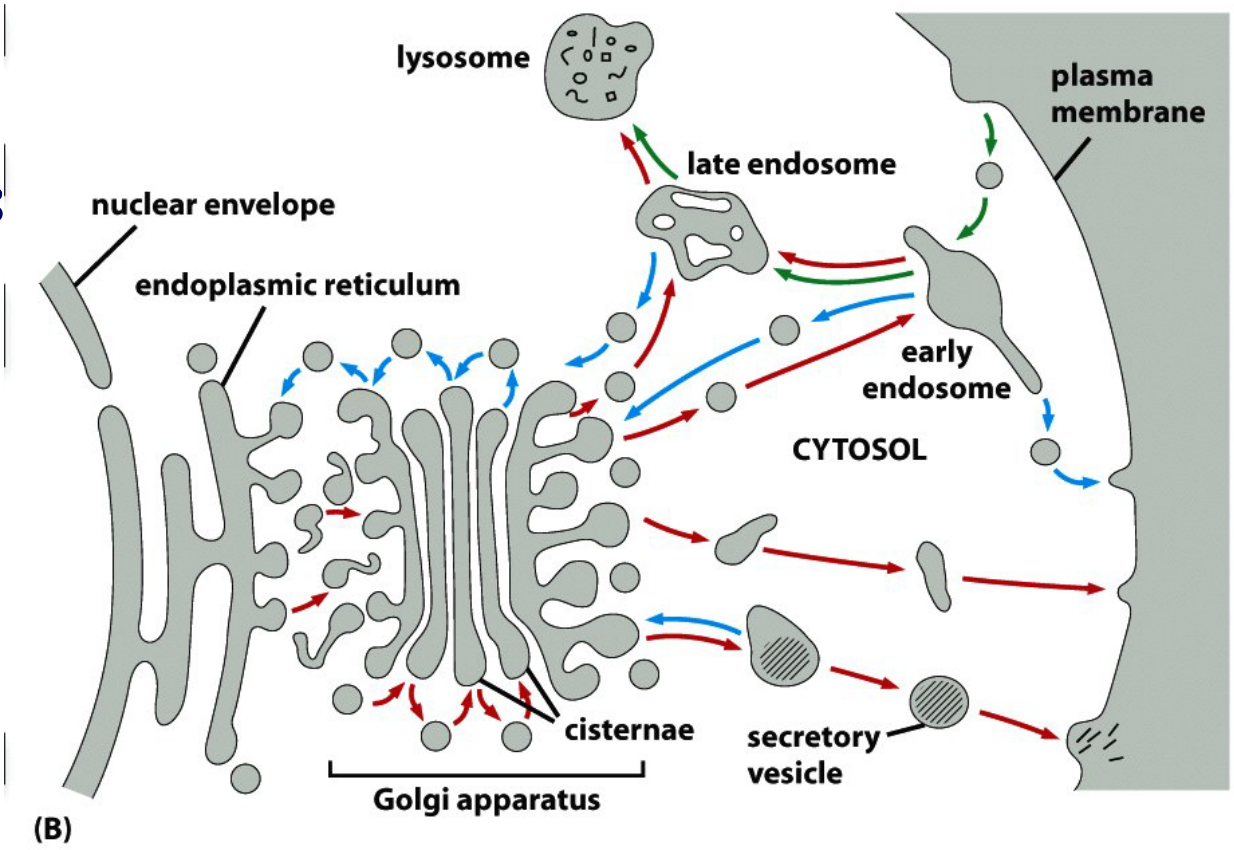
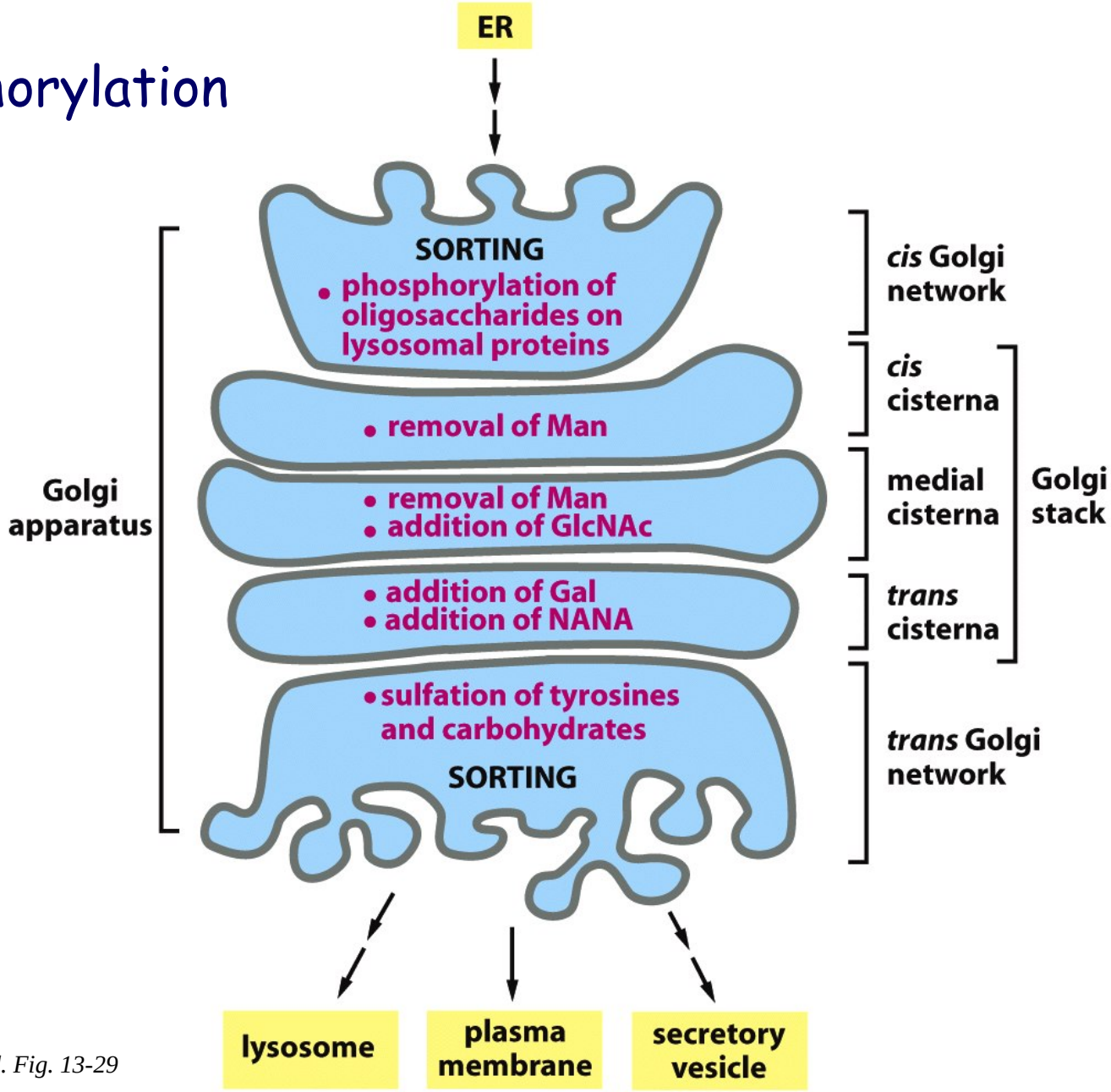


Figure 13-3 *Molecular Biology of the Cell* (© Garland Science 2008)

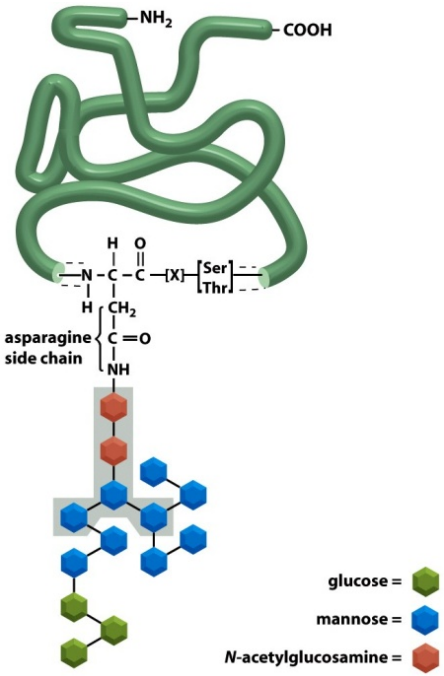
FUNCTIONS: PROTEIN MODIFICATIONS

- Mannose phosphorylation
- Glycosylations
- Sulfations



Alberts, sixth ed. Fig. 13-29

FUNCTIONS: PROTEIN MODIFICATIONS



ENDOPLASMIC RETICULUM

GLYCOSYLATIONS

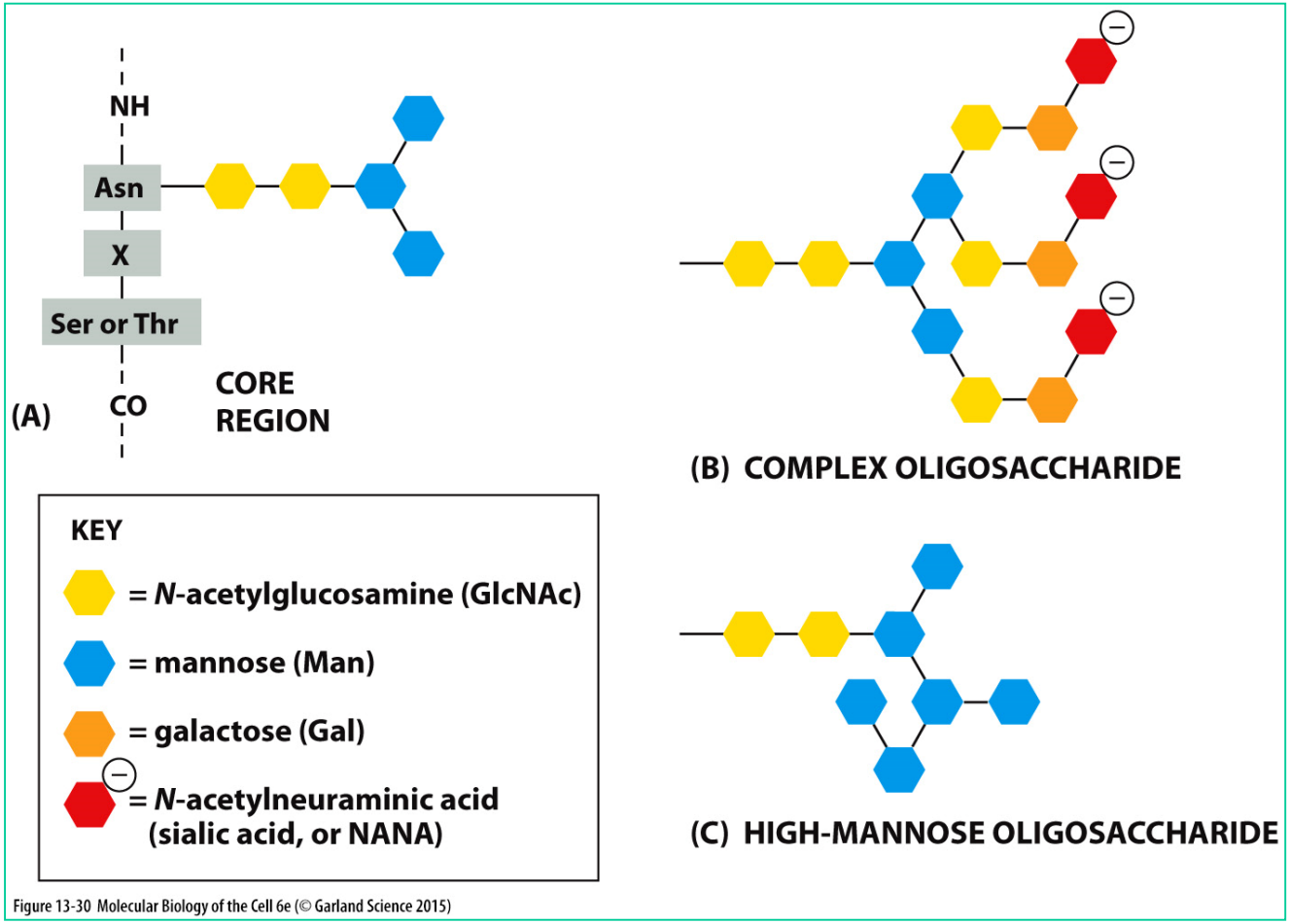
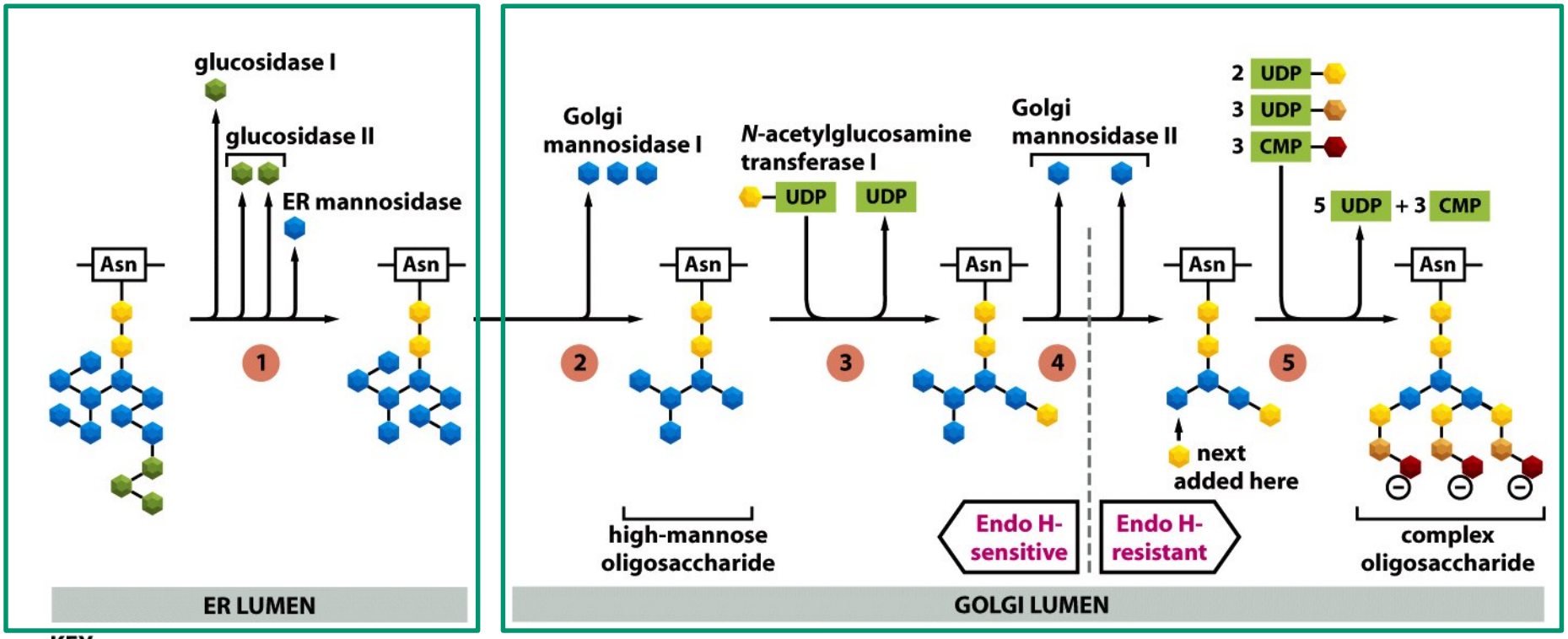


Figure 13-30 Molecular Biology of the Cell 6e (© Garland Science 2015)

FUNCTIONS: PROTEIN MODIFICATIONS

GLYCOSYLATIONS



KEY:

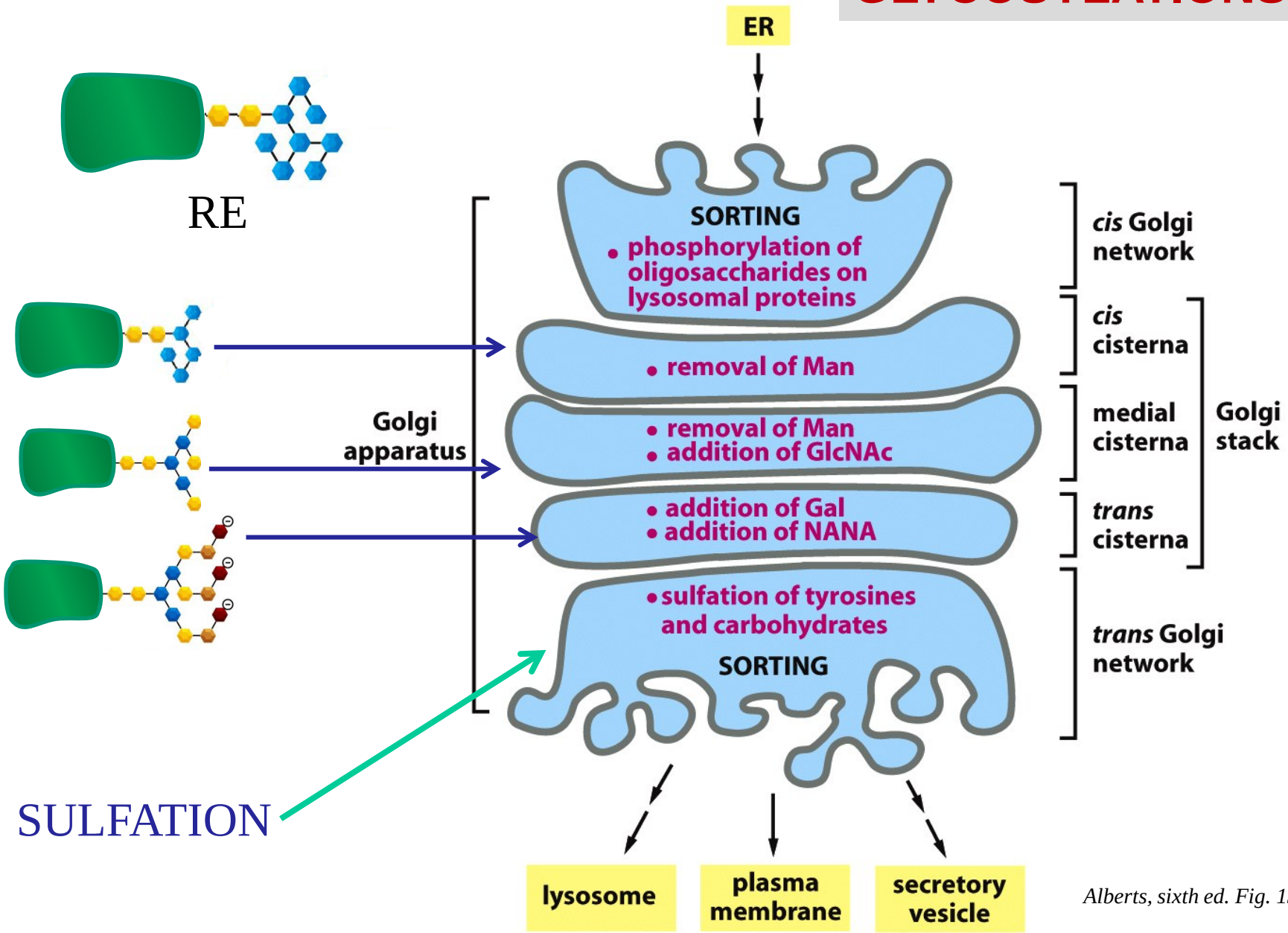
= *N*-acetylglucosamine (GlcNAc) = mannose (Man) = glucose (Glc)

= galactose (Gal) = *N*-acetylneuraminic acid (sialic acid, or NANA)

Figure 13-31 Molecular Biology of the Cell 6e (© Garland Science 2015)

FUNCTIONS: PROTEIN MODIFICATIONS

GLYCOSYLATIONS



Alberts, sixth ed. Fig. 13-29

FUNCTIONS: PROTEIN SECRETION AND MEMBRANE RENEWAL

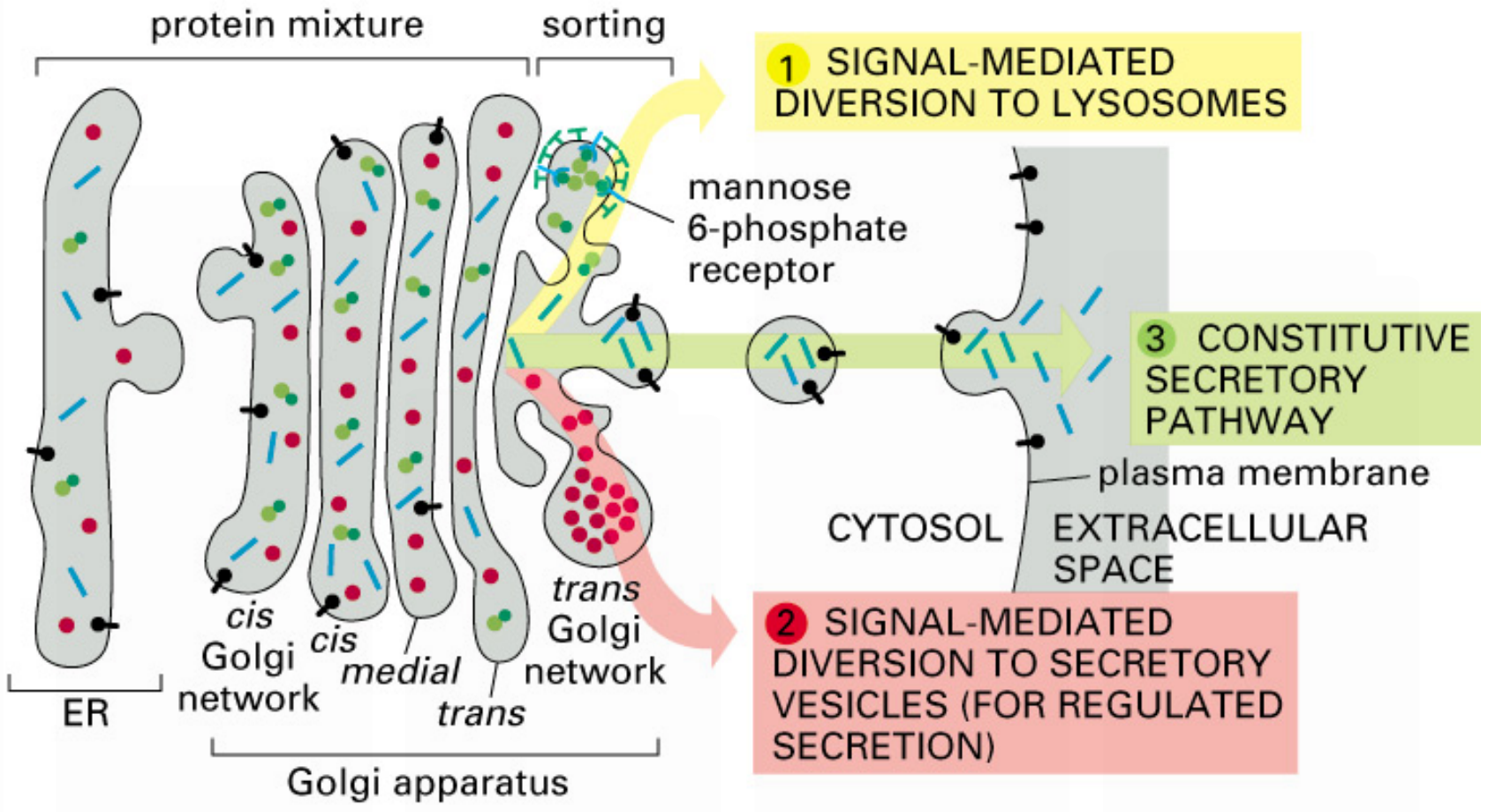
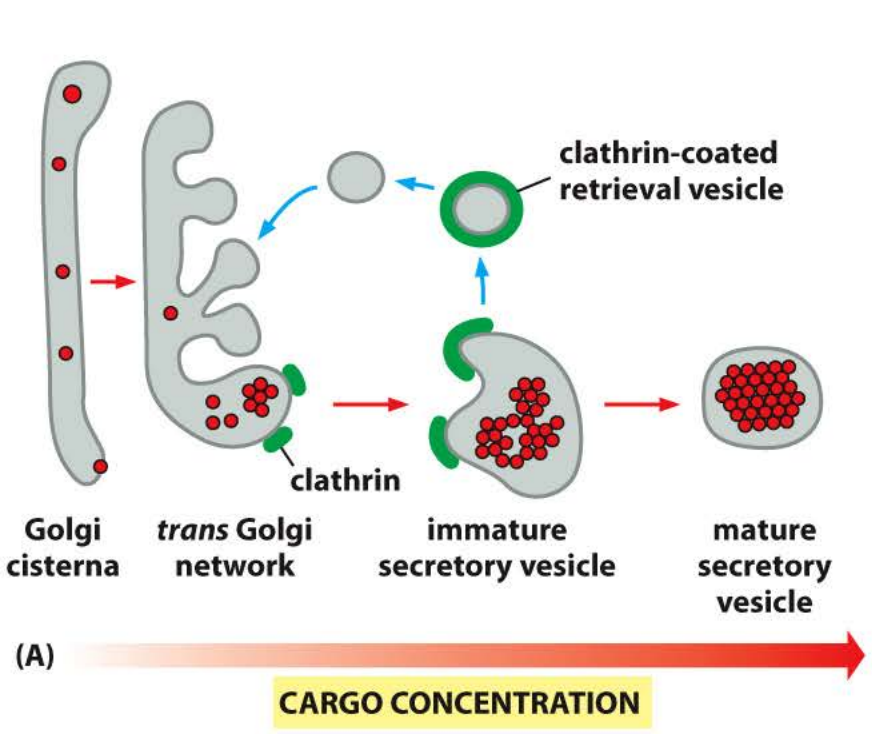


Figure 13–55. Molecular Biology of the Cell, 4th Edition.

FUNCTIONS: PROTEIN SECRETION



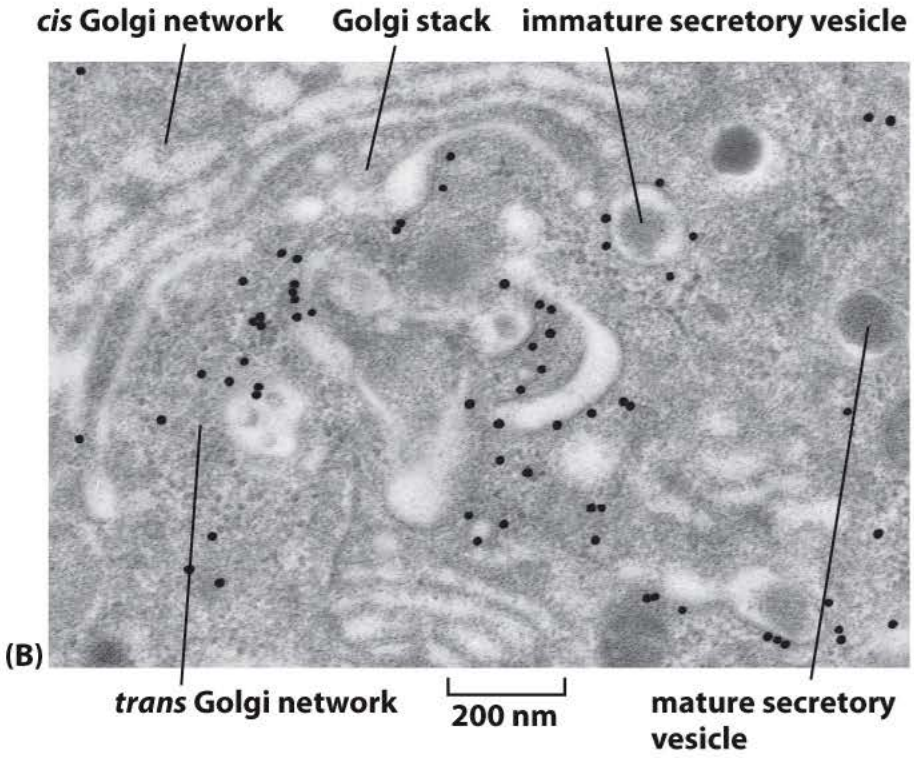


Figure 13-64 Molecular Biology of the Cell 6e (© Garland Science 2015)

VESICLE FORMATION AND TRANSPORT

TYPES OF COATED VESICLES

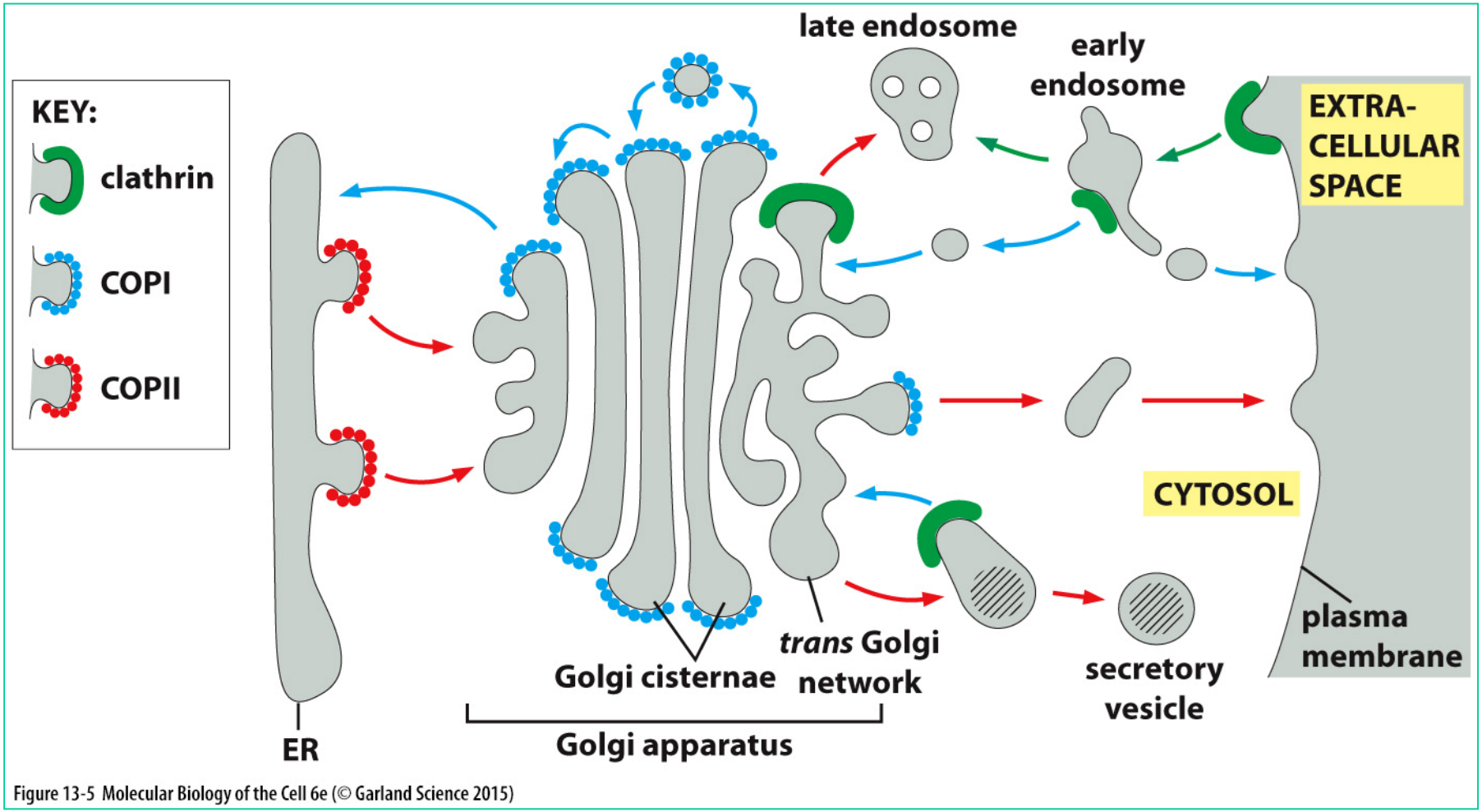
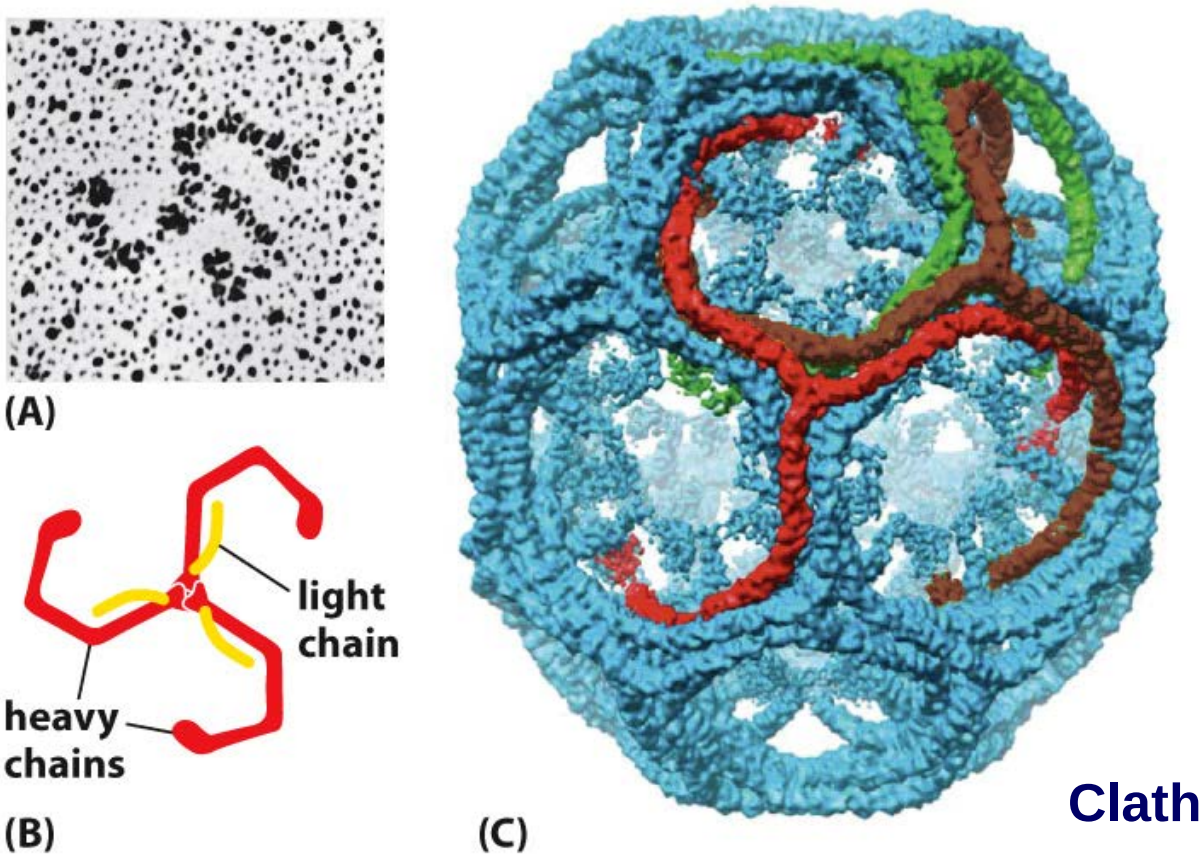


Figure 13-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

VESICLE FORMATION AND TRANSPORT

STRUCTURE OF CLATHRIN COAT

Clathrin triskelion



Clathrin coat

Figure 13-6 Molecular Biology of the Cell 6e (© Garland Science 2015)

VESICLE FORMATION AND TRANSPORT

CLATHRIN COATED VESICLES

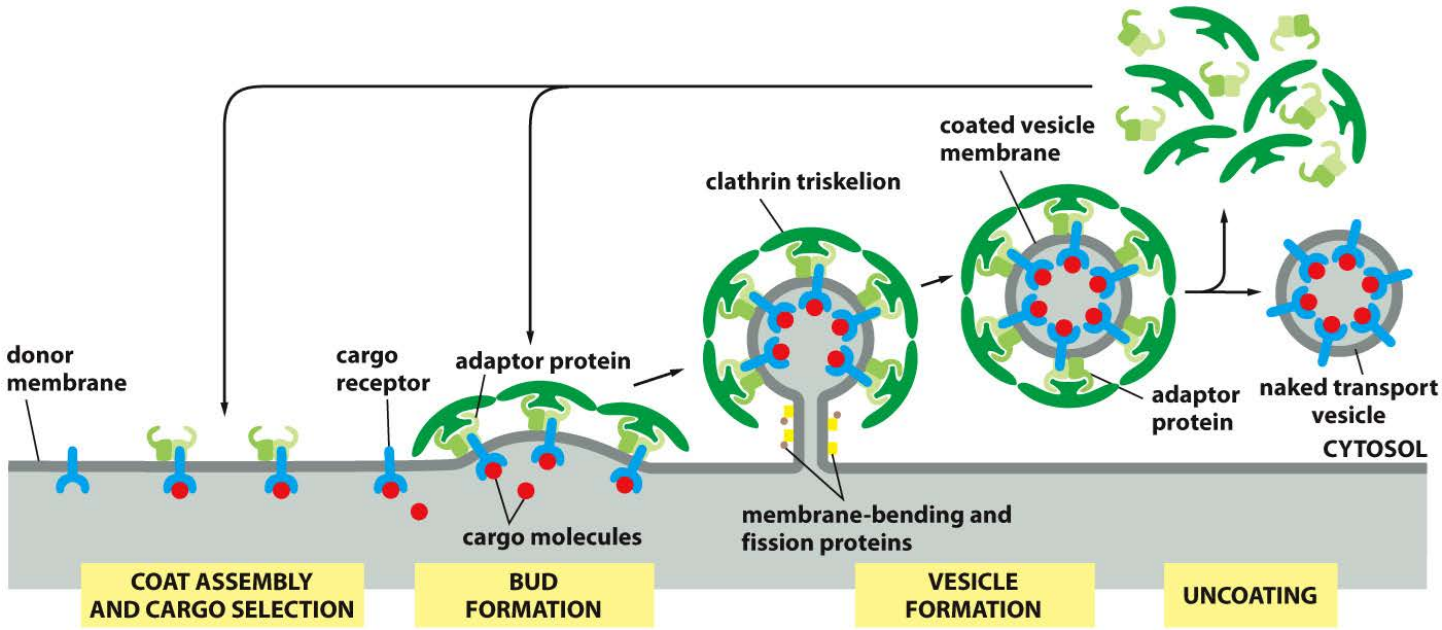
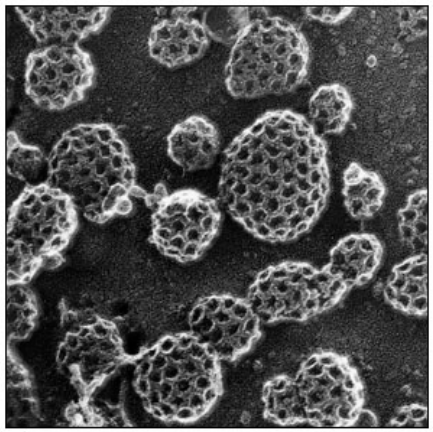
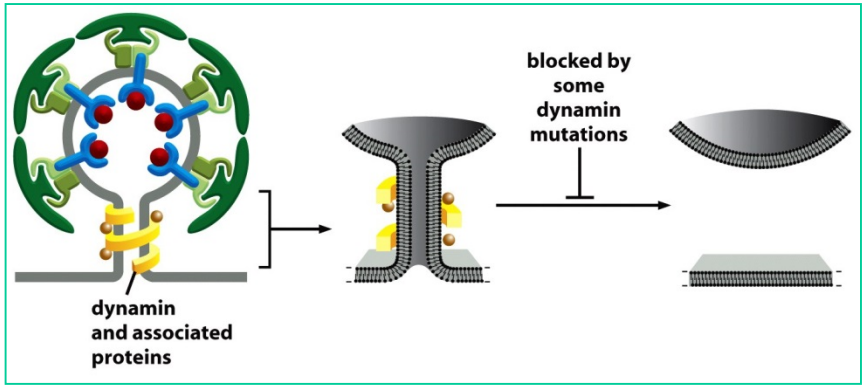


Figure 13-8 Molecular Biology of the Cell 6e (© Garland Science 2015)

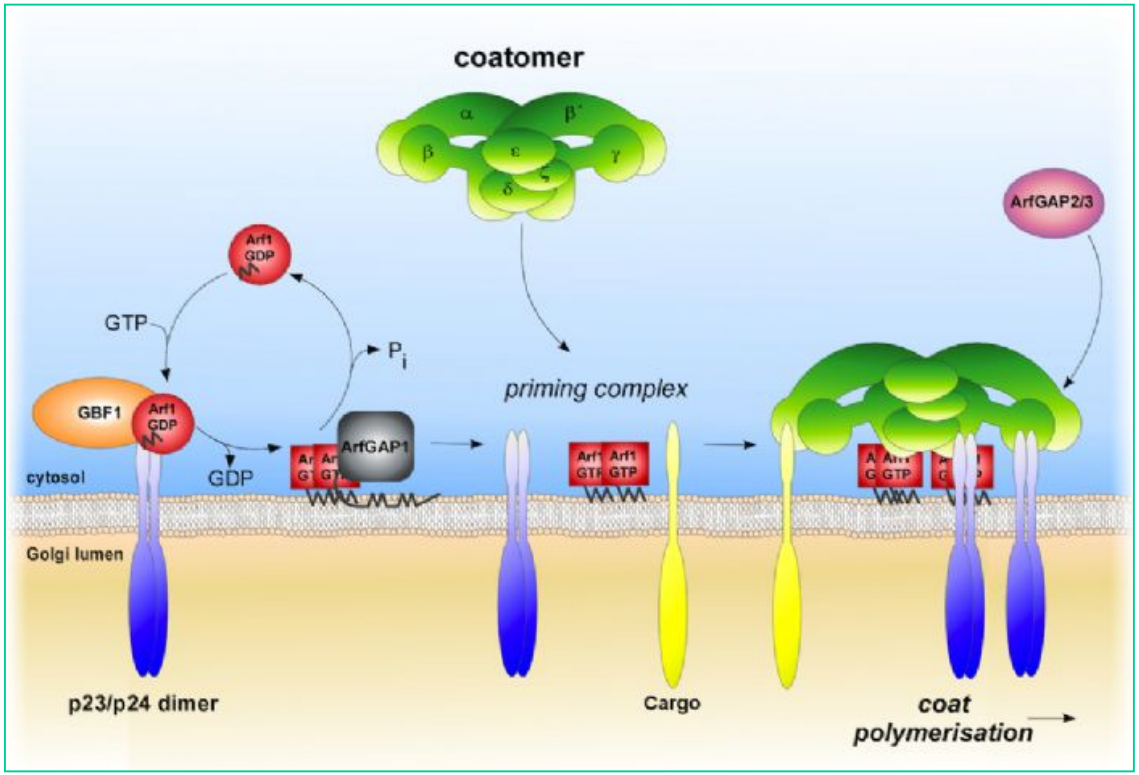




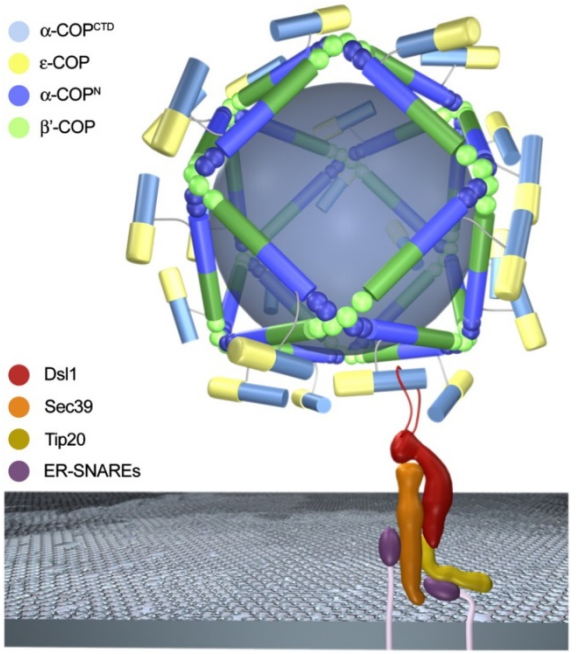
VESICLE FORMATION AND TRANSPORT

COPI

Retrograde transport



A molecular model of the COPI transport-vesicle



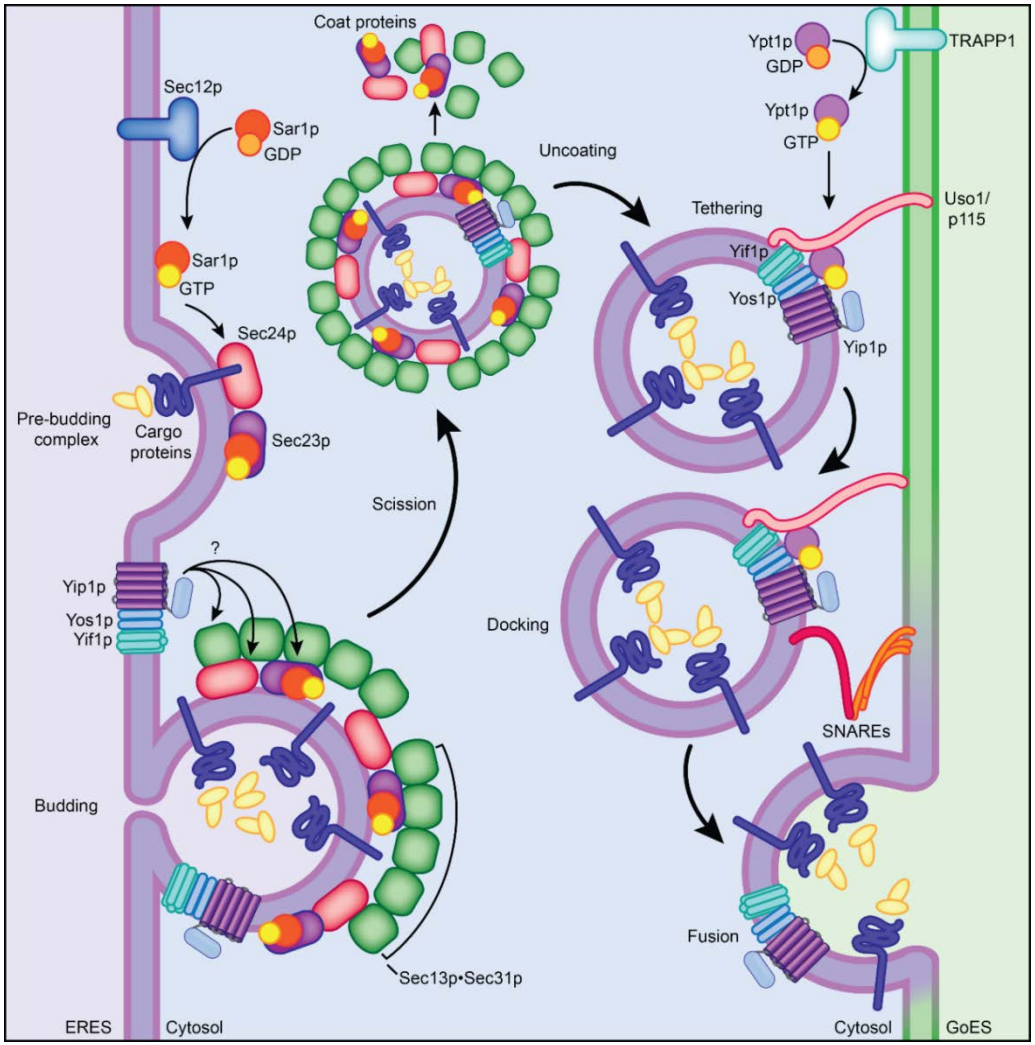
COPI cover is constituted by the cytosolic coatomer which is a protein complex that is formed by seven different protein subunits, and Arf1 GTPase that regulates intracellular vesicle transport modulating the interaction between the cover and the organelle

http://bzh.db-engine.de/default.asp?lfn=2241&fg=2335

VESICLE FORMATION AND TRANSPORT

COPII is a type of vesicle coat protein that transports proteins from the RER to the Golgi appartus. This process is termed ***anterograde transport.***
The coat consists of large protein subcomplexes that are made of four different protein subunits.

There are two protein heterodimers that form the coat complex.
These proteins are:
Sec23p/Sec24p Heterodimer
Sec13p/Sec31p Heterotetramer



http://mutagenetix.org/phenotypic/phenotypic_rec.cfm?pk=433

VESICLE FORMATION AND TRANSPORT

COPII

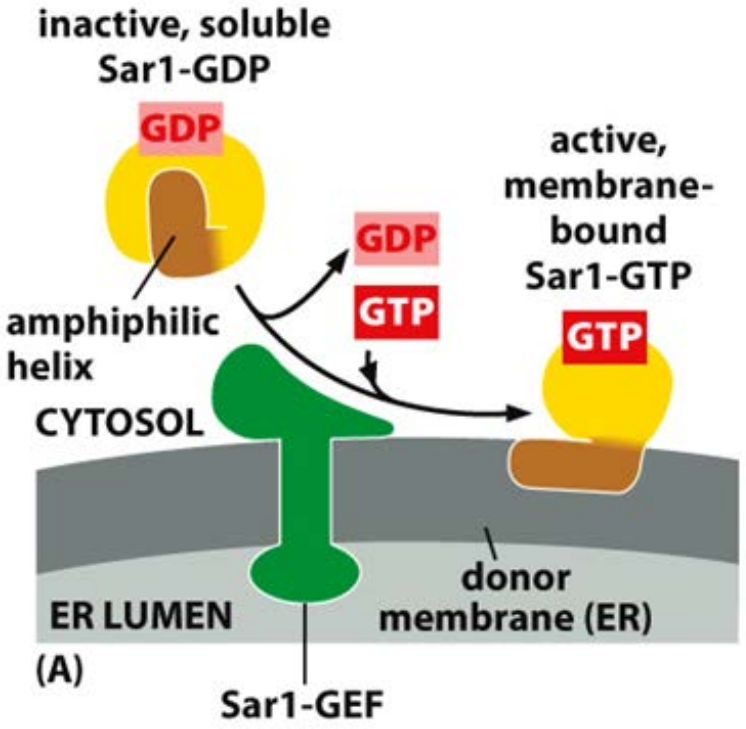
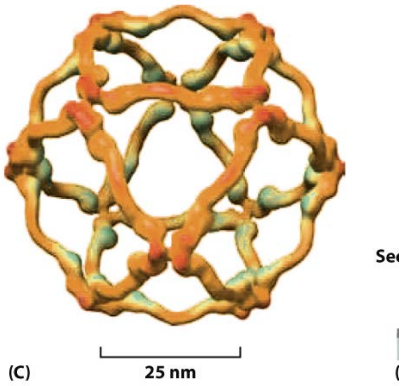


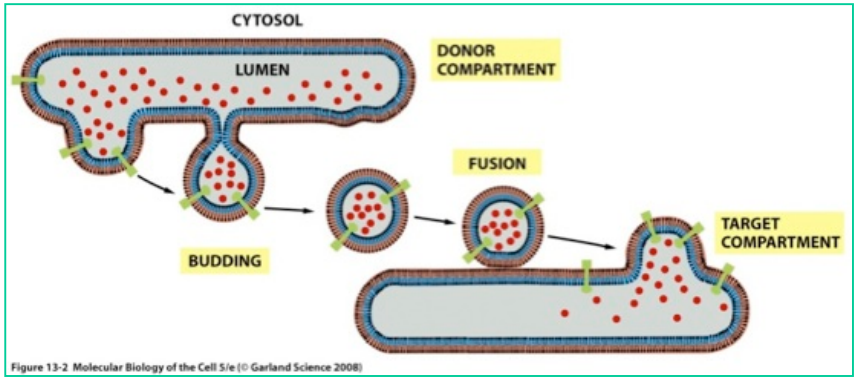
Figure 13-14 Molecular Biology of the Cell 6e (© Garland Science 2015)

Sar1 GTPase that regulates intracellular vesicle transport modulating the interaction between the cover and the organelle



A molecular model of the COPII transport-vesicle

VESICLE FORMATION AND TRANSPORT



v-SNARE

Rab-GTP

TETHERING

DOCKING

Rab effector (tethering protein)

t-SNARE

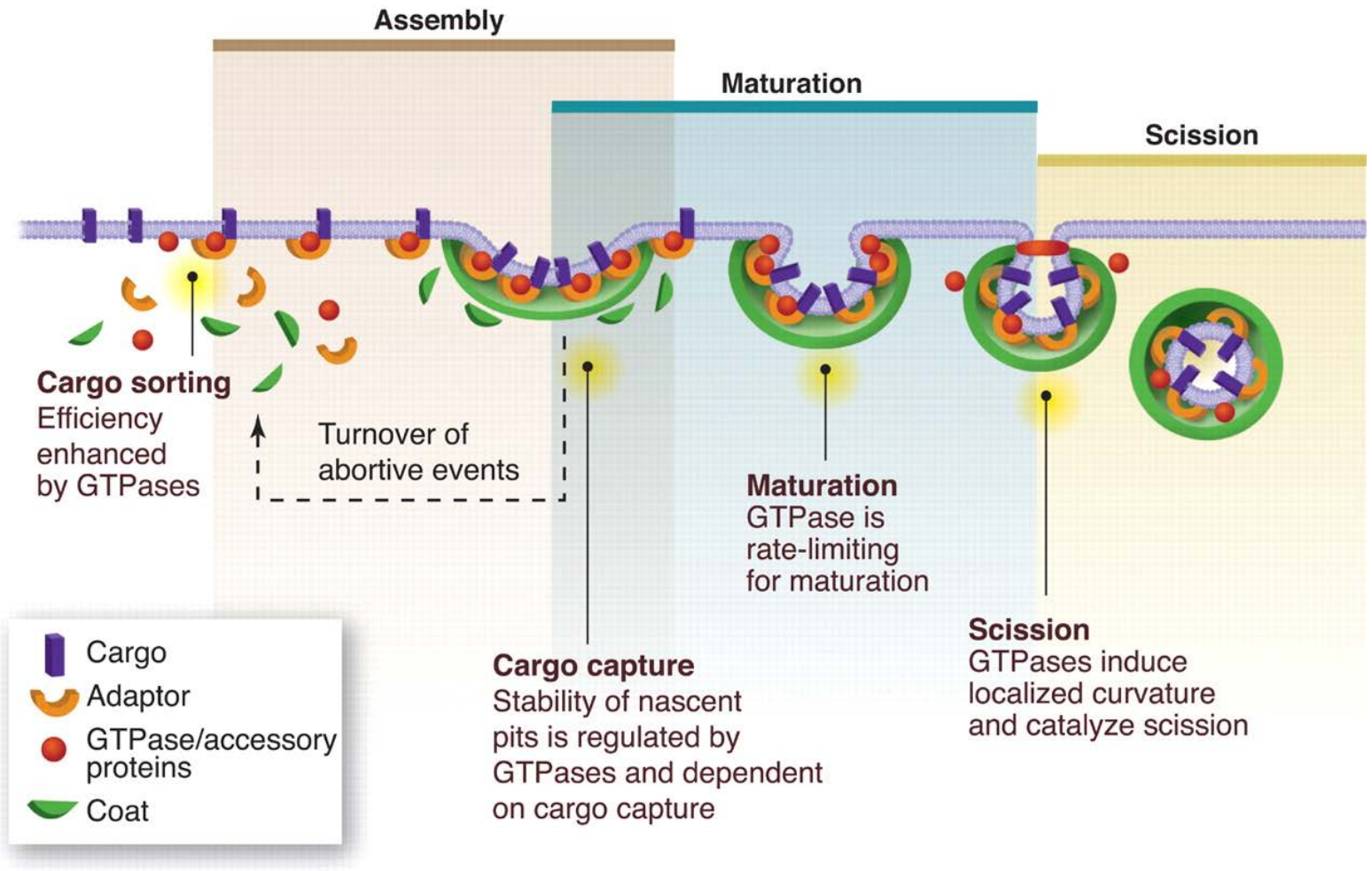
FUSION

target membrane

cargo

Figure 13-14 Molecular Biology of the Cell 5/e (© Garland Science 2008)

VESICLE FORMATION AND TRANSPORT



GTPase: **Arf** (clathrin, COPI)
Sar1 (COPII)

VESICLE FORMATION AND TRANSPORT

TRANSPORT ASOCIATED TO MICROTUBULES

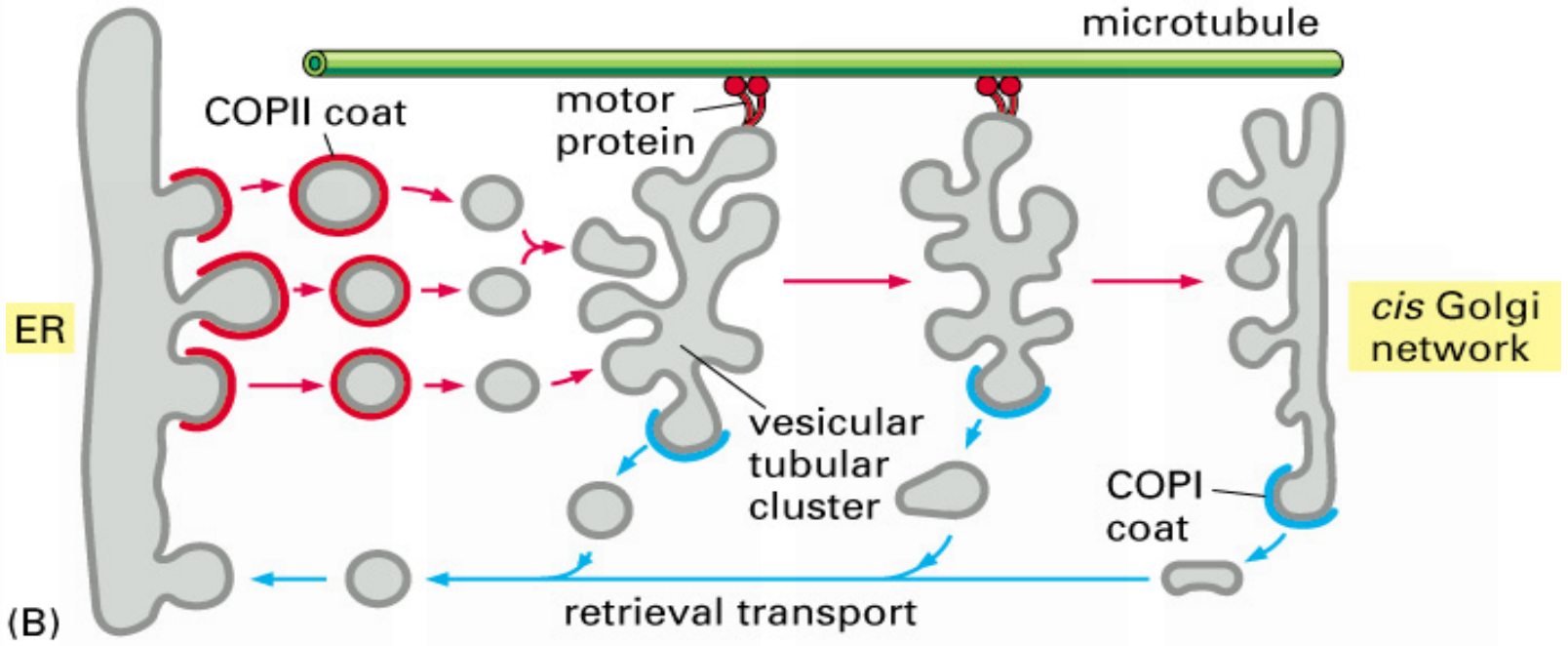


Figure 13–20 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

VESICLE FORMATION AND TRANSPORT

RETRIEVAL OF ER PROTEINS

K—Lysine
D—Aspartic acid
E—Glutamic acid
L—Leucine

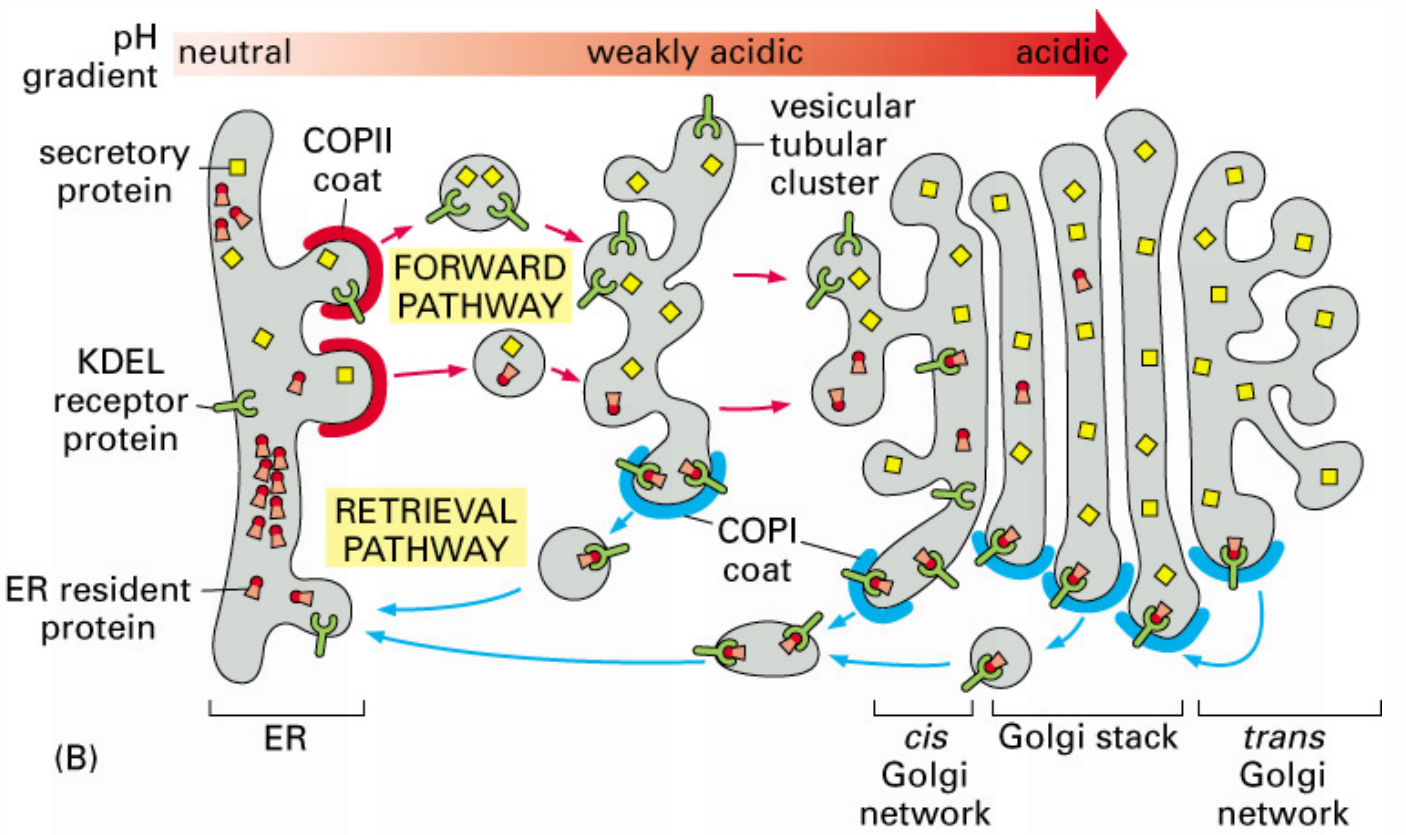
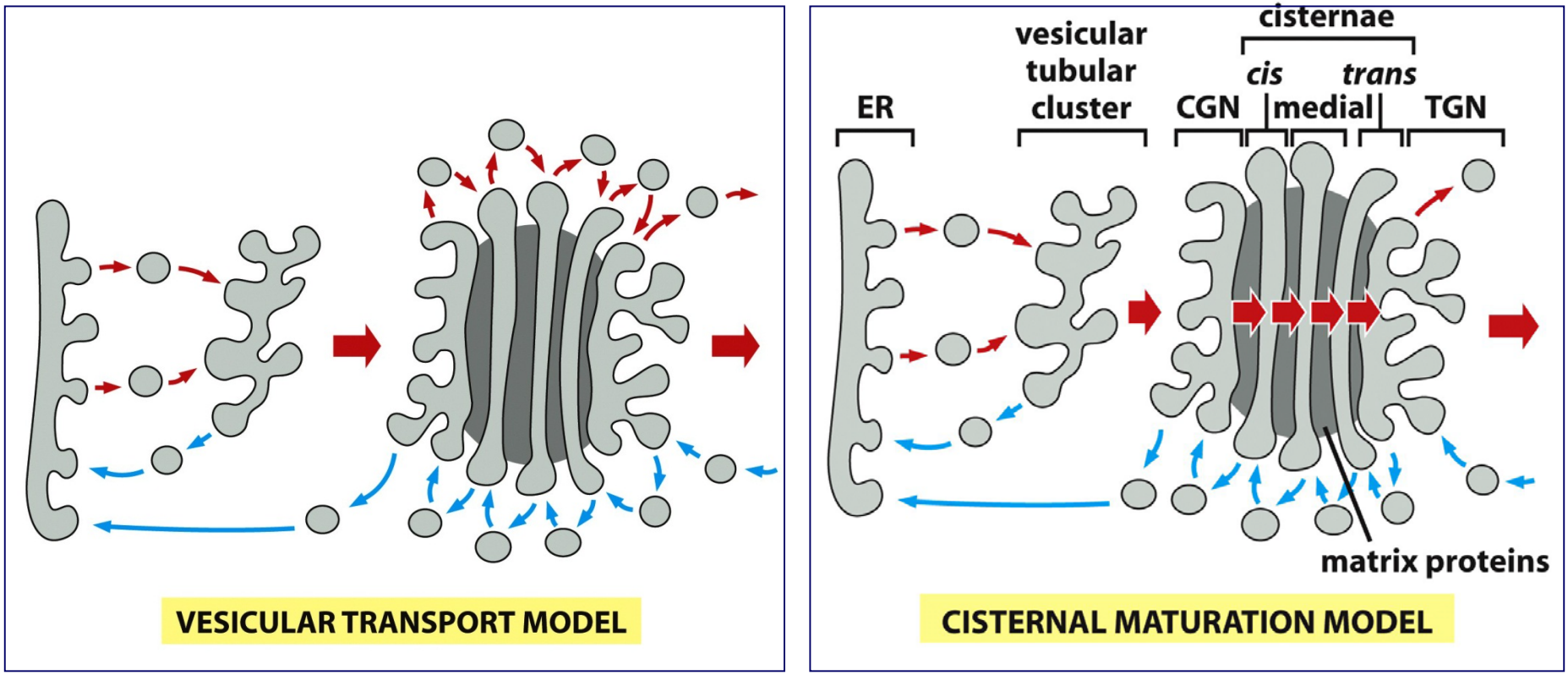


Figure 13–21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

BIOGENESIS

ORGANIZATION MODELS



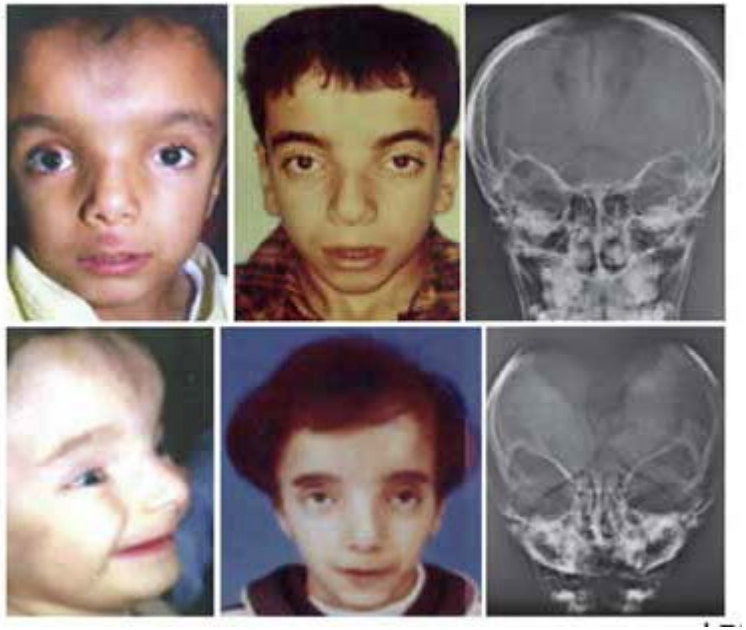
Alberts, sixth ed. Fig. 13-35

BIOPATHOLOGY

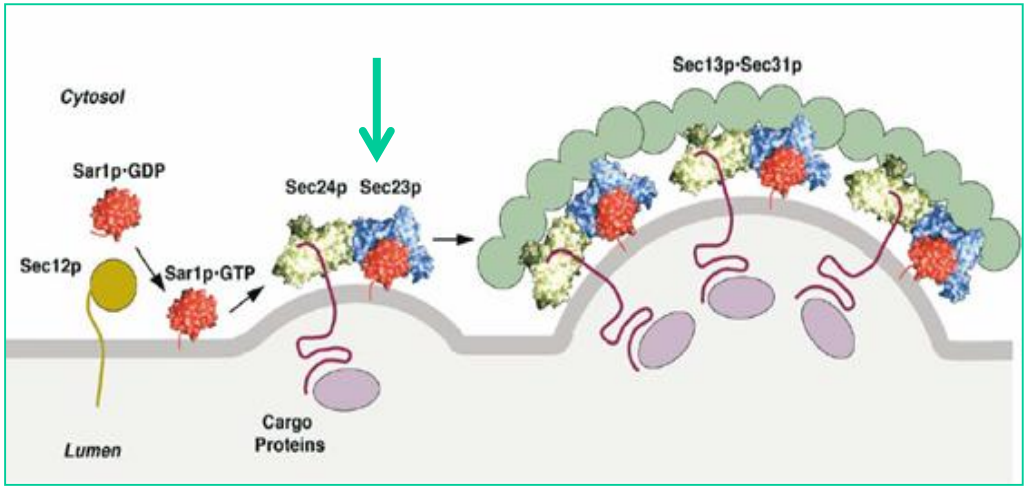
Cranio-lenticulo-sutural dysplasia is caused by a *SEC23A* mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking

Simeon A Boyadjiev[1,2], J Christopher Fromme[3], Jin Ben[4], Samuel S Chong[4], Christopher Nauta[2], David J Hur[1], George Zhang[1], Susan Hamamoto[3], Randy Schekman[3], Mariella Ravazzola[5], Lelio Orci[5] & Wafaa Eyaid[6]

nature genetics



Mutation localized in the 14q13, chromosome 14



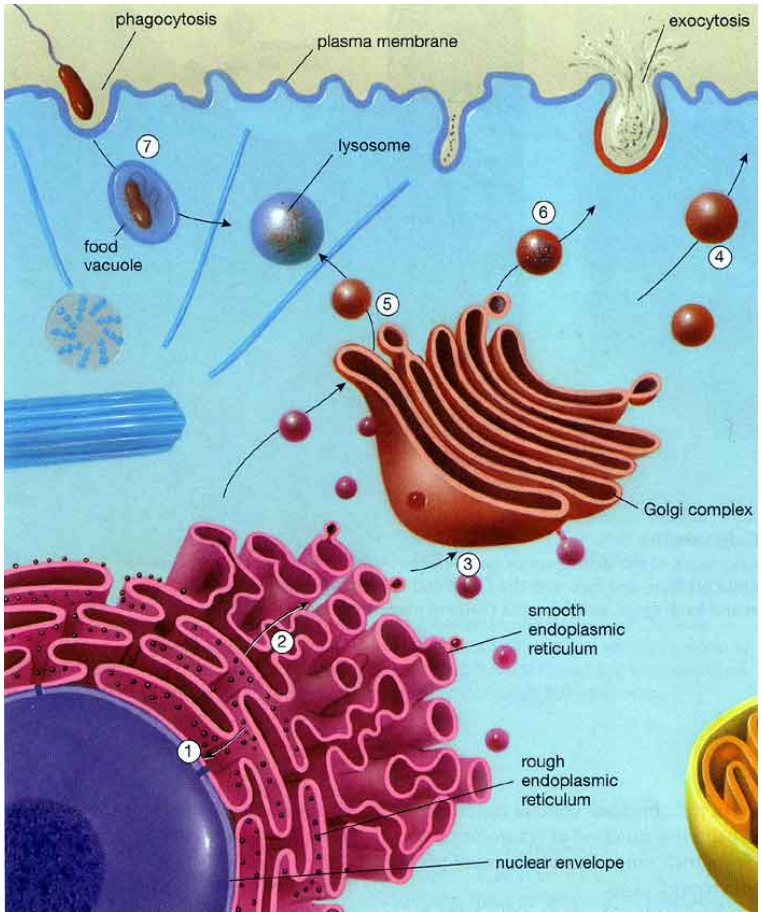
Cranio–lenticulo–sutural is a neonatal/infancy disease caused by a disorder in the 14 chromosome. It is an autosomal recesive disorder.

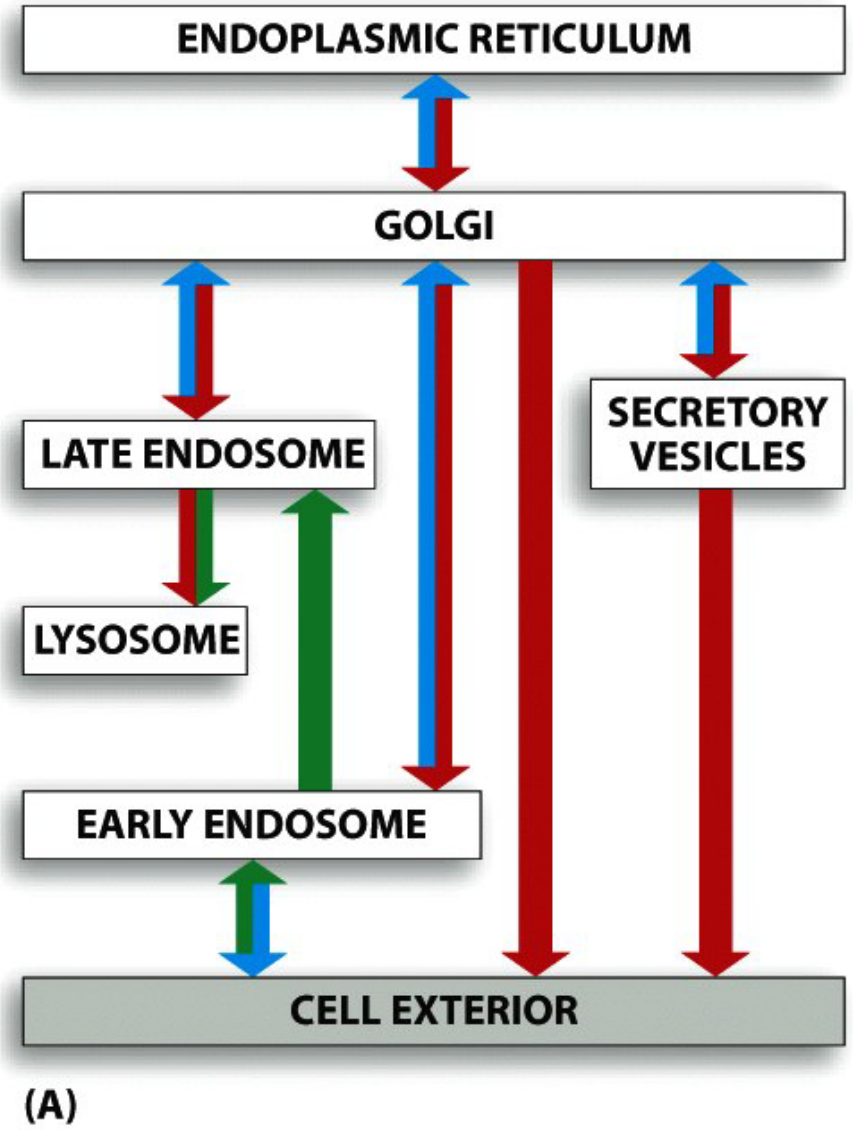
The production of **SEC23A** protein is involved in the pathway of exporting collagen (the COPII pathway), but a **mutation** causes and underproduction of SEC23A which inhibits the pathway, affecting collagen secretion. This decrease in collagen secretion can lead to the bone defects that are also characteristic of the disease, such as skeletal dysplasi and under-ossification.

LYSOSOME AND PEROXISOME

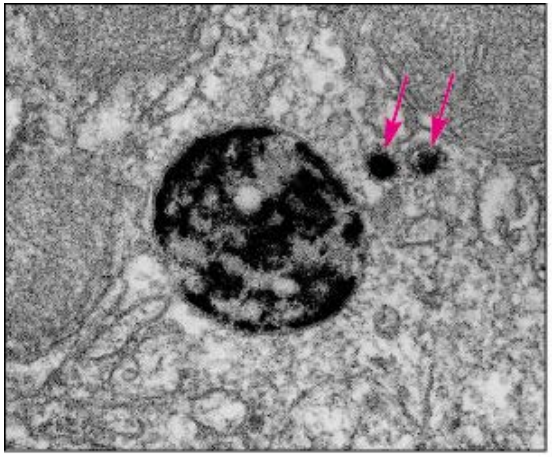
- Lysosome
  - General characteristics
  - Functions
  - Biogenesis
- Peroxisome
  - General characteristics
  - Functions
  - Biogenesis

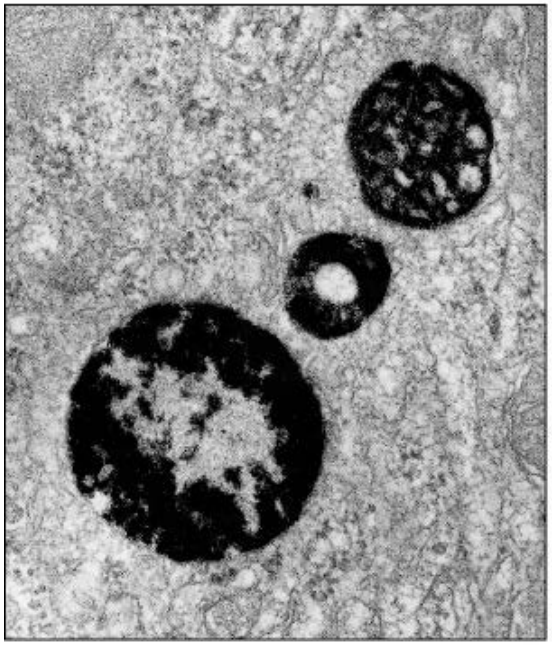
GENERAL CHARACTERISTICS



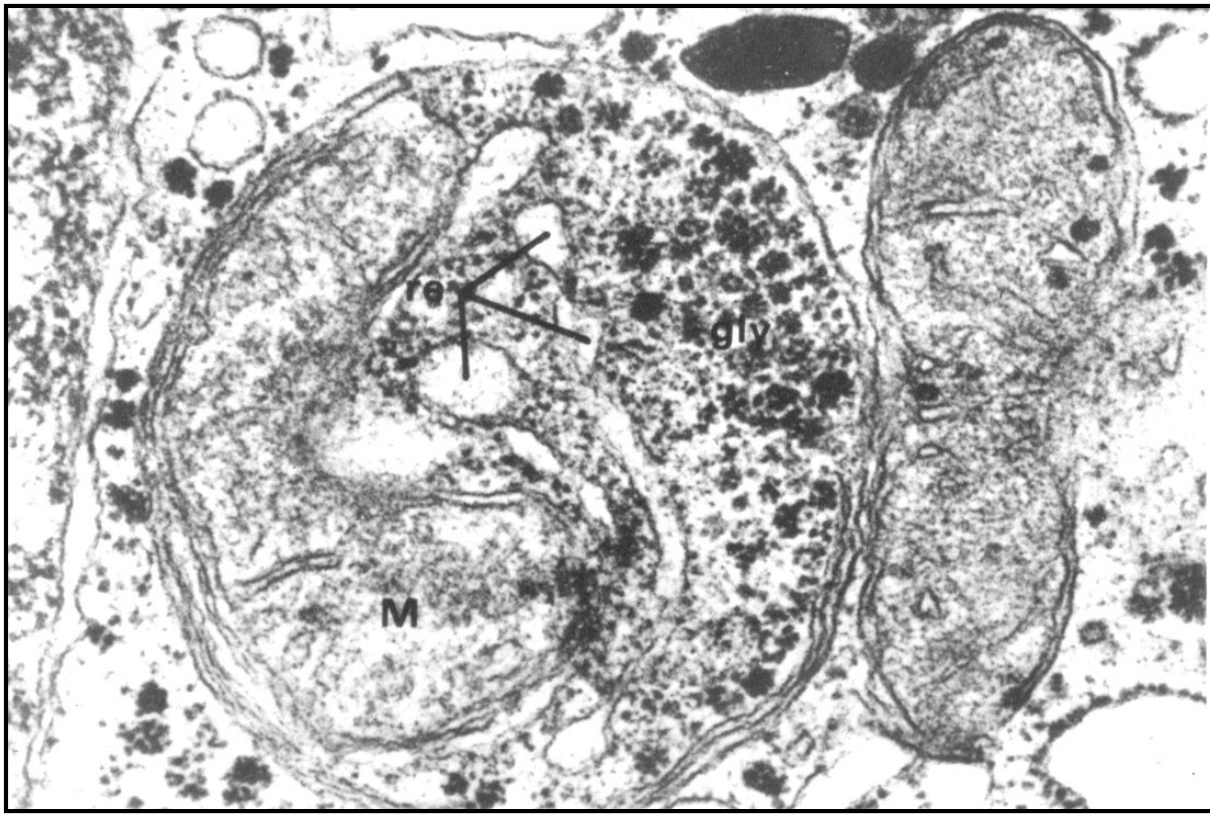


GENERAL CHARACTERISTICS



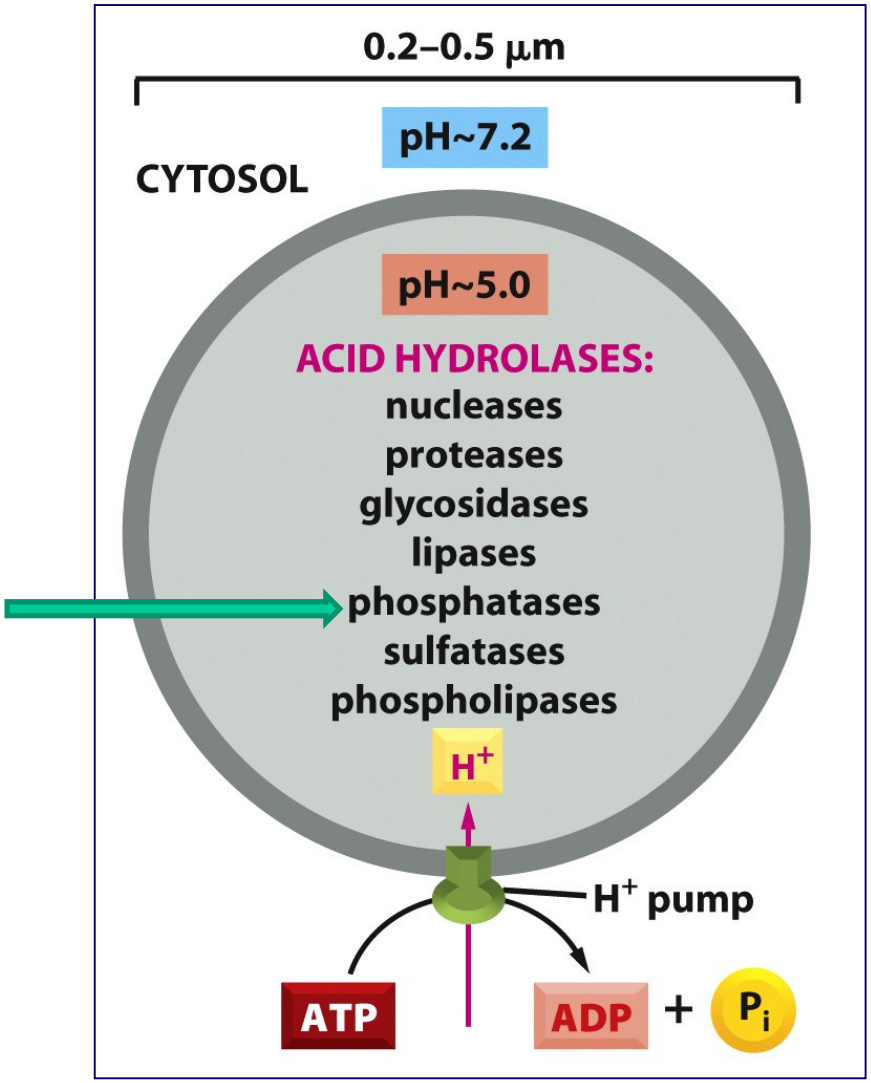


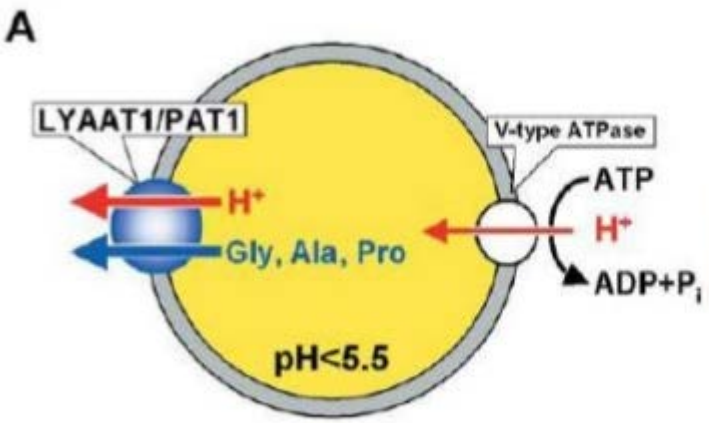
Ultrastructure

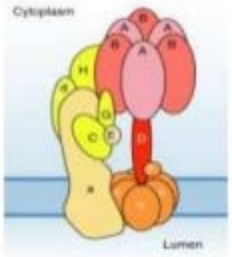


GENERAL CHARACTERISTICS

Chemical composition



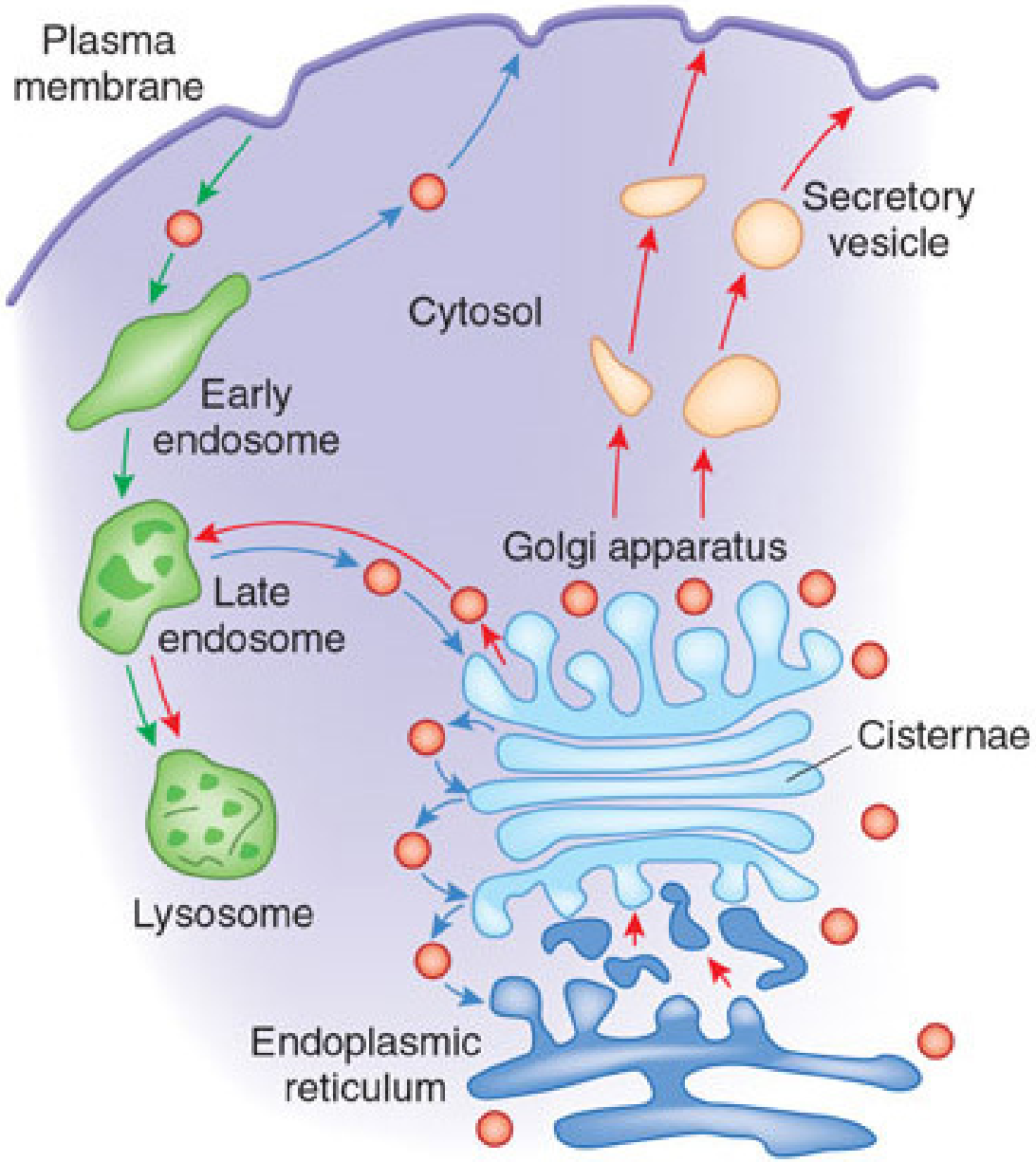




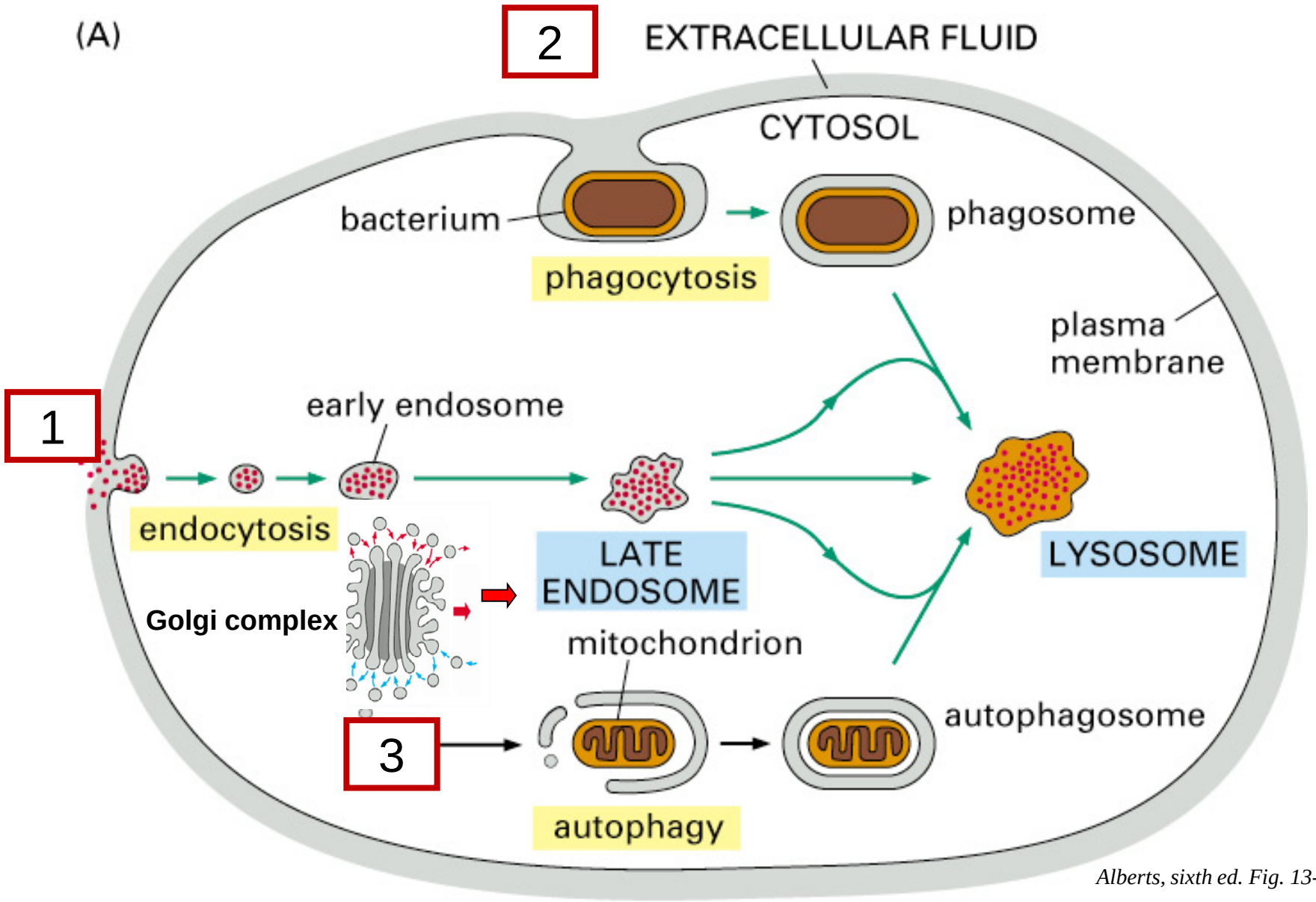
membrane transport protein

Alberts, sixth ed. Fig. 13-37

Functions:intracellular digestion



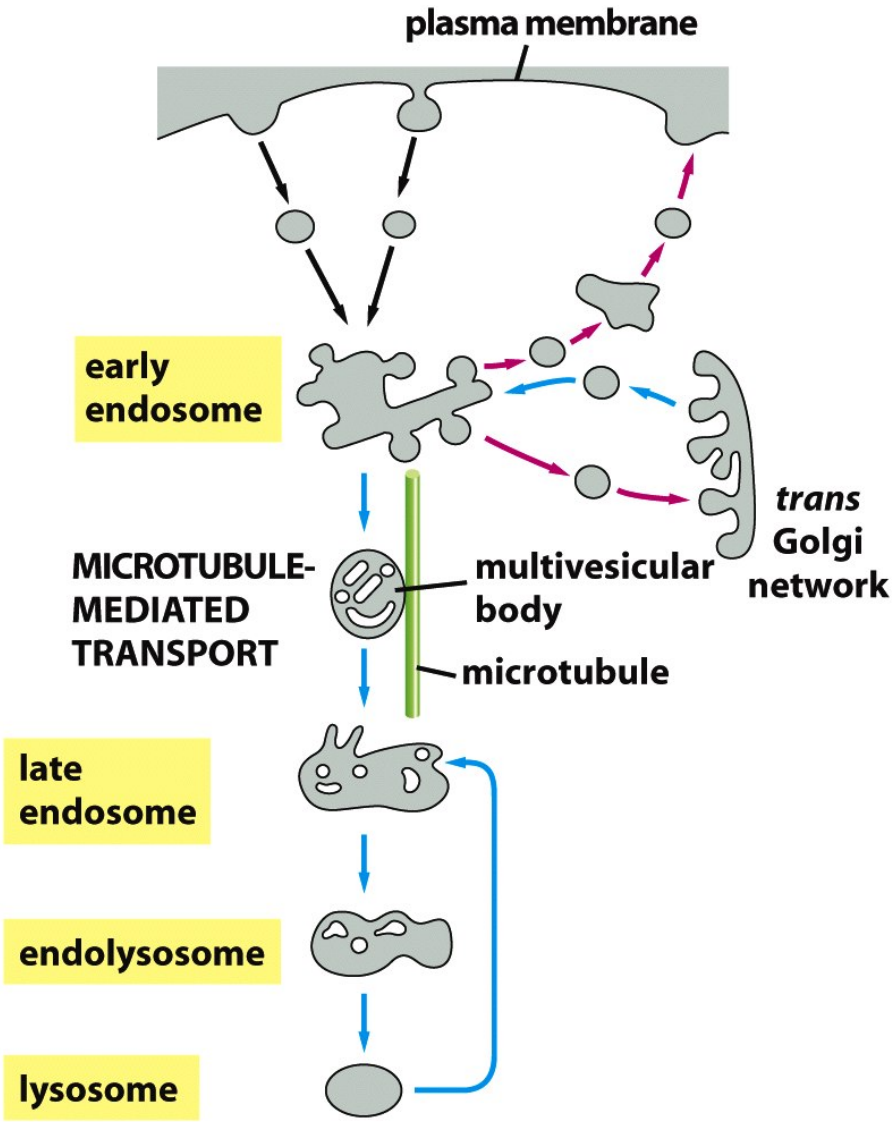
Functions:intracellular digestion



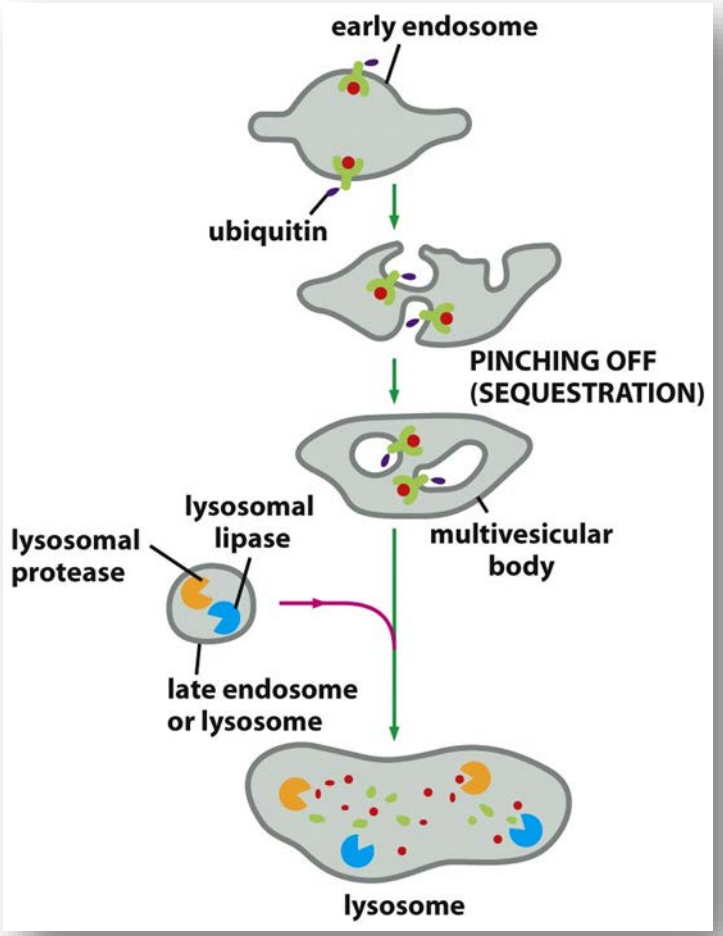
Alberts, sixth ed. Fig. 13-42

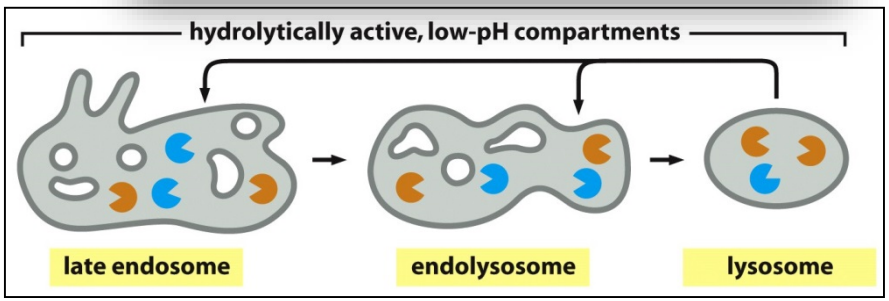
Functions:intracellular digestion

1.- Endocytosis



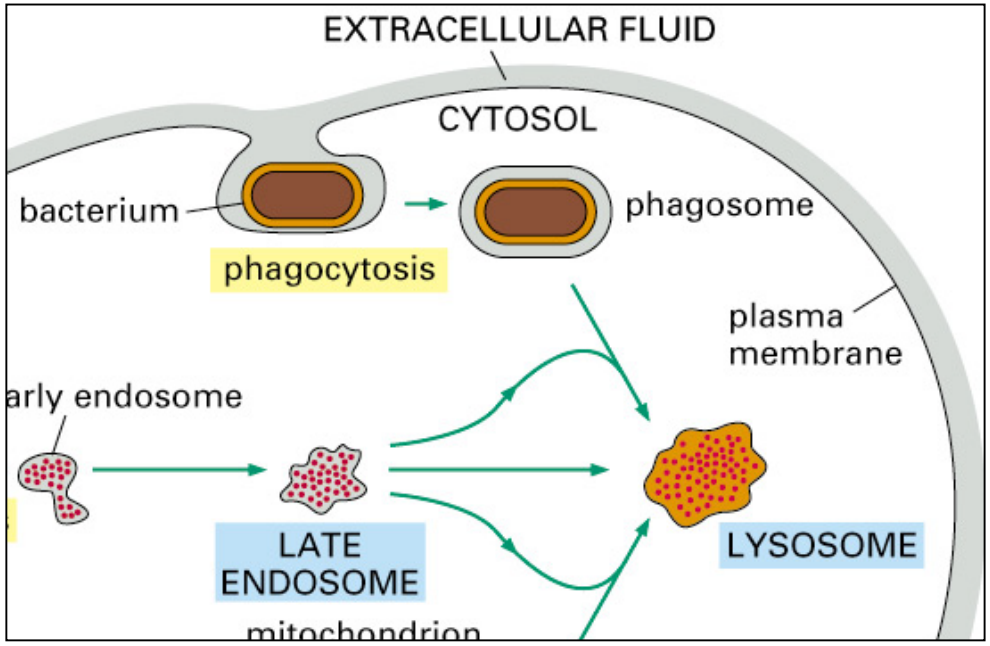
Alberts, Fig. 13-57

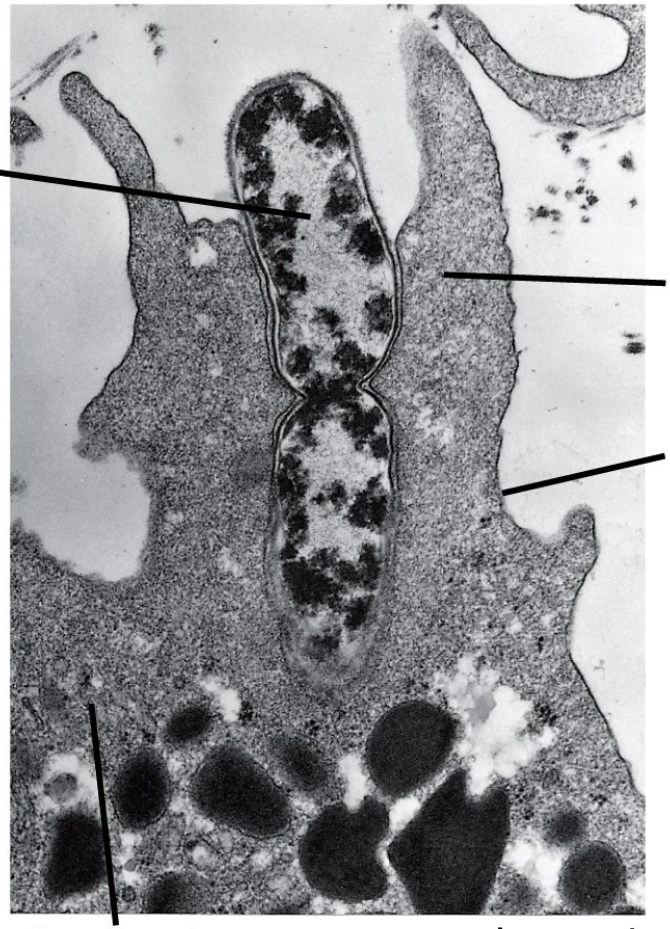




Functions:intracellular digestion

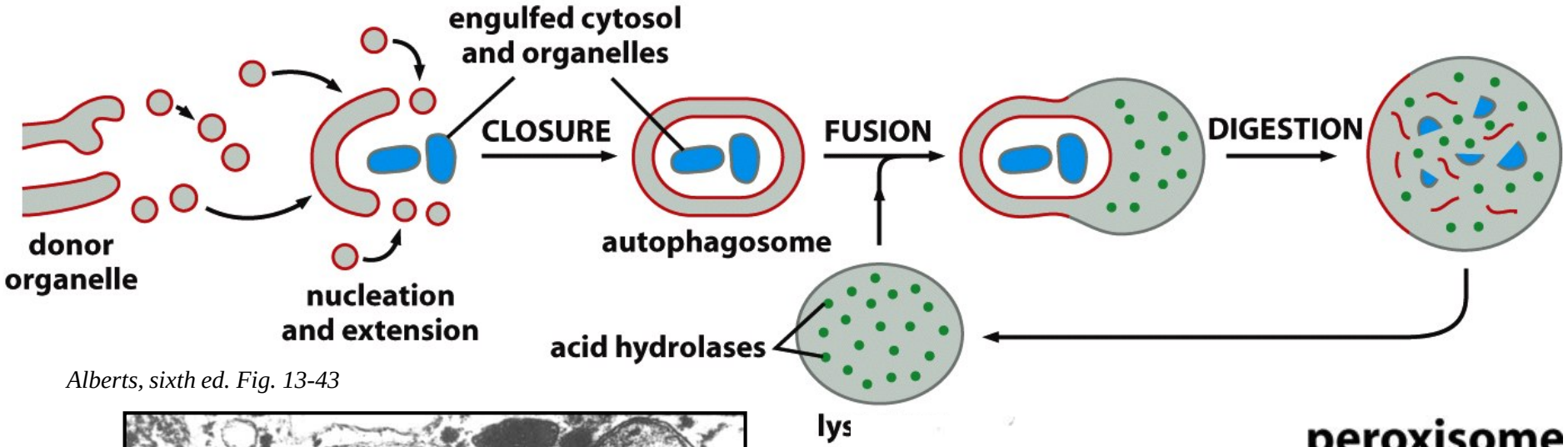
2.- Phagocytosis



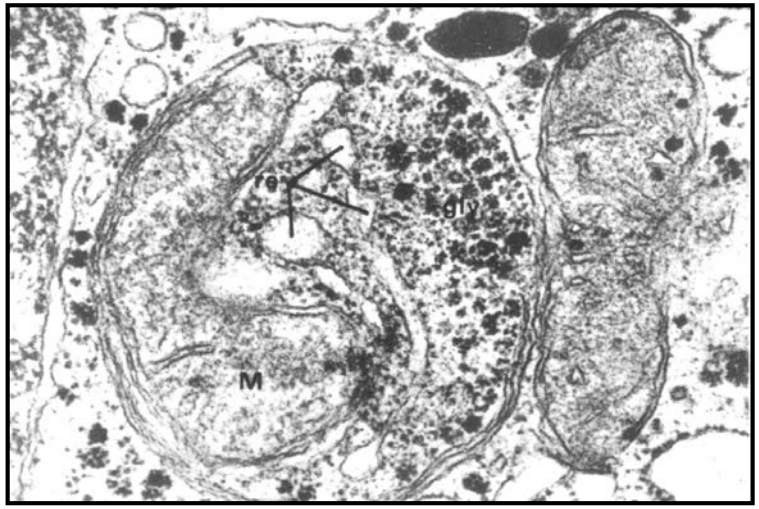


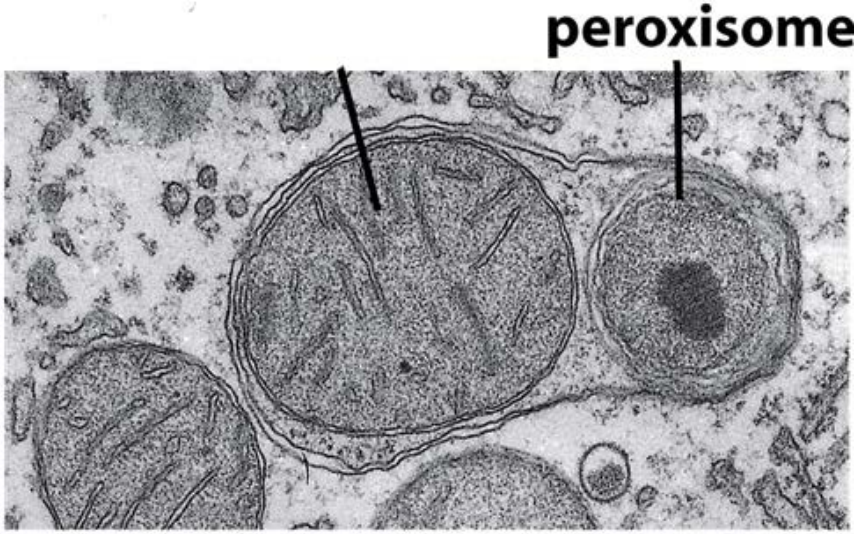
Functions:intracellular digestion

3.- Autophagy



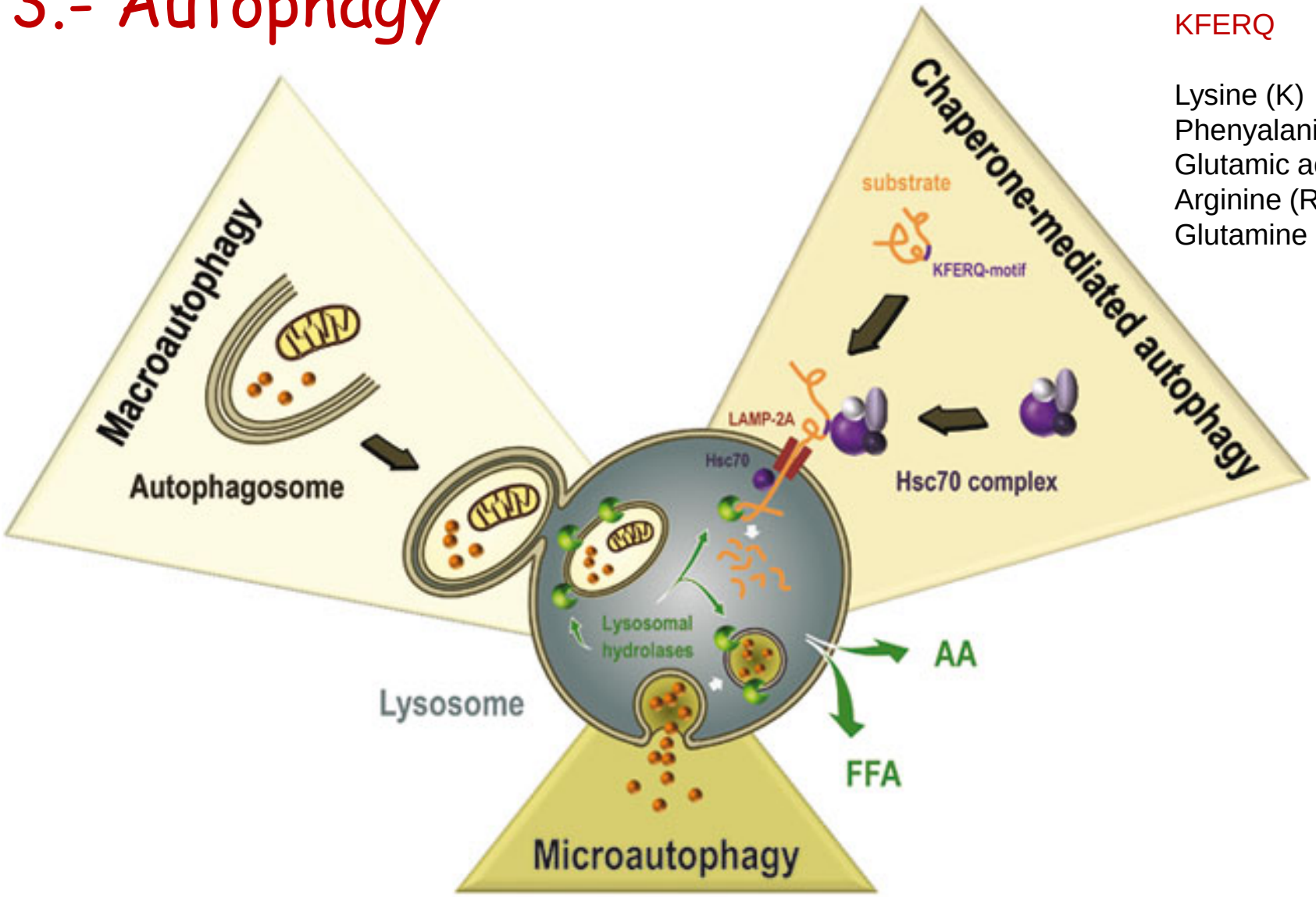
Alberts, sixth ed. Fig. 13-43





Functions:intracellular digestion

3.- Autophagy

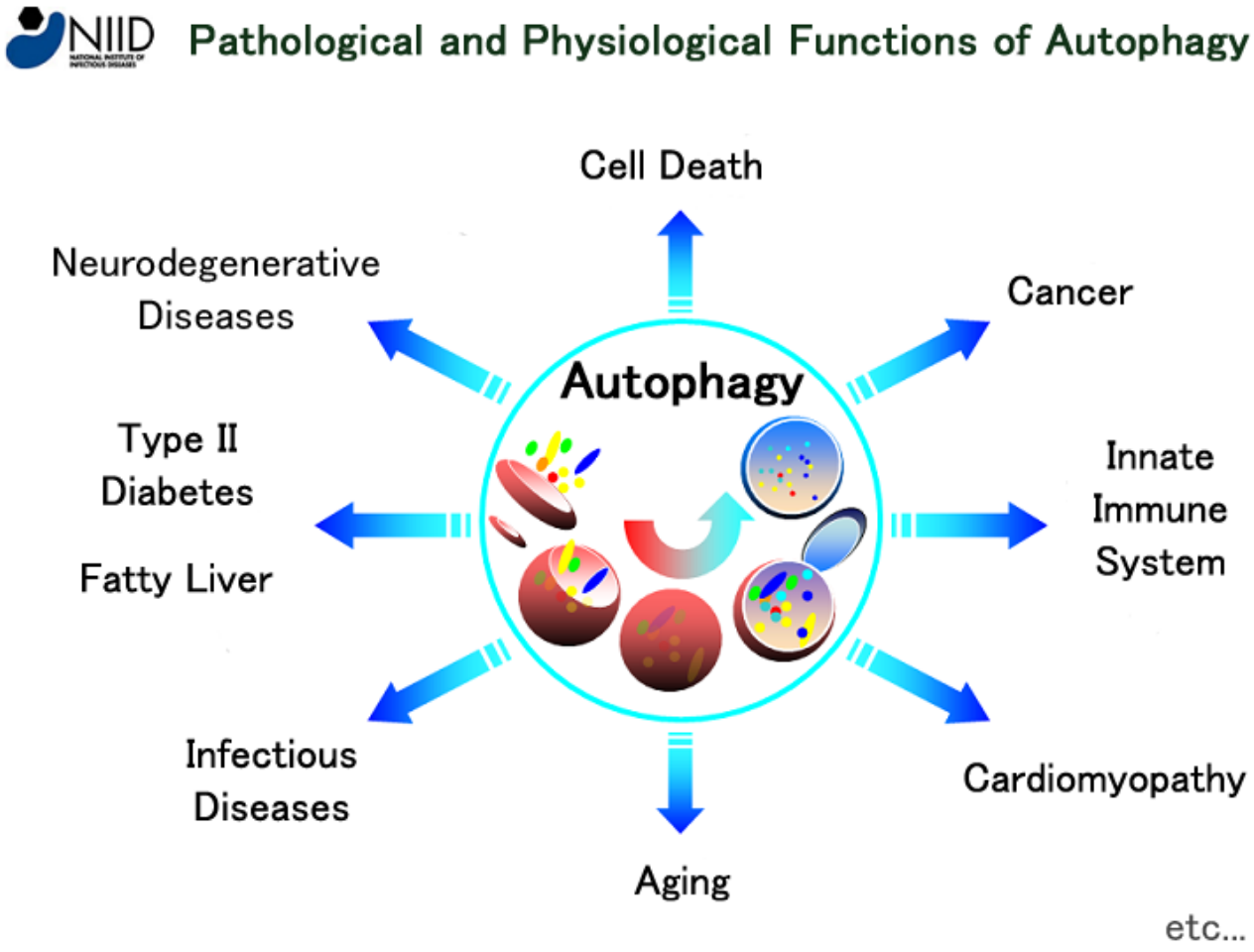


KFERQ

Lysine (K)
Phenyalanine (F)
Glutamic acid (E)
Arginine (R)
Glutamine (Q)

Functions:intracellular digestion

3.- Autophagy



It a key role in preventing diseases such as cancer, neurodegeneration, cardiomyopathy, diabetes, liver disease, autoimmune diseases and infections.

Biogenesis

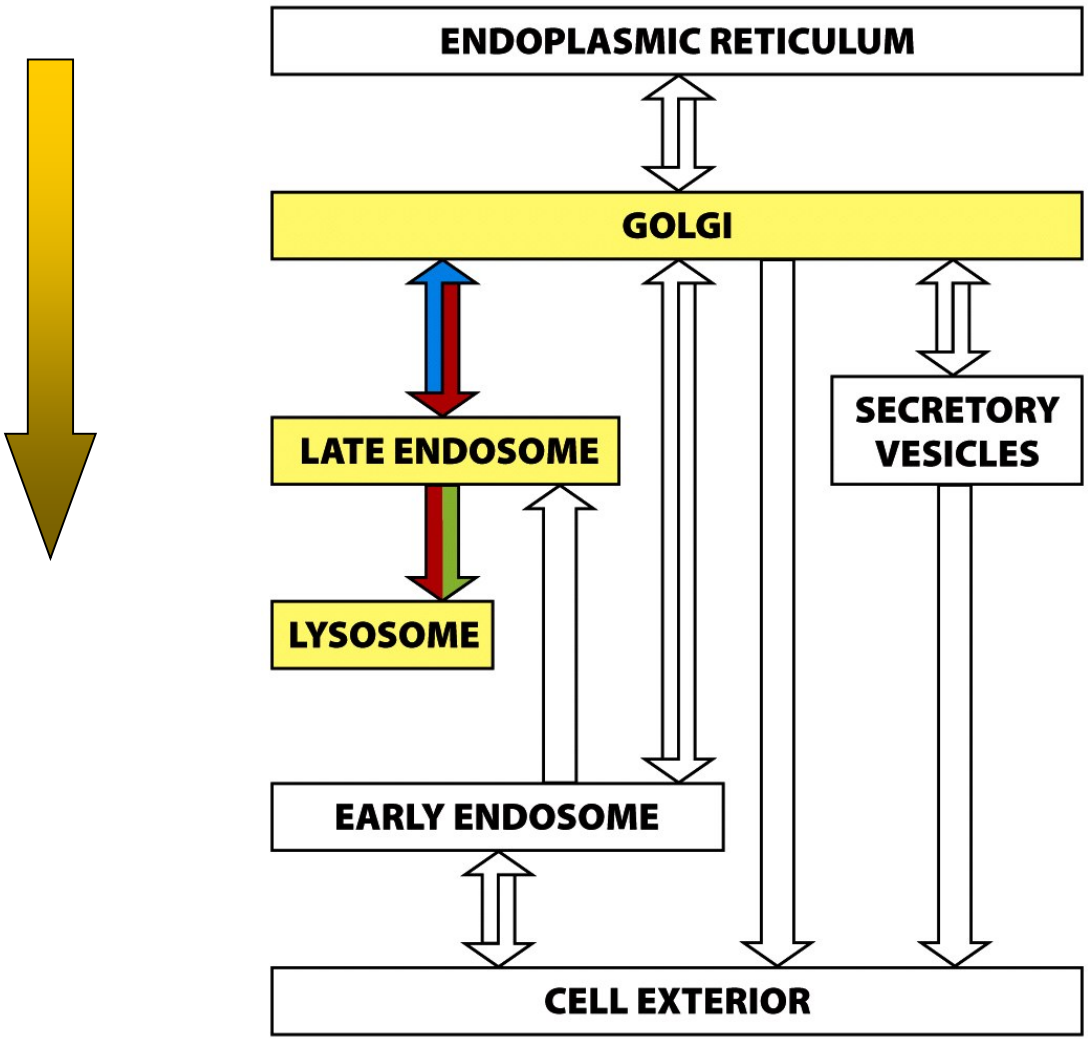


Figure 13-3a *Molecular Biology of the Cell* (© Garland Science 2008)

Biogenesis

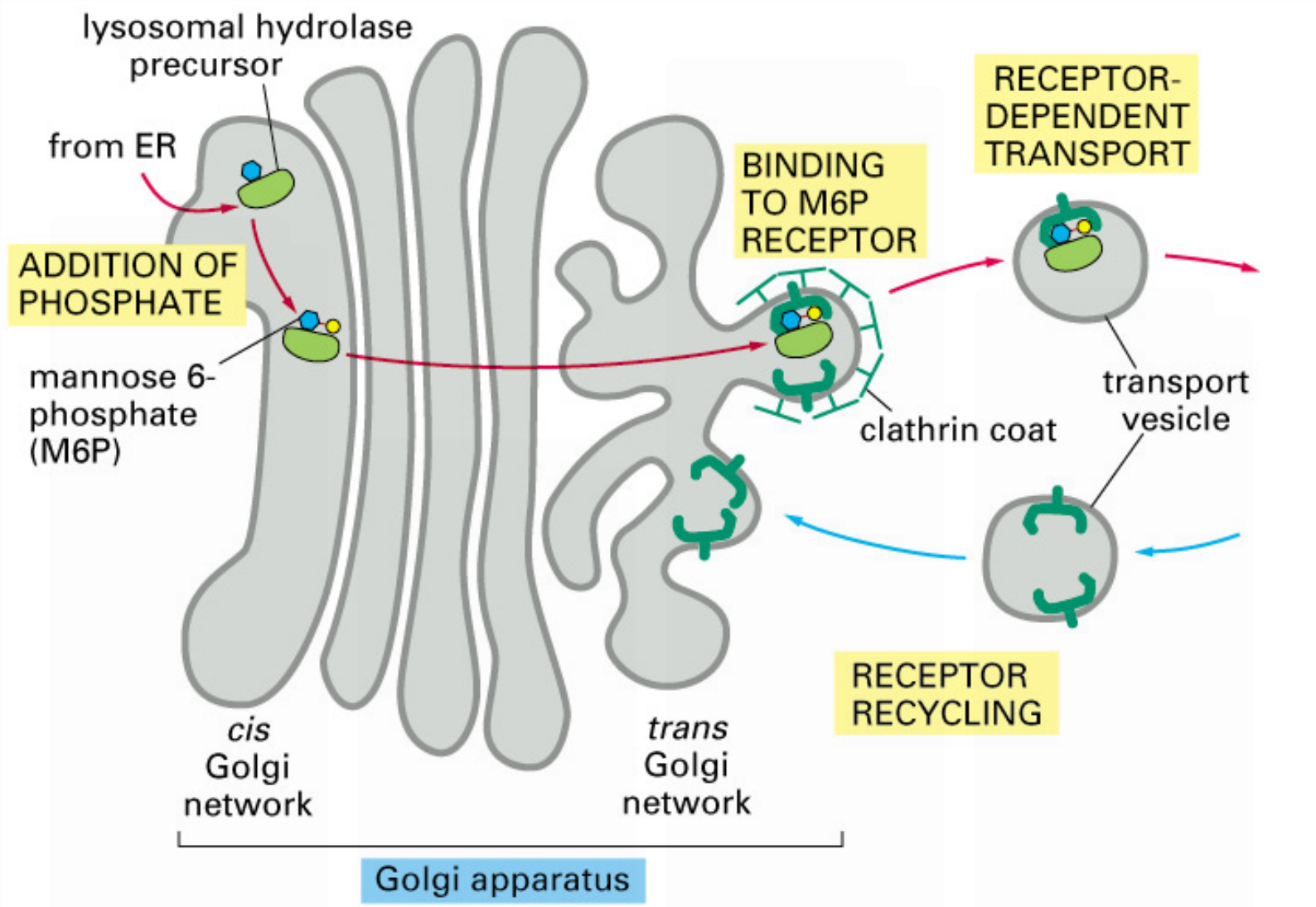
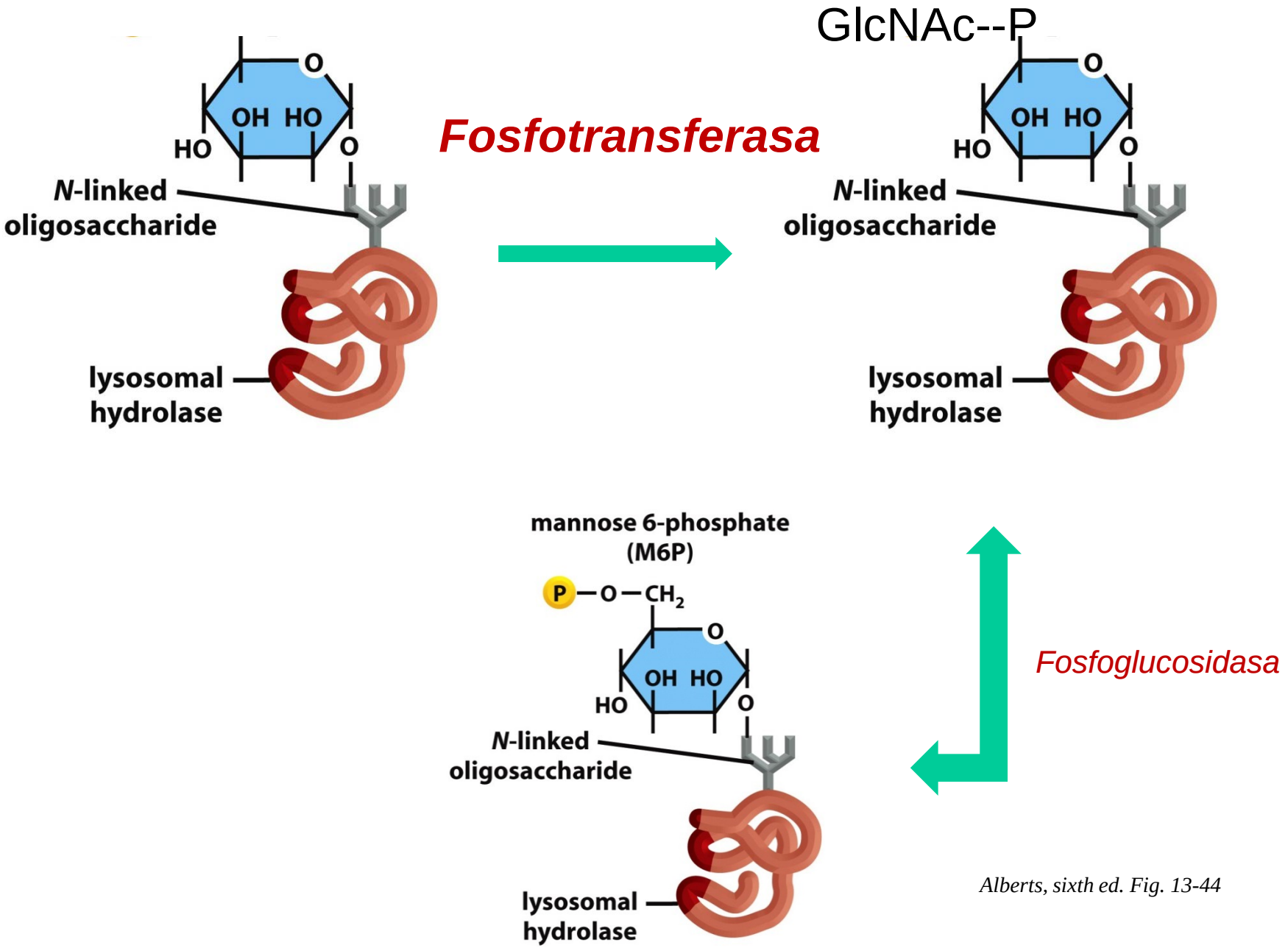


Figure 13–37 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

ADDITION OF PHOSPHATE



Alberts, sixth ed. Fig. 13-44

Biogenesis

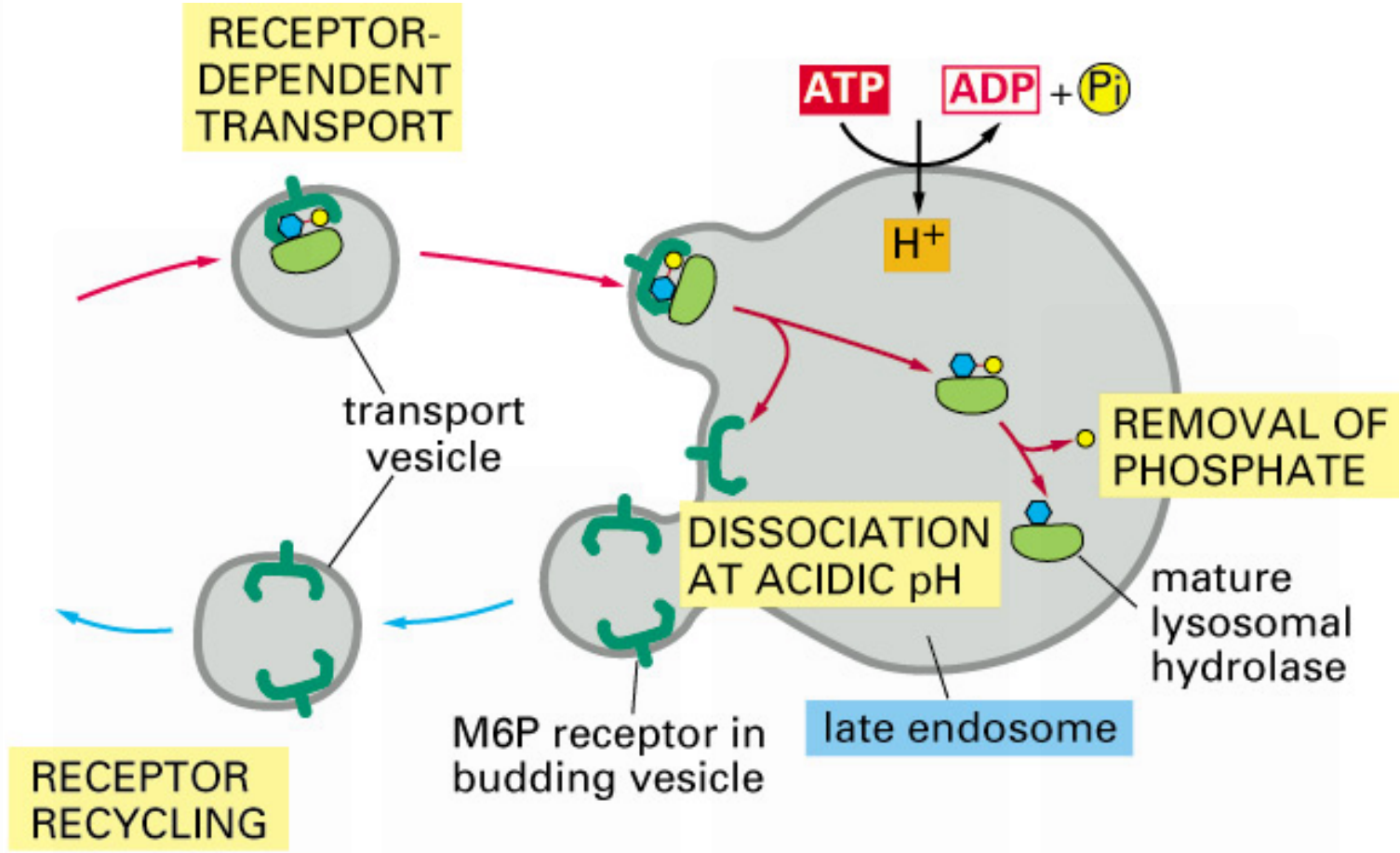
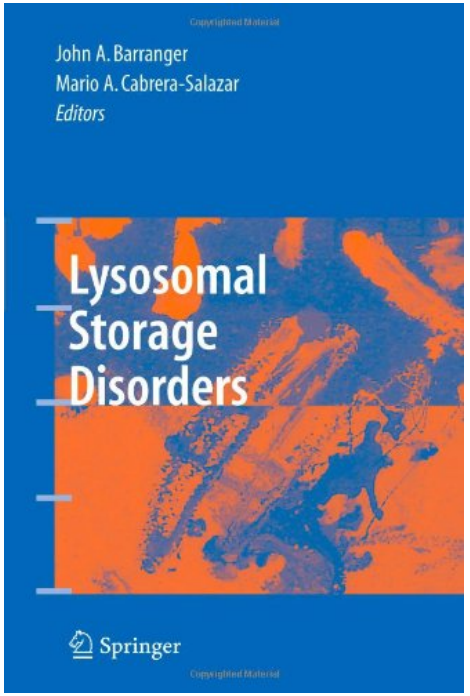


Figure 13–37 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

BIOPATHOLOGY

1. Lysosomal storage diseases

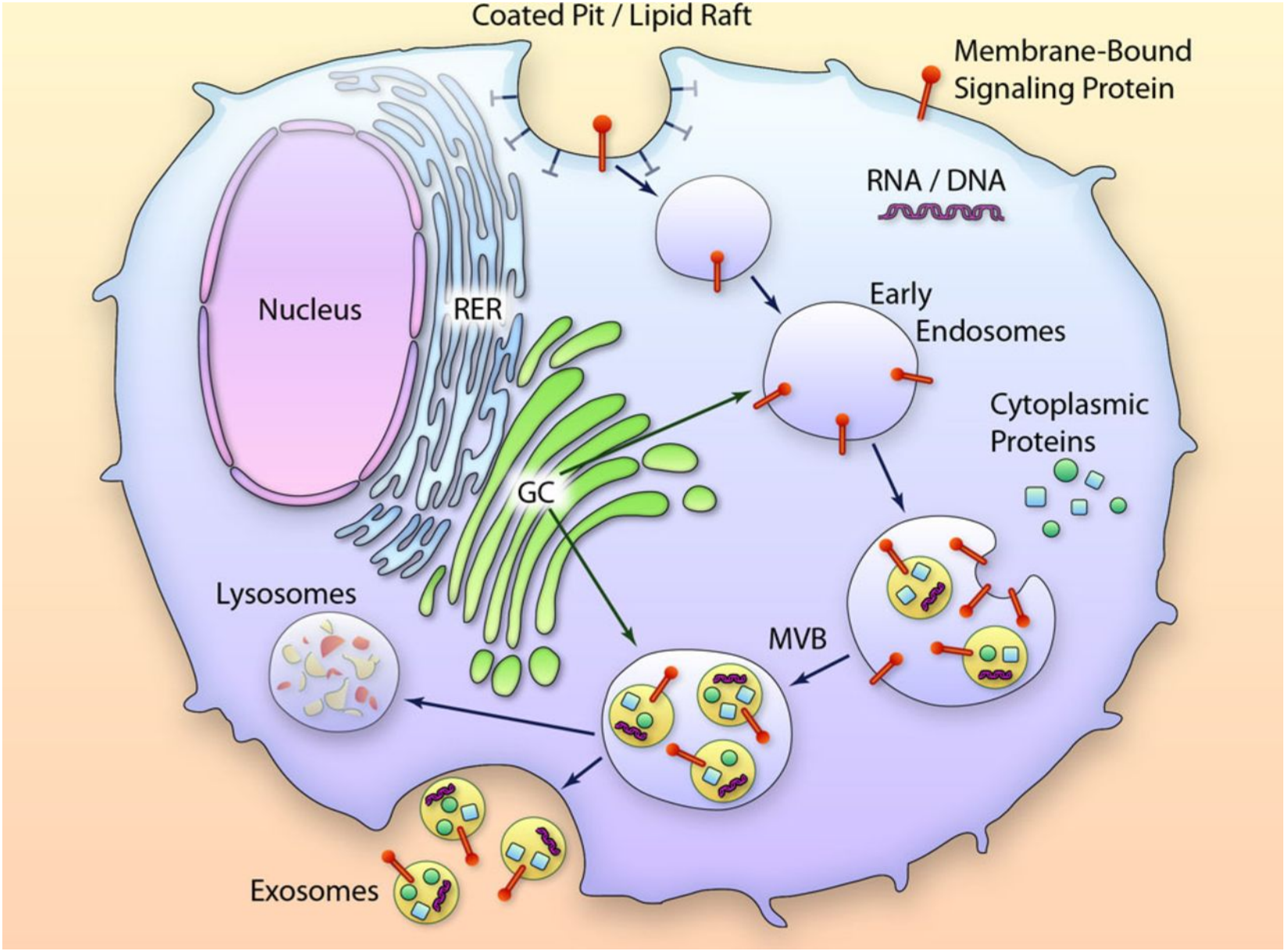
Eg. Pompe disease
Tay-Sachs disease
I-cell disease



2. Diseases caused by the nature of the substrate

Eg. Silicosis disease

EXOSOMES

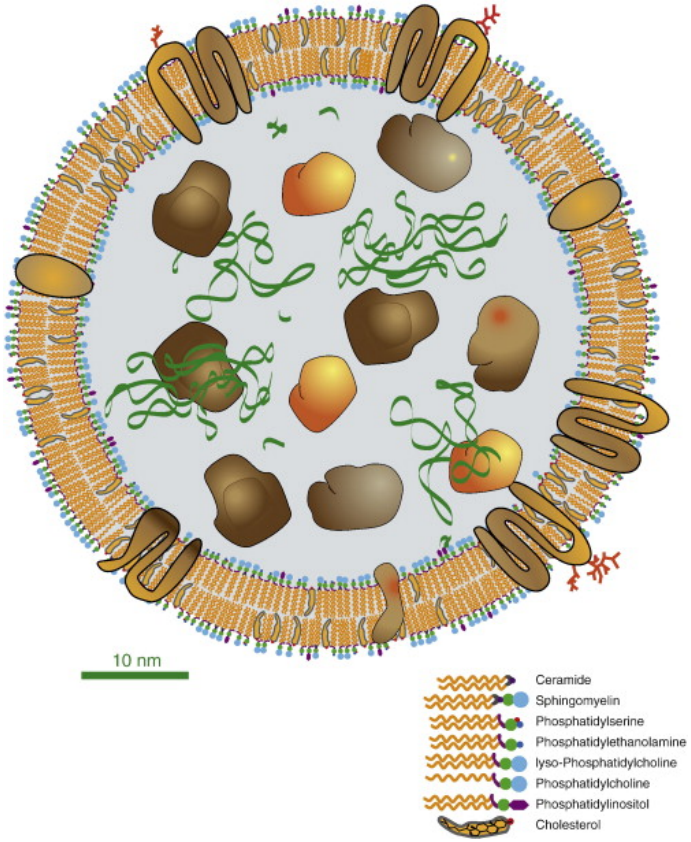

Coated Pit / Lipid Raft
Membrane-Bound Signaling Protein
RNA / DNA
Nucleus
RER
Early Endosomes
Cytoplasmic Proteins
GC
Lysosomes
MVB
Exosomes

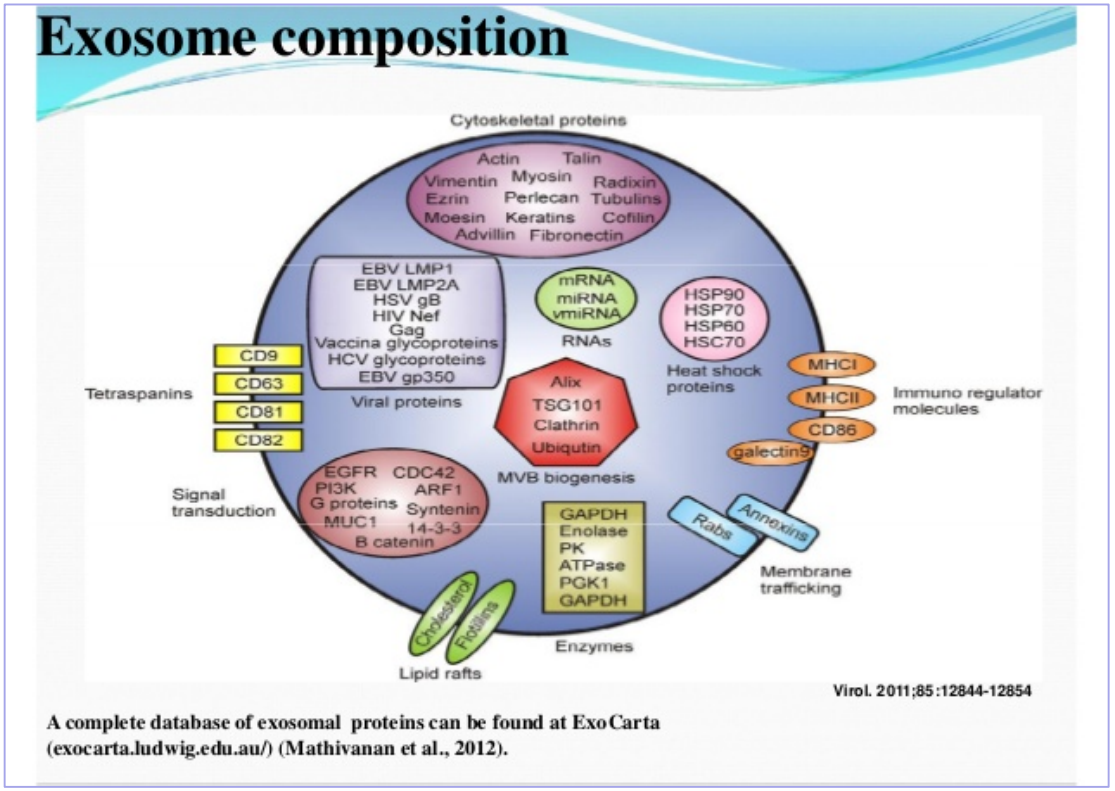
EXOSOMES

The **Exosome** are cell-derived vesicles that are present in many and perhaps all biological fluids, including blood, urine. The reported diameter of exosomes is between 30 and 100 nm, which is larger than LDL, but much smaller than for example red blood cells.

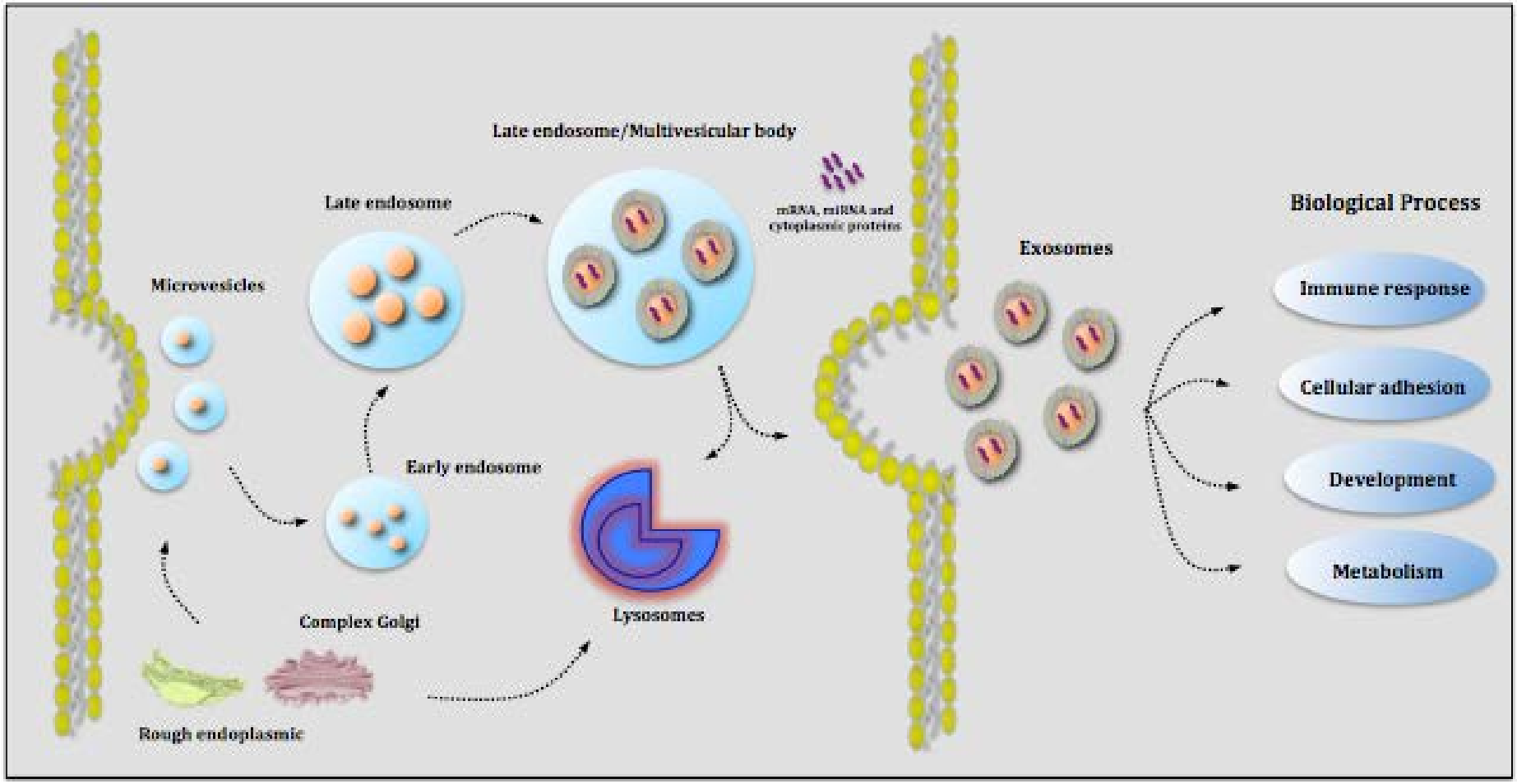
Exosome secretion occurs in a constitutive manner although cellular stress or activation signals modulate their secretion.

Exosomes carry specific repertoires of proteins, mRNAs, small non-coding RNAs, miRNAs….



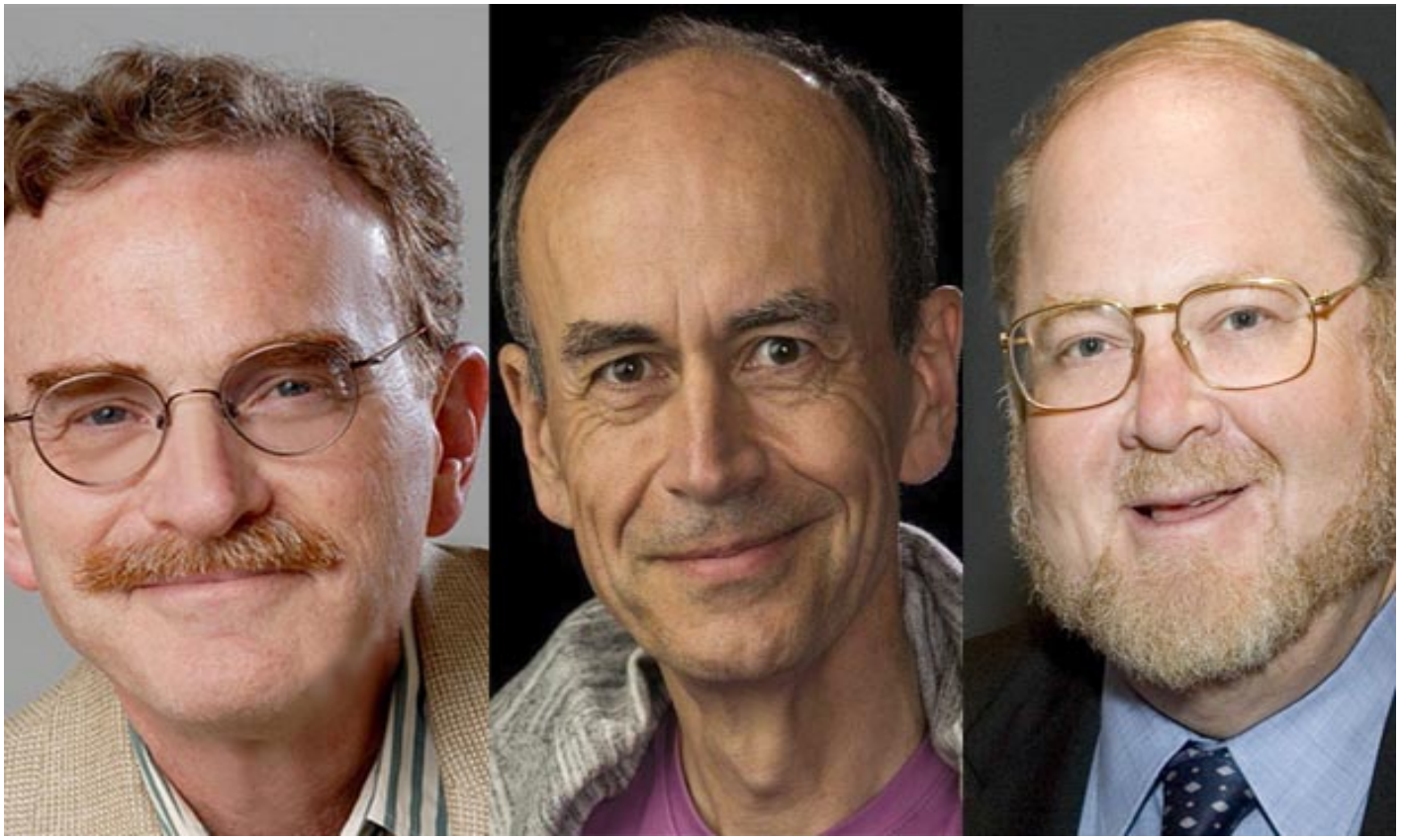


EXOSOMES



- Exosomes have been found to play a role in intercellular communication in several physiological processes, and contribute to organism development, immune responses, neuronal communication, tissue repair…..
- However, they may participate in some pathological disorders, favoring tumor progression.

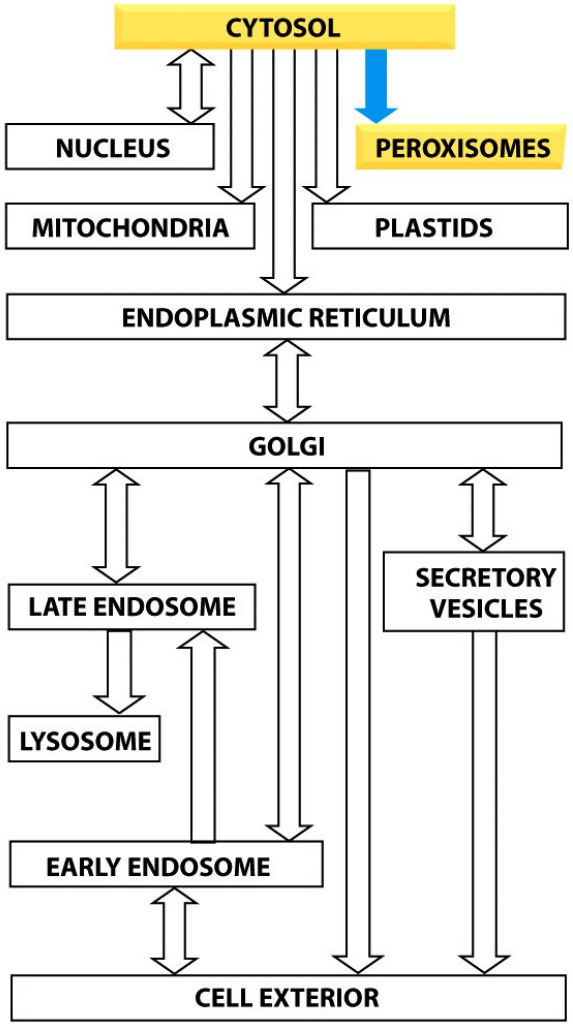
VESICLE FORMATION AND TRANSPORT



Los científicos Randy Shekman, James Rothman (estadunidenses) y Thomas Sudhof (alemán) Premio Nobel de Medicina 2013.

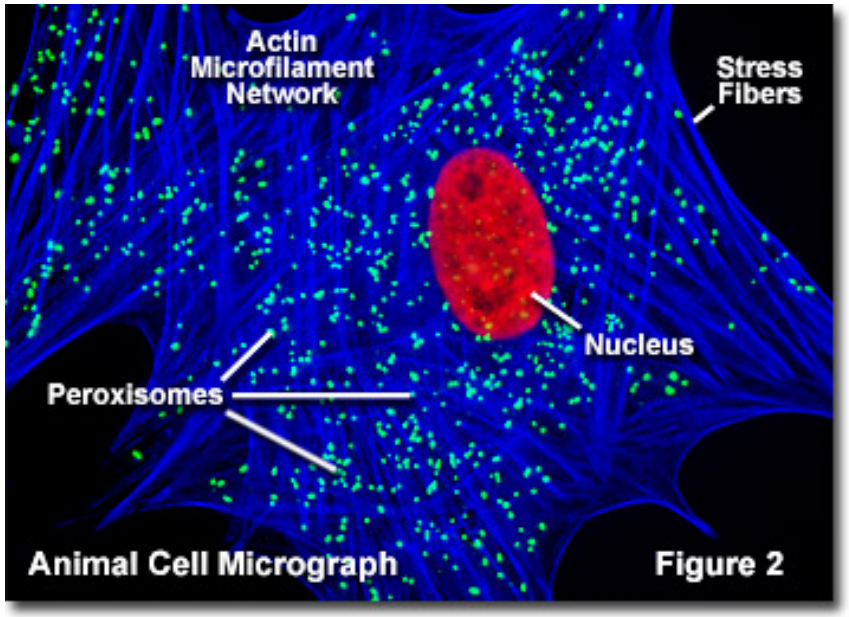
PEROXISOME

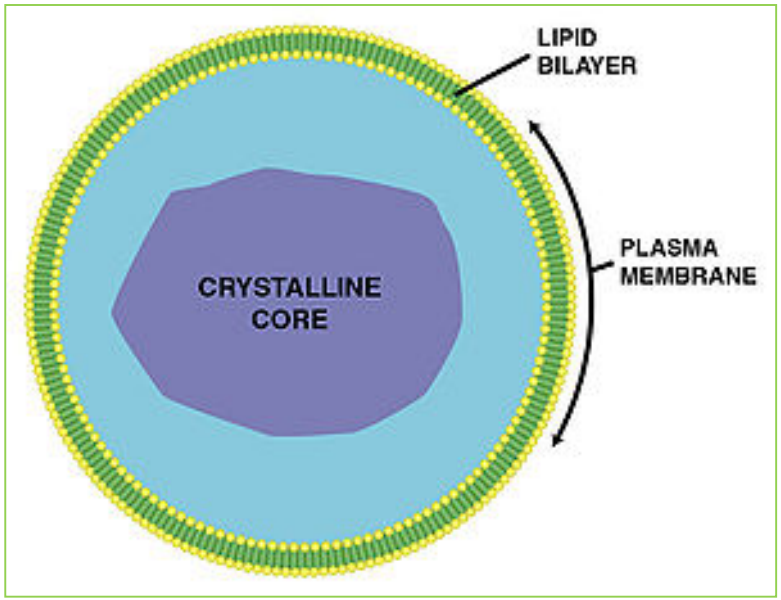
GENERAL CHARACTERISTICS

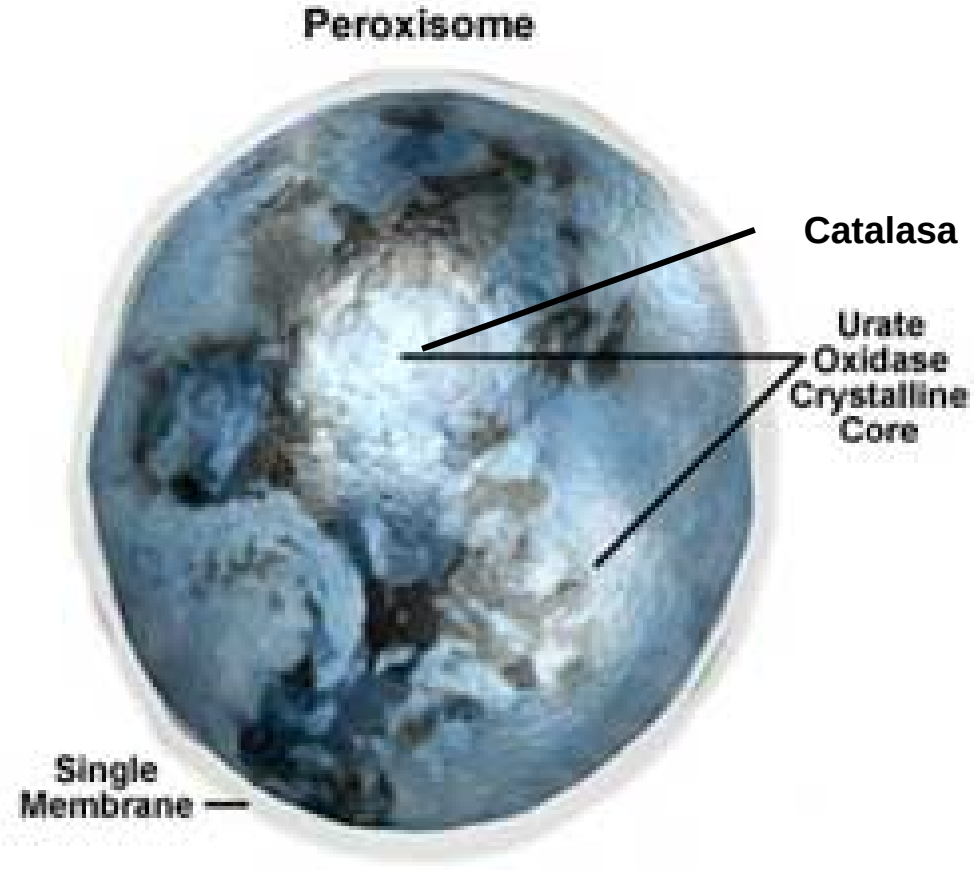


Alberts, sixth ed. Fig. 12-5

GENERAL CHARACTERISTICS

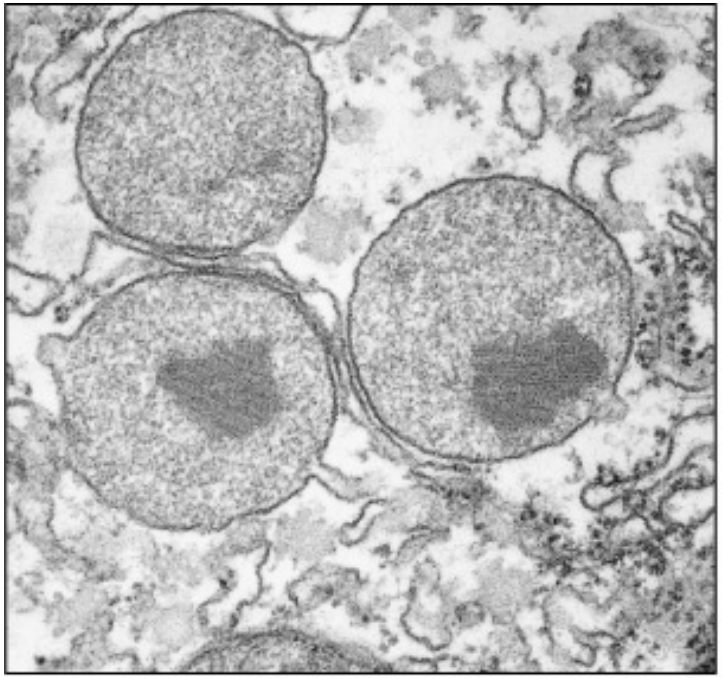


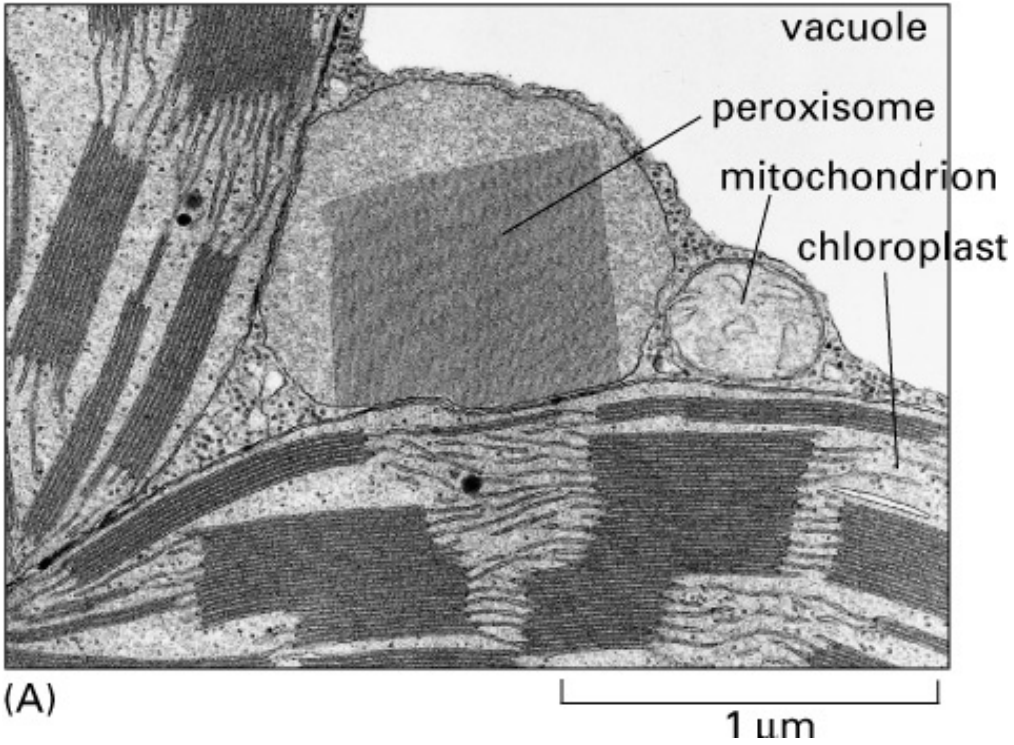




GENERAL CHARACTERISTICS

Ultrastructure





Functions

1. Oxidative reactions

$$RH_2 + O_2 \longrightarrow R + H_2O_2$$

$$H_2O_2 + R'H_2 \xrightarrow{\text{catalase}} R' + 2H_2O$$

2. β-oxidation. Formation of acetyl coenzyme A from fatty acids

3. Participation in the synthesis of plasmalogens (most abundant phospholipids in myelin)

Biogenesis

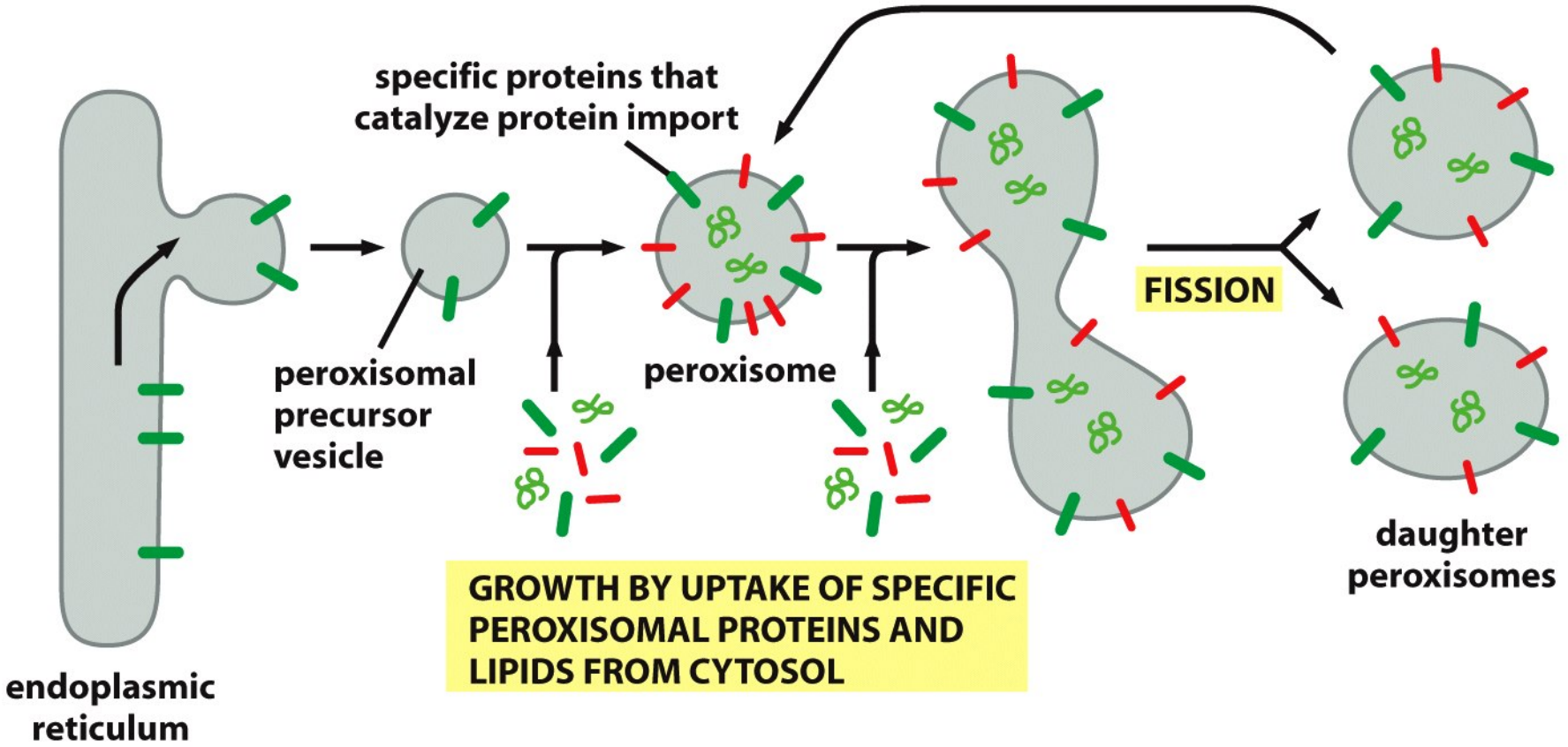


Figure 12-33 *Molecular Biology of the Cell* (© Garland Science 2008)

Biogenesis

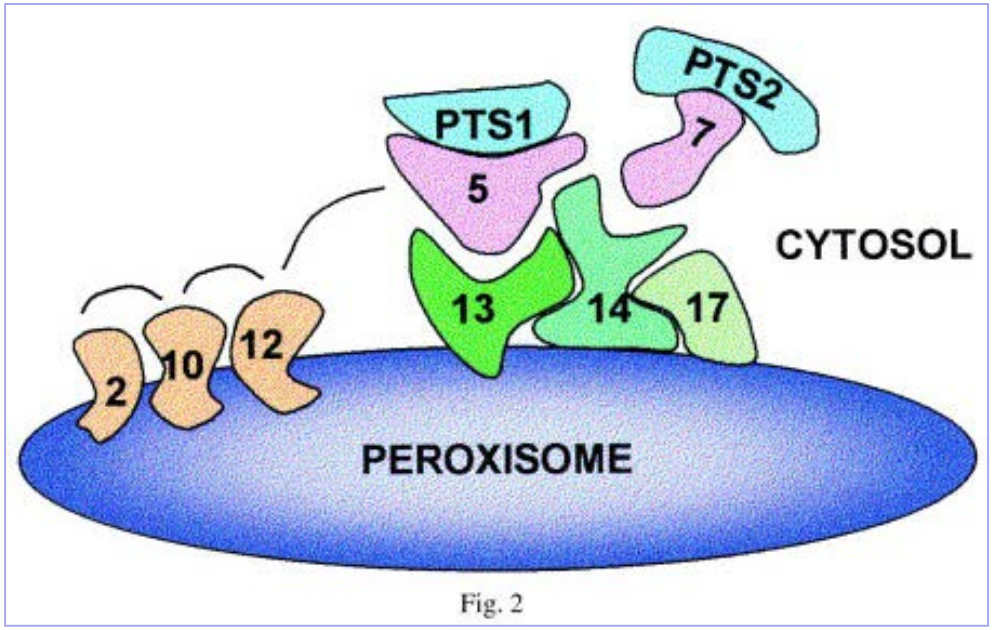
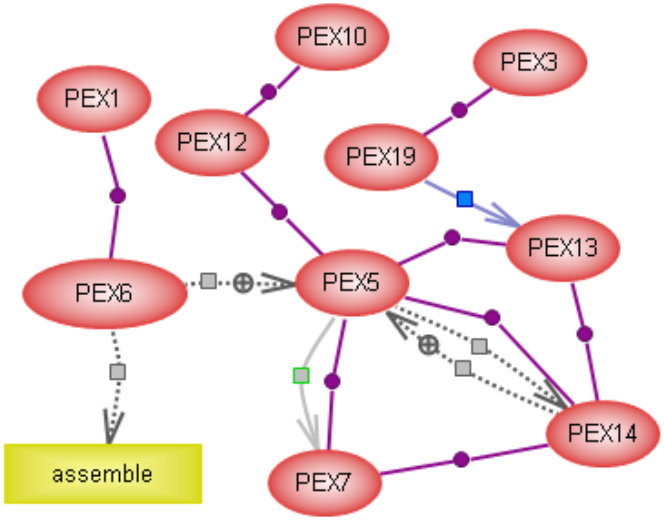


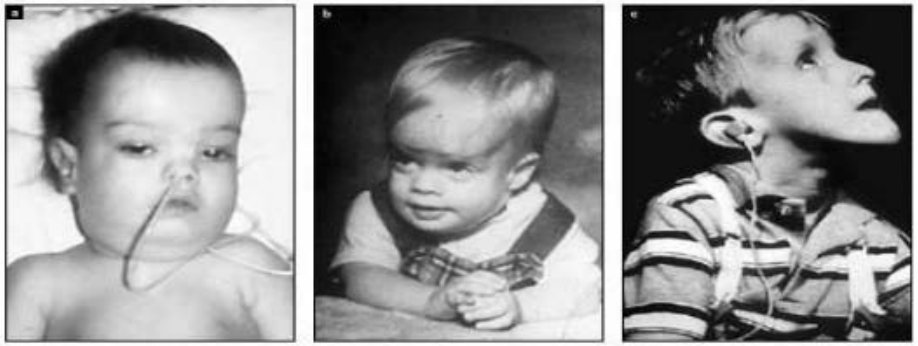
Fig. 2



Genes involved in the synthesis of peroxisomes are known as PEX. Encode the proteins called PEX peroxinas, most of which are located in peroxisomal membrane. Selection of Proteins into peroxisomes depends on two peroxinas which act as receptor peroxisome targeting signals: the PTS-1 and PTS-2, which are composed of a short sequence of amino acids (Ser-Lys-Leu)

BIOPATHOLOGY

ALTERACIONES DE LA BIOGÉNESIS	DEFICIENCIA DE UNA ENZIMA PEROXISOMAL
Sindrome de Zellweger	Deficiencia de proteinas translocadoras de enzimas al peroxisoma
Adrenoleucodistrofia neonatal	Deficiencia de Acil CoA oxidasa
Enfermed. de Refsum infantil	Deficiencia de tiolasa peroxisomal
Condrodisplasia punctata rizomiélica	Defic. de Dihidroxi-acetona-fosfato acil sintetasa (DHAPT)



a) Síndrome Zellweger b) Adrenoleucodristofia neonatal c) Enferm. de Refsum infantil.

Zellweger syndrome, also called cerebrohepatorenal syndrome, is a rare congenital disorder, characterized by the reduction or absence of functional peroxisomas in the cells of an individual. Mutation Pex2

MITOCHONDRIA

General characteristics

Ultrastructure

Chemical components

Functions

Biogenesis

Pathology

GENERAL CHARACTERISTICS

In vivo observation

Location

Number

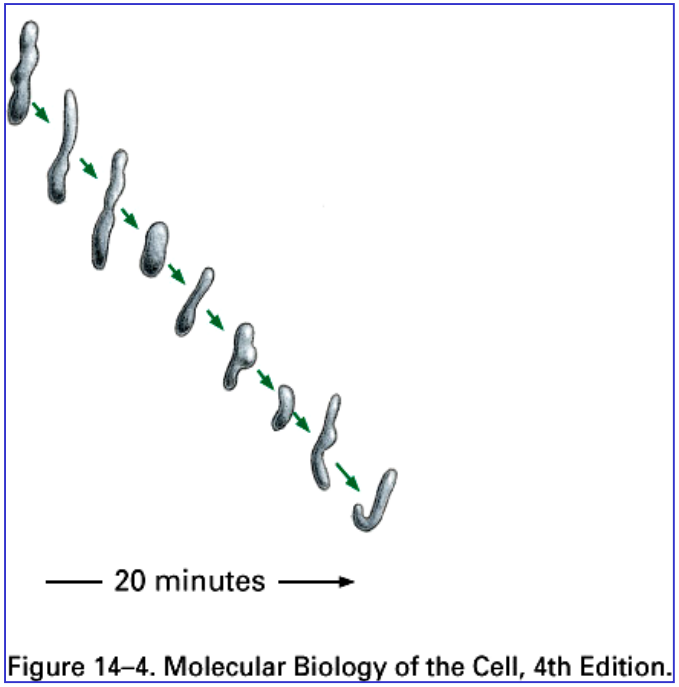


Figure 14–4. Molecular Biology of the Cell, 4th Edition.

plasticity

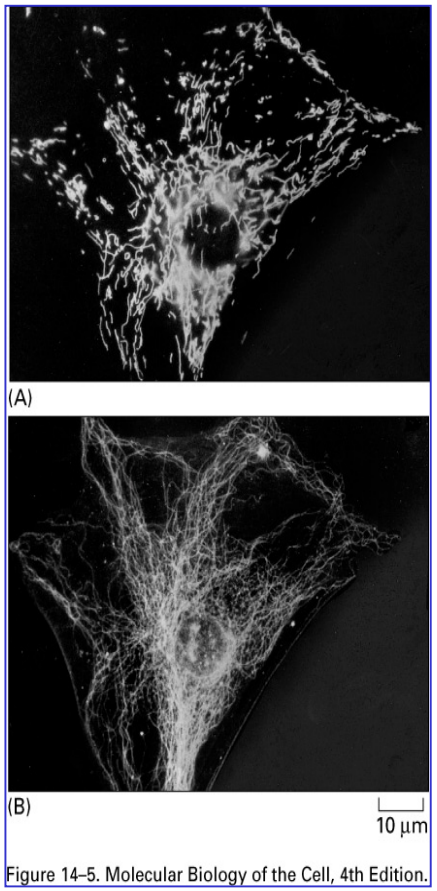
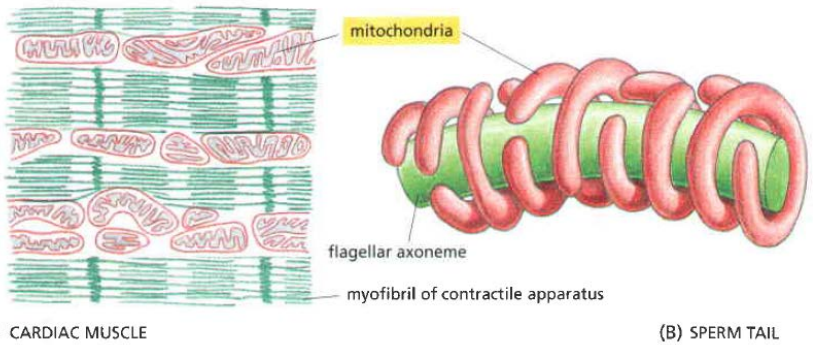
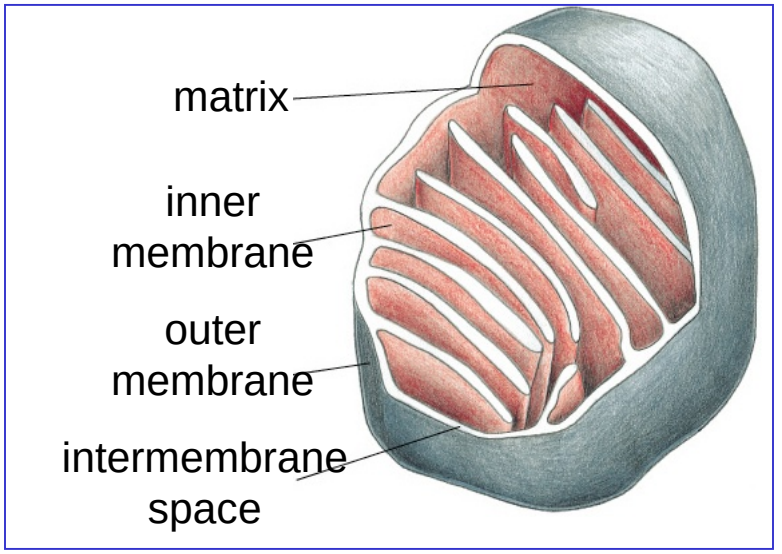


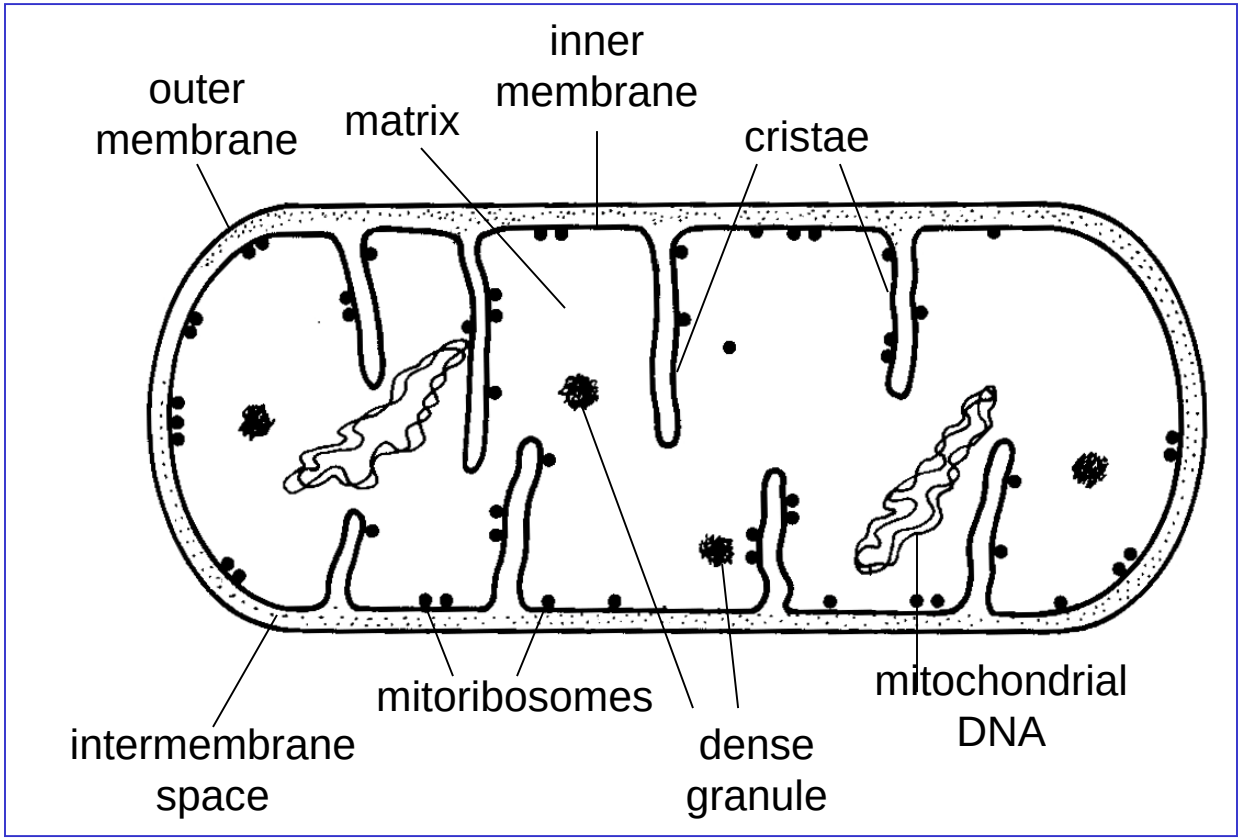
Figure 14–5. Molecular Biology of the Cell, 4th Edition.

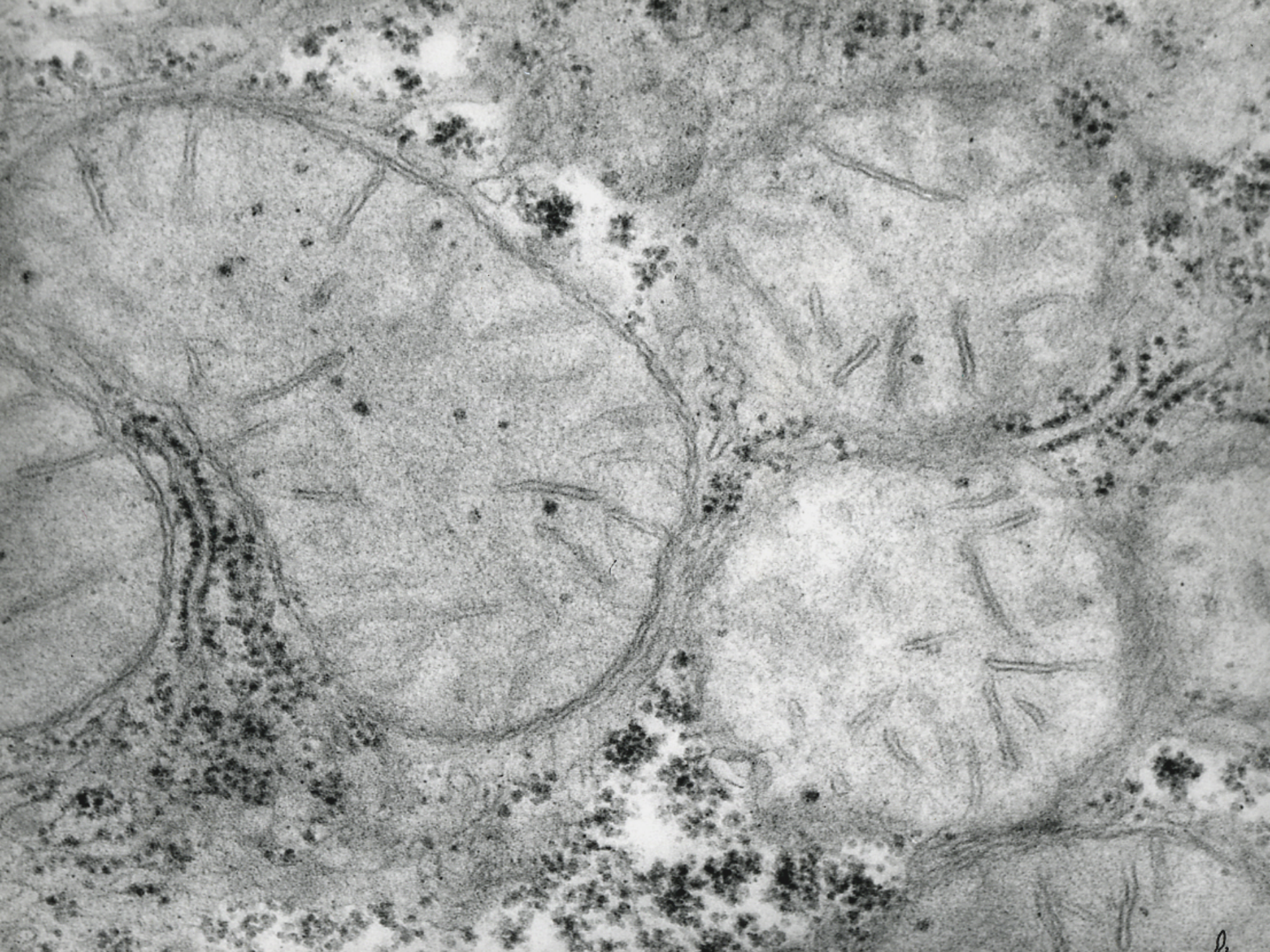
associated to microtubules



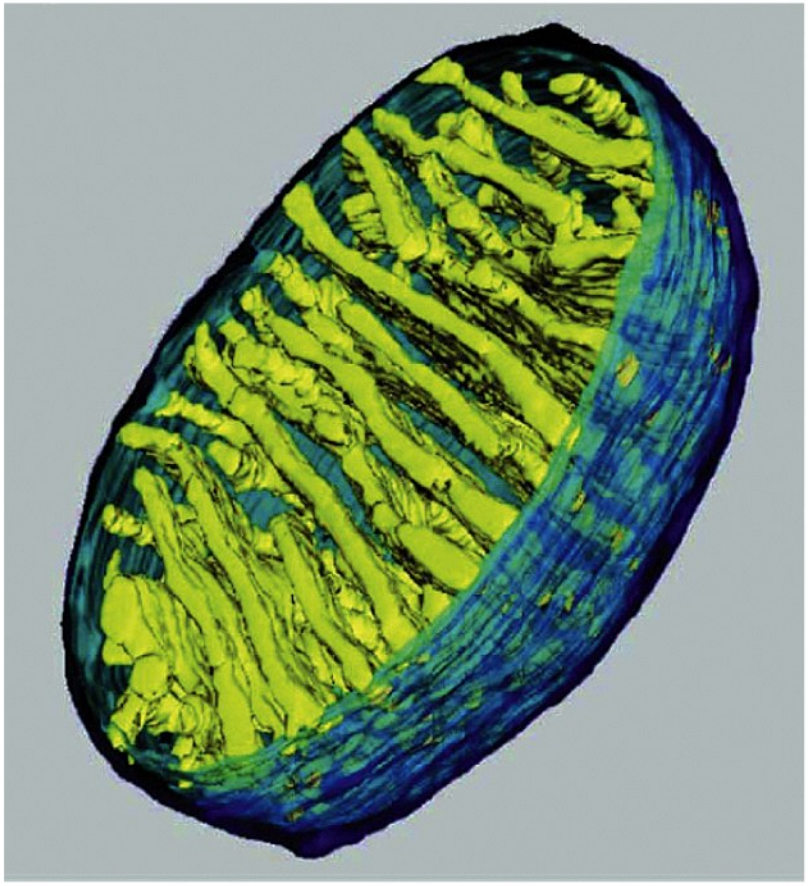
ULTRASTRUCTURE







ULTRASTRUCTURE



CHEMICAL COMPONENTS

Outer membrane

40% lipids and 60% proteins
Lipids: phospholipids (phosphatidylcholine and phosphatidylethanolamine) and little cholesterol
Proteins: porin and enzymes (lipid metabolism)

Inner membrane

20% lipids and 80% proteins
Lipids: phospholipids, cardiolipids and no cholesterol
Proteins:

Components of the electron-transport chain
NADH dehydrogenase complex (more than 40 subunits)
Cytochrome b-c_1 complex (11 subunits, dimer)
Cytochrome oxidase complex (13 subunits, dimer)

ATP synthase (500.000 daltons, F_1/F_0) 9 subunits

Specific transporters (ADP/ATP, phosphate, dicarboxylic acids, tricarboxylic acids, aminoacids, fatty acids, Ca^{++}, etc…)

CHEMICAL COMPONENTS

Components of the respiratory chain

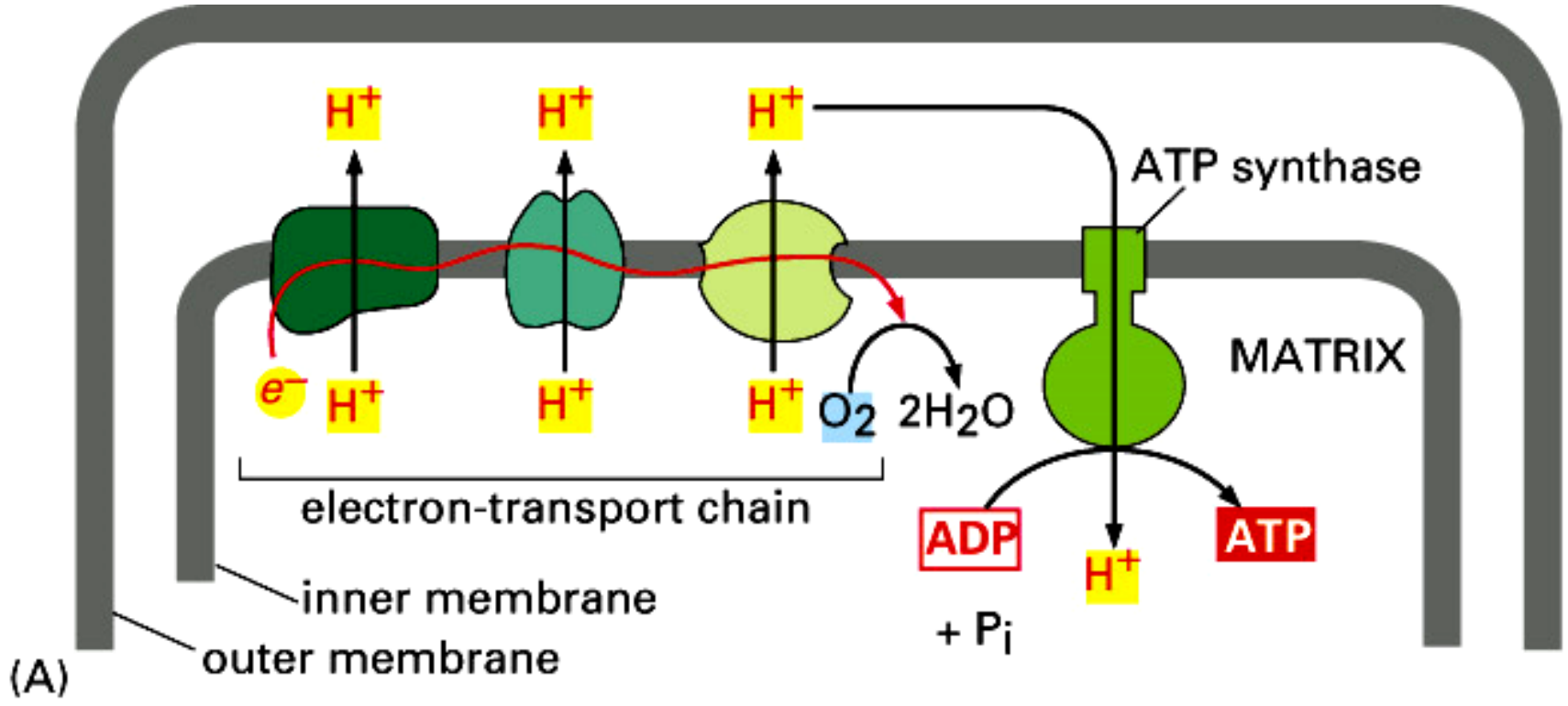
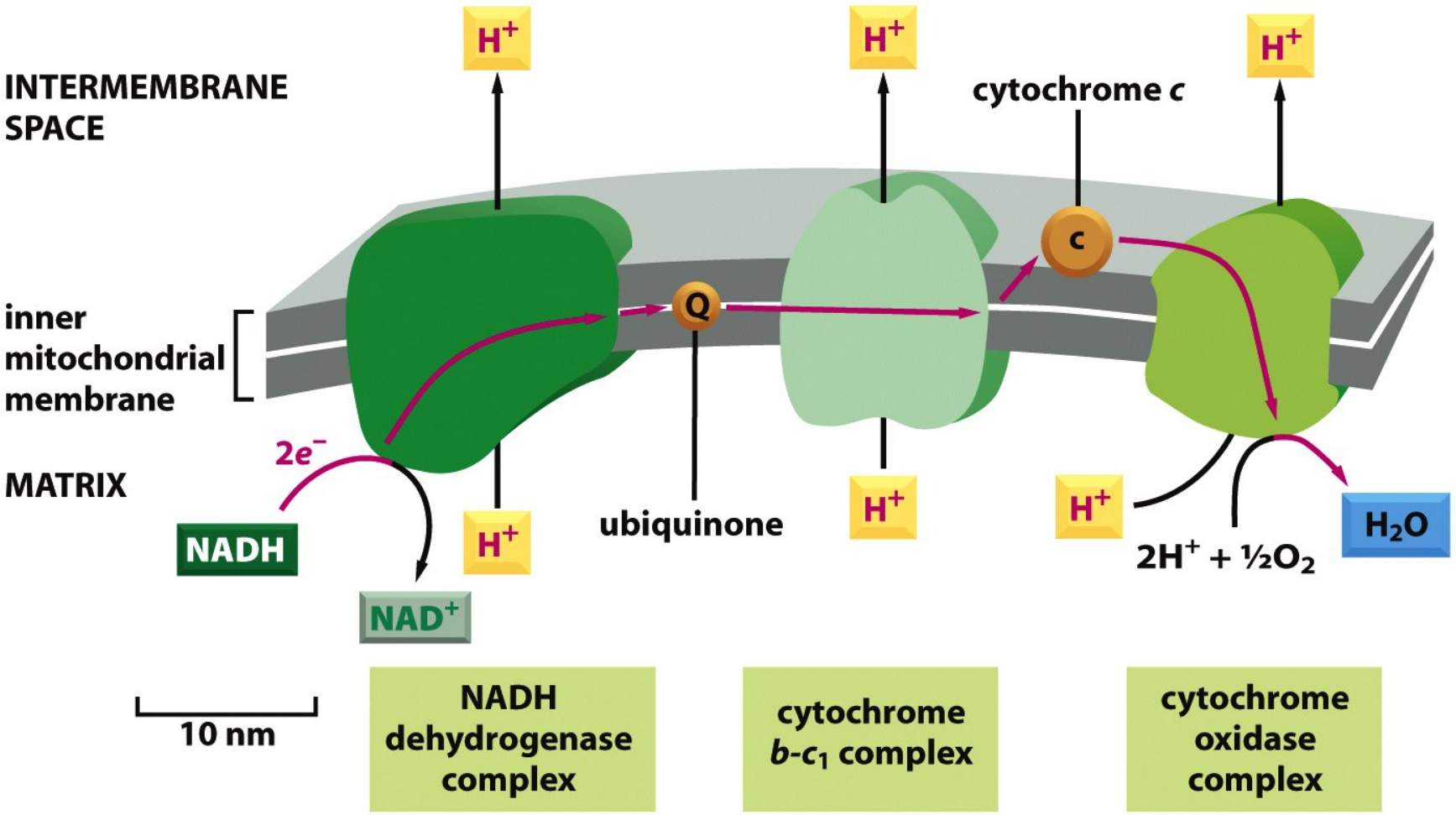


Figure 14–14 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

CHEMICAL COMPONENTS

Components of the respiratory chain



CHEMICAL COMPONENTS

ATP synthase

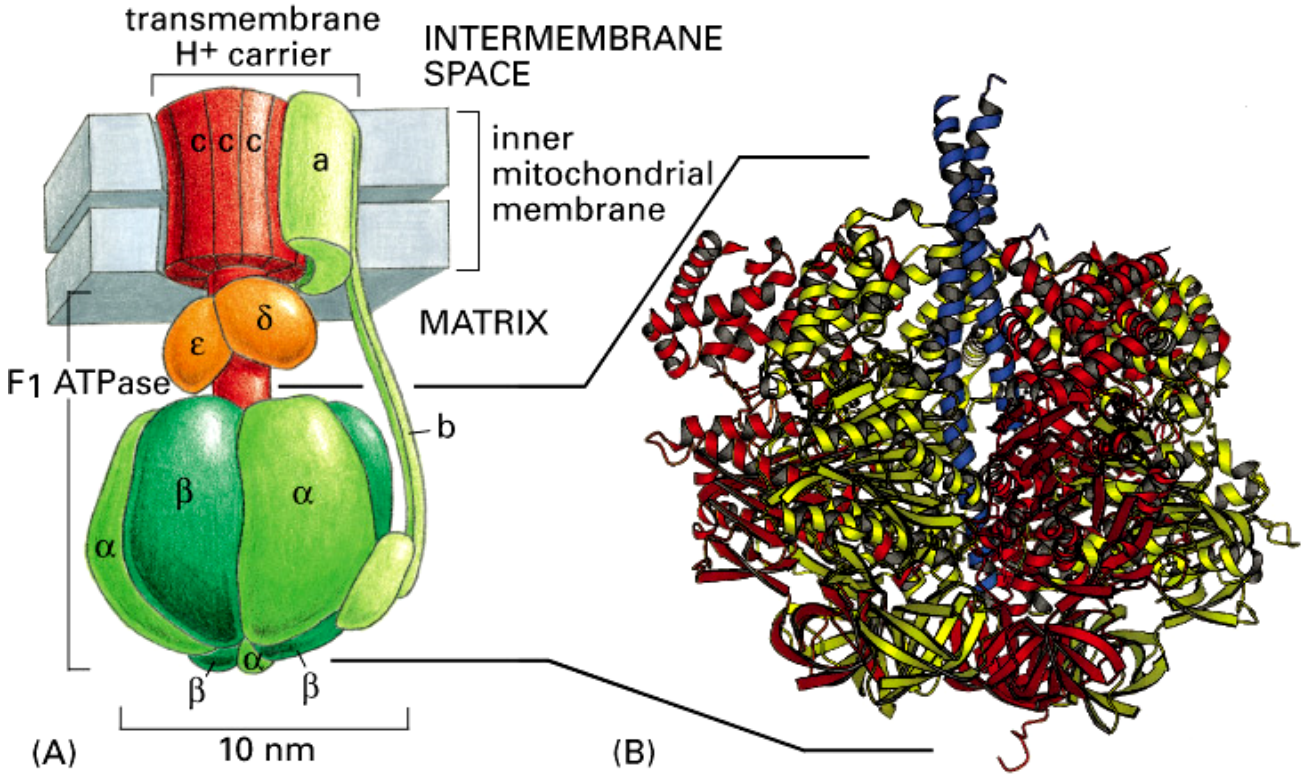
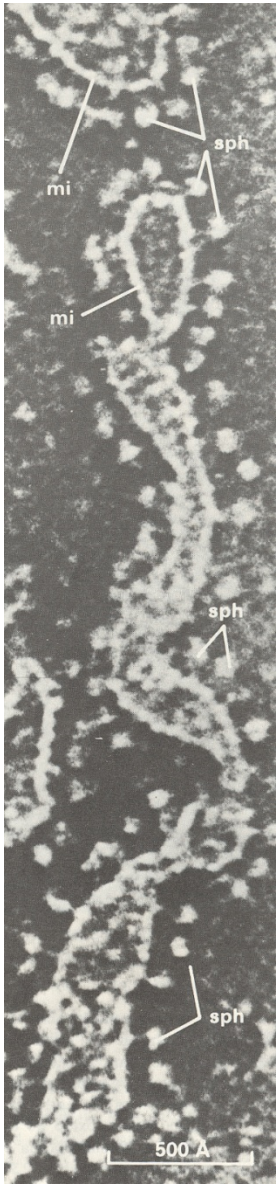
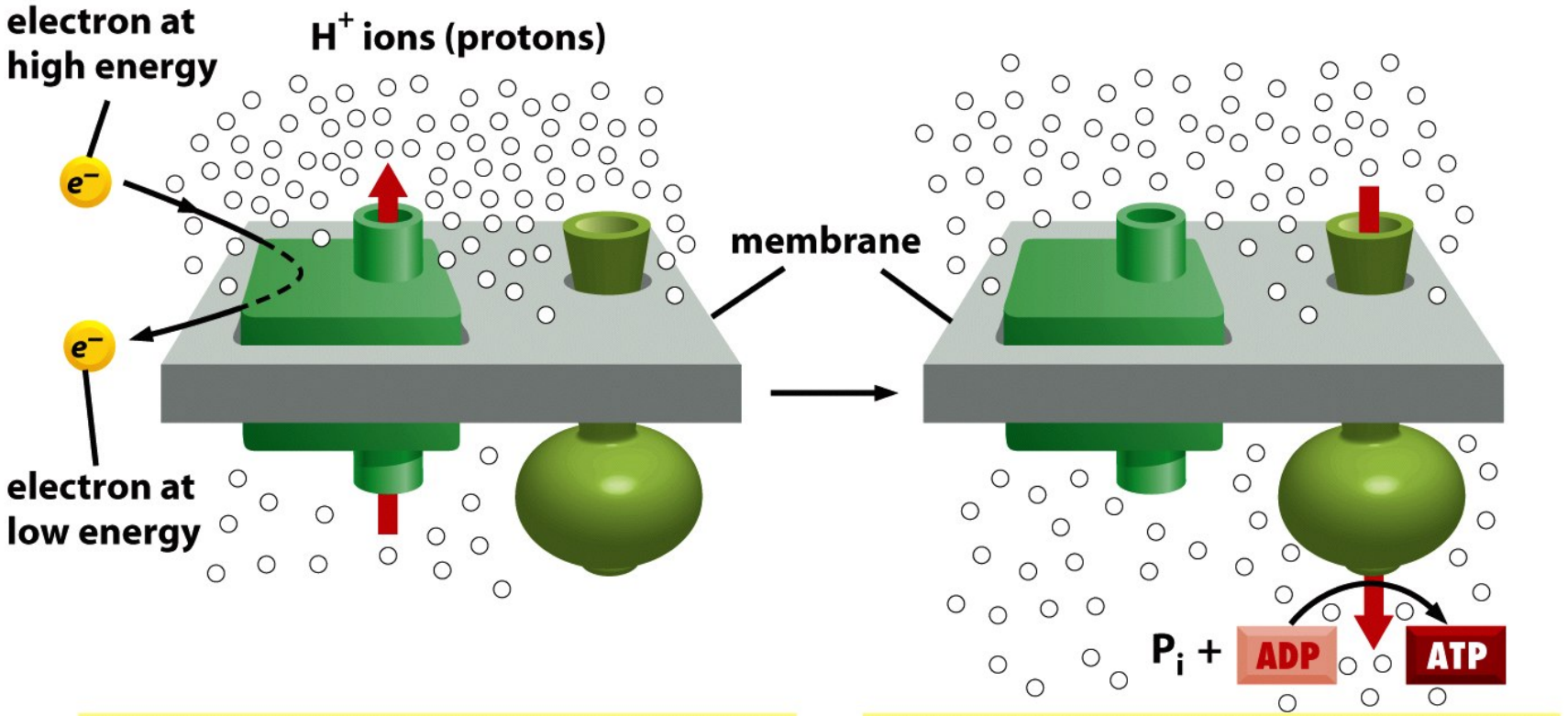


Figure 14–15. Molecular Biology of the Cell, 4th Edition.



CHEMICAL COMPONENTS

ATP synthase



CHEMICAL COMPONENTS

Intermembrane space: enzymes (e.g. adenylate kinase)

Matrix

Mitochondrial DNA

-Double chain, circular, closed
-H chain (rich in A and G) and L chain (rich in T and C)
-16569 bp
-Genetic code different to universal code
-It codifies:13 polypeptides, 22 tRNA and 2 rRNA (12S and 16S)
-1% of cellular DNA
-Great mutability

Ribosomes

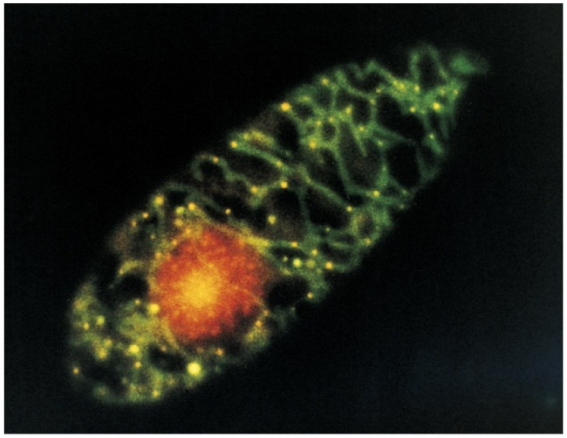
tRNAs

Enzymes for oxidations

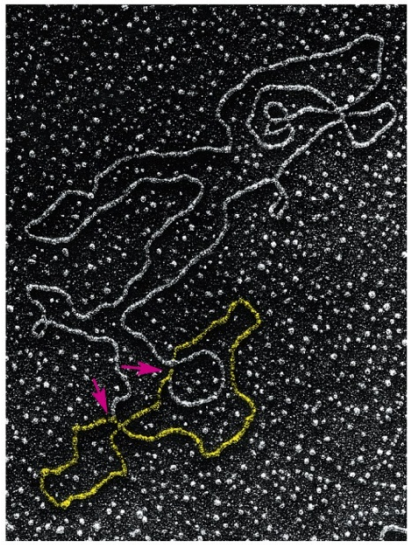
Enzymes related to DNA activity

CHEMICAL COMPONENTS

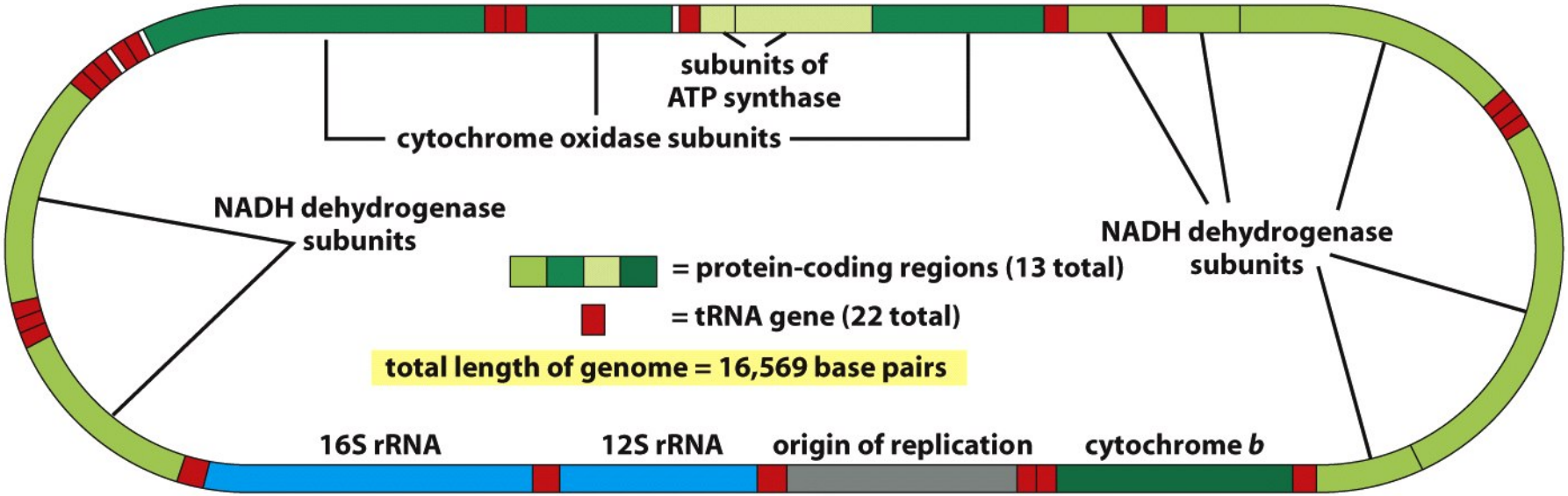
Mitochondrial DNA



25 μm



1 μm



FUNCTIONS

1) Respiratory oxidations

-acetyl CoA formation in the matrix

-acetyl CoA oxidation (Kreb's cycle) in the matrix

-electron transport and oxidative phosphorylation at the inner membrane

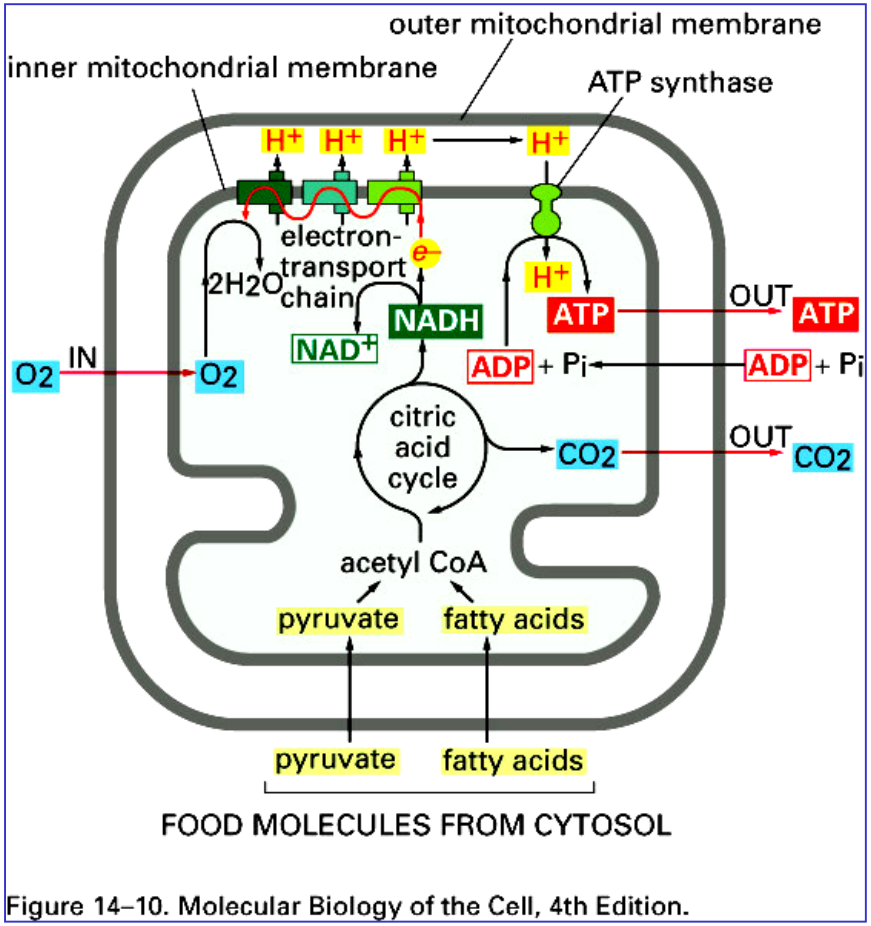


Figure 14–10. Molecular Biology of the Cell, 4th Edition.

FUNCTIONS

2) Generation of precursors

-gluconeogenesis

-fatty acids syntheses

-aminoacids

-ureogenesis

3) Protein synthesis

4) Apoptosis

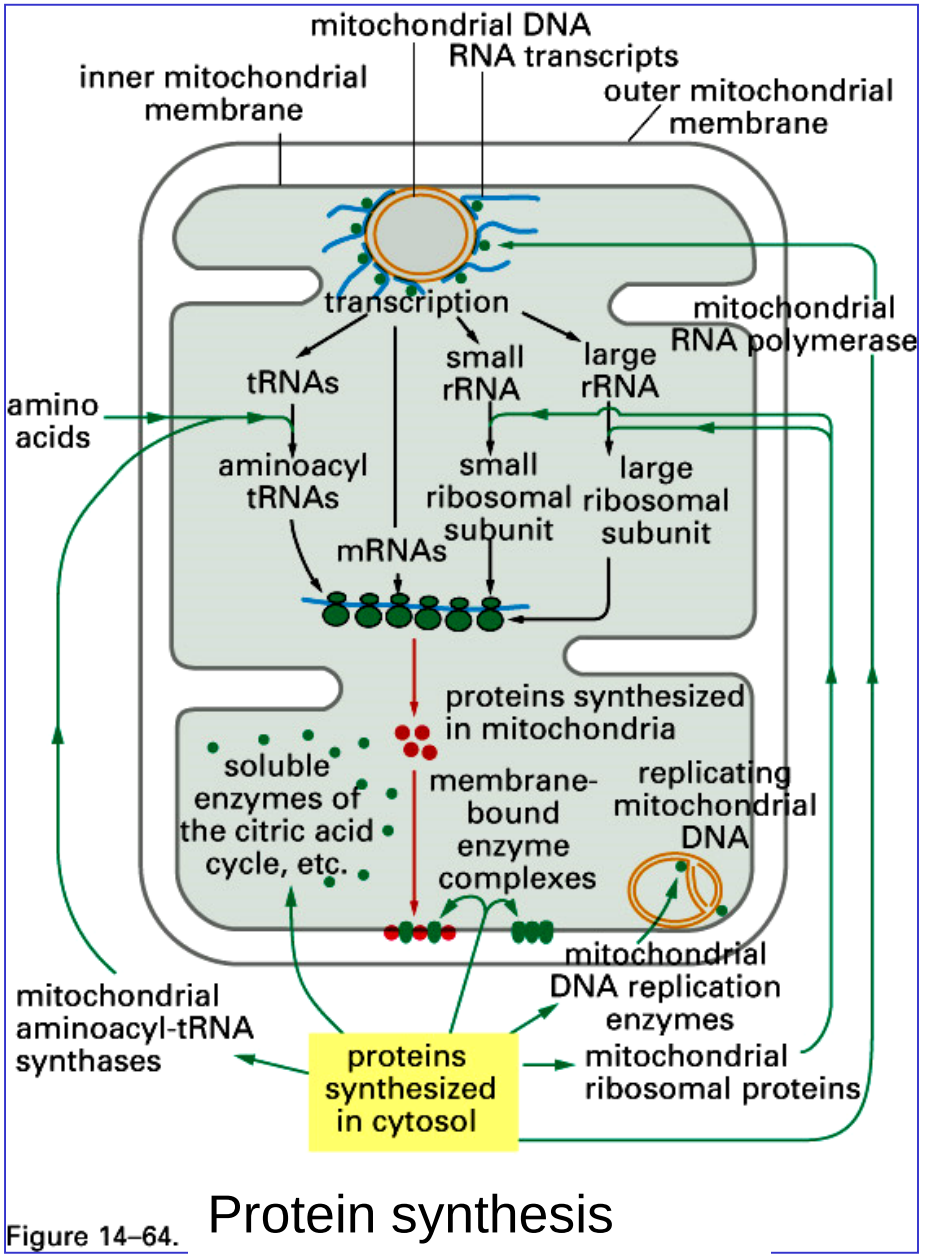
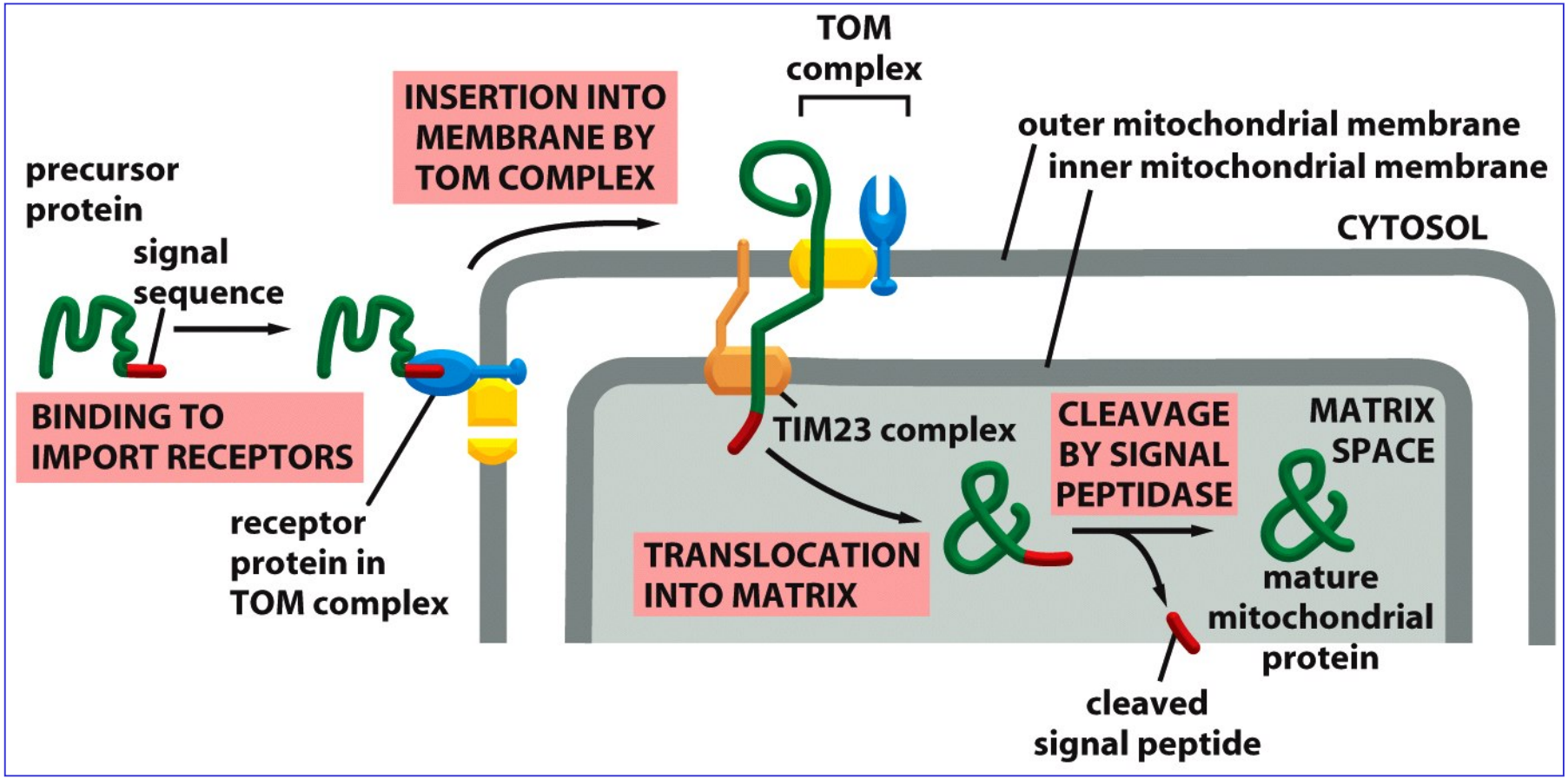


Figure 14–64. Protein synthesis

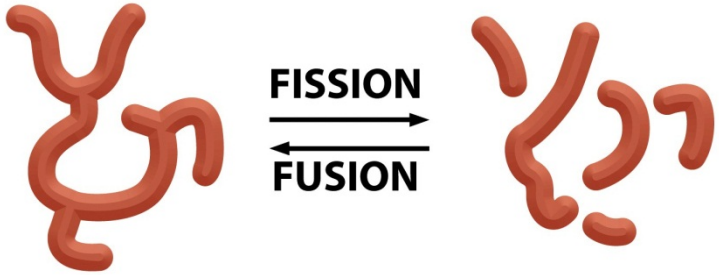
FUNCTIONS/BIOGENESIS

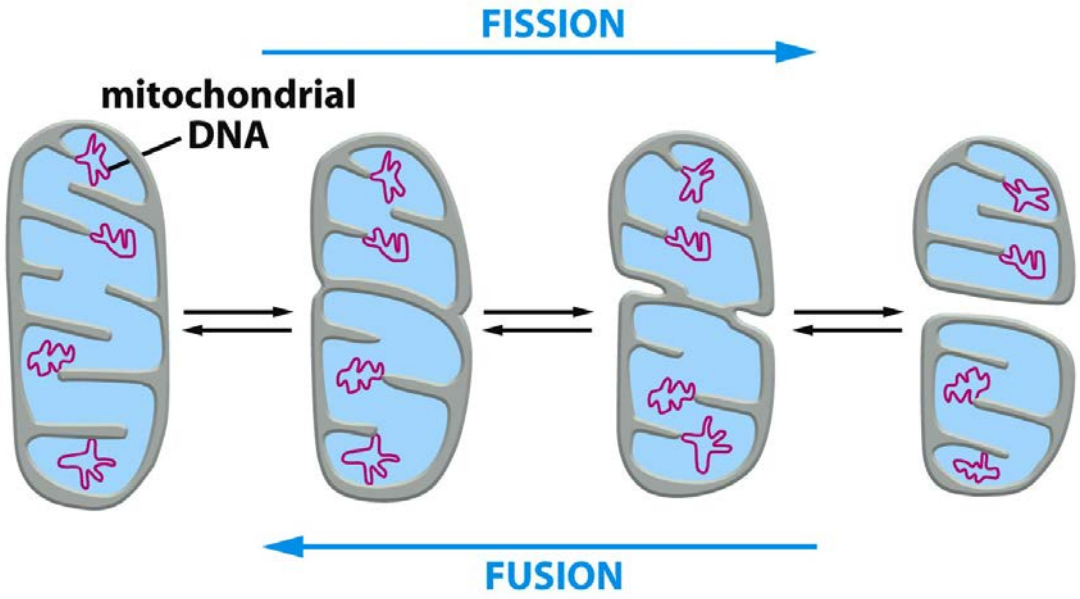
Protein import by mitochondria

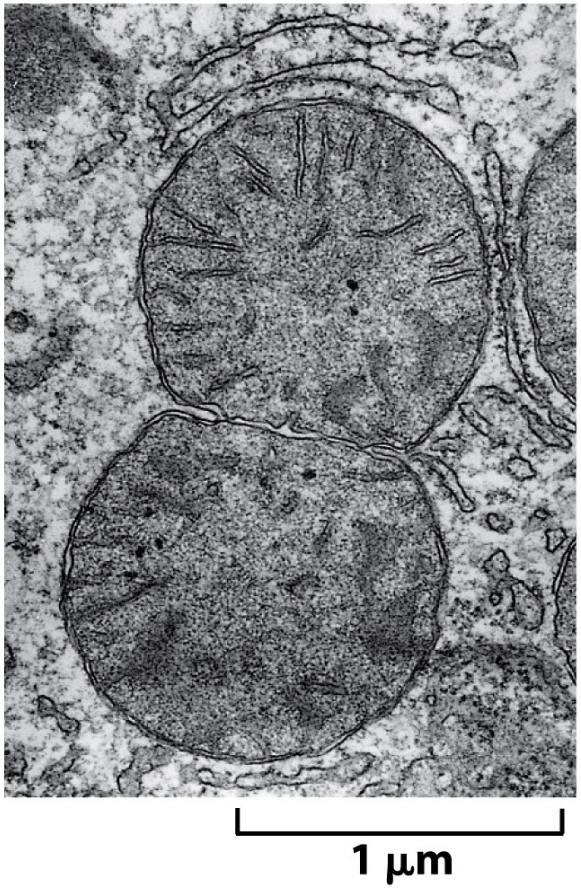


TIM/TOM complex

BIOGENESIS







PATHOLOGY

Causes: Deletion or mutations in mitochondrial or nuclear DNA

Mitochondrial DNA:

- -It is not protected by histones
- -Repair mechanisms not very efficient
- -Exposed to free radicals

Mitochondrial dysfunction is involved in

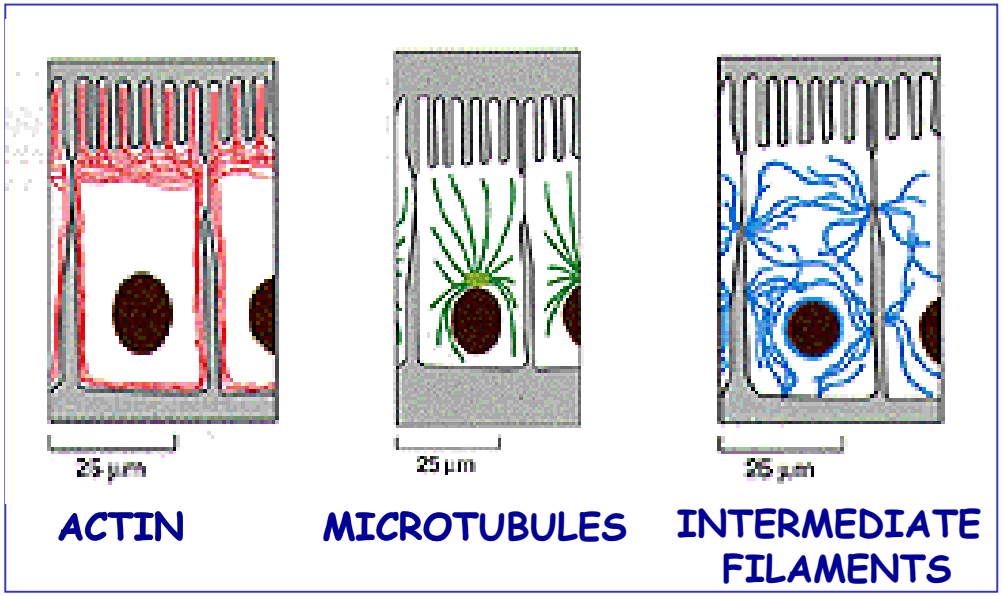
- -Encephalopathies
- -Myopathies
- -Aging

THE CYTOSKELETON

- INTRODUCTION
- MICROTUBULES
  - Morphology
    - Labile microtubules
    - Stable microtubules
      - Axoneme of motile cilia and flagella
      - Primary cilium
      - Centrioles
  - Chemical composition
  - Molecular organization
  - Dynamic equilibrium
  - Biogenesis

INTRODUCTION

- Filaments that extend throughout the cytoplasm
- Highly dynamic structure
- Functions:
  - ➢Maintenance of cell shape
  - ➢Regulation of the position of organelles
  - ➢Cell movement
- Components:
  - ➢Actin (7 nm)
  - ➢Intermediate filaments (10 nm)
  - ➢Microtubules (25 nm)



MICROTUBULES

Morphology

Hollow cylinders with a 25nm outer diameter and a 5nm wall
Variable length

Labile microtubules

Are observed by fixing with glutaraldehyde (>4°C)
Long and straight, forming bundles.
They originate in the microtubule-organizing center (MTOC)

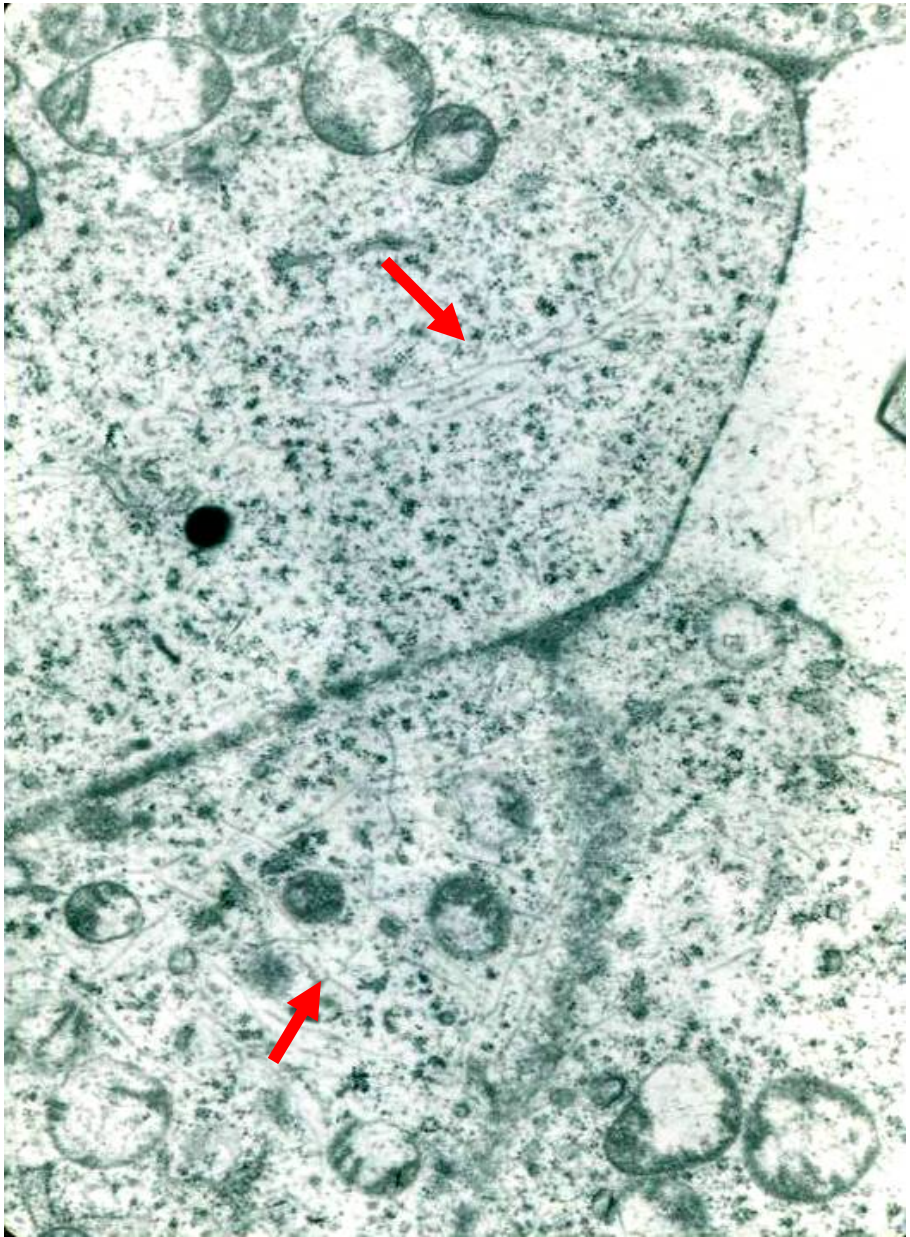
Stable microtubules

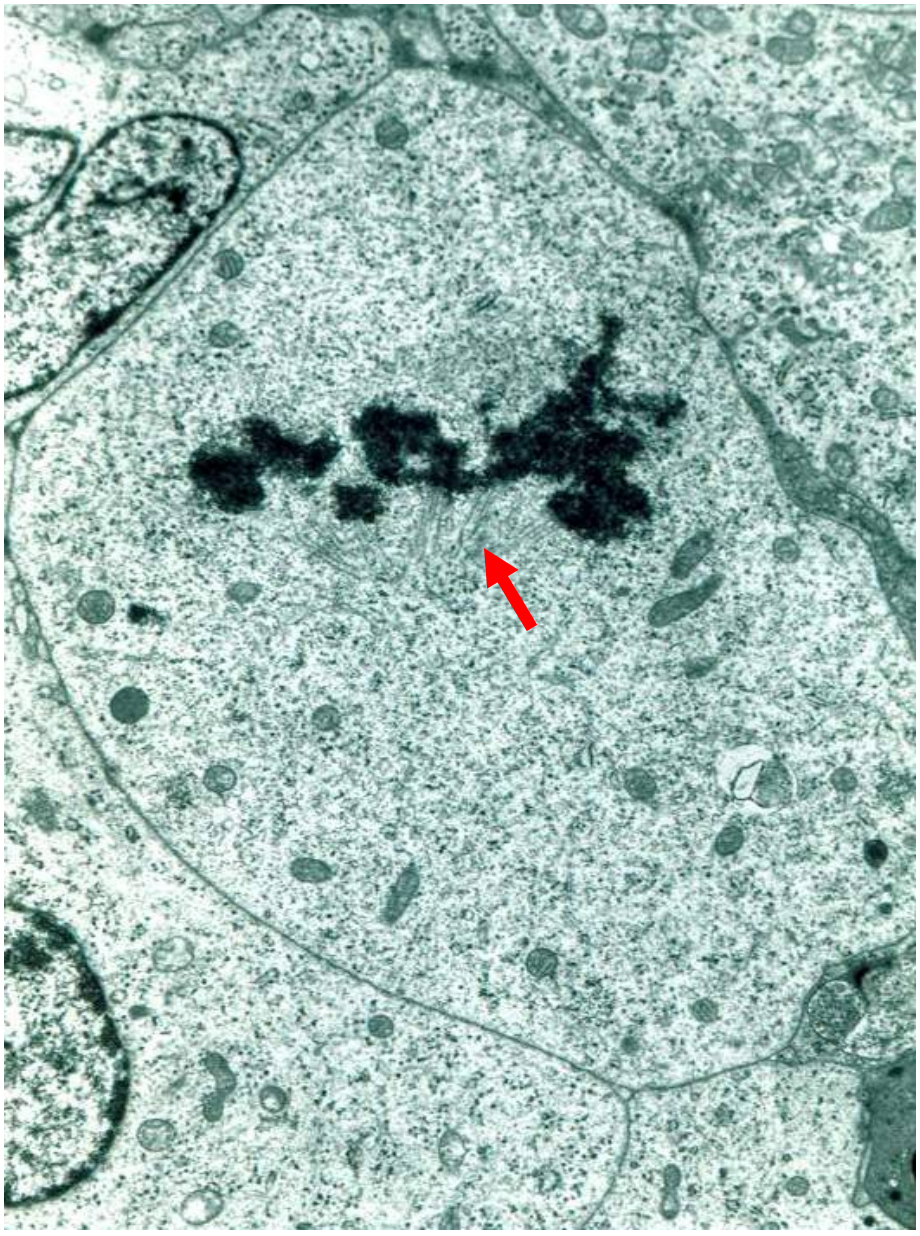
Axoneme of cilia and flagella

Primary cilium

Centrioles

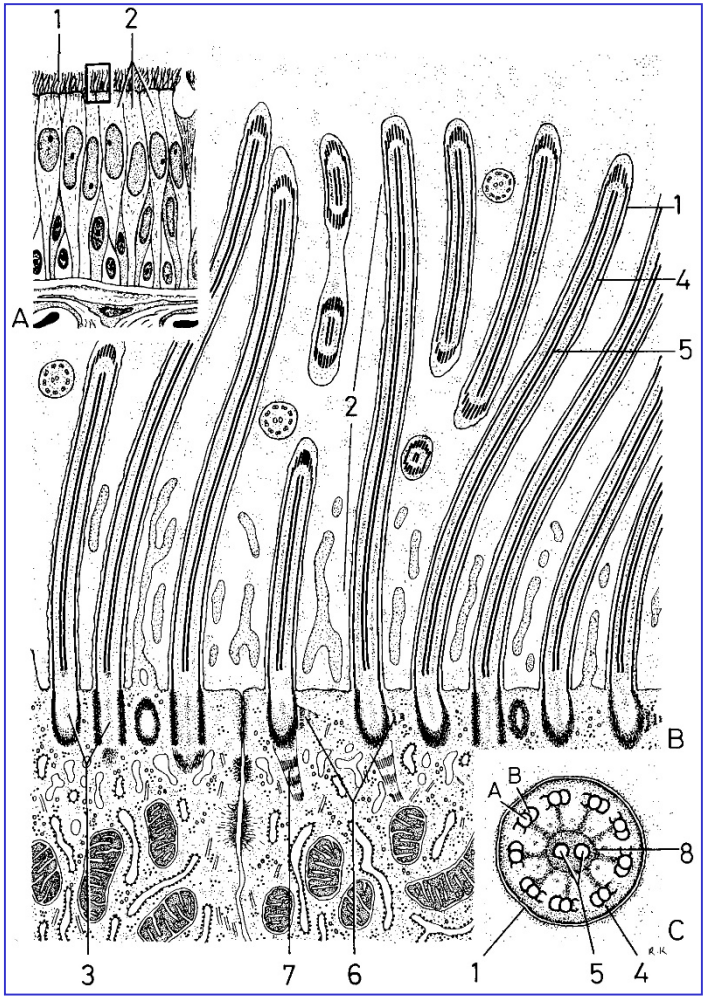
LABILE MICROTUBULES

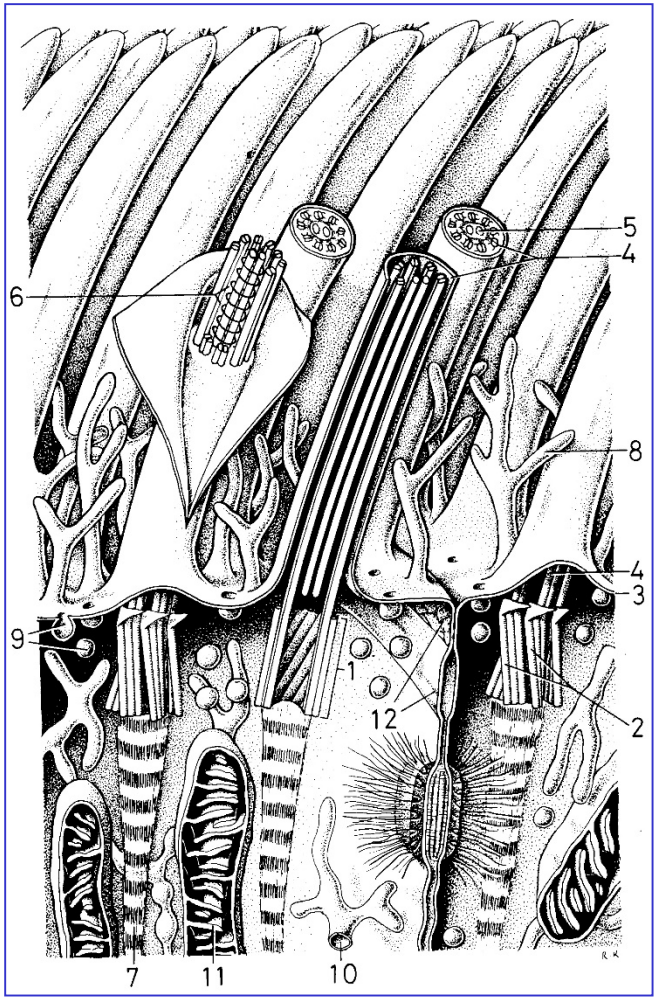




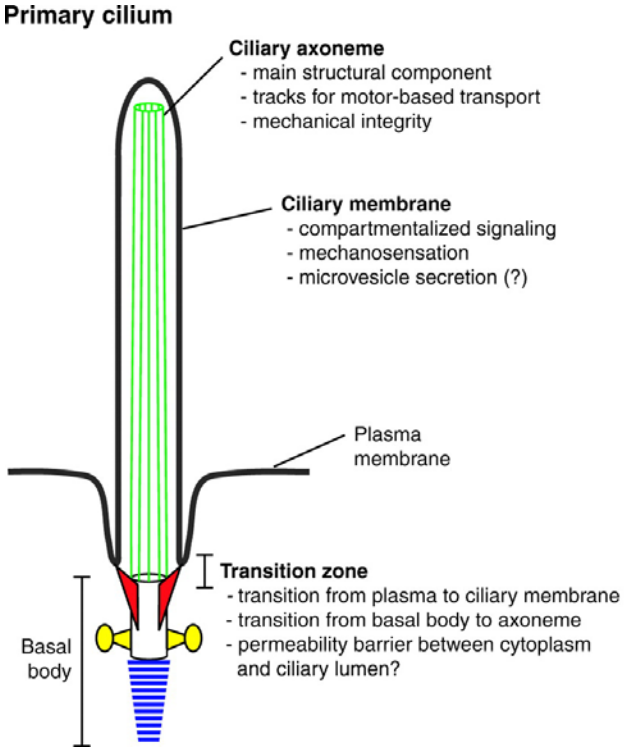
STABLE MICROTUBULES

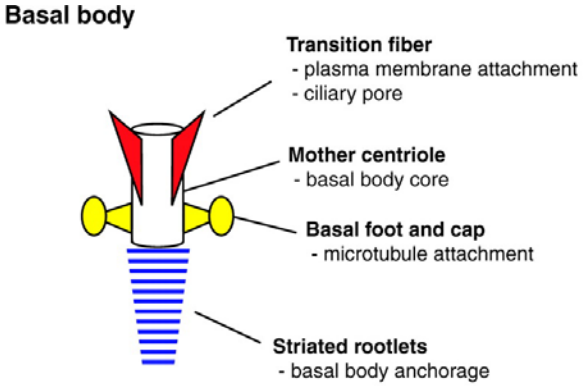
Axoneme of motile cilia and flagella

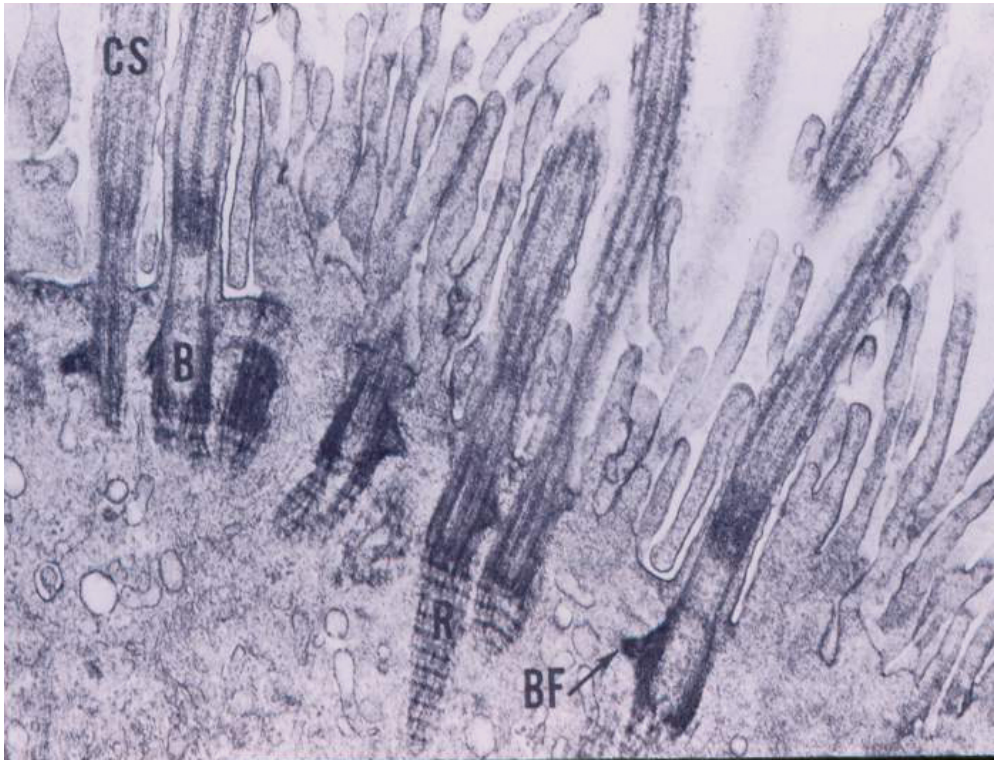




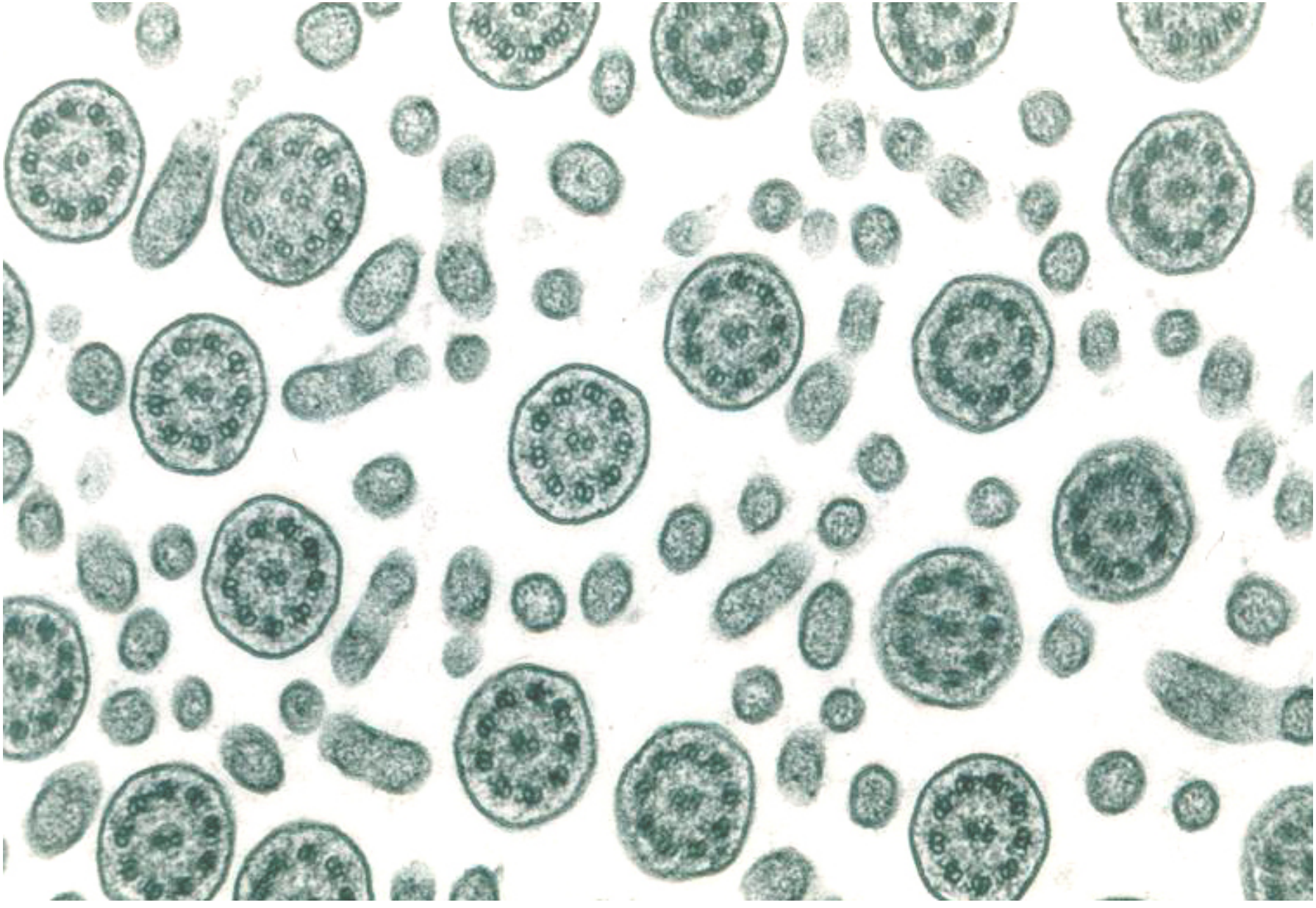
Cilia


Primary cilium
Ciliary axoneme
- main structural component
- tracks for motor-based transport
- mechanical integrity
Ciliary membrane
- compartmentalized signaling
- mechanosensation
- microvesicle secretion (?)
Plasma membrane
Transition zone
- transition from plasma to ciliary membrane
- transition from basal body to axoneme
- permeability barrier between cytoplasm and ciliary lumen?
Basal body

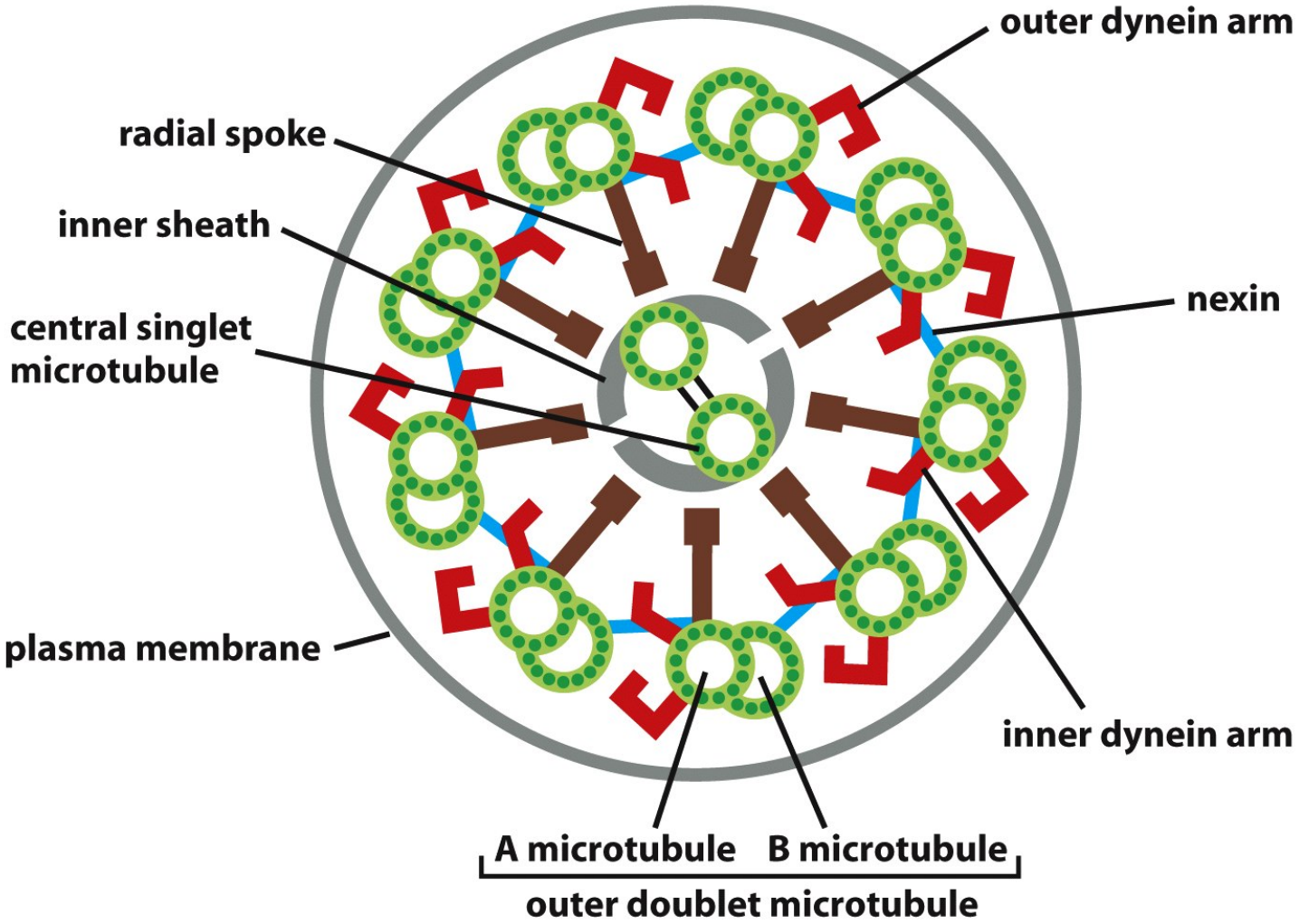

Basal body
Transition fiber
- plasma membrane attachment
- ciliary pore
Mother centriole
- basal body core
Basal foot and cap
- microtubule attachment
Striated rootlets
- basal body anchorage


CS
B
R
BF

Cilia



AXONEME OF MOTILE CILIA AND FLAGELLA



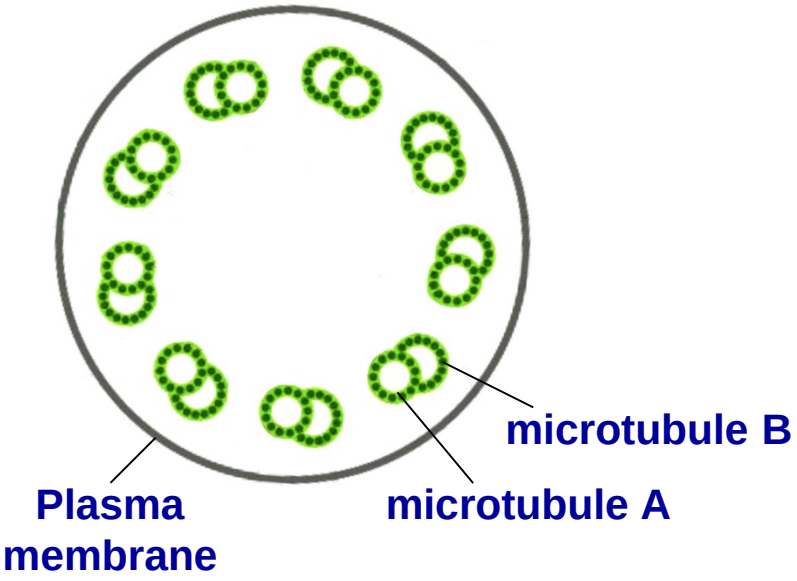
PRIMARY CILIUM

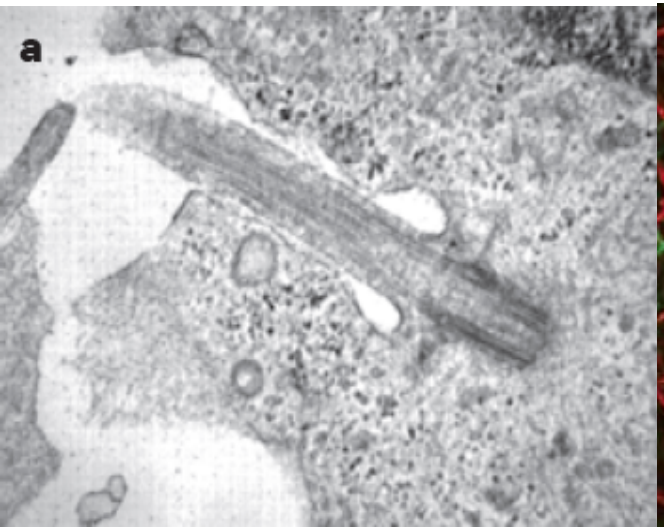
Does not move (non-motile)

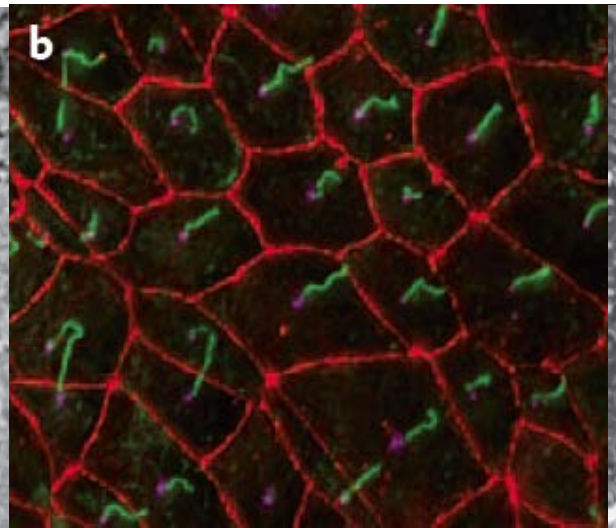
The axoneme is different

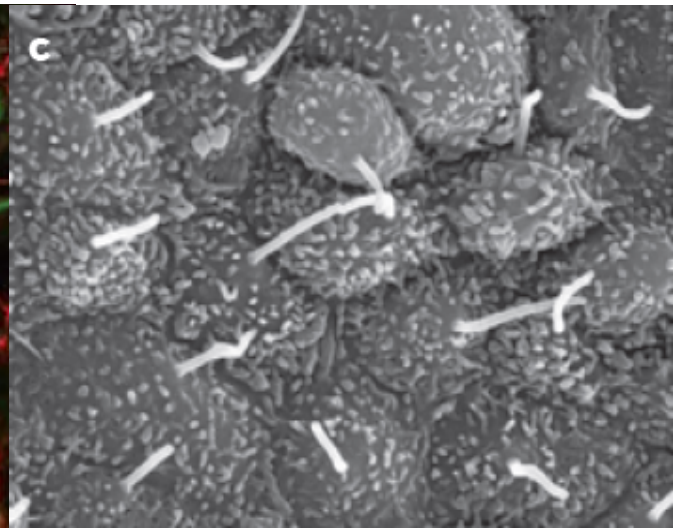
On the apical surface of many cells

Captures extracellular physical and biochemical signals

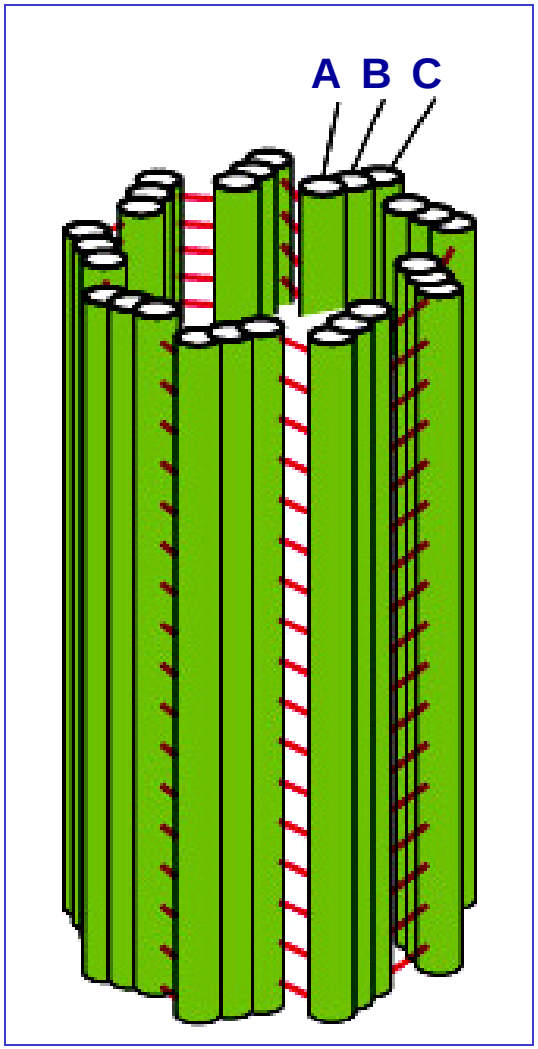


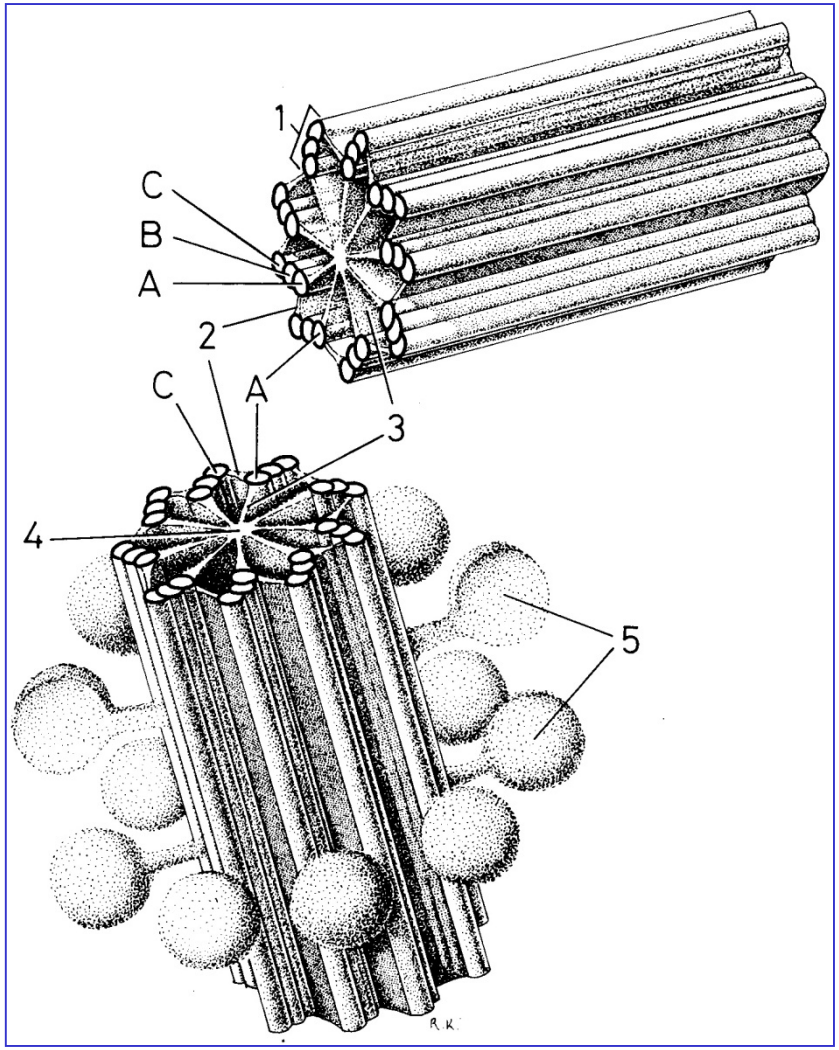






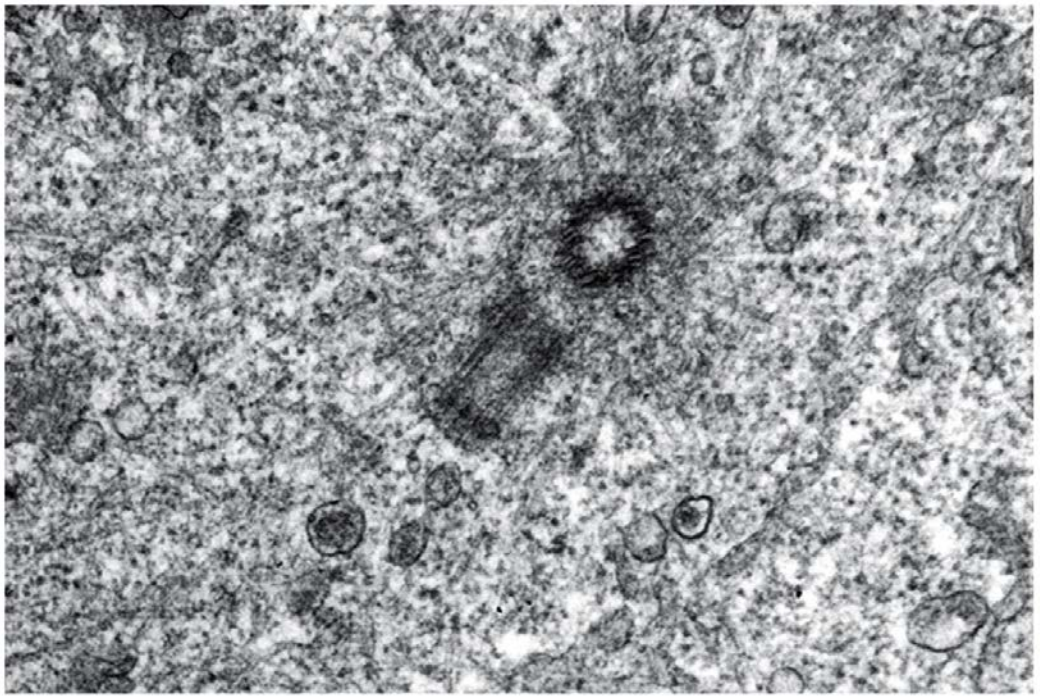
CENTRIOLE (BASAL BODY)



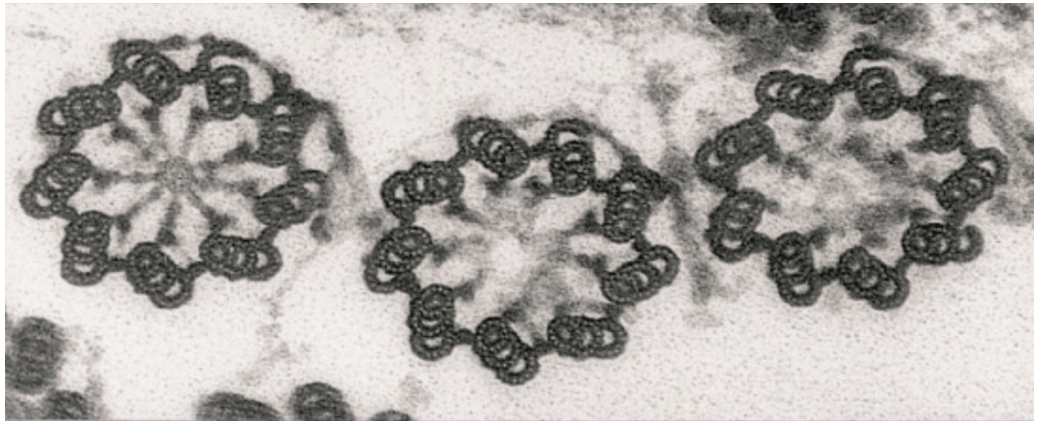


CENTRIOLE (BASAL BODY)

Diplosome
(centriole pair)



Basal bodies



MICROTUBULES
Chemical composition

Studies on cilia:

Cytochemistry:

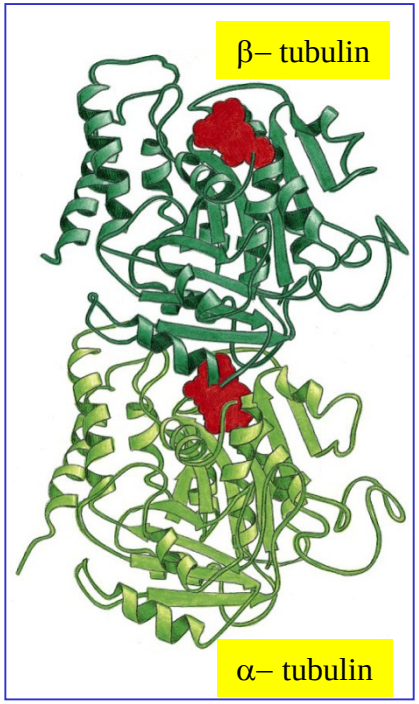
- Digestion with pepsin -> proteic content
- ATPase activity

Chemical analysis:

α-tubulin and β-tubulin

Site of binding to GDP and GTP
Binding to alkaloids: colchicine, vimblastine, taxol …

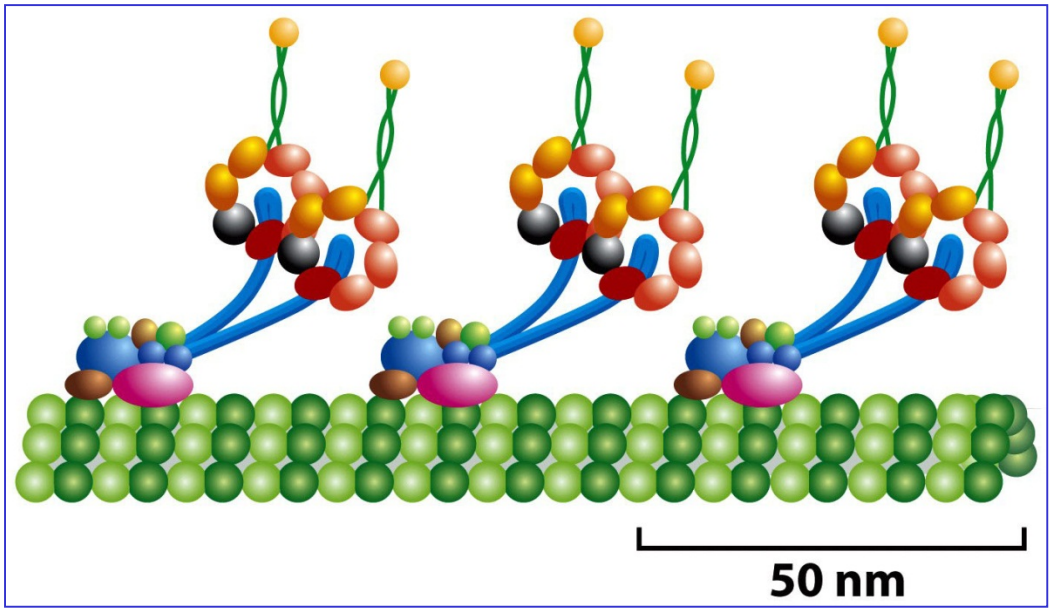
- Microtubule-associated proteins (MAPs): dynein, nexin, etc (about 180 different MAPs)



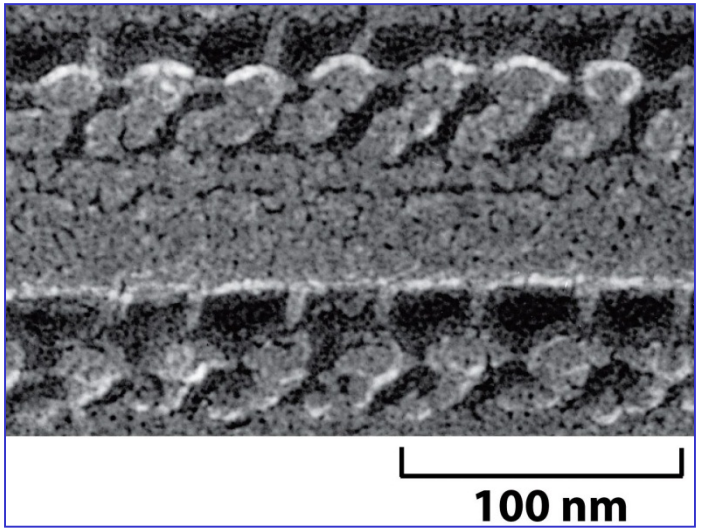
α– tubulin always has a GTP molecule
β– tubulin can be bound to GTP or GDP(**form T o form D**)

MICROTUBULES
Chemical composition

Structure of ciliary dynein

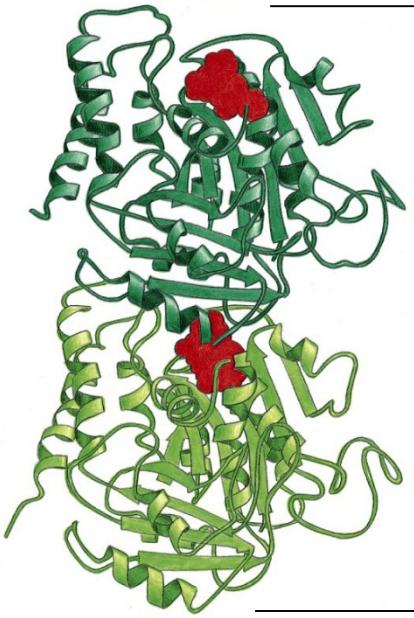


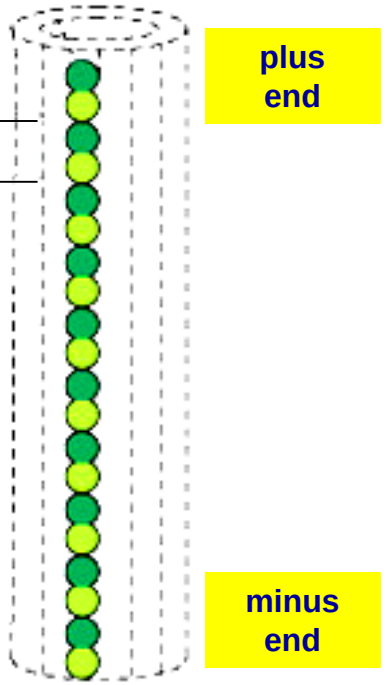
Dynein is a complex formed by 9-12 subunits.
The stem domain binds to an A microtubule.
The globular heads have an ATPase activity.

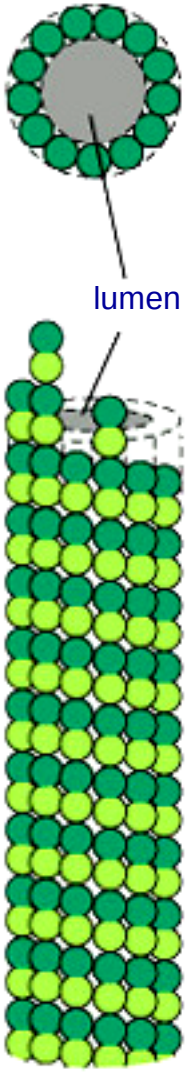


The dynein complexes are distributed in regular intervals.

MICROTUBULES
Molecular organization





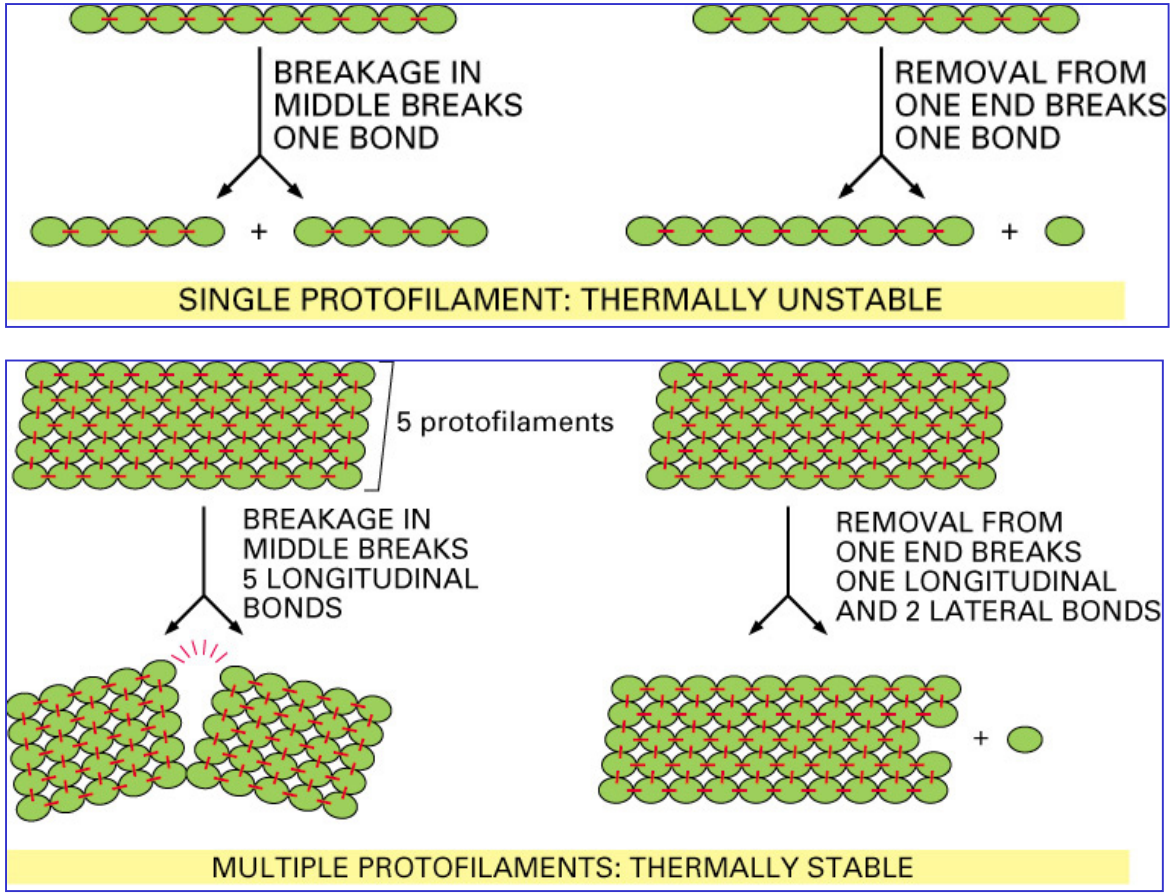


heterodimer

protofilament

microtubule

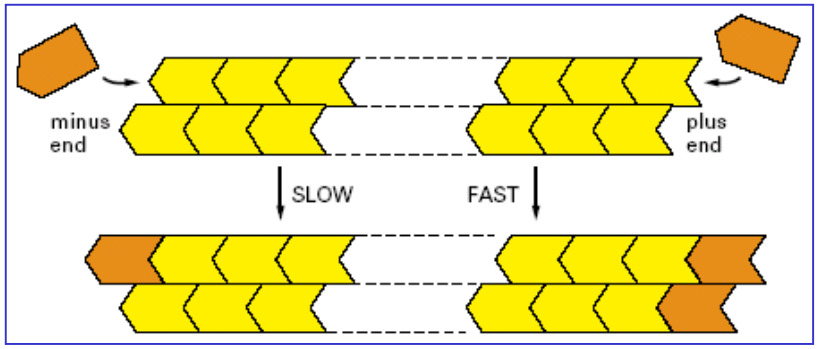
MICROTUBULES
Molecular organization



The parallel alignment of the protofilaments gives a greater stability to the middle of the microtubule and a higher dynamism to the ends

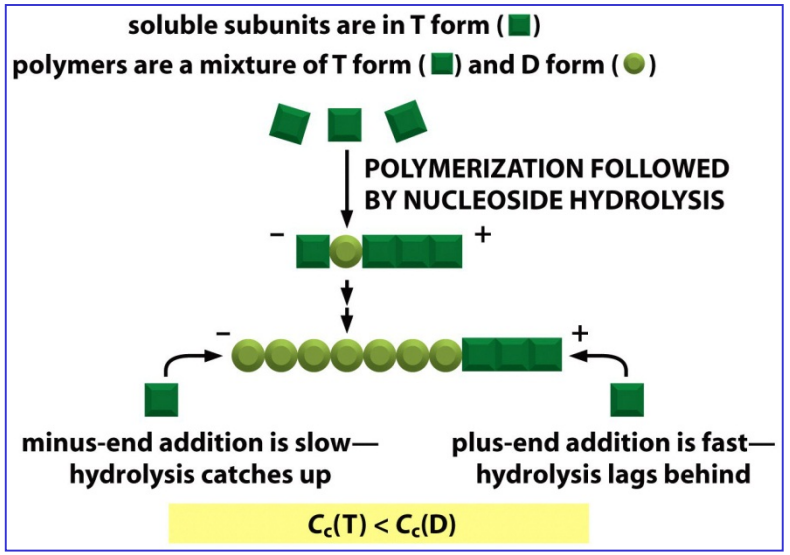
MICROTUBULES
Dynamic equilibrium

The two ends of a microtubule polymerize at different rates:
Plus end → fast growth
Minus end → slow growth



Soon after the T subunits are incorporated to the microtubule GTP is hydrolyzed and they are converted into D subunits.

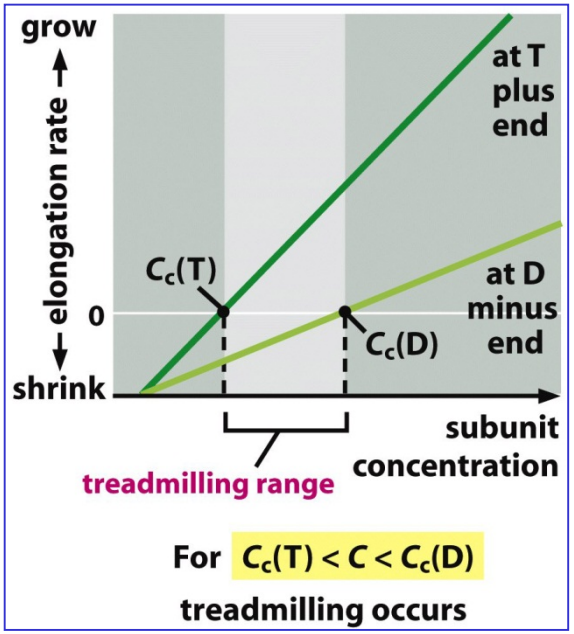
At a given concentration of tubulin in the medium – critical concentration – the rate of subunit addition equals the rate of subunit loss.

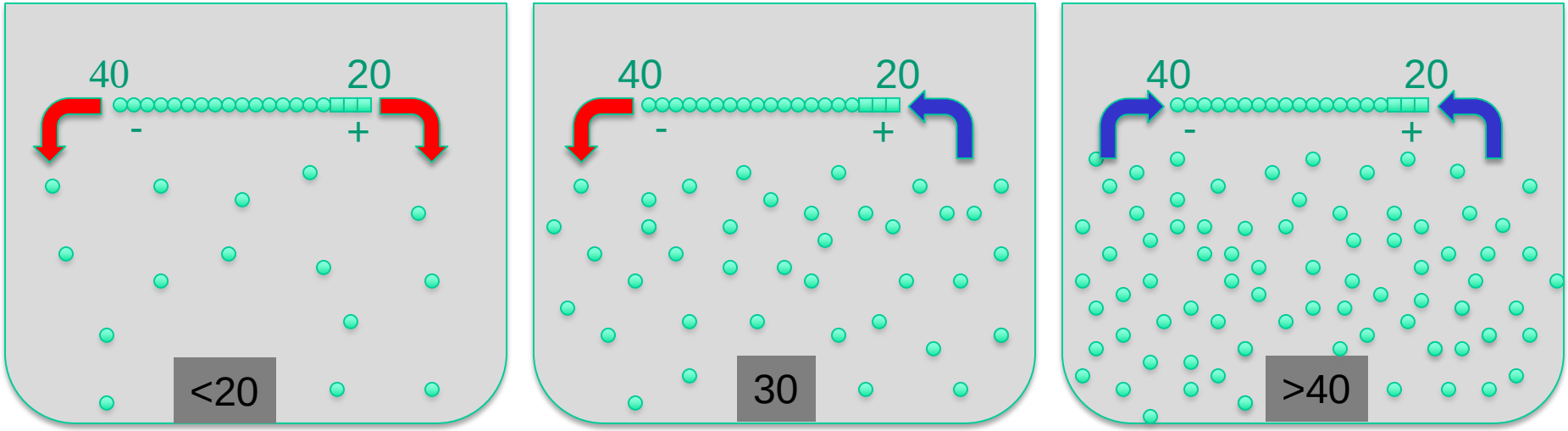


MICROTUBULES
Dynamic equilibrium

The critical concentration for a T form is lower than for a D form, so for a certain concentration of subunits between both critical concentrations, subunits undergo a net assembly at the plus end and a net disassembly at the minus end at an identical rate.

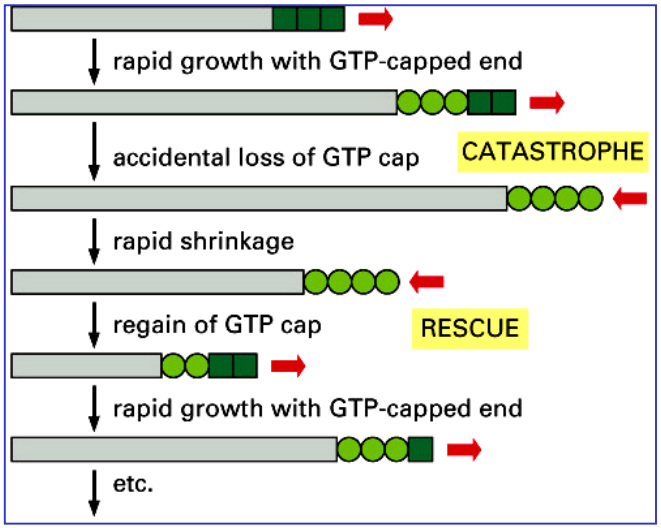
The polymer maintains a constant length, even though there if a net flux of subunits through the polymer, known as treadmilling.

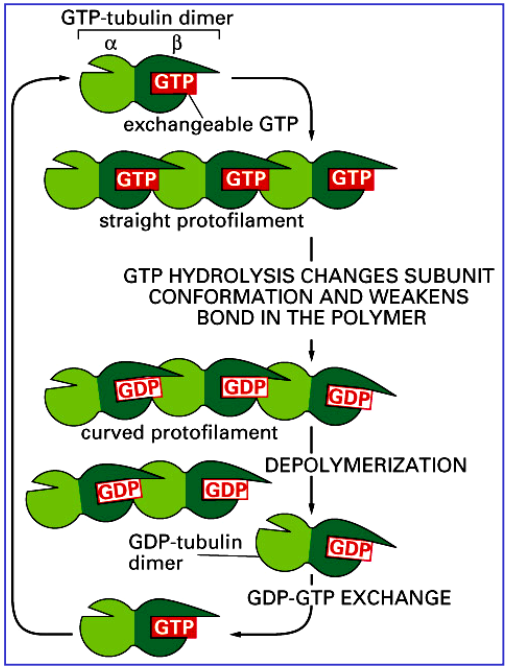


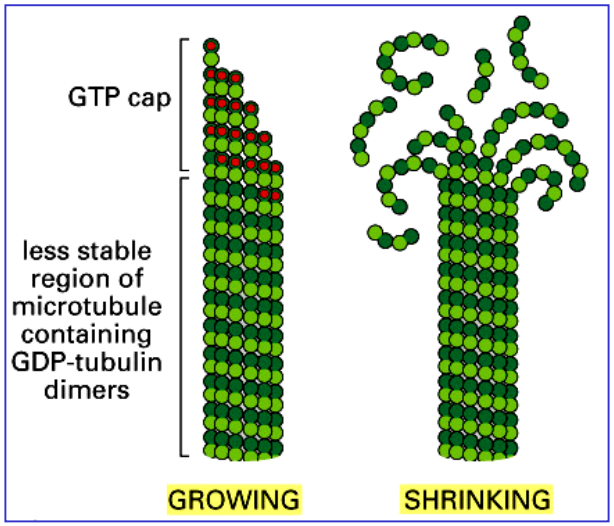


Treadmilling

MICROTUBULES
Dynamic equilibrium



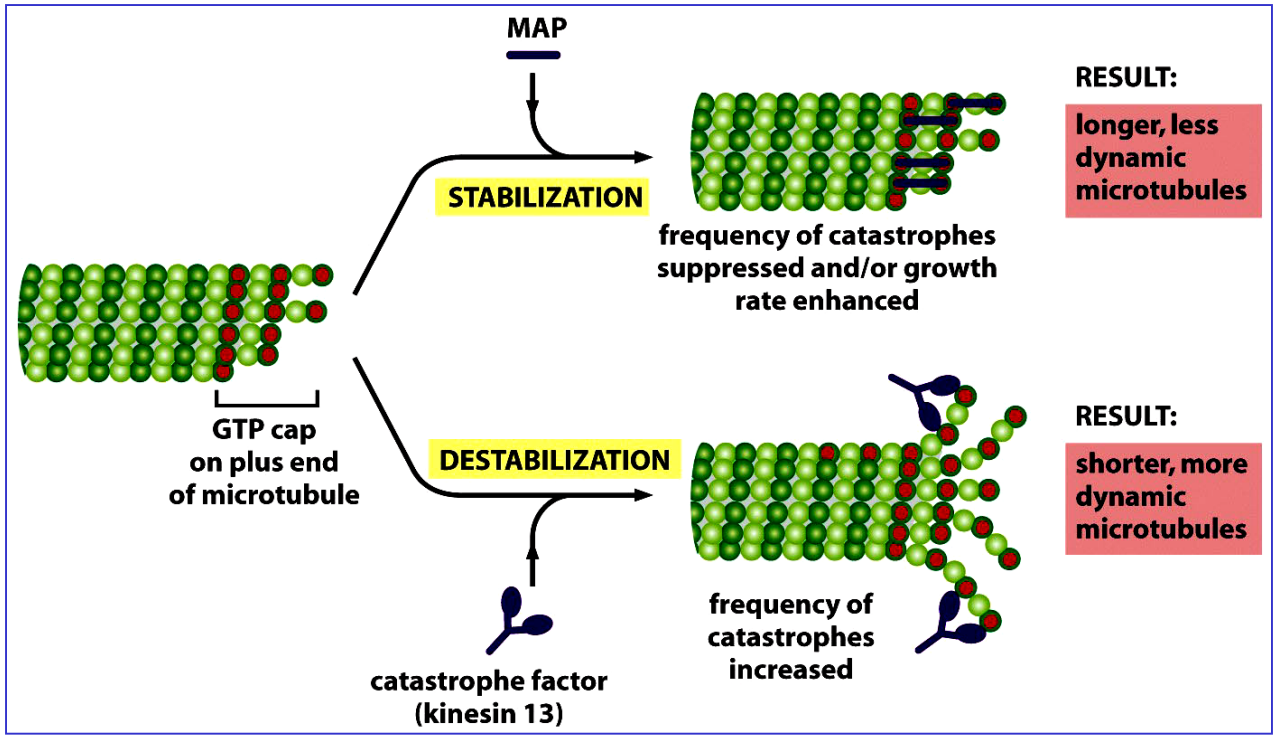




If GTP hydrolysis is faster than the incorporation of subunits, tubulin T at the end (GTP cap) is lost and the microtubule shrinks (catastrophe). But GTP-containing subunits may still add to the shrinking end, than microtubule growth resumes (rescue).

Addition of D subunits seem to force the protofilament into a curved shape that is less able to pack into the microtubule wall.

MICROTUBULES
Dynamic equilibrium



The transition between a growing state and a shrinking state of microtubules is controlled by some proteins.
Protein XMAP215 stabilizes the growth of the microtubule, while kinesin 13 destabilizes it.

MICROTUBULES
Biogenesis

Formation in the
Microtubule Organizing Center **(MTOC)**

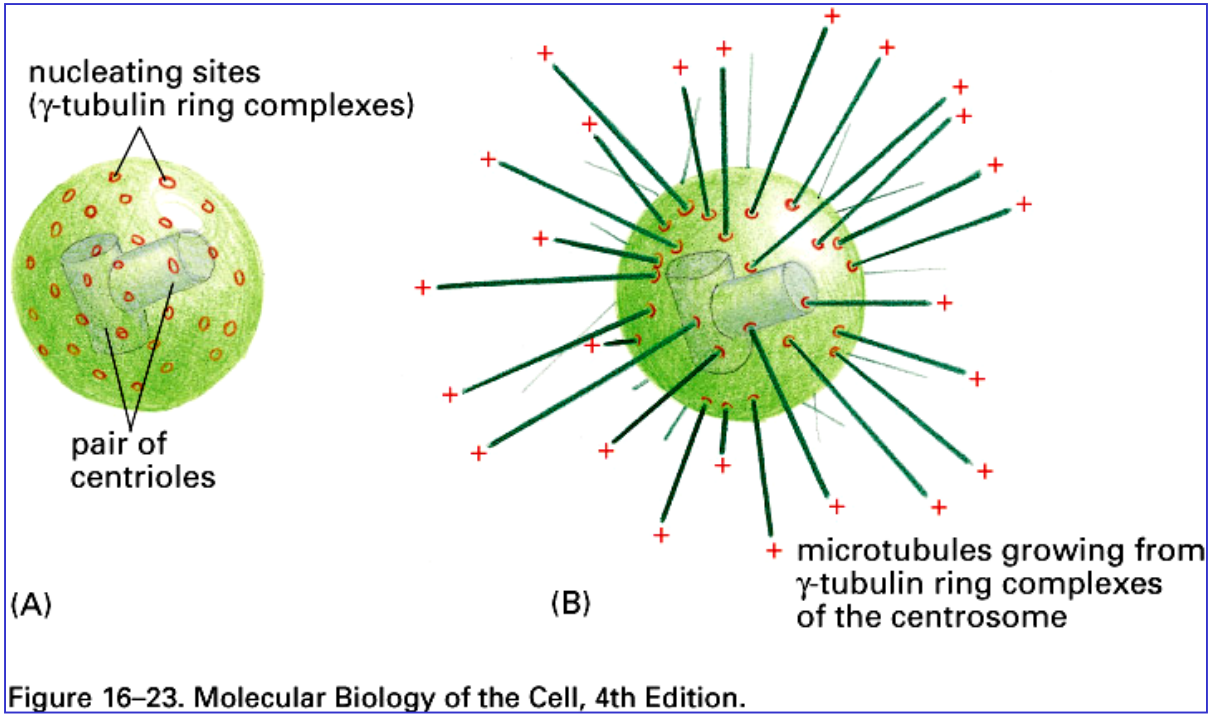
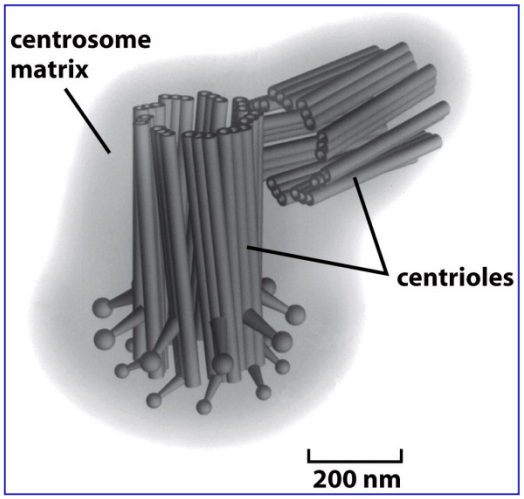
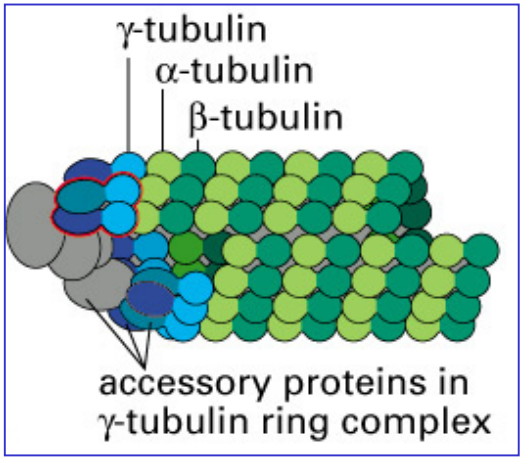
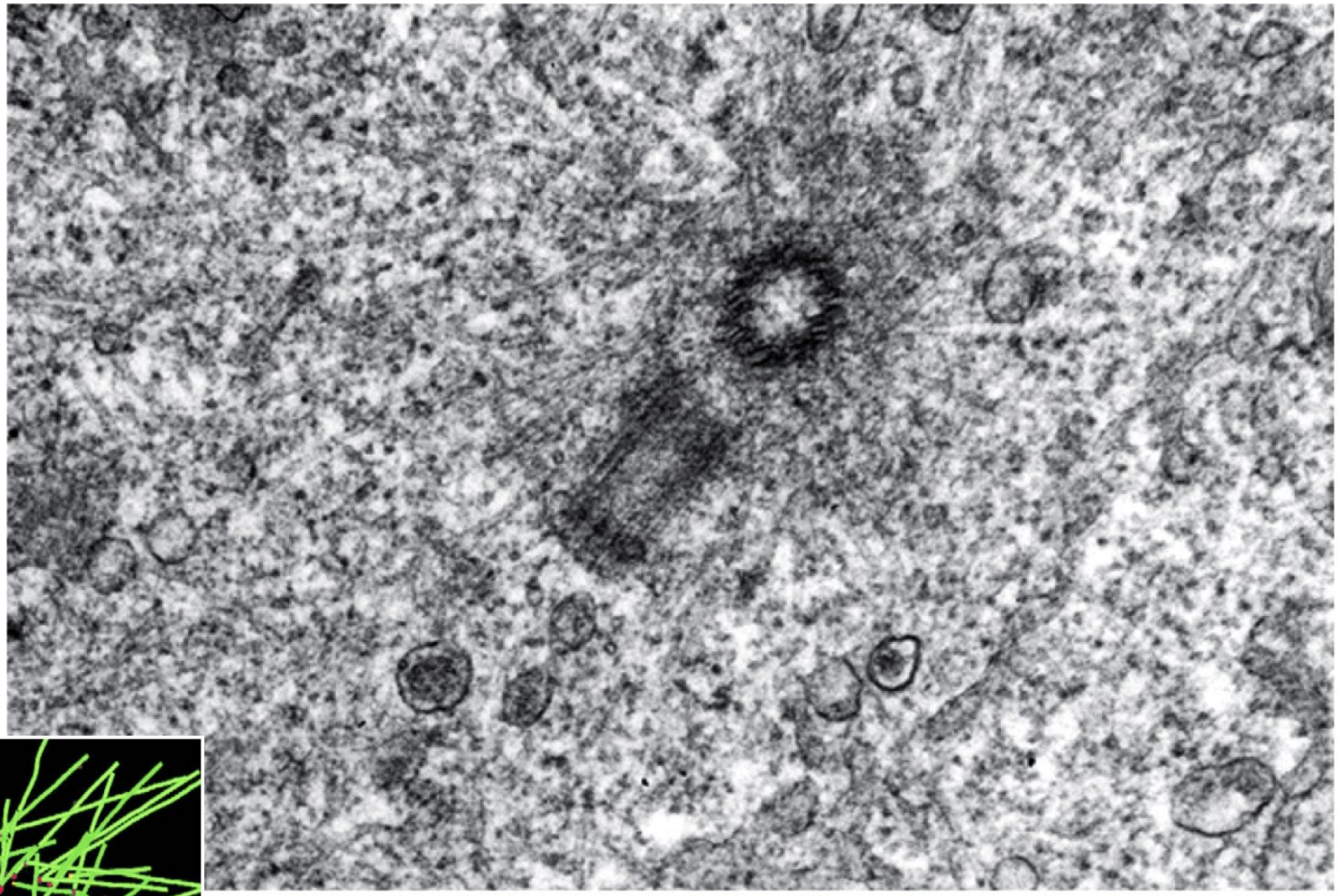


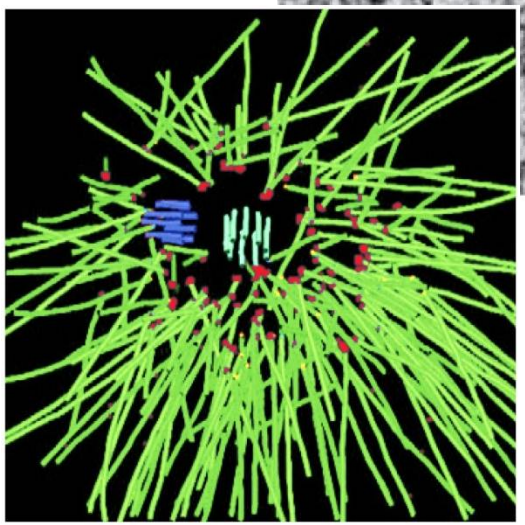
Figure 16–23. Molecular Biology of the Cell, 4th Edition.





In many cells, the minus end is stabilized by association with the centrosome, while the plus end grows or shrinks more freely.





0.5 μm

THE CYTOSKELETON (II)

ACTIN FILAMENTS

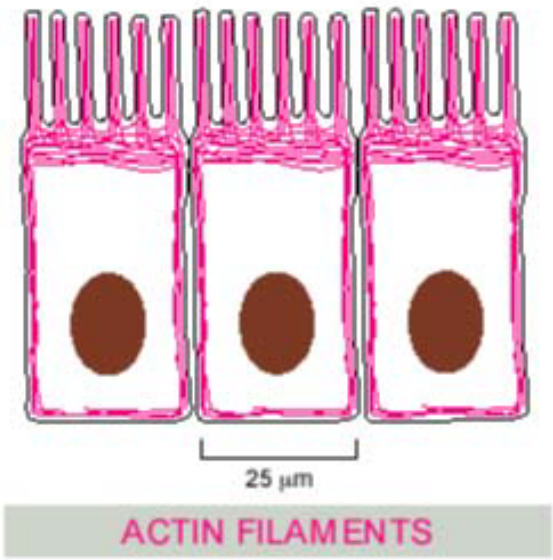
- Morphology
- Molecular organization
- Arrangement in cells

MYOSIN

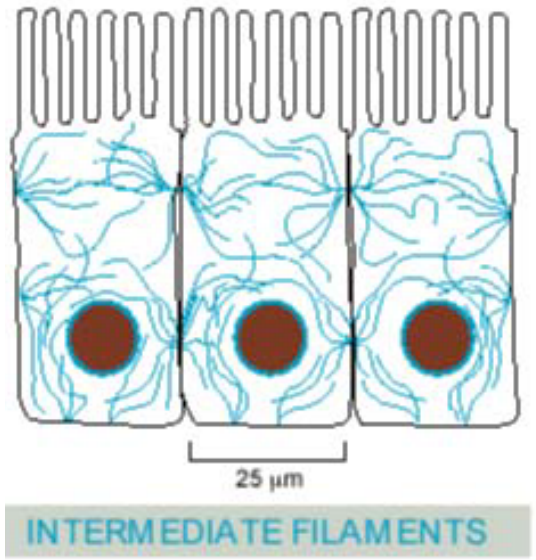
INTERMEDIATE FILAMENTS

- General characteristics
- Molecular organization
- Types

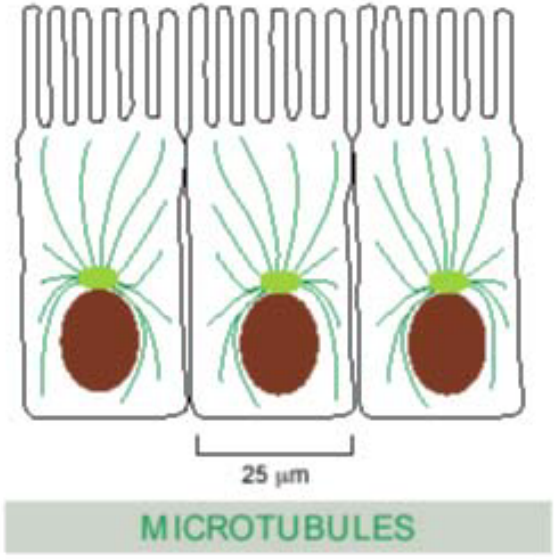
INTRODUCTION



ACTIN (5-9 nm)



INTERMEDIATE FILAMENTS (10 nm)



MICROTUBULES (25 nm)

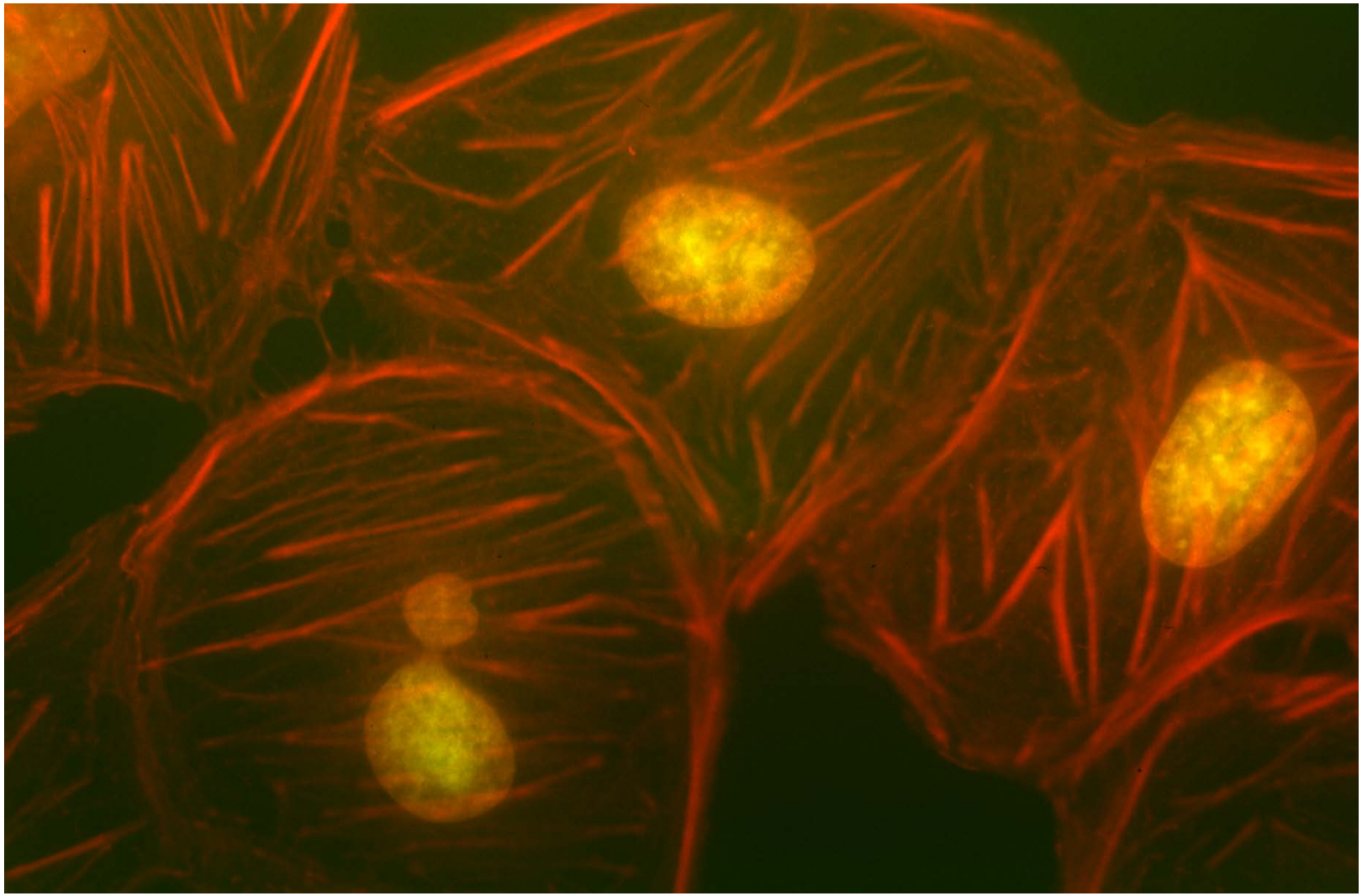
ACTIN FILAMENTS

- Actin filaments or microfilaments (F-actin) are polymers of globular actin (G-actin).
- They are more flexible than microtubules.
- They have a 5 to 9 nm diameter.
- They can be ramified.
- They are usually grouped in bundles.
- These bundles can be observed with fluorescence microscopy using labeled monoclonal antibodies.
- Six different genes

cytoskeleton (ACTB, ACTG1)
skeletal striated muscle (ACTA1)
smooth muscle tissue (ACTA2)
intestinal muscles (ACTG2)
cardiac muscle (ACTC1)

ACTIN FILAMENTS

Morphology

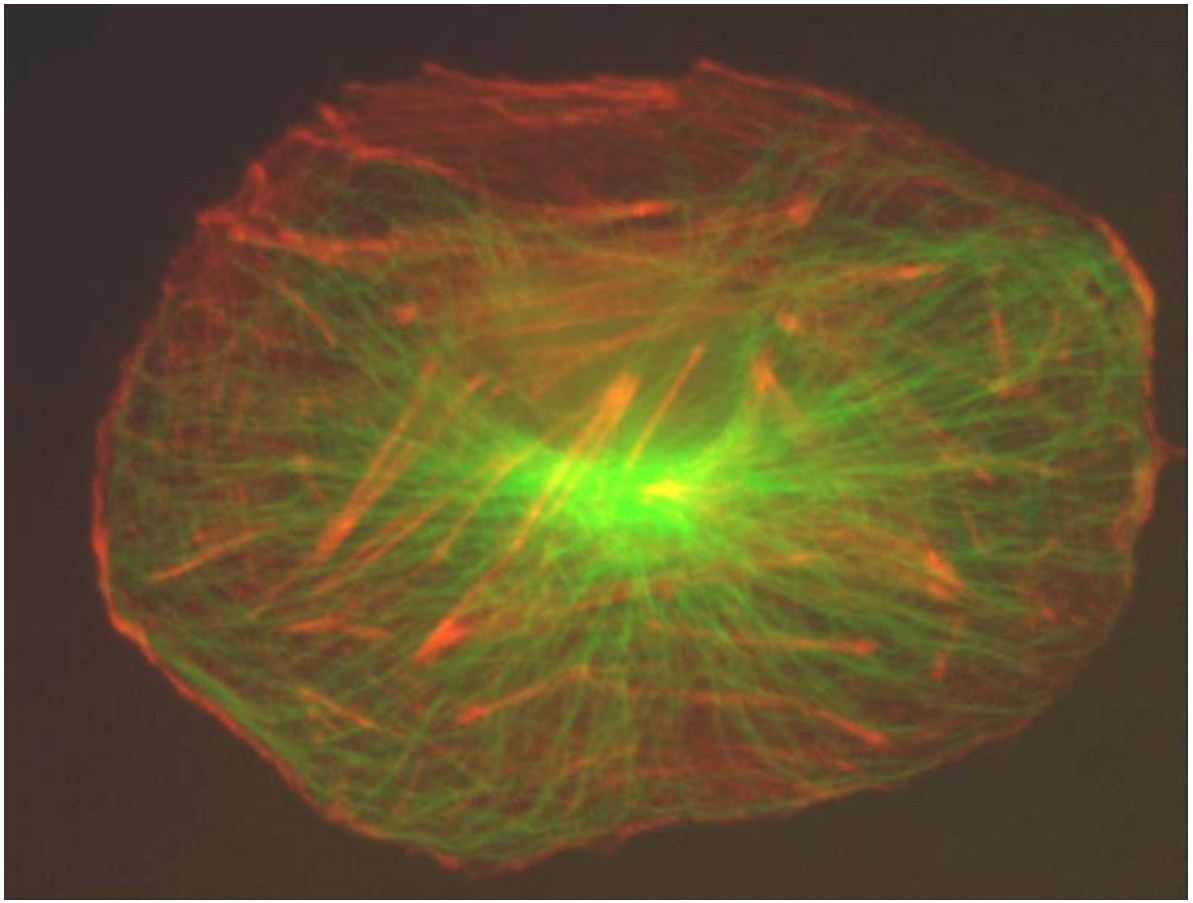


Observation with optical microscopy:
- Immunocytochemistry
- Confocal microscopy

Actin (red)

ACTIN FILAMENTS

Morphology



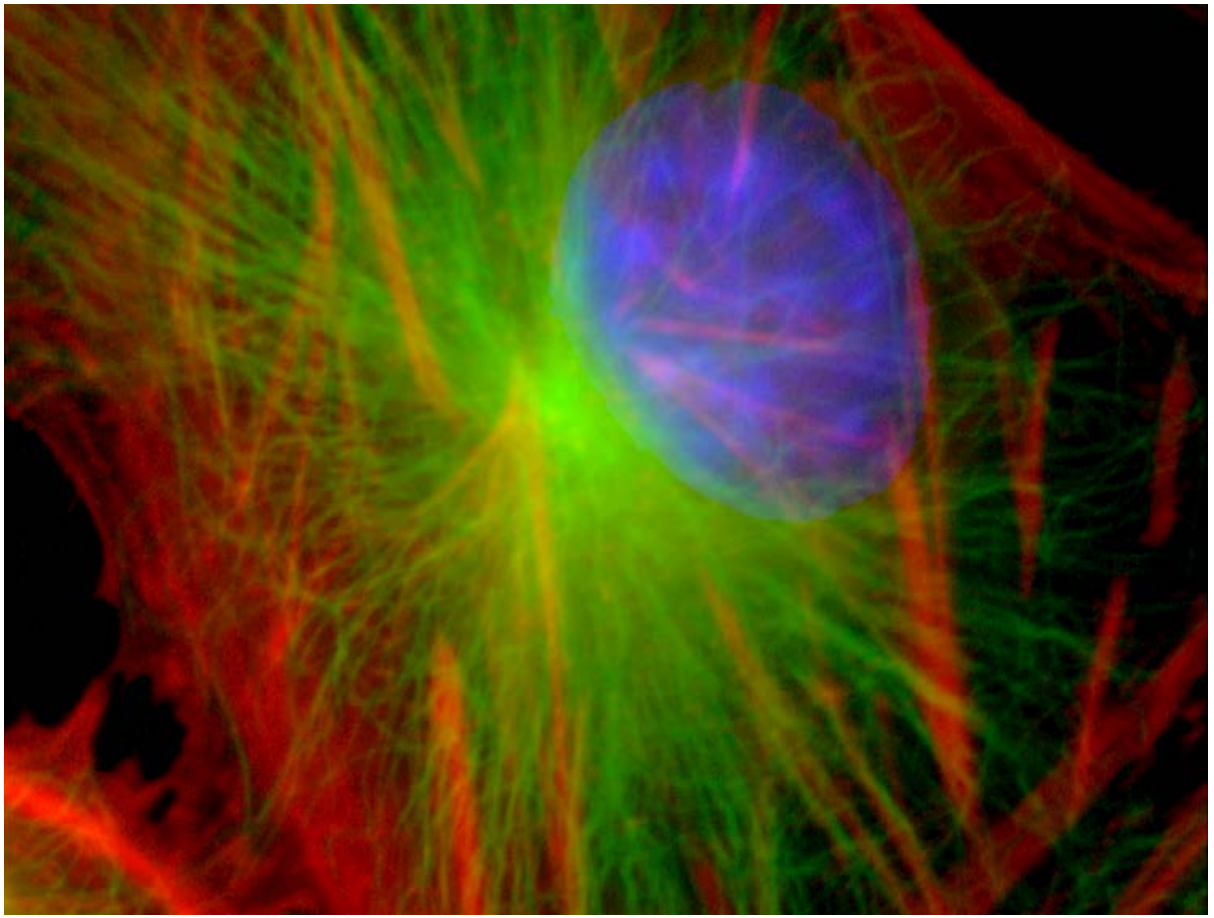
Observation with optical microscopy:
- Immunocytochemistry
- Confocal microscopy

Actin (red)

Microtubules (green)

ACTIN FILAMENTS

Morphology



Observation with optical microscopy:
- Immunocytochemistry
- Confocal microscopy

Actin (red)

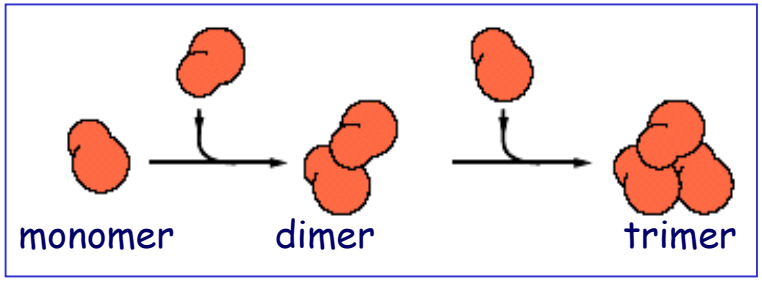
Microtubules (green)

Nucleus (blue)

ACTIN FILAMENTS

Molecular organization

INITIATION OF ACTIN POLYMERIZATION (NUCLEATION)



STRUCTURE OF A MONOMER AND AN ACTIN FILAMENT

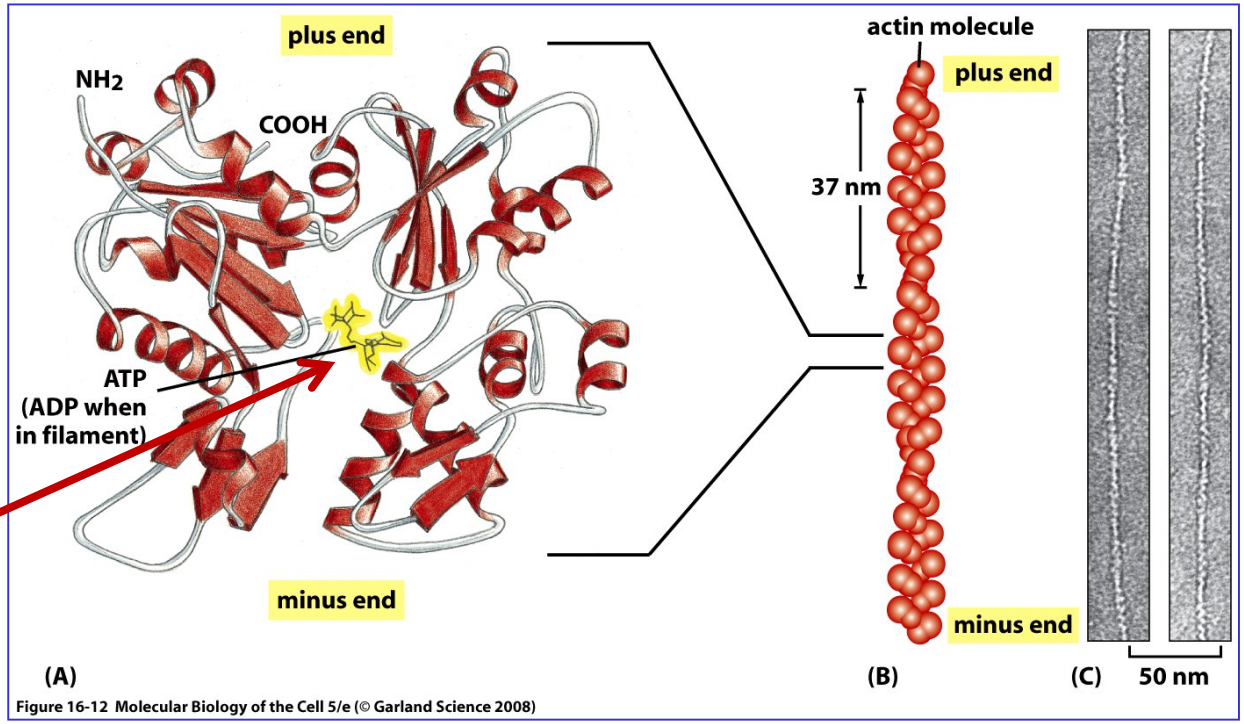


Figure 16-12 Molecular Biology of the Cell 5/e (© Garland Science 2008)

ACTIN FILAMENTS

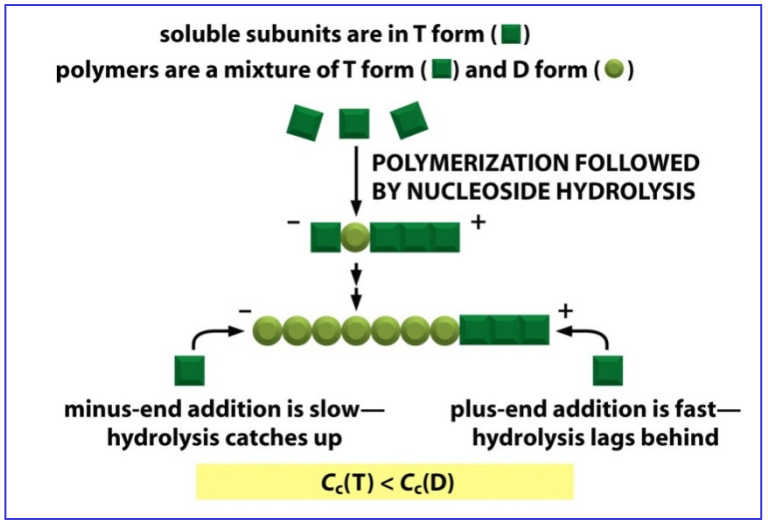
Molecular organization

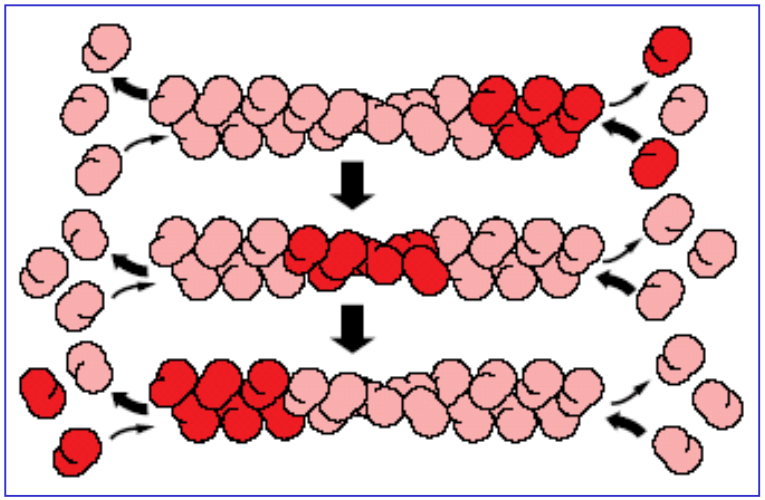
DYNAMIC EQUILIBRIUM

Subunits with ATP polymerize at both ends of the filament. Once incorporated ATP hydrolyzes to ADP. As the filament grows elongation is faster than hydrolysis at the plus end, and subunits are in T form. At the minus end hydrolysis is faster than elongation, and subunits are in D form.

The critical concentration for polymerization at the end with T form is lower than at the end with D form . If the subunit concentration in the cytosol is between both values, the plus end grows and the minus end shrinks.

If no end is protected and the filament reaches a stable length, incorporation of subunits at the plus end is equal to loss at the minus end. There is a continuous renewal of subunits (**treadmilling**) with the same filament length.





TREADMILLING

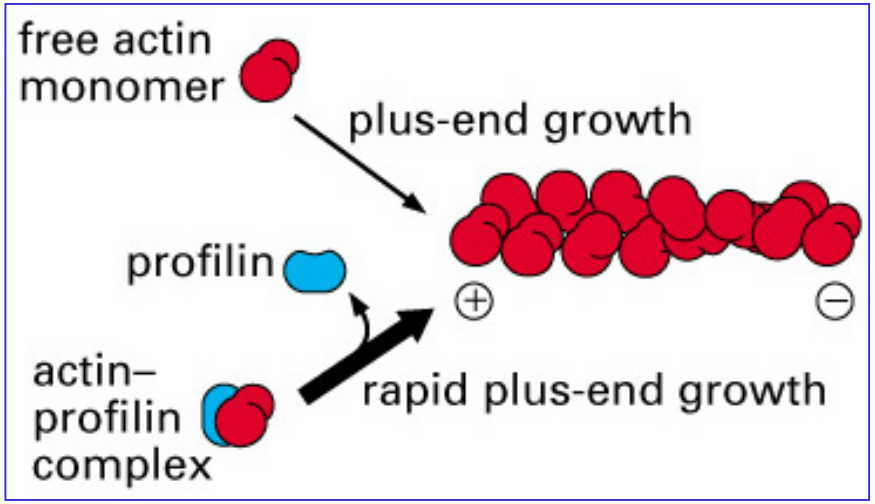
ACTIN FILAMENTS

Molecular organization

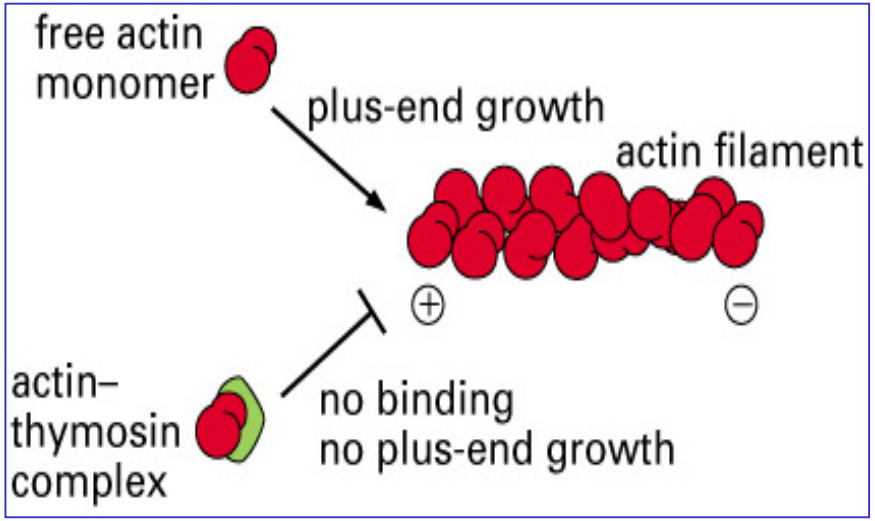
INTERACTION WITH PROTEINS

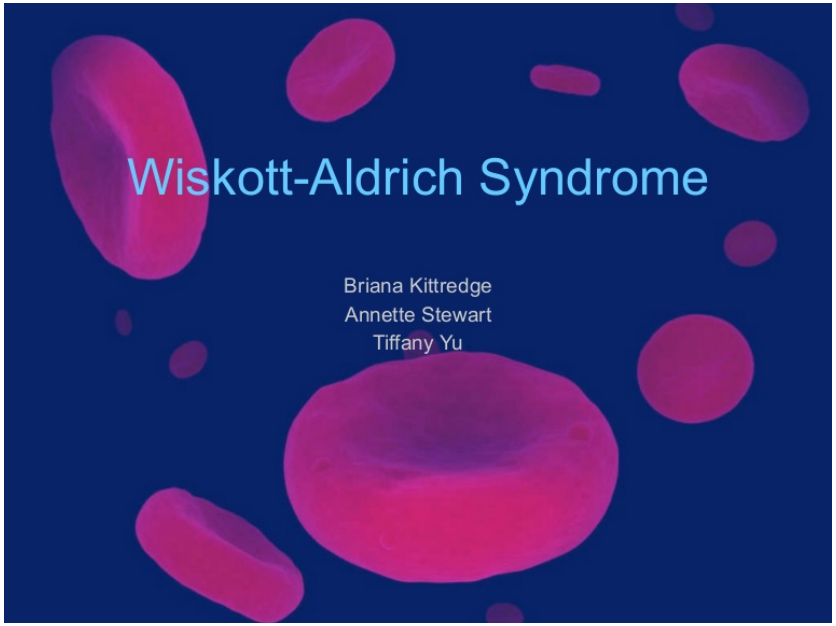
There are proteins that facilitate or impede polymerization of actin.

Profilin binds the monomer and facilitates polymerization of actin.



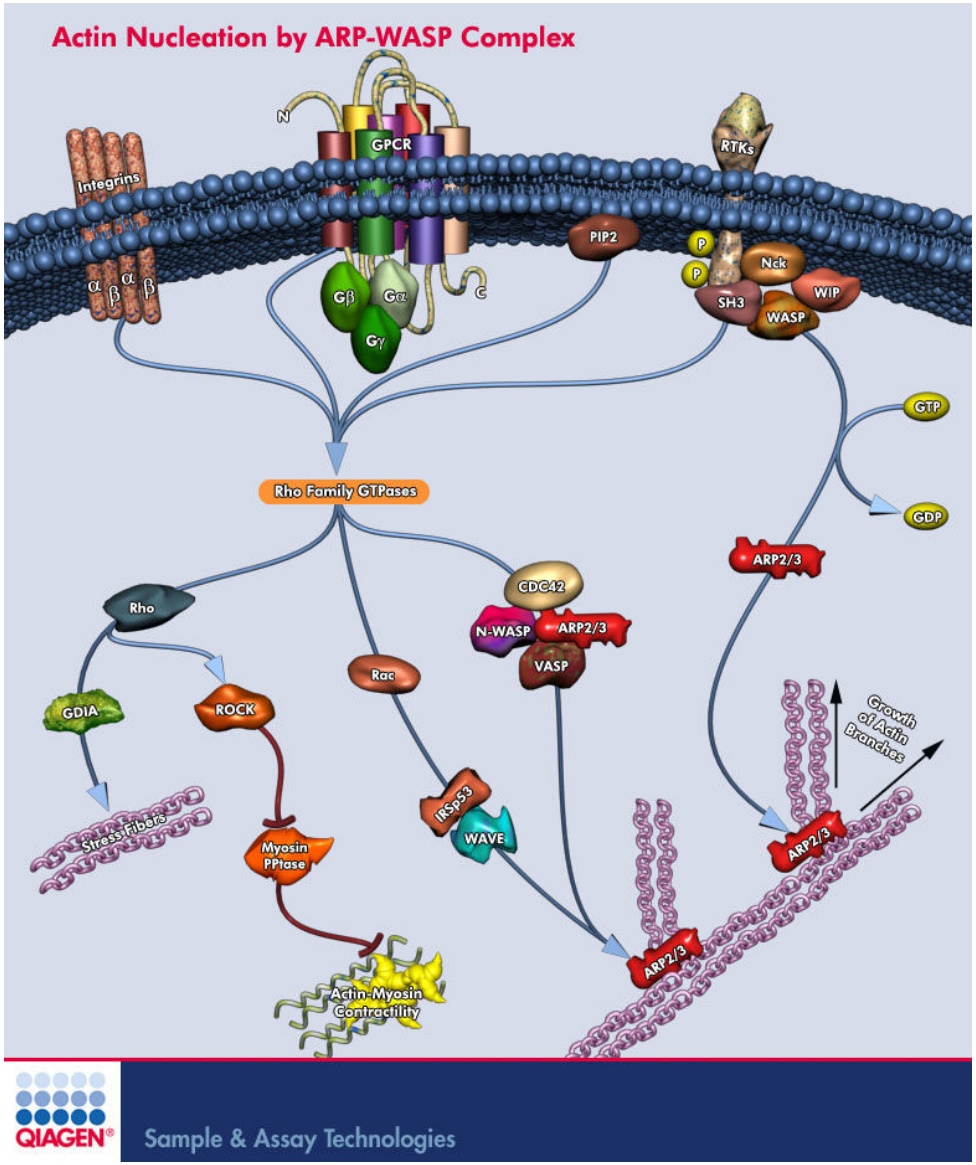
Thymosin impedes incorporation of actin monomers, and polymerization.





Wiskott–Aldrich syndrome (WAS) is a rare X-linked recesive disease

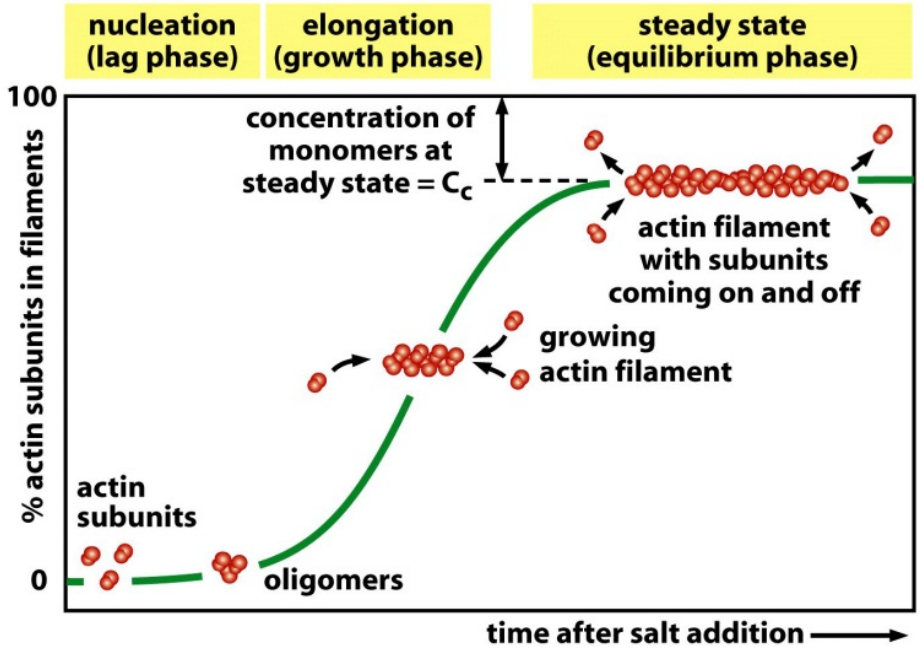
Protein WASp

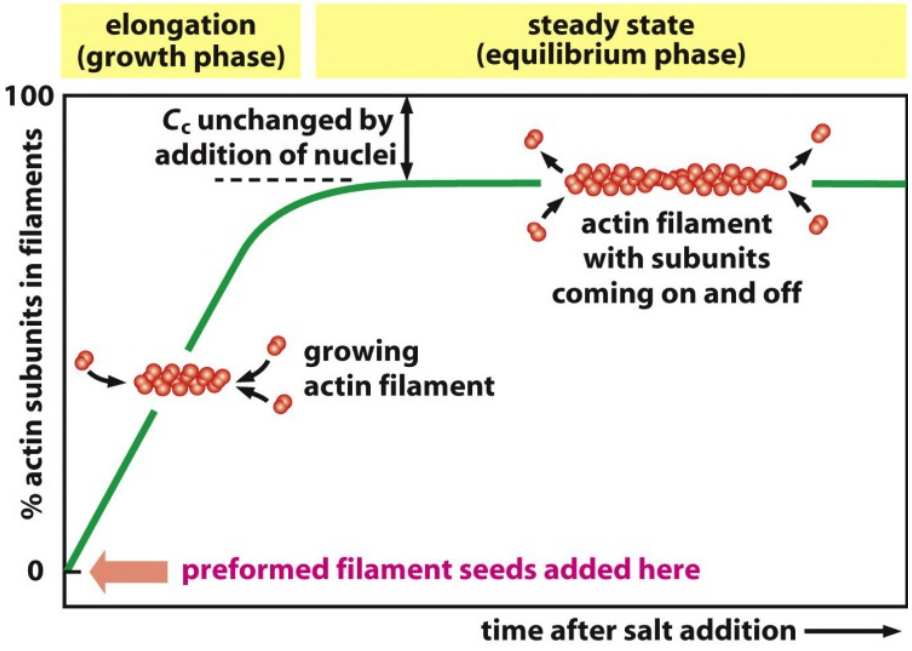


ACTIN FILAMENTS

Molecular organization

NUCLEATION



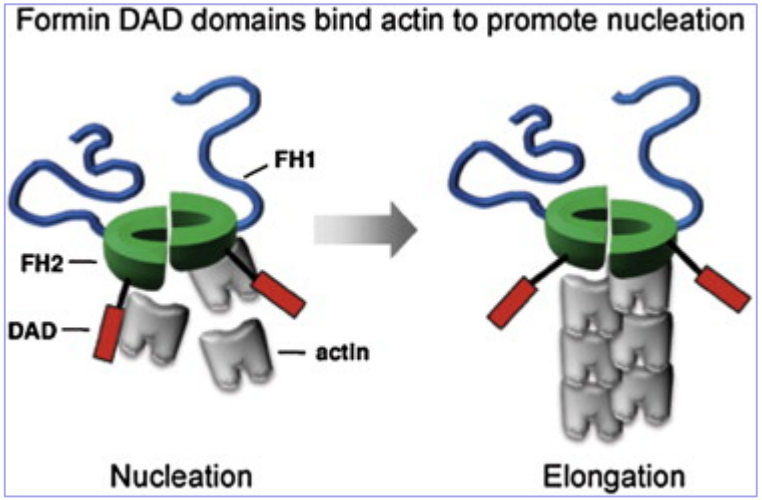


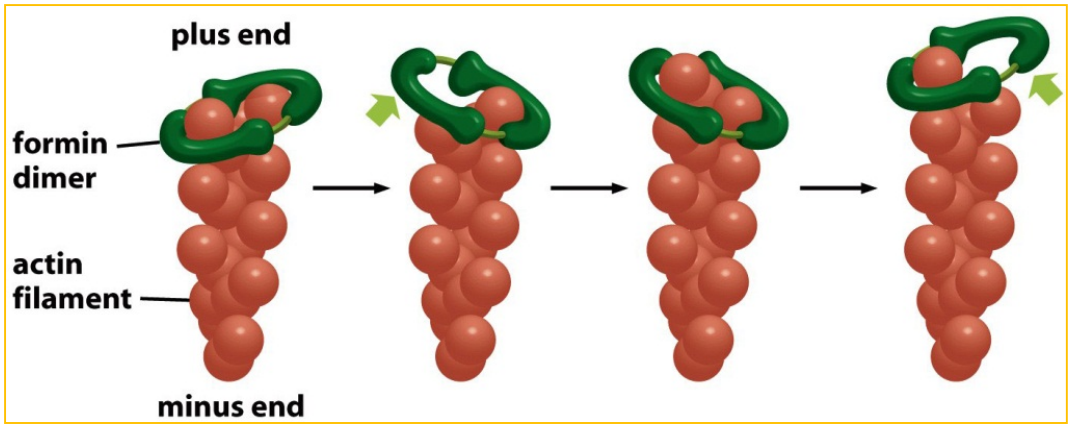
Nucleation is the initial process of subunits polymerization.
It is facilitated if there are preformed small stable filaments.

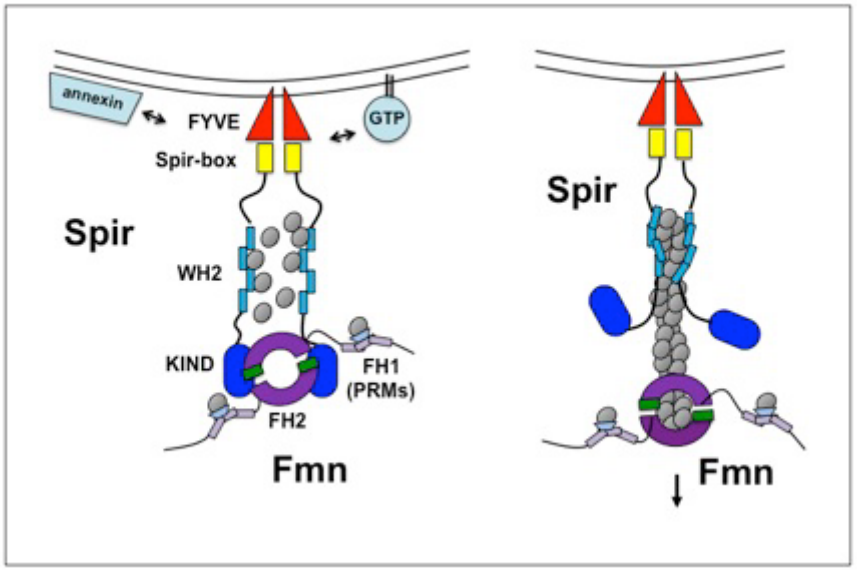
ACTIN FILAMENTS

NUCLEATION

Molecular organization



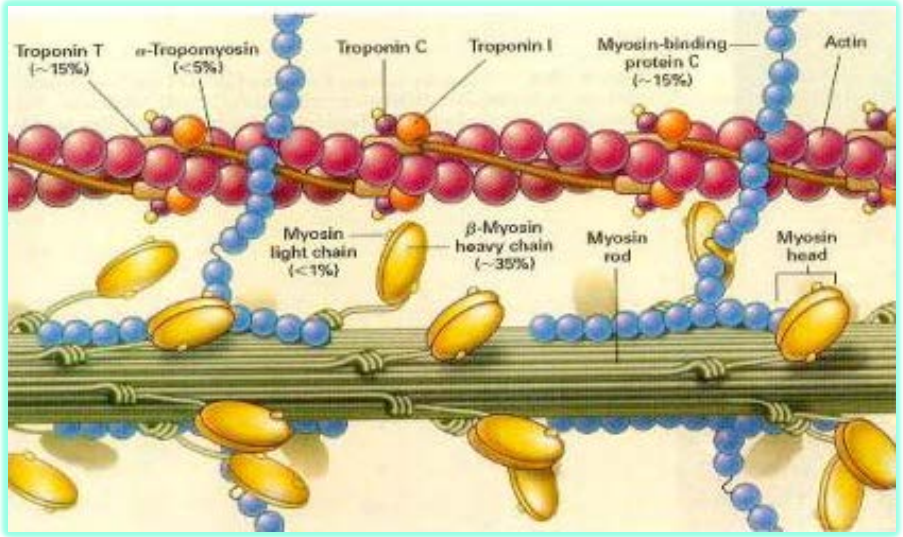


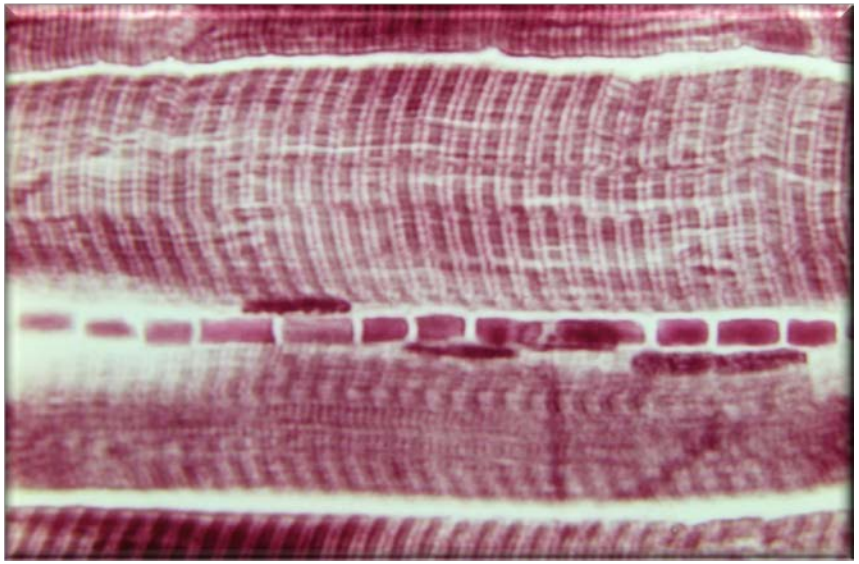


Formins form a dimeric complex that can nucleate the formation of new actin filaments.

ACTIN FILAMENTS: Locations

Muscular contraction. Skeletal fiber

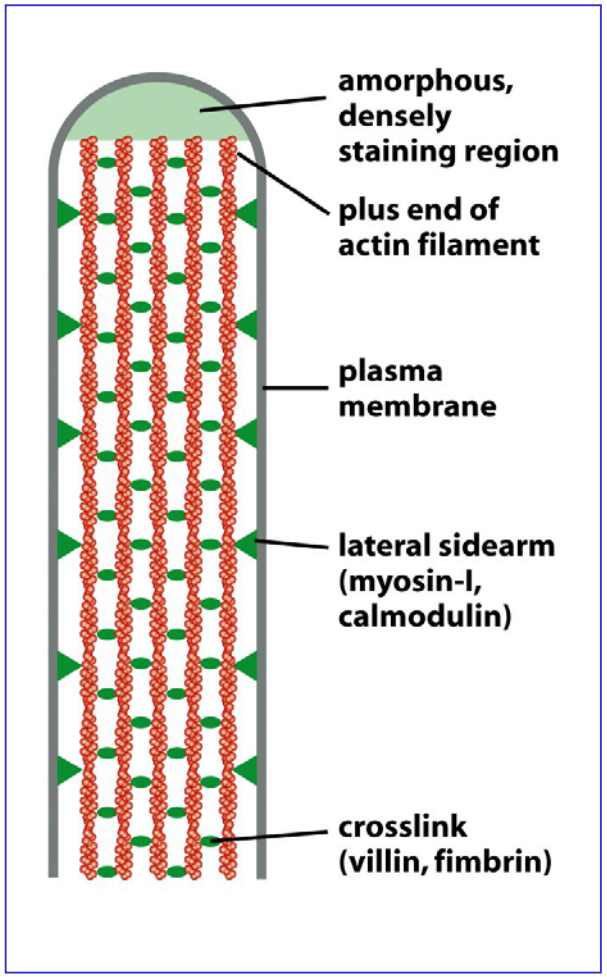




ACTIN FILAMENTS: Locations

Arrangement in cells

MICROVILLI



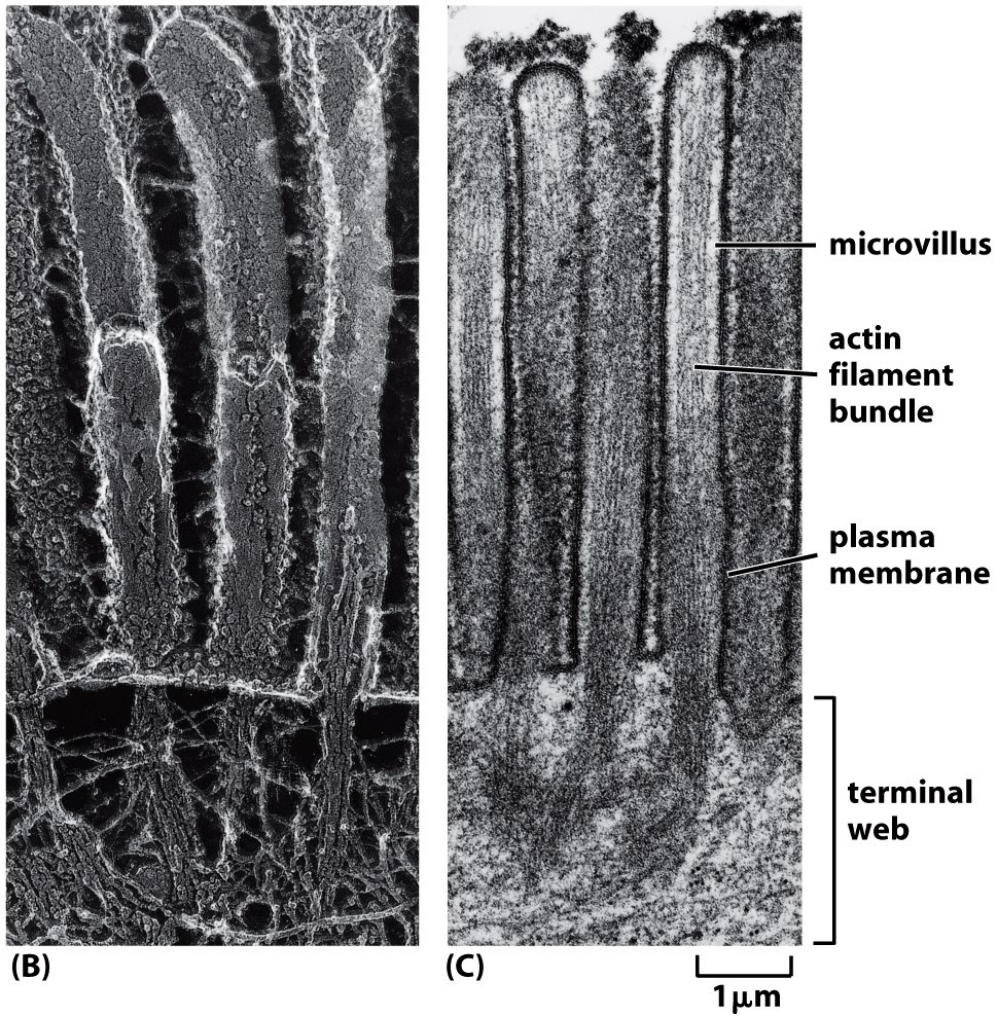
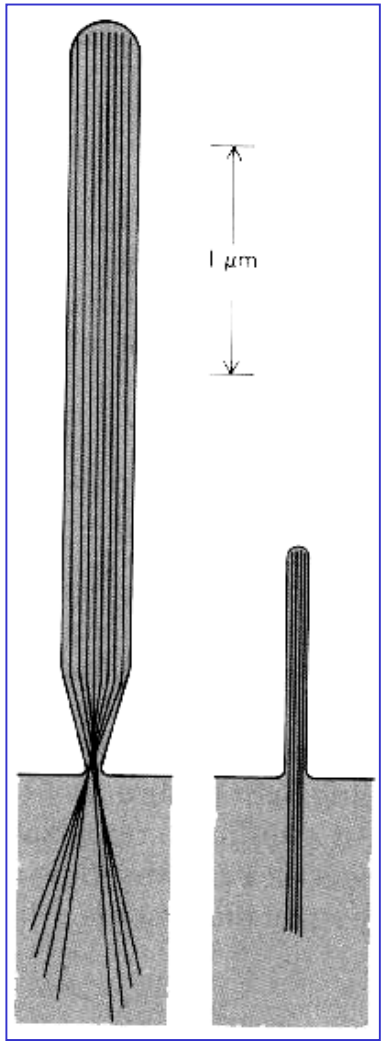


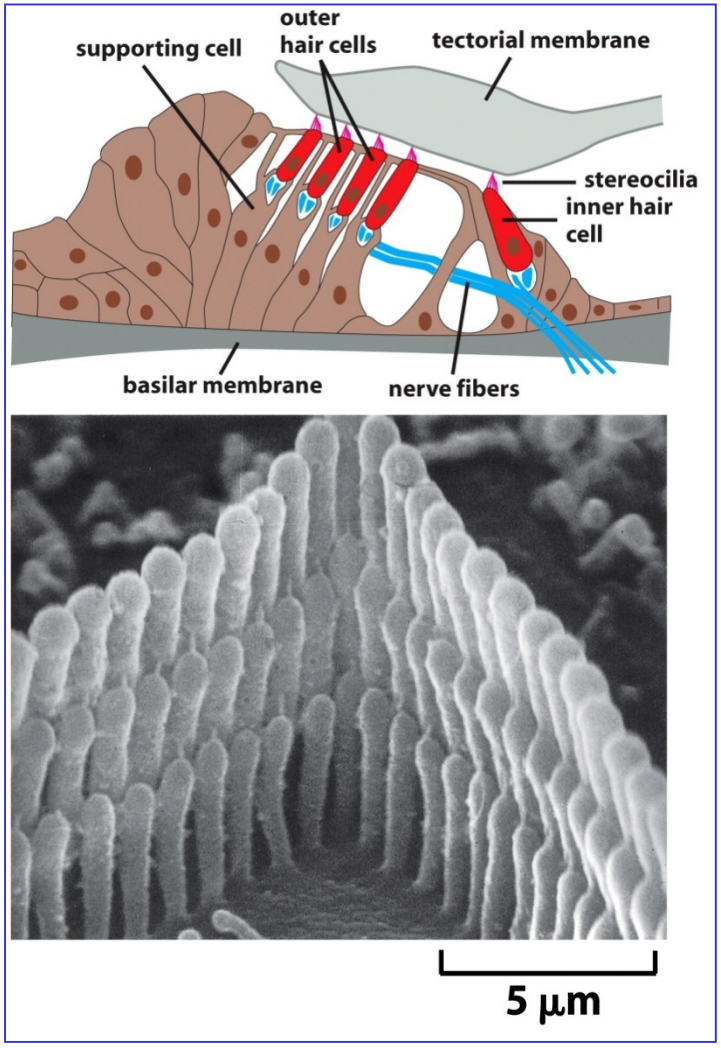
Figure 16-50bc Molecular Biology of the Cell 5/e (© Garland Science 2008)

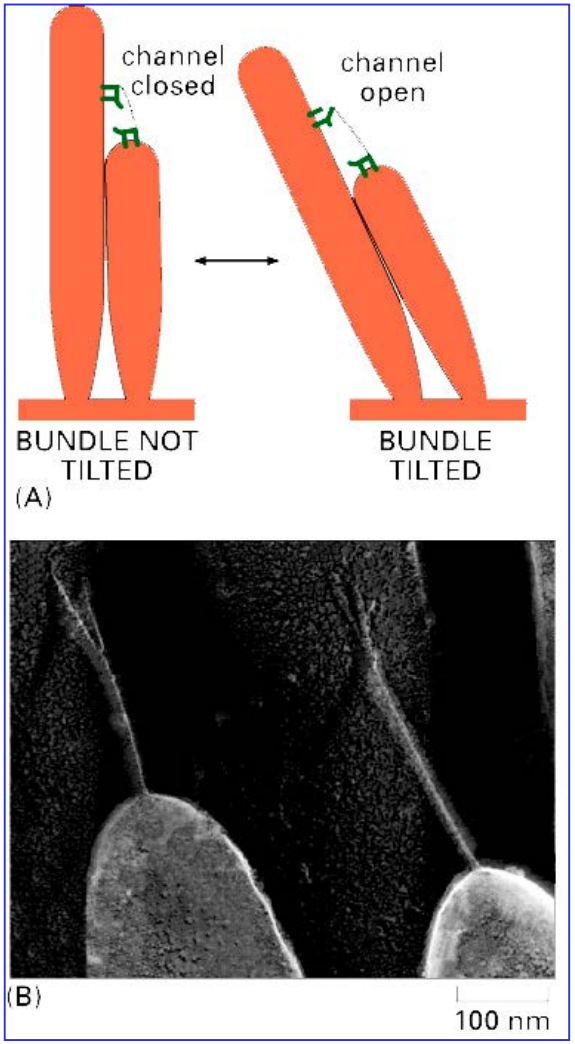
ACTIN FILAMENTS: Locations

Arrangement in cells

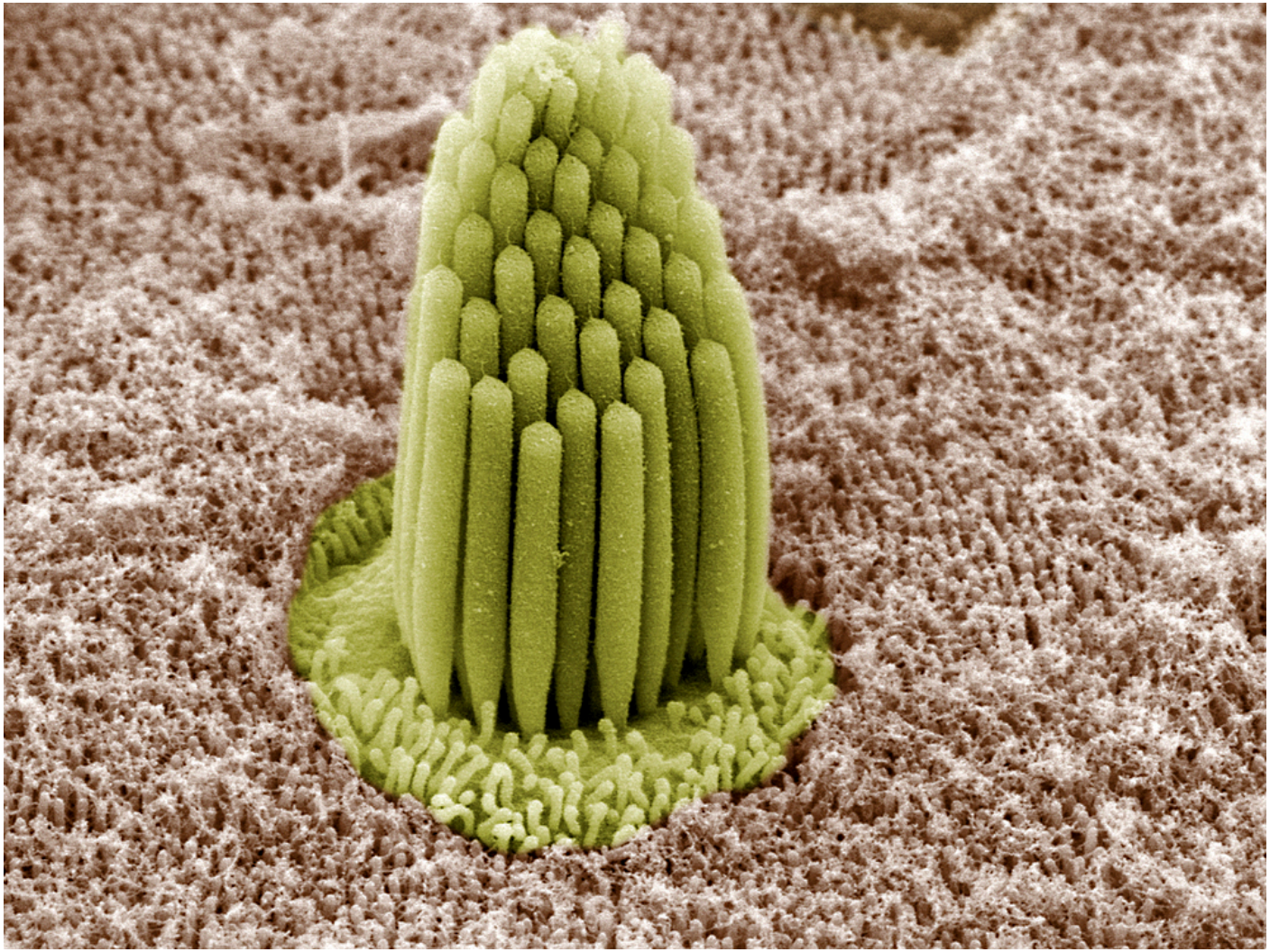
STEREOCILIA







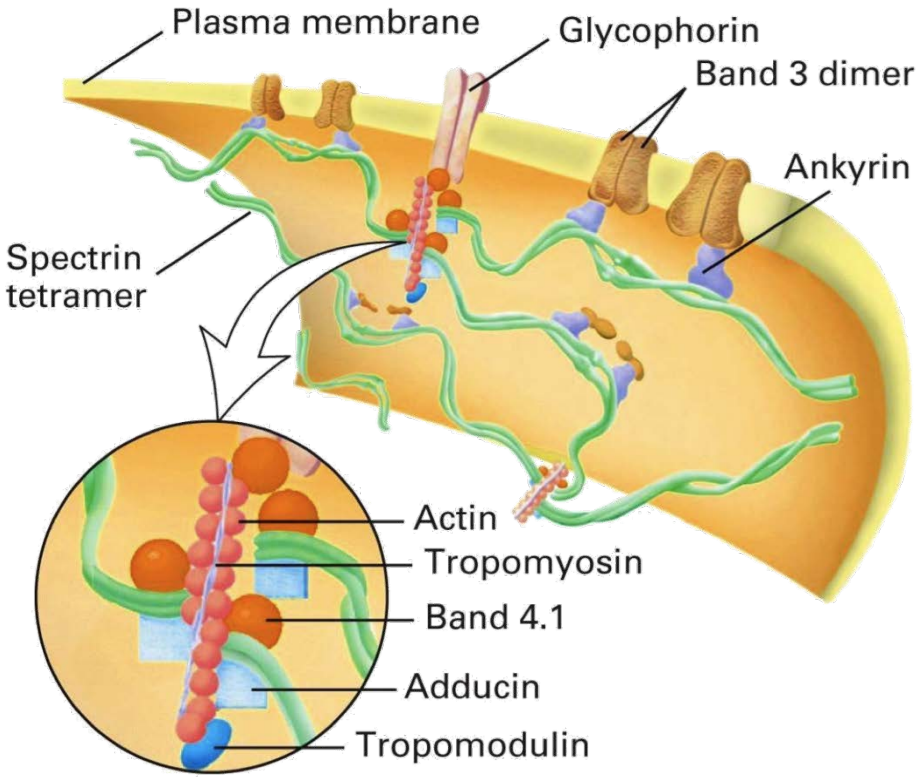
ACTIN FILAMENTS: Locations

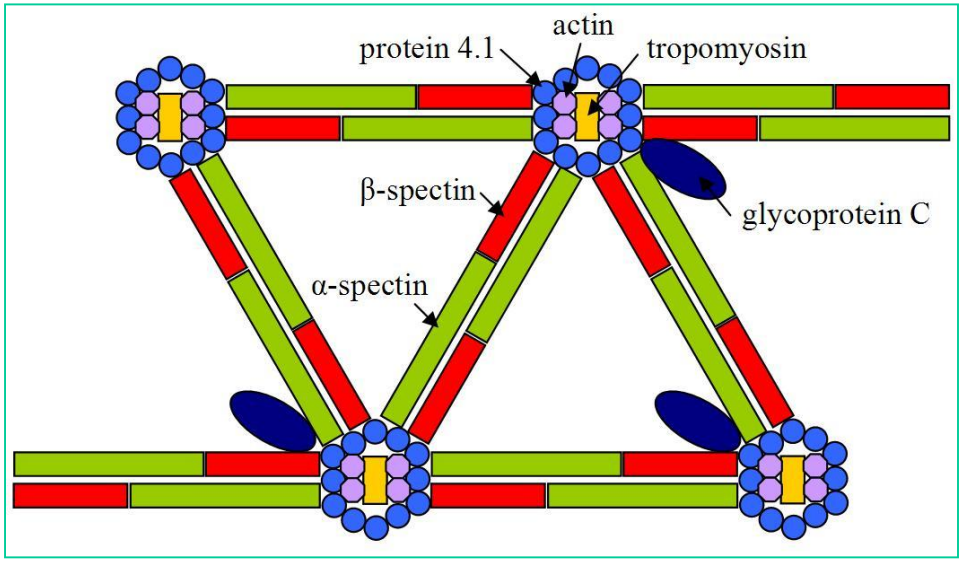


A single bundle of stereocilia projects from the epithelium of the papilla, a sensory patch in amphibian ears. A single sterocilia bundle, false-colored in green, emerges from the apical surface of an inner hair cell. Images were acquired on a Hitachi S-4800.

ACTIN FILAMENTS: Locations

Arrangement in cells



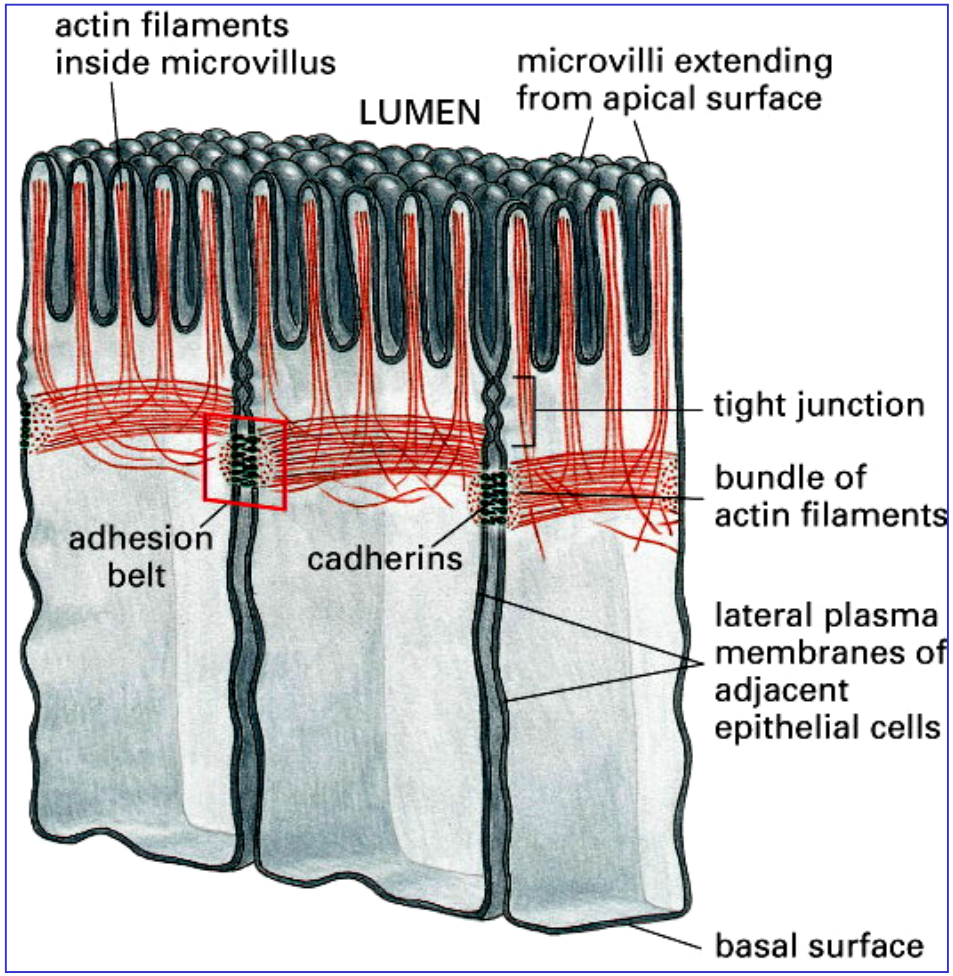


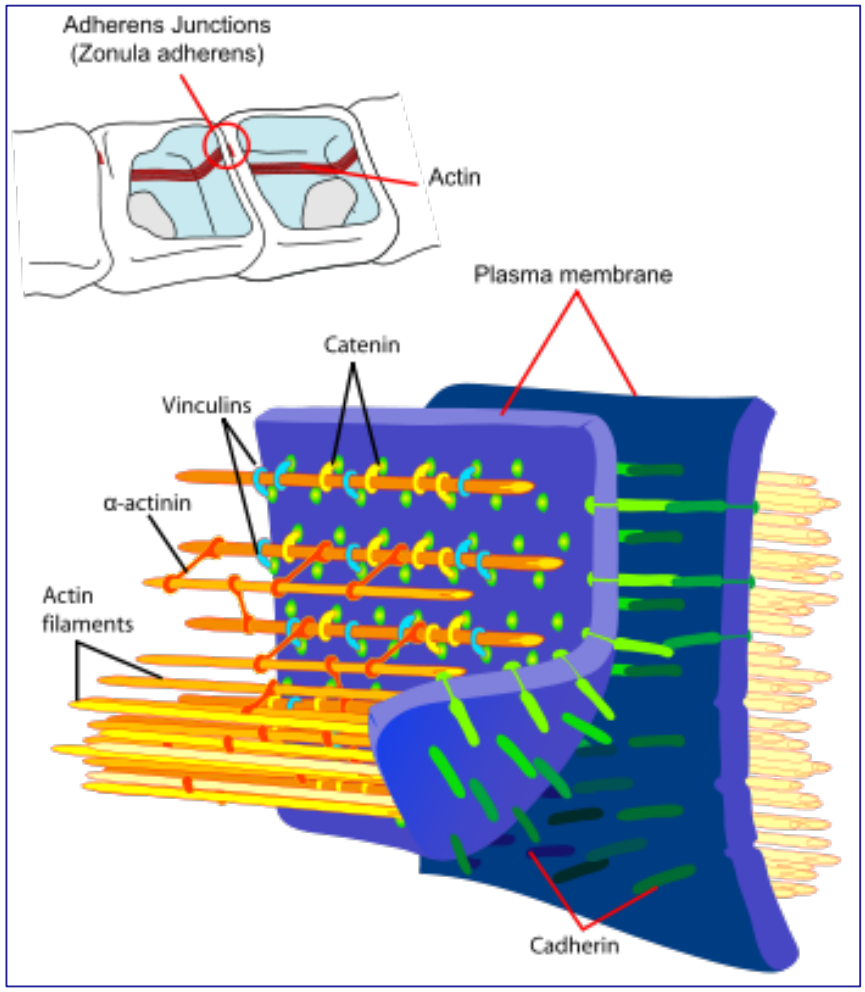
Cellular cortex

ACTIN FILAMENTS: Locations

Arrangement in cells

ADHERENS JUNCTIONS

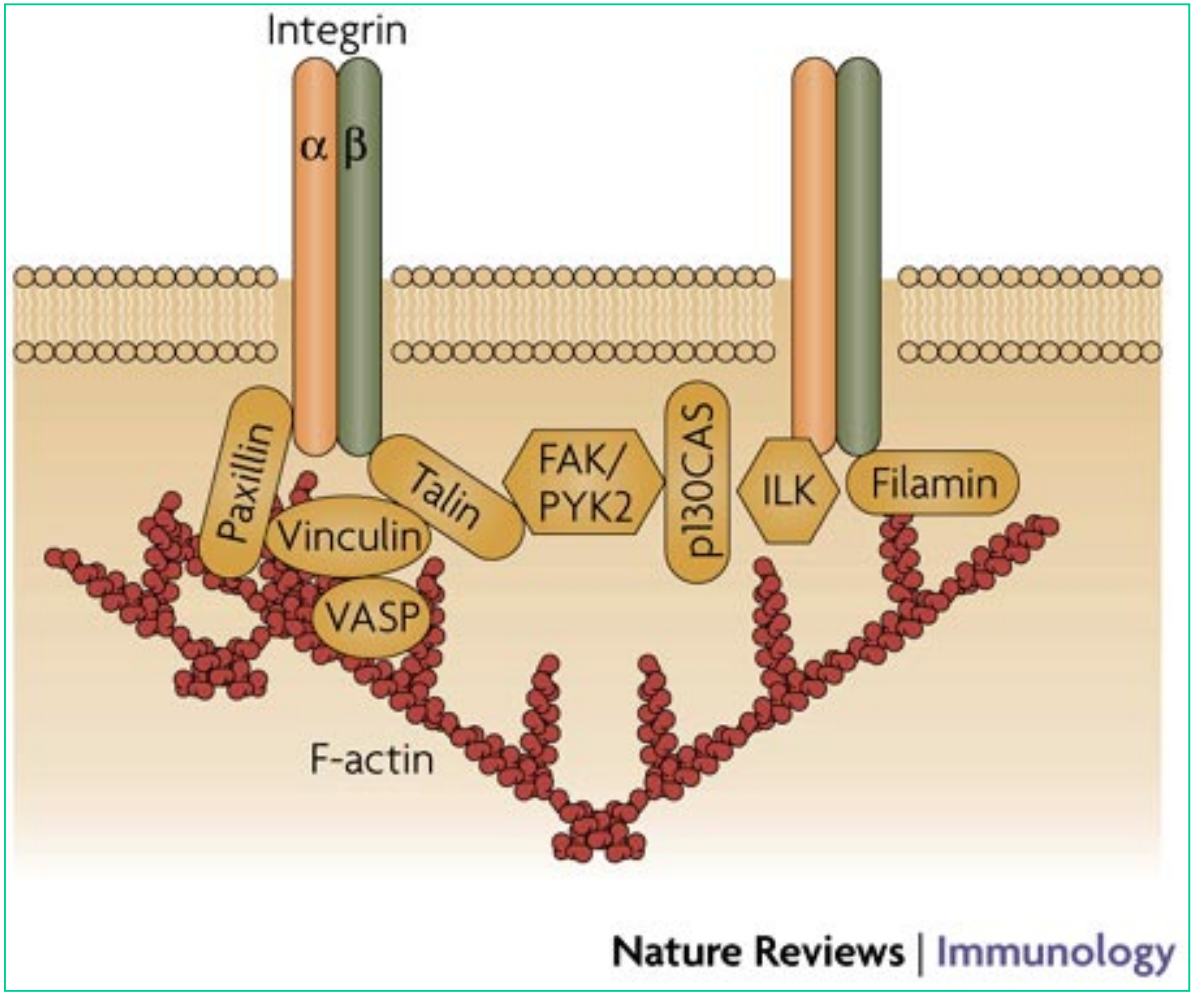




ACTIN FILAMENTS: Locations

Arrangement in cells

Focal adhesions (cell-matrix)



ACTIN FILAMENTS: Locations

Arrangement in cells

CONTRACTILE RING

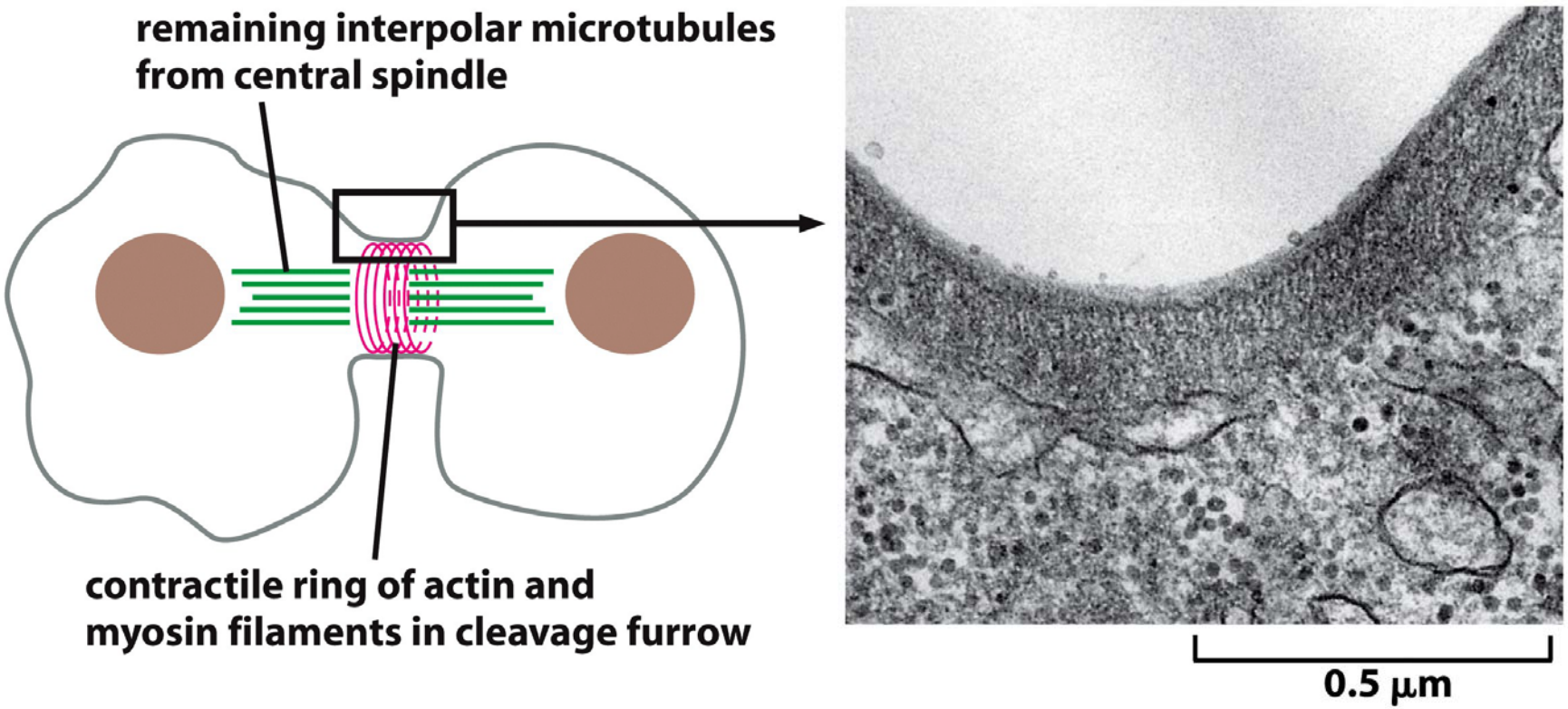
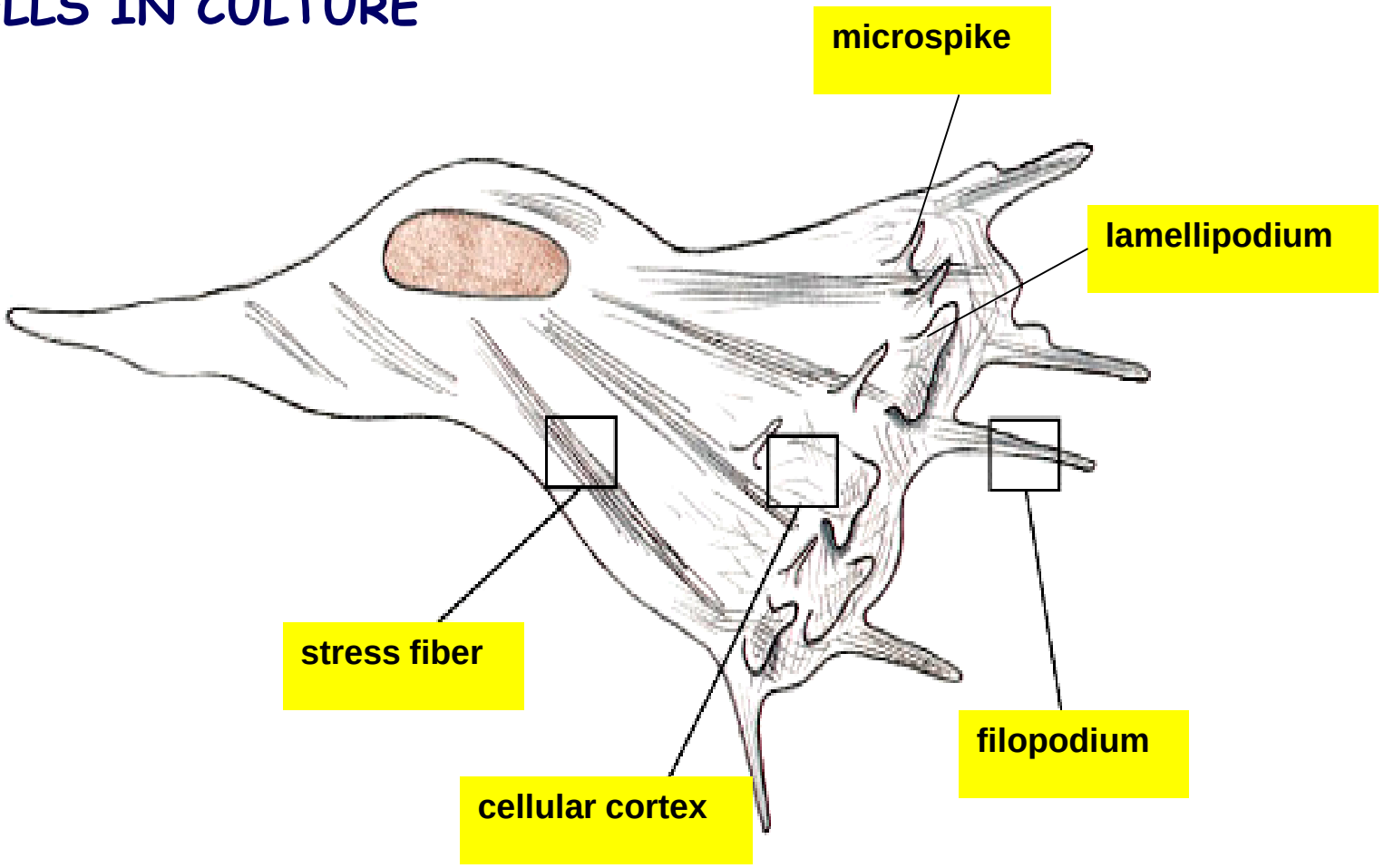


Figure 17-50a *Molecular Biology of the Cell* (© Garland Science 2008)

ACTIN FILAMENTS: Locations

Arrangement in cells

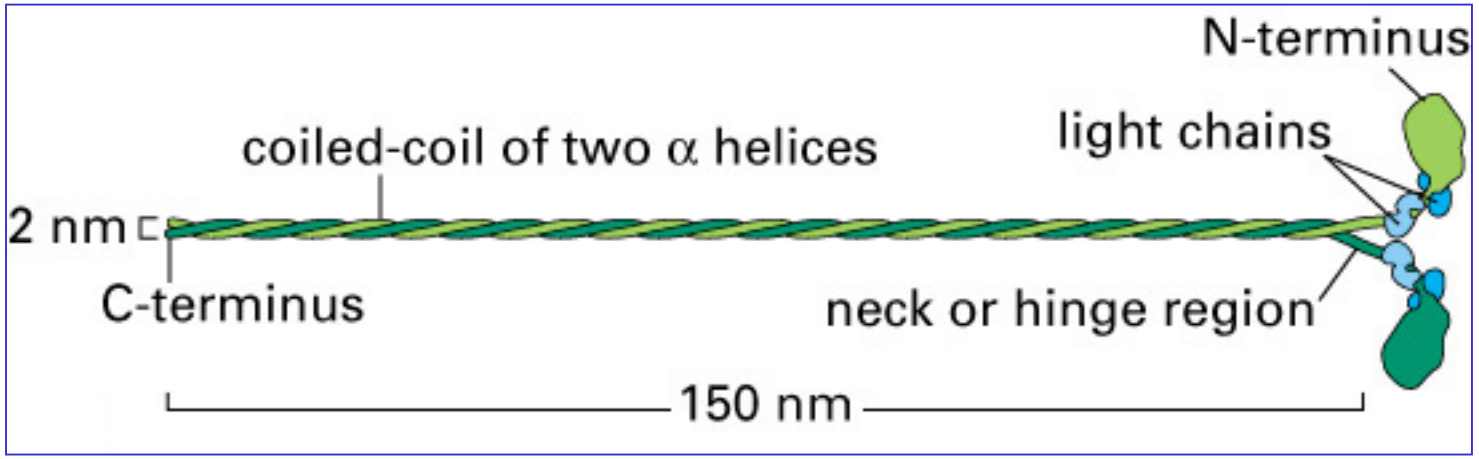
CELLS IN CULTURE

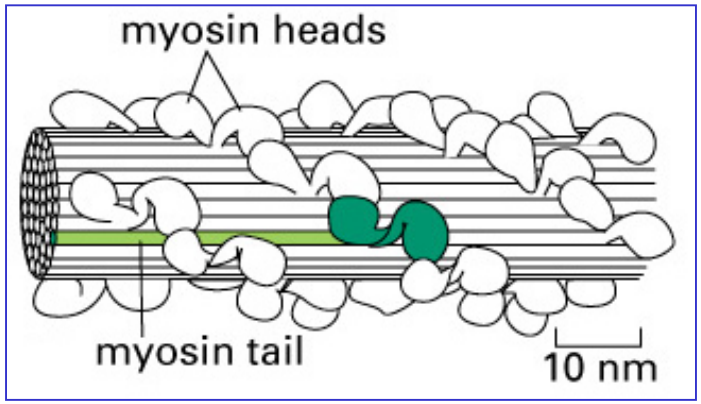


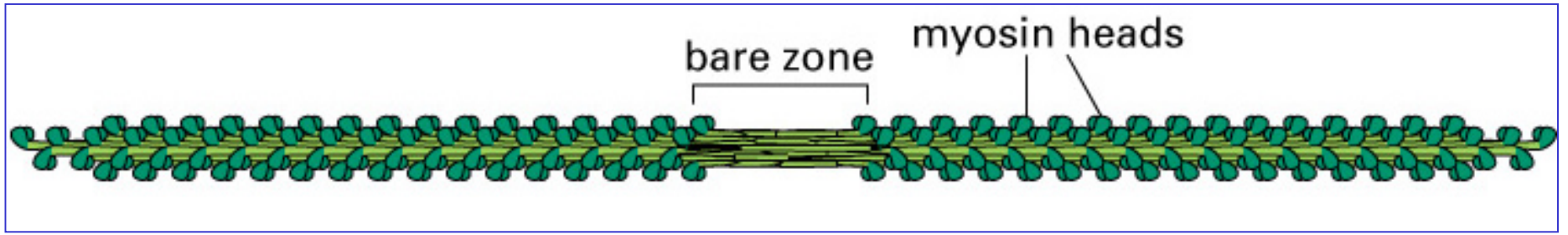
MYOSIN

- Myosin filaments are very abundant in muscle cells.
- Muscular myosin (myosin II) is a dimer formed by two heavy chains and four light chains.
- Hundreds of myosin II molecules form a thick filament of myosin.
- At least 18 different types of myosin exist, that can be monomers or dimers.
- The human genome contains about 40 myosin genes.
- The function of myosin is always related with its contractile activity.

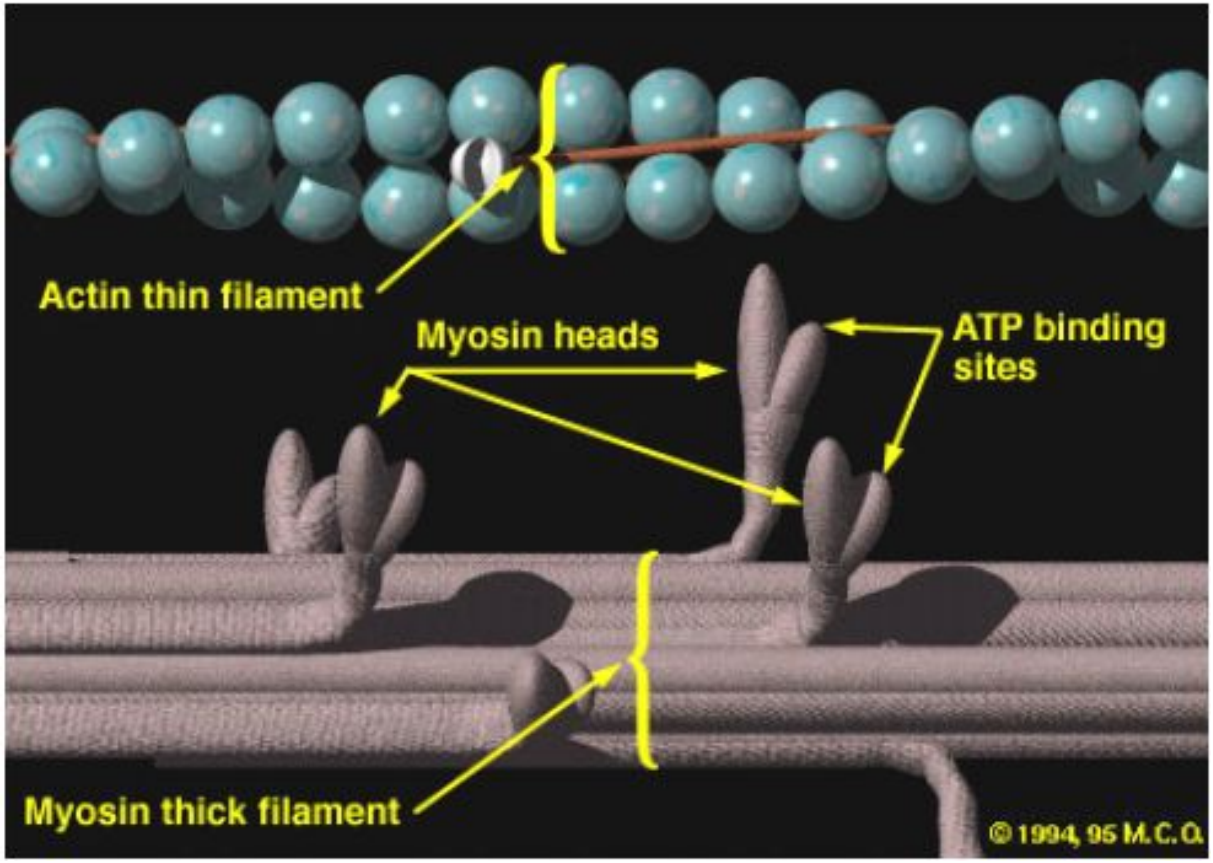
MUSCULAR MYOSIN (Myosin II)



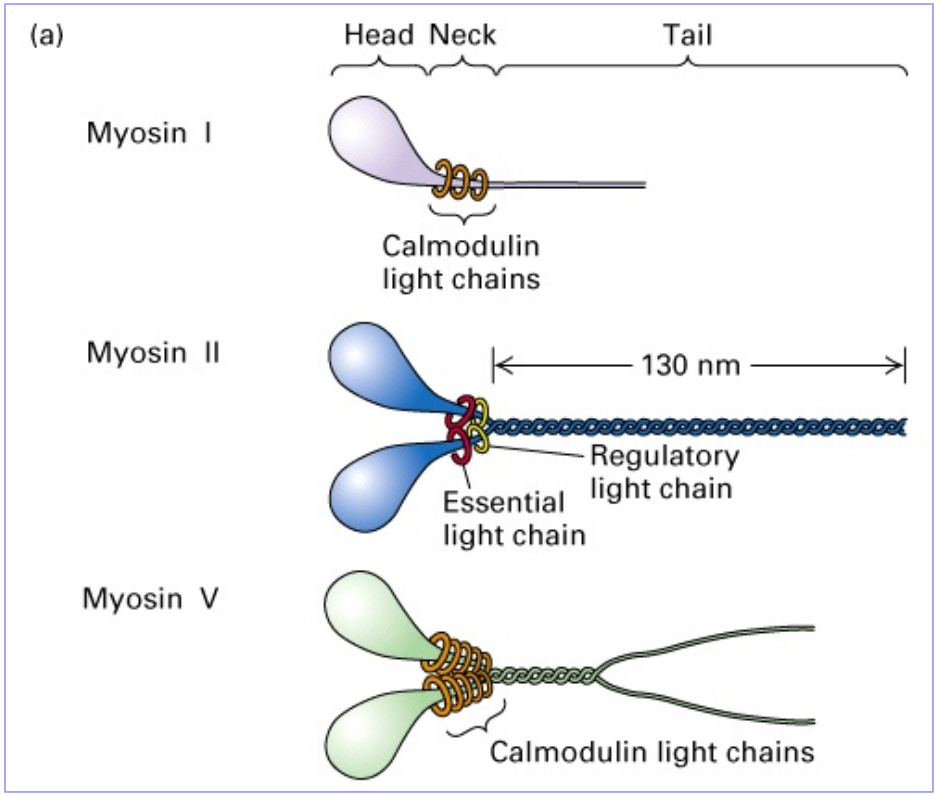


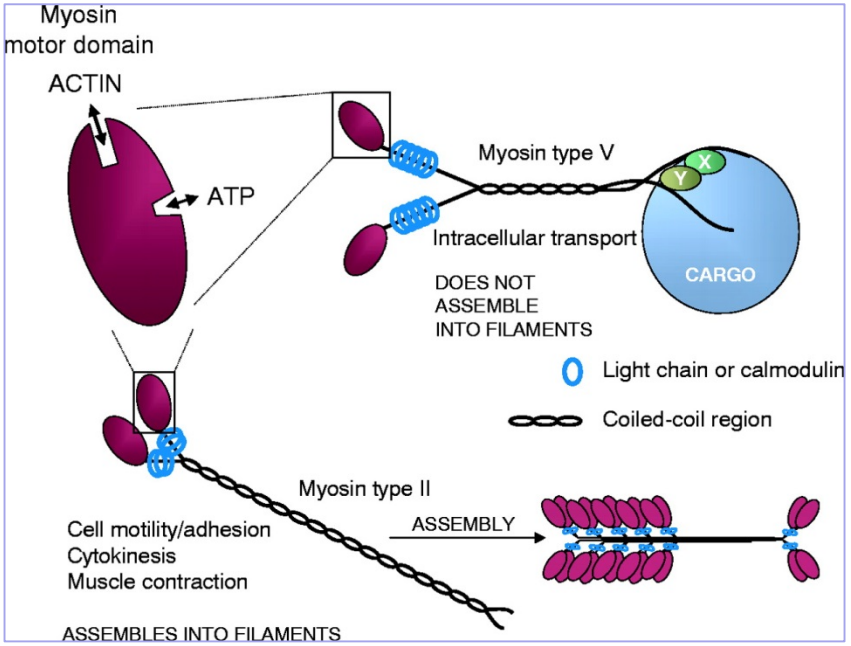


MUSCULAR MYOSIN (Myosin II)



MYOSIN


(a)
Head
Neck
Tail
Myosin I
Calmodulin
light chains
Myosin II
130 nm
Regulatory
light chain
Essential
light chain
Myosin V
Calmodulin light chains


Myosin
motor domain
ACTIN
ATP
Myosin type V
Intracellular transport
DOES NOT
ASSEMBLE
INTO FILAMENTS
X
Y
CARGO
Light chain or calmodulin
Coiled-coil region
Myosin type II
Cell motility/adhesion
Cytokinesis
Muscle contraction
ASSEMBLY
ASSEMBLES INTO FILAMENTS

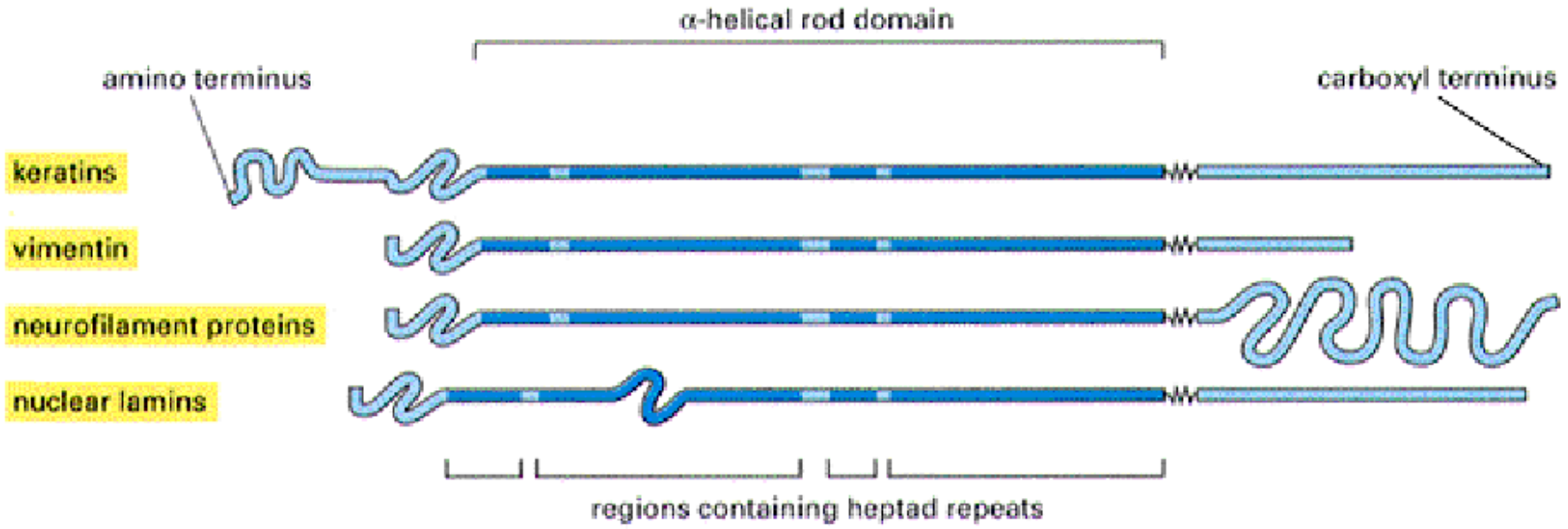
INTERMEDIATE FILAMENTS

General characteristics

- They have an 8 to 12 nm diameter.
- They are found in most eucaryotic cells, not in all.
- They are the most stable elements of the cytoskeleton.
- Intermediate filaments confer mechanical stability to the tissues.
- Different cell types have different intermediate filaments.

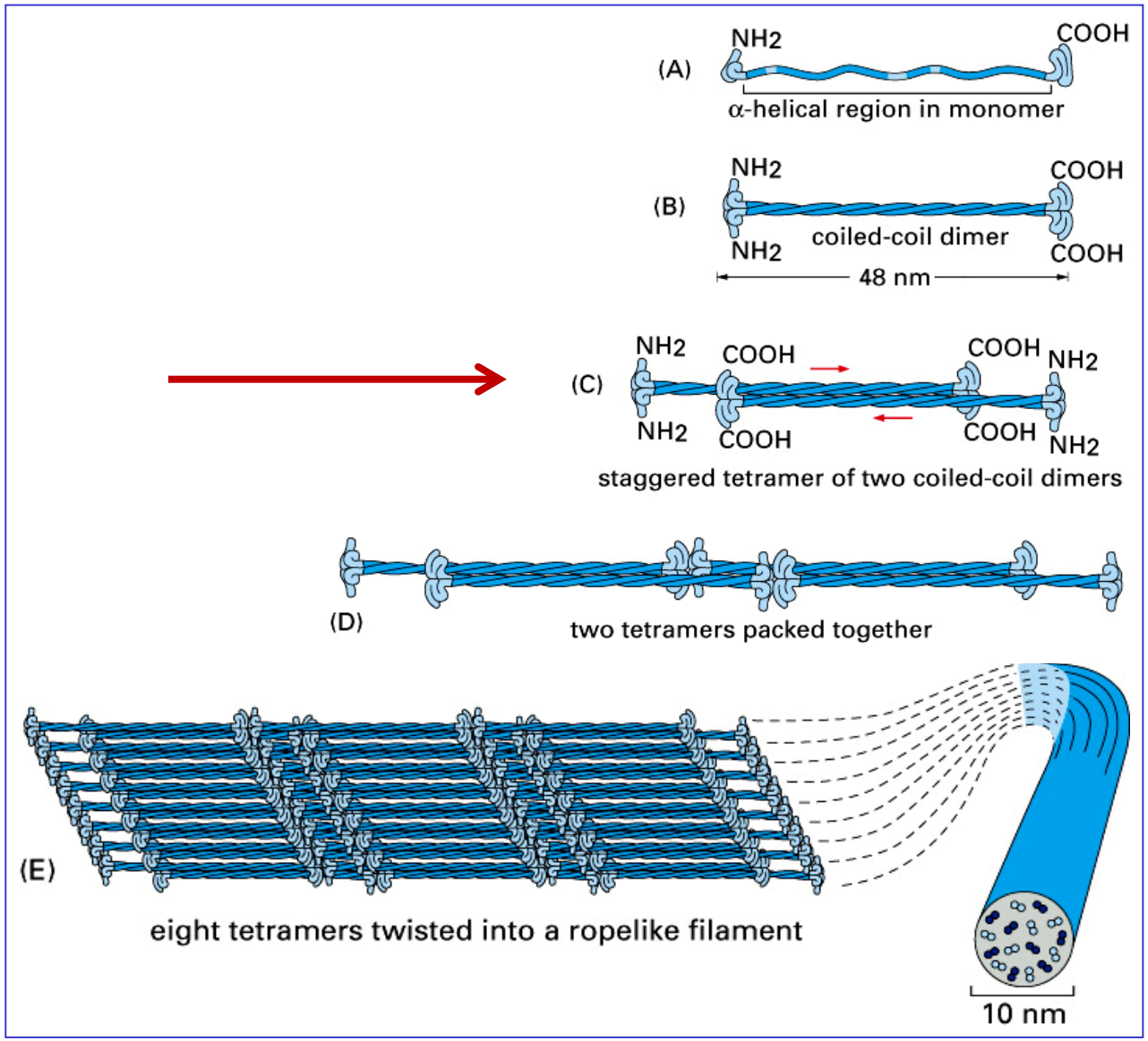
INTERMEDIATE FILAMENTS

Molecular organization



INTERMEDIATE FILAMENTS

Molecular organization



INTERMEDIATE FILAMENTS

Types

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

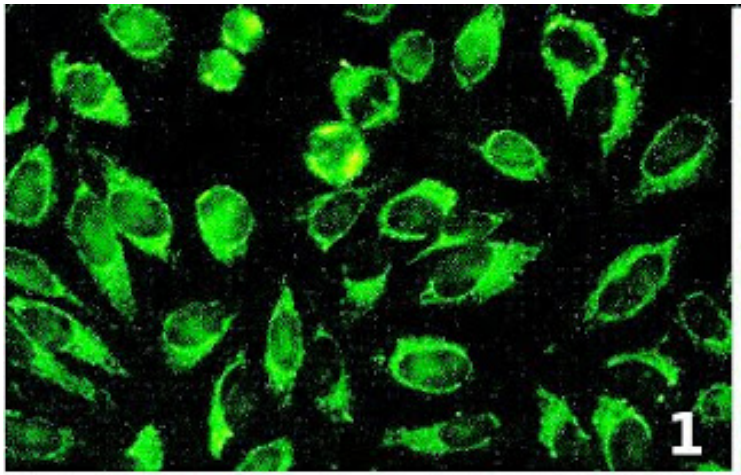
TYPES OF IF	COMPONENT POLYPEPTIDES	LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial	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

Table 16-1 *Molecular Biology of the Cell* (© Garland Science 2008)

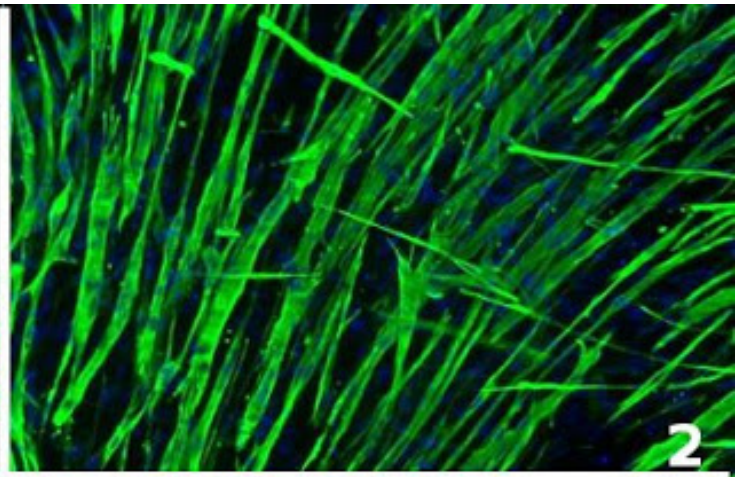
INTERMEDIATE FILAMENTS

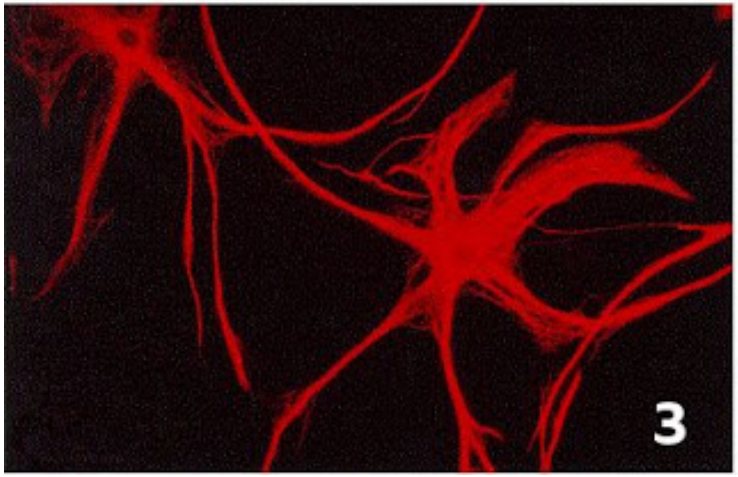
Types

Vimentin (fibroblasts)

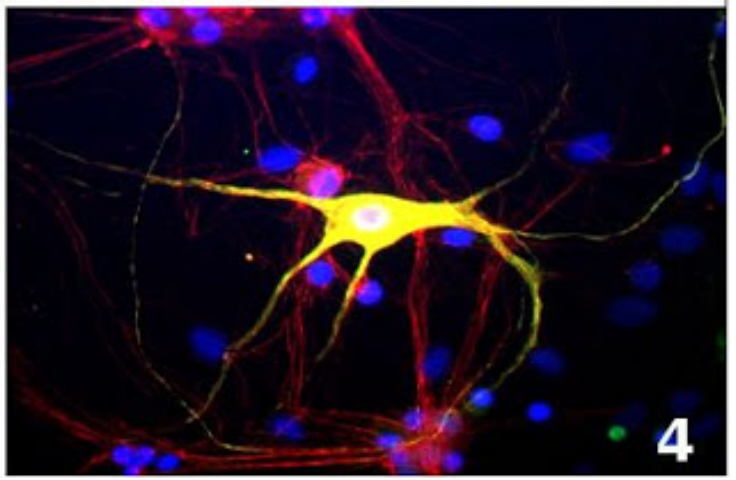


Desmin (muscular cells)





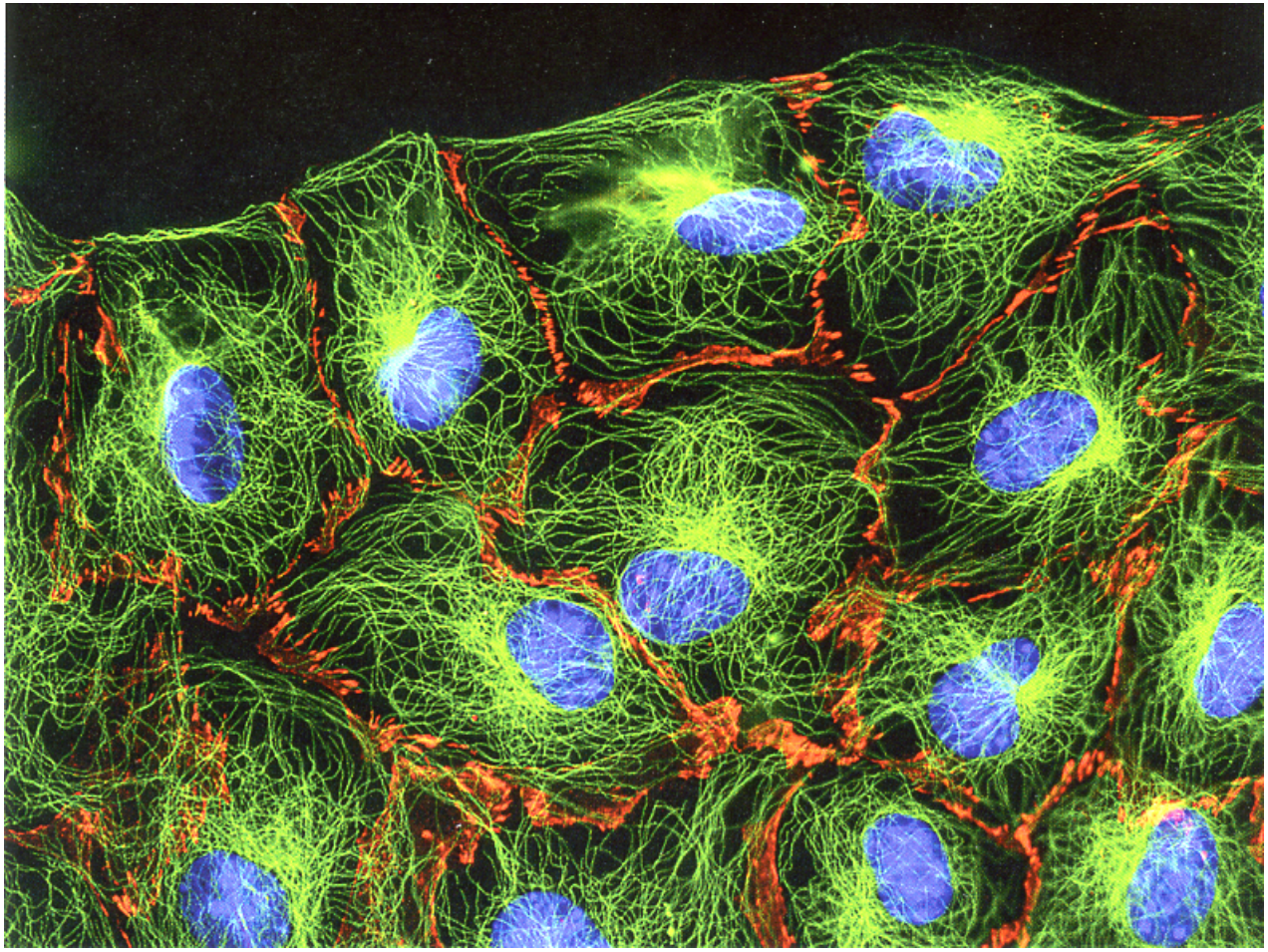
GFAP (astrocytes)



Peripherin (neurons)

INTERMEDIATE FILAMENTS

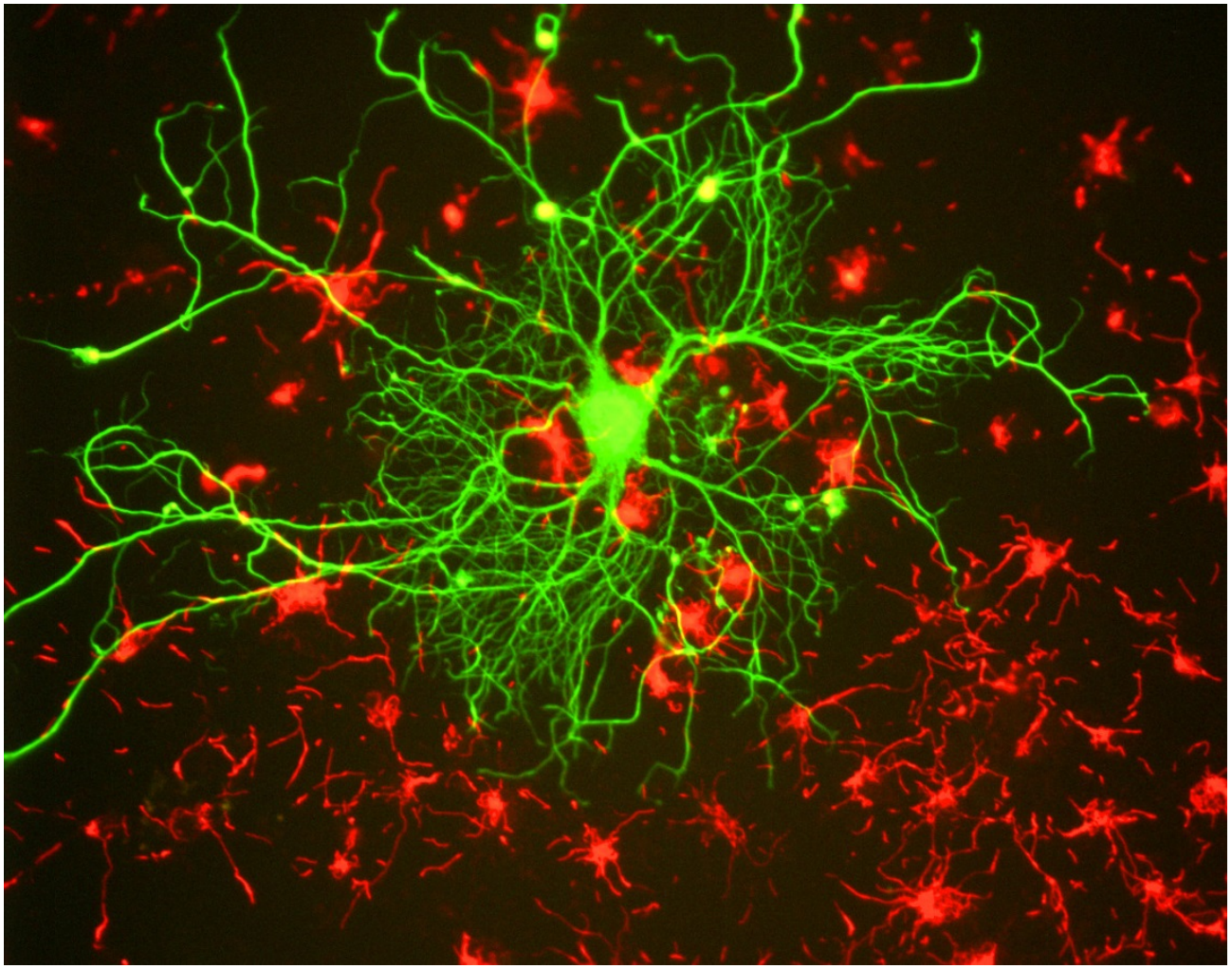
Types



Keratin filaments in epithelial cells

INTERMEDIATE FILAMENTS

Types



Neurofilaments

INTERMEDIATE FILAMENTS

Types

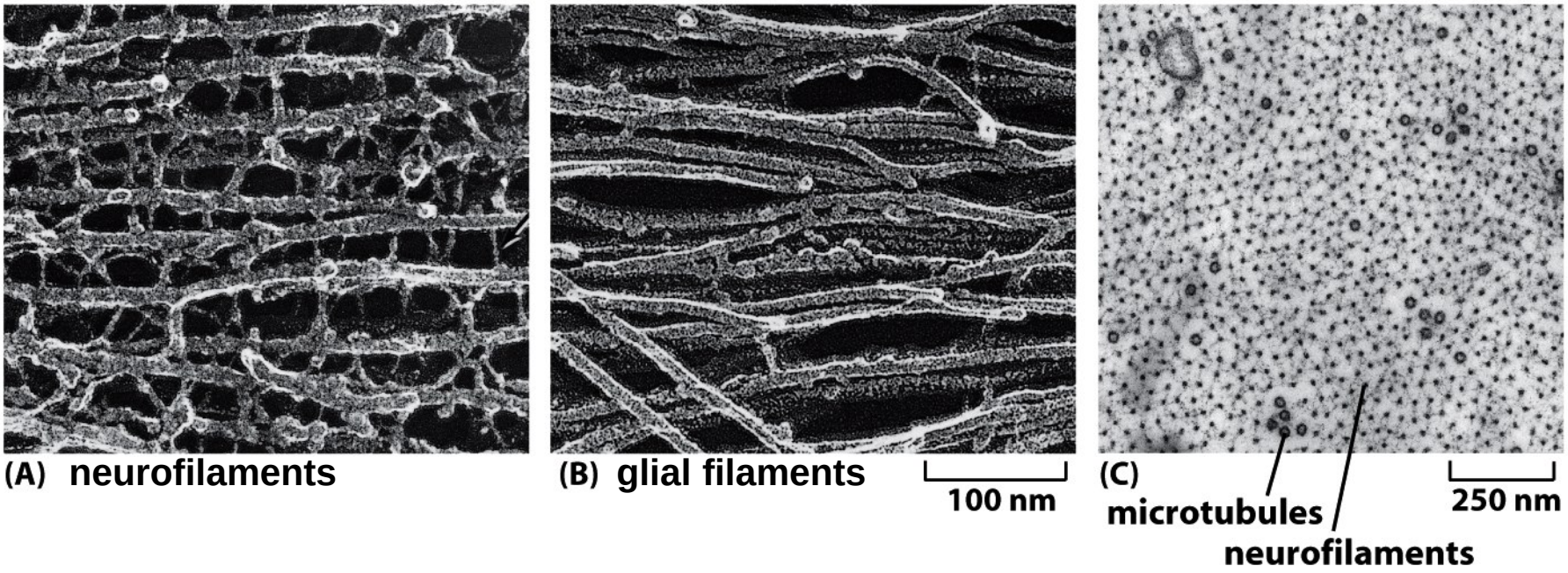
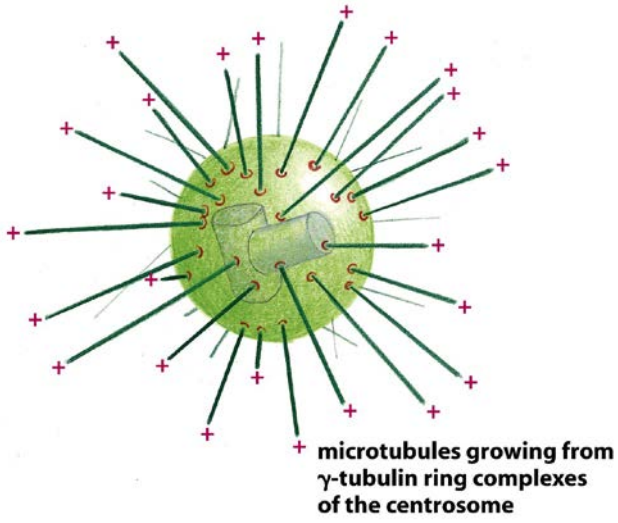


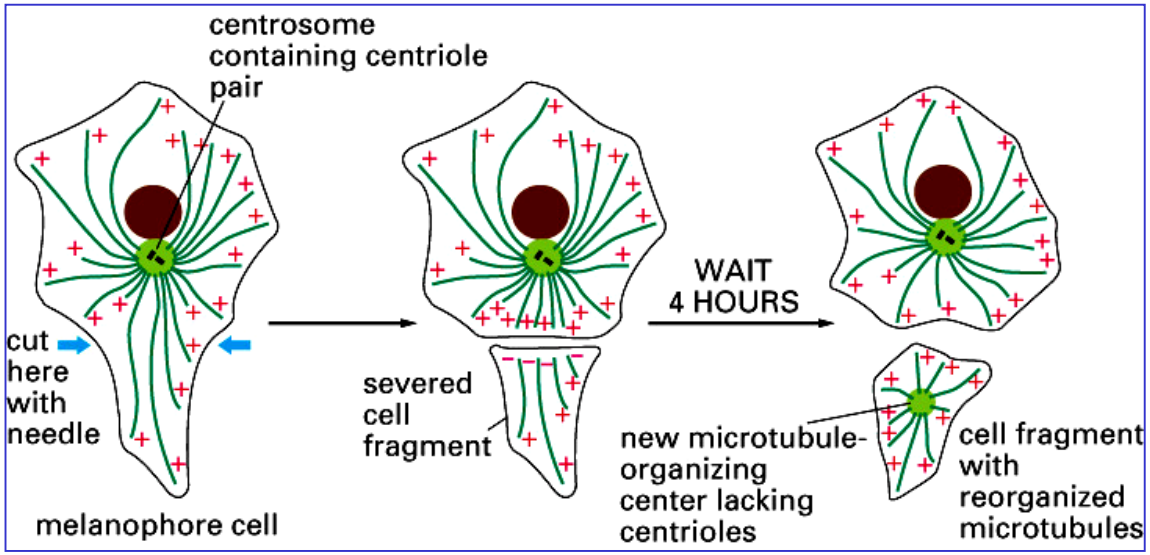
Figure 16-22 Molecular Biology of the Cell 5/e (© Garland Science 2008)

THE CYTOSKELETON: FUNCTIONS

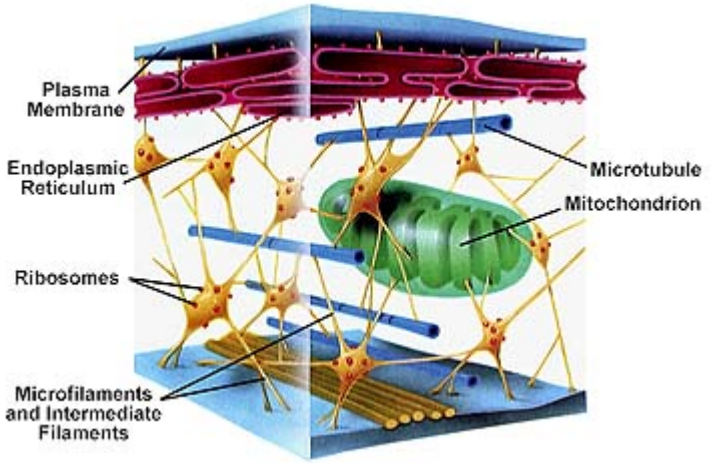
- Introduction
- Functions
  1) Control of the position of the intracellular structures
  2) Transmembrane control of the intracellular organization.
  3) Control of cell shape
  4) Cellular movements
- Motor proteins
- Regulation of the cytoskeletal filaments

FUNCTIONS: CONTROL OF THE POSITION OF INTRACELLULAR STRUCTURES

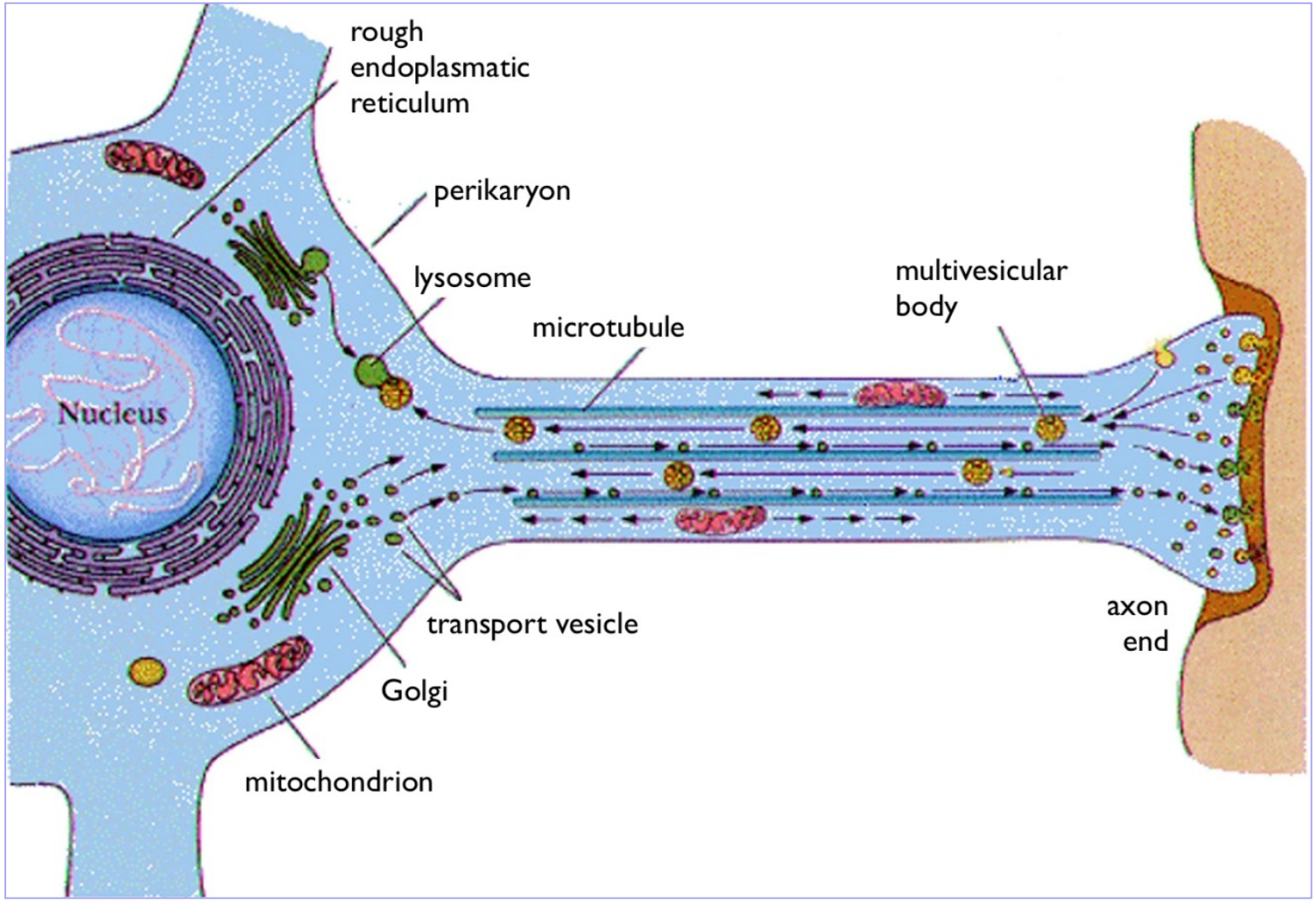




- Mainly microtubules and associated proteins participate
- The microtubules are continuously renewed
- The microtubules can find reference positions within the cell
- The position of ER and Golgi complex depend on microtubules

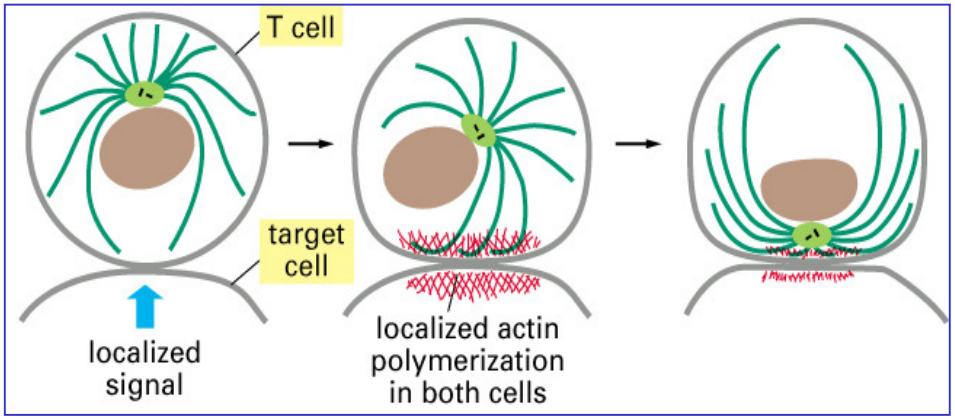


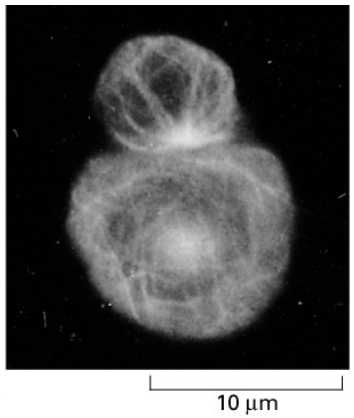
FUNCTIONS: CONTROL OF THE POSITION OF INTRACELLULAR STRUCTURES

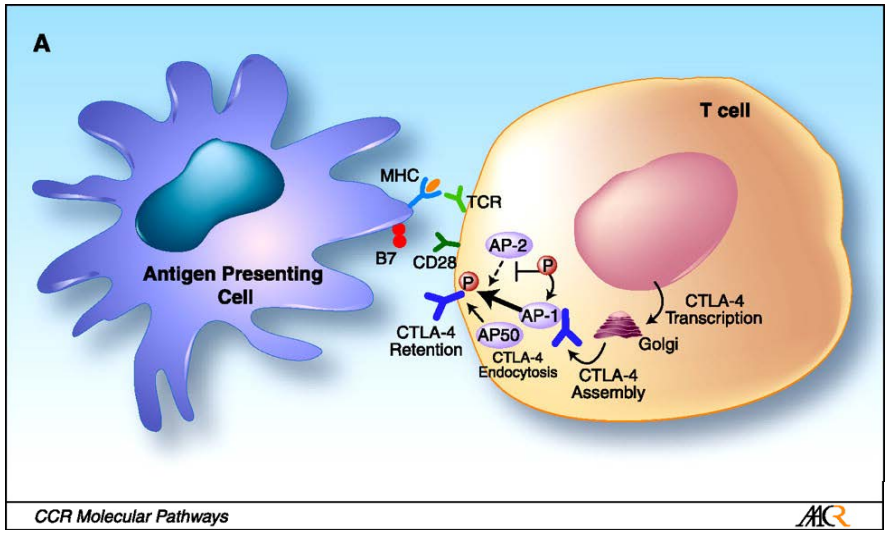


FUNCTIONS: TRANSMEMBRANE CONTROL OF THE INTRACELLULAR ORGANIZATION

Extracellular signals can act on the cytoskeleton through membrane receptors.

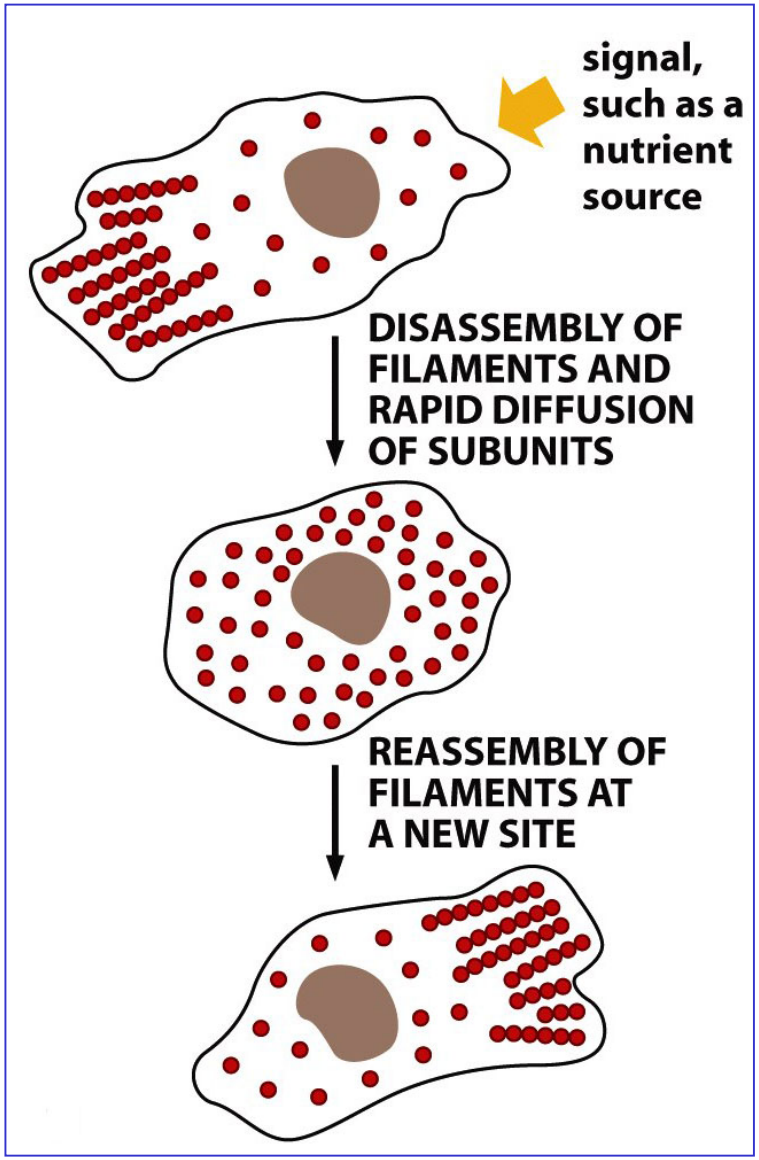




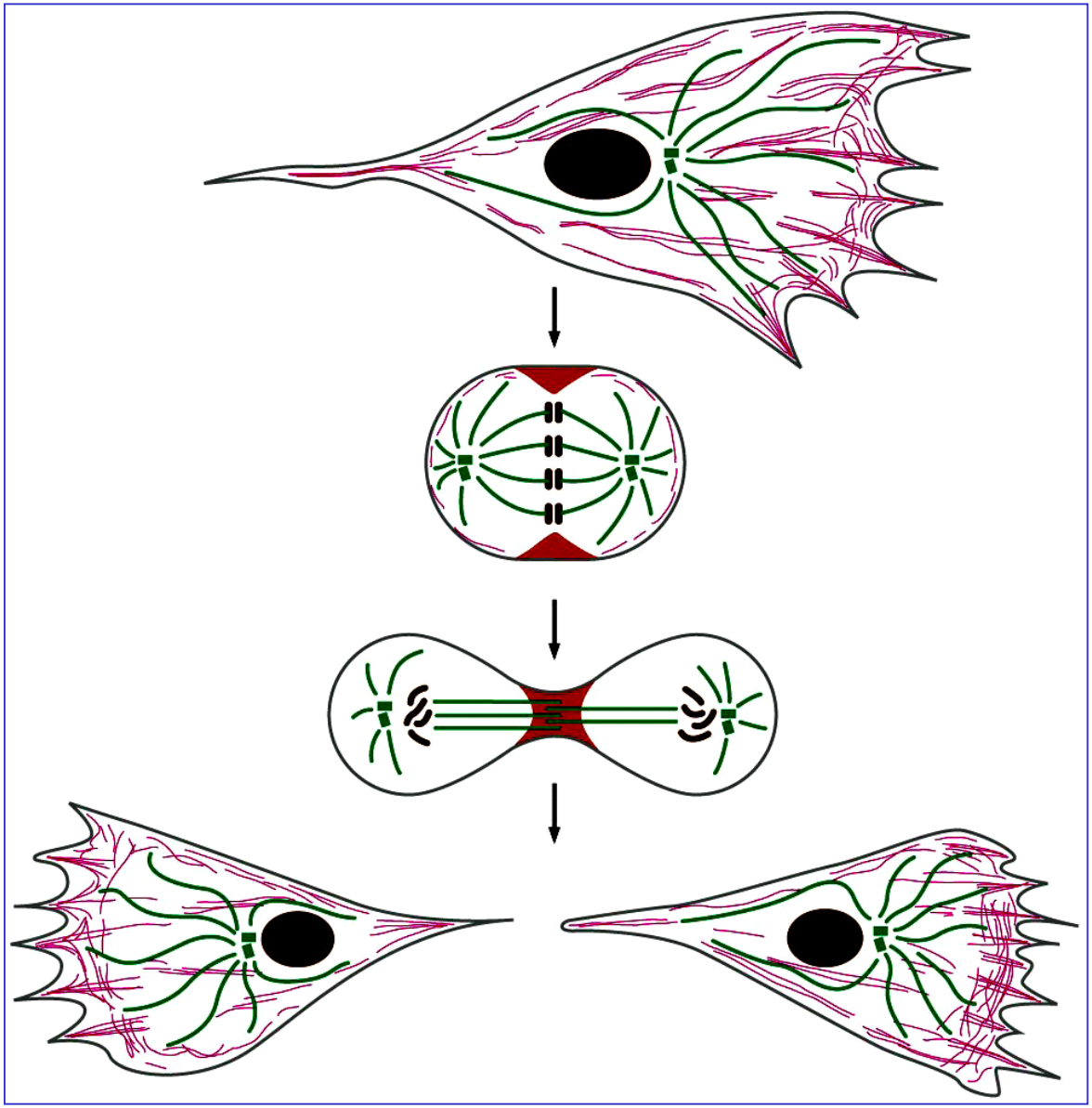


FUNCTIONS: CONTROL OF CELL SHAPE

- Coordinated action of the three types of filament.
- Intermediate filaments: mechanical stability.
- The cell cortex (actin) has a particular importance.
- The capacity to polymerize and depolymerize favors the rapid control of cell shape.

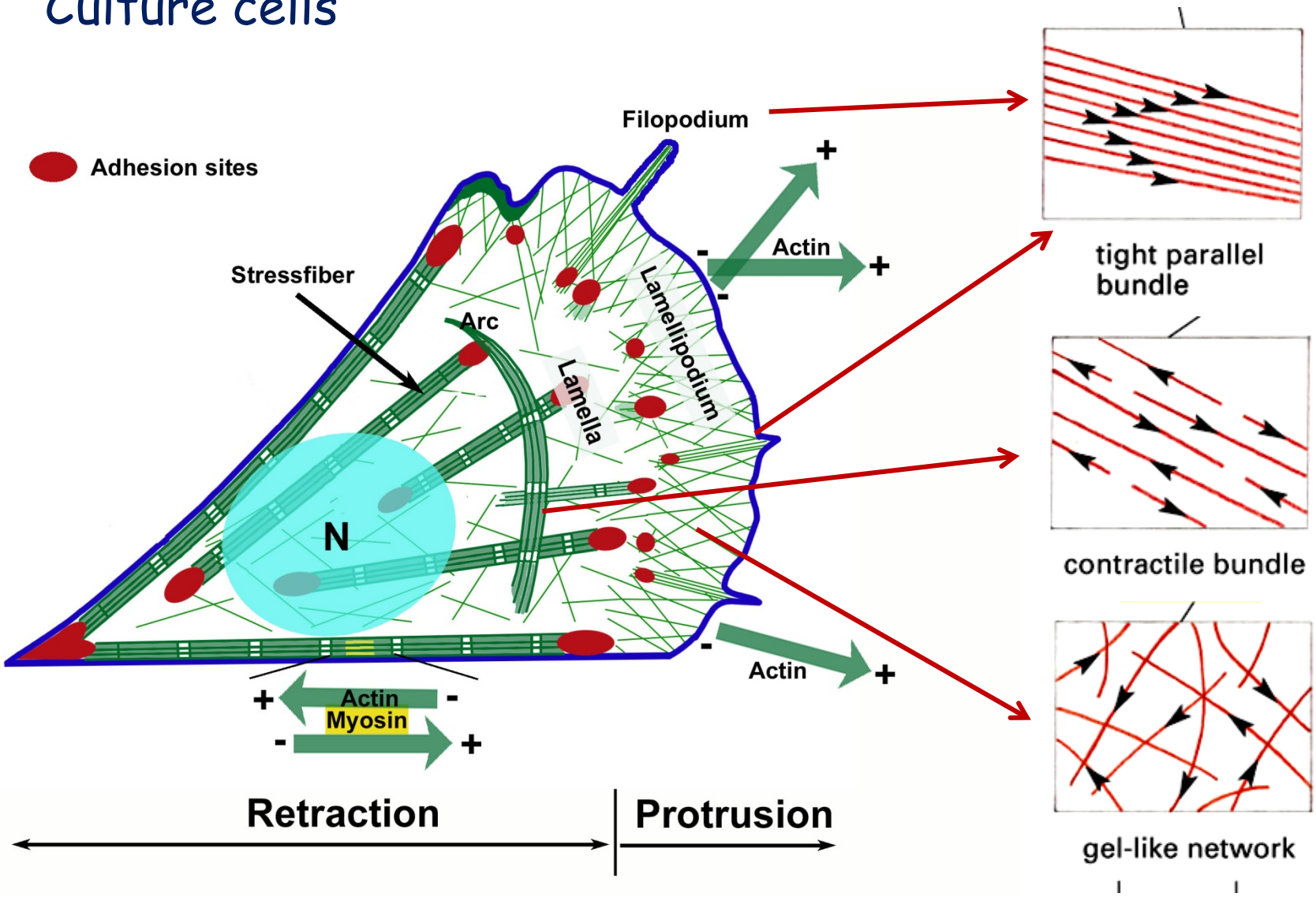


FUNCTIONS: CONTROL OF CELL SHAPE



FUNCTIONS: CELLULAR MOVEMENTS

Culture cells

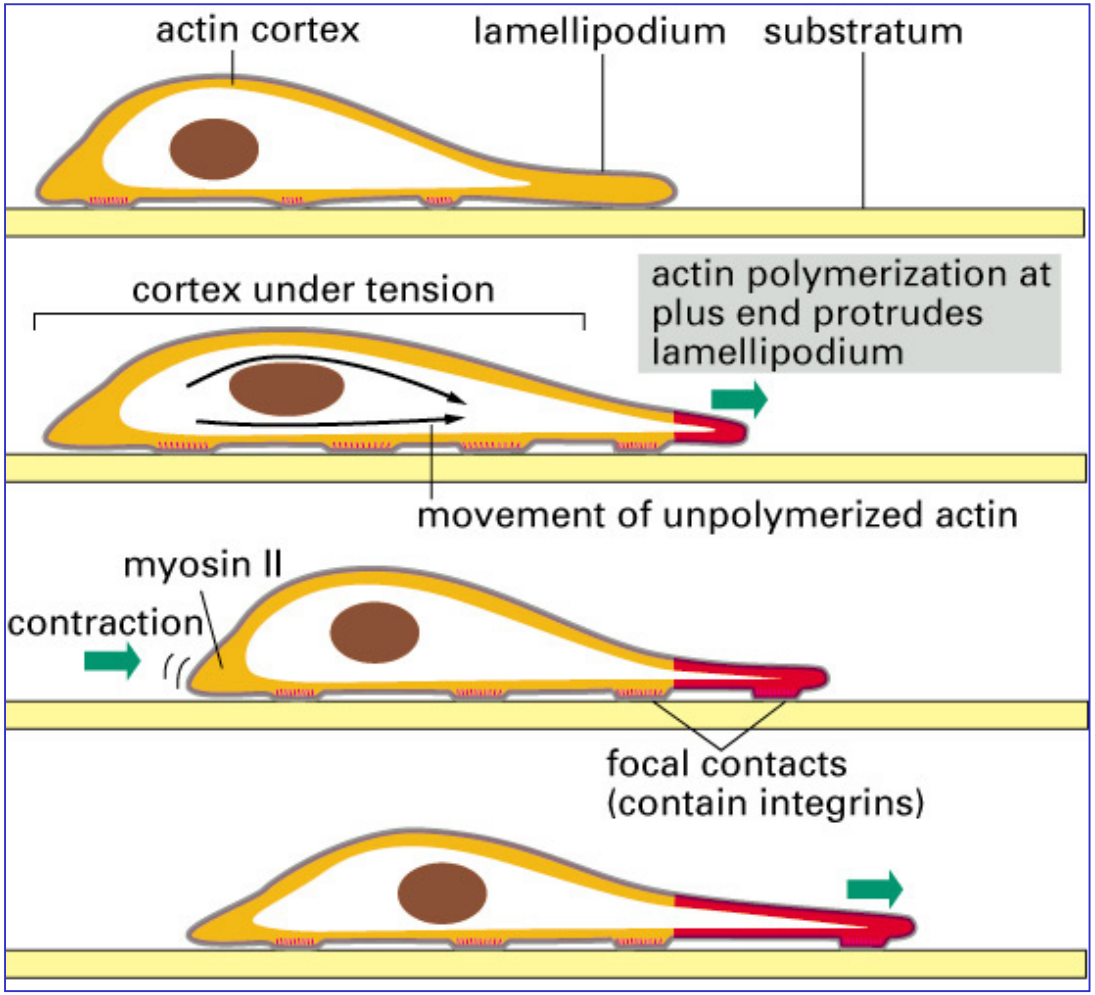


FUNCTIONS: CELLULAR MOVEMENTS

Cell locomotion

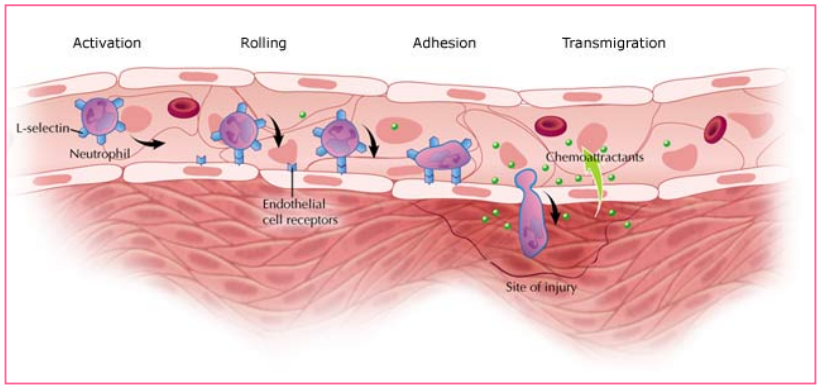
Protrusion

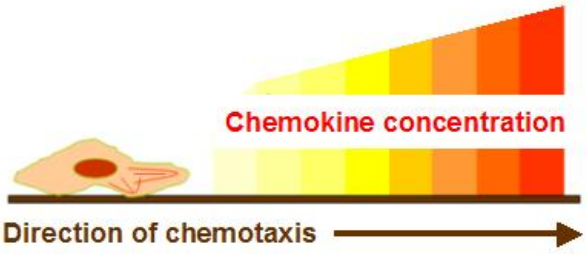
Attachment
Traction

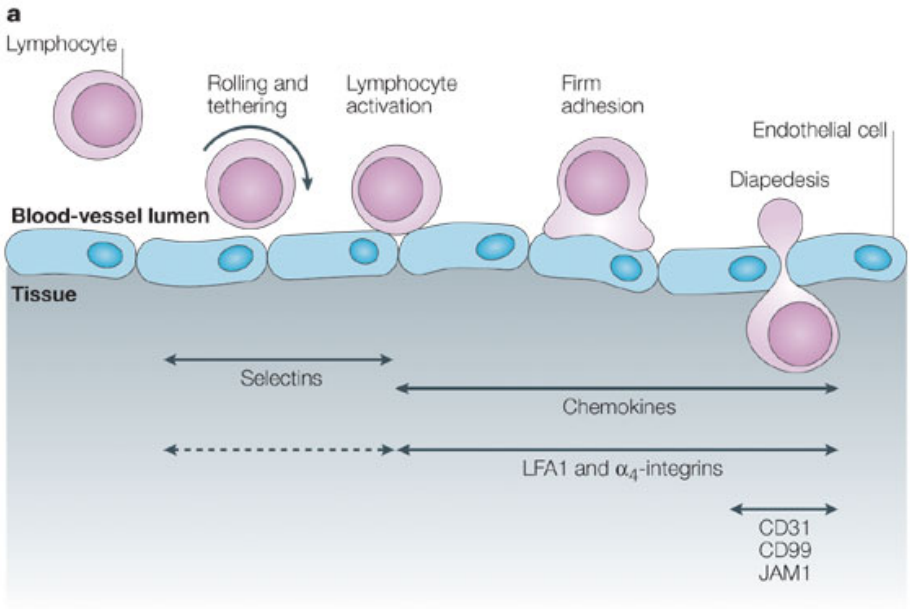


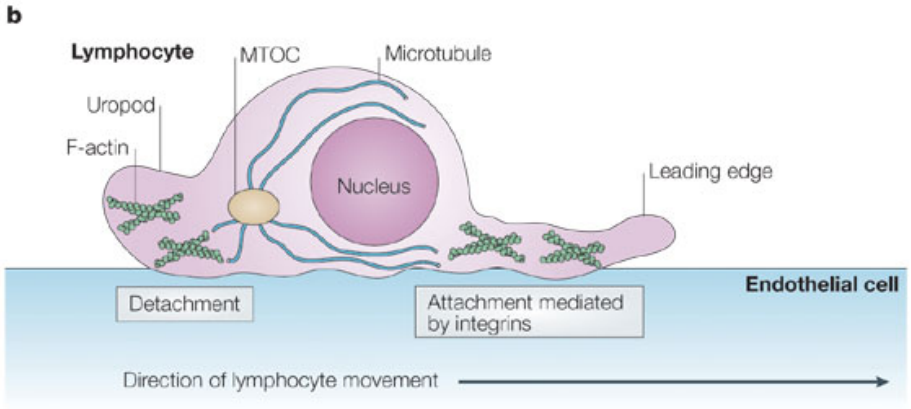
FUNCTIONS: CELLULAR MOVEMENTS

Chemotaxis









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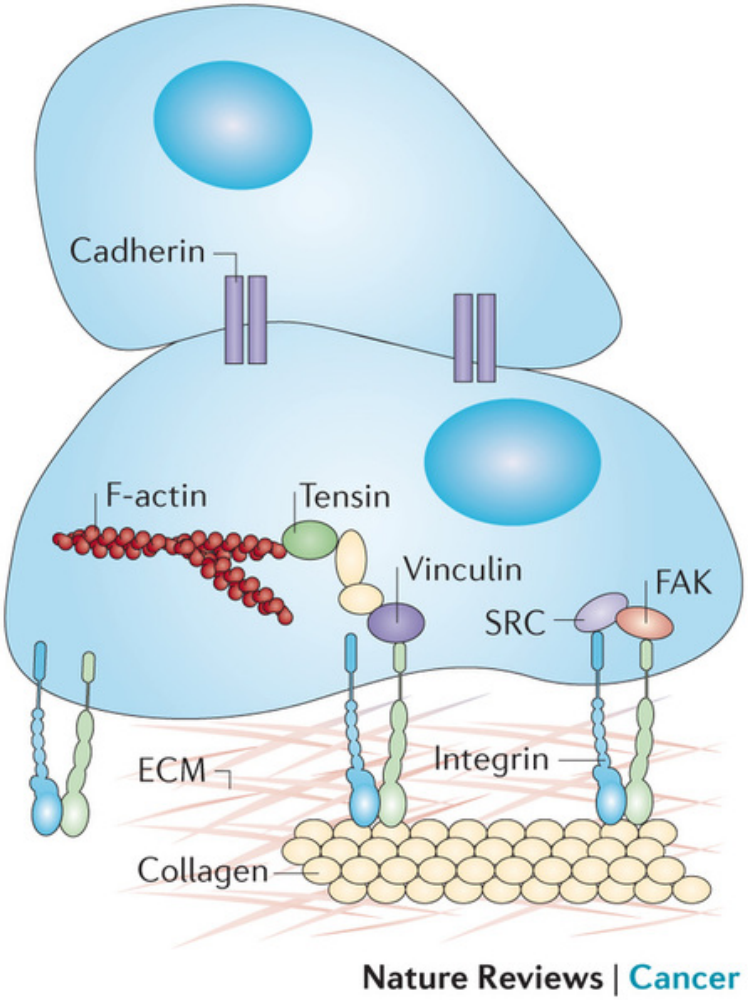
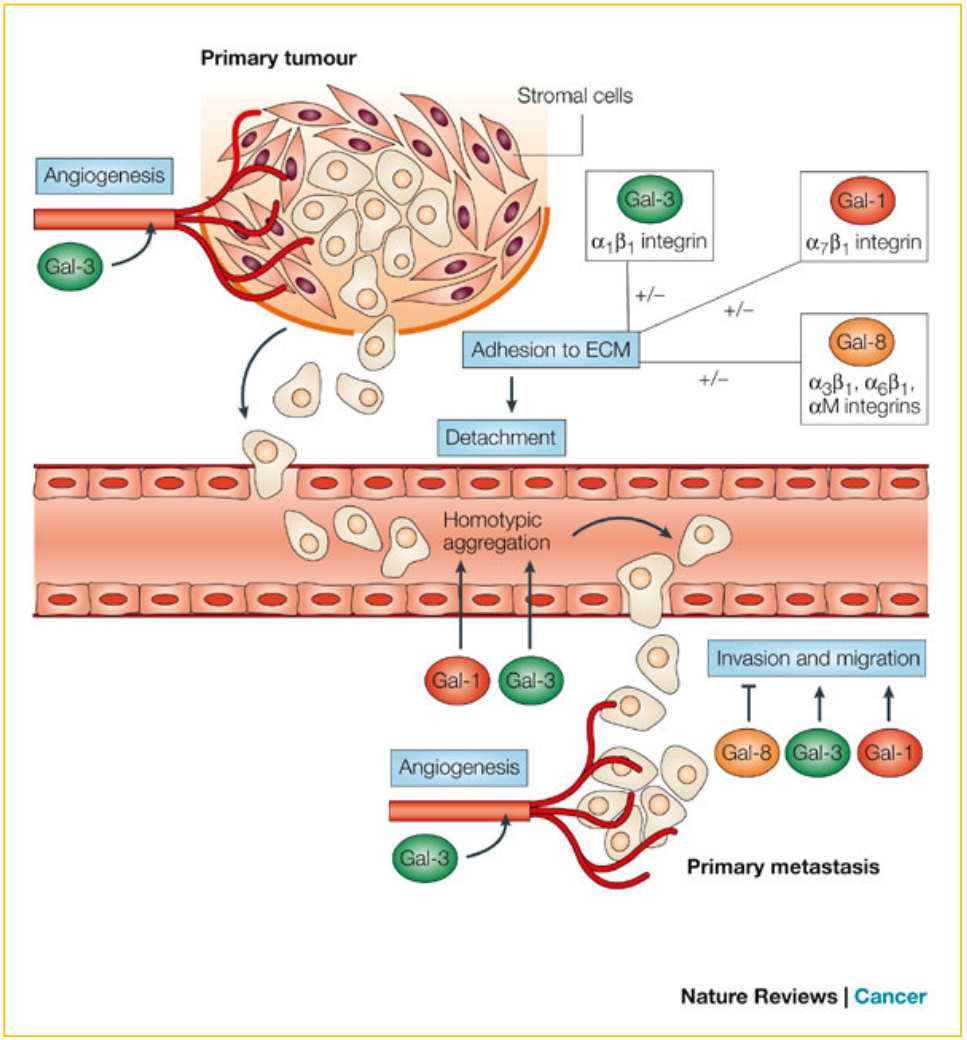
FUNCTIONS: CELLULAR MOVEMENTS

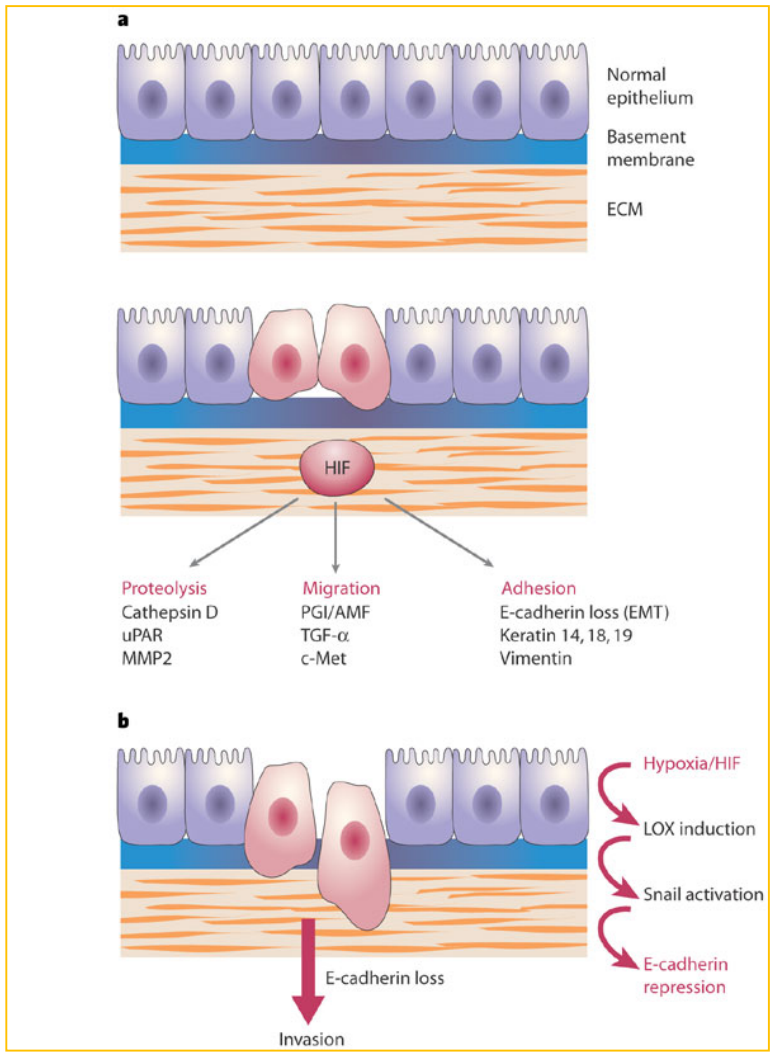
Migration and invasion

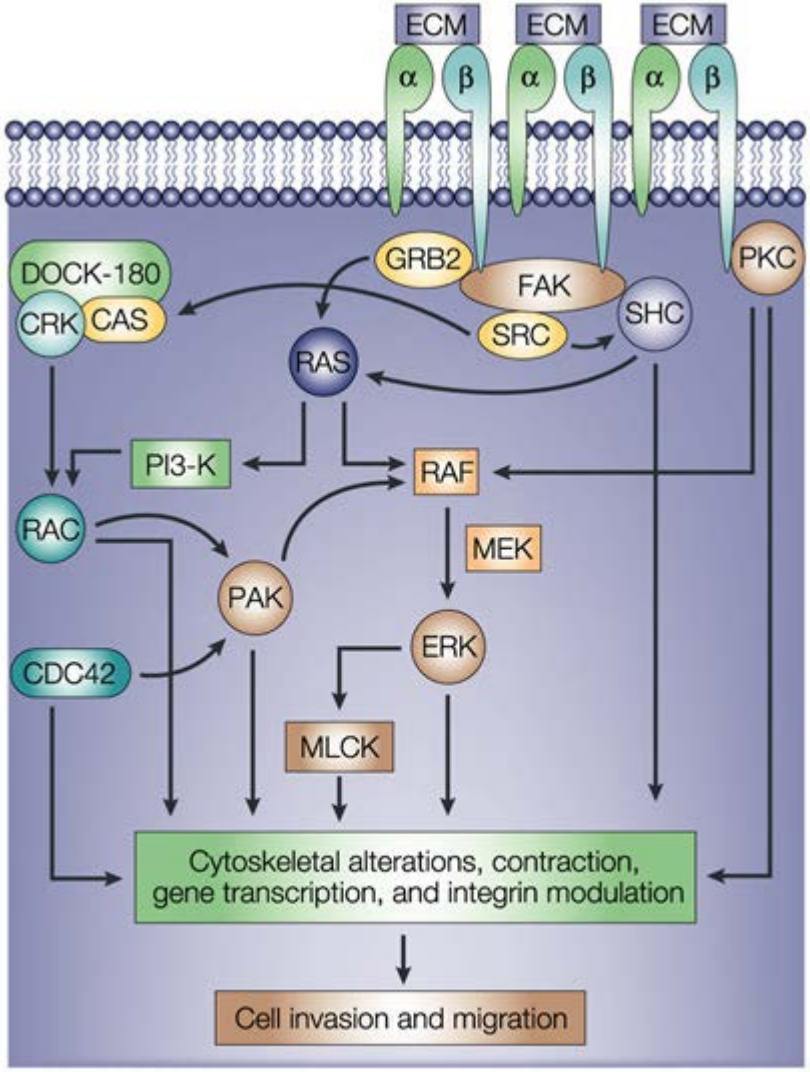




FUNCTIONS: CELLULAR MOVEMENTS

Migration and invasion





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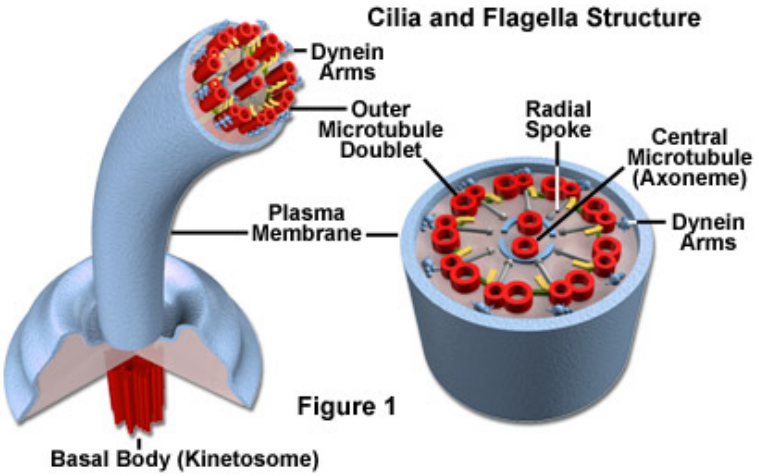
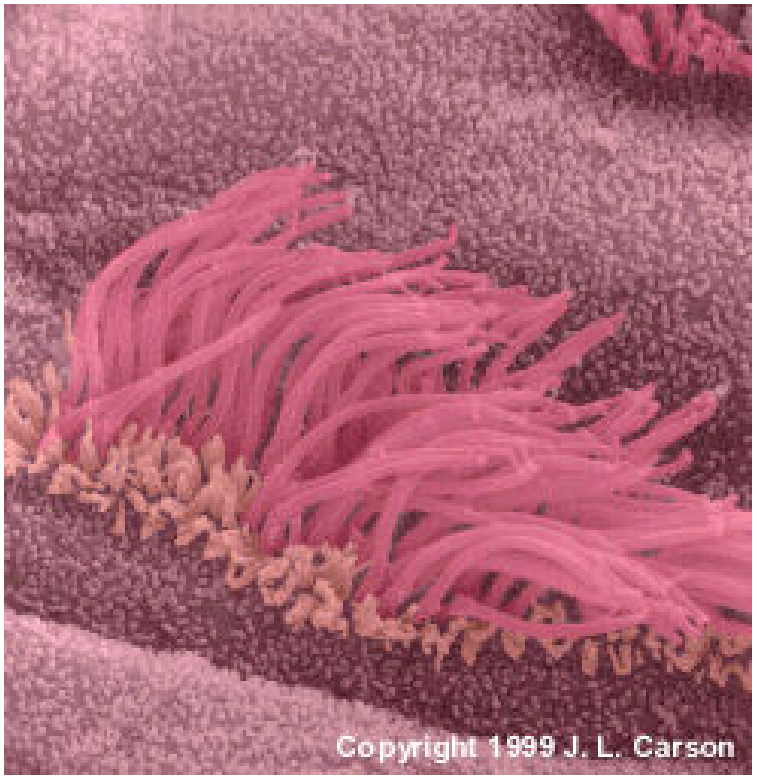
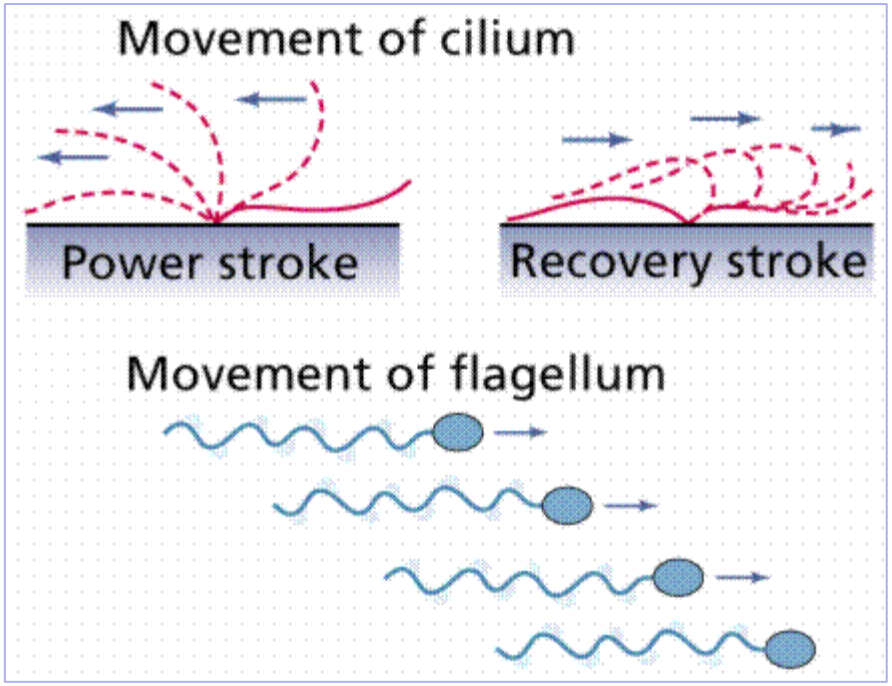
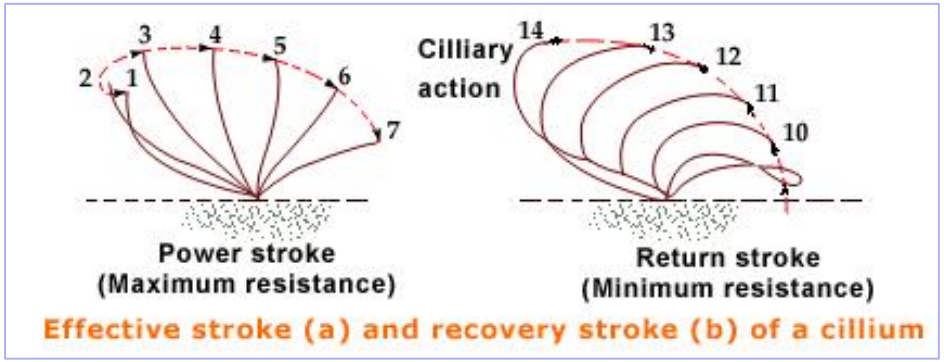
FUNCTIONS: CELLULAR MOVEMENTS

Ciliary movement

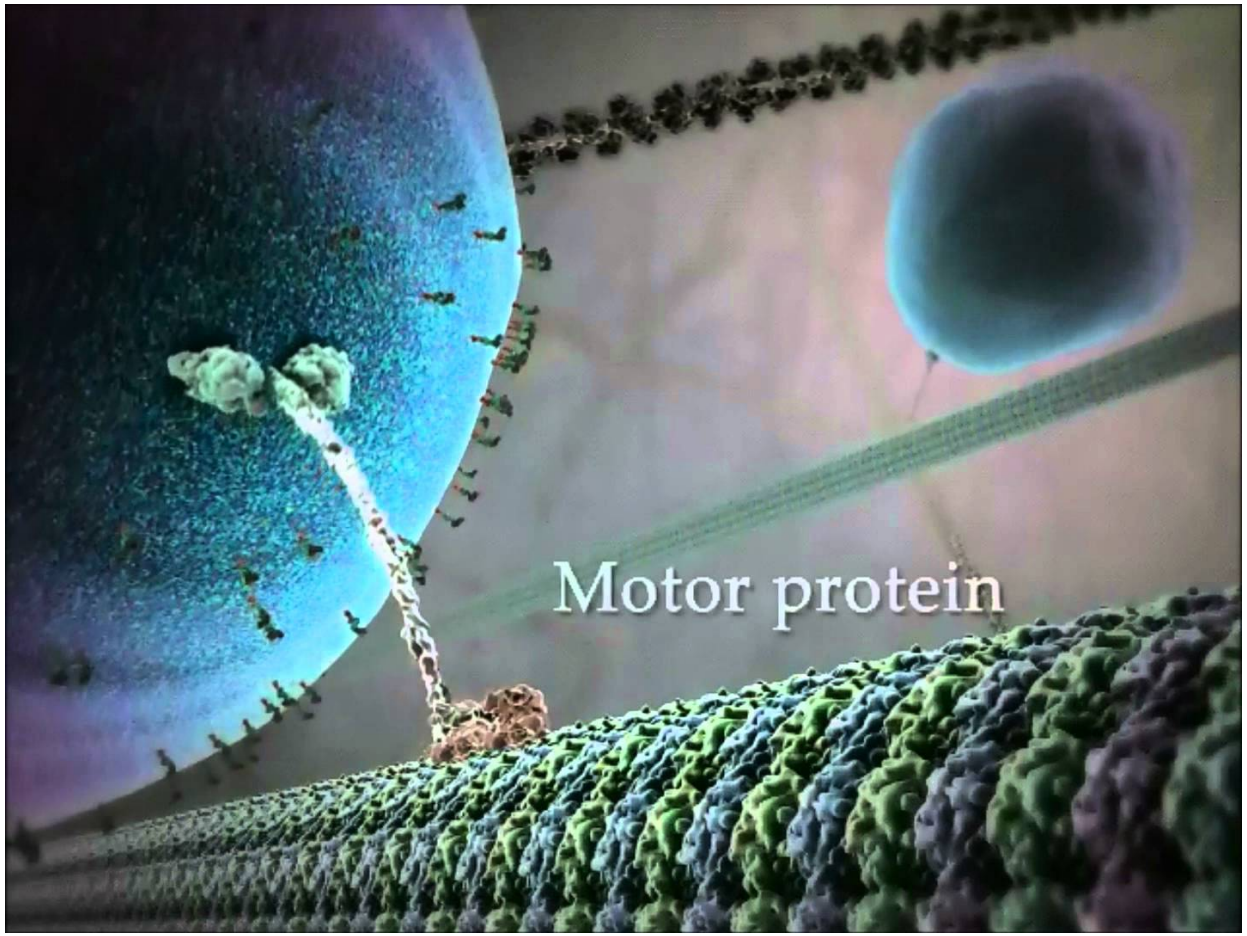






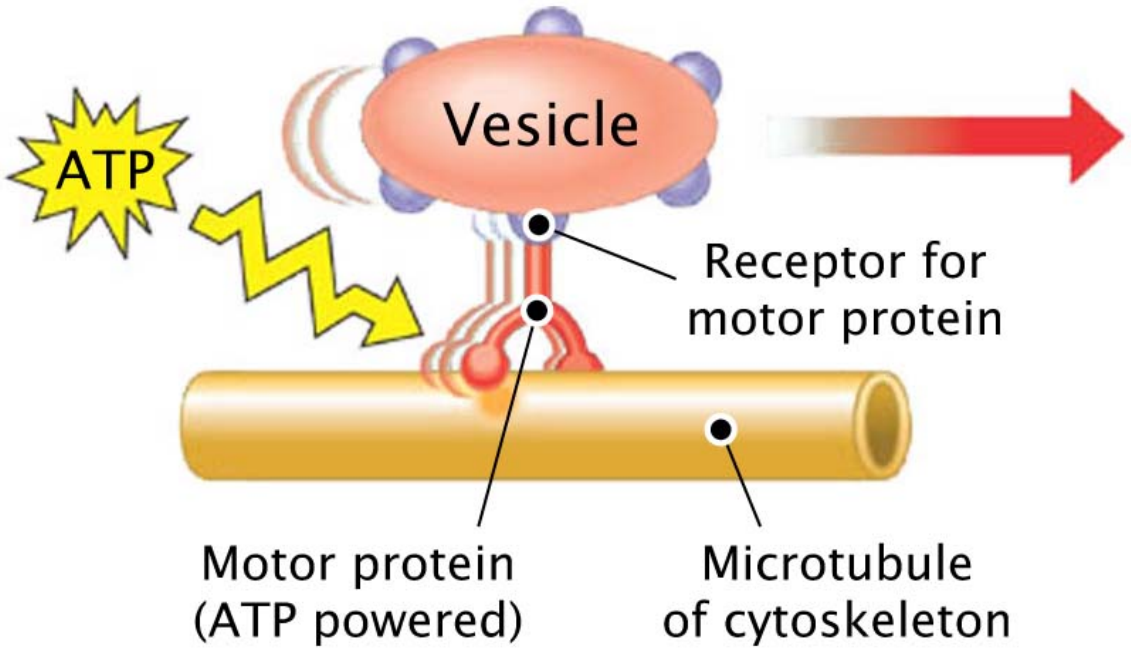


MOTOR PROTEINS



- The type of filament they bind
- Direction in which they move
- Cargo that they carry

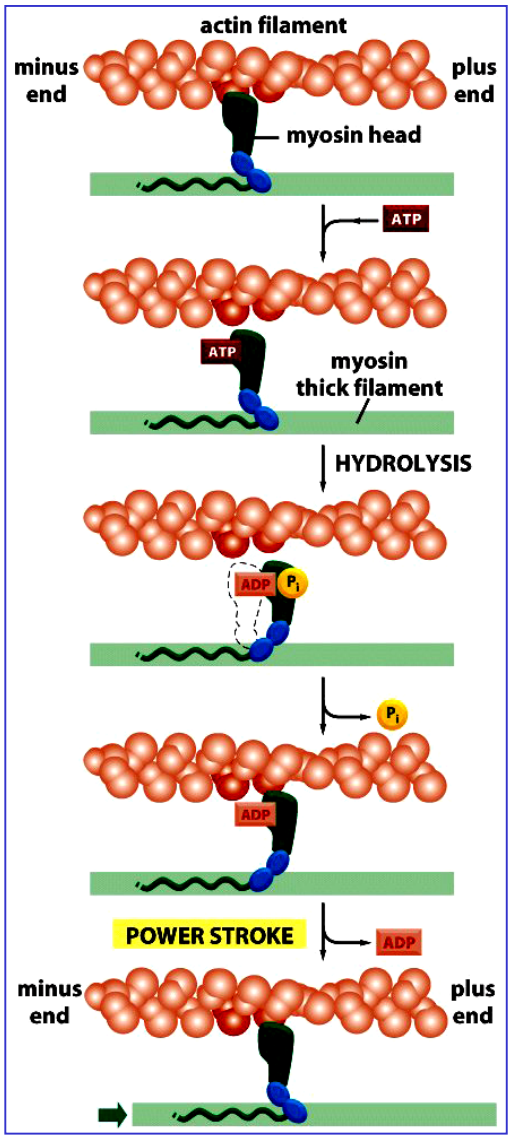
MOTOR PROTEINS

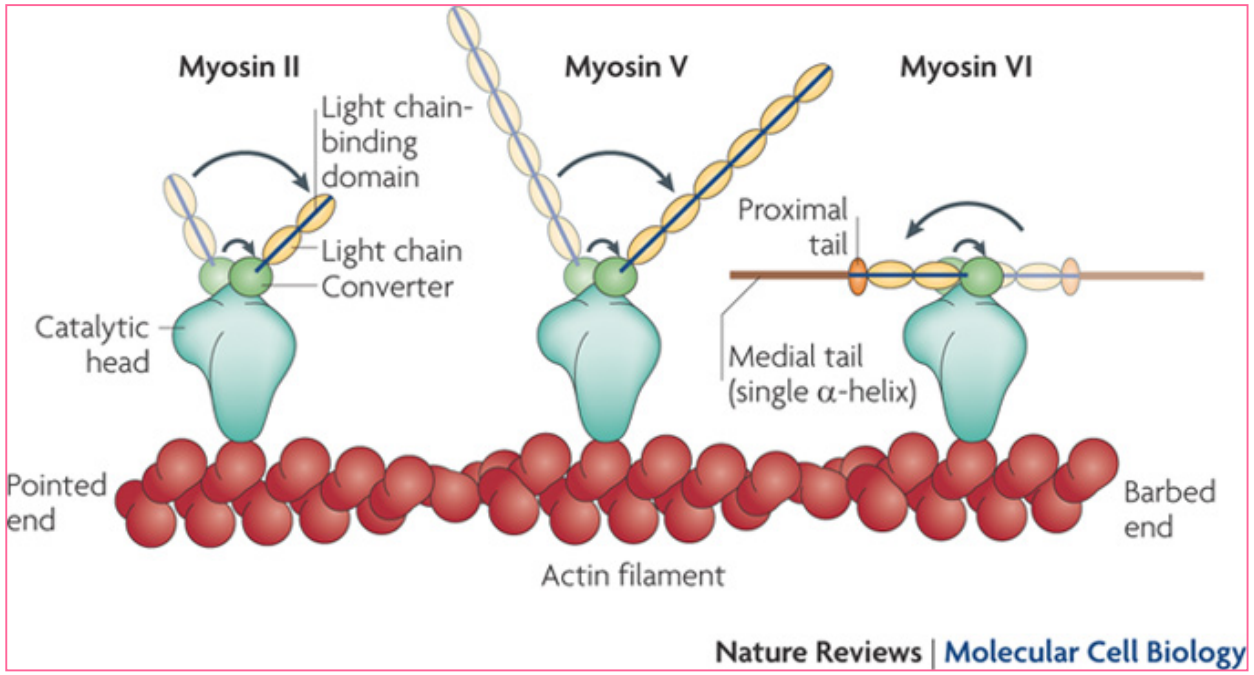


The motor domain identifies the type of filament and the movement direction. The tail determines the identity of the cargo.

MOTOR PROTEINS

actin

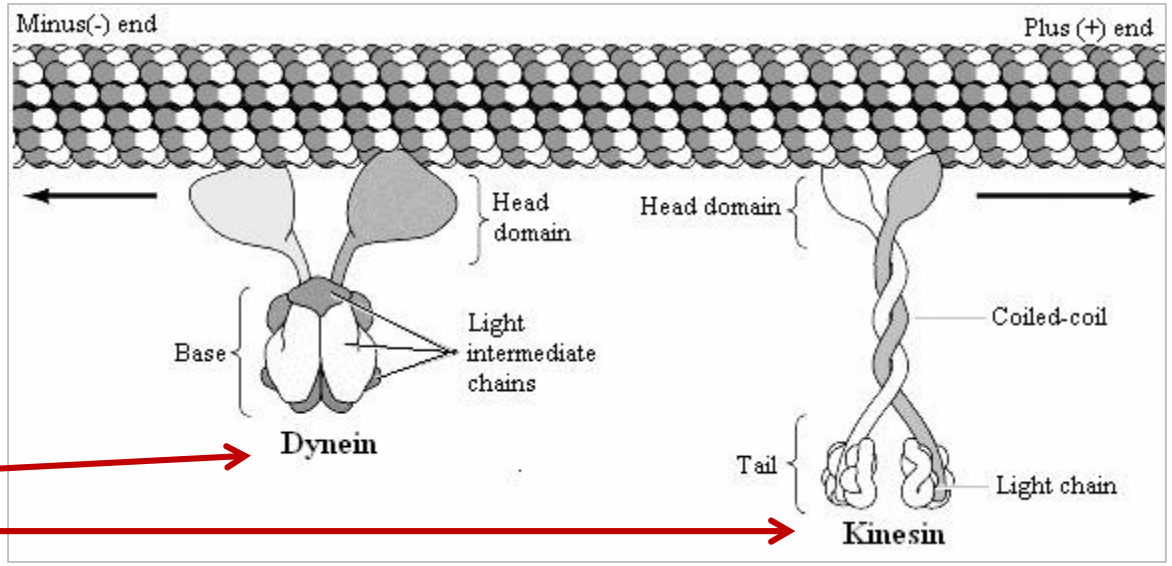


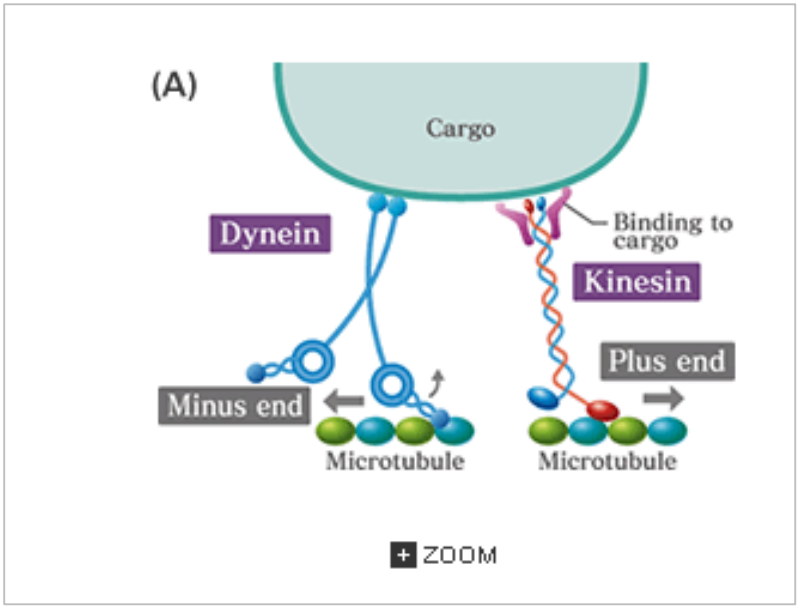


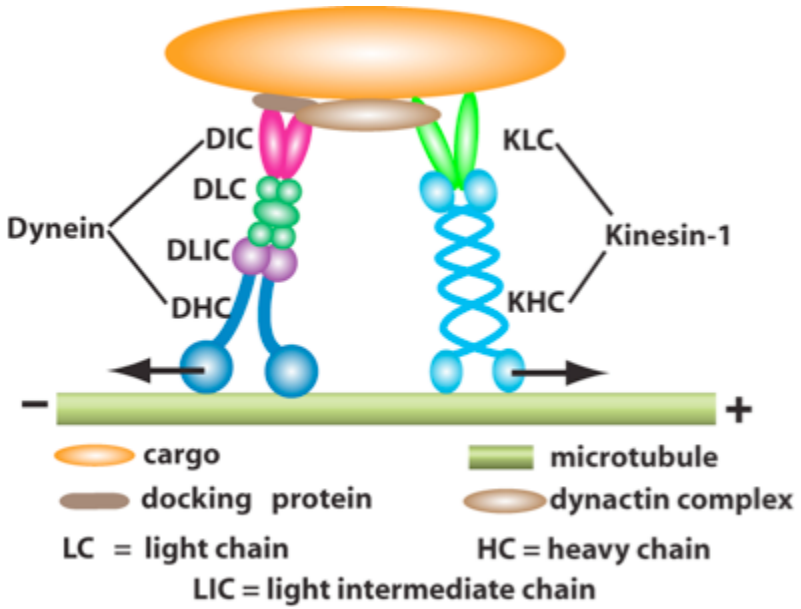
Motor proteins of actin filaments:
Myosins (muscle: myosin II)

MOTOR PROTEINS

microtubules







MOTOR PROTEINS

Kinesin

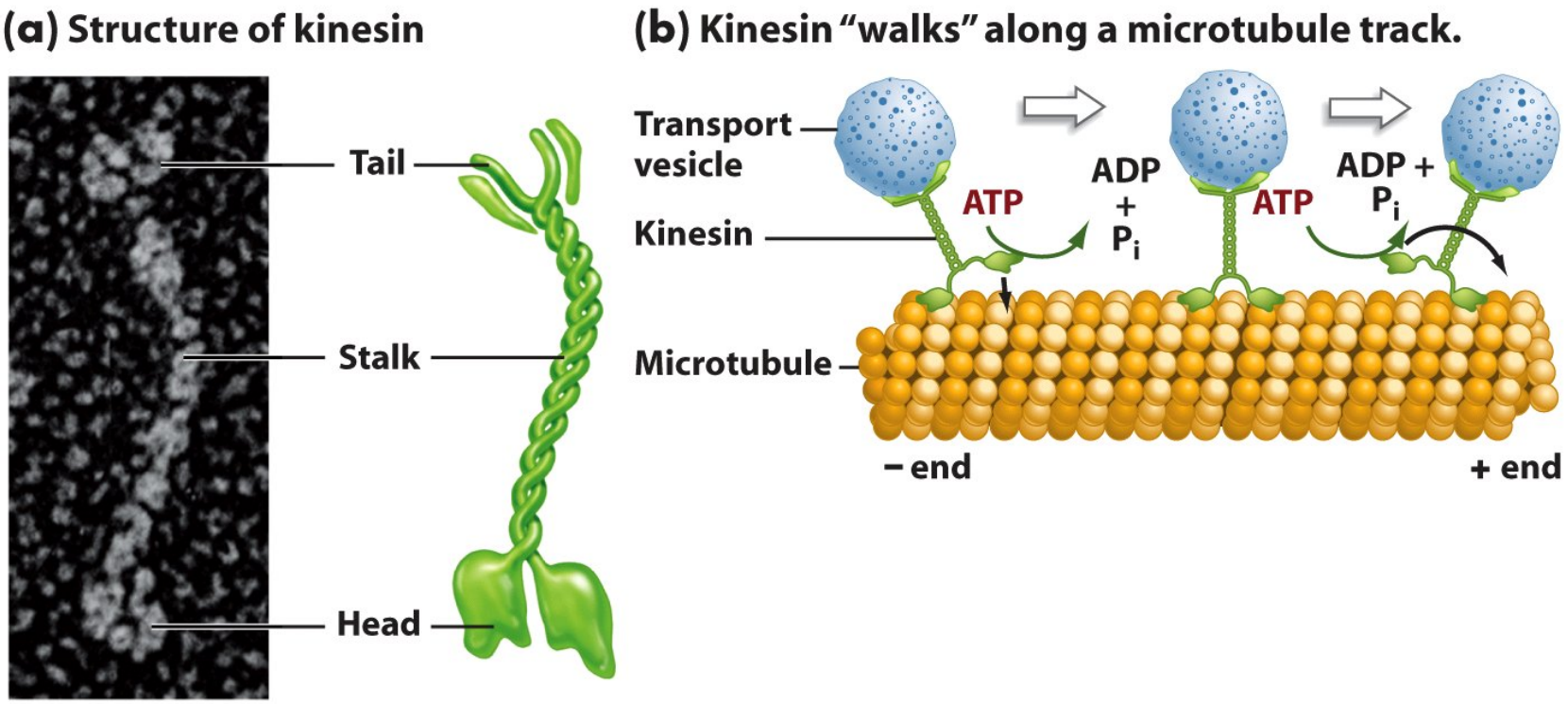
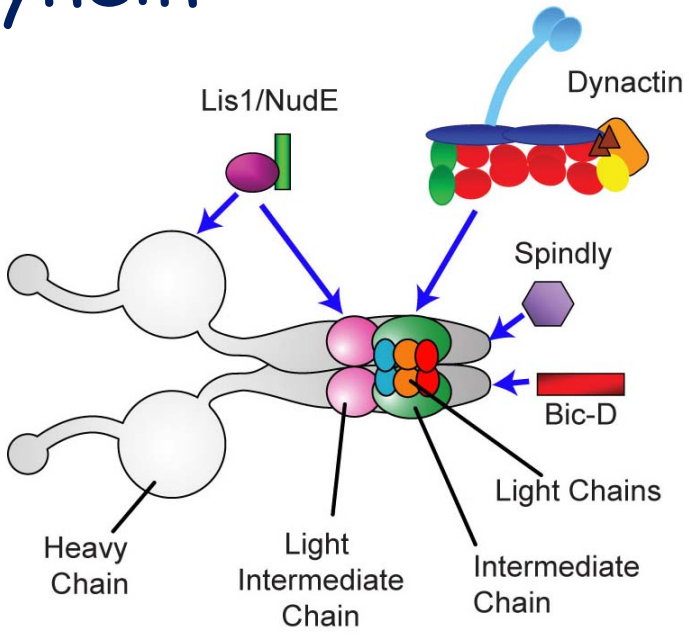
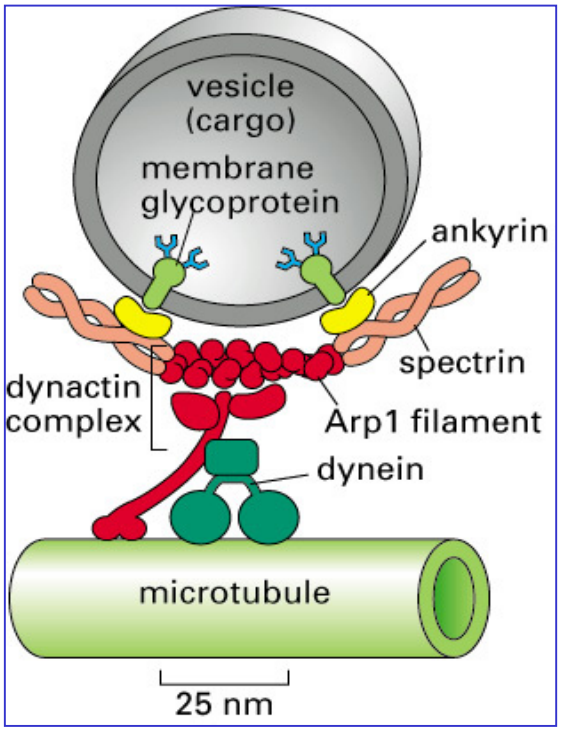


Figure 7-37 Biological Science, 2/e

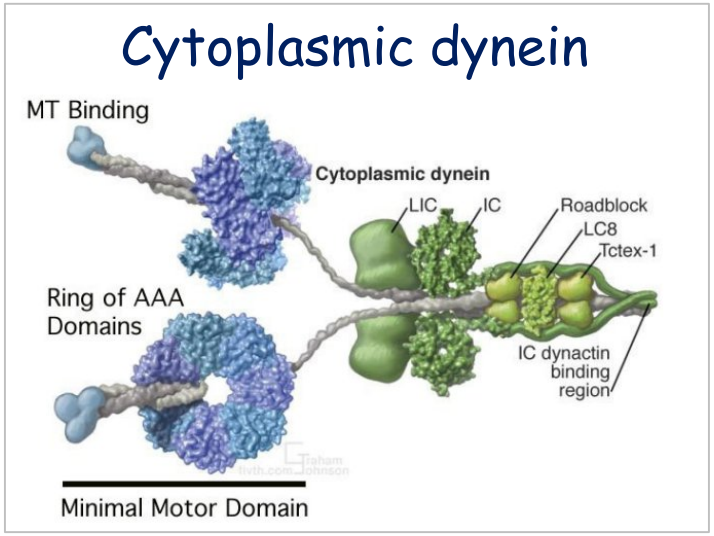
MOTOR PROTEINS

Dynein

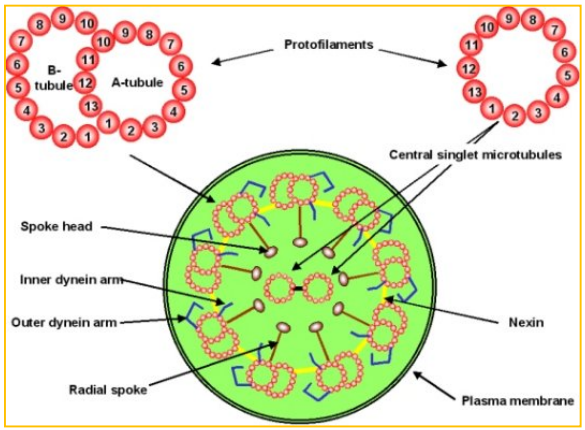




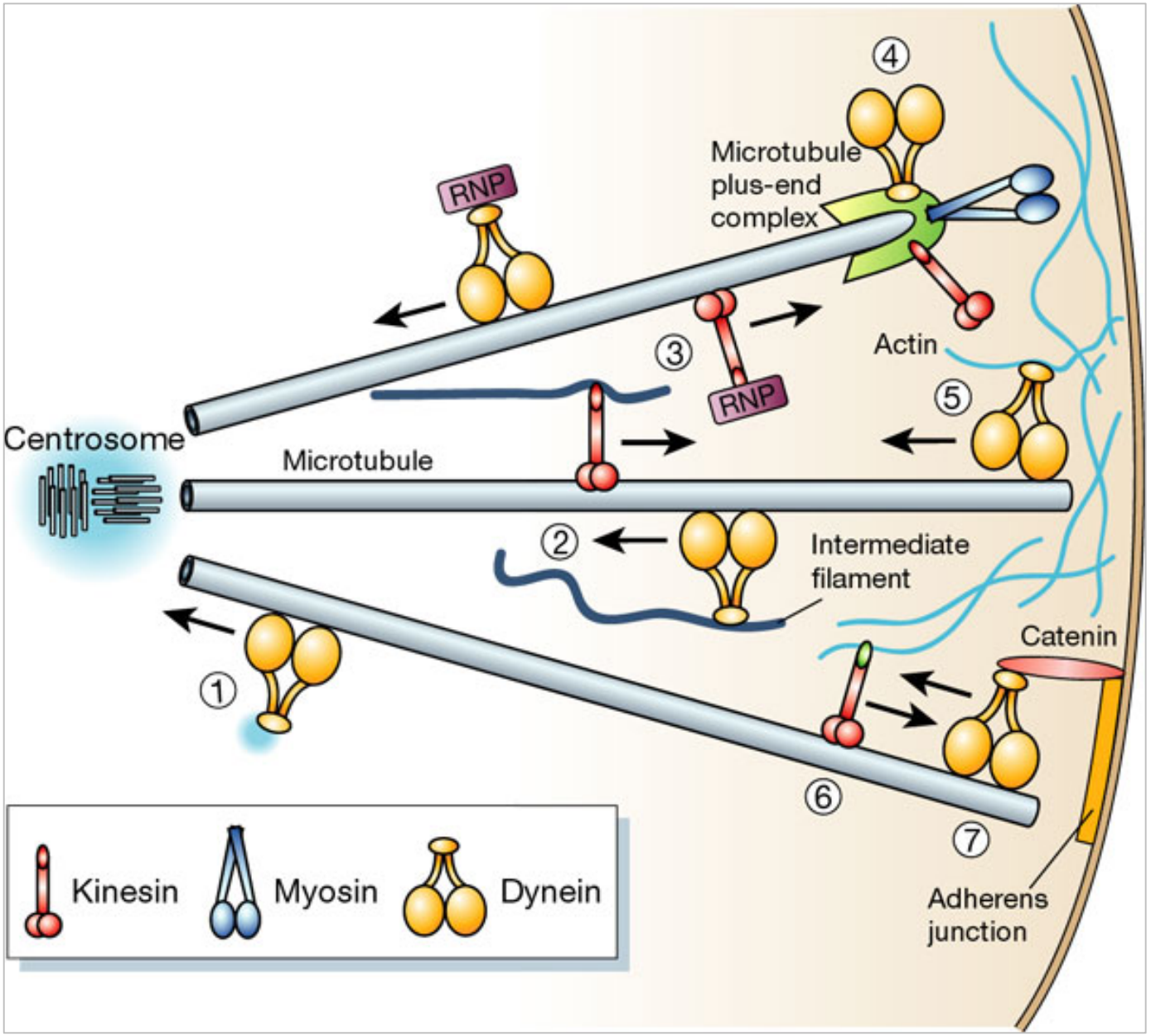
Cytoplasmic dynein



Axoneme dynein



MOTOR PROTEINS



REGULATION OF THE CYTOSKELETAL FILAMENTS

Accessory proteins

Filament nucleation

Filament elongation

Filament stabilization

Filament cross-linking

Filament severing

Binding of filaments with the cell membrane

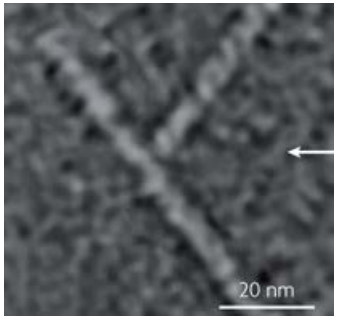
Response to extracellular signals

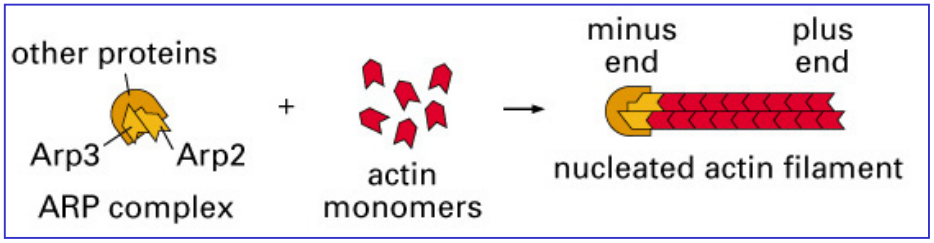
REGULATION OF THE CYTOSKELETAL FILAMENTS

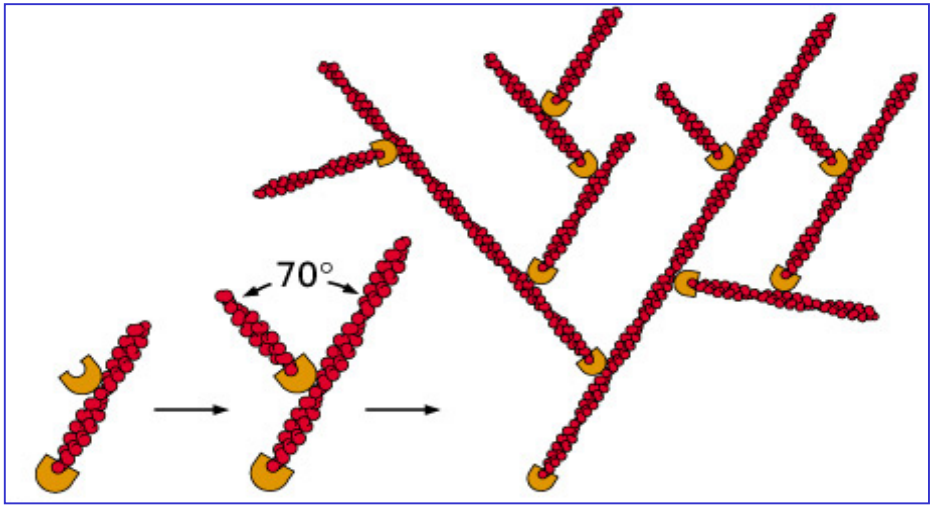
Filament nucleation

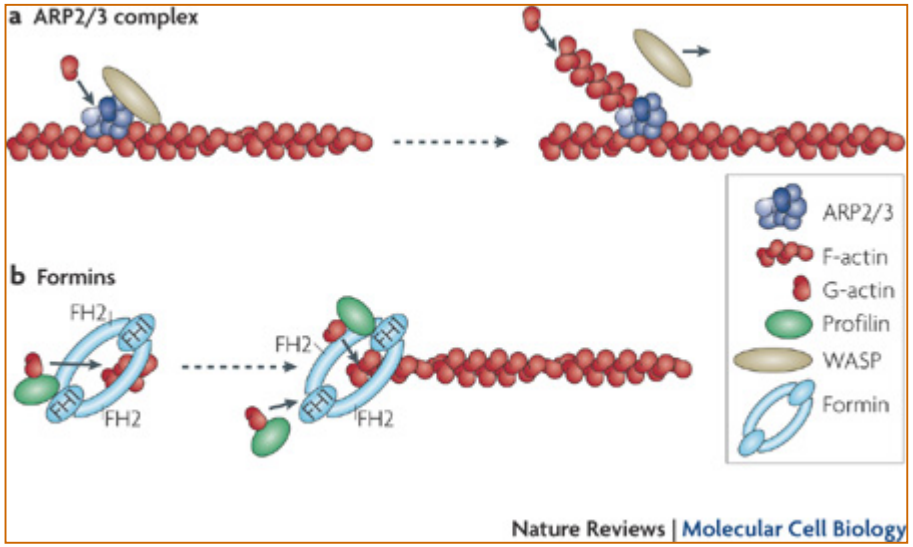
Microtubules (MTOC)

Actin (Formins, ARP complex)





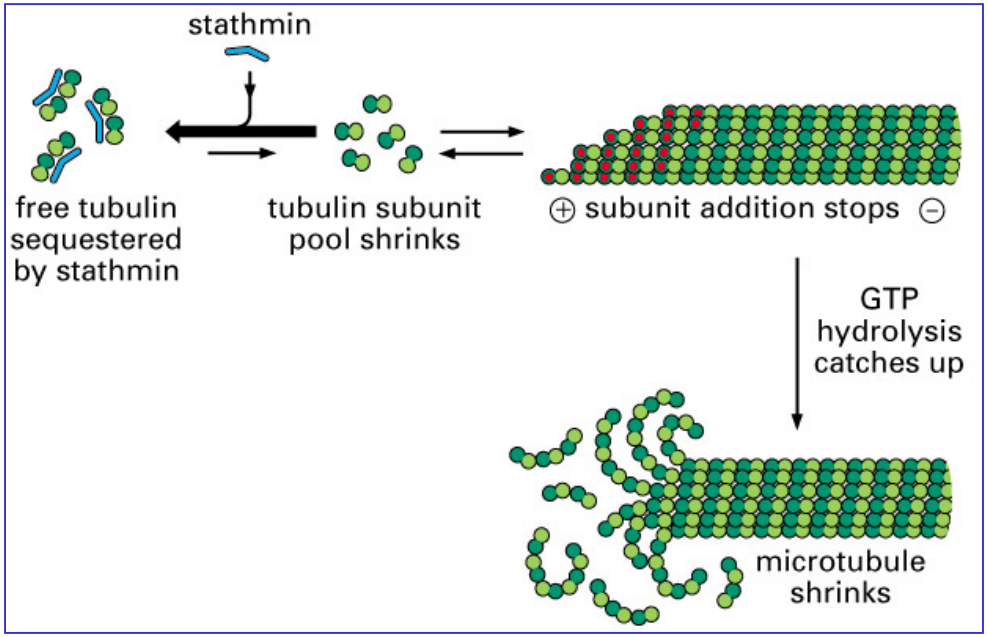


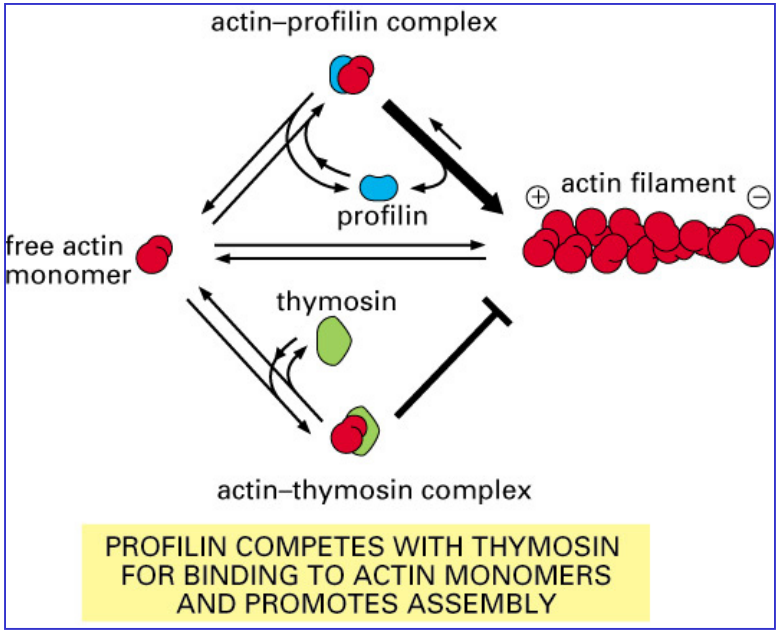


REGULATION OF THE CYTOSKELETAL FILAMENTS

Filament elongation

Microtubules (stathmin)
Actin (profilin, thymosin)

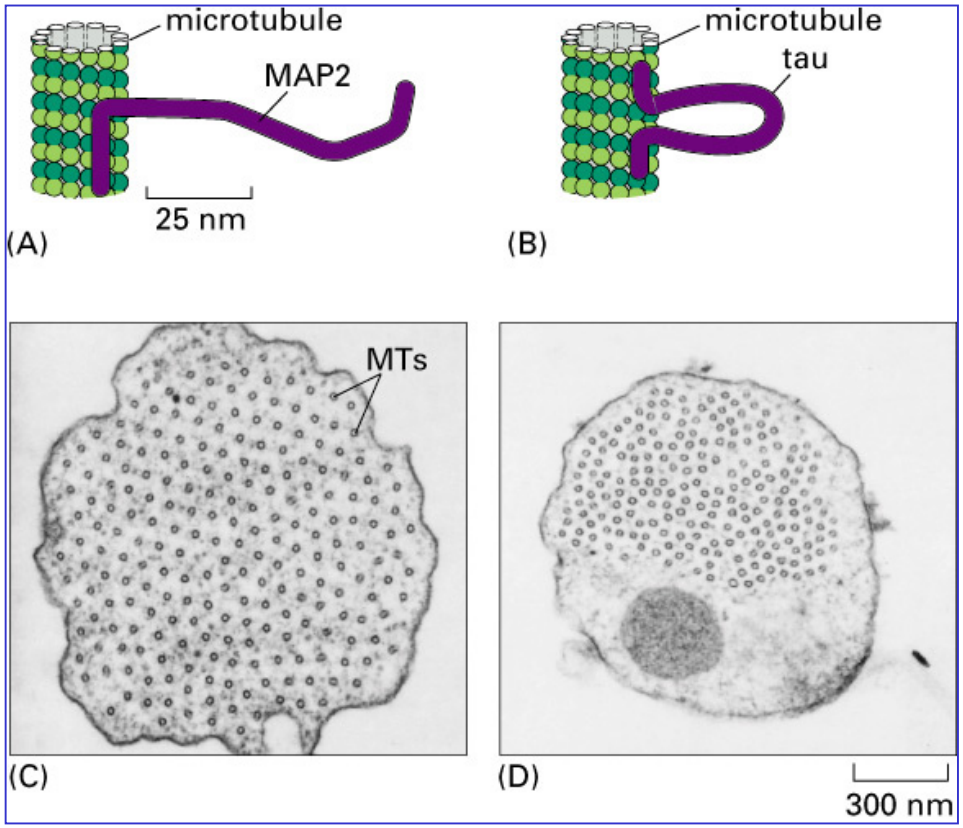




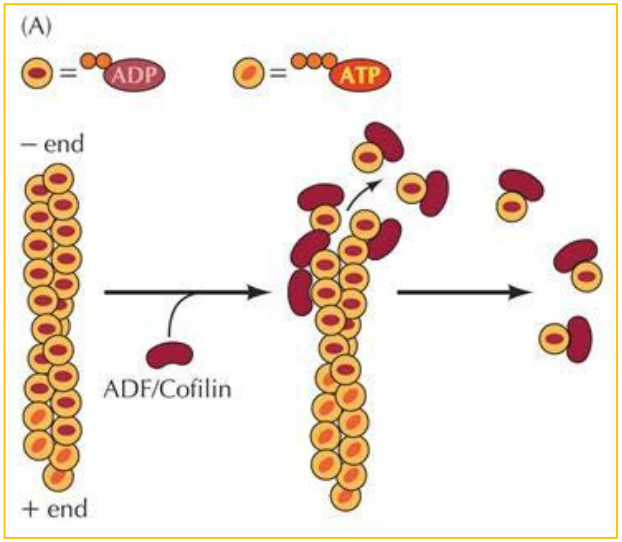
REGULATION OF THE CYTOSKELETAL FILAMENTS

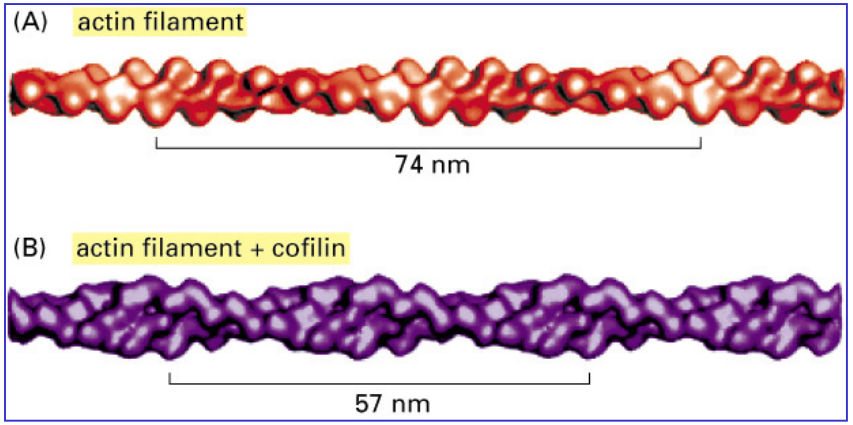
Filament stabilization and destabilization

Microtubules (MAP2, tau)



Actin (cofilin)



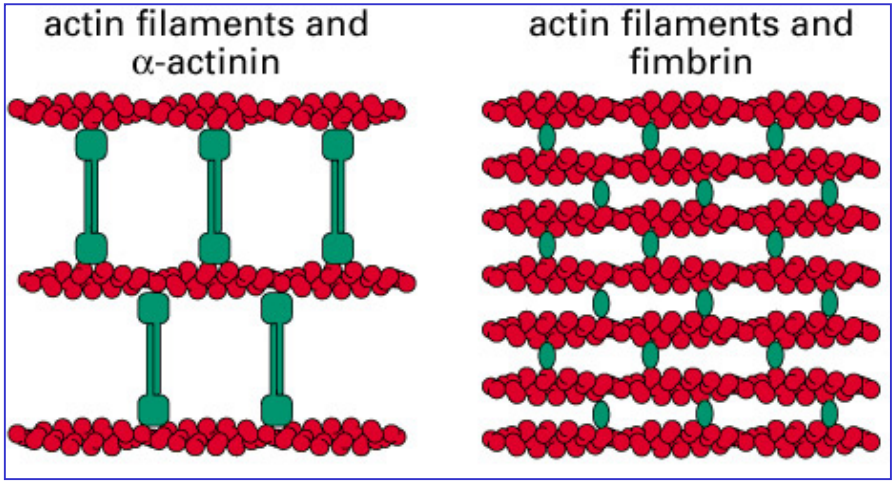


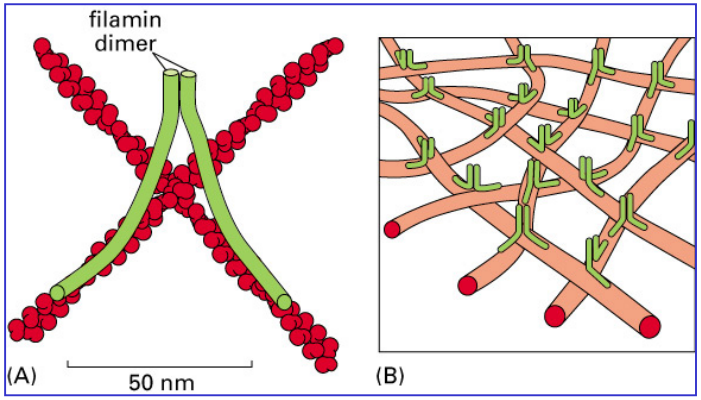
REGULATION OF THE CYTOSKELETAL FILAMENTS

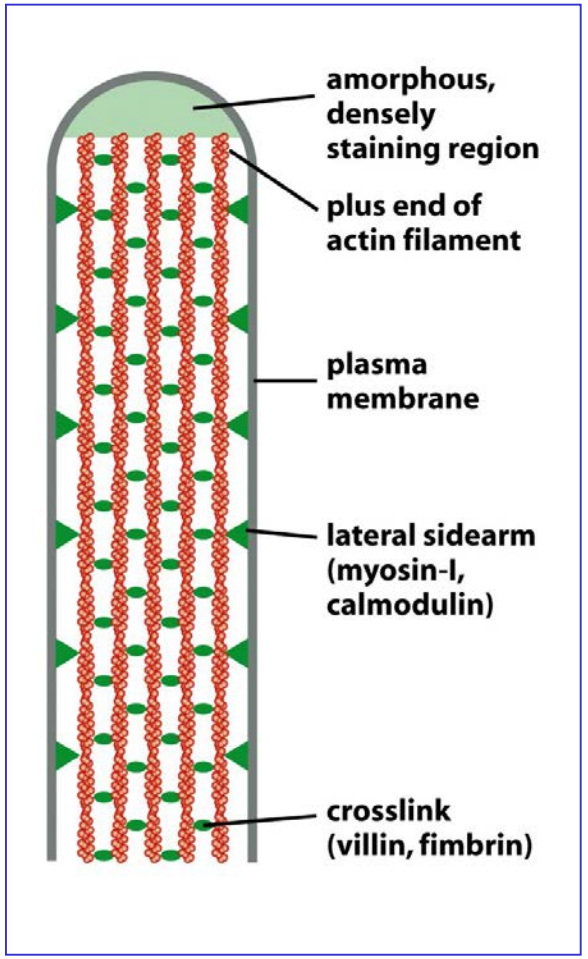
Filament cross-linking

Actin

Bundles (fimbrin, a-actinin, villin, myosin I)
Gel-like networks (spectrin, filamin)





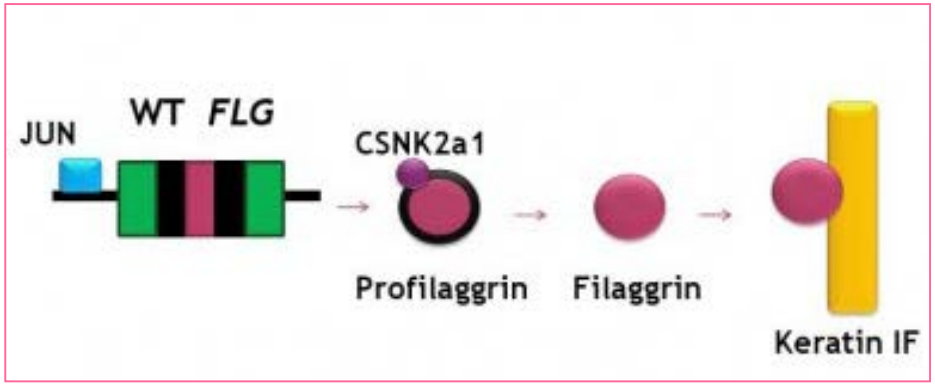


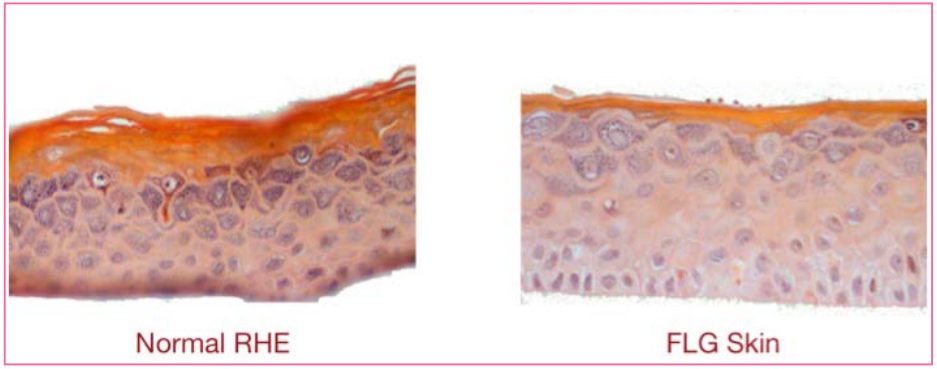
REGULATION OF THE CYTOSKELETAL FILAMENTS

Filament cross-linking

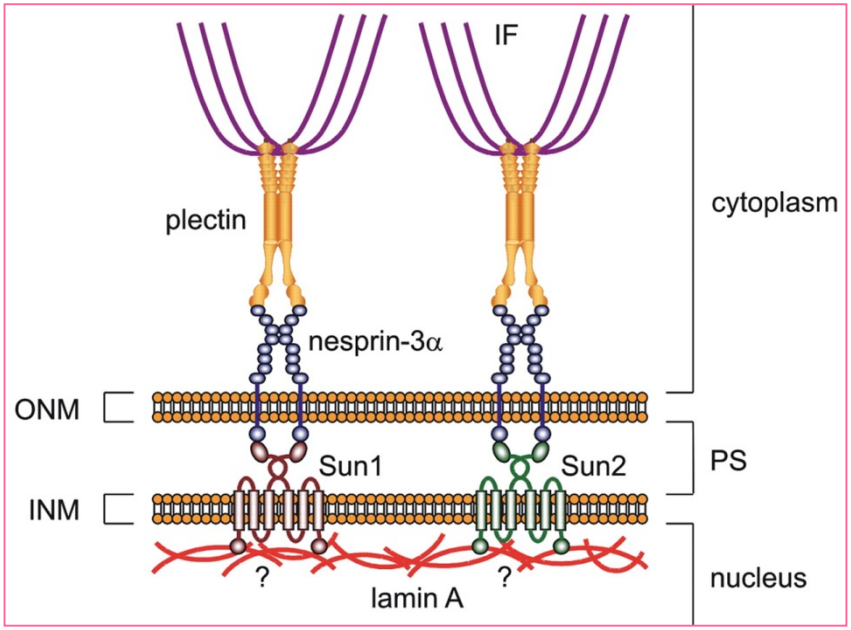
Intermediate filaments

Filaggrin





Plectin

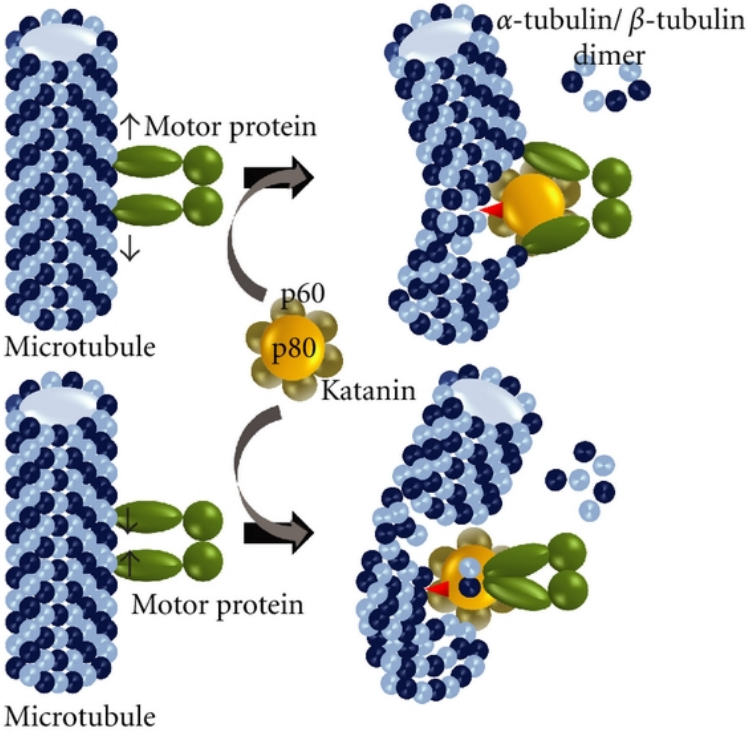


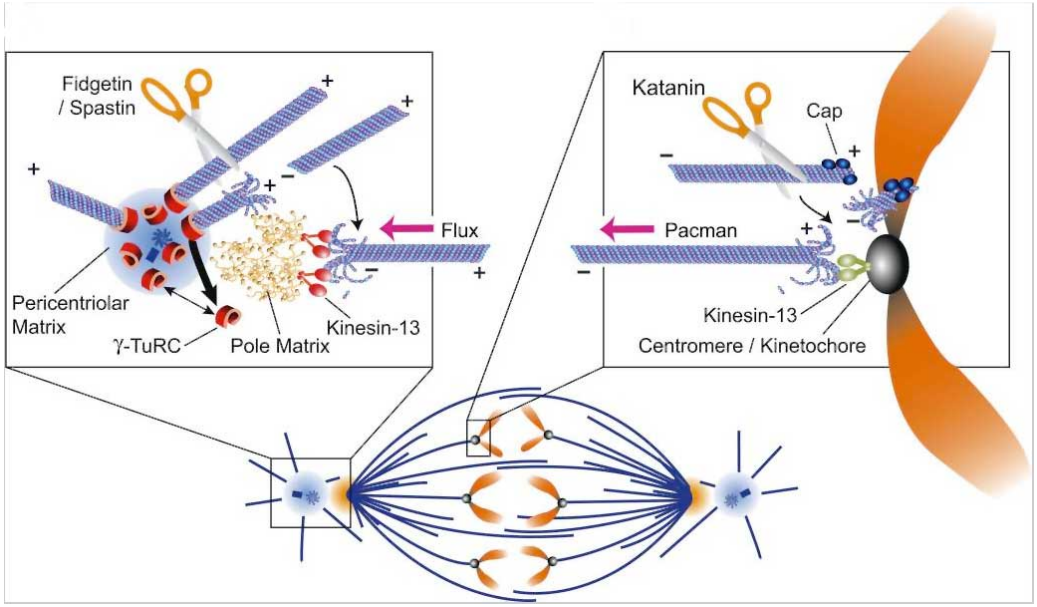
REGULATION OF THE CYTOSKELETAL FILAMENTS

Filament severing

Microtubules

katanin



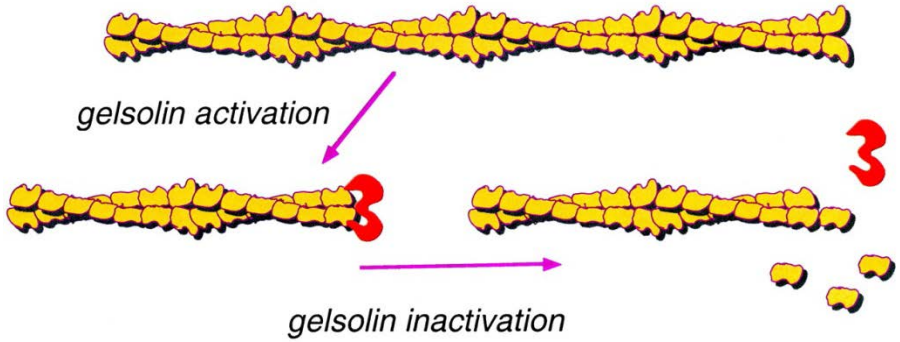


REGULATION OF THE CYTOSKELETAL FILAMENTS

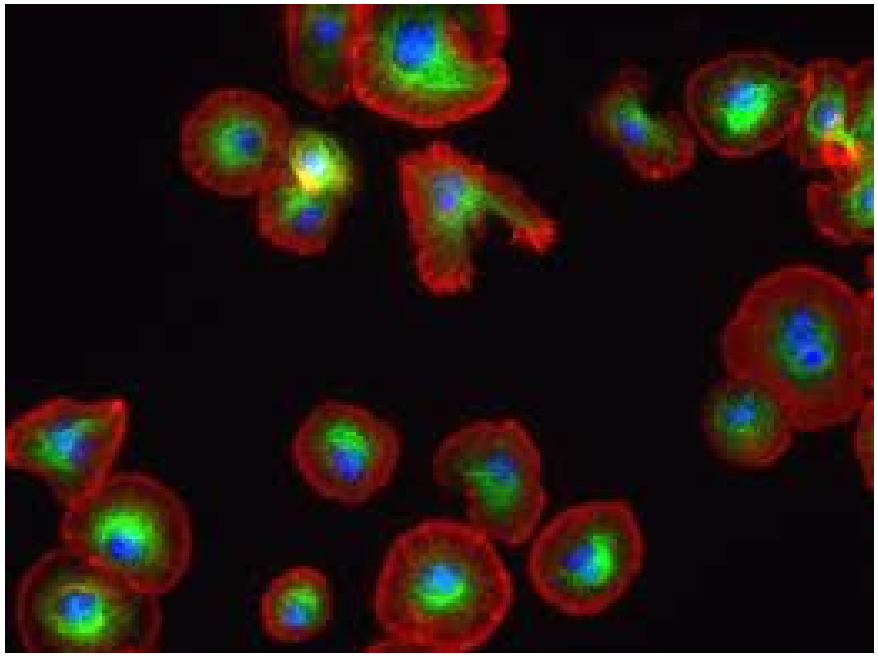
Filament severing

Actin

gelsolins

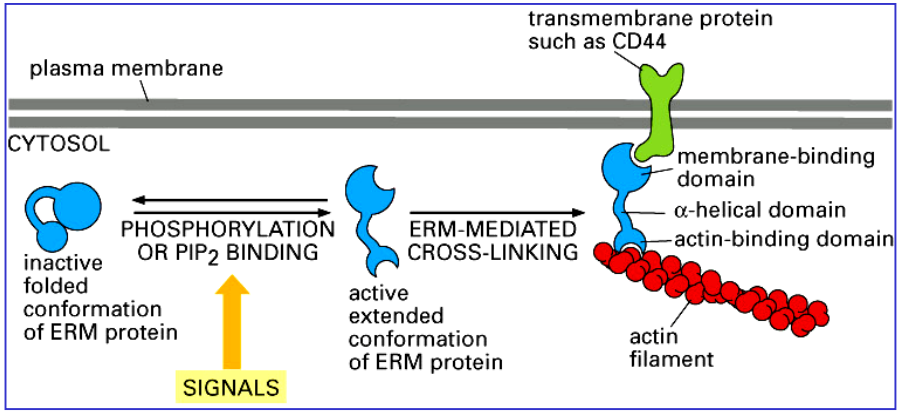


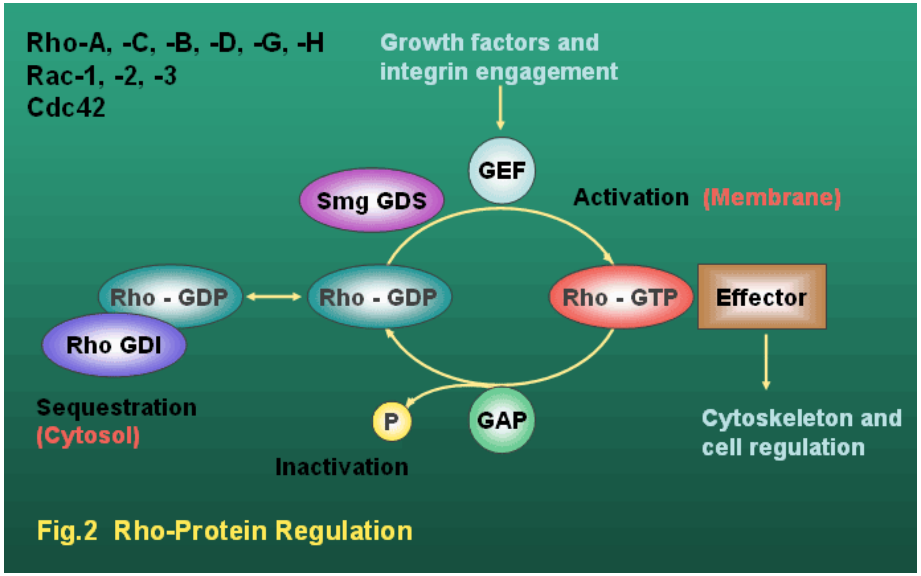
actin filaments
↓ filamin or other cross-linker
cross-linked actin network (gel)
↓ Ca^{++}-activated gelsolin severs & caps (+) end of actin filaments.
smaller filament fragments (sol)



REGULATION OF THE CYTOSKELETAL FILAMENTS

- Filament binding to the cell membrane
  - Actin
    - Junctional complexes: cadherins, integrins
    - ERM family (neurofibromatosis)
- Response to extracellular signals
  - Actin
    - Rho proteins





ASSIGNATURA: BIOLOGIA (34446).
GRAU: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

ASIGNATURA: BIOLOGÍA (34446).
GRADO: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

THE CELL NUCLEUS

1. General characteristics
2. Chemical composition
3. Ultrastructure
    - 3.1. Nuclear envelope
    - 3.2. Chromatin
    - 3.3. Nucleolus
    - 3.4. Extranucleolar RNP
4. Biogenesis

THE CELL NUCLEUS **1. General characteristics**

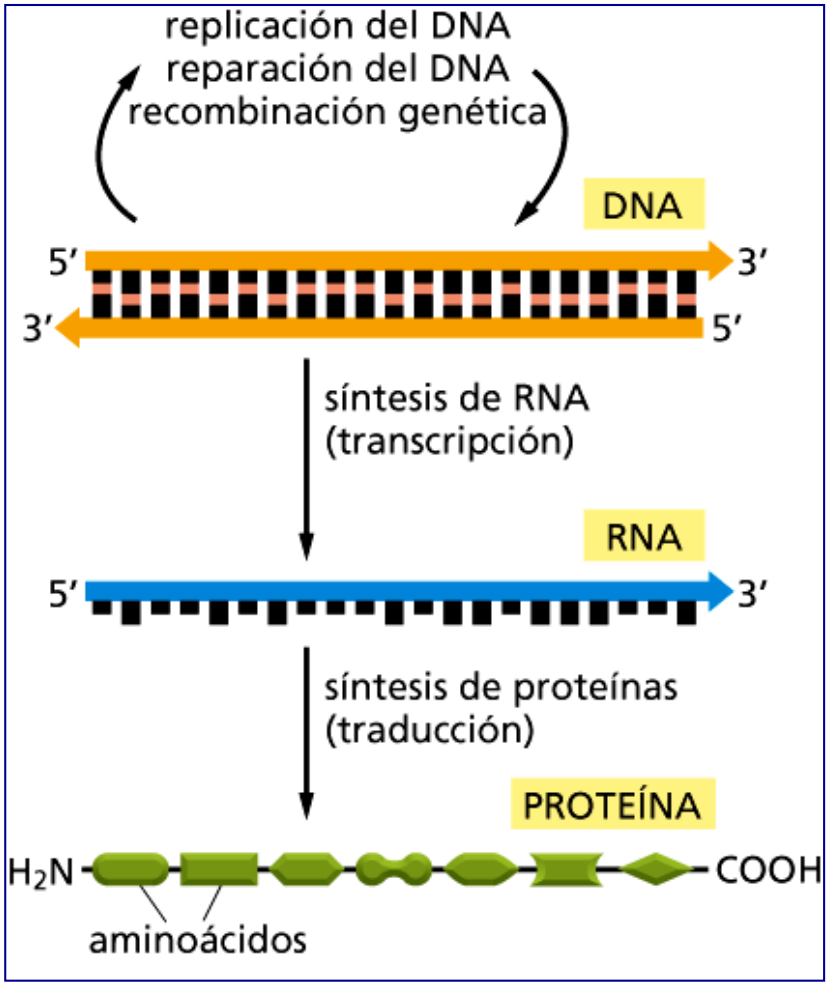
Functions

1. Container of the genetic information of the cell as chromatin

2. Expression of genetic information: DNA transcription, hnRNA maturation, rRNA processing, tRNA, etc

3. Replication of genetic information (S-phase of the cell cycle)

4. Assembling of ribosomes

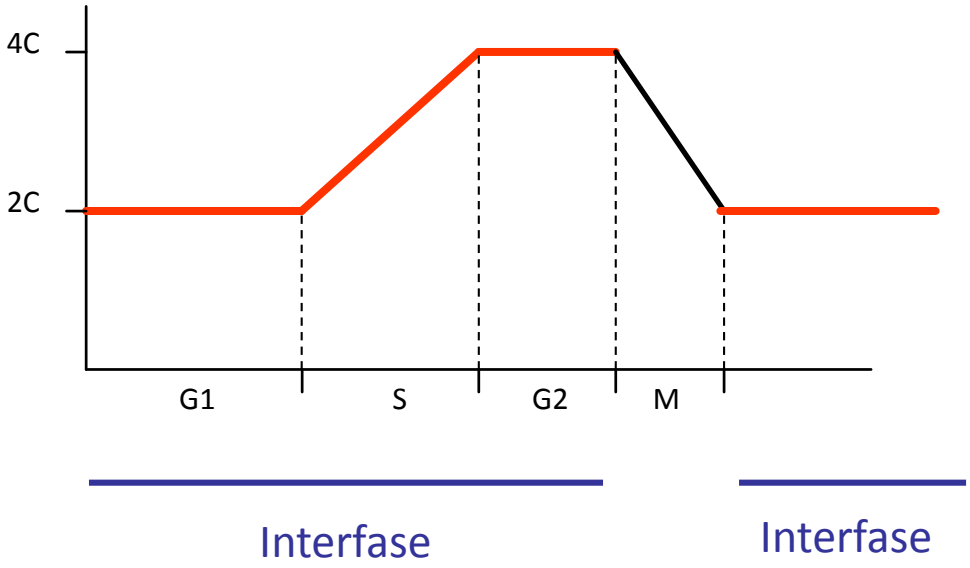


The nucleus requires the existence of a highly elaborated transport system that allows cells to exchange molecules between these compartments in a tightly regulated and efficient manner.

THE CELL NUCLEUS **1. General characteristics**

The structure of the nucleus changes along the cell cyle

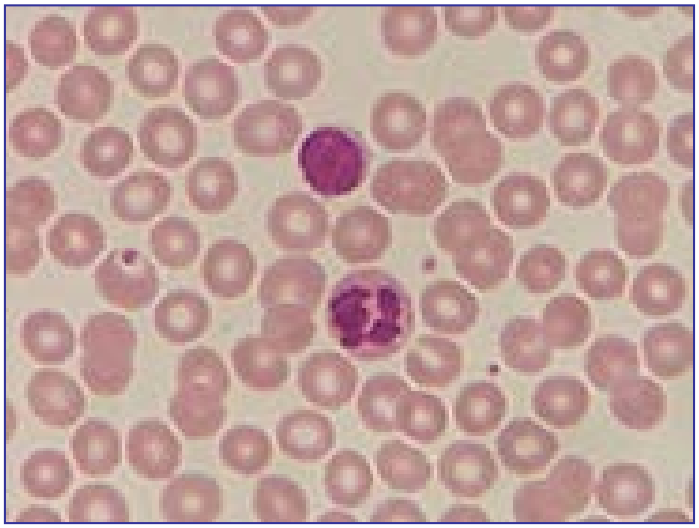
We will discuss this structure during interphase



THE CELL NUCLEUS **1. General characteristics**

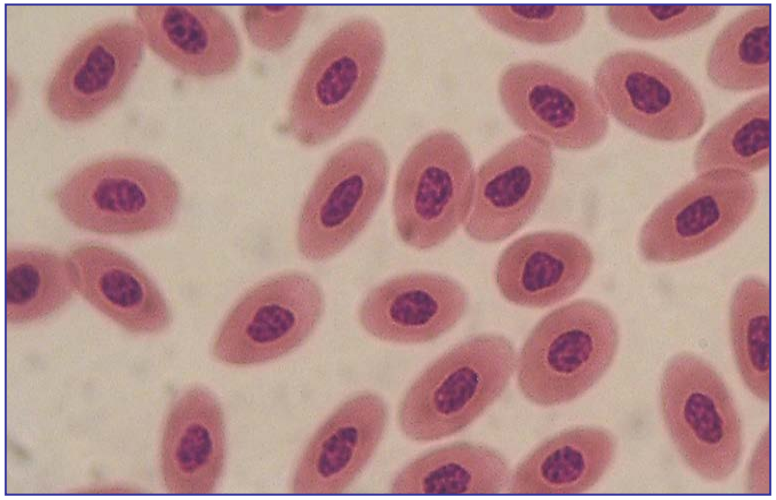
Optical Microscopy

1. Shape
2. Size
3. Number



Syncytium

Plasmodia



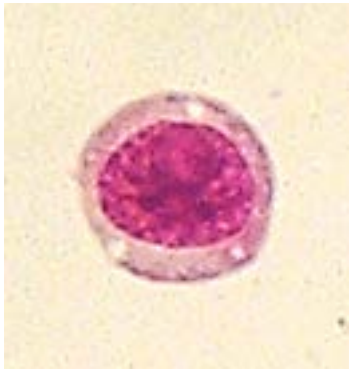
$$NP = V_N / V_C = V_N / V_T - V_N$$

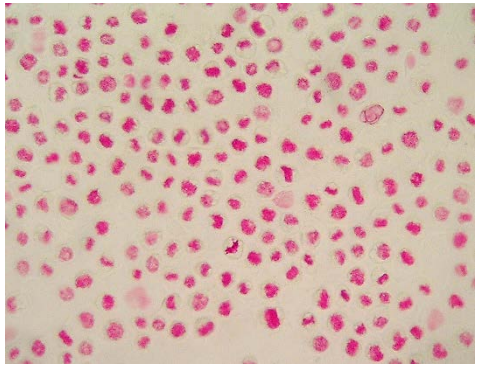
THE CELL NUCLEUS **2. Chemical composition**

Cytochemistry

Basic dyes

Feulgen stain (DNA)





Chemical analysis

DNA: Bound to proteins: histones and no histones

RNA: Codifying (mRNA)

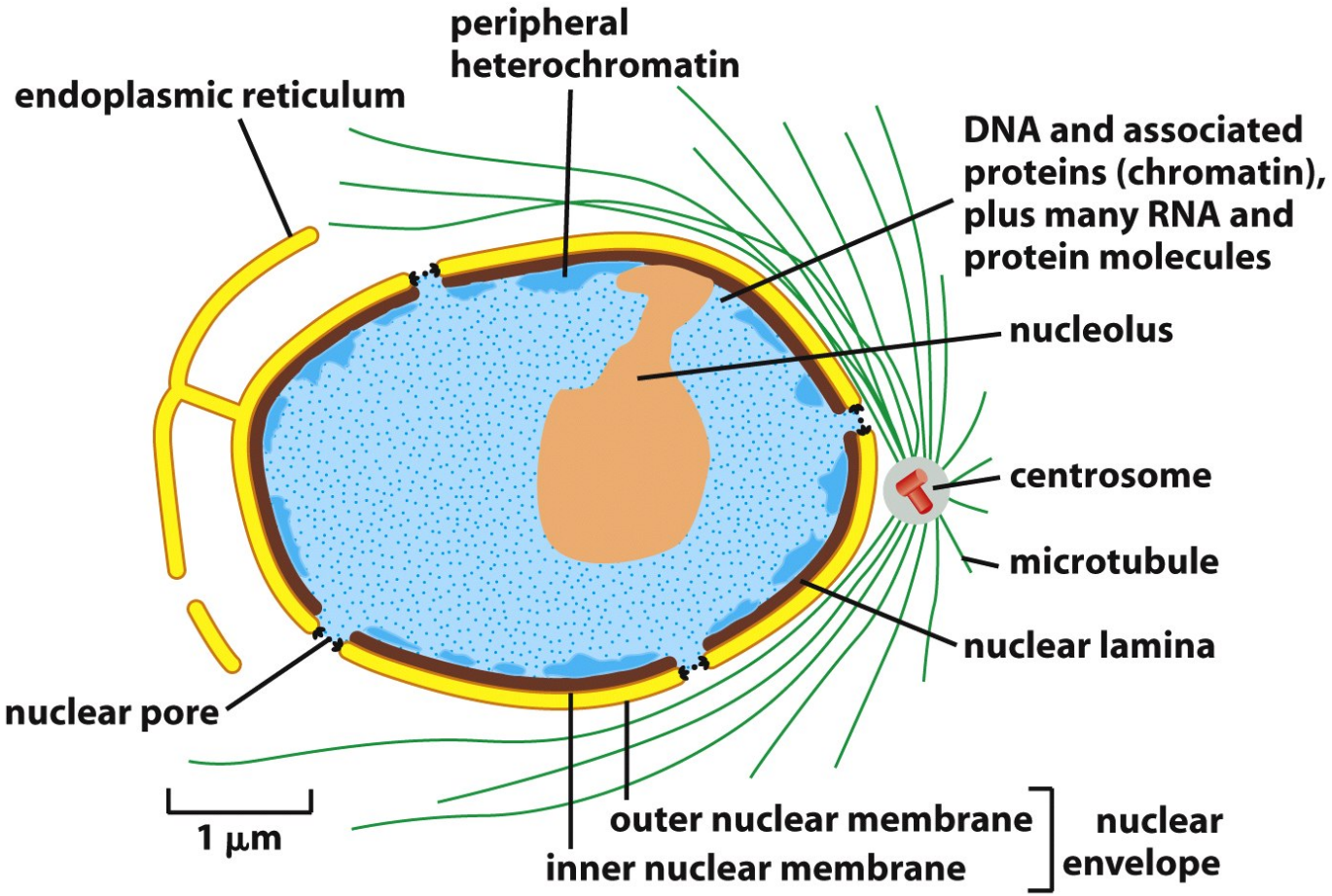
No codifying: rRNA
tRNA
Telomerase RNA
Spliceosome RNA
Silencing RNA
Intron RNA

Proteins: Structural, regulatory, enzymes, transporters, etc

Salts: Mg, Na, K…….

THE CELL NUCLEUS **3. Ultraestructure (EM)**

- Nuclear envelope (karyotheca)
- Chromatin
- Nucleolus
- Extranucleolar RNP



Molecular Biology of the Cell ©

THE CELL NUCLEUS **3. Ultraestructure (EM)**

3.1 Nuclear envelope

- Outer membrane
- Inner membrane
- Perinuclear space
- Nuclear lamina
- Pore complex

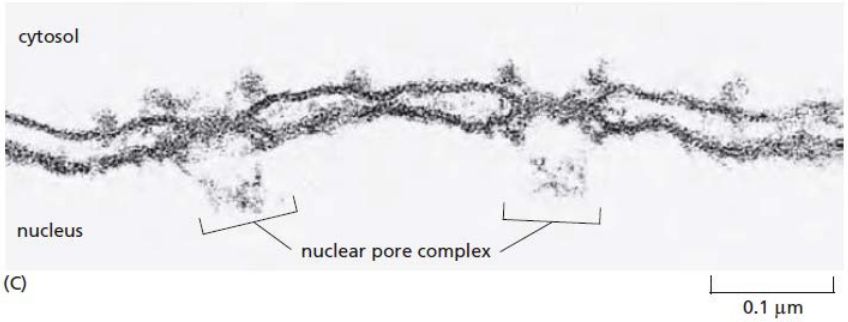


Figure 12-8C *Molecular Biology of the Cell. Sixth edition,* © 2015

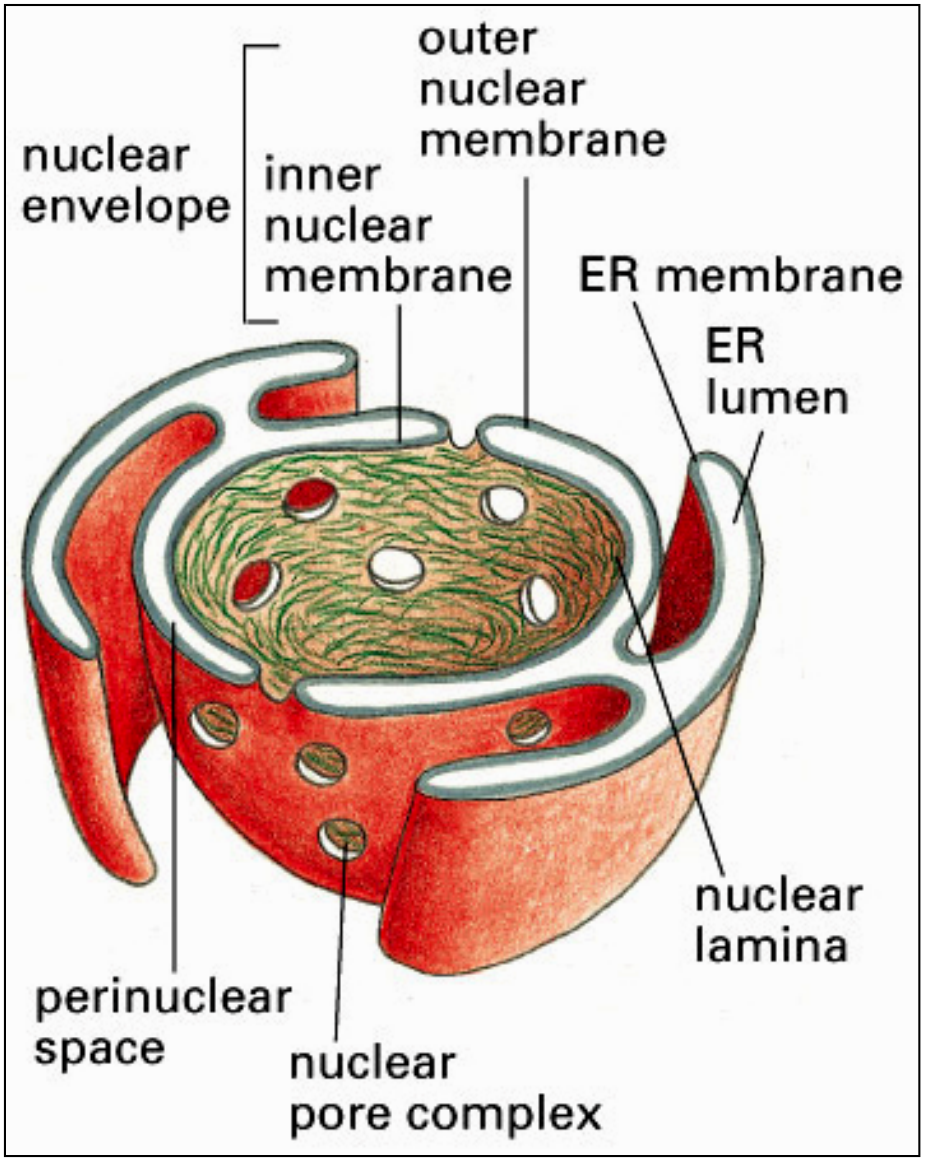


Figure 12-7 *Molecular Biology of the Cell. Sixth edition,* © 2015

THE CELL NUCLEUS. 3.1 Nuclear envelope

Nuclear lamina

A proteic network providing structural support to the nuclear envelope

The nuclear lamina is in contact with the nuclear inner membrane and it contains lamins and associated proteins

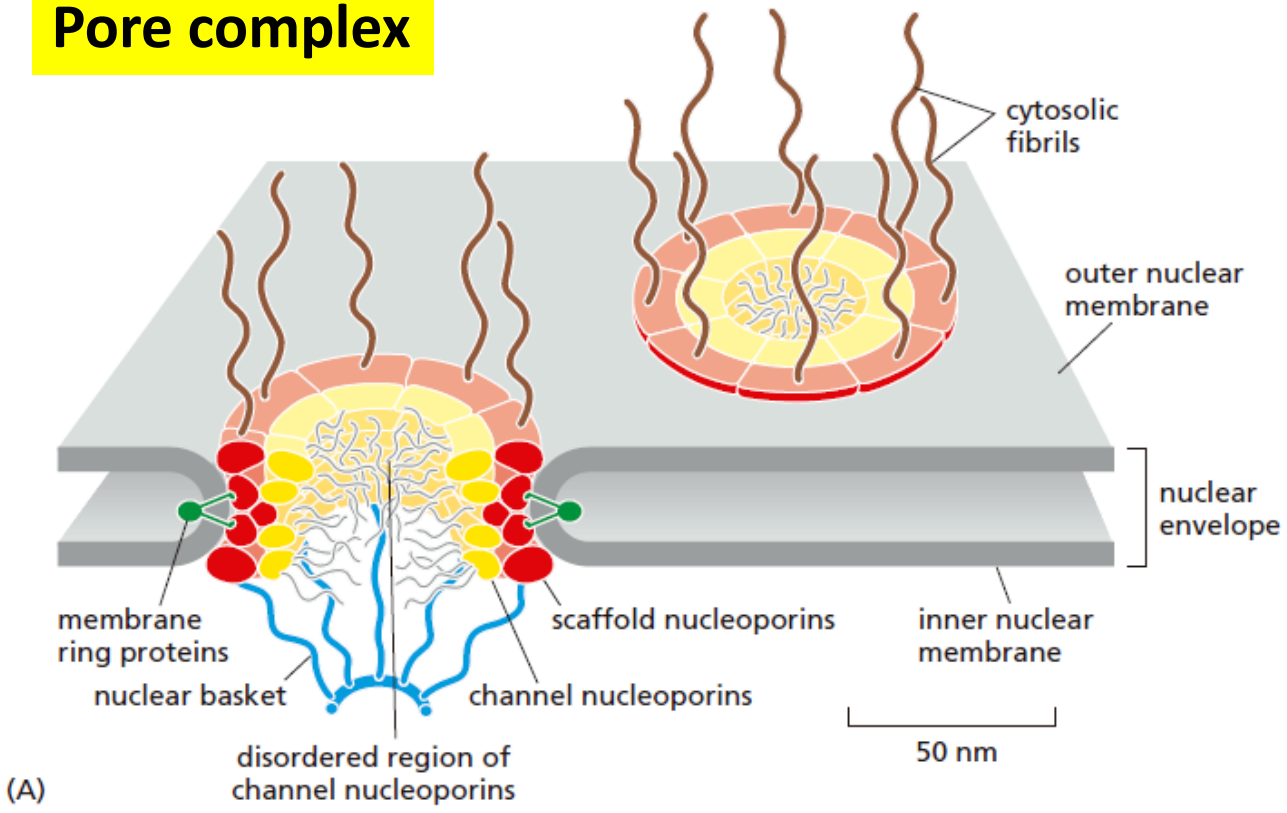
Functions	Nuclear stability Chromatin organitzation Pore Complex linkage

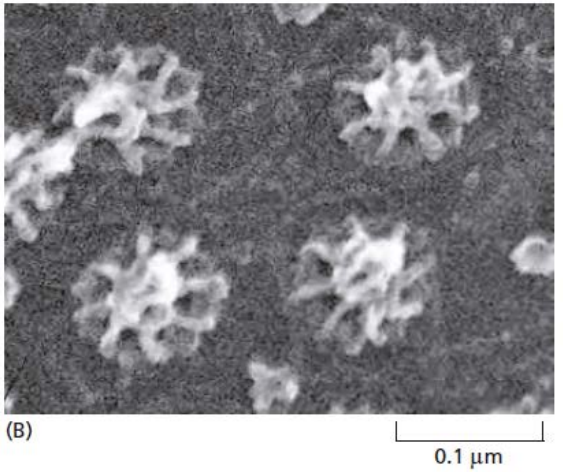
- **Lamins type A: A and C**
- **Lamins type B: B1 and B2**

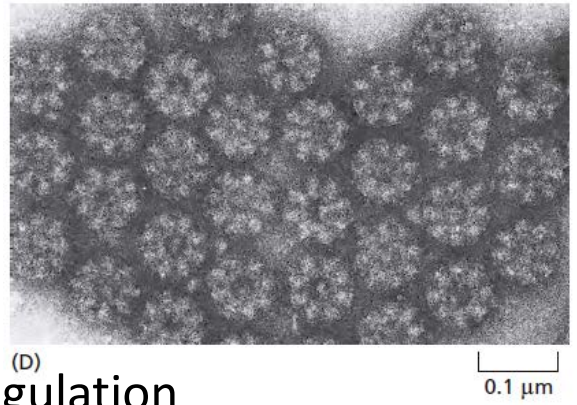
Associated proteins:
LAP1, LAP2
MAN1
Emerin
…

THE CELL NUCLEUS. 3.1 Nuclear envelope

Pore complex







The nucleoporins (NUPs) functions:

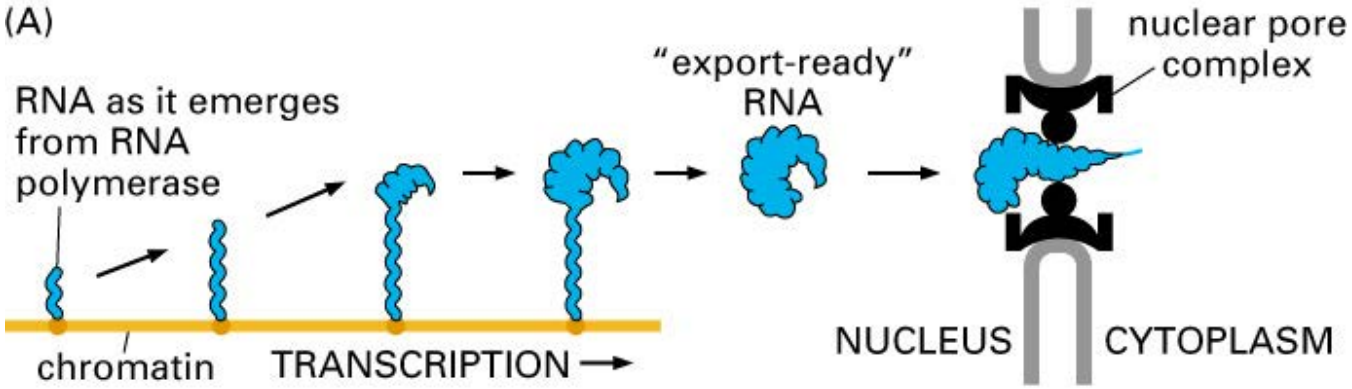
Nuclear transport, genome organization, gene expression regulation

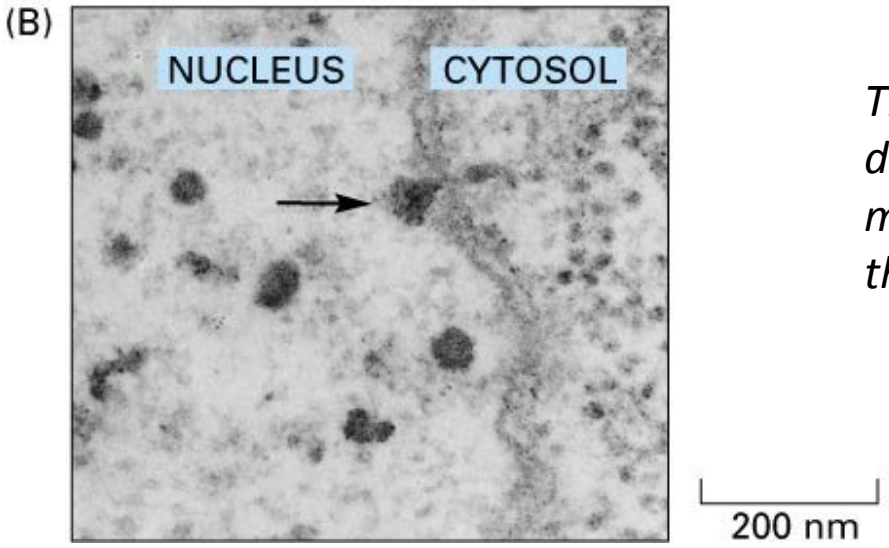
- ***The transport through the pore complex is in both directions***
- ***The pore complex is selective for many molecules***

Figure 12-8 A, B, D *Molecular Biology of the Cell. Sixth edition,* © 2015

THE CELL NUCLEUS. 3.1 Nuclear envelope

Pore complex. Transport





The protein-RNA structure experiments different transitions until mRNA is mature and ready for transport across the pore

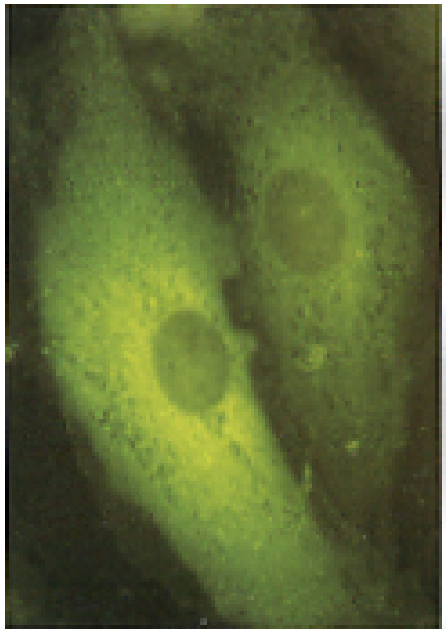
mRNA of the insect Balbiani Ring genes

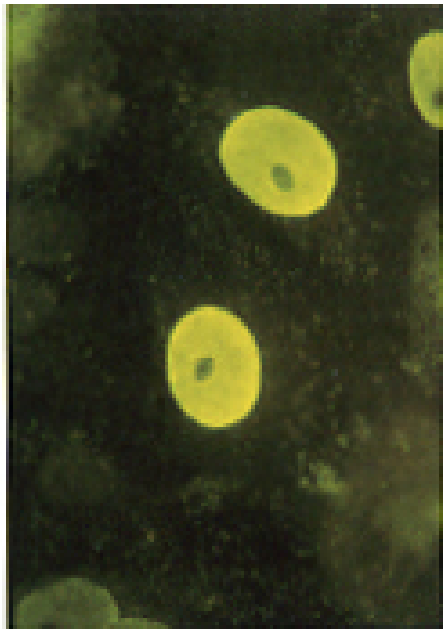
Alberts et al. Fig 6-37

THE CELL NUCLEUS. 3.1 Nuclear envelope

Pore complex. Transport

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-





If a nuclear import **signal sequence** is added to a cytosolic protein, the protein enters the nucleus **(NLSs)**

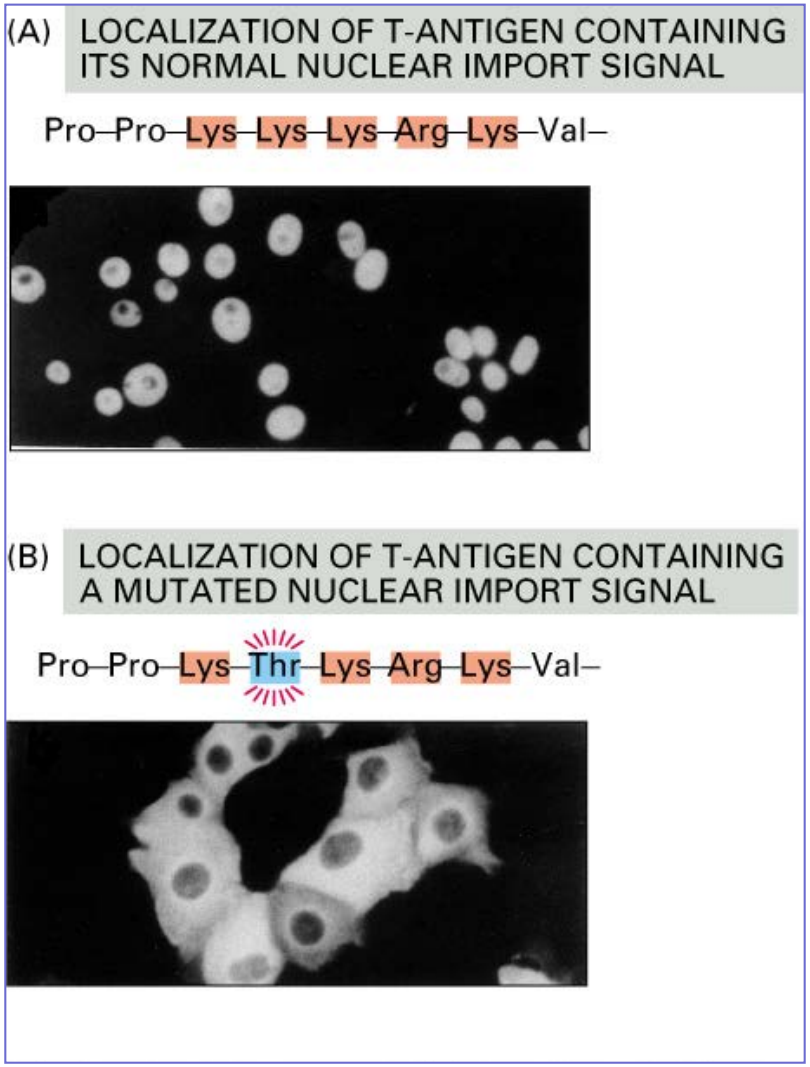


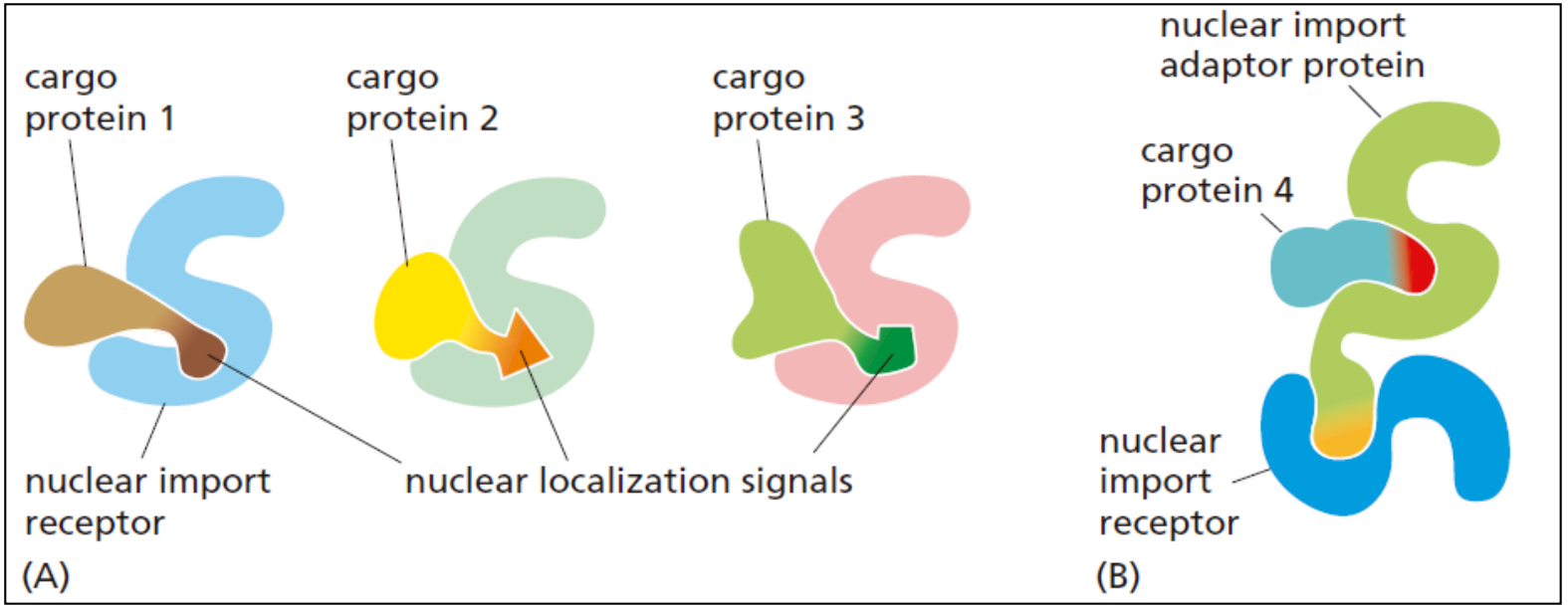
Figure 12-9 *Molecular Biology of the Cell. Sixth edition,* © 2015

Alberts et al. Fig 12-9

THE CELL NUCLEUS. 3.1 Nuclear envelope

Pore complex. Transport

Import receptors



Karyopherins, which may act as importins or exportins

Figure 12-11 *Molecular Biology of the Cell. Sixth edition,* © 2015

THE CELL NUCLEUS. 3.1 Nuclear envelope

Pore complex. Transport

Ran proteins role: monomeric GTPases RAN
specific regulatory proteins: Ran-GAP, Ran-GEF

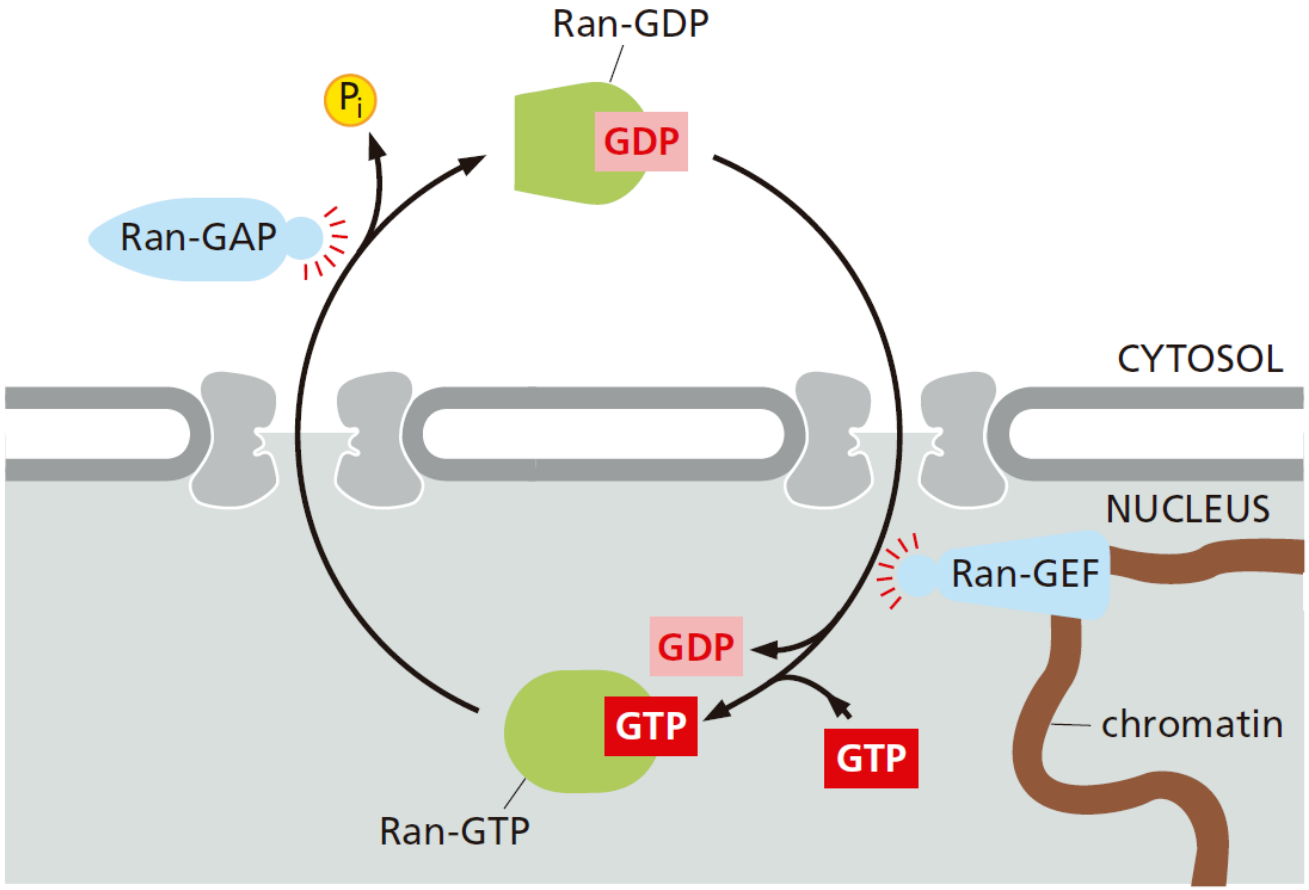
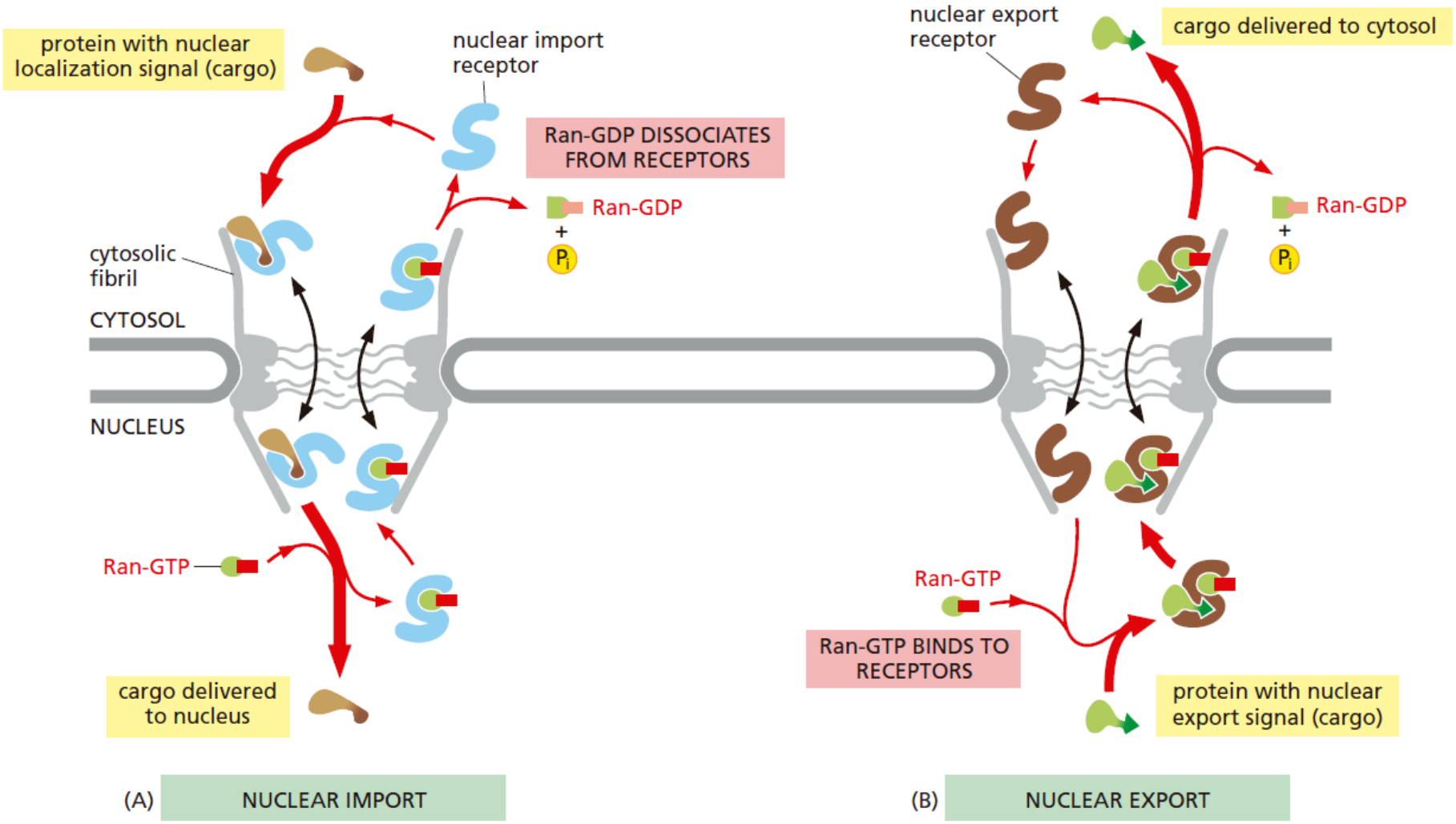


Figure 12-12 *Molecular Biology of the Cell. Sixth edition,* © 2015

THE CELL NUCLEUS. 3.1 Nuclear envelope

Pore complex. Transport



Pore complex. https://www.youtube.com/watch?v=UyhqLpjicZg

Figure 12-13 *Molecular Biology of the Cell. Sixth edition,* © 2015

THE CELL NUCLEUS. 3.2 Chromatin

- It is the morphological form of DNA
- It is stained with basic dyes
- It may be: condensed (heterochromatin)
  extended (euchromatin)
- Condensed chromatin is located preferably by the nuclear lamina, associated to the nucleolus or forming isolated lumps
- Active genes are located at the euchromatin
- When a cell divides, all chromatin is condensed and takes the form of chromosomes

THE CELL NUCLEUS. 3.3 Nucleolus

The Nucleolus Is a Ribosome-Producing Factory

Nucleolus ultrastructure

Components:

- Dense fibrillar components

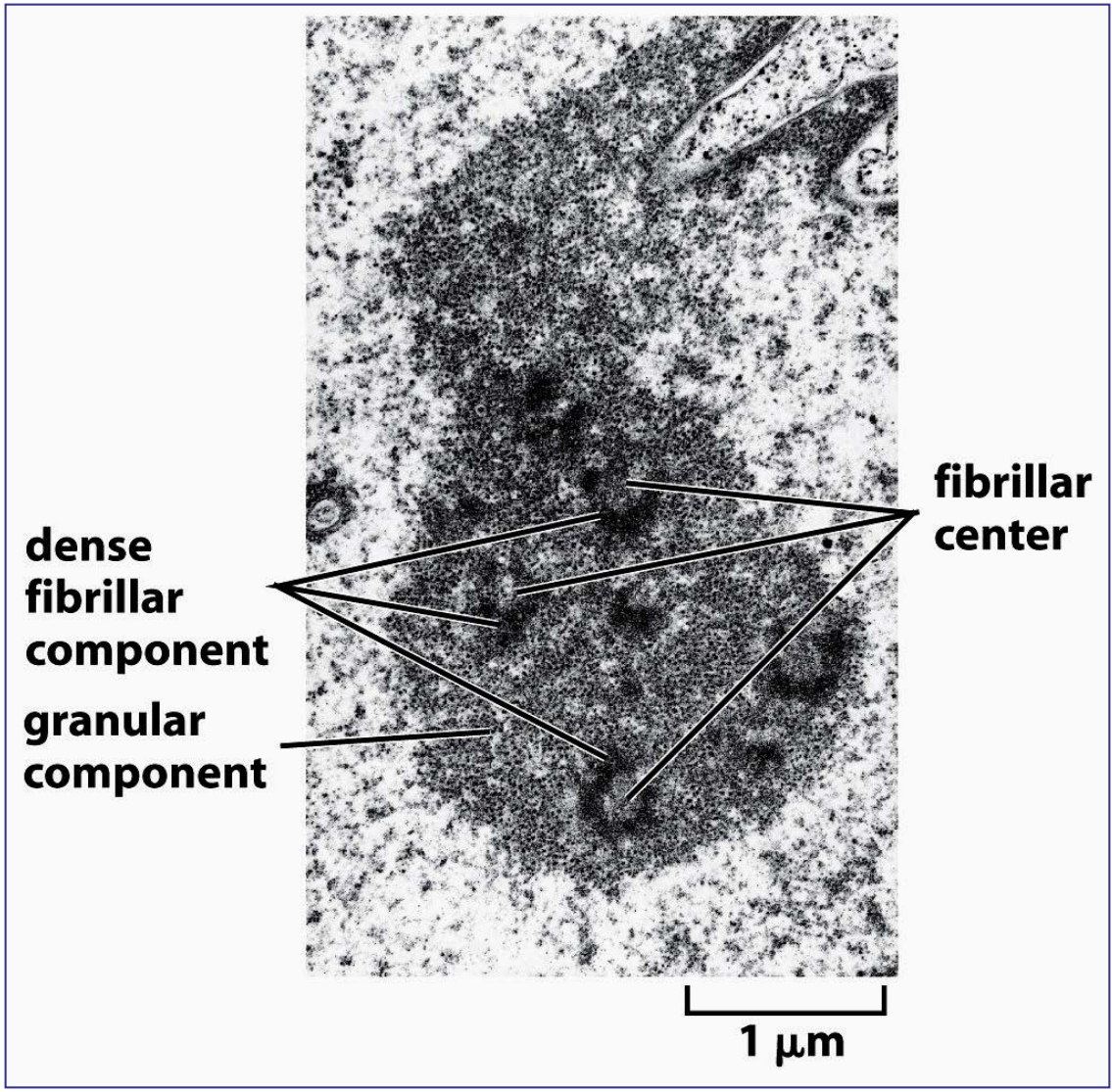
  Particles rRNA transcription process

- Granular components

  Precursors of mature ribosomal particles

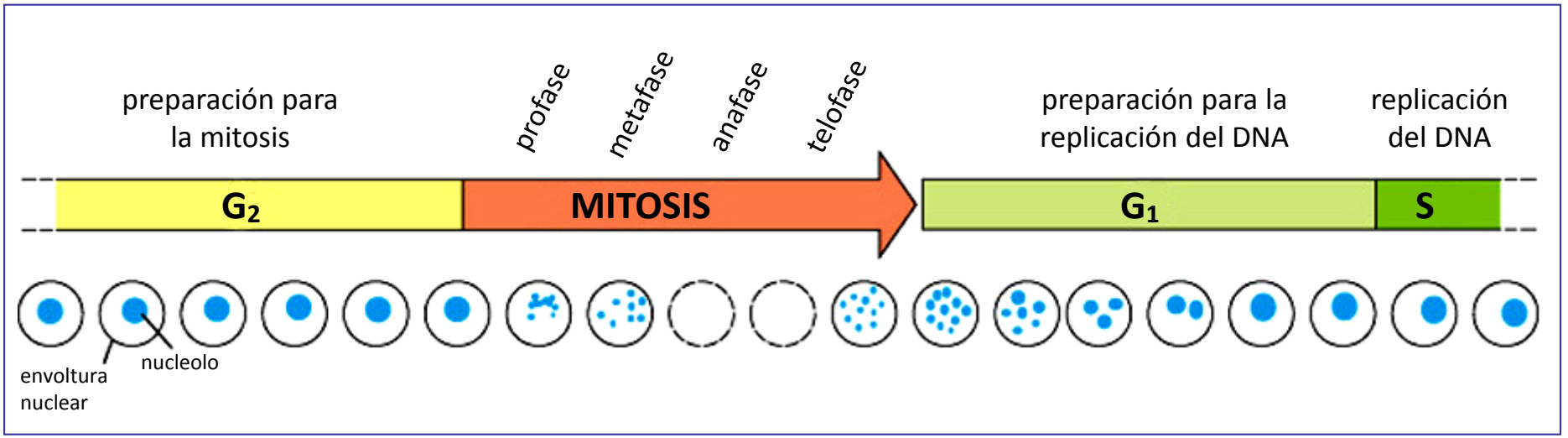
- Fibrillar centers (NOR)

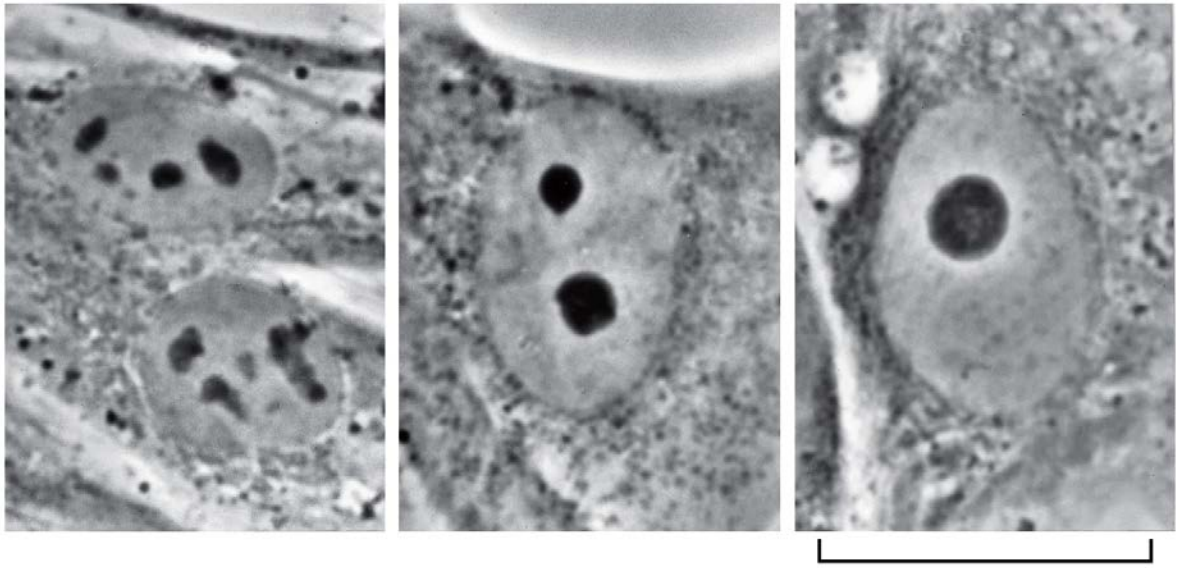
  DNA is being actively transcribed



THE CELL NUCLEUS. 3.3 Nucleolus

Cycle of nucleolus





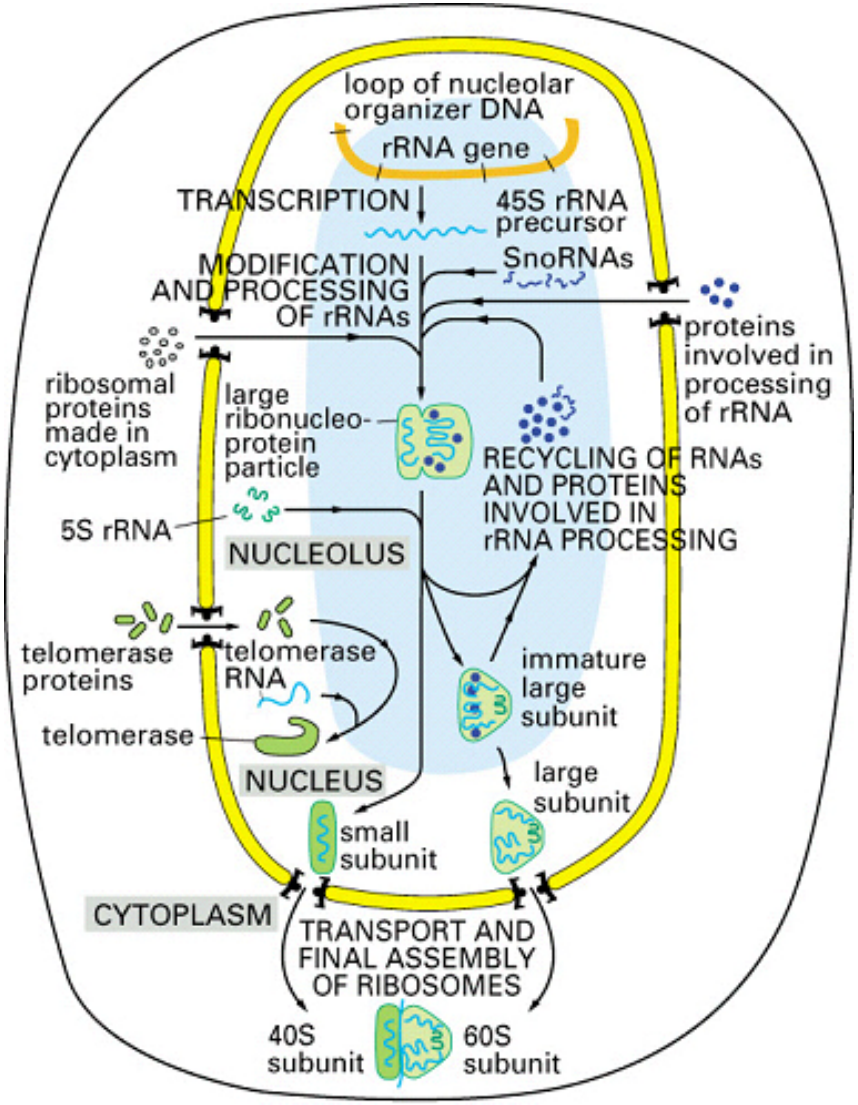
Alberts et al. Fig 6-43

THE CELL NUCLEUS. 3.3 Nucleolus

Nucleolus functions

- Ribosomal syntesis
- Modification of snRNPU6
- Telomerase

……



Alberts et al. Fig 6-45

THE CELL NUCLEUS. 3.4 Extranucleolar RNPs

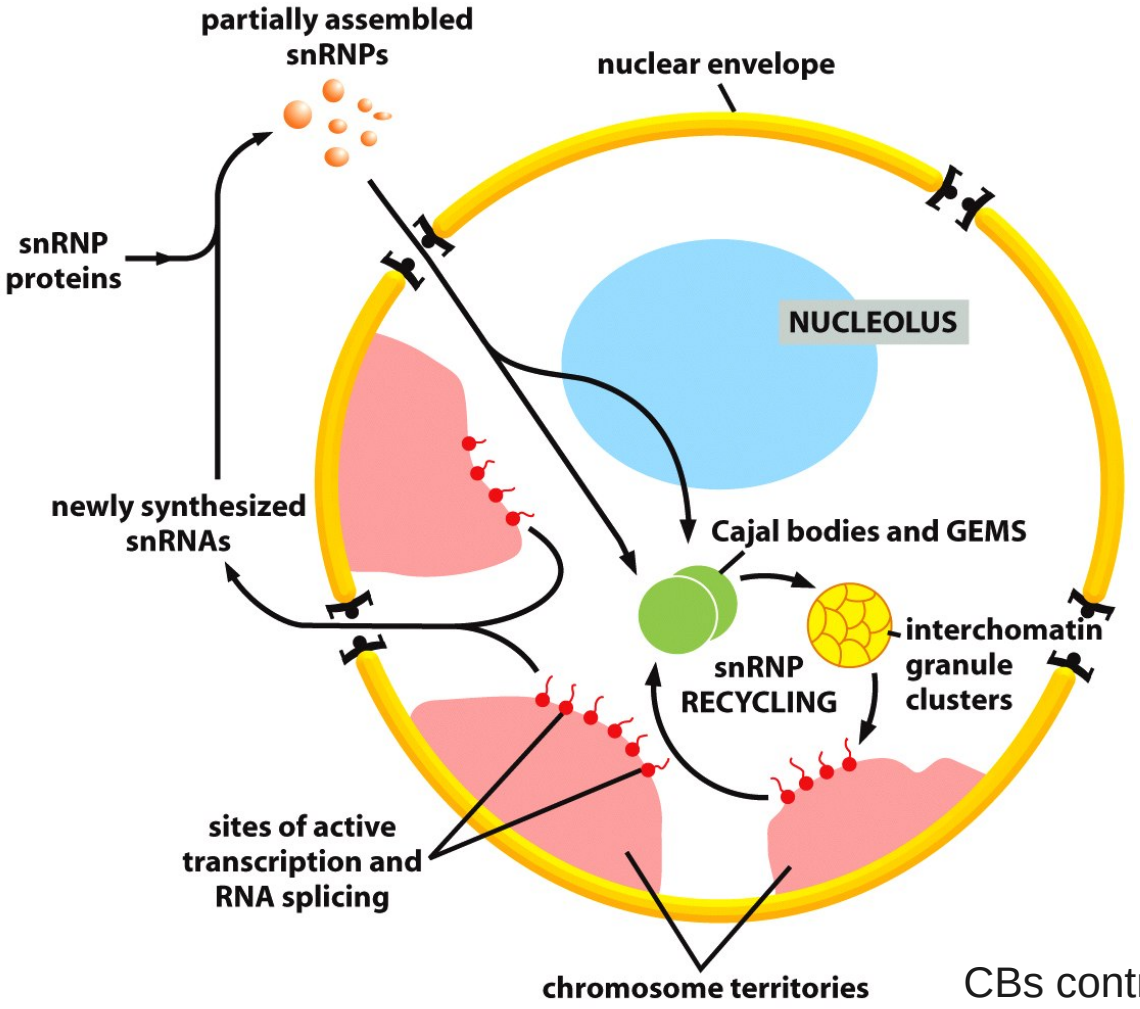
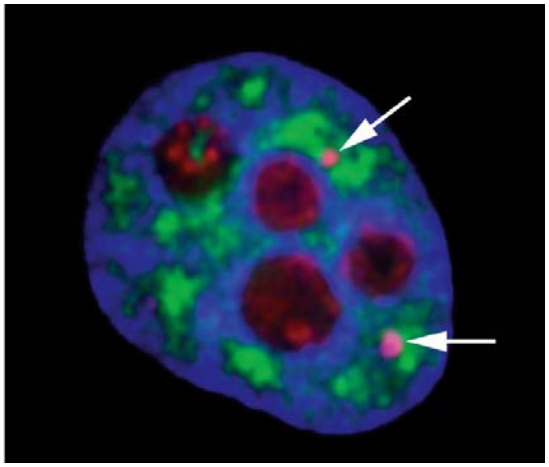


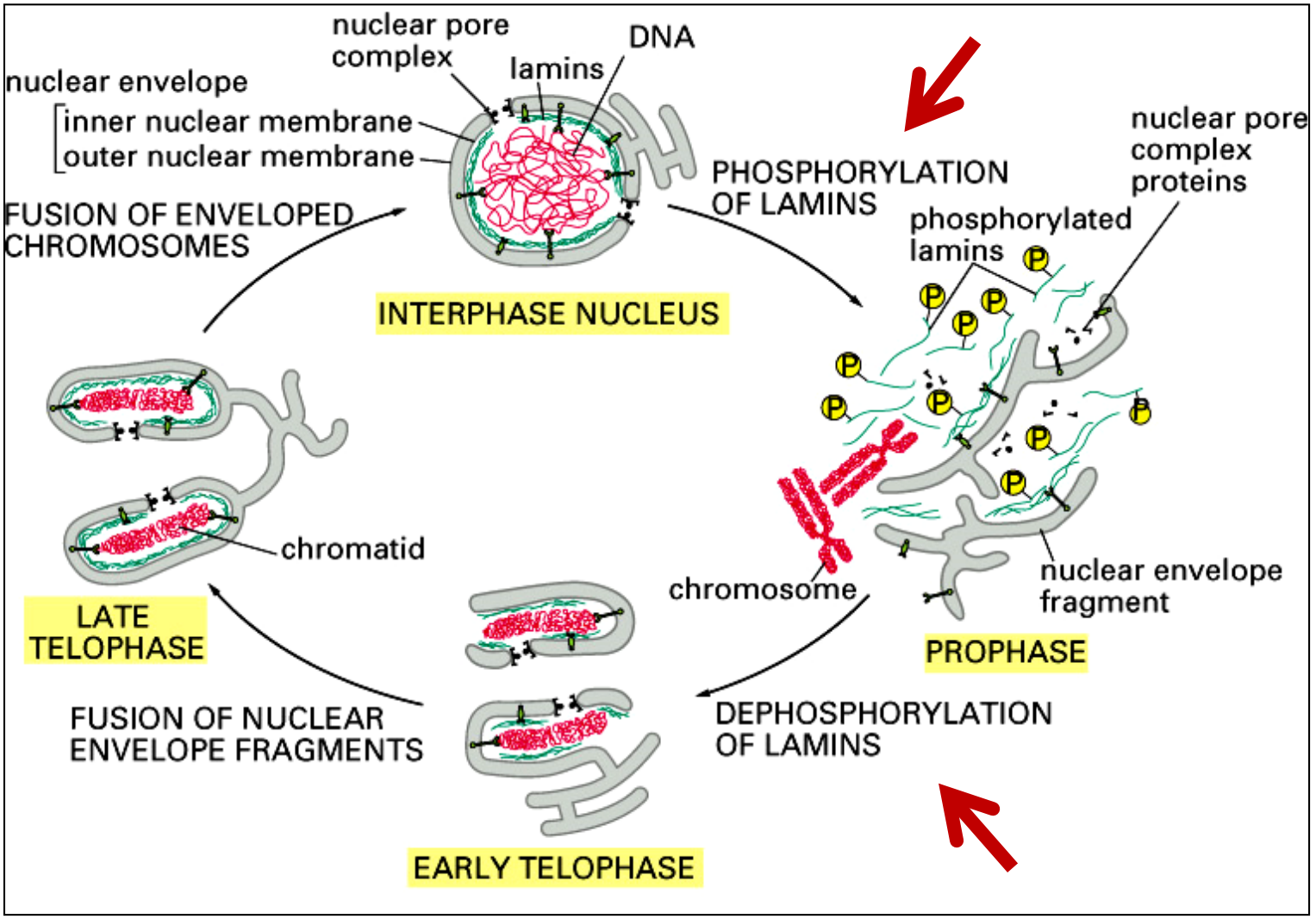
Figure 6-49 Molecular Biology of the Cell 5/e (© Garland Science 2008)



- Perichromatin fibers
- Interchromatin granules
- Cajal bodies
- GEMS
- …

CBs contribute to genome organization with global effects on gene expression and RNA splicing fidelity.

THE CELL NUCLEUS 4. Biogenesis



Alberts et al. Fig 12-18

RIBOSOME

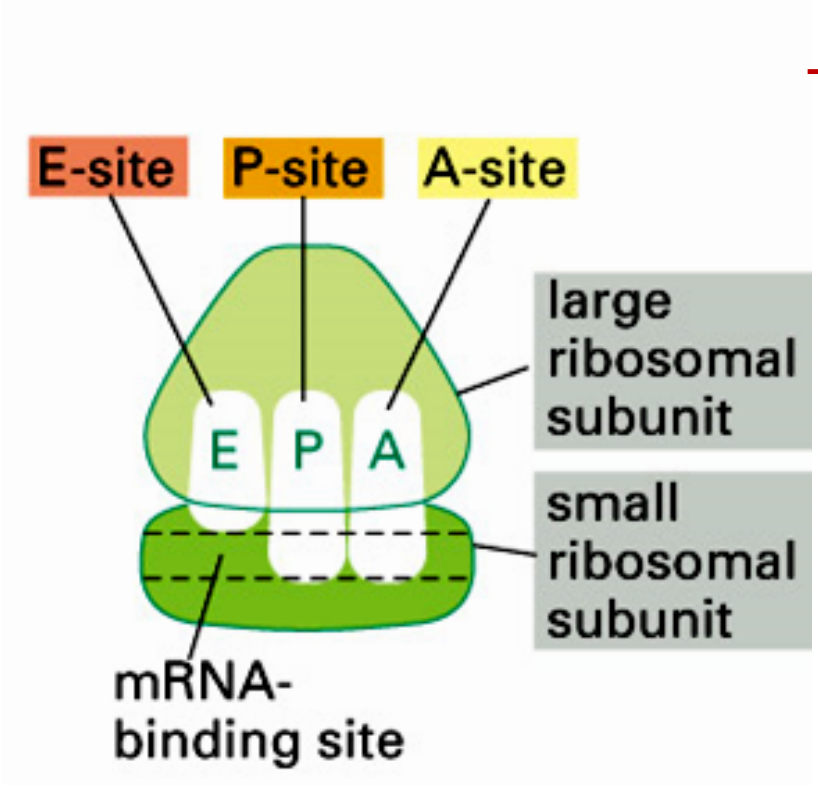
1.- Structure

2.- Chemical composition

3.- Biogenesis

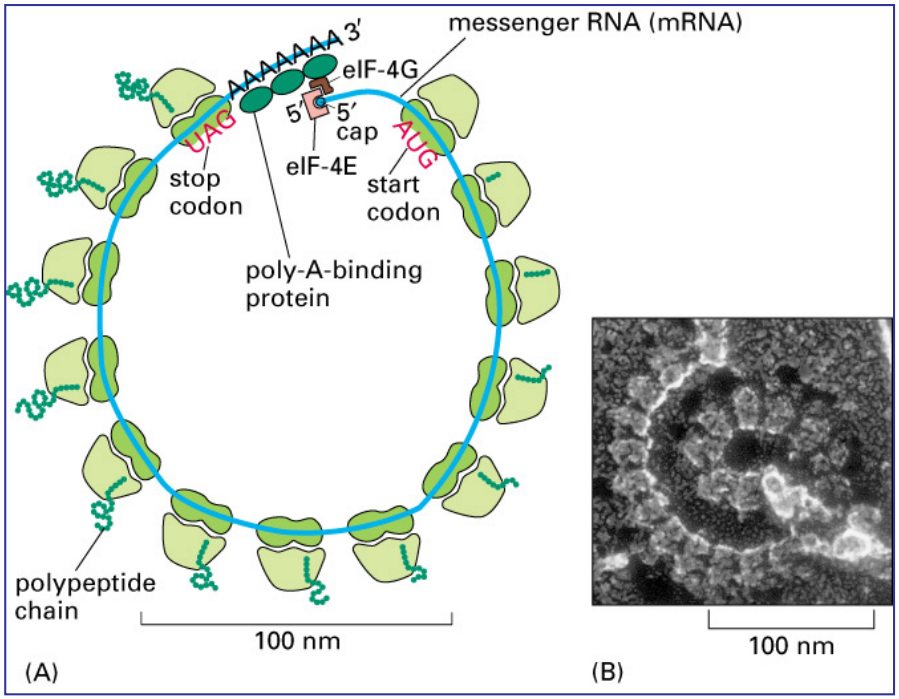
RIBOSOME

Structure



Alberts et al. Fig 6-62

Transmission electronic microscope

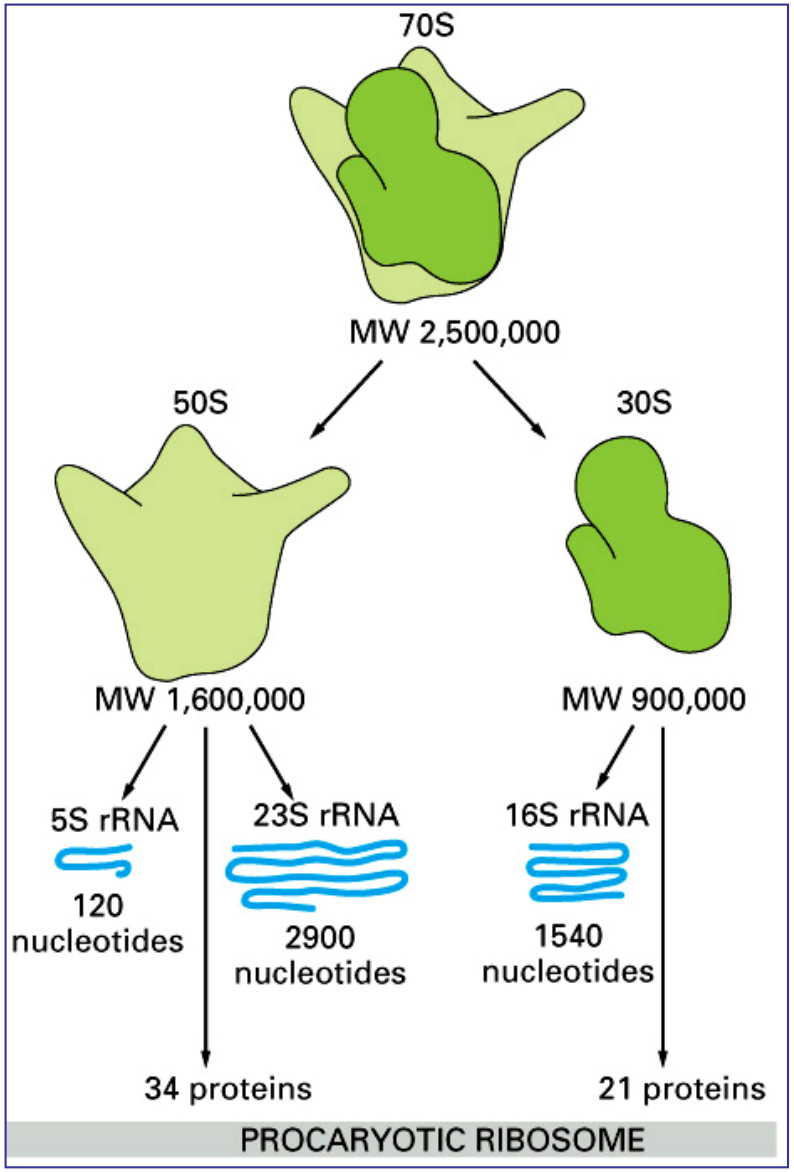


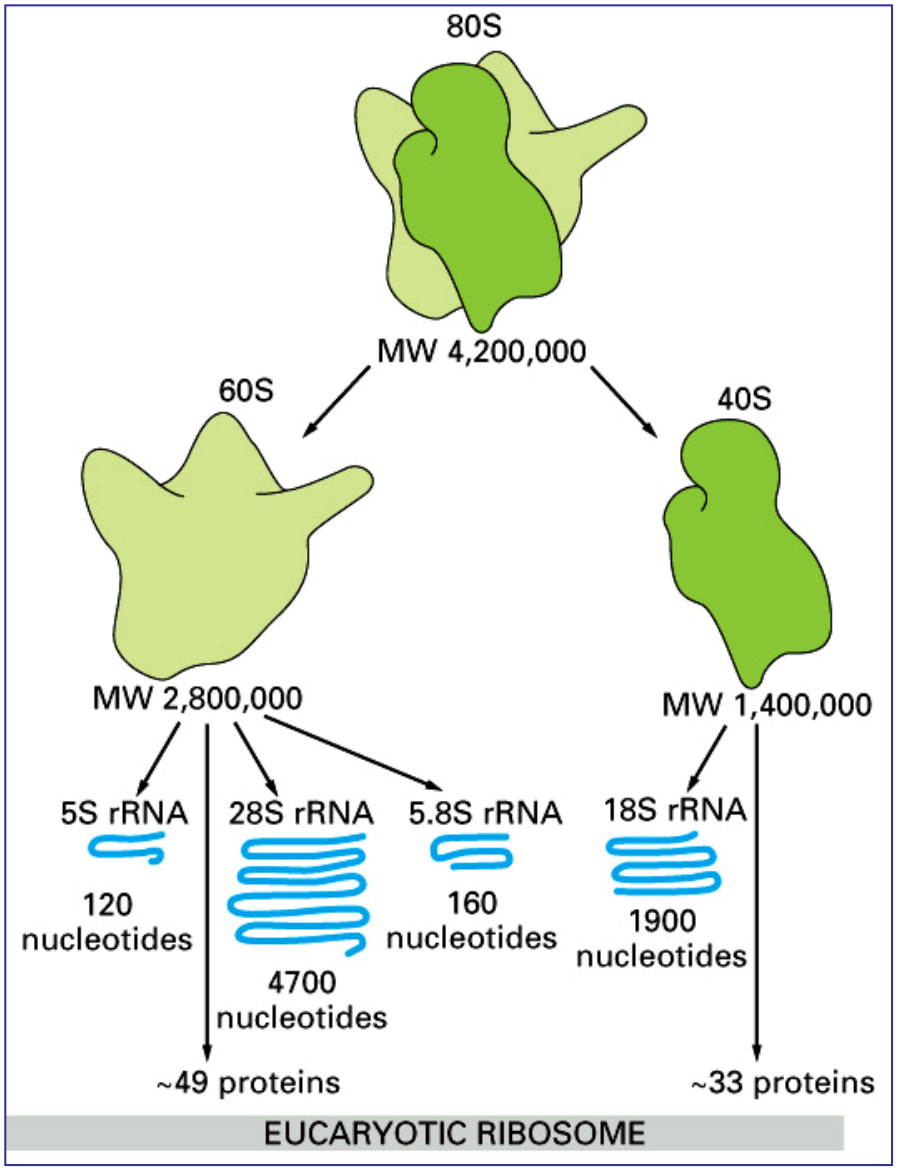
Alberts et al.

RIBOSOME

Chemical composition: H_2O (70%)- RNA 50%, Proteins 50%

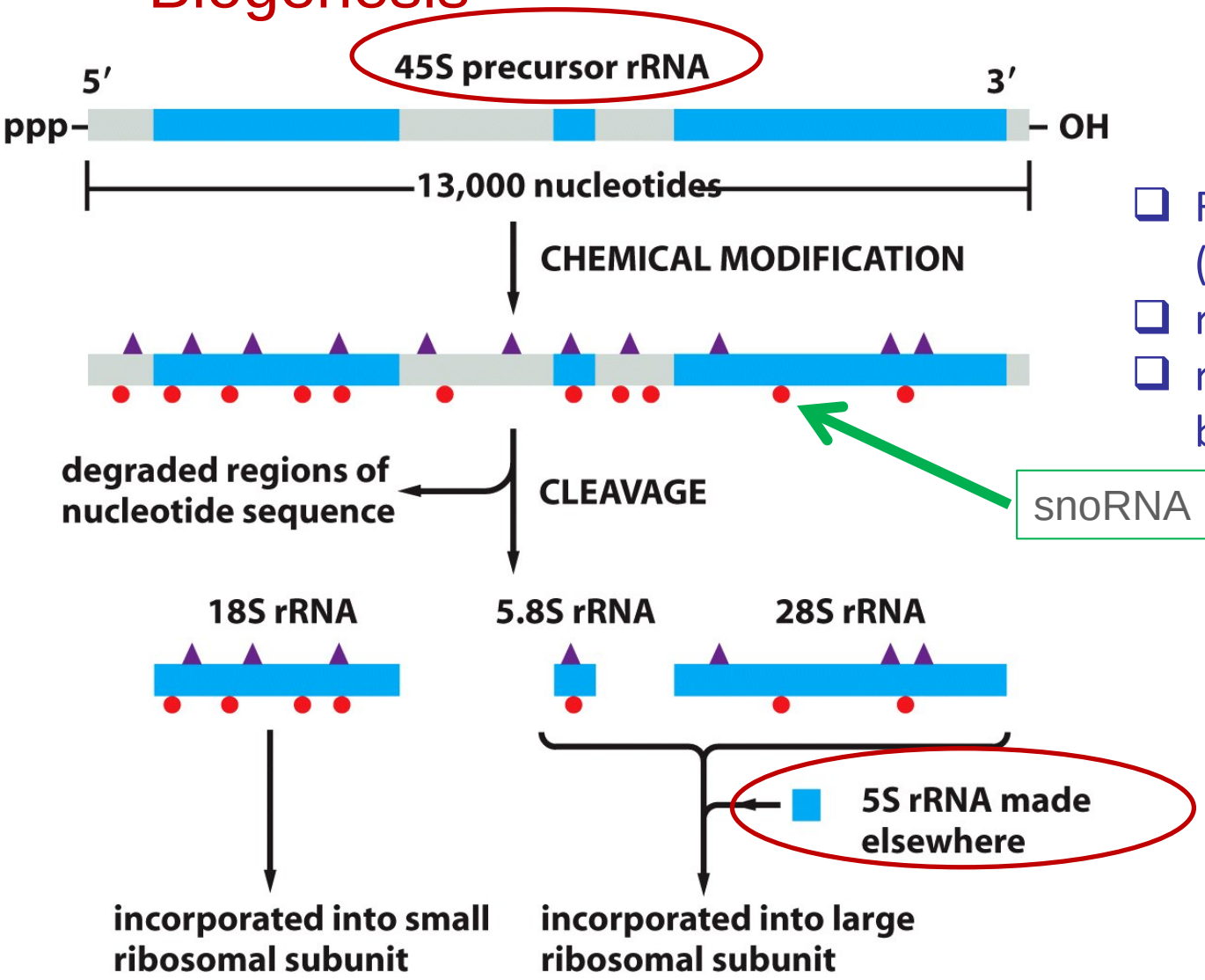
Alberts et al. Fig 6-61





RIBOSOME

Biogenesis



- Ribosome protein genes (RP)
- rRNA genes
- rRNA and Ribosome biosynthesis genes (RRB)

VAULTS CELLULAR ORGANELLES

- *Vaults are multi-subunit **ribonucleoprotein** cellular that may be involved in nucleo-cytoplasmic transport.*
- *Play a role in multiple cellular processes by regulating the MAP kinase, JAK/STAT and phosphoinositide 3-kinase/Akt signaling pathways.*
- *Several reports have indicated a significant association between MVP expression and therapy response/patient prognosis of many types of tumors such as: breast cancer, myeloid leukemia, and non-small-cell lung cancer*.

ASSIGNATURA: BIOLOGIA (34446).
GRAU: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

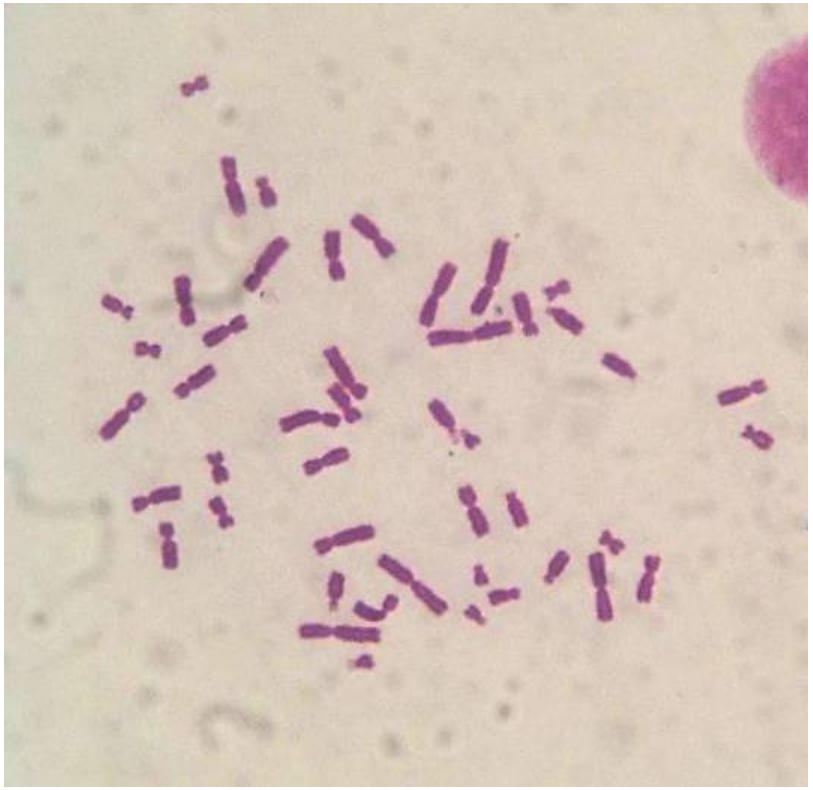
ASIGNATURA: BIOLOGÍA (34446).
GRADO: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

THE KARYOTYPE

1. Introduction
2. Methodology
3. Chromosome classification
4. Chromosome banding techniques
5. Molecular cytogenetics

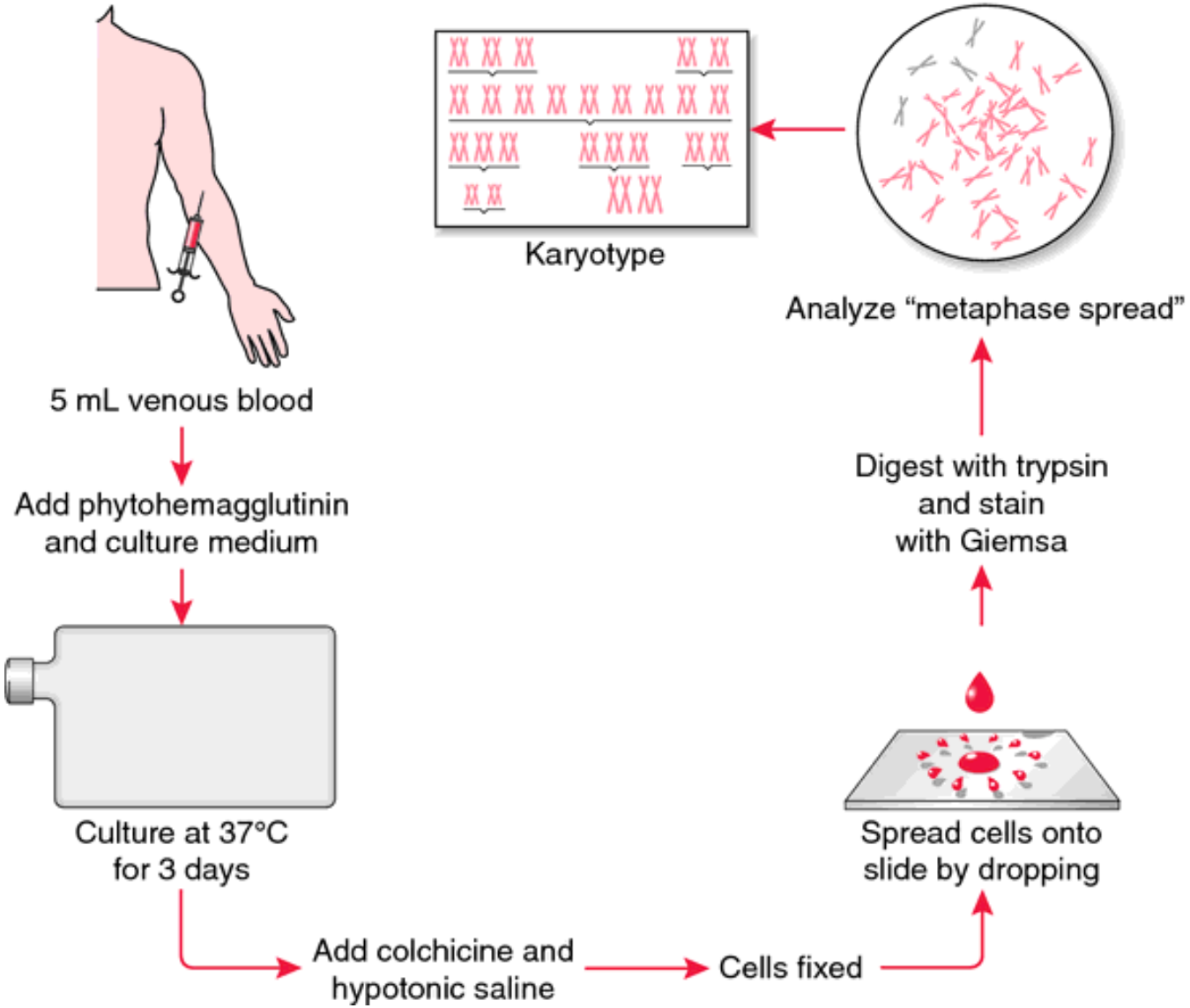
INTRODUCTION

Chromosome (metaphase)



- Karyotype
- Cytogenetics
- Clinical cytogenetics *Leujene et al, 1959*

METHODOLOGY



CHROMOSOME CLASSIFICATION

The chromosomes are classified by pairs of homologues and ordered by:

- Size
- Centromere position (primary constriction)
- Presence of secondary constrictions

Chromosomes are classified into seven groups: A, B, C, D, E, F y G

CHROMOSOME CLASSIFICATION

Group	Chromosomes	Description
A	1–3	Largest; 1 and 3 are metacentric but 2 is submetacentric
B	4,5	Large; submetacentric with two arms very different in size
C	6–12,X	Medium size; submetacentric
D	13–15	Medium size; acrocentric with satellites
E	16–18	Small; 16 is metacentric but 17 and 18 are submetacentric
F	19,20	Small; metacentric
G	21,22,Y	Small; acrocentric, with satellites on 21 and 22 but not on the Y

Autosomes are numbered from largest to smallest, except that chromosome 21 is smaller than chromosome 22.

BANDING TECHNIQUES

1. Bands on the entire chromosome
   - Q-banding
   - G-banding
   - R-banding
   - High-resolution banding
2. Bands at specific regions
   - C-banding
   - T-banding
   - NOR-banding

BANDING TECHNIQUES

Q-BANDING

- Q-bands → Quinacrine → similar pattern to G-banding →fluorescence microscope
- Identification + chromosome study

G-BANDING

- G-bands → Giemsa → chromosomes are treated with trypsin → denatures protein content → stained with Giemsa → light and dark bands
- Identification + cytogenetic diagnostic

Dye gives chromosomes a striped appearance because it stains the regions of DNA that are rich in adenine (A) and thymine (T) base pairs.

- Regions that stain as dark G bands replicate late in S phase of the cell cycle and contain more condensed chromatin,
- While light G bands generally replicate early in S phase, and have less condensed chromatin.

BANDING TECHNIQUES

R-BANDING

- R-bands → Reverse → heat/OH denatured before Giemsa treatment → reverse of those obtained with G-banding
- Identification + cytogenetic diagnostic (telomeres)

HIGH-RESOLUTION BANDING

- High-Resolution banding → Synchronization of cells in prophase/prometaphase (methotrexate) →G-banding
- Identification + cytogenetic diagnostic (microalterations)

C-BANDING

- C-bands → Centromeric bands → stains heterochromatin

T-BANDING

- T-bands → Telomeric bands

NOR-BANDING

- NOR-bands → Nucleolar Organizing Region (NOR)-bands

MOLECULAR CYTOGENETICS

- Application of molecular biology techniques to cytogenetic specimens.
- Identification of rearrangements produced "de novo" and of marker chromosomes.
- Detection of chromosomal abnormalities in interphase cells.
- Study of the structure and function of specific chromosomal regions.

FLUORESCENCE "IN SITU" HYBRIDIZATION (FISH)

•A DNA probe, labeled with fluorescent molecules, is hybridized with chromosomes on a slide and is observed with a fluorescence microscope.

TYPES OF DNA PROBES

CENTROMERIC PROBES

a-satellite probe specific of the centromere

•Interphase: Chromosome counting

•Metaphase: Identification of chromosomes

TYPES OF DNA PROBES

LOCUS SPECIFIC

Locus specific probe with a control probe for the chromosome

Probe to identify the deleted region

Probe to identify the chromosome

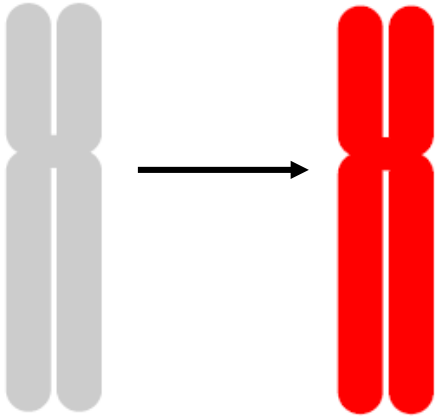
Telomeric and Subtelomeric

- High concentration of genes *microscopic or submicroscopic deletions can have a significant impact*
- They play a critical role in chromosomal pairing during meiosis

TYPES OF DNA PROBES

PAINTING

Marks the entire chromosome: painting



Identifies a determined chromosome

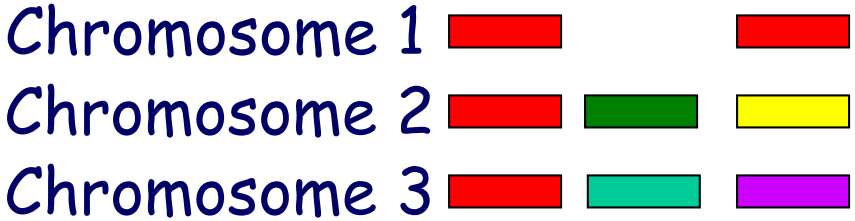
- In translocations

MULTICOLOR FISH

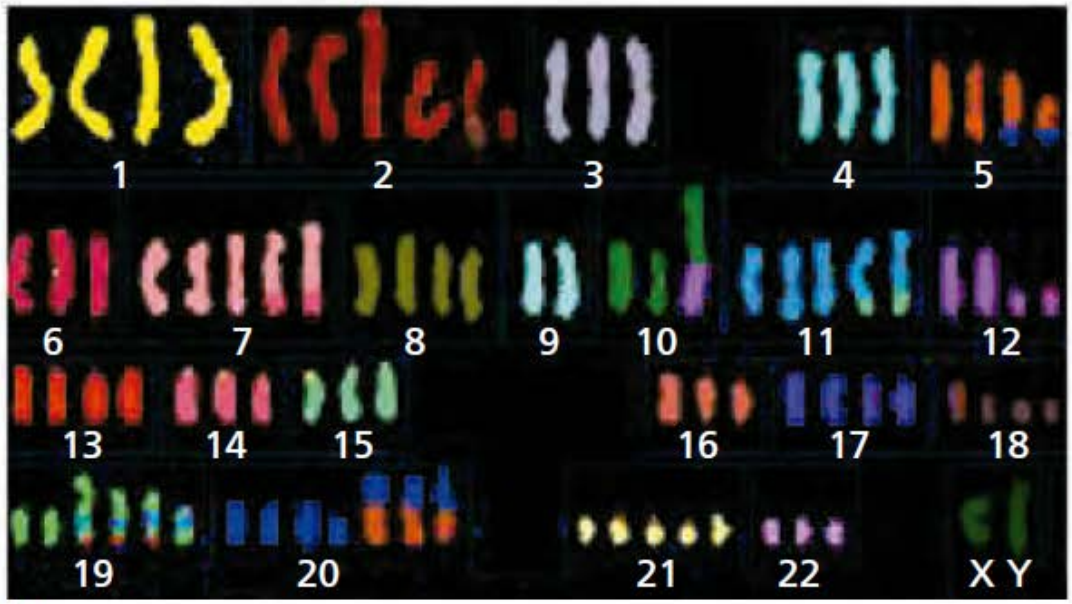
- DNA of each human chromosome is marked directly with one or more fluorochromes, in a combinatorial manner, resulting in an unique combination for each of the 24 chromosomes.

  - Images are captured for each of the fluorochromes using five different filters.

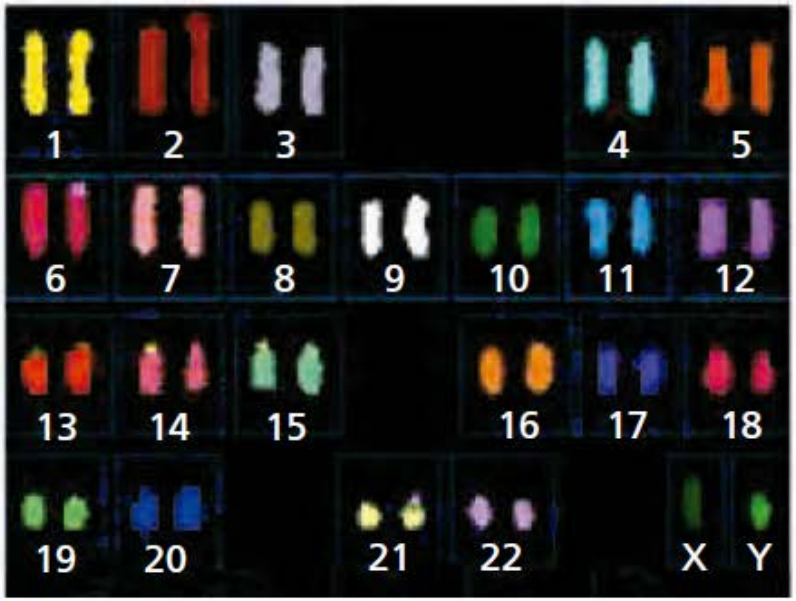
  - The images are processed by a computer to give a pseudo-colored image of the metaphase and the karyotype



MULTICOLOR FISH



(A)



(B)

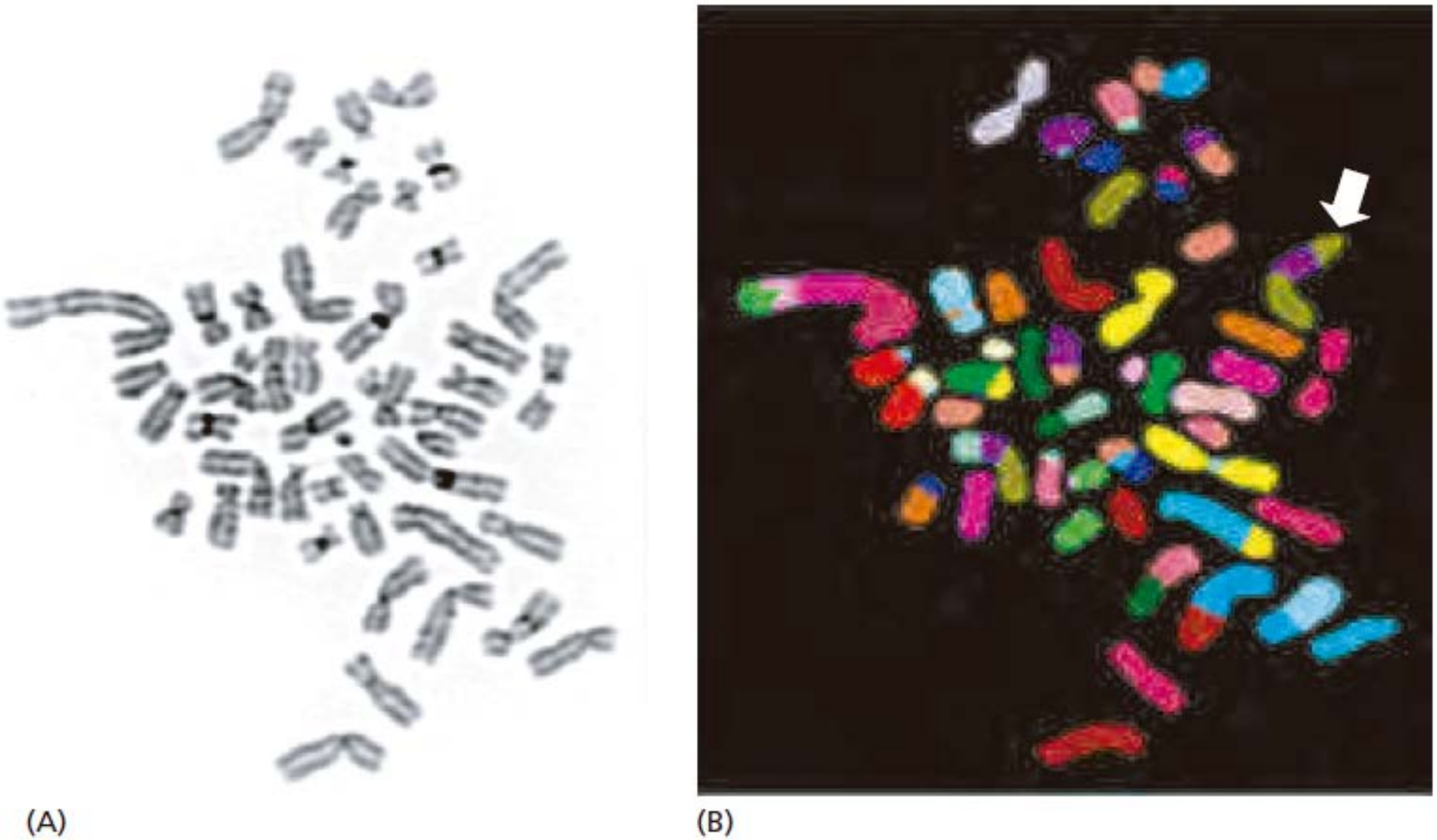
KARYOTYPE

- Numerical abnormalities and translocations
- Cell tumor studies (complex karyotypes)
- Location of interphasic chromosomes

- Expensive technique
- No banding

Figure 20-35 *Molecular Biology of the Cell. Sixth edition,* © 2015

MULTICOLOR FISH



METAPHASE

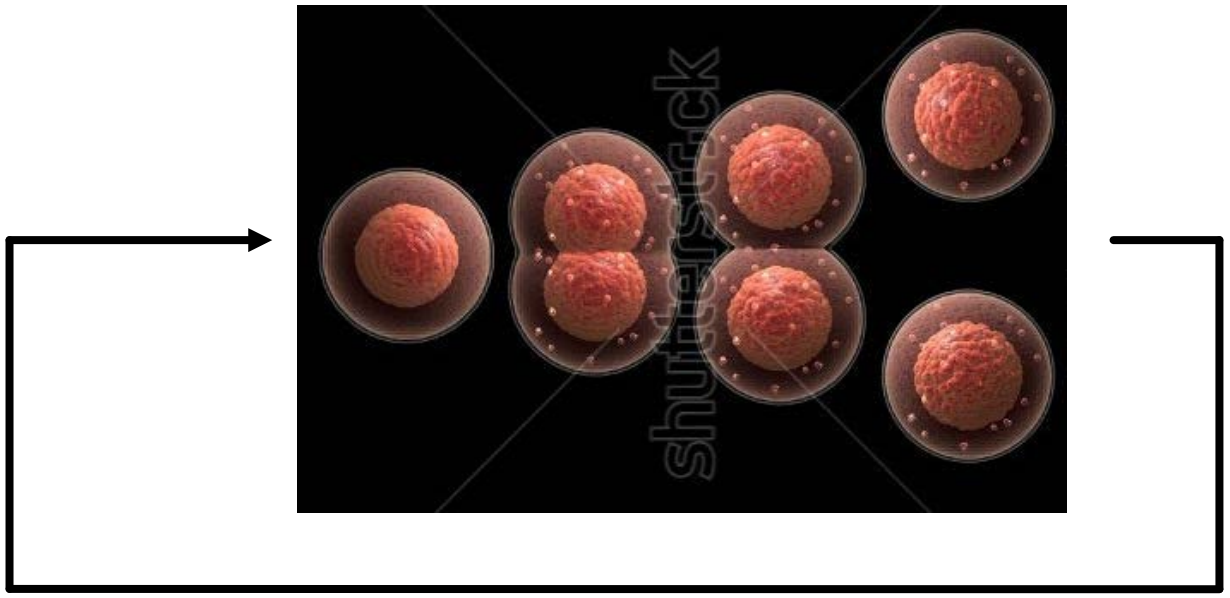
Figure 20-10 *Molecular Biology of the Cell. Sixth edition,* © 2015

THE CELL CYCLE

- Concept of cell cycle
- Cell cycle phases
- Synthetic activities during the cell cycle
- Proliferation in multicellular organisms
- Factors that regulate cell proliferation
- Cell-cycle control system

CONCEPT OF CELL CYCLE

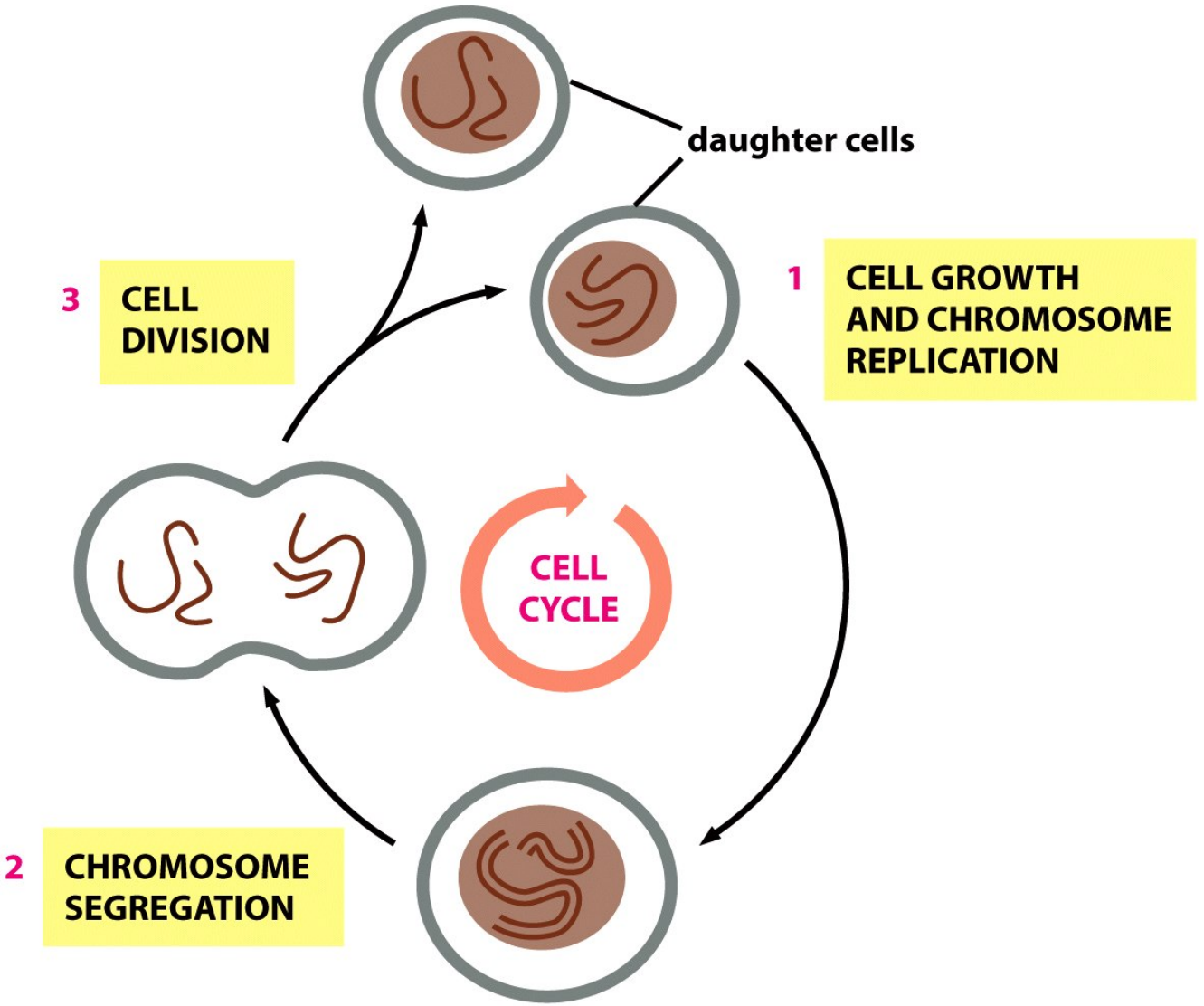
Cell cycle: Cyclic phenomenon by which cells duplicate their content, divide and form two new daughter cells.



Cell growth: Increase of cell mass, previous to cell division.

Proliferation: Increase of cell number produced by cell division.

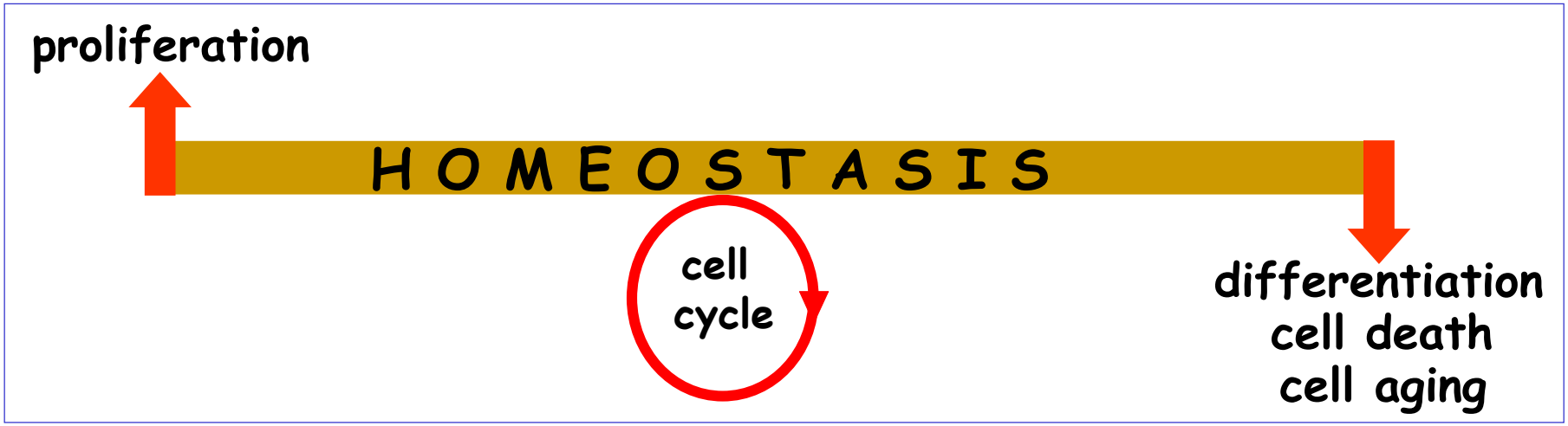
CONCEPT OF CELL CYCLE



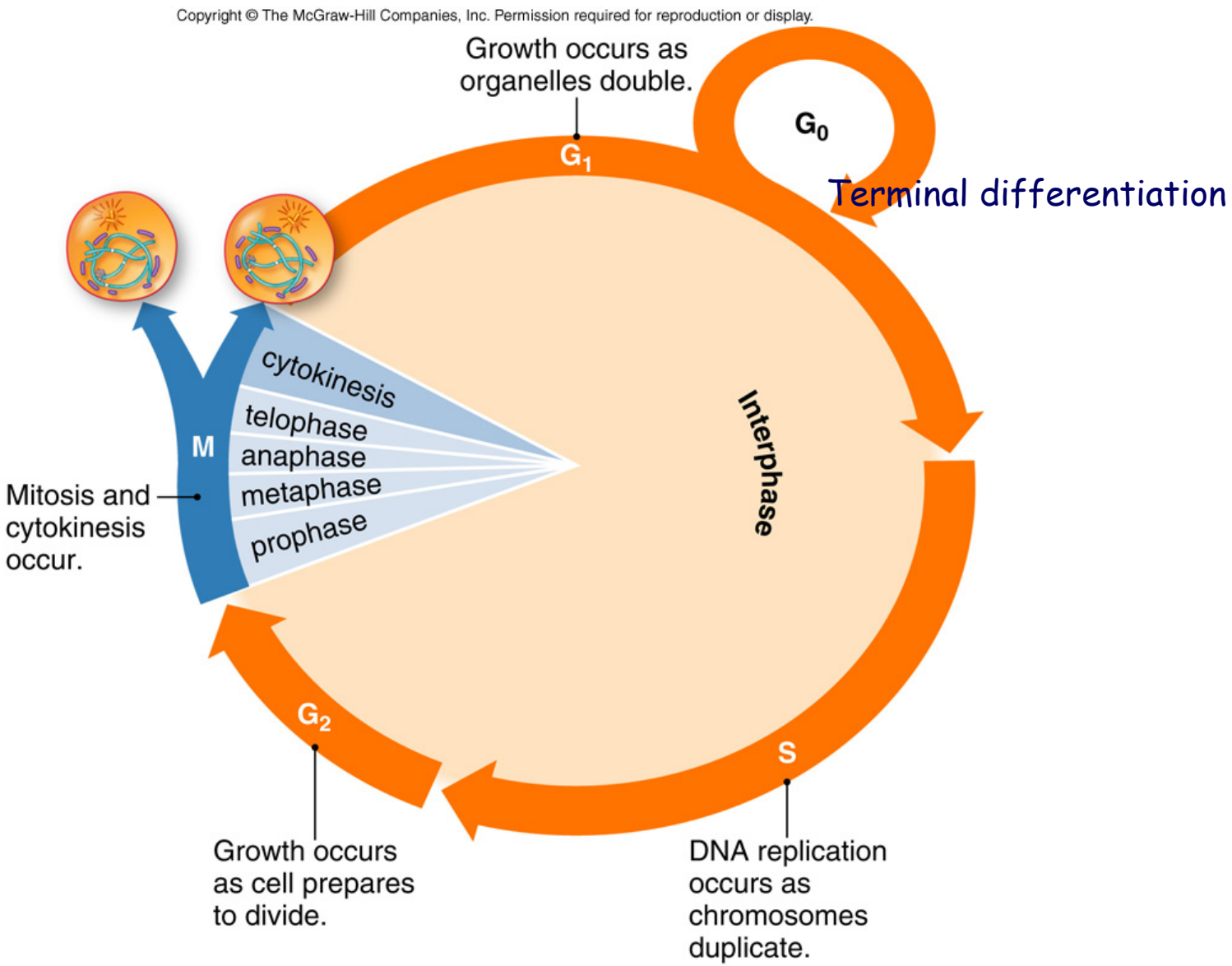
CONCEPT OF CELL CYCLE

The cell cycle constitutes a central point in the regulation of growth and maintenance of the organism.

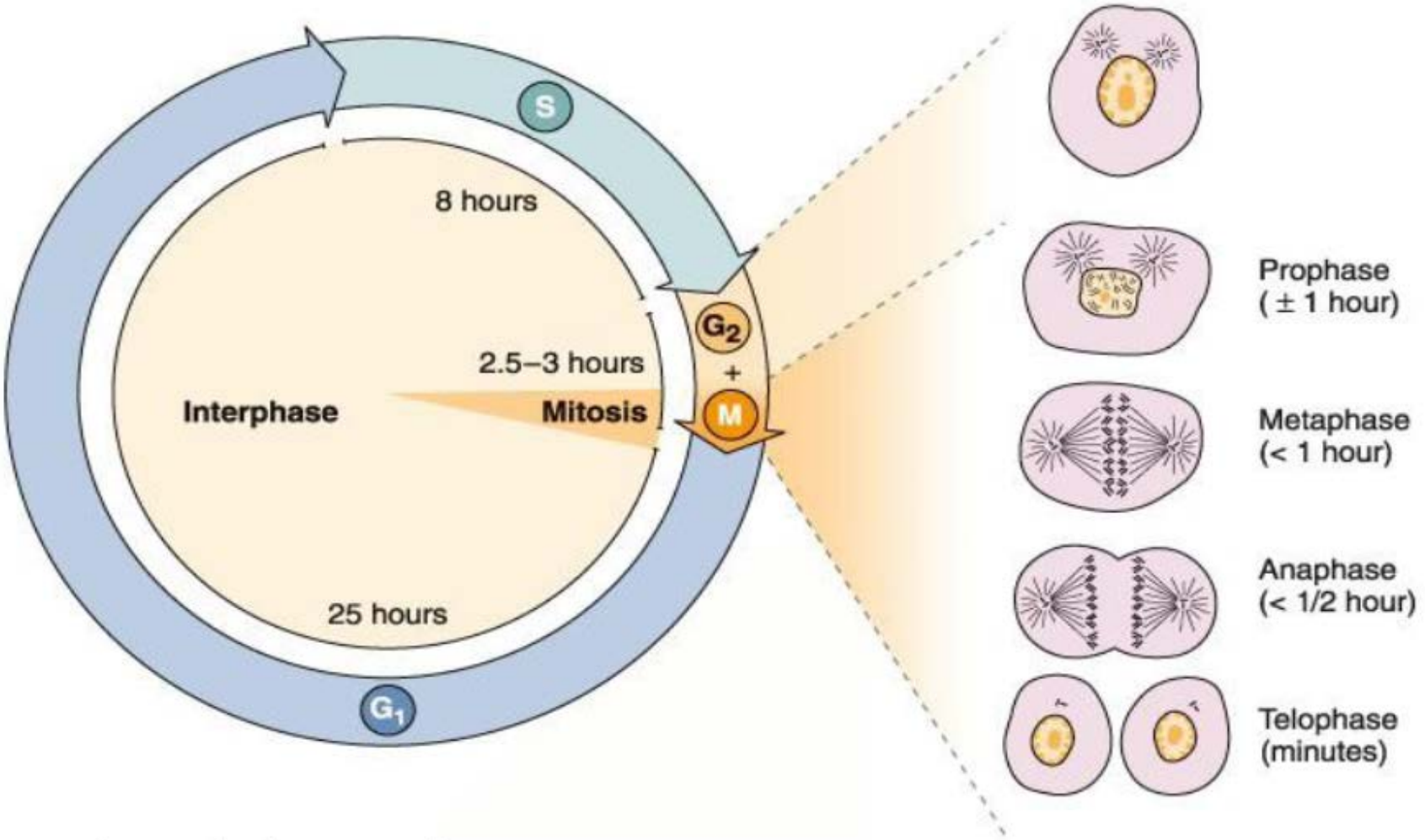
- **Coordinated development of the tissues and organs in the organism.**
- **Stability and equilibrium of structures in the adult individual.**



CELL CYCLE PHASES

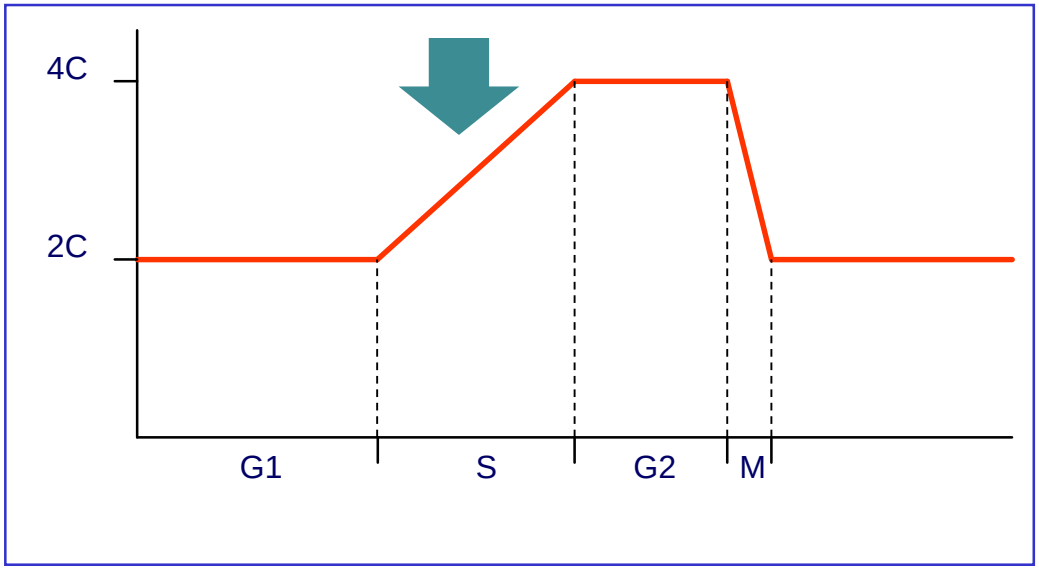


CELL CYCLE PHASES

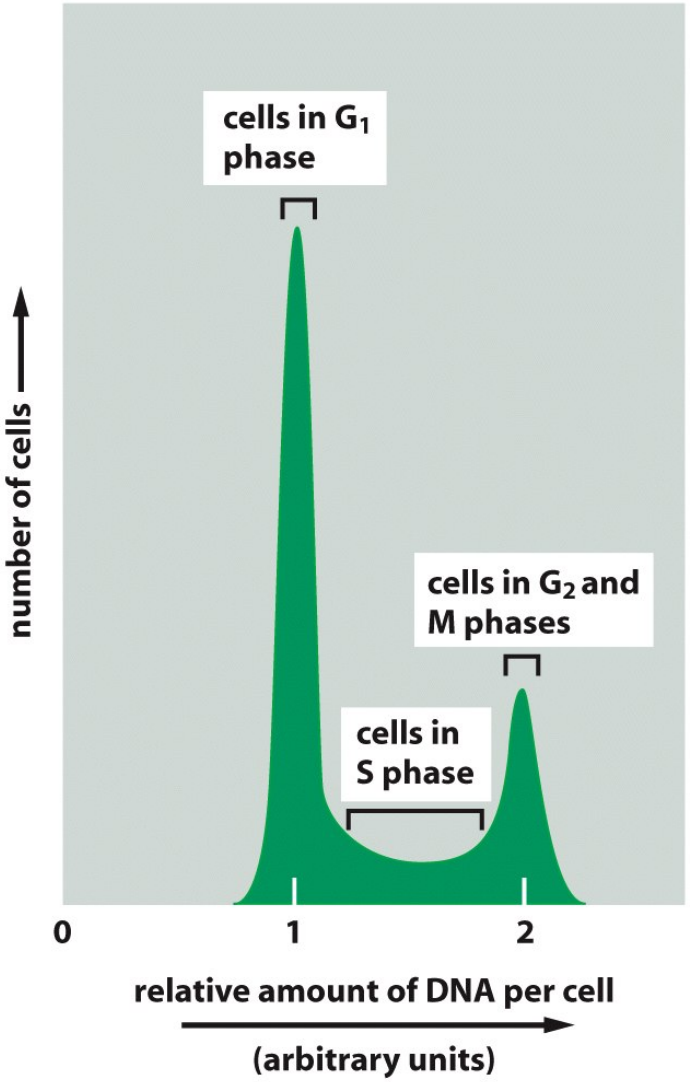


SYNTHETIC ACTIVIES DURING THE CELL CYCLE

- DNA synthesis (only in S phase)
- RNA synthesis (throughout the cycle except in M phase)
- Protein synthesis (throughout the cycle)
- Organelle synthesis (throughout the cycle)



SYNTHETIC ACTIVIES DURING THE CELL CYCLE

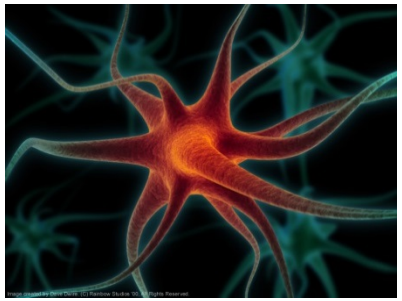


FLOW CYTOMETRY

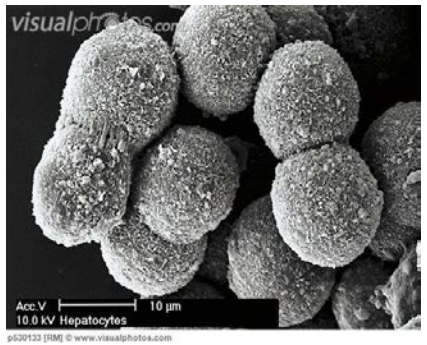
PROLIFERATION IN MULTICELLULAR ORGANISMS

Types of cellular populations based on their proliferative capacity

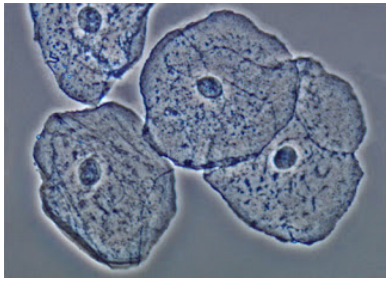
Permanent: Non-proliferating
Terminally differentiated
Cannot be substituted
e.g. neurons, cardiac myocytes



Stable: Low proliferation
Form tissues that renew very slowly
Can proliferate in response to a stimulus
e.g. hepatocytes, endothelial cells

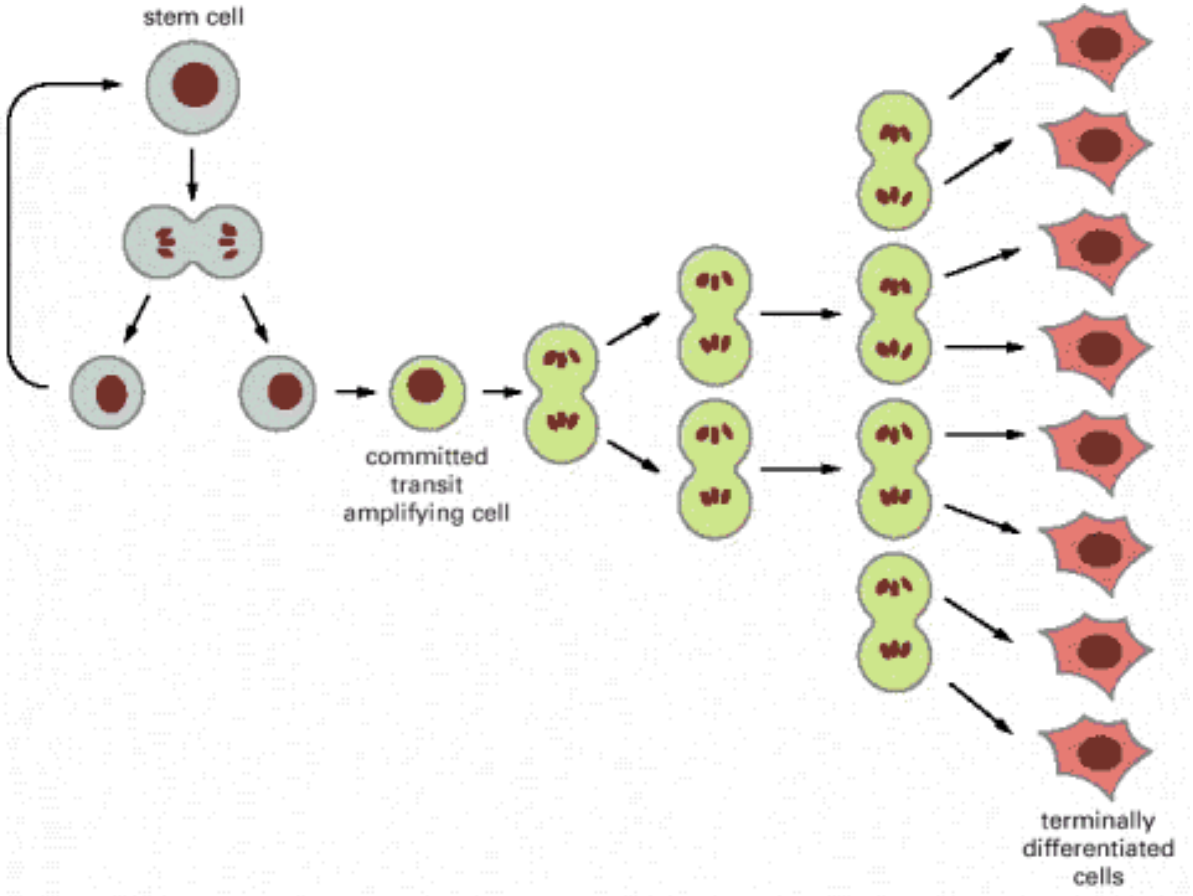


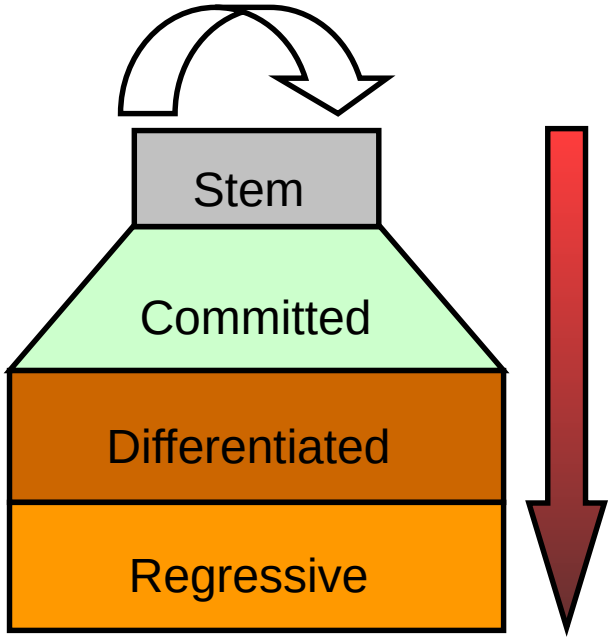
Regenerative: Proliferate actively
Constant renewal
e.g. epithelial cells, blood cells



PROLIFERATION IN MULTICELLULAR ORGANISMS

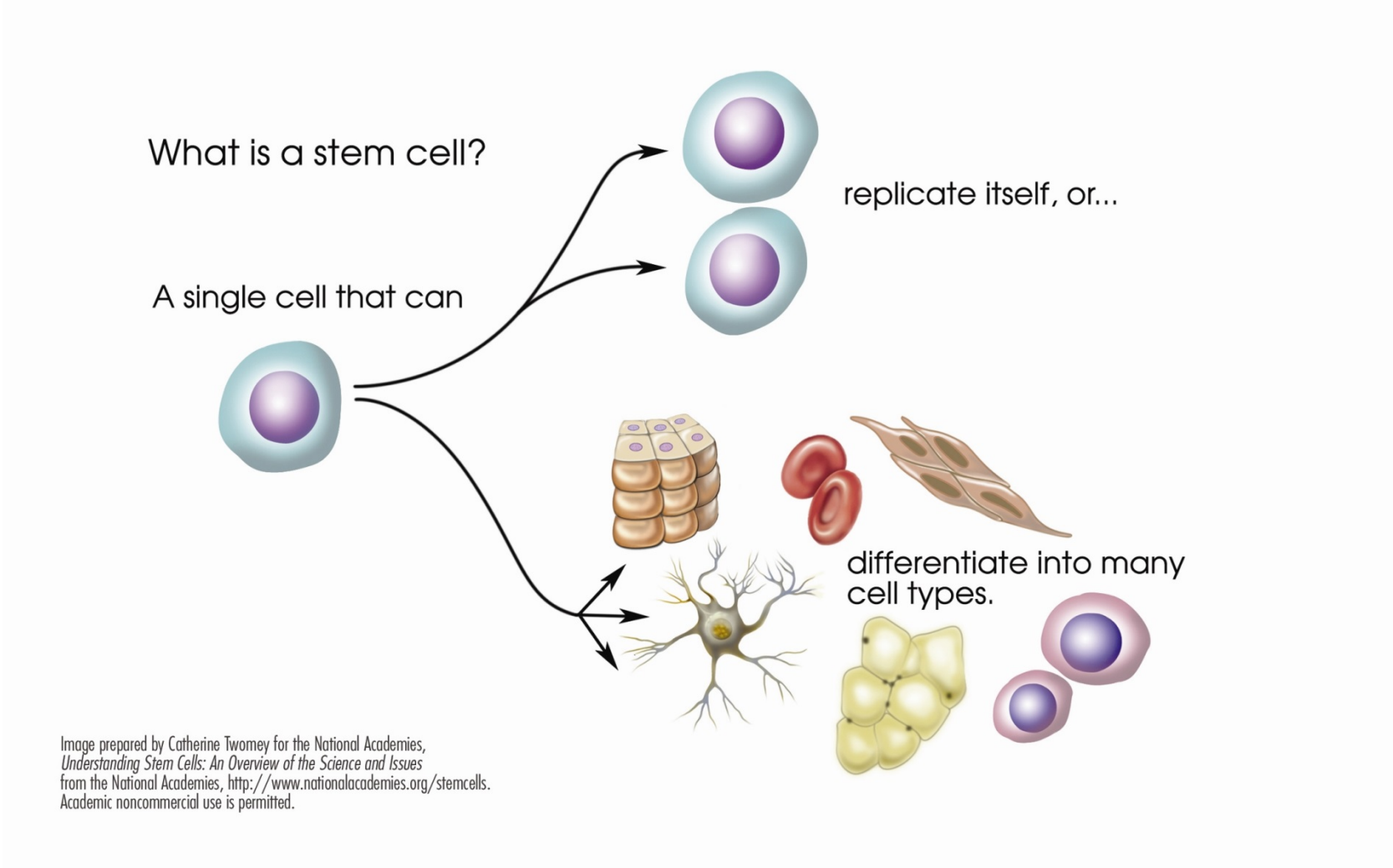
Cell types in proliferative tissues





PROLIFERATION IN MULTICELLULAR ORGANISMS

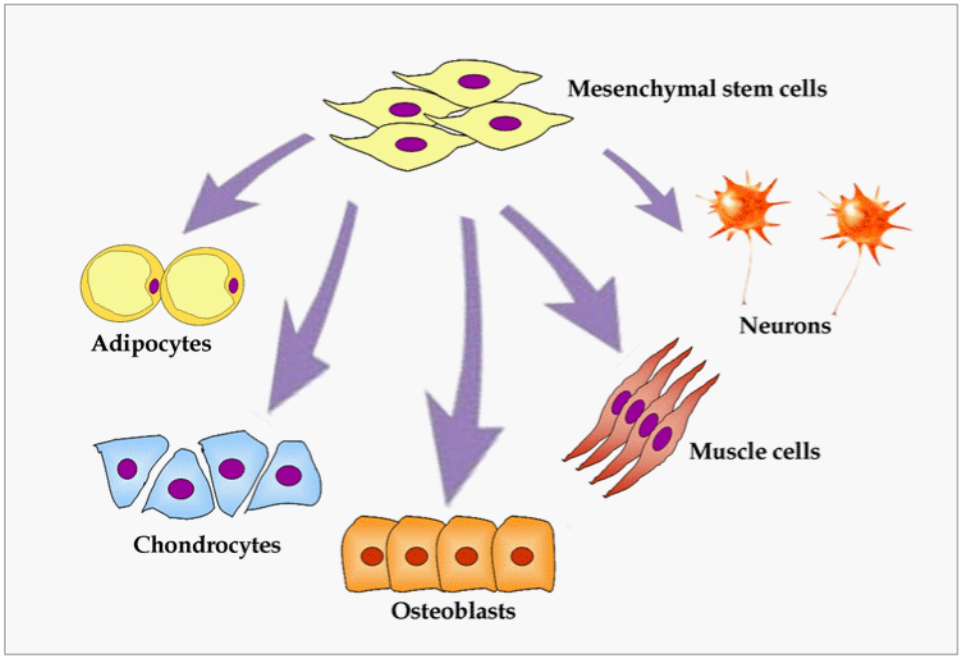
Cell types in proliferative tissues

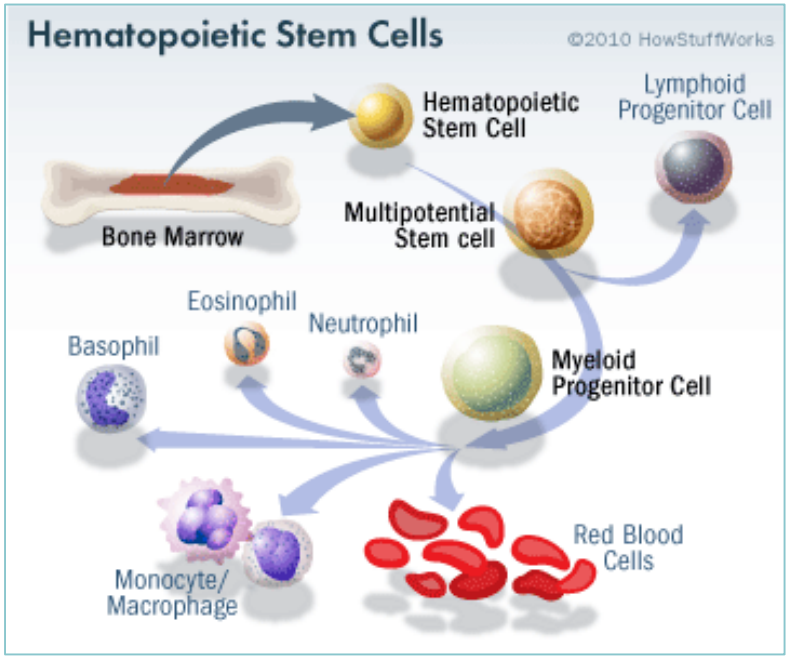


PROLIFERATION IN MULTICELLULAR ORGANISMS

Cell types in proliferative tissues

Adult stem cell

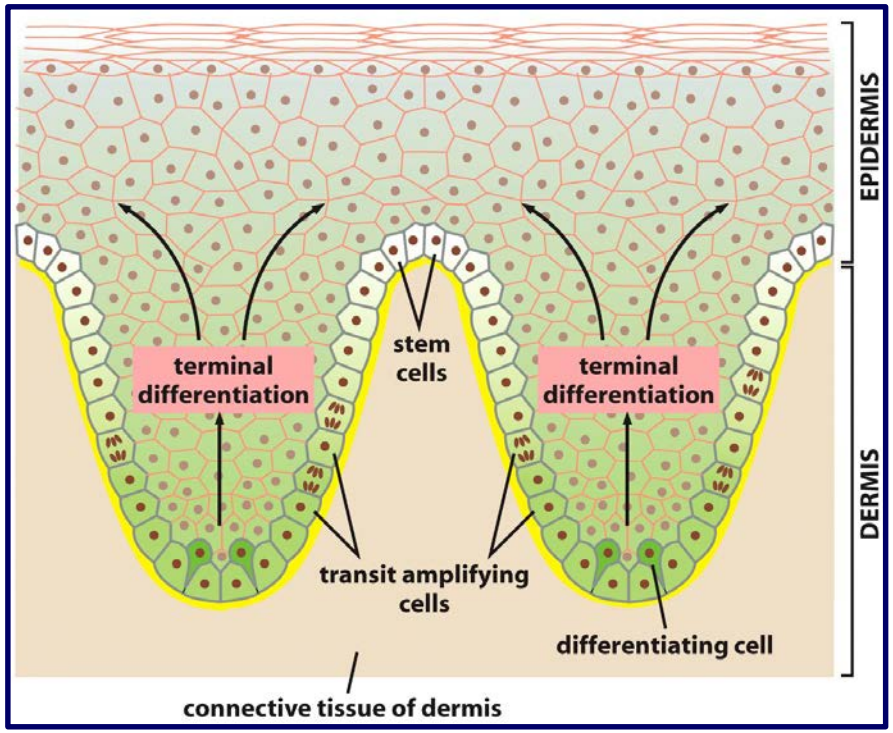


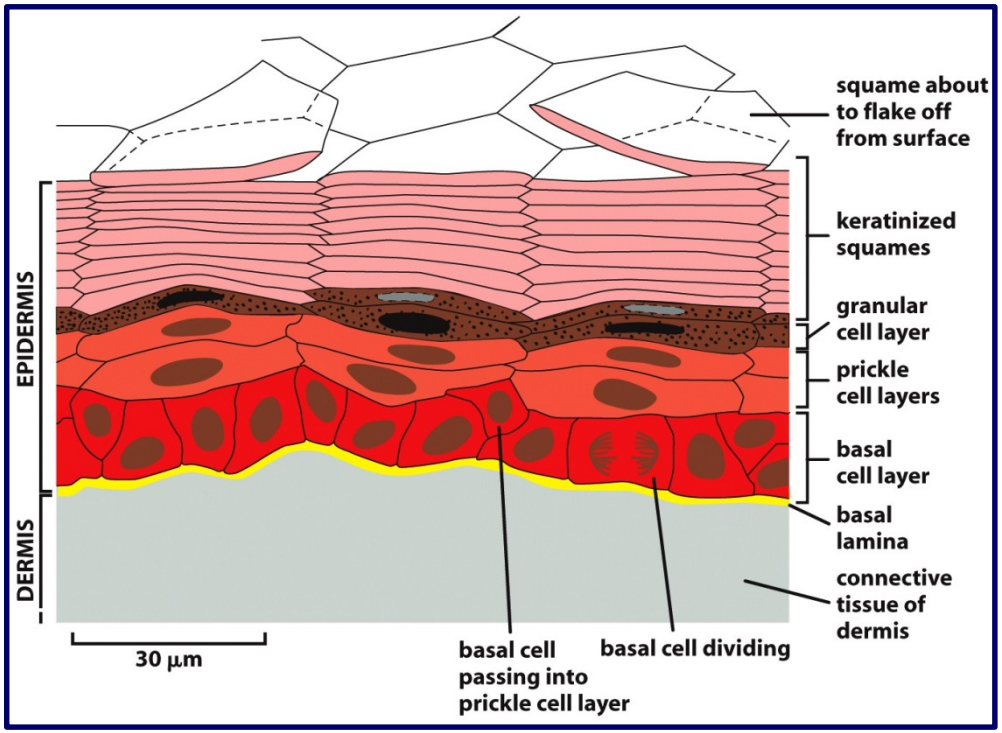


PROLIFERATION IN MULTICELLULAR ORGANISMS

Cell types in proliferative tissues

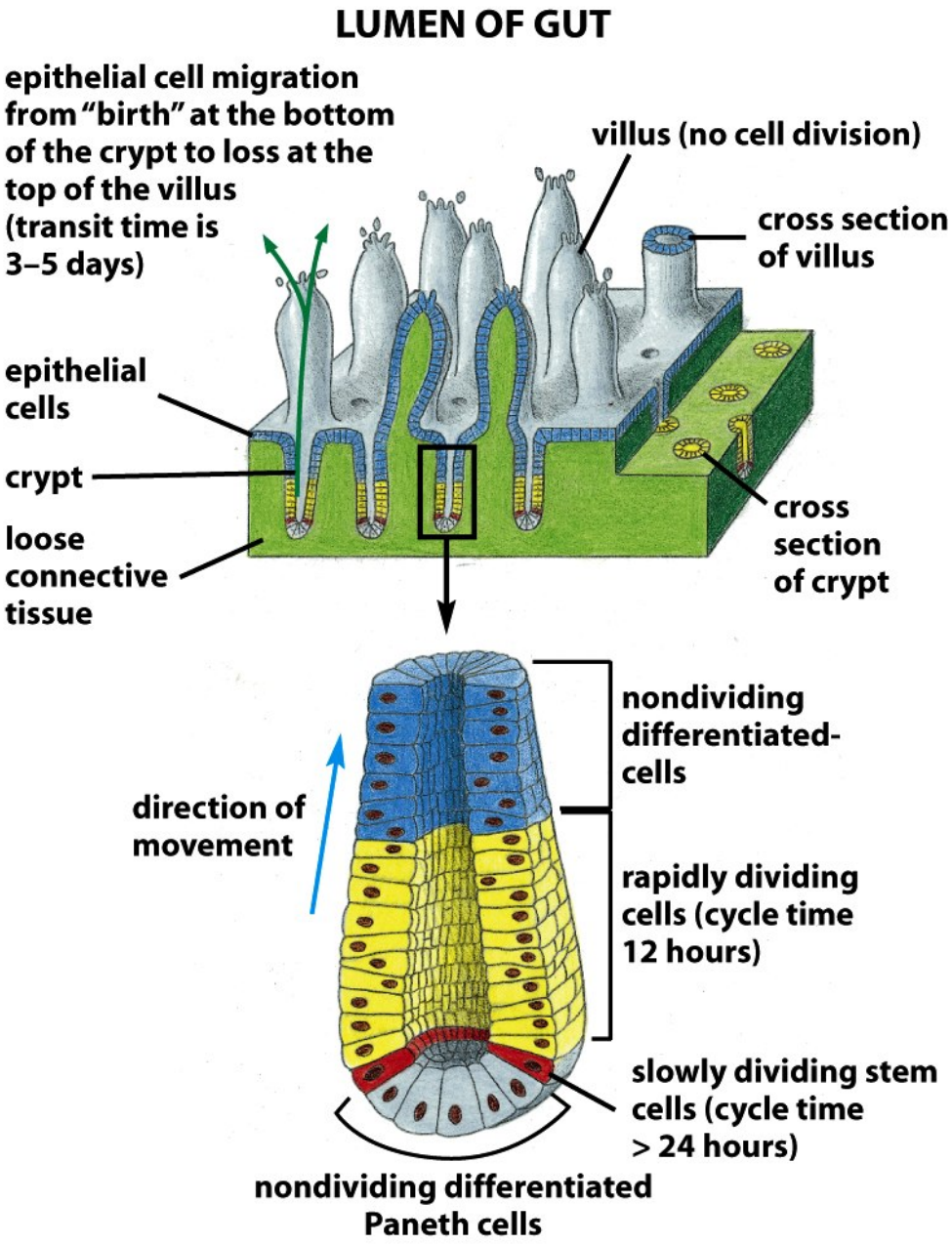
Adult stem cell





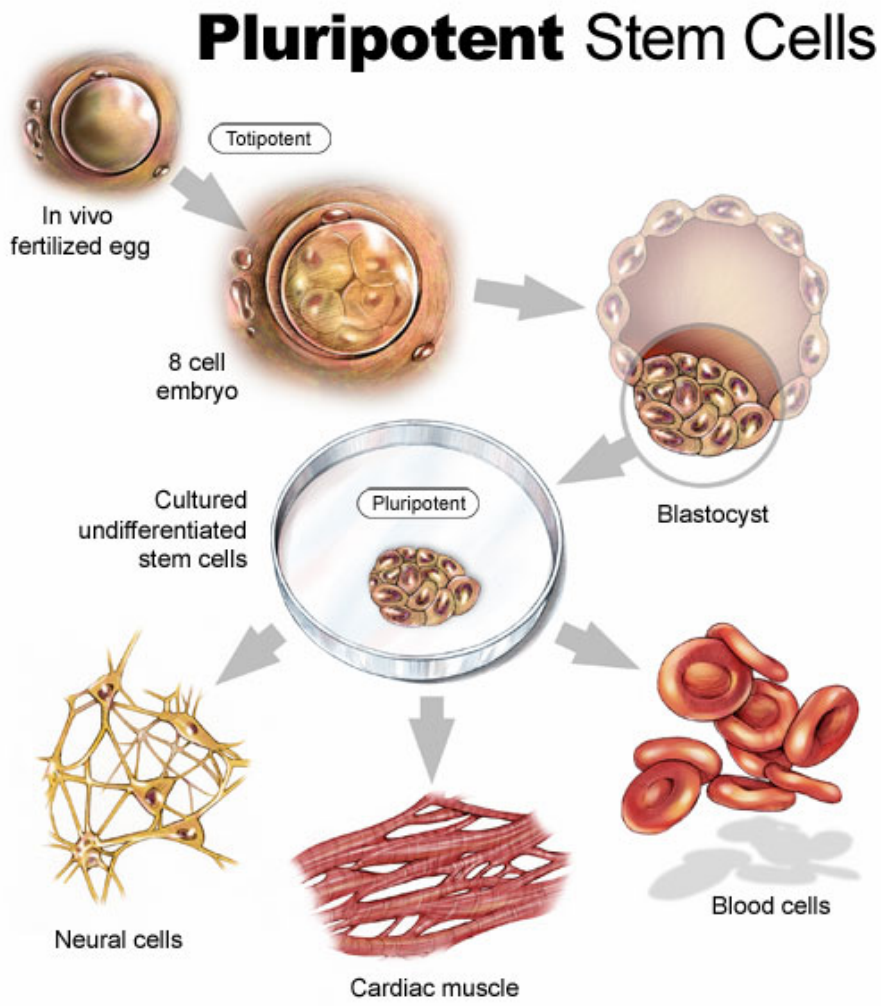
PROLIFERATION IN MULTICELLULAR ORGANISMS

Cell types in proliferative tissues



PROLIFERATION IN MULTICELLULAR ORGANISMS

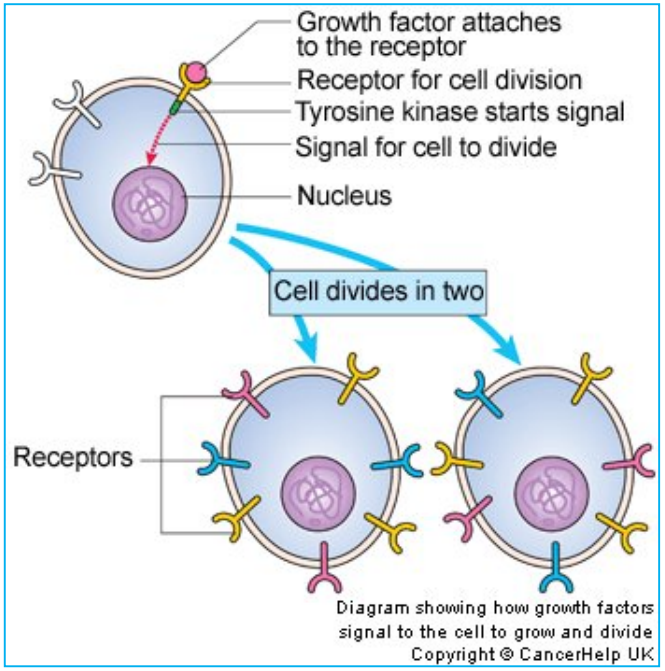
Cell types in proliferative tissues



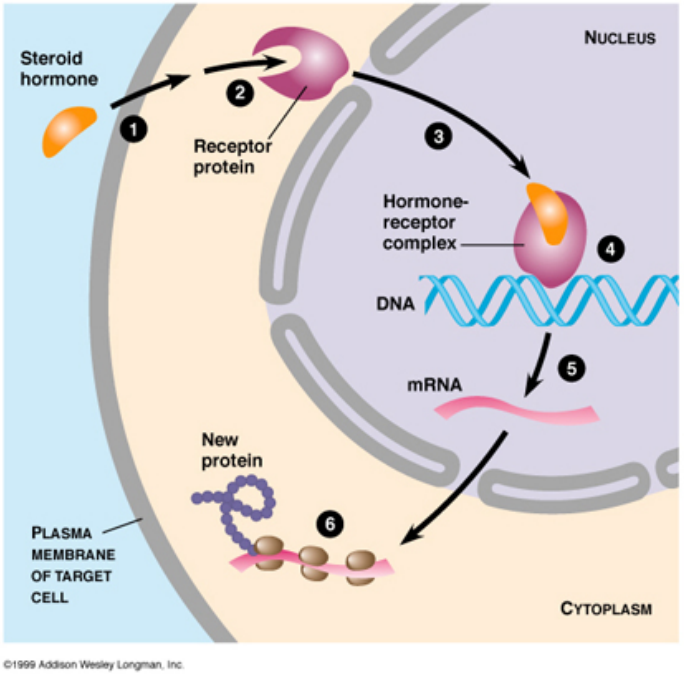
FACTORS THAT REGULATE CELLULAR PROLIFERATION

- Growth factors
- Hormones
- Contact between cells
- Contact with the extracellular matrix

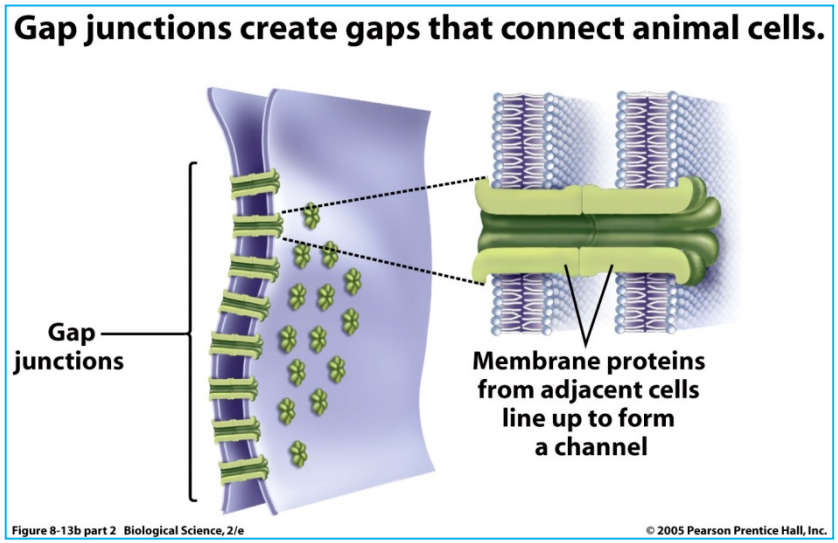
•Growth factors



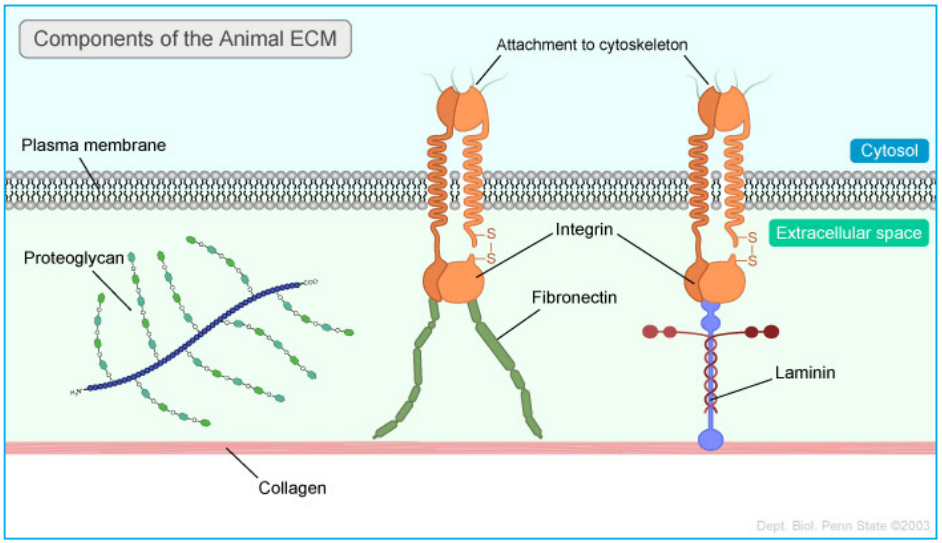
•Hormones



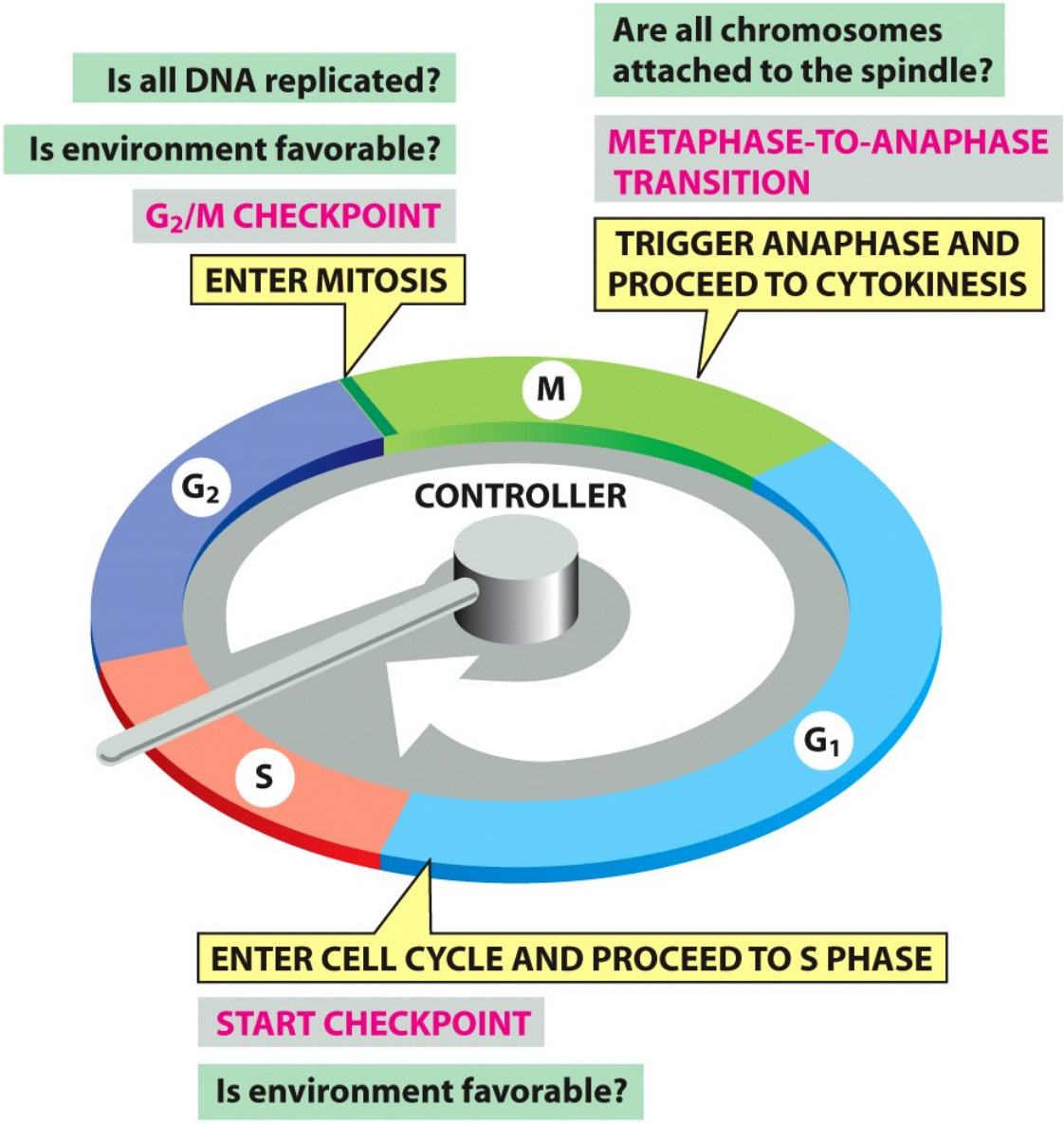
•Contact between cells



•Contact with the extracellular matrix

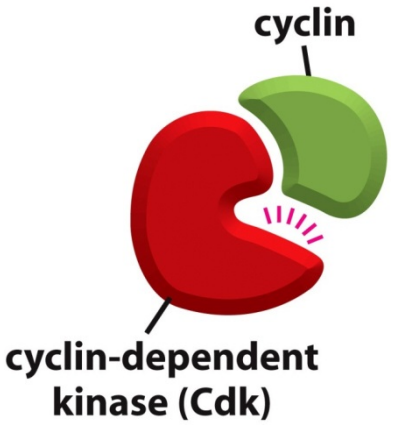


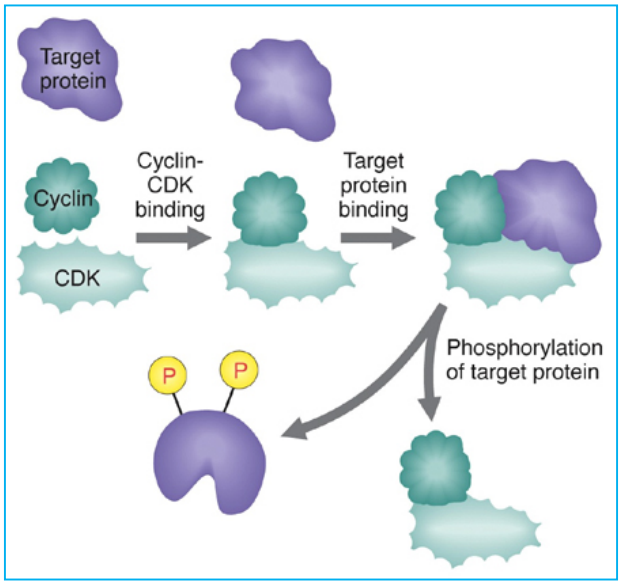
CELL-CYCLE CONTROL SYSTEM



CELL-CYCLE CONTROL SYSTEM

Cyclin-Dependent Protein Kinases (CDKs)





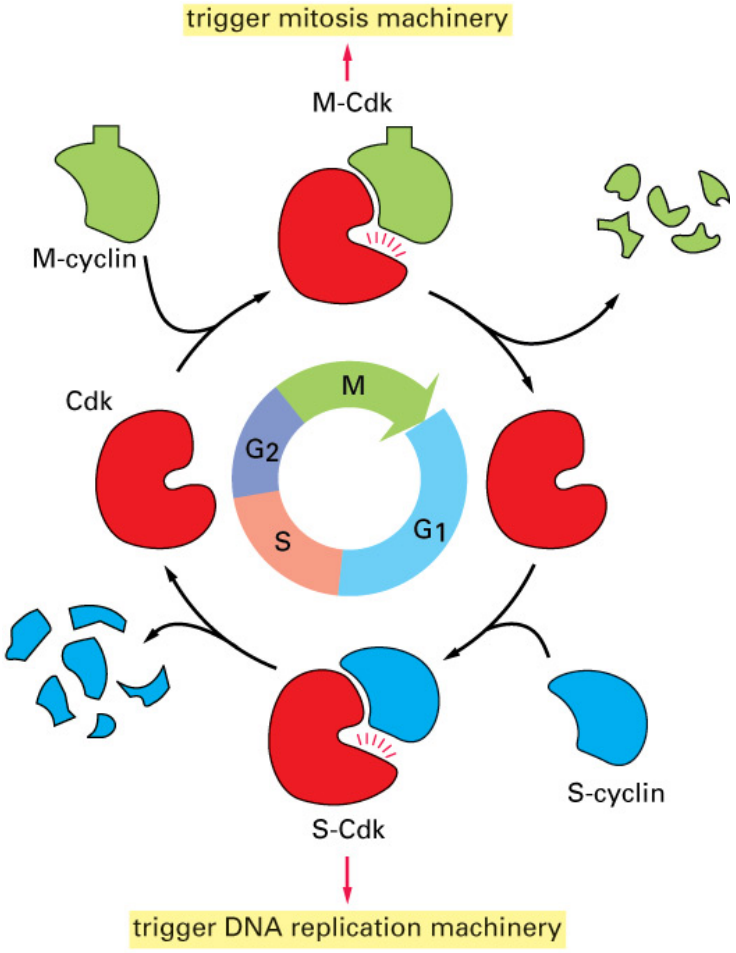


Figure 17–16. Molecular Biology of the Cell, 4th Edition.

CELL-CYCLE CONTROL SYSTEM

Synthesis and degradation of cyclins

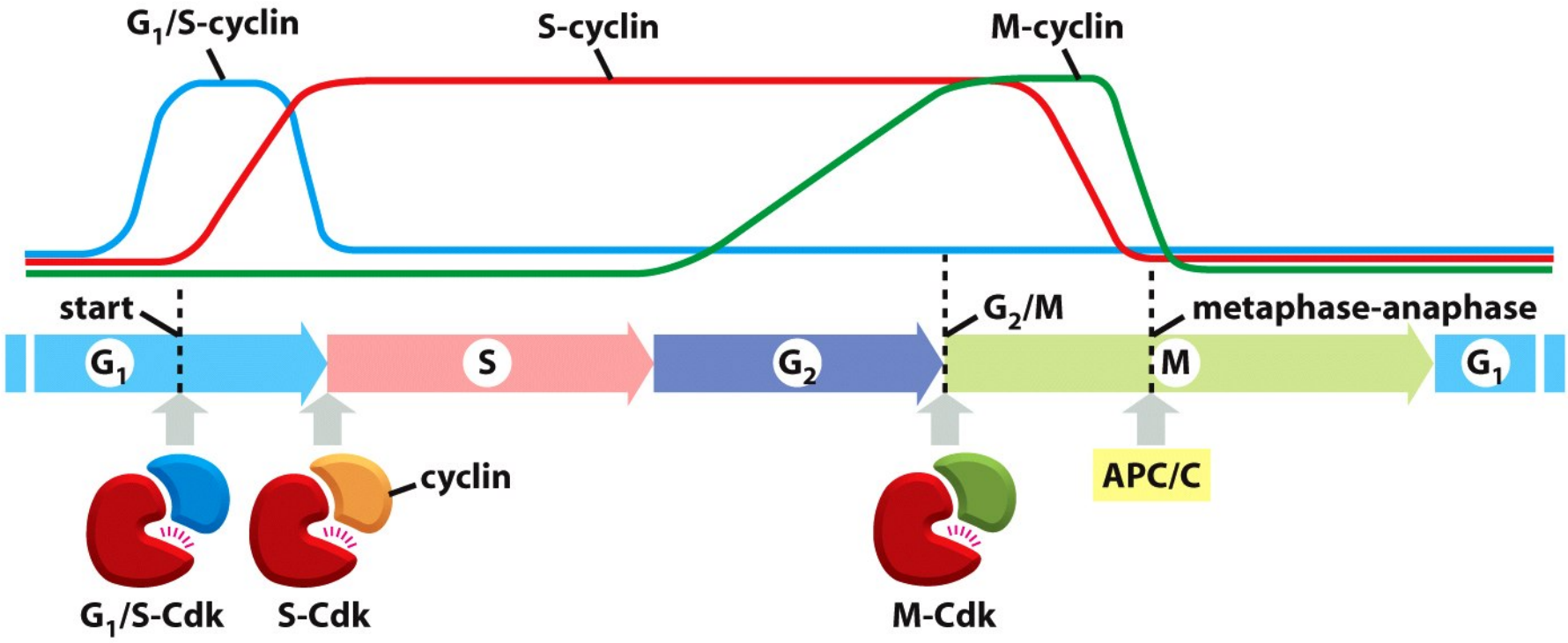
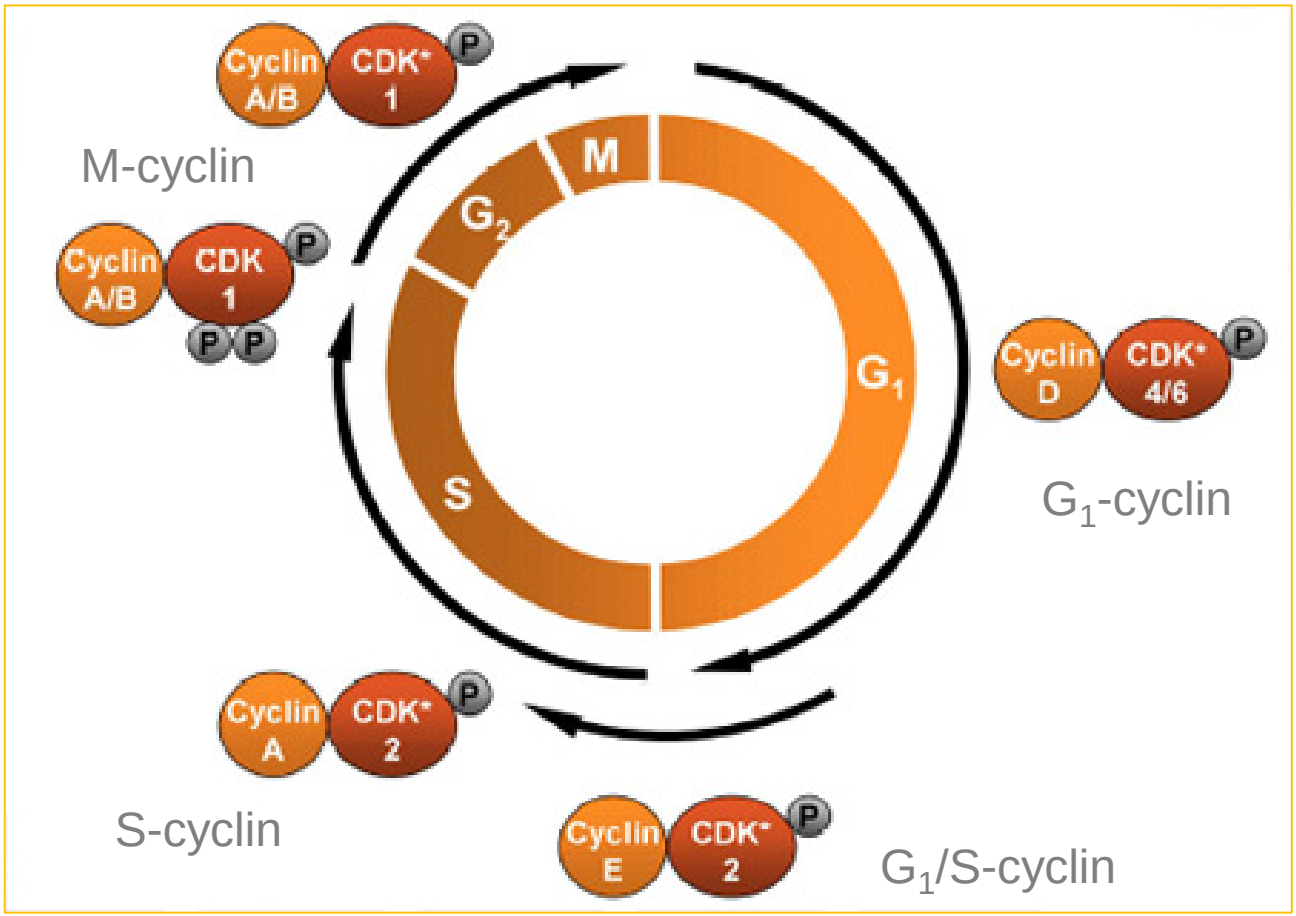


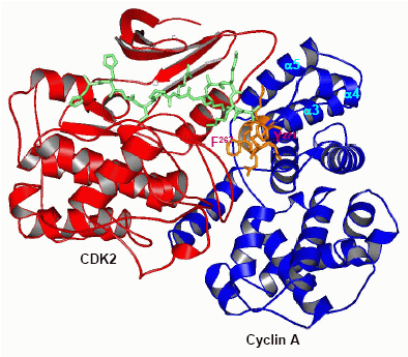
Figure 17-16 *Molecular Biology of the Cell* (© Garland Science 2008)

CELL-CYCLE CONTROL SYSTEM

Cyclin: A,B,C,D,E,F,G,H

Cdk: 1,2,3,4,5,6,7,8





CELL-CYCLE CONTROL SYSTEM

Inhibitory phosphorylation

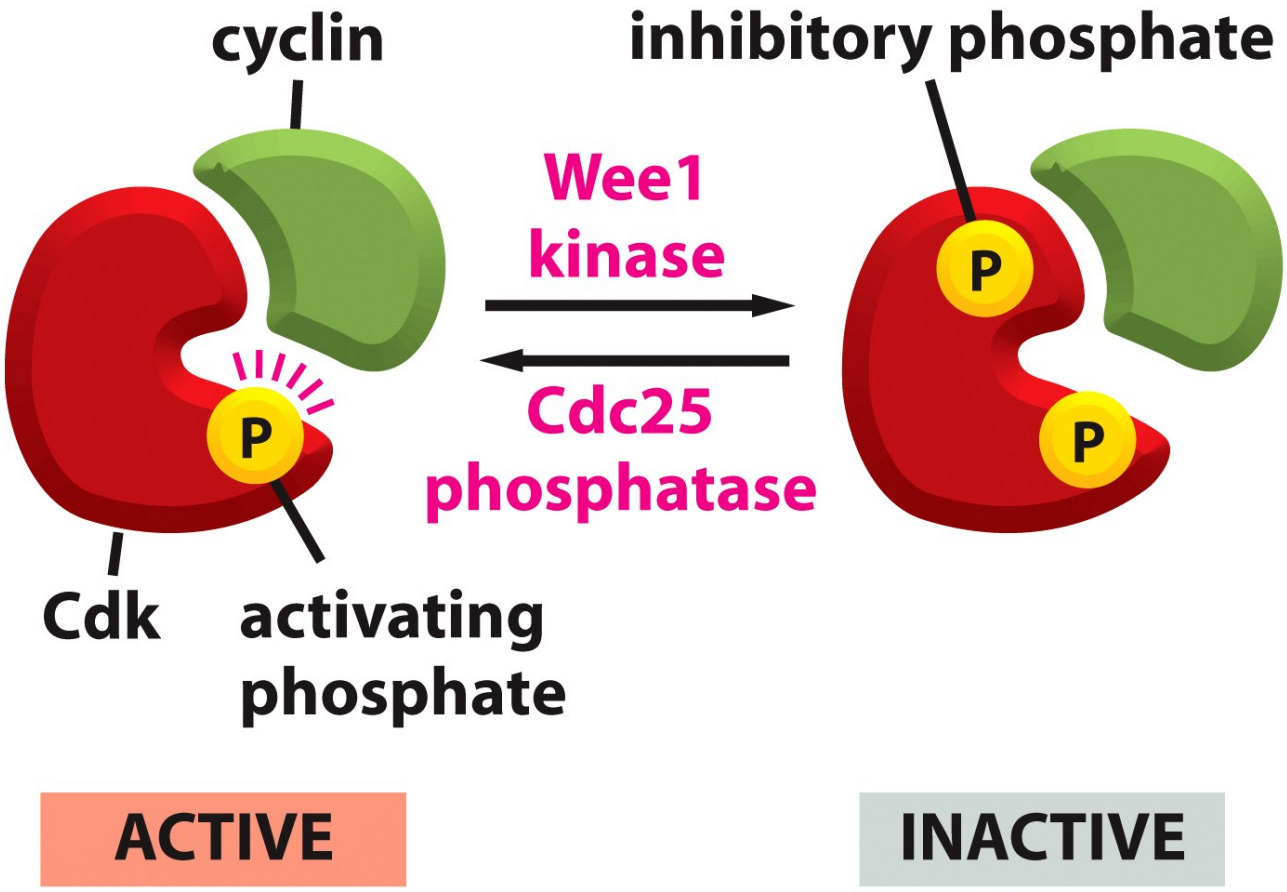


Figure 17-18 *Molecular Biology of the Cell* (© Garland Science 2008)

CELL-CYCLE CONTROL SYSTEM

Cdk Inhibitory Proteins (CKIs)

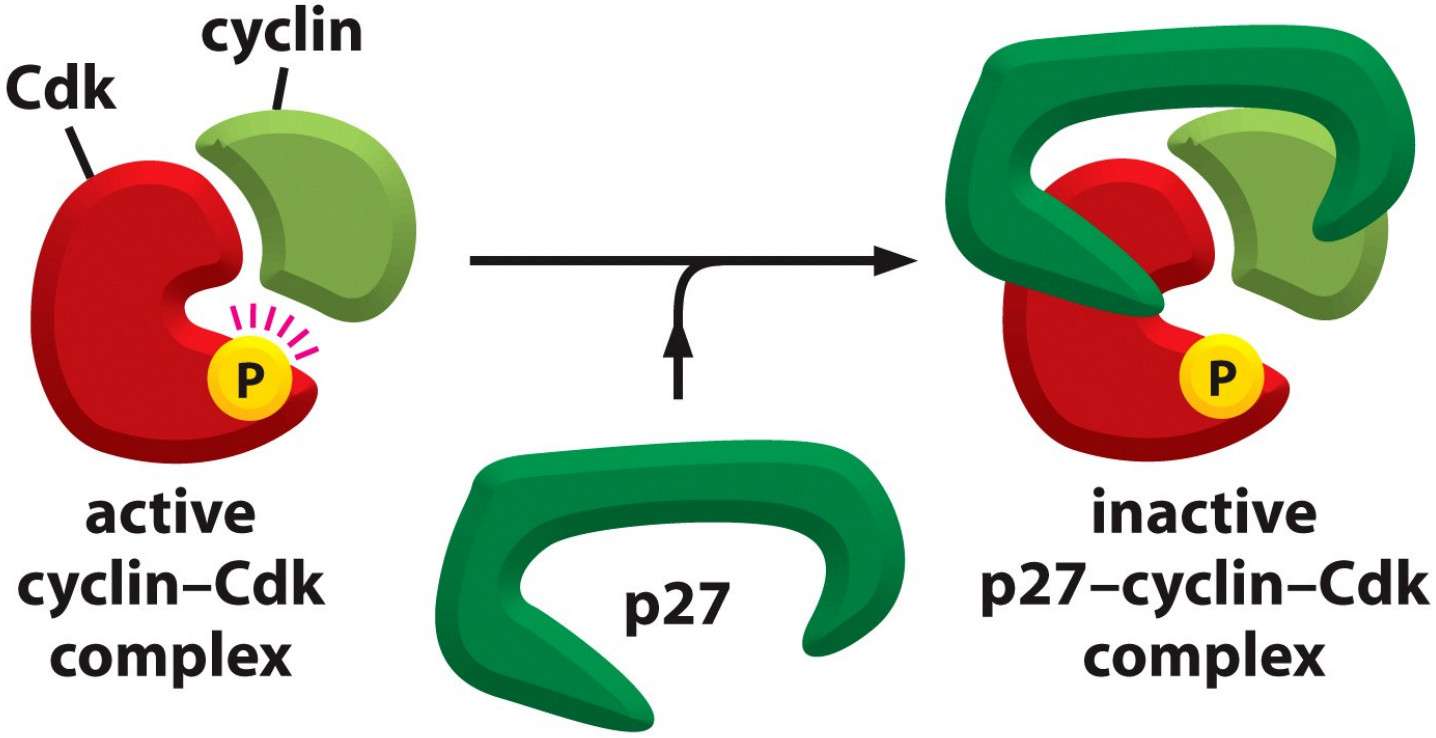
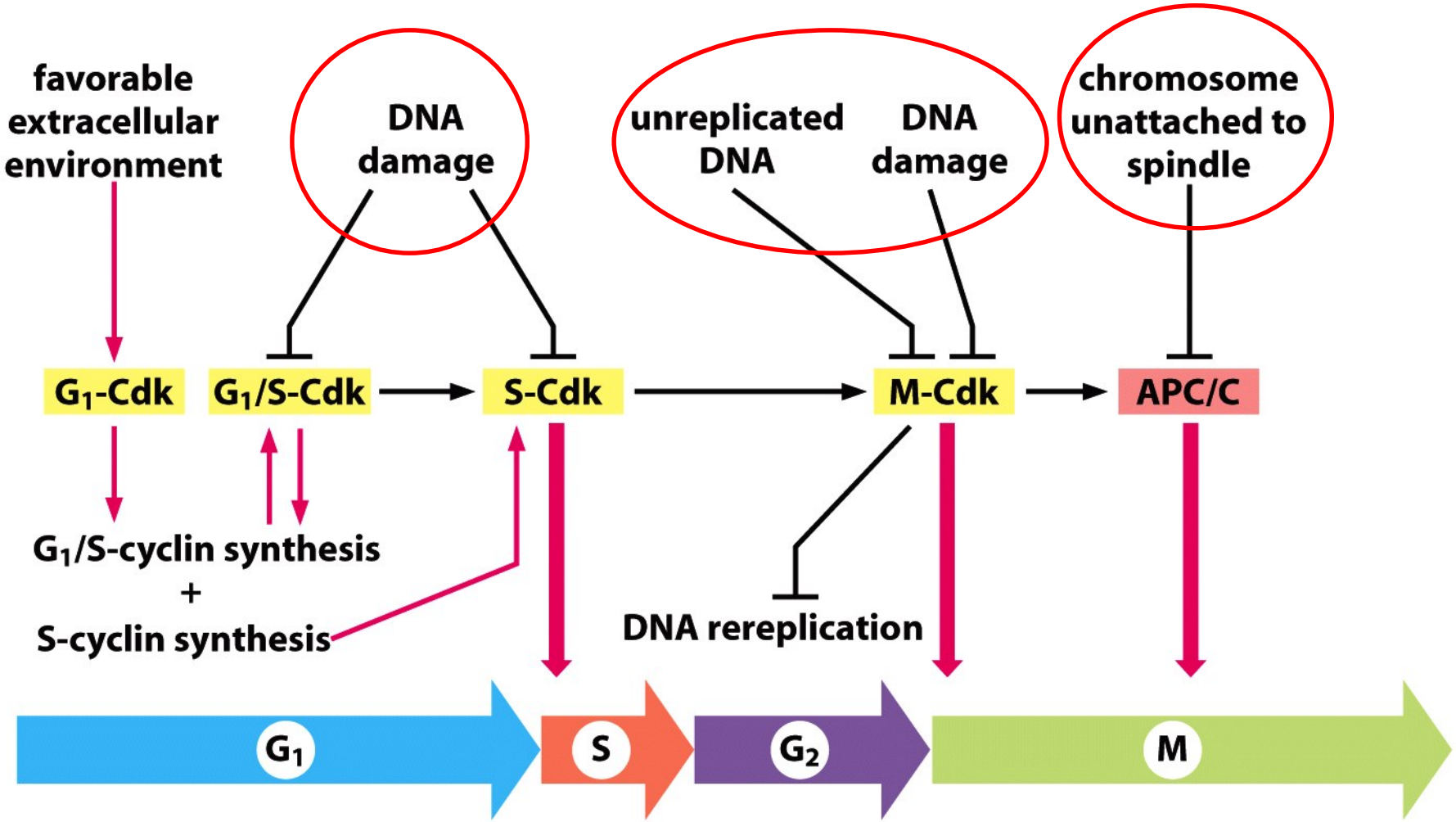
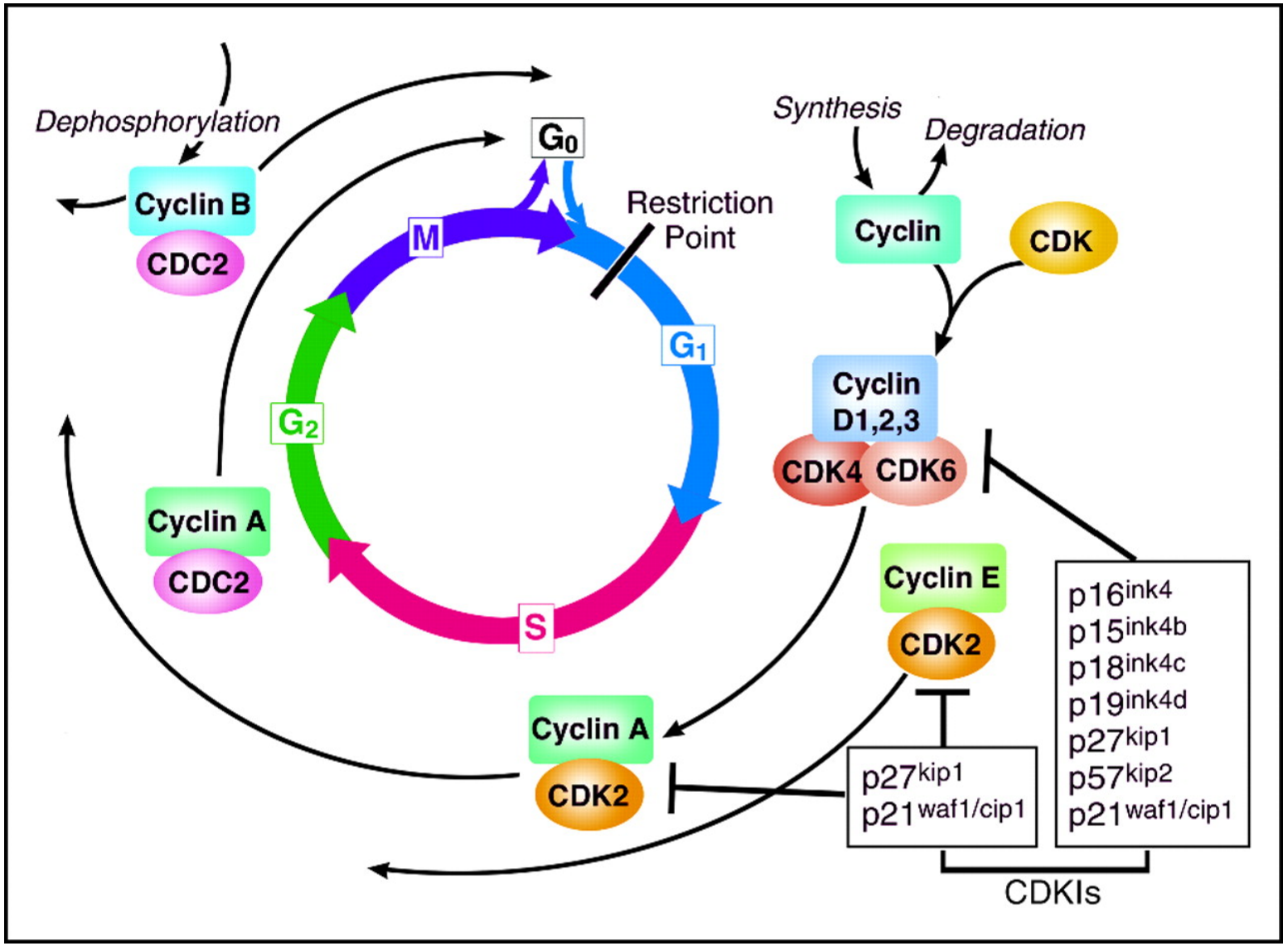


Figure 17-19 *Molecular Biology of the Cell* (© Garland Science 2008)

CELL-CYCLE CONTROL SYSTEM

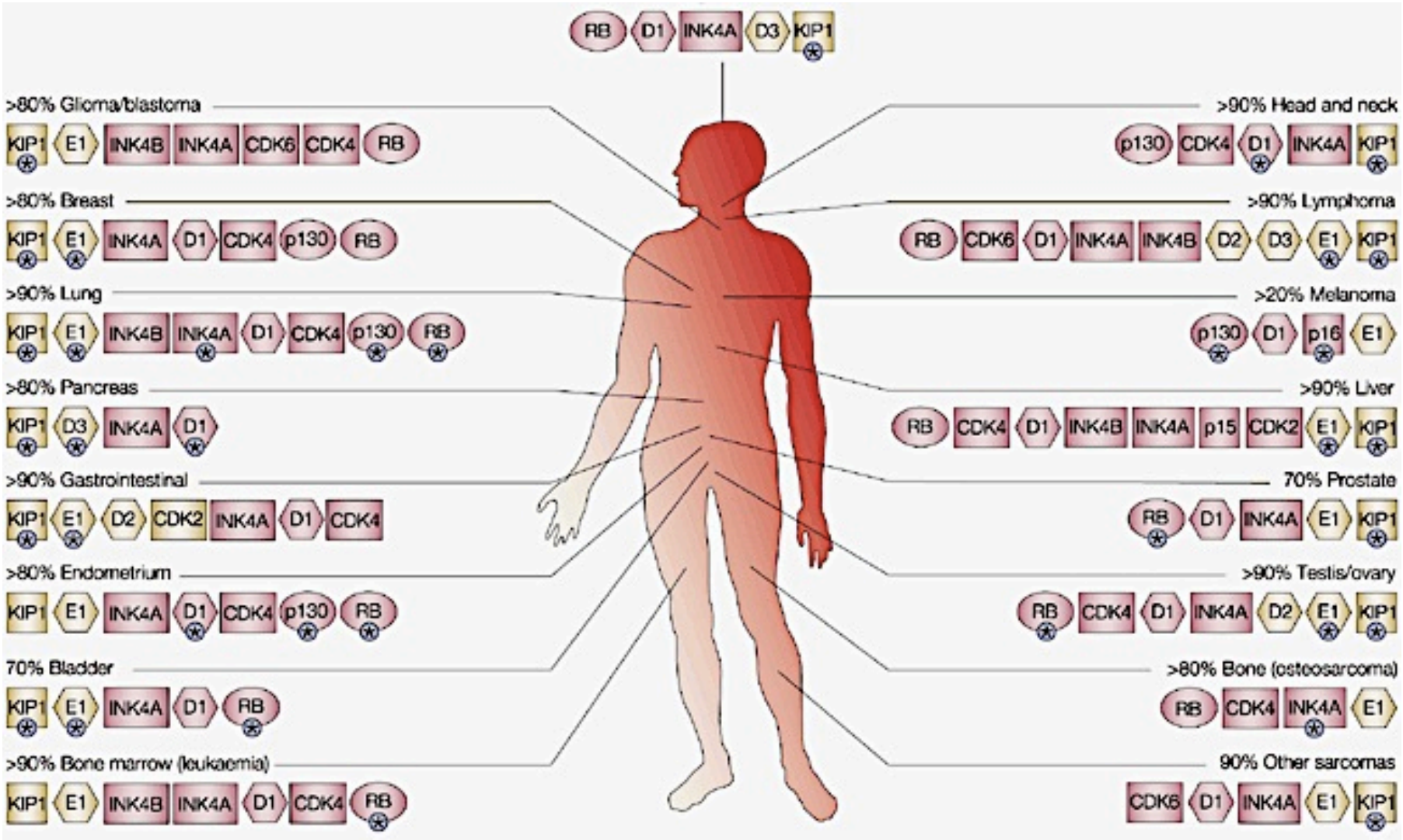


CELL-CYCLE CONTROL SYSTEM



Schwartz G K , Shah M A JCO 2005;23:9408-9421

CELL-CYCLE CONTROL SYSTEM



Alterations of cell cycle regulators in cancer

Nature Rev Cancer 1:222, 2001

CELL DIVISION

1. Introduction
2. Methodology
3. General characteristics
4. Cell division phases
    - Mitosis
        - Prophase
        - Prometaphase
        - Metaphase
        - Anaphase
        - Telophase
    - Cytokinesis
5. Physiological aspects of mitosis

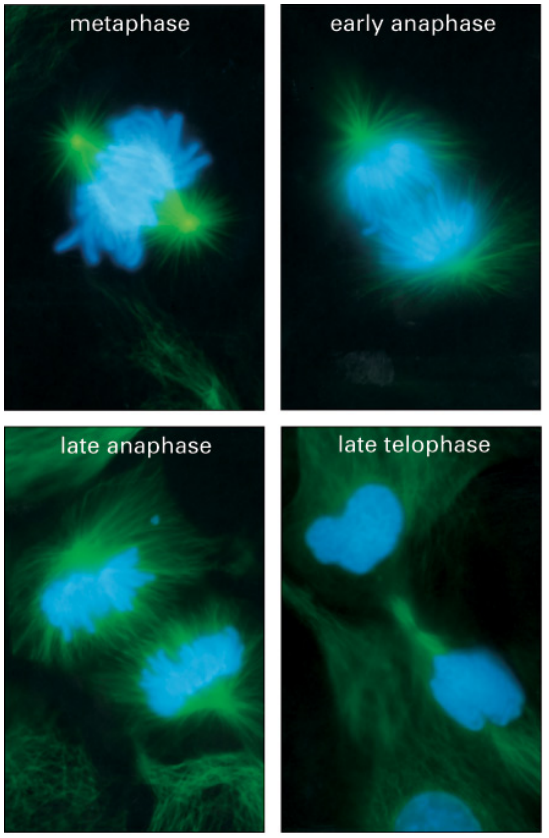
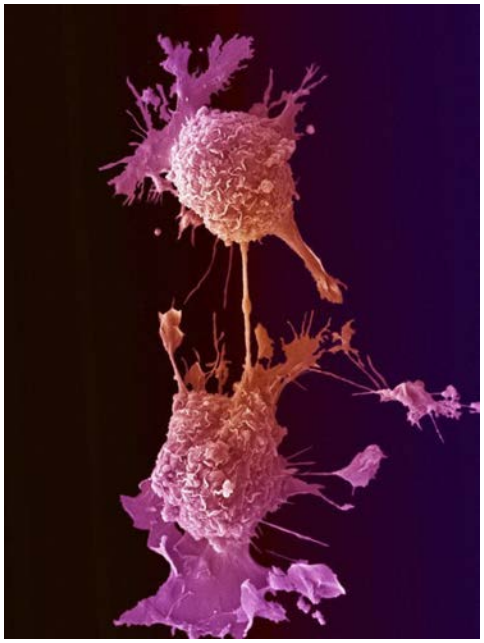
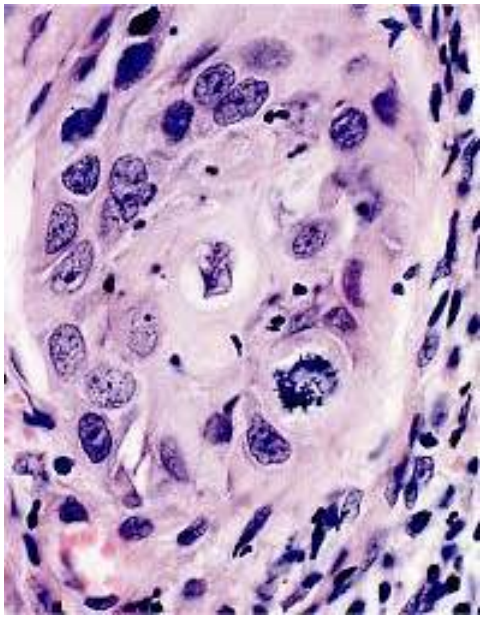


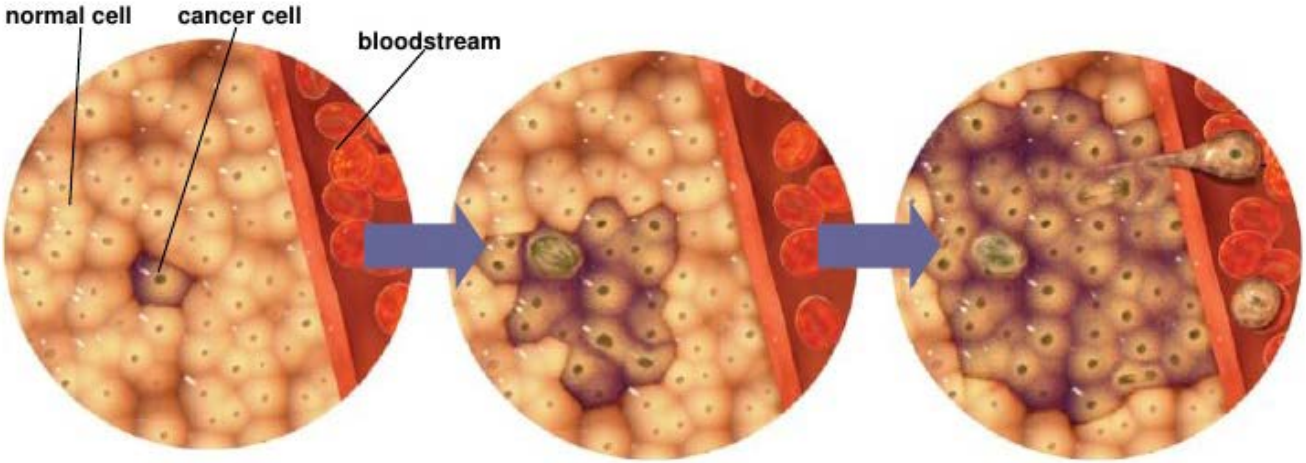
Figure 18–8 part 2 of 2. Molecular Biology of the Cell, 4th Edition.





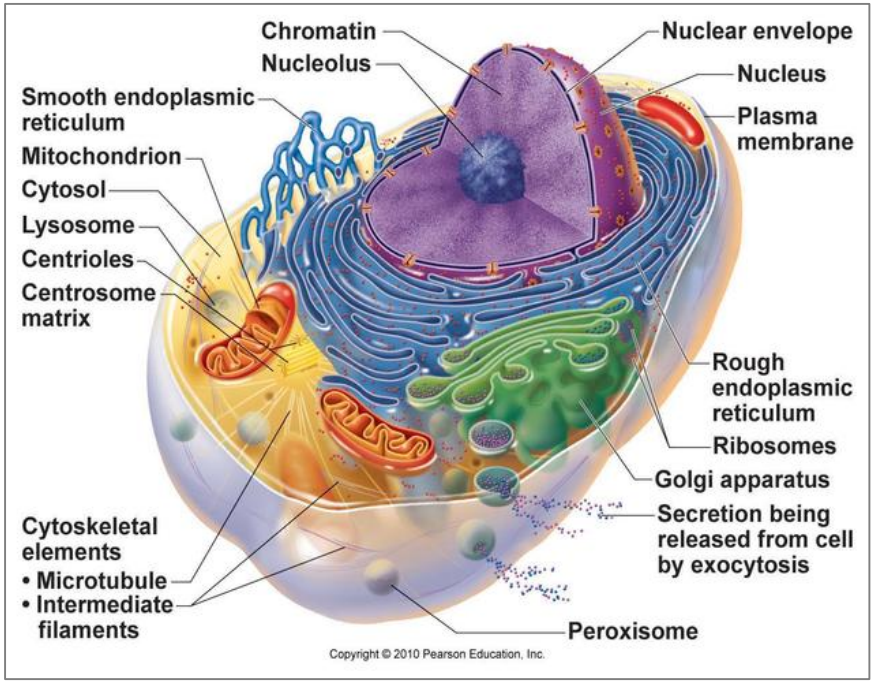
INTRODUCTION

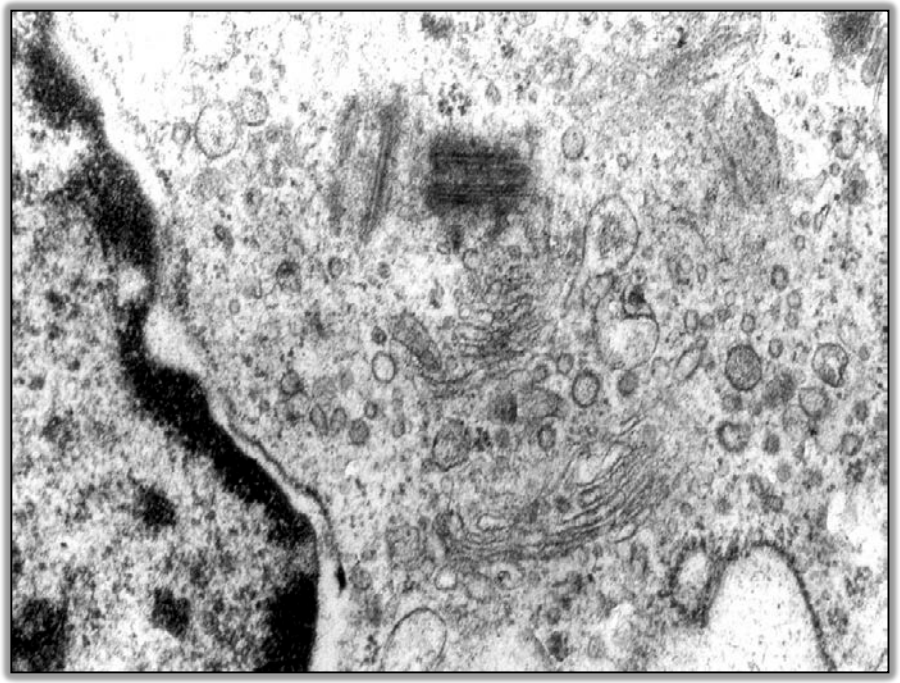
- **Cell division is uncontrolled in cancer.**
  - Cancer cells form disorganized clumps called tumors.
    - Benign tumors remain clustered and can be removed.
    - Malignant tumors metastasize, or break away, and can form more tumors.



INTERPHASE

1. DNA replication (Phase S)
2. Centrosome duplication





CENTRIOLE REPLICATION

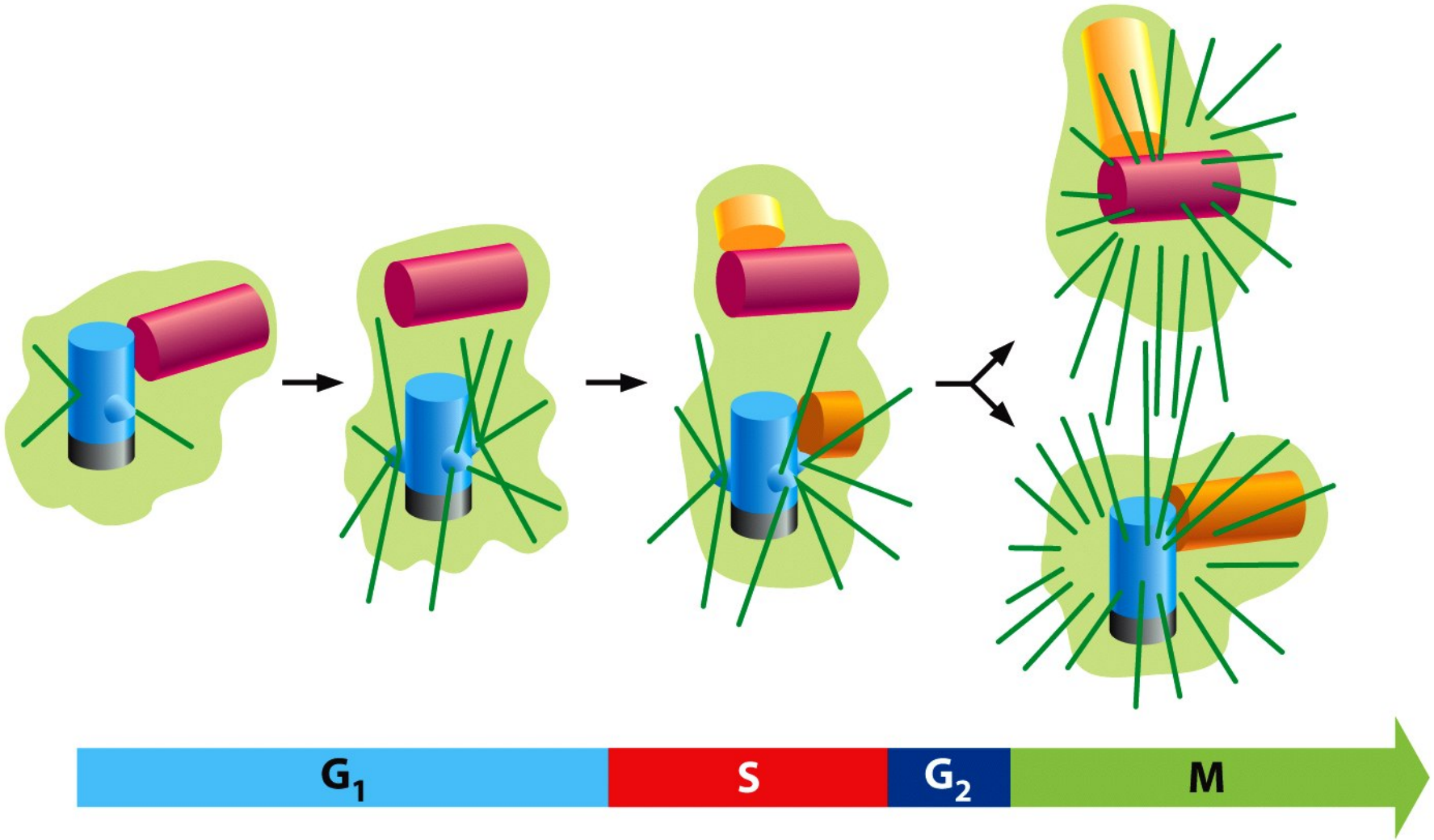
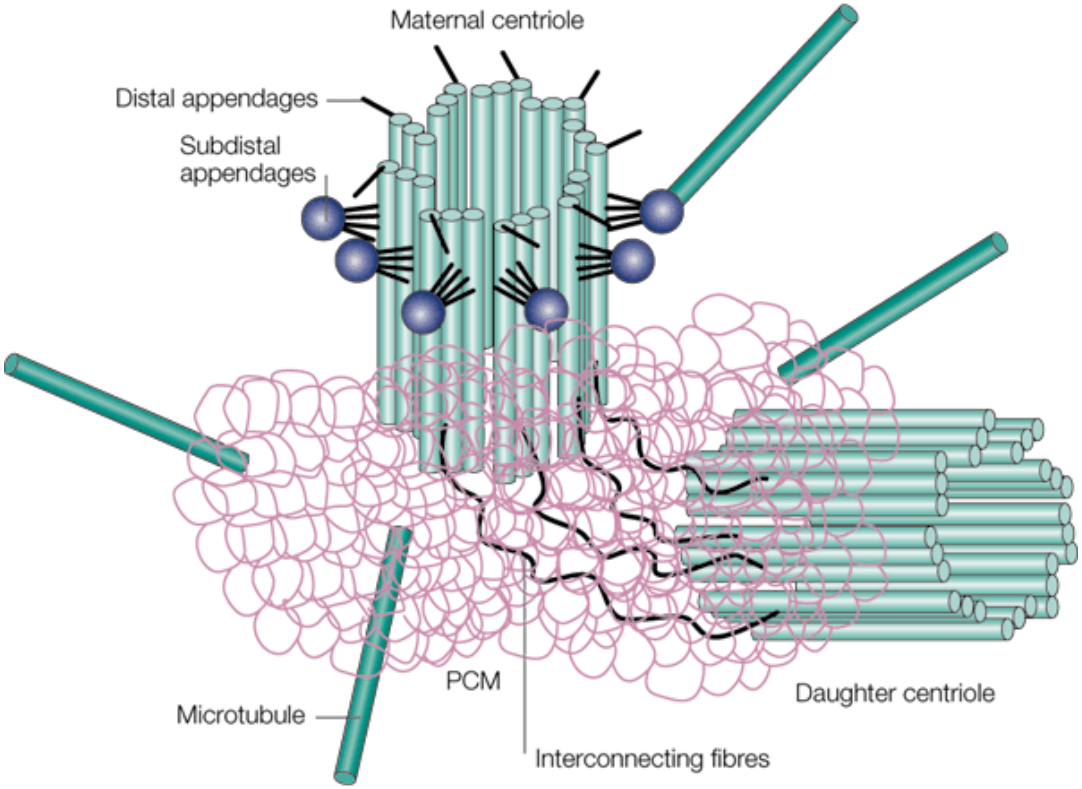
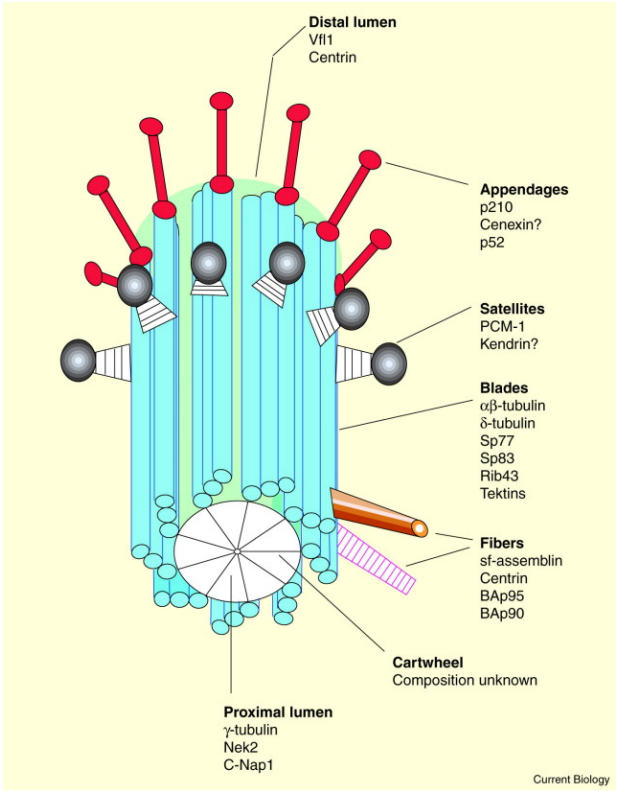


Figure 17-31 *Molecular Biology of the Cell* (© Garland Science 2008)

CENTRIOLE REPLICATION





METHODS

1 Light microscope

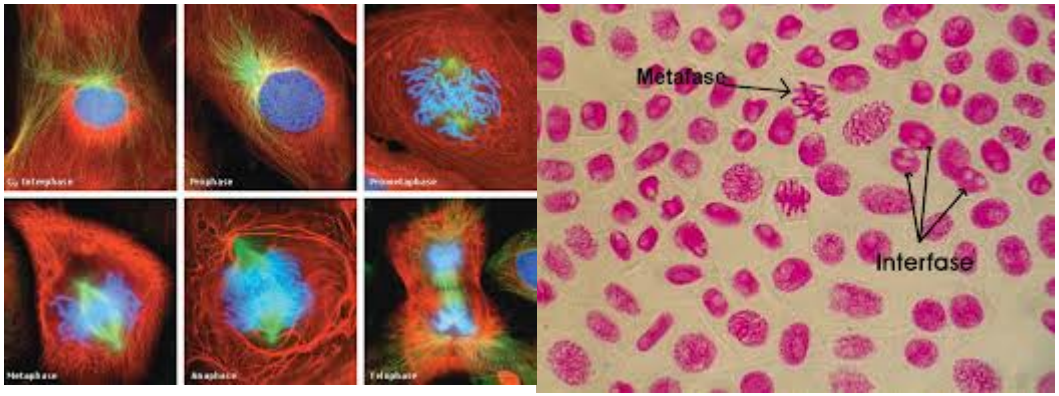
2 Electron microscope

3 Antimitotic compounds

4 Isolating mitotic components and apparatus

METHODS

1



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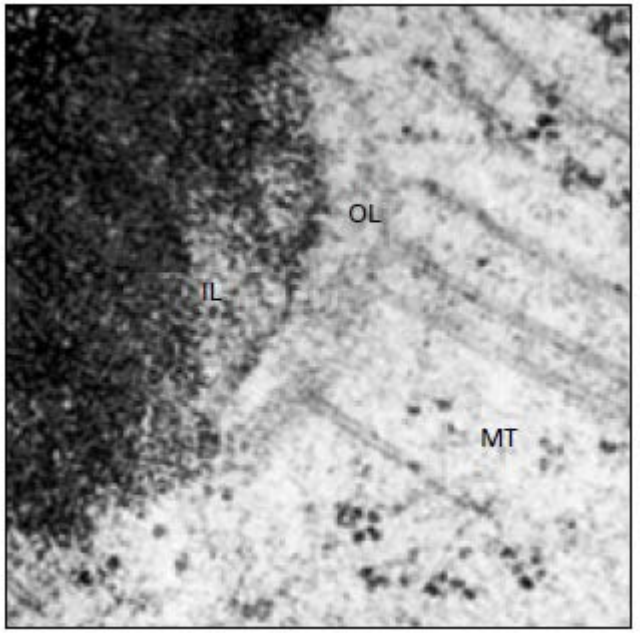

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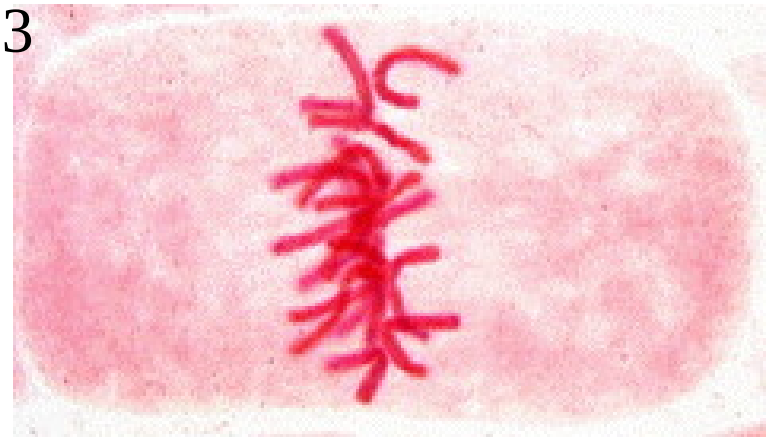

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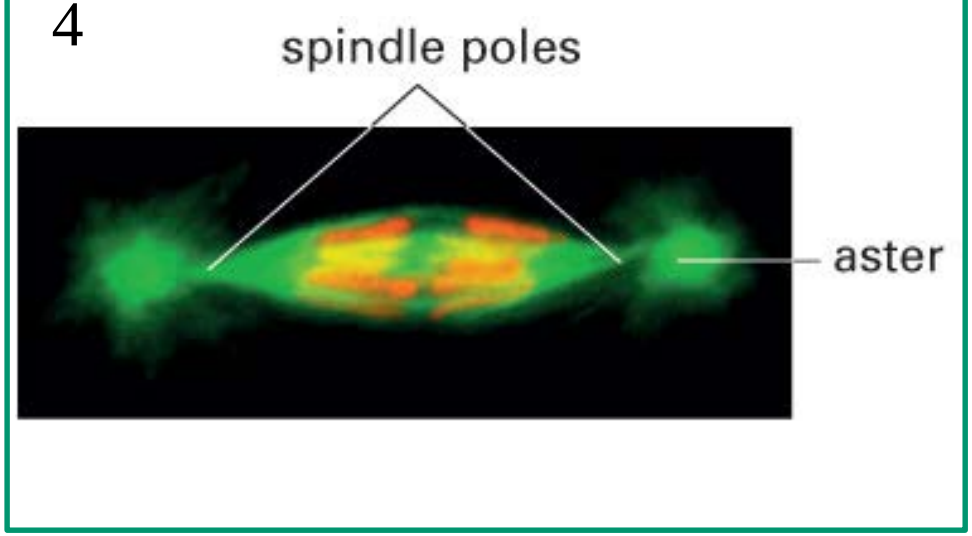


GENERAL CHARACTERISTICS

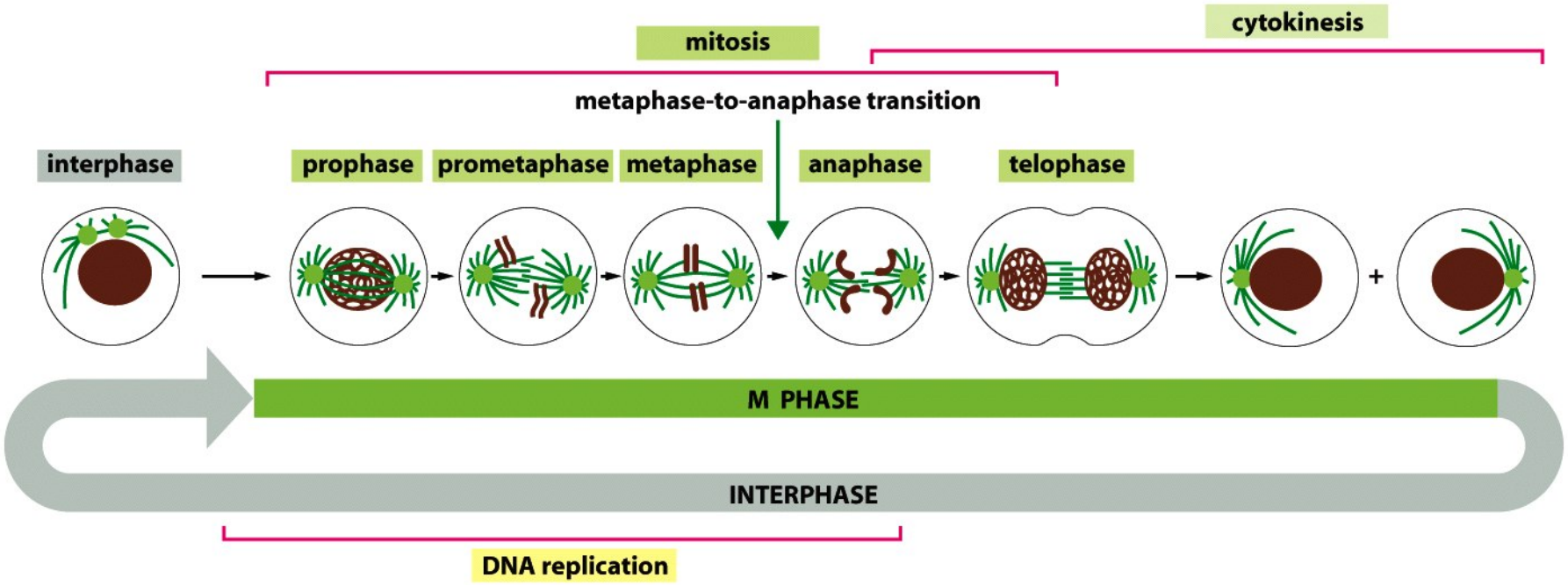
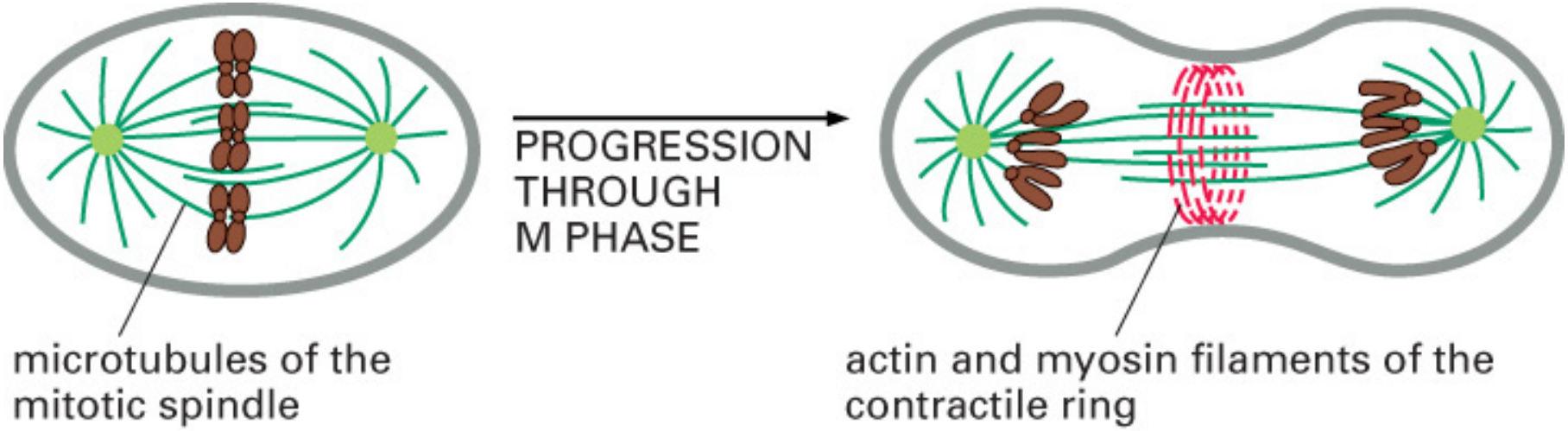
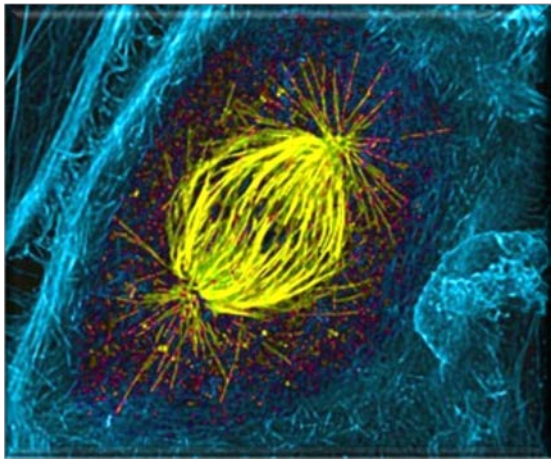


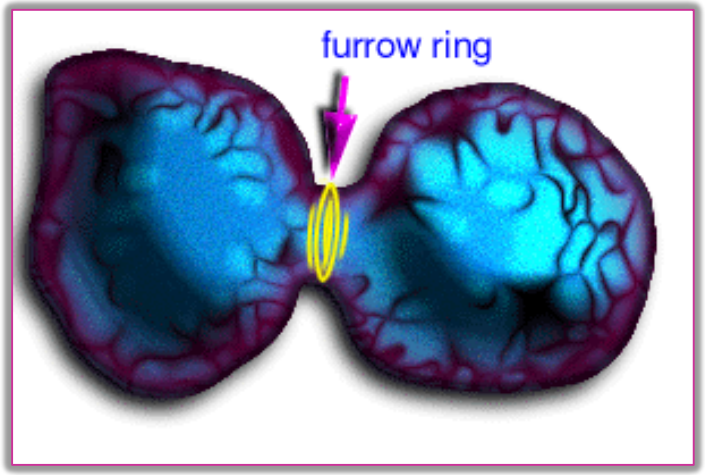
Figure 17-3 *Molecular Biology of the Cell* (© Garland Science 2008)

GENERAL CHARACTERISTICS:
Cytoskeleton components in cell division

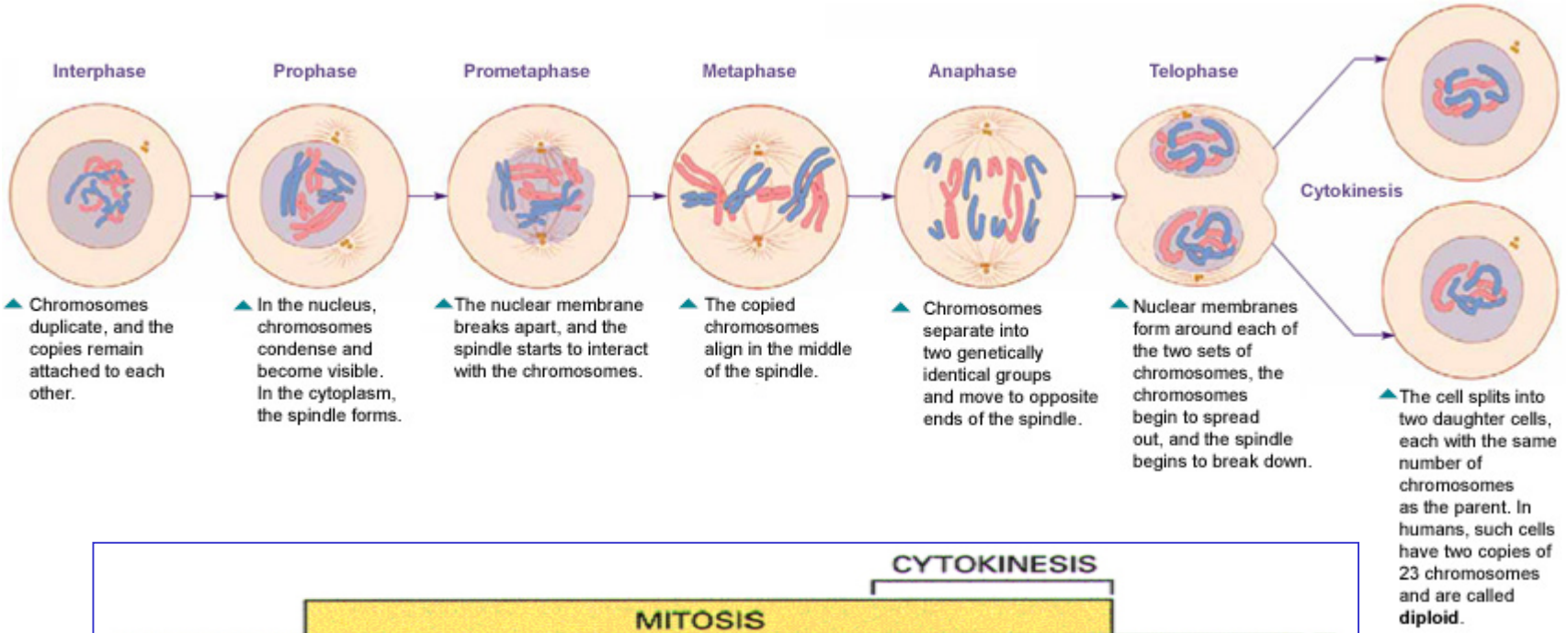


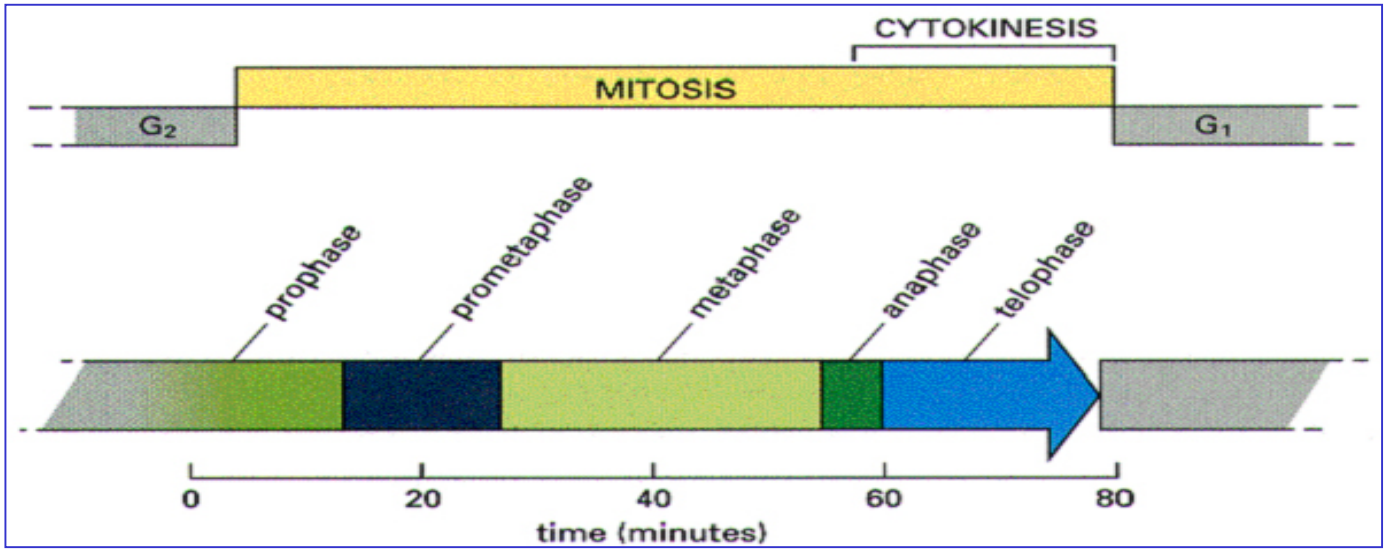
MAPs
Motor proteins
Catastrophins



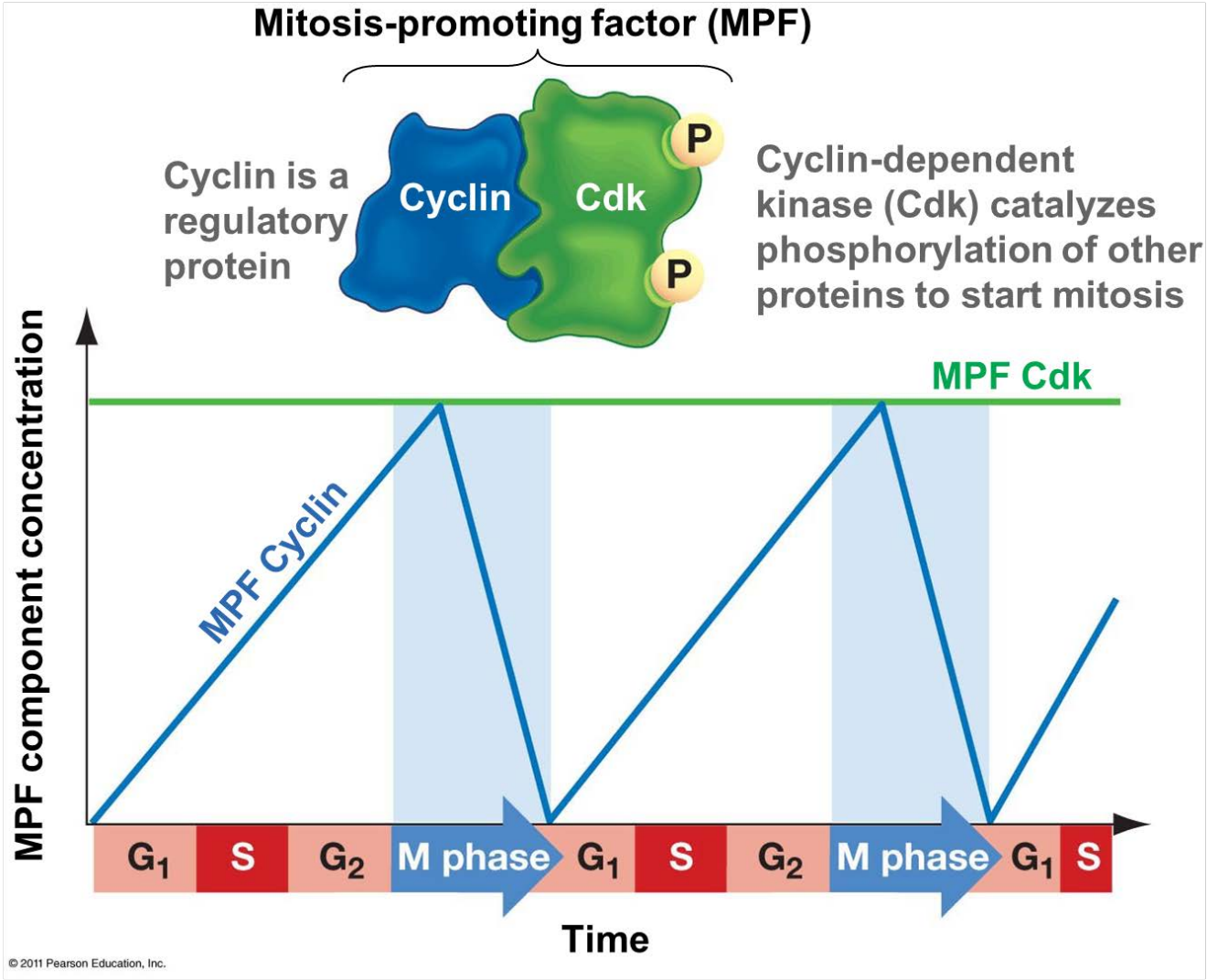


PHASES AND CONTROL OF MITOSIS



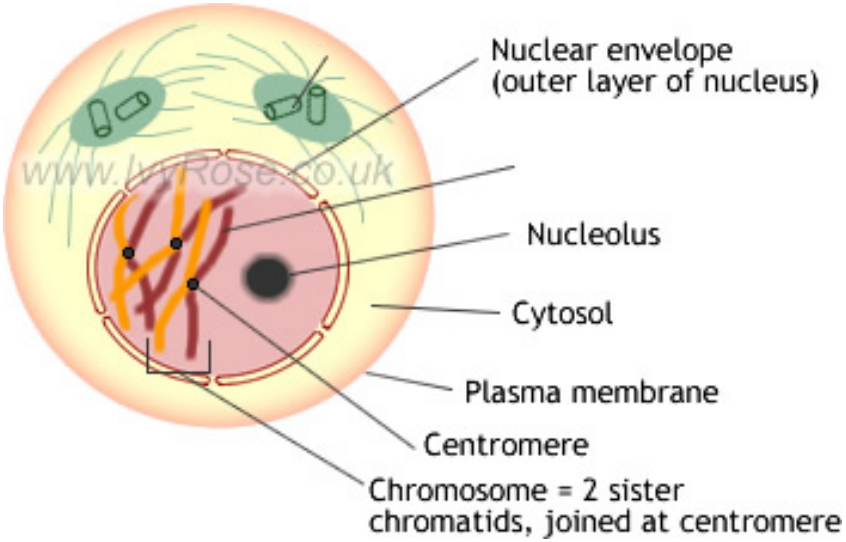


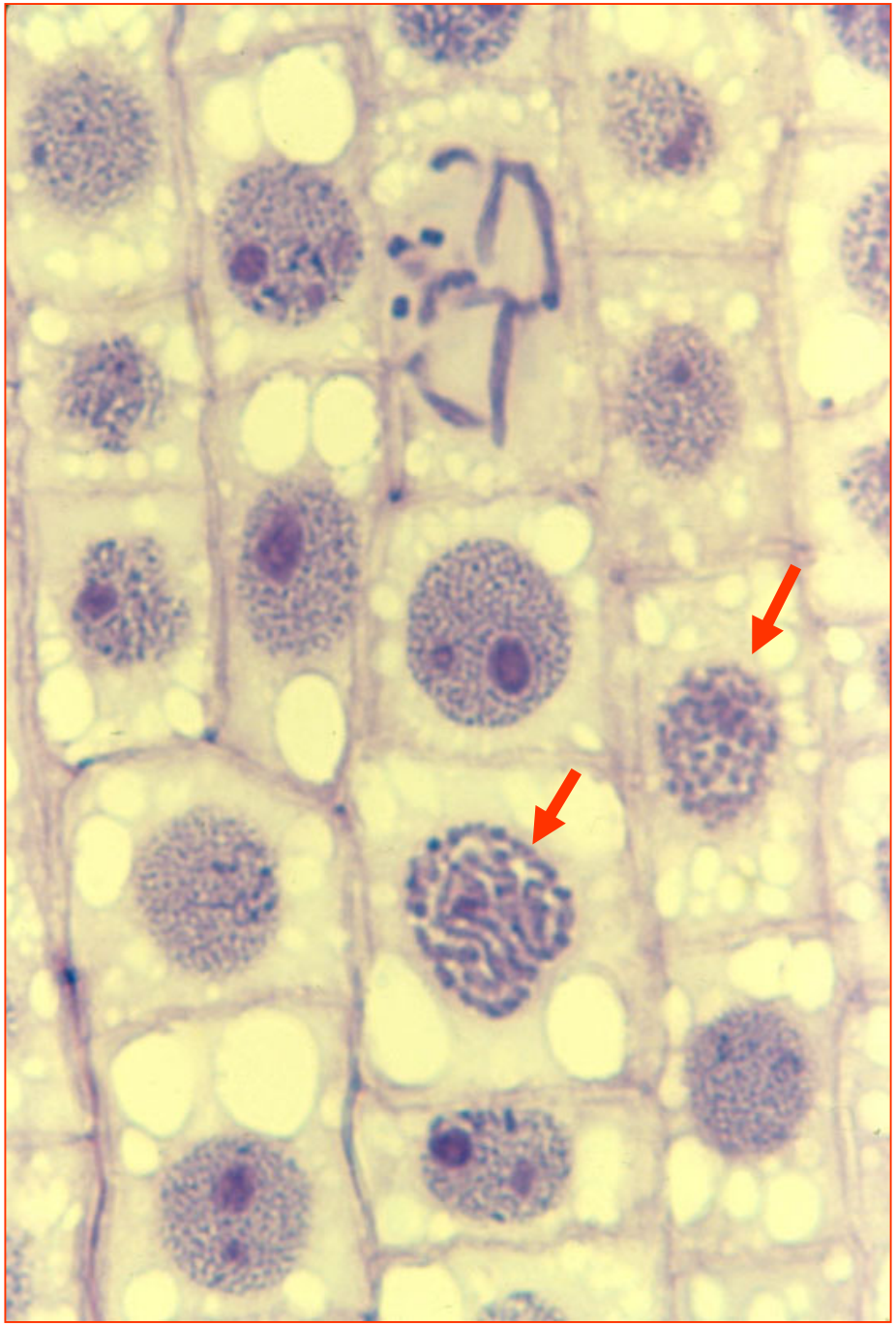
PHASES AND CONTROL OF MITOSIS



Mostly controlled by protein phosphorylation and dephosphorylation by CDKs and other proteins

PROPHASE

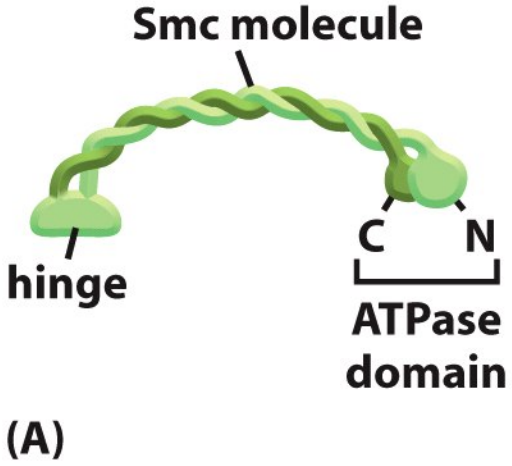

Nuclear envelope
(outer layer of nucleus)
Nucleolus
Cytosol
Plasma membrane
Centromere
Chromosome = 2 sister
chromatids, joined at centromere

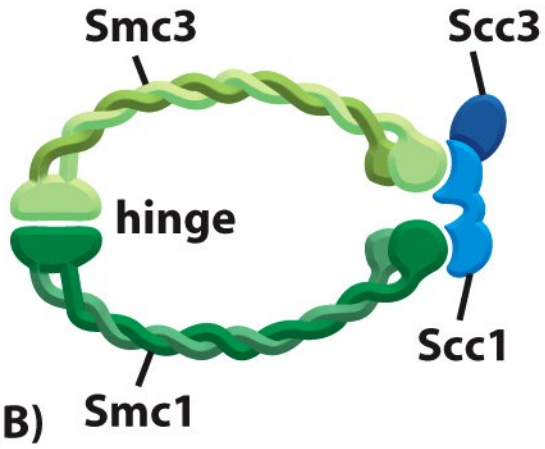


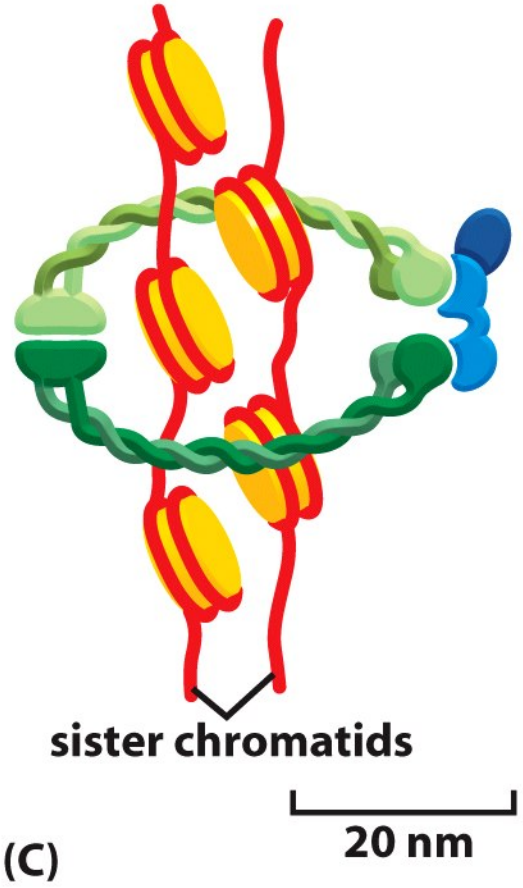
PROPHASE

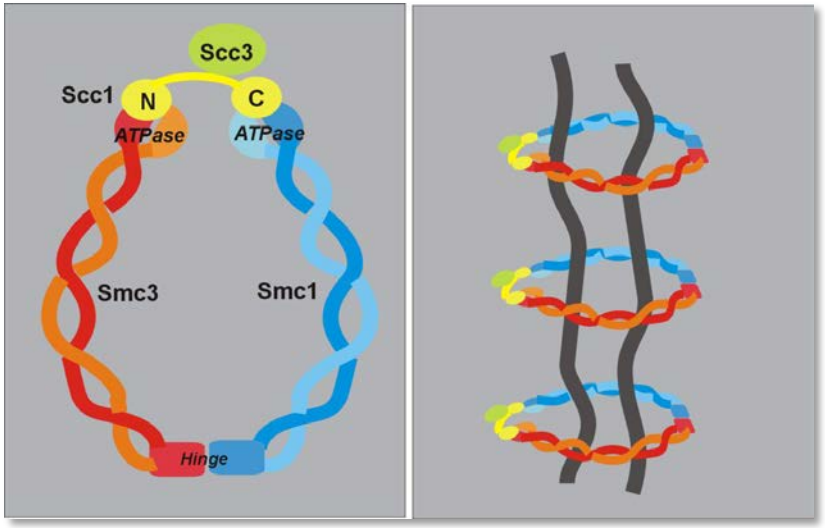
CHROMOSOME CONDENSATION

COHESIN







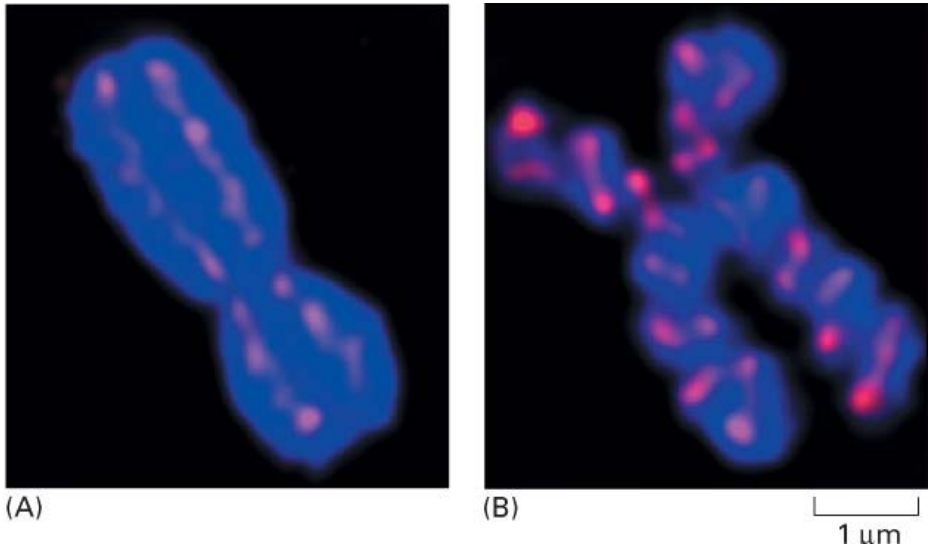


Smc: **S**tructural **M**aintenance of **C**hromosomes

PROPHASE

CHROMOSOME CONDENSATION

CONDENSIN 1



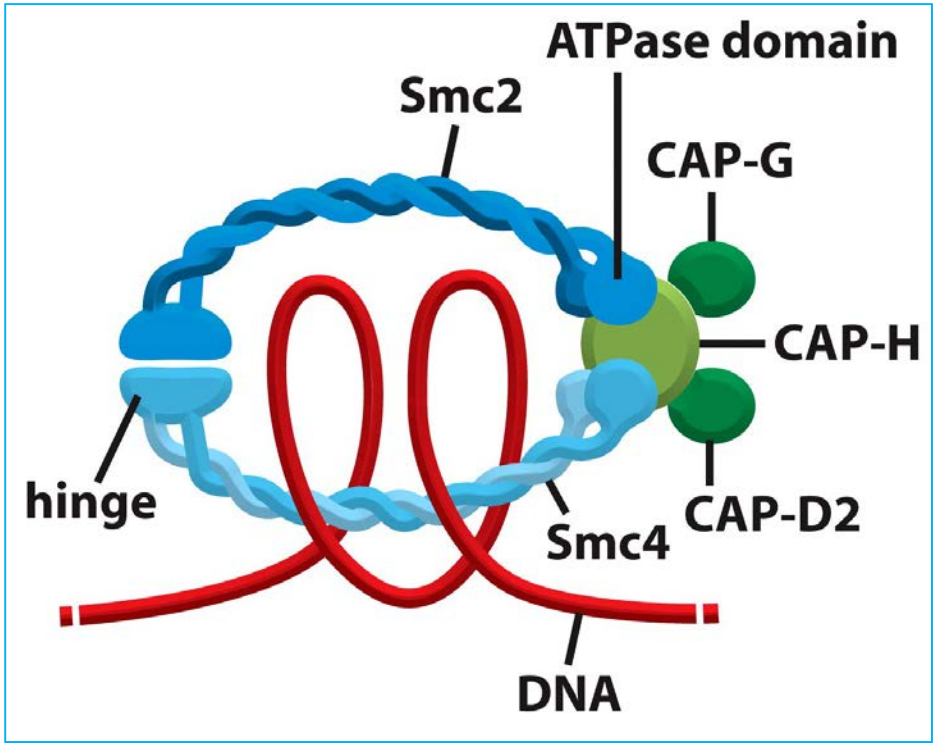
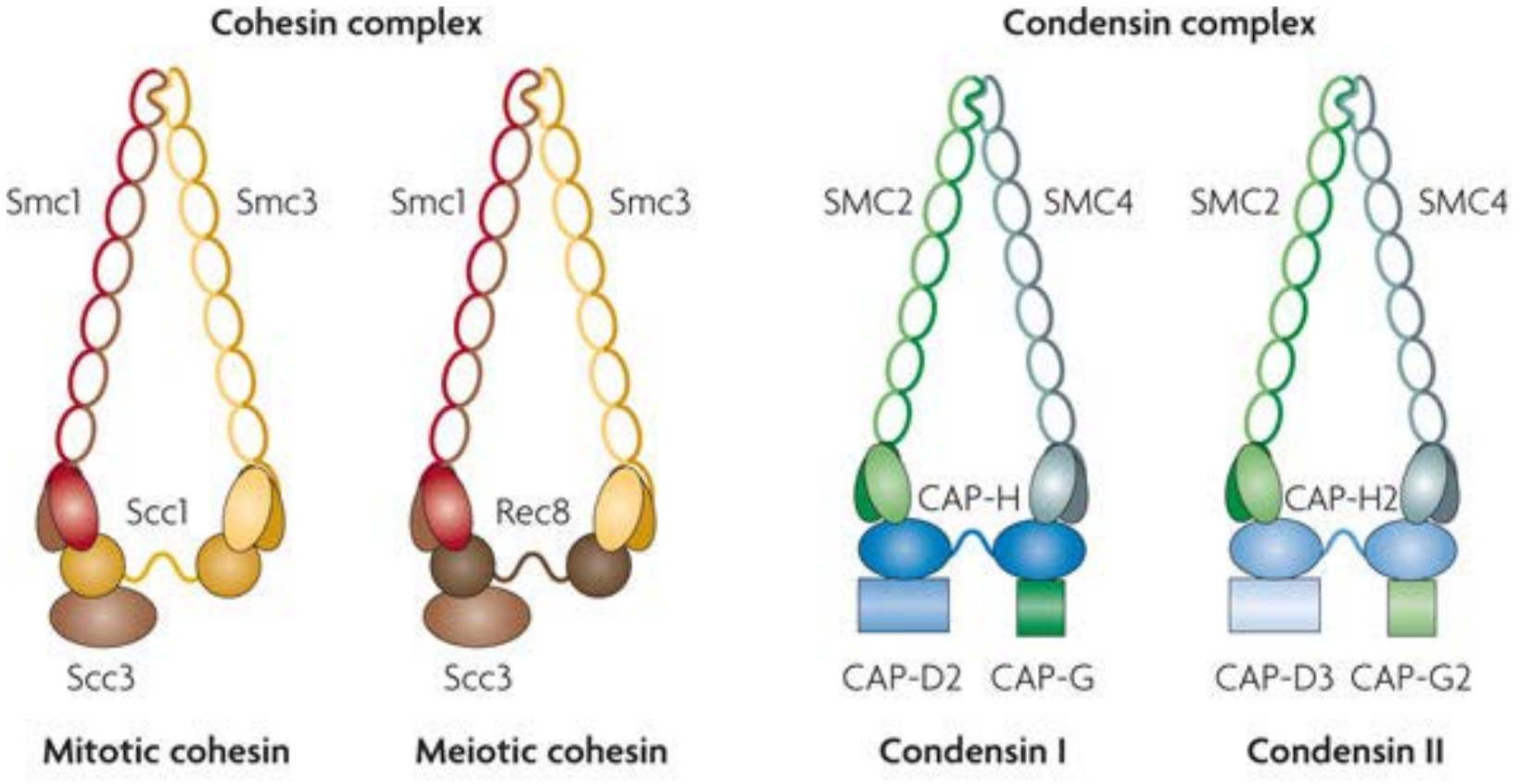


Figure 17-24 *Molecular Biology of the Cell* (© Garland Science 2008)

PROPHASE

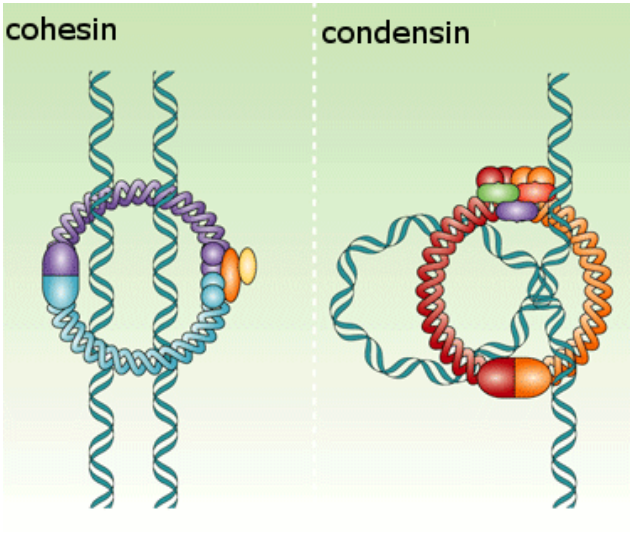
CHROMOSOME CONDENSATION

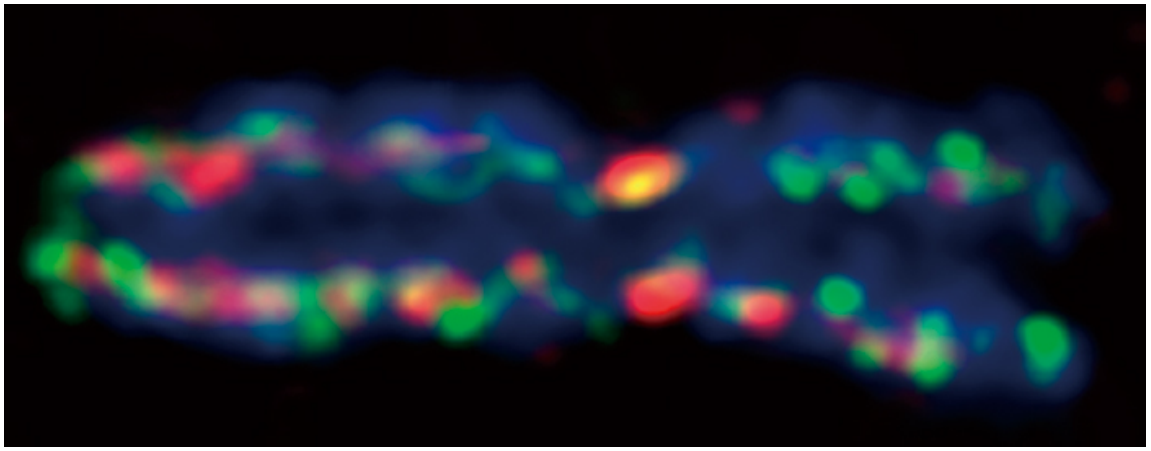


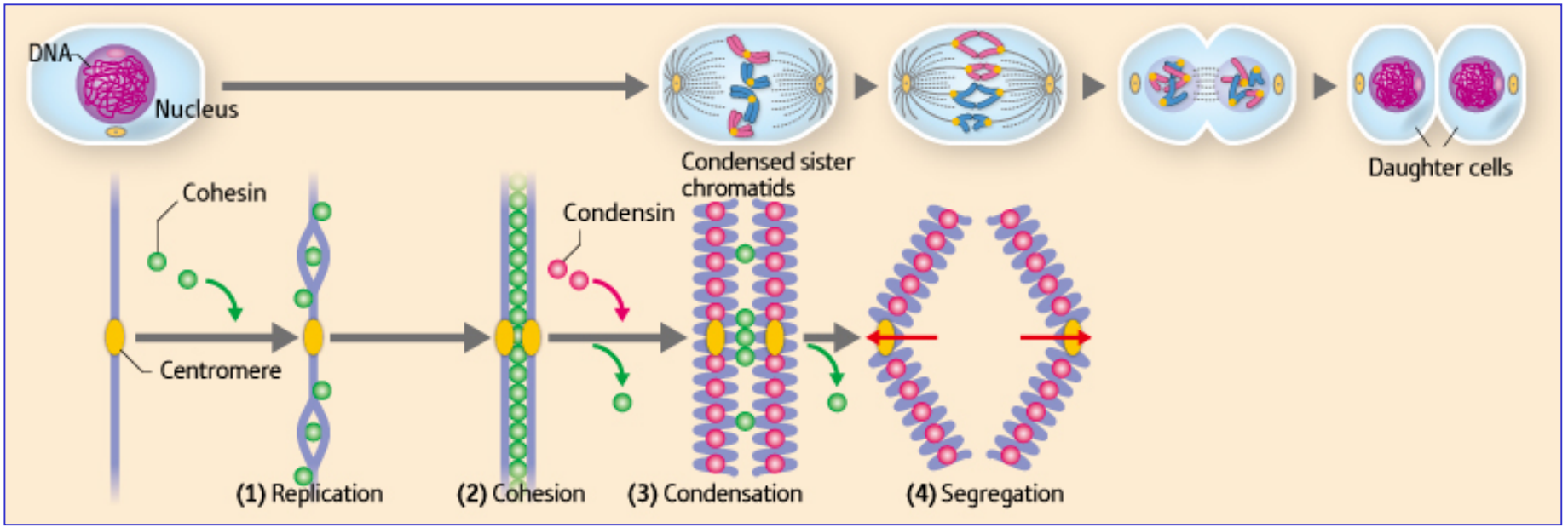
Nature Reviews | Genetics

PROPHASE

CHROMOSOME CONDENSATION

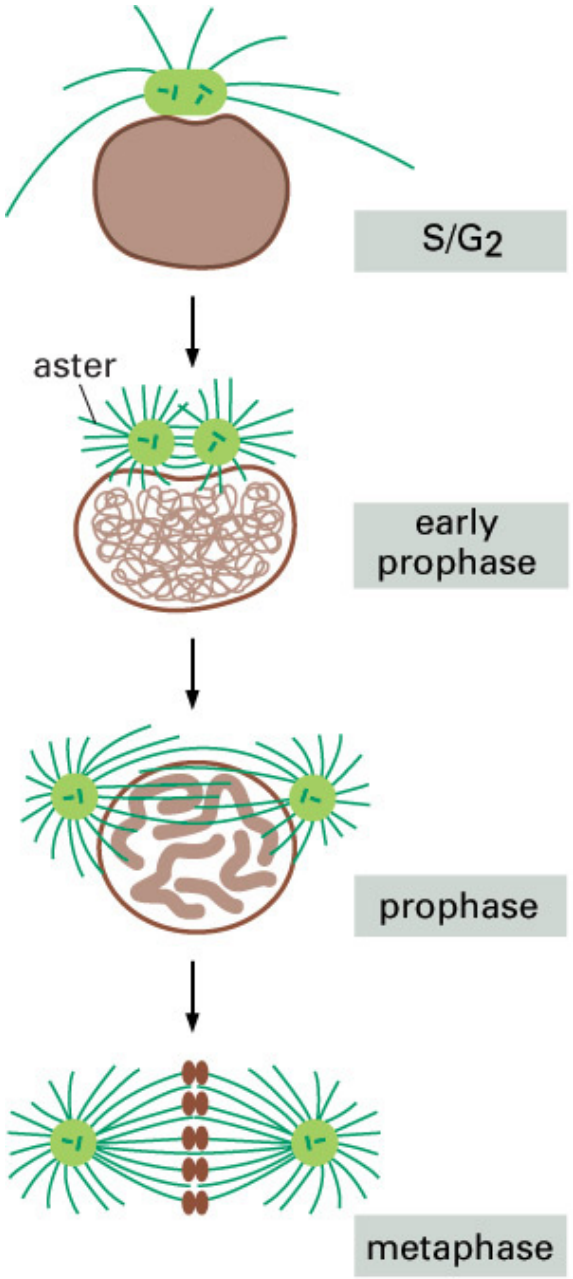


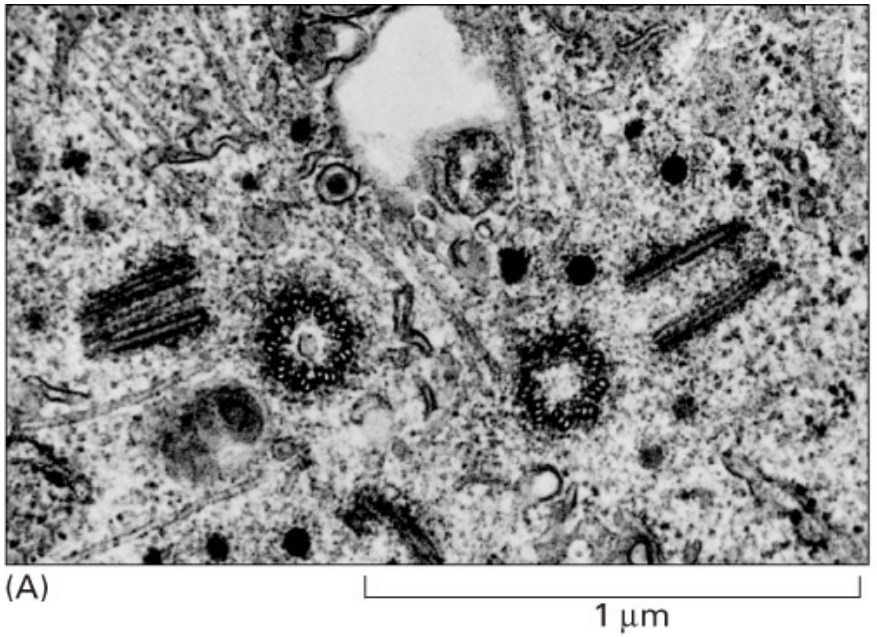


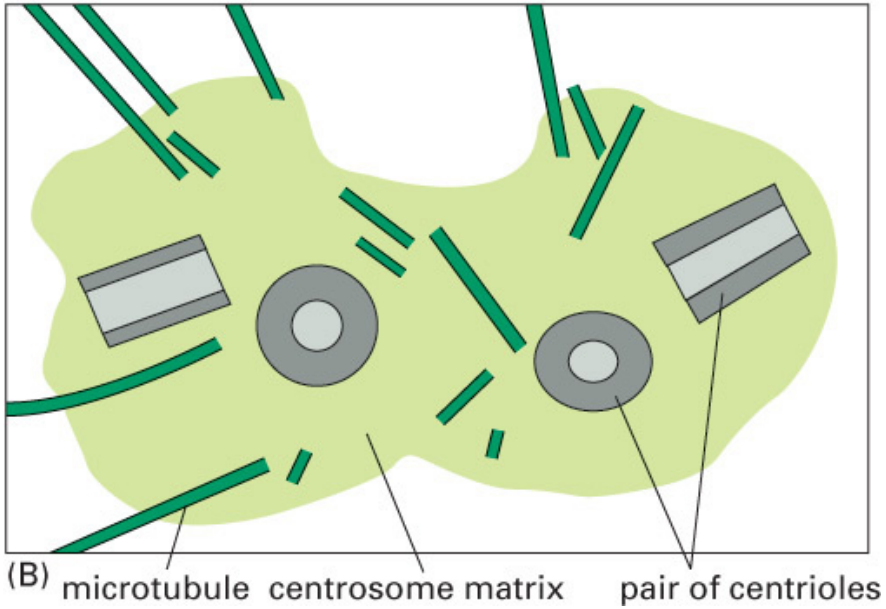


PROPHASE

MITOTIC SPINDLE FORMATION

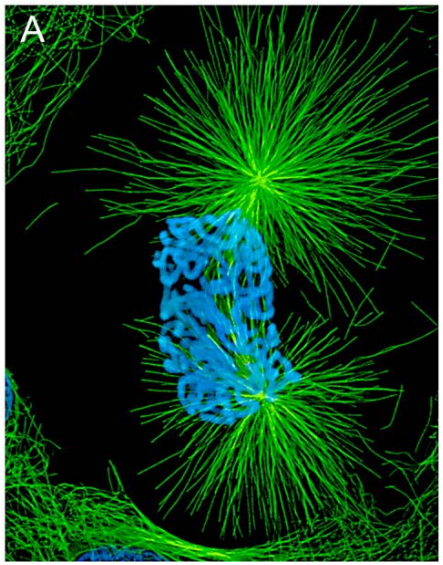


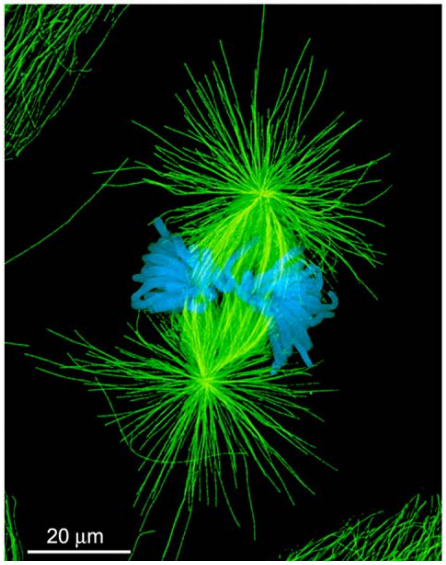


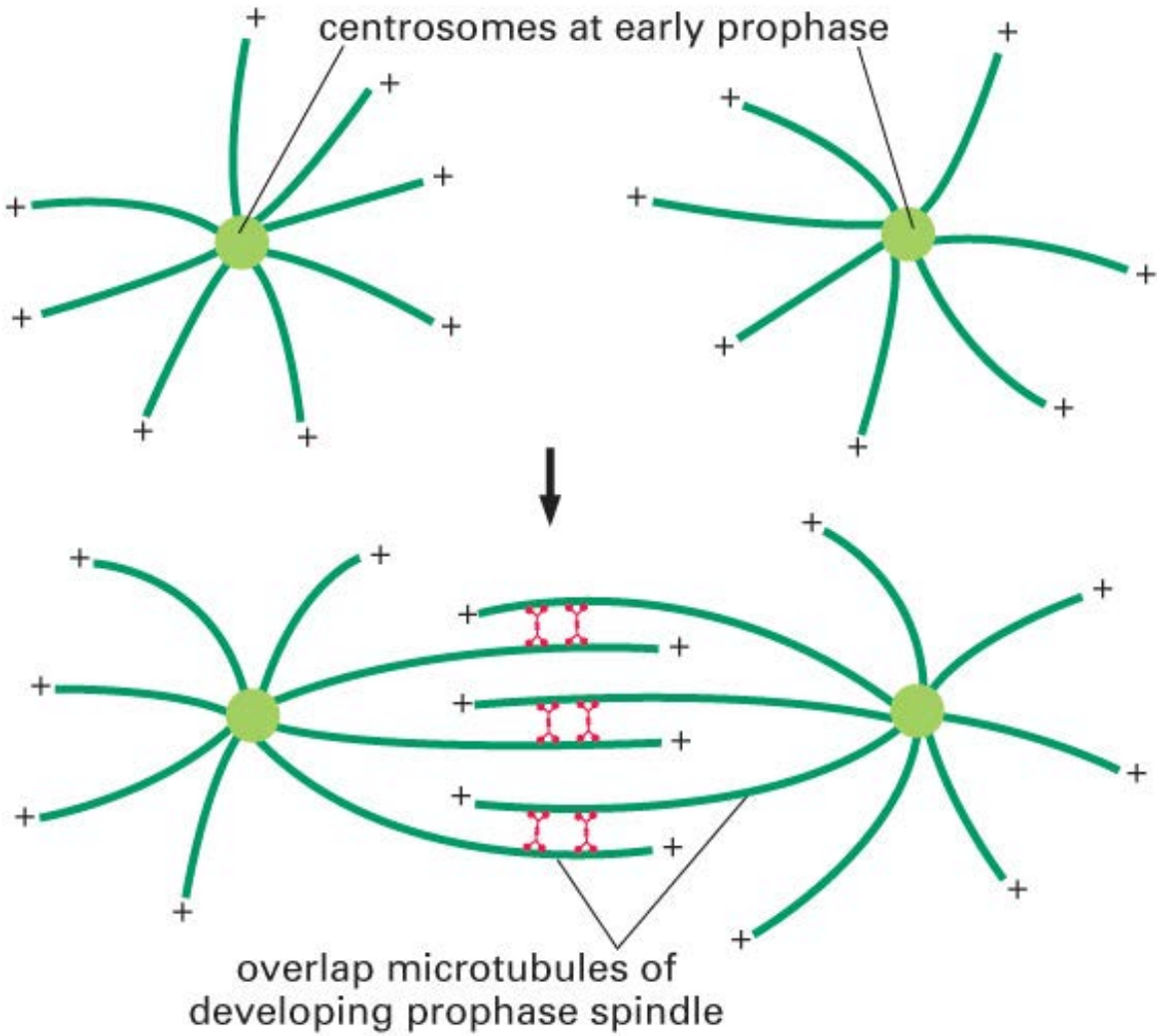


PROPHASE

MITOTIC SPINDLE FORMATION







PROPHASE

MOTOR PROTEINS DEPENDENT ON MICROTUBULES

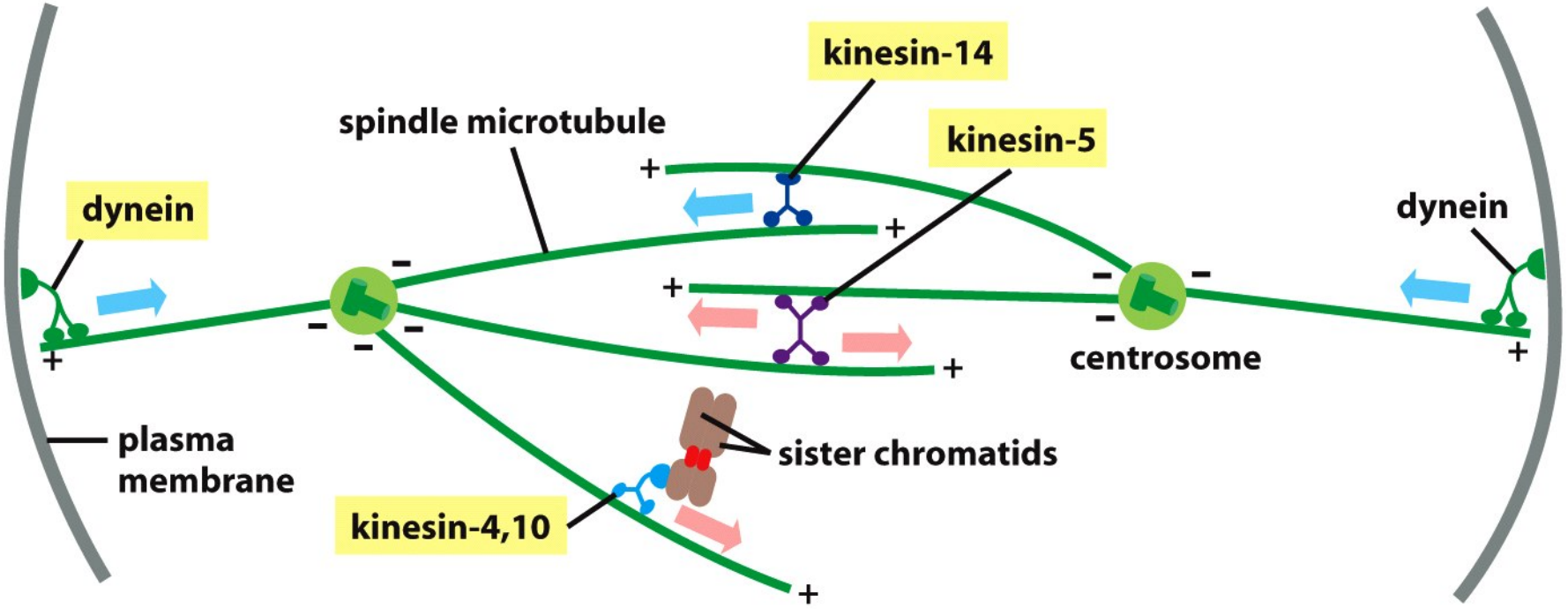
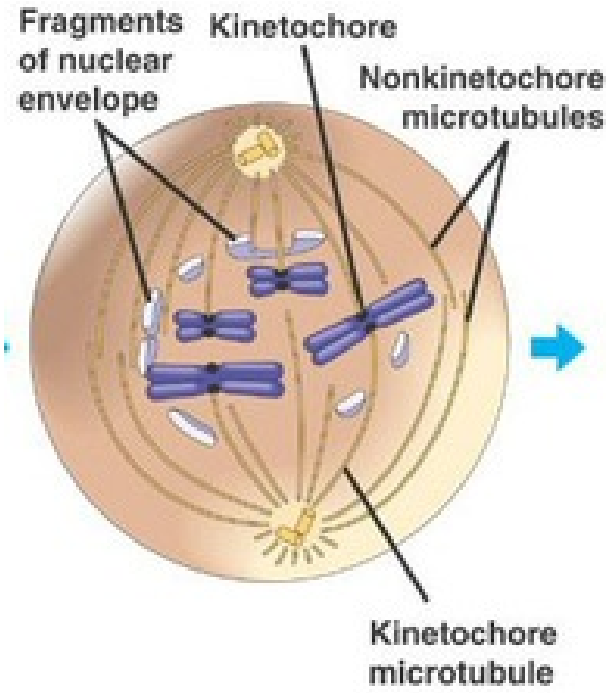
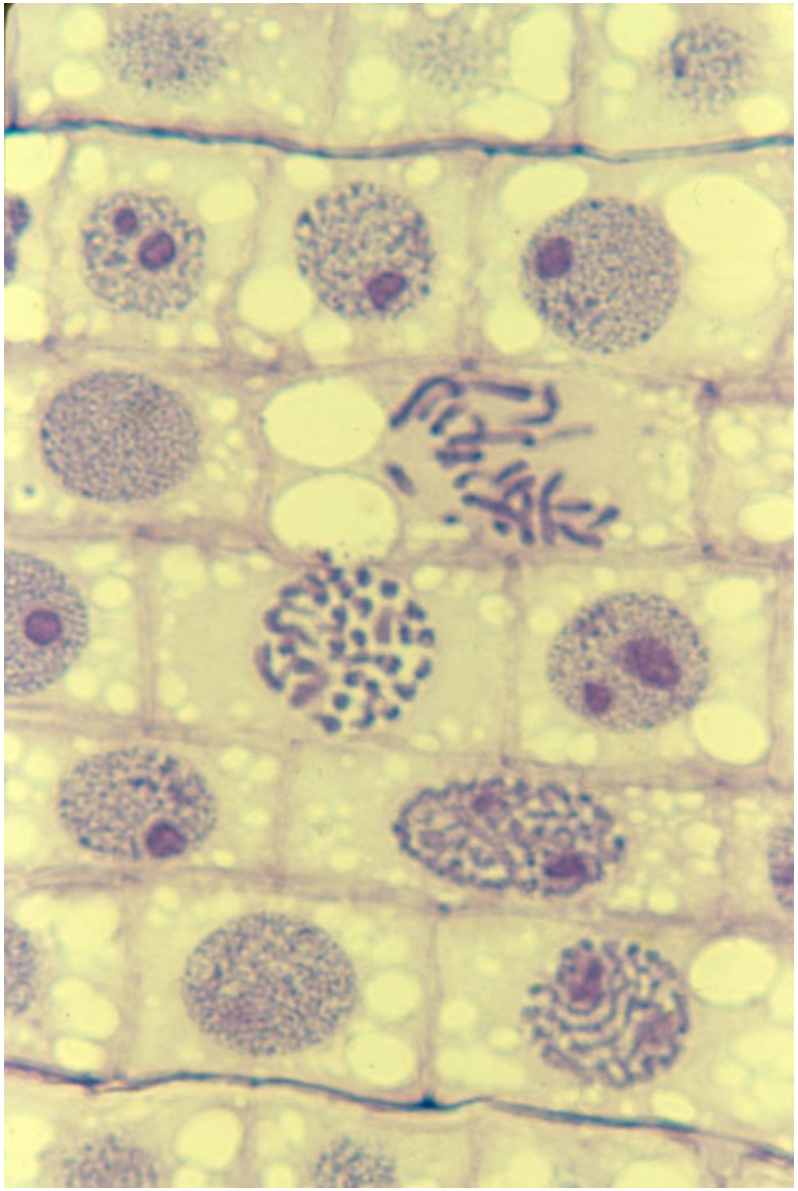


Figure 17-30 *Molecular Biology of the Cell* (© Garland Science 2008)

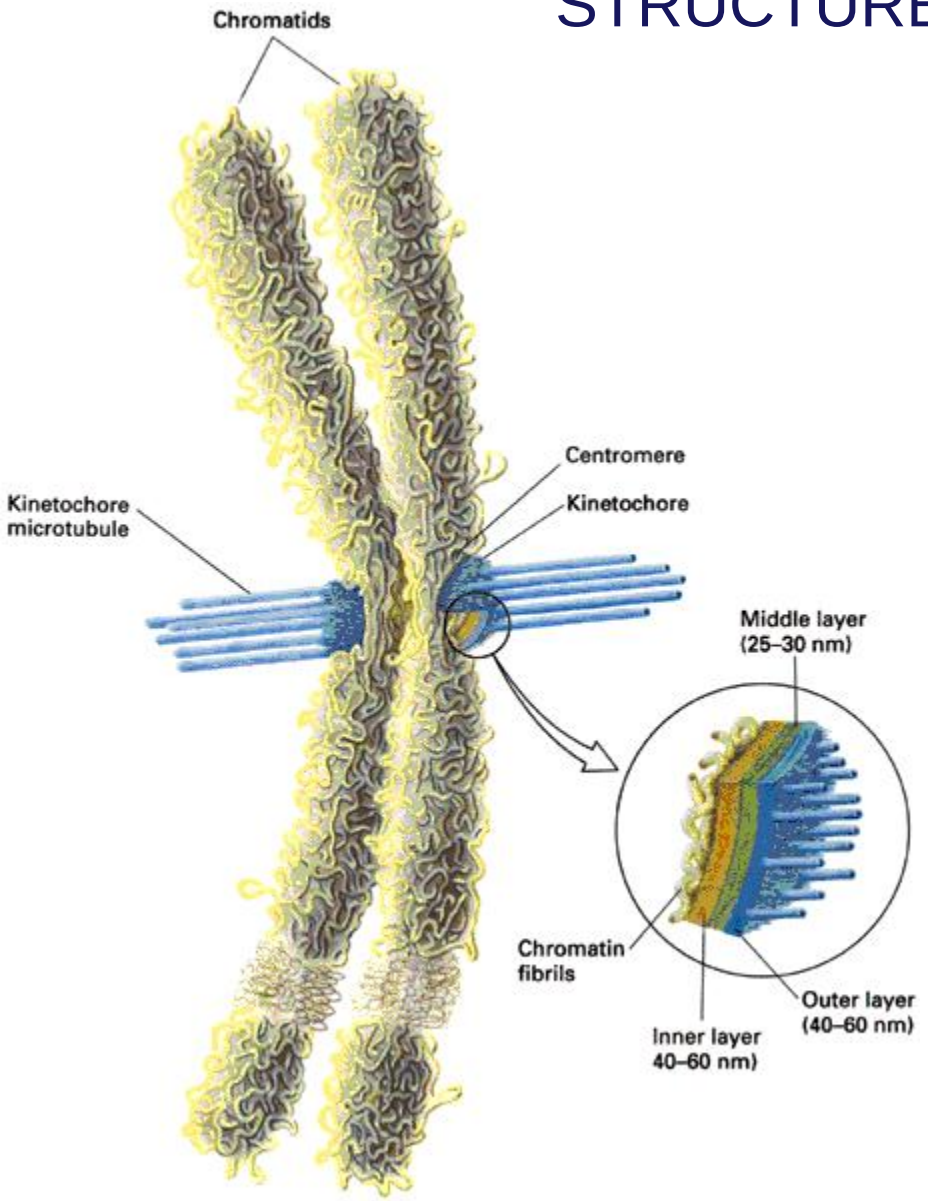
PROMETAPHASE





PROMETAPHASE

STRUCTURE OF THE KINETOCHORE



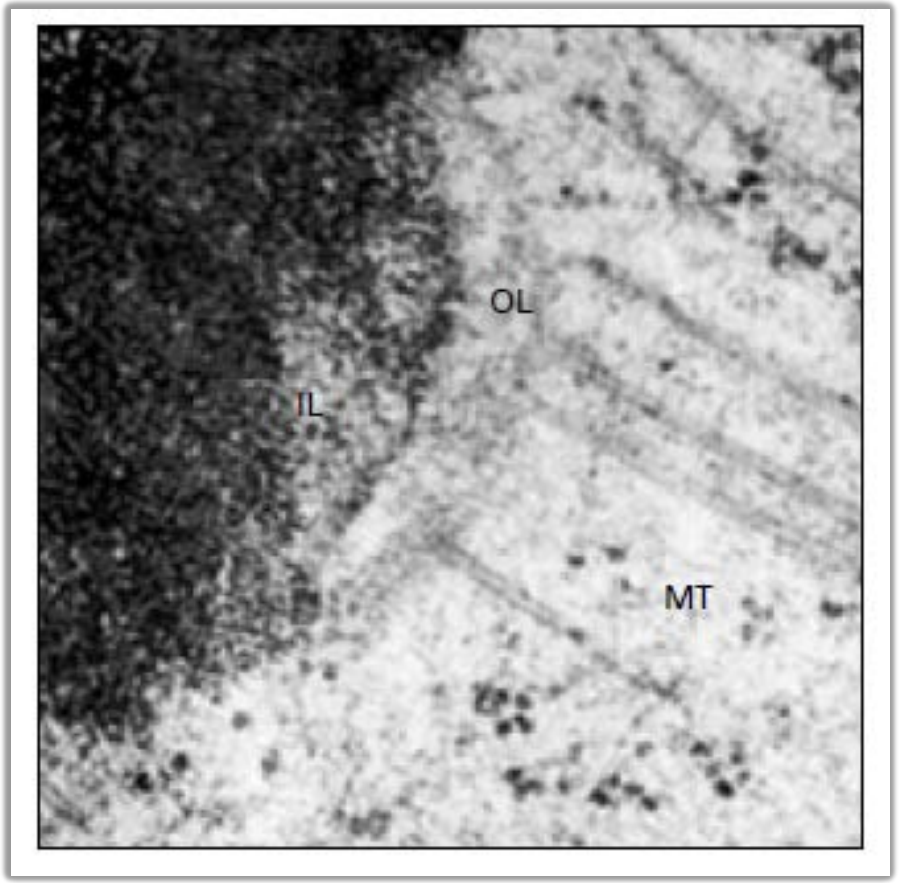
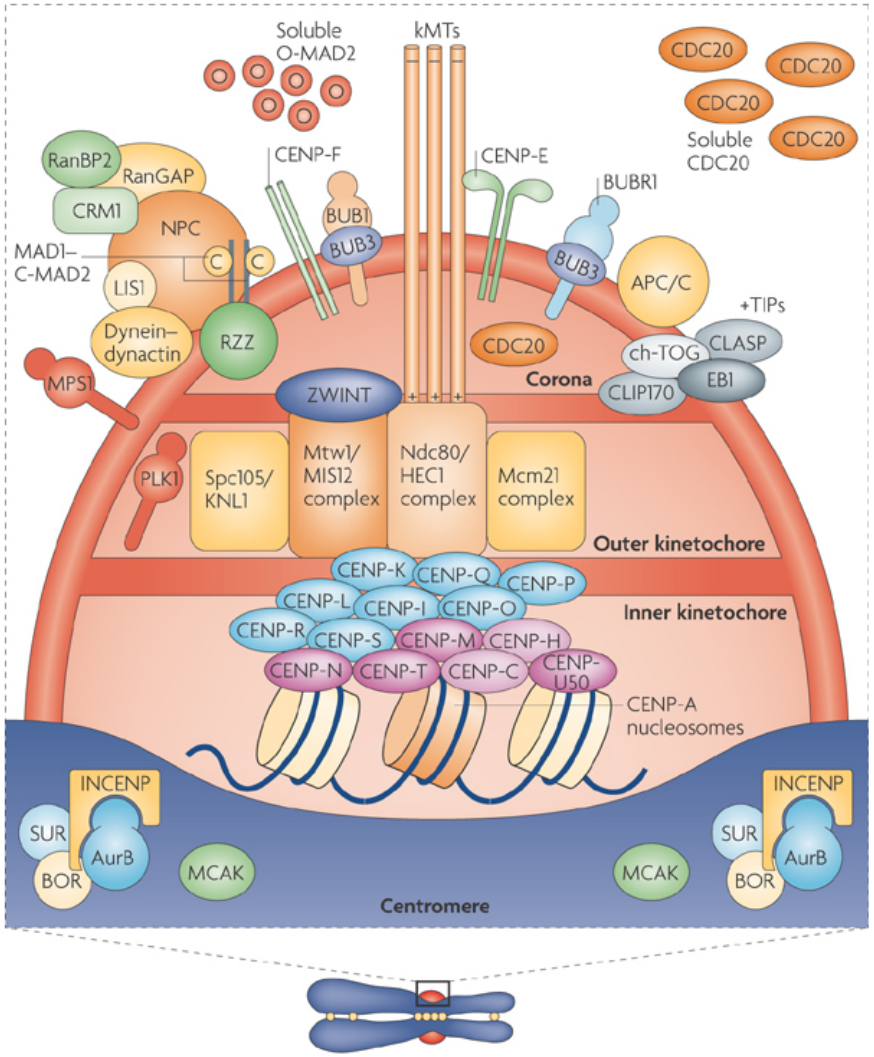


Figure 23-38, p. 1094, Molecular Cell Biology, 3rd ed., Lodish, et al.

PROMETAPHASE

STRUCTURE OF THE KINETOCHORE



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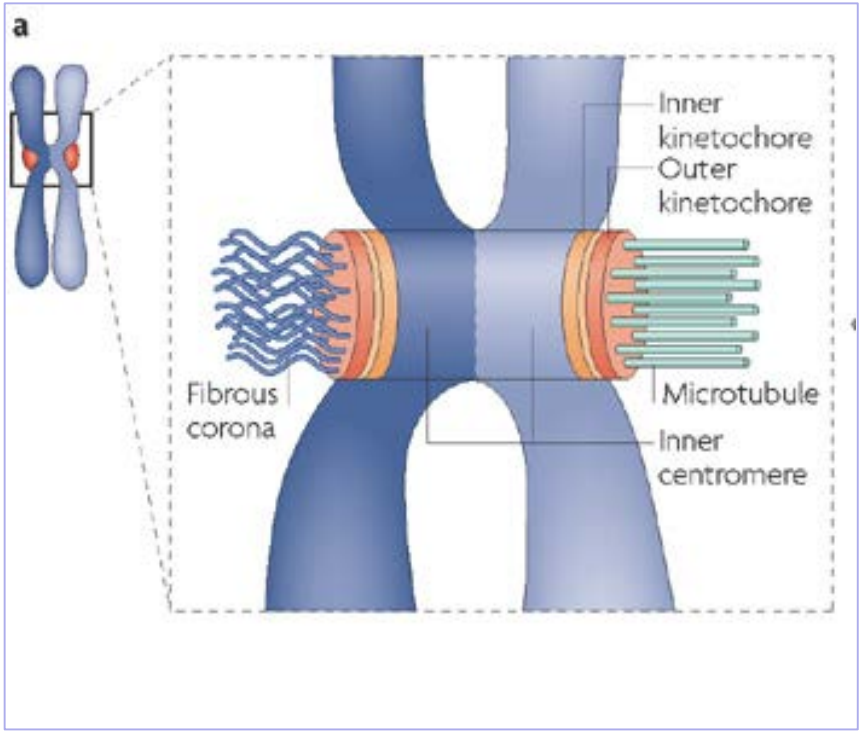
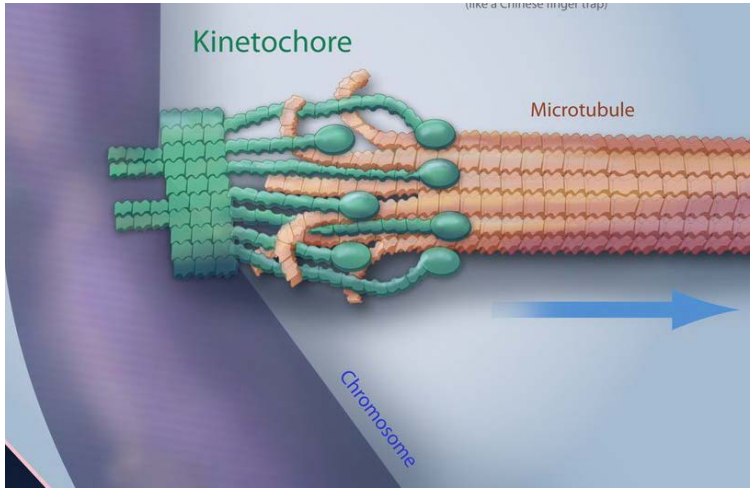
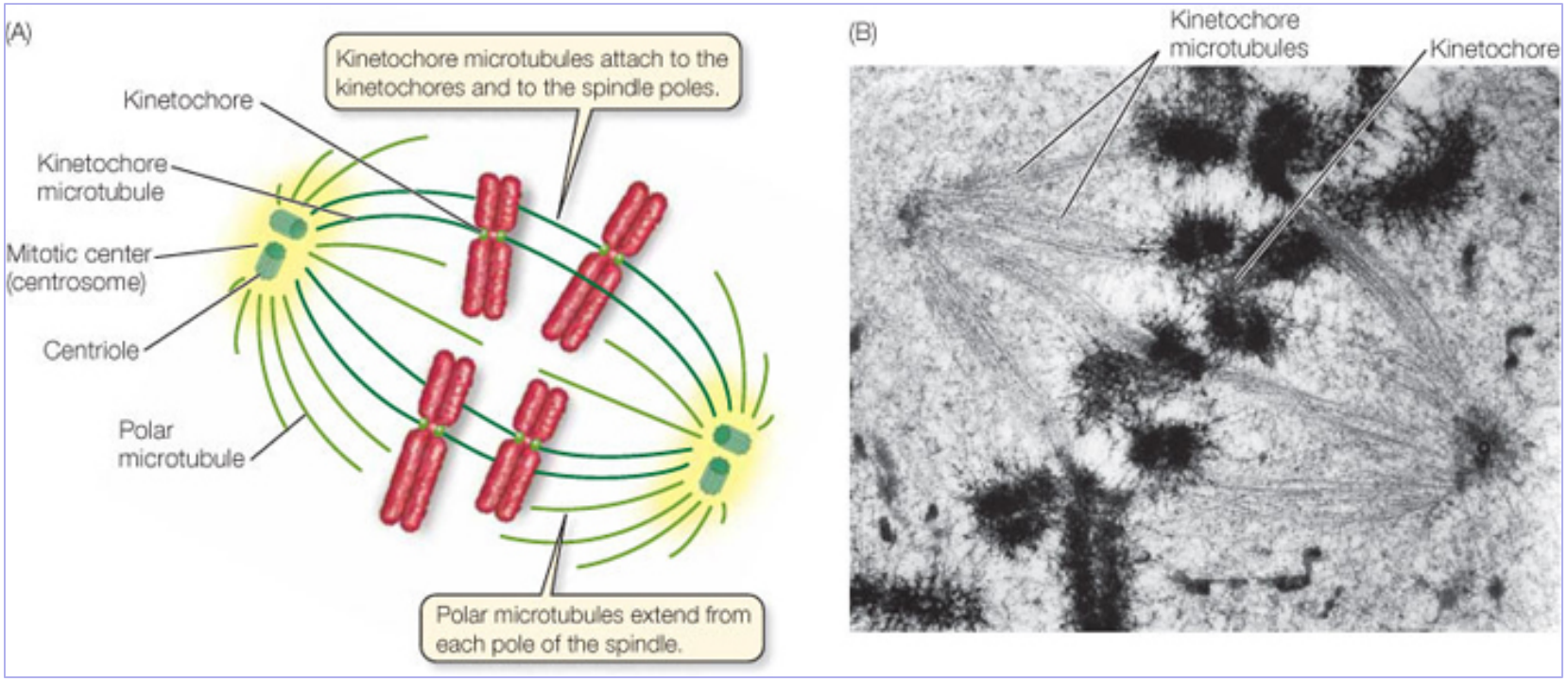
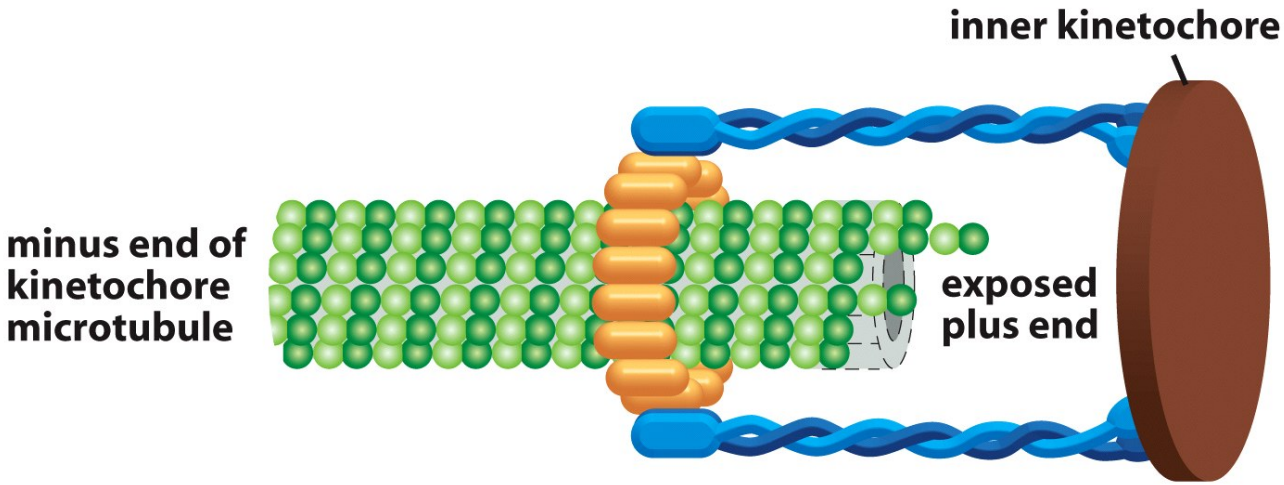




PROMETAPHASE

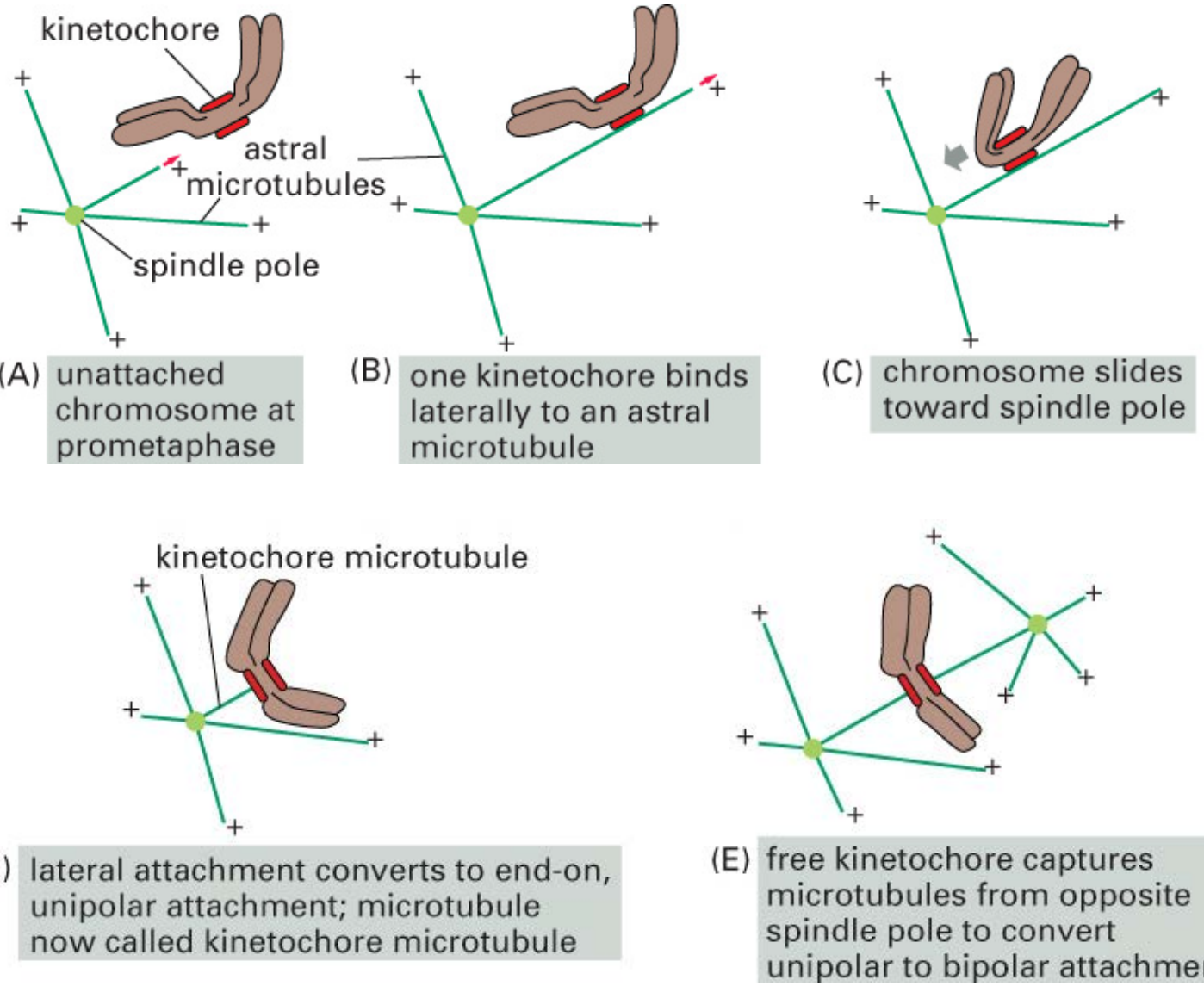
LINKING OF MICROTUBULES TO THE KINETOCHORE





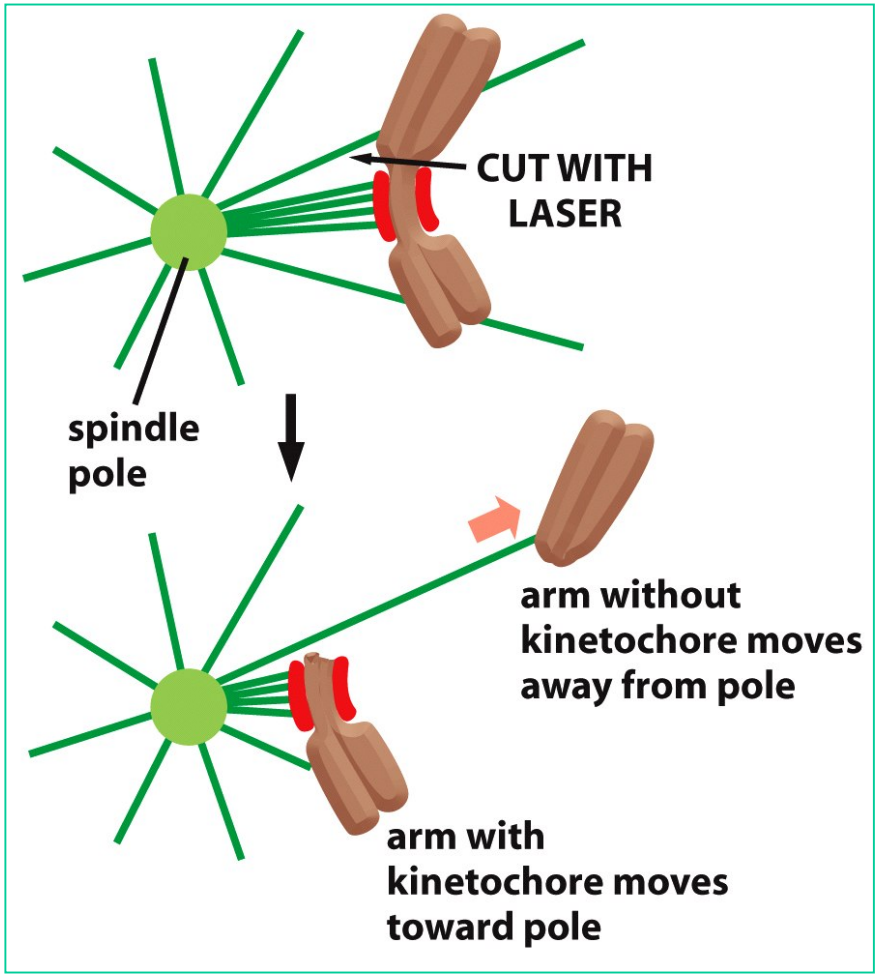
PROMETAPHASE

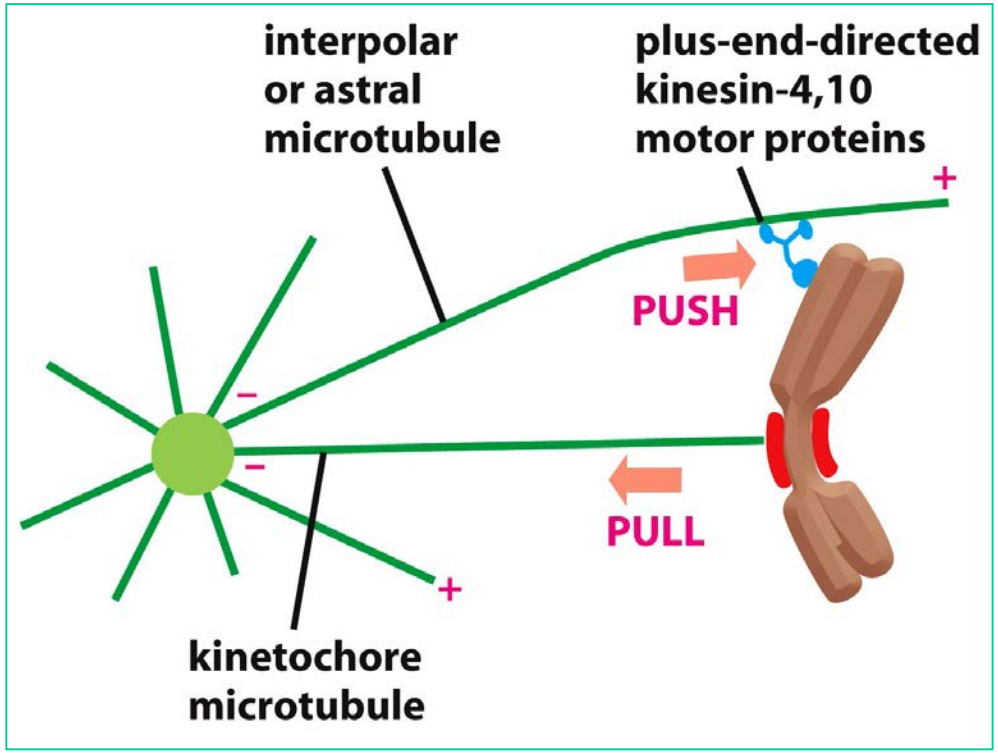
MICROTUBULE CAPTURING BY THE KINETOCHORE



PROMETAPHASE

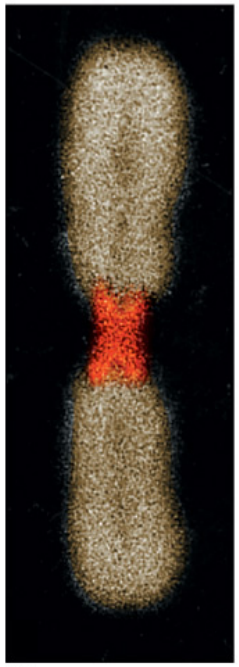
FORCES DRIVING CHROMOSOMES TOWARDS METAPHASIC PLATE





PROMETAPHASE

KINETOCHORE MICROTUBULE



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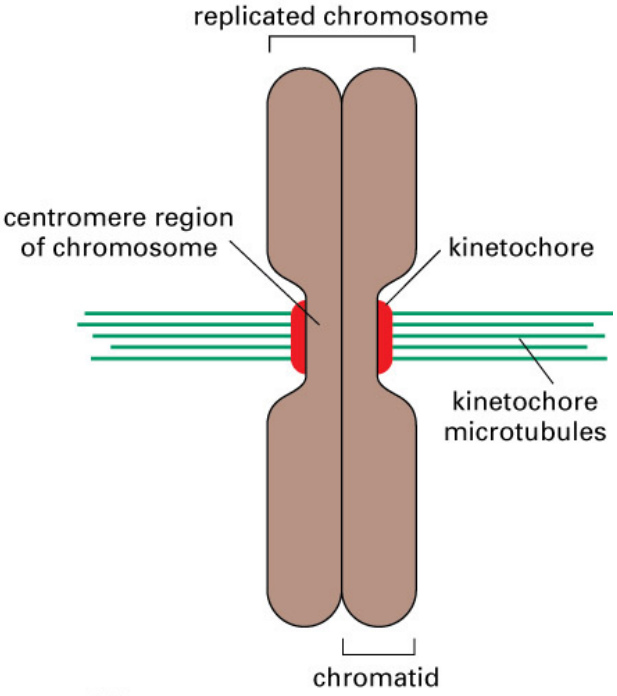
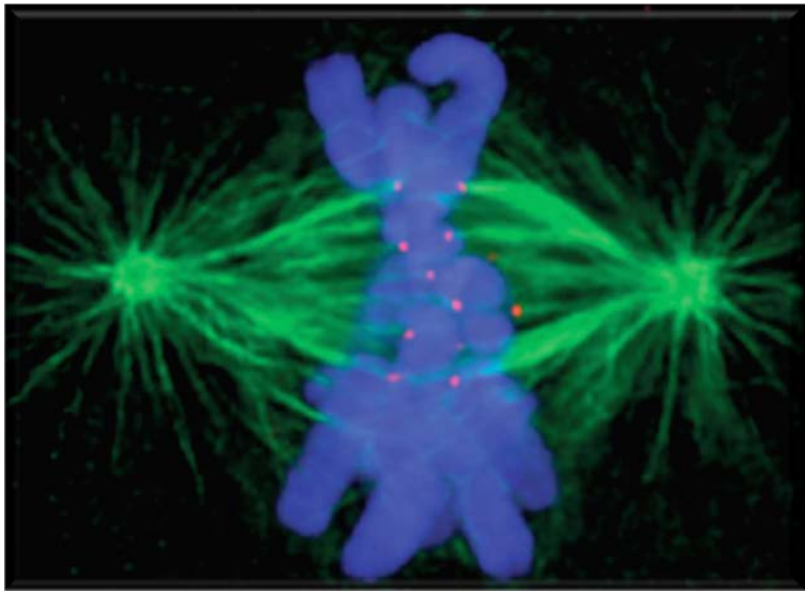
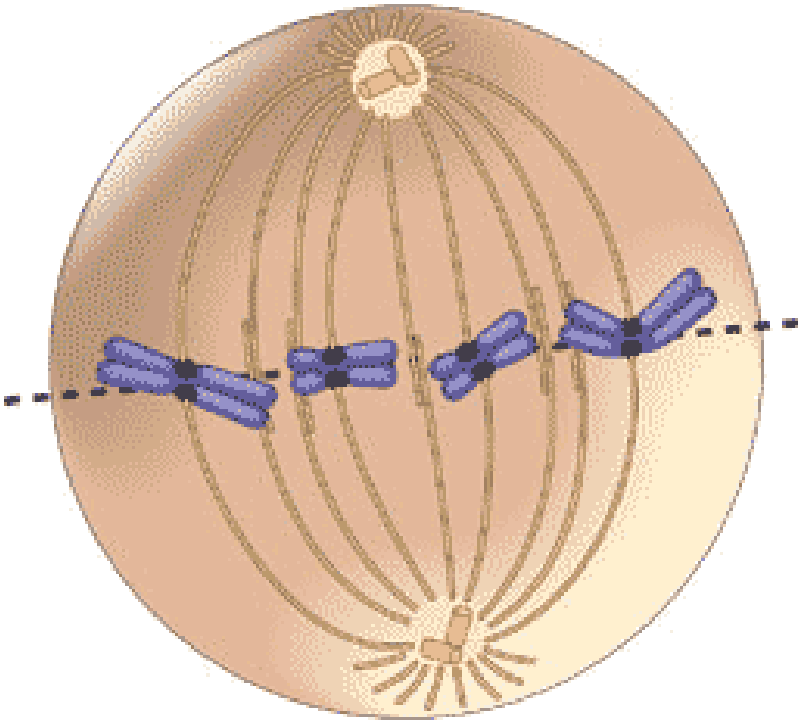
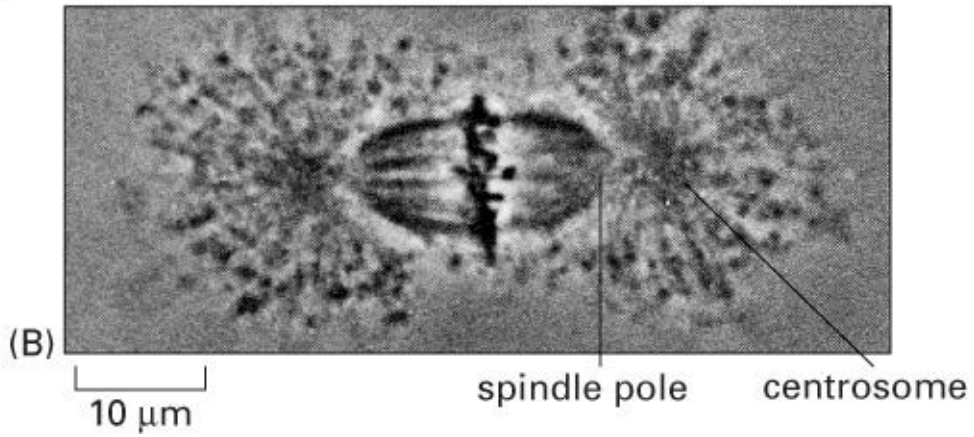


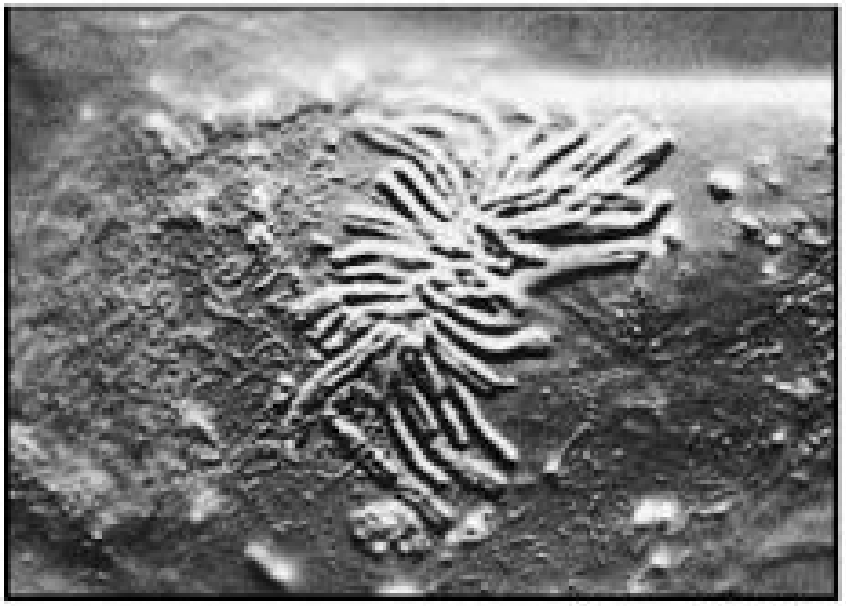
(B)



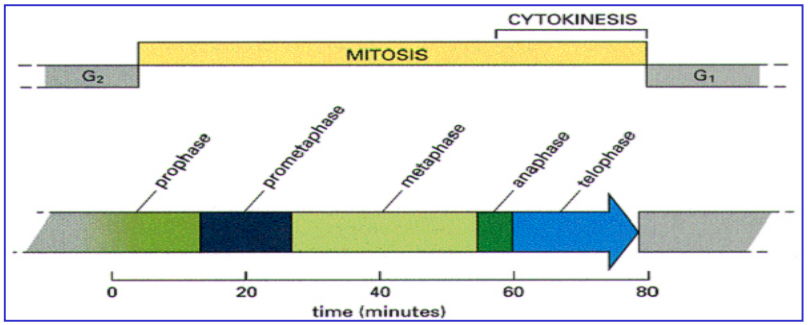
METAPHASE

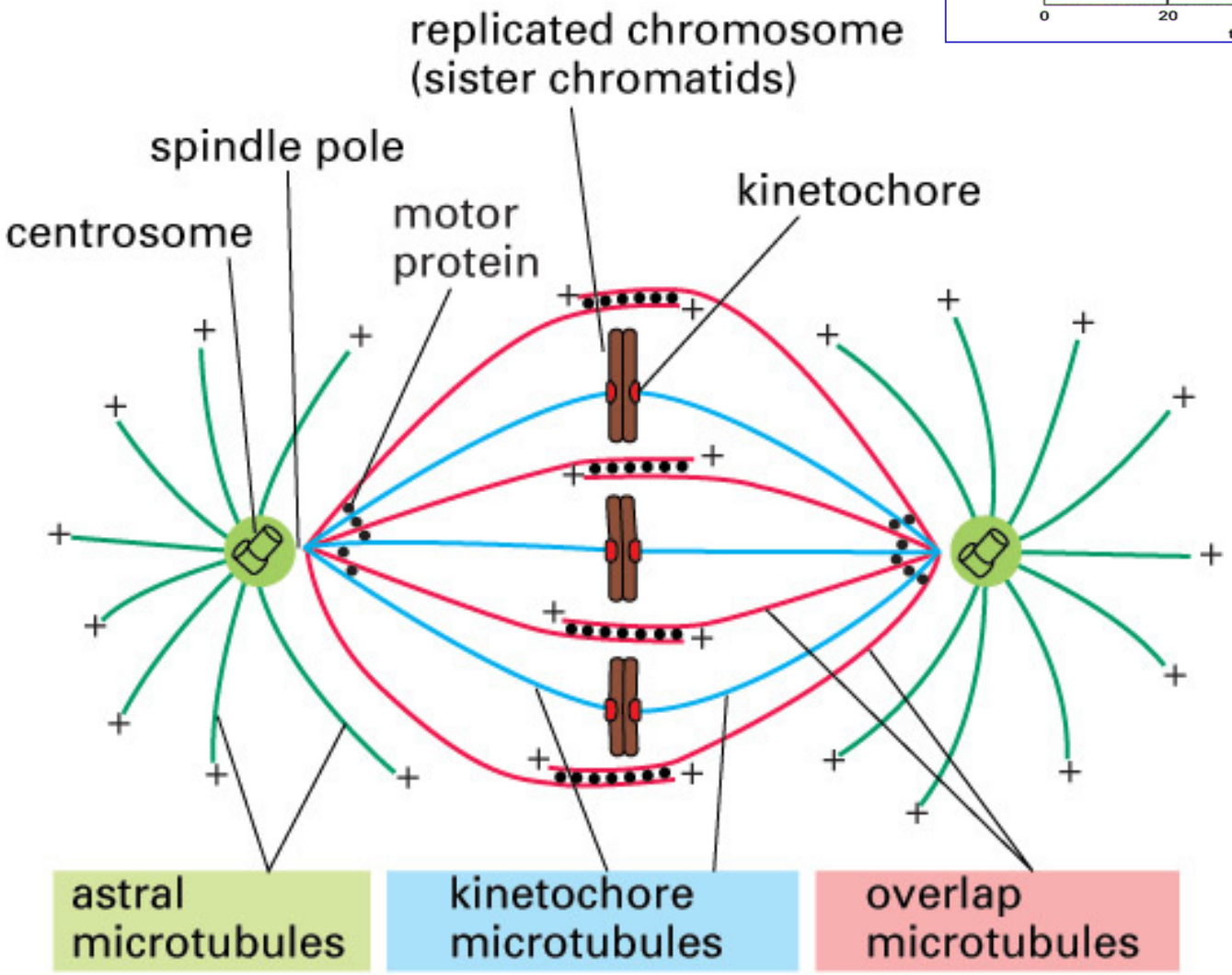




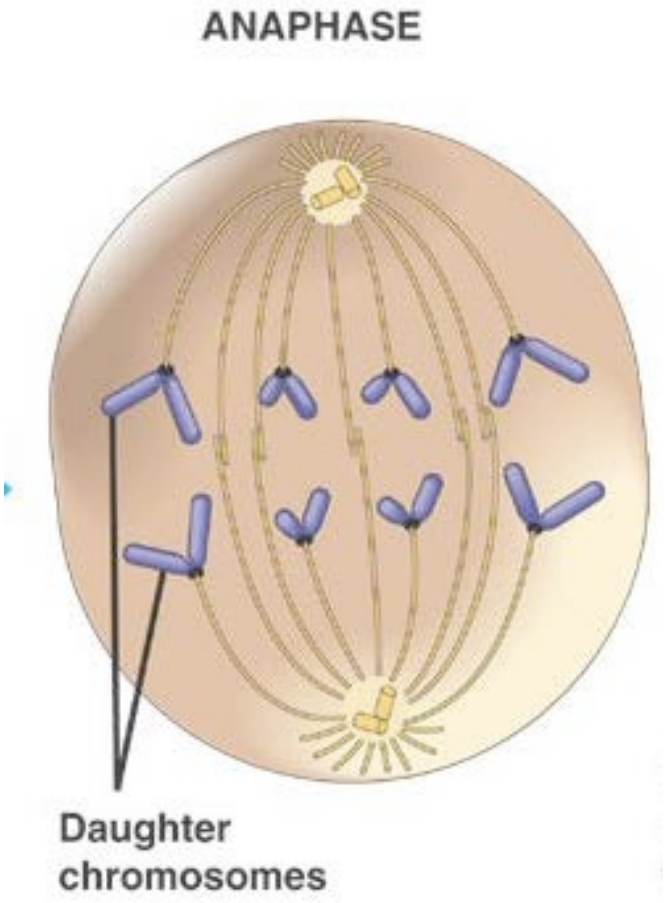


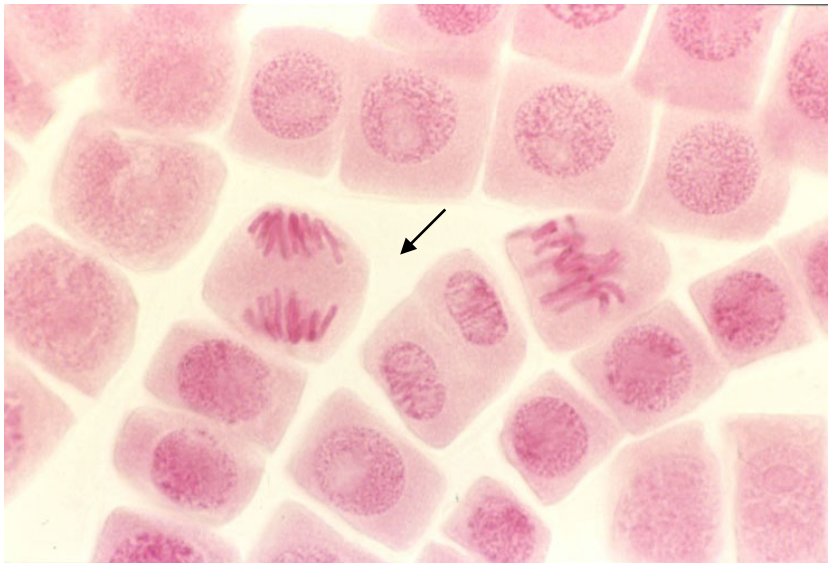
METAPHASE

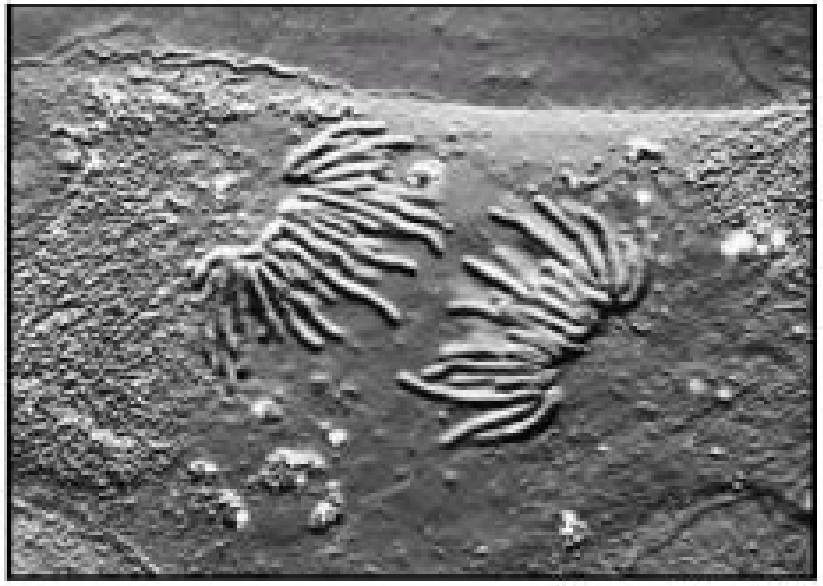

CYTOKINESIS
MITOSIS
G2
G1
prophase
prometaphase
metaphase
anaphase
telophase
0
20
40
60
80
time (minutes)


replicated chromosome
(sister chromatids)
spindle pole
centrosome
motor
protein
kinetochore
astral
microtubules
kinetochore
microtubules
overlap
microtubules

ANAPHASE







ANAPHASE

CHROMATIDS SEPARATION

Activation APC complex

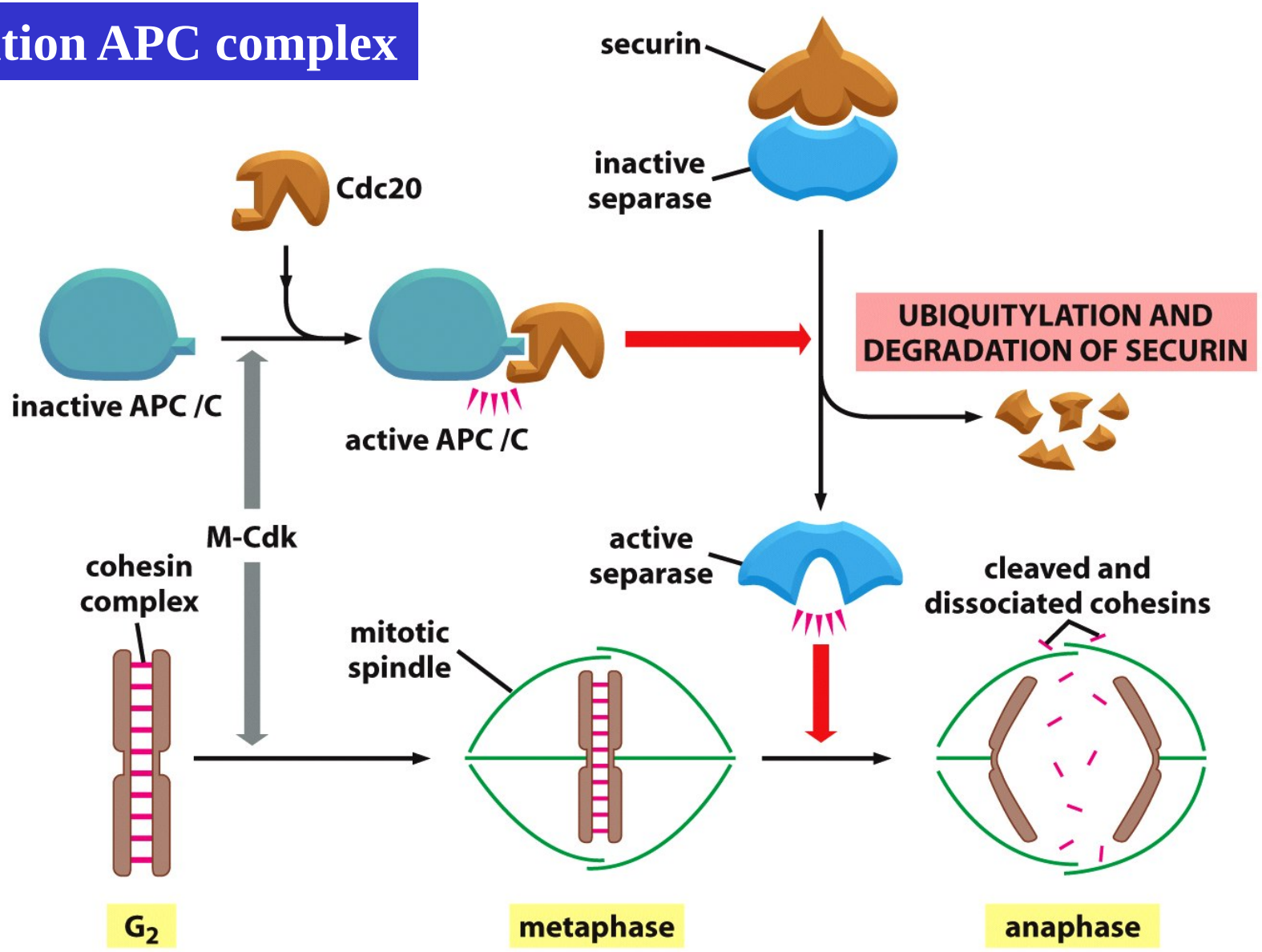
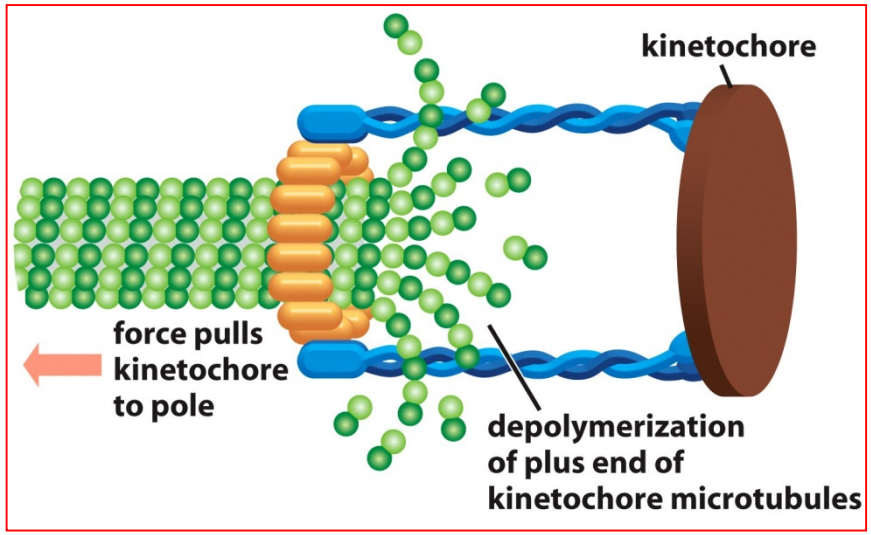
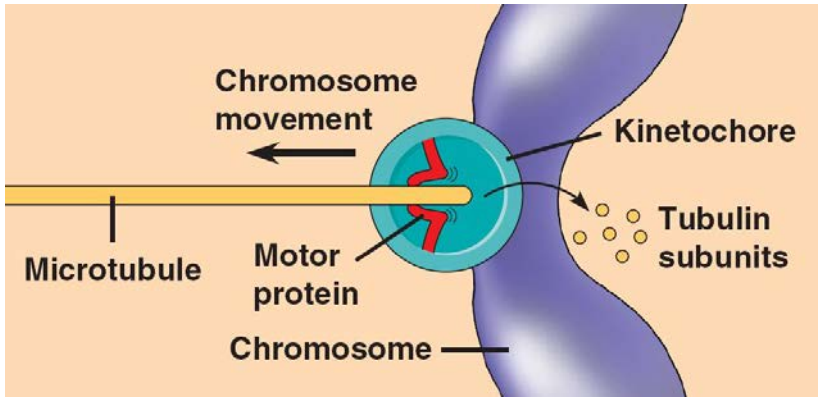


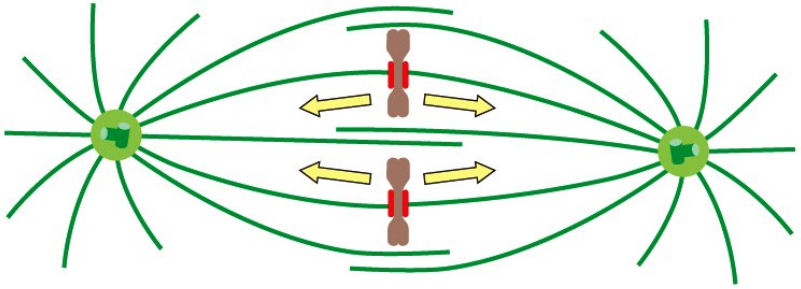
Figure 17-44 *Molecular Biology of the Cell* (© Garland Science 2008)

ANAPHASE A

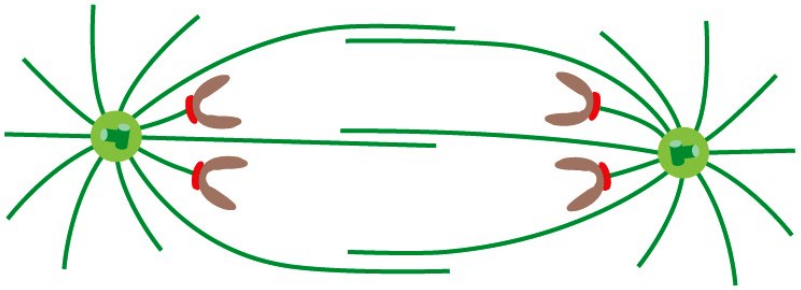




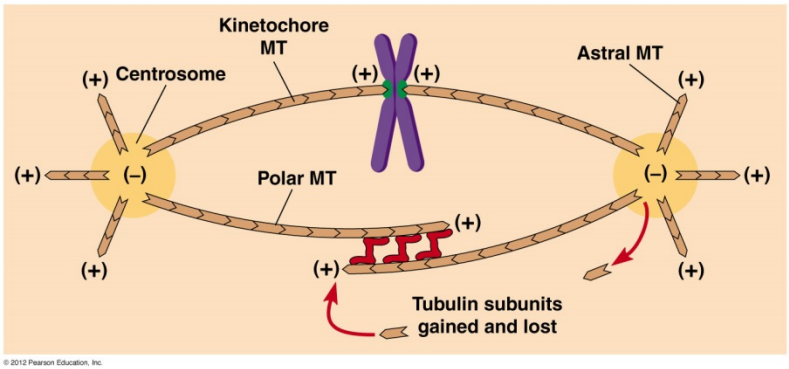
ANAPHASE A

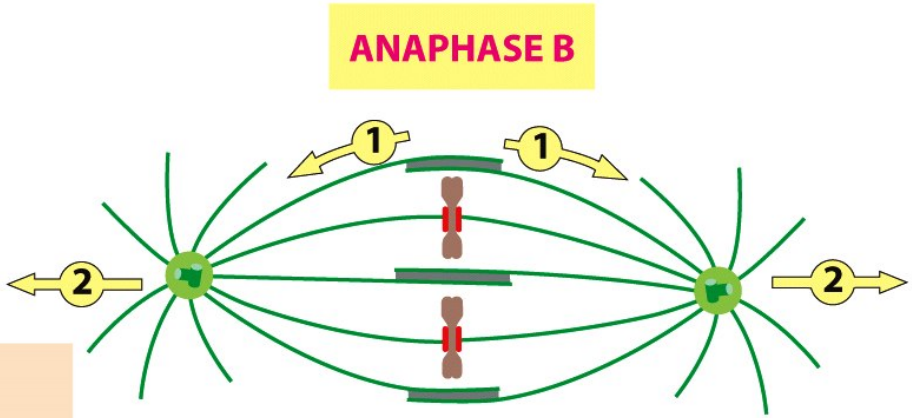


shortening of kinetochore microtubules; movement of daughter chromosomes to poles; forces generated mainly at kinetochores

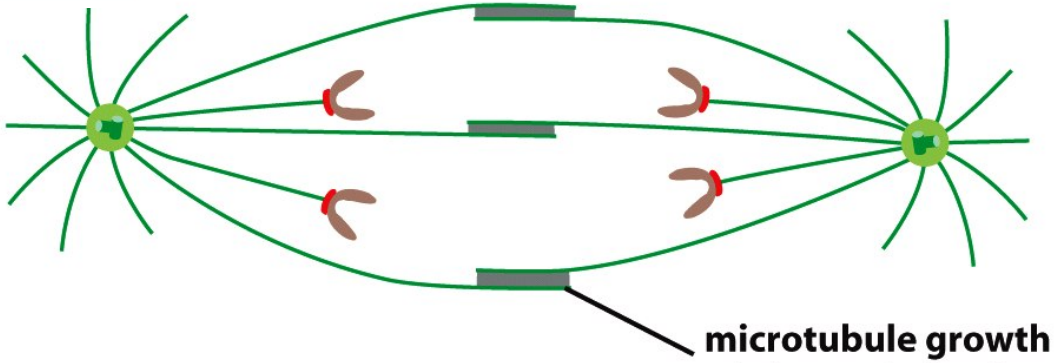


ANAPHASE B



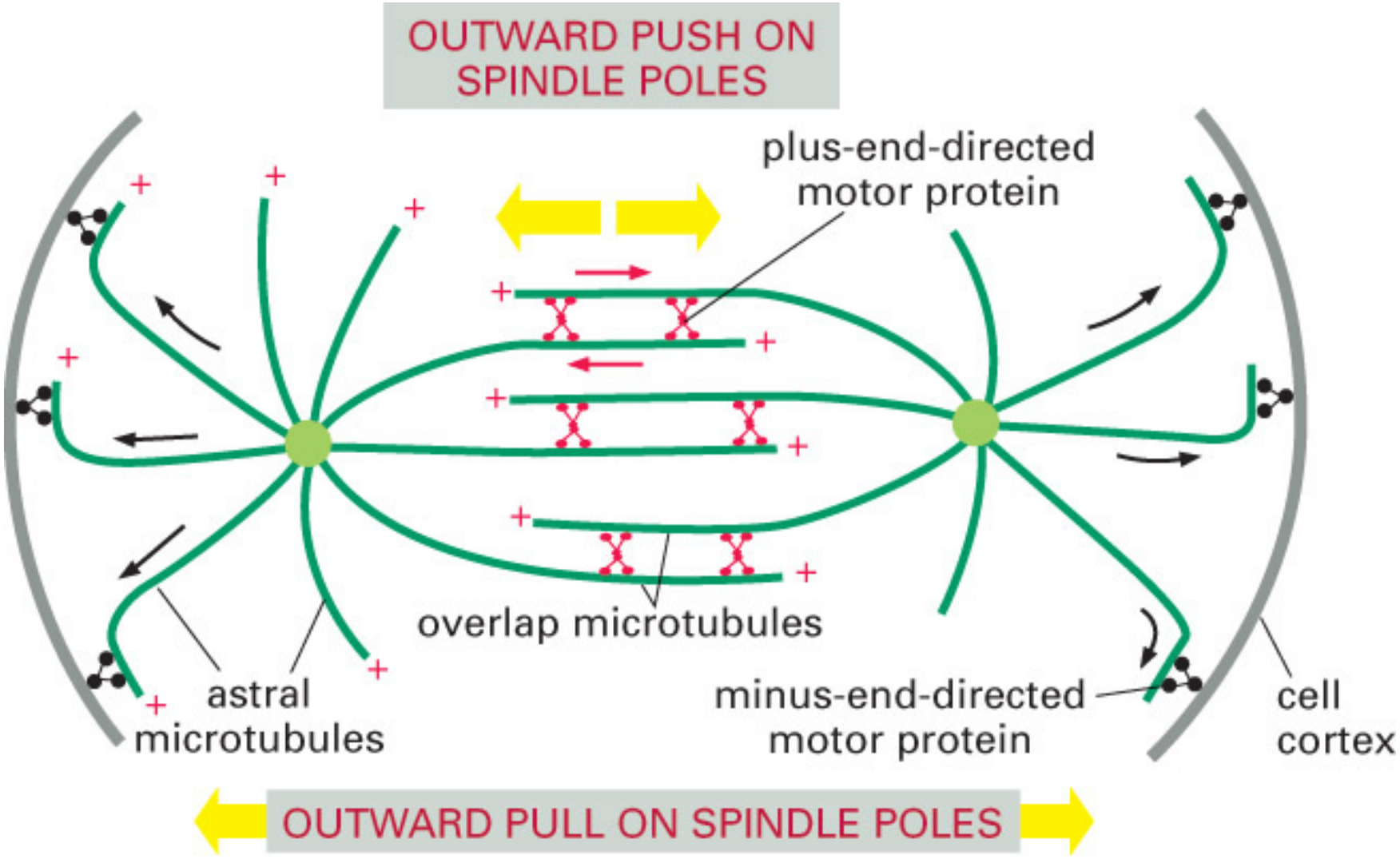


(1) a sliding force is generated between interpolar microtubules from opposite poles to push the poles apart; the interpolar microtubules also elongate; (2) a pulling force acts directly on the poles to move them apart

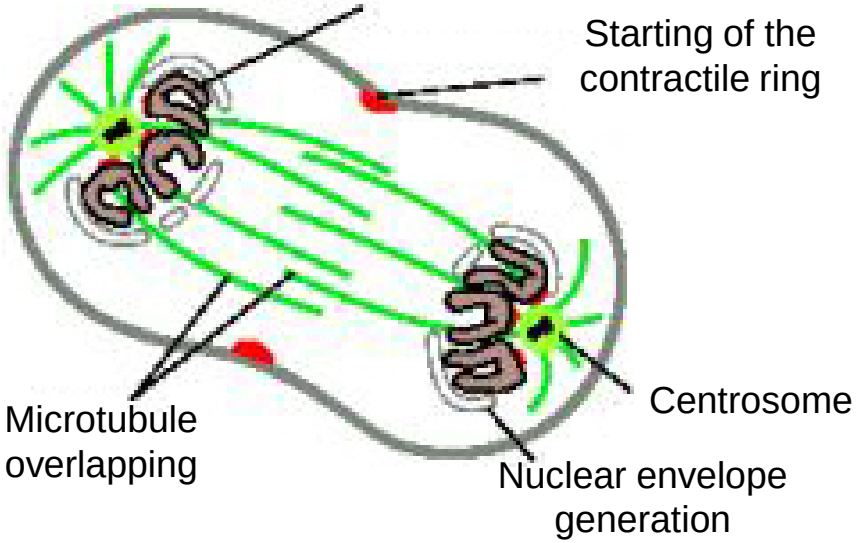


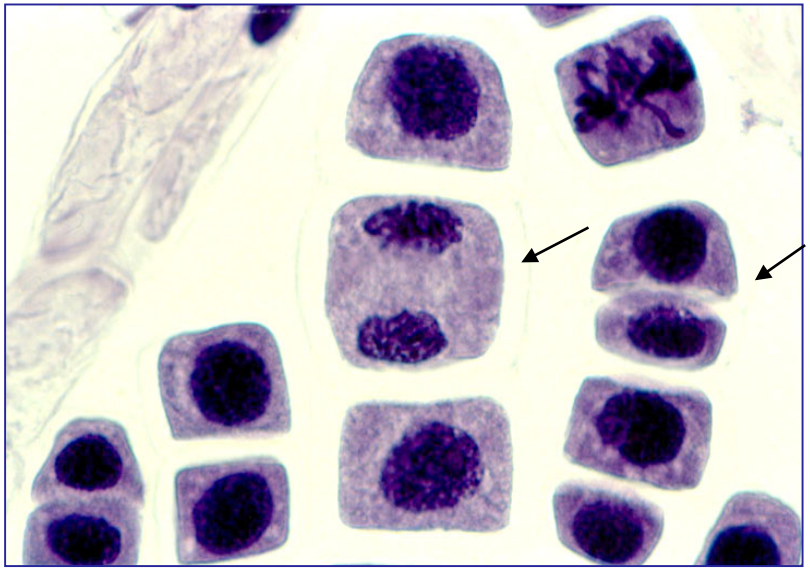
microtubule growth at plus end of polar microtubules

ANAPHASE B

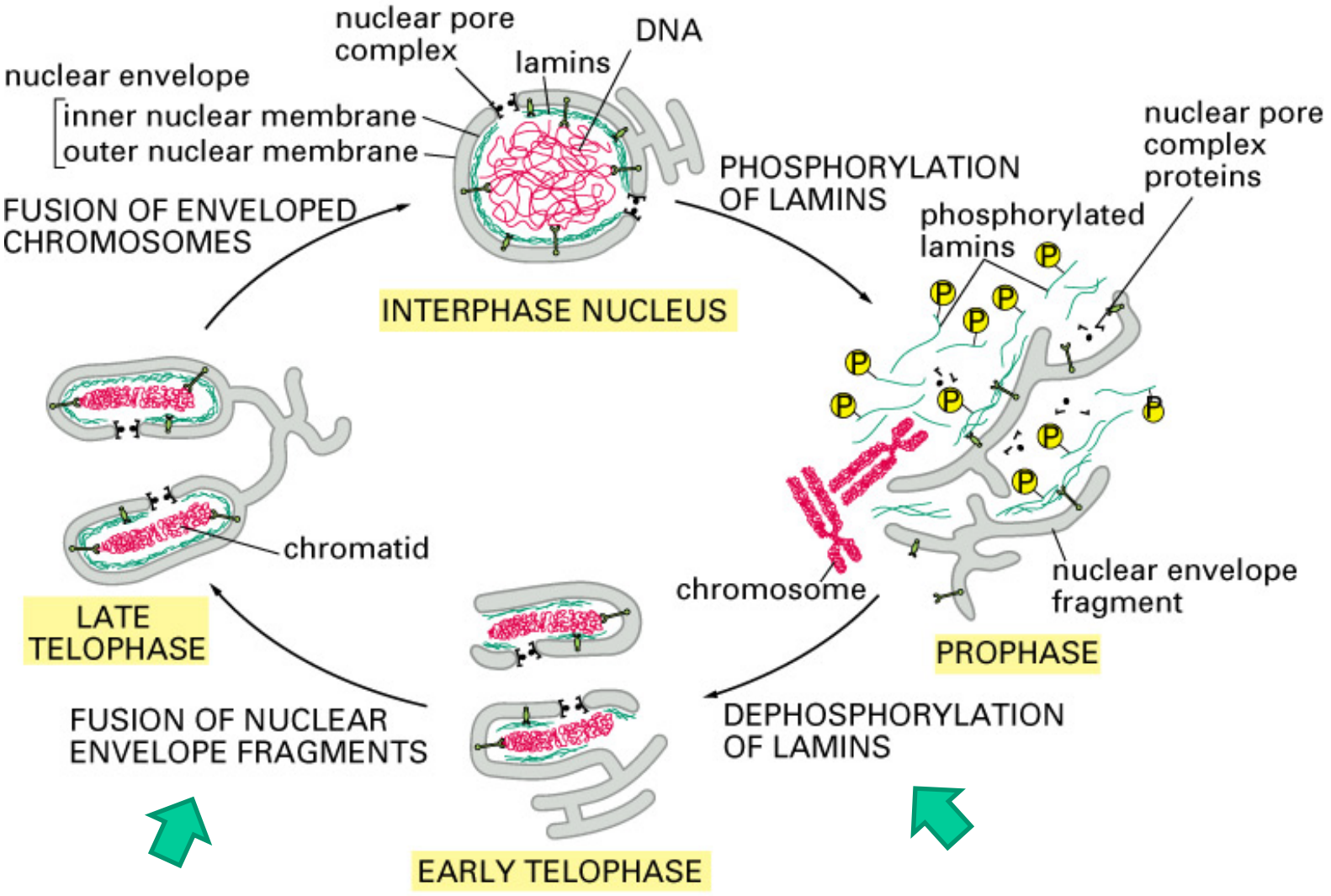

OUTWARD PUSH ON SPINDLE POLES
plus-end-directed motor protein
overlap microtubules
astral microtubules
minus-end-directed motor protein
cell cortex
OUTWARD PULL ON SPINDLE POLES

TELOPHASE

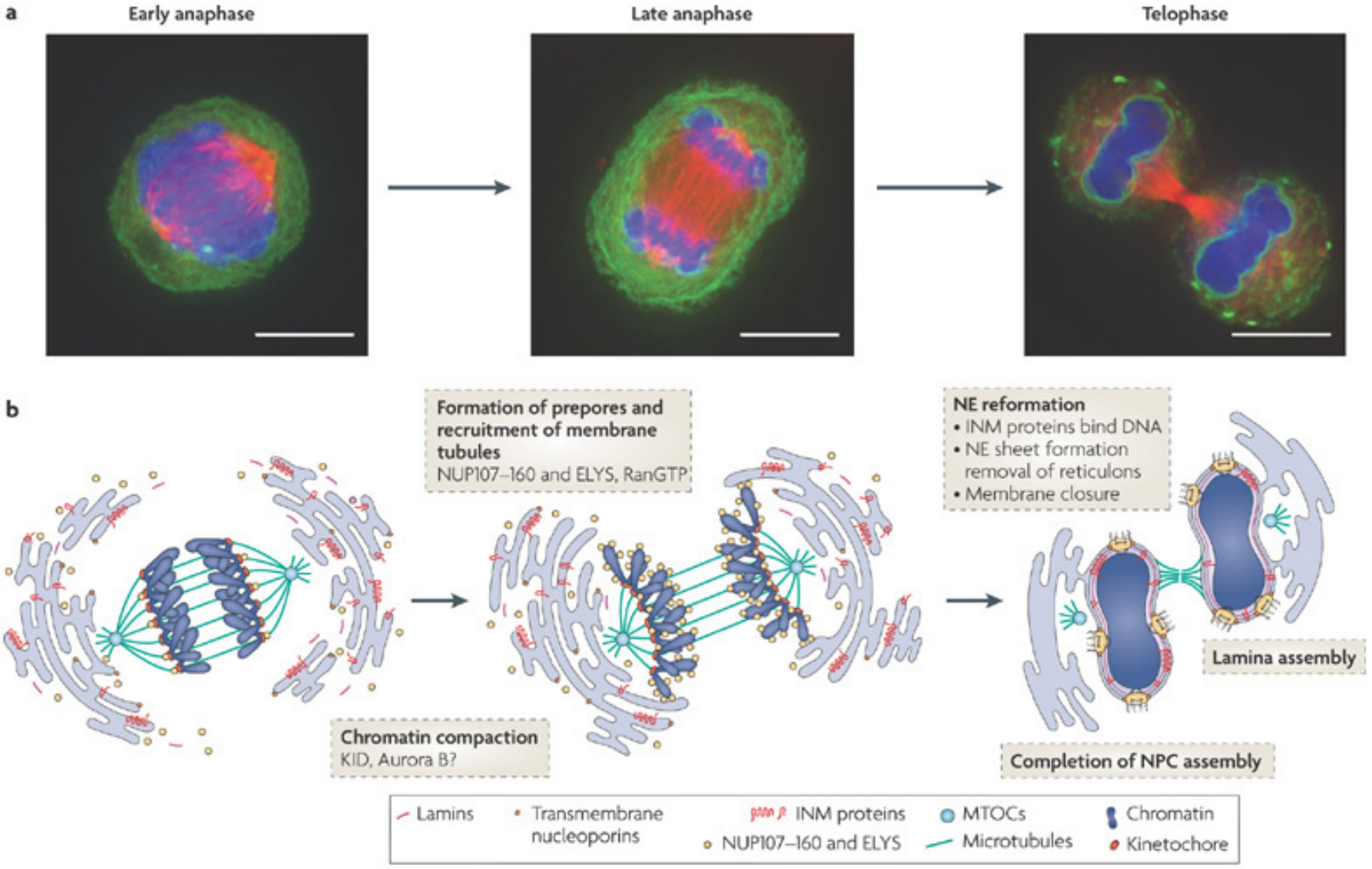

Daughter chromosomes at the spindle pole
Starting of the contractile ring
Microtubule overlapping
Centrosome
Nuclear envelope generation



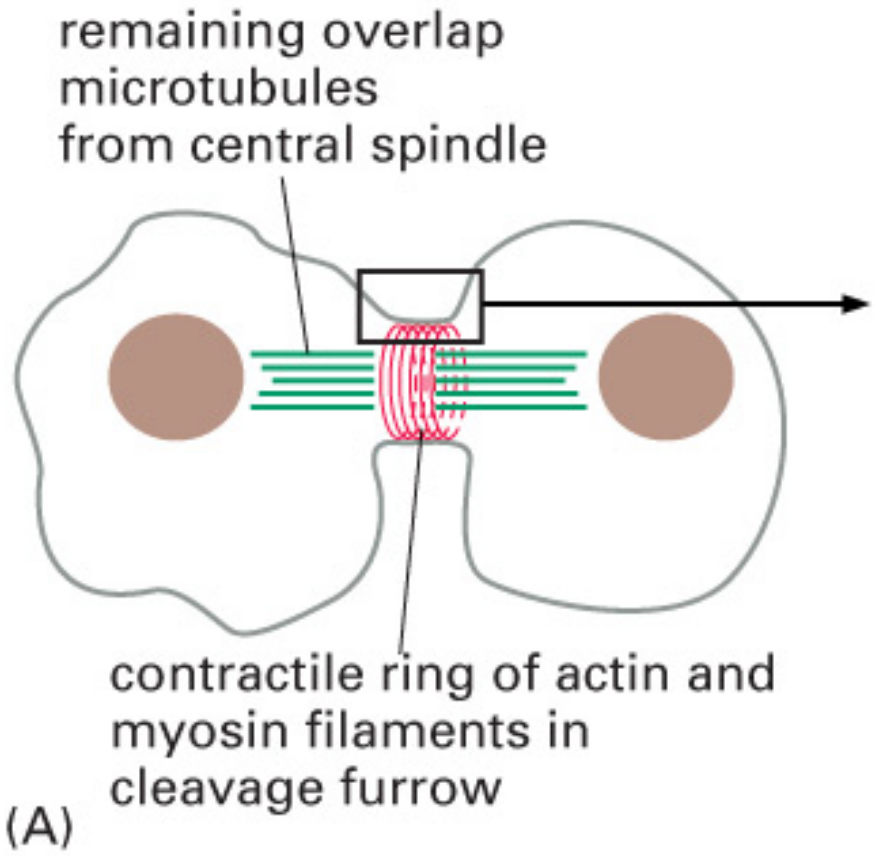
TELOPHASE
Reconstructing the nuclear envelope

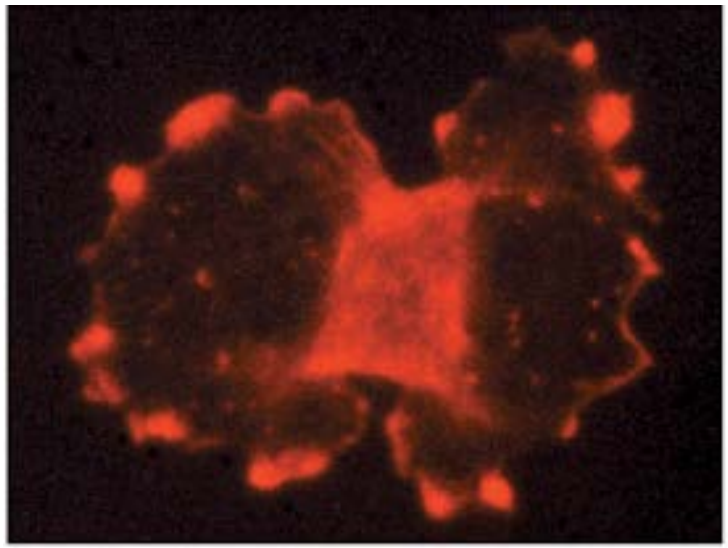


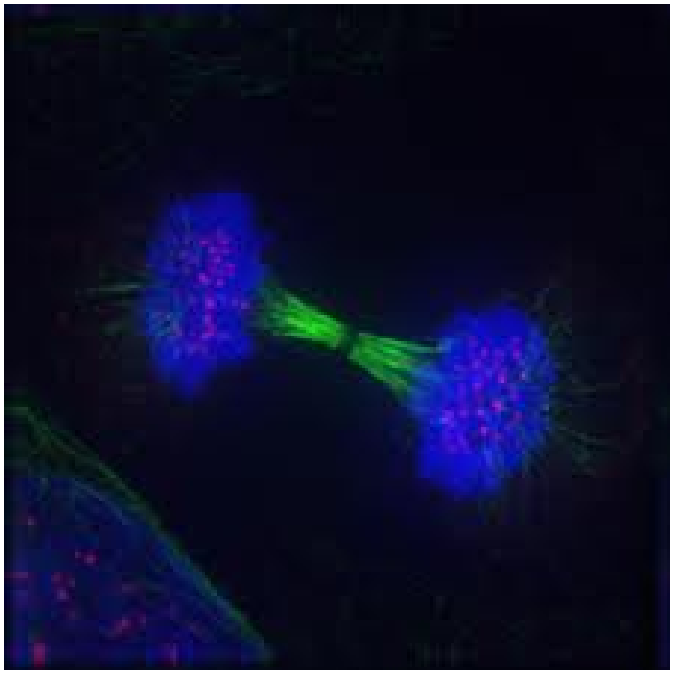
TELOPHASE


a
Early anaphase
Late anaphase
Telophase
b
Formation of prepores and recruitment of membrane tubules
NUP107–160 and ELYS, RanGTP
NE reformation
• INM proteins bind DNA
• NE sheet formation removal of reticulons
• Membrane closure
Chromatin compaction
KID, Aurora B?
Lamina assembly
Completion of NPC assembly
Lamins
Transmembrane nucleoporins
INM proteins
NUP107–160 and ELYS
MTOCs
Microtubules
Chromatin
Kinetochore

CYTOKINESIS

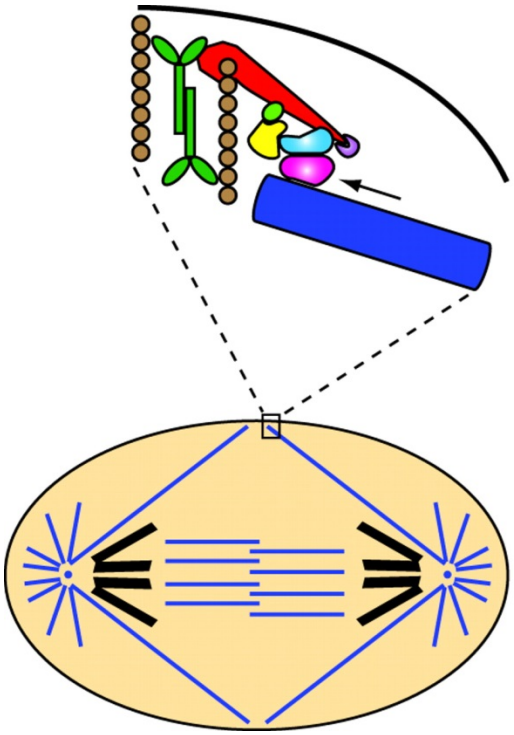


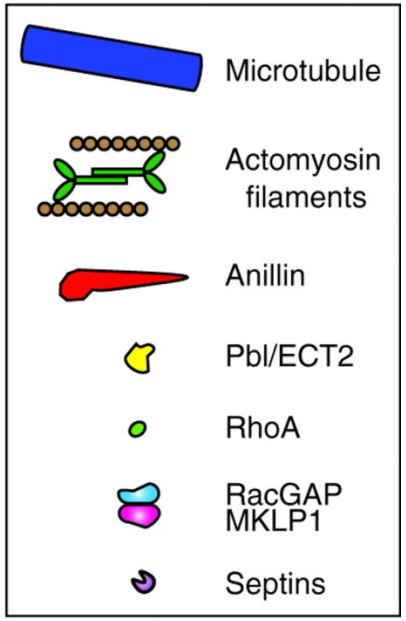


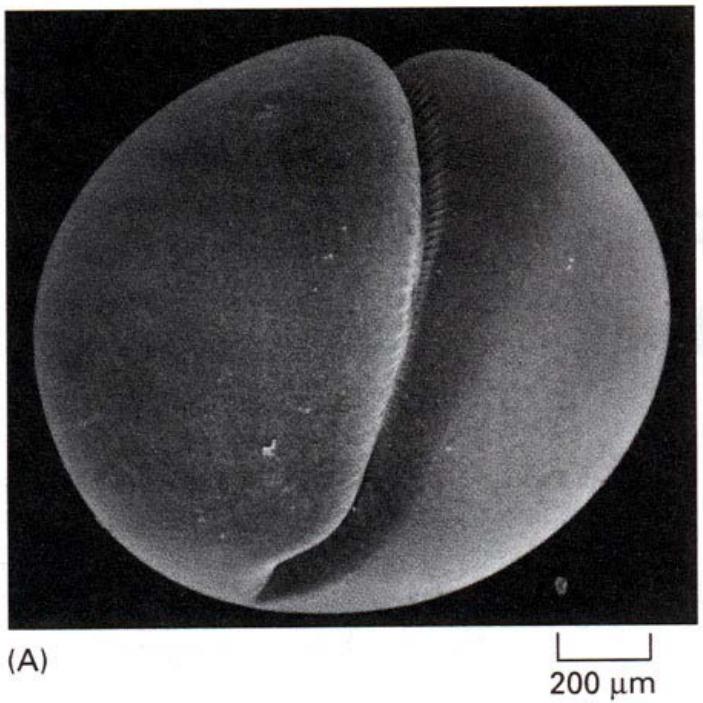


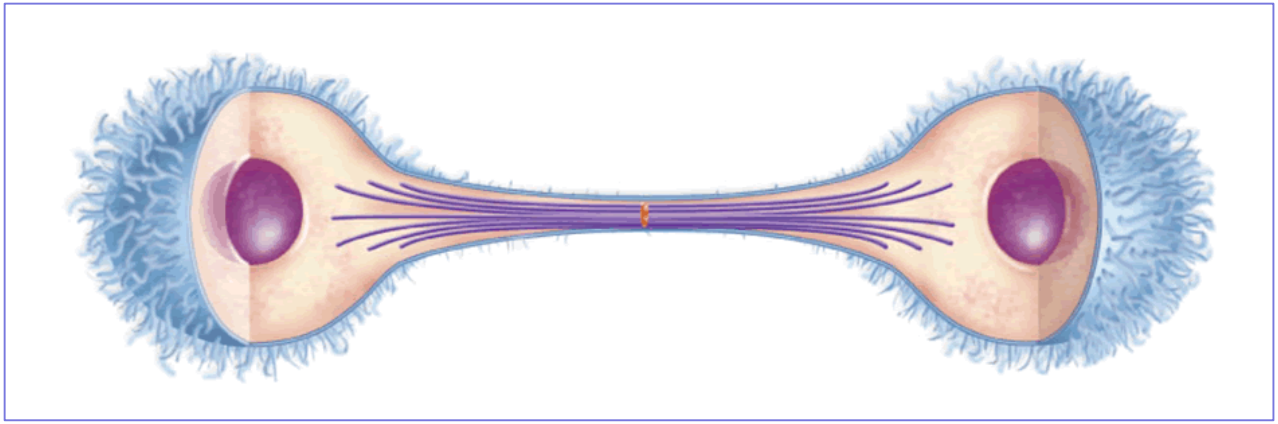
CYTOKINESIS

Contractile ring

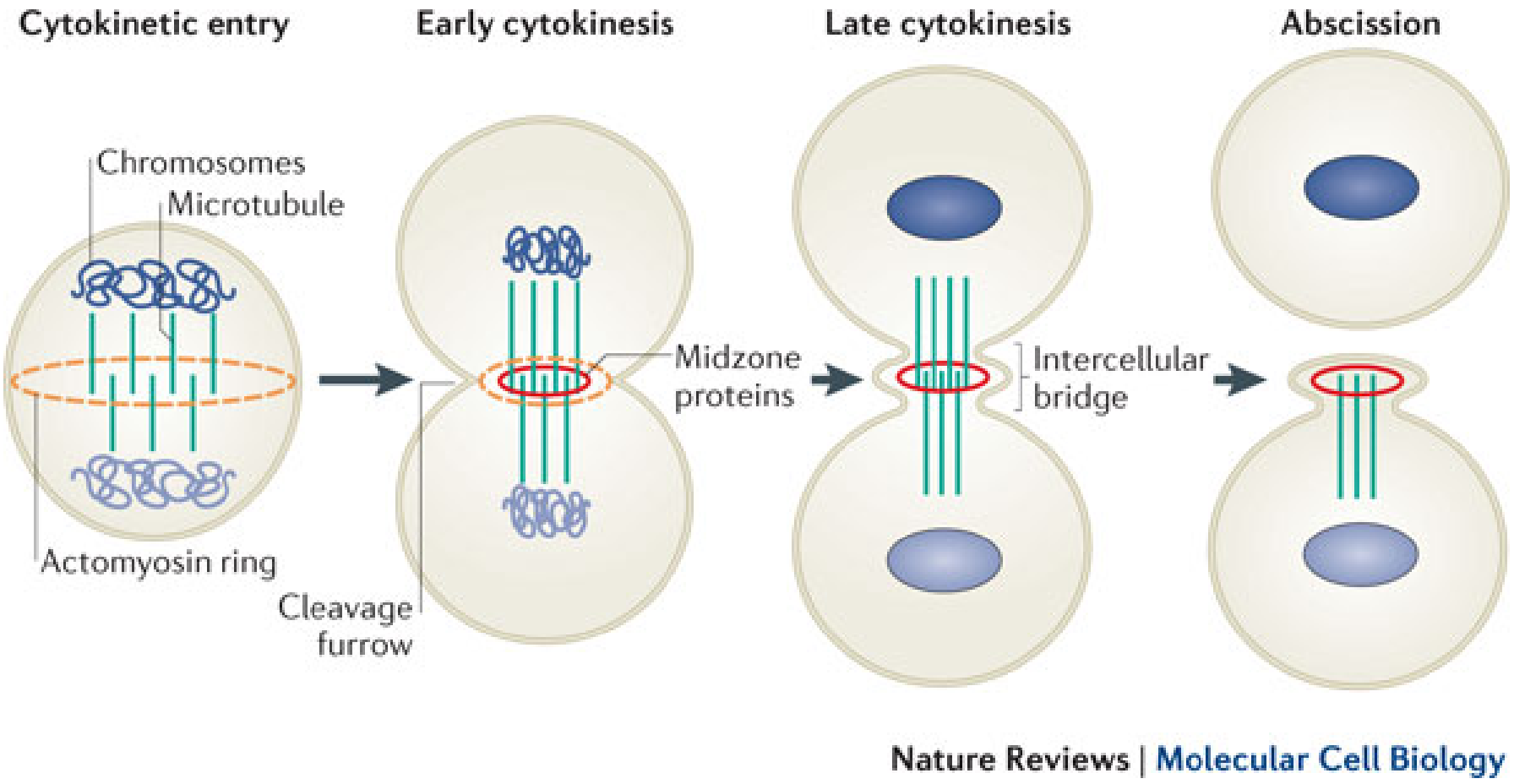




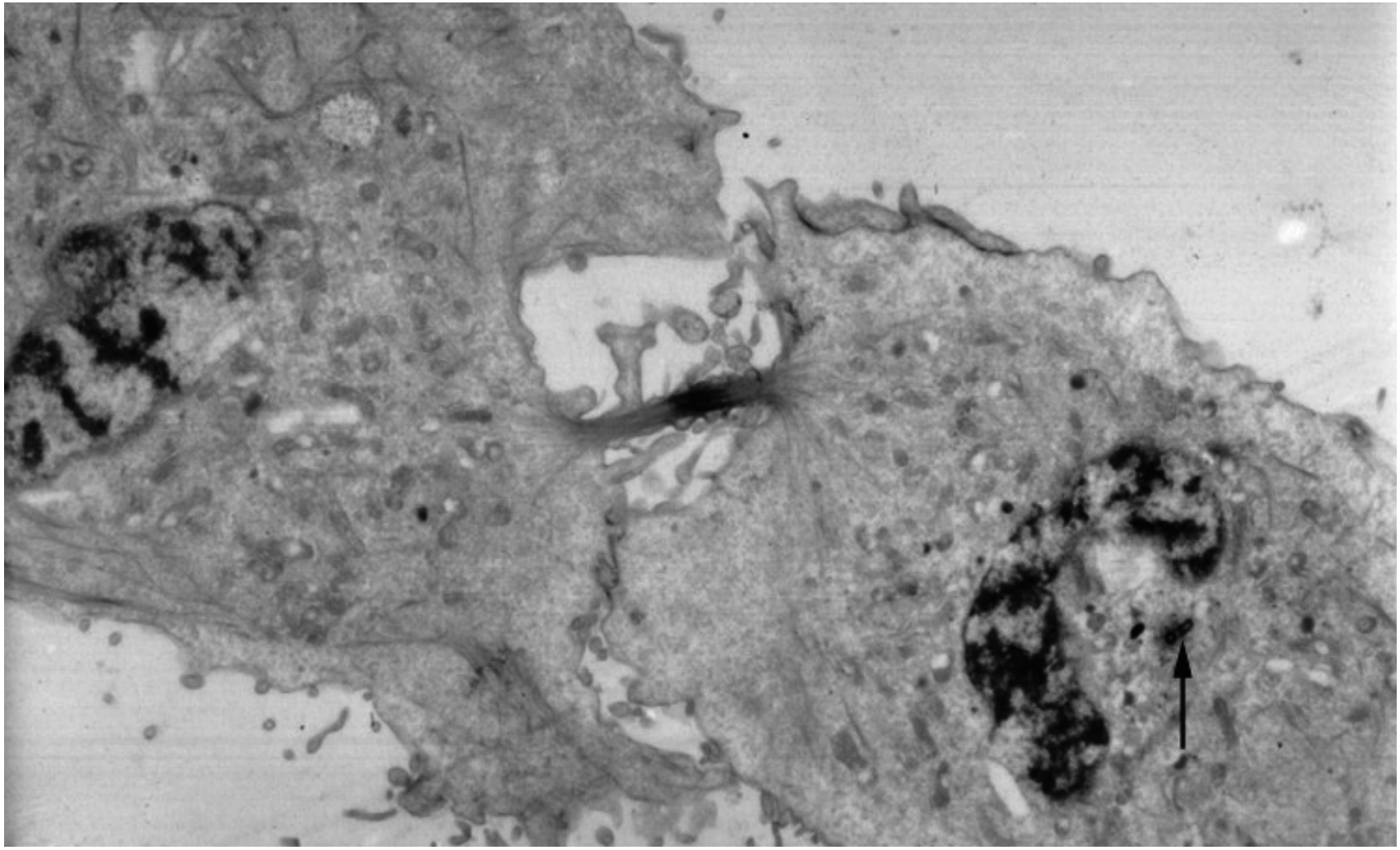




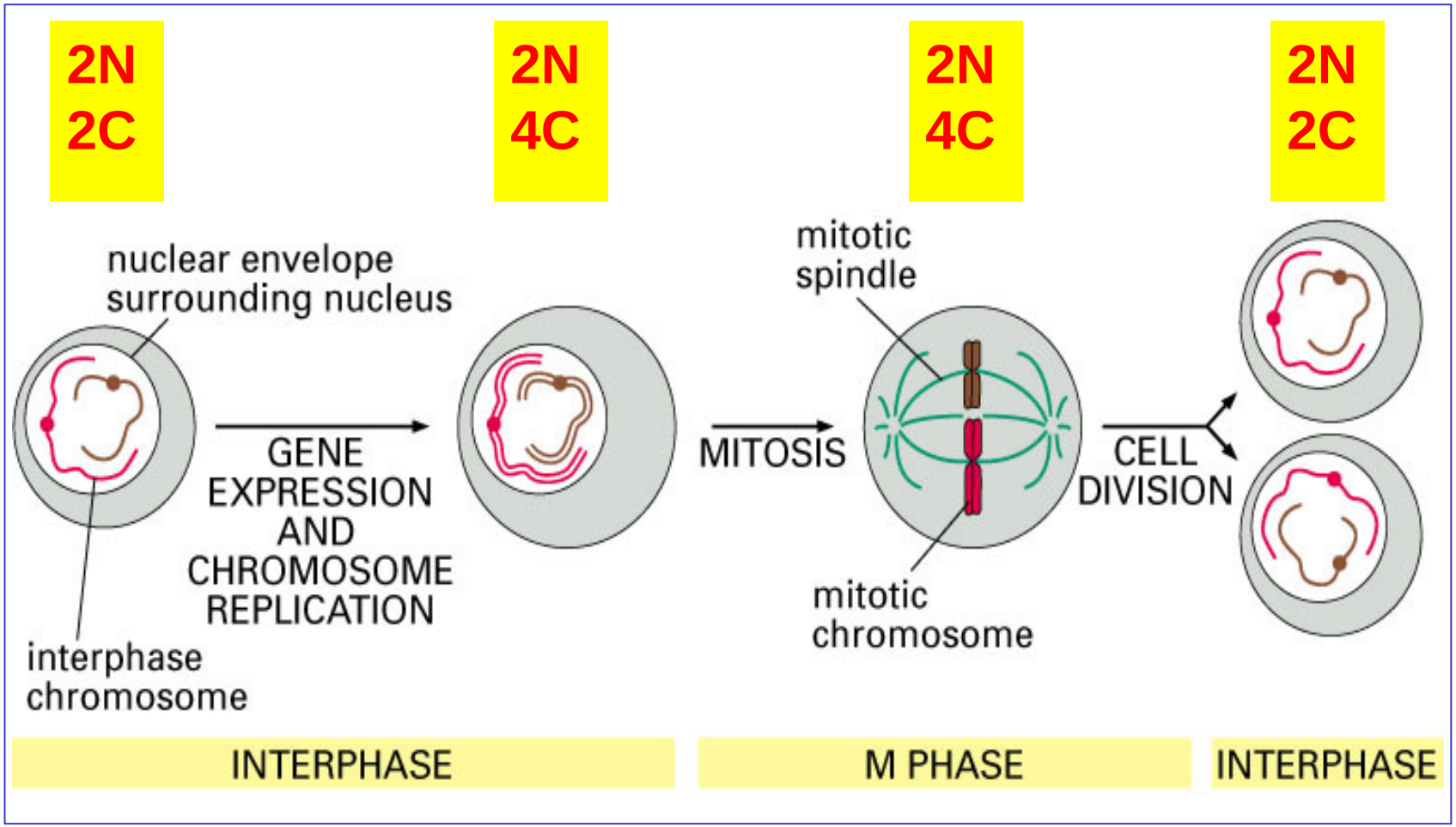
CYTOKINESIS



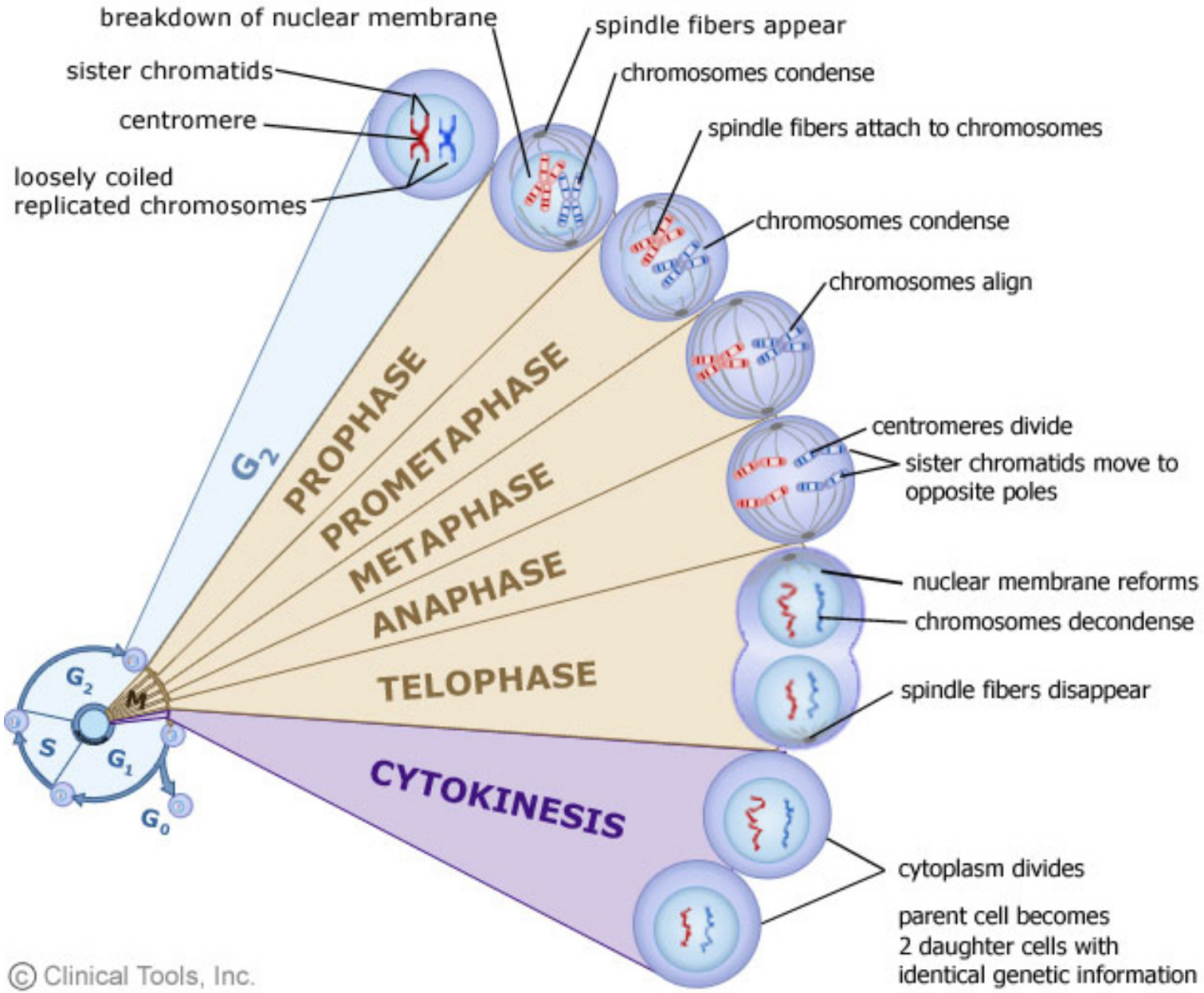
CYTOKINESIS



CHANGES IN CHROMOSOME NUMBER AND DNA AMOUNT ALONG MITOSIS



SUMMARY


breakdown of nuclear membrane
spindle fibers appear
sister chromatids
chromosomes condense
centromere
spindle fibers attach to chromosomes
loosely coiled
replicated chromosomes
chromosomes condense
chromosomes align
G2
PROPHASE
PROMETAPHASE
METAPHASE
ANAPHASE
TELOPHASE
centromeres divide
sister chromatids move to
opposite poles
nuclear membrane reforms
chromosomes decondense
spindle fibers disappear
CYTOKINESIS
cytoplasm divides
parent cell becomes
2 daughter cells with
identical genetic information
G2
M
S
G1
G0
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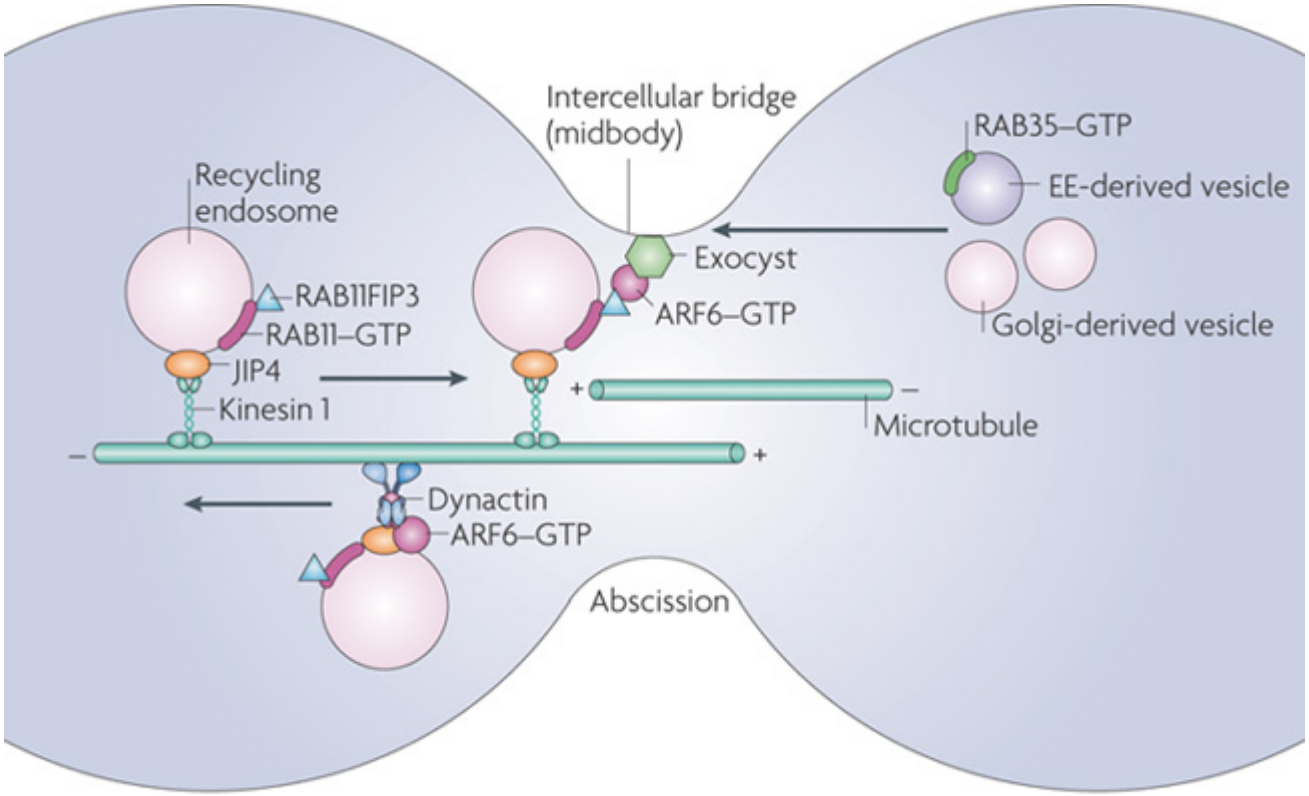
SUMMARY



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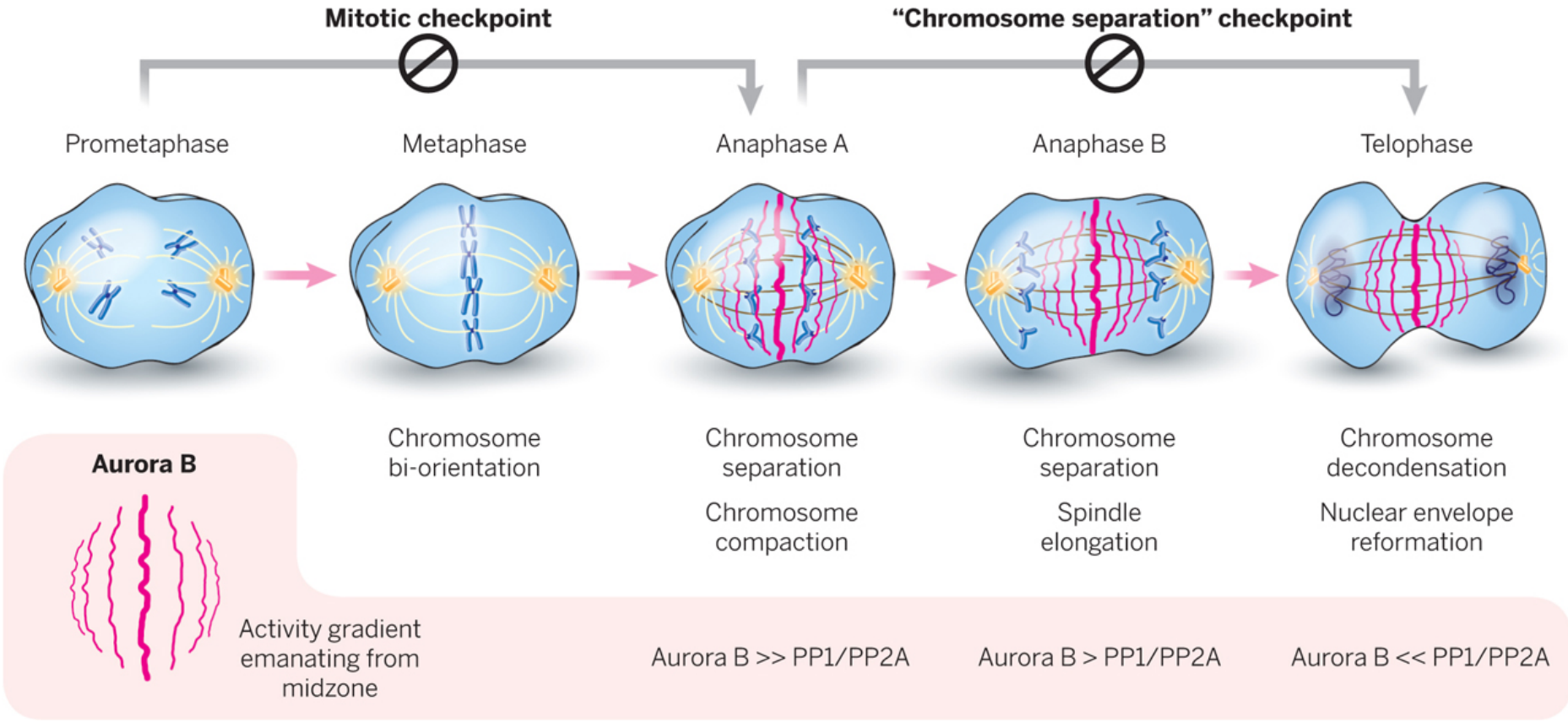
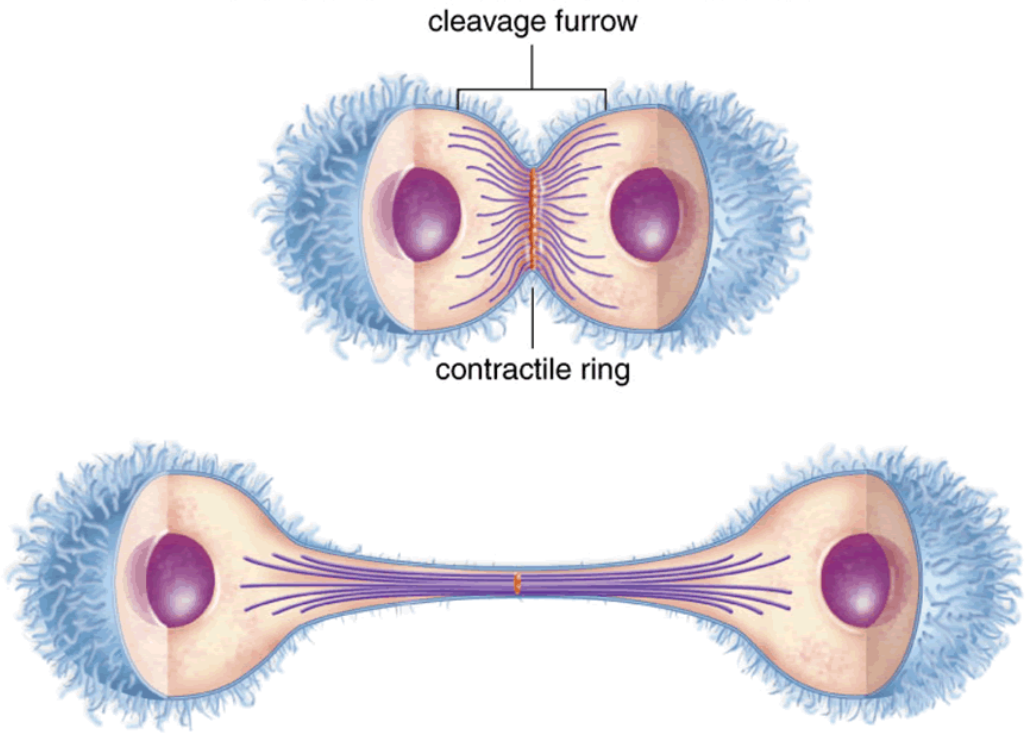
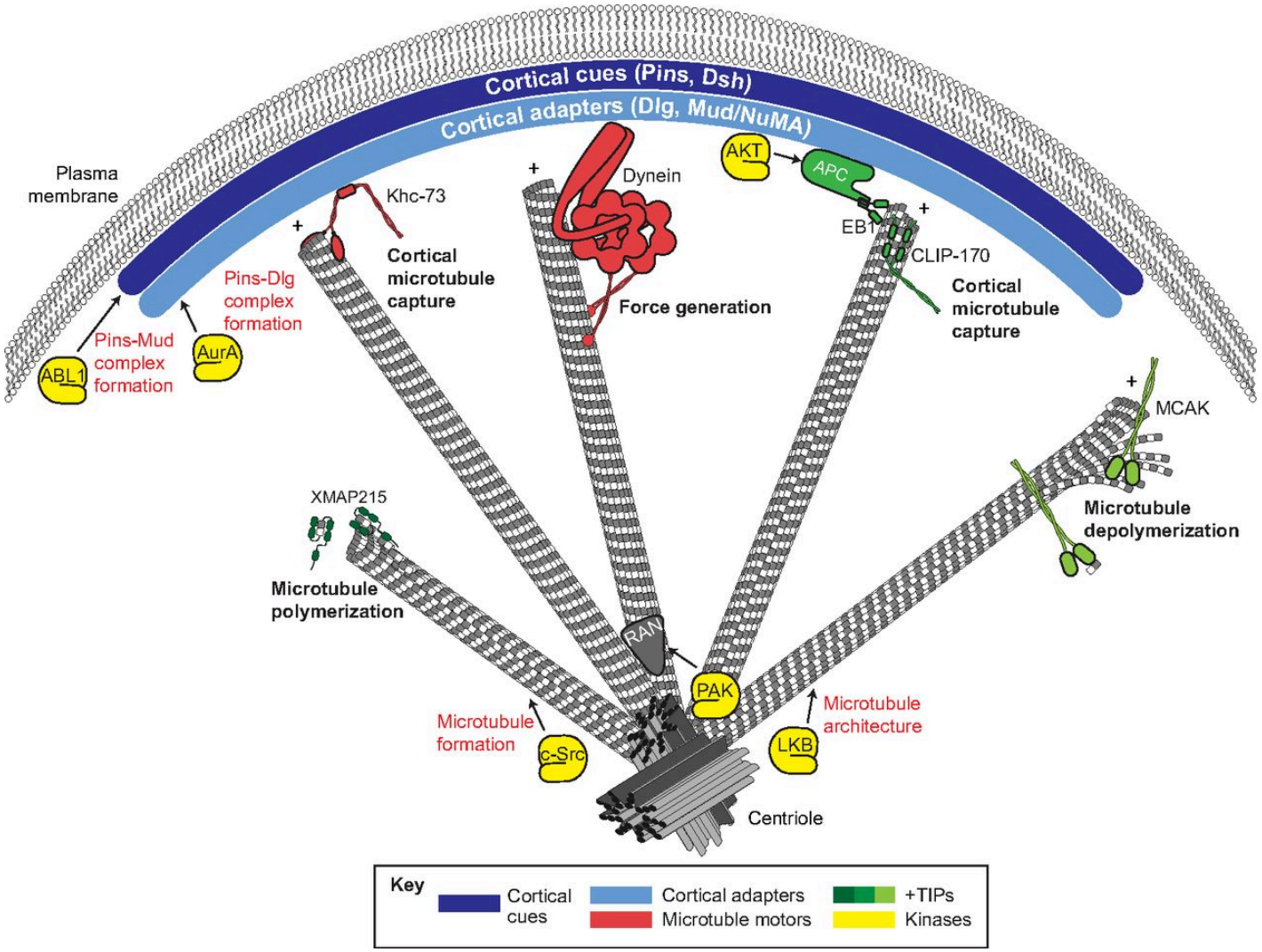

Mitotic checkpoint
"Chromosome separation" checkpoint
Prometaphase
Metaphase
Anaphase A
Anaphase B
Telophase
Chromosome bi-orientation
Chromosome separation
Chromosome compaction
Chromosome separation
Spindle elongation
Chromosome decondensation
Nuclear envelope reformation
Aurora B
Activity gradient emanating from midzone
Aurora B >> PP1/PP2A
Aurora B > PP1/PP2A
Aurora B << PP1/PP2A

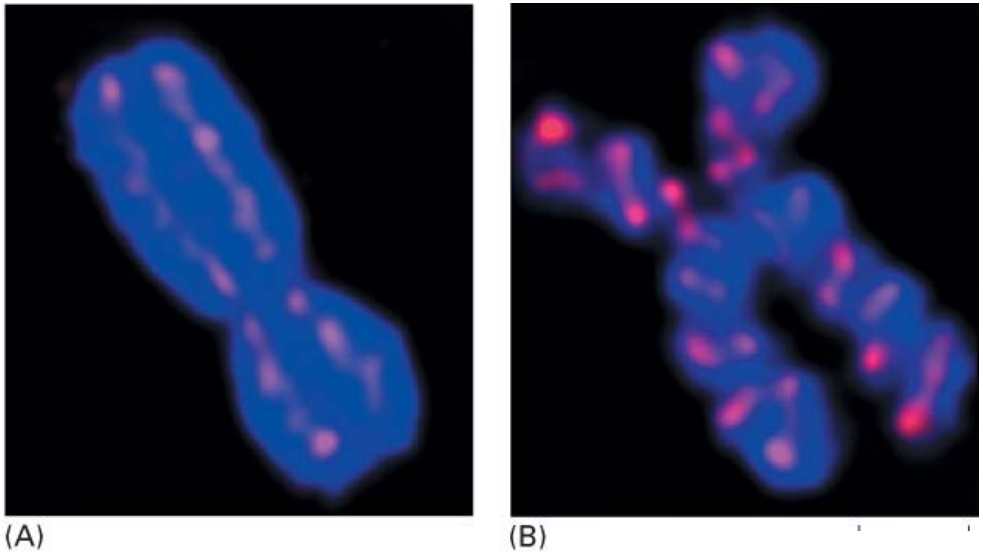


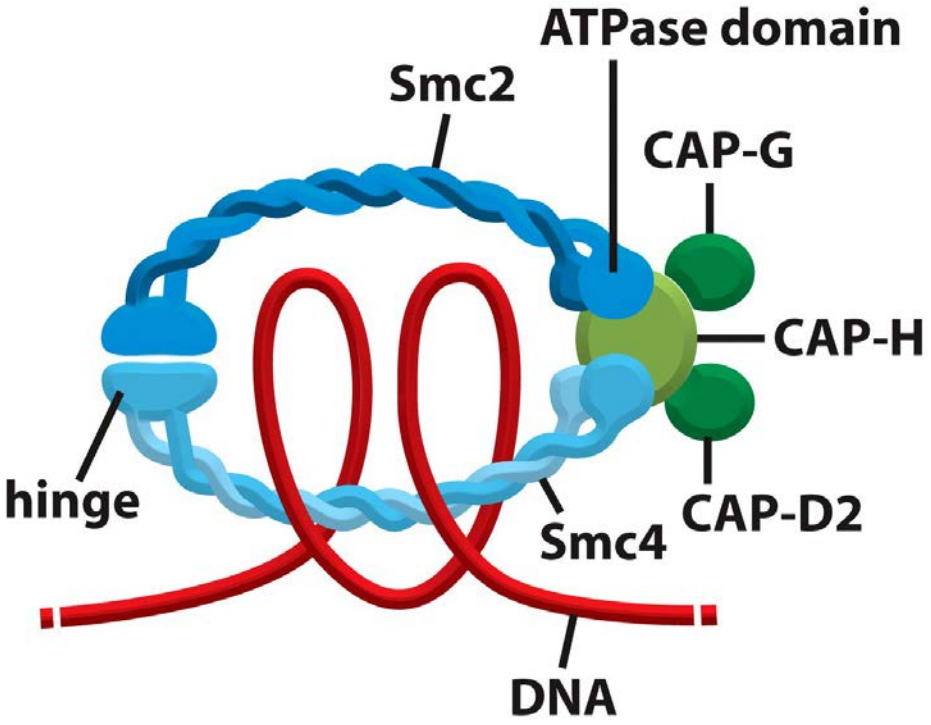
PROPHASE

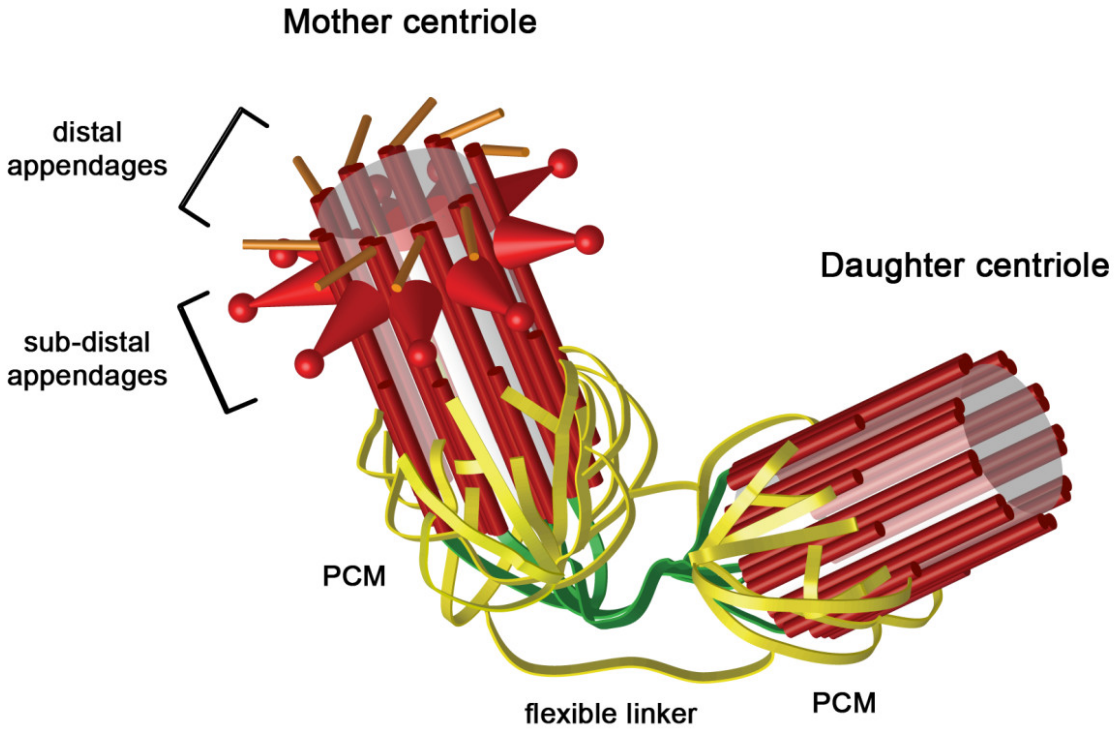
MOTOR PROTEINS DEPENDENT ON MICROTUBULES

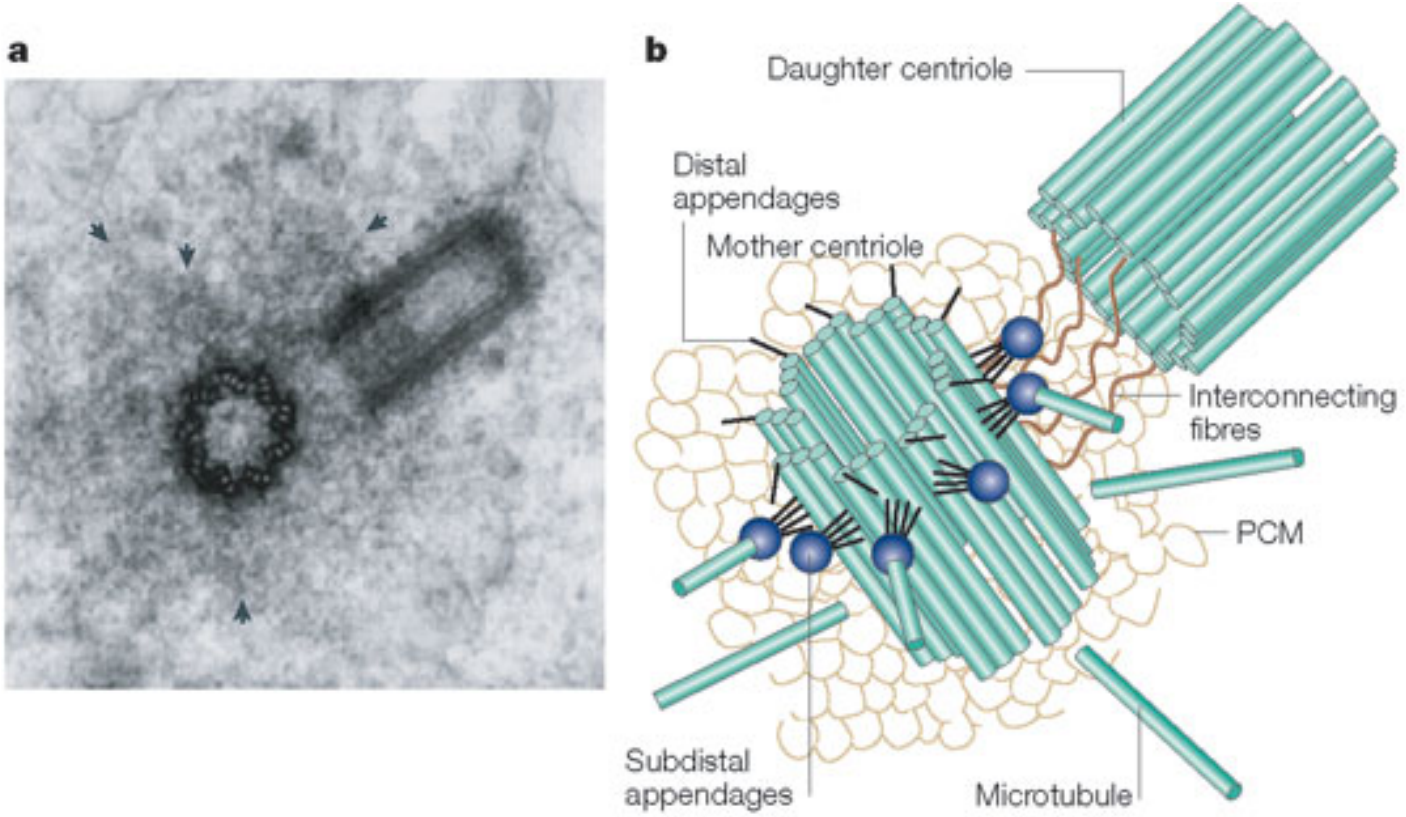


CHROMOSOME CONDENSATION

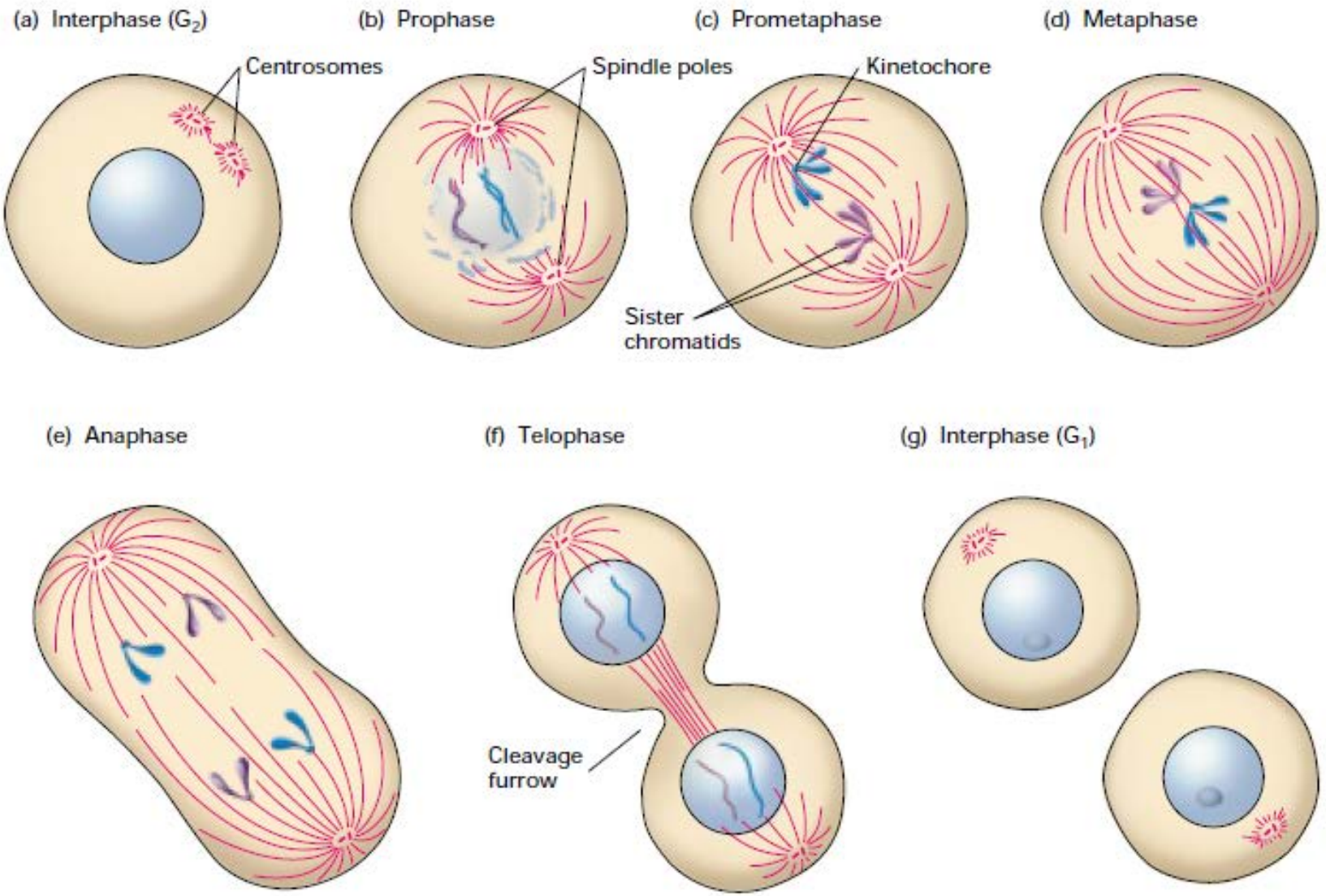




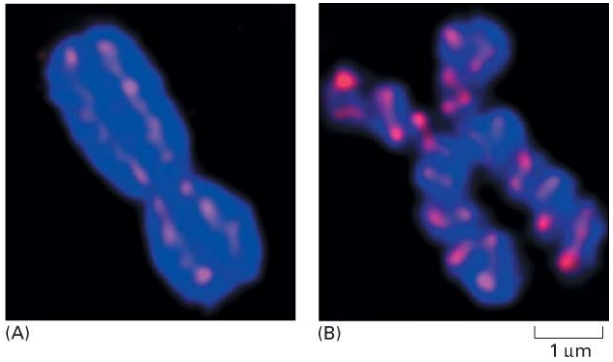

Mother centriole
distal appendages
sub-distal appendages
Daughter centriole
PCM
flexible linker
PCM

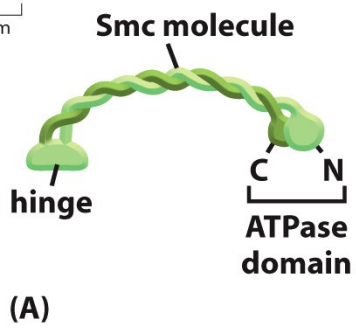


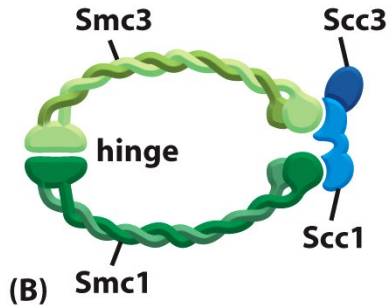
PHASES OF MITOSIS

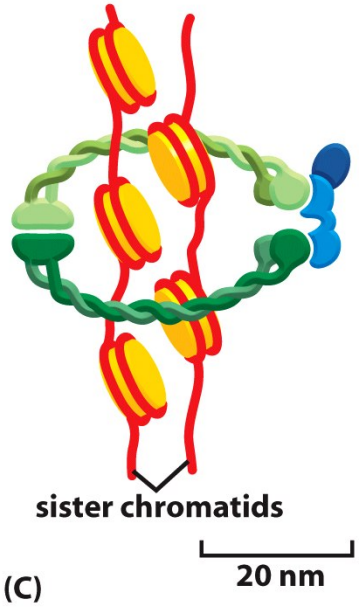


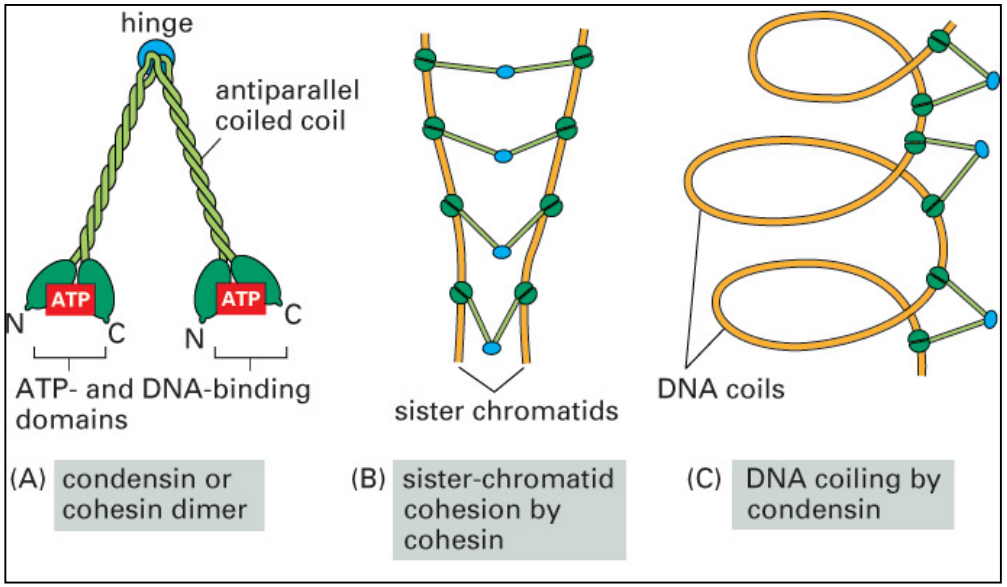
CHROMOSOME CONDENSATION



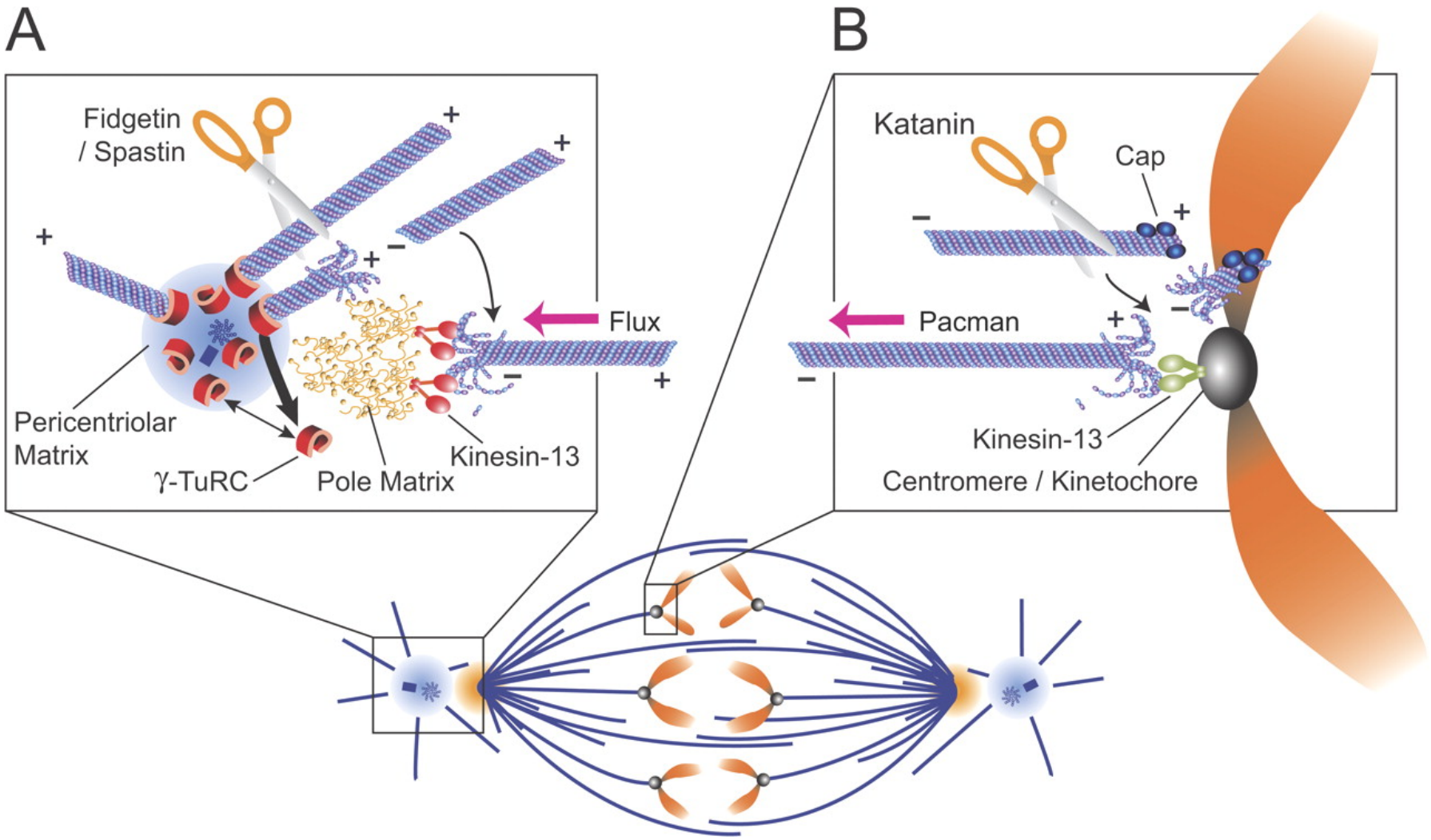


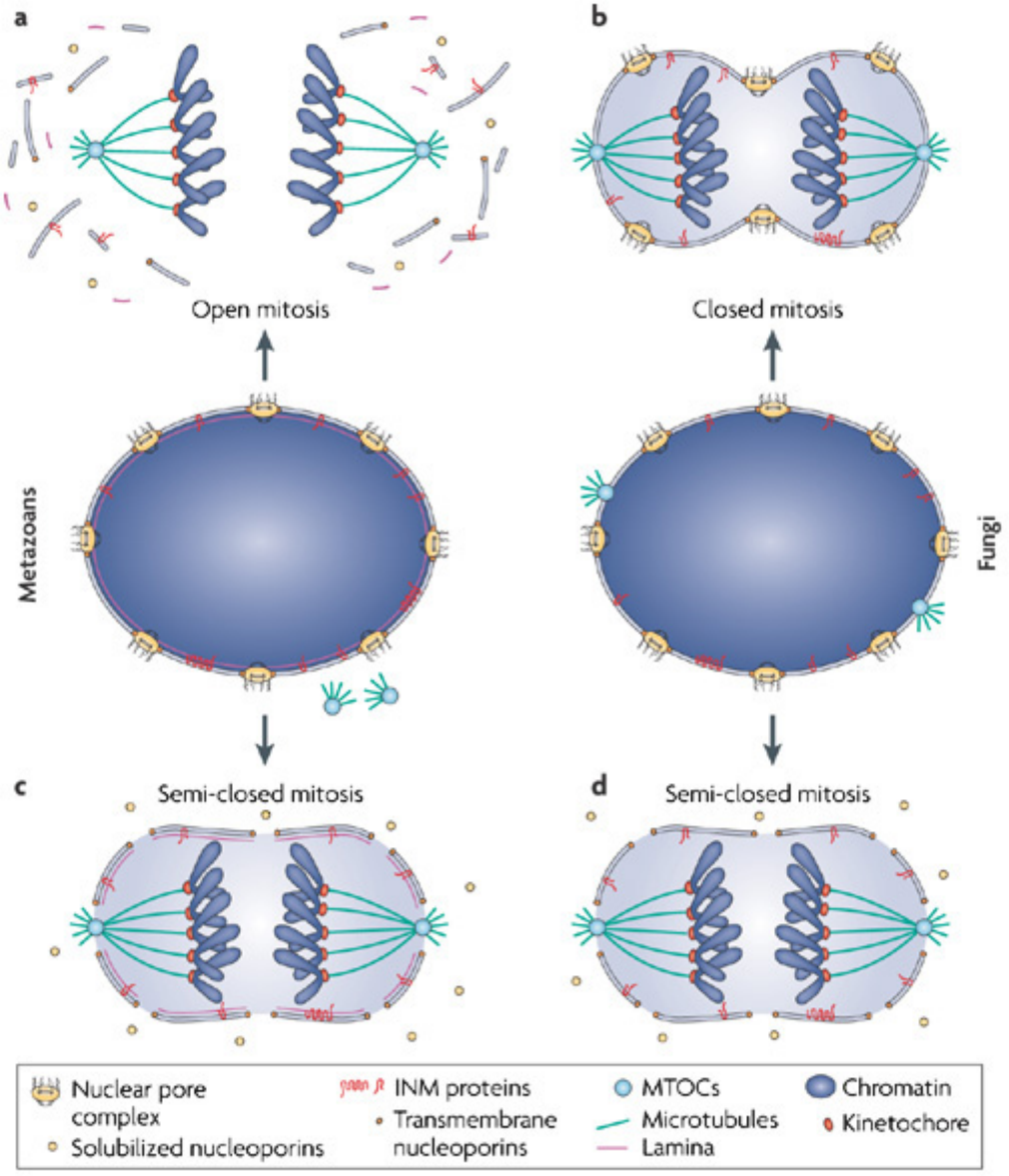






COHESINS


A
Fidgetin
/ Spastin
+
+
+
+
−
−
Flux
Pericentriolar
Matrix
γ-TuRC
Pole Matrix
Kinesin-13
B
Katanin
Cap
−
+
Pacman
+
−
−
Kinesin-13
Centromere / Kinetochore



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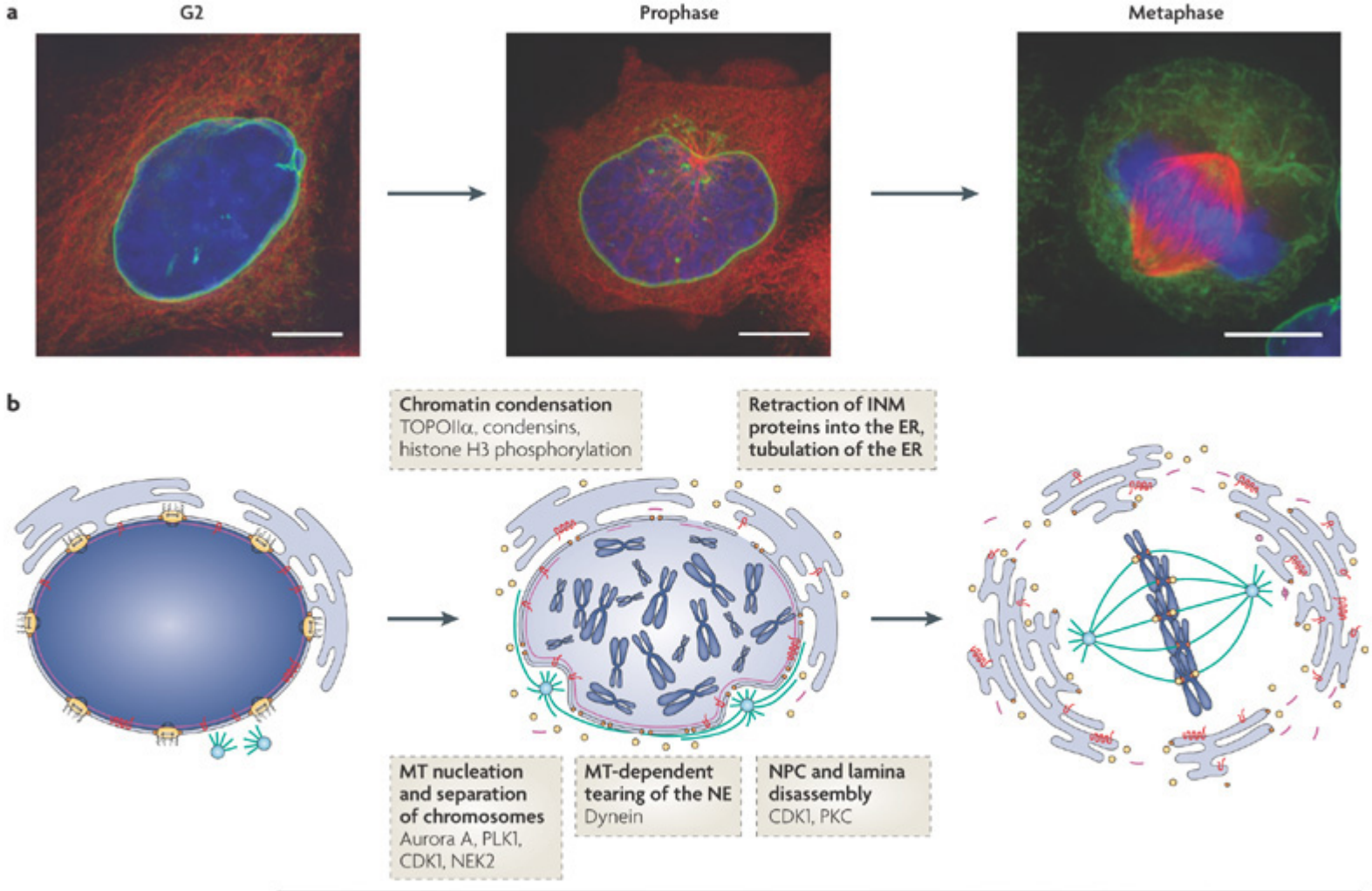
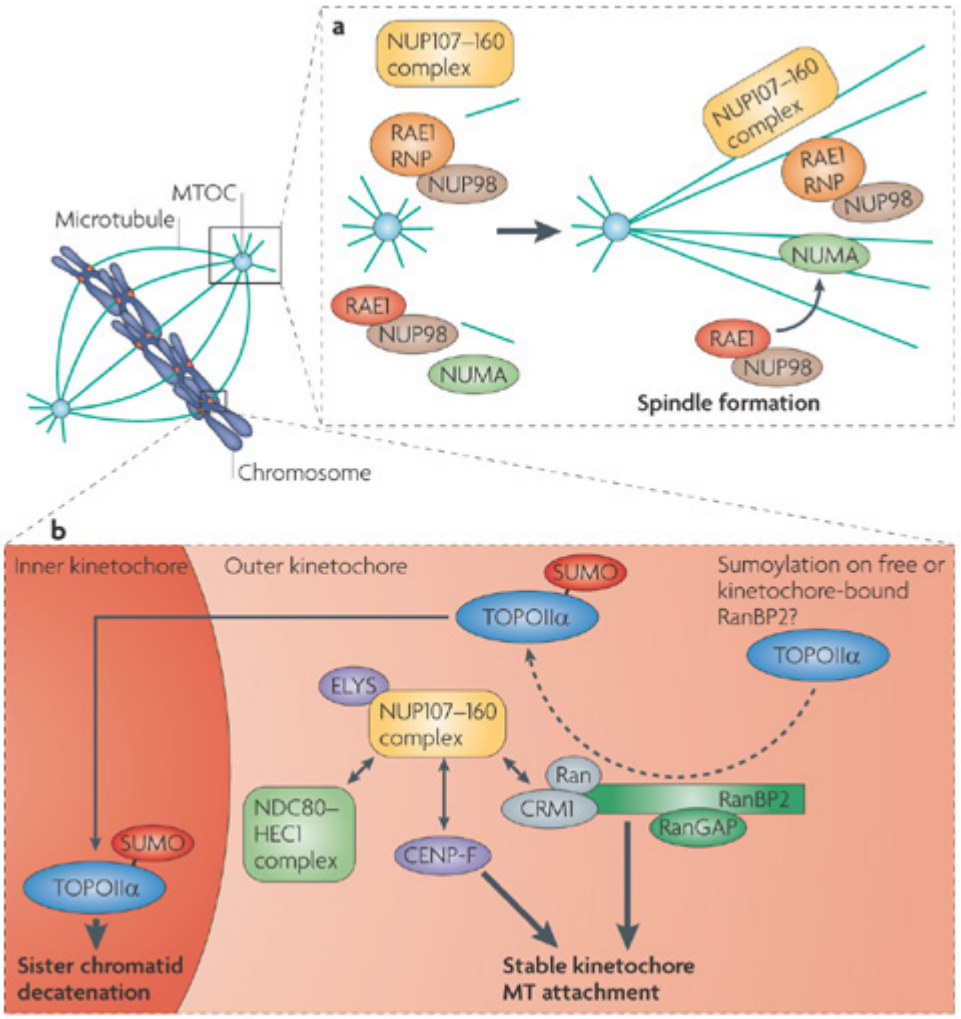

a
G2
Prophase
Metaphase
b
Chromatin condensation
TOPOIIα, condensins,
histone H3 phosphorylation
Retraction of INM
proteins into the ER,
tubulation of the ER
MT nucleation
and separation
of chromosomes
Aurora A, PLK1,
CDK1, NEK2
MT-dependent
tearing of the NE
Dynein
NPC and lamina
disassembly
CDK1, PKC
Chromatin
INM proteins
Kinetochore
Lamins
Microtubules
MTOCs
Nuclear pore complex
NUP107–160 and ELYS
Transmembrane nucleoporins



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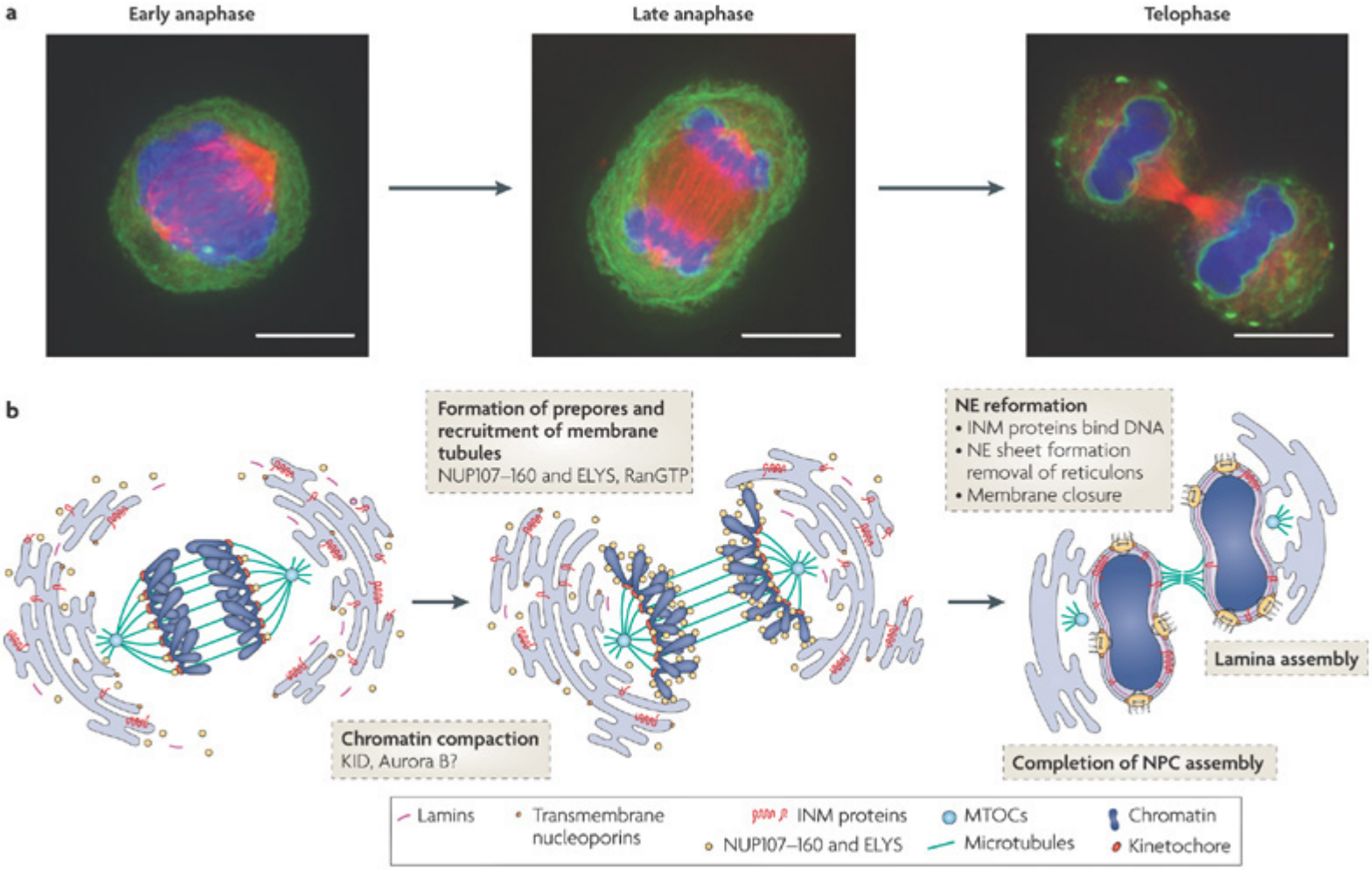
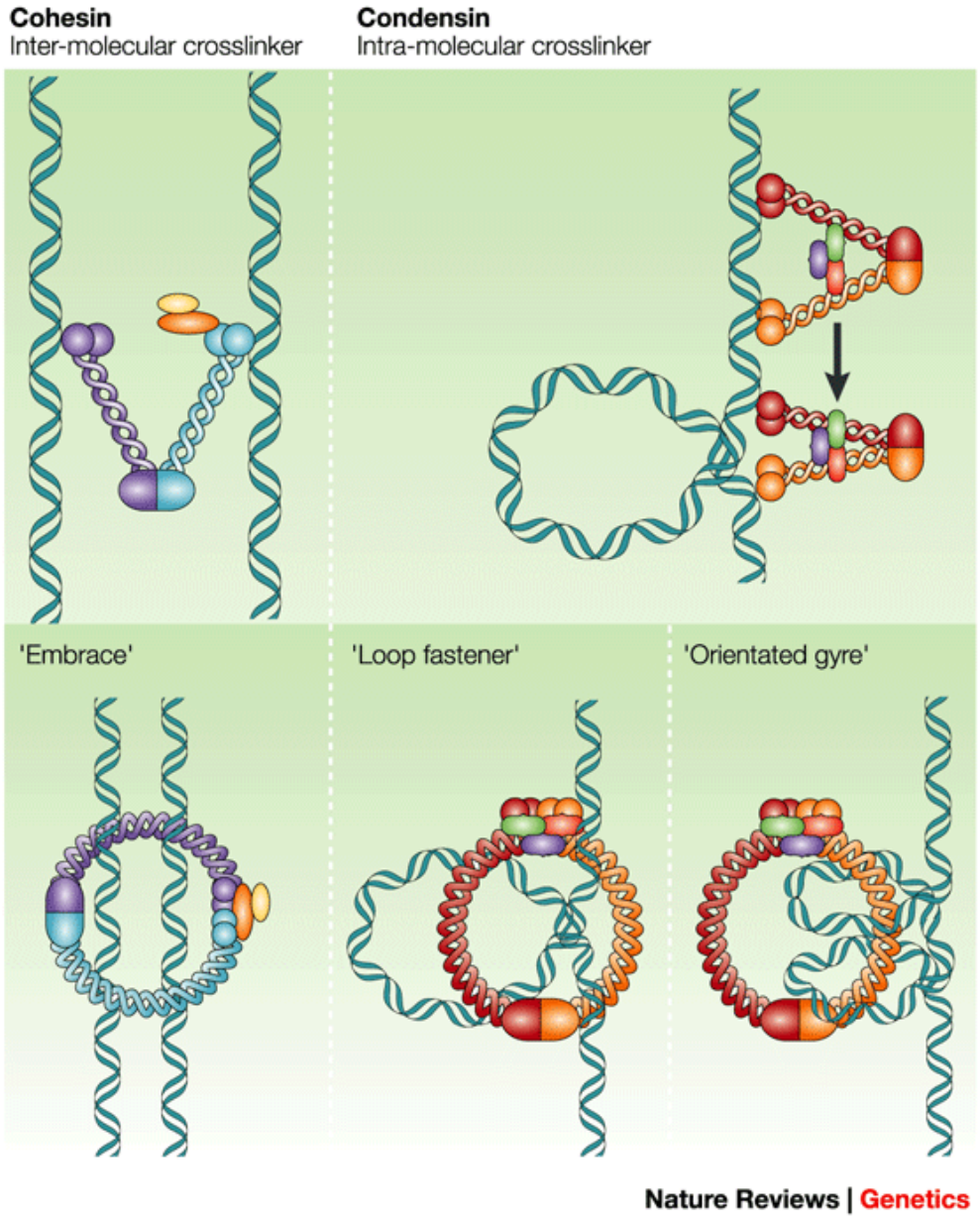
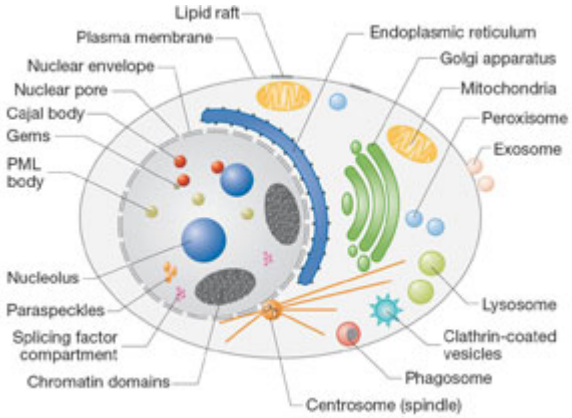
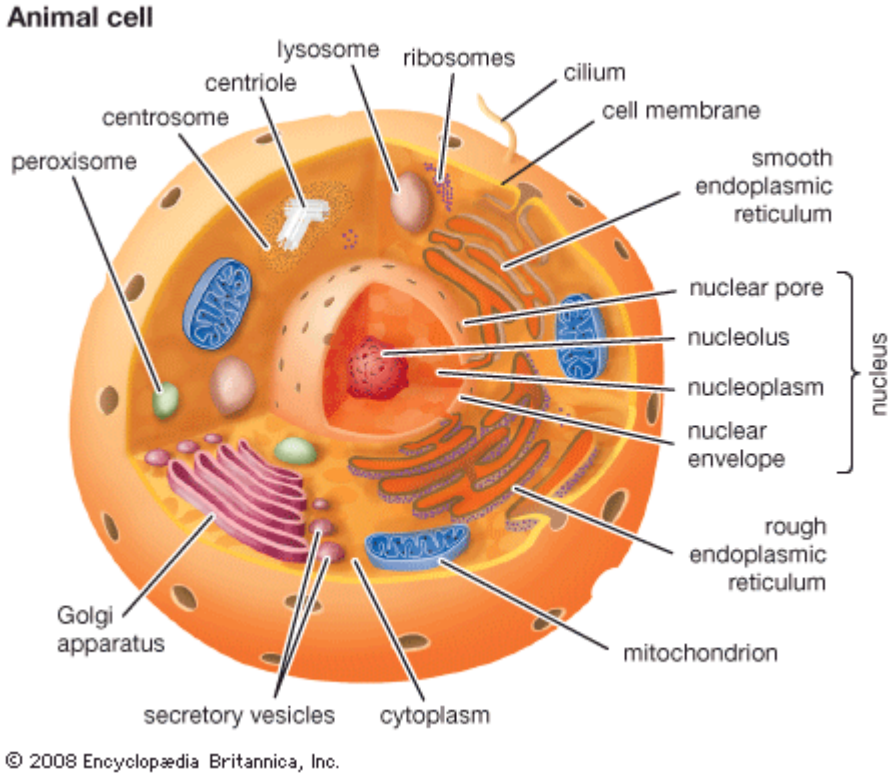

a
Early anaphase
Late anaphase
Telophase
b
Formation of prepores and recruitment of membrane tubules
NUP107–160 and ELYS, RanGTP
NE reformation
• INM proteins bind DNA
• NE sheet formation removal of reticulons
• Membrane closure
Lamina assembly
Chromatin compaction
KID, Aurora B?
Completion of NPC assembly
Lamins
Transmembrane nucleoporins
INM proteins
NUP107–160 and ELYS
MTOCs
Microtubules
Chromatin
Kinetochore

PROPHASE

CHROMOSOME CONDENSATION




Lipid raft
Plasma membrane
Nuclear envelope
Nuclear pore
Cajal body
Gems
PML body
Nucleolus
Paraspeckles
Splicing factor compartment
Chromatin domains
Endoplasmic reticulum
Golgi apparatus
Mitochondria
Peroxisome
Exosome
Lysosome
Clathrin-coated vesicles
Phagosome
Centrosome (spindle)


Animal cell
lysosome
ribosomes
cilium
centriole
centrosome
cell membrane
peroxisome
smooth endoplasmic reticulum
nuclear pore
nucleolus
nucleoplasm
nuclear envelope
nucleus
rough endoplasmic reticulum
Golgi apparatus
mitochondrion
secretory vesicles
cytoplasm
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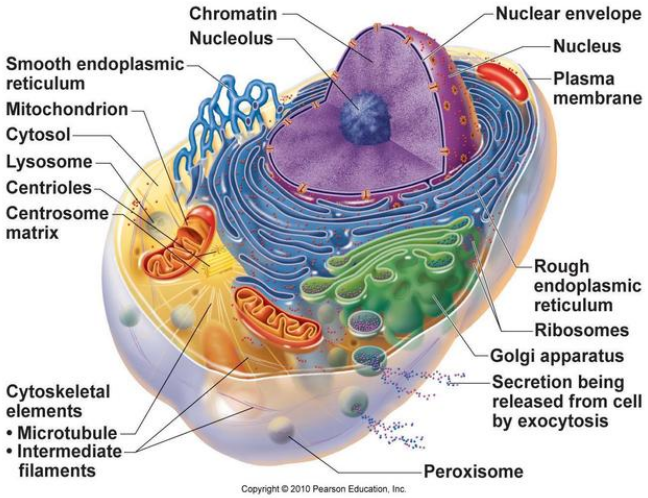
Chromatin
Nucleolus
Smooth endoplasmic reticulum
Mitochondrion
Cytosol
Lysosome
Centrioles
Centrosome matrix
Nuclear envelope
Nucleus
Plasma membrane
Rough endoplasmic reticulum
Ribosomes
Golgi apparatus
Secretion being released from cell by exocytosis
Cytoskeletal elements
• Microtubule
• Intermediate filaments
Peroxisome
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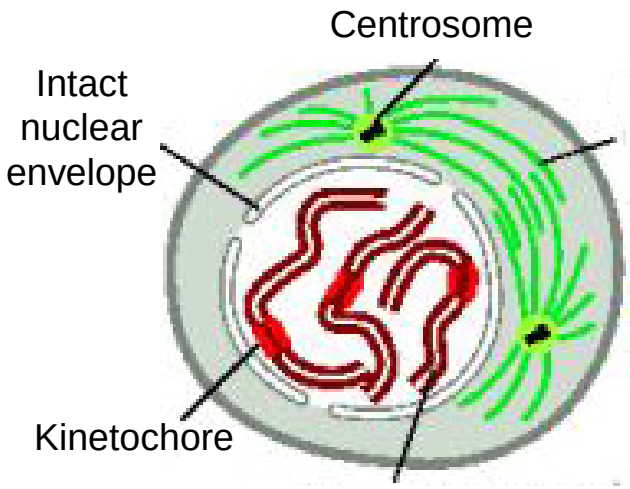
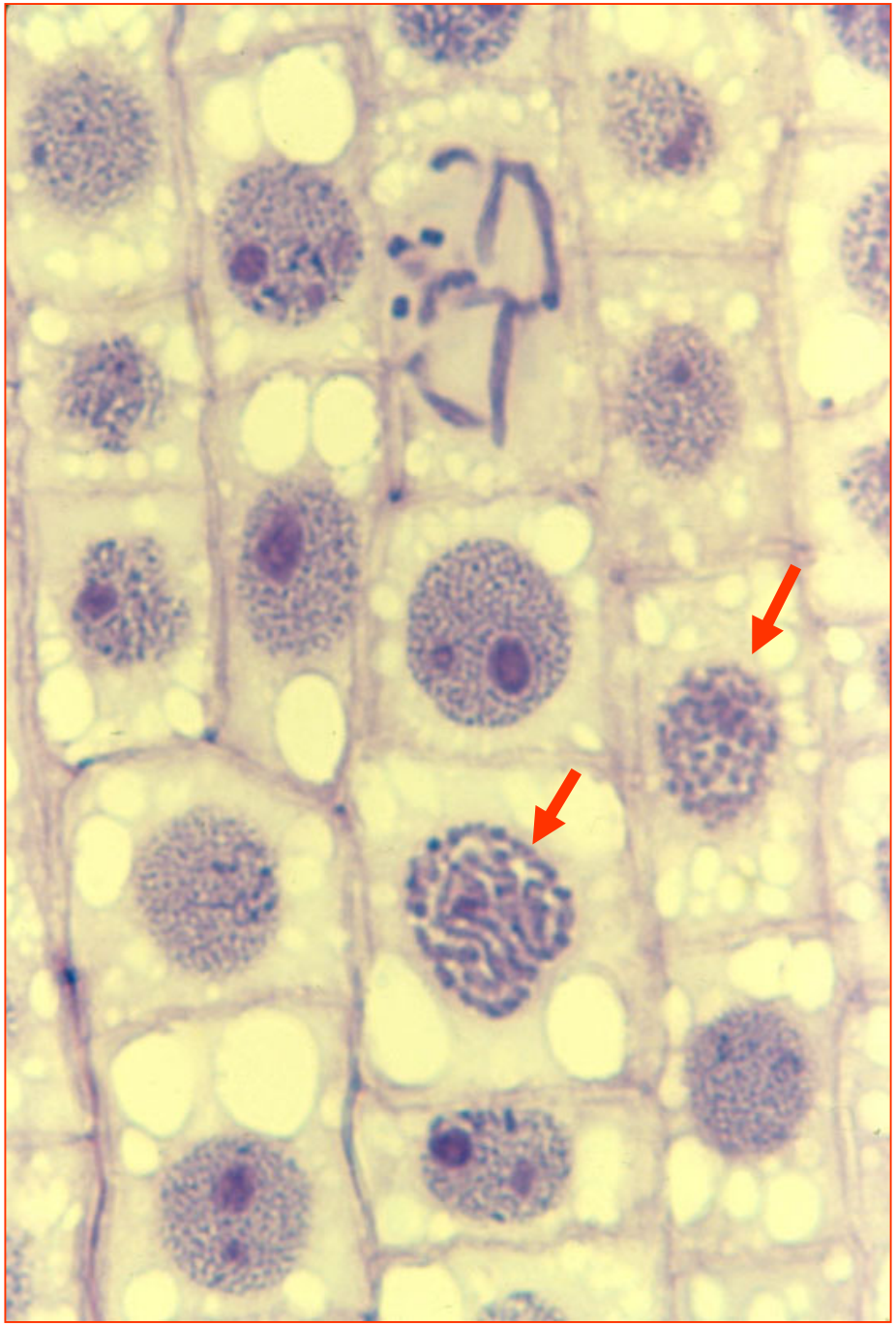
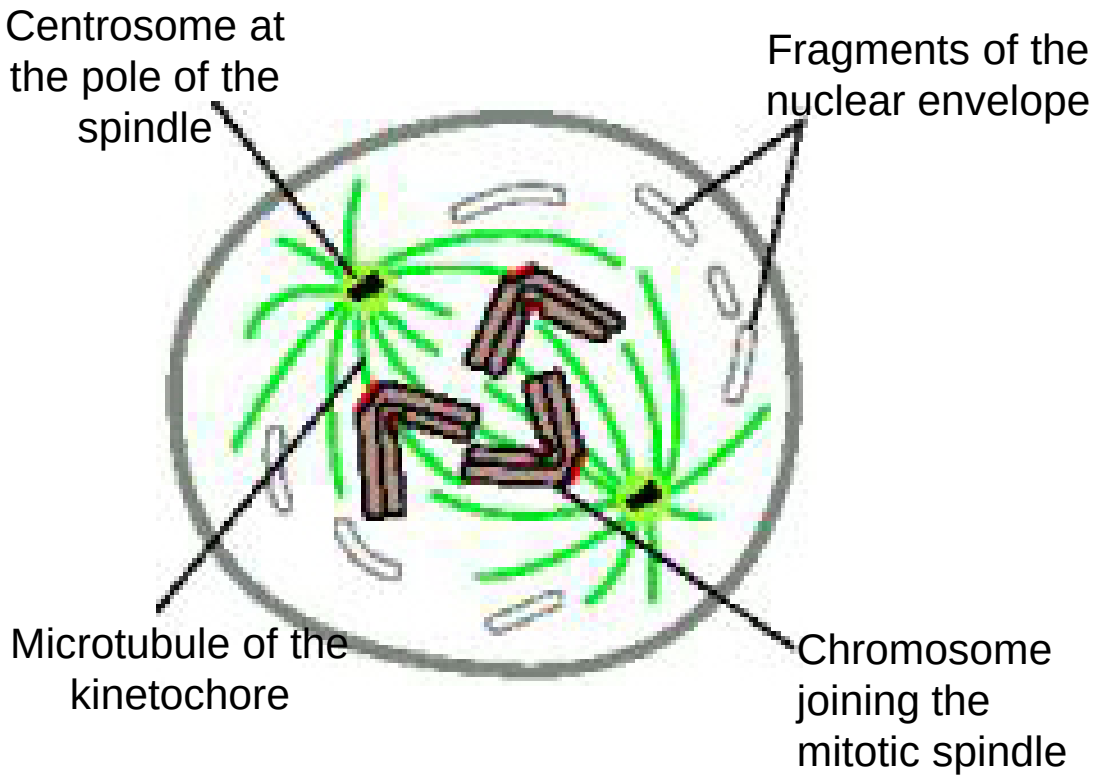
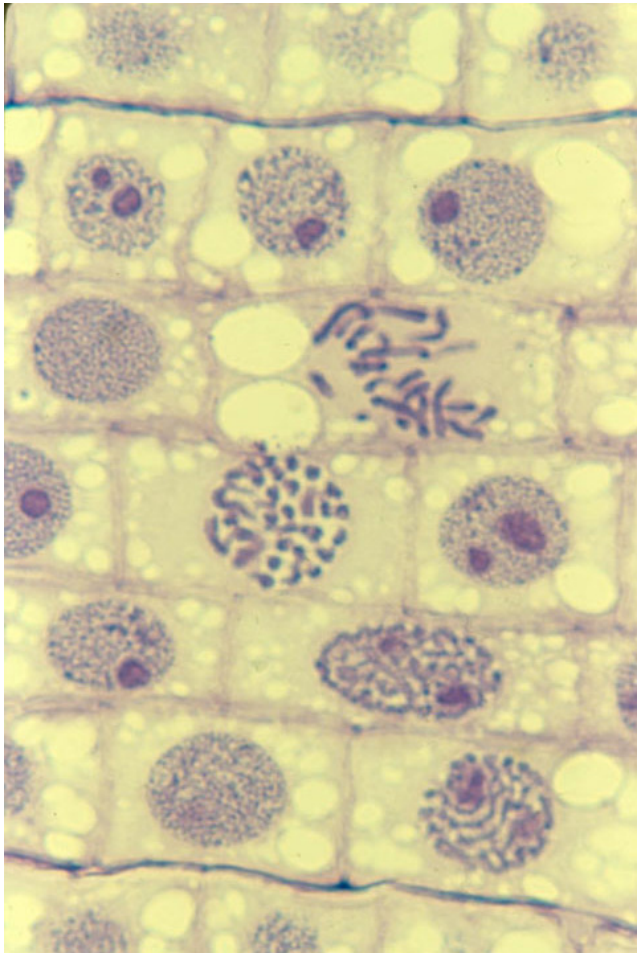
PROPHASE


Centrosome
Intact nuclear envelope
Mitotic spindle generation
Kinetochore
Chromosomes in condensation. It is possible to see both chromatids



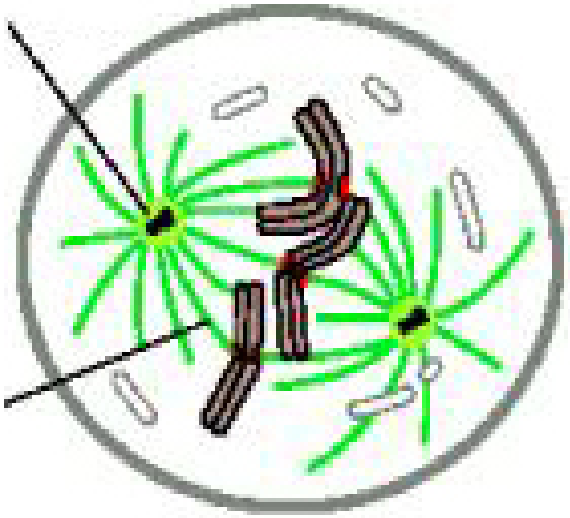
PROMETAPHASE



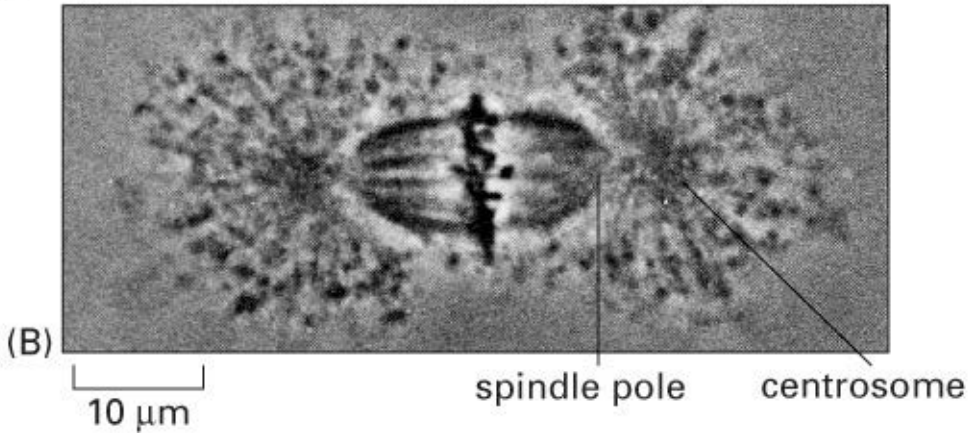


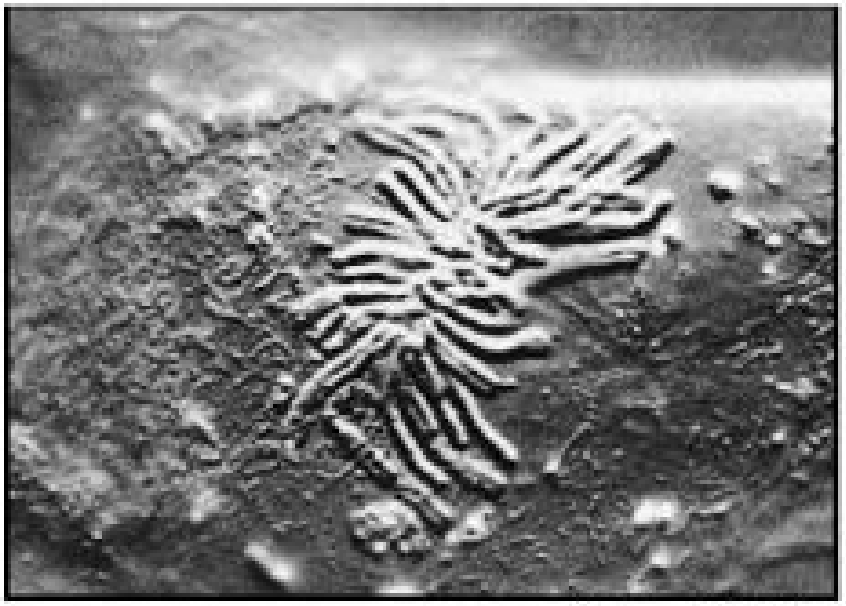
METAPHASE

Centrosome at the pole of the spindle

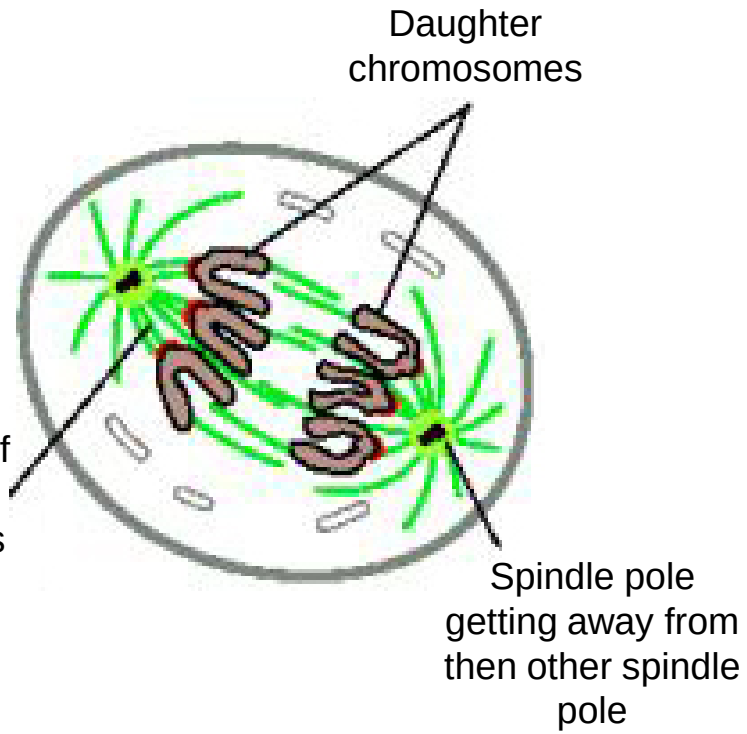


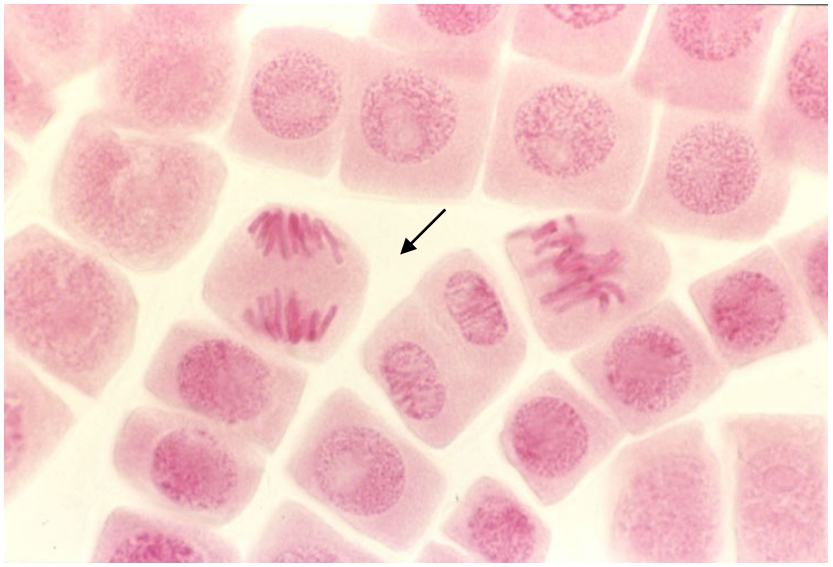
Kinetochore microtubules

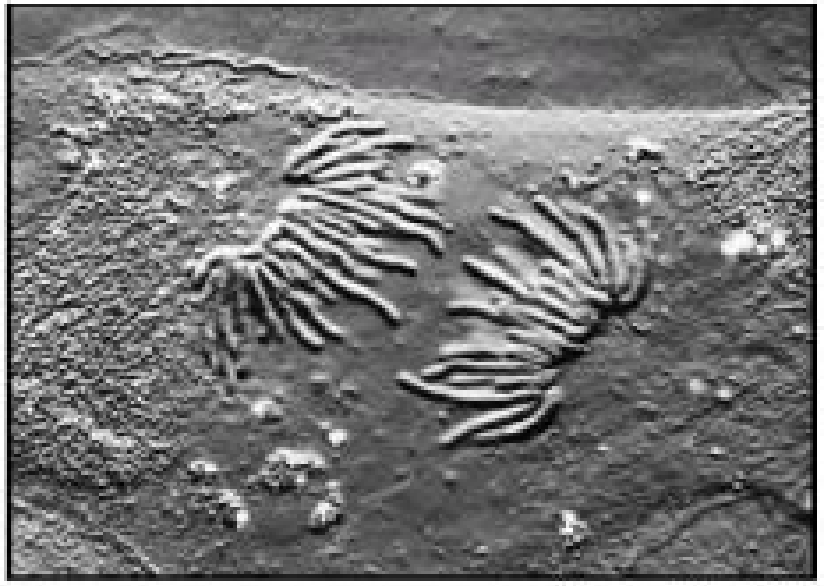




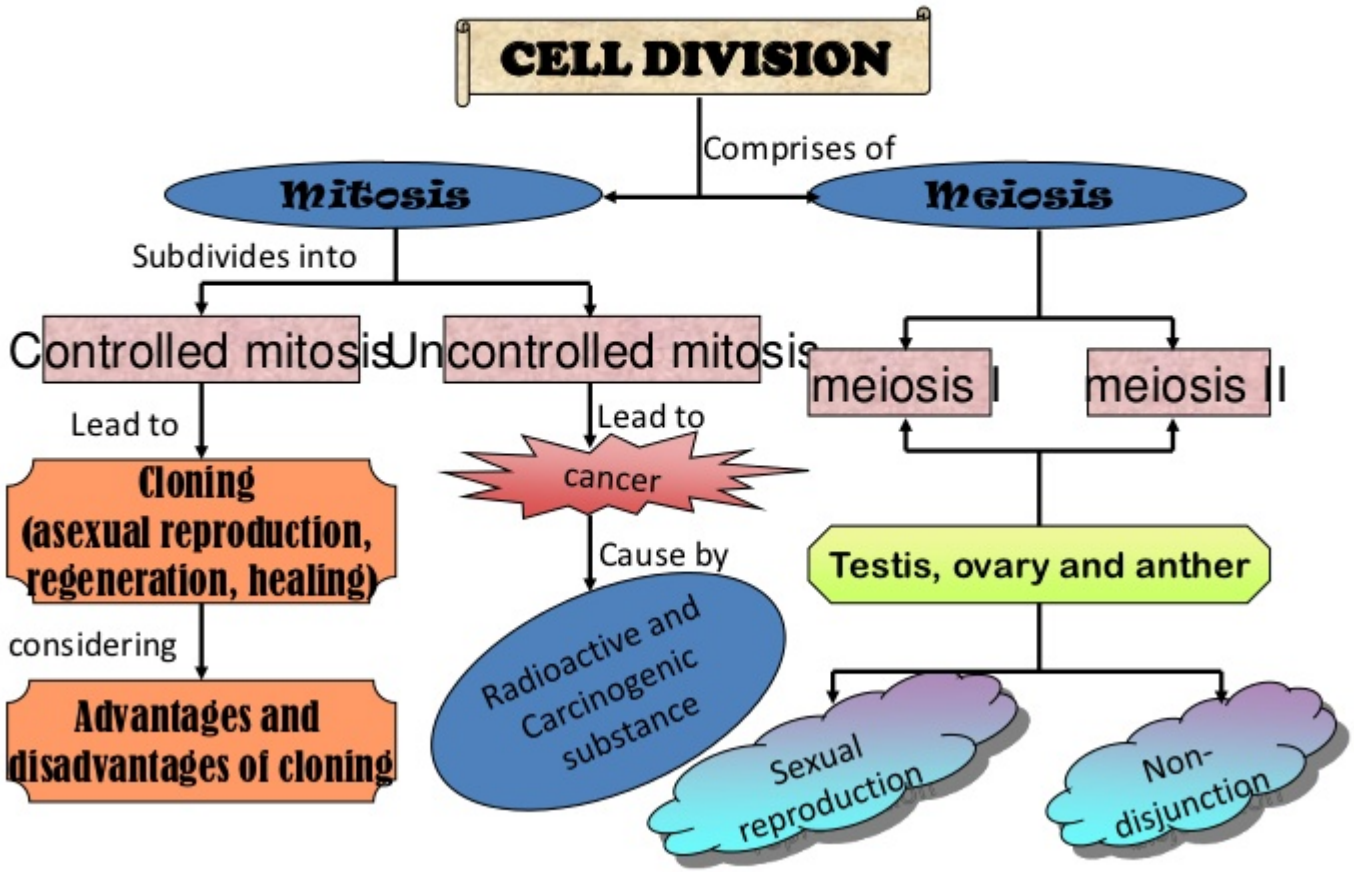
ANAPHASE


Daughter chromosomes
Shortening of kinetochore microtubules
Spindle pole getting away from then other spindle pole





Cell division: Concept map



ASSIGNATURA: BIOLOGIA (34446).
GRAU: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

ASIGNATURA: BIOLOGÍA (34446).
GRADO: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

THE HUMAN GENOME

Introduction

The **genome** is the complete set of genes that all the cells of an organism possess.

Genes are the basis of the functions that we consider specific of living beings:

- They are, in the last term, the regulators of the equilibrium state of cells and organisms (health-disease).
- They are responsible for the structure and function of cells, and for the maintenance of the vital characteristics during time.
- This *hereditary information* is transmitted from a cell to its daughter cells by cell division and from an organism to another by germ cells.

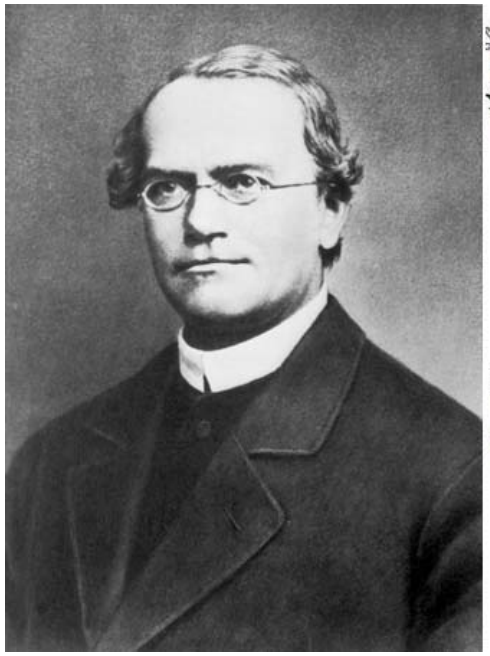
HUMAN GENOME

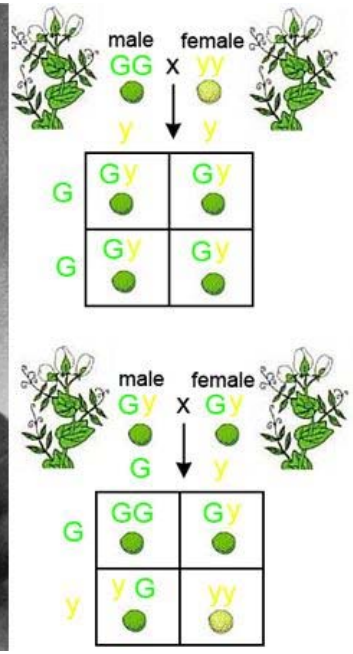
Introduction
Historical outline
Experiments
The structure of DNA
Function of genetic material
Human Genome Project
- Objectives
- Results
- Types of sequences in human genome
- Structure of a typical human gene
- Future prospects

Historical outline

1865. Mendel: discrete *factors* exist that do not mix but combine ones with the other when they are transmitted from one generation to the next.

They are statistically abstract entities. Their chemical nature is unknown.





Historical outline

1868. Miescher: Isolation of phosphorous rich material (he called it nuclein) from cells nucleus.

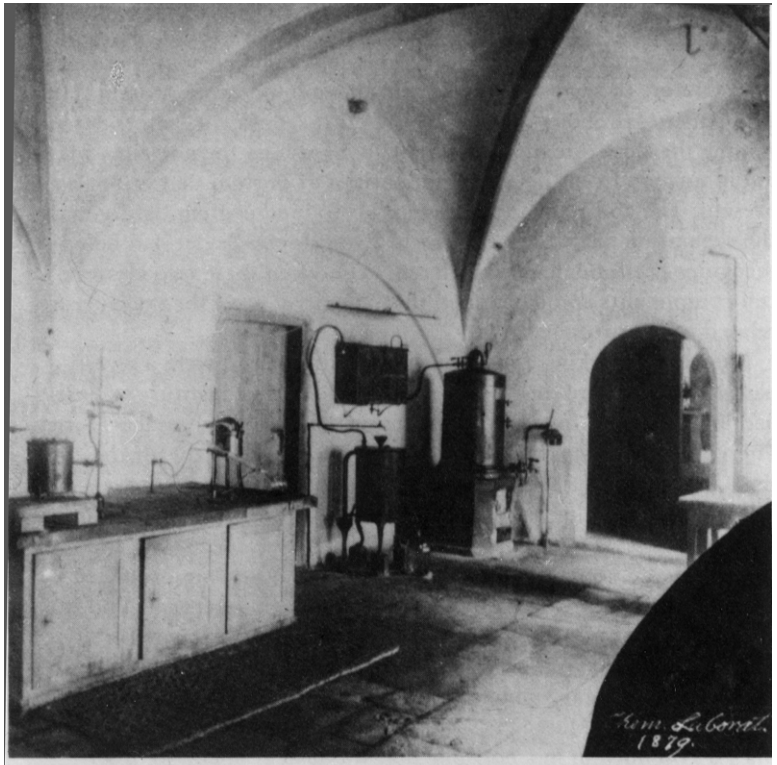


Figure 1.5 The laboratory at Tübingen where Miescher isolated nuclein (courtesy of the University of Tübingen Library, Tübingen, Federal Republic of Germany).

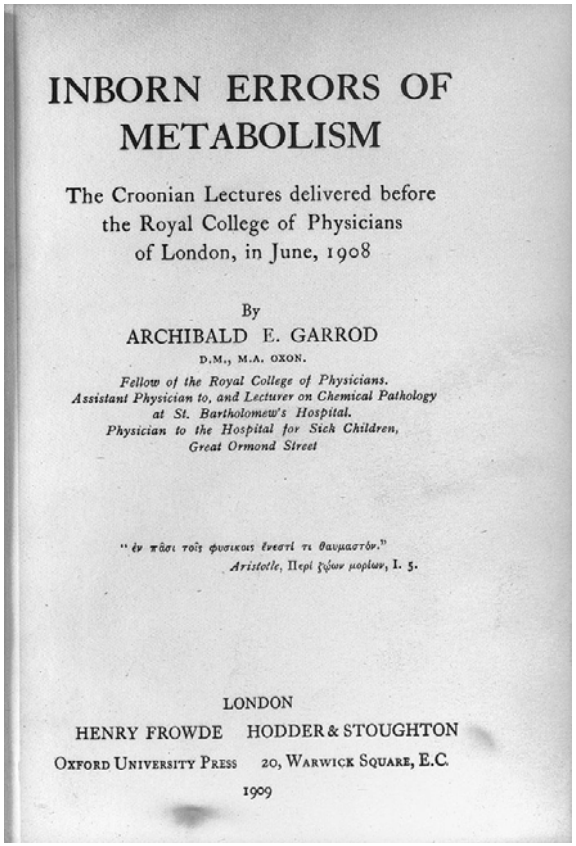
He isolated nucleic acids from neutrophils nuclei in pus using only chemical methods.

The material was resistant to proteases, acidic and rich in phosphorous.

Historical outline

1900-1910. First geneticists: genes are on chromosomes (chromosomal theory of heredity).
A determined gene occupies a concrete place (*locus*) on the chromosome.

Garrod and Bateson
- They revisited Mendel's work
- Inborn errors of metabolism
- Gene and genetics as abstract concepts



INBORN ERRORS OF METABOLISM

The Croonian Lectures delivered before the Royal College of Physicians of London, in June, 1908

By
ARCHIBALD E. GARROD
D.M., M.A. OXON.
Fellow of the Royal College of Physicians.
Assistant Physician to, and Lecturer on Chemical Pathology at St. Bartholomew's Hospital.
Physician to the Hospital for Sick Children, Great Ormond Street

"ἐν πᾶσι τοῖς φυσικοῖς ἔνεστί τι θαυμαστόν."
Aristotle, Περὶ ζῴων μορίων, I. 5.

LONDON
HENRY FROWDE HODDER & STOUGHTON
OXFORD UNIVERSITY PRESS 20, WARWICK SQUARE, E.C.
1909

Historical outline

1928. Griffith: bacterial transformation.

Strain S: Smooth pathogenic bacteria produce pneumonia.

Strain R: Rough mutants are not pathogenic.

Heat-killed smooth bacteria are not pathogenic .

Heat-killed smooth bacteria can ***transform*** strain R bacteria and make them pathogenic.

Transforming element

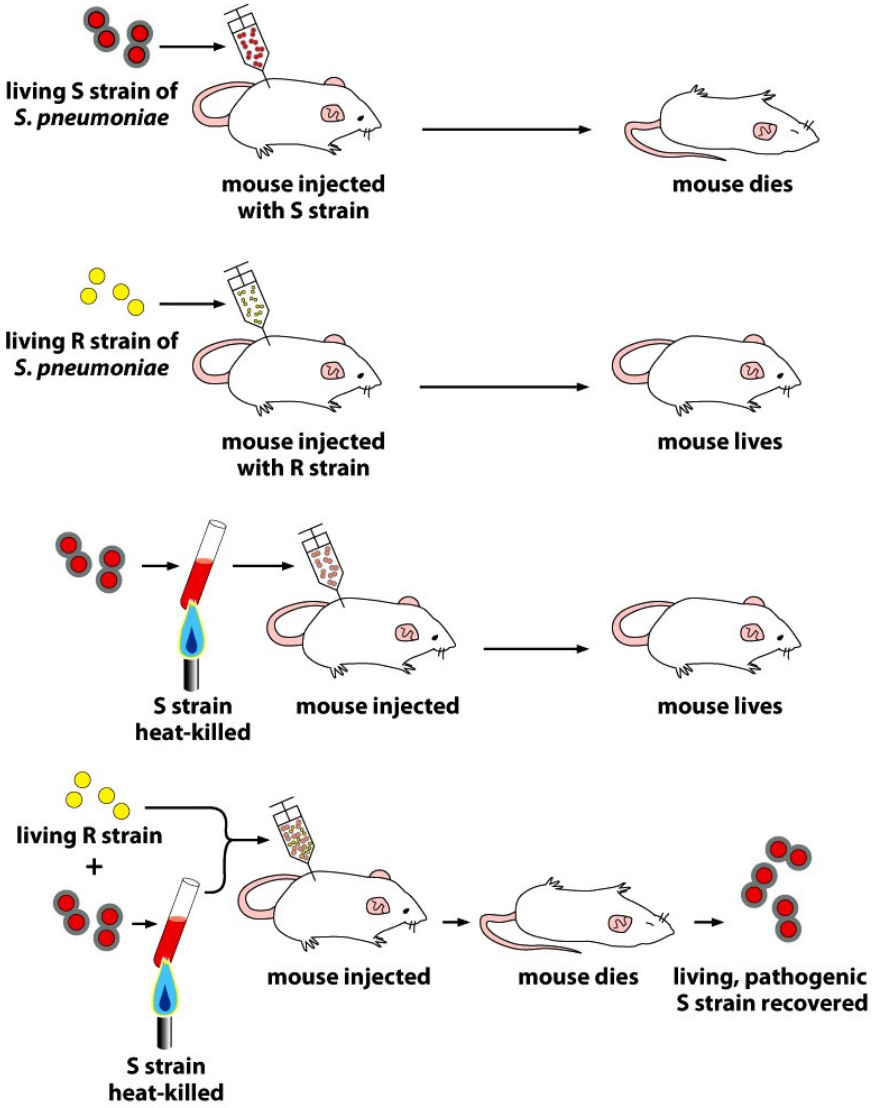


Figure 5-3 Essential Cell Biology (© Garland Science 2010)

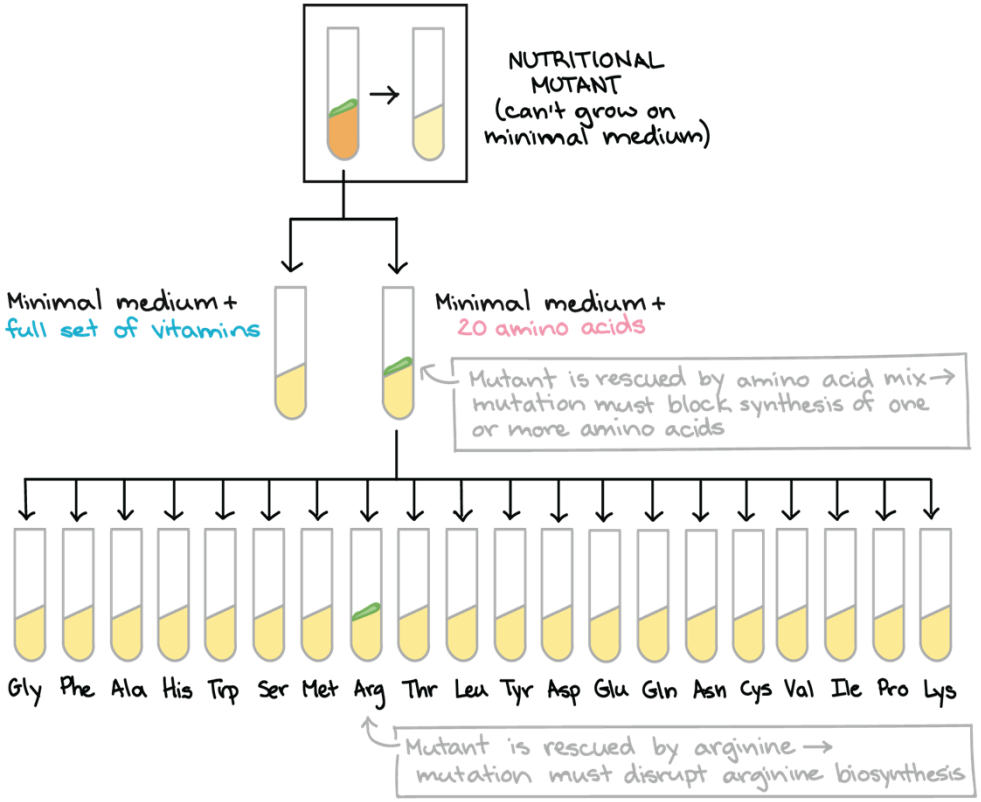
Historical outline

1941. Beadle and Tatum: one-gene-one-enzyme hypothesis.

Genetic information carries instructions to produce proteins.

In a wider sense to produce proteins that are the molecules responsible for most cellular functions:

- enzymes for catalysis of chemical reactions
- building blocks for cell structures
- regulators of gene expression
- signaling molecules
- controllers of cell movements
- …

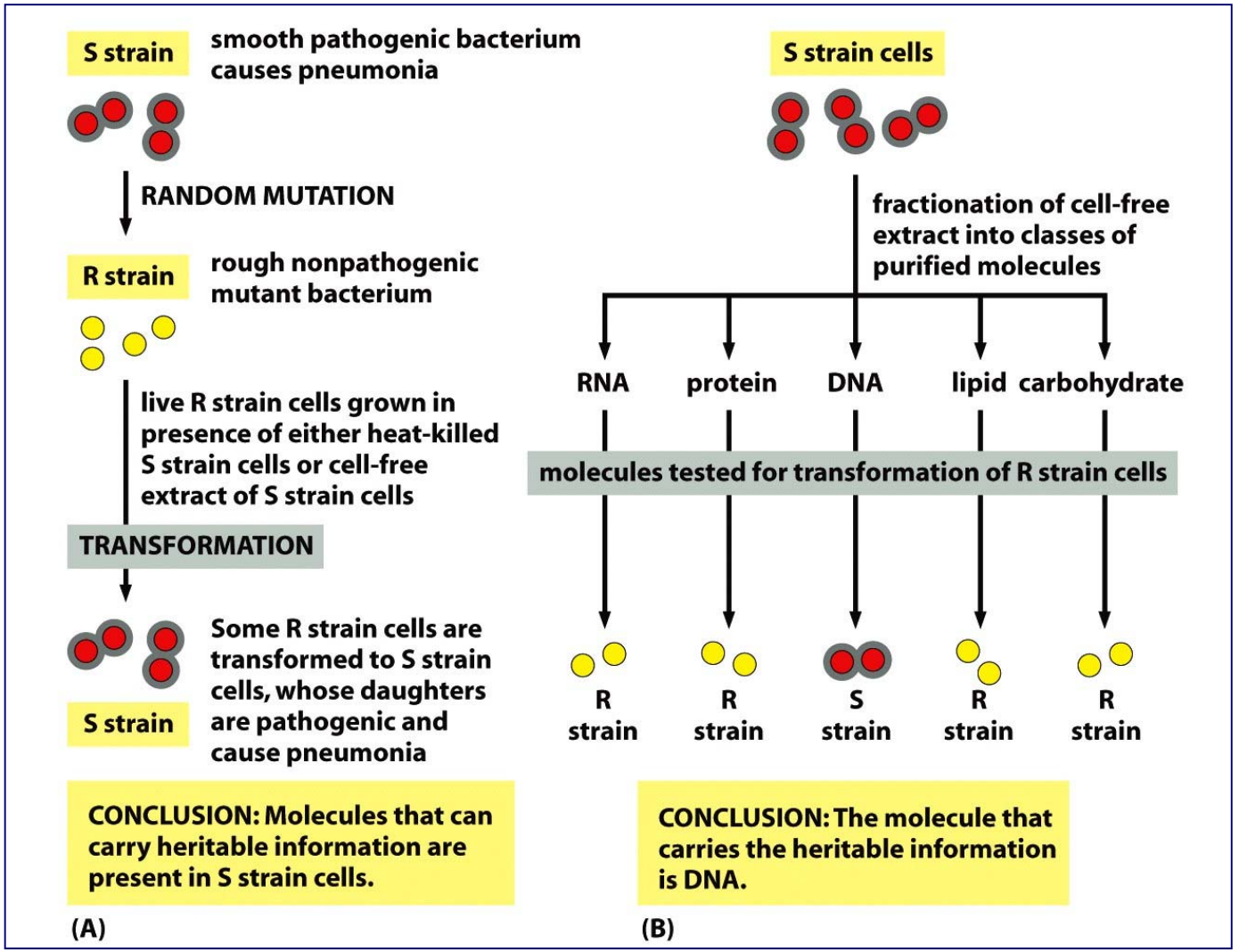


Historical outline

1944. Avery, MacLeod and McCarty: DNA carries the genetic information.

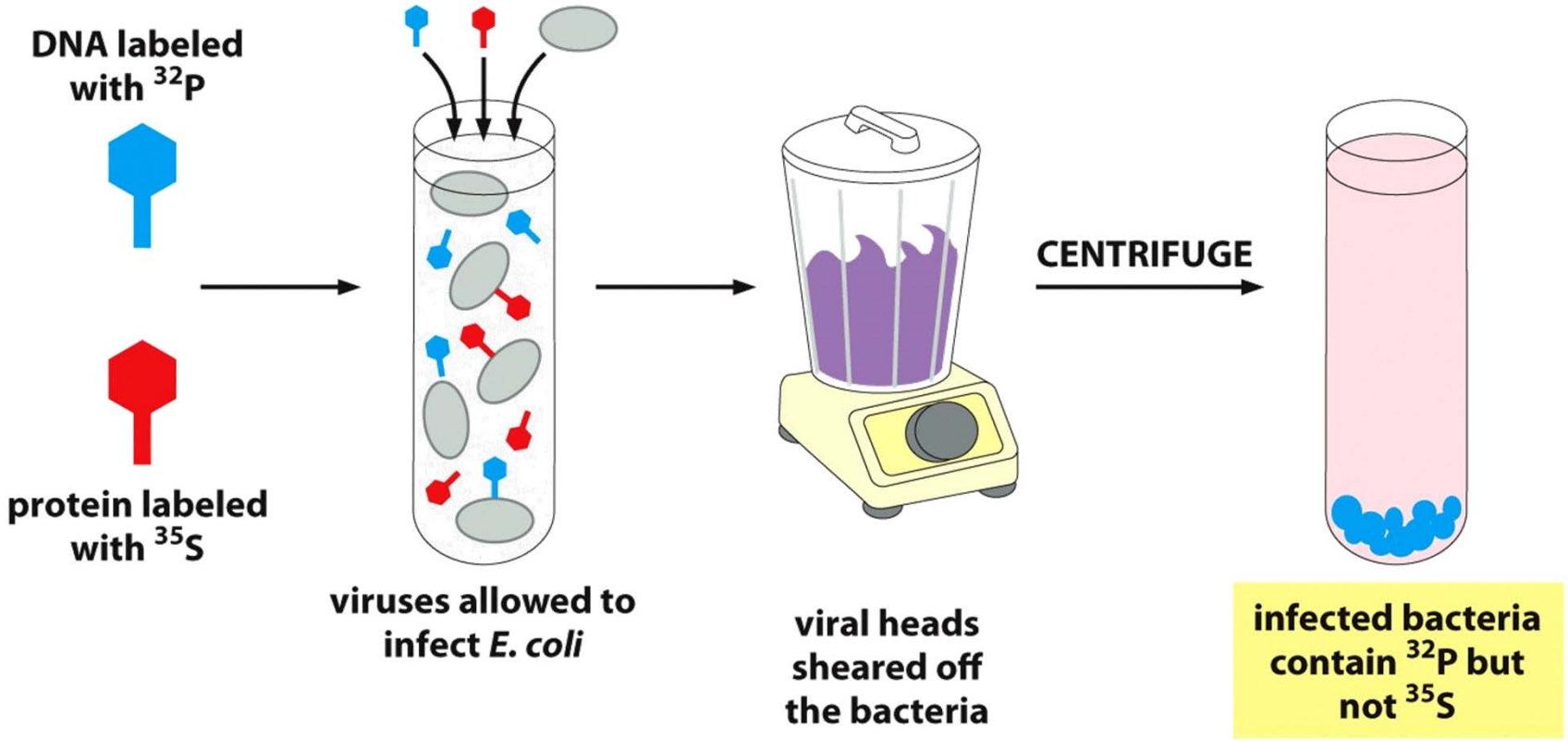
Griffith experiments extension

DNA as transforming principle



3. Experiencias

1952. Hersey and Chase. DNA and no protein is the genetic material.

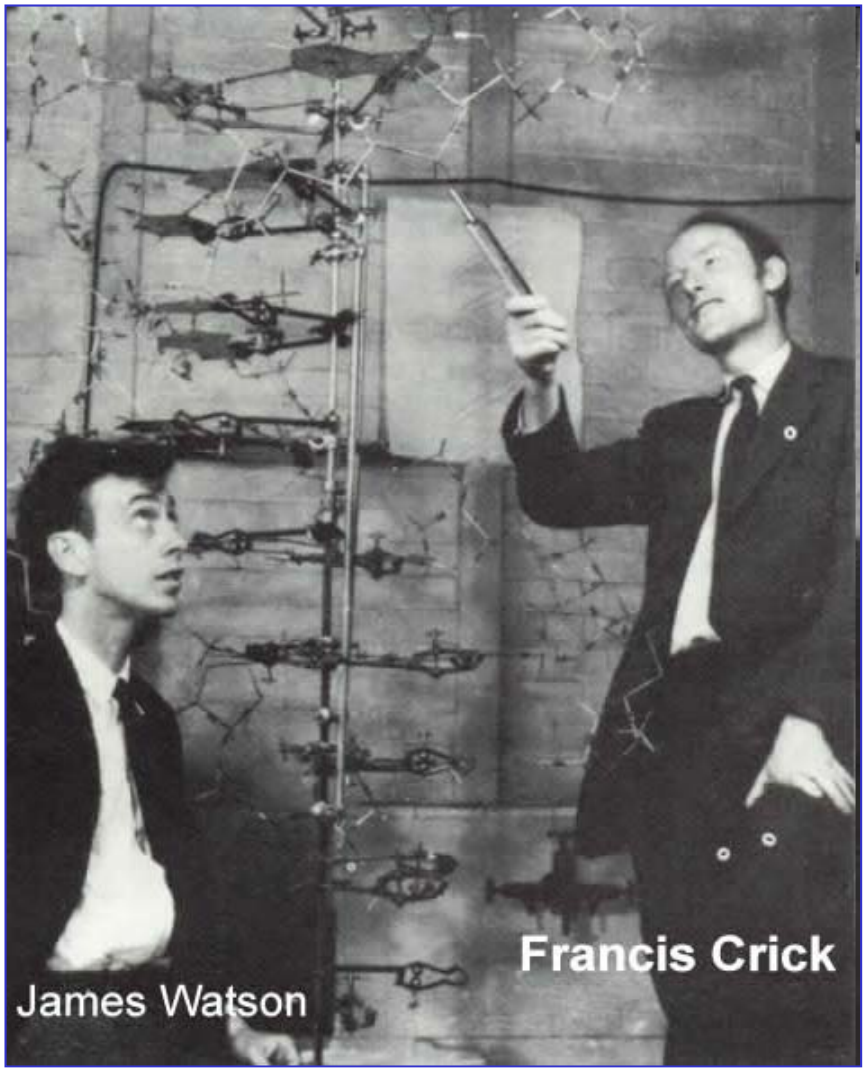


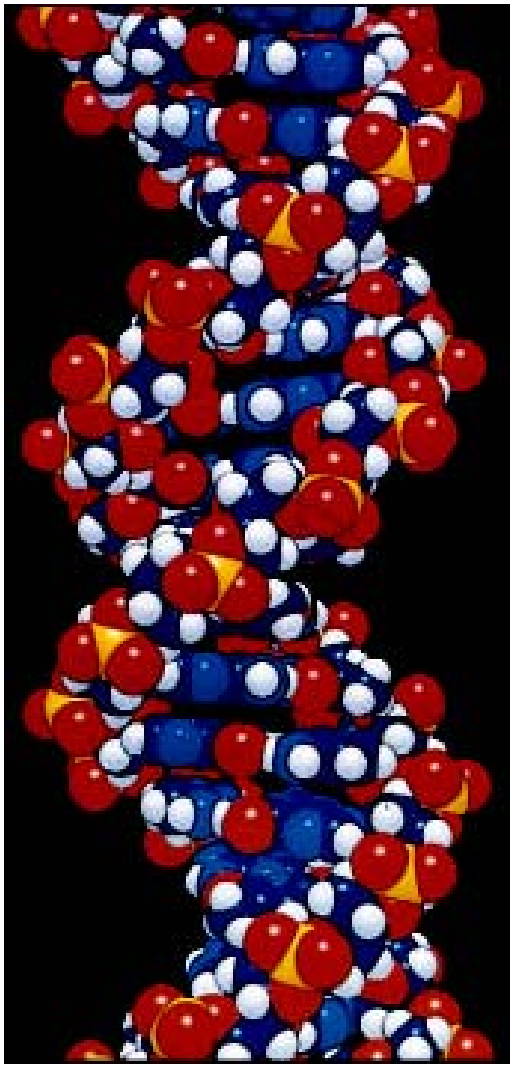
Bacteriophages

Figure 5-5b *Essential Cell Biology* (© Garland Science 2010)

Historical outline

1953. Watson and Crick: Structure of DNA. Initiation of molecular genetics.





equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

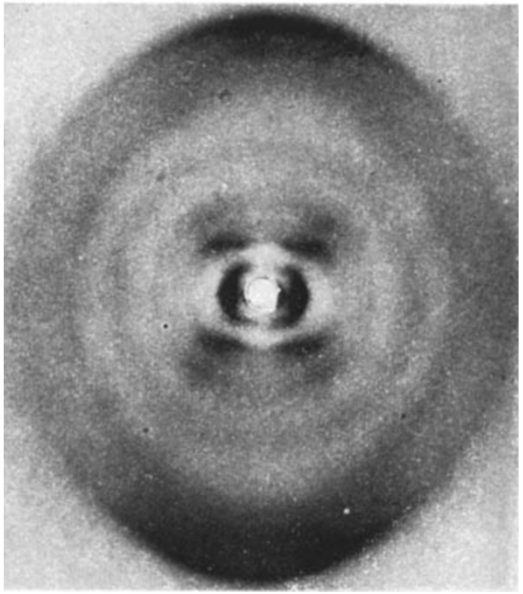
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

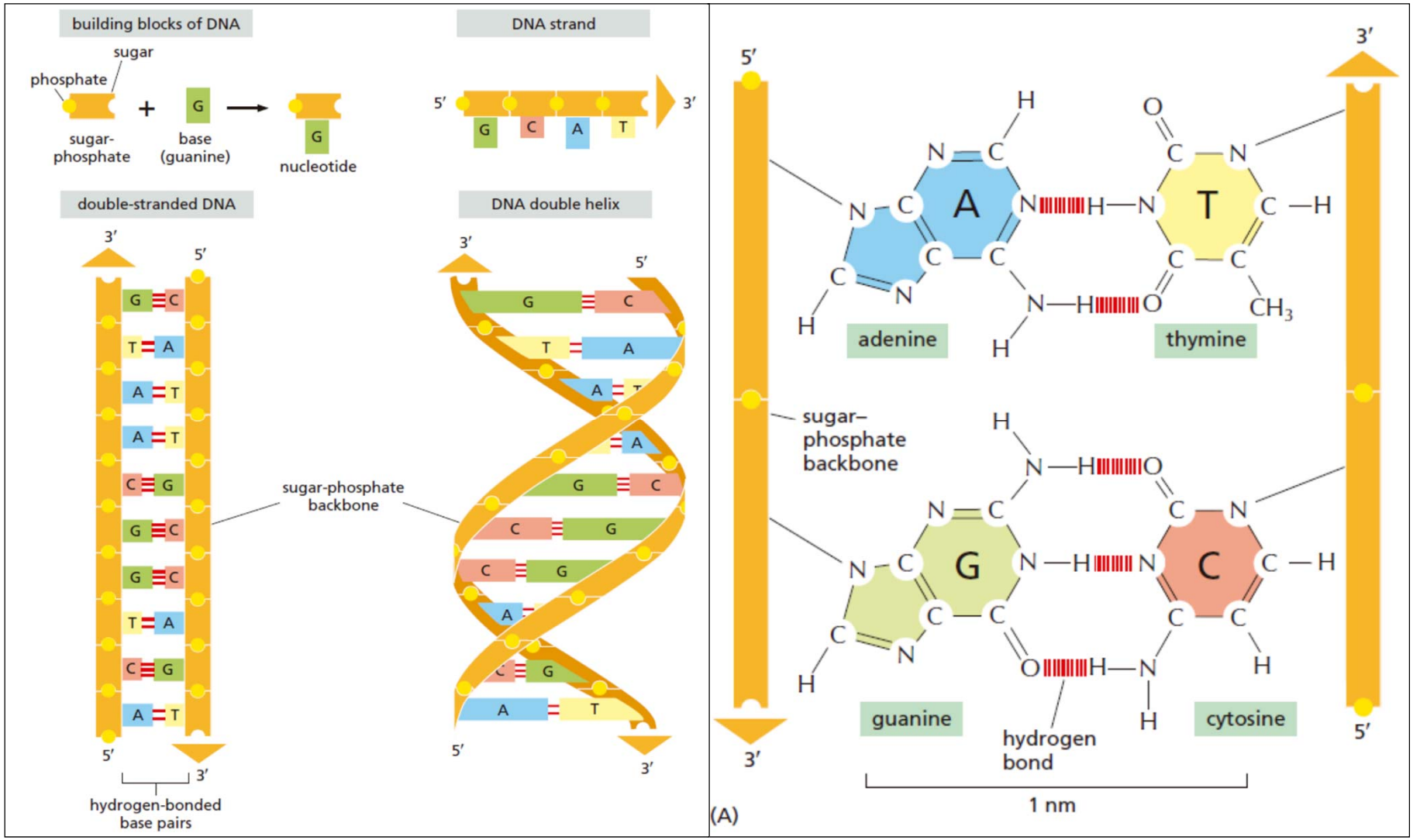
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK
Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

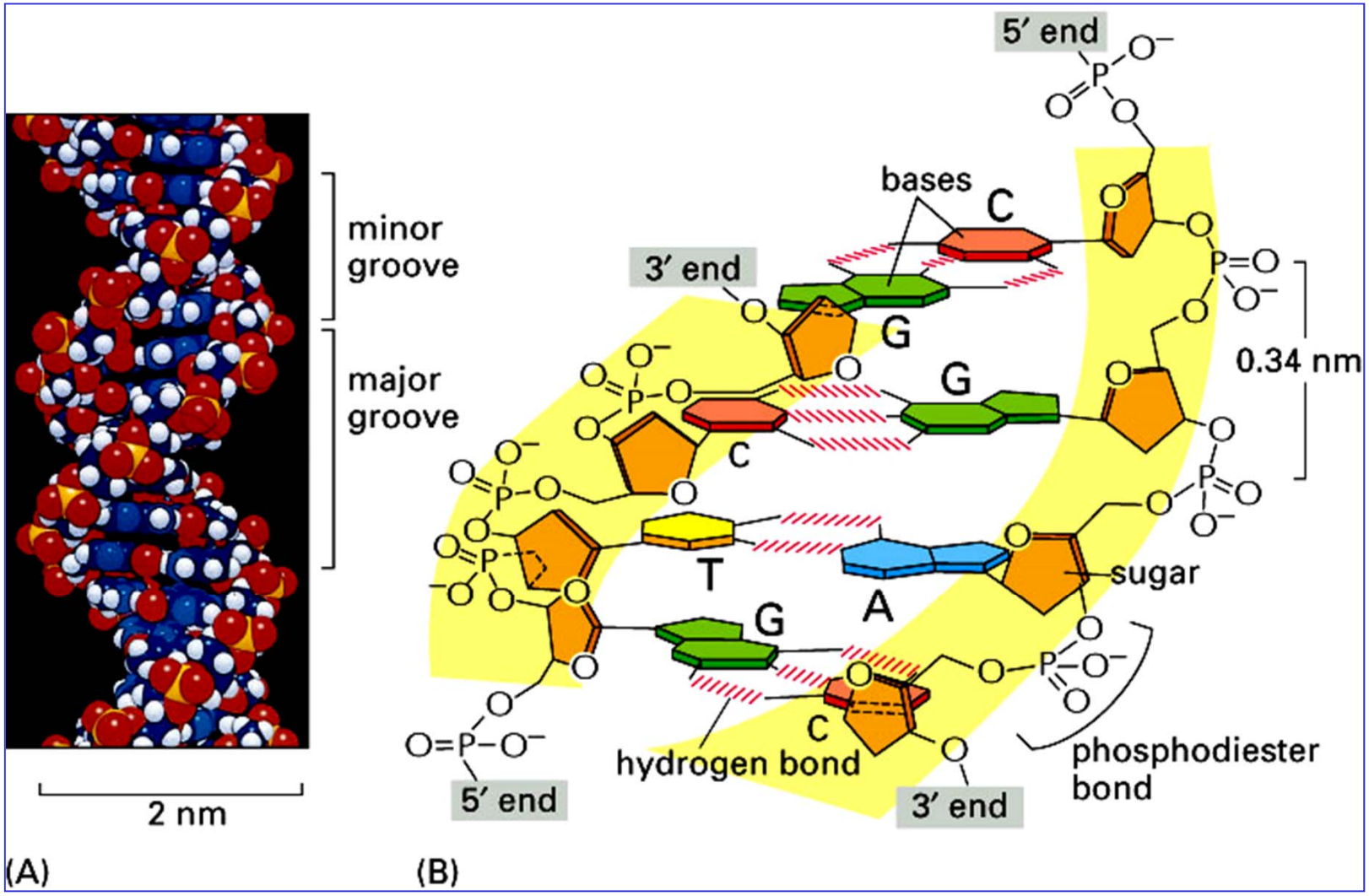
[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
[5] Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).



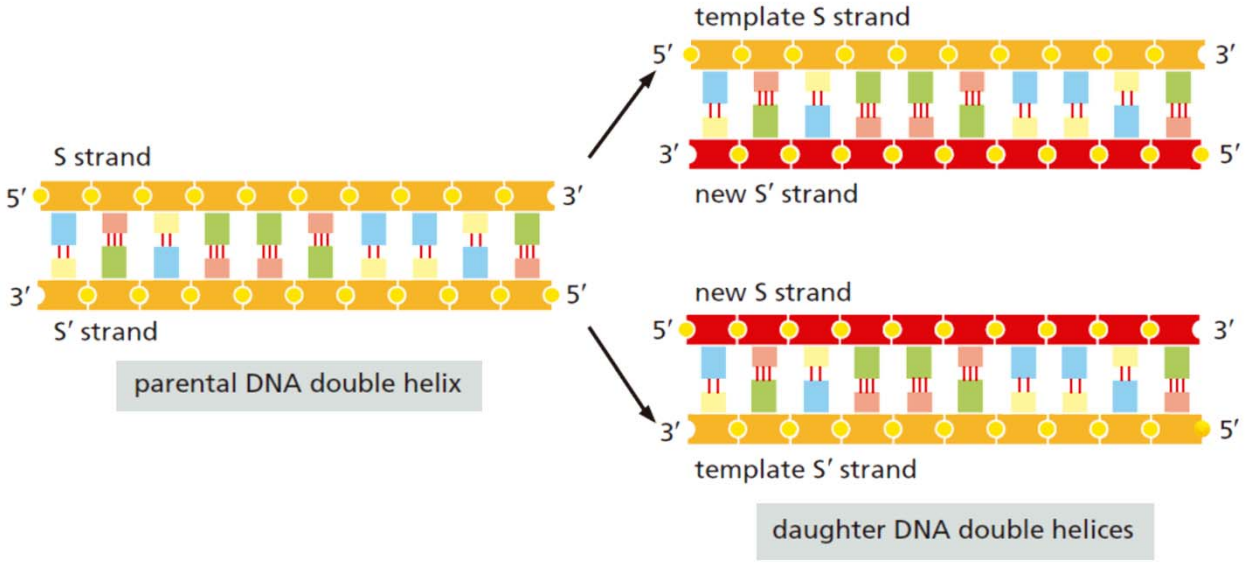
The structure of DNA



The structure of DNA

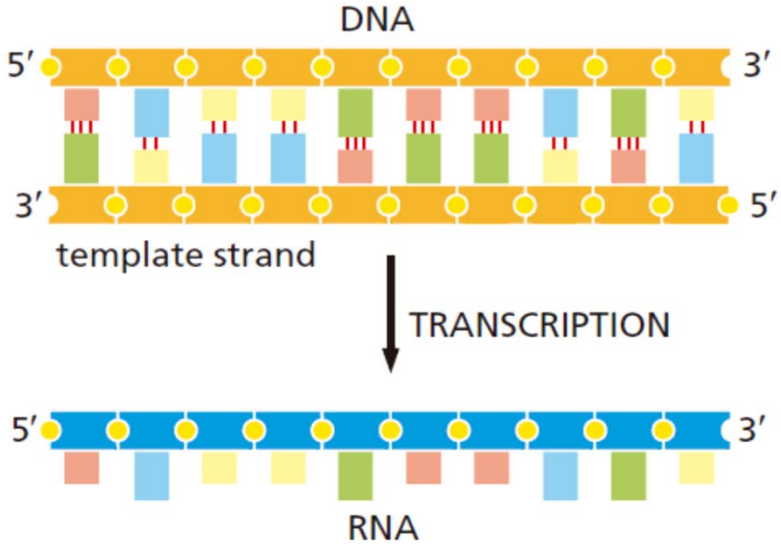


The structure of DNA

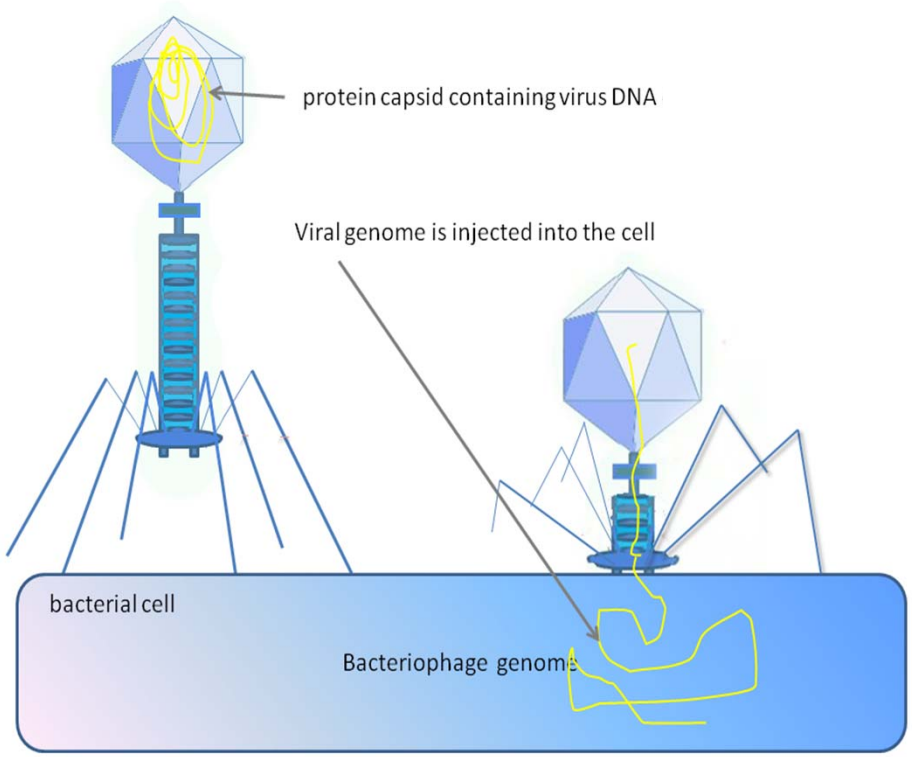


DNA structure explains inheritance

DNA is transcribed into RNA



The structure of DNA



- Some phages
- nº T ≠ nº A and nº G ≠ nº C
- It inserts in infected DNA as double chain
- Acts as a template for the other chain

Single chain DNA

The structure of DNA

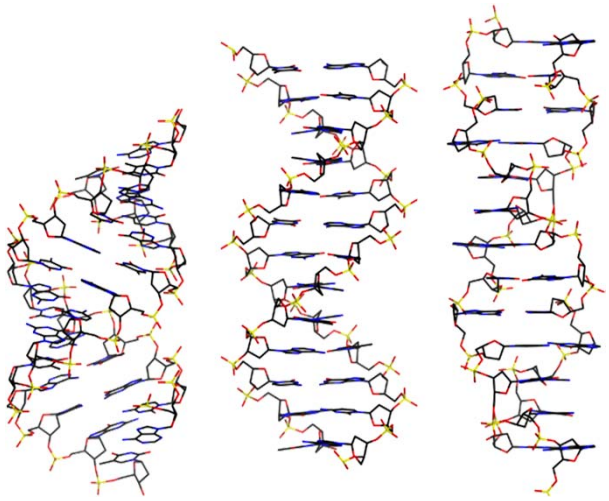
Dextro

- **Form B**: 0.34nm (solution)
- **Form A**: 0.27nm
  - o DNA-DNA, RNA-RNA or DNA-RNA
  - o Polypurines heteromalous regions

Levo

- **Form Z:** 0.38nm
  - o Pirimidine ANTI – purine SIN
  - o High cationic concentration
  - o Unfrequent

Double chain DNA conformations

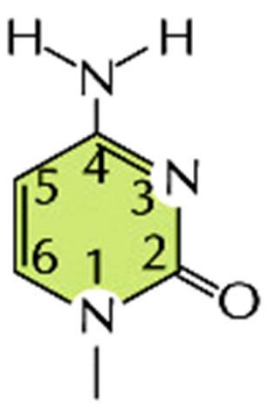


The structure of DNA

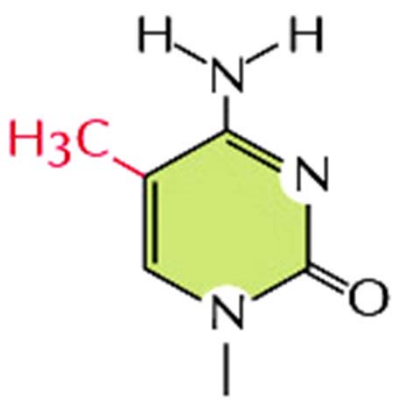
- DNA metilases add CH3 to C5 (m5C) in regions called CpG islands
- B-DNA -> A-DNA
- Regulates the expression of the genes
- *In prokaryotes it happens in A*

DNA methylation

cytosine

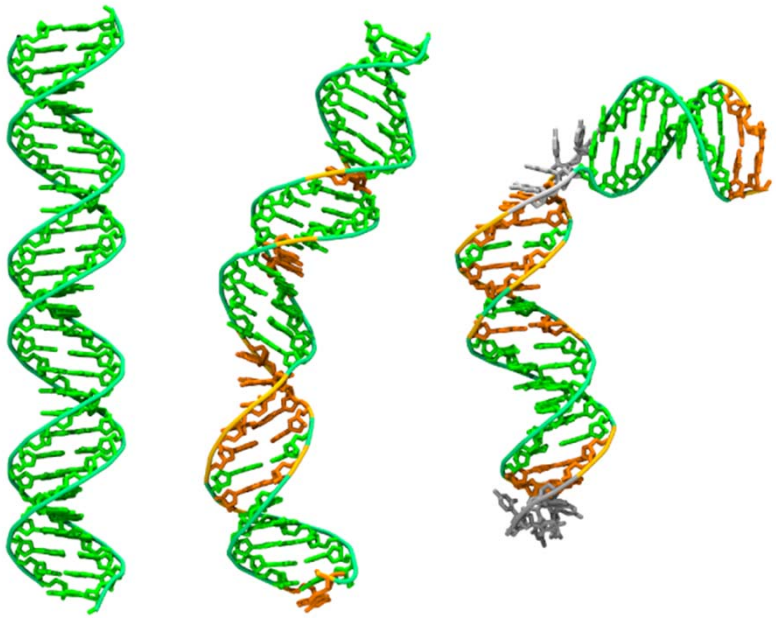


5-methylcytosine



The structure of DNA

DNA deformation



In solution

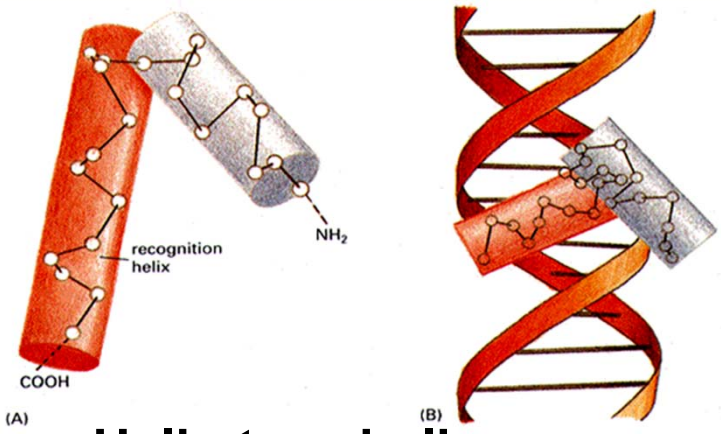
- DNA: plastic in solution
- "bending“ y "kinking” without loss of Hbonds

Because specific sequences

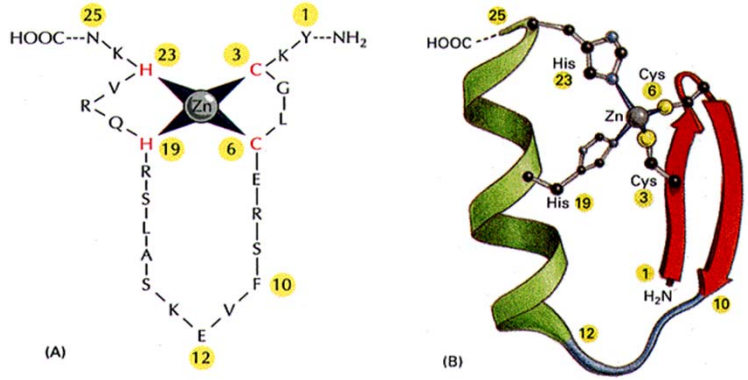
- Ex. CAAAAAT (or CAAAAAAT) repeated 4x and separated by 10nt it provides fast motion in eletrophoresis (different shape)

The structure of DNA

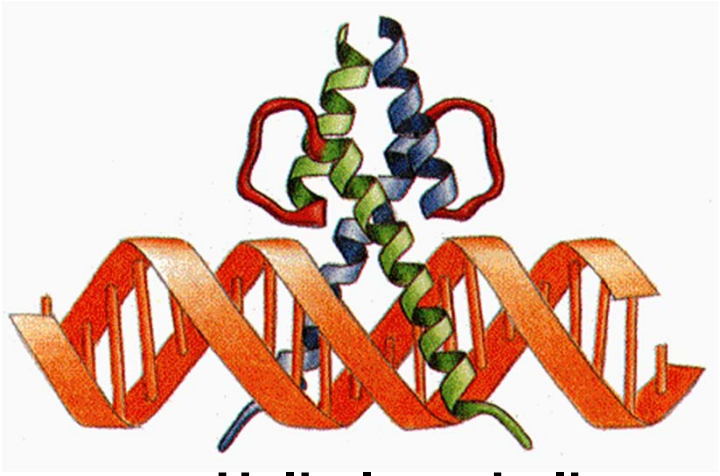
DNA structure controls protein binding



Helix-turn-helix



Zn fingers

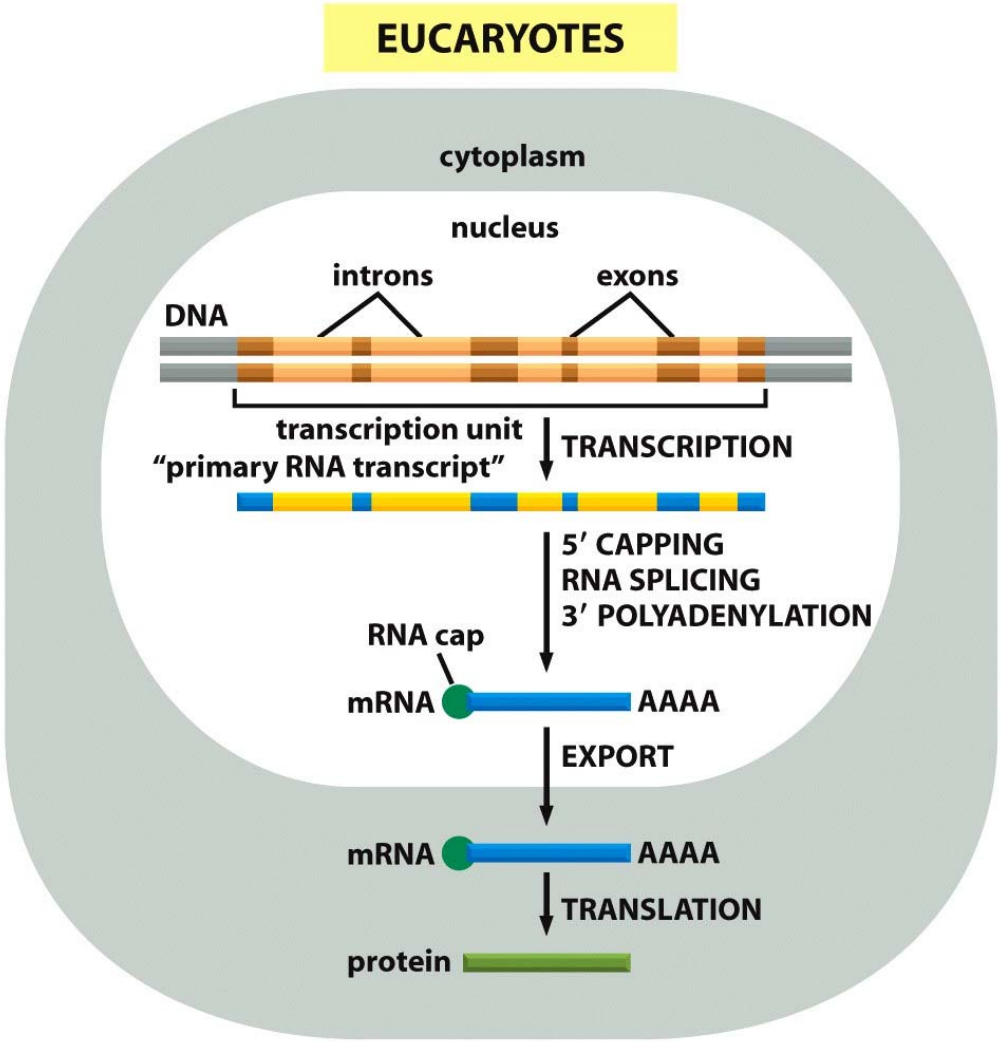


Helix-loop-helix

Leucine zipper

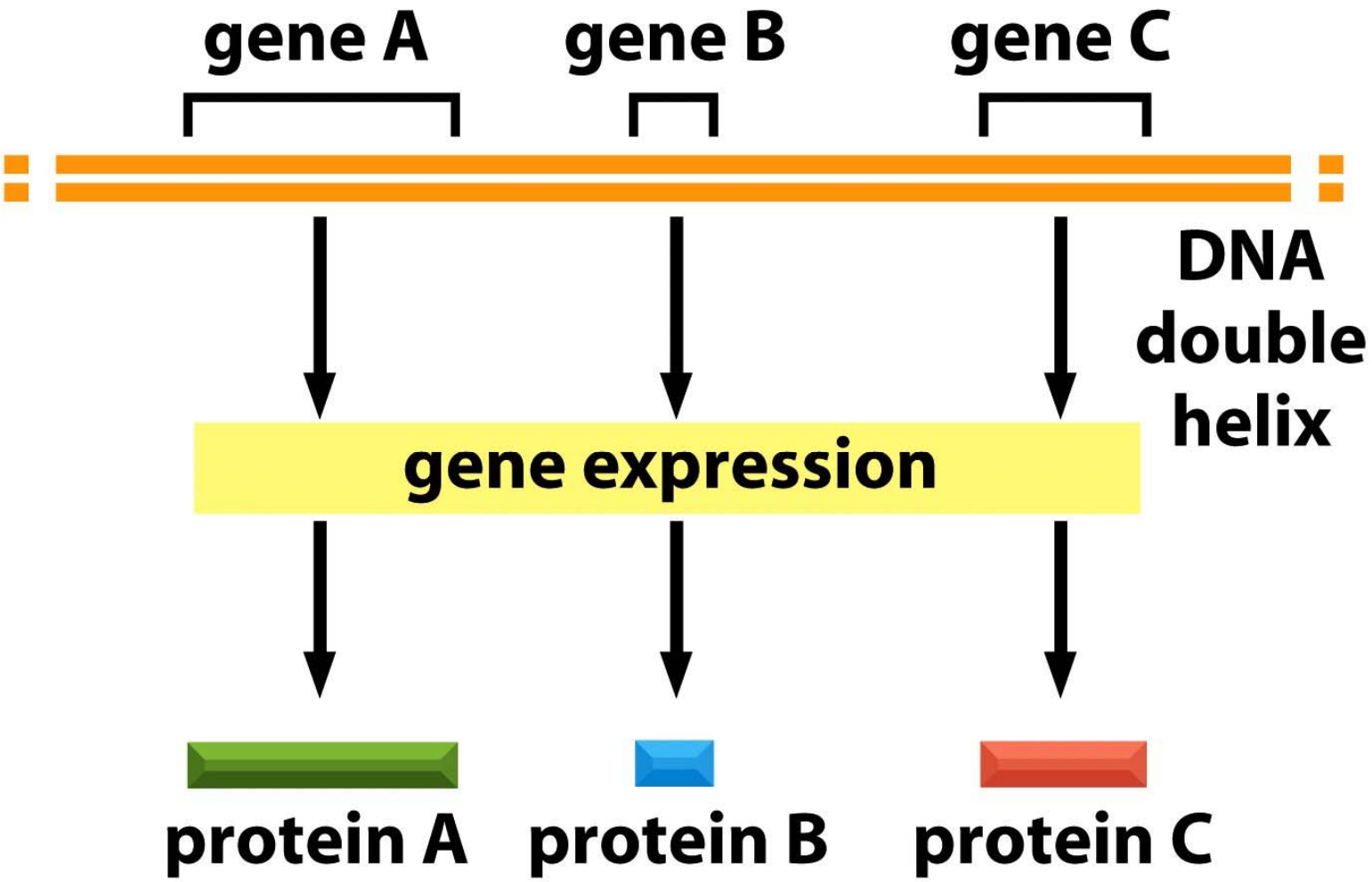
Function of genetic material

Cell distribution



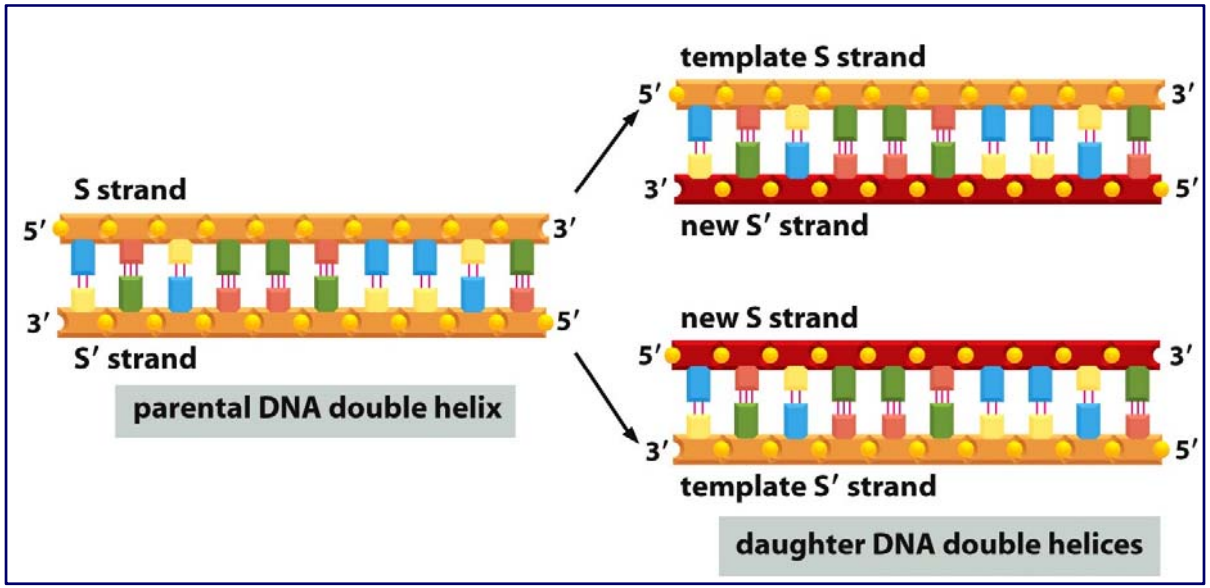
Function of genetic material

Genes in DNA code proteins

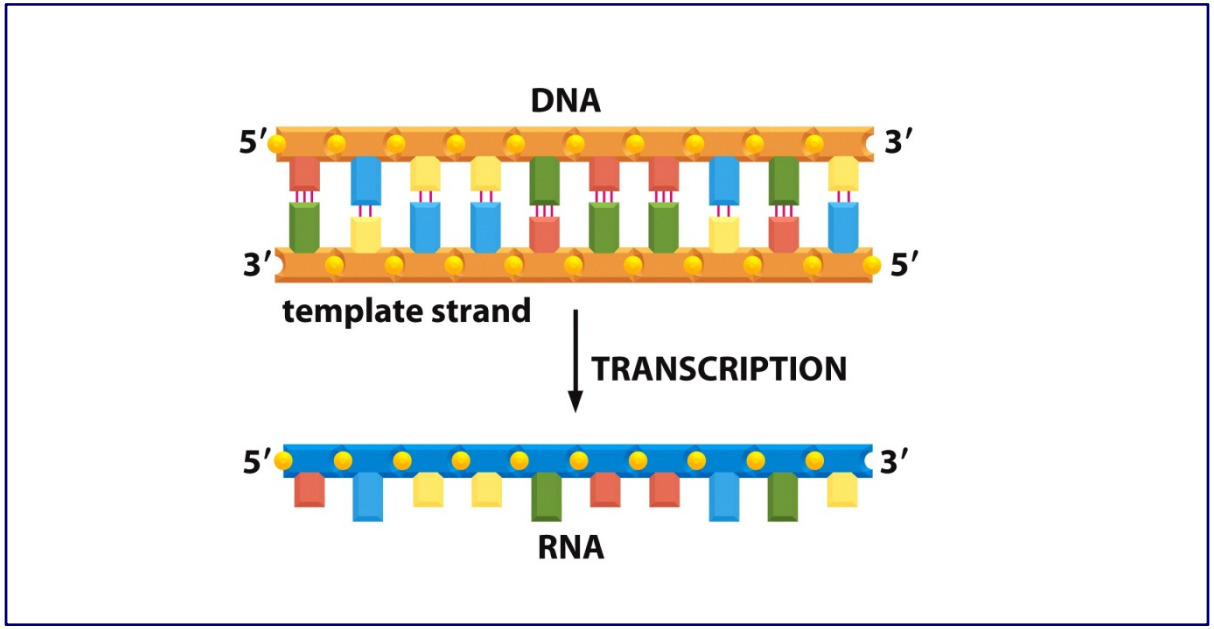


Function of genetic material

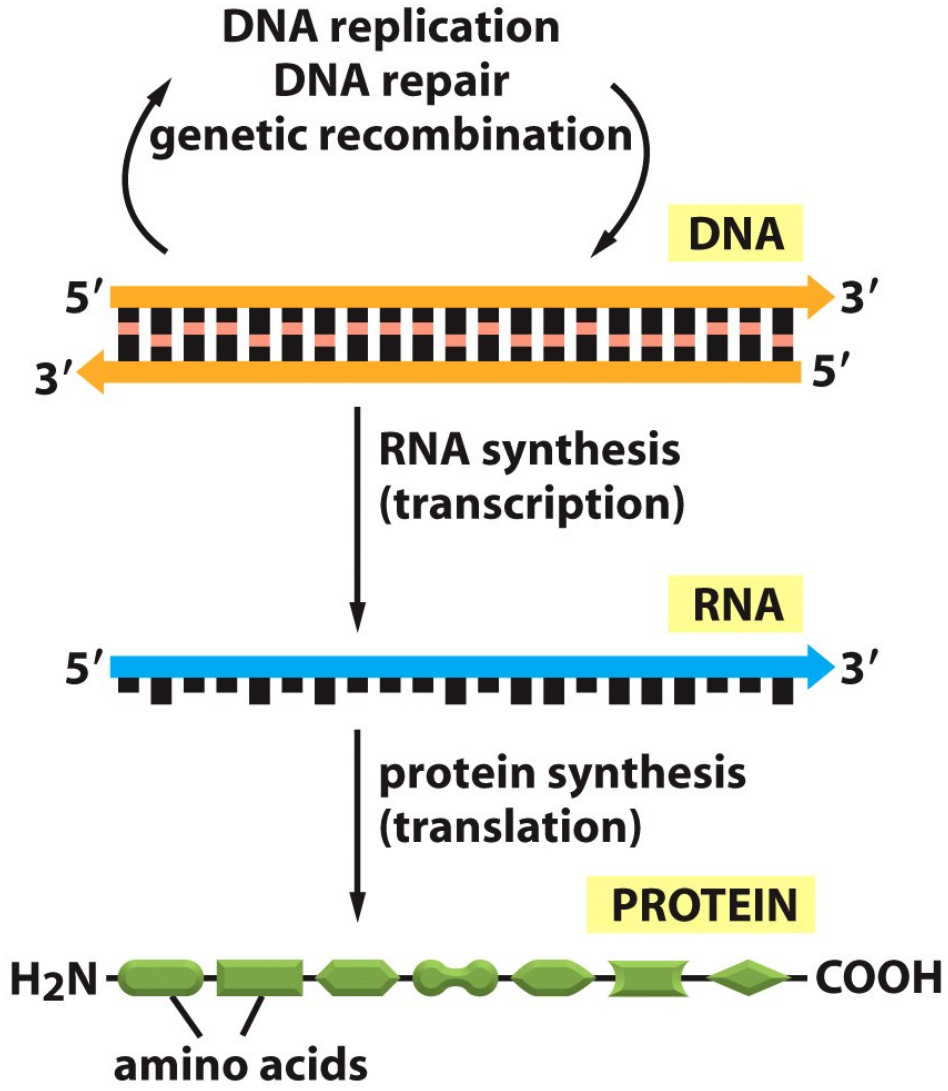
Autosynthetic



Heterosynthetic



Function of genetic material

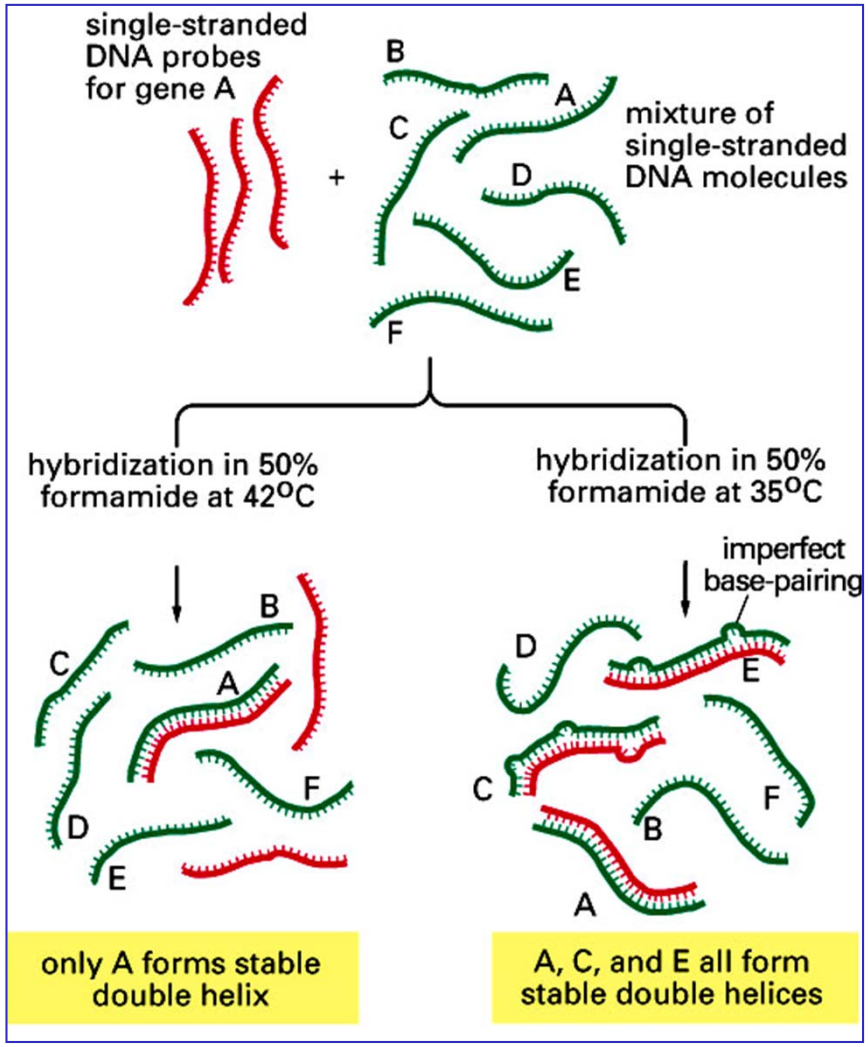


GENOME

TRANSCRIPTOME

PROTEOME

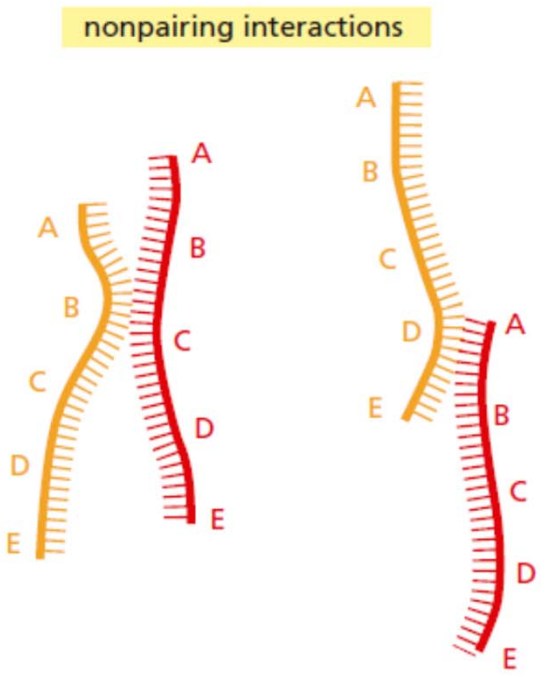
Function of genetic material

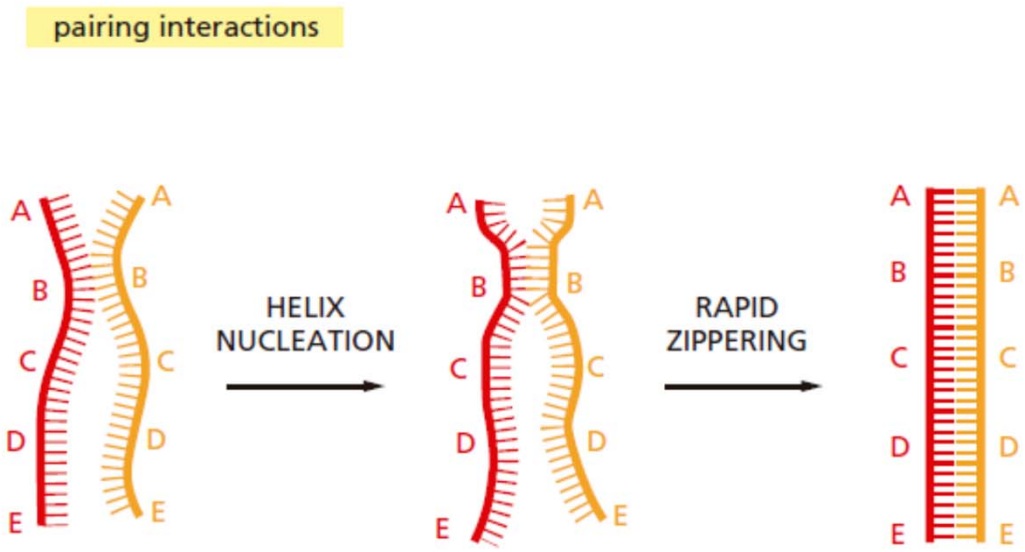


An important property of DNA:

Denaturation } Hybridization
Renaturation

Function of genetic material





The basis for many molecular techniques;

Base complementarity

Desnaturalization - renaturalization

TA and CG different strength

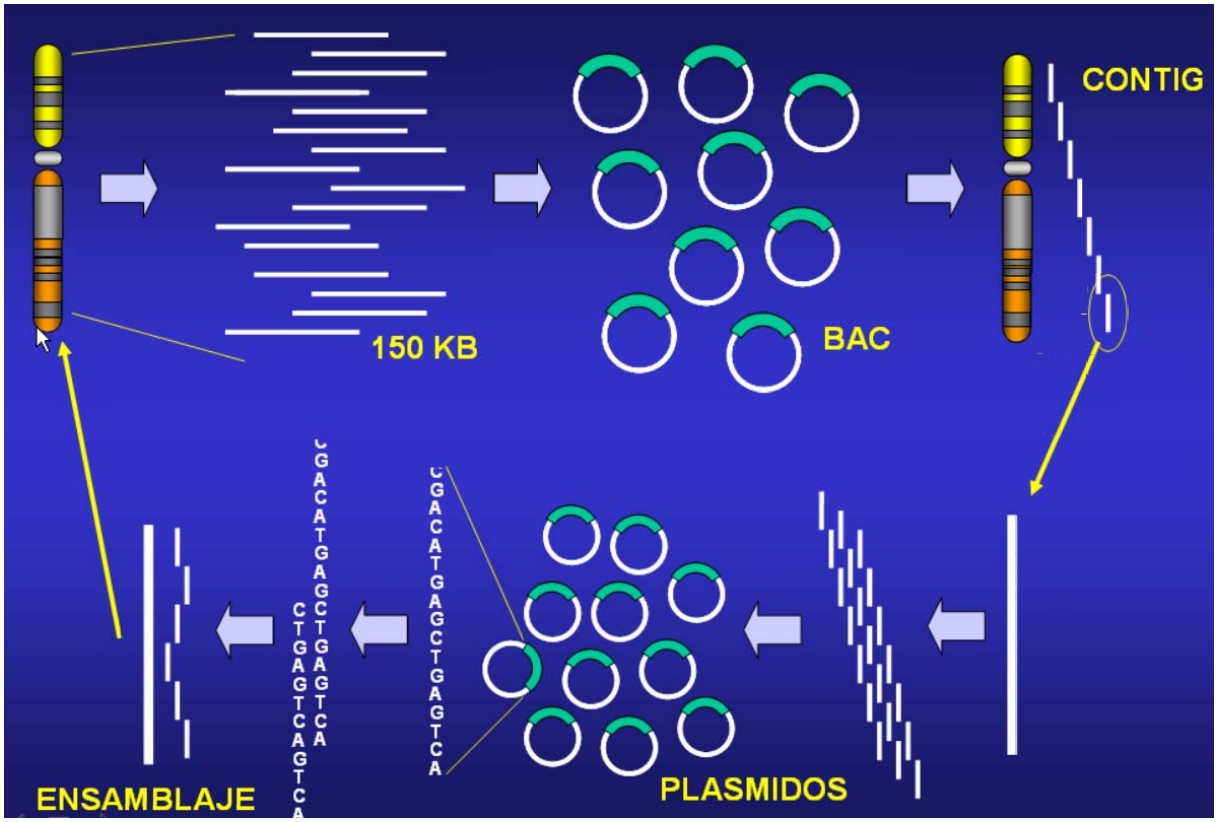
Historical outline

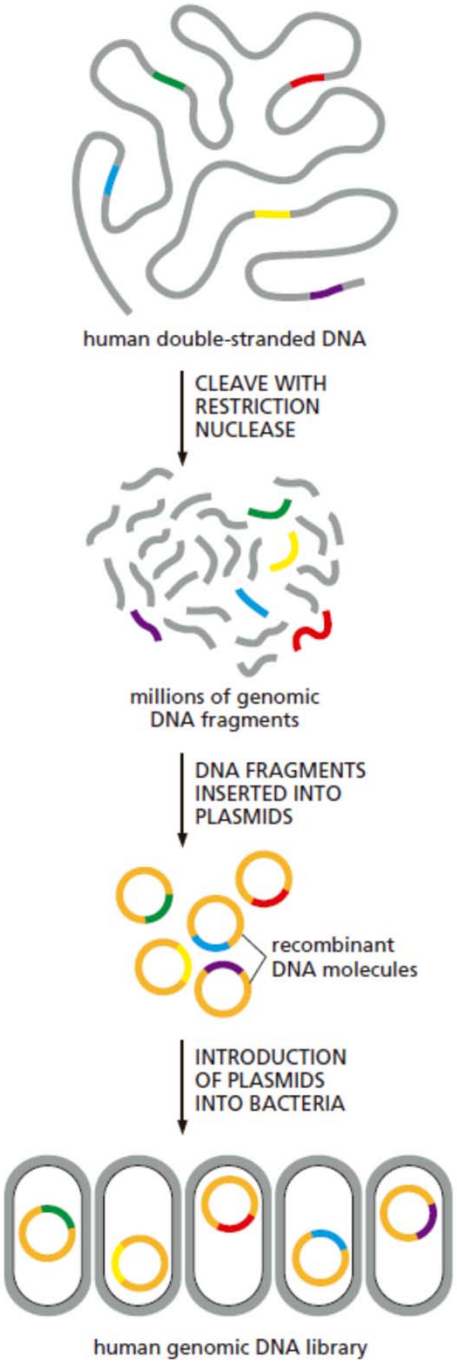
1966, Nirenberg, Ochoa and Khorana elucidate the genetic code.

CODIGO GENETICO					
5'end	2nd position				3'end
	T	C	A	G	
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Lys	Arg	A
	Leu	Pro	Lys	Arg	G
G	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
A	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Historical outline

1972, Bacterial gene cloning- Genomic libraries.

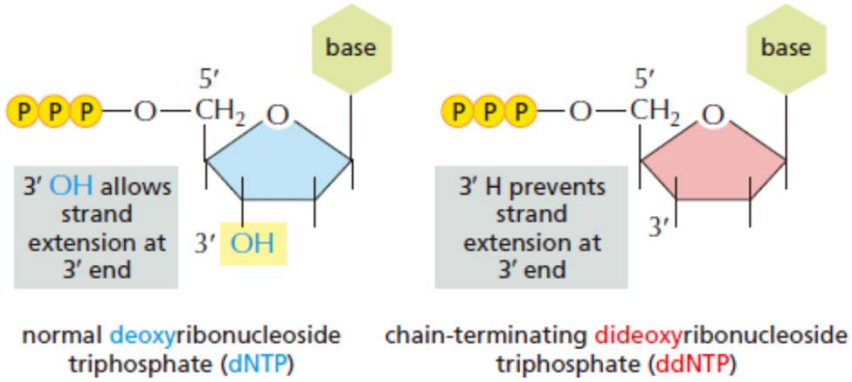


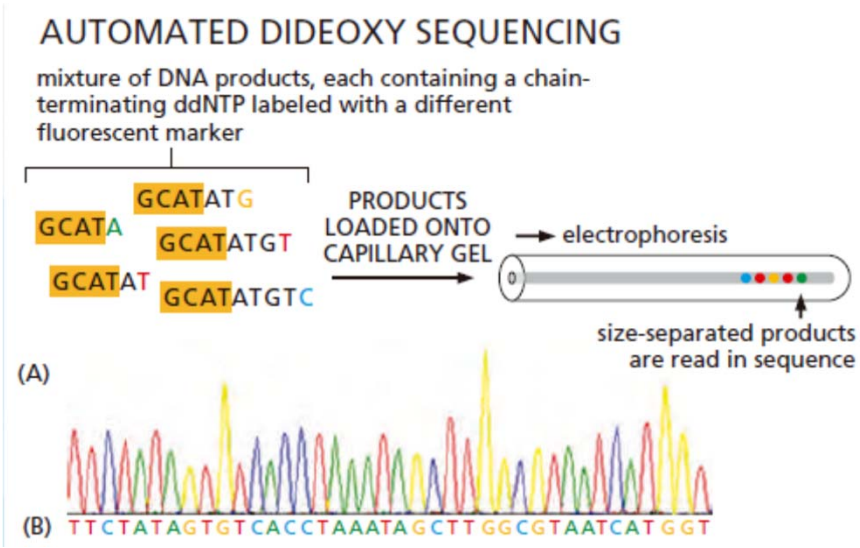


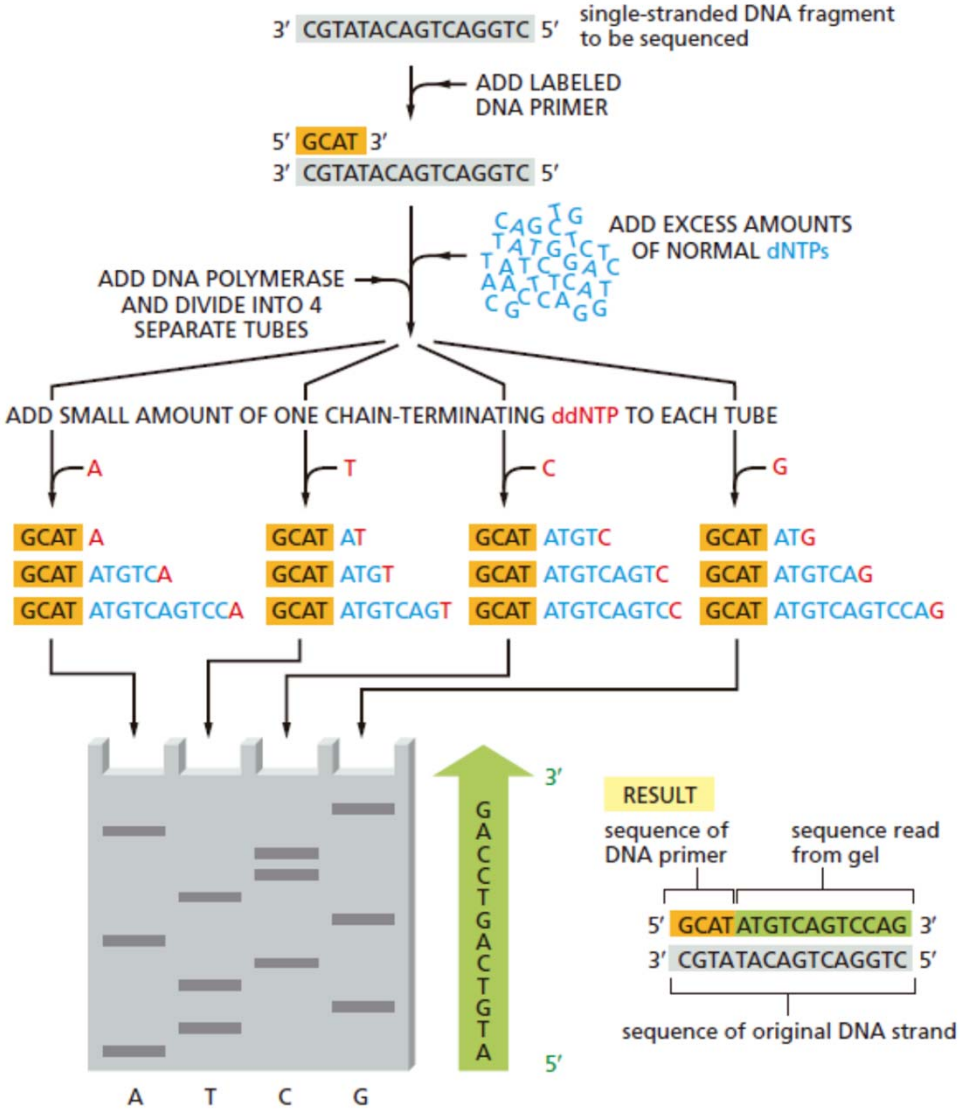
Panel 8-1 *Molecular Biology of the Cell. Sixth edition,* © 2015

Historical outline

1977, Sanger develops an efficient DNA sequencing method.







Panel 8-1 *Molecular Biology of the Cell. Sixth edition,* © 2015

Historical outline

1983. Mullis. PCR.

2000. Celera and Public Consortium: publish the first draft of human genome.

2003. Celera and Public Consortium: finish the complete sequencing of human genome.

Human Genome Project

Objectives:

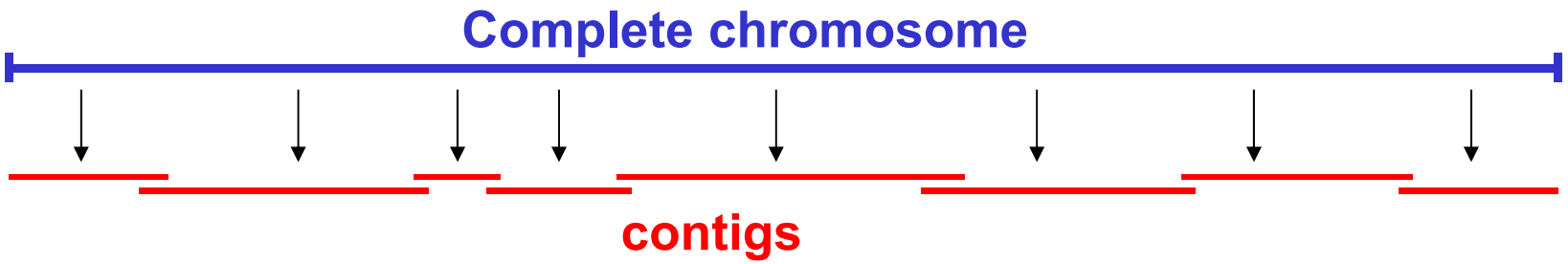
- Determine the complete nucleotide sequence
- Store all the information in databases
- Improve the methods of analysis
- Identify the genes that exist in the human genome

Important dates:

- 1990: The project begins leaded by the Department of Energy and the National institute of Health (USA)
- June 2000: The Human Genome draft is completed
- February 2001: Analysis of the draft is made public
- April 2003: Sequencing is completed and the Project is declared finished

Human Genome Project

Sequencing method



Sequencing of each contig

Computer search of overlapping sequences

Ordering and alignment of all the contigs

Human Genome Project

Table 4–1 Some Vital Statistics for the Human Genome

	HUMAN GENOME
DNA length	3.2×10^9 nucleotide pairs*
Number of genes	approximately 25,000
Largest gene	2.4×10^6 nucleotide pairs
Mean gene size	27,000 nucleotide pairs
Smallest number of exons per gene	1
Largest number of exons per gene	178
Mean number of exons per gene	10.4
Largest exon size	17,106 nucleotide pairs
Mean exon size	145 nucleotide pairs
Number of pseudogenes**	more than 20,000
Percentage of DNA sequence in exons (protein coding sequences)	1.5%
Percentage of DNA in other highly conserved sequences***	3.5%
Percentage of DNA in high-copy repetitive elements	approximately 50%

• There are genes with a very similar sequence within the same specie (*paralogues*), or between different species (*ortologues*).

Human Genome Project

Results:

Gene disposition

• Human genome has gene-rich zones, where G and C nucleotides predominate.

• There are zones with few genes ("deserts"), where A and T nucleotides predominate.

• Genes concentrate randomly in genome regions, with large spaces of non-coding DNA between them.

• There are regions with thousands (30K) of C and G repetitions (CG islands) near genes.

• Chromosome 1 has the most genes (3141) and chromosome Y the least (231).

Human Genome Project

Results:

Gene disposition

•Very small part of the genome encodes proteins., (*Most are short, mobile sequences, inserted into chromosomes during evolution as transposable elements)*

•The genes are very large. The difference in gene-protein size shows that there are long extensions of non-coding DNA (introns) that interrupts the coding sequences (exons) *(Difference with organisms with more compact genomes)*

•Introns and exons join long regulatory sequences.

•The comparison of genomes reveals DNA sequences PRESERVED (5% of the genome, 1/3 coding of proteins)

Human Genome Project

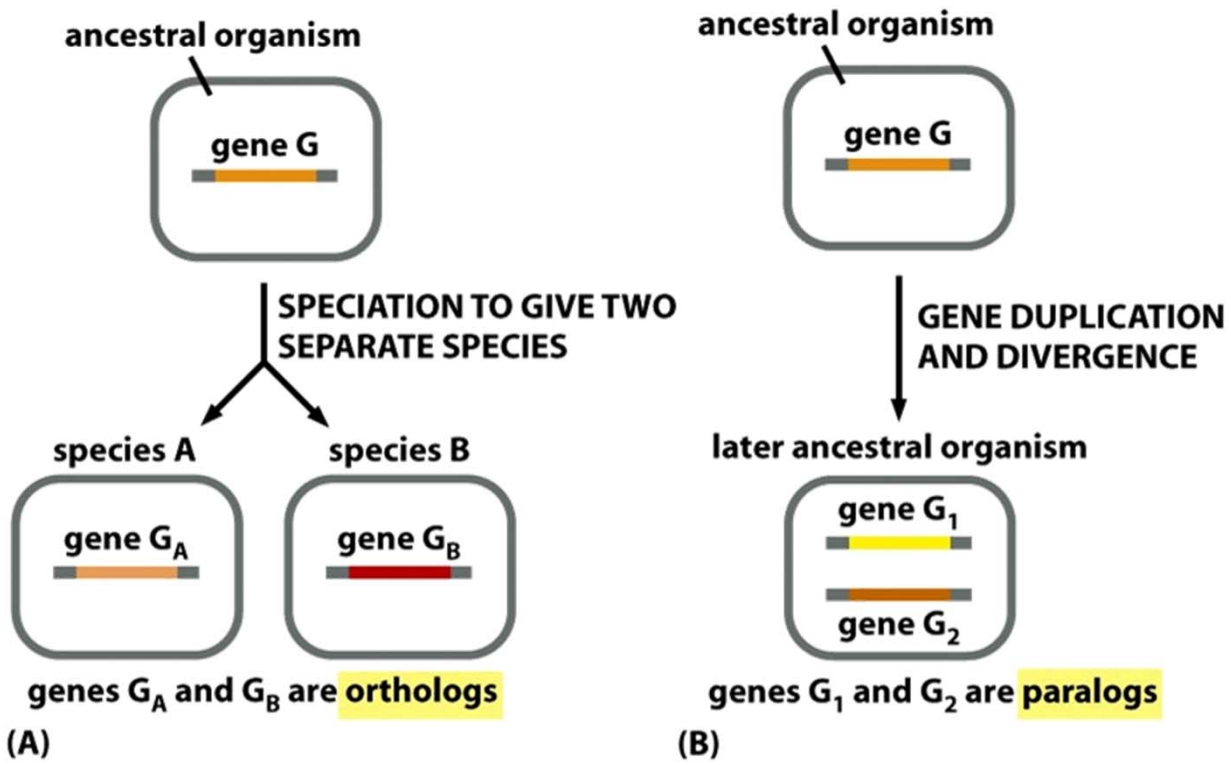
Results:

Some completely sequenced genomes

Organism	Size (Bases)	Estimated genes
Homo sapiens	3.200 million	25.000
Mus musculus	2.600 million	25.000
Arabidopsis thaliana	100 million	25.000
Caenorhabditis elegans	97 million	19.000
Drosophila melanogaster	137 million	13.000
Saccharomyces cerevisiae	12.1 million	6.000
Escherichia coli	4.6 million	4.200
Human Immunodeficiency Virus (HIV)	9.700	9

Human Genome Project

Comparative genomics:



There are genes with very similar sequences within the same species (paralogs) or between different species (orthologs)

Figure 1-25 Molecular Biology of the Cell 5/e (© Garland Science 2008)

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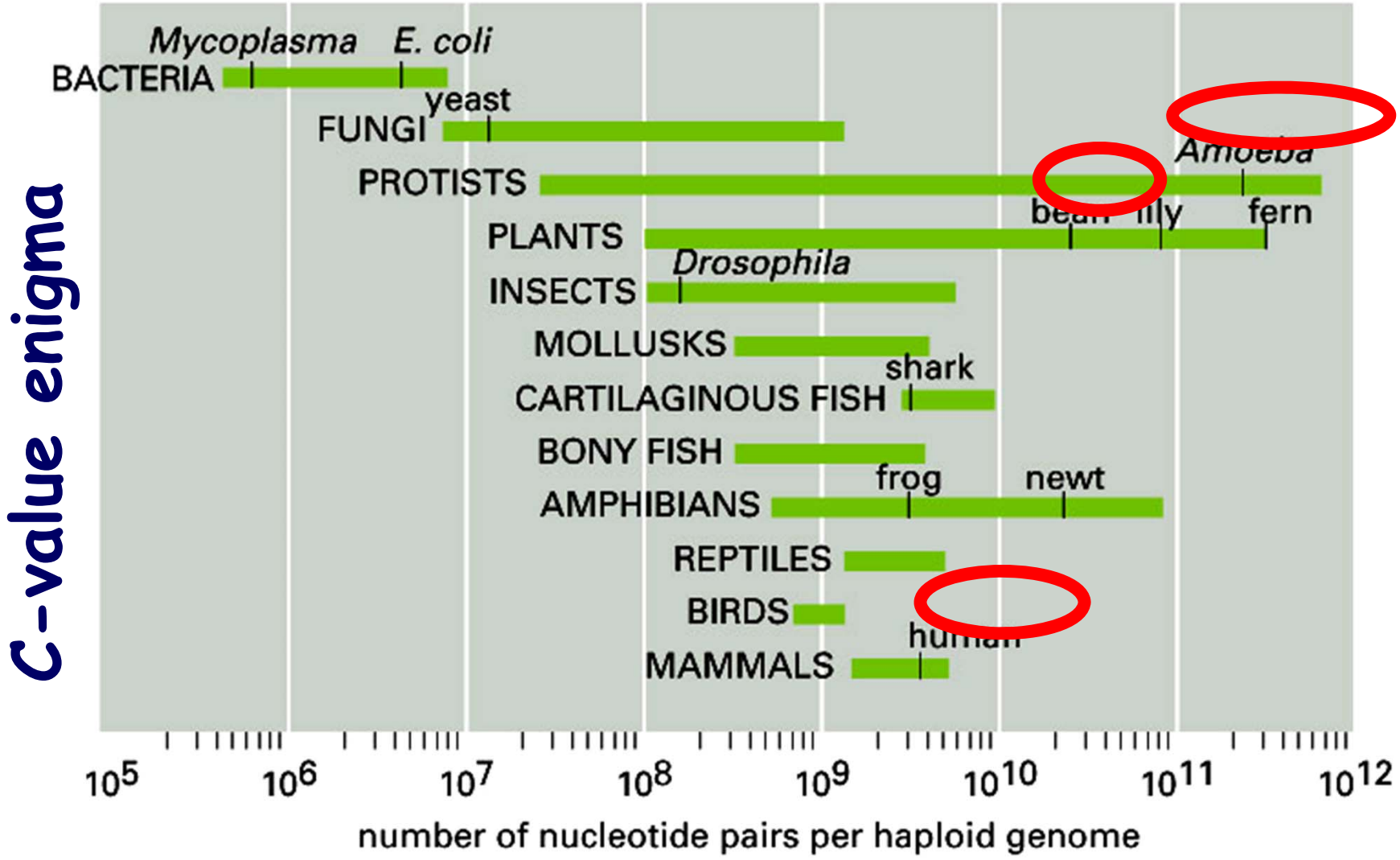
Comparative genomics:



Human Genome Project

Comparative genomics:

C-value enigma

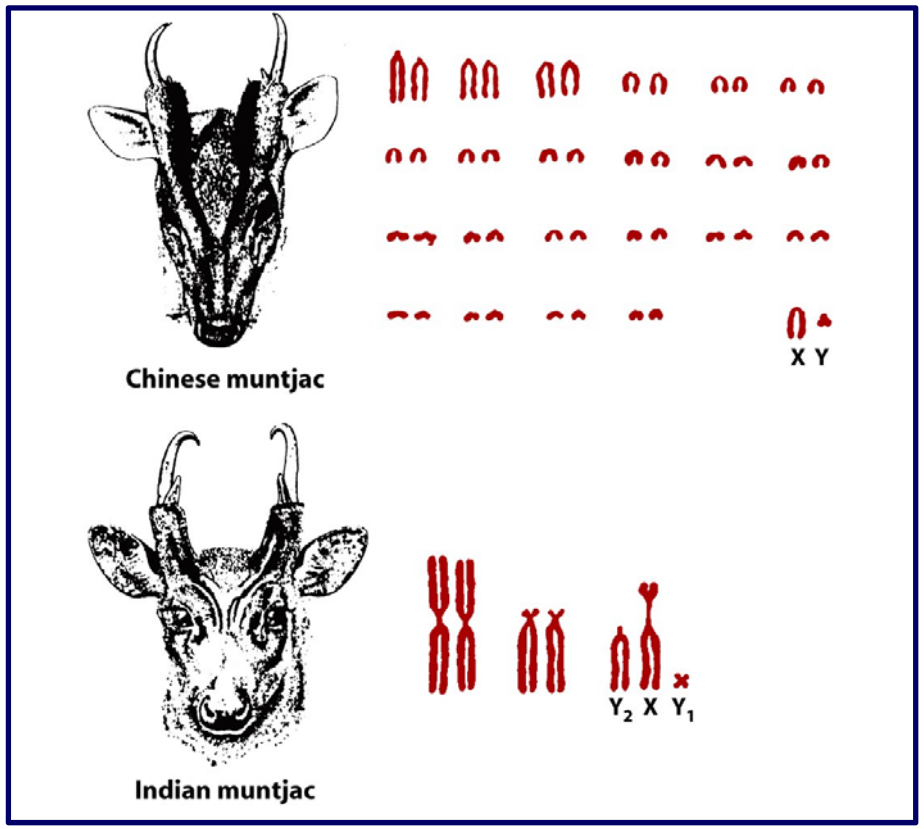
There is no direct relationship between the number of chromosomes, the complexity of species and the size of their genome.

Human Genome Project

Comparative genomics:

Two similar species can have very different karyotypes

C-value enigma



It seems that each of the genomes and chromosomes of the current species has arisen by a unique historical process of genetic events, performed at random and subjected to the pressures of selection throughout evolution

Human Genome Project

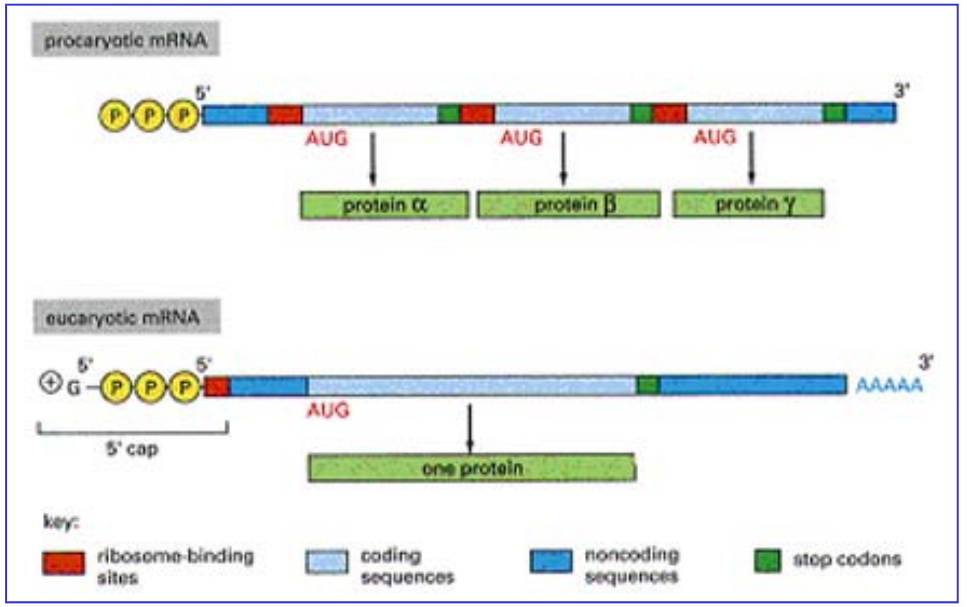
Comparative genomics:

Genome of viruses

- Small
- Compact
- Some overlapping genes

Genome of bacteria

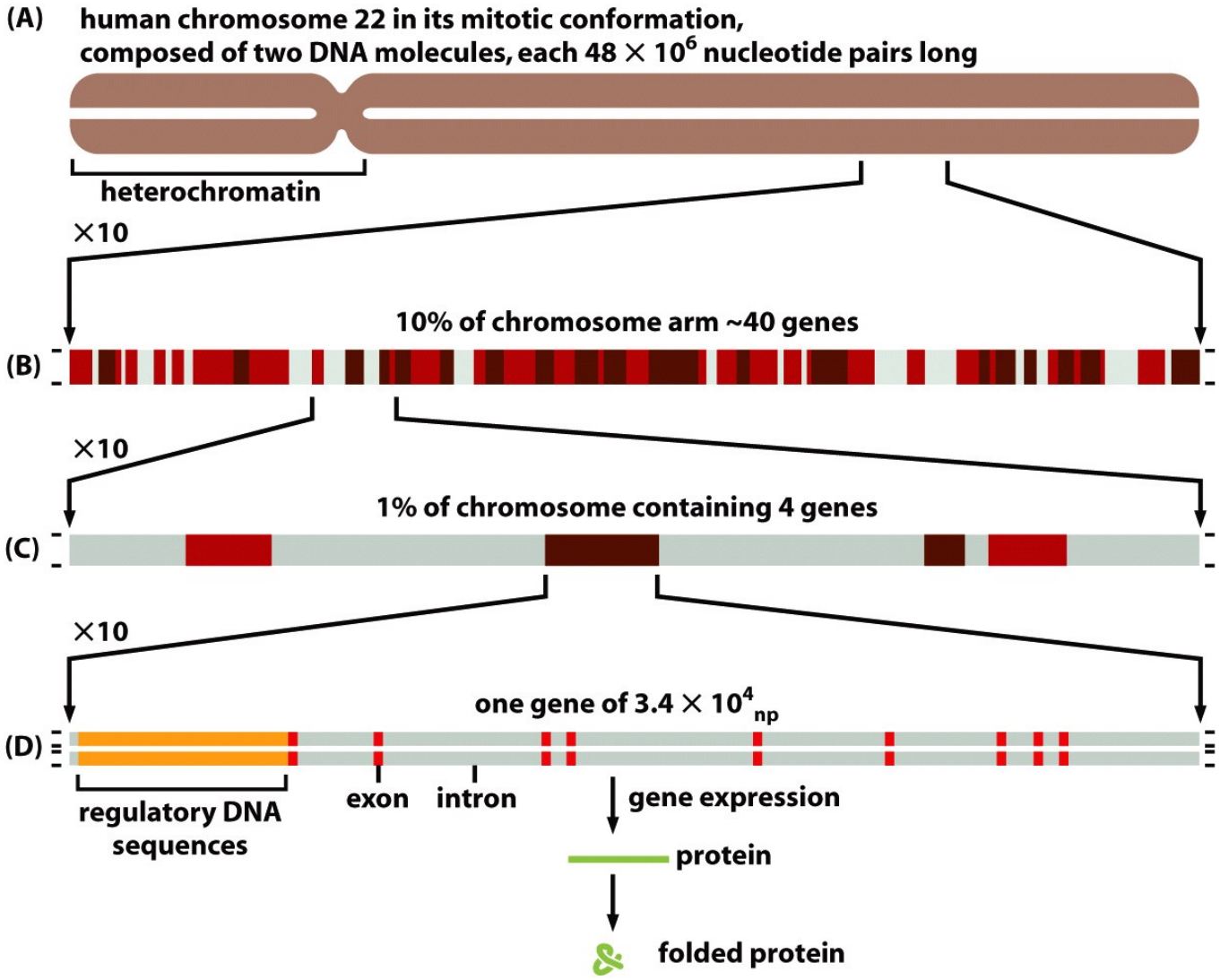
- Smaller than in eucaryotes
- Compact
- Very little repeated DNA
- Functionally related genes are found together in groups



Human Genome Project

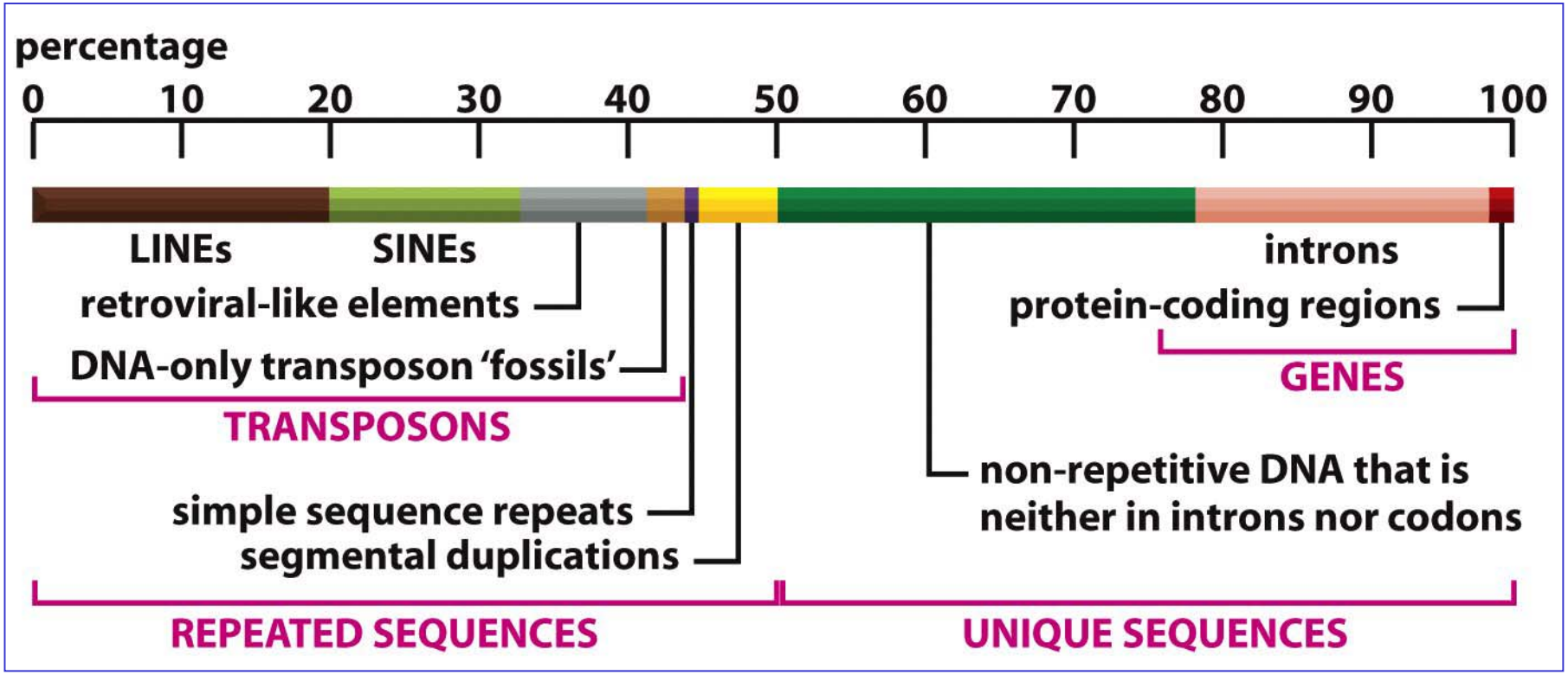
Genome of eucaryotes

- Large
- Much repeated DNA
- Genes formed by exons and introns



Human Genome Project

Types of sequences in human genome



Human Genome Project

Types of sequences in human genome

REPEATED

Localized

Centromeres (DNA alpha satellite 25-100 nt tandem repeats).
CENP-A Histone

Telomeres (DNA minisatellite: 6 nt tandem repeats)

Interspersed

Retrotrasposons

- LINEs (L1 6 Kb, 800.000 copies)
- SINEs (Alu 300pb, 1.500.000 copies)
- Endogenous retroviruses (HERV). Truncated copies

Tandem repeats: Minisatellites: 5-25 nt / Microsatellites: 1-4 nt.

Human Genome Project

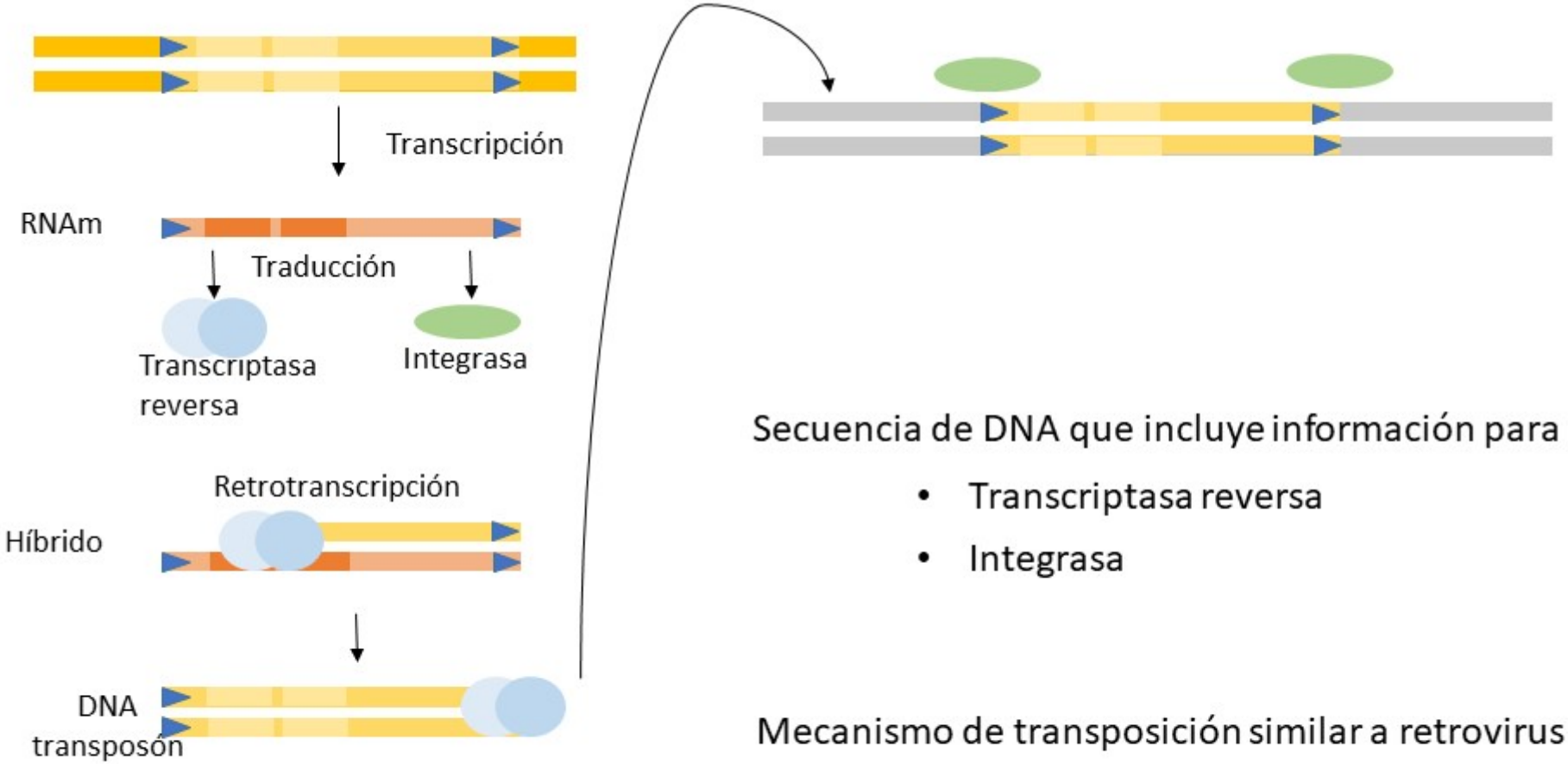
Retrotrasposons

TABLE 5–4 Three Major Classes of Transposable Elements

Class description and structure	Specialized enzymes required for movement	Mode of movement	Examples
DNA-only transposons			
 Short inverted repeats at each end	Transposase	Moves as DNA, either by cut-and-paste or replicative pathways	P element *(Drosophila)*, Ac-Ds (maize), Tn3 and Tn10 *(E. coli)*, Tam3 (snapdragon)
Retroviral-like retrotransposons			
 Directly repeated long terminal repeats (LTRs) at each end	Reverse transcriptase and integrase	Moves via an RNA intermediate whose production is driven by a promoter in the LTR	Copia *(Drosophila)*, Ty1 (yeast), THE1 (human), Bs1 (maize)
Nonretroviral retrotransposons			
 Poly A at 3′ end of RNA transcript; 5′ end is often truncated	Reverse transcriptase and endonuclease	Moves via an RNA intermediate that is often synthesized from a neighboring promoter	F element *(Drosophila)*, *L1* (human), Cin4 (maize)

These elements range in length from 1000 to about 12,000 nucleotide pairs. Each family contains many members, only a few of which are listed here. Some viruses can also move in and out of host-cell chromosomes by transpositional mechanisms. These viruses are related to the first two classes of transposons.

Human Genome Project
Retroviral-like retrotrasposons



Secuencia de DNA que incluye información para

- Transcriptasa reversa
- Integrasa

Mecanismo de transposición similar a retrovirus

Imagen Unidad de Biología

Human Genome Project

No retroviral-like retrotrasposons

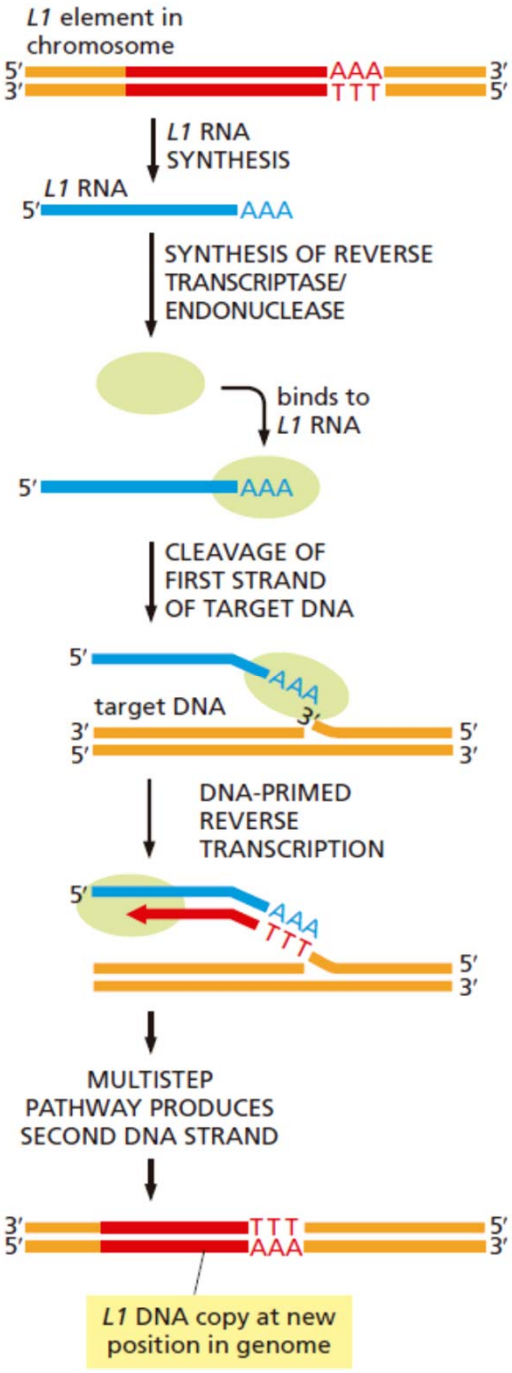
DNA sequence including information for endonuclease/reverse transcriptase complex

It requires many enzymes

Examples:

- LINE L1 (hemophilia cause because interruption gen Factor VIII)
- SINE: some enzymes lost (transposon dependent)

40% genome



Human Genome Project

Types of sequences in human genome

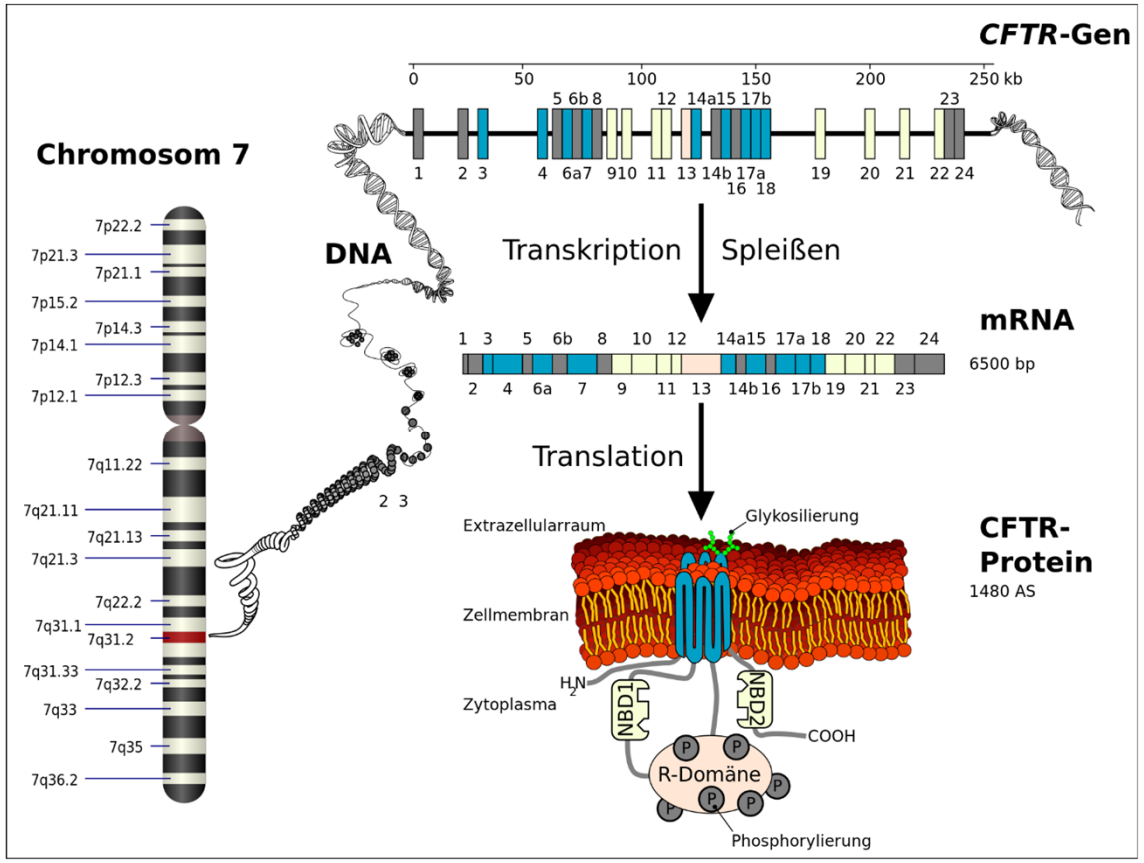
UNIQUE

Genes

- Exons
- Introns
- Regulatory sequences

Pseudogenes

- Nucleotide sequences similar to a functional gene, which contains multiple mutations / changes that prevent its expression. Duplication of a functional gene.



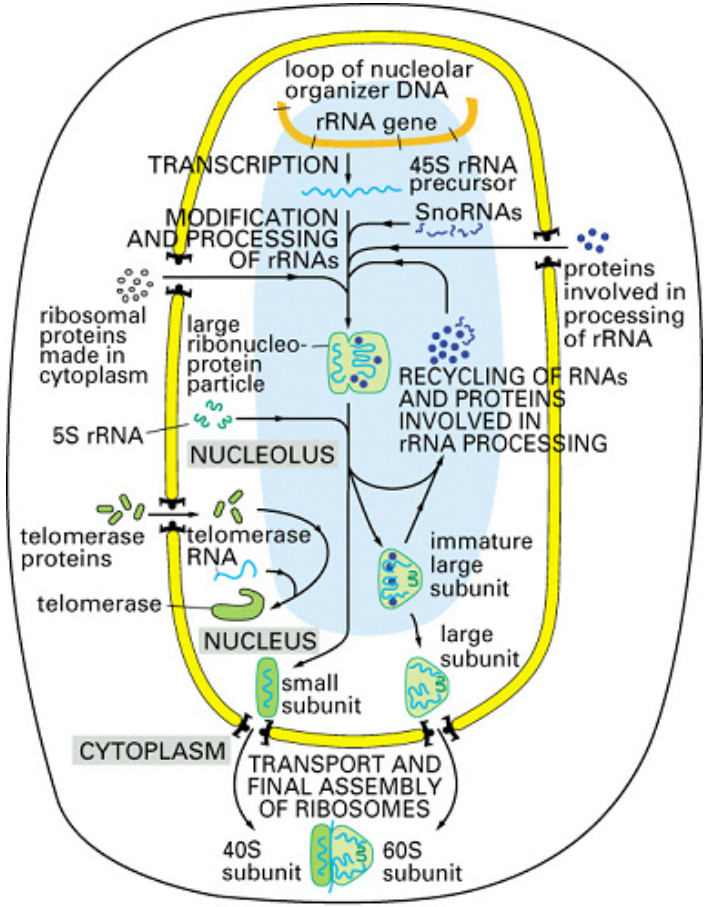
Human Genome Project

Types of RNA in human genome

mRNA (messenger RNA: code for protein)

rRNA (ribosomal RNA: form the basic structure of the ribosome and catalyze protein synthesis)

tRNA (transfer RNA: adaptors between mRNA and amino acids)



Human Genome Project

Types of RNA in human genome

snRNA (small nuclear RNA: functions in splicing of pre-mRNA)

snoRNA (small nucleolar RNA: used to process and chemically modify rRNAs)

scaRNA (small cajal RNA: used to modify snoRNAs and snRNAs)

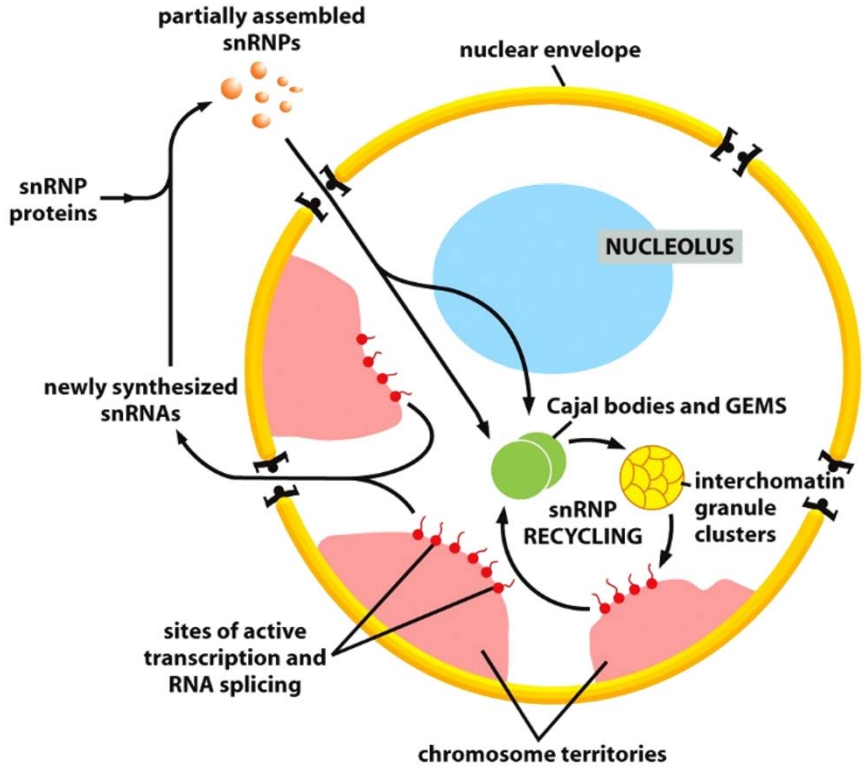
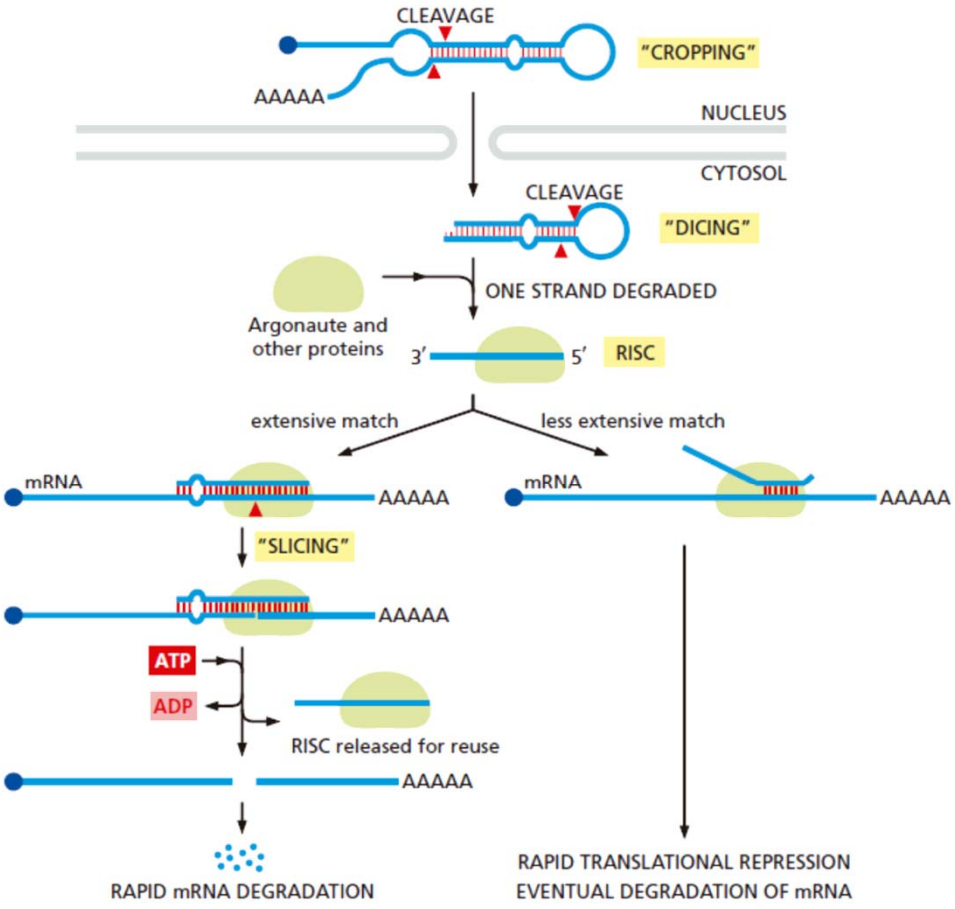


Figure 6-49 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Human Genome Project

Types of RNA in human genome

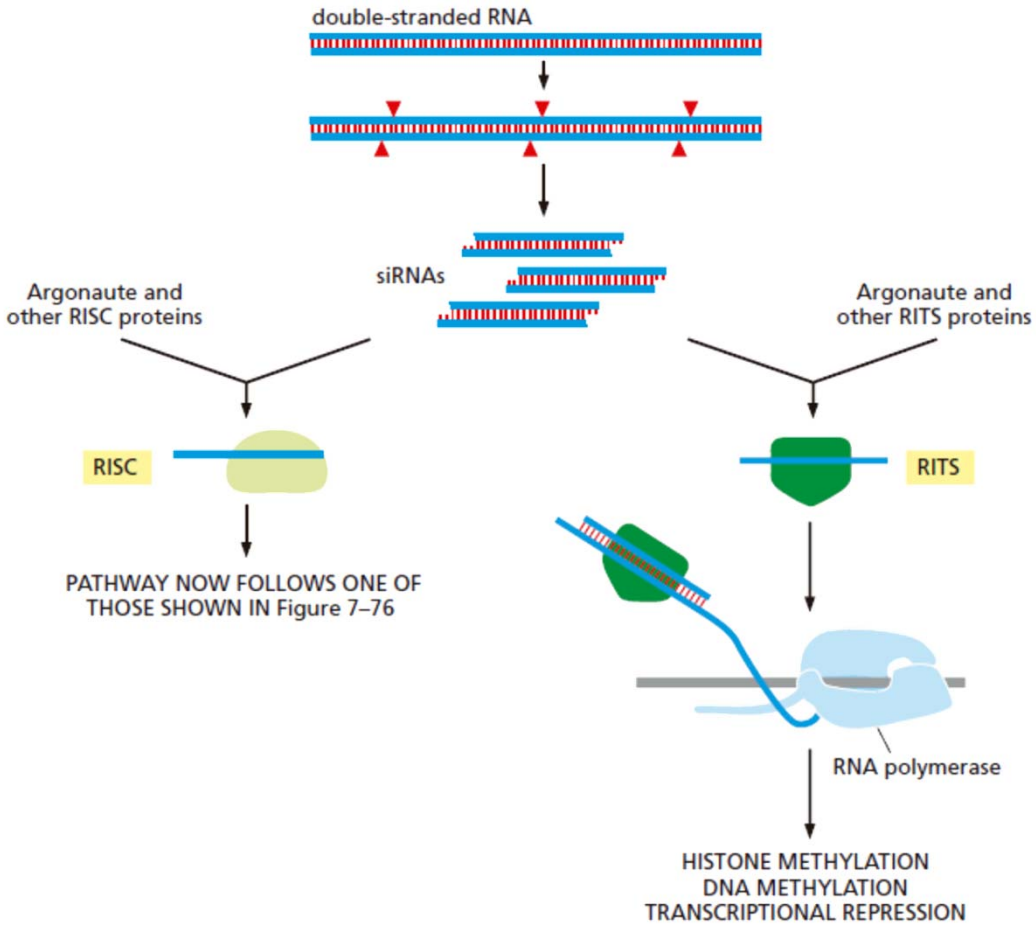
miRNA - micro RNA:Precursor: pri-miRNA, self-complementary -> hairpin. Processed: DROSHA (nucleus) forms the pre-miRNA. DICER (cytoplasm) forms the miRNA. It regulates gene expression by blocking the translation and inducing degradation of certain mRNAs.



Human Genome Project

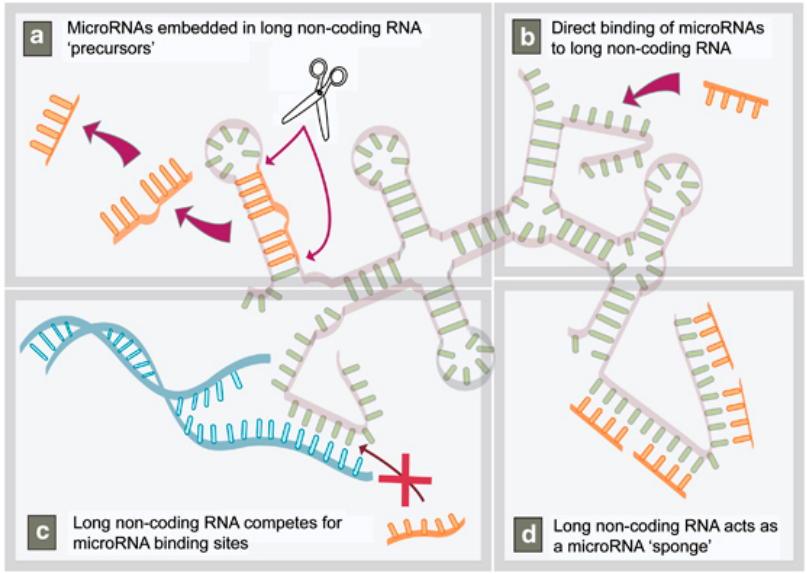
Types of RNA in human genome

siRNA - small interfering RNA: Precursor: dsRNA; processed: DICER (cytoplasm). Similar to miRNA and also blocks gene expression by inducing compact chromatin structures.



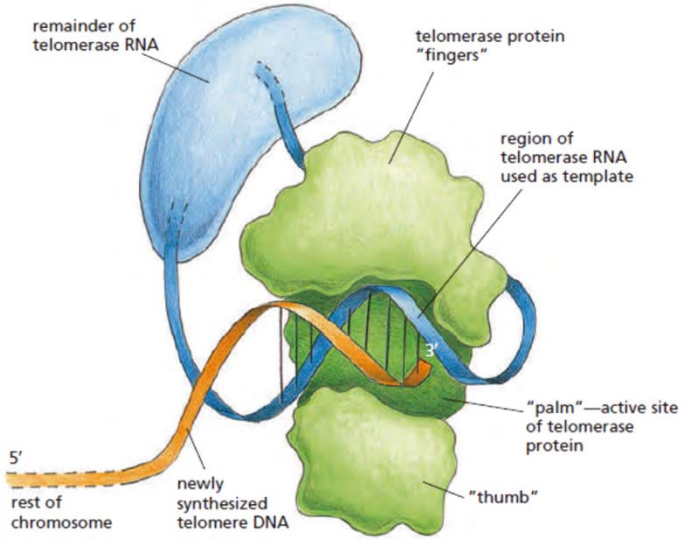
Human Genome Project
Types of RNA in human genome

lncRNA (long non-coding RNA: can act in the activation or repression of genes)



Integrating the roles of long and small non-coding RNA in brain function and disease. **Article** *in Molecular Psychiatry 19(4) · January 2014. DOI: 10.1038/mp.2013.196 · Source: PubMed*

TER (telomerase RNA: functions in telomere synthesis)



Xist (X-inactive specific transcript: Inactivation of chromosome X)

Human Genome Project

C. DARKIN

WHAT IS A GENE?

The idea of genes as beads on a DNA string is fast fading. Protein-coding sequences have no clear beginning or end and RNA is a key part of the information package, reports **Helen Pearson**.

'Gene' is not a typical four-letter word. It is not offensive. It is never bleeped out of TV shows. And where the meaning of most four-letter words is all too clear, that of gene is not. The more expert scientists become in molecular genetics, the less easy it is to be sure about what, if anything, a gene actually is.

Rick Young, a geneticist at the Whitehead Institute in Cambridge, Massachusetts, says that when he first started teaching as a young professor two decades ago, it took him about two hours to teach fresh-faced undergraduates what a gene was and the nuts and bolts of how it worked. Today, he and his colleagues need three months of lectures to convey the concept of the gene, and that's not because the students are any less bright. "It takes a whole semester to teach this stuff to talented graduates," Young says. "It used to be we could give a one-off definition and now it's much more complicated."

In classical genetics, a gene was an abstract concept — a unit of inheritance that ferried a characteristic from parent to child. As biochemistry came into its own, those characteristics were associated with enzymes or proteins, one for each gene. And with the advent of molecular biology, genes became real, physical things — sequences of DNA which when converted into strands of so-called messenger RNA could be used as the basis for building

Laurence Hurst at the University of Bath, UK.

"All of that information seriously challenges our conventional definition of a gene," says molecular biologist Bing Ren at the University of California, San Diego. And the information challenge is about to get even tougher. Later this year, a glut of data will be released from the international Encyclopedia of DNA Elements (ENCODE) project. The pilot phase of ENCODE involves scrutinizing roughly 1% of the human genome in unprecedented detail; the aim is to find all the sequences that serve a useful purpose and explain what that purpose is. "When we started the ENCODE project I had a different view of what a gene was," says contributing researcher Roderic Guigo at the Center for Genomic Regulation in Barcelona. "The degree of complexity we've seen was not anticipated."

> "We've come to the realization that the genome is full of overlapping transcripts."
> — Phillip Kapranov

Under fire

The first of the complexities to challenge molecular biology's paradigm of a single DNA sequence encoding a single protein was alternative splicing, discovered in viruses in 1977 (see 'Hard to track', overleaf). Most of the DNA sequences describing proteins in humans have a modular arrangement in which exons, which

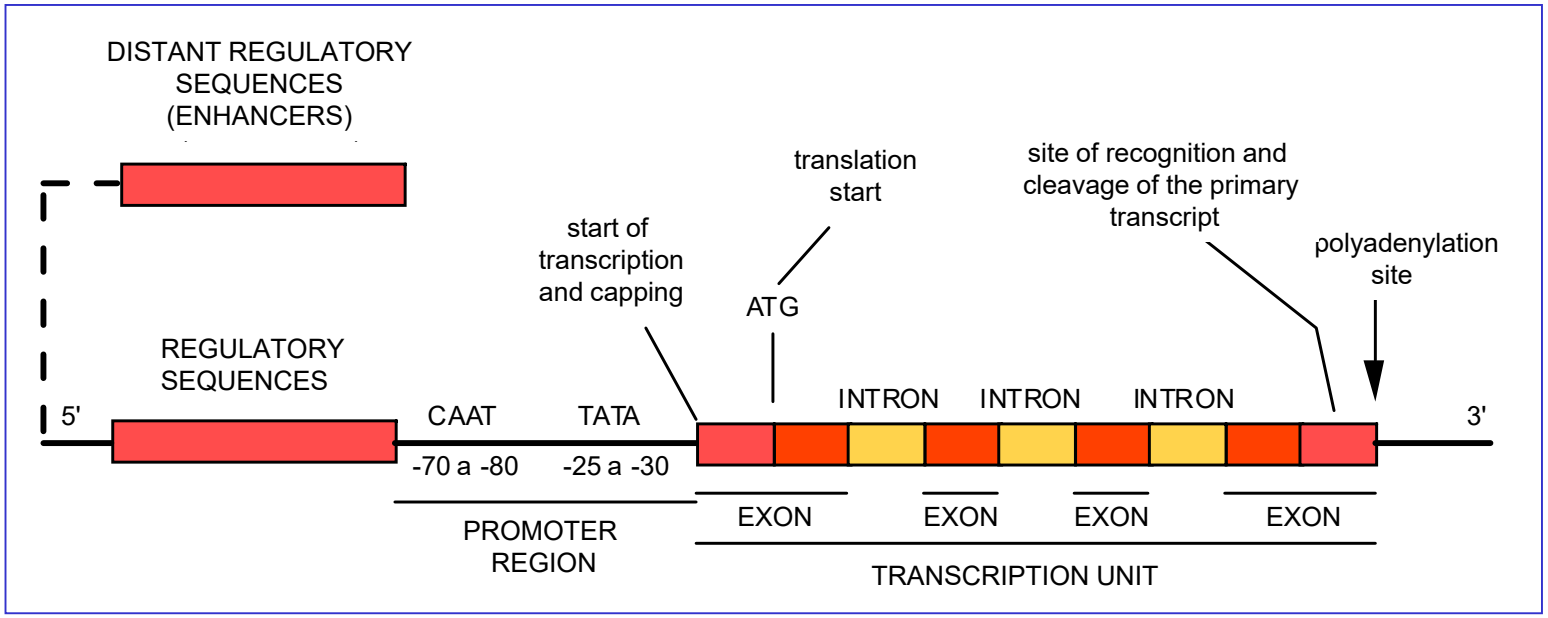
viously unimagined scope of RNA.

The one gene, one protein idea is coming under particular assault from researchers who are comprehensively extracting and analysing the RNA messages, or transcripts, manufactured by genomes, including the human and mouse genome. Researchers led by Thomas Gingeras at the company Affymetrix in Santa Clara, California, for example, recently studied all the transcripts from ten chromosomes across eight human cell lines and worked out precisely where on the chromosomes each of the transcripts came from[3].

The picture these studies paint is one of mind-boggling complexity. Instead of discrete genes dutifully mass-producing identical RNA transcripts, a teeming mass of transcription converts many segments of the genome into multiple RNA ribbons of differing lengths. These ribbons can be generated from both strands of DNA, rather than from just one as was conventionally thought. Some of these transcripts come from regions of DNA previously identified as holding protein-coding genes. But many do not. "It's somewhat revolutionary," says Gingeras's colleague Phillip Kapranov. "We've come to the realization that the genome is full of overlapping transcripts."

Human Genome Project

Structure of a typical human gene



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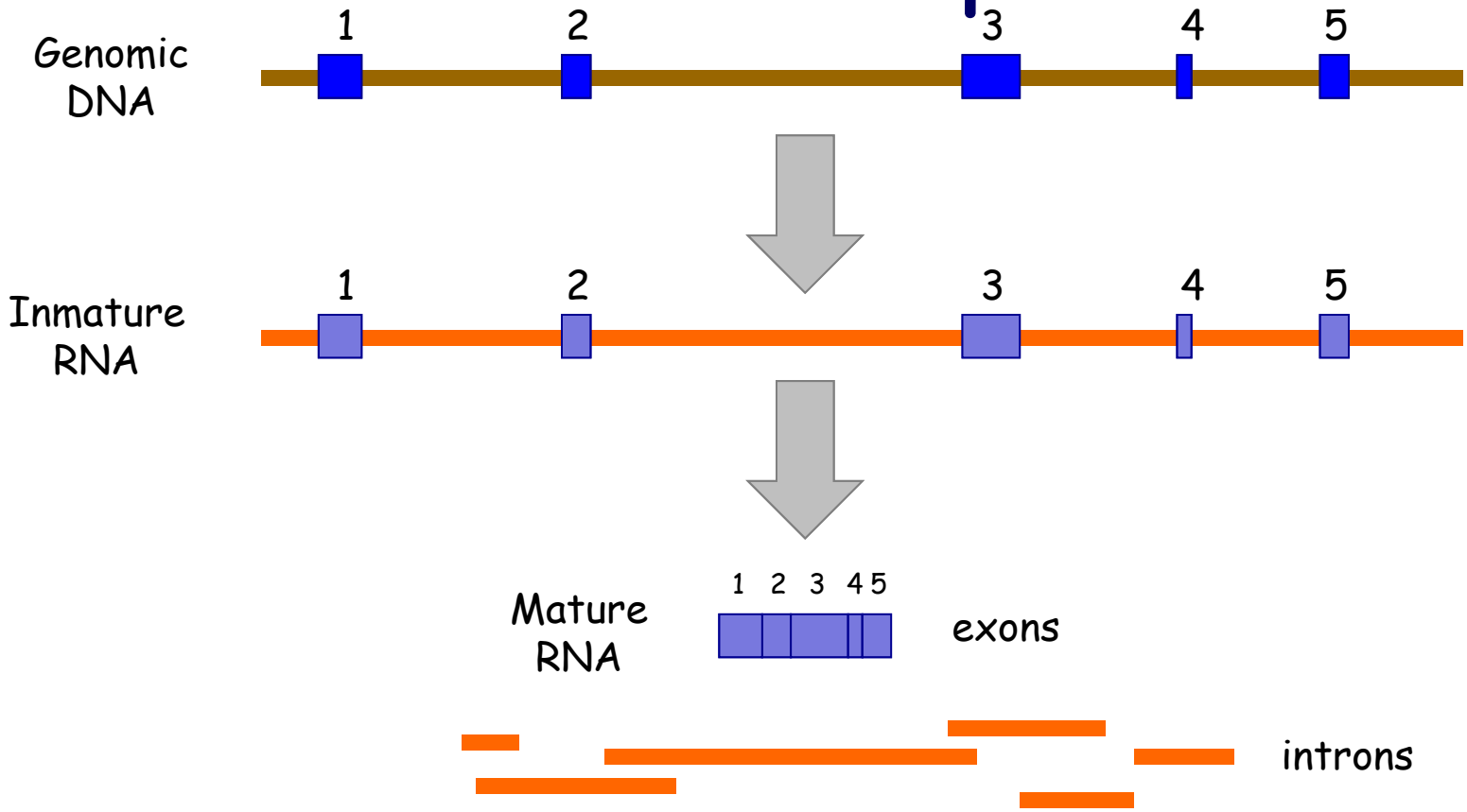
Gene transcription



SUNBFHTGONYONLKJIJFJMLBKNFJCOMOMNHSYIEWRBVDDFH
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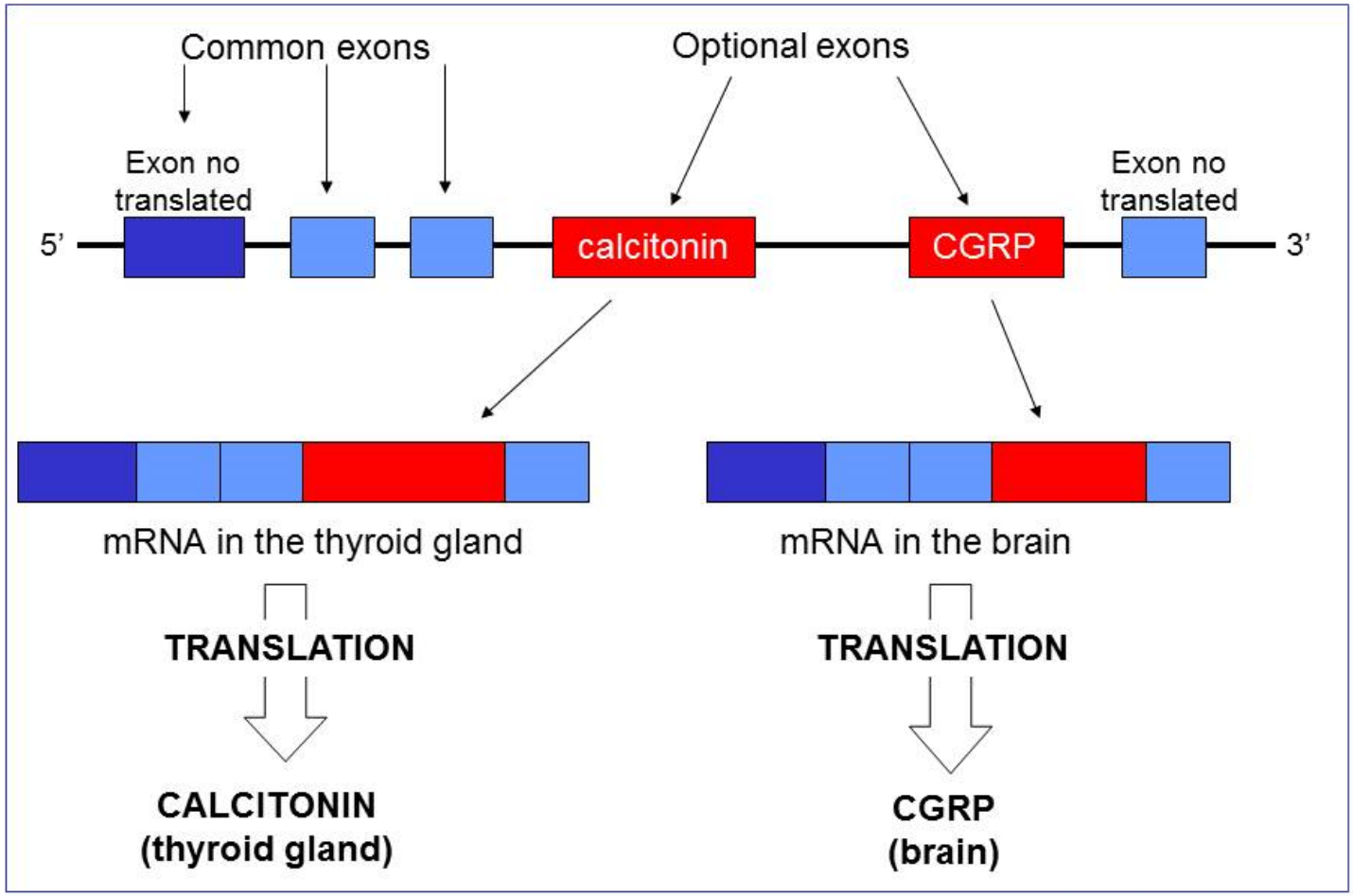
Human Genome Project
Gene splicing



SUNBFHTGONYONLKJIJFJMLBKNFJCOMOMNHSYIEWRBVDDFH
JGDGVPANMRNBHYQBAGDKIRBMVHFIKFBBCDJNHGCARNECHI

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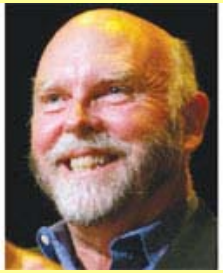
Historical outline

Post-genomic era (since 2003): study of the regulation of genetic activity.
ENCODE Project
HapMap project
Other projects…
First personal genome (2007 Venter)


JAMES WATSON


CRAIG VENTER

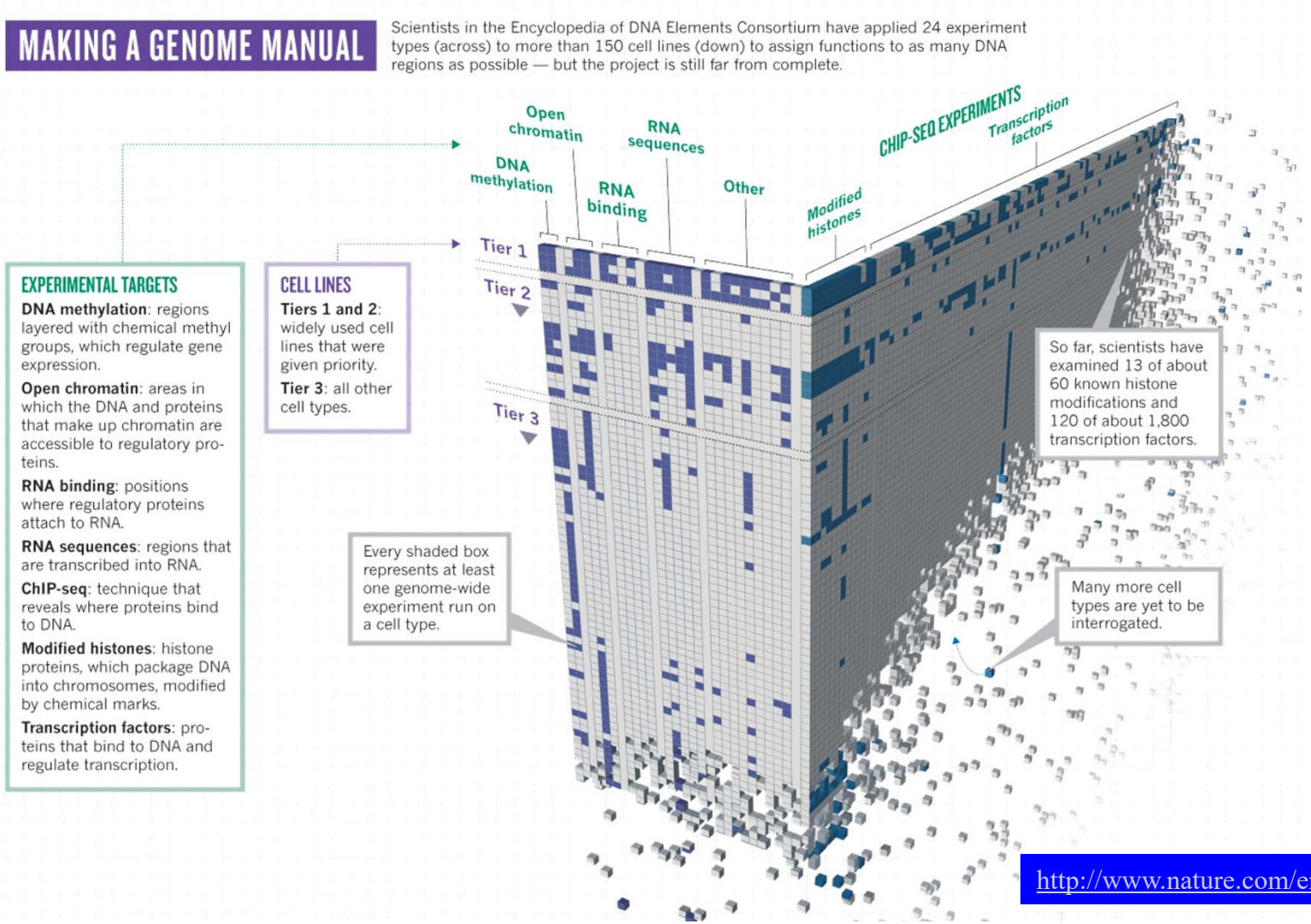

GEORGE CHURCH

News & Opinion

Getting to Know the Genome

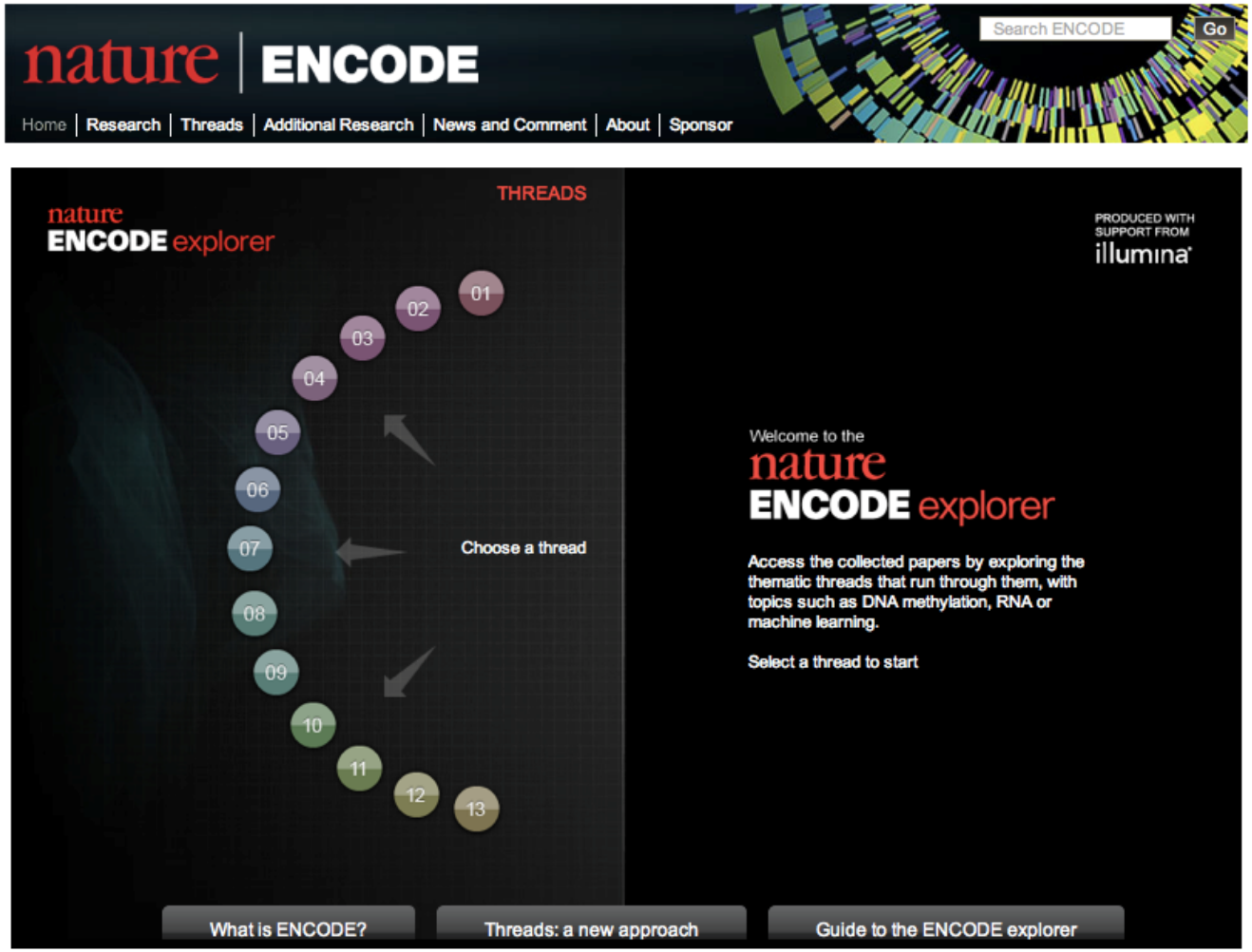
A massive project involving hundreds of scientists suggests that very little—if any—of the human genome is truly non-functional.

By Ed Yong | September 5, 2012



http://www.nature.com/encode/#/threads

Volume 489 Number 7414 pp5-170
6 September 2012



http://www.nature.com/encode/#/threads

ENCODE (Encyclopedia Of DNA Elements)

Goal: to describe the functional elements in the genome

442 scientists
10 years of work
They study 147 different cell types (there are 2000)

Results: more than 80% of human genome with an assigned function

Some functions of the non codifying elements:
- Sites for protein binding which affect the expression of proximal or distal genes
- Genes for transcription of RNA molecules never translated into protein
- DNA folding and packing elements

ARTICLE

Nature 1 noviembre 2012

doi:10.1038/nature11632

An integrated map of genetic variation from 1,092 human genomes

The 1000 Genomes Project Consortium*

By characterizing the geographic and functional spectrum of human genetic variation, the 1000 Genomes Project aims to build a resource to help to understand the genetic contribution to disease. Here we describe the genomes of 1,092 individuals from 14 populations, constructed using a combination of low-coverage whole-genome and exome sequencing. By developing methods to integrate information across several algorithms and diverse data sources, we provide a validated haplotype map of 38 million single nucleotide polymorphisms, 1.4 million short insertions and deletions, and more than 14,000 larger deletions. We show that individuals from different populations carry different profiles of rare and common variants, and that low-frequency variants show substantial geographic differentiation, which is further increased by the action of purifying selection. We show that evolutionary conservation and coding consequence are key determinants of the strength of purifying selection, that rare-variant load varies substantially across biological pathways, and that each individual contains hundreds of rare non-coding variants at conserved sites, such as motif-disrupting changes in transcription-factor-binding sites. This resource, which captures up to 98% of accessible single nucleotide polymorphisms at a frequency of 1% in related populations, enables analysis of common and low-frequency variants in individuals from diverse, including admixed, populations.

Human Genome Project

Future prospects

What we still don't know

- Number of genes, exact location and function.
- Genetic regulation. Coordination of genetic expression, protein synthesis and post-translational events.
- Structure and organization of chromosomes.
- Types of non-coding DNA, amount, distribution and functions.
- Interaction of proteins in macromolecular complexes.
- Evolutionary conservation among different organisms.
- Proteomes (content and function of all proteins) of the organisms.
- Identification of susceptibility genes to the different diseases, based on sequence variations.
- Identification of genes implied in complex diseases (multi-factorial).

Human Genome Project

Future prospects

Possible benefits

Molecular Medicine

- Improve diagnosis of disease
- Detect genetic predispositions to disease
- Gene therapy
- Design "custom drugs" (pharmacogenomics) based on individual genetic profiles
- Personal medicine
- DNA identification

GENETIC VARIABILITY: POLYMORPHISM AND MUTATION

- Introduction
- Genetic polymorphism
  - Types of genetic locus
  - Inheritance of polymorphisms
  - Types of polymorphisms
  - ABO system
- Mutation
  - Types
    - By cell affected
      - Germinal
      - Somatic
    - By size
      - Large (chromosomal anomalies)
      - Small

INTRODUCTION

- In general terms, any nucleotide change in DNA may be called mutation. However, the term mutation is typically applied to changes that have as a consequence a pathological outcome. In the case of changes producing structural variations which keep some normal function, they are called polymorphisms.

- Most genetic variations found at the genome level do not include codifying sequences but extragenic sequences or non codifying heterochromatic chromosomal regions. By evolution, the constant influence of new nucleotide variations has ensured a high degree of diversity and genetic individuality.

INTRODUCTION

- Human beings show a high degree of variability. Many variations are just personal features without pathological implications. For example, heght, skin color, etc. Medical genetics study variations which involve some alteration of the healht status, and its possible impact.

- These phenotypic pathological differences may be due to genetic changes or non genetic changes (environment). Medical genetics study those due to permanent genetic variations.

- Not all changes produced in the DNA are pathological. The genetic disease is the extreme and most obvious expression of genetic changes, within a context of normal genetic variablity. Progenies are not clones of progenitors. Genetic variability is the base of evolution.

INTRODUCTION

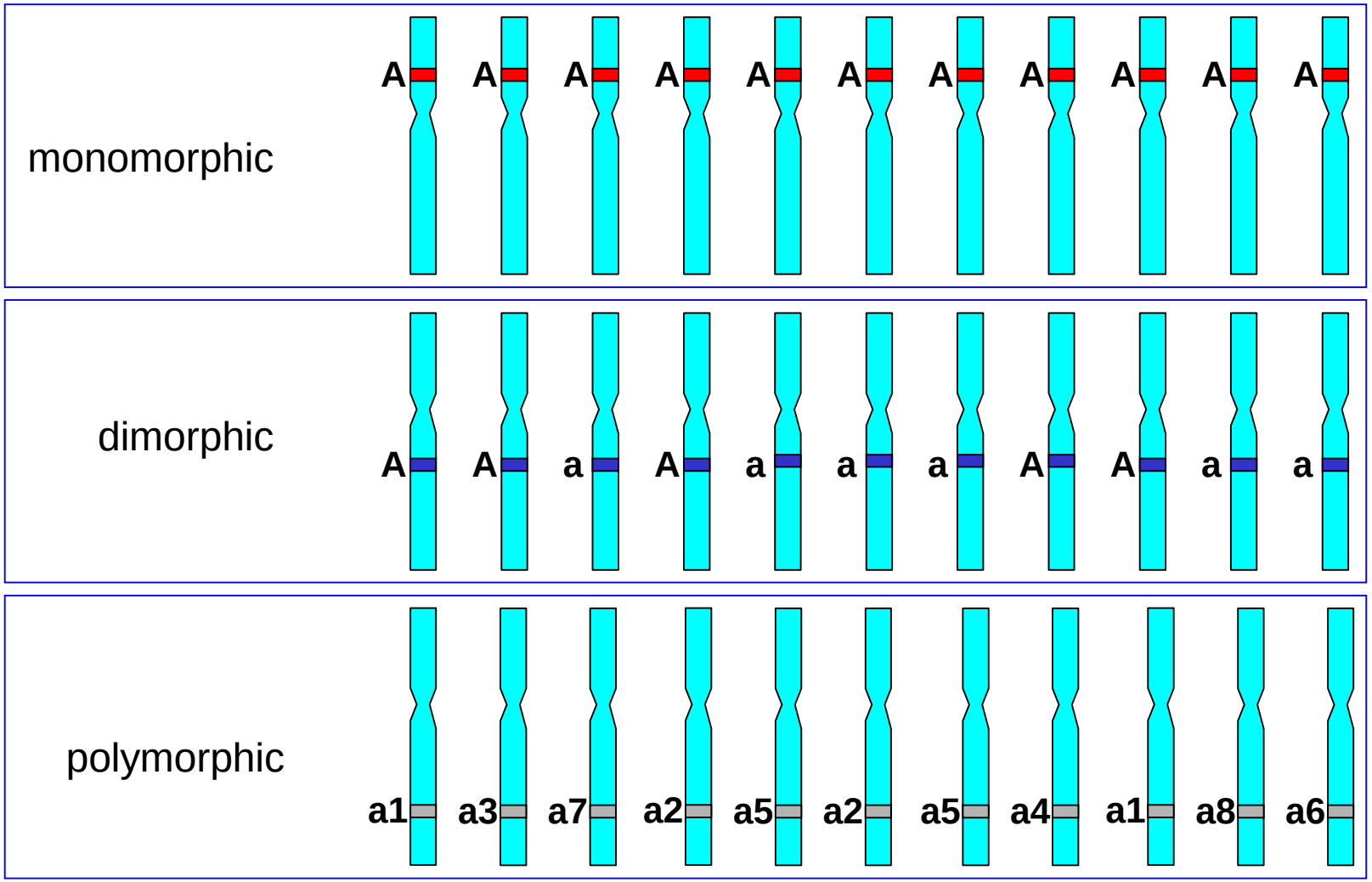
At the molecular level, DNA ensures the constancy of fundamental characteristics allowing simyultaneously some variability

- DNA constancy:
  - Replication
  - Repairing

- DNA variations:
  - Mutation
  - Recombination
  - Transposition

TYPES OF GENETIC LOCUS

Chromosomal locus : a part of a chromosome at a specific, constant and well defined position within the chromosome itself

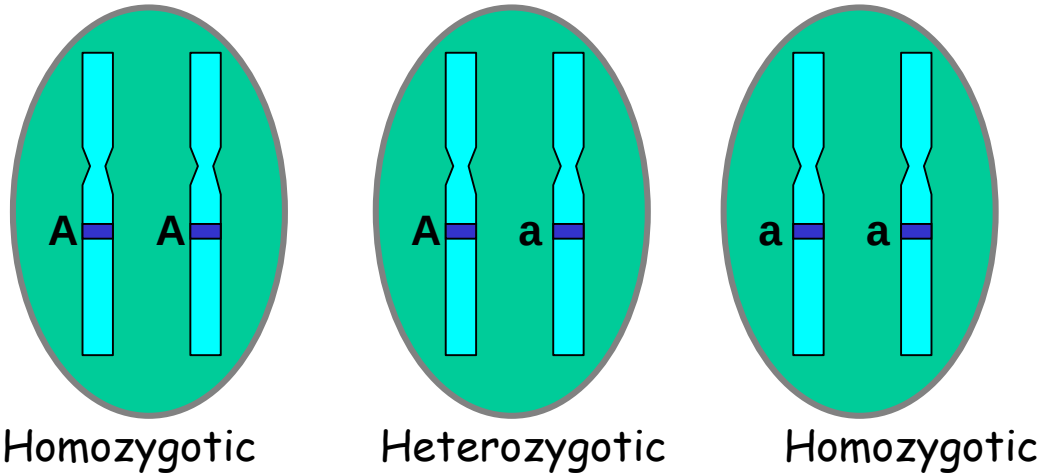


Alelle genes are the different alternative forms that can a genetic locus can contain

ALLELES

Mendel experiments were performed over diallelic characters dialélicos

In diploid cells, we can find the following combinations of two alleles:



INHERITANCE OF POLYMORPHISMS

- Multiple allelism occurs at a particular genetic locus if it can contain three or more different alternatives forms (alleles)
- The complete set of alleles that can be at a same locus is called multiallelic serie or multiple alleles
- In a diploid cell, there are many possible genotypes: all the possible combinations of pairs of alleles

Three alleles

a1a1	a2a2	a3a3
a1a2	a1a3	
a2a3		

6 possible genotypes

Four alleles

a1a1	a2a2	a3a3	a4a4
a1a2	a1a3	a1a4	
a2a3	a2a4		
a3a4			

10 possible genotypes

- Multiple genotypes produce multiple phenotypes. That is the reason for the name polymorphism for those characters controlles by multiple alleles
- As a general agreement, a polymorphism must be present in at least 1% of the population. Otherwise, they are called rare variants

TYPES OF GENETIC POLYMORPHISMS

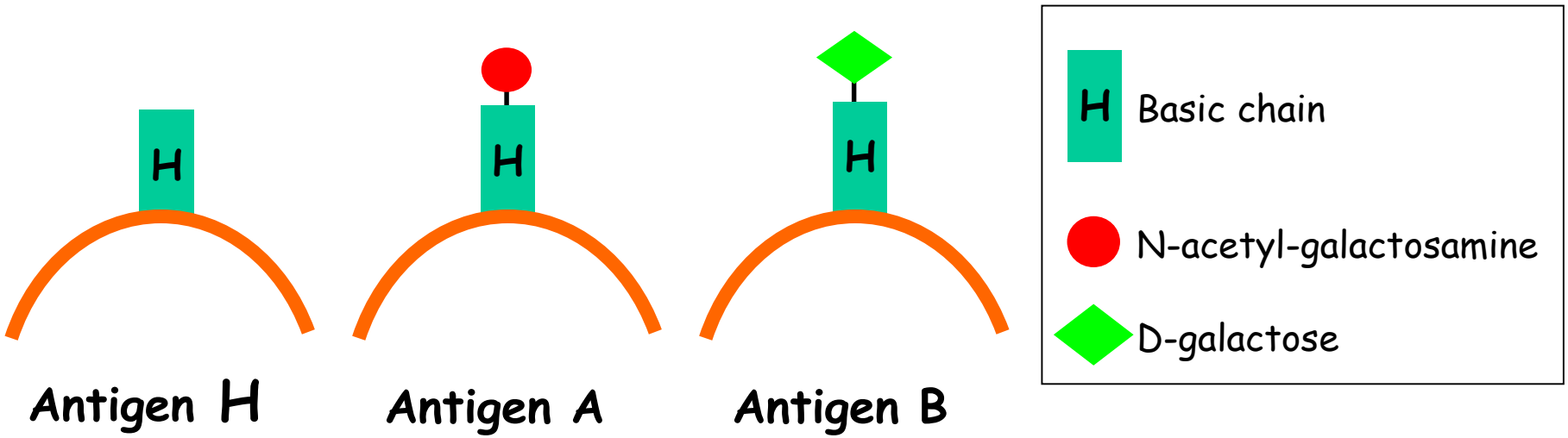
- Chromosomal (heteromorphisms: Y, 1, 9, 16)
- Proteic (isoforms: structural variants showing all the same function: ABO, etc)
- DNA (intragenic and extragenic)

INHERITANCE OF POLYMORPHISMS

Blood groups system

- Blood group is determined by the membrane antigens of the erythrocytes and the serum antibodies
- Antigens are glycolipids or glycoproteins at the membrane
- More than 100 different antigens grouped in 14 blood groups have been identified.
- The presence of antigens is detected by the antigen-antibody interaction in vitro or in vivo.

ABO system - Phenotype



Group A: Antigen A
Group B: Antigen B
Group AB: Antigen A + Antigen B
Group O: Nor Antigen A neither Antigen B, only unmodified Antigen H

ABO systems - Genotype

Two different locus involved:

Locus H (at chromosome 19): 2 alelles

H (dominant)

h (recessive)

Locus I (at chromosome 9): 3 allelic forms

I^A (co-dominant) → antigen A

I^B (co-dominant) → antigen B

i (recessive) → unmodified antigen H

ABO system - Genotype

Group	Genotype	Phenotype
Group A	$I^A I^A$ $I^A i$	antigen A
Group B	$I^B I^B$ $I^B i$	antigen B
Group AB	$I^A I^B$	antigen A antigen B
Group O	i i	antigen H

ABO system - Genotype

Examples of genetic posibilities

Group A x Group 0

	I^A	I^A
i	I^A /i	I^A / i
i	I^A /i	I^A / i

100% group A

	I^A	i
i	I^A / i	i / i
i	I^A / i	i / i

50% group A
50% group 0

Group A x Group AB

	I^A	I^A
I^A	I^A / I^A	I^A / I^A
I^B	I^A / I^B	I^A / I^B

50% group A
50% group AB

	I^A	i
I^A	I^A / I^A	I^A / i
I^B	I^A / I^B	I^B / i

50% group A
25% group AB
25% group B

ABO System – Bombay phenotype

- It is an exceptional situation in which H antigen is not present (~1 case per 10.000 individuals in India; ~1 case per million in Europe)
- Homozygosis h/h. H antigen is not synthesized and therefore cannot by modified by genes IA or IB. Phenotypically, individuals show 0 group
- Casoe: woman with 0 blood group, married to man with A blood group mujer. Children of groups AB and 0.
- Normal group 0 phenotype [i/i], and assuming locus H genotype [H/H] or [H/h], possible children genotypes and phenotypes would be:

	I^A	I^A
i	I^A / i	I^A / i
i	I^A / i	I^A / i

	I^A	i
i	I^A / i	i / i
i	I^A / i	i / i

- Real genotype was [h/h, IB/i]. Children with a man [H/H, IA/i] would be:

	H	H
h	H / h	H / h
h	H / h	H / h

	I^A	i
I^B	I^A / I^B	I^B / i
i	I^A / i	i / i

- In locus H, all children would be [H/h]. Father allele is H (dominant), antigen H would always be expressed and could be modified by antigen A or B. In that case, children could be A, AB, B and 0.

POLYMORPHISMS
VARIABLE NUMBER OF TANDEM REPEATS (VNTR)

P. ej., ...gcaacttc$[cgtgg]_n$accgttatt...

5′...gcaacttc**cgtgg** **cgtgg** **cgtgg** **cgtgg** **cgtgg**accgttatt...3′

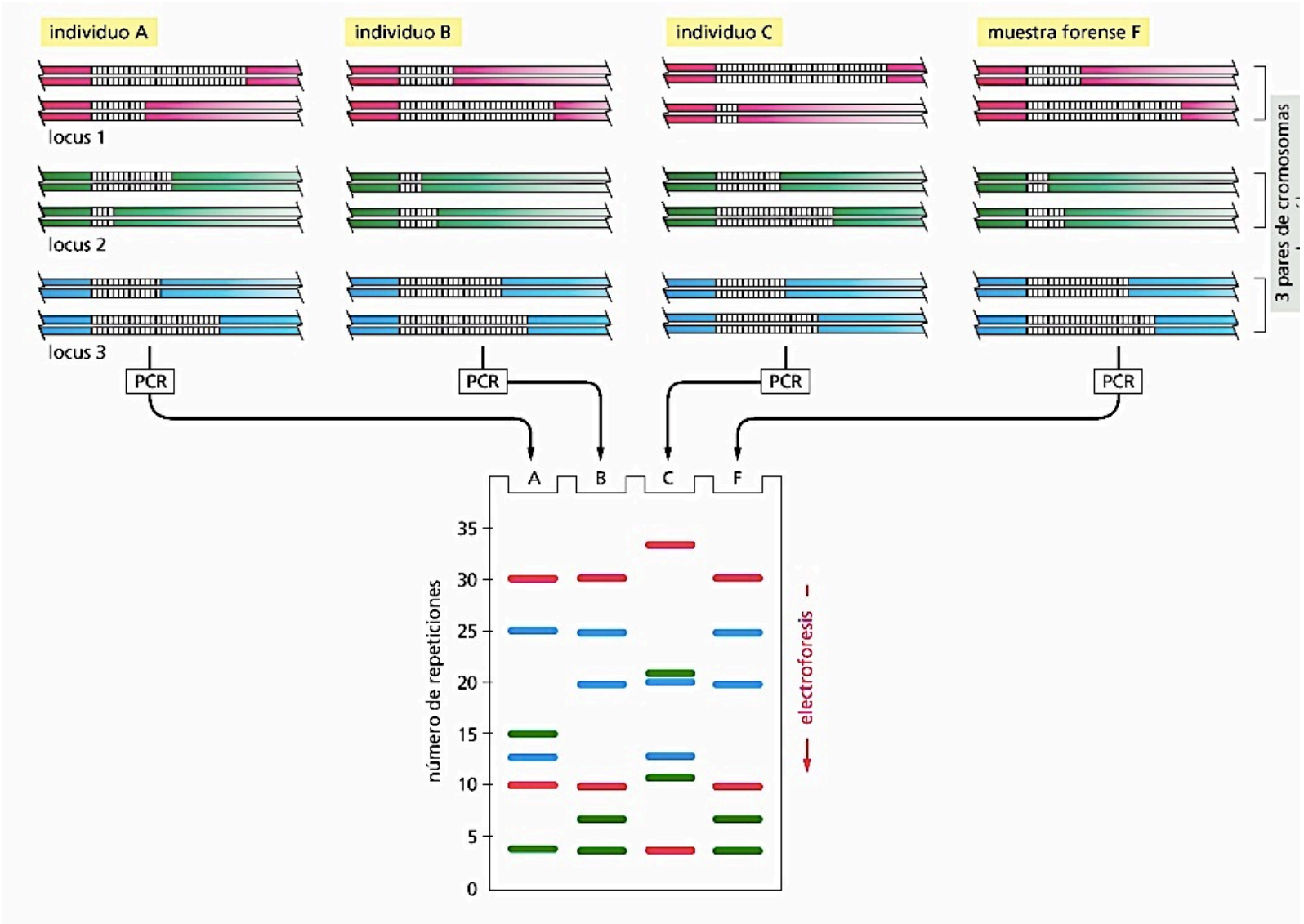
n=5

5′...gcaacttc**cgtgg** **cgtgg**accgttatt... 3′

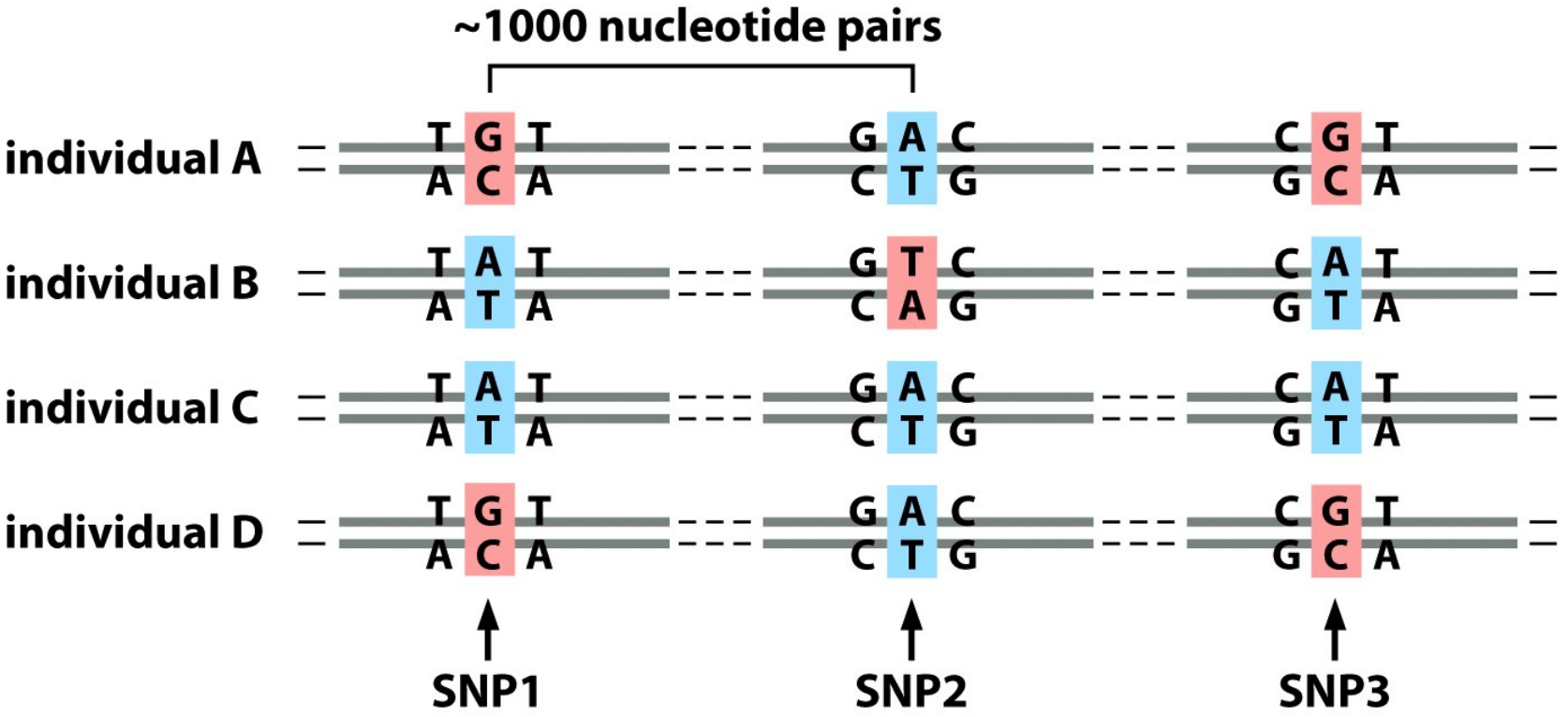
n=2

POLYMORPHISMS
VARIABLE NUMBER OF TANDEM REPEATS (VNTR)

Forensic tests

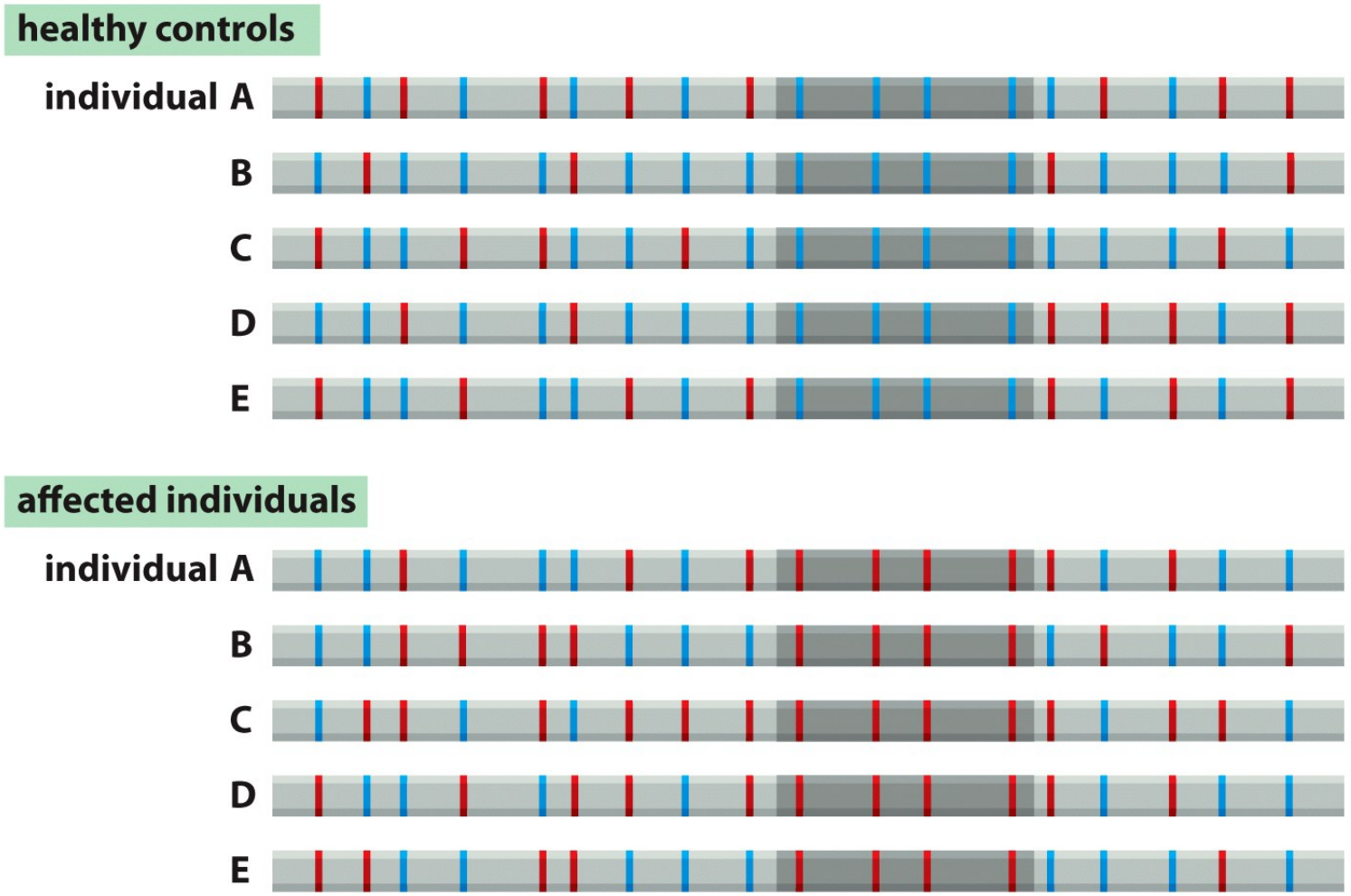


SINGLE NUCLEOTIDE POLYMORPHISM (SNP)



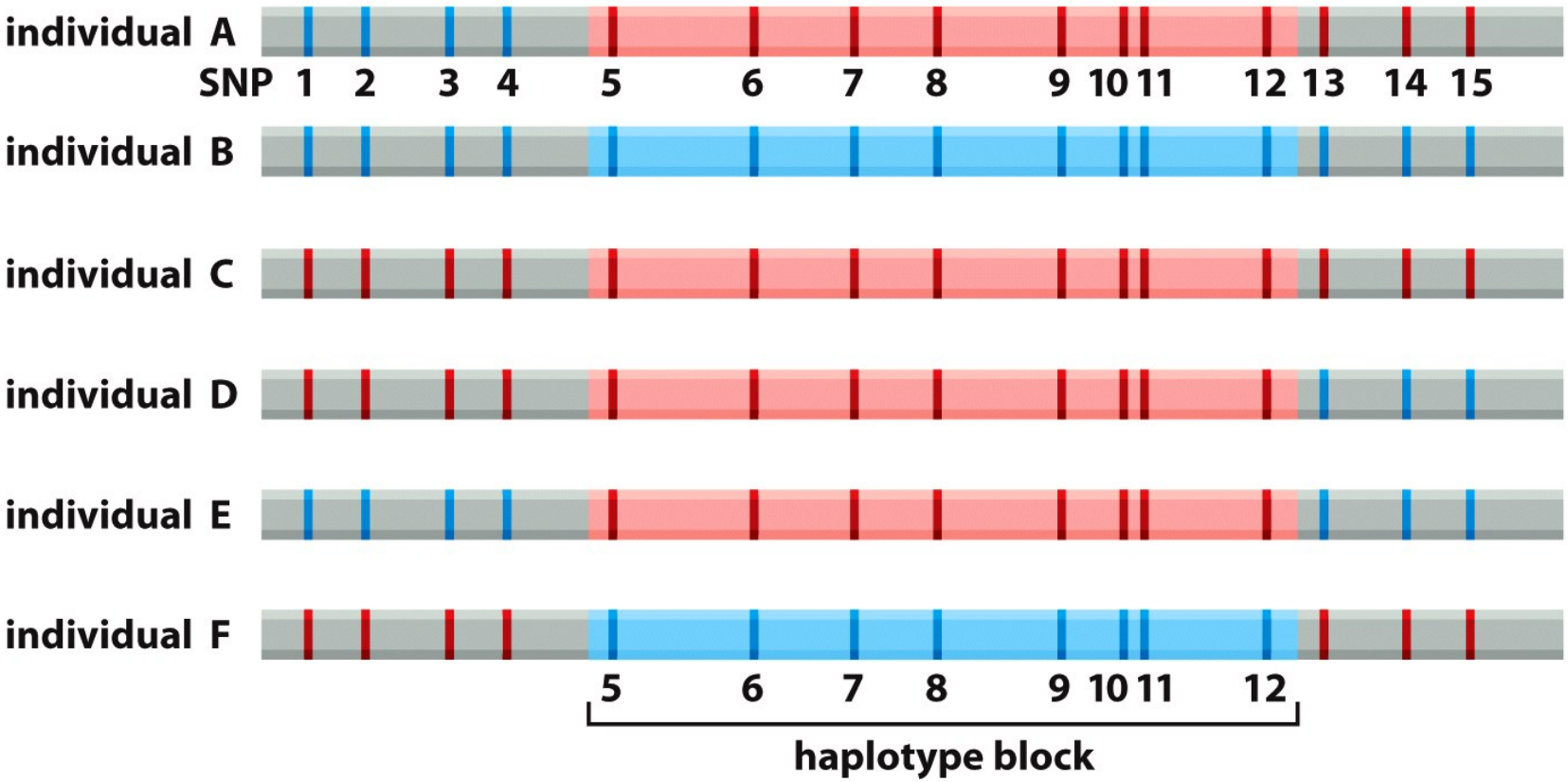
SNP are locations in the genoma with two or more alternative variants in a single nucleotide which are common in general population

SINGLE NUCLEOTIDE POLYMORPHISM (SNP)



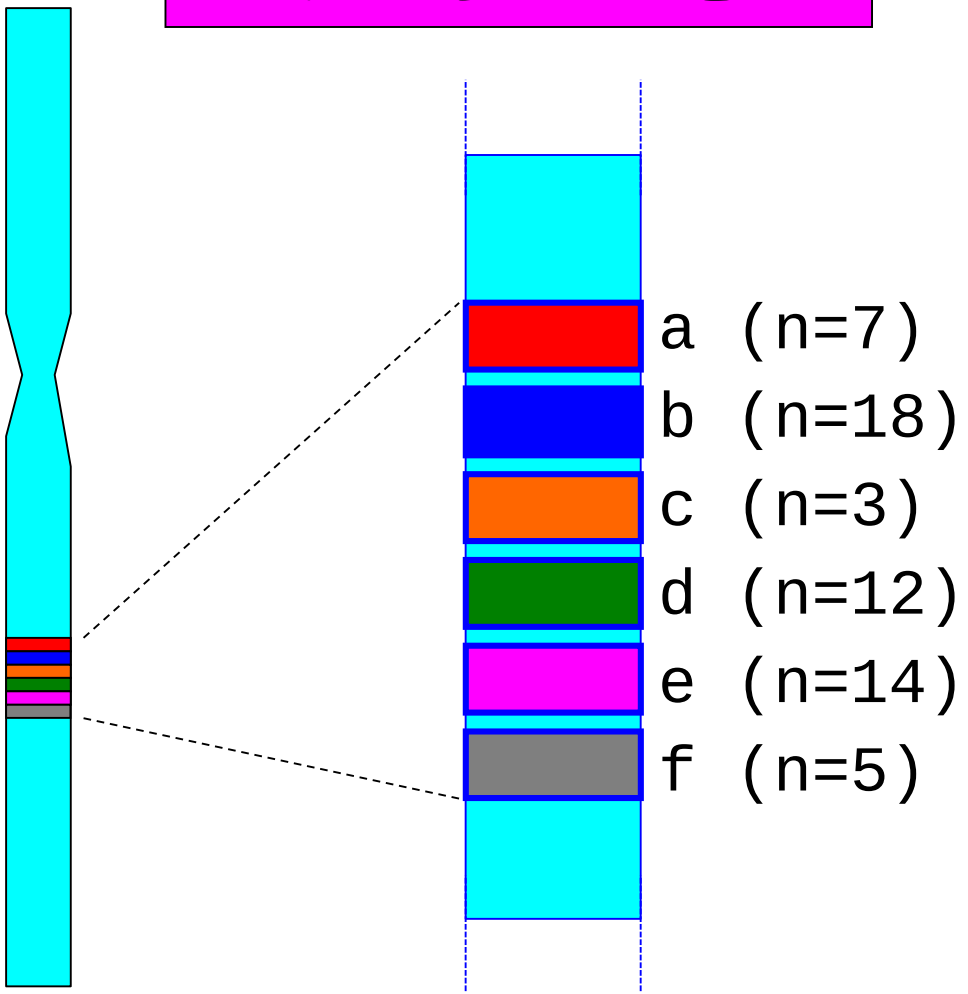
Genes affecting common diseases predisposition may be located by a binding to a SNP

POLIMORPHISMS HAPLOTYPE

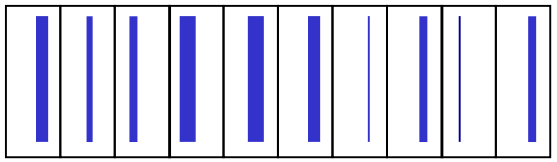


Loci group, with multiple allelic variants, which are inherited as a unit. Never (almost never) recombine during meiosis.

POLIMORPHISMS
HAPLOTYPE

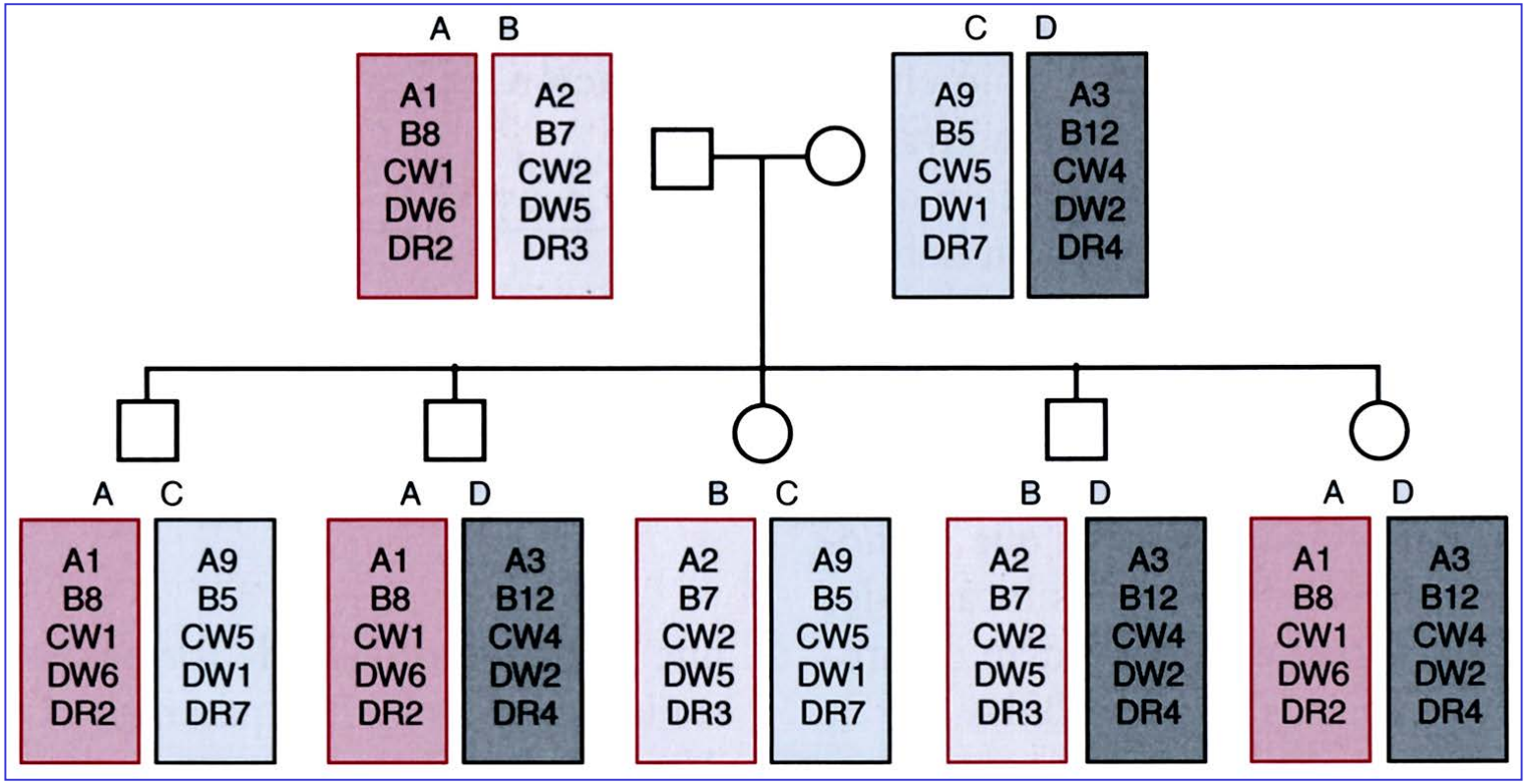


They form a unique and distinctive genetic pattern for each individual



Barcode

POLIMORPHISMS HAPLOTYPE



HLA haplotype inheritance

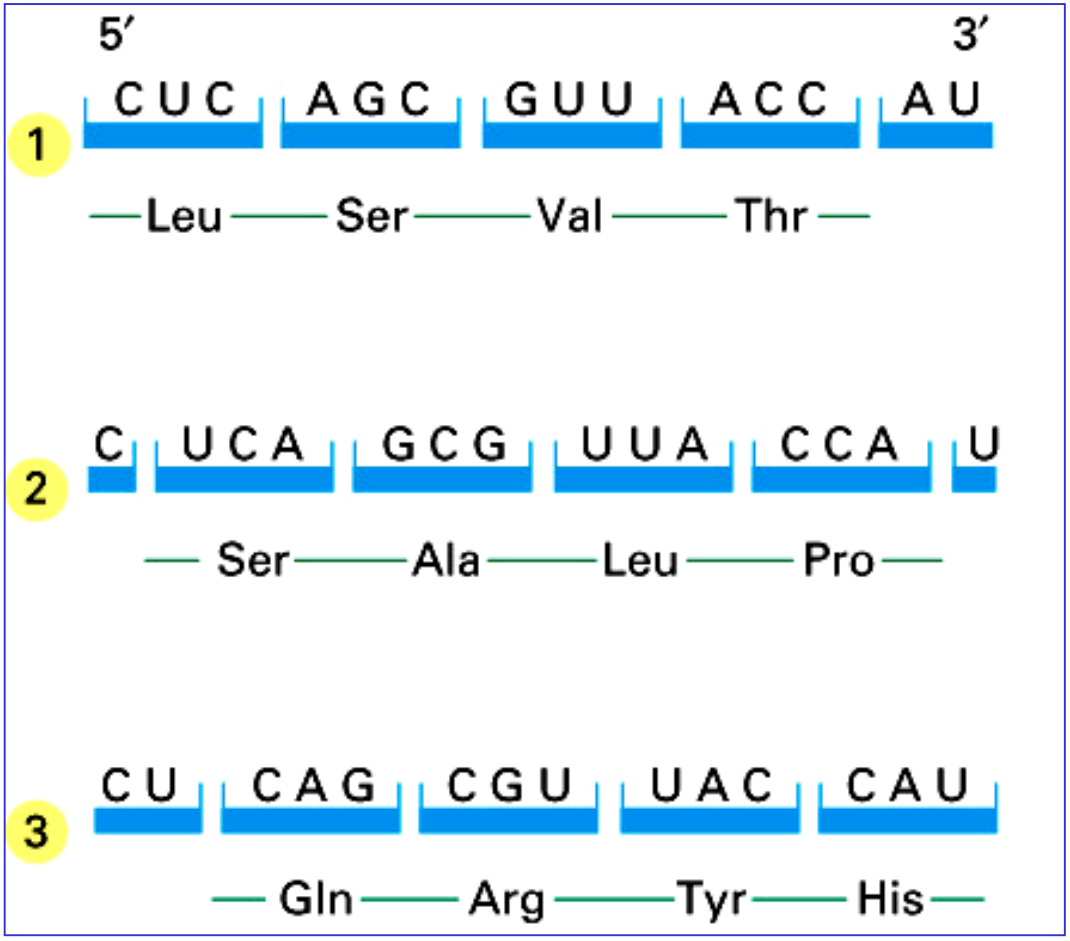
MUTATIONS

Genetic code

	AGA AGG									
GCA	CGA						GGA			
GCC	CGC						GGC		AUA	
GCG	CGG	GAC	AAC	UGC	GAA	CAA	GGG	CAC	AUC	
GCU	CGU	GAU	AAU	UGU	GAG	CAG	GGU	CAU	AUU	
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	
A	R	D	N	C	E	Q	G	H	I	
UUA					AGC					
UUG					AGU					
CUA				CCA	UCA	ACA			GUA	
CUC				CCC	UCC	ACC			GUC	UAA
CUG	AAA		UUC	CCG	UCG	ACG		UAC	GUG	UAG
CUU	AAG	AUG	UUU	CCU	UCU	ACU	UGG	UAU	GUU	UGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

MUTATIONS

Variations in the reading pattern of a DNA sequence



One DNA sequence may be read in three different patterns (can code three different polypeptides) depending on the initial reading point

TYPES OF MUTATIONS

By type of cell affected

Germinal

- They are originated at meiosis (gametogenesis)
- Through porter gamete, it is transmitted to all cells of progenie cells
- Germinal cells of the progenie also contain the mutation and transmit to descendents
- The impact dependes on the function affected by the mutation (if essential, probably early abortion, sometimes undetected; if not essential but important, a inherited disease or sub-normality)

Somatic

- They appear in a somatic diploid cell at some organ, tissue or isolated cell, during development or at adult stage
- They affect only descendent cells, not all the organism
- The earlier the mutation appears, the larger the affected area
- The adult individual is a genetic mosaic: presence of two or more cell lines genetically different, coming from a unique zygote
- Non inheritable

TYPES OF MUTATIONS

By mutation size

- Large mutations (chromosomal anomalies)
- Small or puntual mutations

Changes in the DNA sequence

Substitution
Loss (deletion)
Insertion

By consequences on expressed protein

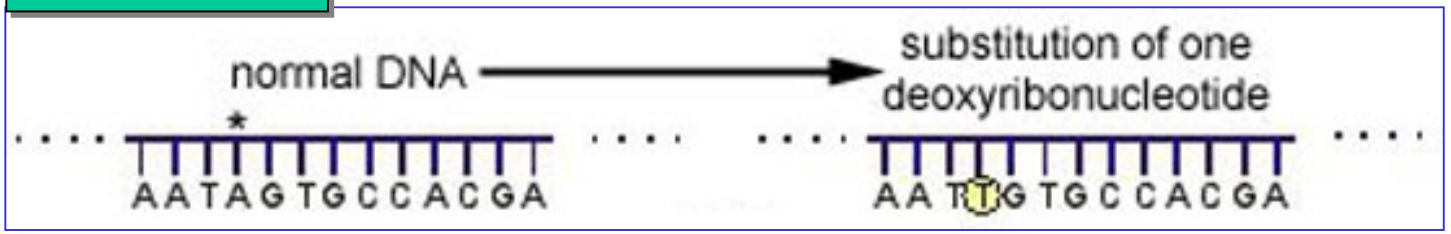
- Silent mutations
- No silent mutations

Mutations affecting the meaning
Mutations affecting the reading frame
Mutations with premature protein end
Mutations with delayed protein end

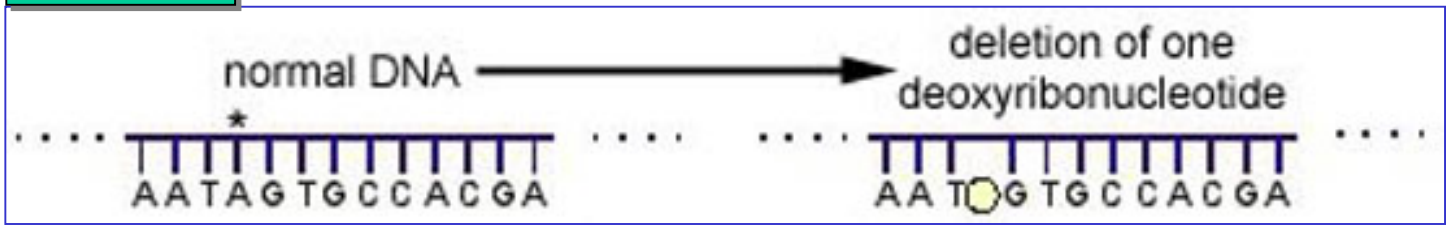
MUTATIONS

Changes in the DNA sequences

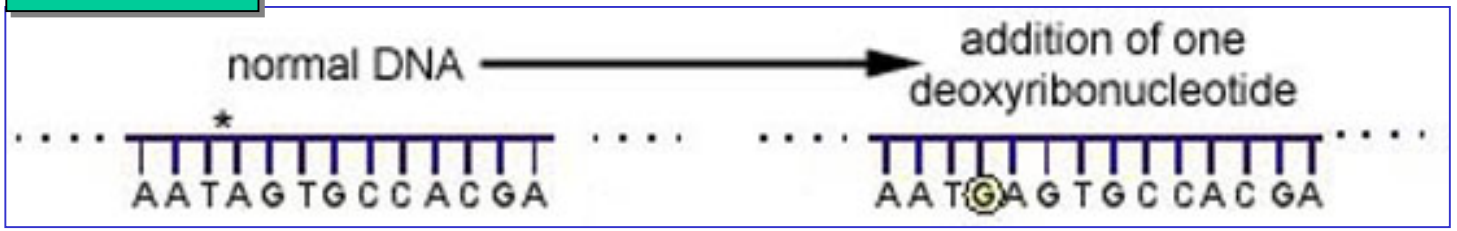
Substitution



Deletion



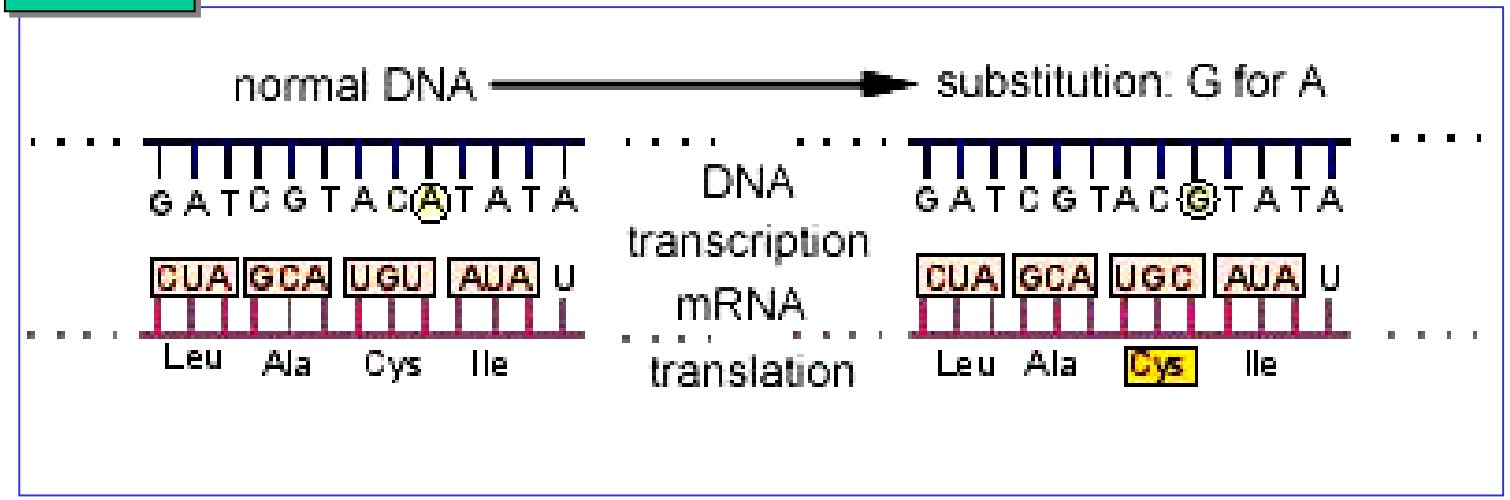
Insertion



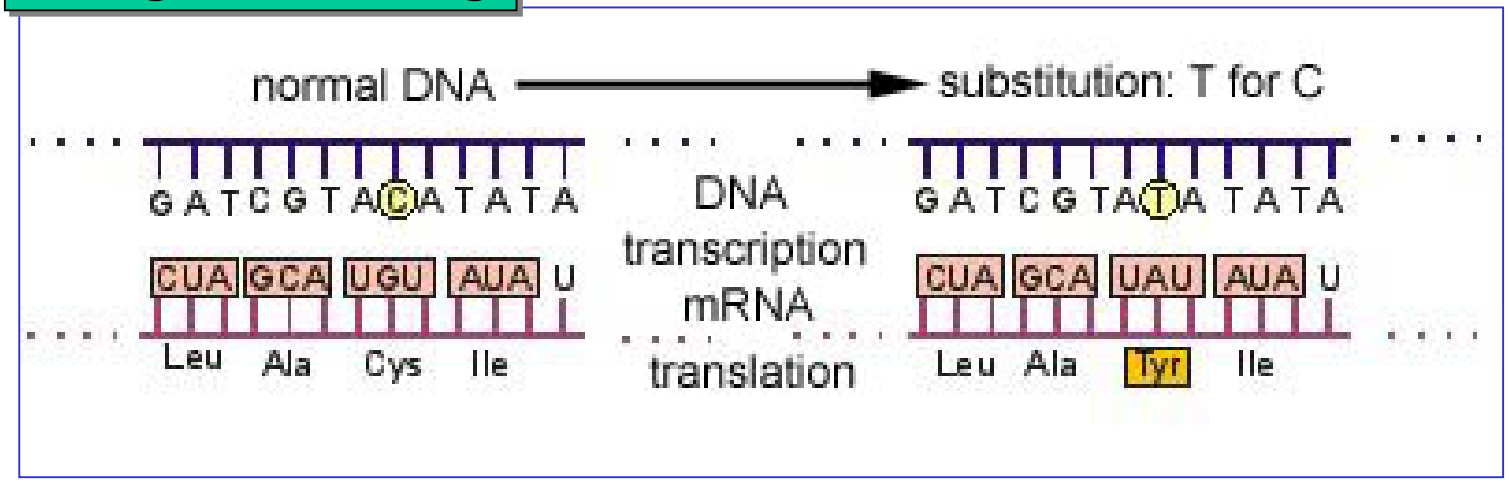
MUTATIONS

Consequences

Silent



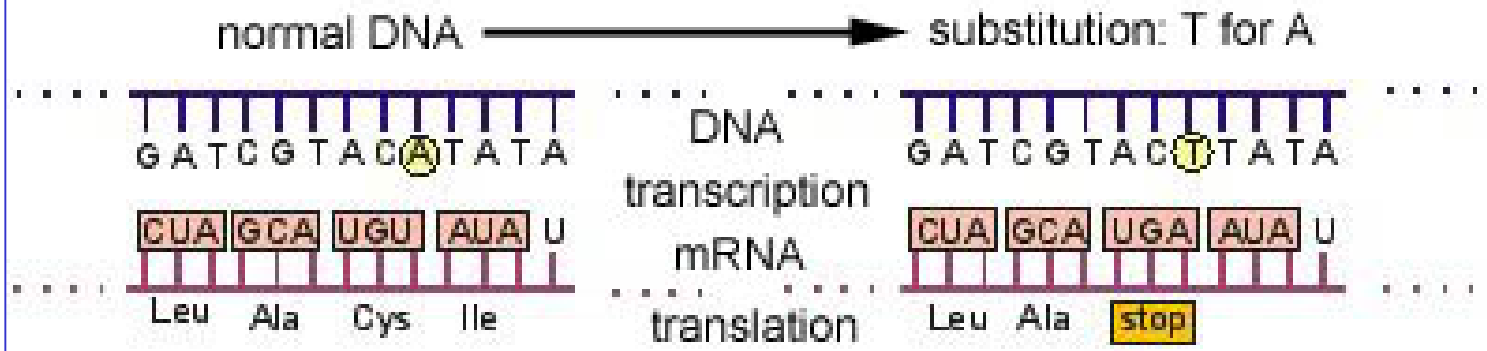
Change in meaning



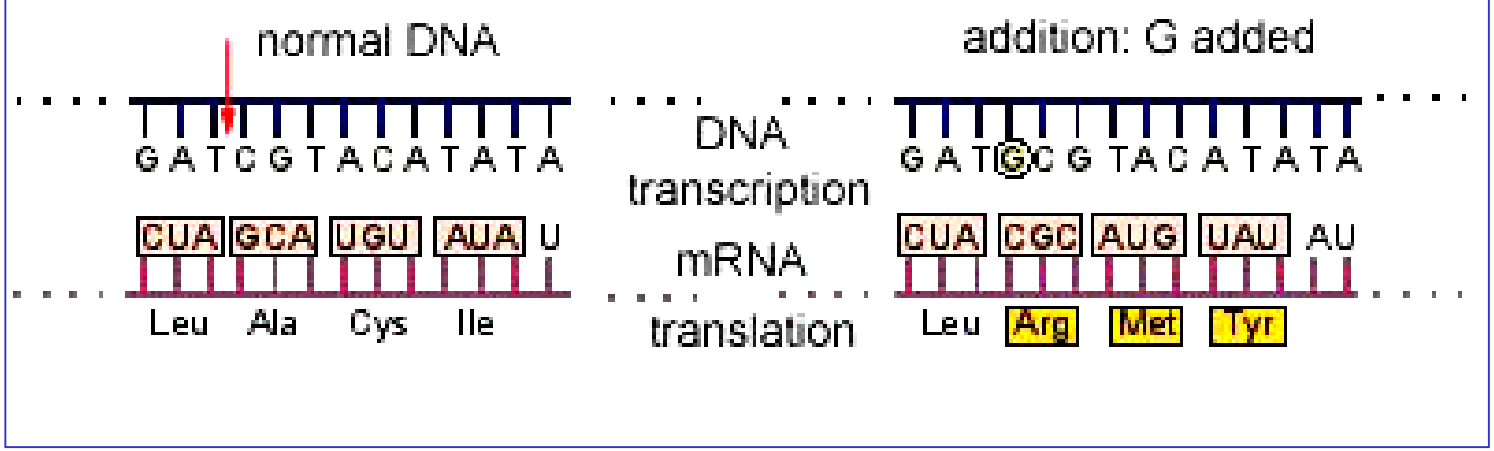
MUTATIONS

Consequences

No meaning (premature end)



Change in reading frame

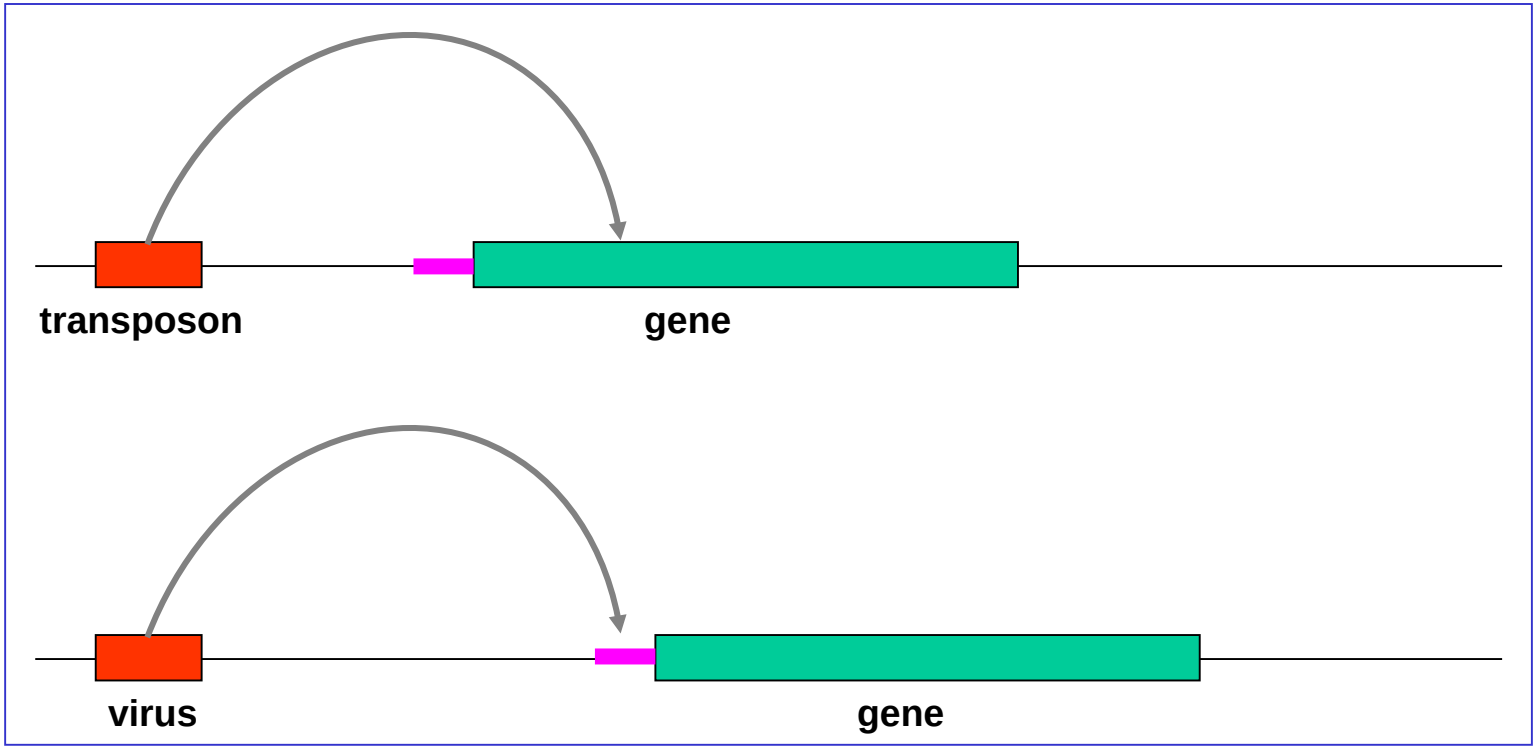


MUTATIONS

Insertions

Expansive (trinucleotides)

Points with increased mutation rate



ASSIGNATURA: BIOLOGIA (34446).
GRAU: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

ASIGNATURA: BIOLOGÍA (34446).
GRADO: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

DNA REPAIR

- Sources of DNA damage

- Types of DNA lesions

- Repair mechanisms

1. Direct reversal
2. Base excision repair (BER)
3. Nucleotide excision repair (NER)
4. Mismatch repair (MMR)
5. Double-strand break repair

Sources of DNA damage

- Normal cell metabolism and biochemistry (e.g. endogenous production of ROS)
- Some radiation wavelengths:
  - Ionizing radiation (e.g. gamma radiation or X-rays)
  - UV radiation
- Free radicals
- Natural or man-made environmental chemicals:
  - Hydrocarbons, smoke from tobaco, so on.
  - Natural products, like aflatoxins

DNA damage vs mutation

- **DNA damage** involves physical abnormalities in the DNA (e.g. strand breaks, new residues like 8-hydroxydeoxyguanosine, and so on).
  - It can be recognized by enzymes and repaired if complementary information is present
  - If not repaired, transcription of the affected gene may be prevented and even replication can be blocked

- **Mutation** involves a change in the genetic sequence.
  - It is not repaired and therefore causes changes in protein function
  - Mutations are replicated and propagated

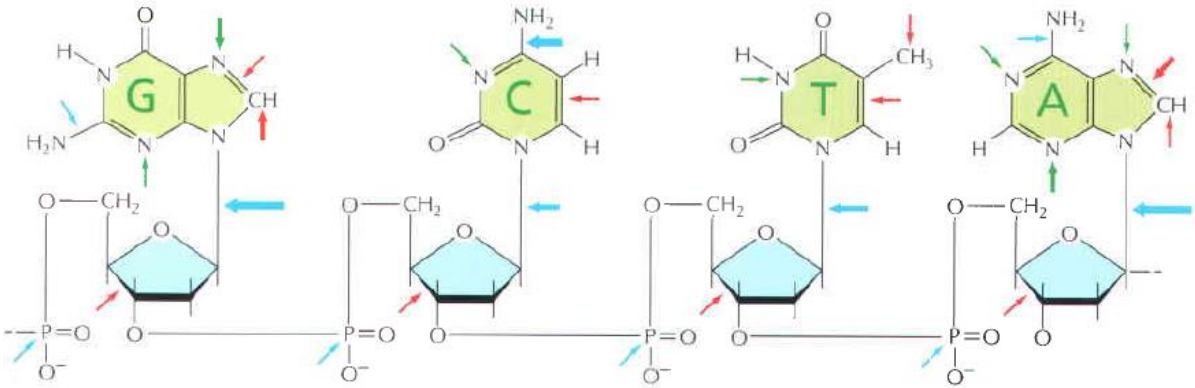
Types of DNA lesions

- Covalent binding between bases (crosslinks)
  - Intrastrand
  - Interstrand

- Chemical modifications or base loss
  - The four bases (A, T, G, C) may experience chemical modifications (e.g. deamination, methylation, so on). …
  - Loss of bases, mostly purines (A, G)

- Mismatch of bases, due to errors in DNA replication

- Strand breaks
  - Single strand break
  - Double strand break



Oxidation hydrolysis alkylation

Figure 5-37 *Molecular Biology of the Cell. Sixth edition*, © 2015

Types of DNA lesions

Crosslinks

Covalent binding between bases

UV irradiation (sunlight)

- Intrastrand
- Adjacent pyrimidine bases

↓ Replication

Deletion

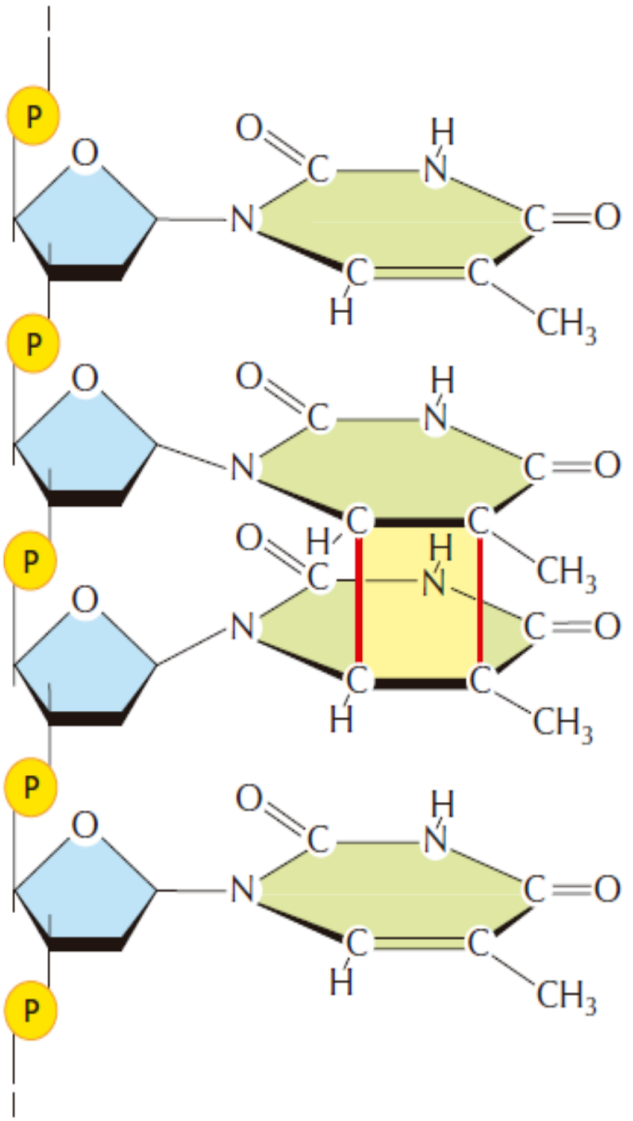


Figure 5-39 *Molecular Biology of the Cell. Sixth edition,* © 2015

Types of DNA lesions

Chemical modifications

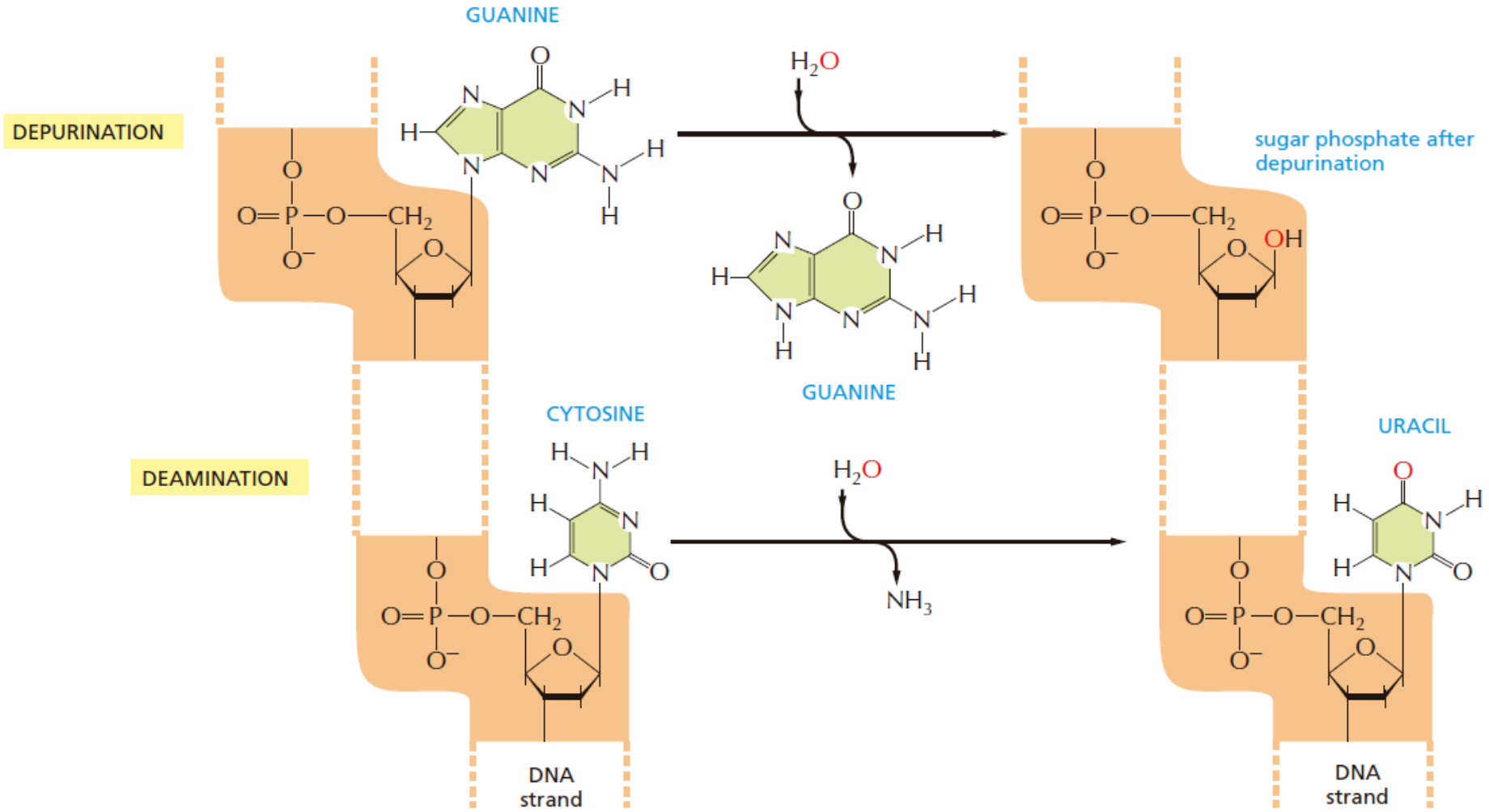


Figure 5-38 *Molecular Biology of the Cell. Sixth edition,* © 2015

Types of DNA lesions

Chemical modifications

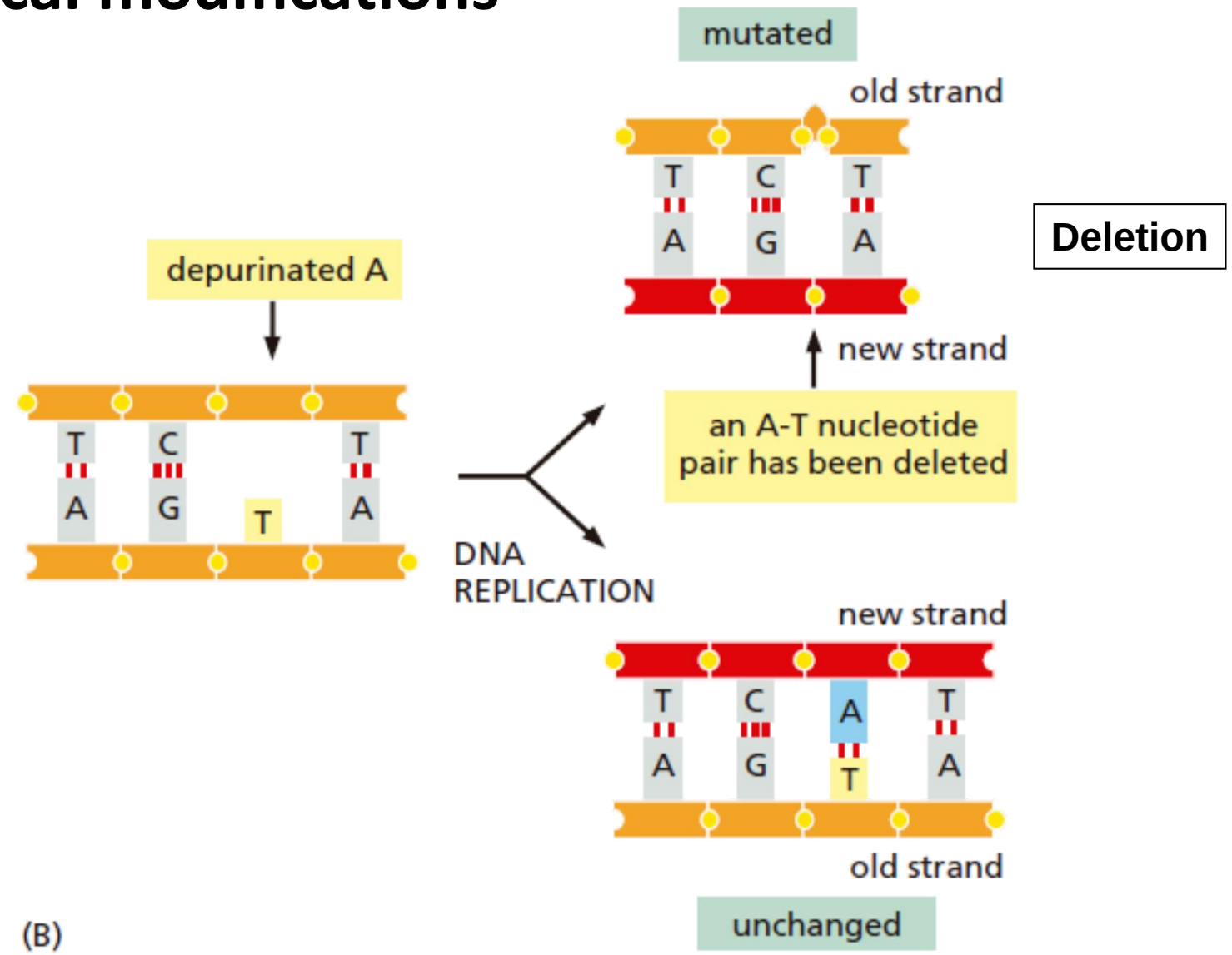


Figure 5-40B *Molecular Biology of the Cell. Sixth edition,* © 2015

Types of DNA lesions

Chemical modifications

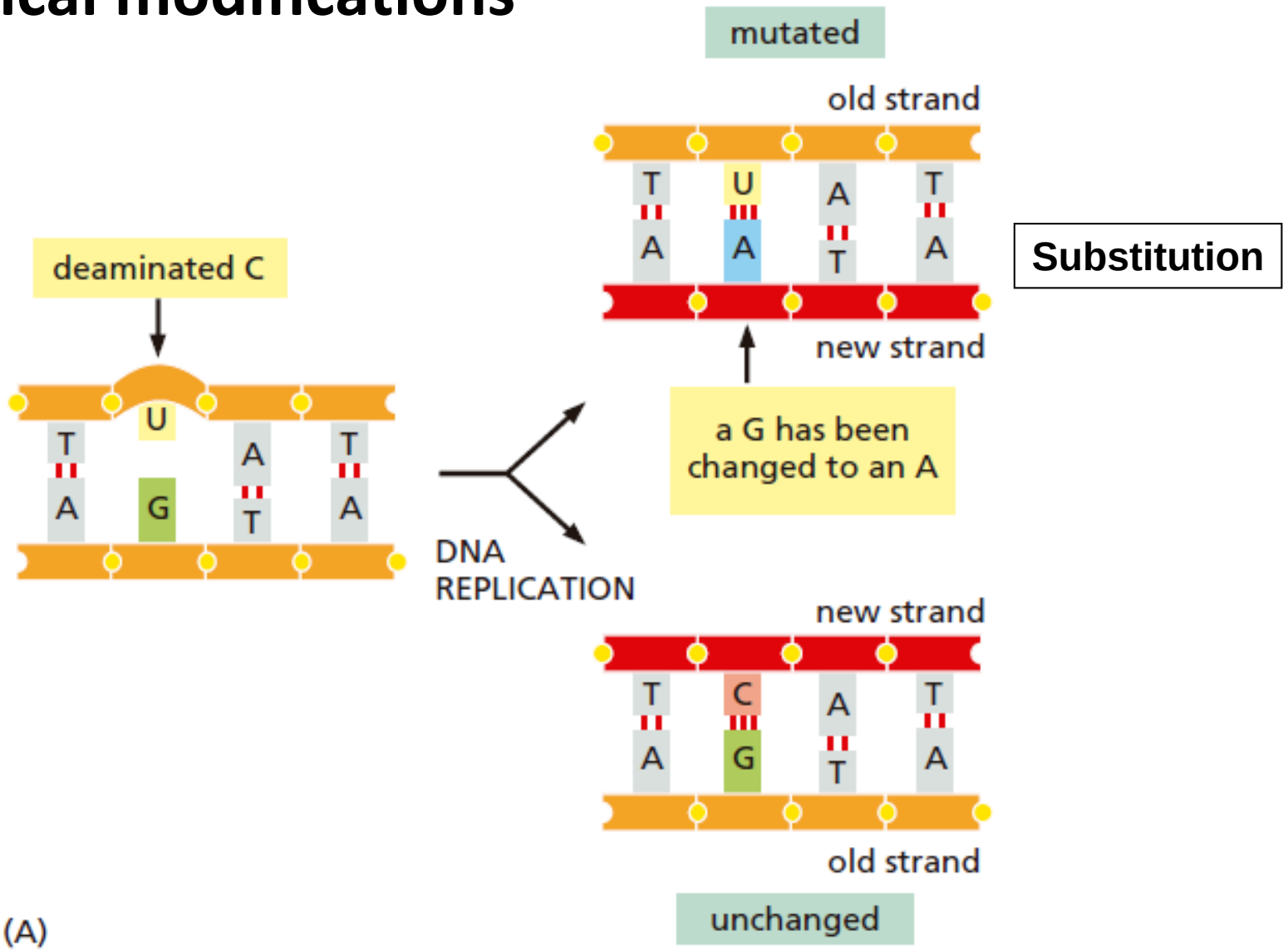


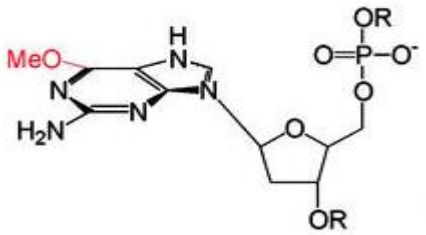
Figure 5-40A *Molecular Biology of the Cell. Sixth edition,* © 2015

1. Direct reversal

• It does <u>not need template</u> as damage occurs in specific bases

• <u>Alkylation of bases:</u> usually methylation of guanine, e.g. O(6) methylguanine, 7-methylguanine, 1-methyladenine). This change produces <u>GT pairing</u> on replication.

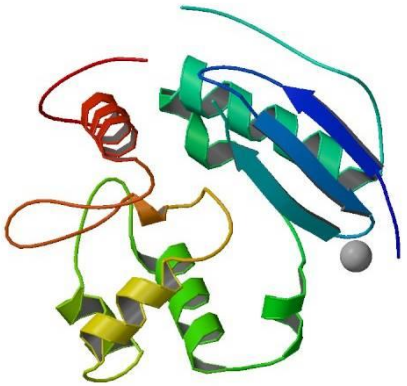
• Methylguanine DNA methyltransferase (<u>MGMT</u>) can remove the methyl group of methylated guanines by transferring it to its own cysteine active site, in a methyl-cysteine stable link.
However, the protein is irreversibly inactivated and needs to be degraded at the proteosome (suicide protein).



Pairs with T instead of C

O^6MeGua

MGMT

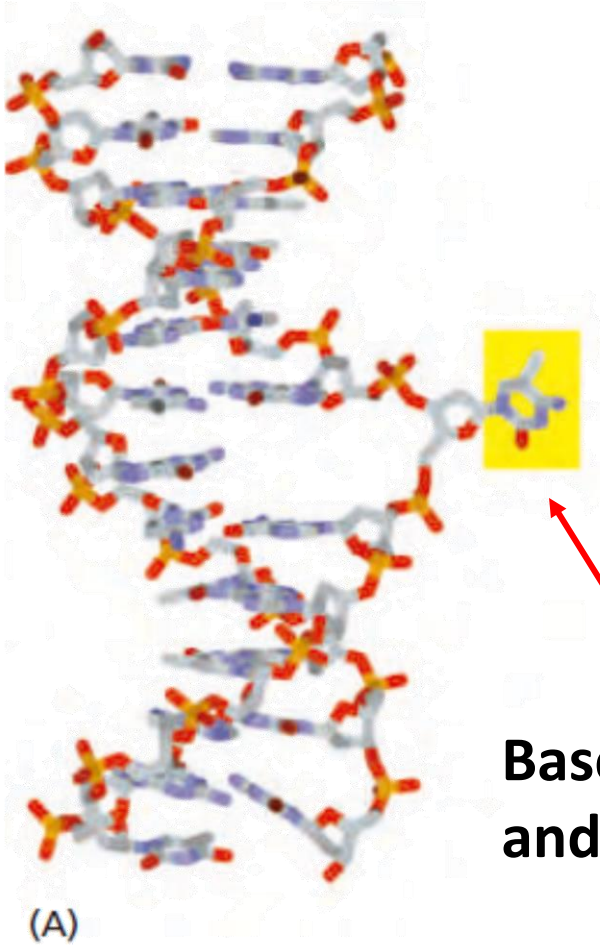


2. Base excision repair (BER)

This process repairs DNA with **modified bases** (deaminated citosines and adenines, alkylated or oxidized bases, etc) or DNA with **bases loss** (apurinic or apyrimidinic sites).

- Even coupled to transcription

DNA glycosylases: specific (+6 variants). They recognize deaminated Cs, deaminated As, alkylated/oxidized bases, bases with opened ring…



Base-flipping and checking

Figure 5-42A *Molecular Biology of the Cell. Sixth edition*, © 2015

Repair mechanisms

2. Base excision repair (BER)

1. Recognition and removal of damaged bases by **specific DNA glycosylase**

2. Cut and removal of deoxyribophosphate residue from the DNA strand by **AP endonuclease** and **phosphodiesterase**
(in a depurination case this is step 1)

3. Substitution for the correct nucleotide by **DNA β polymerase**

4. Linking of the strand by **DNA ligase**

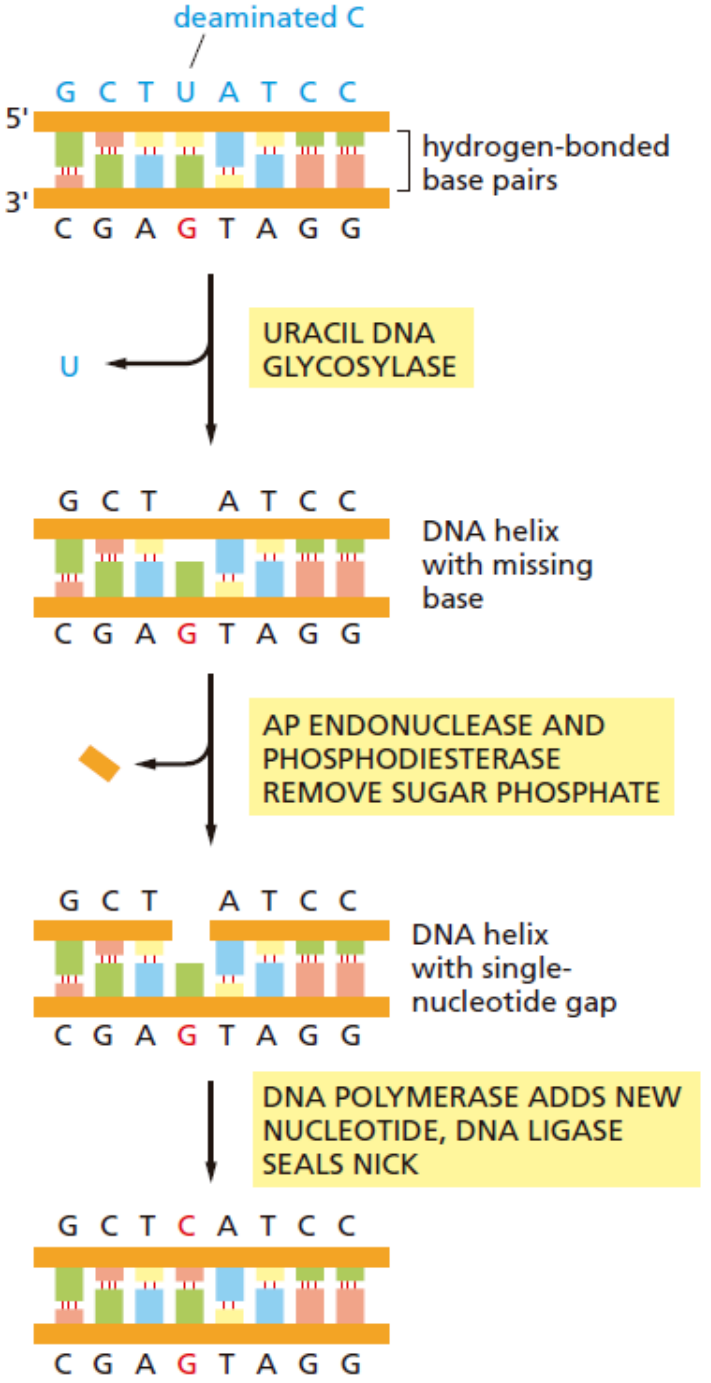


Figure 5-41A *Molecular Biology of the Cell. Sixth edition*, © 2015

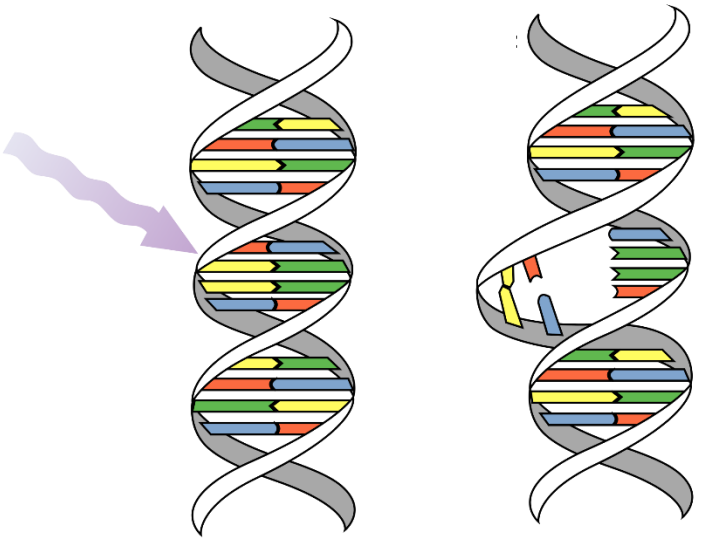
3. Nucleotide excision repair (NER)

This process repairs **prominent DNA structural lesions** (pyrimidine dimers, covalent binding with hydrocarbons like benzopyrene, so on).

- NER may happen even coupled to transcription

In humans (simplification):

- Detection of the structural change in the duplex DNA (DNA helicases or other proteins)
- **Helicases** unwind the DNA duplex locally
- Excision **nucleases** cut extremes 5' and 3' (about 30 nt)
- **DNA polymerase** and **ligase** repair the segment



Wikimedia commons

Repair mechanisms

3. Nucleotide excision repair (NER)

Bacterial mechanism:

1. Recognition of the DNA lesion and cut of extremes 5’ and 3’ by **nuclease** protein complex

2. Recruiting by the protein **complex** of **DNA helicase** proteins which remove altered segment

3. Substitution by correct nucleotides by **DNA polymerase.**

4. Linking of the strands by **DNA ligase**

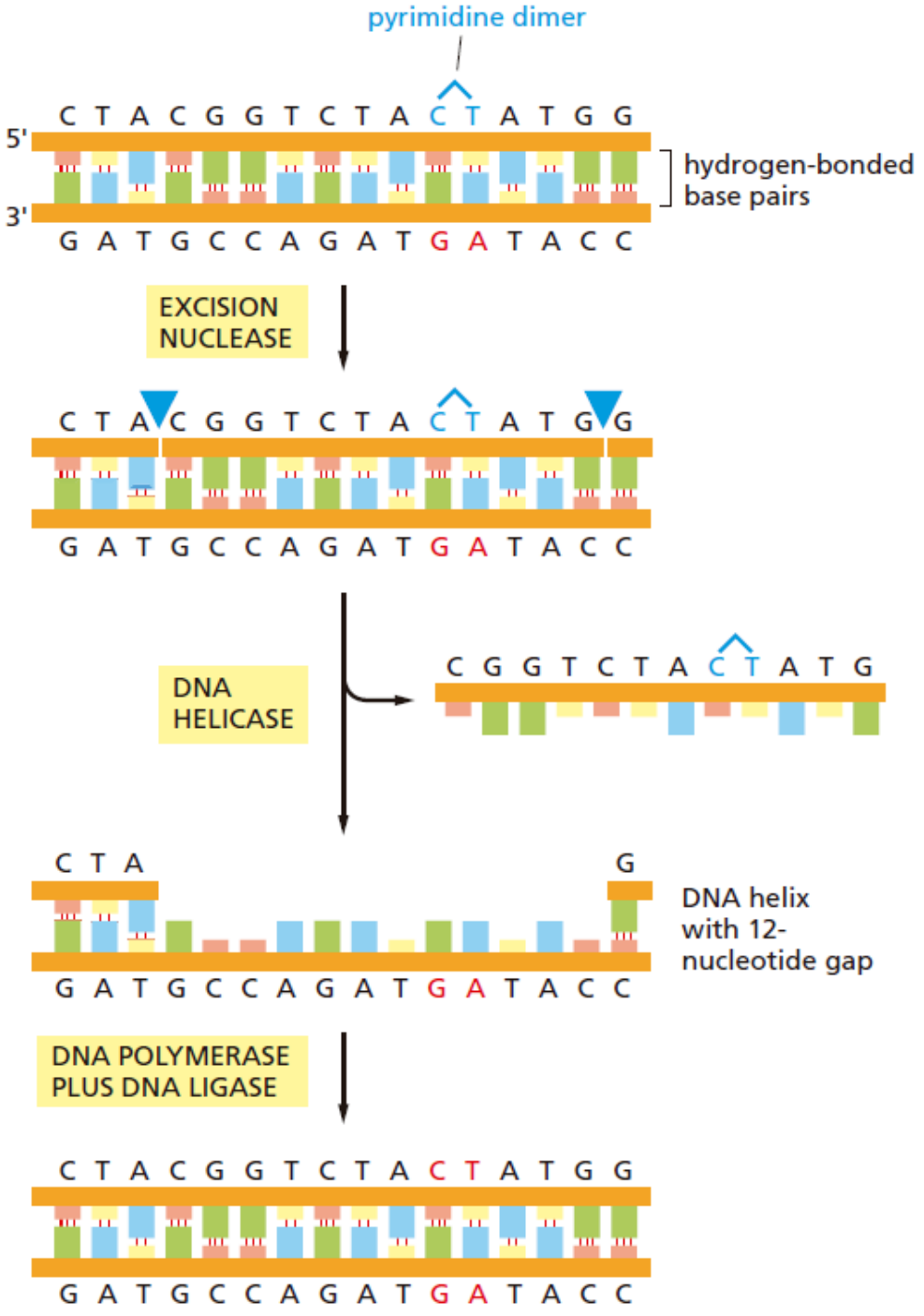


Figure 5-41B *Molecular Biology of the Cell. Sixth edition,* © 2015

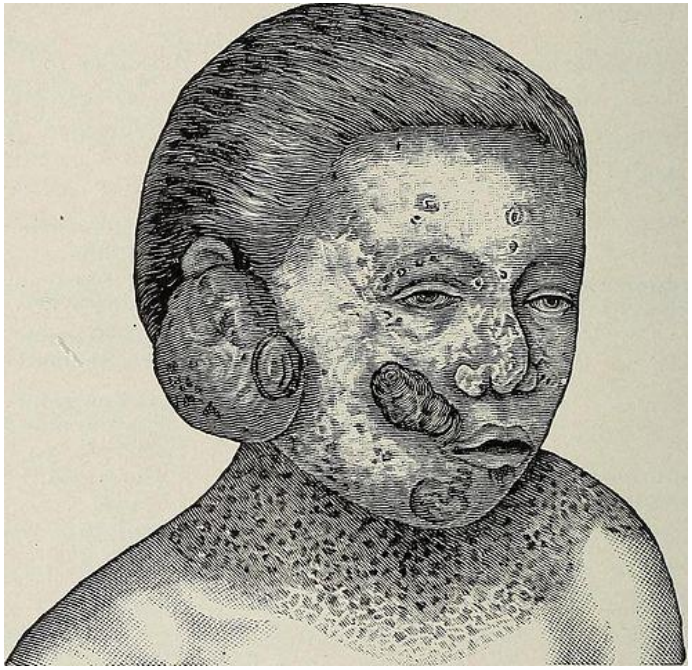
3. Nucleotide excision repair (NER)

Xeroderma pigmentosum (XP)

XP is a inherited disease which predisposes patients to skin cancer and to pigmentary lesions in skin areas exposed to the sun. It is produced by mutations in genes involved in NER mechanisms.

Some of these proteins are:

- XPA and XPC, which **recognize** and bind to lesion area and recruit NER needed proteins
- XPB and XPD, which are subunits if the transcription factor IIH with **helicase** activity
- XPF **cuts** the strand at the lesion 5' side
- XPG **cuts** the strand at the lesion 3' side



Wikimedia commons

4. Mismatch repair

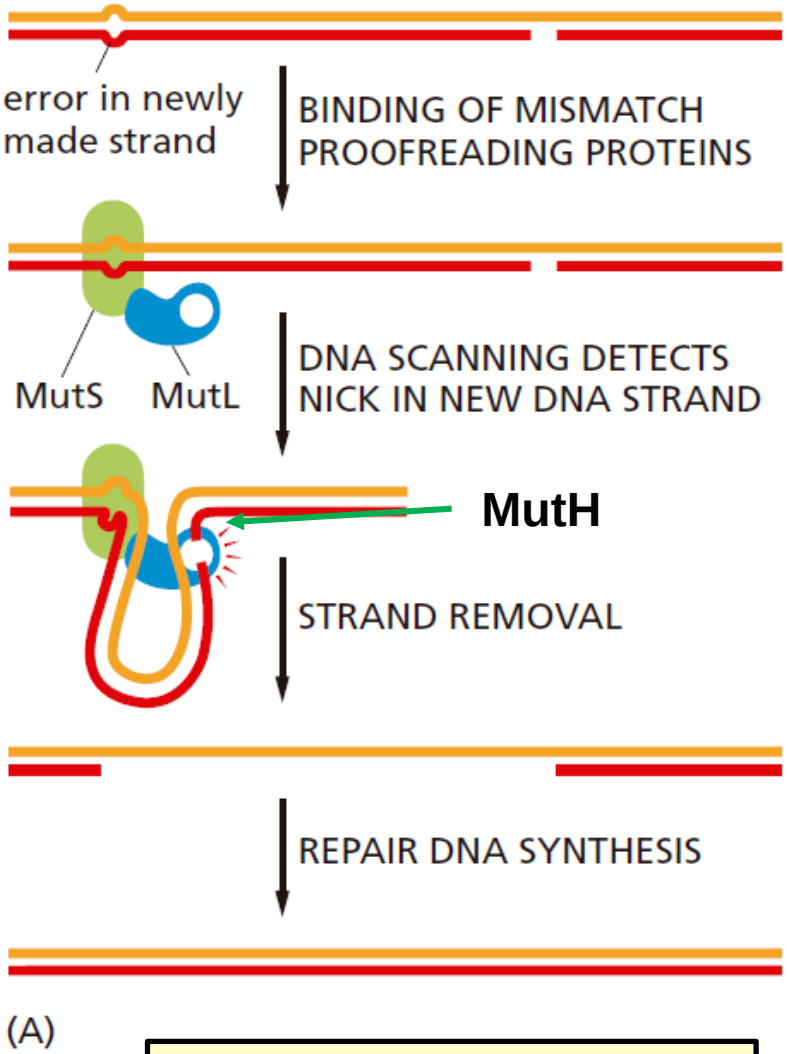
This process repairs mismatches between the base pairs (no complementary bases) introduced in the DNA during replication

1. Recognition of the lesion in the DNA by **MutS complex**

2. Addition and recognition of the new strand by **MutL** complex

3. Cut and removal of damaged fragment by **exonuclease I** and substitution by correct nucleotides by d **DNA polymerase**

4. Linking of the strand by **DNA ligase**

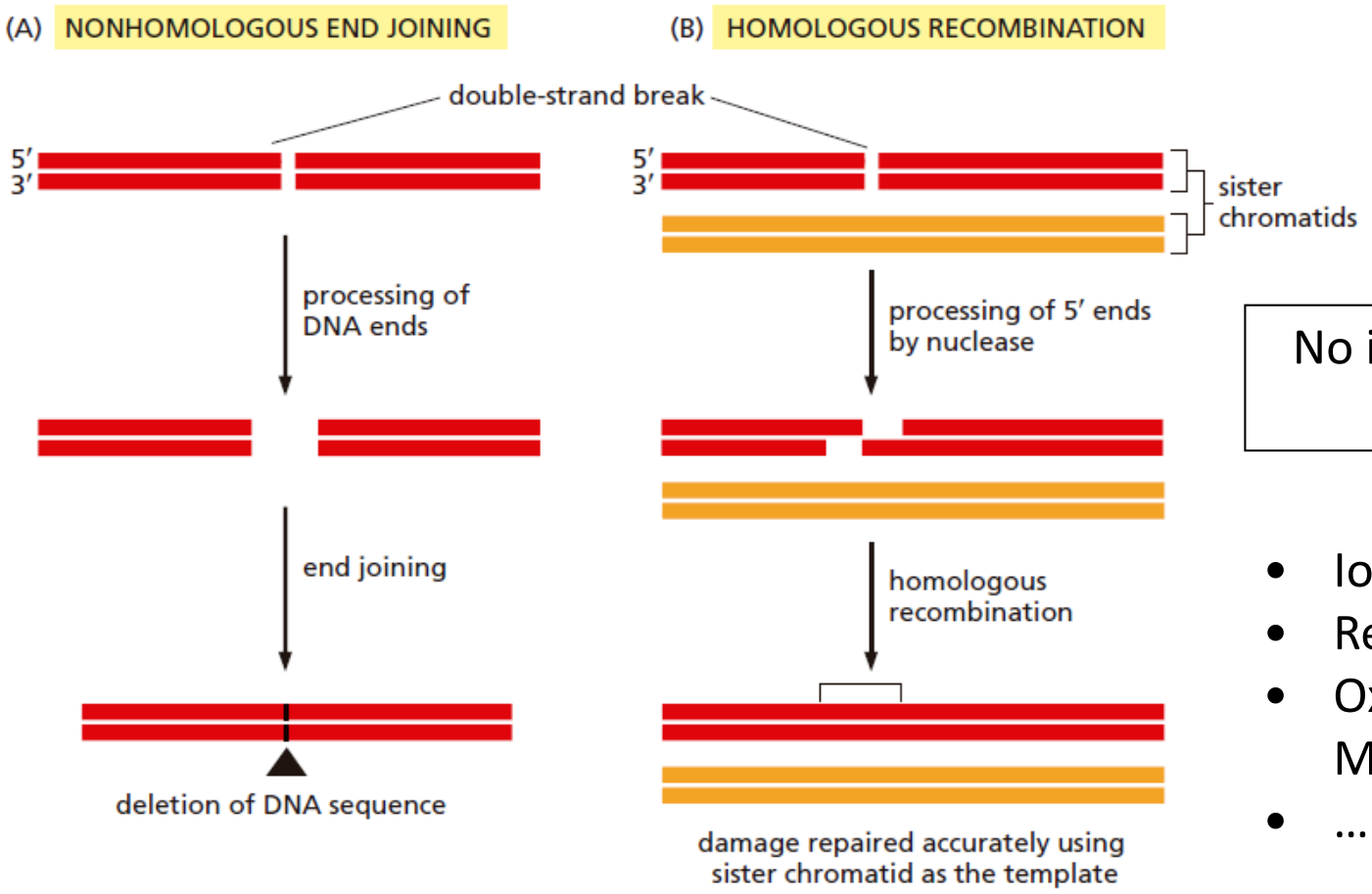


E. coli: MutS, MutL, MutH
Humans: MSH, MLH, PMS.

Figure 5-19 *Molecular Biology of the Cell. Sixth edition*, © 2015

5. Double-strand break repair

This process allows repairing of DNA with broken double strand. These are specially dangerous as they can lead to chromosomal rearrangements.



No intact template strand

- Ionizing radiation
- Replication errors
- Oxidizing agents Metabolism
- …

Figure 5-45 *Molecular Biology of the Cell. Sixth edition,* © 2015

5. Double-strand break repair

Nonhomologous end joining (NEHJ)

Direct binding of broken ends.

Common in somatic cells.

It may produces loss of some nucleotides.

- Ku heterodimers grasps the broken chromosome ends
- Variety of repair proteins hold, process and join the breaking ends

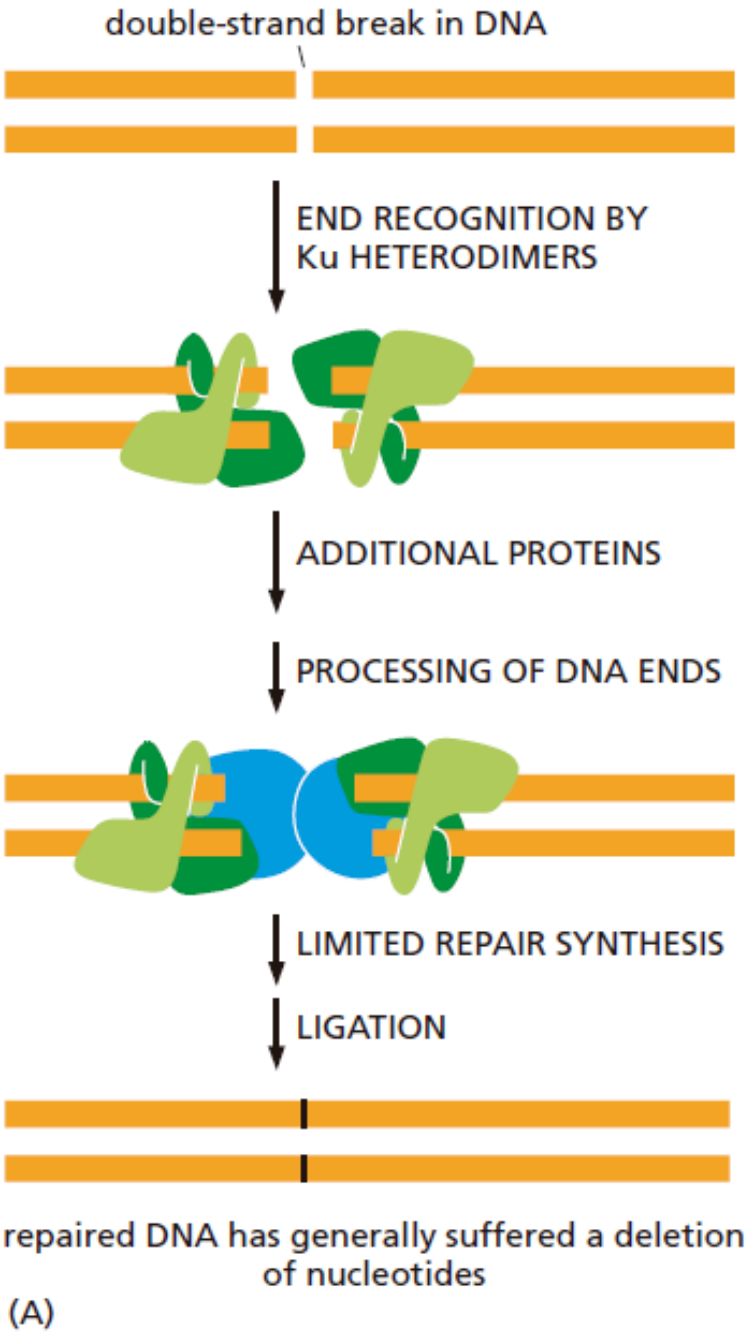


Figure 5-46A *Molecular Biology of the Cell. Sixth edition,* © 2015

5. Double-strand break repair

Homologous recombination

Similar process to homologous recombination in meiosis

Similar proteins involved in NEHJ

1.DURING replication:

Replication fork collapses

- Undamaged chromatid
- Incomplete chromatid

1.Nuclease degradation of 5' end

2.One strand invasion

3.DNA polymerization

4.Strand breakage (from undamaged chromatid)

5.Replication fork restarts

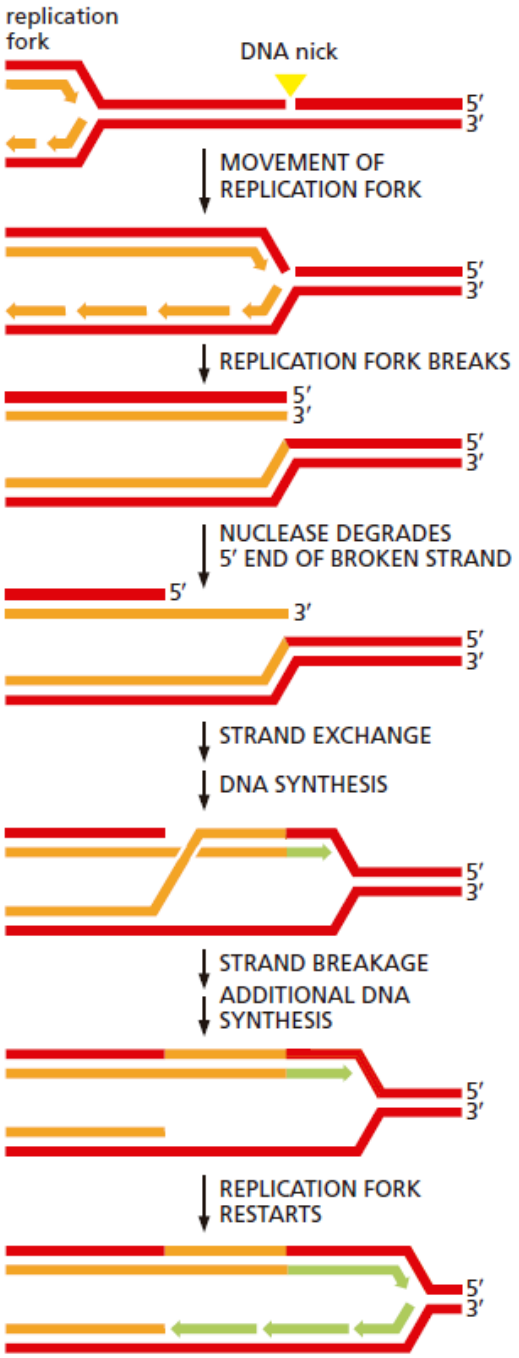


Figure 5-50 *Molecular Biology of the Cell. Sixth edition,* © 2015

5. Double-strand break repair

Homologous recombination

Accidental breakages “pre” or “post” replication

2. POST replication:

Sister chromatids are joined

Undamaged chromatid acts as mold

3. PRE replication:

No sister chromatide

Homologous chromosome can be used

No information is lost

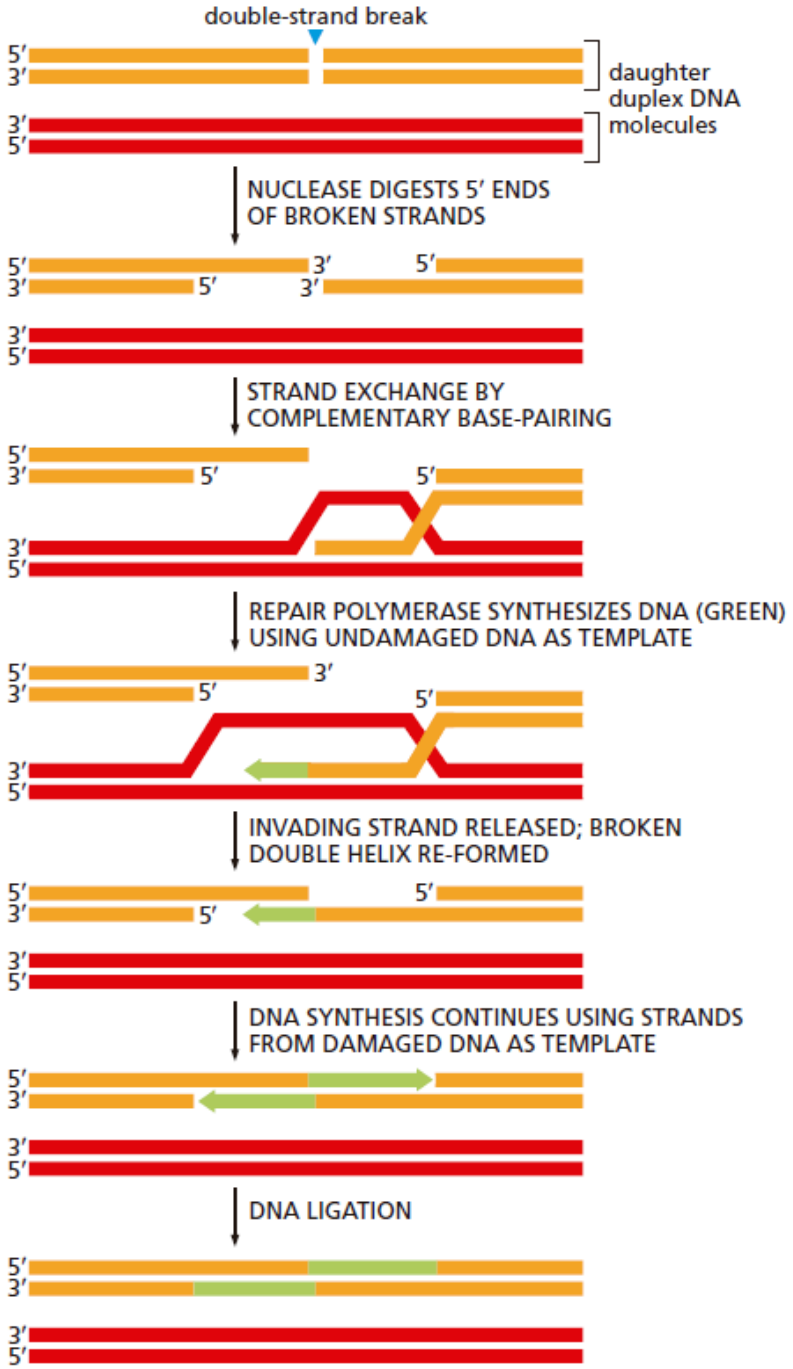
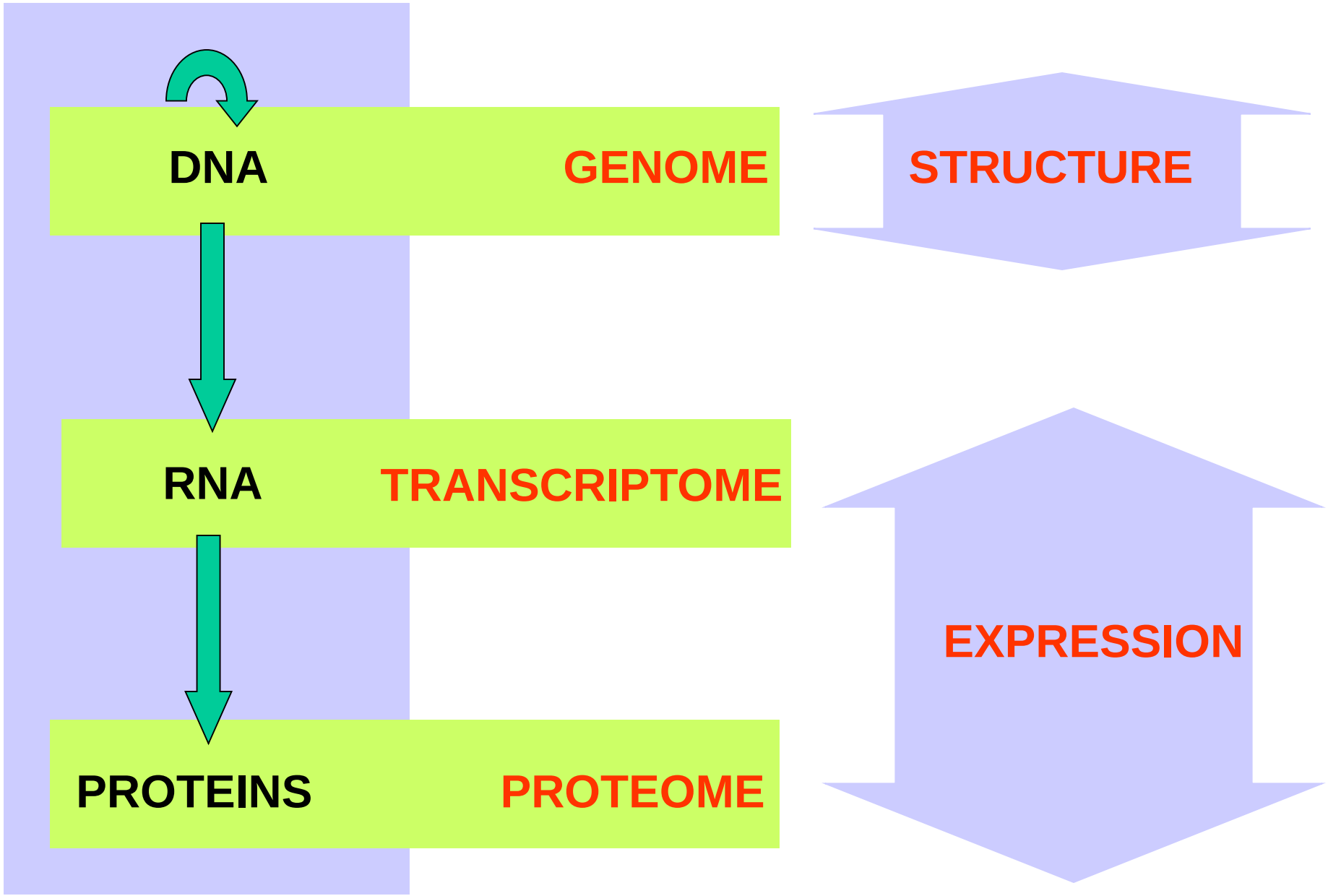


Figure 5-50 *Molecular Biology of the Cell. Sixth edition,* © 2015

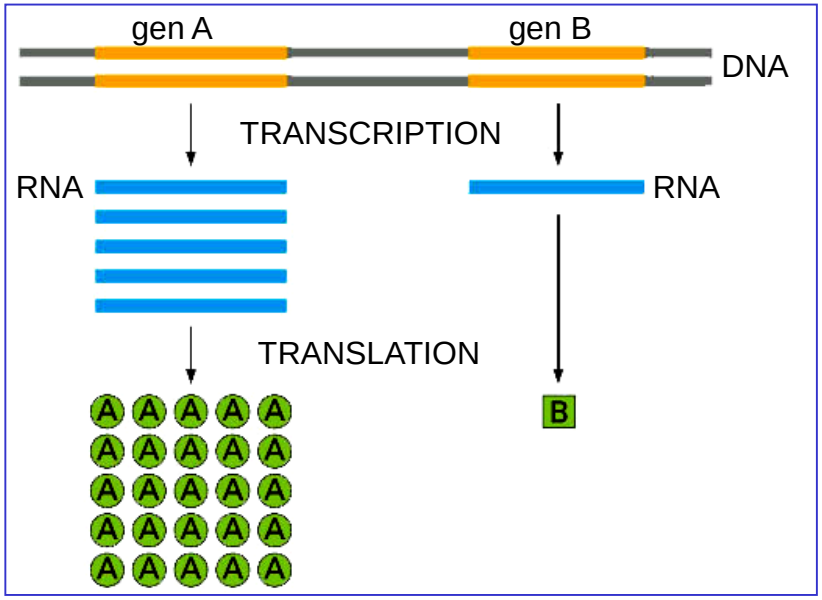
GENE REGULATION IN EUCARYOTES

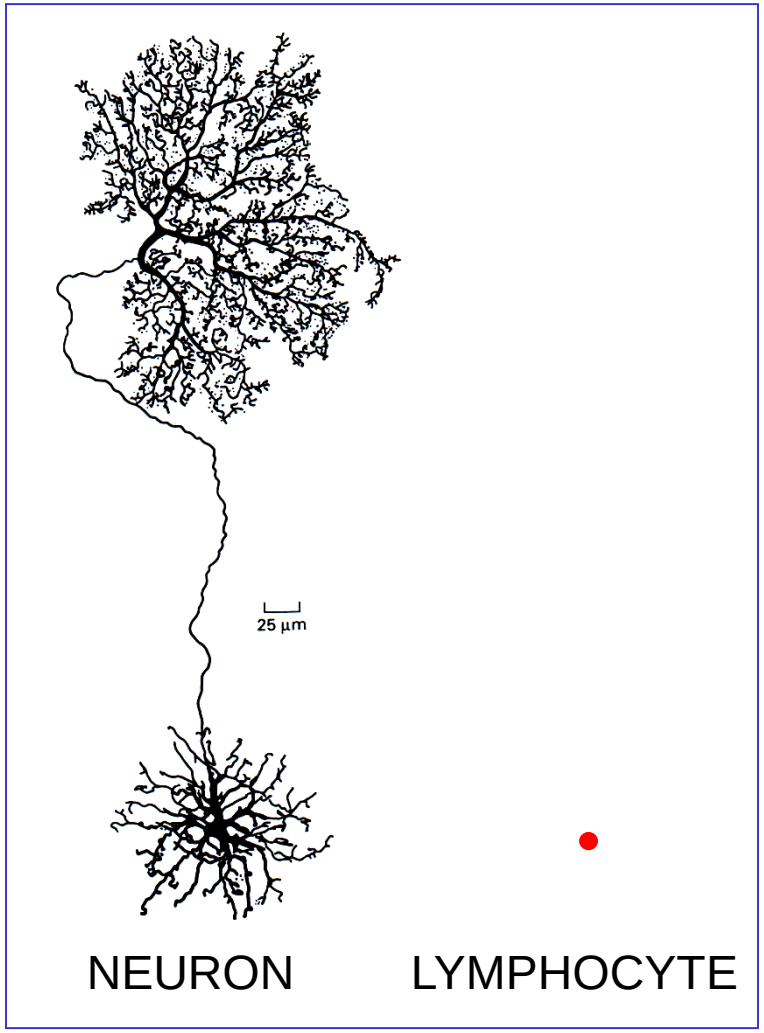
GENETIC INFORMATION



CONTROL OF GENE EXPRESSION

- In a multicellular organism there are many cell types (differentiation)
  - Different genes are activated
  - At different moments
  - With different intensity
- Different cell types use partially a common information





CELL DIFFERENTIATION

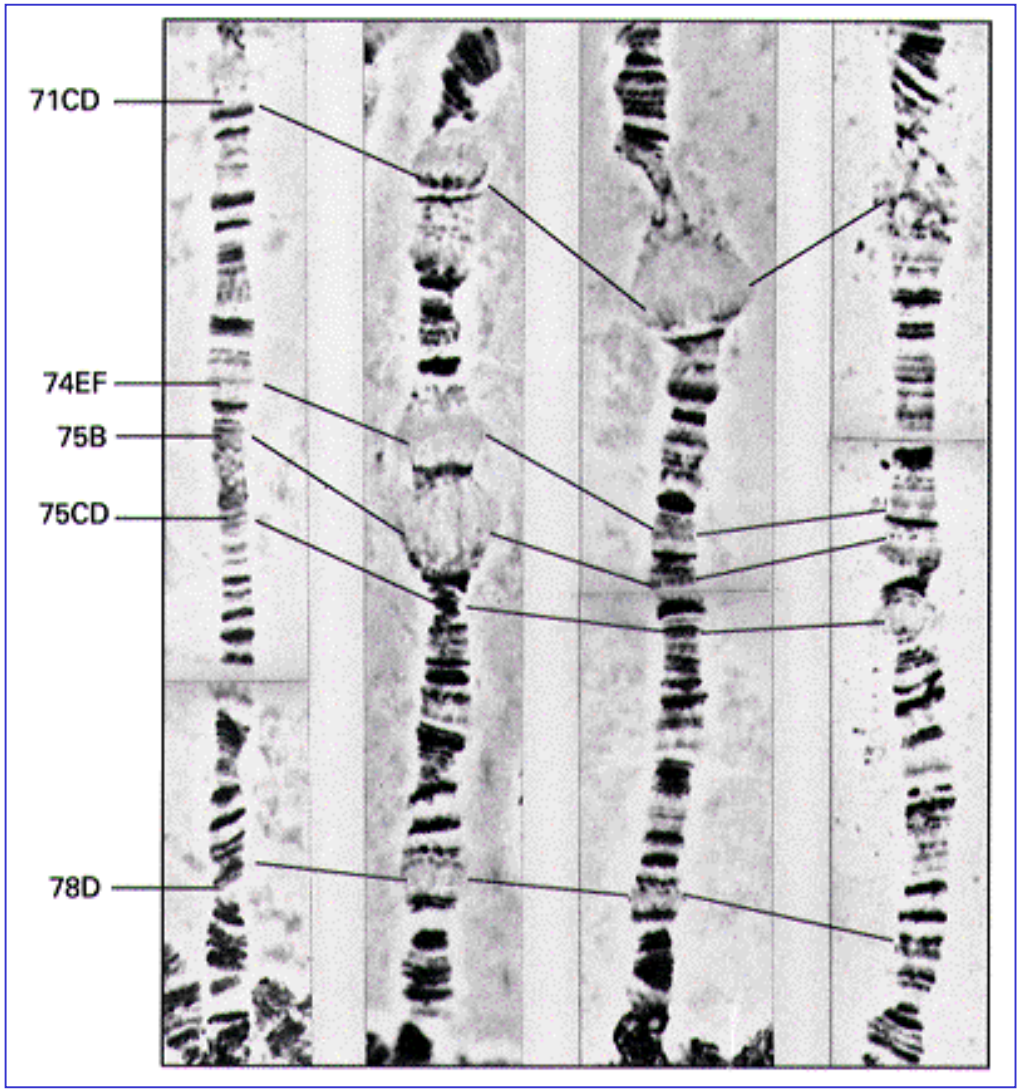
Cell differentiation: Cells are especialized to perform a particular function

Basis of cell differentiation:

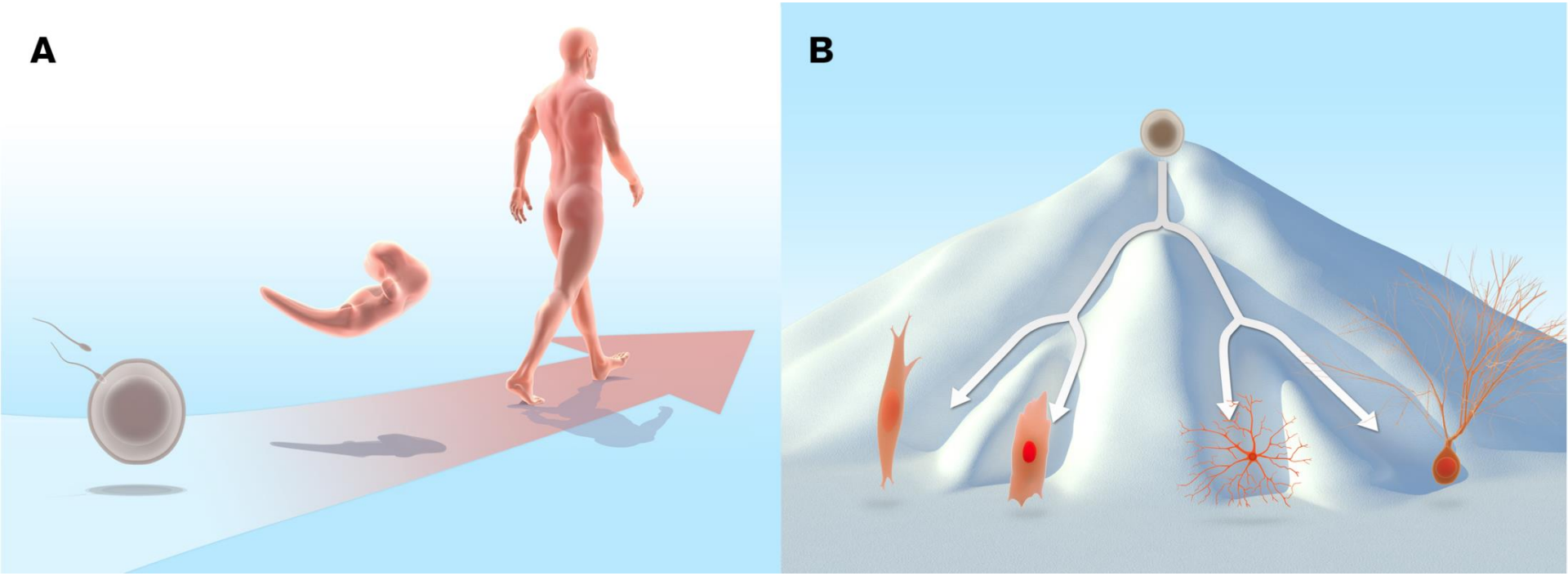
1) All cells of an organism contain **all** the genetic information
2) Differentiated cells only use **part** of this information
3) Differentiation is **reversible**

CONSERVATION OF ALL INFORMATION AND PARTIAL USE OF IT

Polytene chromosomes : different transcription units are activated

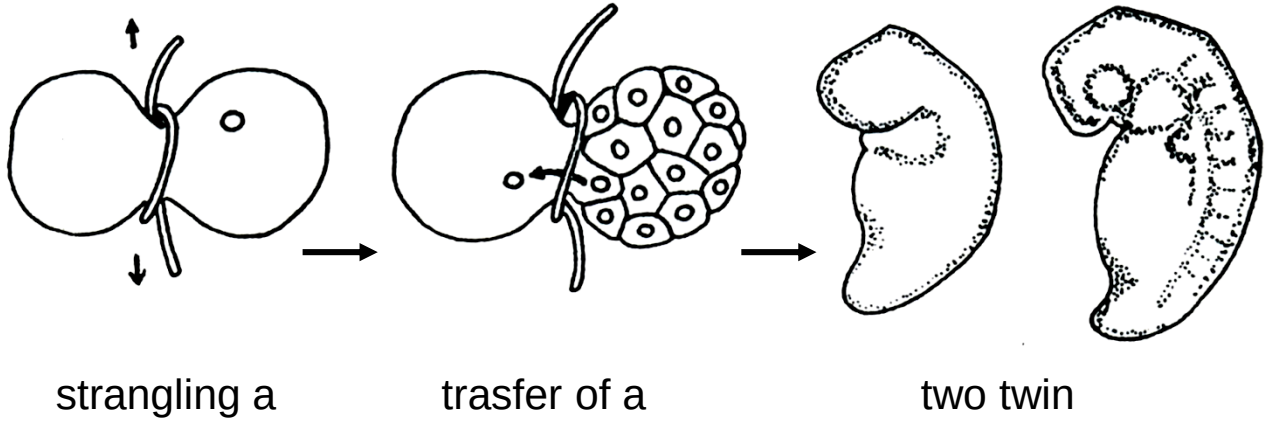


Differentiation is a unidirectional and irreversible process



REVERSIBILITY OF DIFFERENTIATION

Spemann experiments



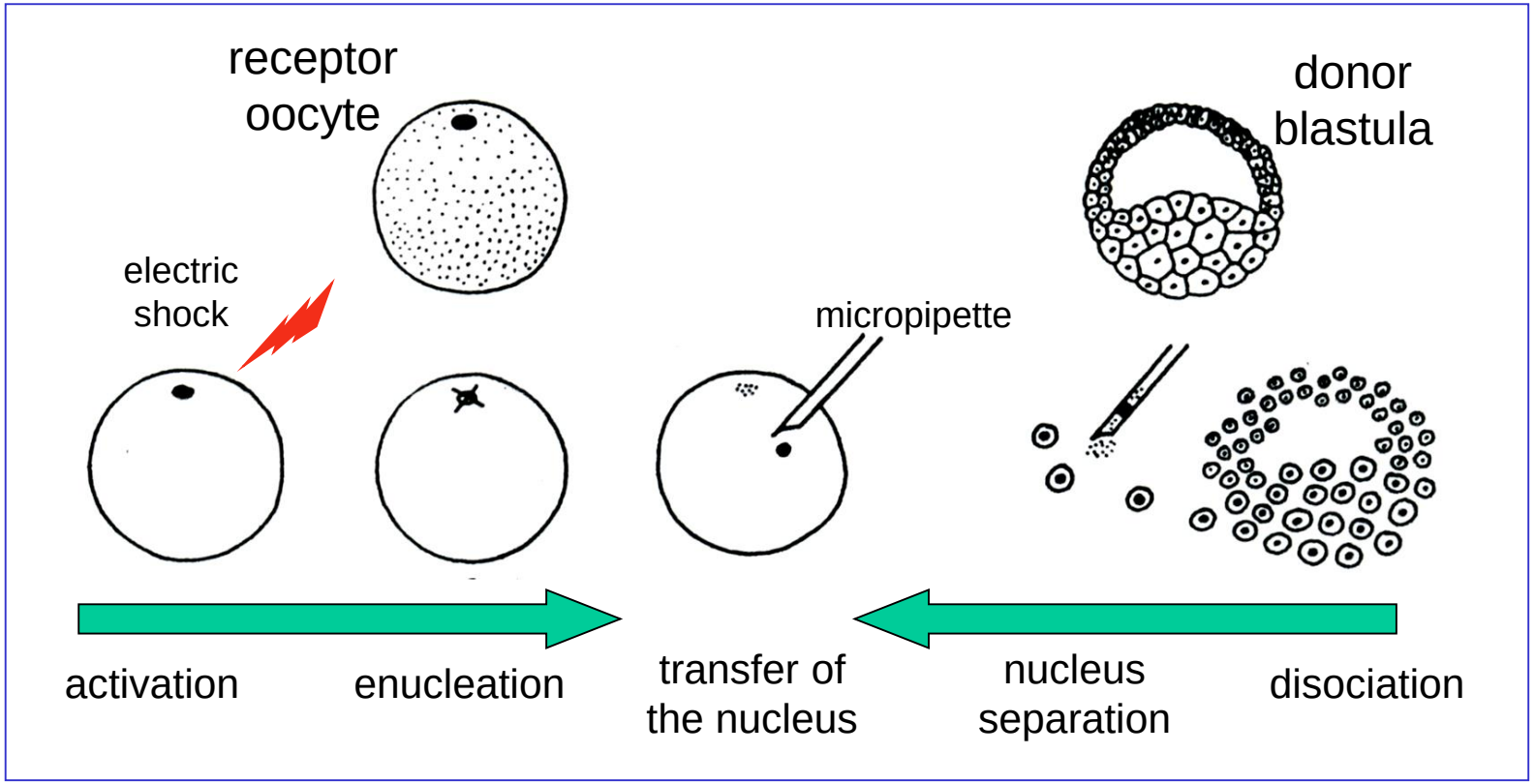
strangling a fertilized oocyte

trasfer of a nucleus from the blastula

two twin embryos (with sligh dephase)

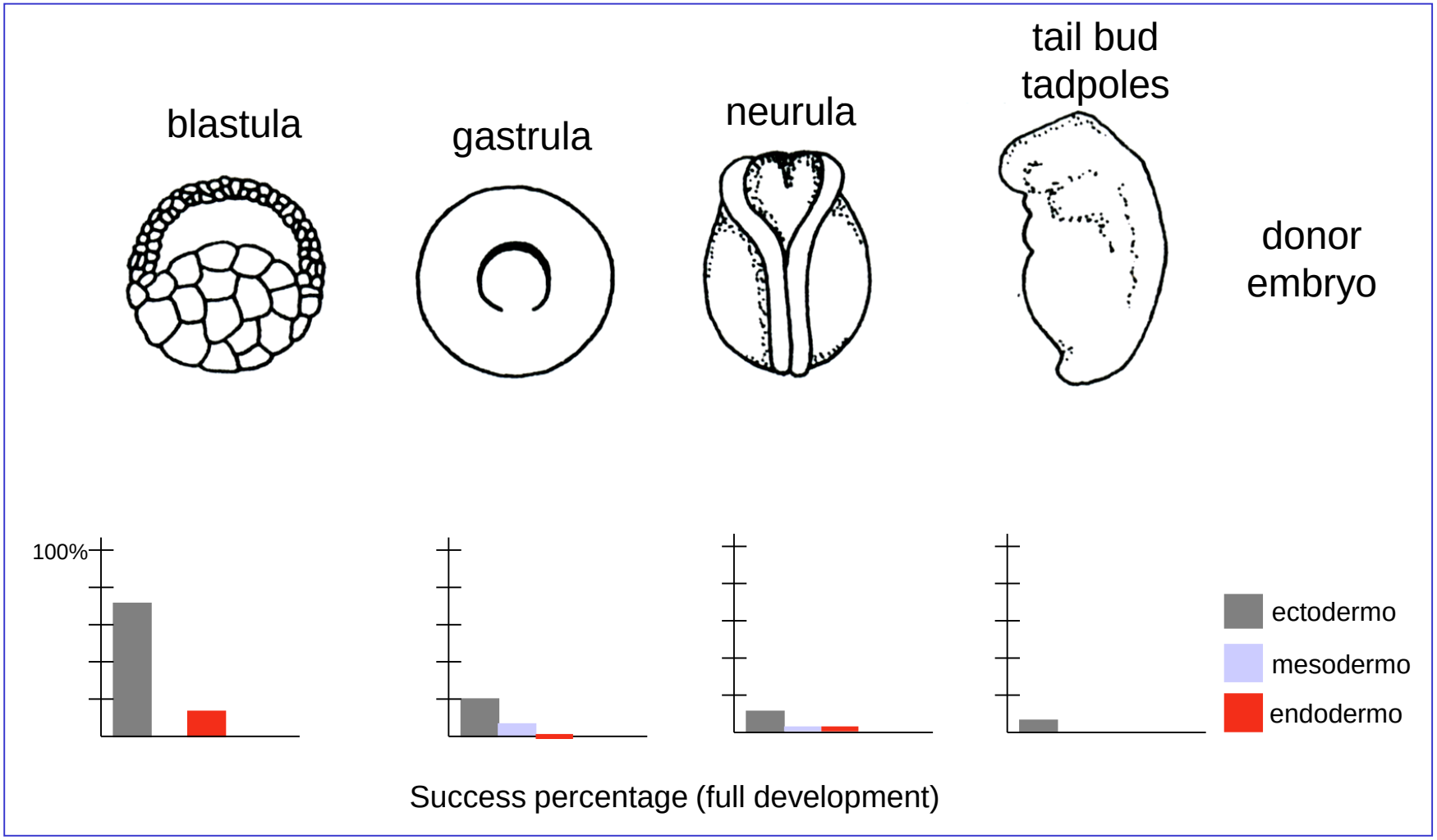
REVERSIBILITY OF DIFFERENTIATION

Nuclear transfer technique



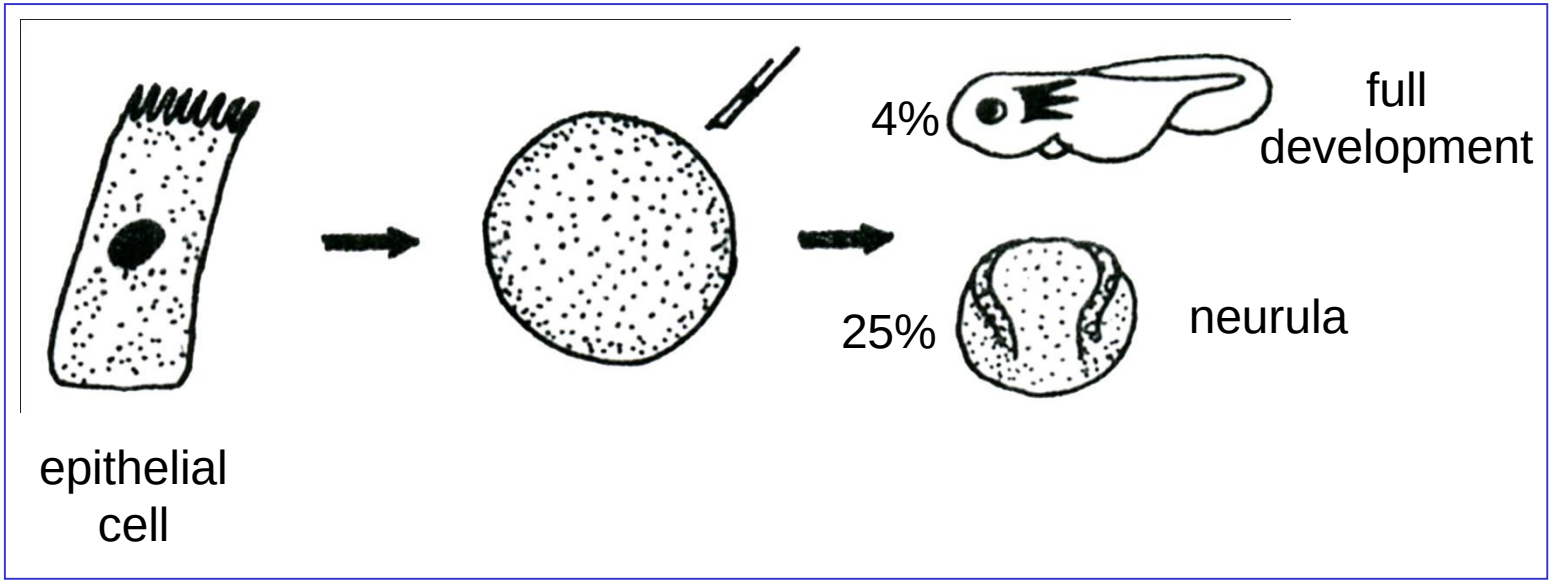
REVERSIBILITY OF DIFFERENTIATION

Briggs and King experiments (*Rana pipiens*)



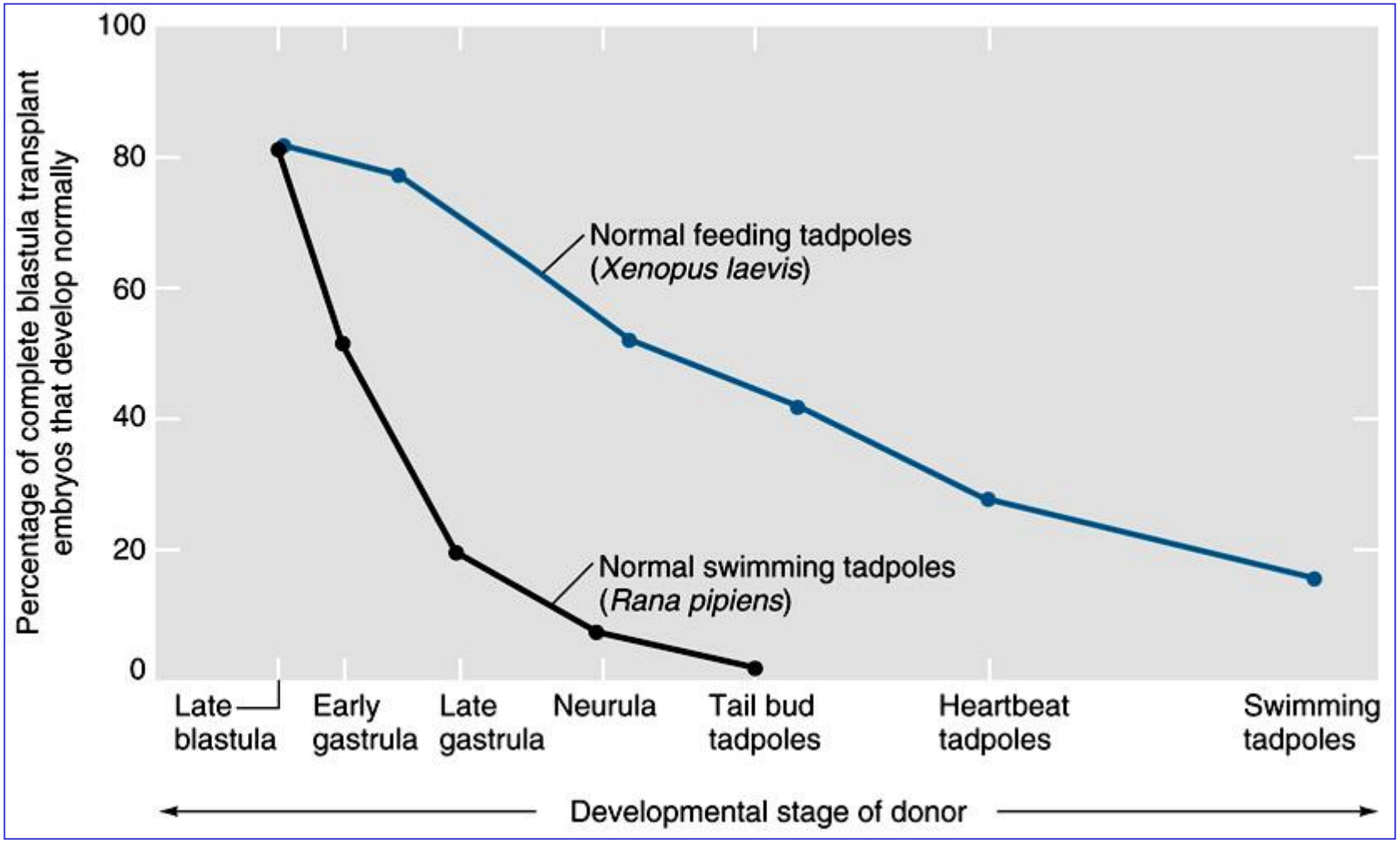
REVERSIBILITY OF DIFFERENTIATION

Gurdon experiments (*Xenopus laevis*)



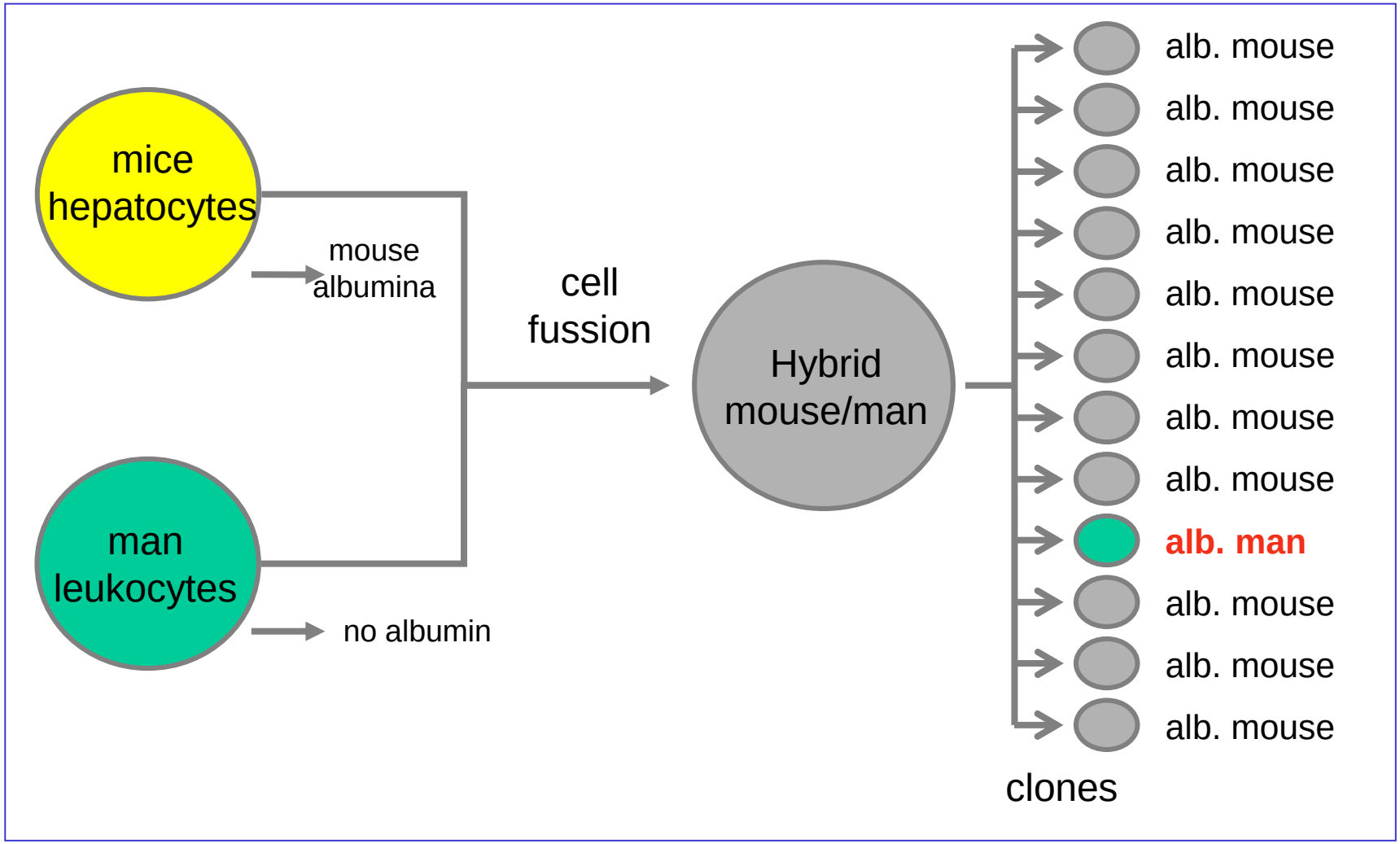
REVERSIBILITY OF DIFFERENTIATION

Percentage of succes in both experiments



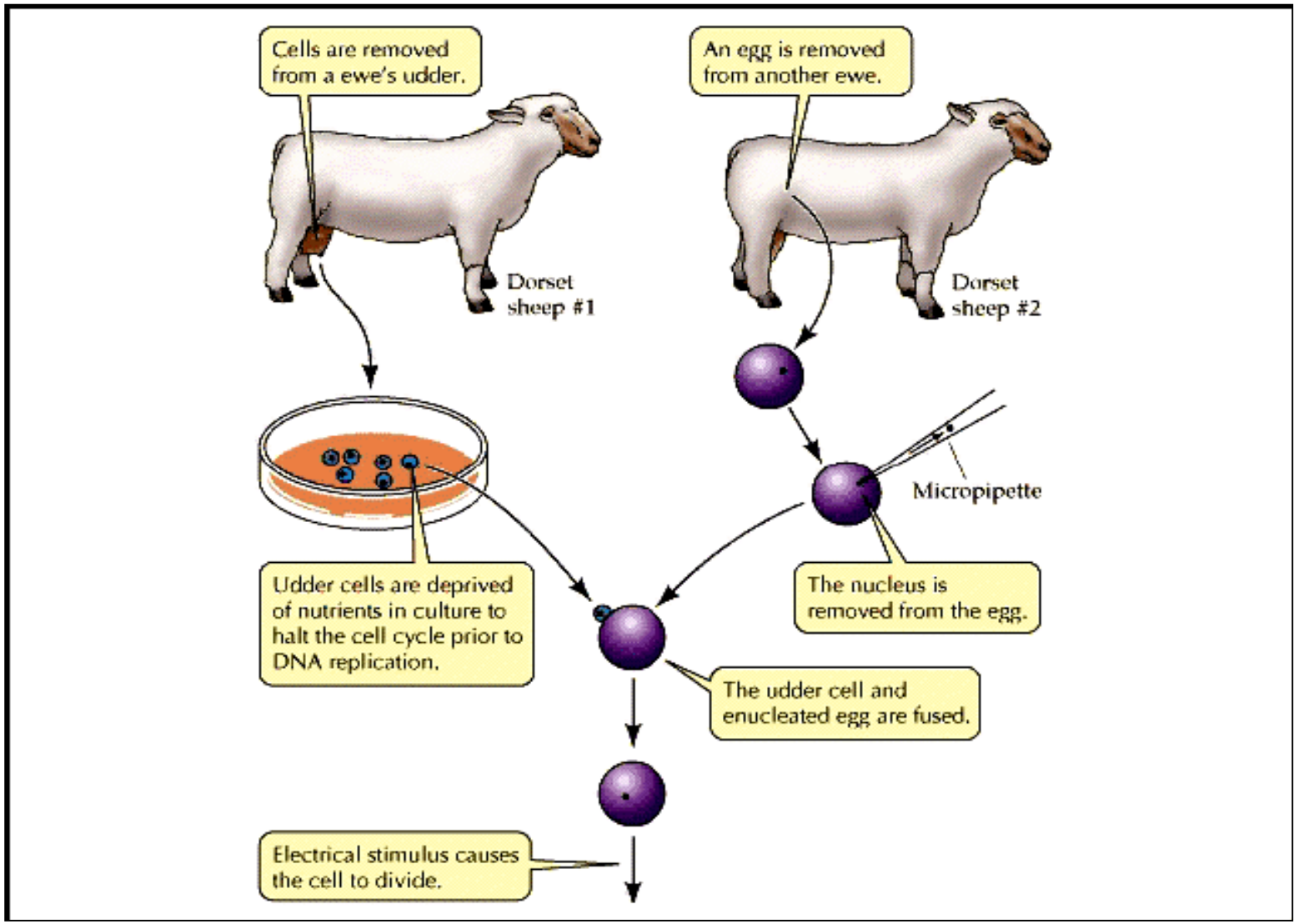
REVERSIBILITY OF DIFFERENTIATION

Somatic hybridization experiments



REVERSIBILITY OF DIFFERENTIATION

Wilmut et al experiments



REVERSIBILITY OF DIFFERENTIATION

Wilmut et al experiments

table 7.1 Development of Cloned Sheep

Cell Type	No. (%) of Fused Couplets	No. (%) Recovered from Oviduct	No. Cultured In Vitro	No. (%) of Morulae/ Blastocysts	No. of Morulae/ Blastocysts Transferred	No. (%) of Pregnancies/ Recipients	No. (%) of Live lambs Born*
Mammary epithelium	277 (63.8)	247 (89.2)	0	29 (11.7)	29	1/13 (7.7)	1 (3.4)
Fetal fibroblast	172 (84.7)	124 (86.7)	—	34 (27.4)	34	4/10 (40.0)	2 (5.9)
		—	24	13 (54.2)	6	1/6 (16.6)	1 (16.6)†
Embryo-derived	385 (82.8)	231 (85.3)	—	90 (39.0)	72	14/27 (51.8)	4 (5.6)
		—	92	36 (39.0)	15	1/5 (20.0)	0

*As a proportion of morulae or blastocysts transferred

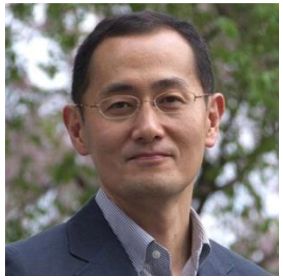
†This lamb died within a few minutes from birth.

Data from Wilmut et al. (1997).

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

Cell 126:663, 25 Agosto 2006

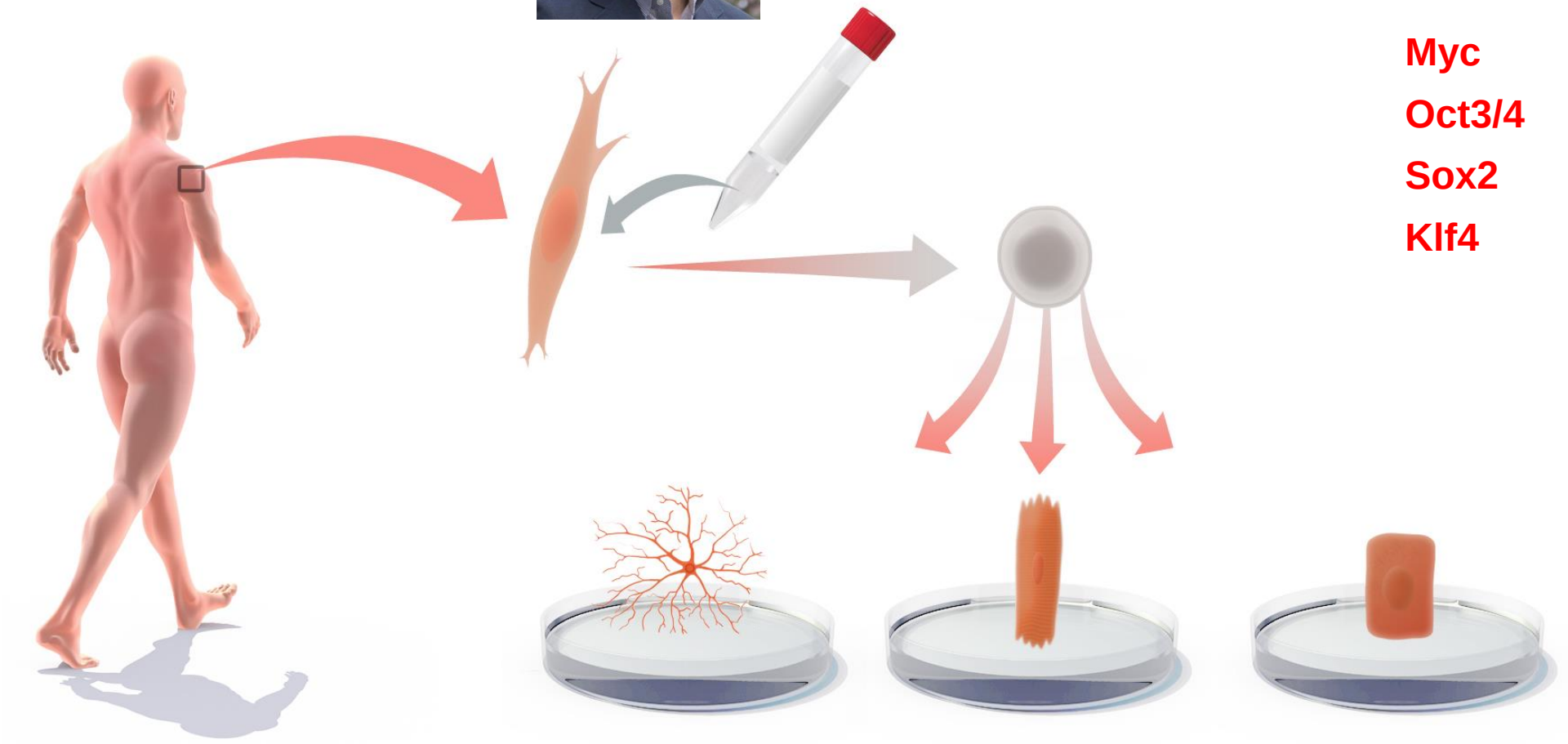


Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
[4]Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

Cell, 131:861 30 Noviembre 2007)

Myc
Oct3/4
Sox2
Klf4



CELL REPROGRAMMING

Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors

Masaki Ieda,[1,2,3,6,*] Ji-Dong Fu,[1,2,3] Paul Delgado-Olguin,[1,2,4] Vasanth Vedantham,[1,5] Yohei Hayashi,[1] Benoit G. Bruneau,[1,2,4] and Deepak Srivastava[1,2,3,*]
[1]Gladstone Institute of Cardiovascular Disease
[2]Department of Pediatrics
[3]Department of Biochemistry and Biophysics
[4]Cardiovascular Research Institute
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Cell 142:375, August 6th 2010

Heart repair by reprogramming non-myocytes with cardiac transcription factors

Kunhua Song[1], Young-Jae Nam[1,2], Xiang Luo[2], Xiaoxia Qi[1], Wei Tan[2], Guo N. Huang[1], Asha Acharya[1], Christopher L. Smith[1], Michelle D. Tallquist[1], Eric G. Neilson[3], Joseph A. Hill[1,2], Rhonda Bassel-Duby[1] & Eric N. Olson[1]

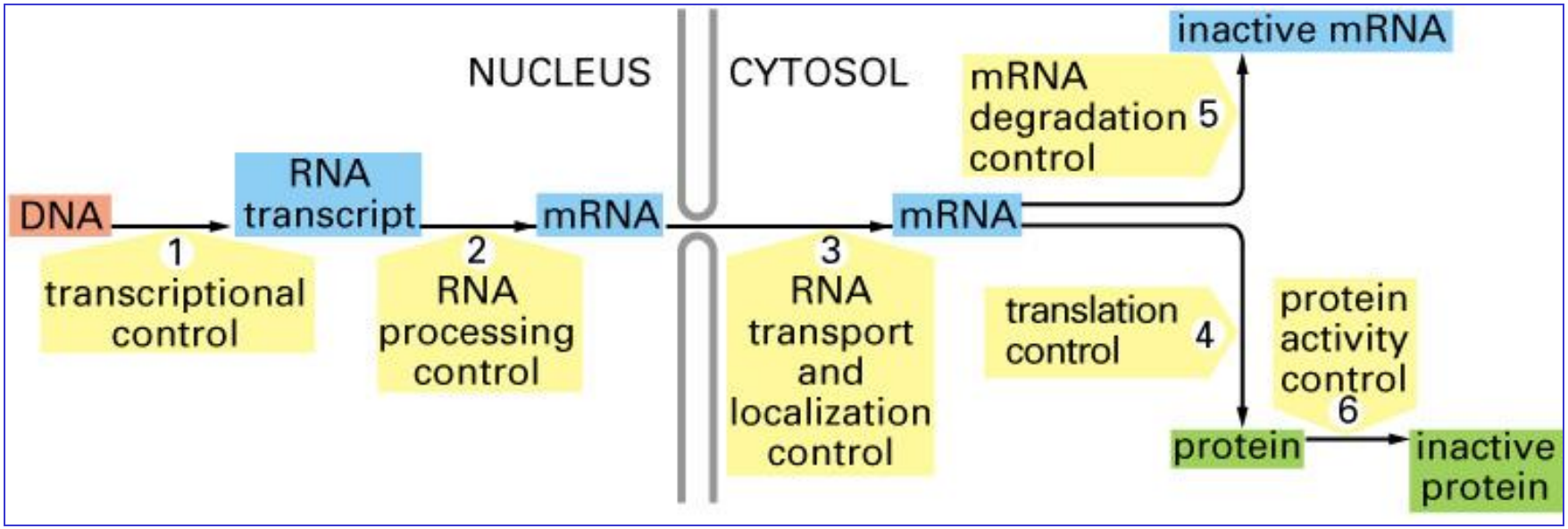
The adult mammalian heart possesses little regenerative potential following injury. Fibrosis due to activation of cardiac fibroblasts impedes cardiac regeneration and contributes to loss of contractile function, pathological remodelling and susceptibility to arrhythmias. Cardiac fibroblasts account for a majority of cells in the heart and represent a potential cellular source for restoration of cardiac function following injury through phenotypic reprogramming to a myocardial cell fate. Here we show that four transcription factors, GATA4, HAND2, MEF2C and TBX5, can cooperatively reprogram adult mouse tail-tip and cardiac fibroblasts into beating cardiac-like myocytes *in vitro*. Forced expression of these factors in dividing non-cardiomyocytes in mice reprograms these cells into functional cardiac-like myocytes, improves cardiac function and reduces adverse ventricular remodelling following myocardial infarction. Our results suggest a strategy for cardiac repair through reprogramming fibroblasts resident in the heart with cardiogenic transcription factors or other molecules.

Nature 485:599, May 31st 2012

CONTROL OF GENE EXPRESSION

Control levels:

- 1. Chromatin
- 2. Transcription
- 3. Post-transcription
- 4. Translation
- 5. Post-translation



CONTROL OF GENE EXPRESSION

1. Chromatin

Genetic control

Based on inheritable variations in the DNA sequence

Epigenetics control

Based on inheritable regulation of gene expression without variations in nucleotide sequence

CONTROL OF GENE EXPRESSION

1. Chromatin

DNA sequence reconfiguration

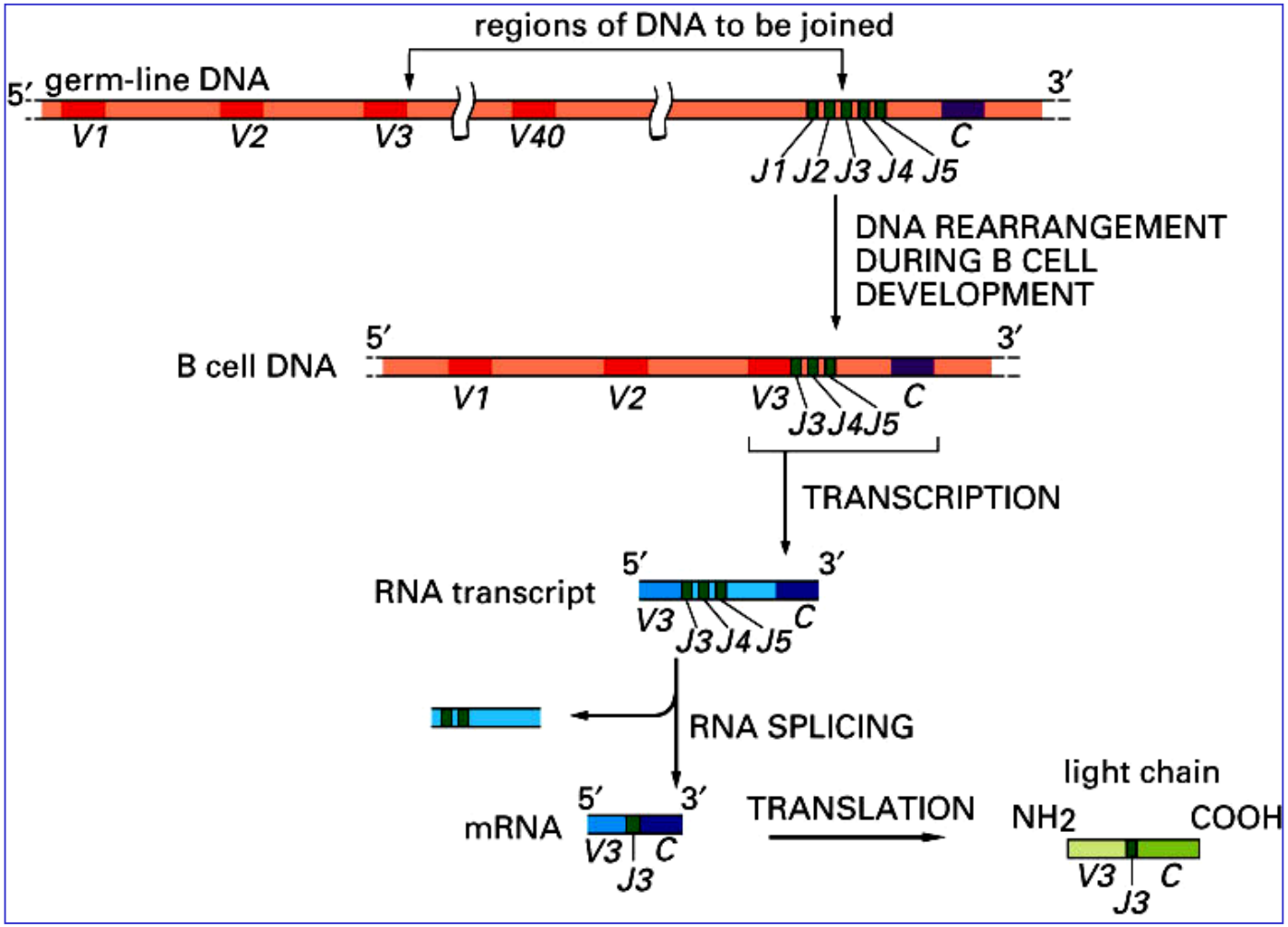
Chromatin: permissive structure

- Cytosine methylation
- Histone modification
- No codifying RNAs

CHROMATIN CONTROL

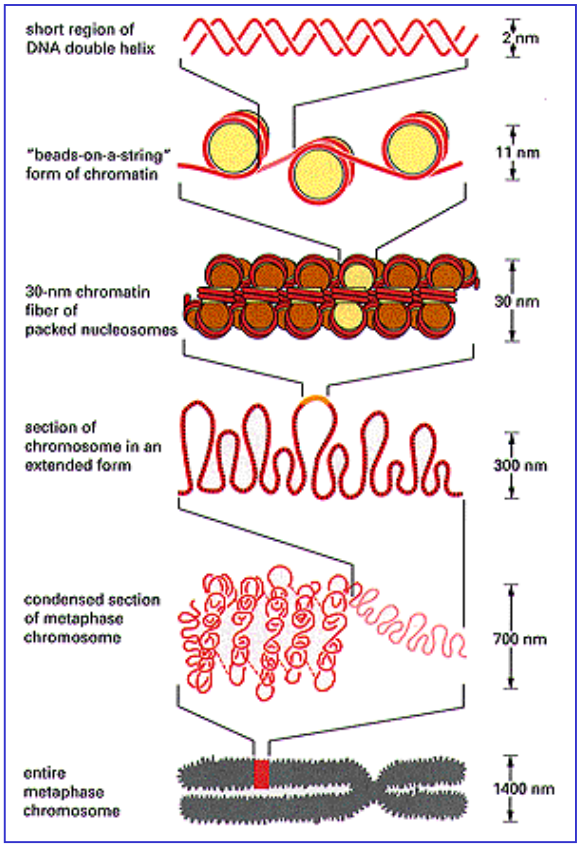
Genetic regulation

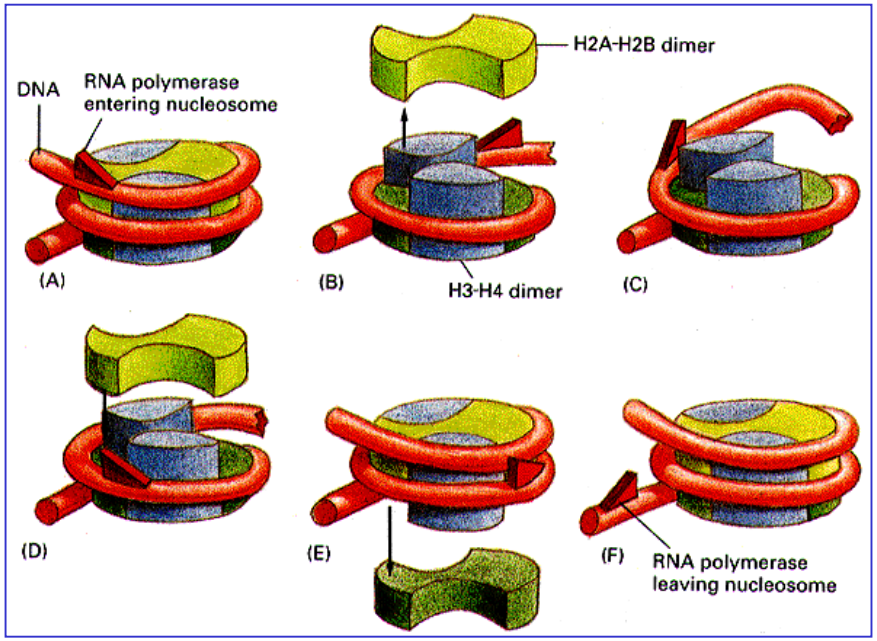
DNA sequence reconfiguration by irreversible loss of nucleotide fragments

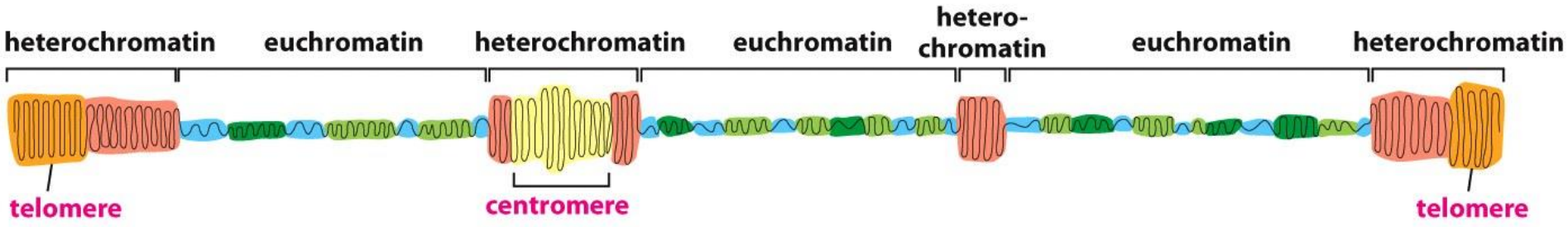


CHROMATIN CONTROL

Different levels of packing

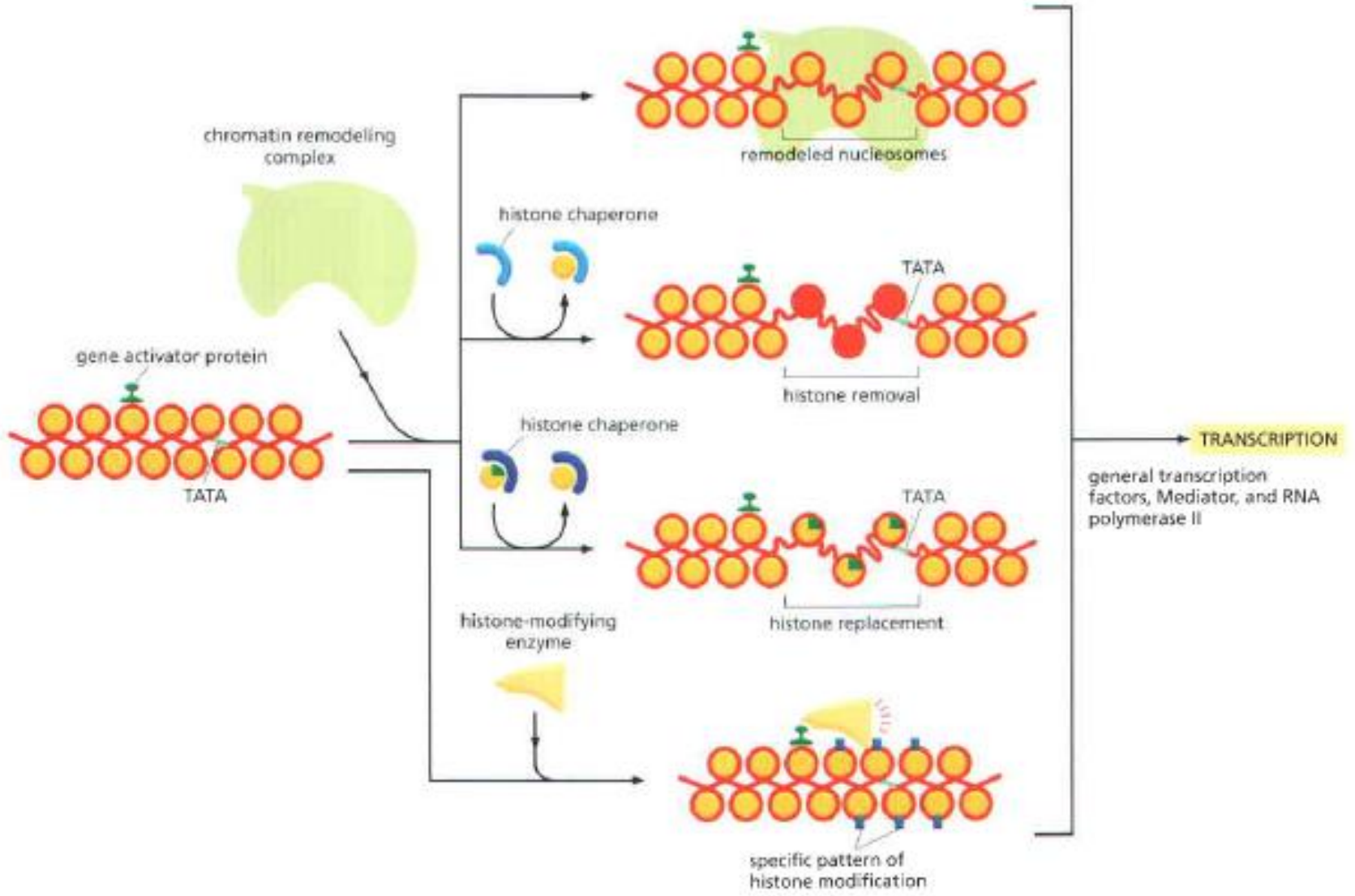






CHROMATIN CONTROL

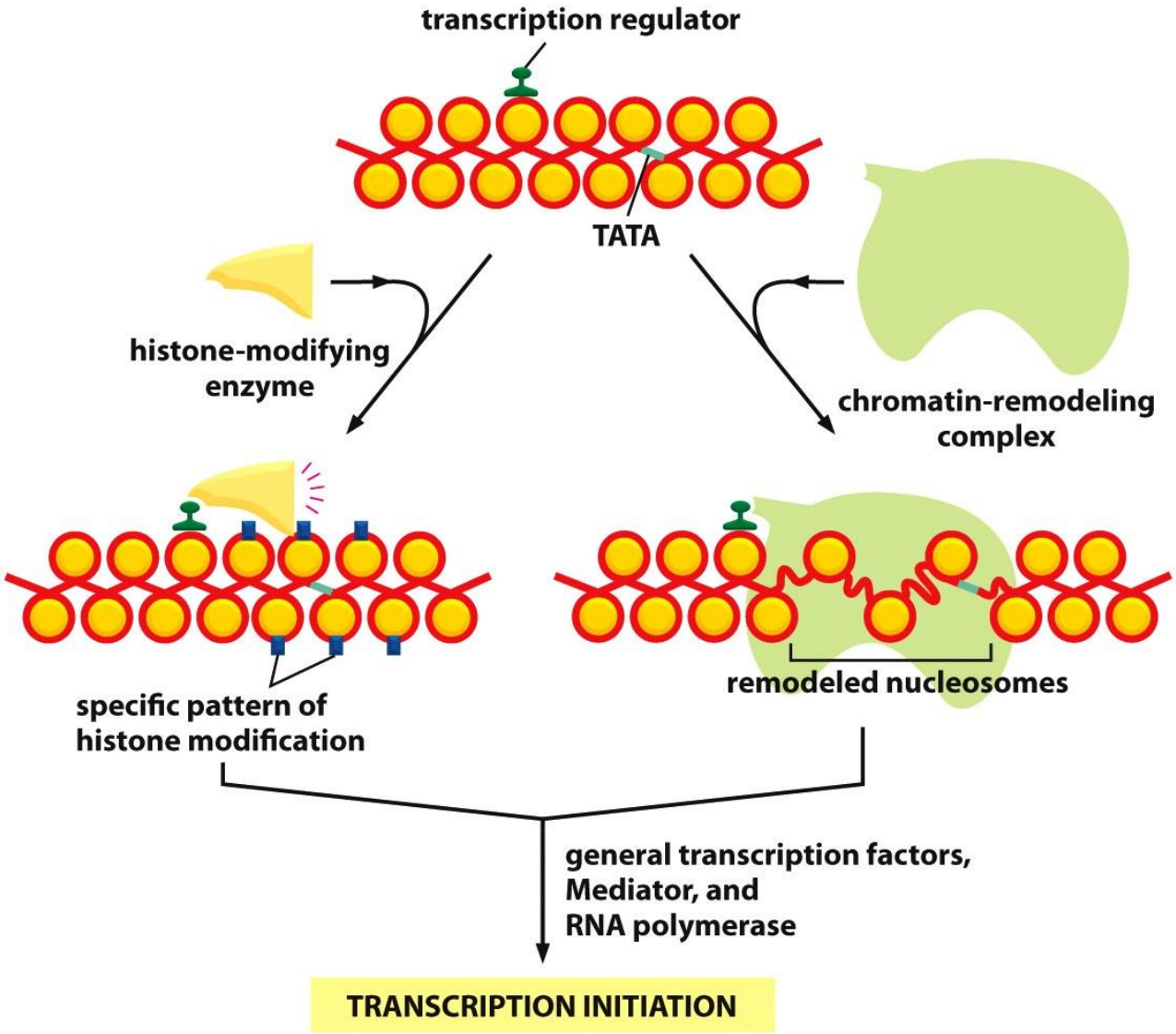
Different levels of packing



CHROMATIN CONTROL

Different levels of packing

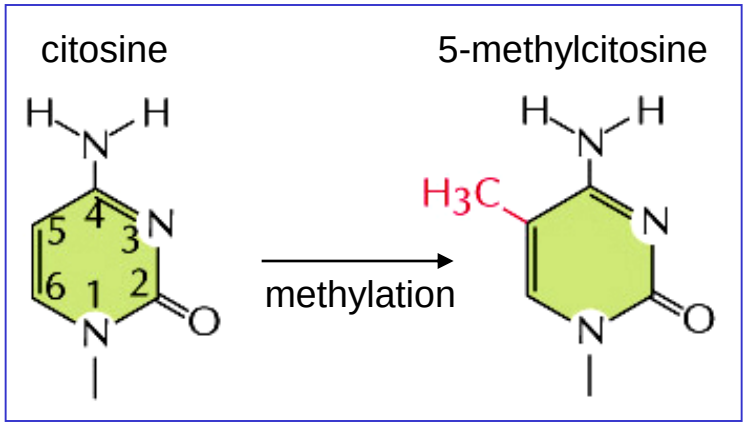
Chromatin remodelling complexes

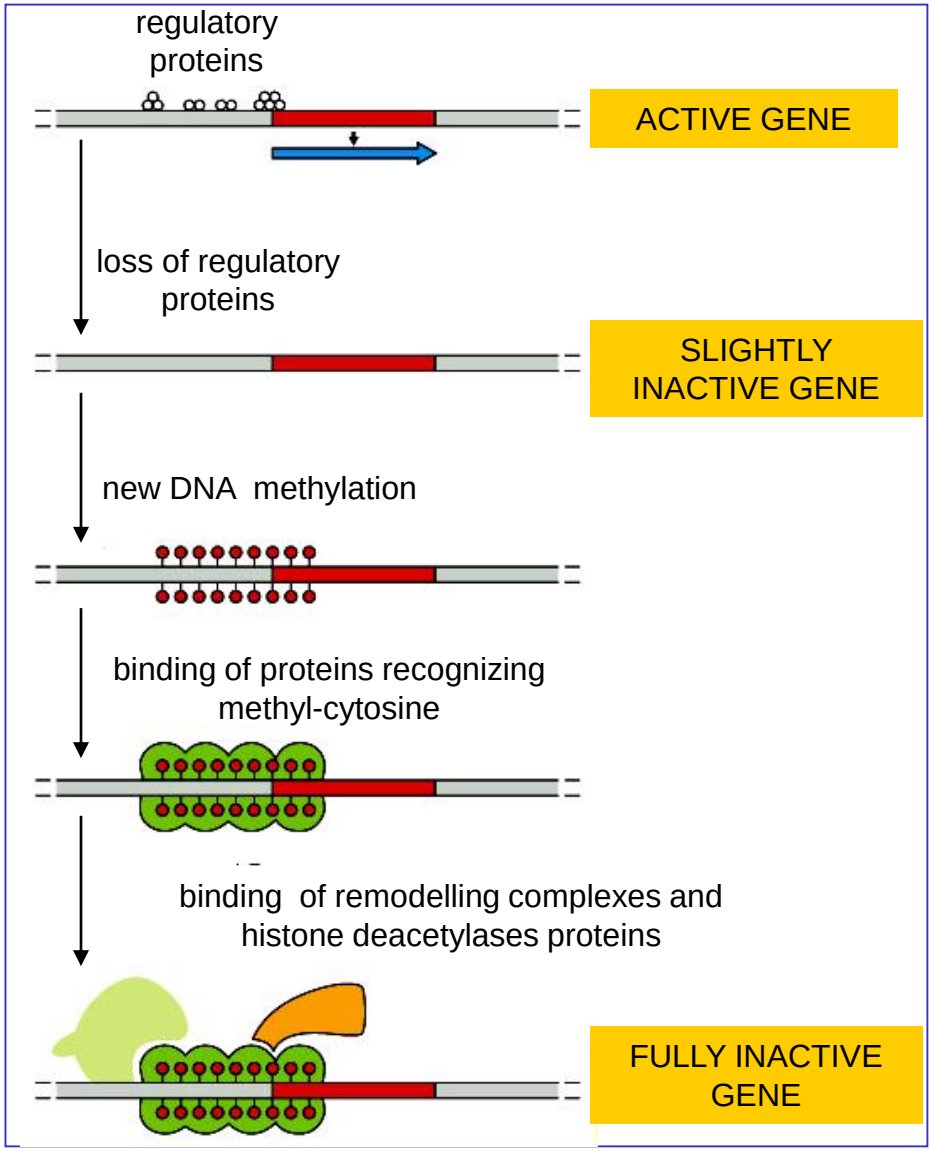


CHROMATIN CONTROL

Epigenetic regulation

Cytosine methylation

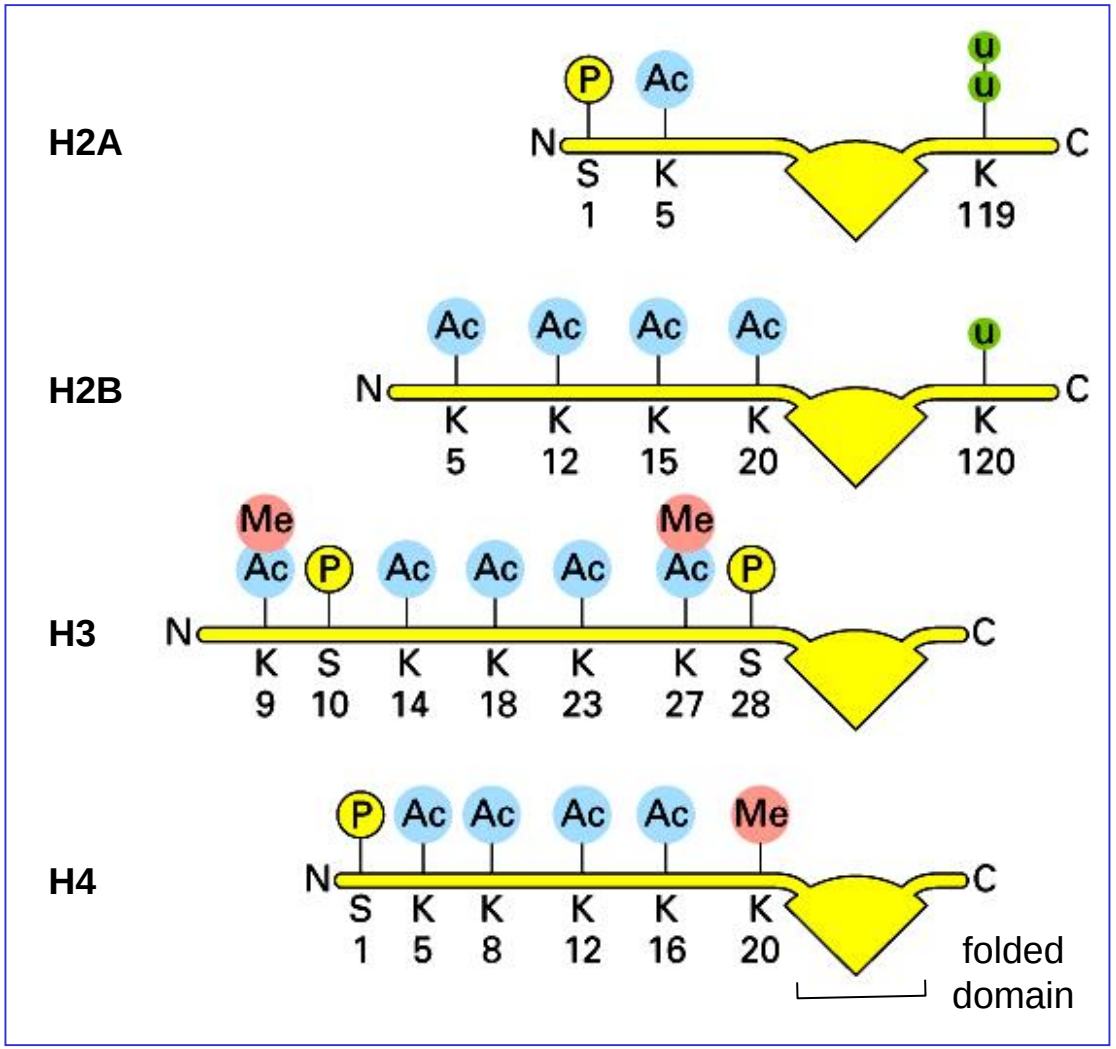


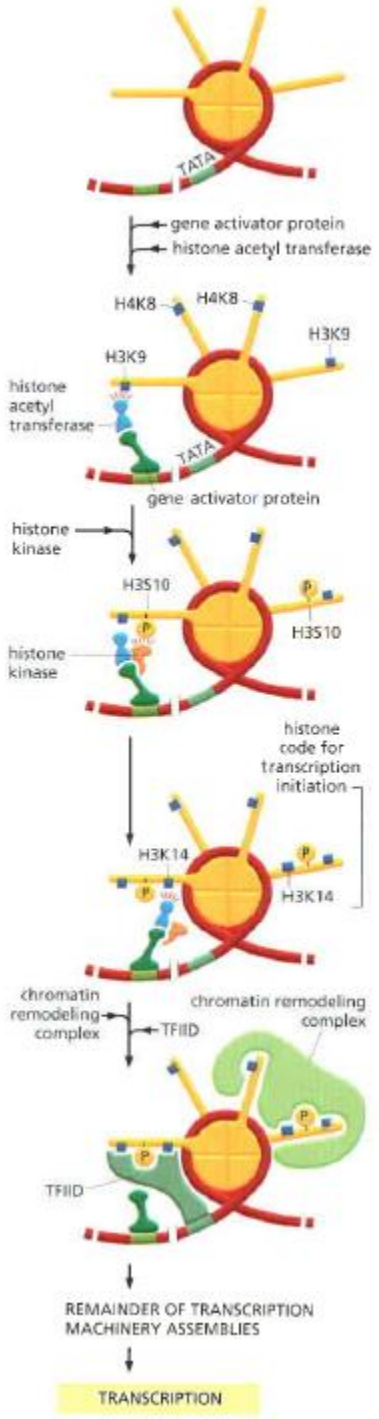


CHROMATIN CONTROL

Epigenetic regulation

Histone modification (histone code)

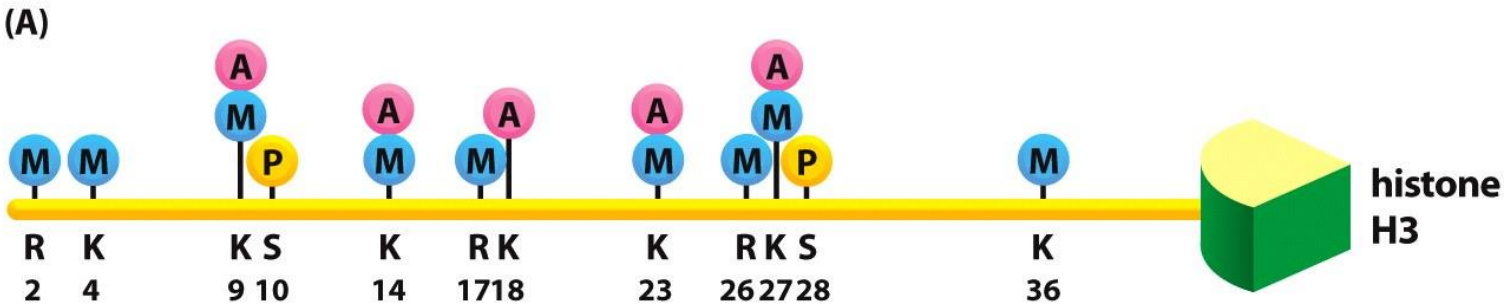


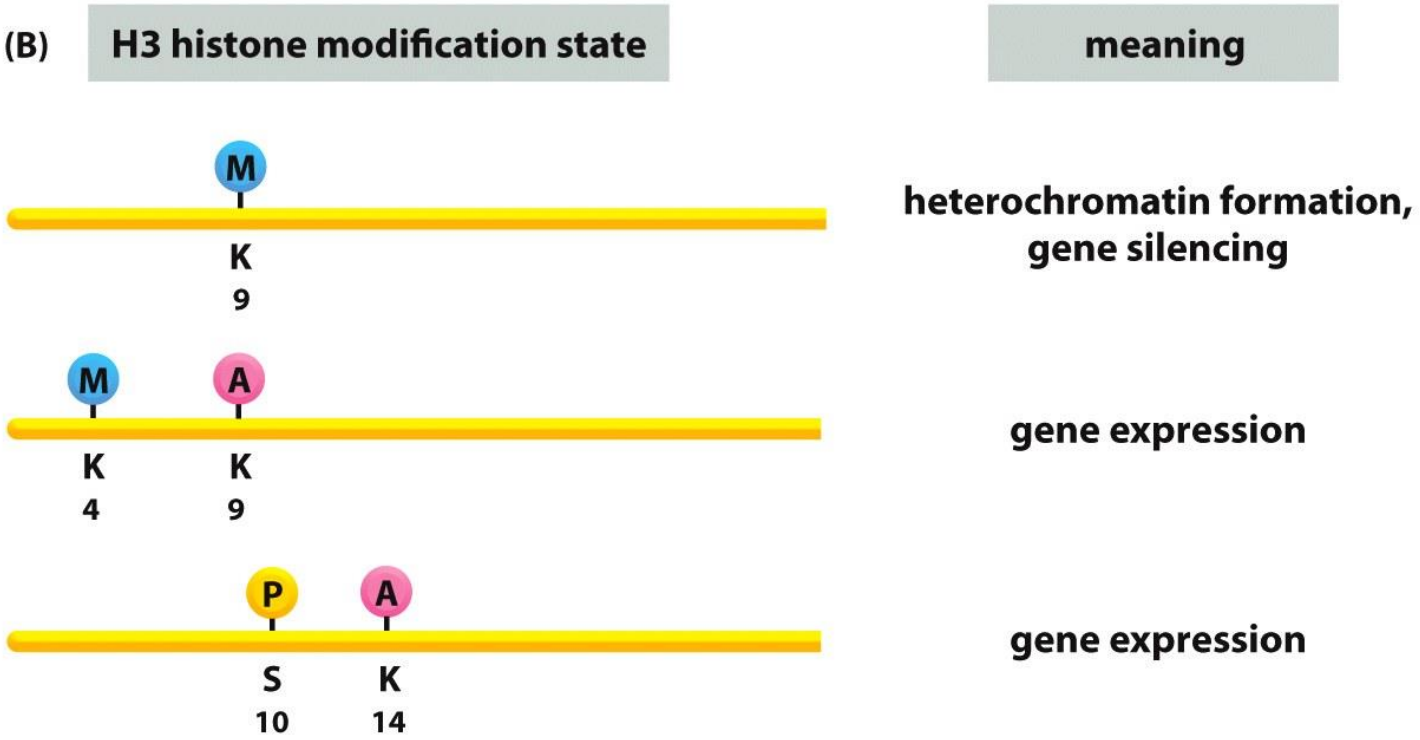


CHROMATIN CONTROL

Epigenetic regulation

Histone modification



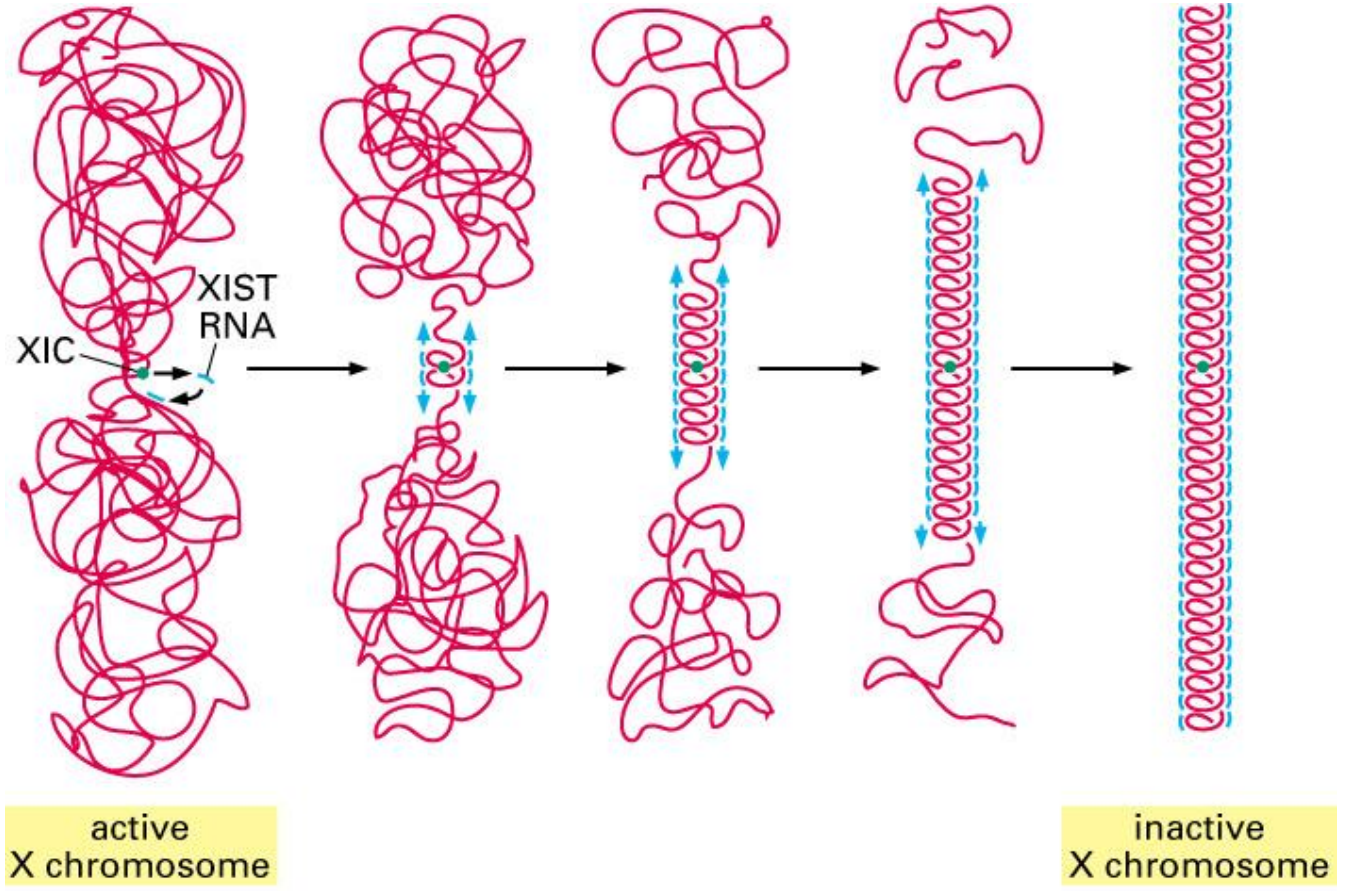


CHROMATIN CONTROL

Epigenetic regulation

No codifying RNAs

(Example: XIST RNA)

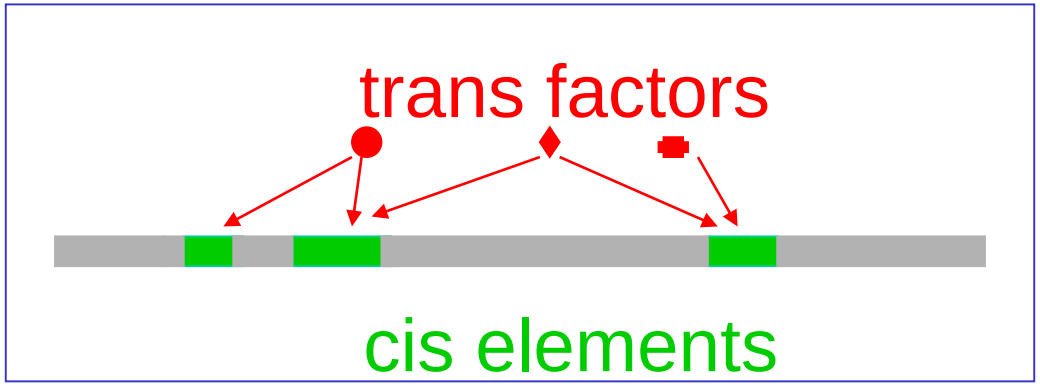


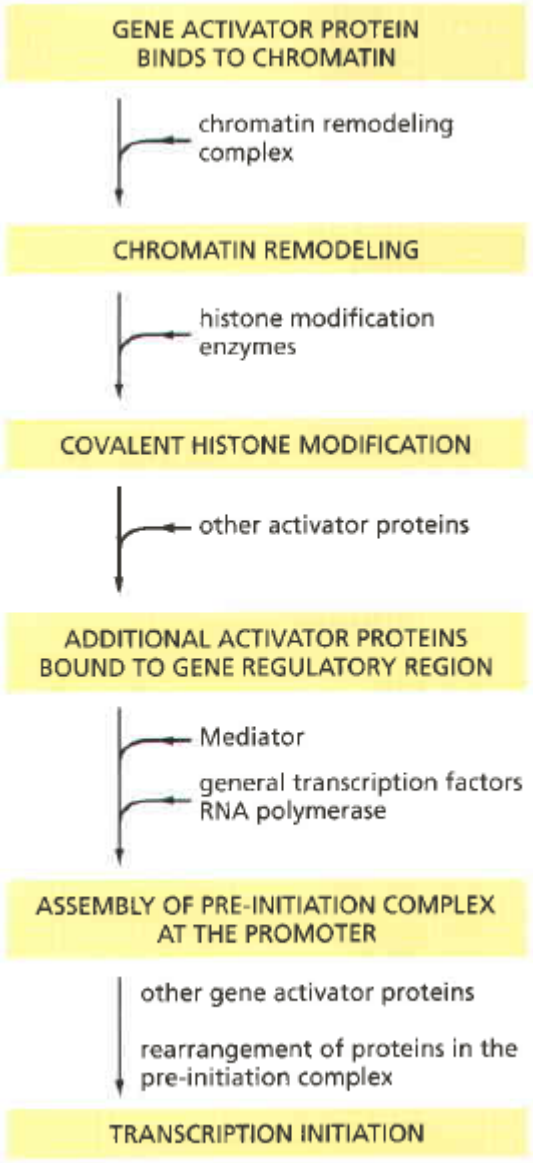
Chromosome X inactivation

CONTROL OF GENE EXPRESSION

2. Transcription

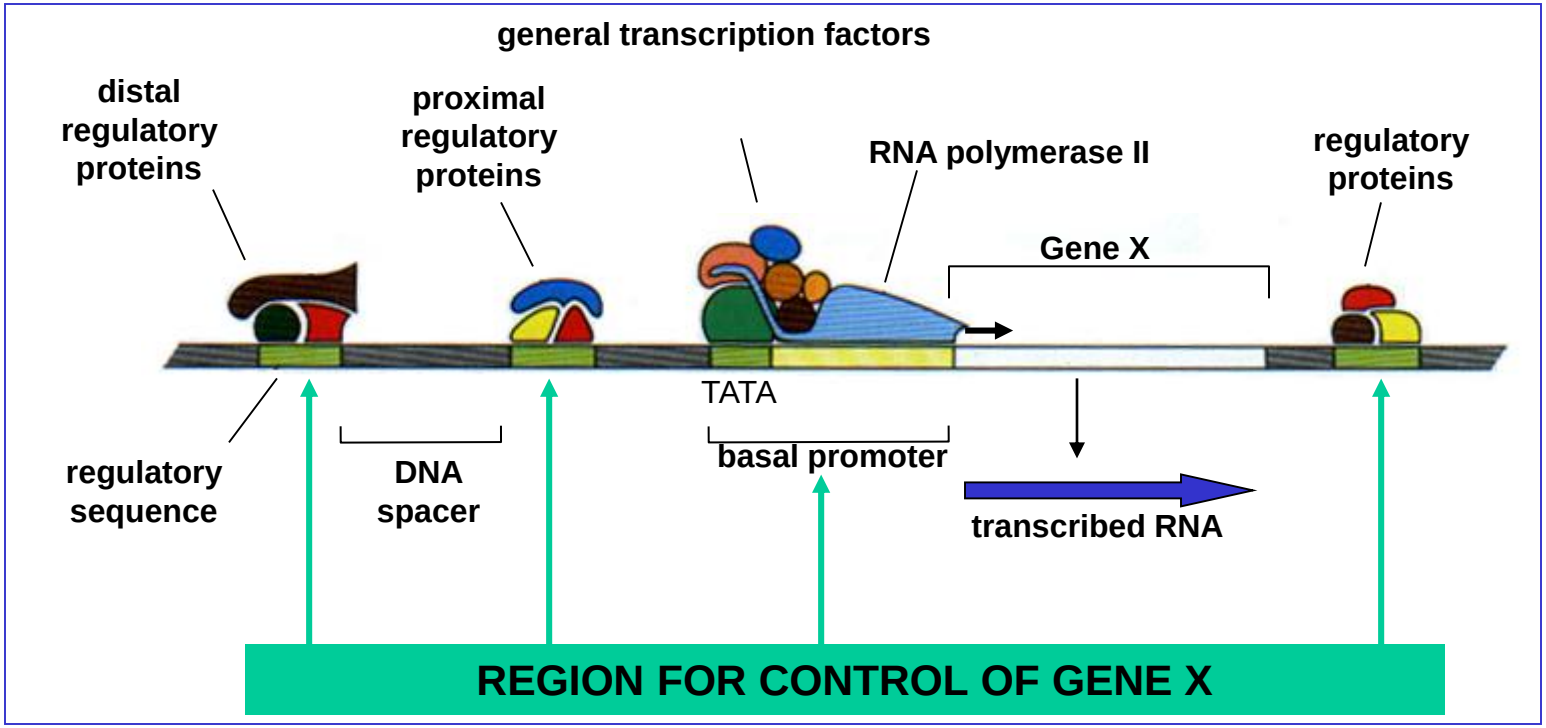
- Cis regulation: regulatory sequences
- Trans regulation: transcription factors and regulatory proteins





TRANSCRIPTION CONTROL

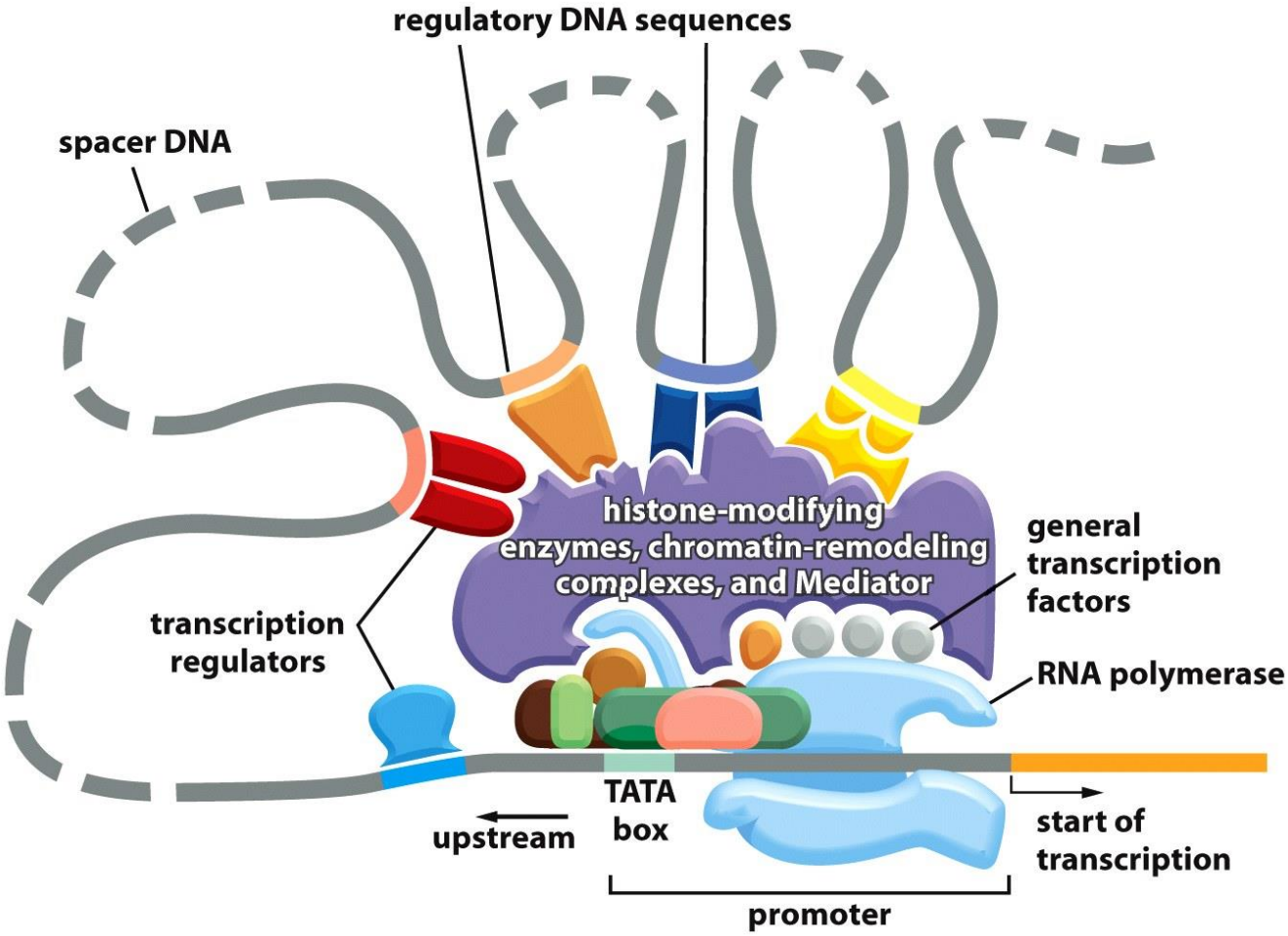
Gene control regions



- **Promoters which are only recognized by general and proximal transcription factors regulate housekeeping genes, i.e. those expressed in all cells of the organism**

- **Transcription factors acting over distal promoters regulate the expression of inducible genes, which are only expressed in specific tissues**

CONTROL OF GENE EXPRESSION



TRANSCRIPTION CONTROL

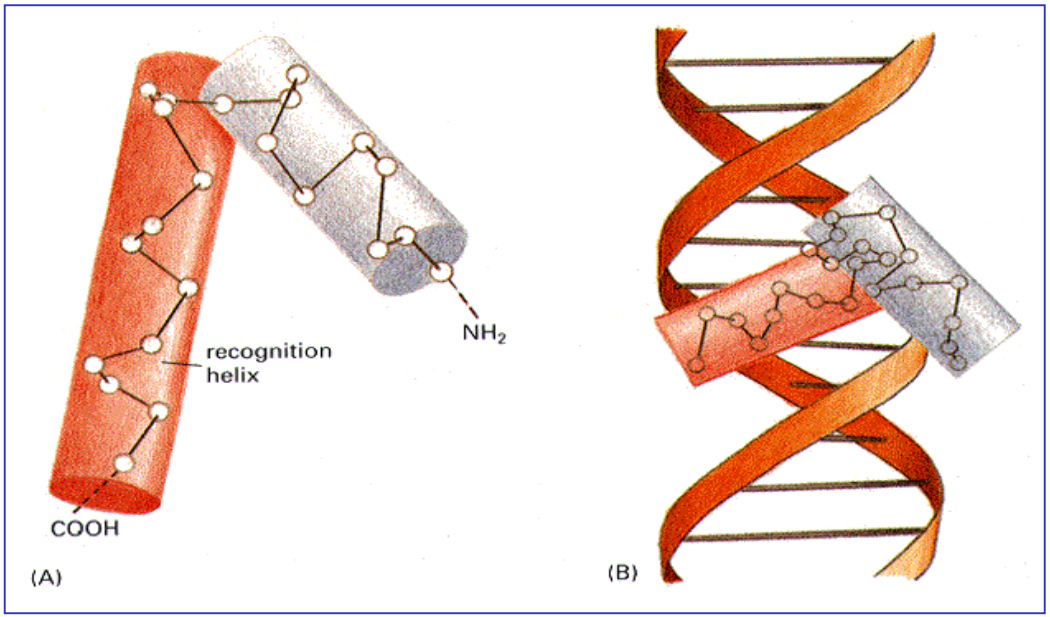
Cis regulation: response elements in DNA

	Name	Recognized sequence
Yeast	Gal4	CGGAGGACTGTCCTCCG GCCTCCTGACATTCGGC
	Matα2	CATGTAATT GTACATTAA
	Gcn4	ATGACTCAT TACTGAGTA
Drosophila	Kruppel	AACGGGTTAA TTGCCCAATT
	Bicoid	GGGATTAGA CCCTAATCT
Mammals	Sp1	GGGCGG CCCGCC
	Oct-1 Pou	ATGCAAAT TACGTTTA
	GATA-1	TGATAG ACTATC
	MyoD	CAAATG GTTTAC
	p53	GGGCAAGTCT CCCGTTCAGA

TRANSCRIPTION CONTROL

Trans regulation

Transcription factors: structure

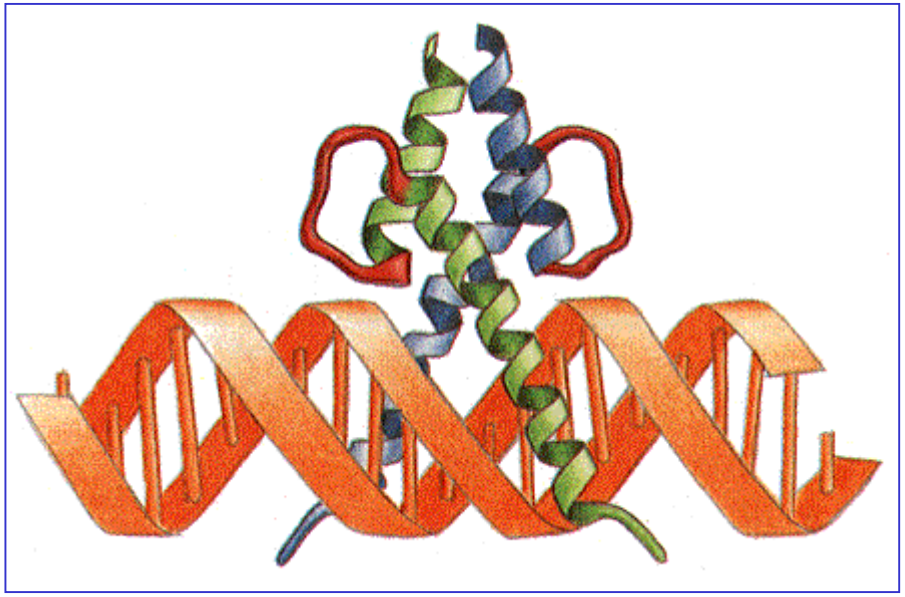


Helix-turn-helix

TRANSCRIPTION CONTROL

Trans regulation

Transcription factors: structure



Helix-loop-helix

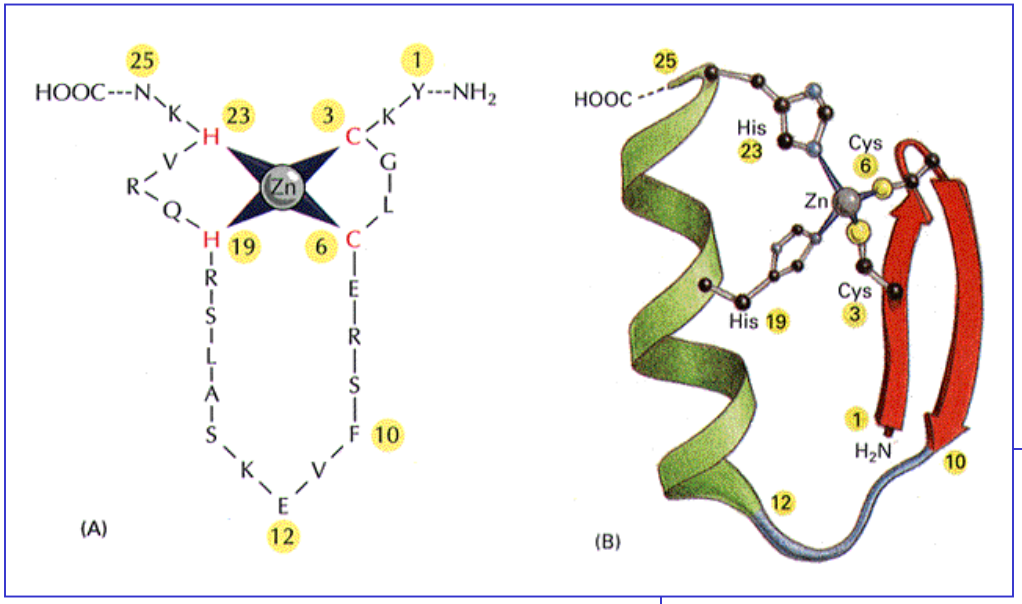
MyoD1: specific genes for muscle cells

E12/E47: sequence stimulating immunoglobulin synthesis

TRANSCRIPTION CONTROL

Trans regulation

Transcription factors: structure

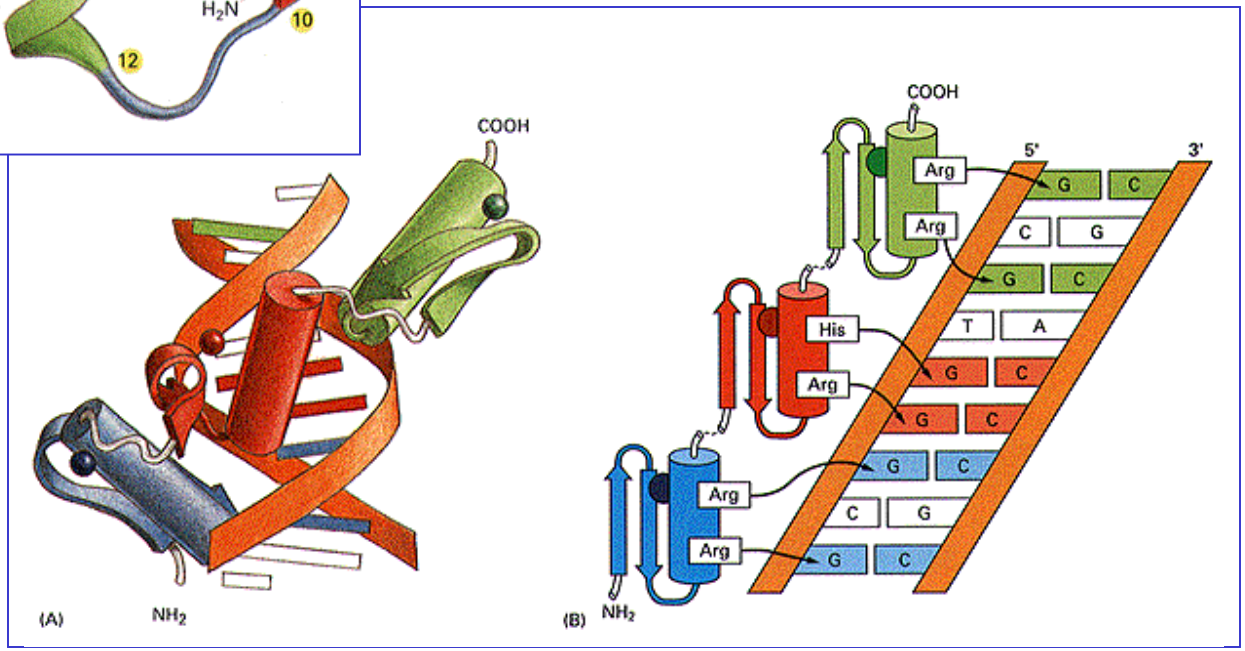


Zinc fingers

Hormone receptors:

Thyroids, steroids, retinoic acid, etc

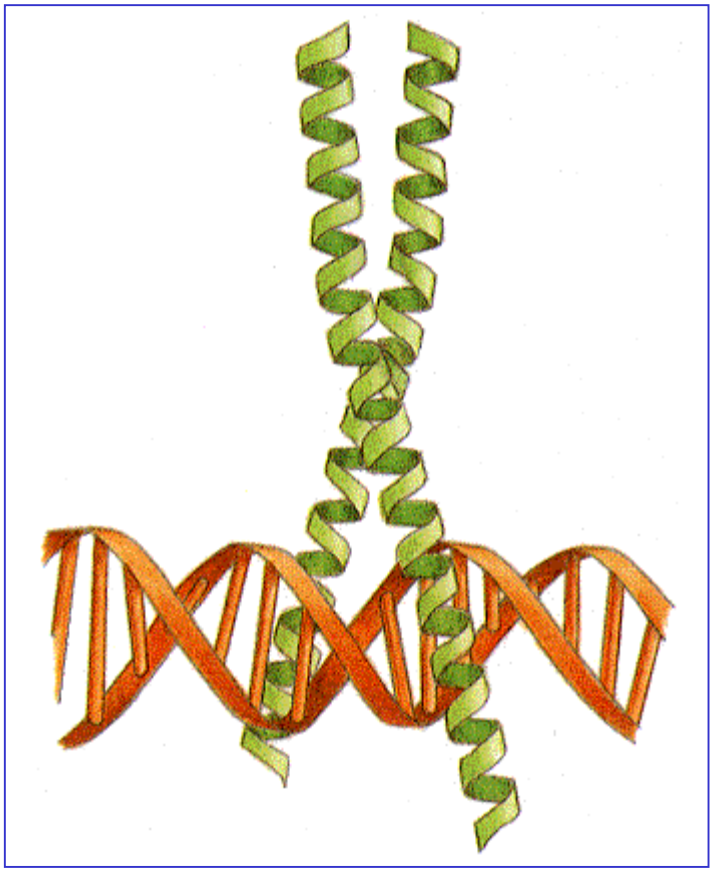
RNA 5S promoter



TRANSCRIPTION CONTROL

Trans regulation

Transcription factors: structure



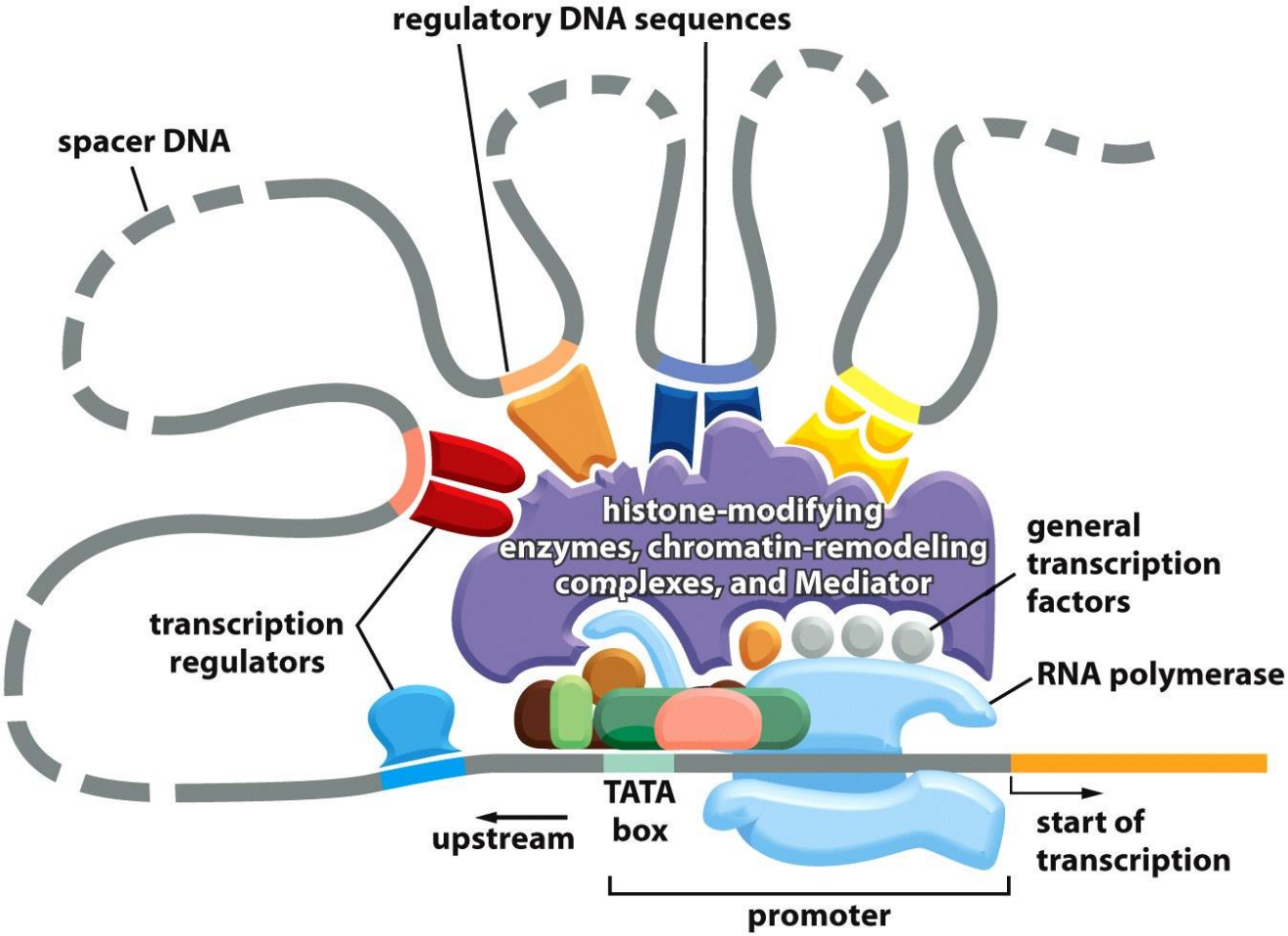
Leucine zipper

Oncogenes fos/jun

CREB: binds a cAMP response element

TRANSCRIPTION CONTROL

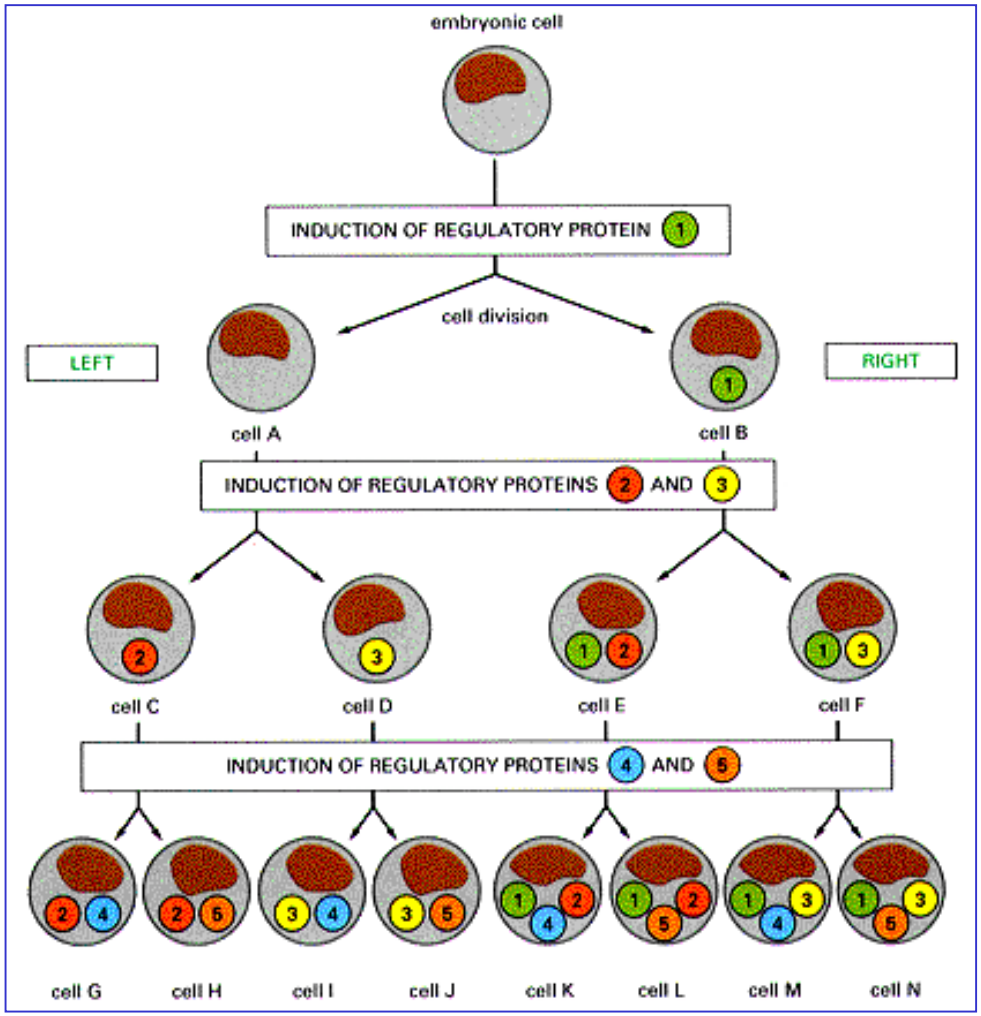
Trans regulation: combinatory control



TRANSCRIPTION CONTROL

Trans regulation: combinatory control

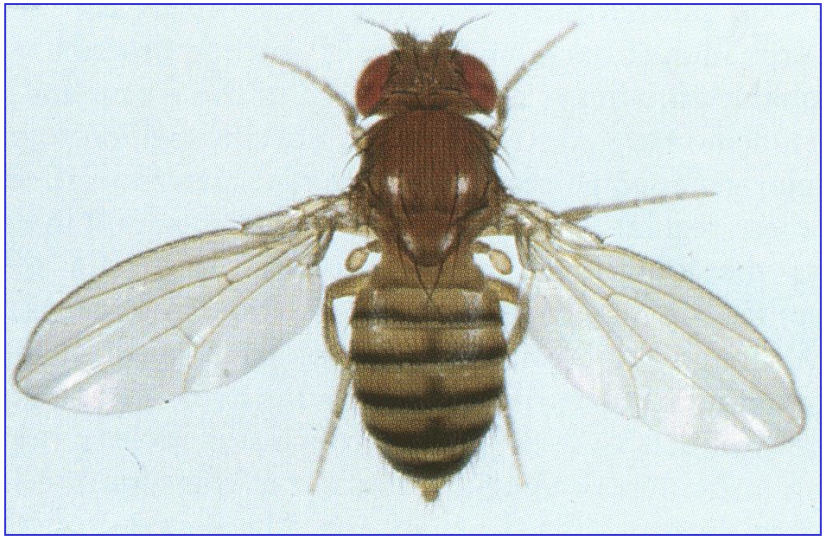
The combination of a few regulatory proteins may produce many different cell types during development



TRANSCRIPTION CONTROL

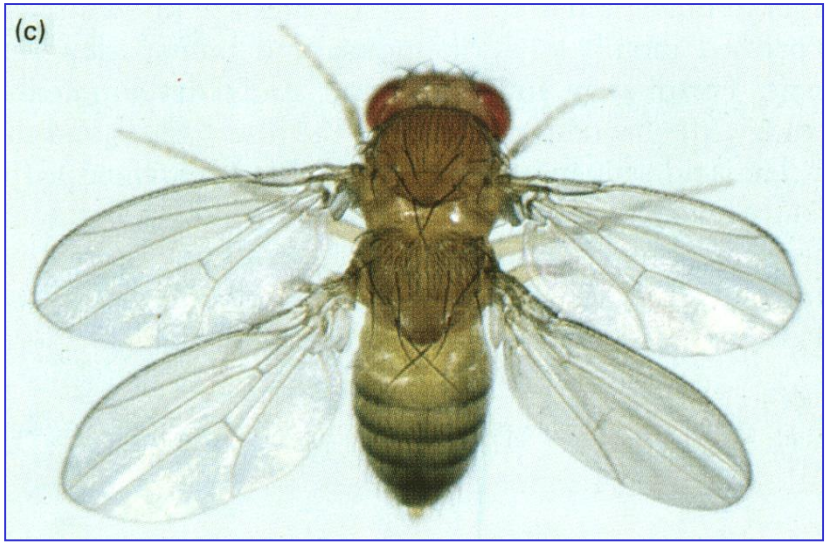
Trans regulation: combinatory control

Normal drosophila



Bitorax mutation: three mutant alleles in homozygosis:

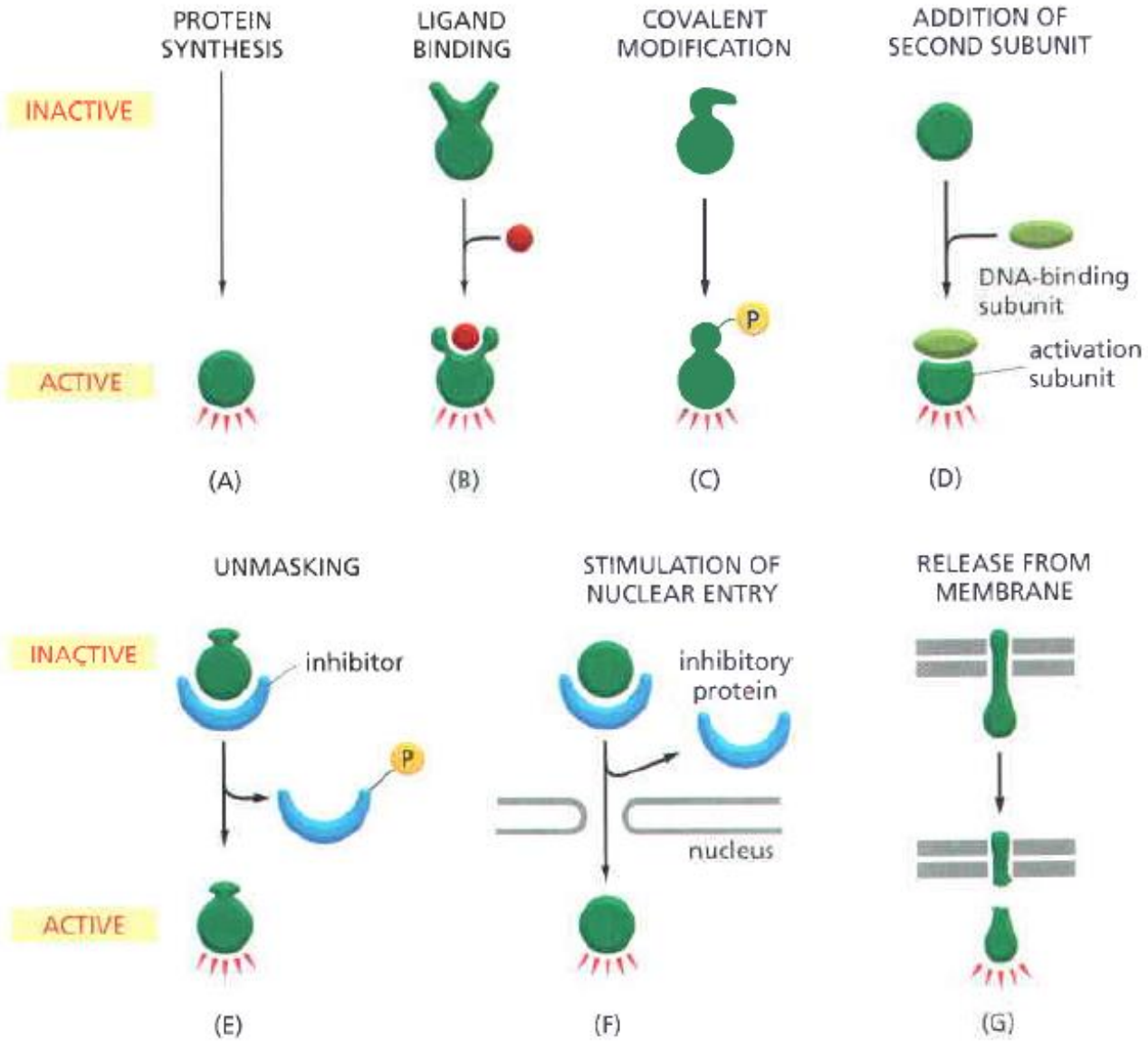
bx*, *abx* and *pbx



TRANSCRIPTION CONTROL

Trans regulation: combinatory control

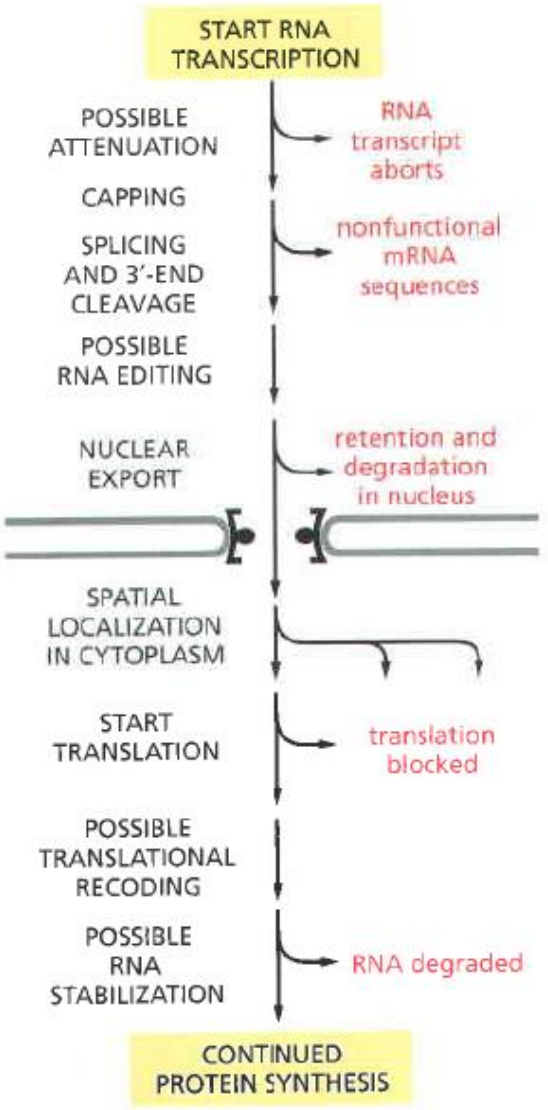
Mechanisms activating regulatory proteins



CONTROL OF GENE EXPRESSION

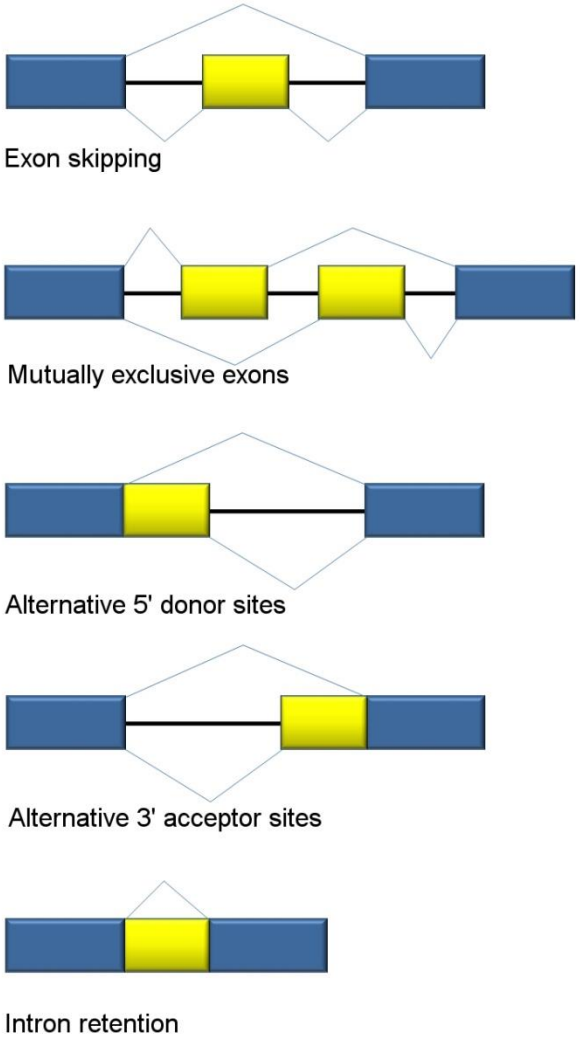
3. Post-transcription

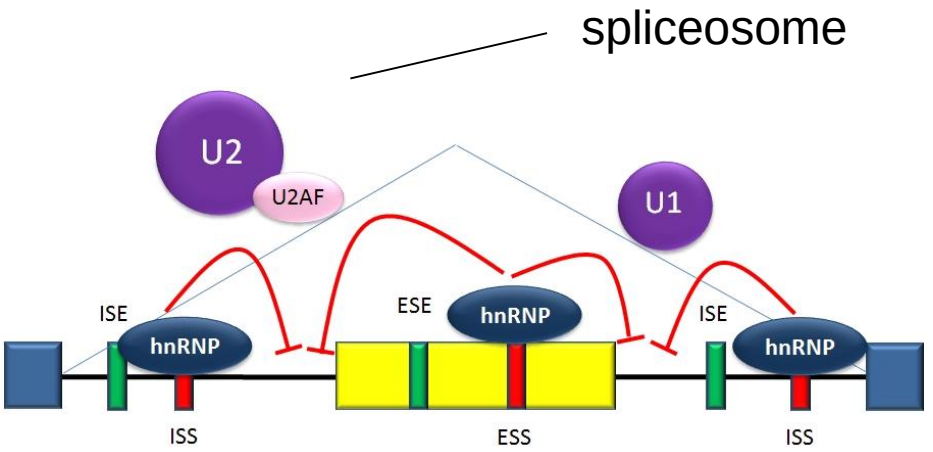
- Alternative splicing
- mRNA edition
- Cytoplasm transport
- mRNA average lifetime
- Interference RNA (RNAi)



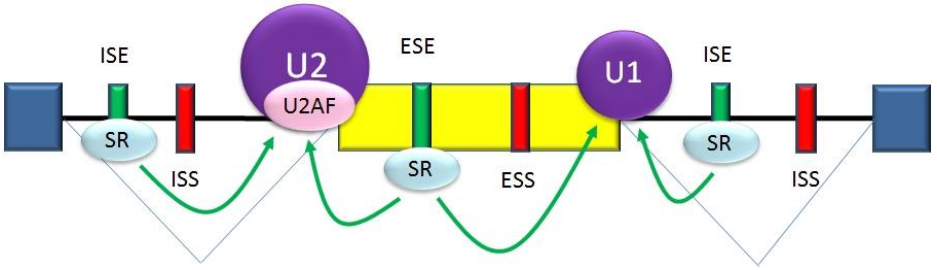
POSTRANSCRIPTIONAL CONTROL

Alternative splicing





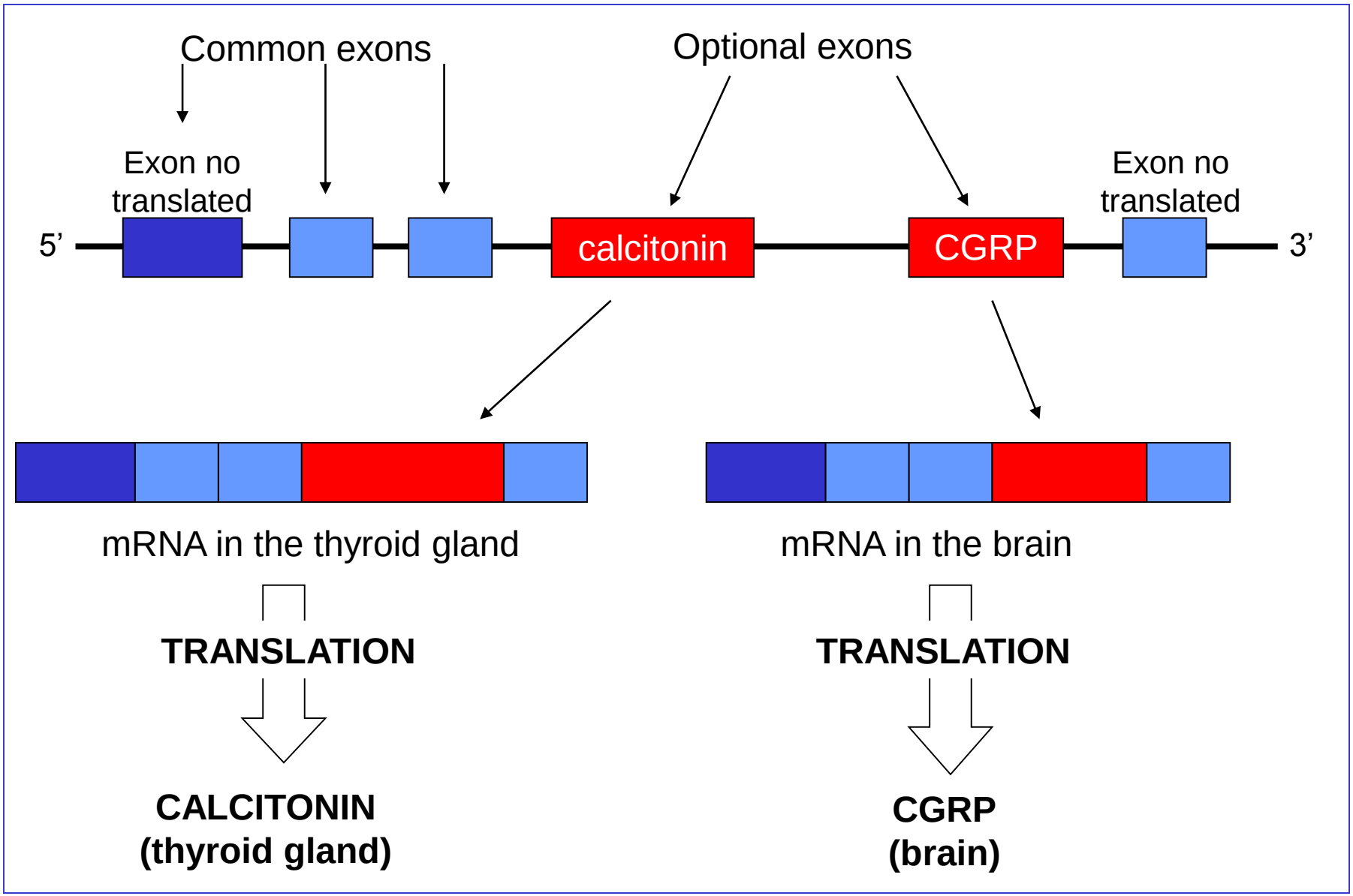
repression



activation

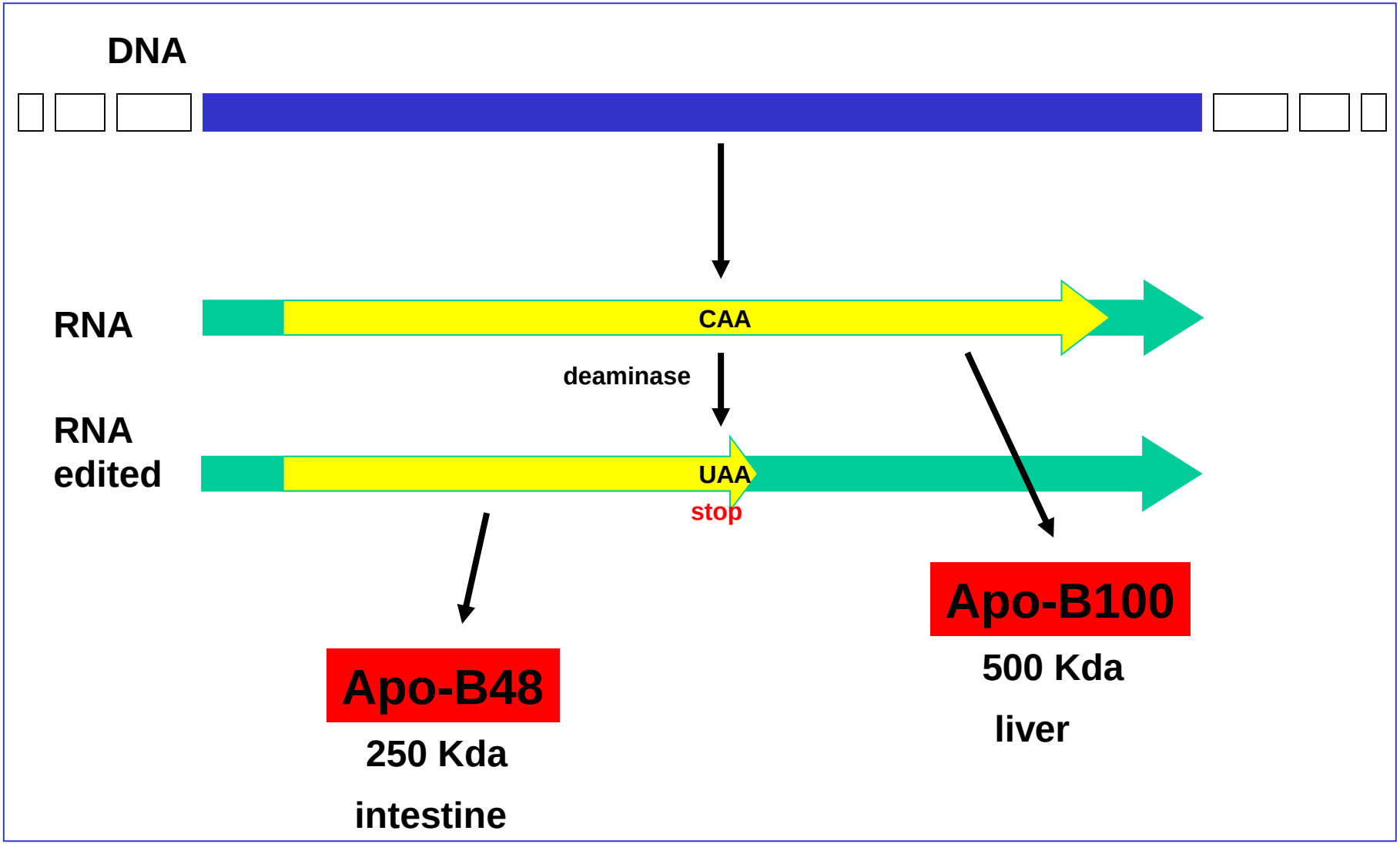
POSTRANSCRIPTIONAL CONTROL

Alternative splicing



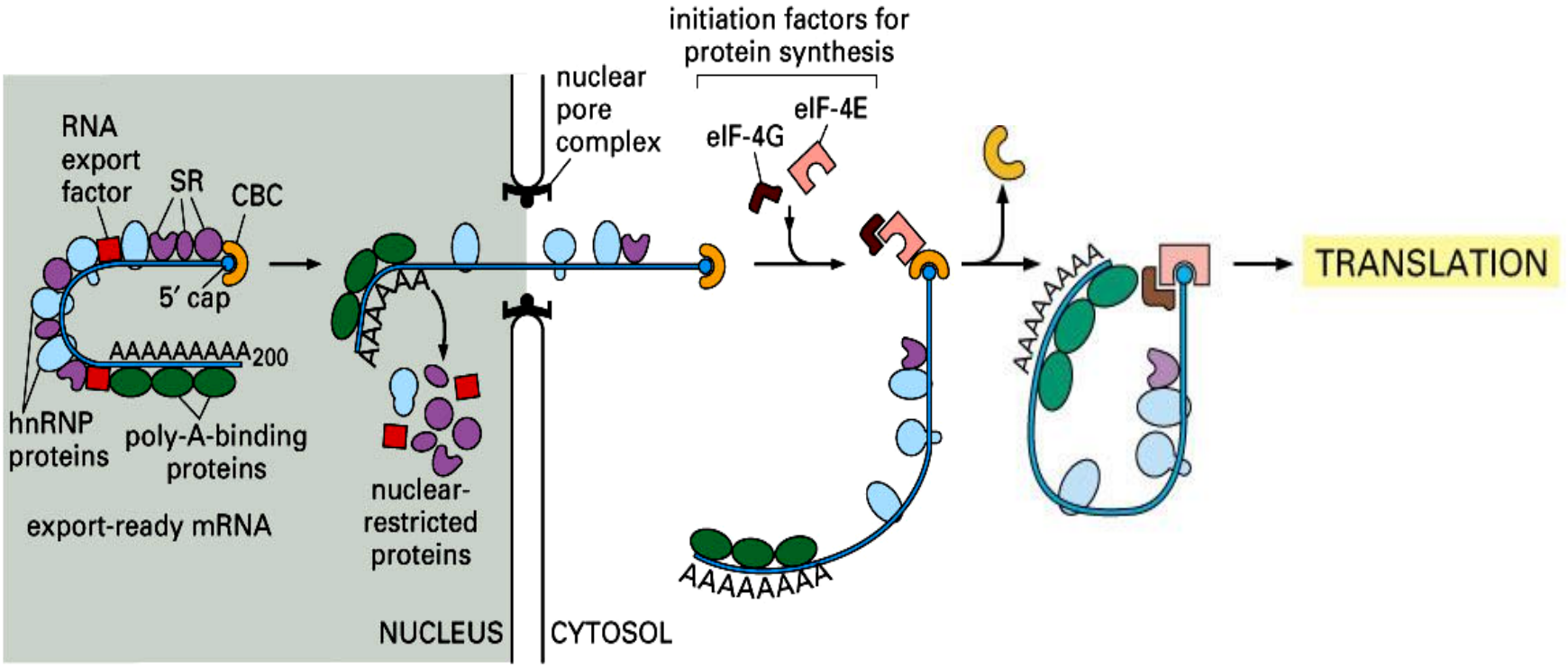
POSTRANSCRIPTIONAL CONTROL

RNA edition



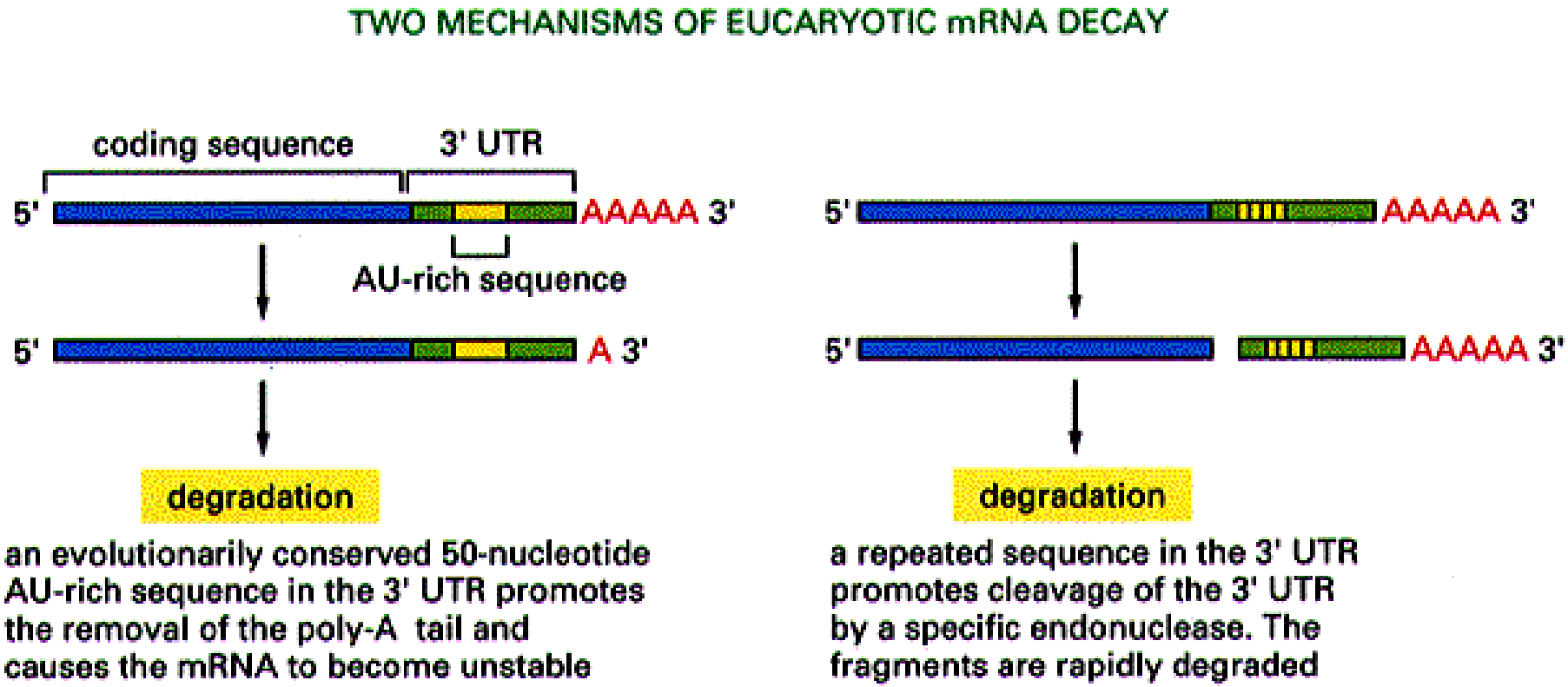
POSTRANSCRIPTIONAL CONTROL

Transport of RNA to cytoplasm



POSTRANSCRIPTIONAL CONTROL

mRNA average lifetime

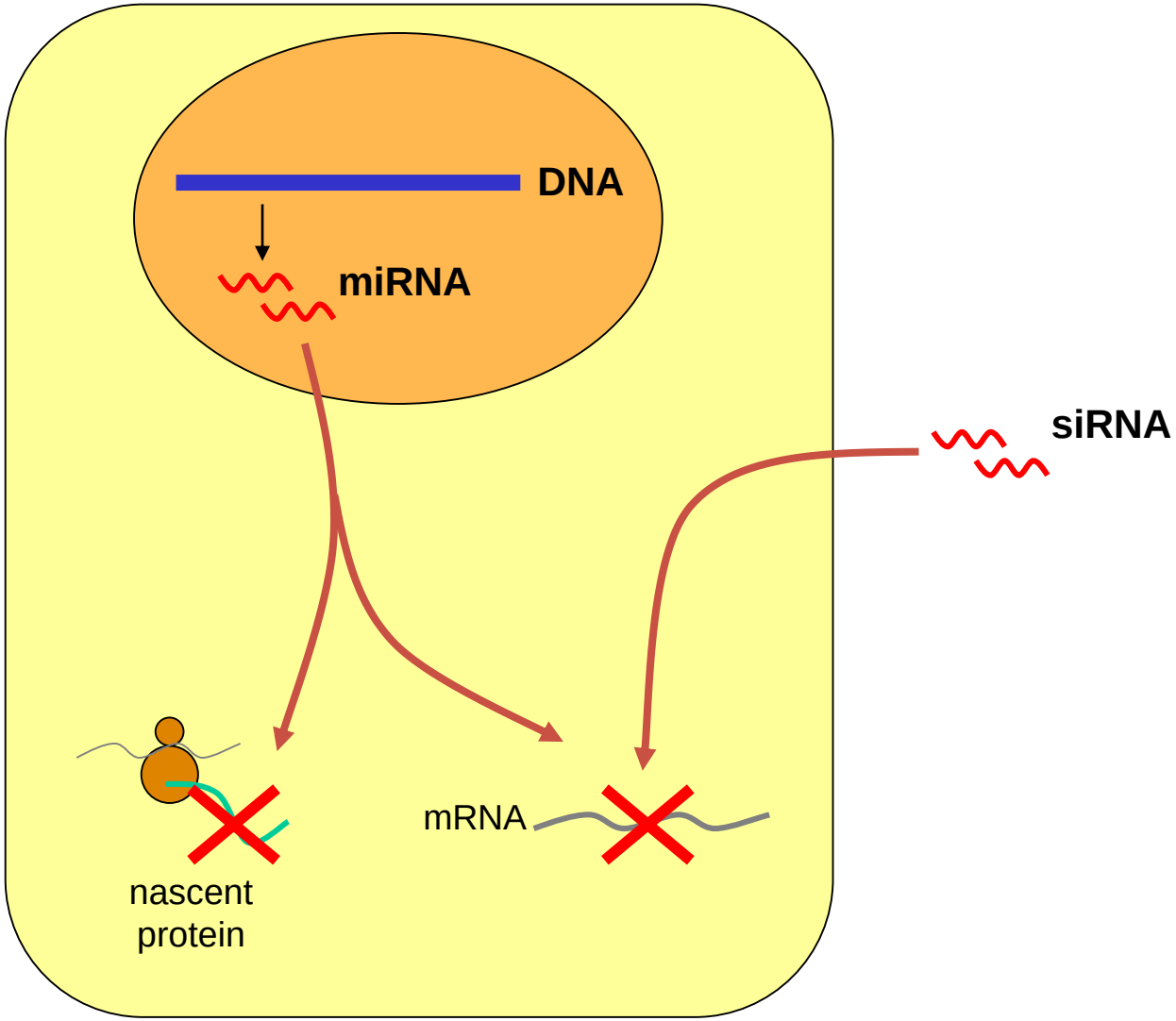


POSTRANSCRIPTIONAL CONTROL

Interference RNA (RNAi)

Andrew Fire

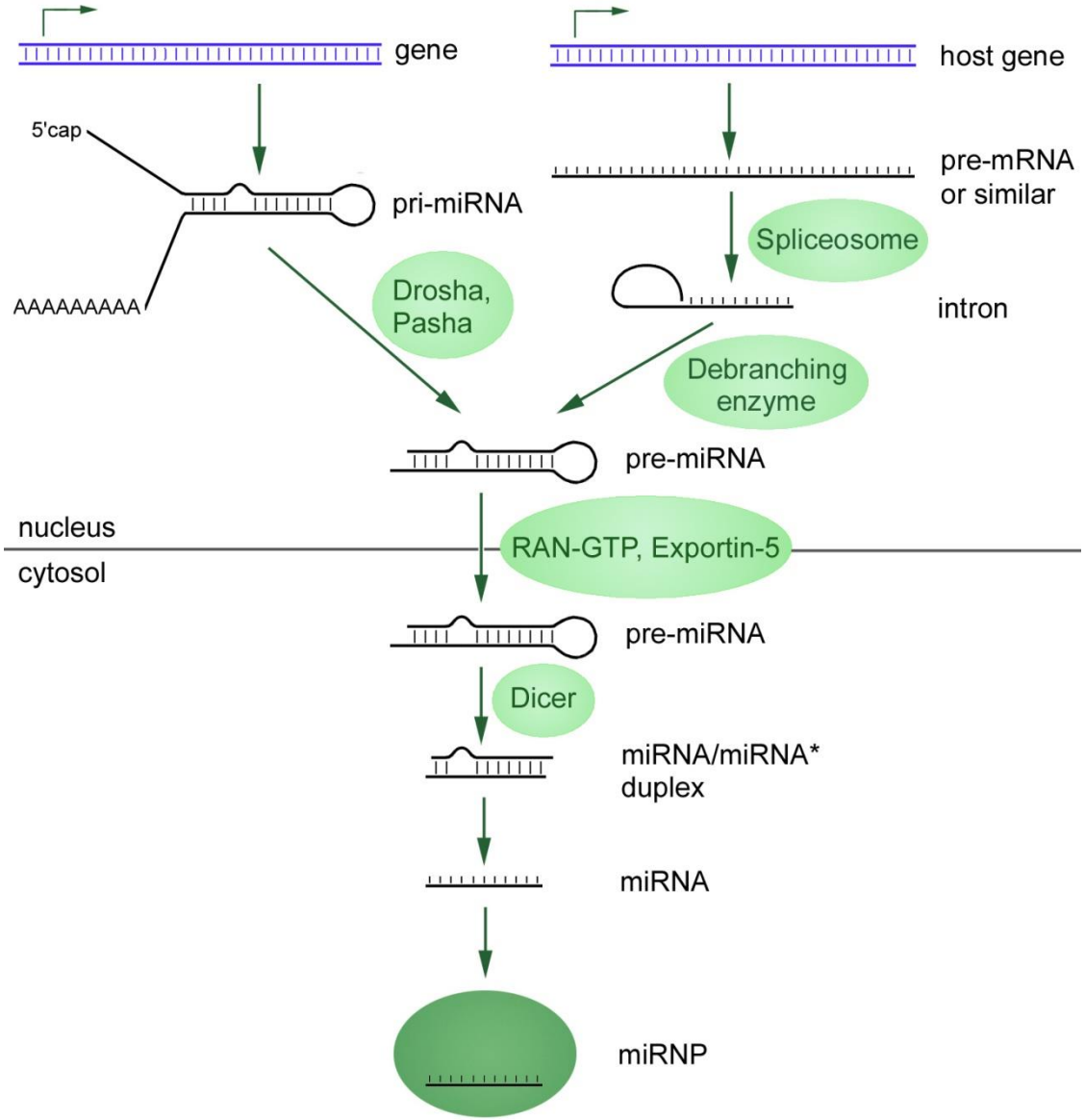
Craig Mello



2006 Nobel Prize in Medicine

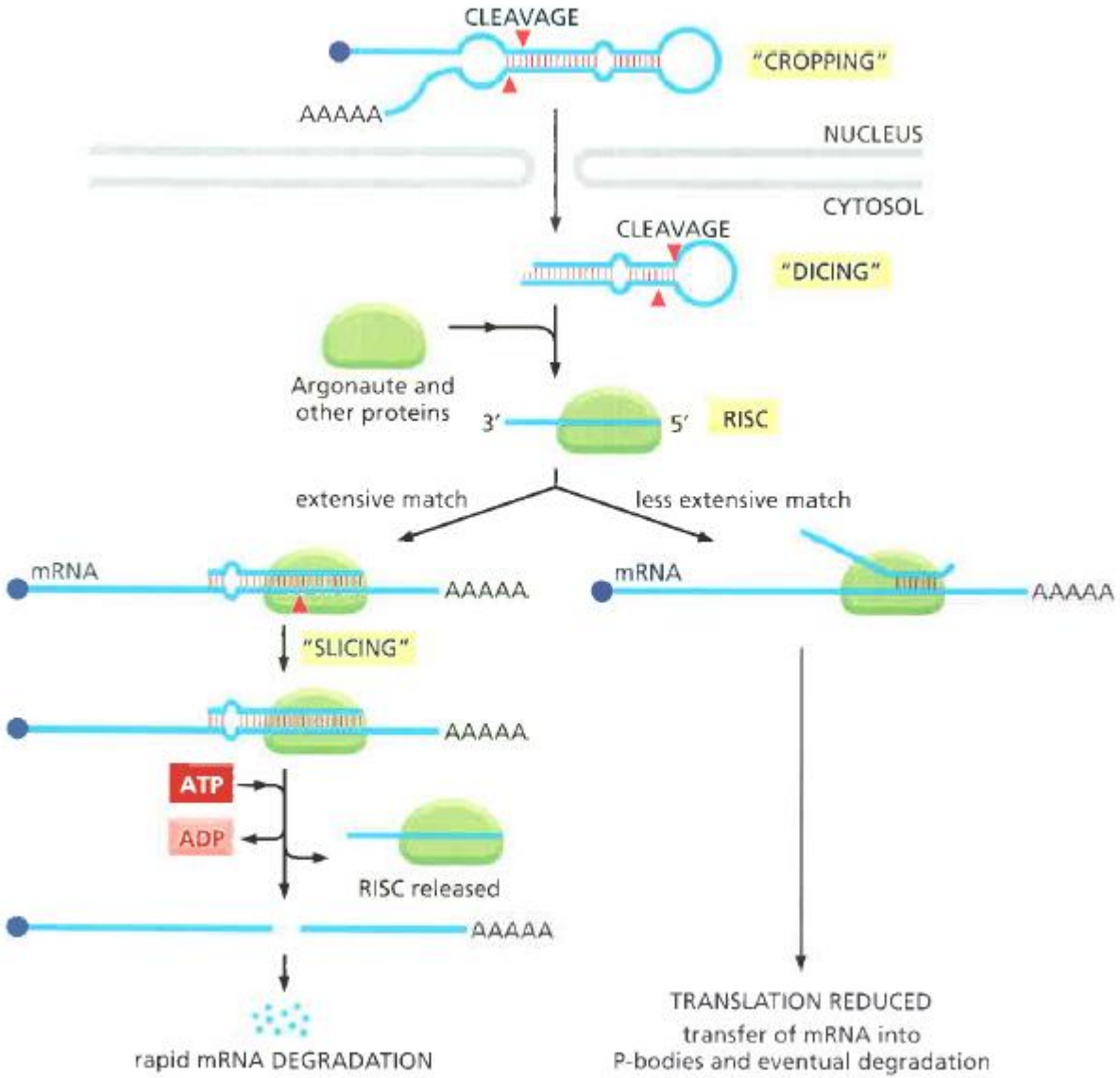
POSTRANSCRIPTIONAL CONTROL

miRNA



POSTRANSCRIPTIONAL CONTROL

miRNA



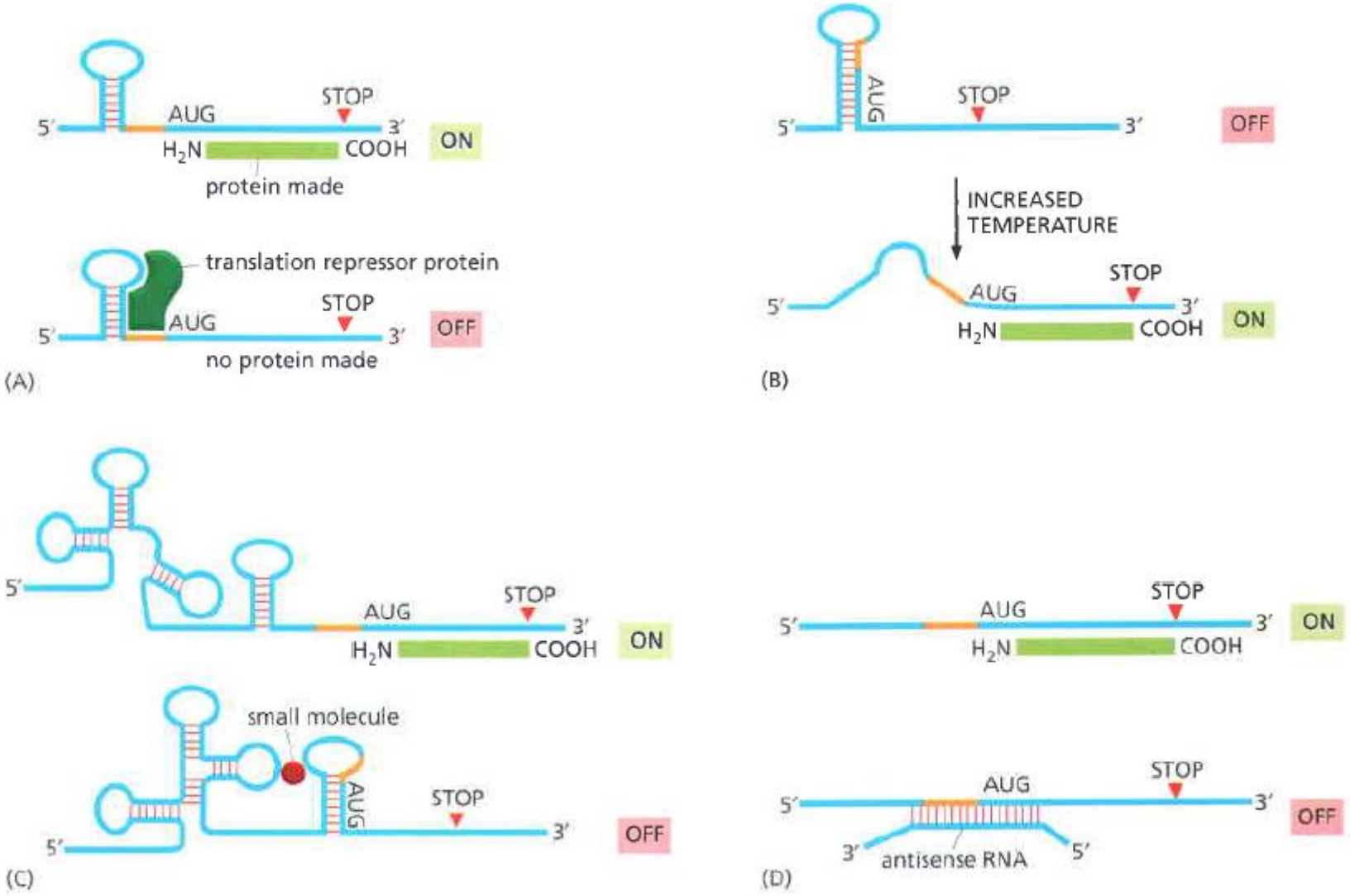
CONTROL OF GENE EXPRESSION

4. Translation

- Binding of proteins to 5’ or 3’ regions no translated
- Modification of initiation factor
- Internal Ribosomal Entry Sites (IRES)

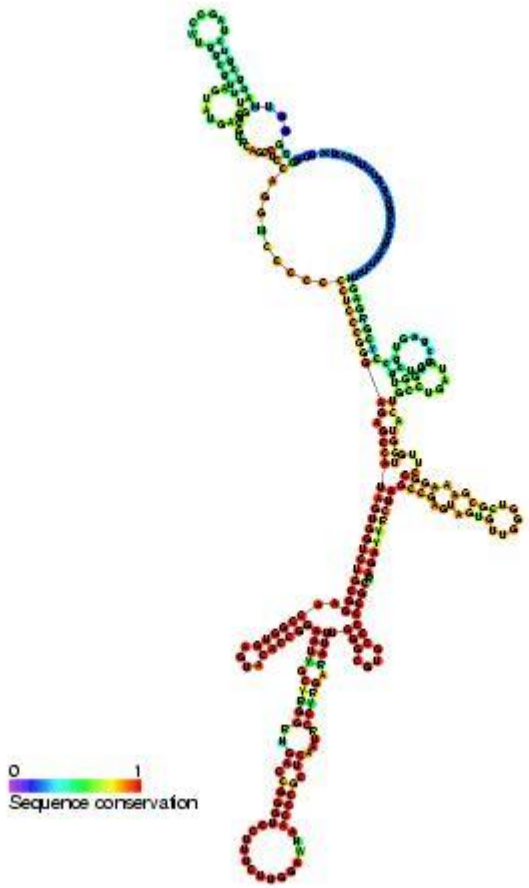
TRANSLATION

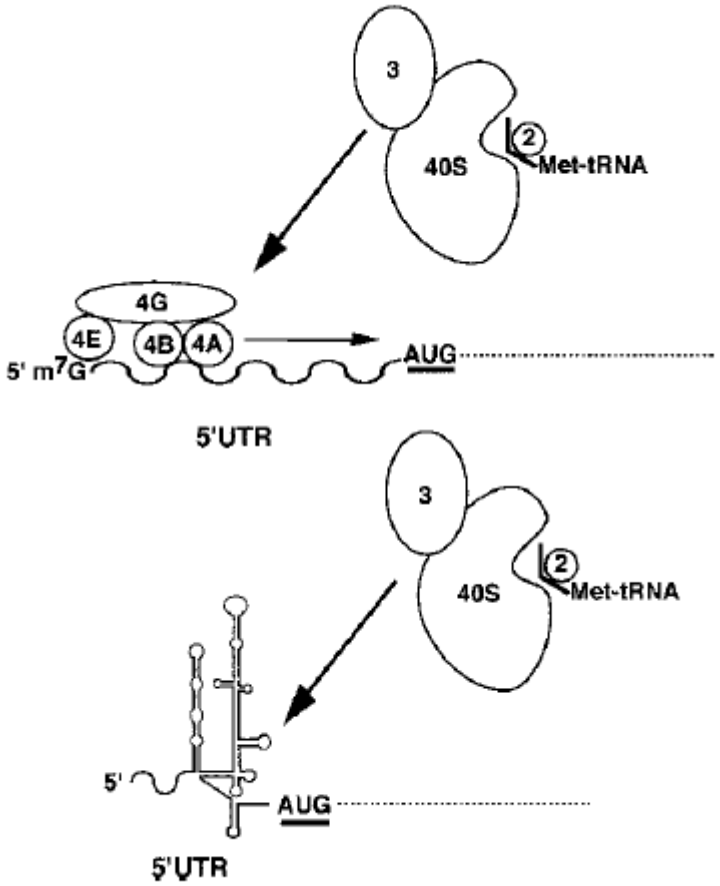
Binding and/or modification of initiation factor



TRANSLATION

Hepatitis C Virus IRES





CAP

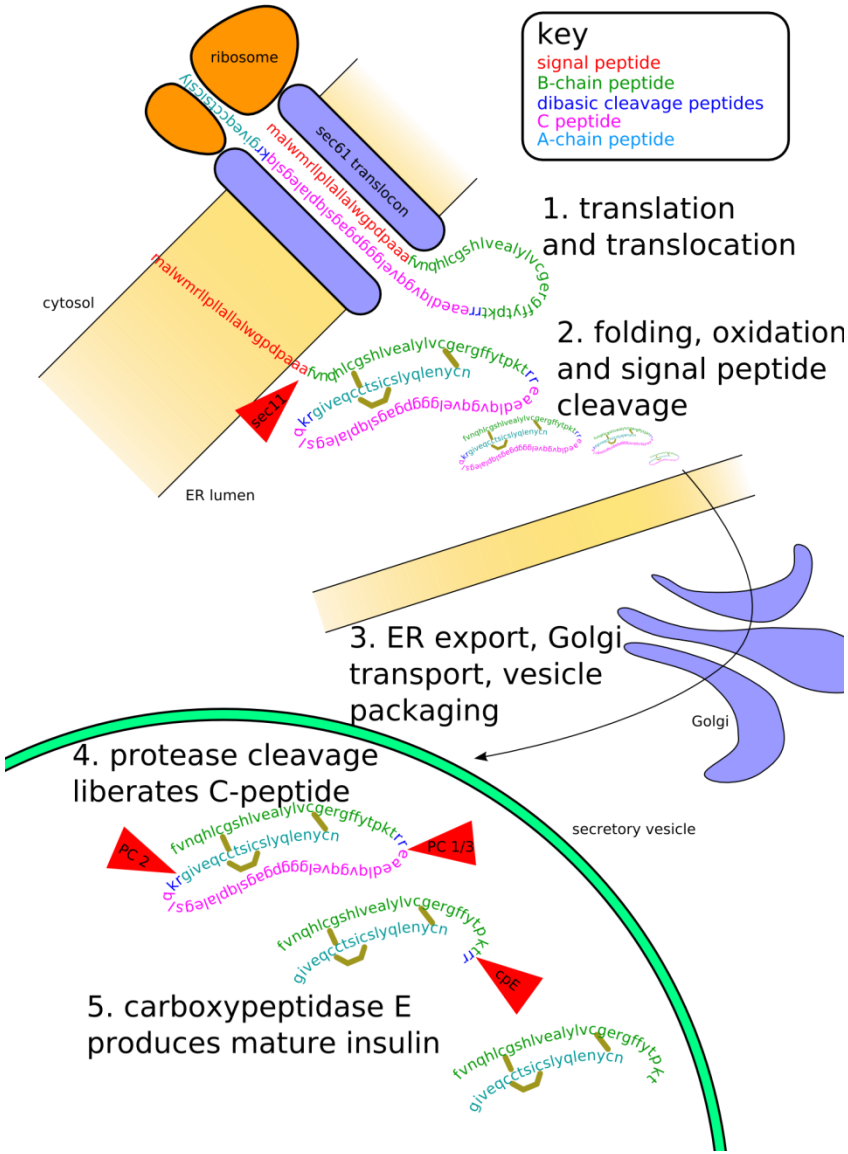
IRES

CONTROL OF GENE EXPRESSIOn

5. Post translational modifications

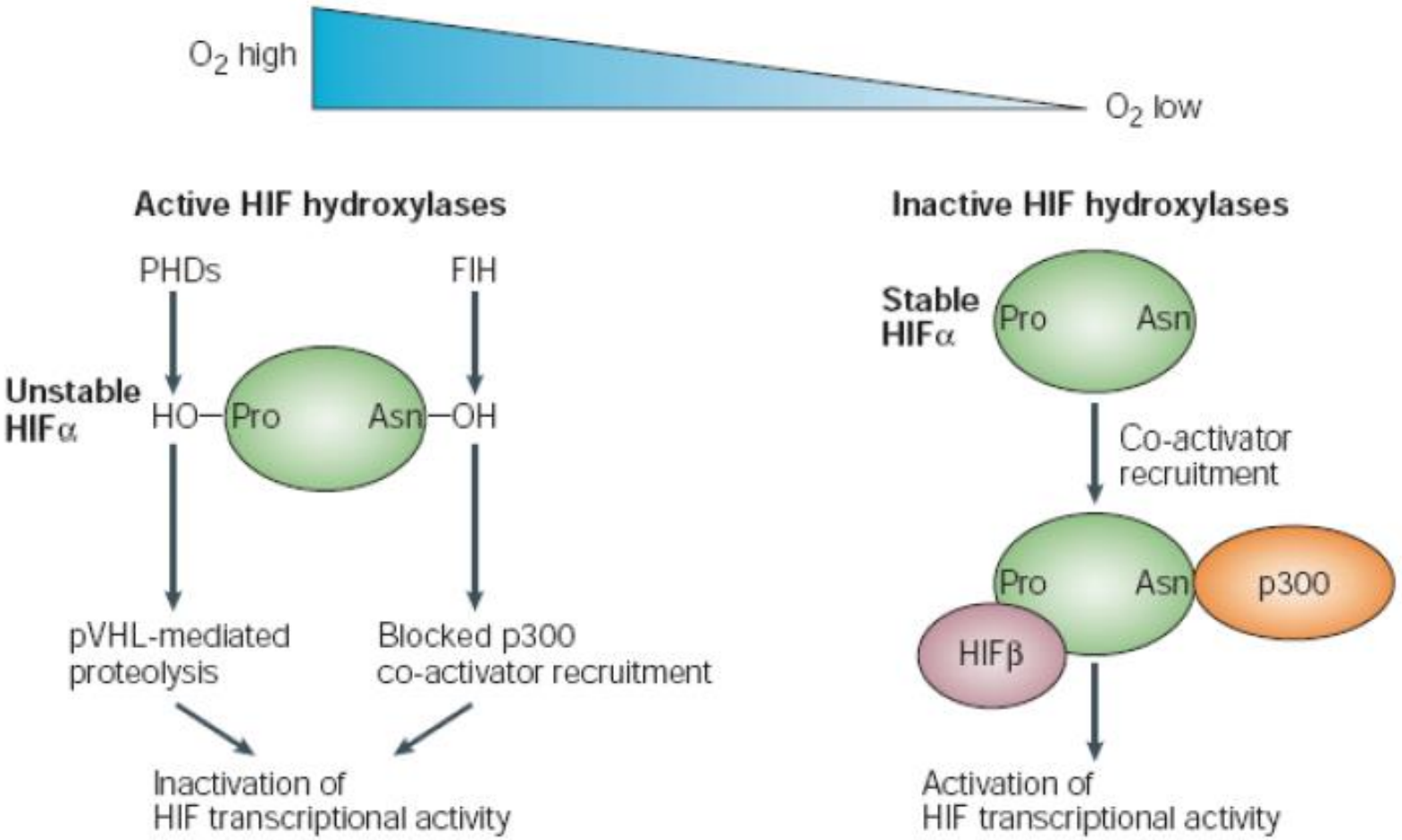
- Chemical remodelling
- Acetylation
- Carboxylation
- Phosphorylation
- Hydroxylation
- Glycosylation
- Acylation
- Disulfide bridge formation
- **Folding**
- **Degradation**

POSTTRANSLATIONAL MODIFICATIONS



Insulin post-translational modifications

POSTTRANSLATIONAL MODIFICATIONS



GENETIC ENGINEERING

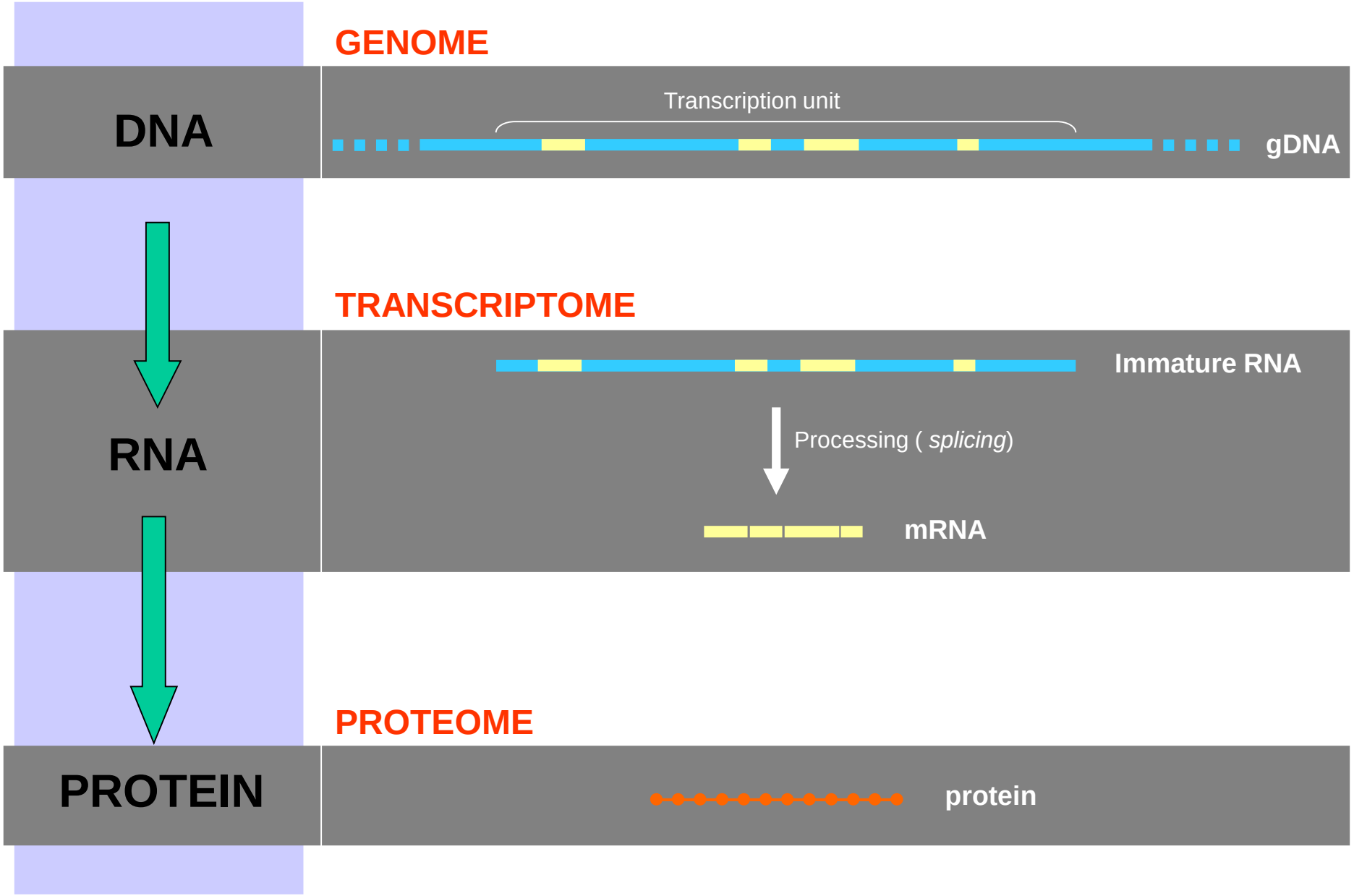
Formation of new combinations of genetic material by insertion of nucleic acid molecules –isolated from the rest of organism components – in any vectorial system that permits its continuous propagation.

Synonyms:

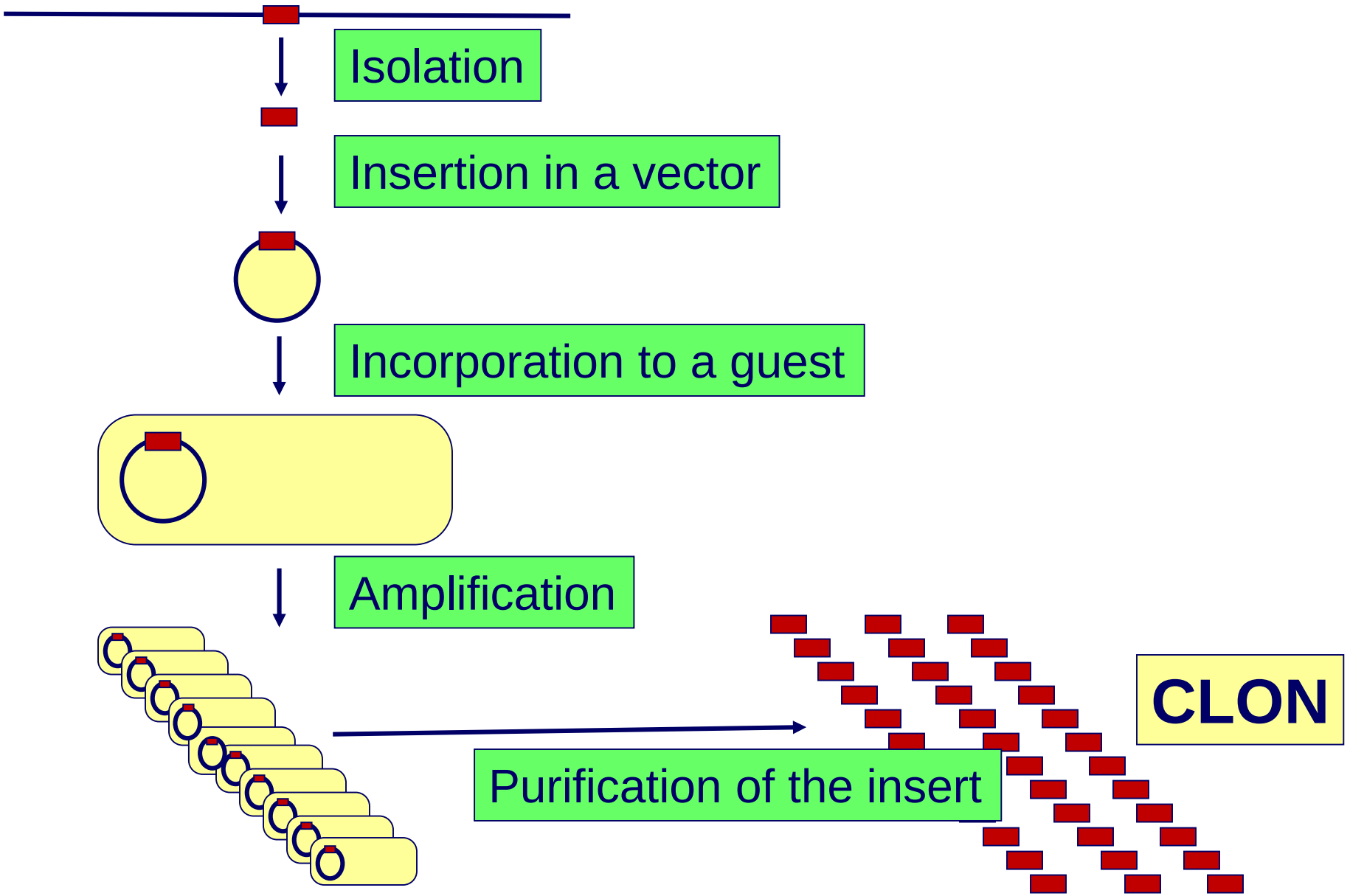
- **Recombinant DNA technology**
- **Gene cloning**
- **Genetic manipulation**

Genetic engineering: important advances	
1953	Watson y Crick: structure of DNA double helix
1957	Kornberg: DNA polymerase
1961	Marmur y Doty: renaturation and hybridization of DNA
1962	Arber, Nathans y H.Smith: restriction enzymes
1966	Niremberg, Ochoa, Khorana: genetic code
1967	Gellert: DNA ligase
1972-73	Boyer, Cohen y Berg: DNA cloning
1975	Southern: hybridization from genes
1975-77	Sanger, Barrel, Maxam y Gilbert: DNA sequencing
1981-82	Palmiter y Brinster: transgenic mice
1985	Mullis: polymerase chain reaction
1990	Several: Fluorescent in situ hybridization (FISH)
1996-97	Several: DNA microarrays
2001	Venter y Collins: human genome project

MOLECULAR ANALYSIS



GENE CLONING



GENE ISOLATION

1) Physical methods

- Agitation
- Sonication

2) Chemical synthesis

- Small oligonucleotides

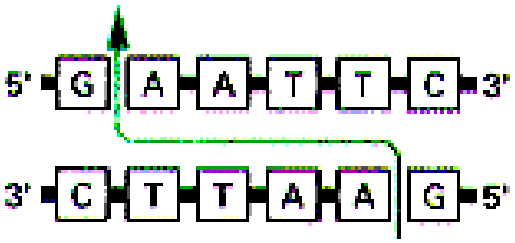
3) Restriction enzymes

4) Obtaining cDNA

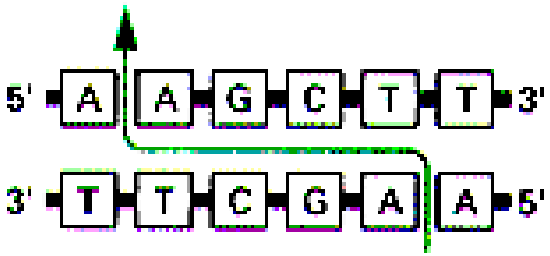
ENZYMES

Restriction nucleases

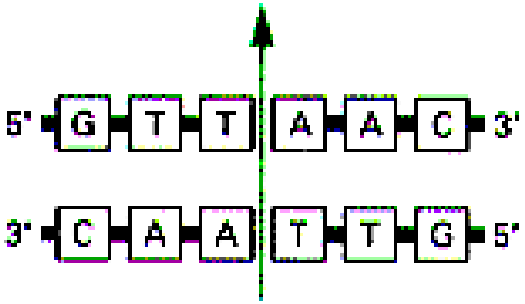
EcoR I



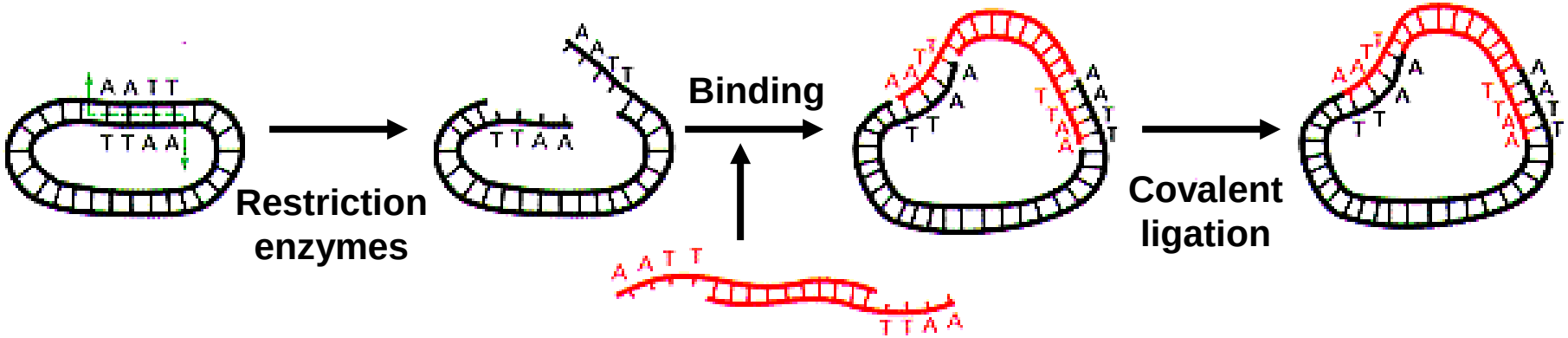
Hind III



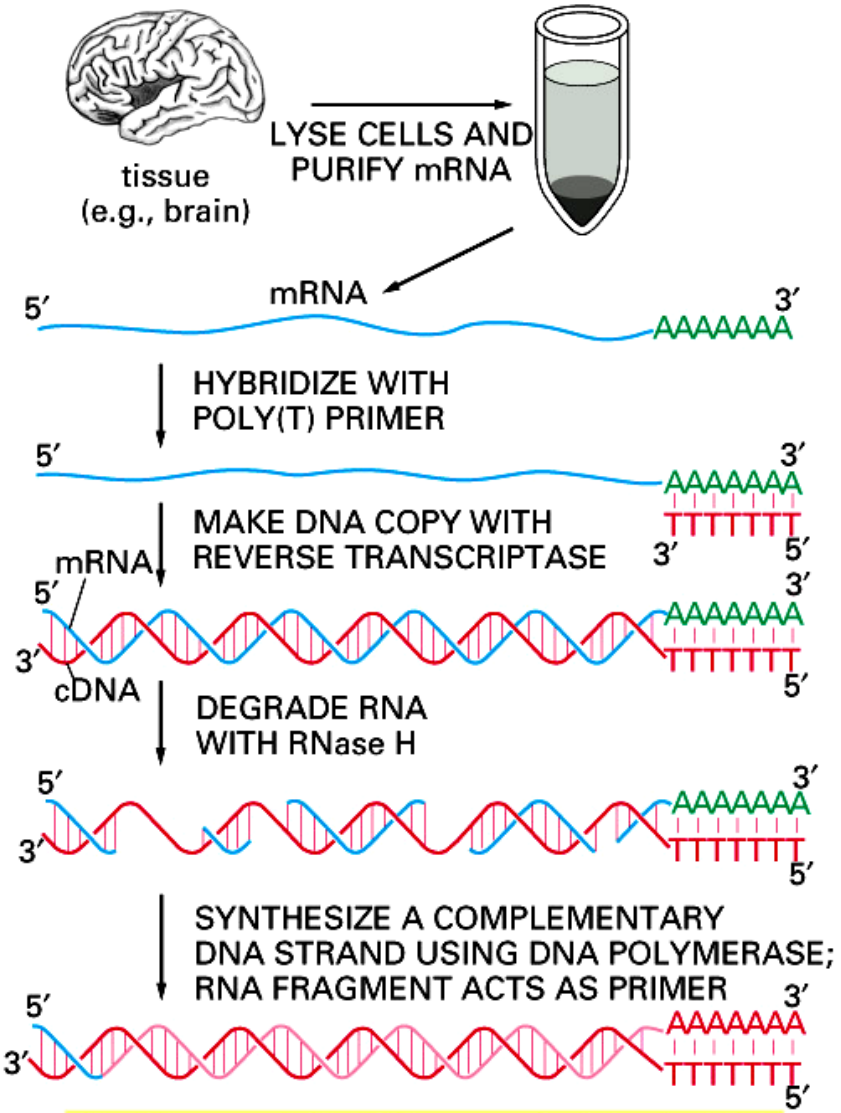
Hpa I



Ligases



Obtaining cDNA

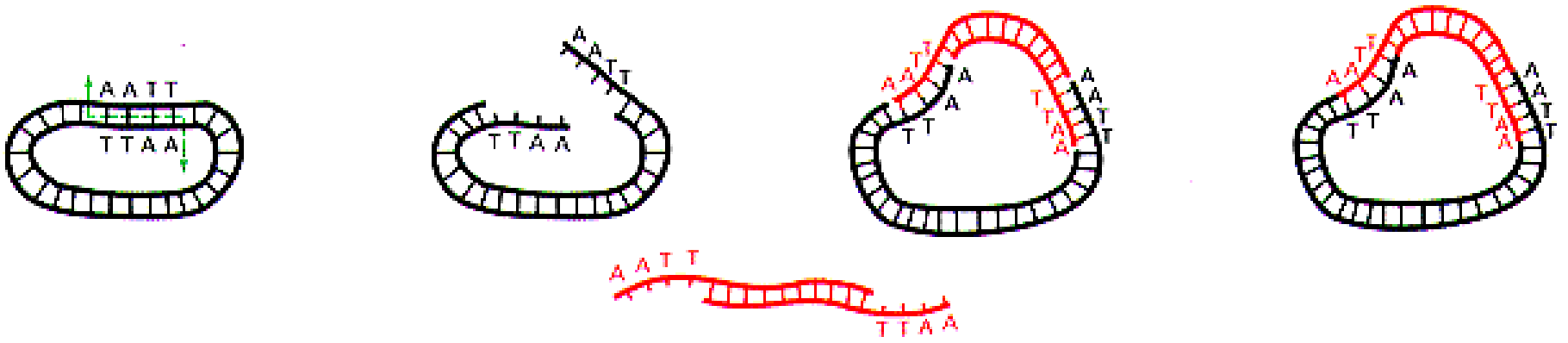


double-stranded cDNA copy of original mRNA

GENE VECTORS

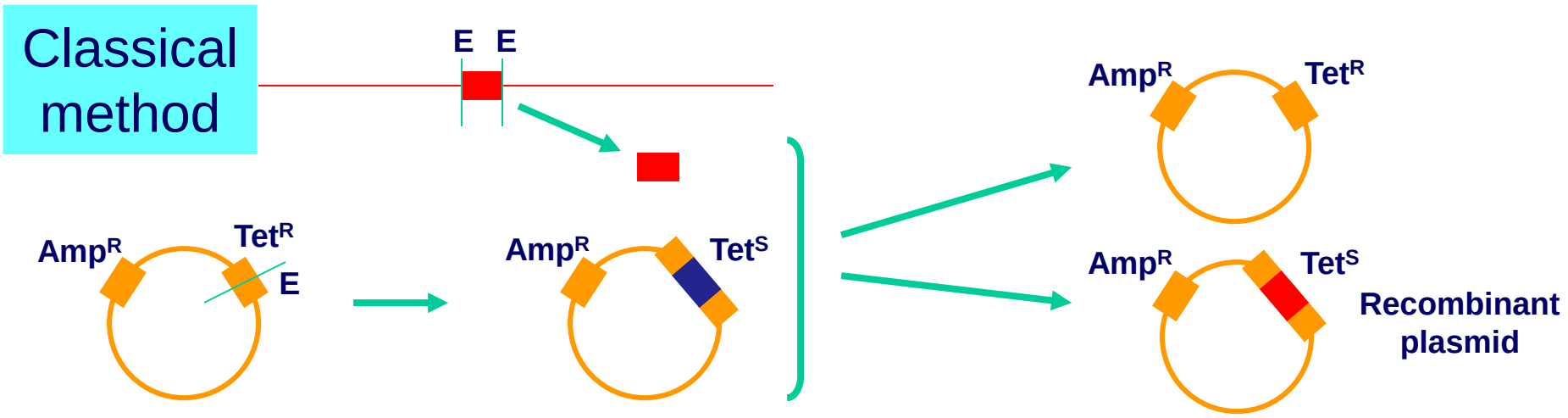
Vector	Capacity (kb)	Guest
Plasmid	8-9	E.Coli
Phage λ	Insertion: 12 Deletion-substitution: 22	E.Coli
Cosmids	45	E.Coli
Phage P1	70-100	E.Coli
PAC	130-150	E.Coli
BAC	120-300	E.Coli
YAC	250-400	Yeast

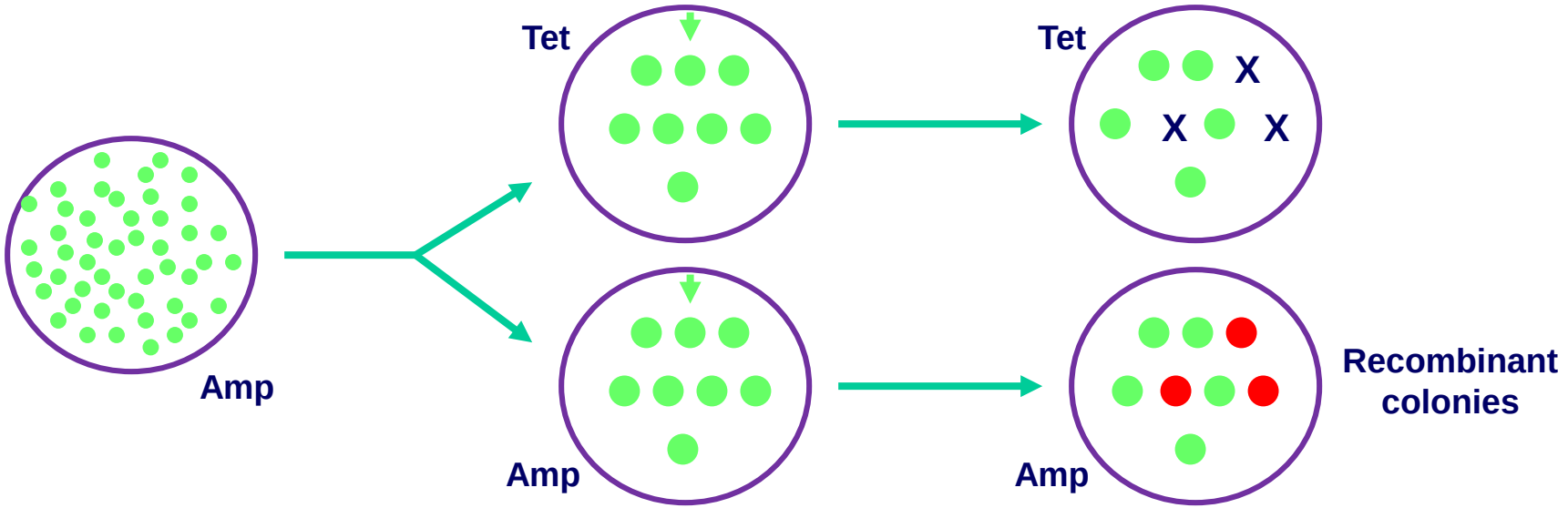
LIGATION BETWEEN HOST AND PASSENGER DNA



RECUPERATION OF RECOMBINANT COLONIES

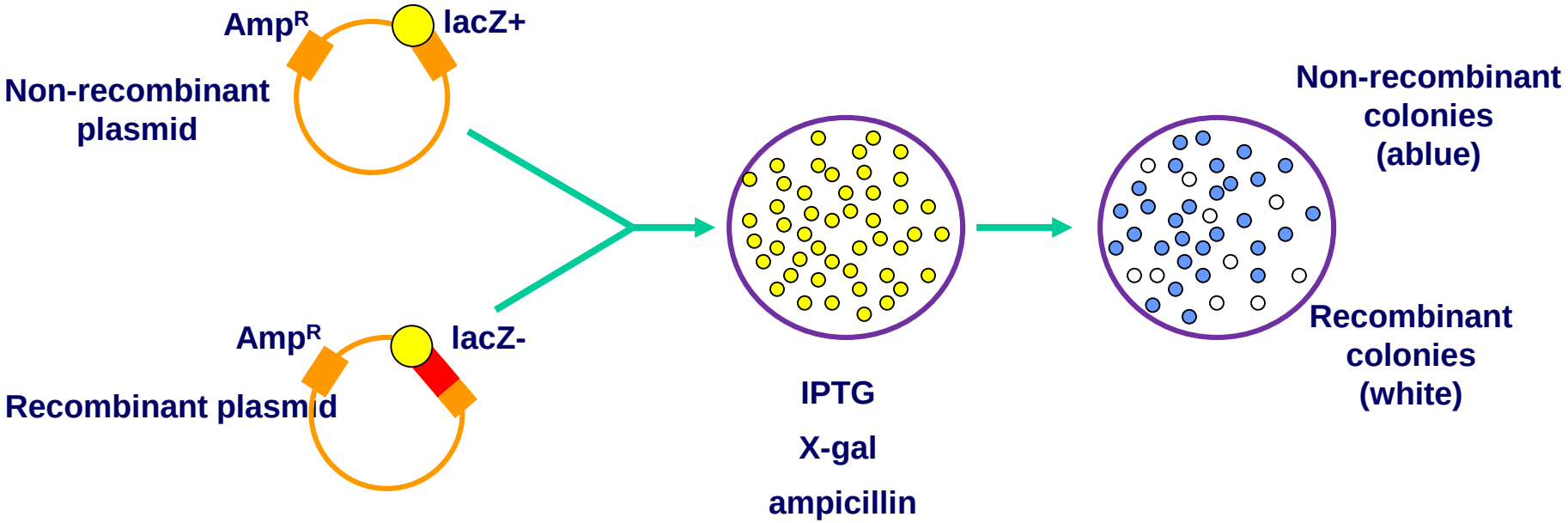
Classical method





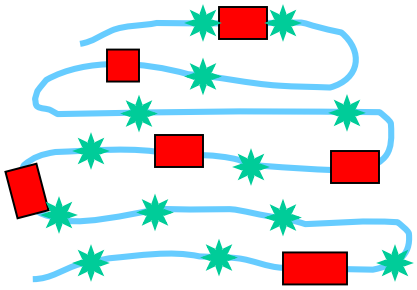
RECUPERATION OF RECOMBINANT COLONIES

Plasmids with the gene lacZ



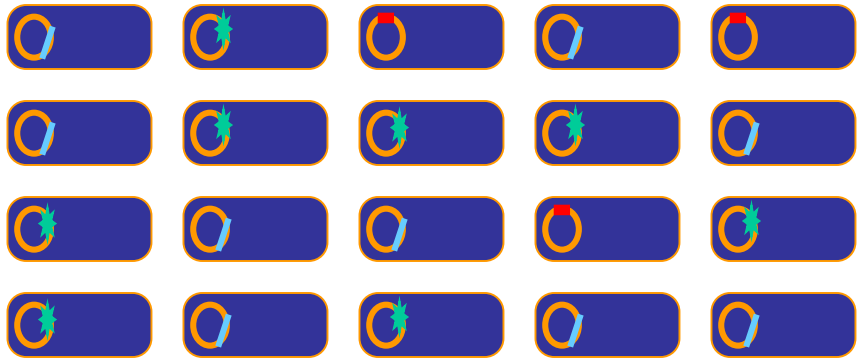
LIBRARIES

Genomic DNA (gDNA)



STS (sequence taged sites)

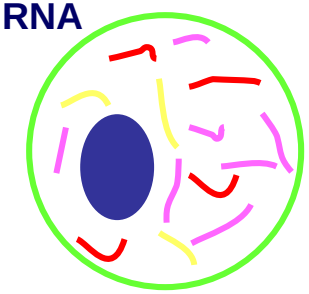
cloning



gDNA library

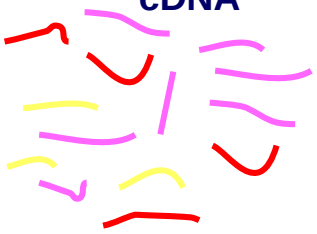
Complementary DNA (cDNA)

mRNA

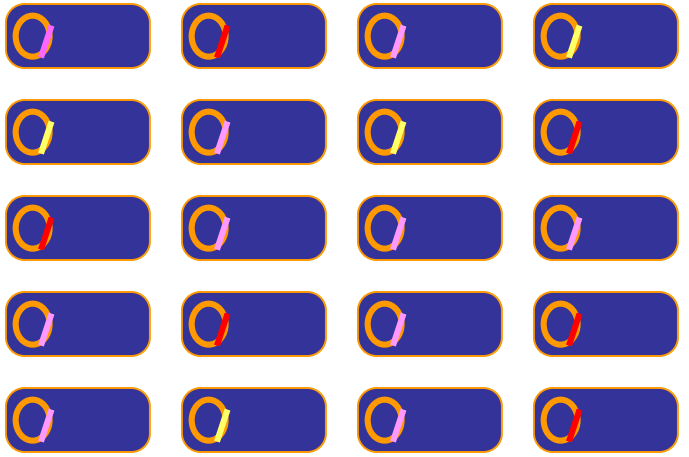


RT

cDNA



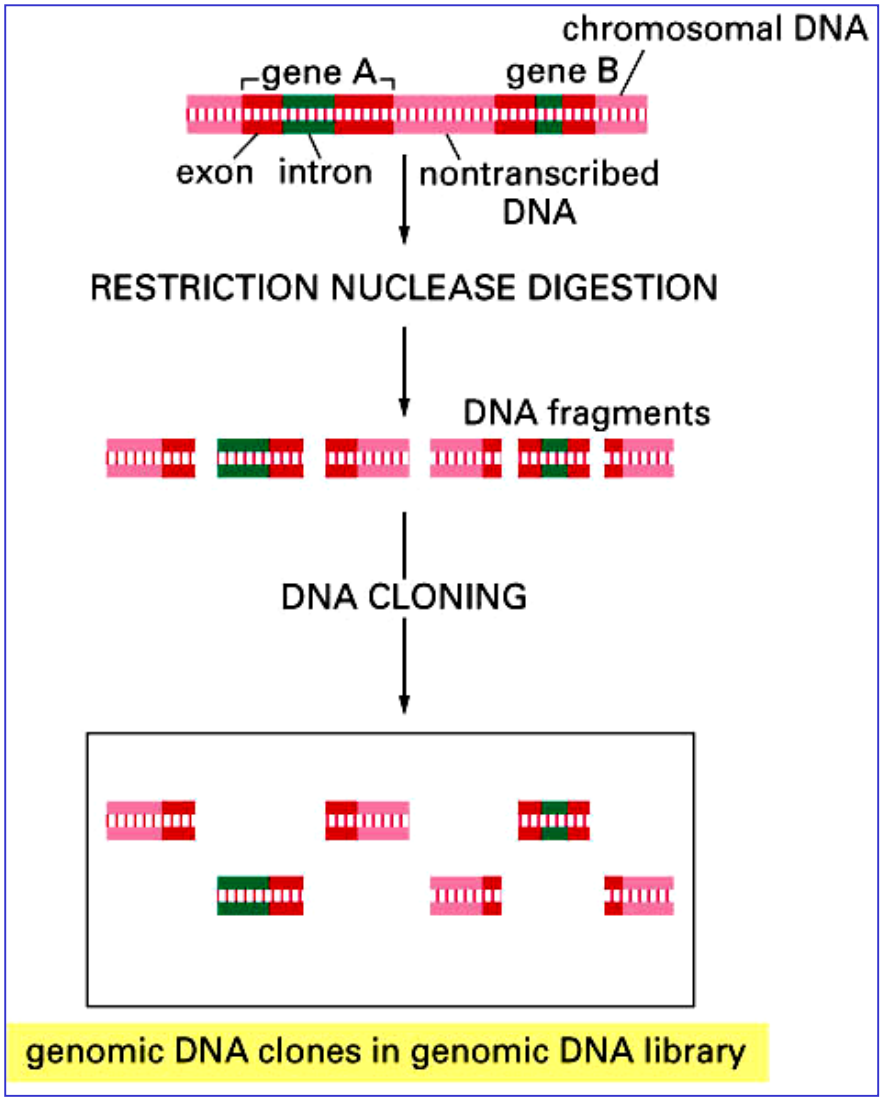
cloning

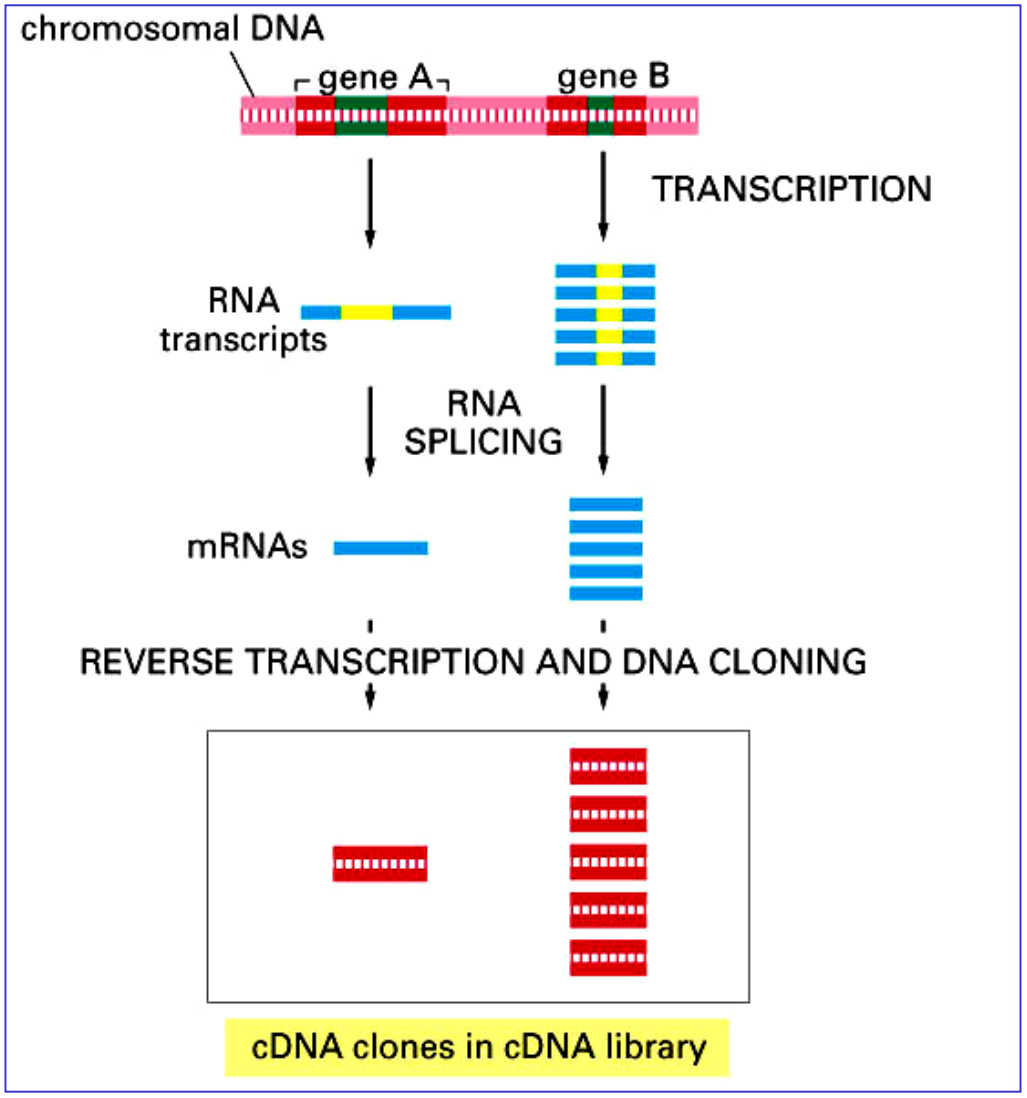


EST (expresed sequence tags)

cDNA library

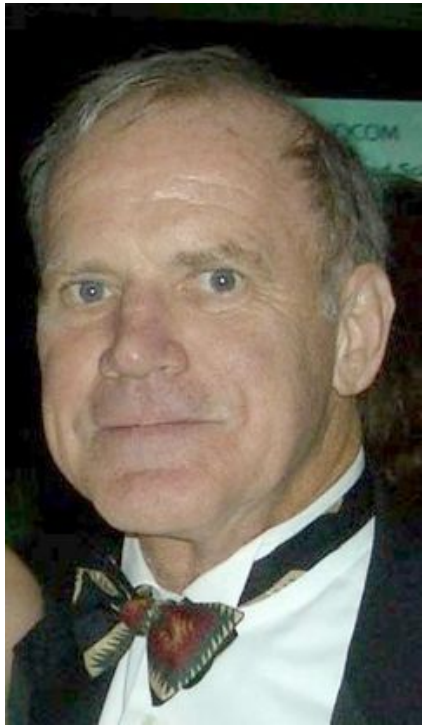
Clones of gDNA and cDNA derived from the same region of DNA



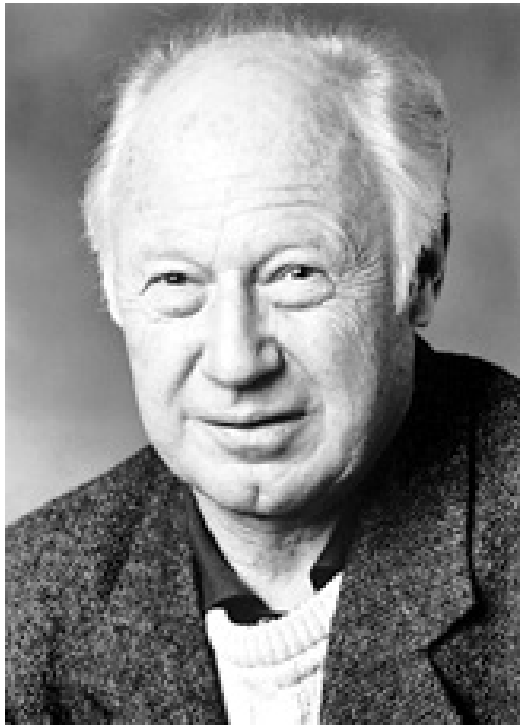


MEDICINE AND PHYSIOLOGY NOBEL PRIZE

1993



Kary Mullis



Michael Smith

for contributions to the developments of methods within DNA-based chemistry

CLONING IN VITRO: PCR

1) Denaturation

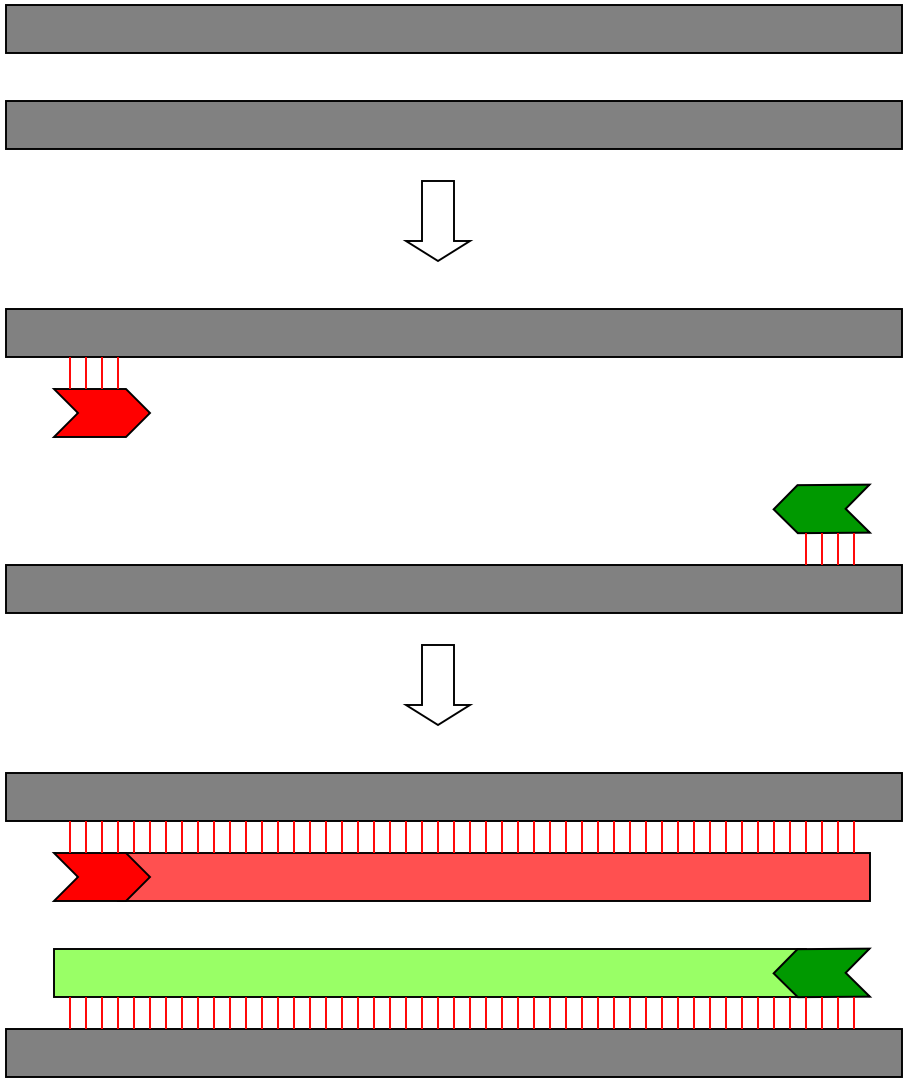
94ºC 1 min.

2) Primer hybridization

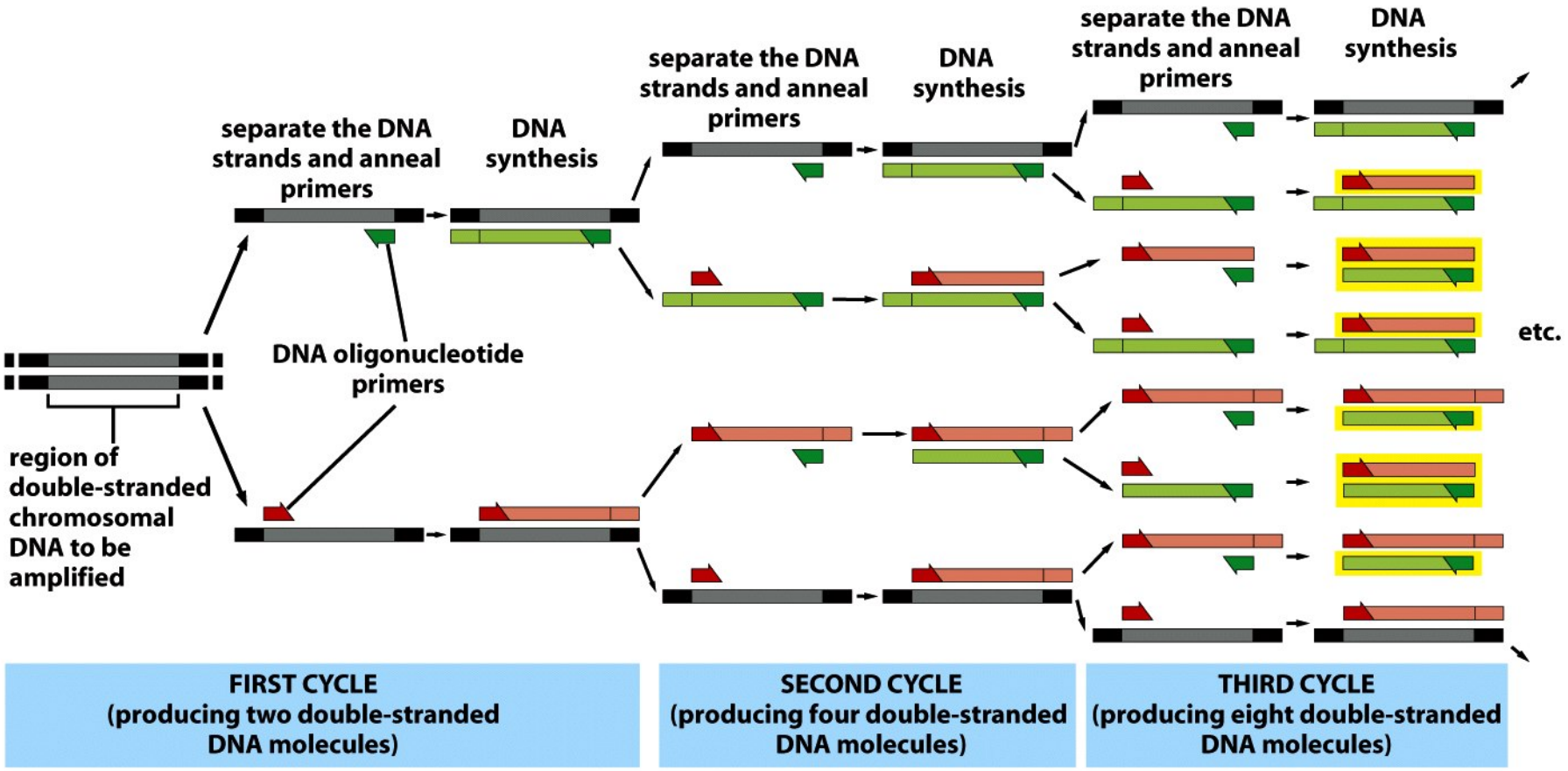
58ºC 1 min.

3) Polymerization (Taq polymerase)

72ºC 1 min.



CLONING IN VITRO: PCR



PCR

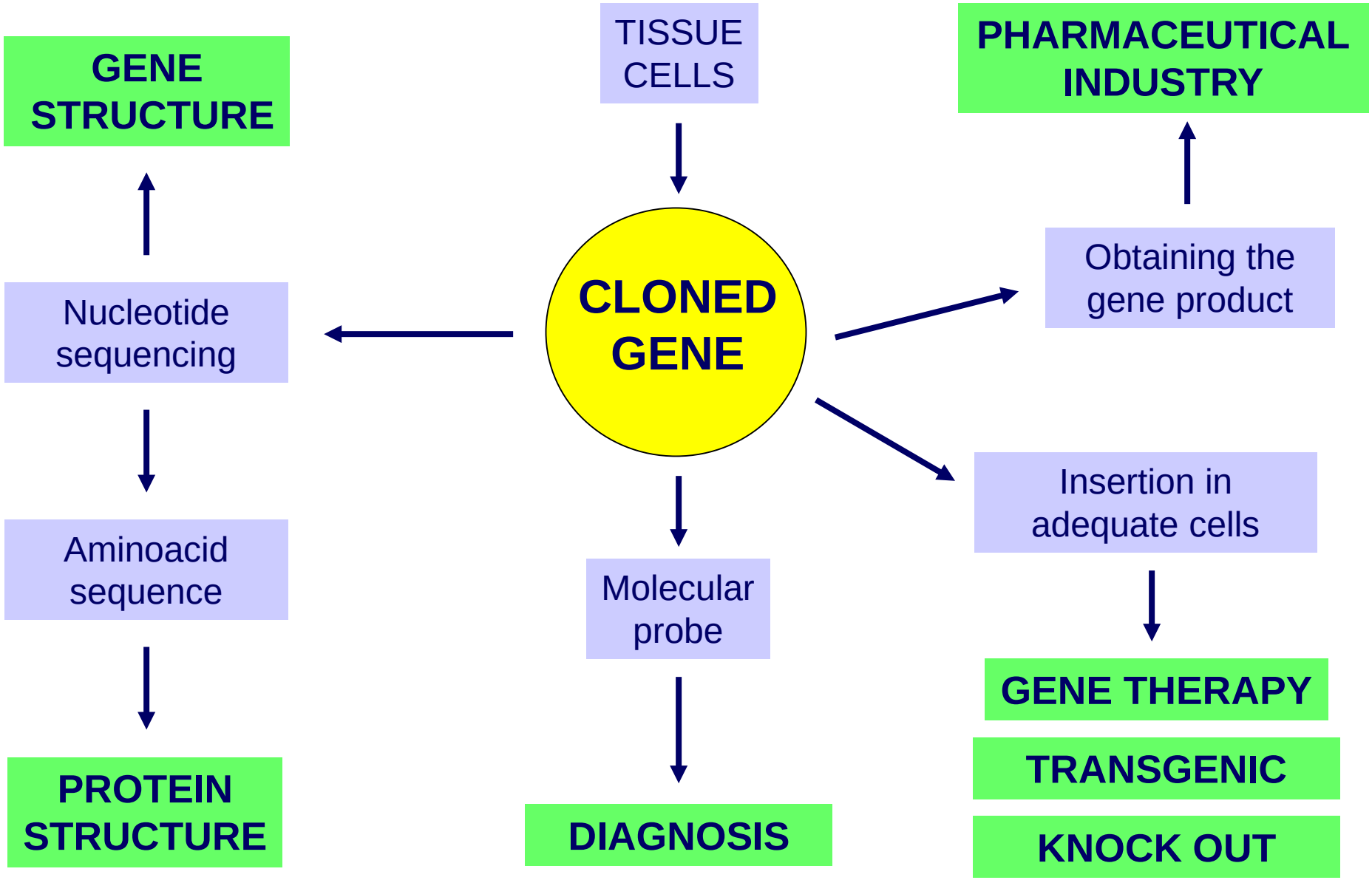
1) The sequence of the DNA fragment that we want to amplify must be prevously known.

2) High sensitivity
   Theoretically, it is possible to amplify DNA fragments starting from a single DNA molecule.
   In any case, with only a very small amount of sample (just a few cells) DNA can be amplified.

3) High specicifity
   If the correct primers are choosen, only the desired fragment is amplified.

4) Not costly
   It is performed in a short time
   Reactives are not expensive

APPLICATIONS



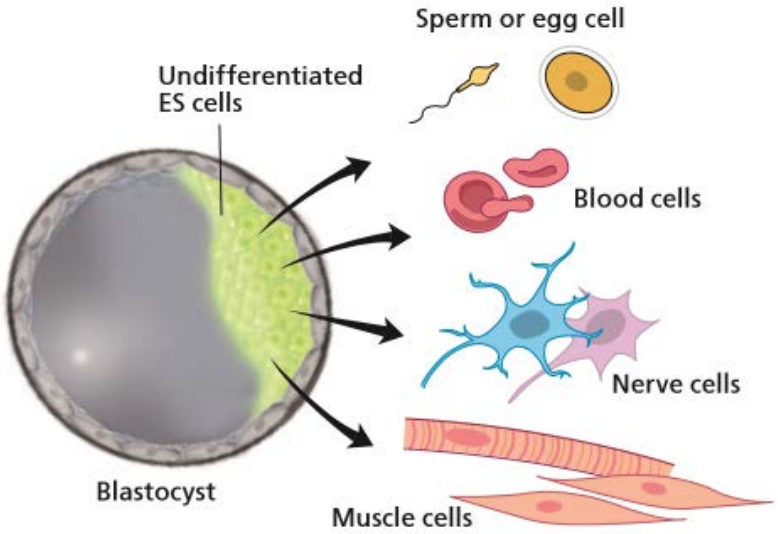
MEDICINE AND PHYSIOLOGY NOBEL PRIZE

2007

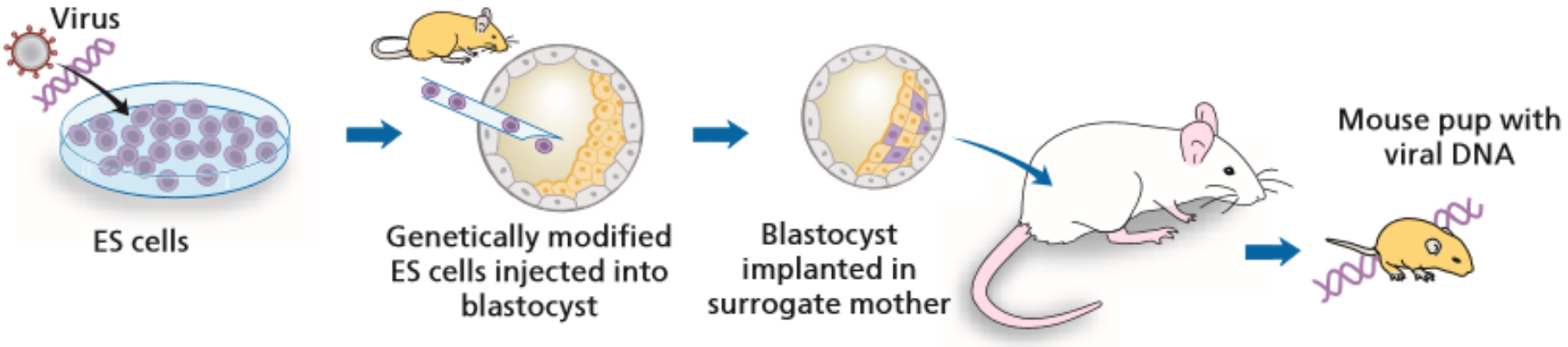
Mario R. Capecchi **Martin J. Evans** **Olivier Smithies**

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

TRANSGENIC MICE



- Infect blastocyst cells/sperm with viral vector with the gene of interest.
- Hope that in some cells homologous recombination will insert the DNA section of interest into the target cell's chromosome.
- Select chimeric organisms.
- Breed until the transformed DNA is found in a germ line.



TRANSGENIC MICE

Knock-out mouse without some olfactory neurons. Turns mice fearless.

1868	**F. Miescher**, Switzerland, first isolates nucleic acid from biological material.
1940	G. Beadle* and E. Tatum, USA, put forward the "one gene – one enzyme" hypothesis. (*1958)
1944	**O. Avery**, USA, shows that genetic material does not consist of proteins but of deoxyribonucleic acid (DNA).
1953	J. Watson*, USA, and F. Crick*, UK, show that the DNA molecule consists of a double helix, thus making one of the most important discoveries of this century. (*1962)
1956	A. Kornberg*, USA, discovers the enzyme DNA polymerase, which is needed for copying DNA. (*1959)
1957	A. Todd*, UK, receives the Nobel Prize in Chemistry for synthesis DNA's building blocks. Later G. Khorana and his coworkers in the USA develop these chemical methods further and, for the first time (1970), synthesise a biologically active gene.
1961-65	Work by M. Nirenberg*, **J. Matthei**, G. Khorana*, S. Ochoa and their co-workers in the USA leads to an understanding of the genetic code. (*1969)
1961-69	W. Arber*, Switzerland, D. Nathans* and H. Smith*, USA, discover restriction enzymes, which can cleave DNA molecules in a predetermined way and can hence function as important tools in gene technology. (*1978)
1972	P. Berg*, USA, lays the foundation of recombinant-DNA technology. (*1980)
1975-77	W. Gilbert*, USA, and F. Sanger*, UK, develop methods for determining the sequence of DNA. (*1980)
1978	**Michael Smith***, Canada, and his co-workers manage to induce a site-directed mutation in a bacteriophage DNA molecule.
1982	**Michael Smith*** together with **A. Fehrst** and **G. Winter**, UK, manages to produce large quantities of an enzyme in which, using site-directed mutagenesis, one pre-determined amino acid is exchanged for another. (*1993)
1985	The PCR method developed by **Kary B. Mullis***, USA, for mass-copying of DNA is presented for the first time. (*1993)

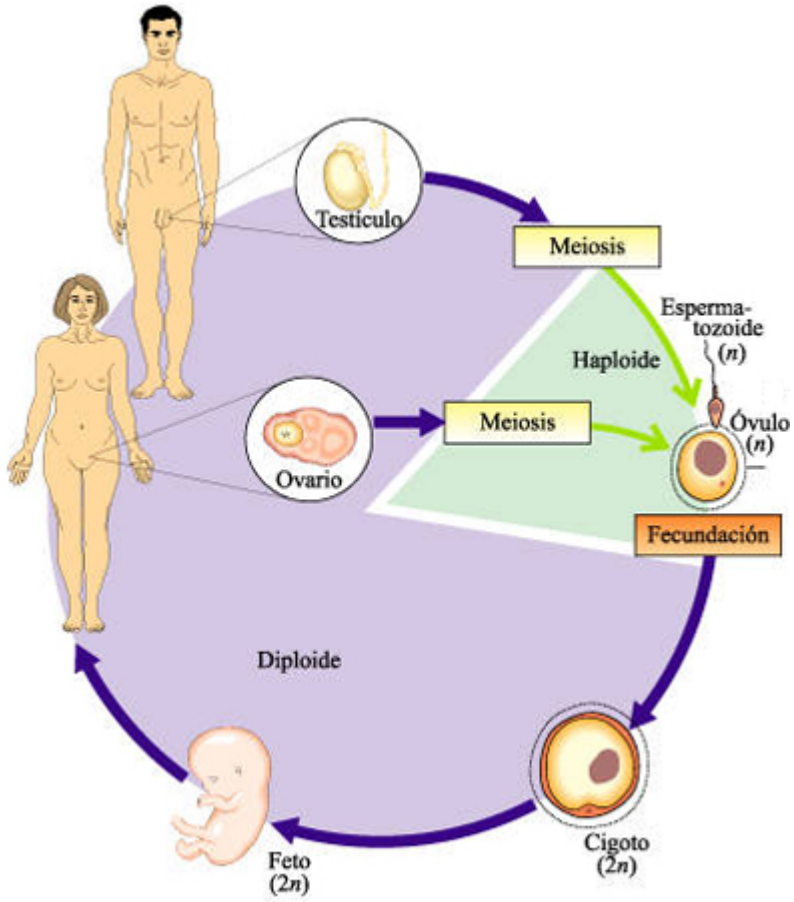
*Nobel Prize

CELLULAR AGING AND DEATH

- Introduction
- Cellular aging
- Types of cell death (apoptosis and necrosis)
- Characteristics of cell death
- Control of apoptosis
- Diseases associated with apoptosis

ETAPAS DEL CICLO VITAL

1. Fecundación
2. Desarrollo embrionario
3. Nacimiento
4. Desarrollo postnatal
5. Madurez
6. Envejecimiento
7. Muerte



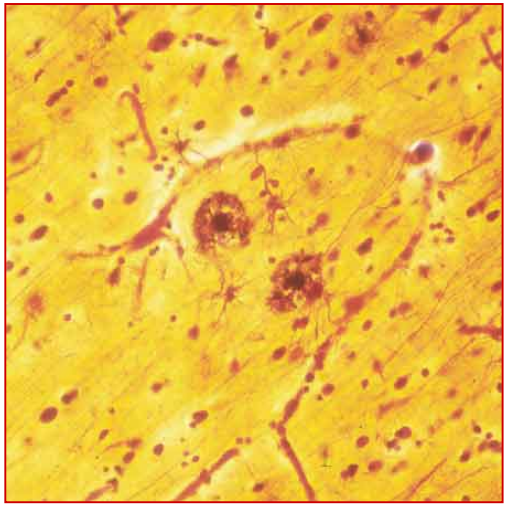
CELLULAR AGING (SENESCENCE)

MORPHOLOGY

- Morphological changes are not very evident: cell size increases, the cell flattens, becomes irregular and looses contact with other cells.

- Intermediate filament accumulation (keratinocytes…)

- Pigment accumulation:



Lipofucsin (neurons, cerdiac myocytes…)

Ceroid (hepatocytes, macrophages, …)

Lipofucsin in neurons

CELLULAR AGING

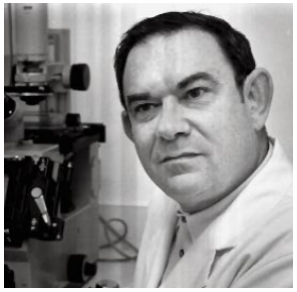
HAYFLICK'S EXPERIMENTS

When normal human fibroblasts are cultured they divide during a limited period (months) and finally they die

It seems to be that aging is an intrinsic property of cells

Experiment:

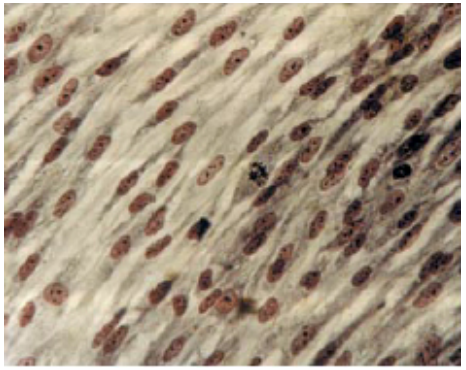
- *A primary cell culture is obtained from embrionary pulmonary tissue(cut in pieces, trypsinize,wash and seed)*
- *When cultures reach confluence(typically 1 week) it is subcultured*
- *While cells divide new subcultures are made*
- *After about 50 subcultures cells stop dividing, degenerate and die*
- *Soon before there are signs of aging: cultures reach confluence later and do so irregularly*



Hayflick's limit

WI-38 cells:

a) Passage 20
b) Passage 50

a   b

CELLULAR AGING

HAYFLICK'S EXPERIMENTS

To know if aging is an intrisical property of cells

Experiment:
Three types of cell cultures are used:

- Male cells (XY) at subculture 40 → can be subcultured 10 times more
- Female cells (XX) at subculture10 → can be subcultured 40 times more
- A mix of both cultures → they are subcultured 20 times more and only the female cells (XX) are left

Experiment:
Cells can be frozen at very low temperatures (liquid nitrogen) and live again when unfrozen

- Cells frozen after 20 subcultures → are unfrozen and can be subcultured 30 times more
- Cells frozen after 40 subcultures → are unfrozen and can be subcultured10 times more

Conclussion The limited life duration is an intrinsical property of cells

CELLULAR AGING

HAYFLICK'S EXPERIMENTS

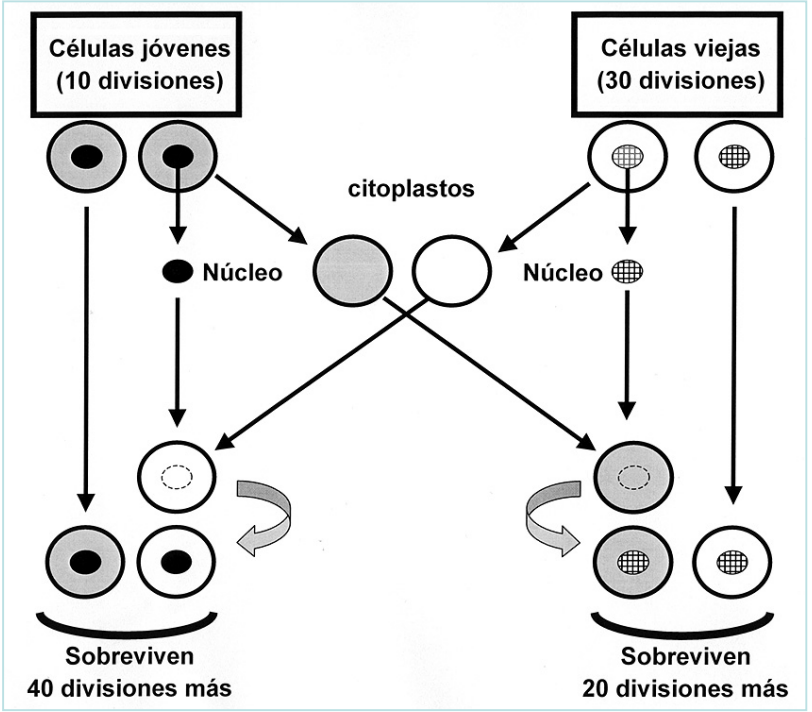
Other experiments demonstrate that the regulator factor of **replicative senescence** is in the nucleus

Experiments:
Cells treated with cytochalasin B expel their nucleus and become cytoplasts that remain viable several days
- Fusion of young cytoplasts+ old cells
- Fusion of old cytoplasts + young cells

The age of the cytoplasts does not modify cellular aging

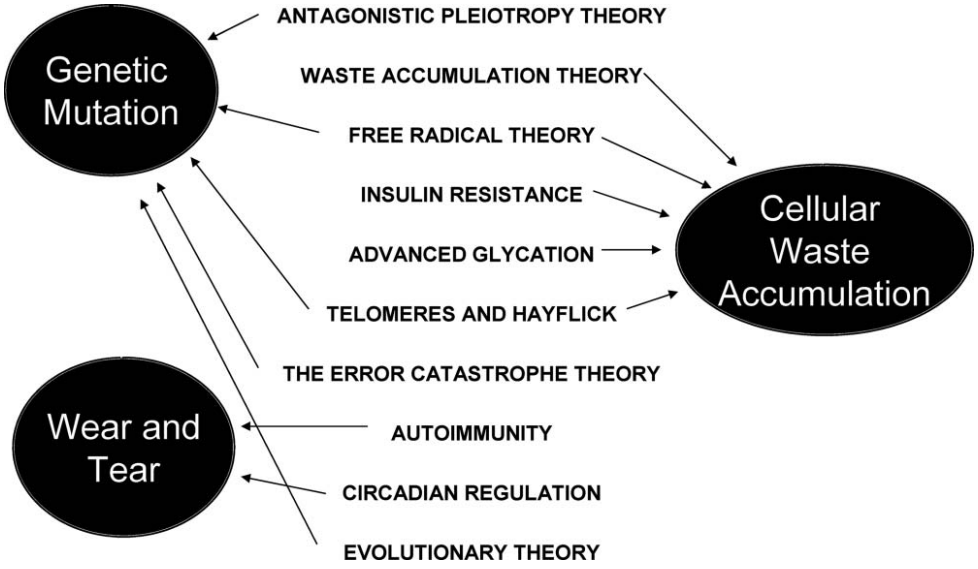
Experiment:
Nuclei can be transferred to cytoplasts
- Nuclei of young cells + old cytoplasts
- Nuclei of old cells + young cytoplasts

The nucleus determines cellular aging



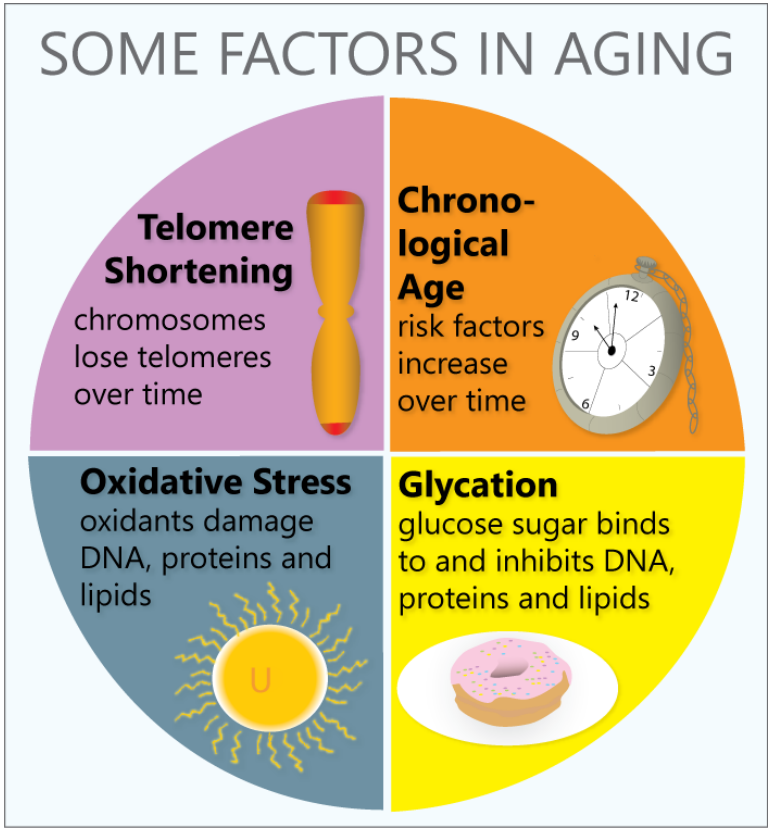
Conclussion:
There must be some genetic mechanism that regulates cellular aging

THEORIES OF AGING

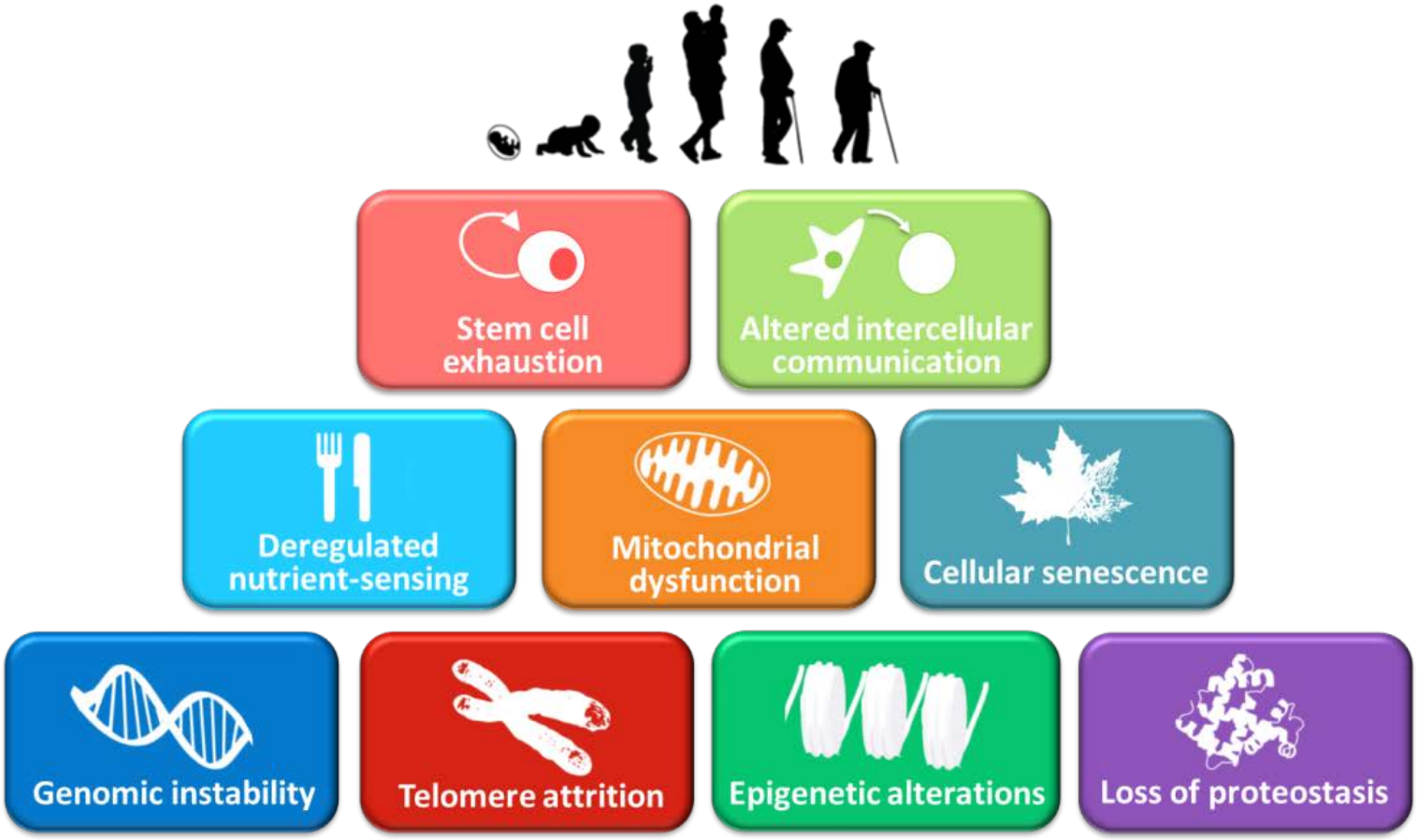


Viña et al. Theories of ageing. IUBMB Life. 2007

More than 300 in 1990
and increasing…



THEORIES OF AGING



The hallmarks of aging, López-Otín et al., Cell 2013

THEORIES OF AGING

OPEN ACCESS Freely available online

PLoS GENETICS

Editorial

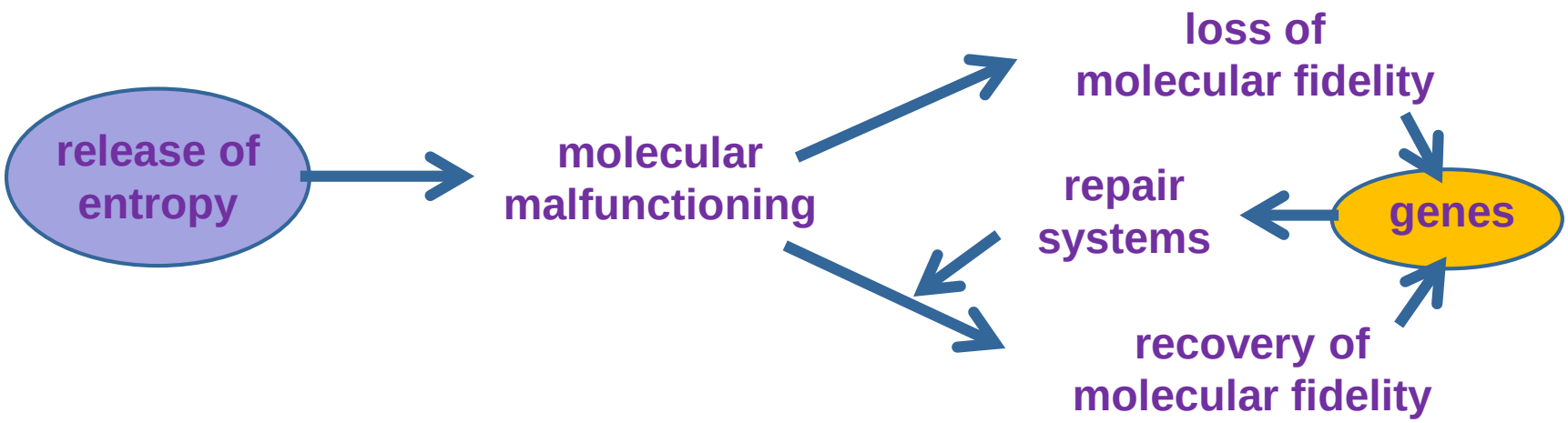
Entropy Explains Aging, Genetic Determinism Explains Longevity, and Undefined Terminology Explains Misunderstanding Both

Leonard Hayflick

Acknowledgments ?????

Funding. These studies were supported by Grant 200701 from the Center for Mediocrity in Biogerontology.

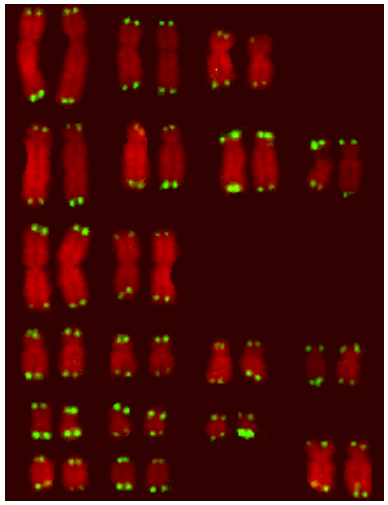
Competing interests. The author has declared that no competing interests exist.

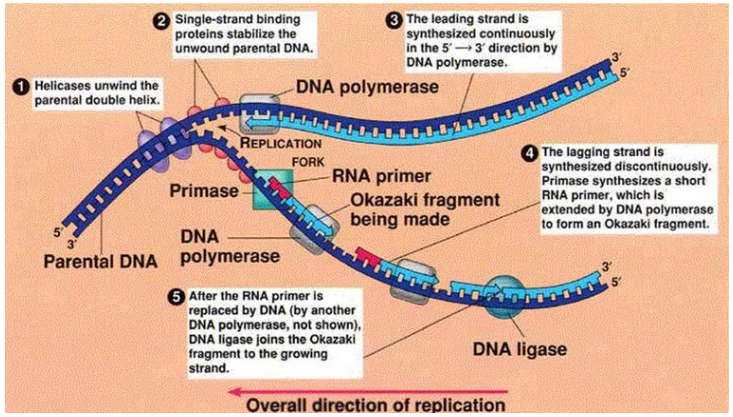


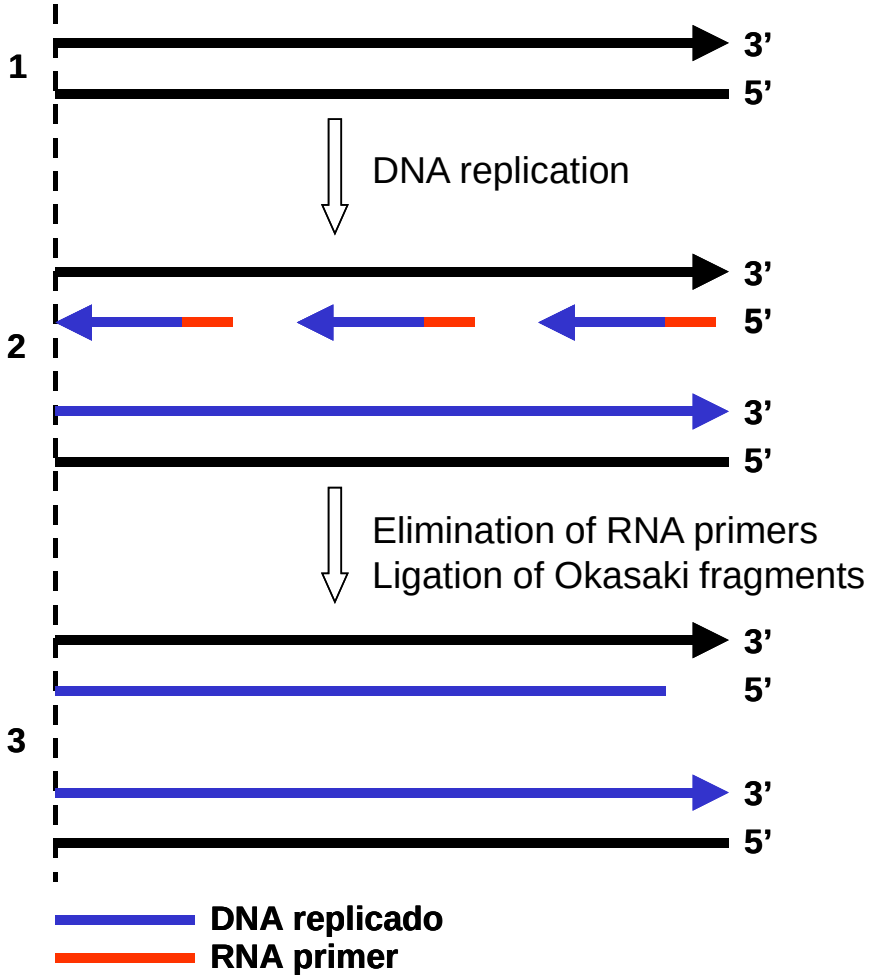
CELLULAR AGING

EXPLICATIVE HYPOTHESES

Telomere shortening





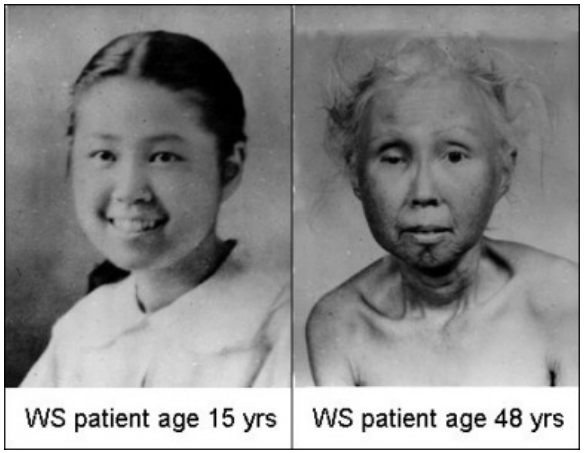


Repetitive sequence: TTAGGG
Lack of telomerase activity

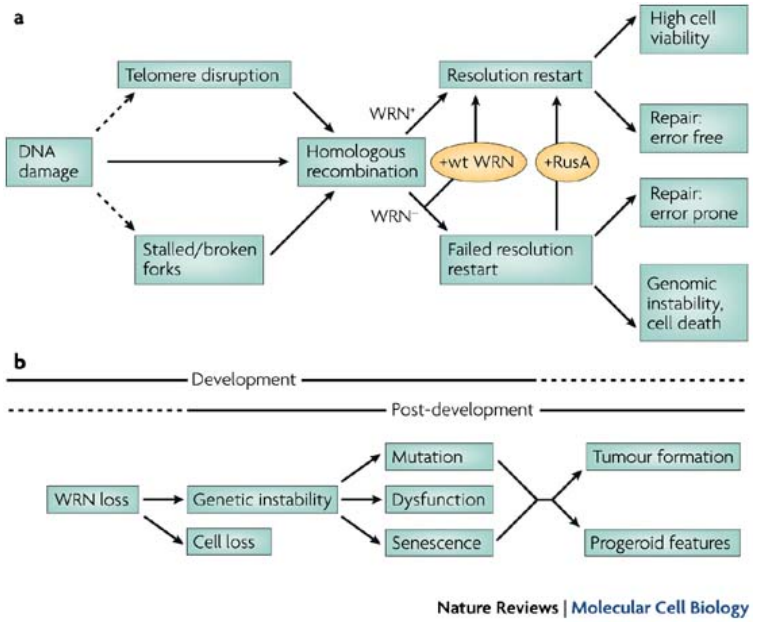
CELLULAR AGING

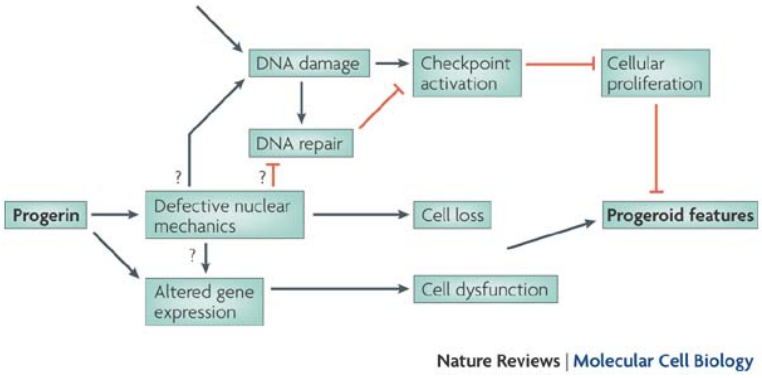
EXPLICATIVE HYPOTHESES

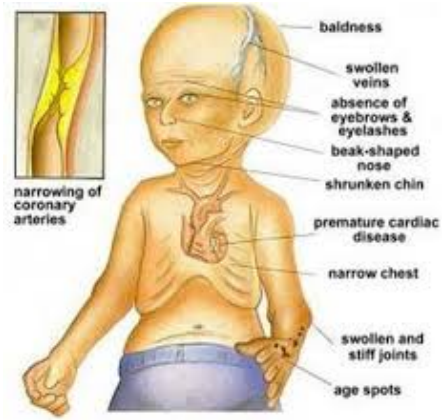
Aging genes



Werner





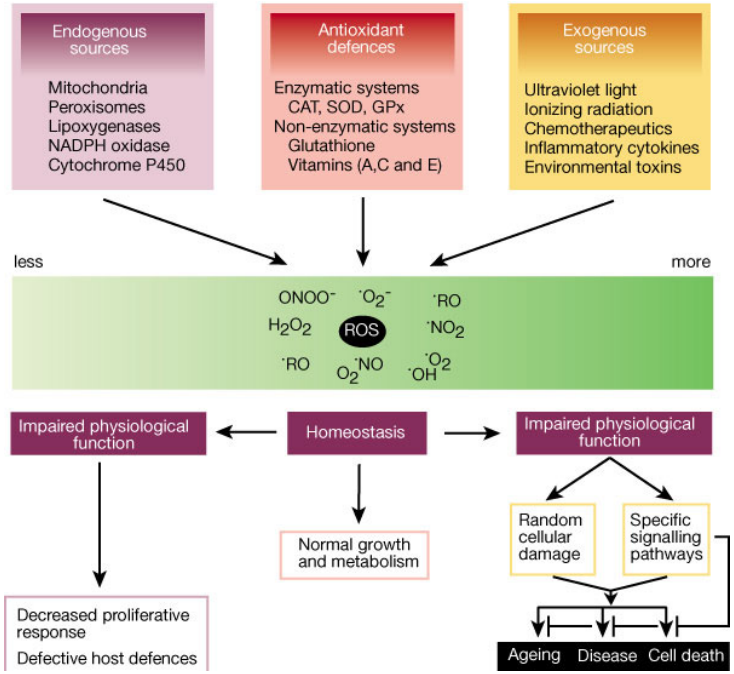


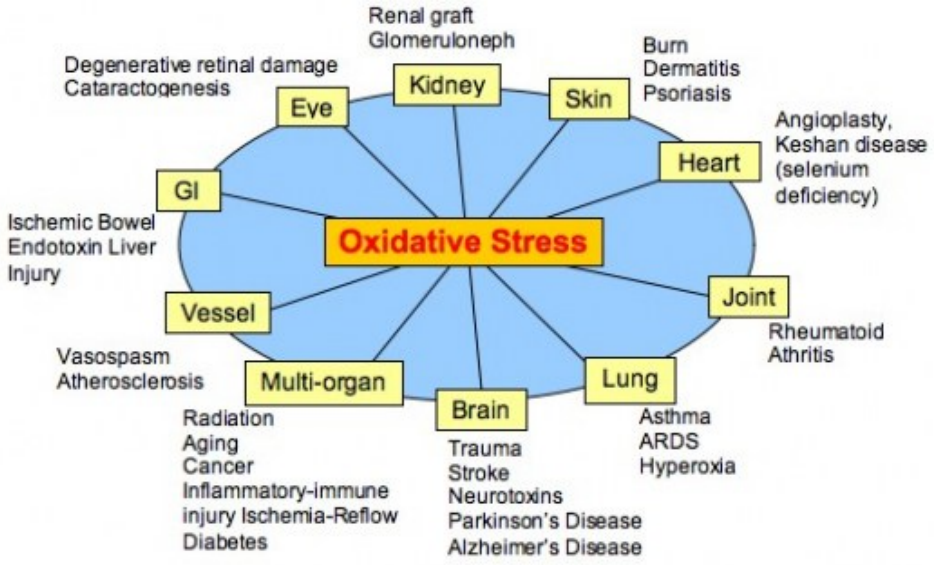
Progeria

CELLULAR AGING

EXPLICATIVE HYPOTHESES

Oxidative damage

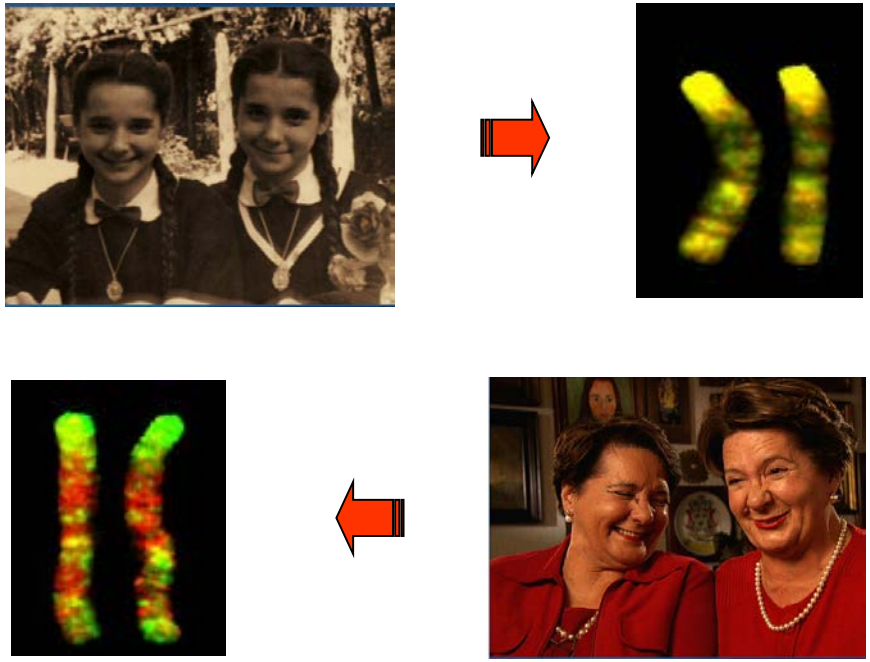


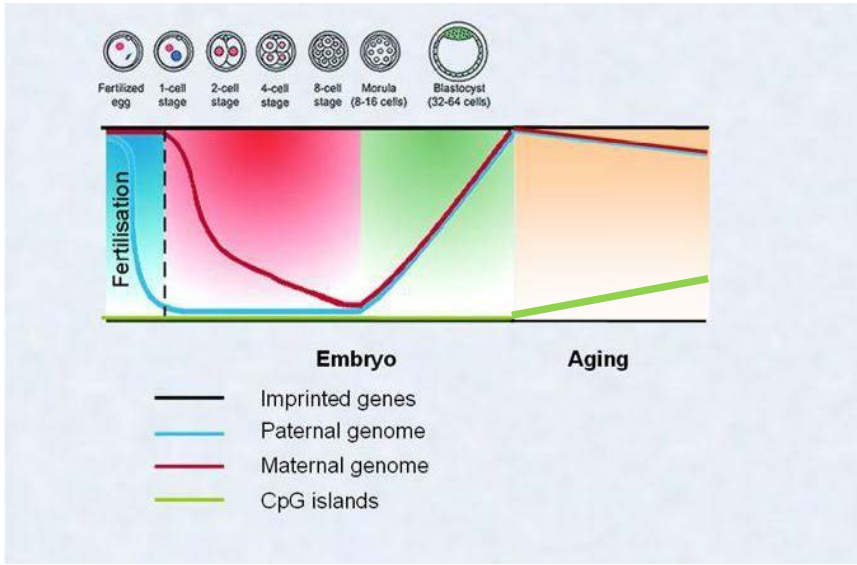


CELLULAR AGING

EXPLICATIVE HYPOTHESES

DNA methylation



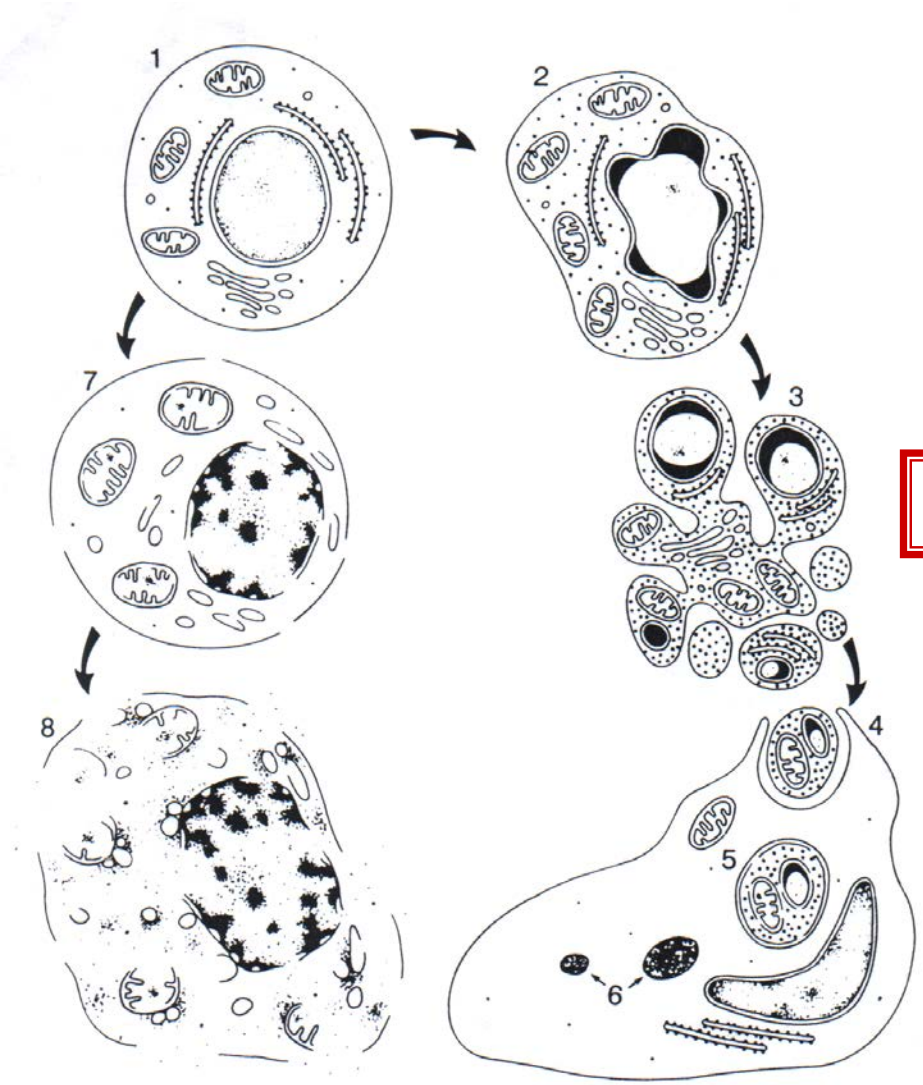


Hovarth clock

CELLULAR DEATH

TYPES OF CELL DEATH

NECROSIS



APOPTOSIS

CELLULAR DEATH

GENERAL CHARACTERISTICS

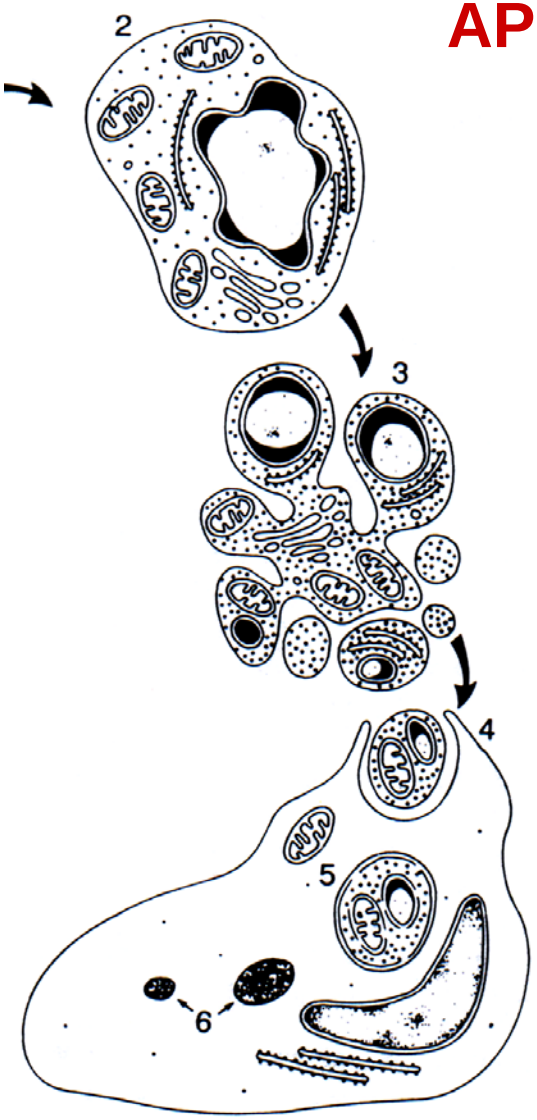
APOPTOSIS

- Physiological
- Affects individual cells
- Does not produce inflammation
- Does not alter the structure nor the function of the cell

NECROSIS

- Accidental
- Affects groups of cells
- Produces inflammation
- Can alter the structure and function of the tissue

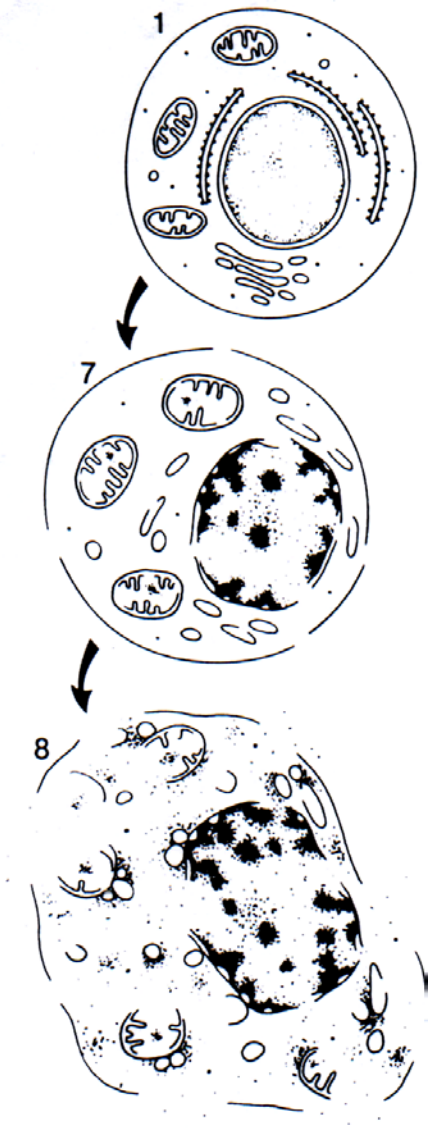
CELLULAR DEATH



APOPTOSIS: Morphological characteristics

- Decrease in cellular volume
- Well conserved organelles
- Condensation and margination of chromatin (picnosis)
- Fragmentation of the nucleus
- Fragmentation of the cell into apoptotic bodies
- Phagocytosis by neighboring cells

CELLULAR DEATH

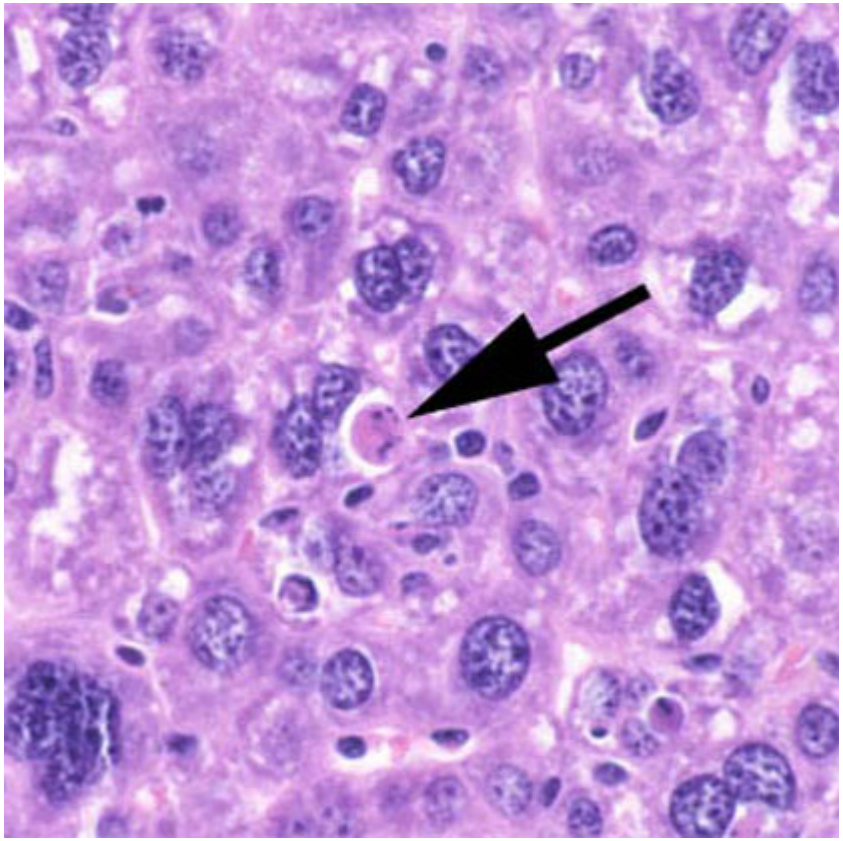


NECROSIS: Morphological characteristics

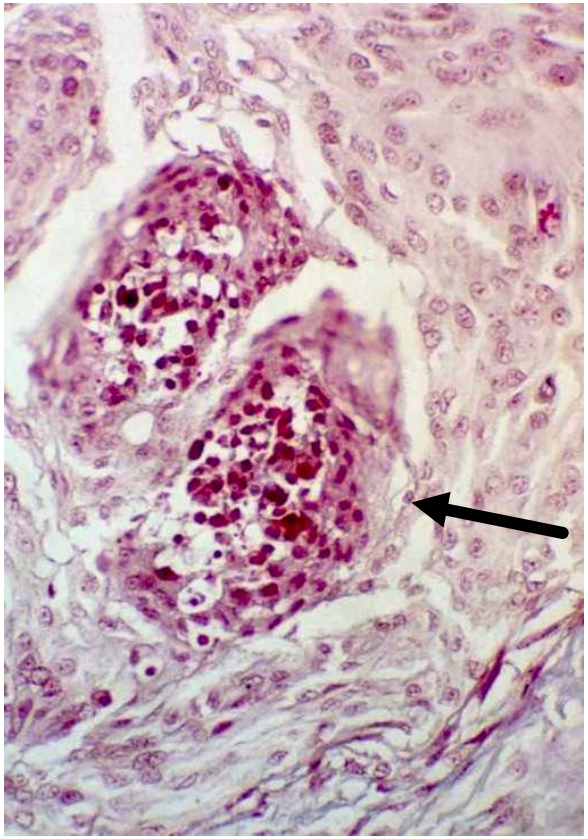
- Increase of cellular volume
- Altered cell organelles
- Condensation of the nucleus in non-defined clumps
- Rupture of plasma membrane
- Phagocytosis by specialized phagocytes

CELLULAR DEATH

MORPHOLOGY



Apoptosis



Necrosis

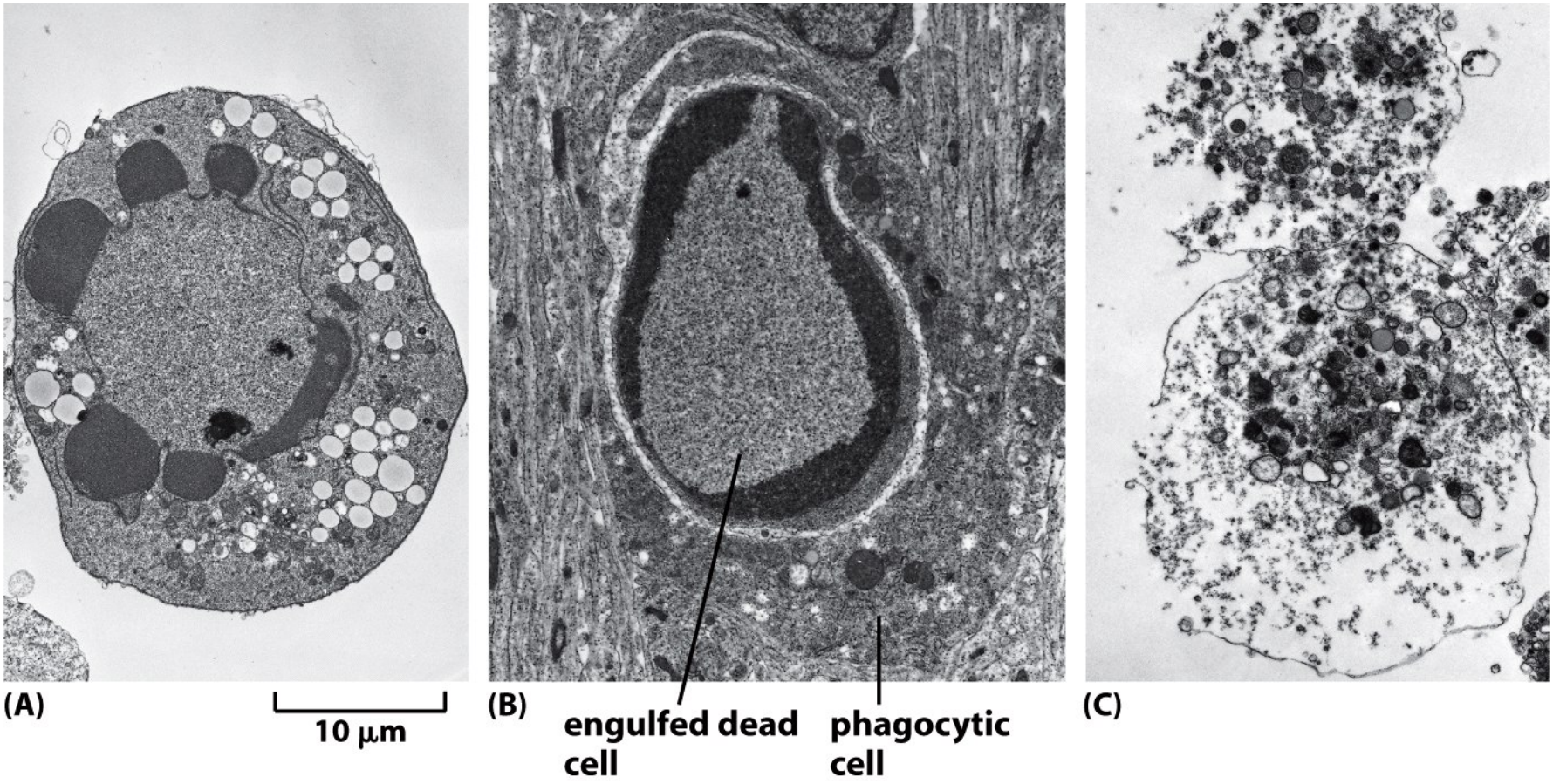
CELLULAR DEATH

MORPHOLOGY

apoptosis

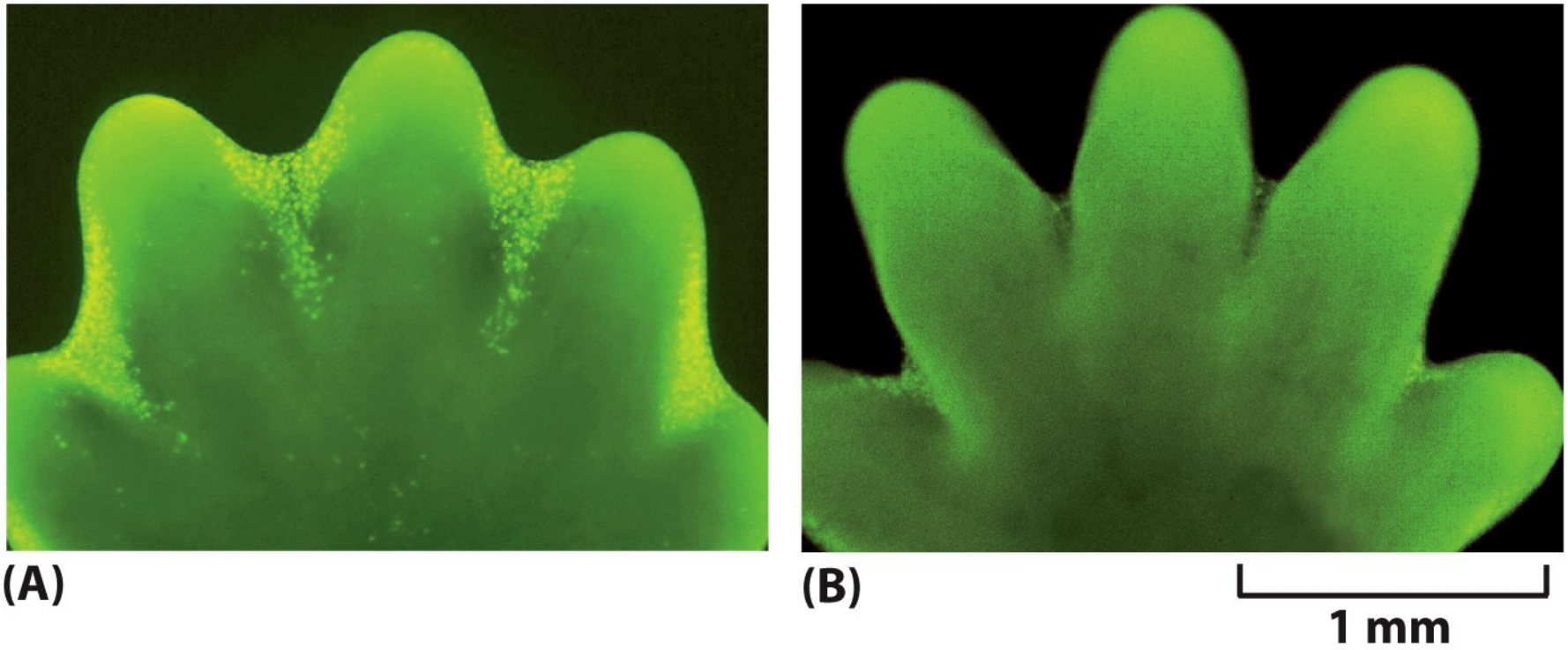
apoptosis

necrosis

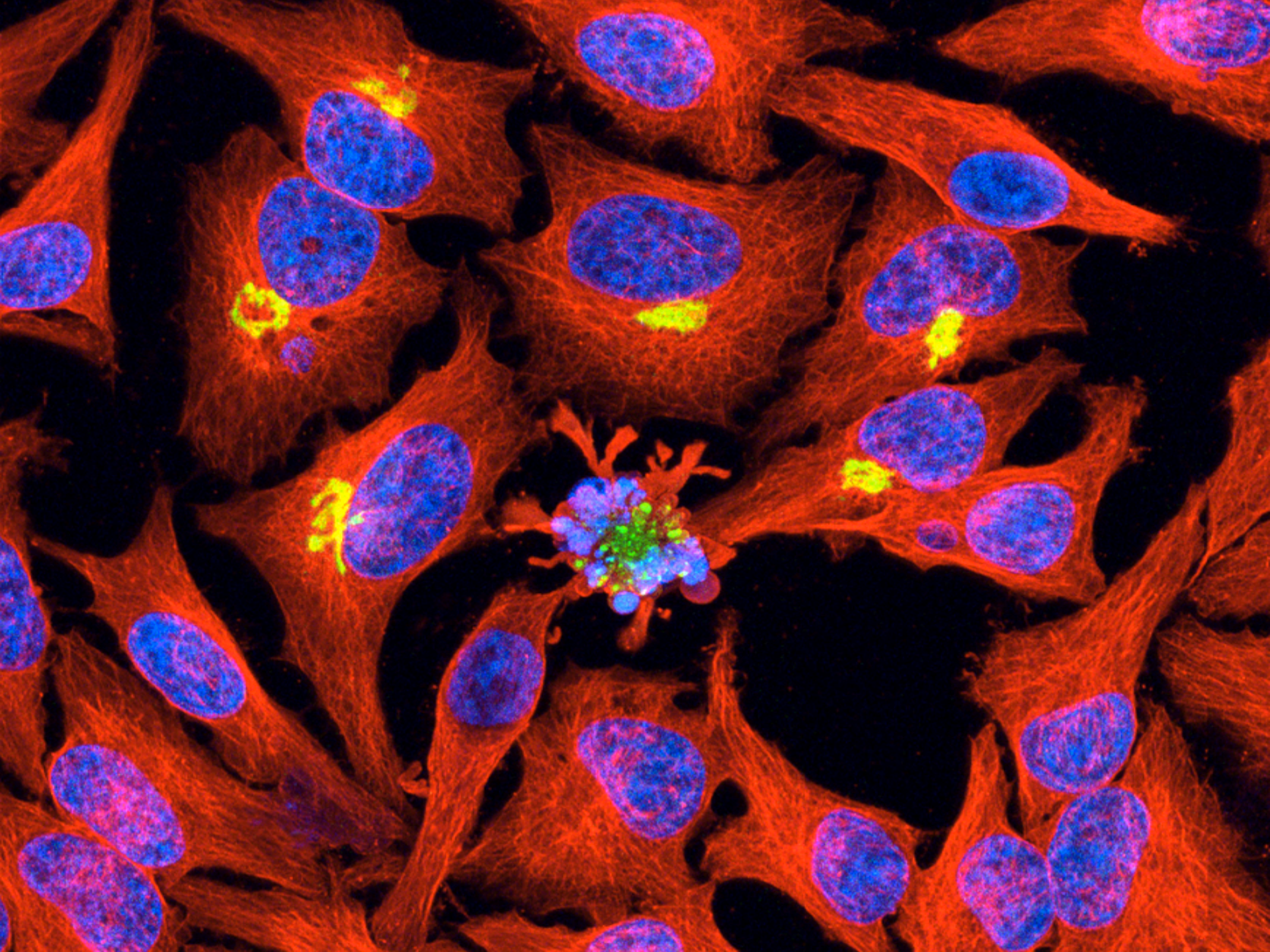


CELLULAR DEATH

APOPTOSIS

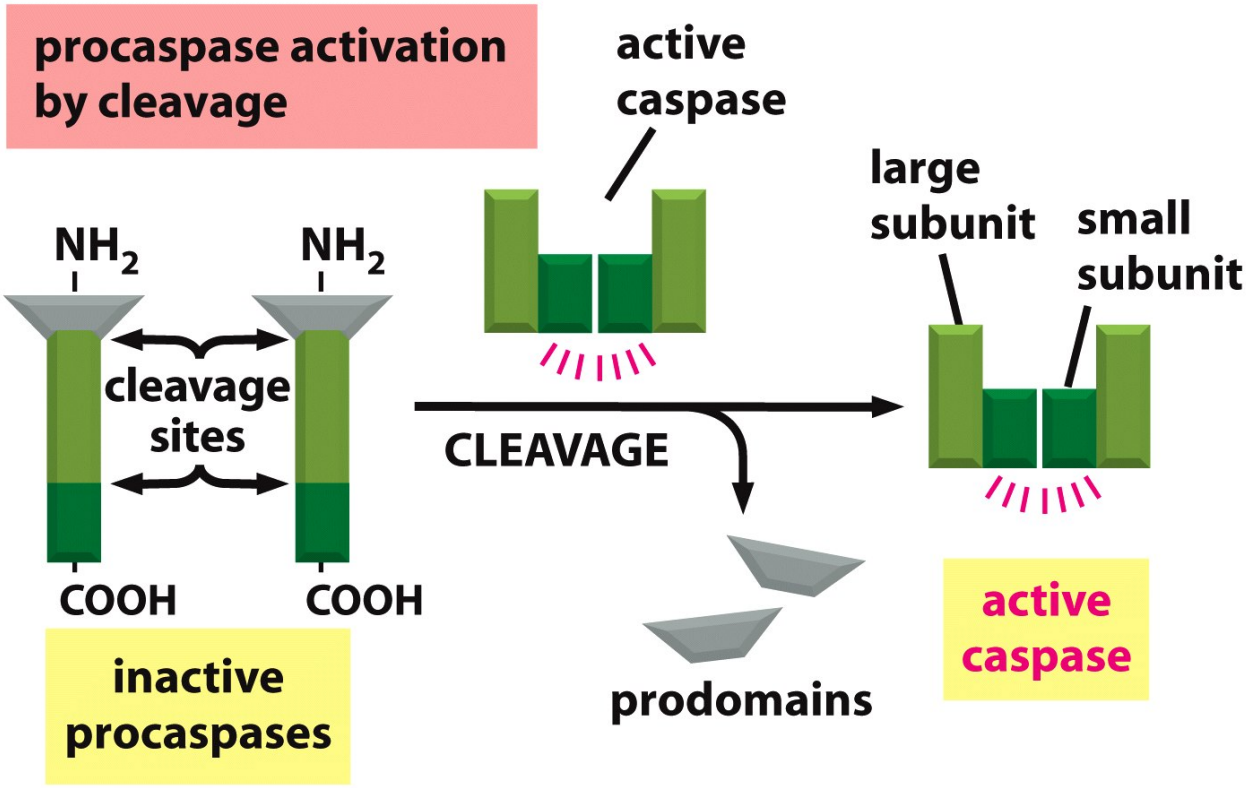


Tissue remodelation during embryonic development
The interdigital cells (bright green) die by apoptosis



CONTROL OF APOPTOSIS

ACTIVATION OF INACTIVE CASPASES



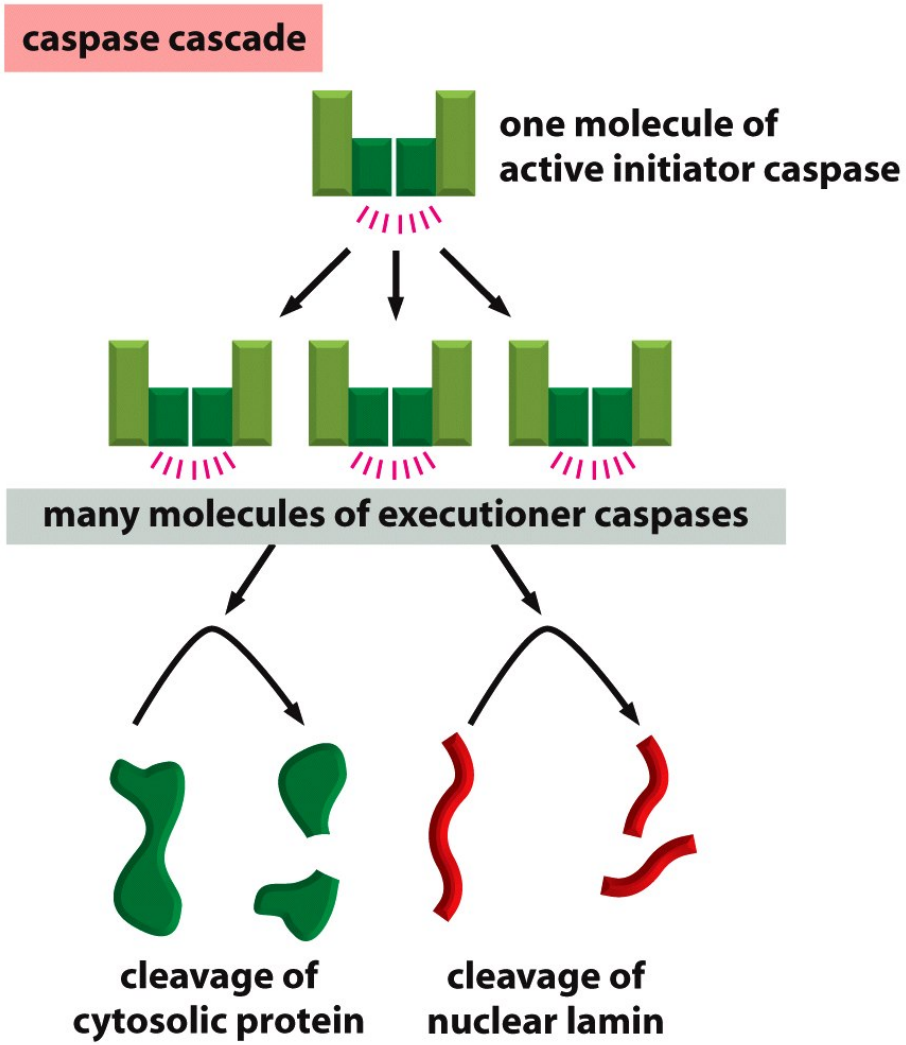
***CASPASE: cystein**-prote**ase** hydrolizing sustrate at the **ASP** residue*

CONTROL OF APOPTOSIS

CASPASE CASCADE ACTIVATION

Initiator caspases (8, 9, 10)
Executioner caspases (3)

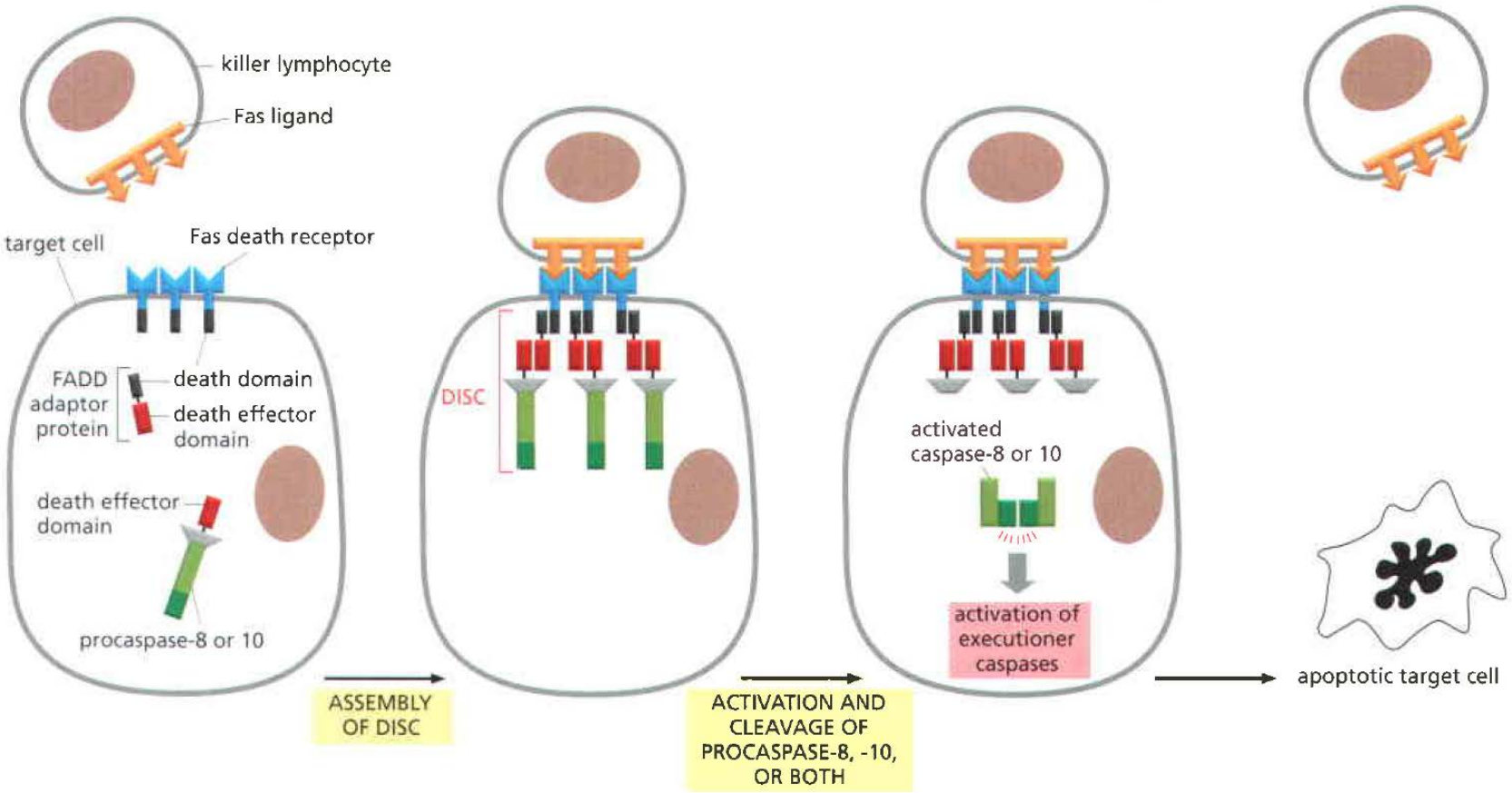
Procaspases are produced since early embriogenesis



CONTROL OF APOPTOSIS

ACTIVATION OF APOPTOSIS FROM OUTSIDE THE CELL

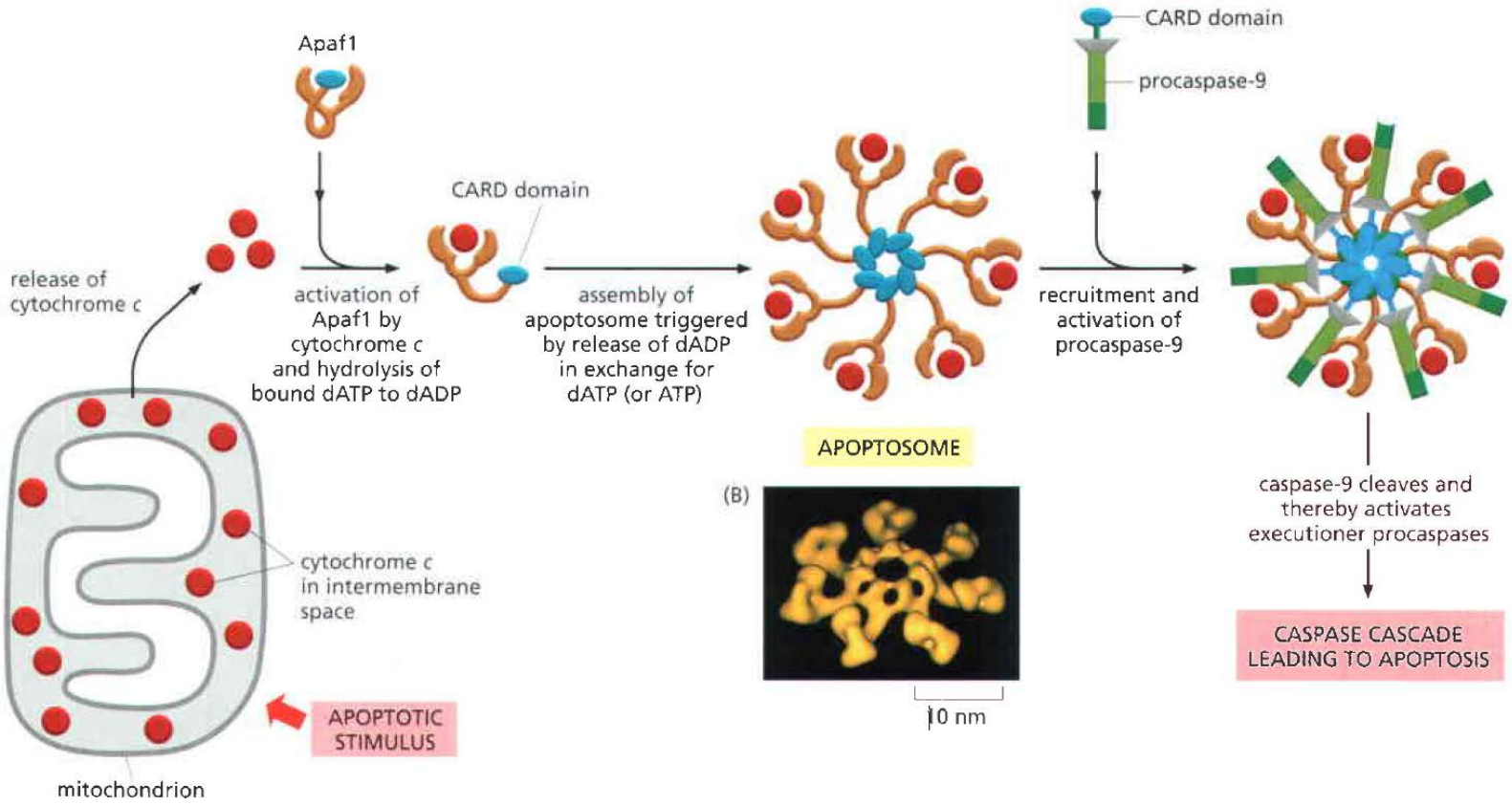
(EXTRINSIC PATHWAY)



CONTROL OF APOPTOSIS

ACTIVATION OF APOPTOSIS FROM INSIDE THE CELL

(INTRINSIC PATHWAY)



DISEASES ASSOCIATED WITH APOPTOSIS

Diseases associated with a decrease of apoptosis

- **Cancer:** follicular lymphoma (bcl-2 +), hormone-dependent tumors
- **Autoimmune diseases**: systemic lupus erythematosus
- **Viral infections**: Herpesvirus, Poxvirus, Adenovirus

Diseases associated with an increase of apoptosis

- **AIDS**
- **Neurodegenerative diseases**: Alzheimer's disease, Parkinson's disease
- **Myelodysplastic syndromes:** Refractory anemia
- **Ischaemic injury:** myocardial infarction, stroke

CHROMOSOME

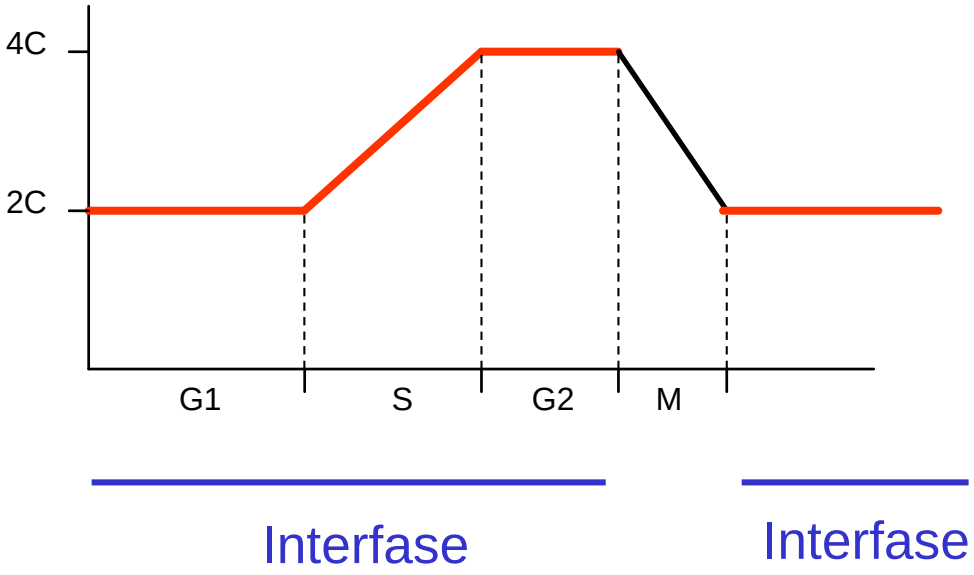
1-General characteristics

2- Ultraestructure

3- Chemical composition

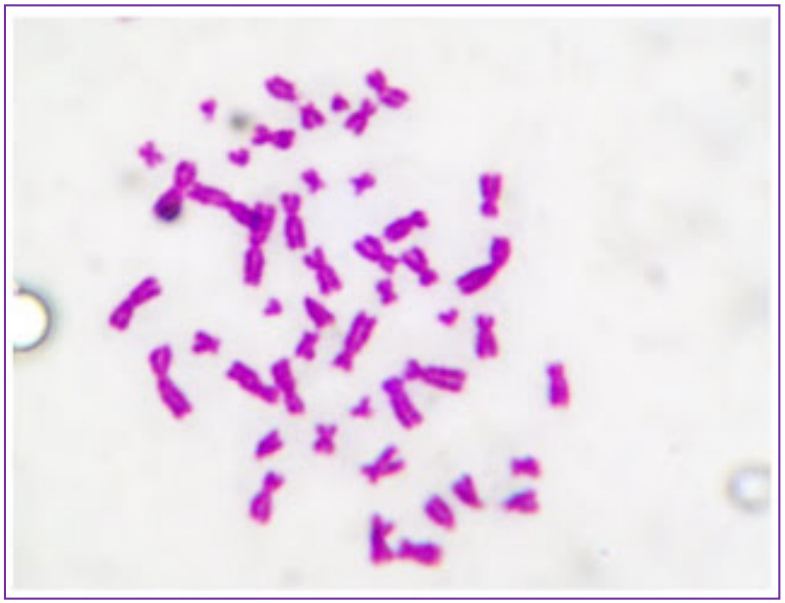
4- Molecular organization

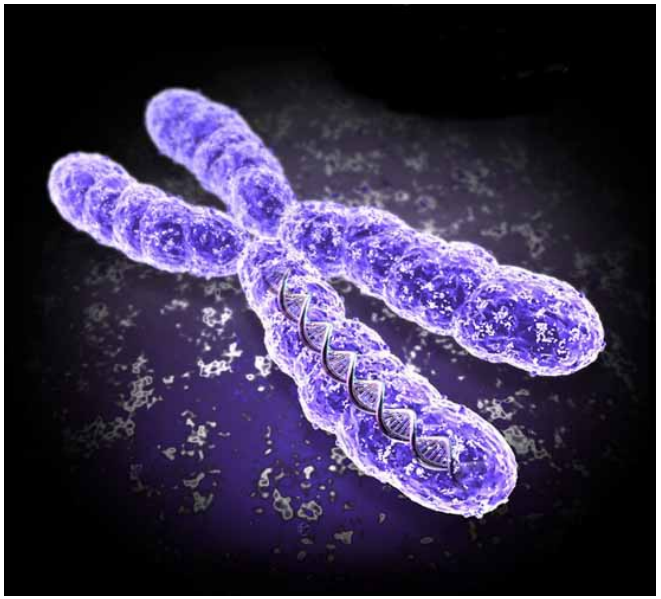
METAPHASIC CHROMOSOME



Definition

Why?





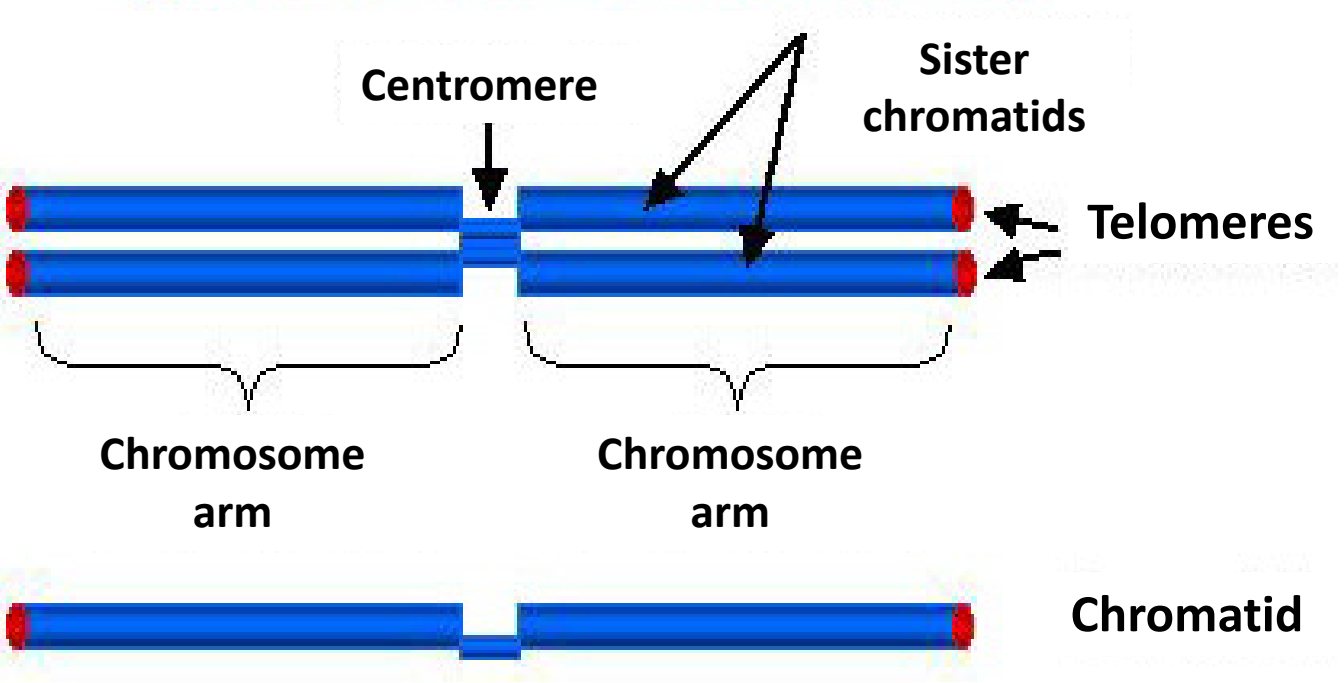
Molecular Biology of the Cell ©. Wikimedia Commons. Unidad de Biología

How does colchicine work?

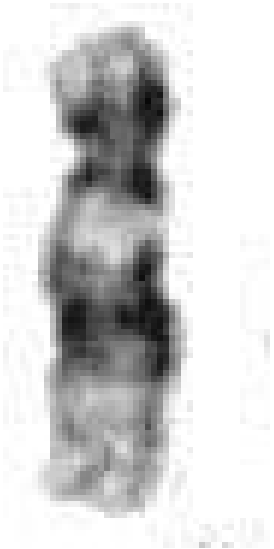
- Colchicine is an alhkloid produced by *Colchicum autumnale*
- It works as mitotic inhibitor: by binding to tubulin during **mitosis** it inhibits spindle formation so that the cell cannot split into two daughter cells

General characteristics

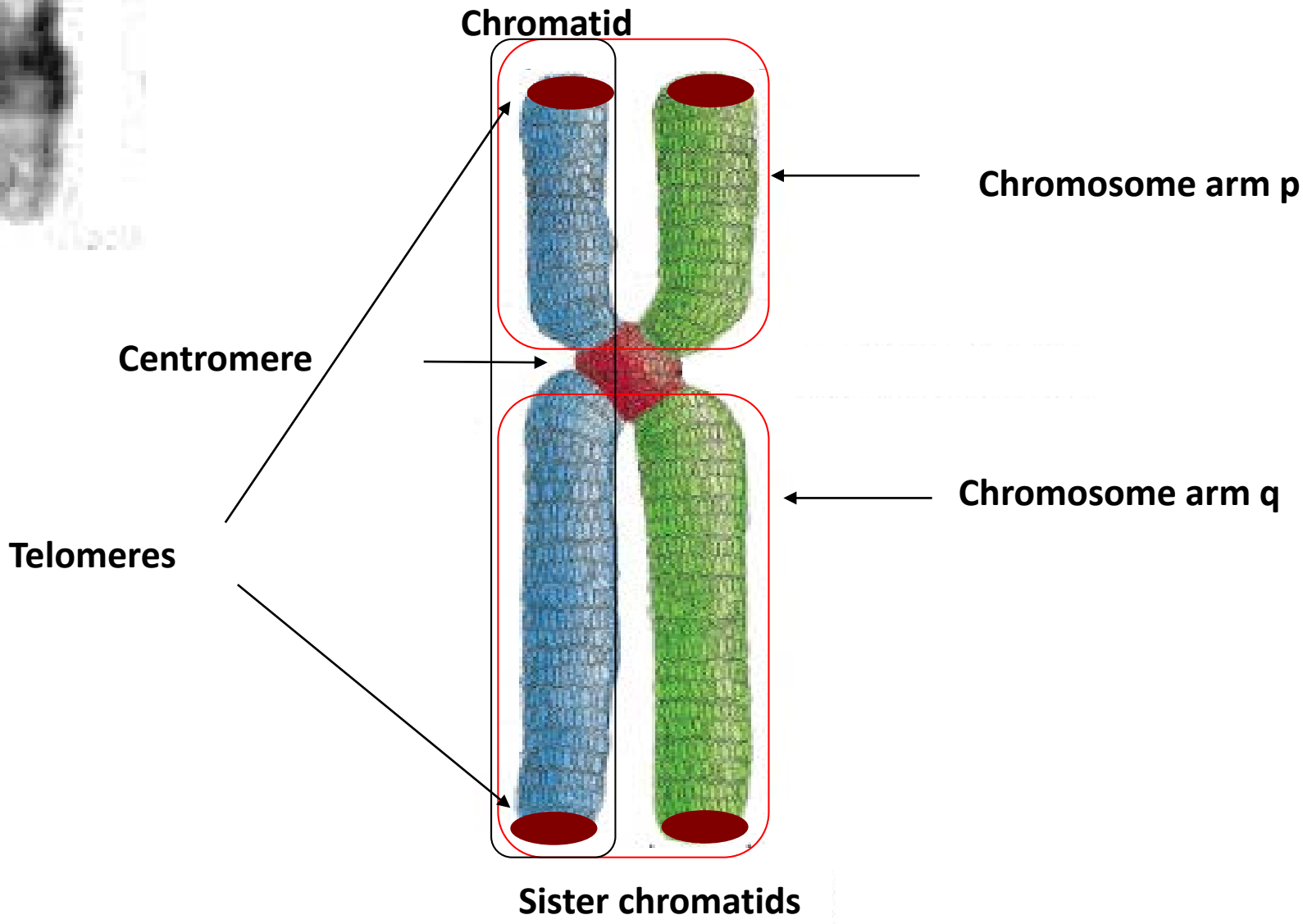
Typical metaphasic chromosome



General characteristics

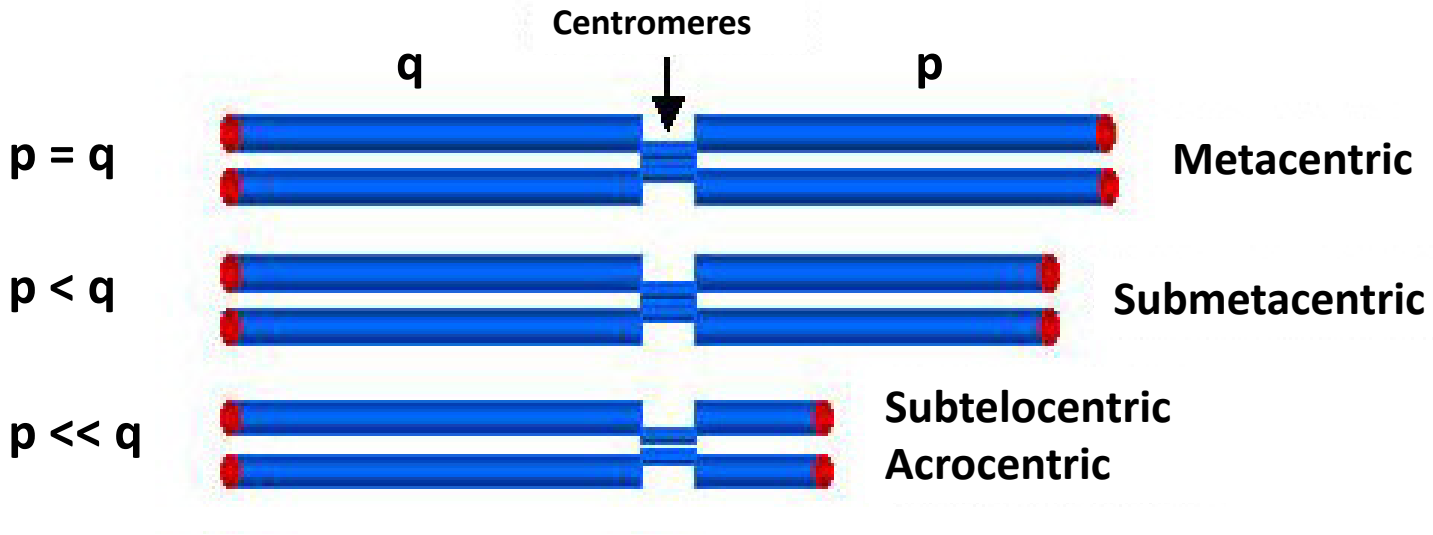


Typical metaphasic chromosome



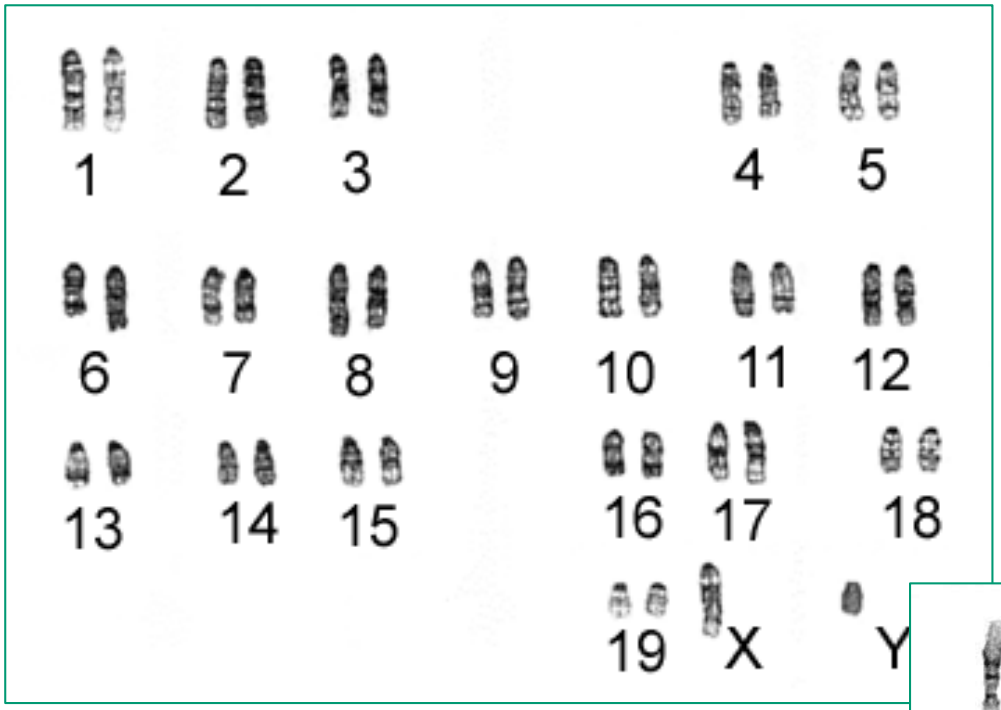
General characteristics

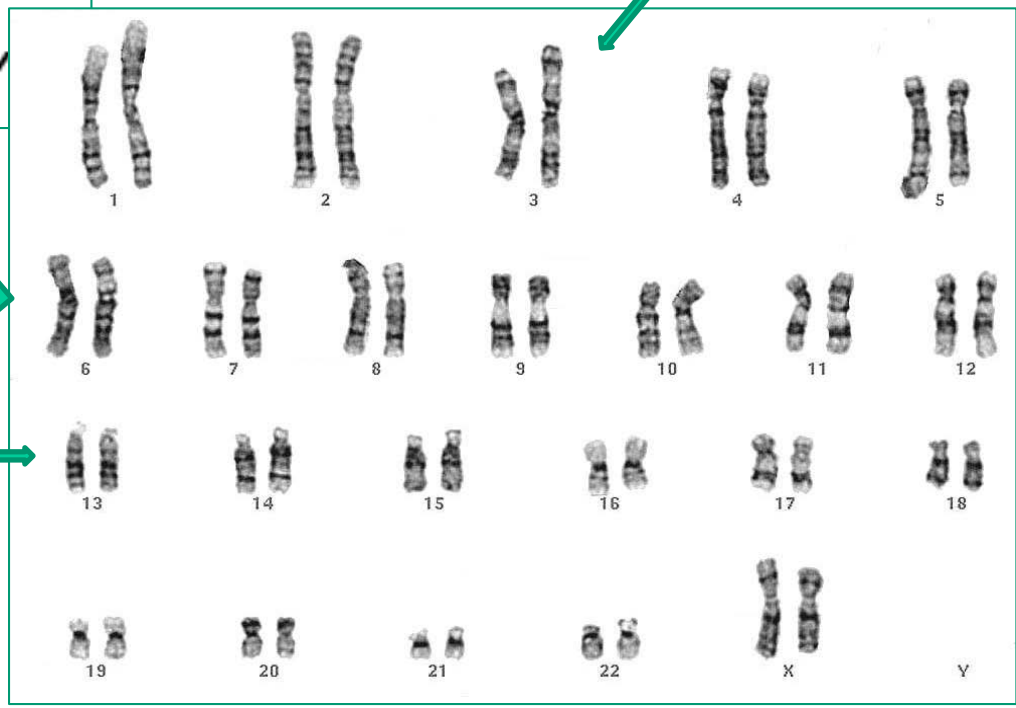
Chromosome classification according to shape



Centromere Index= $\frac{\text{P lenght}}{\text{Total lenght}}$

General characteristics





General characteristics

1.1 Centromeres

- No genes. Heterochromatin
- DNA α-satellite (tandem repeats <100kb)
- CENP-A: protein variant of histone H3 in centromere's nucleosomes

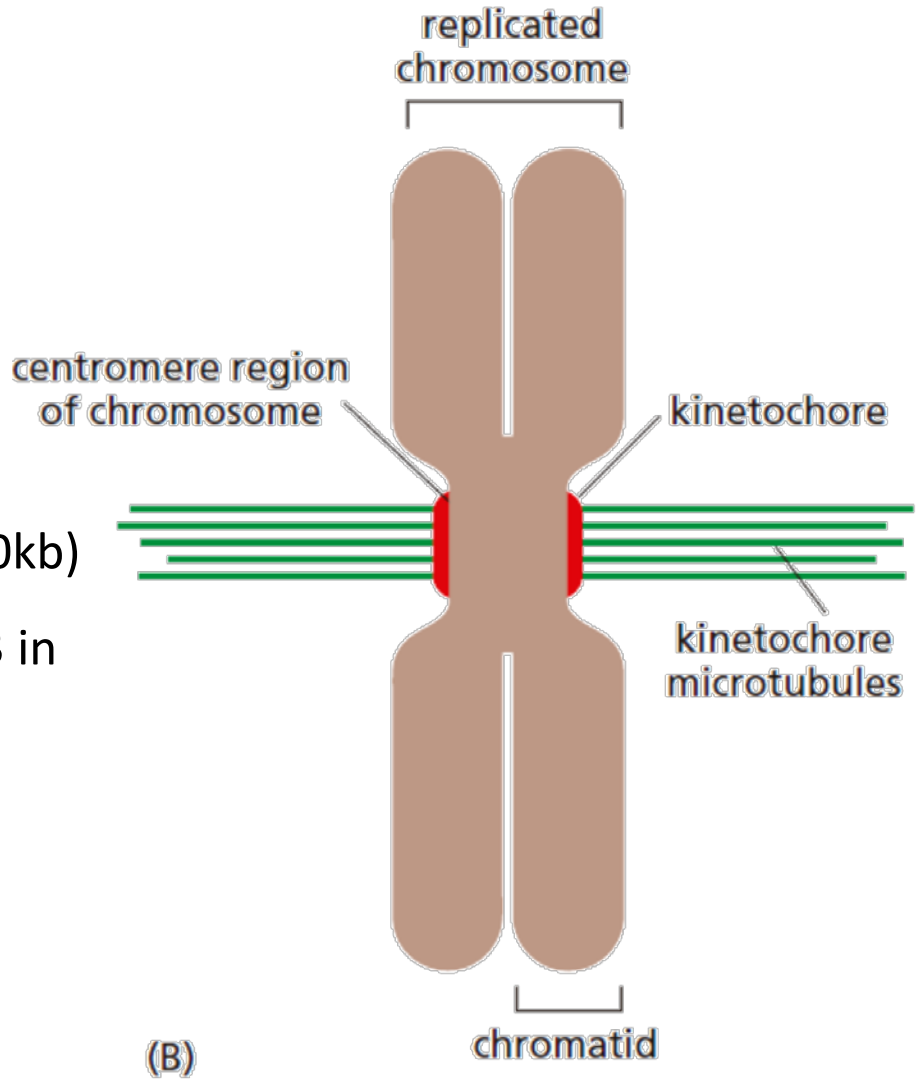


Figure 17-30 *Molecular Biology of the Cell. Sixth edition,* © 2015

General characteristics

1.2 Telomeres

- Lagging strand at a replication fork -> discontinuously
- Problem when the replication fork reaches an end of a linear chromosome (there is no 3′-OH end available for the repair polymerase)
- DNA would be lost from the ends of all chromosomes each time a cell divides

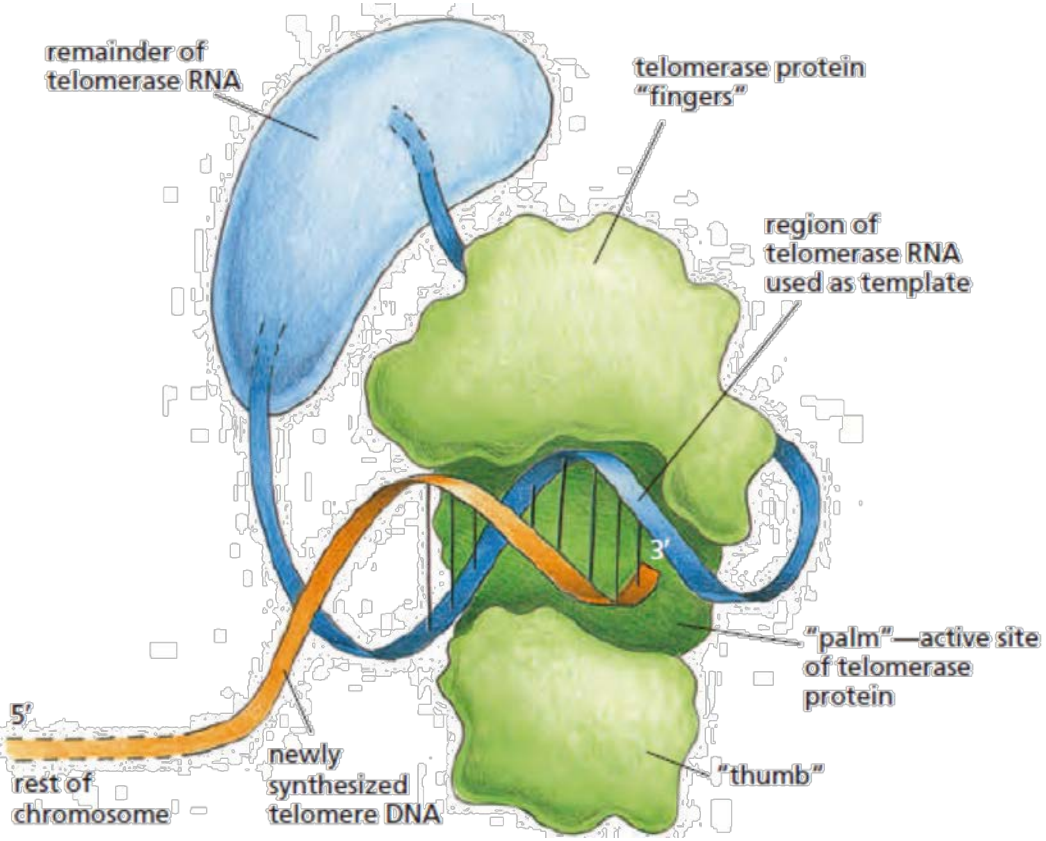


Figure 5-33 *Molecular Biology of the Cell. Sixth edition,* © 2015

- Specialized nucleotide sequences at the ends of the chromosomes.
- NO GENES
- Tandem repeats of DNA minisatellite: a short sequence GGGTTA recognized by sequence-specific DNA-binding proteins (TELOMERASE)

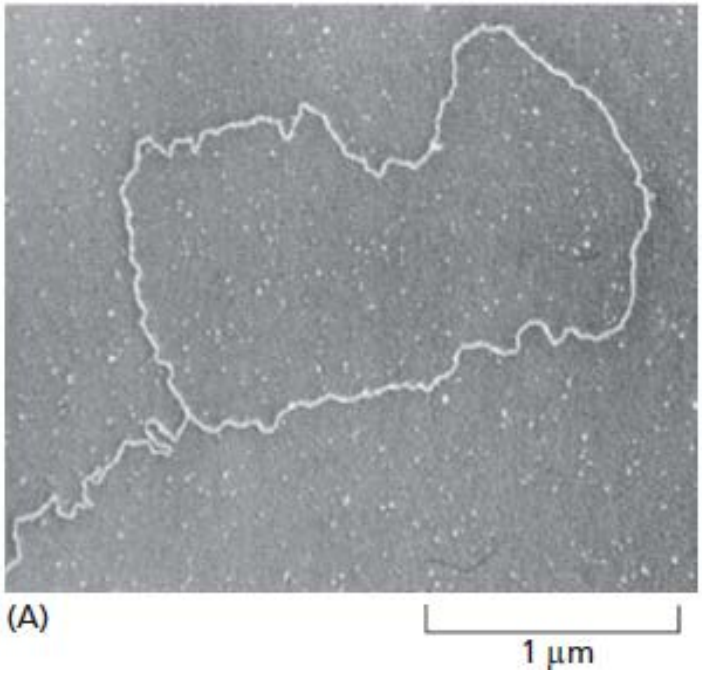
❑ T. replenishes these sequences each time a cell divides.

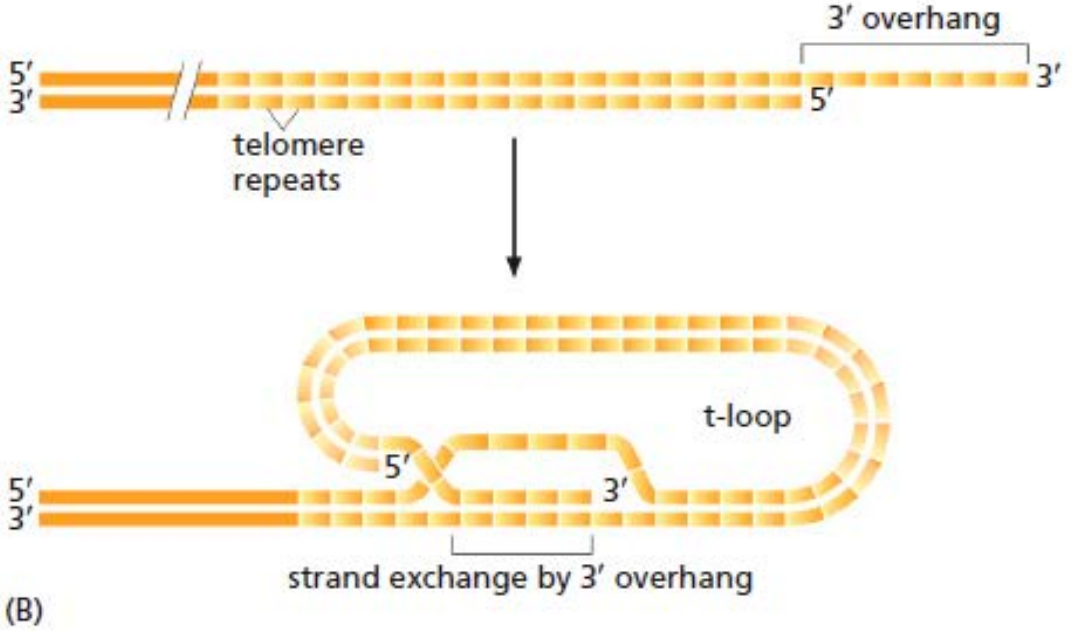
❑ T. elongates in the 5′-to-3′ direction using an RNA template that is a component of the enzyme itself to synthesize new copies of the repeat

General characteristics

1.2 Telomeres

Telomeres must clearly be distin-guished from these accidental breaks; the cell will attempt to “repair” telomeres, causing chromosome fusions and other genetic abnormalities





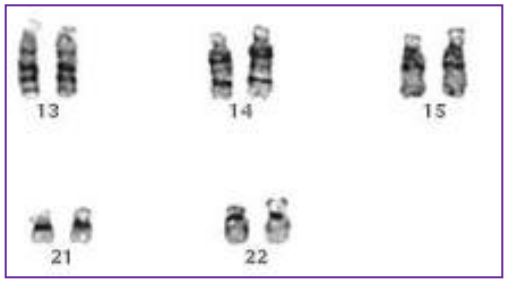
Shelterin “telosome”. TRF1, TRF2, RAP1, POT1

+ Replicative cell senescence

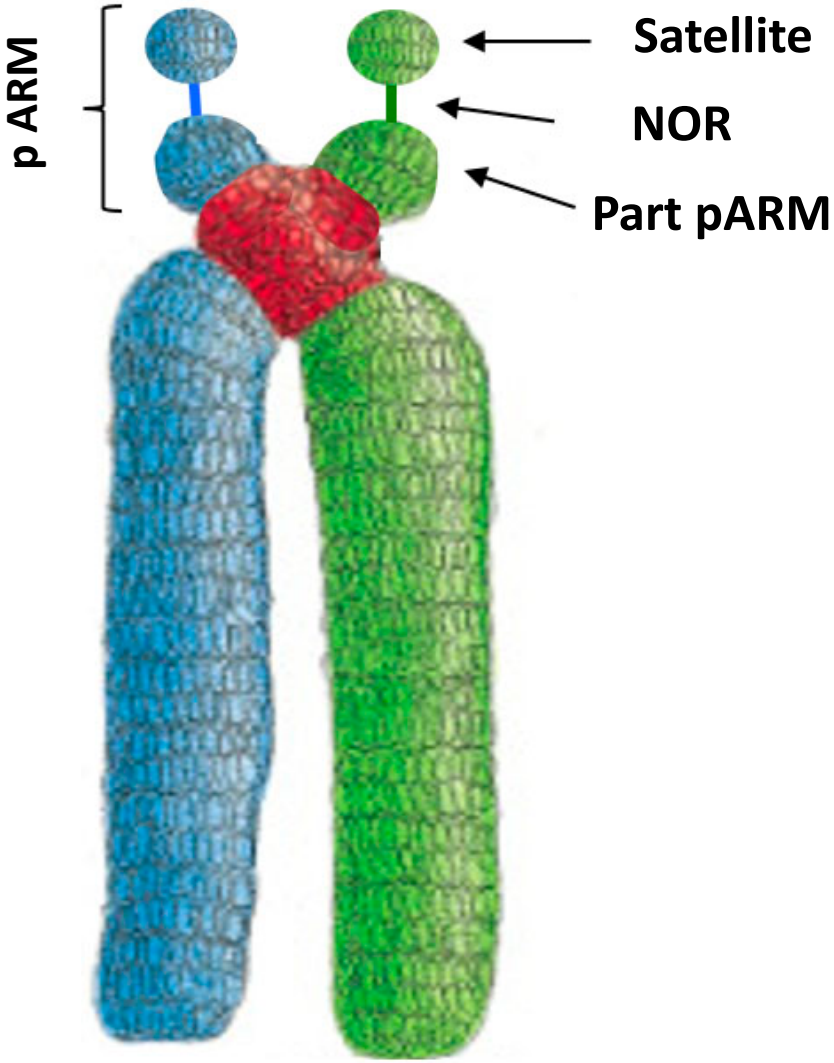
Figure 5-35 *Molecular Biology of the Cell. Sixth edition*, © 2015

General characteristics

1.3 Secondary constriction



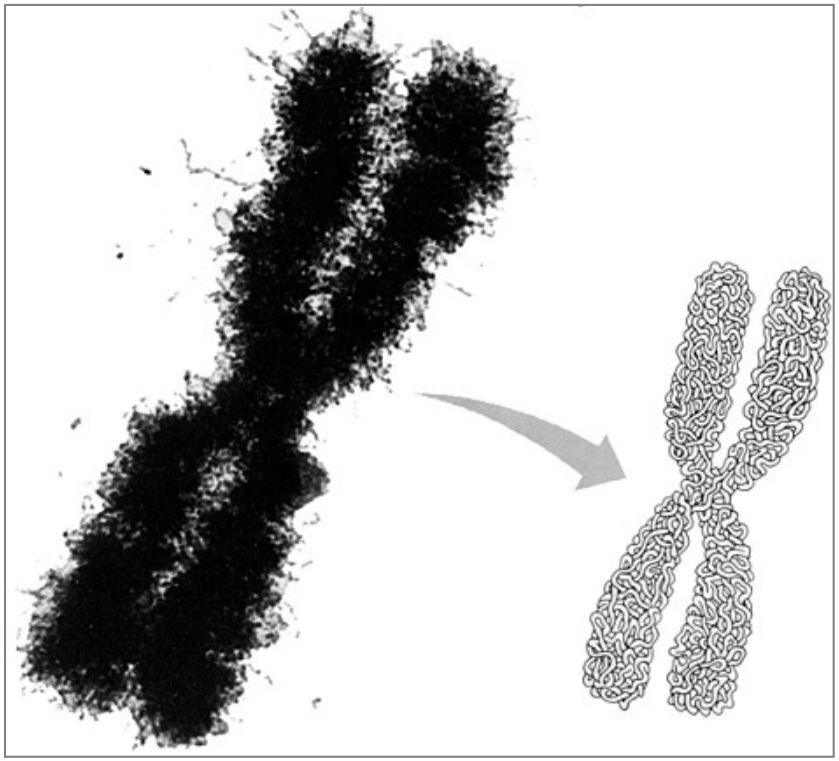
Chromosome acrocentric



Chromosome numbers (2n) in some animals

Species	#	Species	#
Common fruit fly	8	Guinea Pig	64
Dove	16	Garden snail	54
Earthworm	36	Tibetan fox	36
Domestic cat	38	Domestic pig	38
Laboratory mouse	40	Laboratory rat	42
Rabbit	44	Syrian hamster	44
Hare	46	Human	46
Gorillas, Chimpanzees	48	Domestic sheep	54
Elephants	56	Cow	60
Donkey	62	Horse	64
Dog	78	Kingfisher	132
Goldfish	100-104	silkworm	56

Ultraestructure



DU PRAW technique

Only partial views of the chromosome are possible.

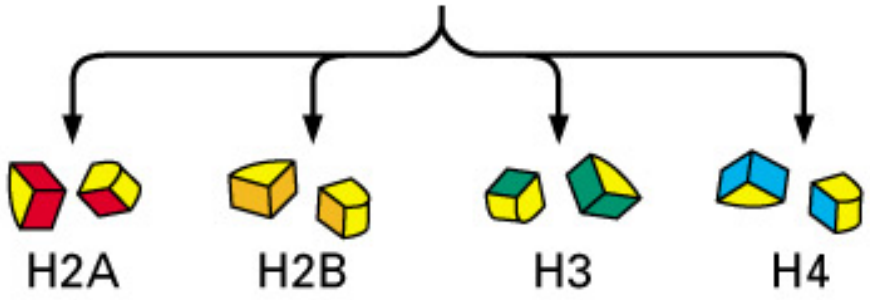
They are conglomerates of tight fibers sliced in multiple points (30nm)

Chemical composition

- DNA
- Proteins:

  Histones

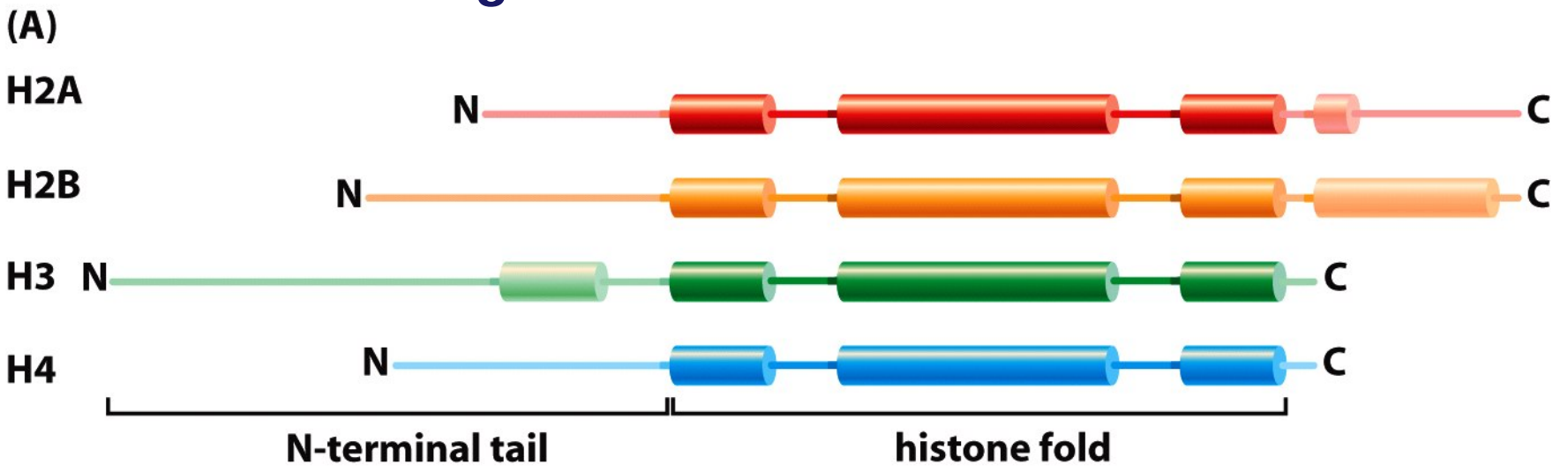
  No-histones



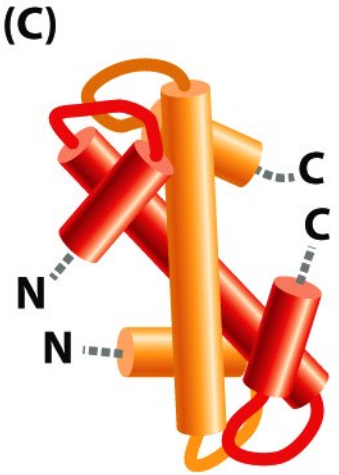
Histones

Molecular organization

Structural organization of histones in a nucleosome



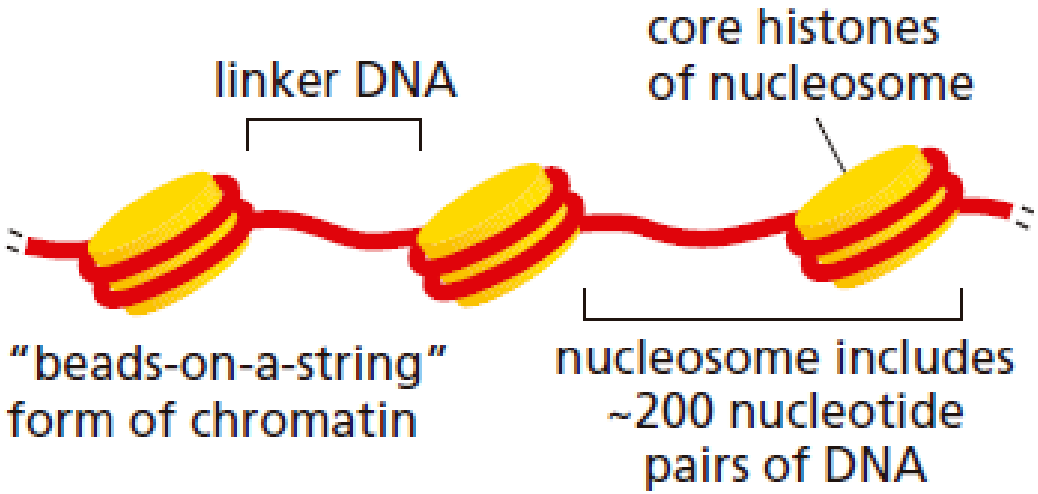
Histone fold

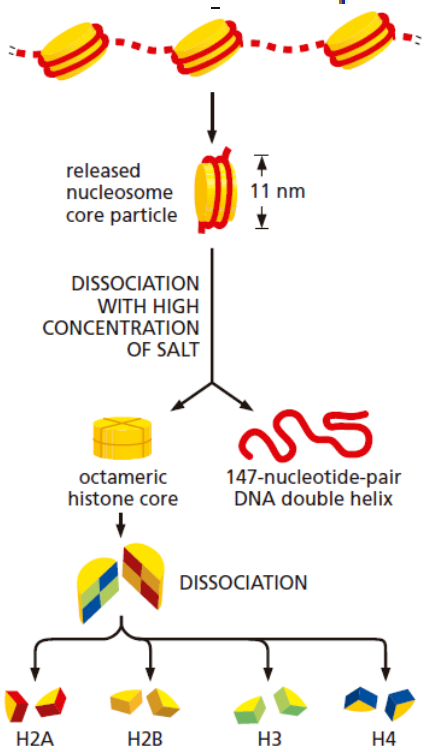


Dimer

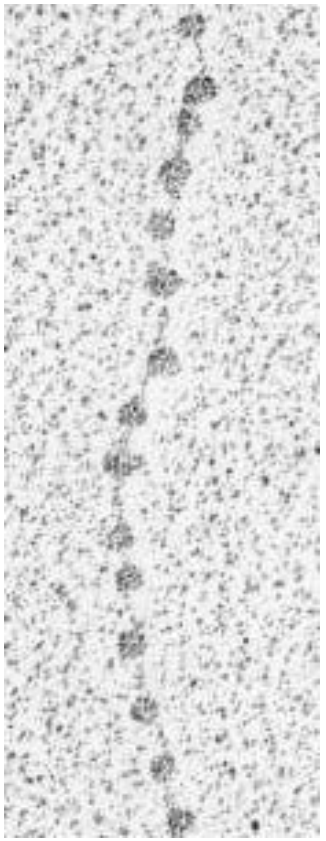
Figure 4-25 *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization





First level of packing



Nucleosome fiber (11nm)

Figure 4-22 *Molecular Biology of the Cell. Sixth edition,* © 2015

Molecular organization

Histone assembling

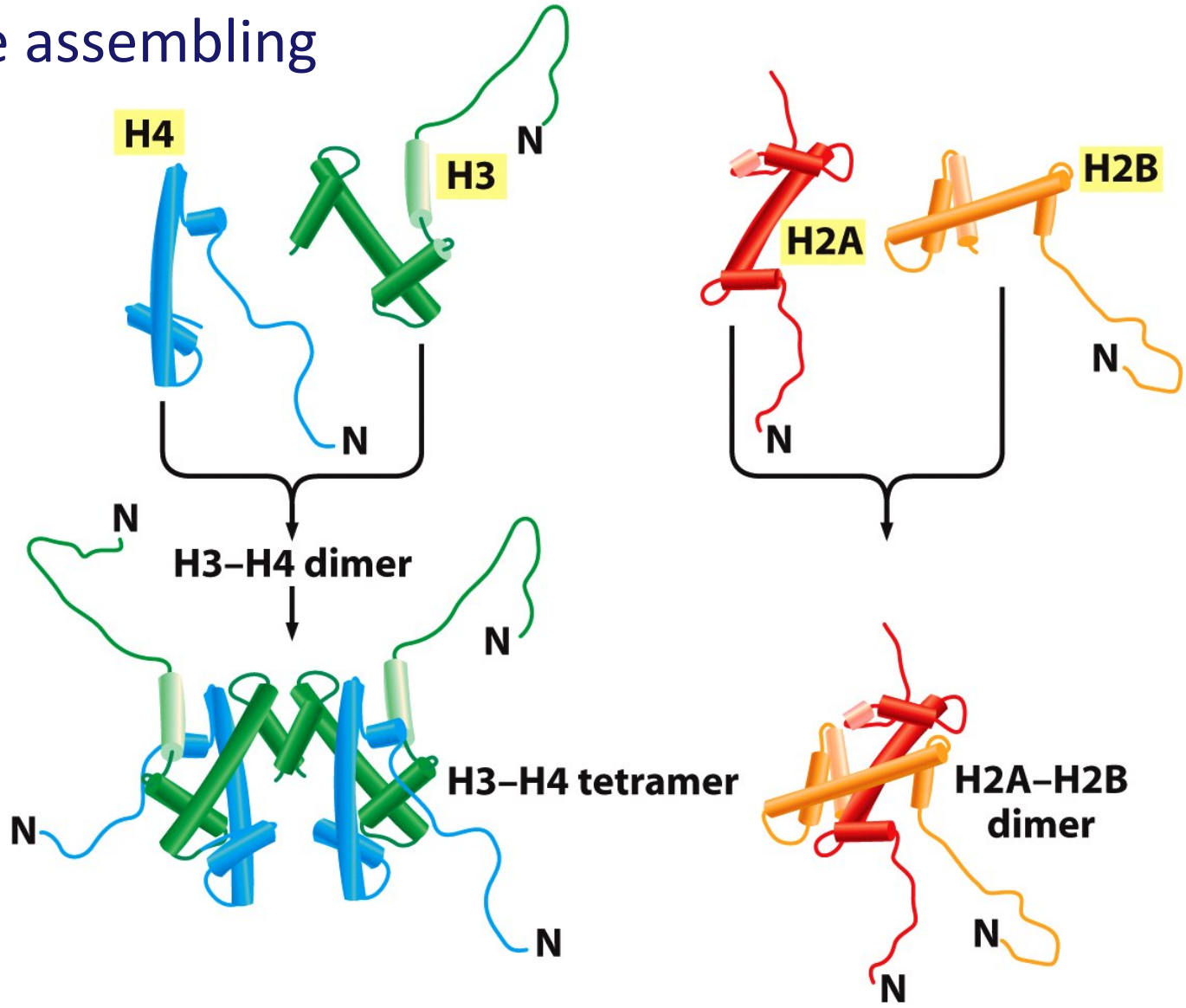
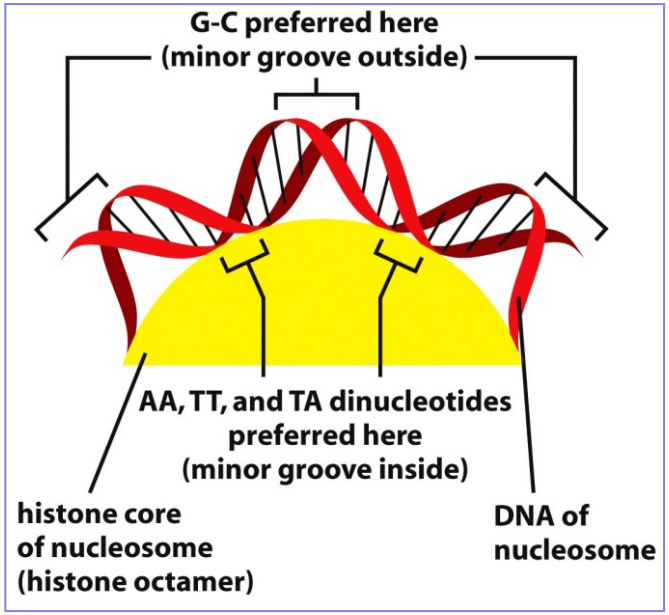


Figure 4-26 (part 1 of 2) *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization

Histone assembling



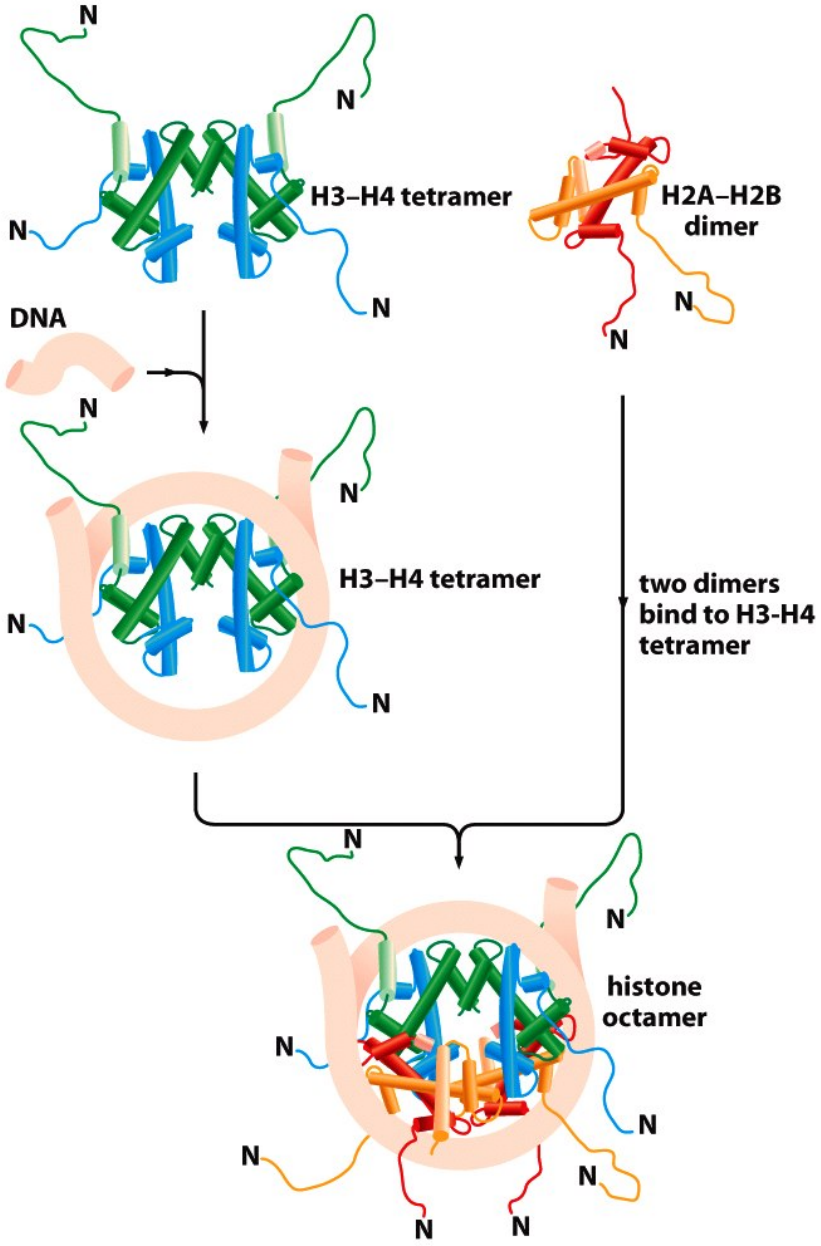


Figure 4-25 *Molecular Biology of the Cell. Sixth edition*, © 2015

Molecular organization

Nucleosome nucleus structure

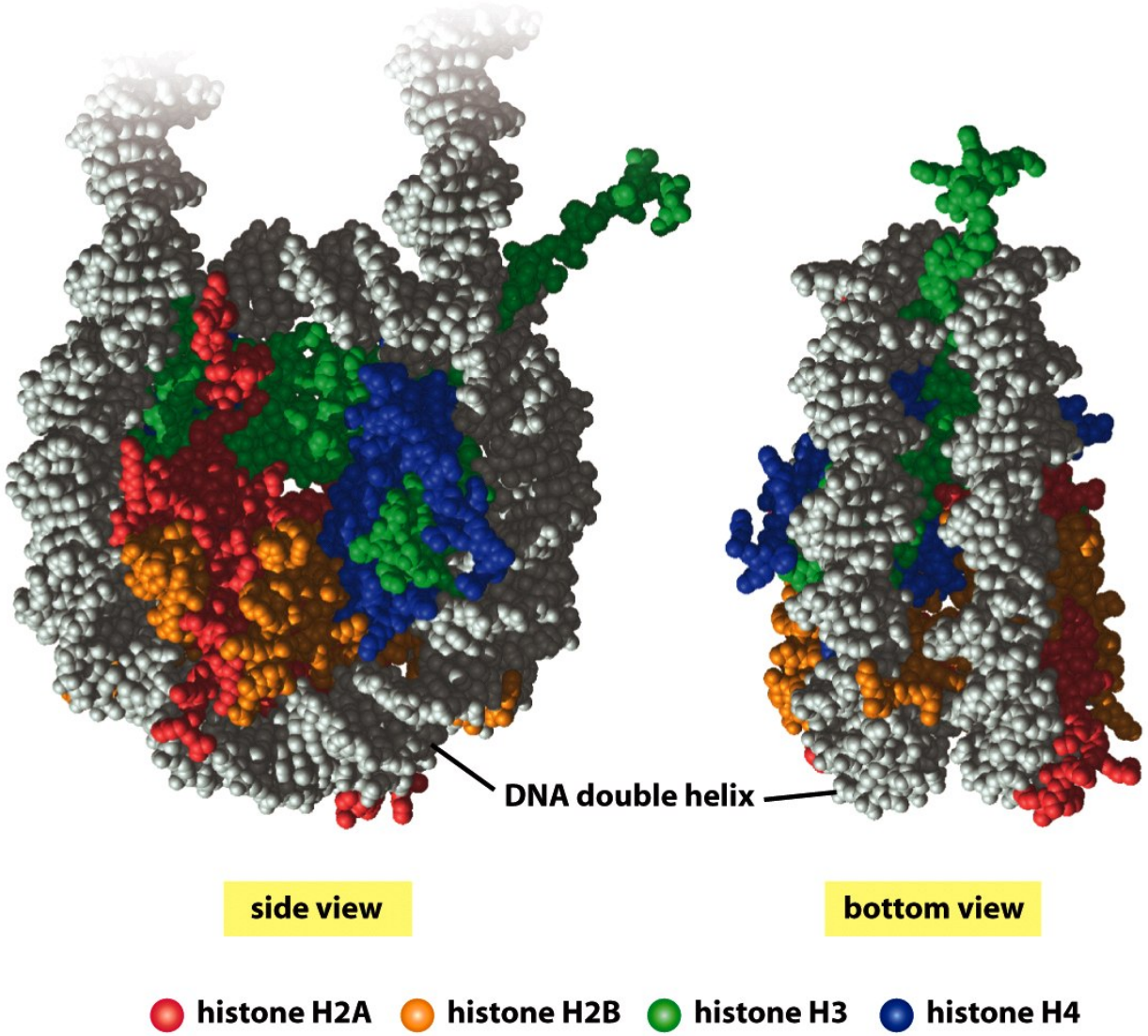
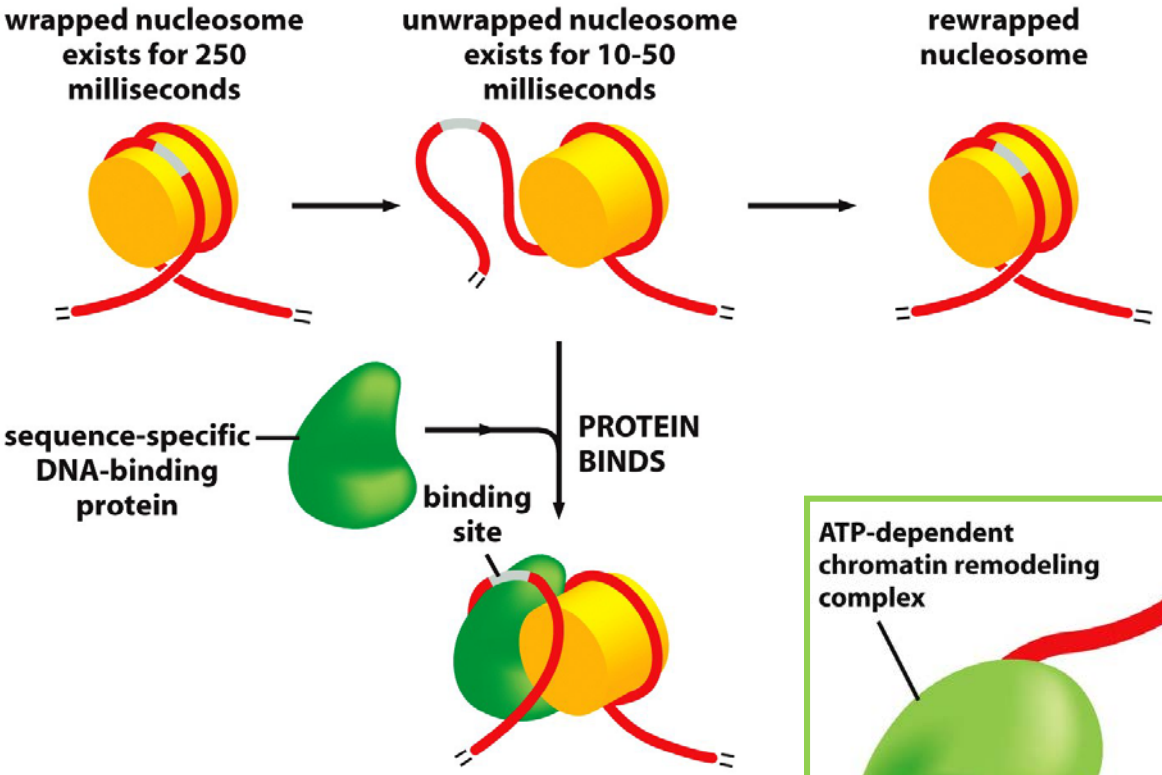


Figure 4-23 *Molecular Biology of the Cell. Sixth edition,* © 2015

Molecular organization



Nucleosomes are dynamic

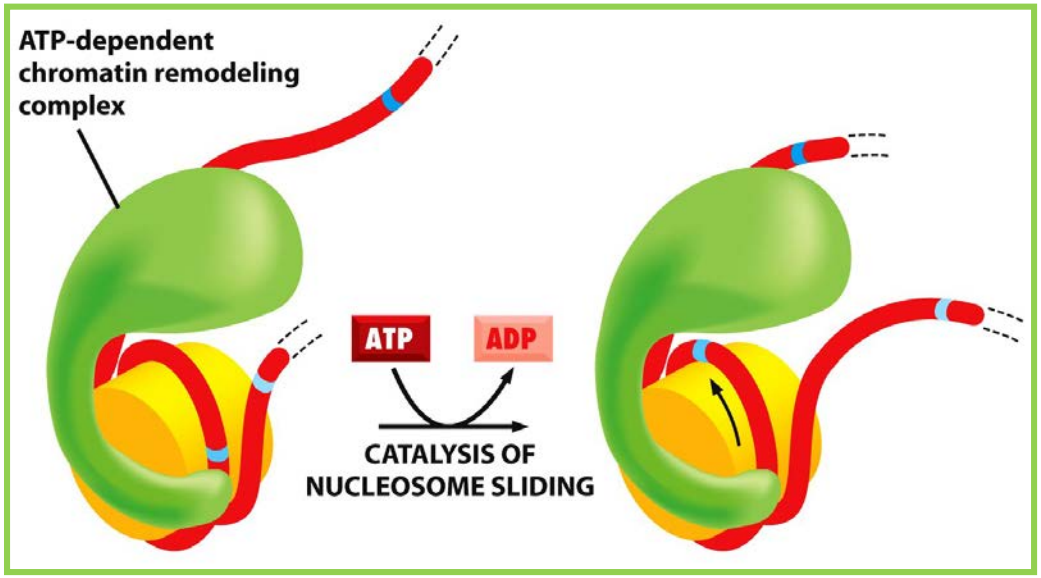


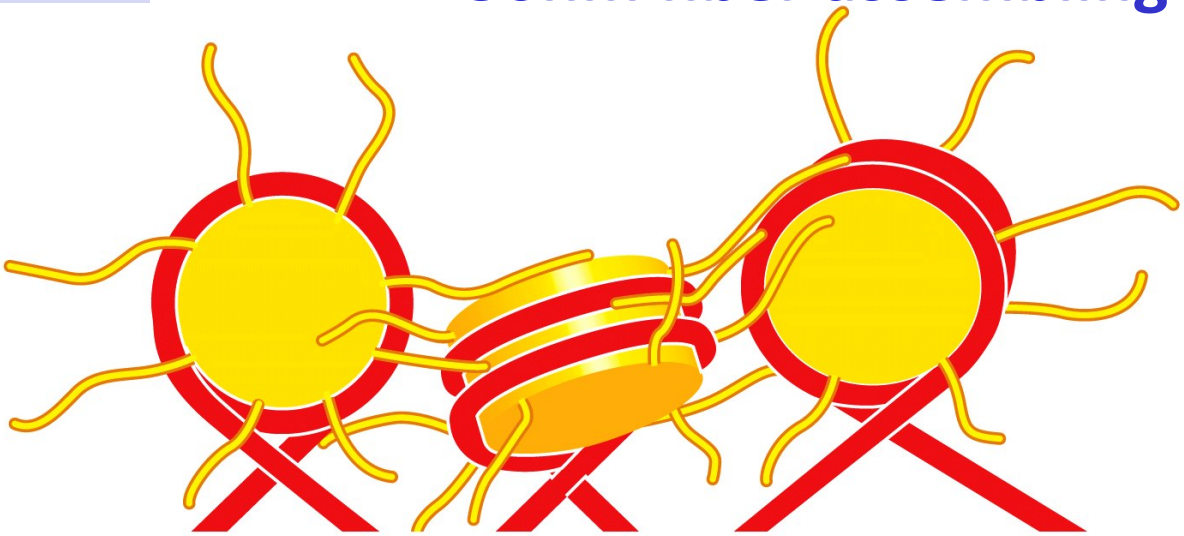
Figure 4-28 *Molecular Biology of the Cell* (© Garland Science 2008)

Figure 4-29 *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization

Second level of packing

30nm fiber assembling



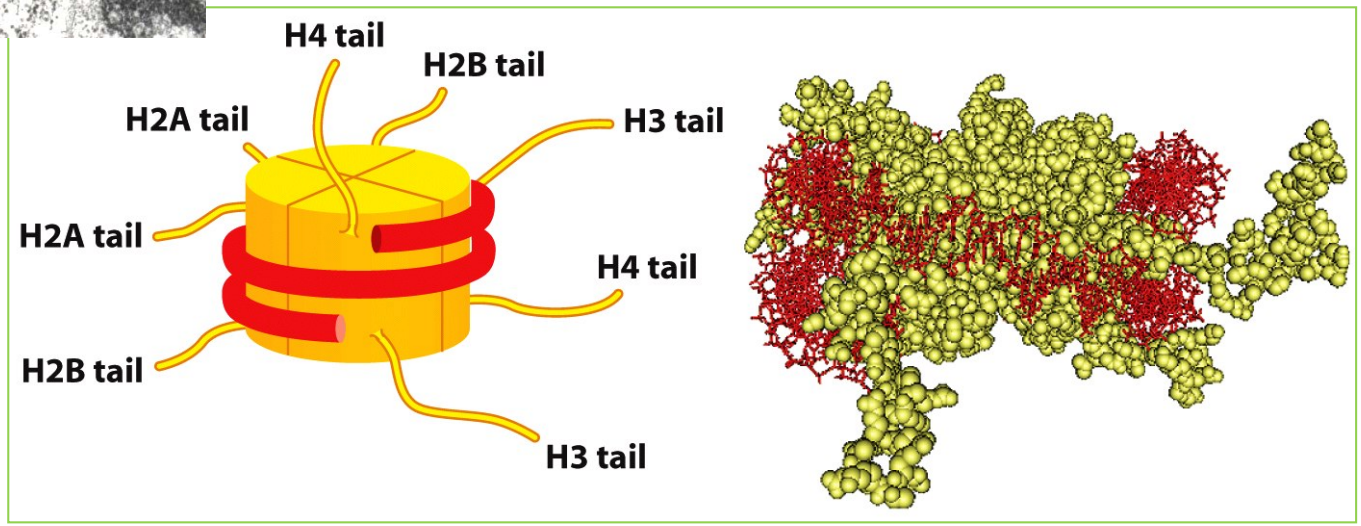


Figure 4-33b *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization

Second level of packing

H1 histone position

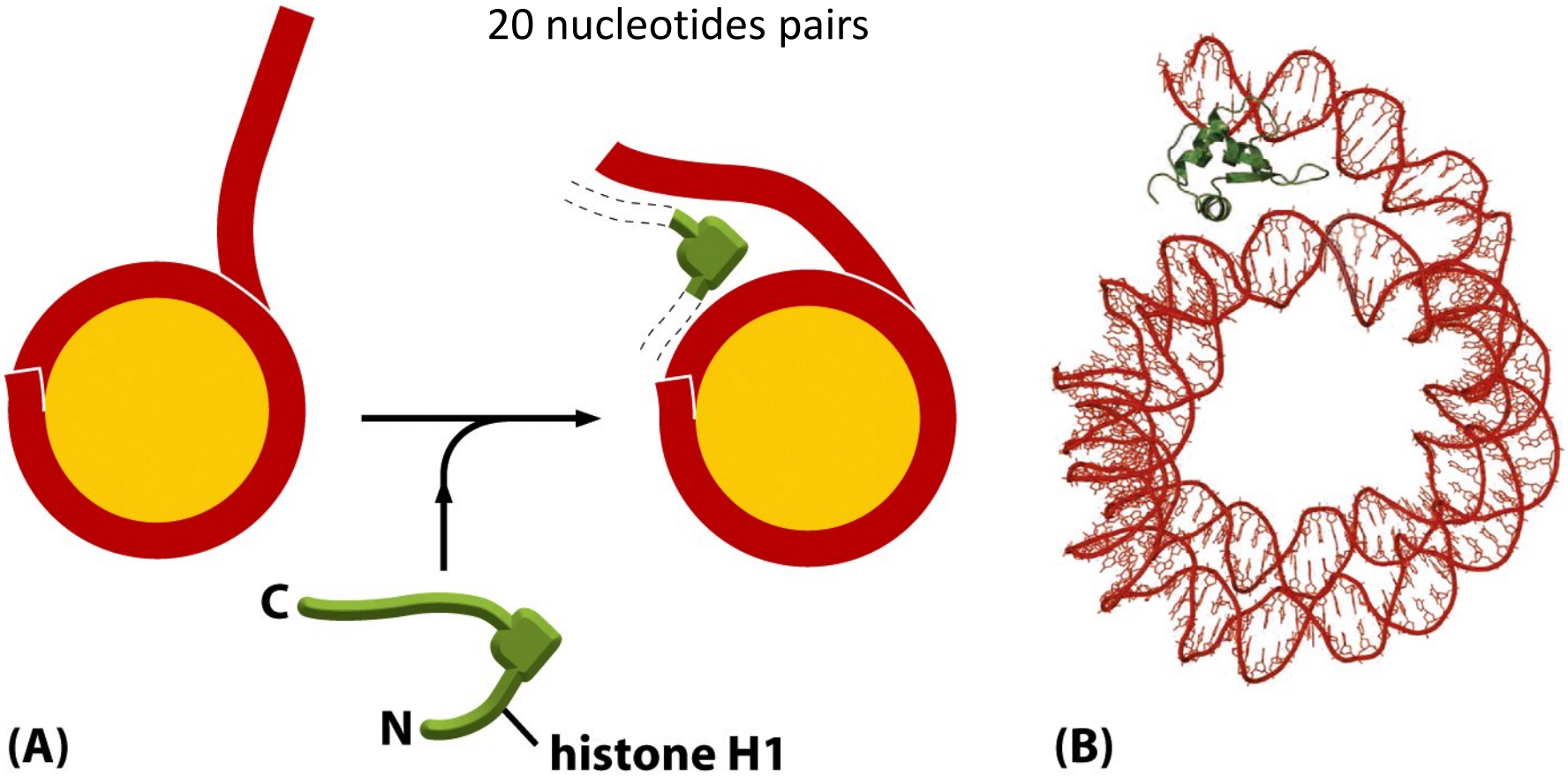


Figure 4-34 *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization

Second level of packing

30nm chromatin fiber

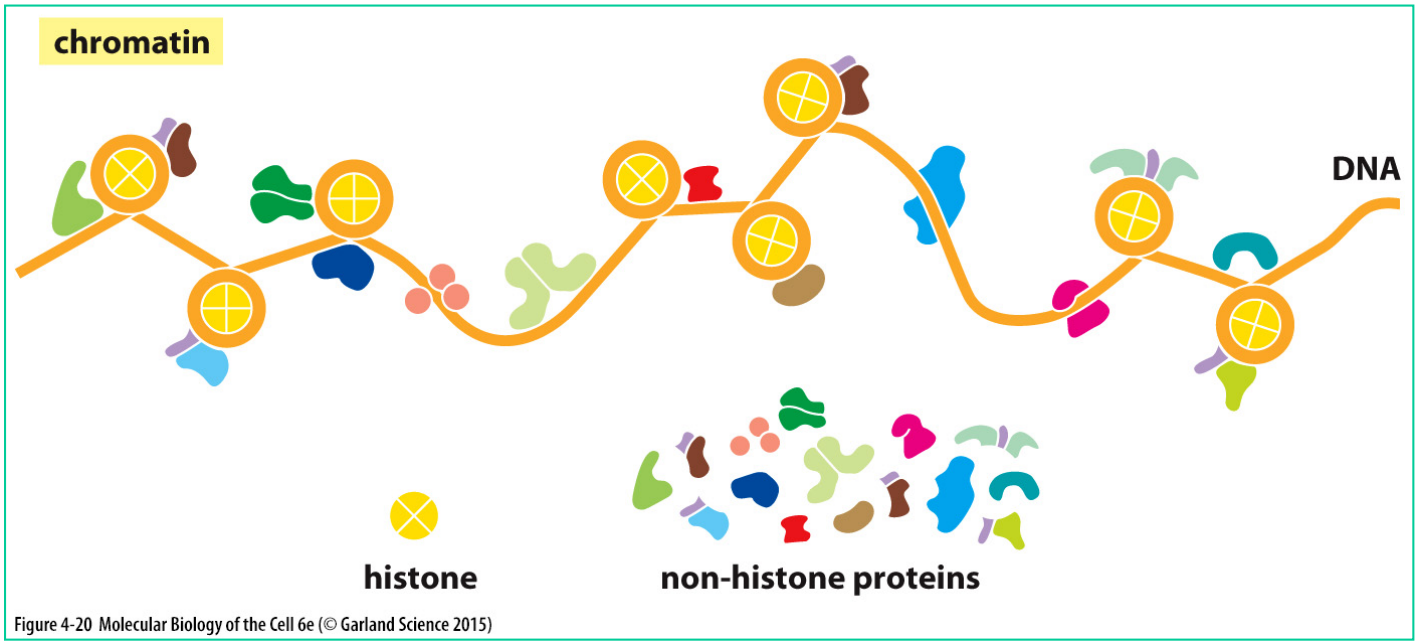


Figure 4-20 Molecular Biology of the Cell 6e (© Garland Science 2015)

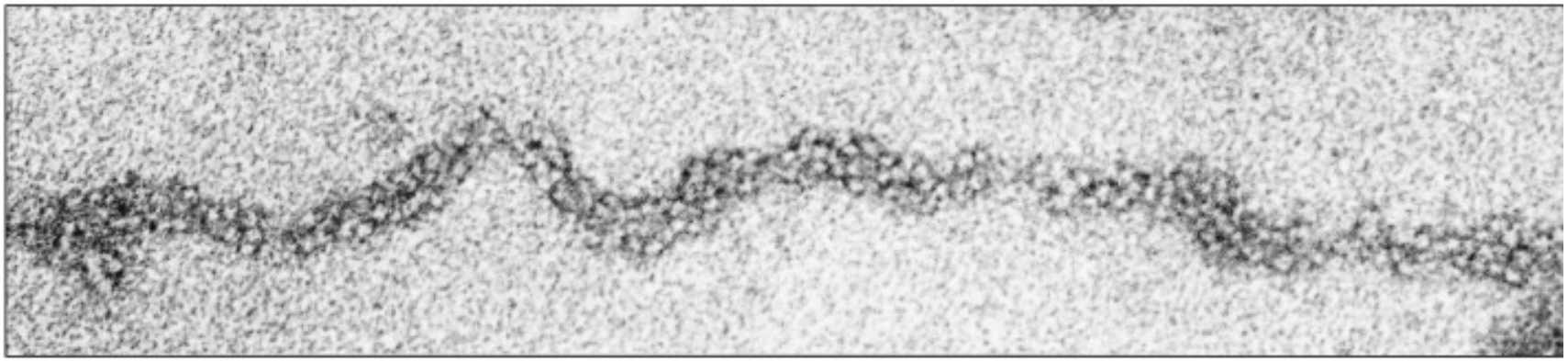
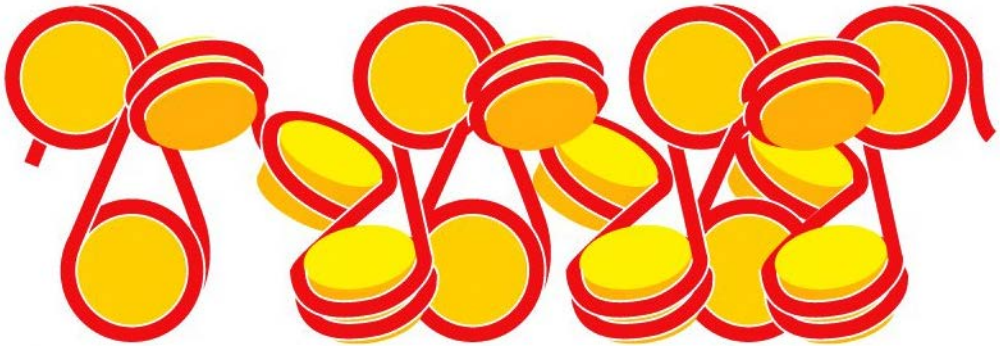
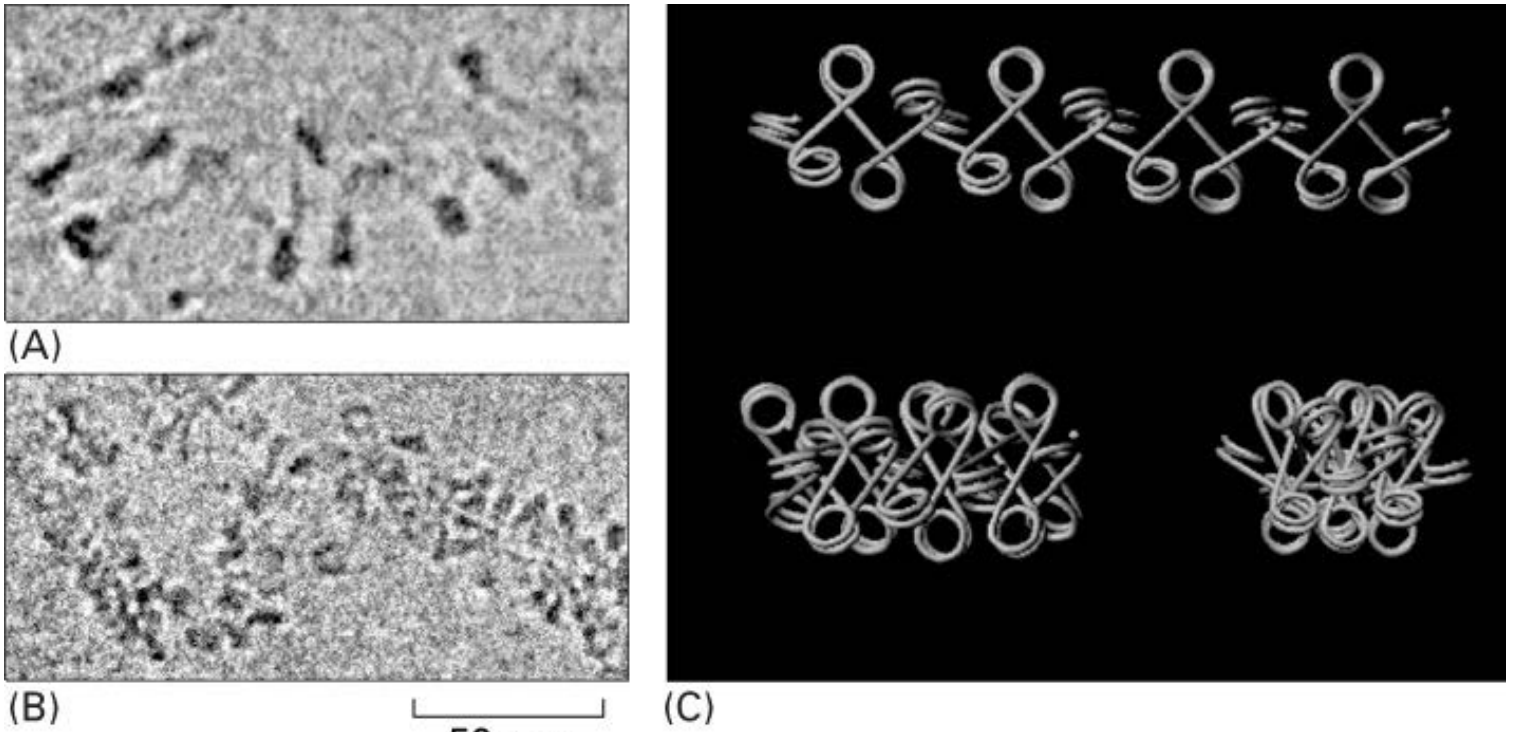


Figure 4-20 *Molecular Biology of the Cell. Sixth edition,* © 2015

Molecular organization

Zig-zag model for the 30nm fiber





Molecular organization

Solenoid model for the 30nm fiber

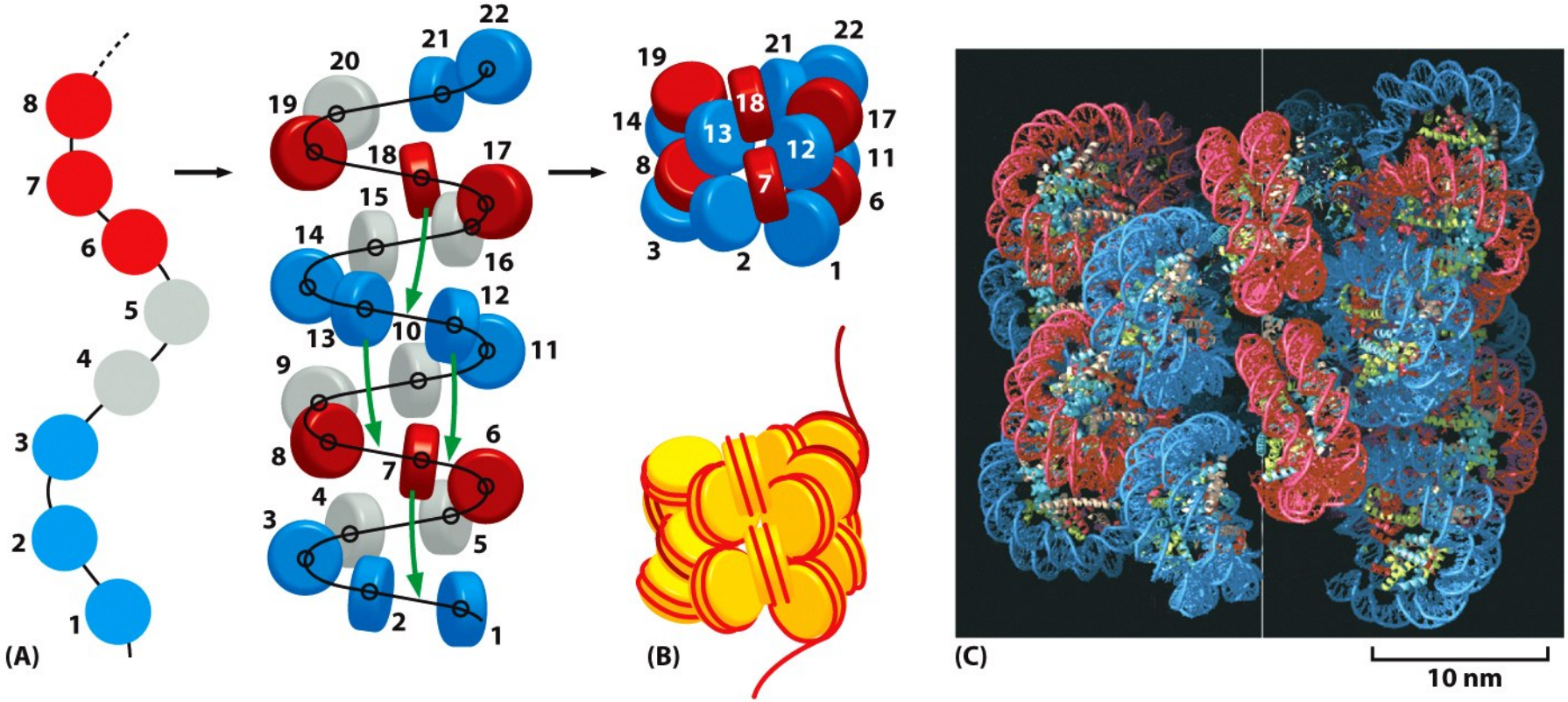
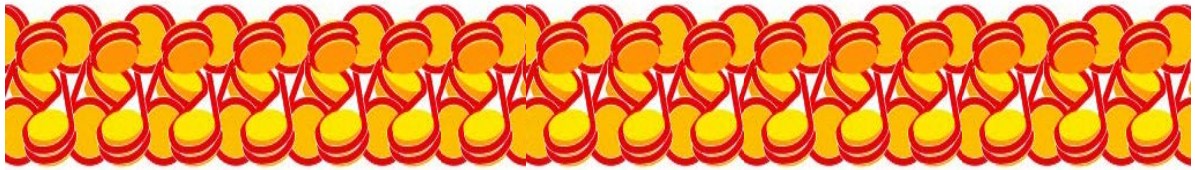
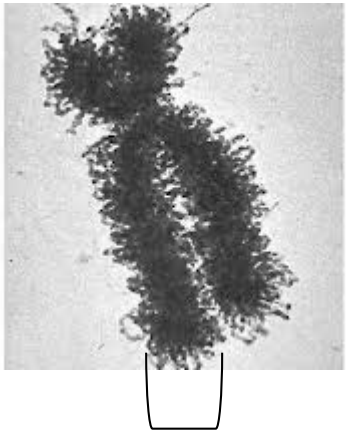


Figure 4-32 *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization

Nucleosome fiber 300nm





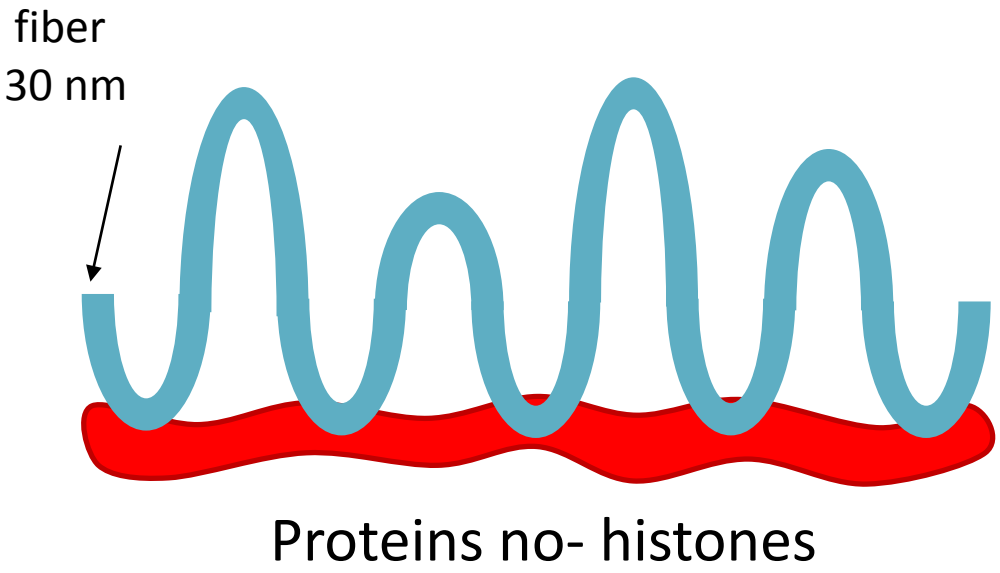


Figure 4-33b *Molecular Biology of the Cell* (© Garland Science 2008)

Molecular organization

Nucleosome fiber 700nm

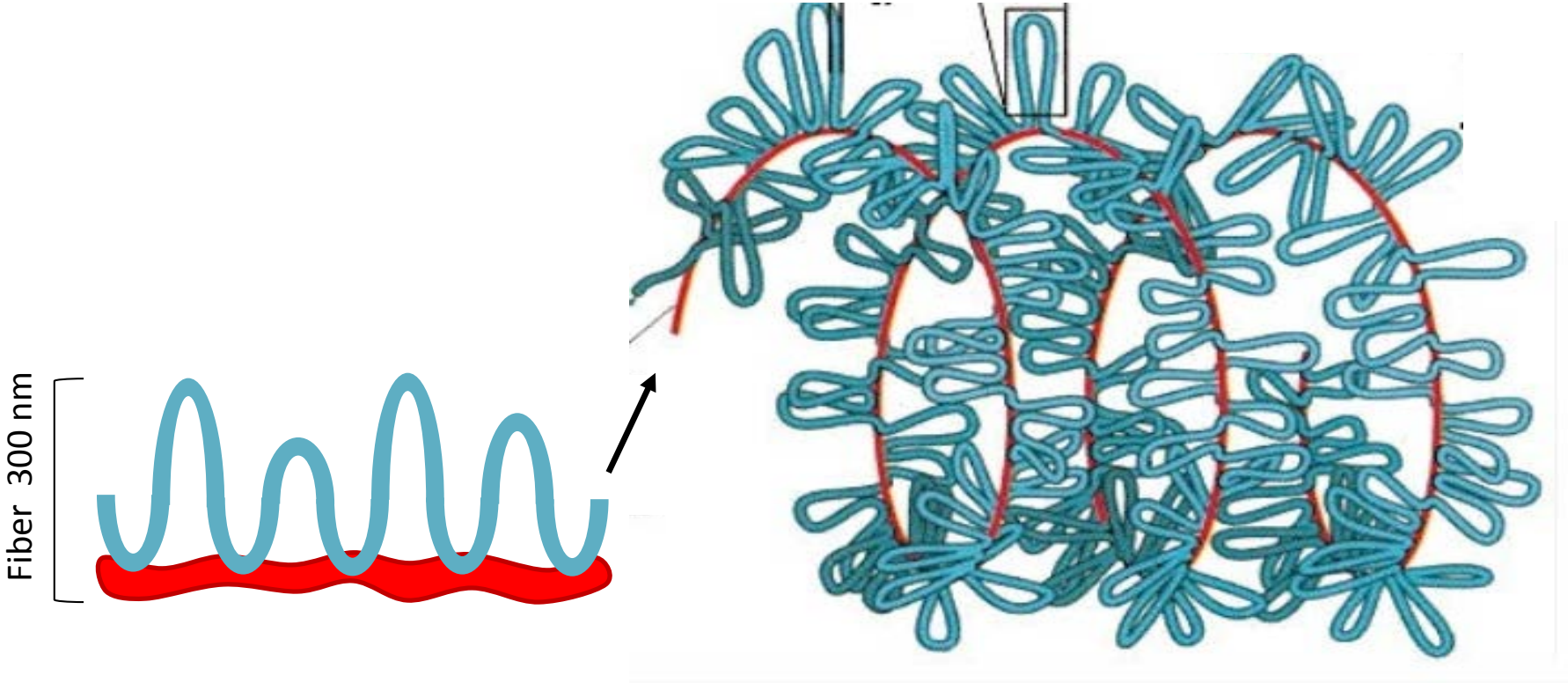
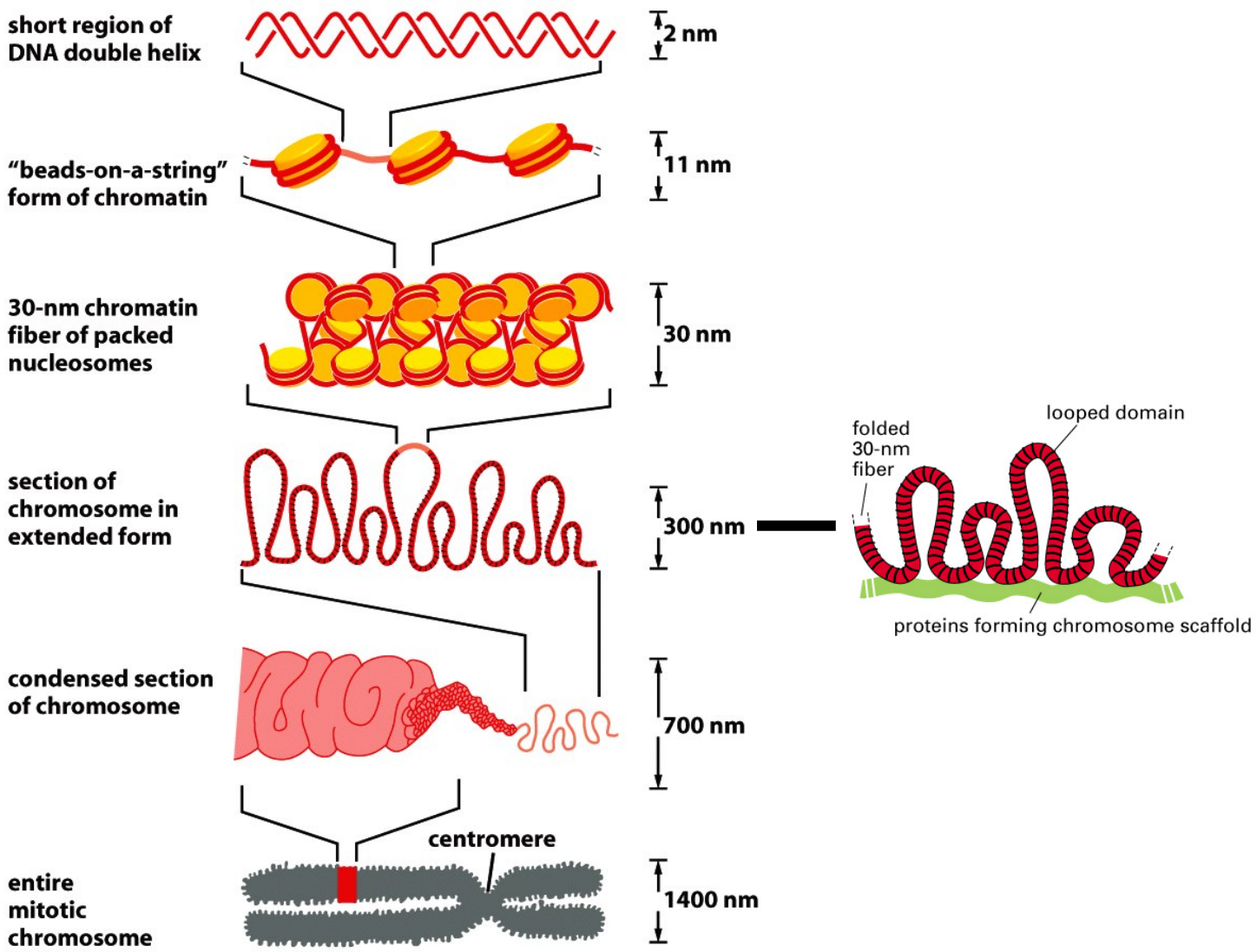


Figure 4-33b *Molecular Biology of the Cell* (© Garland Science 2008)

Chromatin packing levels



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

MEIOSIS

- Living beings reproduction. Biological cycles.
- Phases of meiosis.
- Genetic variability in meiosis.
- Evolution of chromosome number and DNA amount during meiosis
- Comparison mitosis-meiosis

SEXUAL REPRODUCTION

Special division: MEIOSIS **Cell fusion: FECUNDATION**

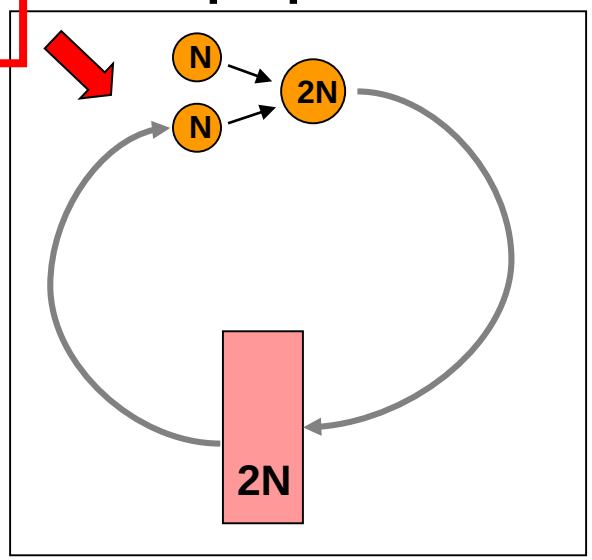
Mixing the genome of two cells (gametes)
Descendents differ genetically from the parents and also among them

Advantages:

- **The mixture of genes helps to species to survive in a variable and unpredictable environment**
- **It also helps to eliminate dangerous genes from a population**

BIOLOGICAL CYCLES

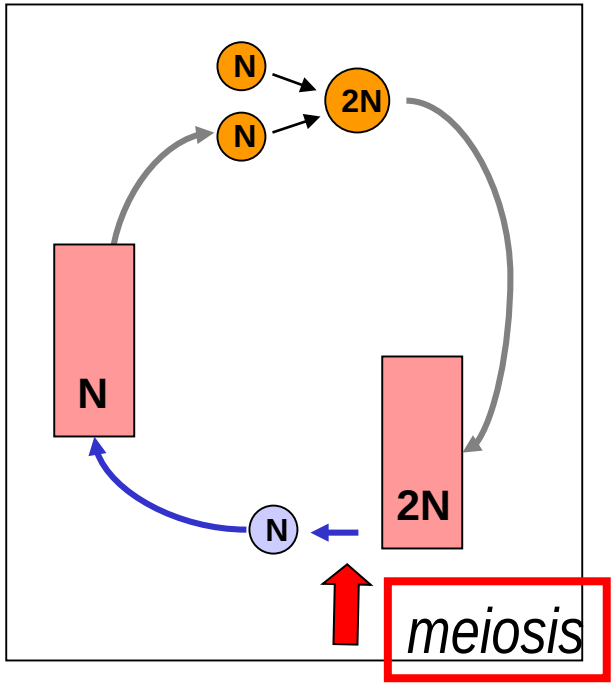
Diplophasic



Diploid cells proliferate by mitosis

Haploid cells formed by meiosis do not proliferate anymore

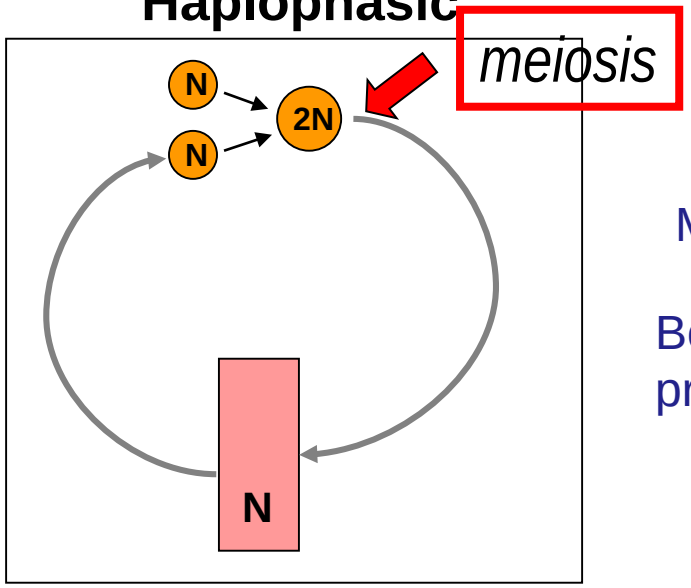
Haplo-diplophasic



Meiosis happens during sporulation

Both haploid and diploid cells proliferate by mitosis

Haplophasic



Diploid cell formed by fusion divides directly by meiosis

Resulting haploid cells proliferate by mitosis

MEIOSIS: PHASES

1ª

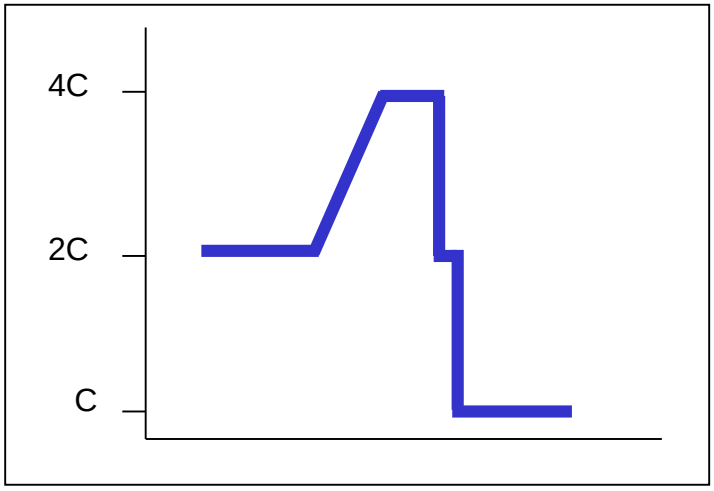
Prophase I
- leptotene
- zygotene
- pachitene
- diplotene
- diakinesis

Prometaphase I
Metaphase I
Anaphase I
Telophase I

INTERPHASE

2ª

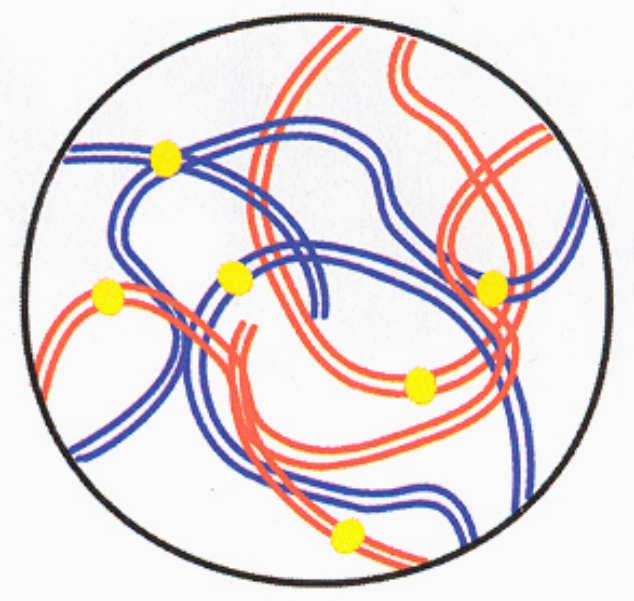
Prophase II
Prometaphase II
Metaphase II
Anaphase I
Telophase II



MEIOSIS: PHOPHASE I

Leptotene

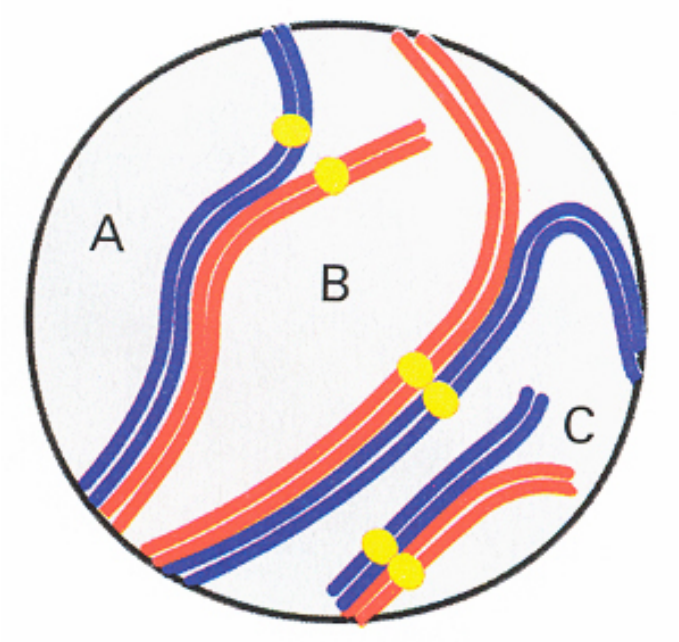
- The nucleus increases its volume
- Chromosomes get individual
- Telomeres contact with nuclear envelope
- Sometimes, chromomeres appear

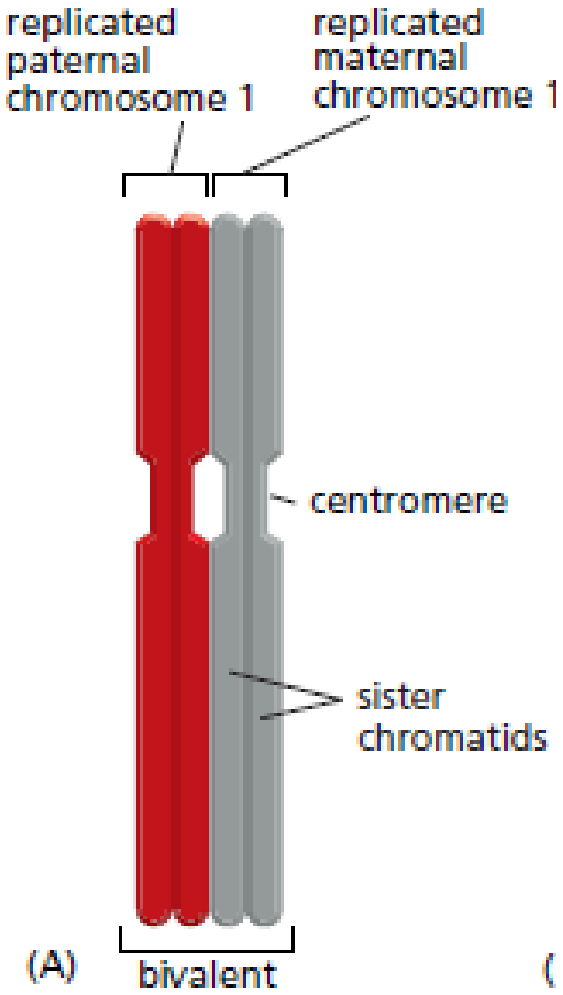


MEIOSIS: PHOPHASE I

Zygotene

- Pairing of homolog chromosomes
- Partial pairing between X and Y chromosomes
- Bivalent or tetrads are formed
- Sinaptonemal complex is formed



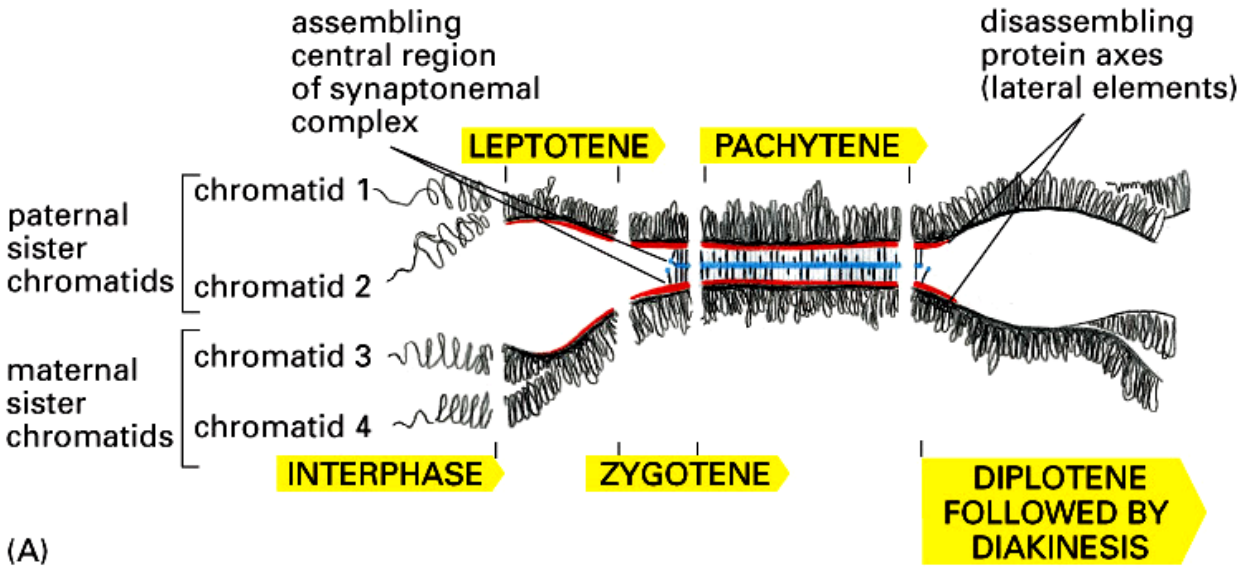


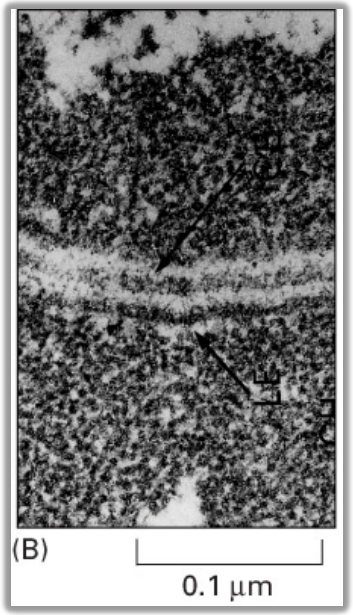
Alberts et al., Fig. 17-54

MEIOSIS: PHOPHASE I

Zygotene

Synaptonemal complex





Model of the synaptonemal complex structure

•**Lateral element** (LE): cohesin, structural proteins SYCP2 y SYCP3

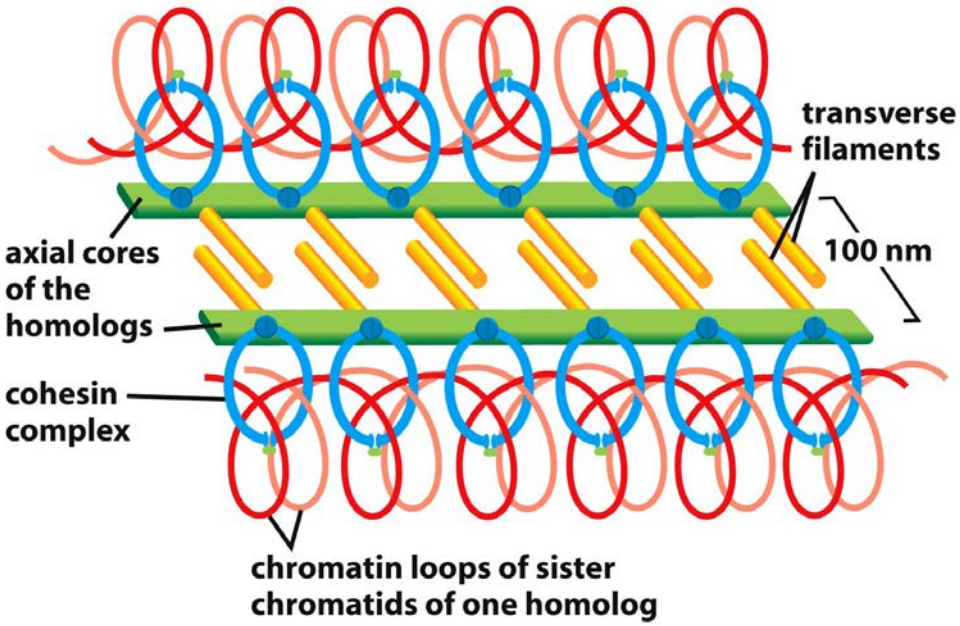
• **Central element:** (CE) with transverse filaments: SYCP1

Alberts et al., Fig. 17-56

MEIOSIS: PHOPHASE I

Pachitene

- Genetic recombination
- Recombination complexes

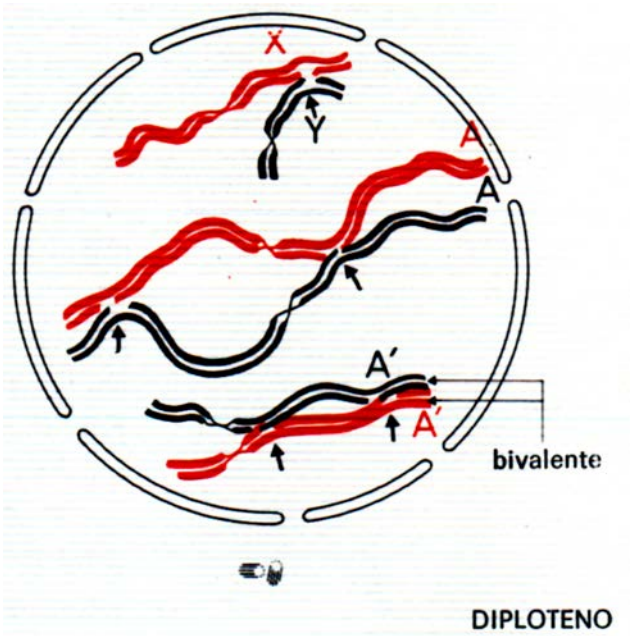


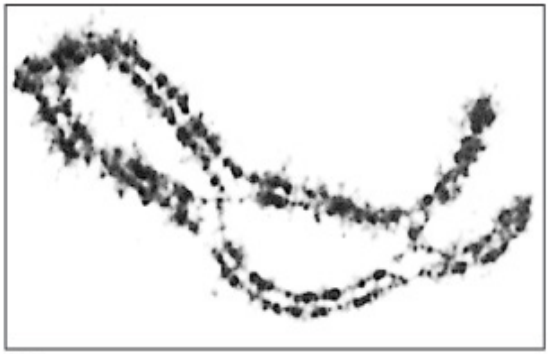
Alberts et al., Fig. 17-55

MEIOSIS: PHOPHASE I

Diplotene

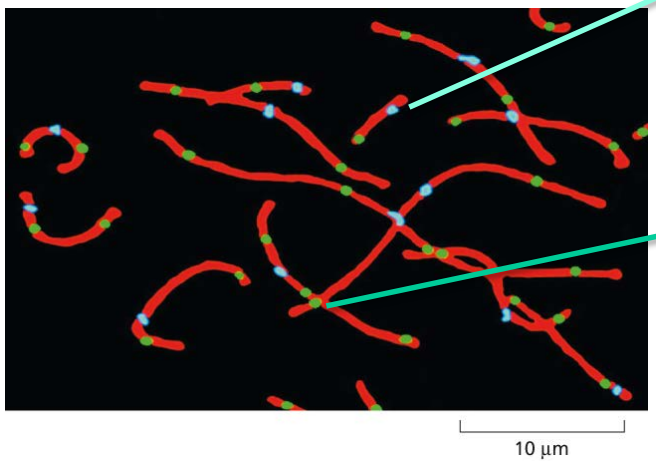
- Paired chromosomes separati
- Chiasmas appear (stabilizing b





Alberts et al., Fig. 17-57

Three separated intercrossing produce three chiasmas



Alberts et al., Fig. 17-59

MEIOSIS: PHOPHASE I

Diakinesis

- Chromosomes maximum degree of condensation and movements to ecuador cell
- Paired chromosomes continue separation
- Chiasmas shift towards the extreme of the chromosomes (terminalization)
- The nucleolus disappears
- The nuclear envelope breaks down

MEIOSIS: PHASES

Prometaphase I

- The nuclear envelope disappears
- Chromosomes bind to mi. kinetochoric
- Chromosomes are heading to ecuador

Metaphase I

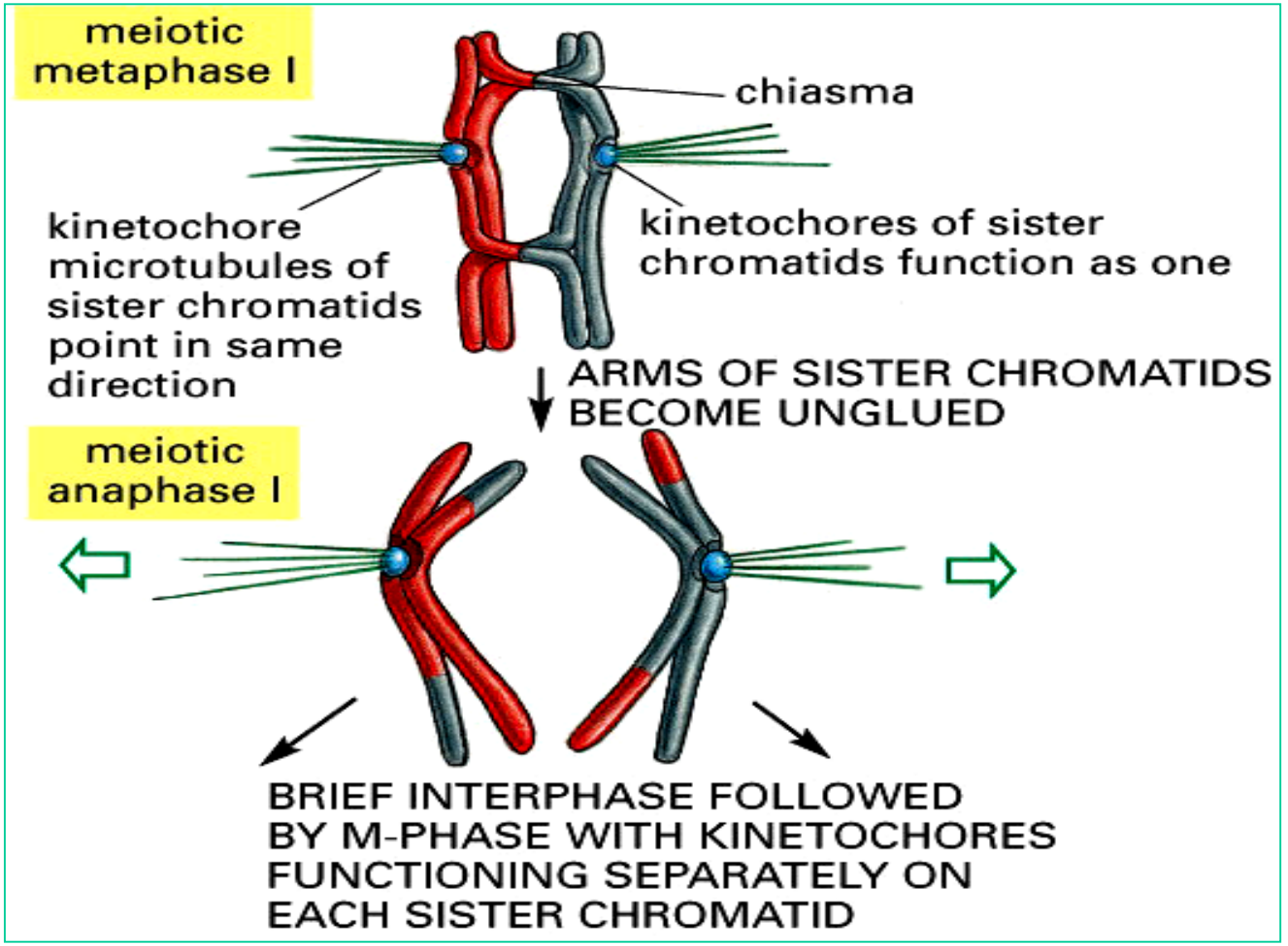
- Both kinetochores of a chromosome go to the same pole. Therefore, kinetochoric microtubules go to only one of the poles of the spindle
- Random orientation of homologs (paternal and maternal) in the metaphasic plate.

MEIOSIS: PHASES

Anaphase I

Homolog chromosomes separation during first stage of meiosis

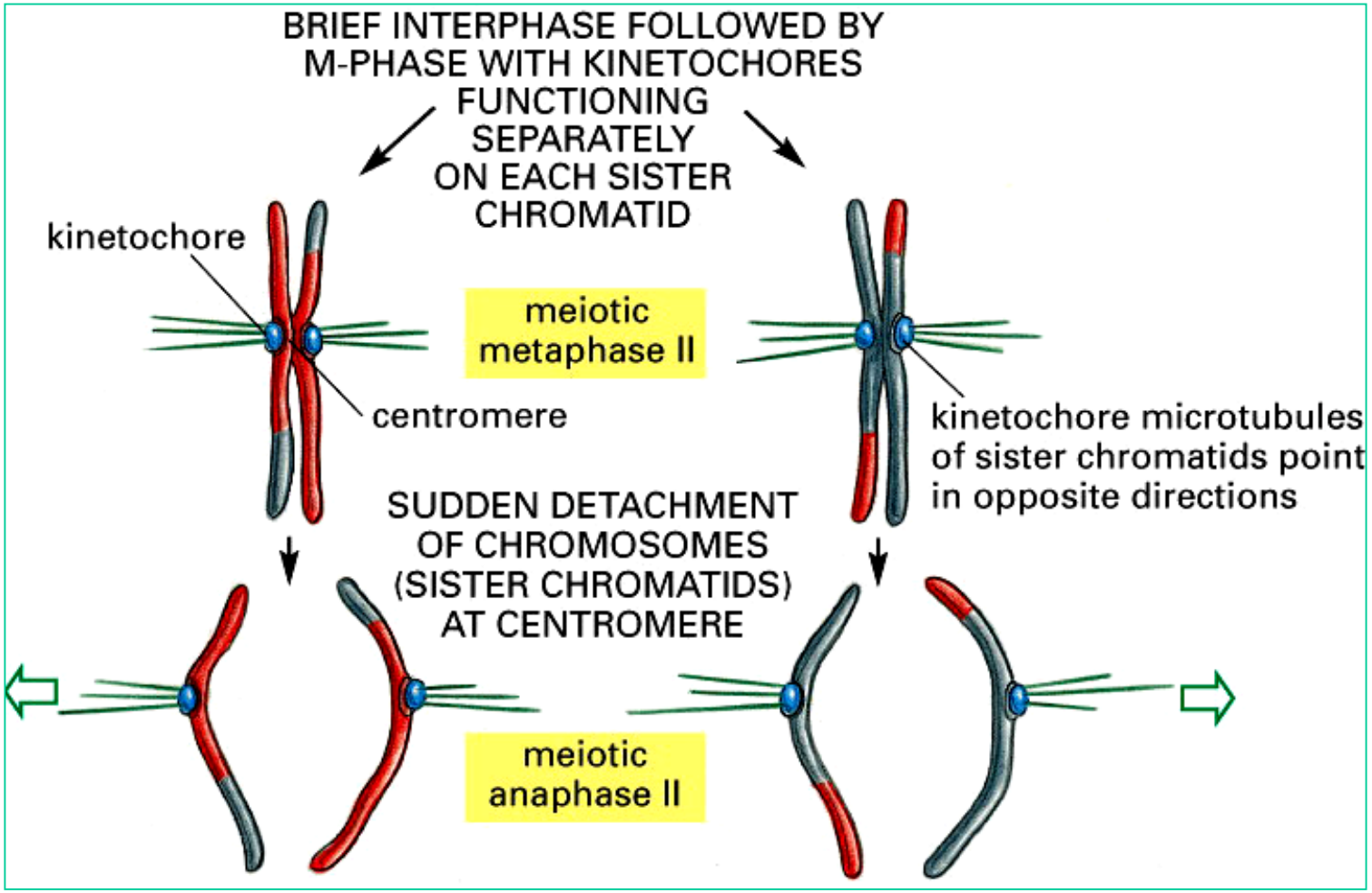
- Homolog separation during anaphase will also be random.
- Centromeres do not break



Alberts et al., Fig. 20-11

MEIOSIS: PHASES

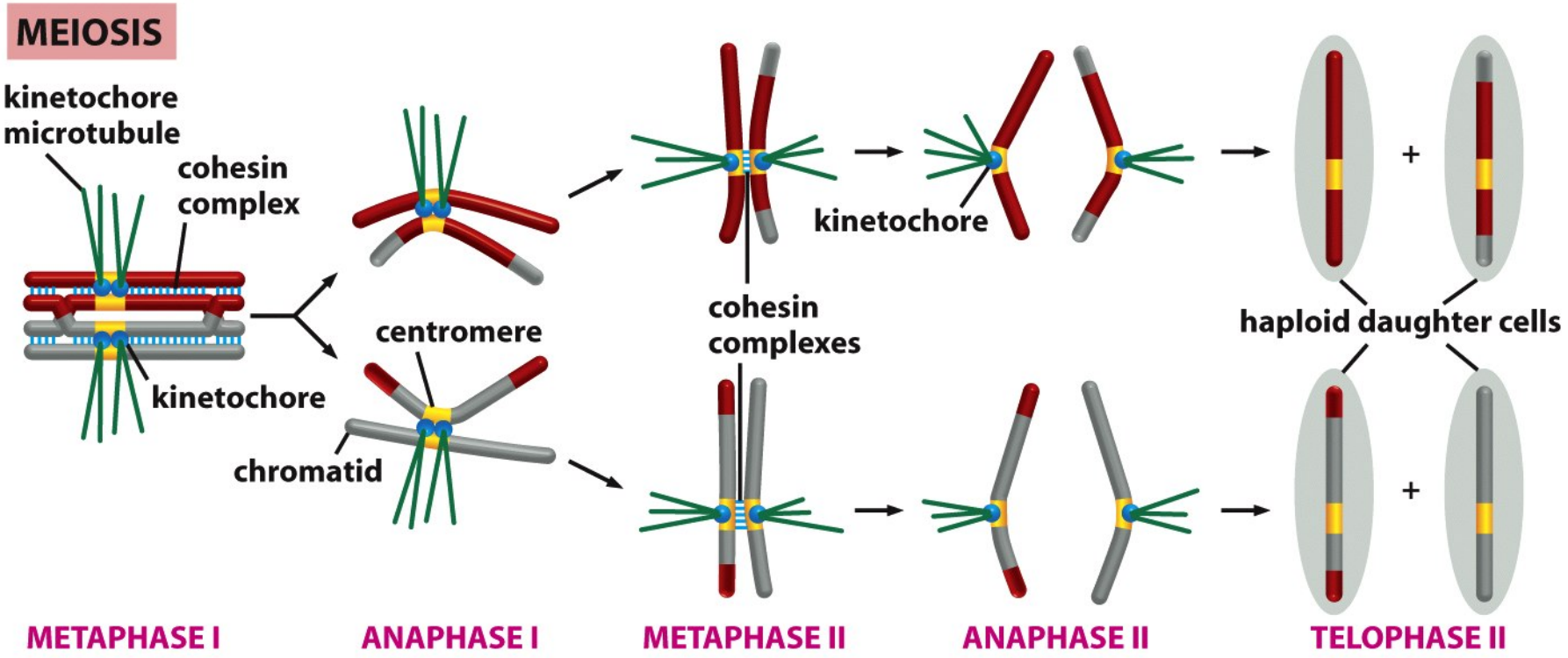
Homolog chromosomes separation during second stage of meiosis



Alberts et al., Fig. 20-11

MEIOSIS: PHASES

Homolog chromosomes separation during first and second stages of meiosis



Alberts et al., Fig. 17-58

MEIOSIS: PHASES

Chromosomes separation

- The cohesin complex mediates cohesion between sister chromatids during both mitosis and meiosis. According to the ‘ring model’, cohesin mediates sister chromatid cohesion by topologically entrapping sister chromatids. At the onset of anaphase, cohesin is cleaved by a protease called separase, which opens the cohesin ring. During meiosis, the chromosome number is halved because two rounds of chromosome segregation, called meiosis I and meiosis II, follow a single round of DNA replication.
- During the first meiotic division, segregation of recombined homologous chromosomes is triggered by separase cleavage of cohesin’s kleisin subunit Rec8 along chromosome arms. A key feature of meiosis I is that centromeric cohesin is protected from separase cleavage by shugoshin proteins in complex with PP2A.
- In meiosis I, centromeric cohesin carrying Rec8 is protected from separase cleavage. For Rec8 to be cleaved by separase, it must be phosphorylated by casein kinase 1 (CK1) in yeast. Shugoshin 1 (Sgo1) recruits protein phosphatase 2A (PP2A) to the pericentric heterochromatin regions and antagonizes this phosphorylation, thus preventing the cleavage of pericentric cohesin.

Sugosina prevents the degradation of pericentromeric cohesins and ensures the correct biorientation of the chromosomes

Nature Reviews | Molecular Cell Biology

Genetic consequences of meiosis

1. Reduction of chromosomes number to half

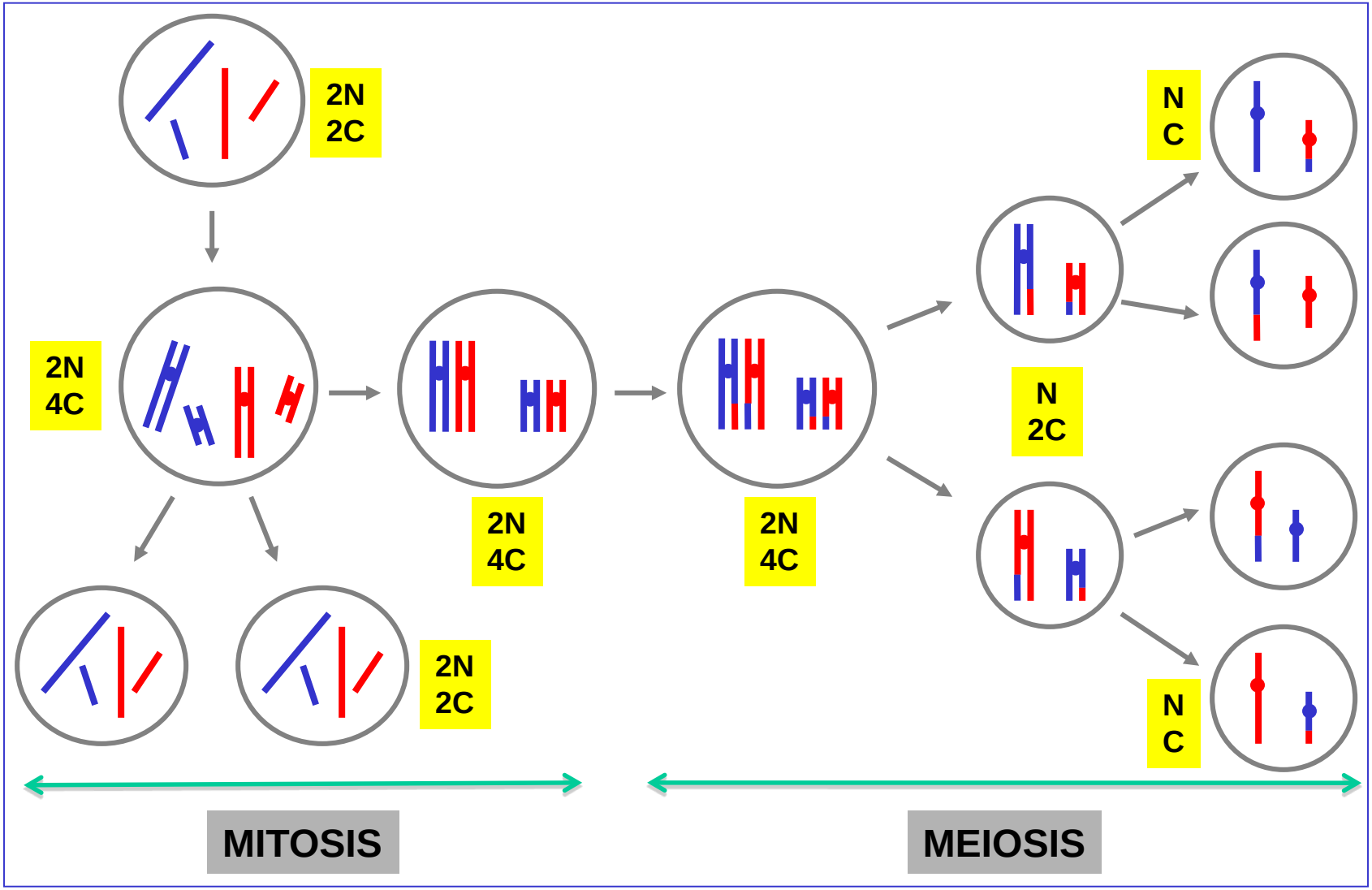
2. Generation of genetic diversity in the gametes
   - Recombination between homolog chromatid during prophase of first meiotic division
   - Random distribution of chromosomes from paternal and maternal origin during anaphase of first meiotic division

3. Cells generated from meiosis are genetically different to each other and different to progenitor cell

4. Errors in meiosis raise important pathologies

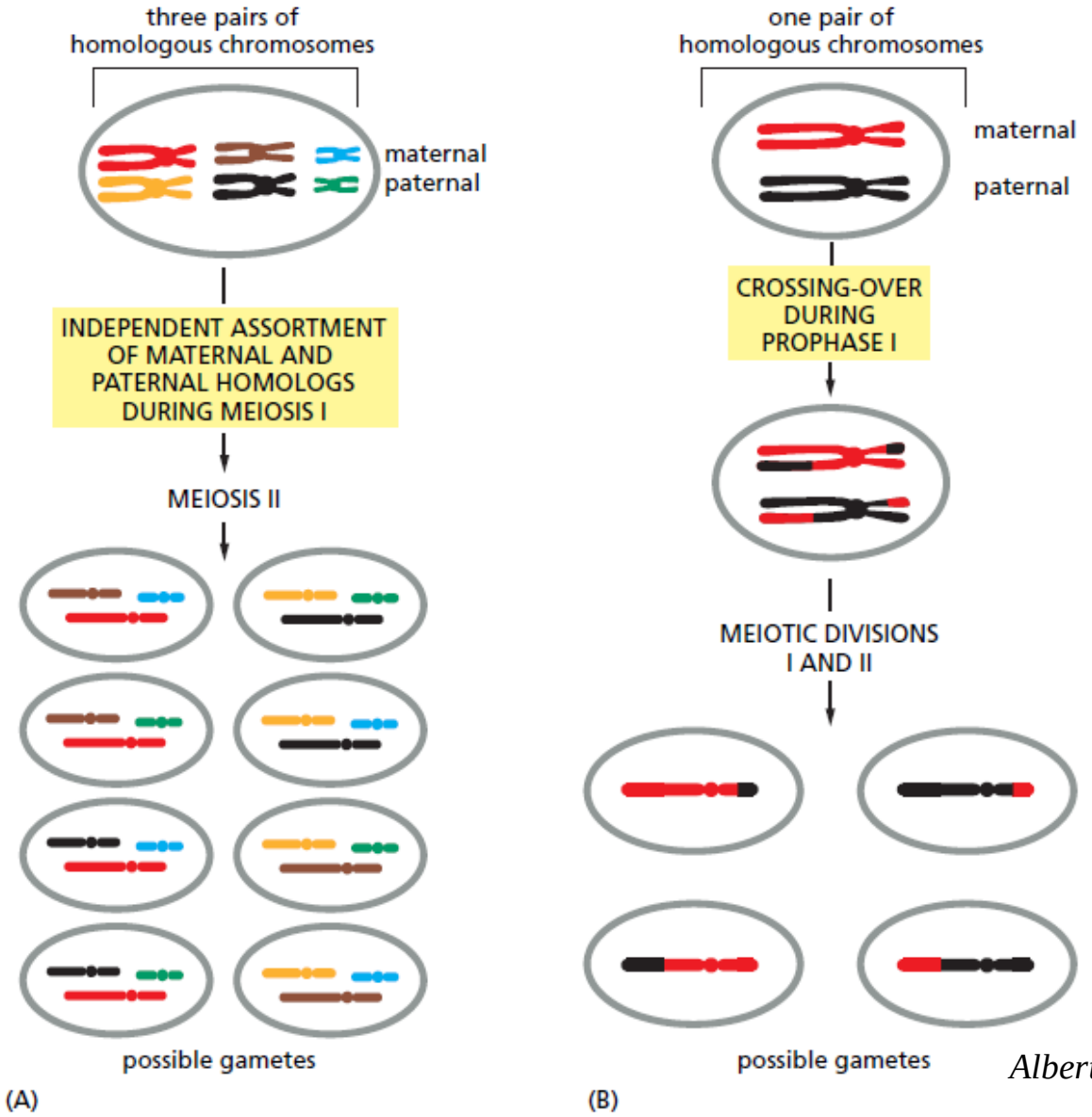
Genetic consequences of meiosis

1. Evolution of number of chromosomes and DNA amount during meiosis



Genetic consequences of meiosis

2. Origin of genetic variability in meiosis



Alberts et al., Fig. 20-8

Genetic consequences of meiosis

3. Cells generated from meiosis are genetically different to each other and different to progenitor cell

4. Errors in meiosis raise important pathologies

COMPARISON MITOSIS-MEIOSIS

Similarities:

- chromosomes appear
- the spindle (cytoskeleton) drives the chromosome motions
- nucleus and cytoplasm divides

Differences:

- Mitosis: one DNA duplication followed by one cell division
  Meiosis: one DNA duplication followed by two cell divisions

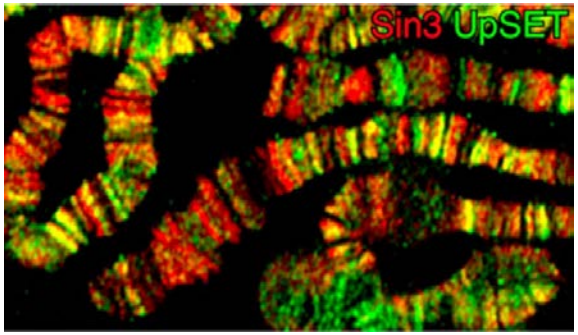
- Mitosis: short in time
  Meiosis: it may happen during years

- Mitosis: each chromosome follows a motion independently from the others
  Meiosis: homologs keep close binding at the beginning of division

- Mitosis: an exact copy of the genome is transmitted to daughter cells
  Meiosis: there are genome variation in daughter cells

SPECIAL CHROMOSOMES

- Lampbrush chromosomes
- Polytene chromosomes
- Interphasic chromosomes
- The chromosome cycle



Nucleus

Interphase

Chromosome

Division

- Lampbrush chromosomes
- Polytene chromosomes

Lampbrush chromosomes

Meiosis
Diplotene

- **Chromatin loop**
- **Chromosome axis**
- **RNAs and so on**

Lampbrush chromosomes

Molecular organization

Axis

Two chromatids

Chromomeres

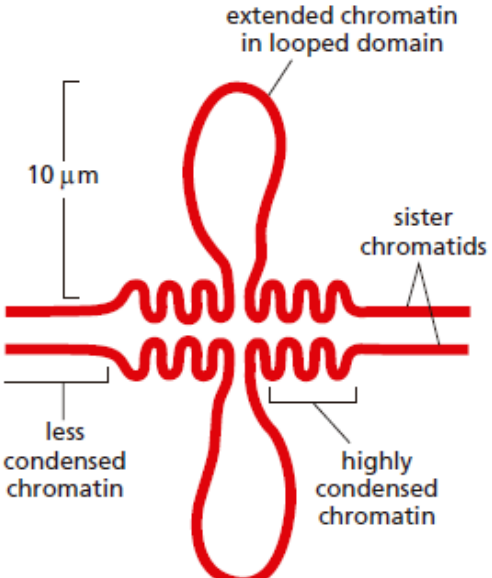
Condensations

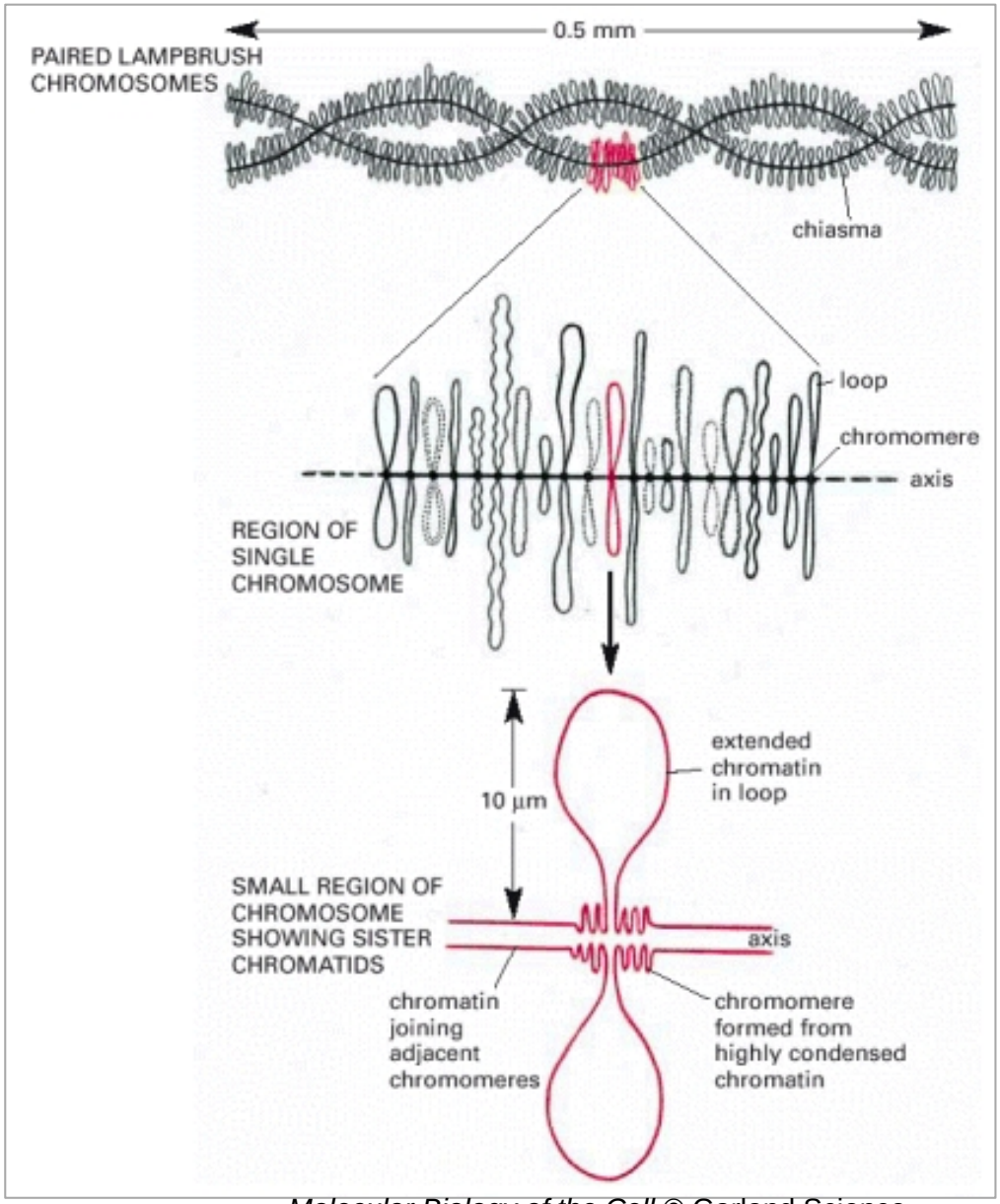
Always at the same position

Loops

Identical and symmetrical

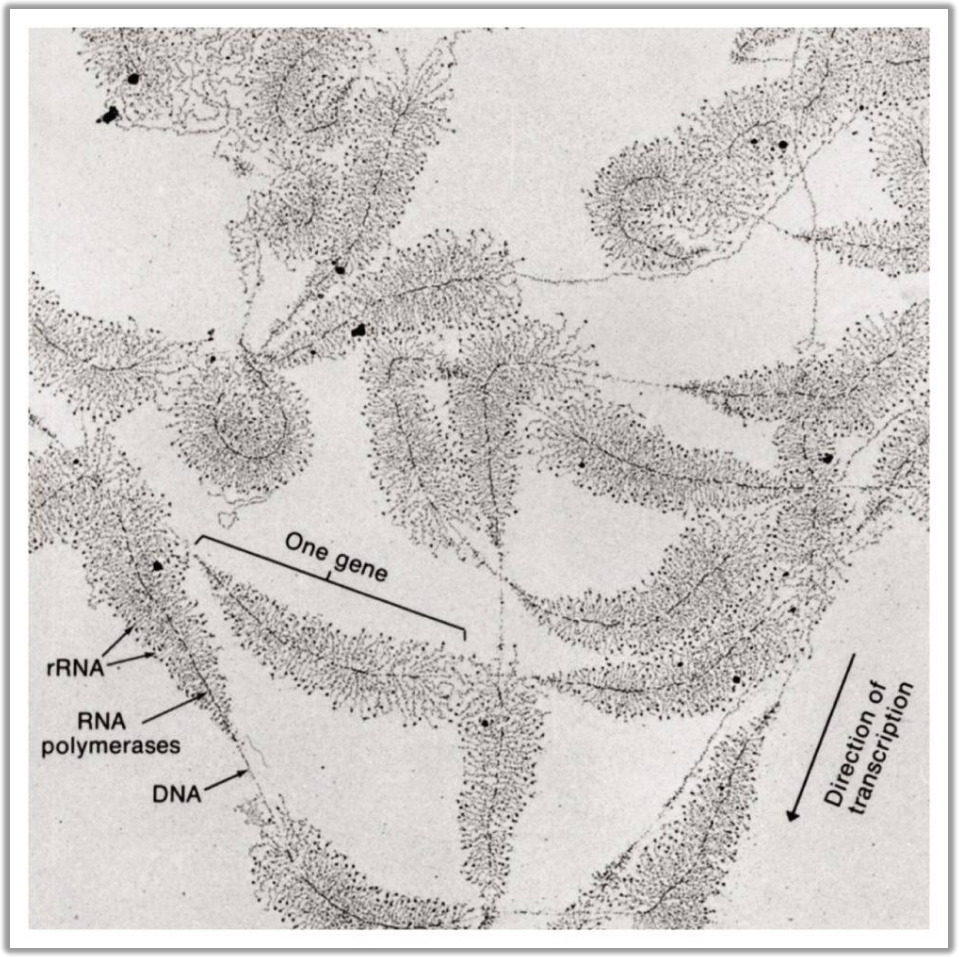
Characteristic size, position and shape





Molecular Biology of the Cell © Garland Science

Lampbrush chromosomes



Trasnscription

a very high transcription rate

Functional meaning

Loops are the places where transcription takes place

Loop size changes according to the physiological state, age, etc. They appear and disappear as they are needed

Polytene chromosomes

They stay during the interphase as chromosomes and they have transcription activity

E.G. Balbiani en 1881

Polytenization

- Homolog chromosomes get paired and extended
- The chromosomes divide several times without nucleus division (polyploidism)
- The level and state of polytenization is variable among tissues
- Filaments stay together

In *Drosophila*, interphase chromosomes replicate 10 times without going through mitosis

- **Each chromosome has 2^{10} double helices**

Banding patterns are reproducible and provide detailed physical guide to gene mapping

- **Total ~5000 bands, size of each band is 3-150 kb**

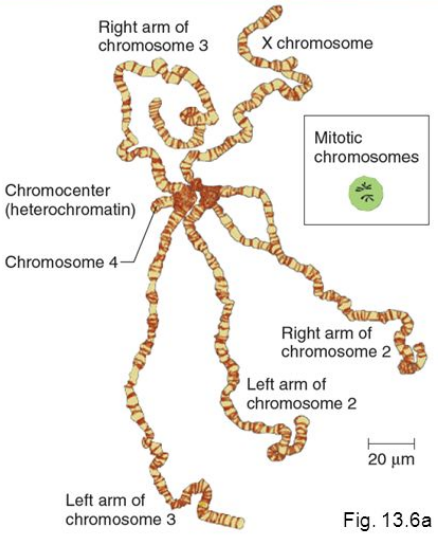
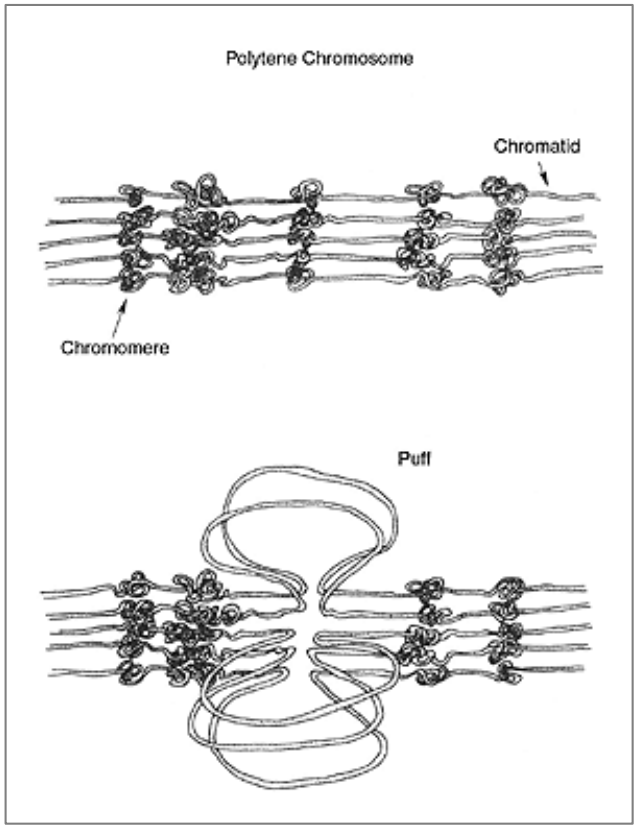
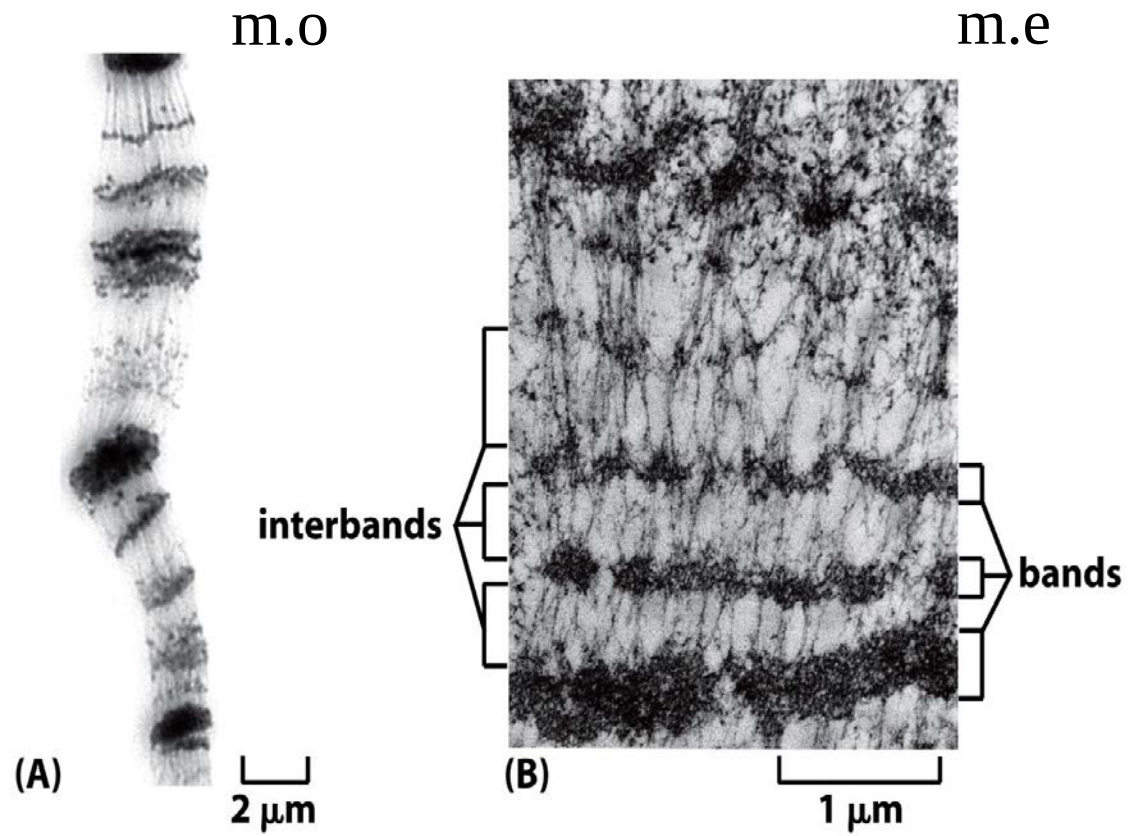


Fig. 13.6a

Polytene chromosomes

Molecular organization





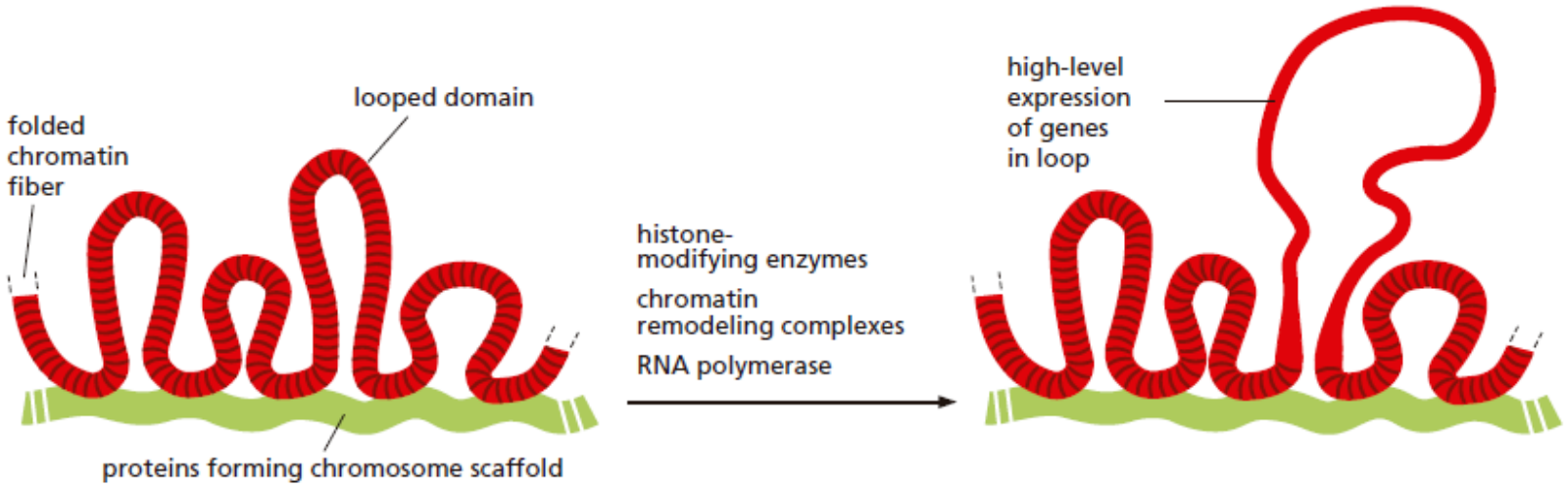
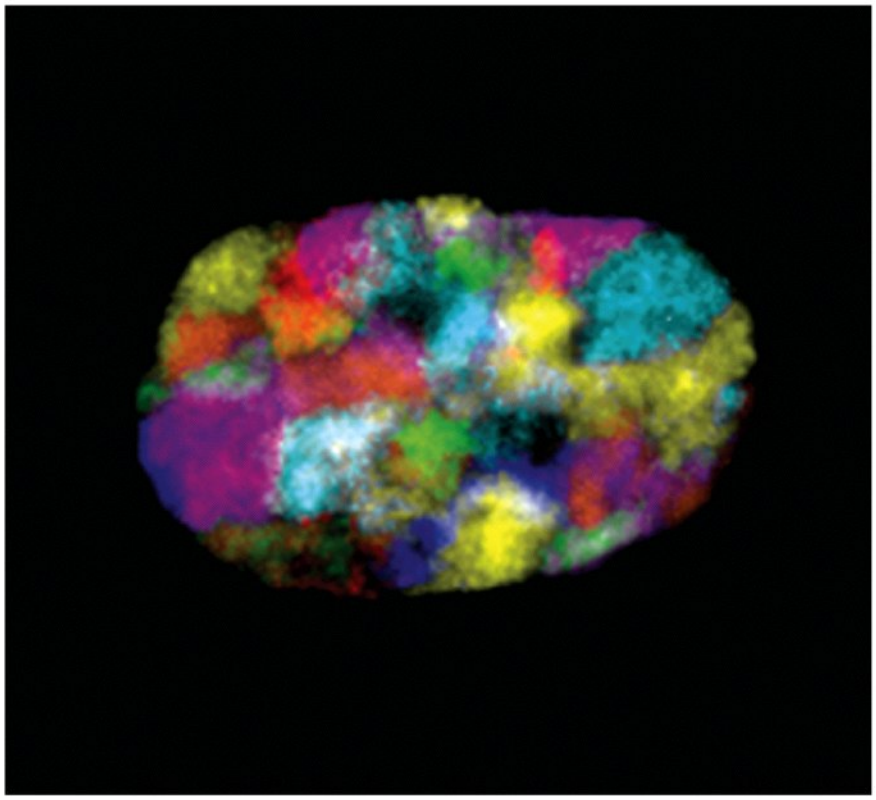


Figure 4–49 A model for the organization of an interphase chromosome. A section of an interphase chromosome is shown folded into a series of looped domains, each containing perhaps 50,000–200,000 or more nucleotide pairs of double-helical DNA condensed into a chromatin fiber. The chromatin in each individual loop is further condensed through poorly understood folding processes that are reversed when the cell requires direct access to the DNA packaged in the loop. Neither the composition of the postulated chromosomal axis nor how the folded chromatin fiber is anchored to it is clear. However, in mitotic chromosomes, the bases of the chromosomal loops are enriched both in condensins (discussed below) and in DNA topoisomerase II enzymes (discussed in Chapter 5), two proteins that may form much of the axis at metaphase.

Interphasic chromosomes



M-FISH

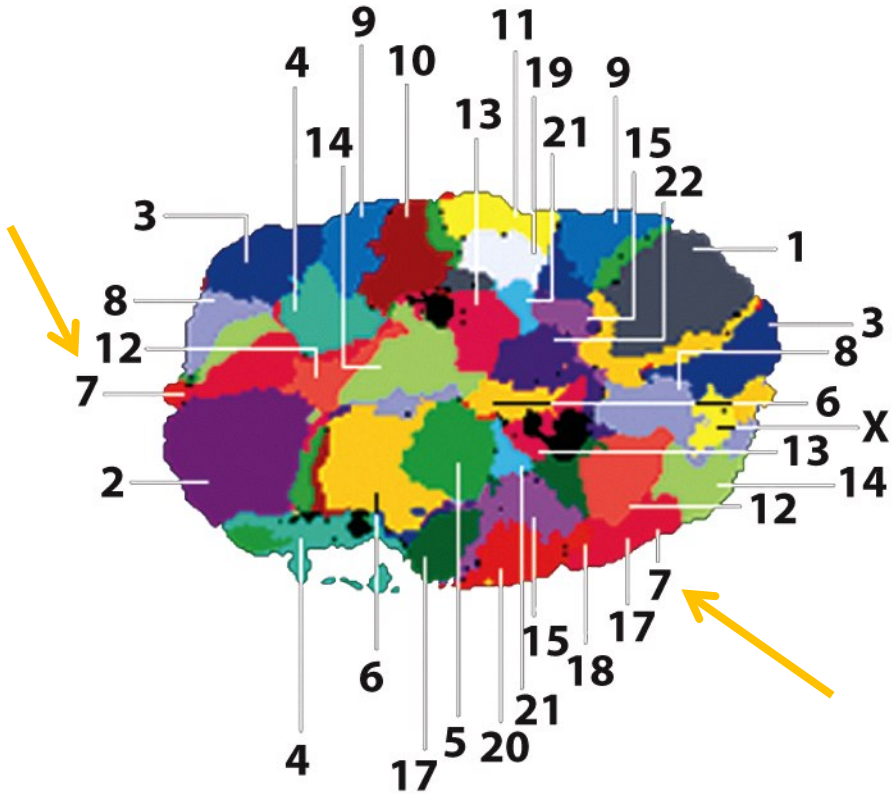
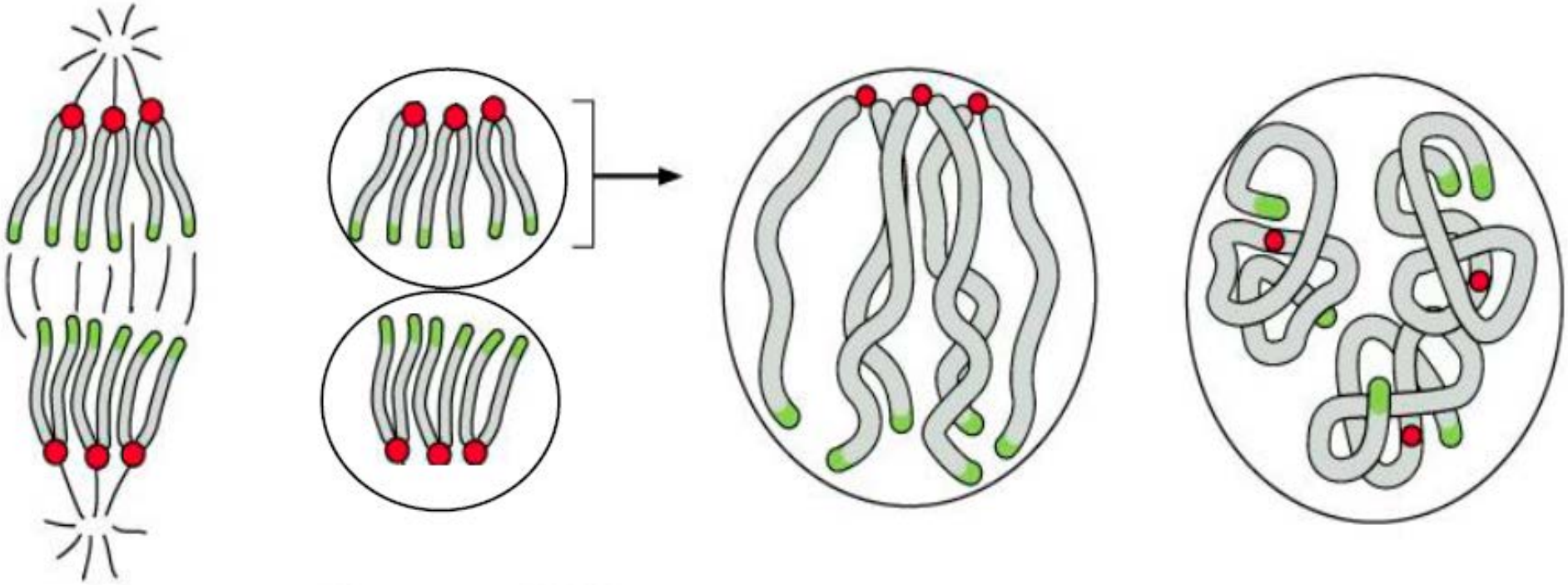


Figure 4-63 *Molecular Biology of the Cell* (© Garland Science 2008)

Interphasic chromosomes



anaphase

Short interphase
in embryo

Long interphase
in larval tissues

Interphasic chromosomes

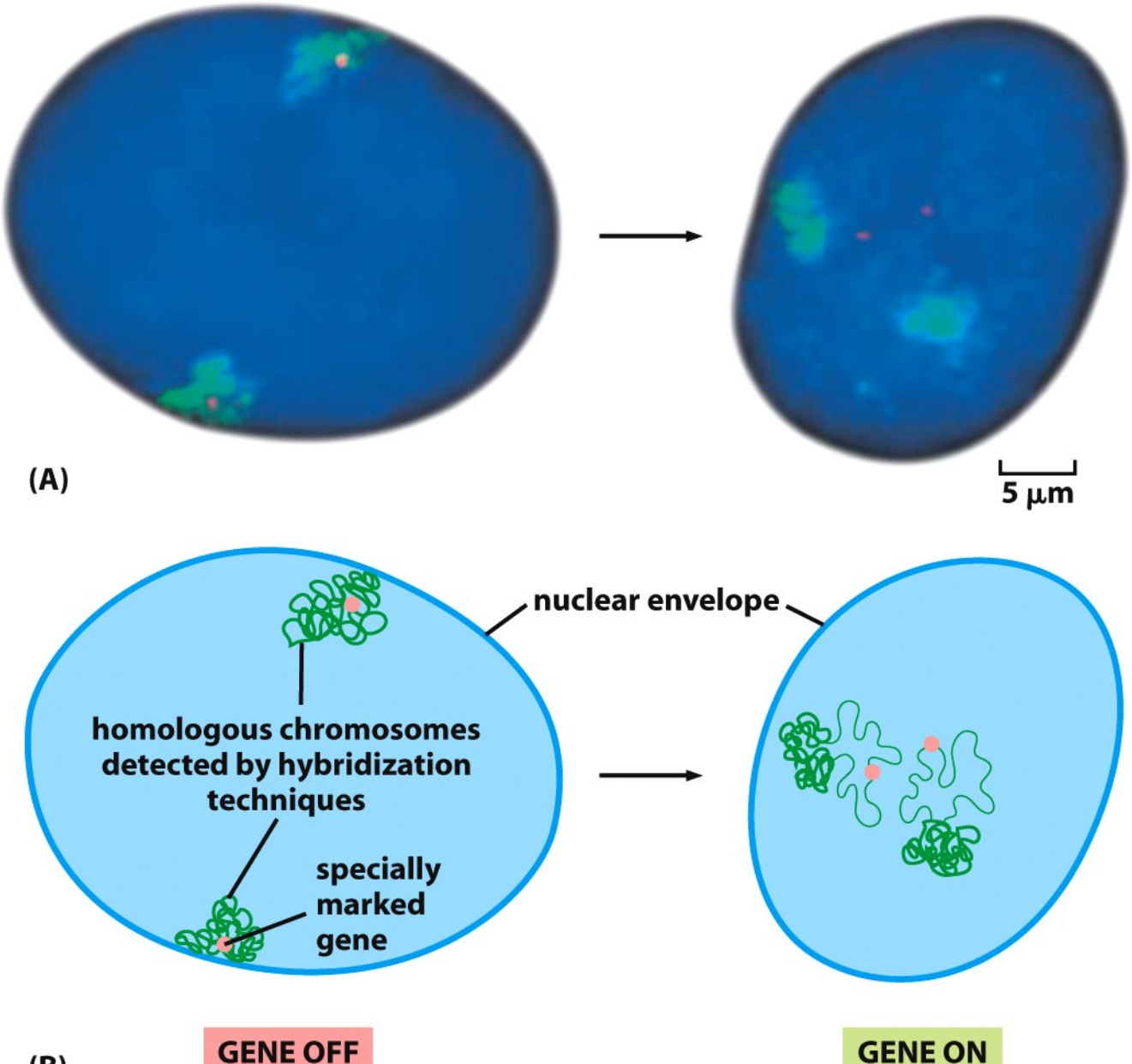


Figure 4-65 *Molecular Biology of the Cell* (© Garland Science 2008)

Interphasic chromosomes

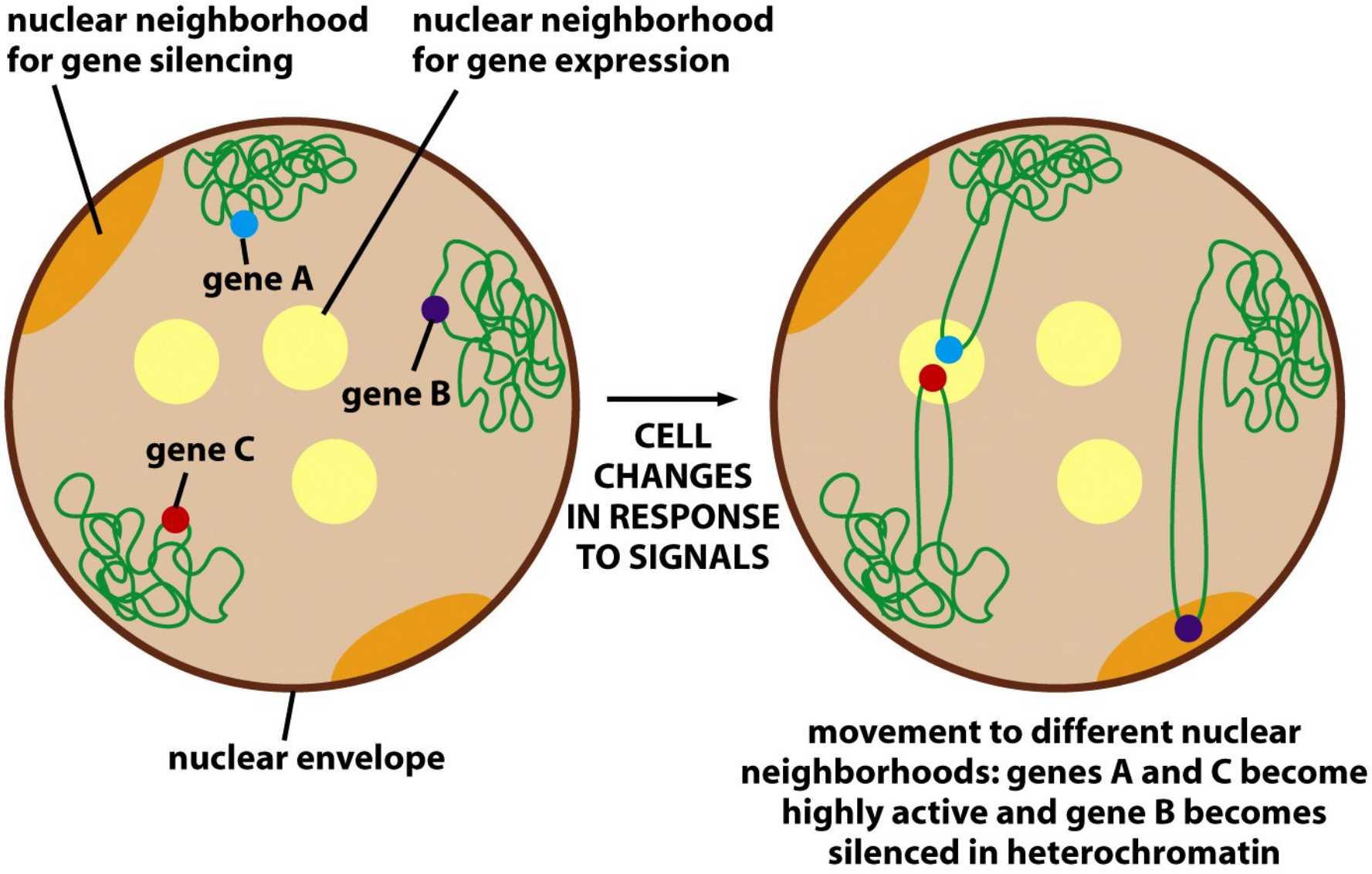


Figure 4-66 *Molecular Biology of the Cell* (© Garland Science 2008)

DNA sequences in a eukaryote chromosome

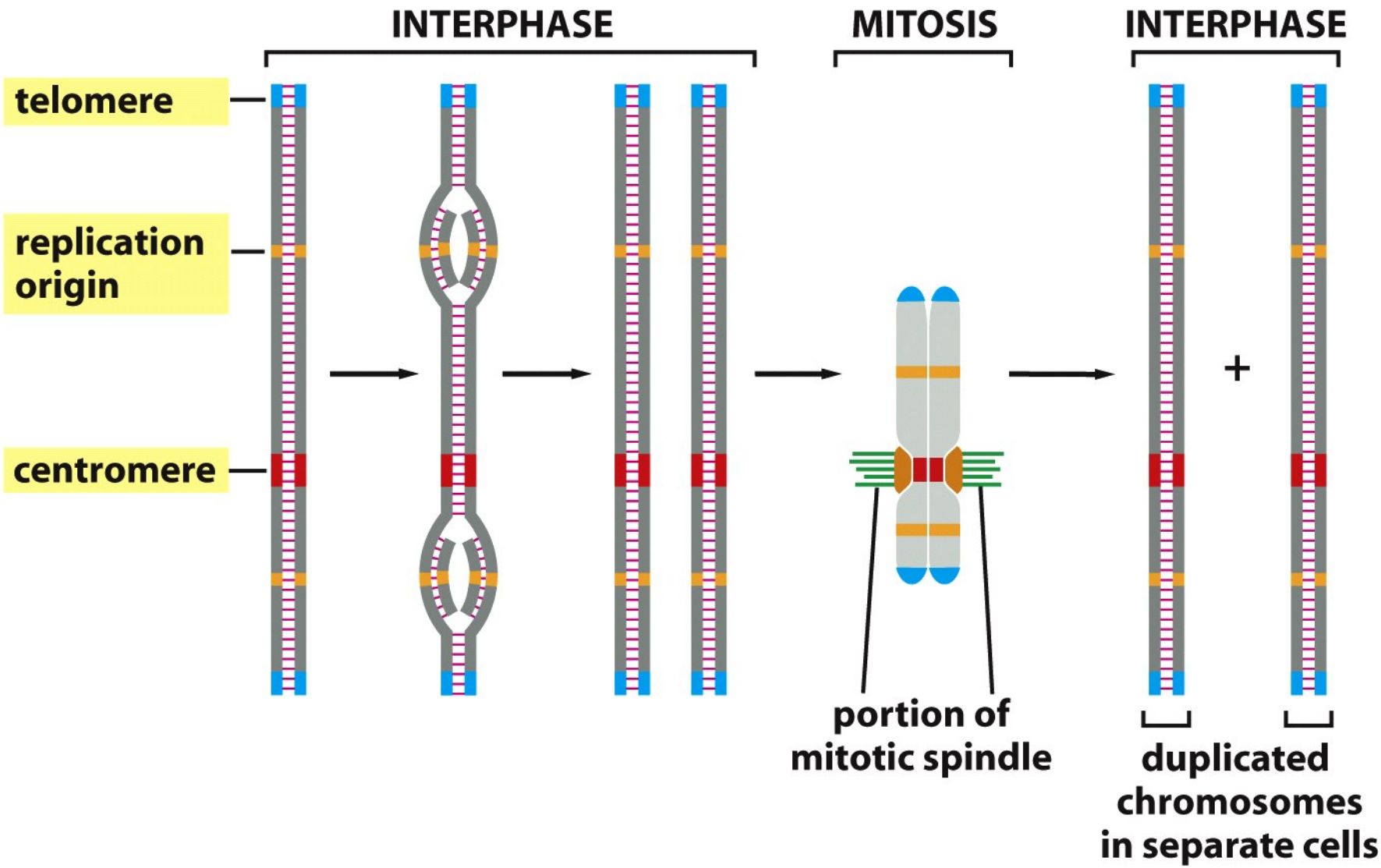
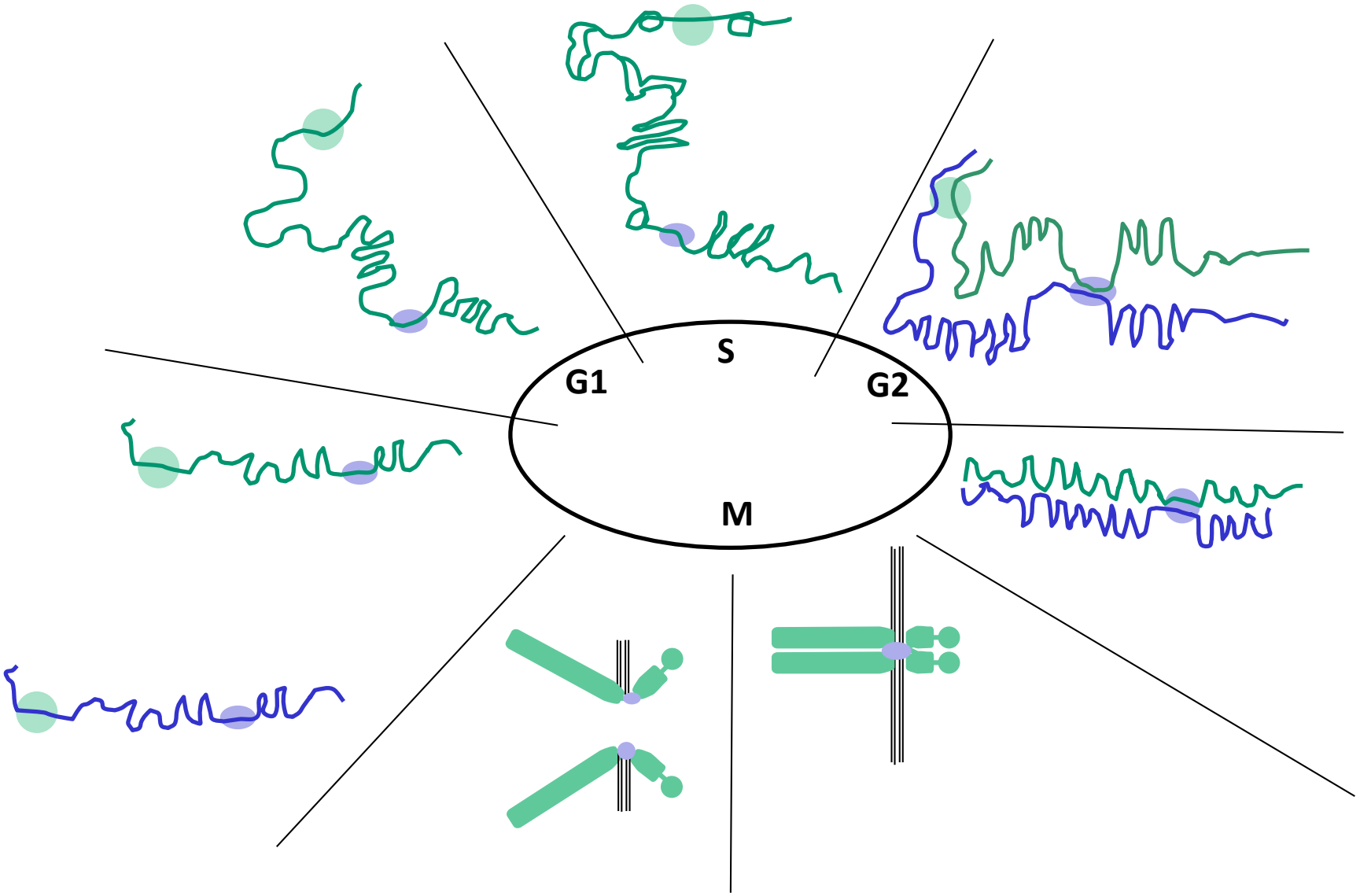


Figure 4-21 *Molecular Biology of the Cell* (© Garland Science 2008)

The chromosome cycle



Medicine is an experimental science

Scientific method:

1. Observation
2. Question
3. Hypothesis
4. Experimentation
5. Theory

THE OPTICAL MICROSCOPE

Components:

- Light source
- Optical elements (lenses)
- Mechanical elements

Optical elements

Condenser lens

The purpose of the condenser is to focus the light from the illumination source onto the sample. It improves the image quality.

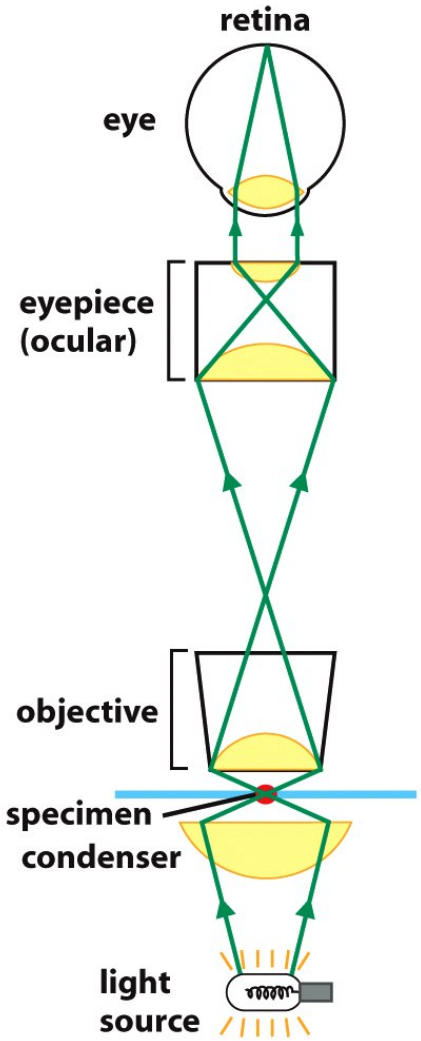
Objective lens

Magnifies and projects the image to the oculars.

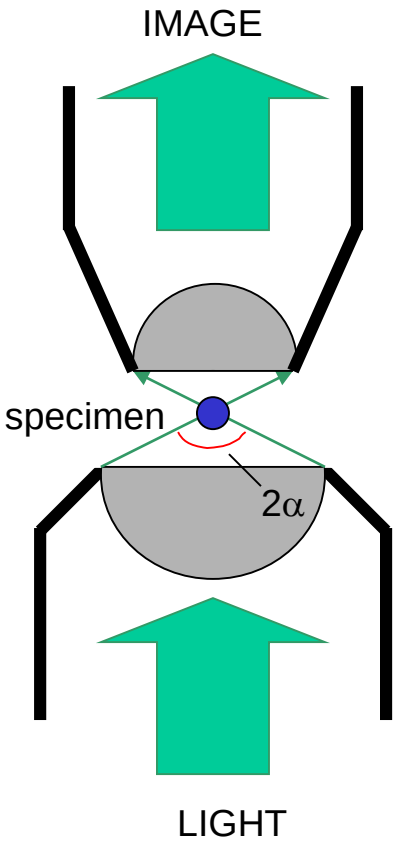
Ocular lens

Magnifies the image and brings the image into focus for the eye.

Compound microscope



Resolution limit of the microscope. Resolving power



Resolving power: 1/d

Resolution limit: d

$$d = \kappa \cdot \lambda / AN$$

$$d = \kappa \cdot \lambda / n \cdot \text{sen}\ \alpha$$

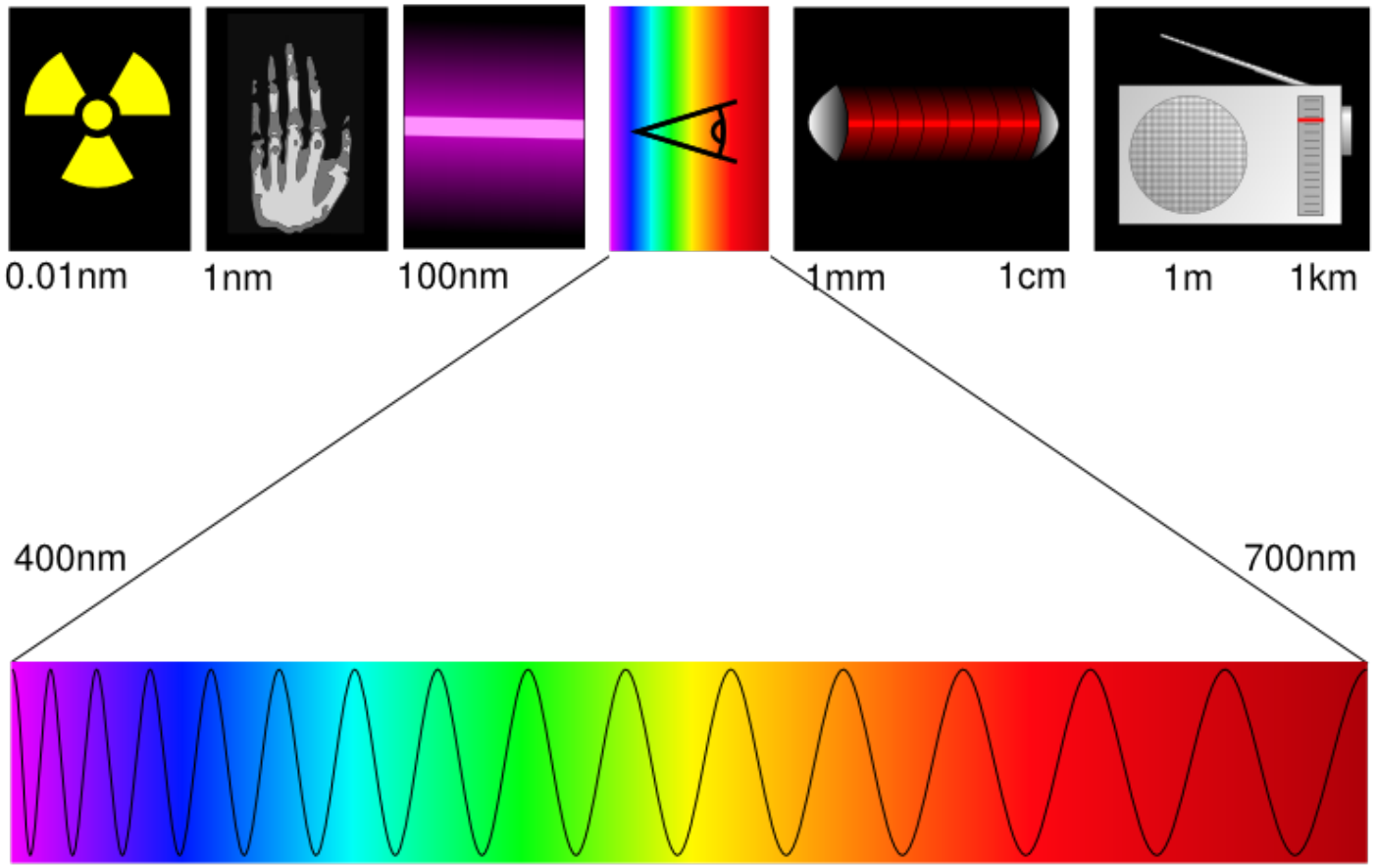
$\kappa = 0.61$

λ = wavelength of the light used

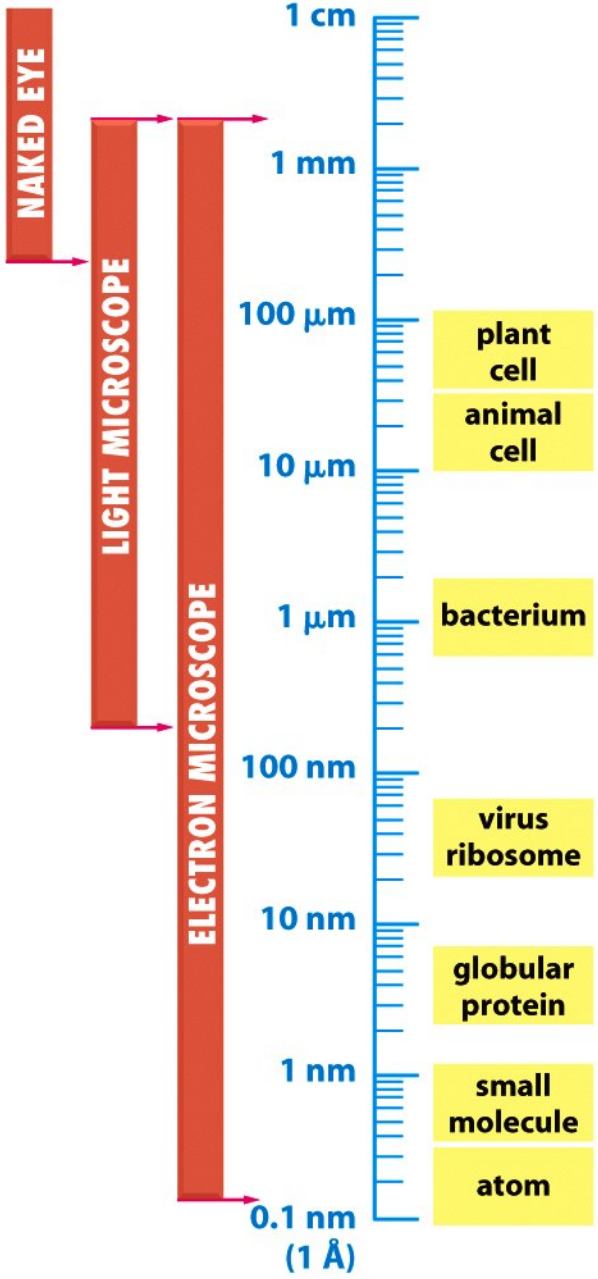
n = refractive index of the medium separating the specimen from the objective

α = half of the angular width of the cone of rays collected by the objective lens from the specimen

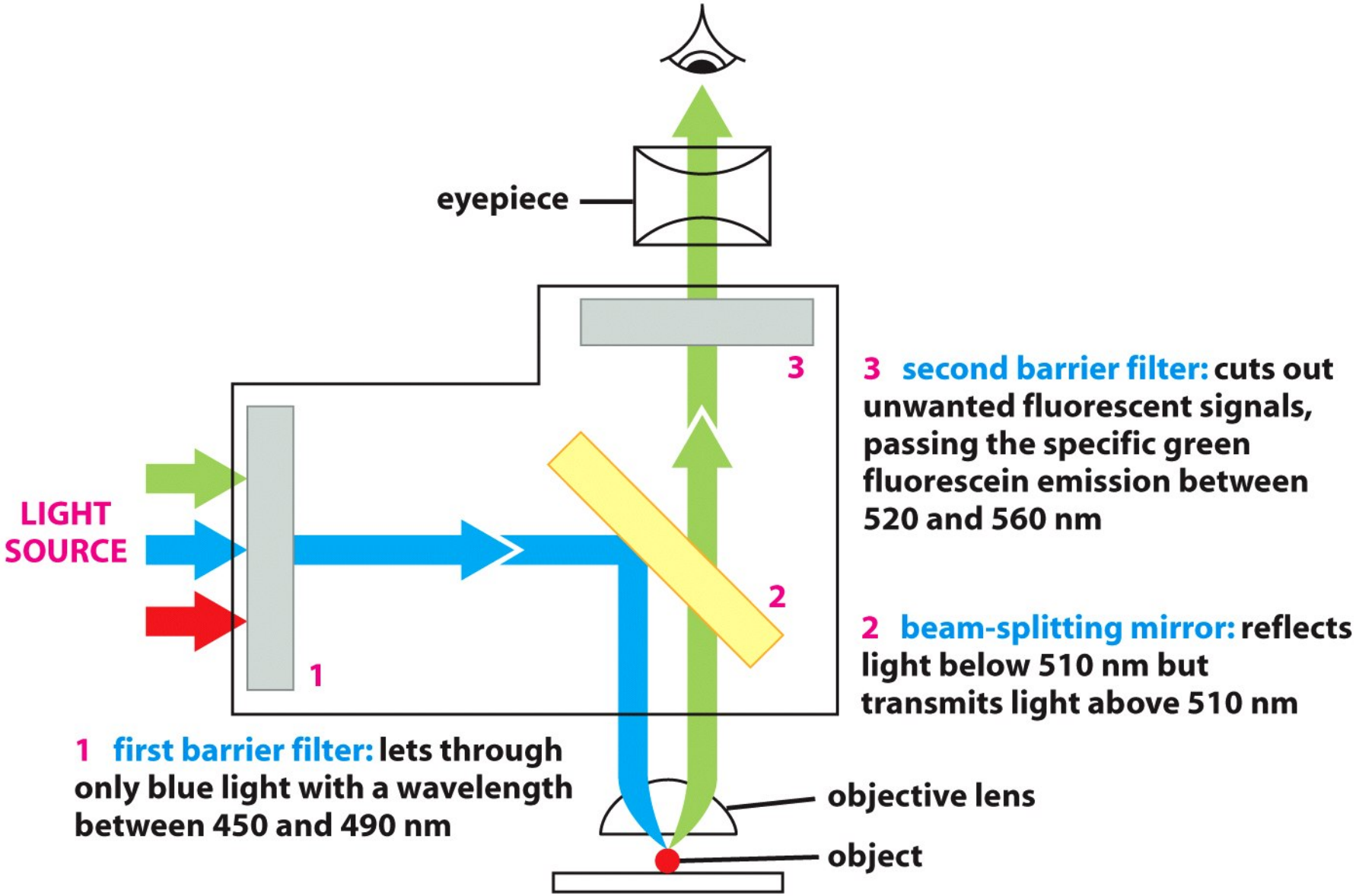

$d = \frac{\lambda}{2n \sin\alpha}$
ERNST ABBE
1840–1905
FRIEDRICH SCHILLER
UNIVERSITÄT JENA



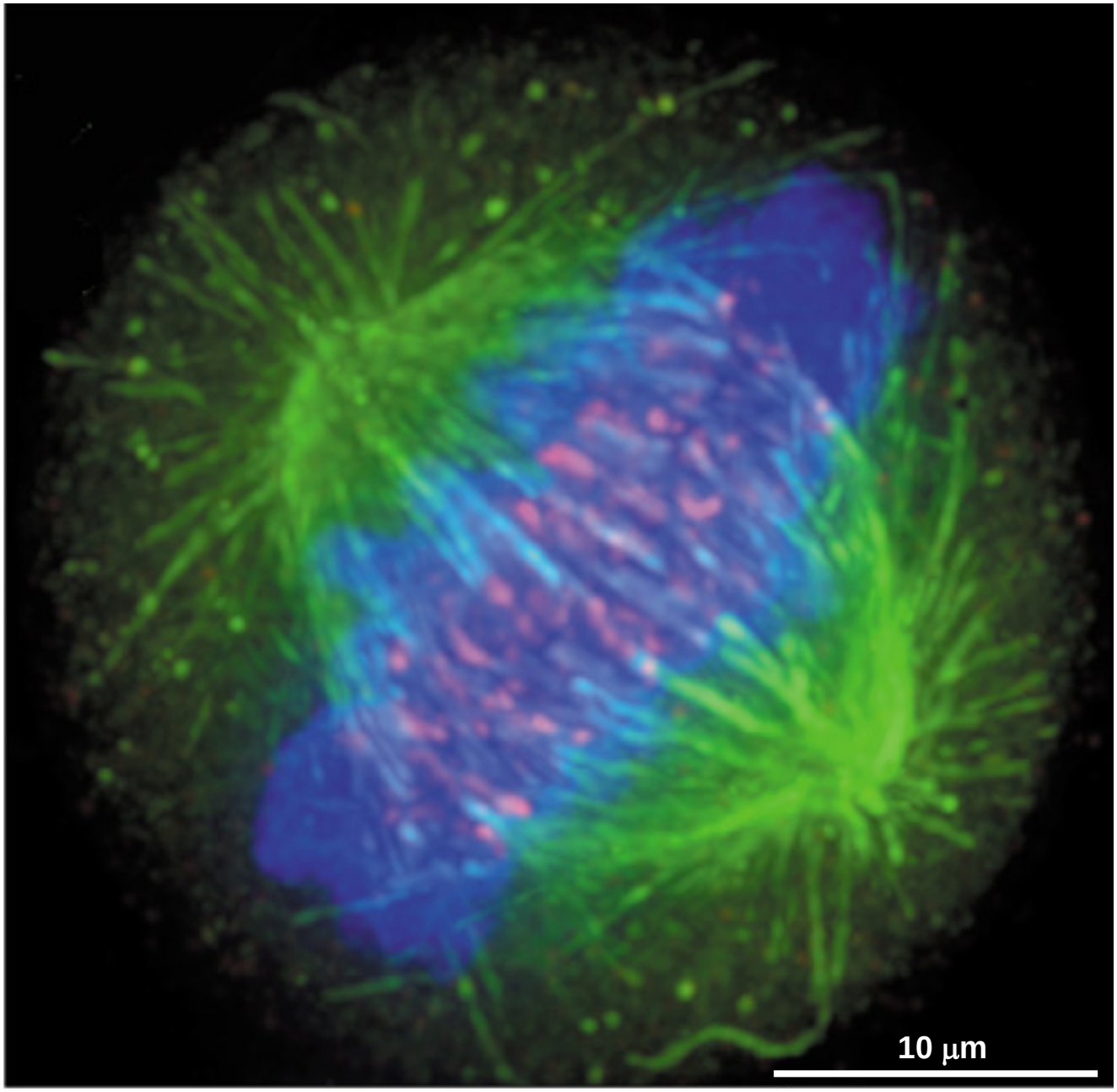
White light: 550 nm (d = 0.25 µm)
Violet light: 400 nm (d = 0.17 µm)
Ultraviolet light: 200 nm – 300 nm (d = 0.1 µm)
Infrared light: 800 nm (d = 0.4 µm)
Electrons: 0.005 nm (theoretical d = 0.002 nm
real d = 0.1 nm – 0.2 nm)
Human eye: approximately 0.2 mm


NAKED EYE
LIGHT MICROSCOPE
ELECTRON MICROSCOPE
1 cm
1 mm
100 μm
10 μm
1 μm
100 nm
10 nm
1 nm
0.1 nm
(1 Å)
plant cell
animal cell
bacterium
virus
ribosome
globular protein
small molecule
atom

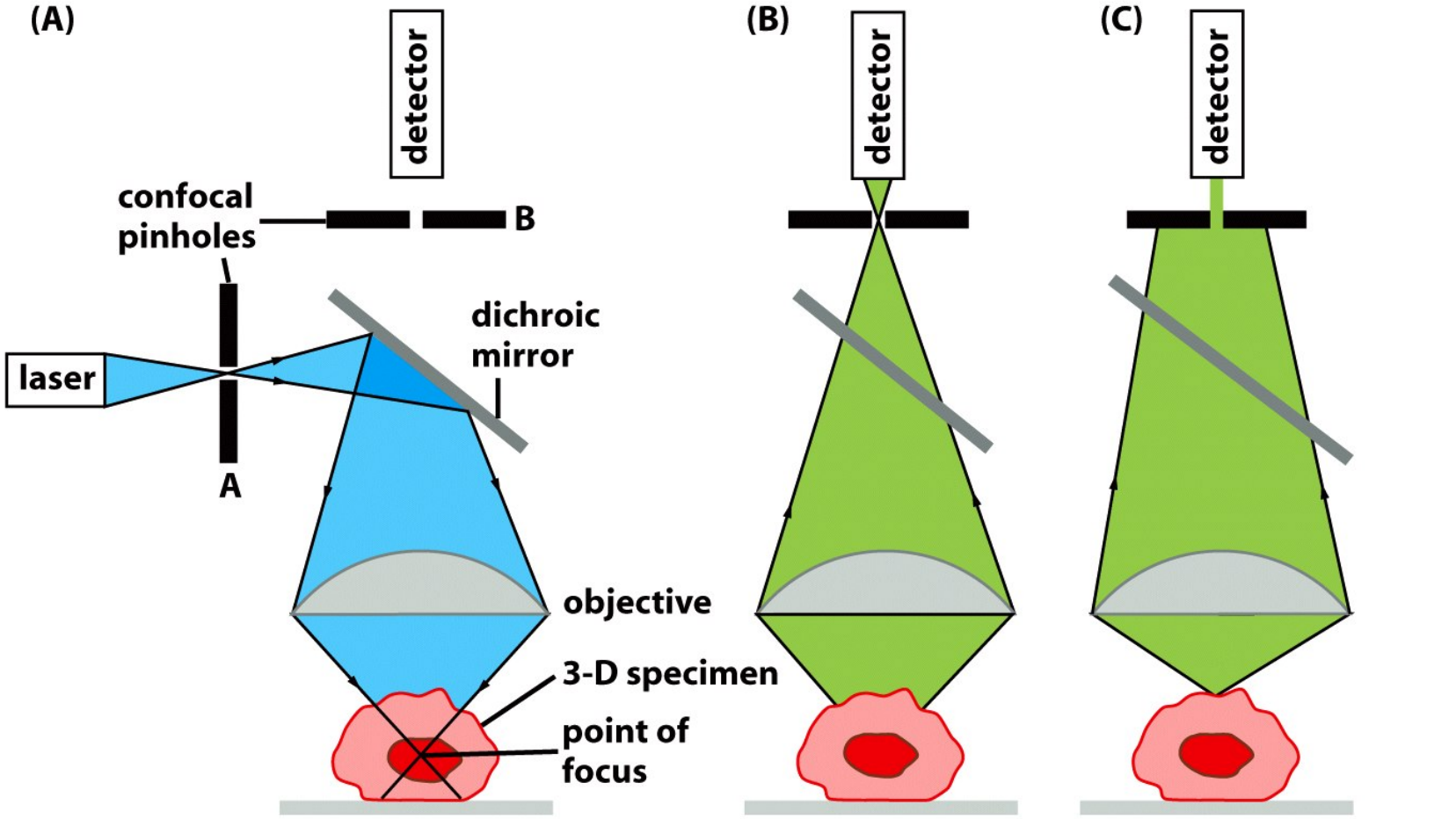
Types of optical microscopes: Fluorescence microscope



Fluorescence microscopy



Types of optical microscopes: Confocal microscope



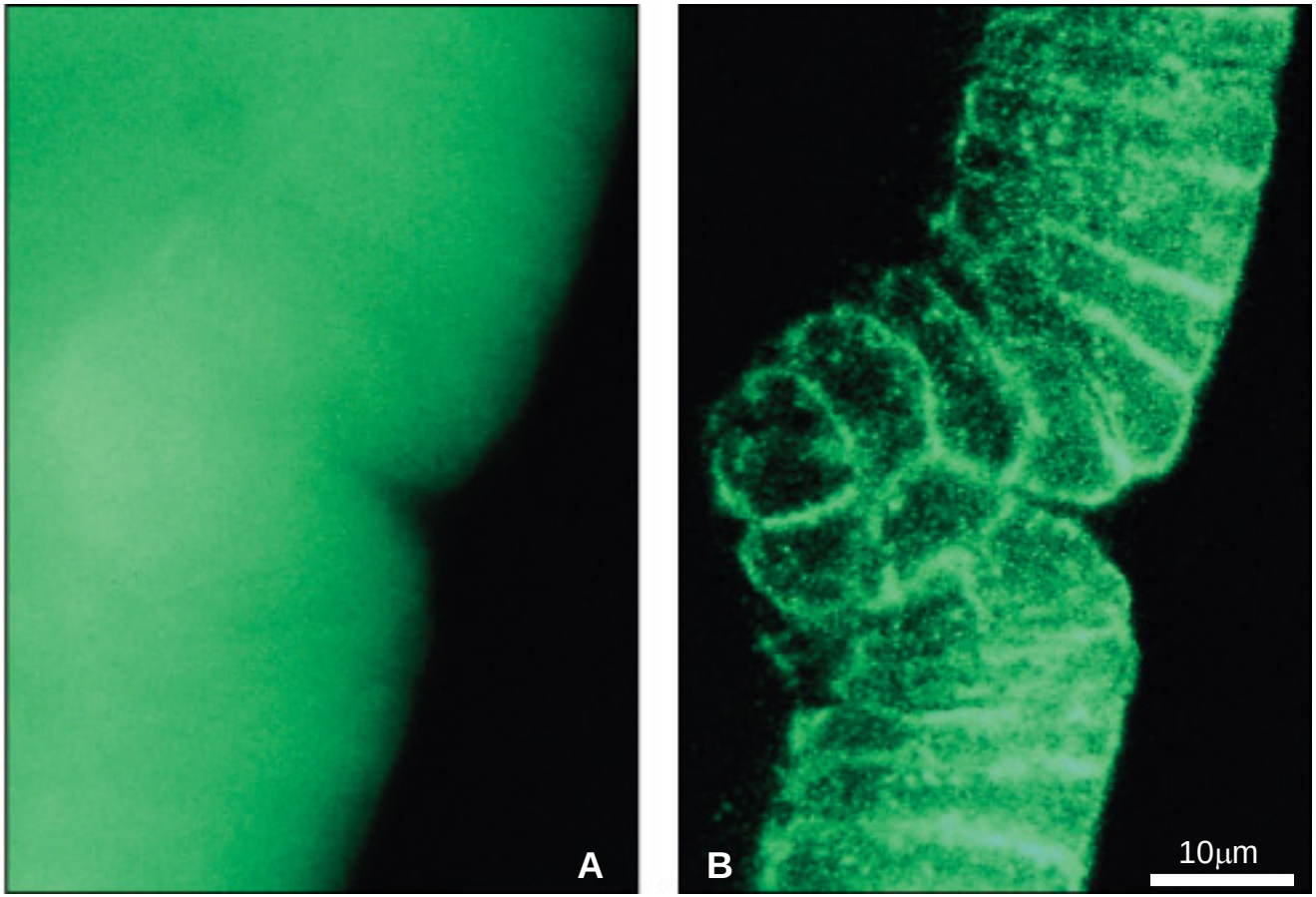
fluorescent specimen is illuminated with a focused point of light from a pinhole

emitted fluorescent light from in-focus point is focused at pinhole and reaches detector

emitted light from out-of-focus point is out of focus at pinhole and is largely excluded from detector

Fluorescence microscope

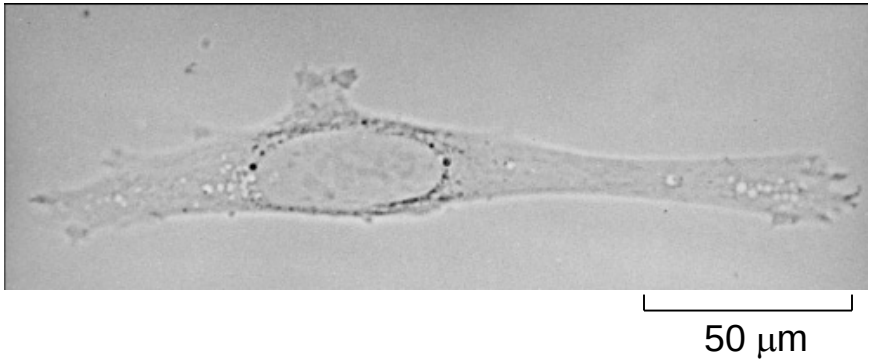
Confocal microscope



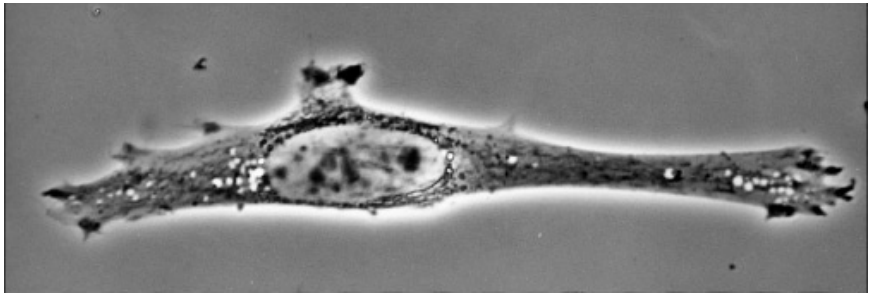
Types of optical microscopes

Observation of live cells

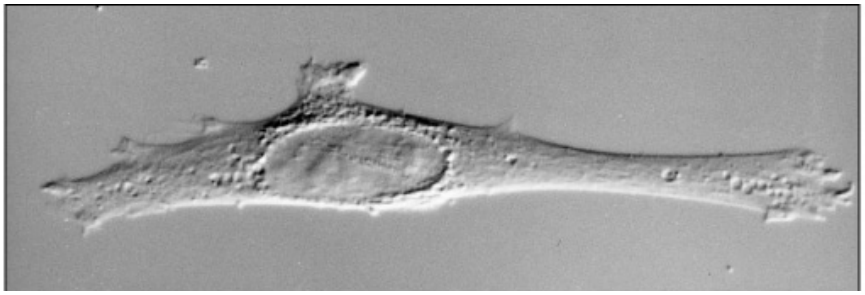
A. Bright-field



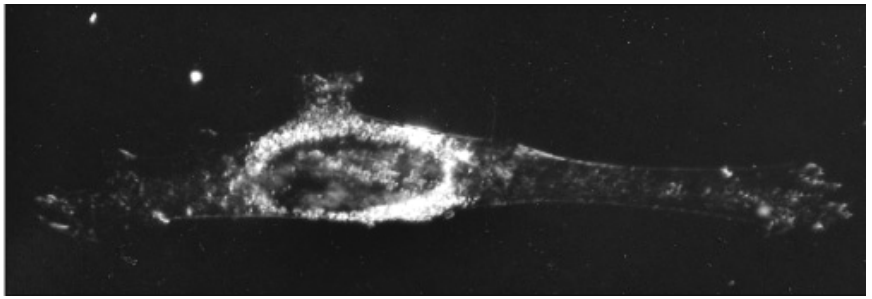
B. Phase-contrast



C. Differential-interference contrast



D. Dark field



Techniques of adaptation of biological material for observation with the optical microscope

Fixation

- Impedes the autolysis and decomposition due to microorganisms
- Conserves the structural and molecular composition of the sample.
- Fixative agents: chemical (formaldehyde, ethanol, etc) and physical (freezing, heat, etc)

Embedding

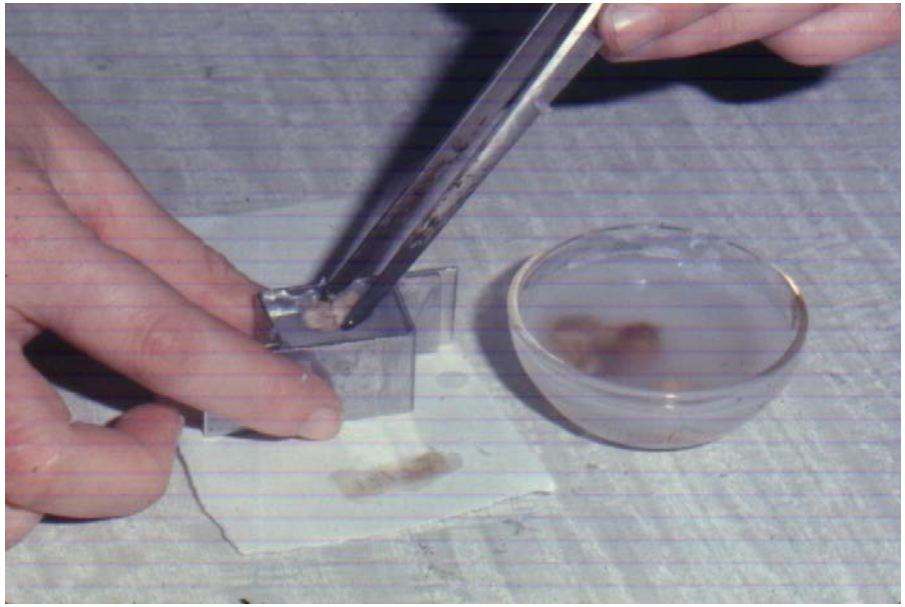
- Gives consistency to the sample so that thin sections can be obtained.
- Water, the major component of cells and tissues, is substituted by paraffin (solid at room temperature and liquid at 60ºC)
- This substitution is performed progressively:
  Water → ethanol → xylene → paraffin 60º → cool to room temperature
- Resins are also used as embedding media.

Sectioning

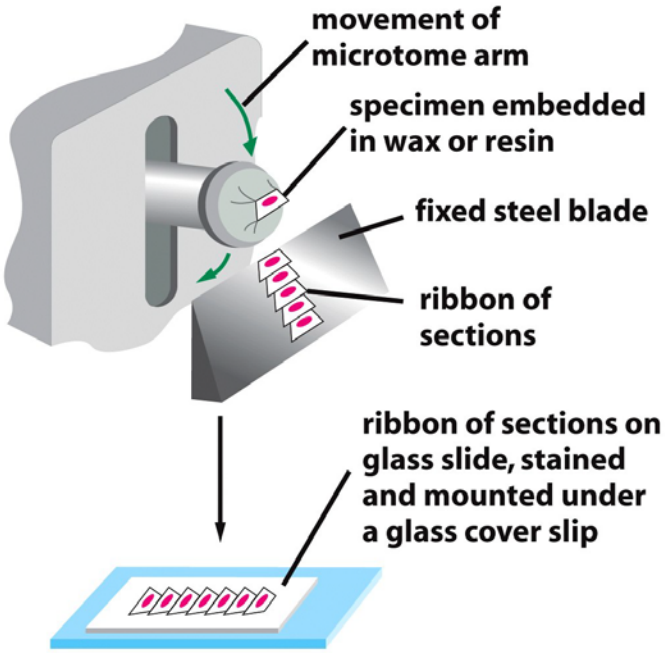
- Microtomes are used to obtain thin sections of the sample.
- Sections of approximately 5 to 10 μm are obtained.

Staining

•Embedding

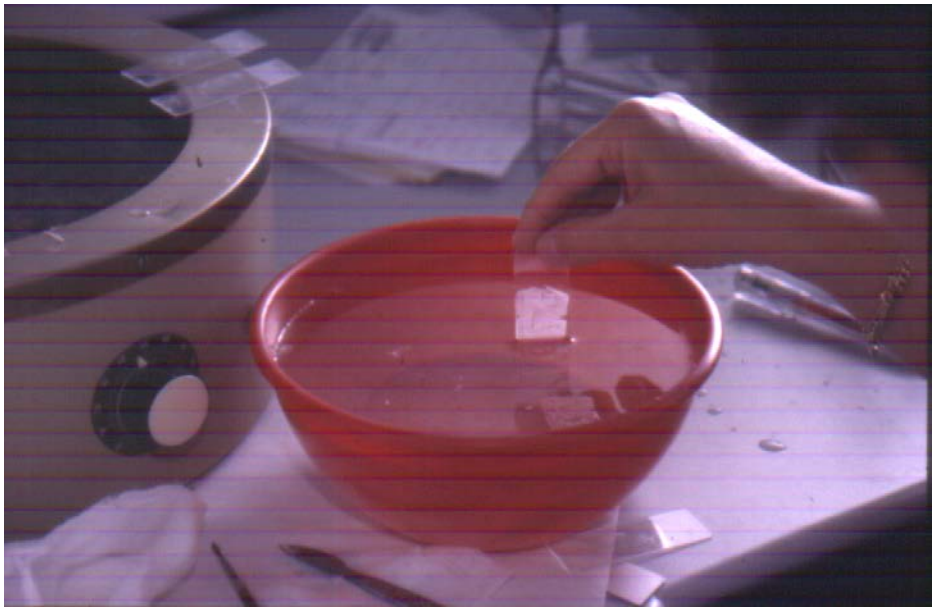


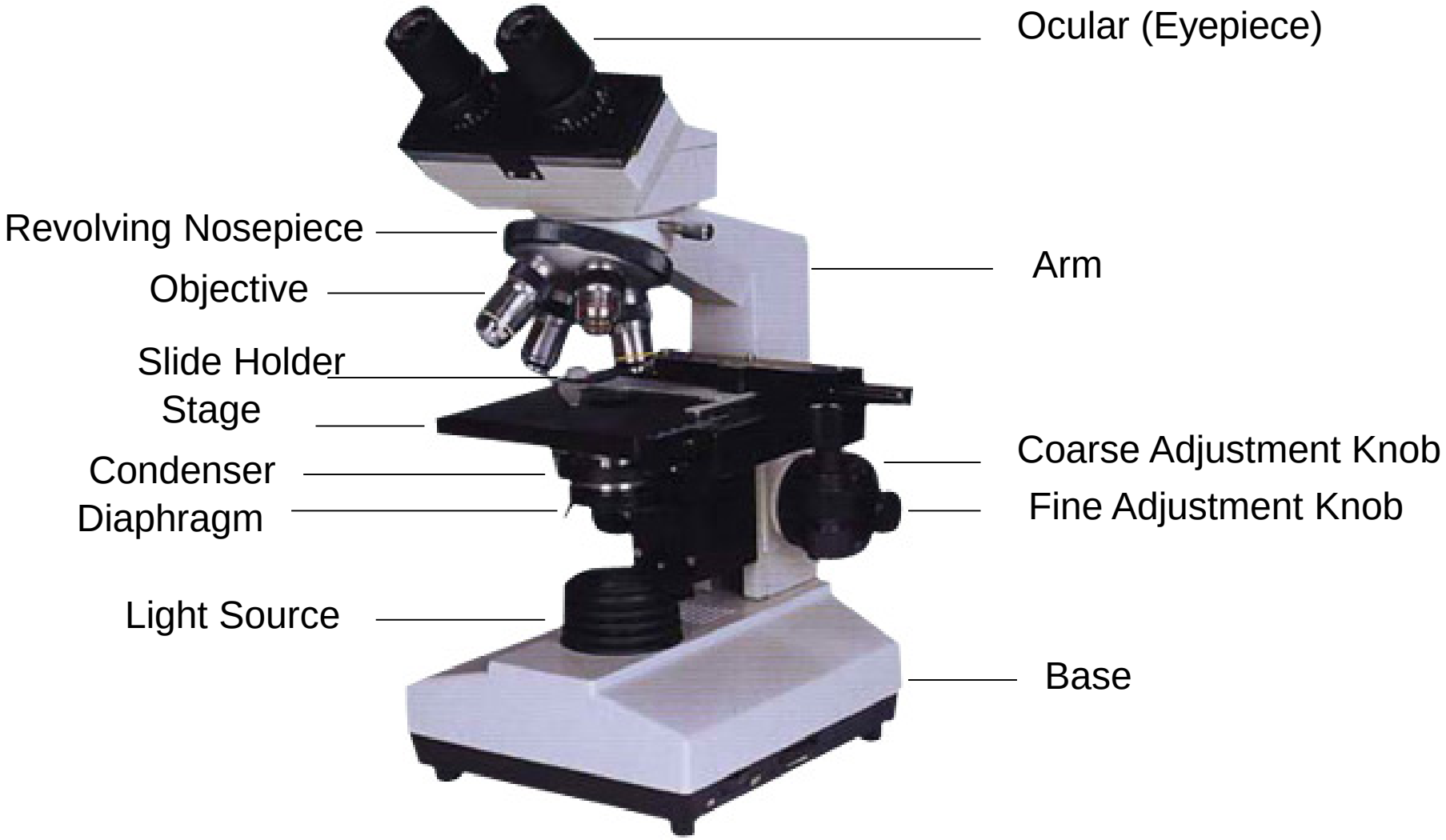
•Sectioning



Specimen preparation

•Section extension




Ocular (Eyepiece)
Revolving Nosepiece
Arm
Objective
Slide Holder
Stage
Coarse Adjustment Knob
Condenser
Diaphragm
Fine Adjustment Knob
Light Source
Base

QUESTIONS

Practical session 1

1. Does the wavelength of the used light change the resolving power of the microscope?
2. What is the diaphragm of the microscope used for?
3. What advantages does the confocal microscope have versus the conventional fluorescence microscope?
4. Why do we have to harden biological samples that have to be observed with the optical microscope?
5. How is the final magnification of an optical microscope calculated?

Practical session 2
Staining techniques

Cell Biology Unit
Department of Pathology
School of Medicine and Dentistry

The aim of this practical session is to become familiarized with the concept of staining and its applications. We have chosen two types of tissue and applied on them several histological stains.

The students have to examine the slides using the microscope. They have to identify elements of interest, perceiving how the different stains provide complementary information about the studied system.

Observations obtained from these slides must be both written and drawn down in the lab notebook. The aim is not to perform an histological study, but to check how the different stains change the aspect of the tissue and the observable information.

Slide II 1 Liver section – hematoxylin-eosin
Slide II 2 Liver section – reticulin
Slide II 3 Liver section – Masson's trichrome
Slide II 4 Intestine section – hematoxylin-eosin
Slide II 5 Intestine section – Alcian blue
Slide II 6 Intestine section – Masson's trichrome

Techniques of adaptation of biological material for observation with the optical microscope

Fixation

- Impedes the autolysis and decomposition due to microorganisms
- Conserves the structural and molecular composition of the sample.
- Fixative agents: chemical (formaldehyde, ethanol, etc) and physical (freezing, heat, etc)

Embedding

- Gives consistency to the sample so that thin sections can be obtained.
- Water, the major component of cells and tissues, is substituted by paraffin (solid at room temperature and liquid at 60ºC)
- This substitution is performed progressively:
  Water → ethanol → xylene → paraffin 60º → cool to room temperature
- Resins are also used as embedding media.

Sectioning

- Microtomes are used to obtain thin sections of the sample.
- Sections of approximately 5 to 10 μm are obtained.

Staining

- The sections are progressively hydrated (deparaffination):
  paraffin → xylene → ethanol (decreasing concentrations) → water
- Perform the different staining methods

Introduction

Unstained cells are 70% water and are mostly transparent.

Staining is an auxiliary technique used in microscopy to improve the contrast of the microscopic image.

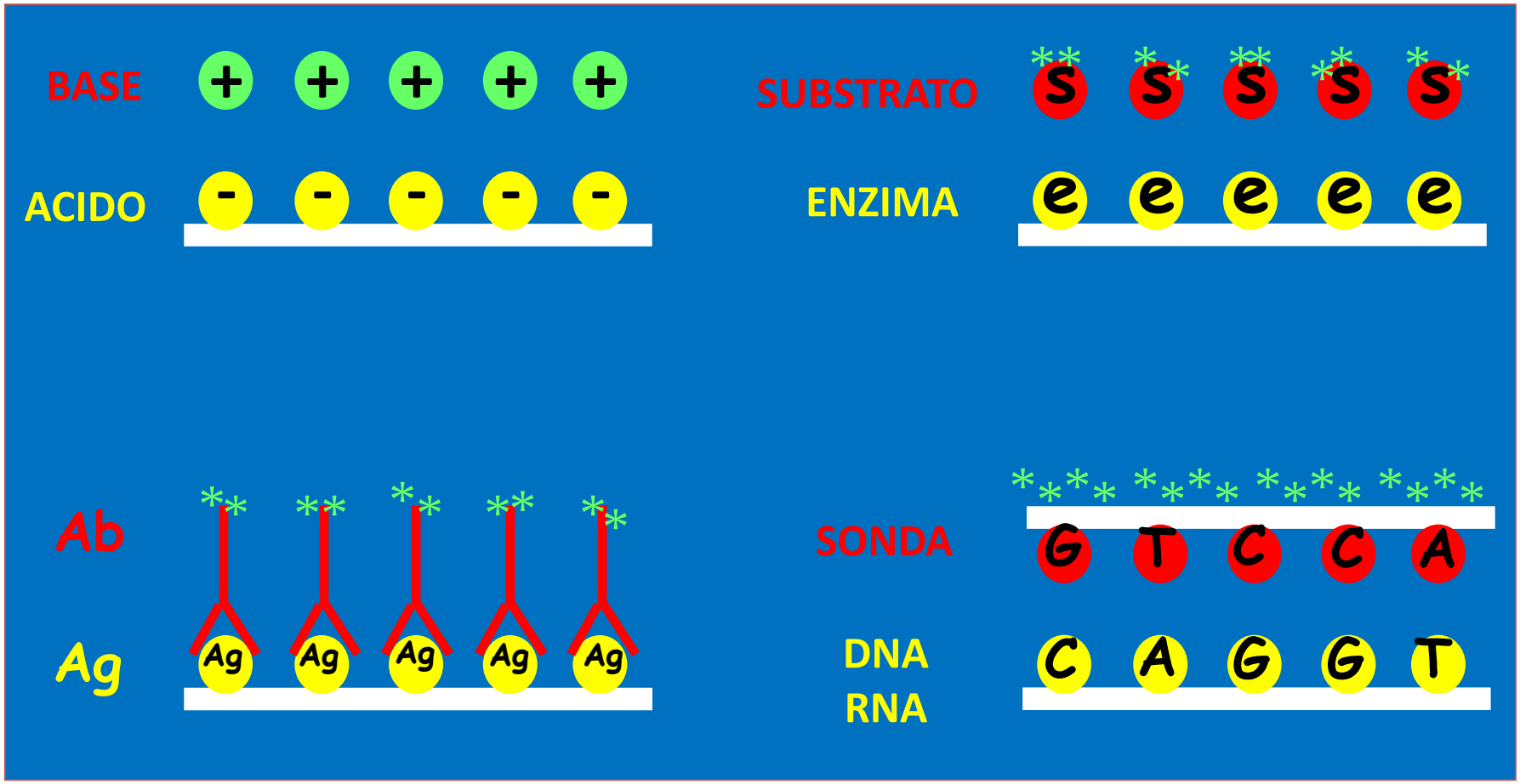
Staining allows us to obtain information about the composition and location of different cellular components.

The color of a staining is derived from a chromophore which interacts with the components to observe.

The ‘in vivo’ stains or vital stains allow the study live cells (neutral red, Janus green, trypan blue)

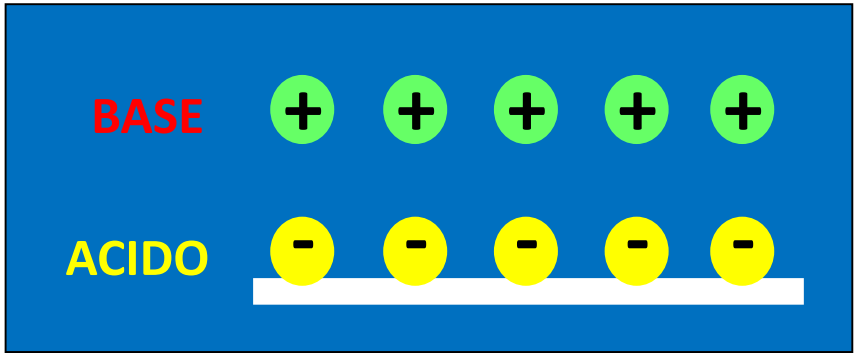
Staining

Chemical affinity reactions



Staining

Chemical affinity reactions



Stains

Acid: Eosin, acid fuchsin

Basic: Hematoxylin, toluidine blue

Example: Hematoxylin-eosin

Hematoxylin-eosin stain, usually written H&E, is probably the stain most widely used in clinical diagnosis.

The stain has a mixture of hematoxylin, which stains the nuclei in blue, and eosin, which stains cytoplasm in pink.

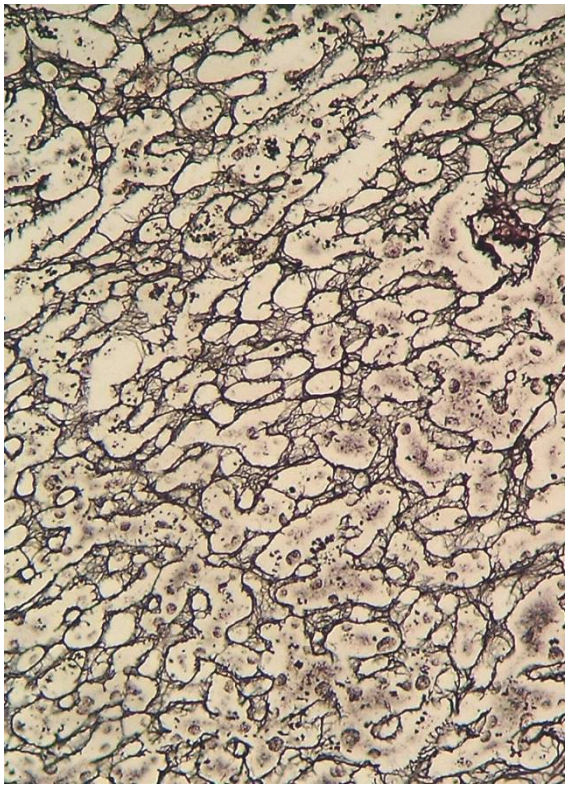
Structures stained with hematoxylin are basophilic, whereas those stained with eosin are acidophilic.

Basophilic structures are slightly acidic, whereas acidophilic structures are slightly basic.

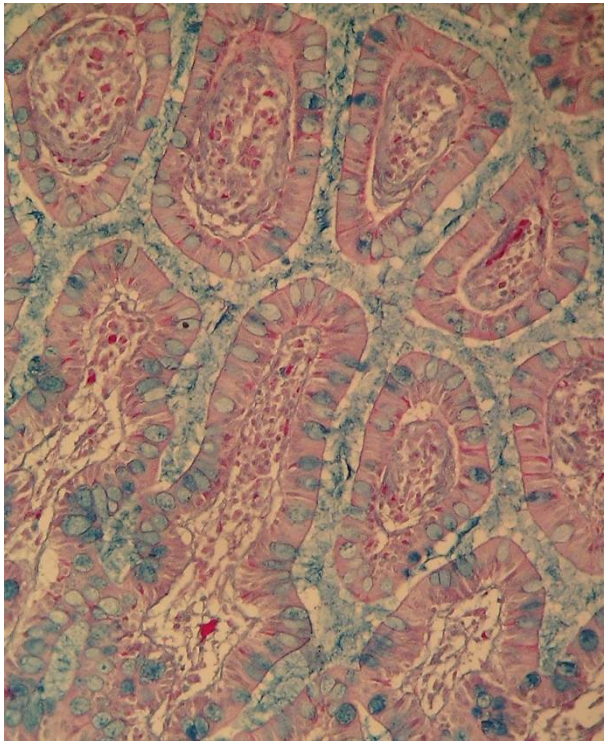
Ej: Hematoxylin (nuclei) - - - Eosin (cytoplasm)

Example: Reticulin

Staining with reticulin allows observation of collagen III fibers in a brown color. The stain contains Ag+ salts which bind to the hydrocarbonated residues providing a characteristic aspect to the fibers.



Example: Alcian blue



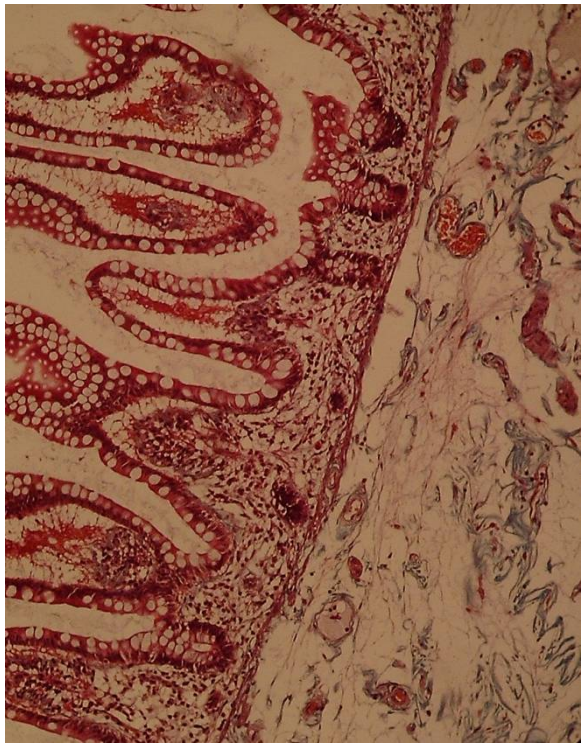
Alcian blue is a stain with copper salts used in microscopy. This stain is mostly used for detecting mucopolysaccharides among other molecules.

Example: Masson's trichrome

Masson's trichrome stain is mostly used to identify cells surrounded by connective tissue.

The stain solution contains acid fuchsin, which colors cytoplasm in red, aniline blue, which colors collagen in blue, and hematoxylin, which colors nuclei in dark blue..

In our case, the stain also contains orange G, that stains blood red cells in orange.



Questions

1. What type of substances can we observe with the hematoxilin-eosin stain? Explain the chemical affinity of both reagents

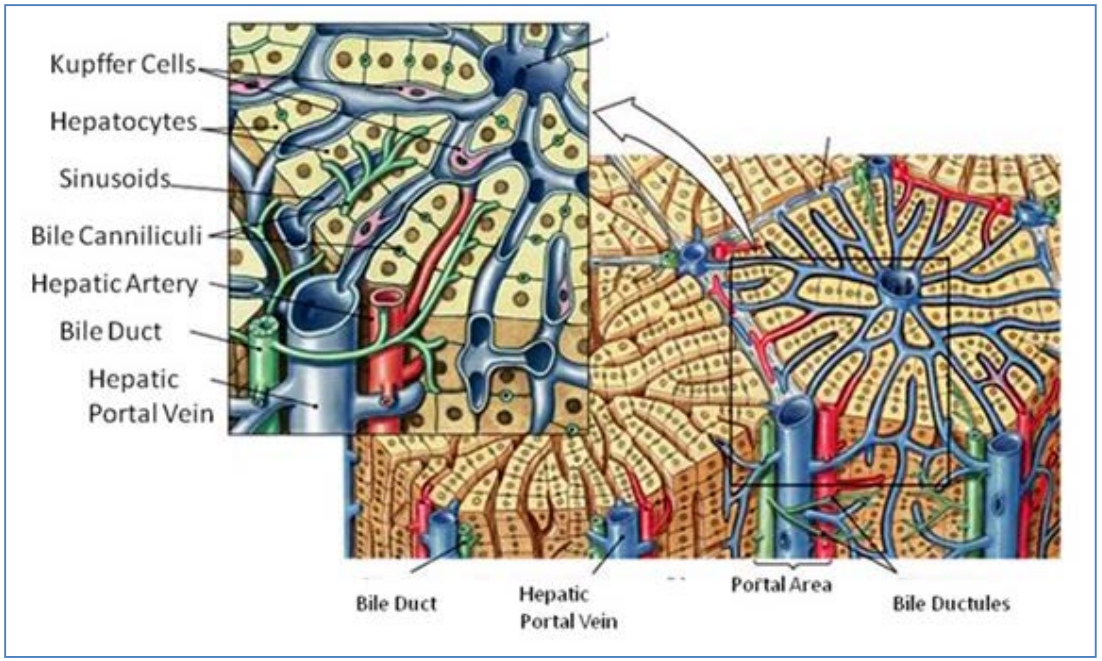
2. Why do we stain biological samples?

3. Which of the used stains allows us to observe mucopolysaccharides?

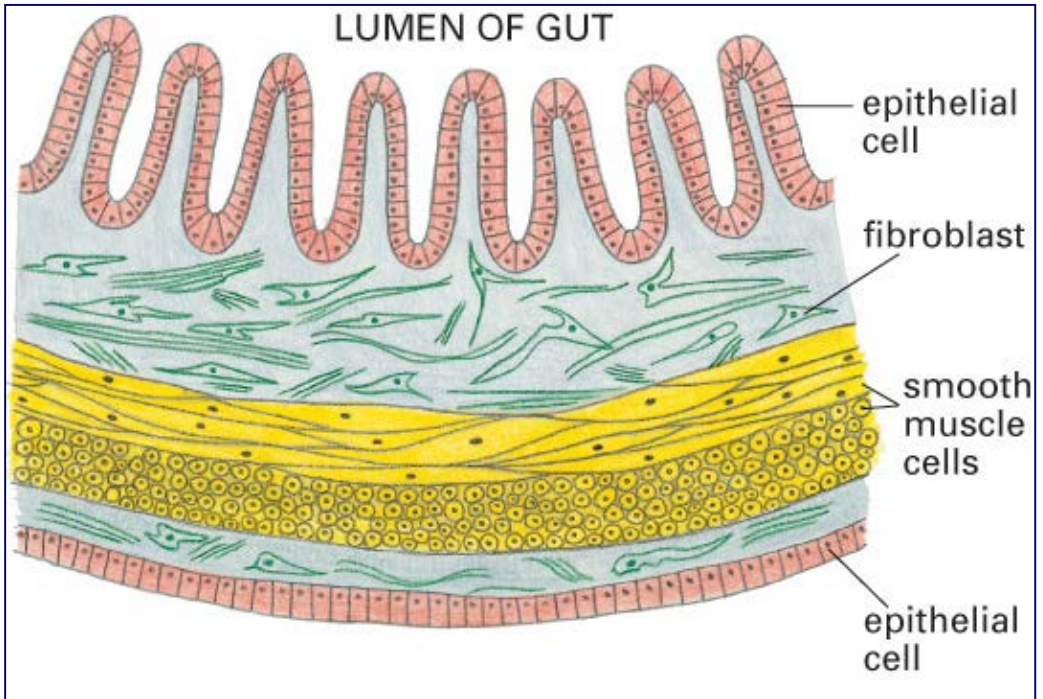
4. Which of the utilized stains allow us to observe collagen and in what color do we see it in each case?

5. What substances do we observe with an acidophilic stain?

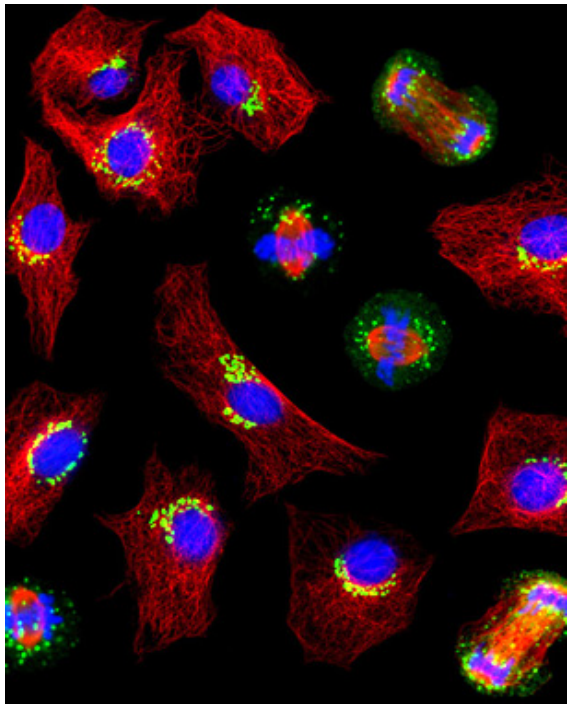
Liver



Intestine



Practical session 3
Cytochemical techniques



Cell Biology Unit
Department of Pathology
School of Medicine and Dentistry

Cytochemistry applies chemical and biochemical reactions to the cytological techniques, in order to locate and determine specifically certain substances or their activity. When applied to histological sections this field is called histochemistry.

In these reactions the substance is stained directly or after chemical modification.

Slide III 1 Ascites tumor – *Giemsa*
Slide III 2 Ascites tumor – *P.A.S.*
Slide III 3 Ascites tumor – *Feulgen*
Slide III 4 Ascites tumor – *Lactate dehydrogenase*
Slide III 5 Intestine section – *P.A.S*

CYTOCHEMICAL TECHNIQUES:

CONDITIONS FOR A CYTOCHEMICAL STAINING

- The analyzed components must not diffuse. Therefore, they must not be soluble in the fixation agent.

- The staining product must be insoluble, remaining in the analyzed location.

- The fixation must be adequate for the studied substance. For instance, if the activity of enzymes is studied most samples must be frozen.

- The method has to be specific for the analyzed substance or chemical group.

CYTOCHEMICAL TECHNIQUES

PAS stain: Detects sugars, mucoproteins, glycogen

Feulgen stain: Detects DNA

Enzymatic techniques: Detects activity of enzymatic proteins

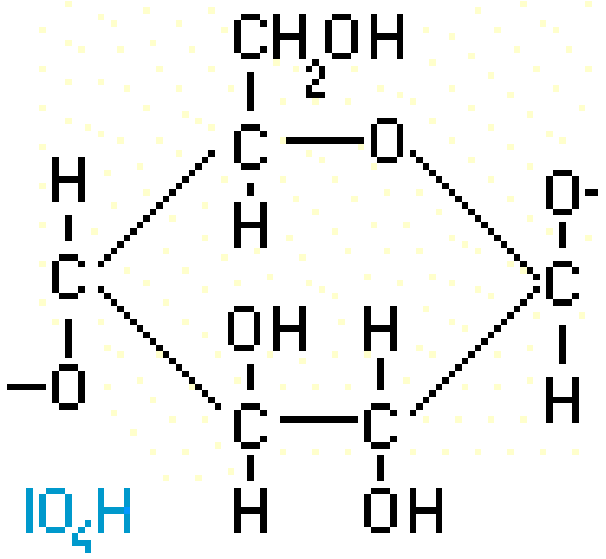
Immunocytochemical techniques: Detects specific proteins

Lipids: They are detected by liposoluble stains.

DETECTION OF SUGARS: PERIODIC ACID-SCHIFF (PAS) STAIN

- This method is performed in two steps. First, periodic acid **(IO_4H)** acts oxidizing the 1-2 glycol bonds (-CHOH-OHHC-) transforming them in aldehydes (-COH HOC-). Secondly, these aldehyde groups are detected with the Schiff reagent **F(SO3H)$_2$**

Periodic acid does not act when glycol groups are blocked by acid radicals.



DETECTION OF DNA: FEULGEN STAIN

- In the FEULGEN stain, cells are first subjected to hydrolysis with hydrochloric acid (HCl). RNA is not hydrolyzed.

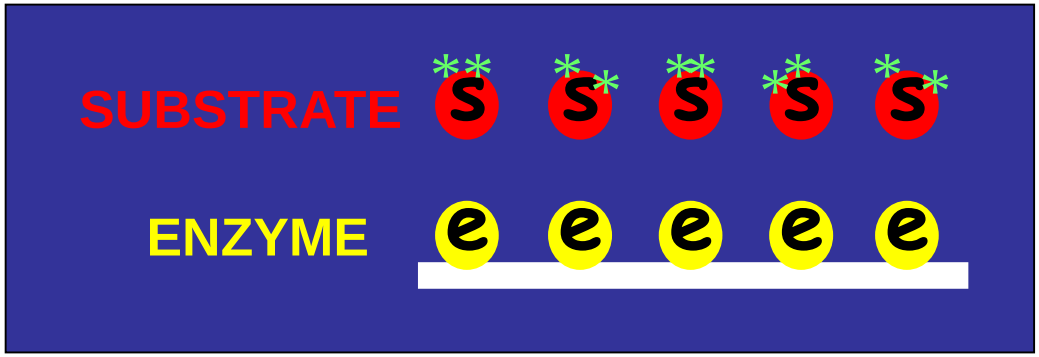
- This hydrolysis extracts the purines from DNA, leaving free aldehyde groups on the deoxyribose chain.

- The free aldehyde groups react with the Schiff reagent, giving an intense red color.

- The time of hydrolysis is critical. A too long hydrolysis gives a low staining, due to chemical alteration, depolymerization and destruction of DNA.

ENZYMATIC CYTOCHEMISTRY

Chemical affinity reactions



$$S + E \rightarrow P$$

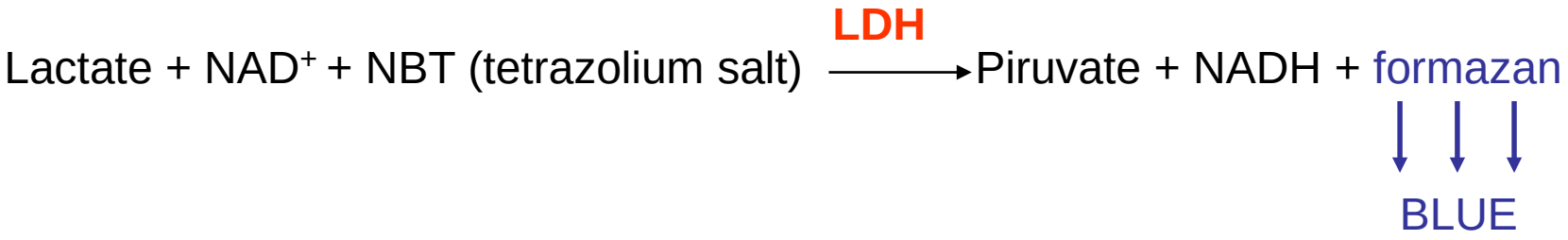
$$+$$

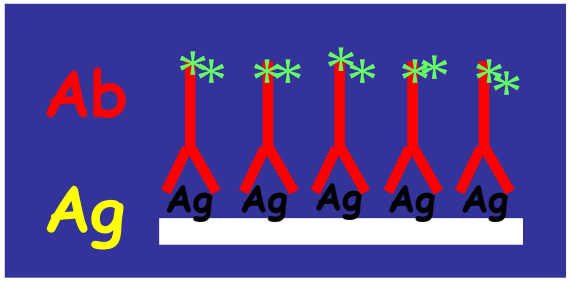
$$R \rightarrow \text{Color}$$

Phosphatases
Esterases
Oxidases
Dehydrogenases

LACTATE DEHYDROGENASE (LDH)

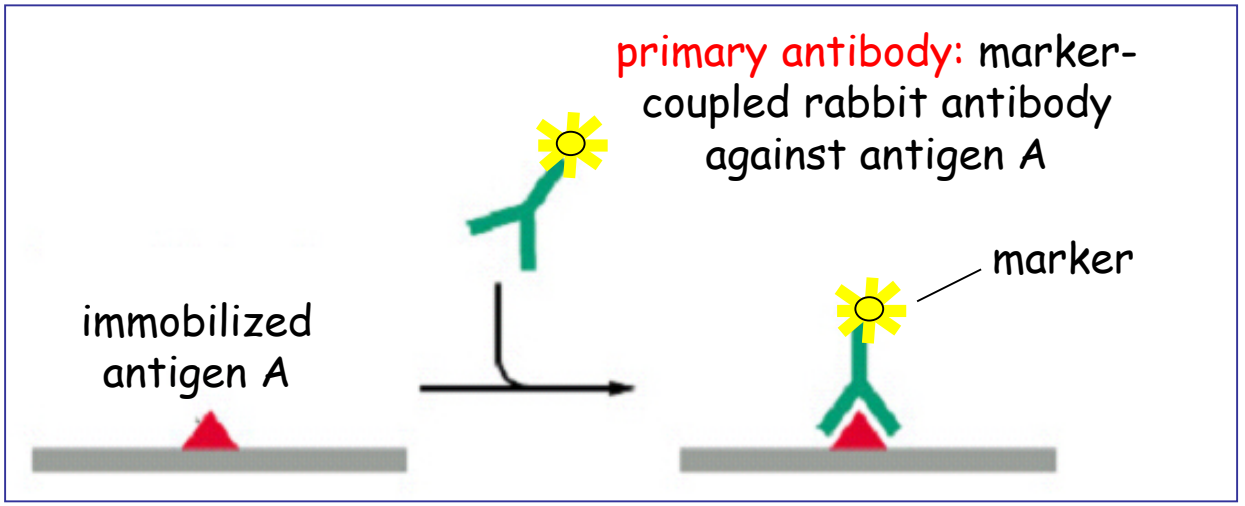
The activity of the enzyme lactate dehydrogenase is detected within the cytoplasm of cells as a blue insoluble deposit, formazan.



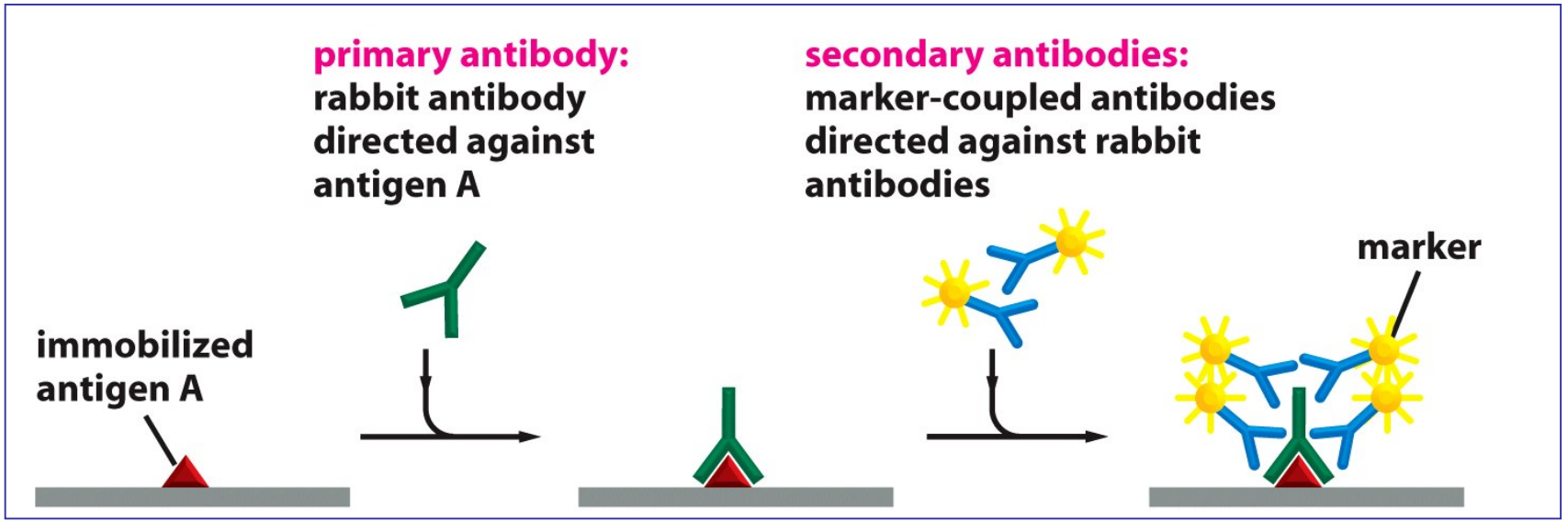


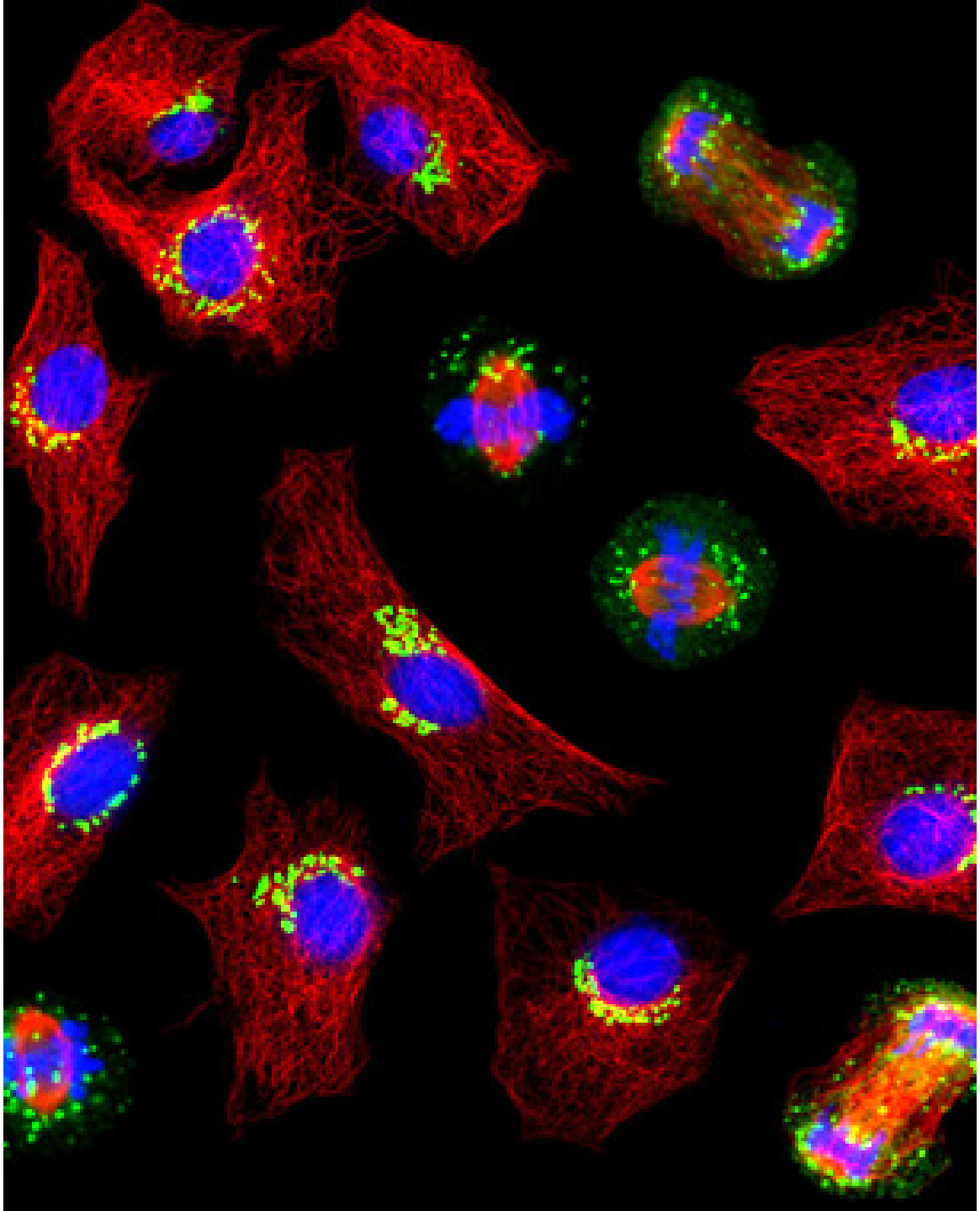
Immunocytochemistry

Direct method

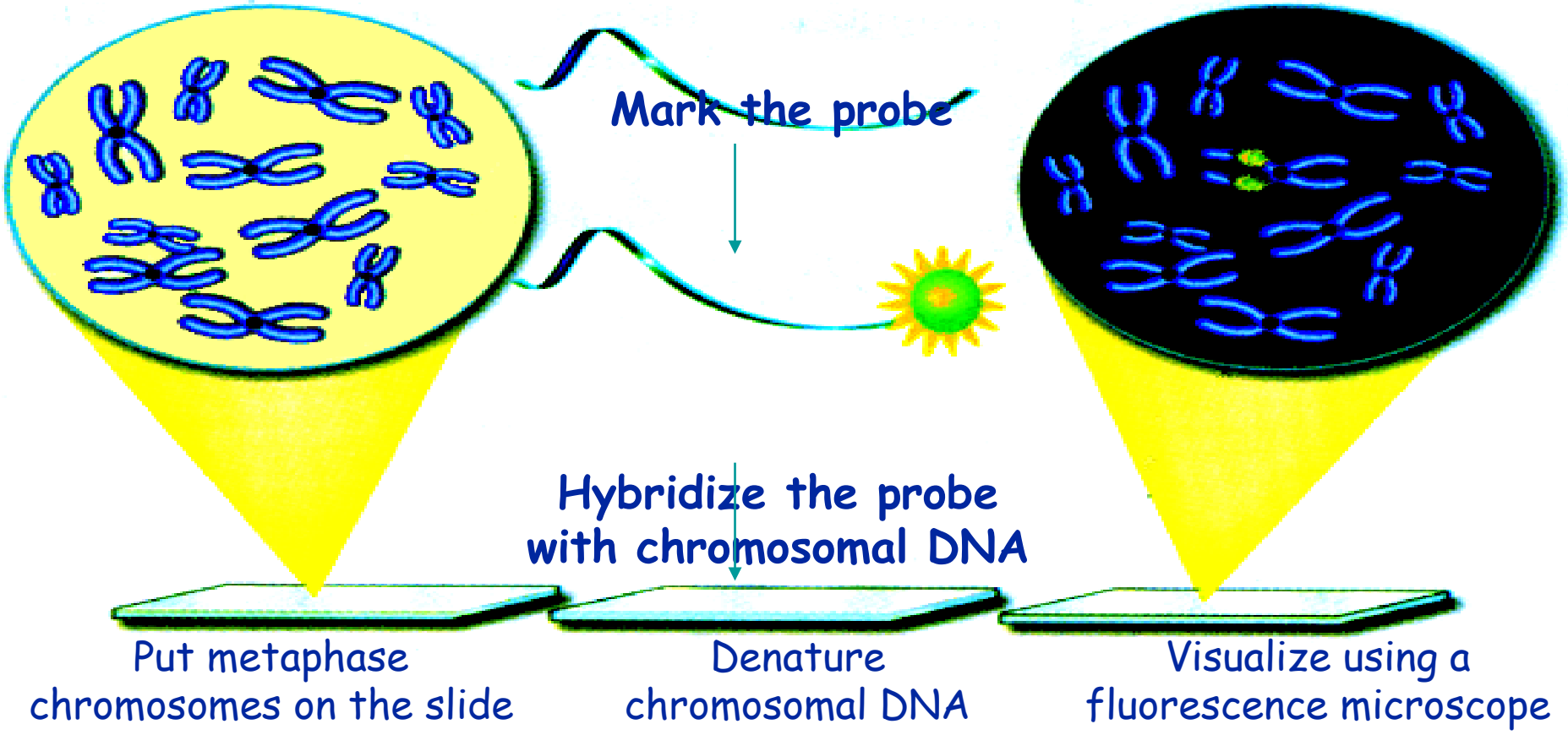


Indirect method

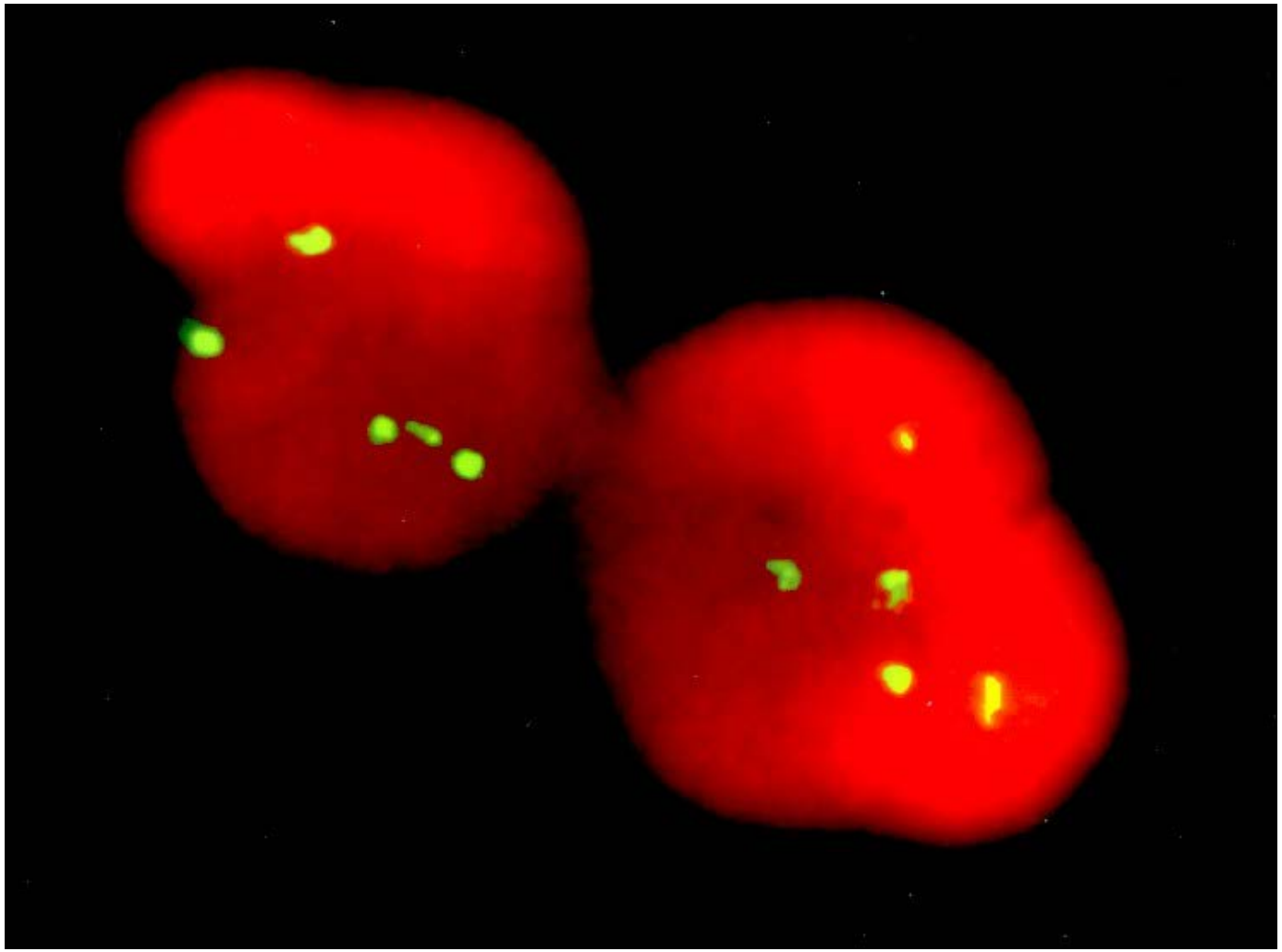




Fluorescent “in situ” hybridization (FISH)



Fluorescent “in situ” hybridization (FISH)



Centromere chromosome 7

QUESTIONS

1. What do we observe in a dividing cell using the Feulgen technique?

2. What staining method allows us to see the glycocalix?

3. Why can the PAS-positive content be variable in different cells?

4. What differences exist between the direct and indirect immunocytochemical methods?

5. What fixation method would you use to perform an enzymatic cytochemical technique? Why?

PRACTICAL SESSION 4

CELL TYPES

Department of Pathology
Biology Unit

2012-2013

OBJECTIVE

Study of different cell types and their components.

COMPETENCES

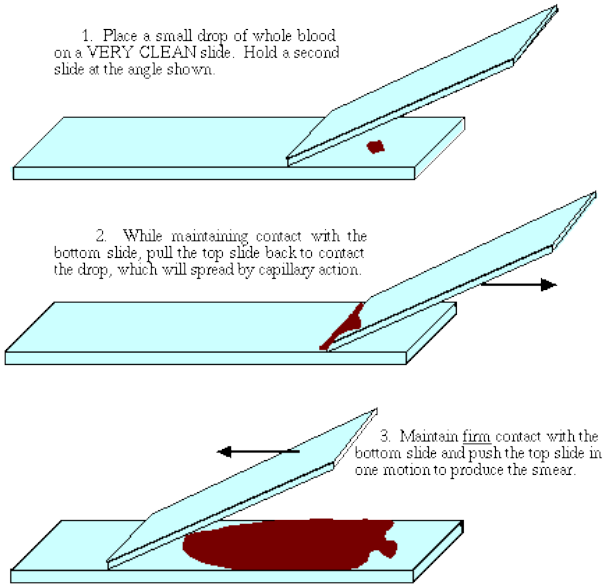
•Use of the optical microscope.
•Identification of the different cell types.
•Recognition of the characteristics of eucaryotic cells: Shape, Size (nucleus/cytoplasm ratio), Number.
•Identification of the cellular components: Cytoplasm, nucleus, nucleolus.
•Observation of the staining affinity of cellular components depending on the stain.

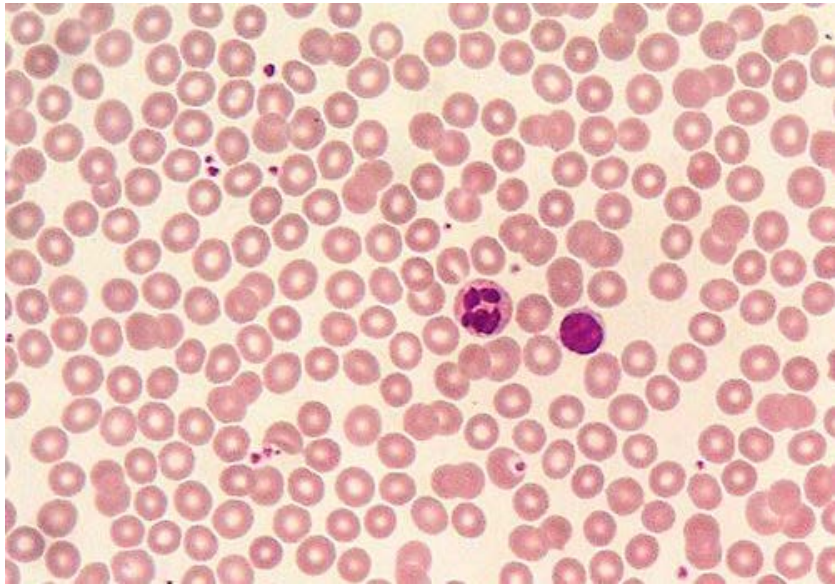
SLIDES

4.1- Human blood: Giemsa stain
4.2- Eel blood: Giemsa stain
4.3- Cervical cytology: Papanicolaou stain (Pap test or Pap smear)

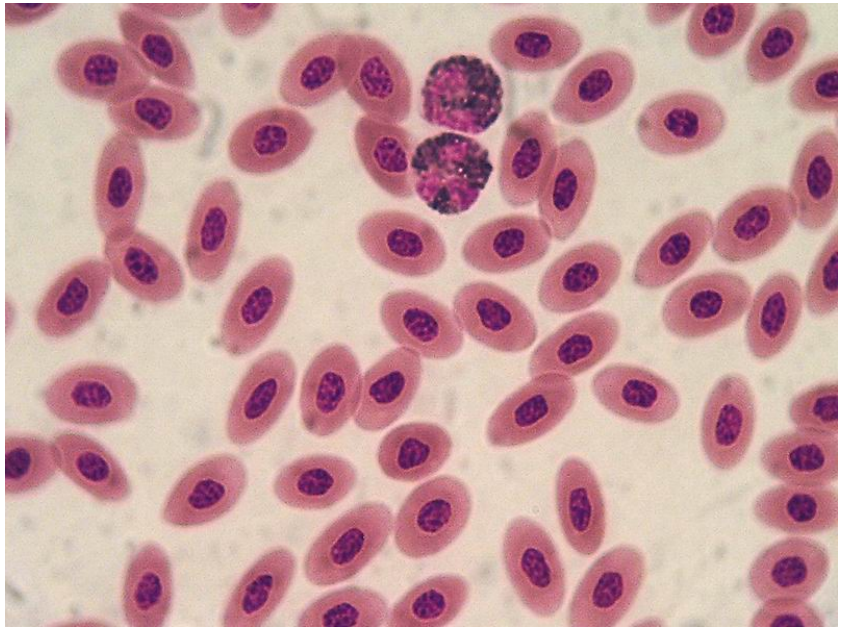
PRACTICAL EXERCISE

Blood smear



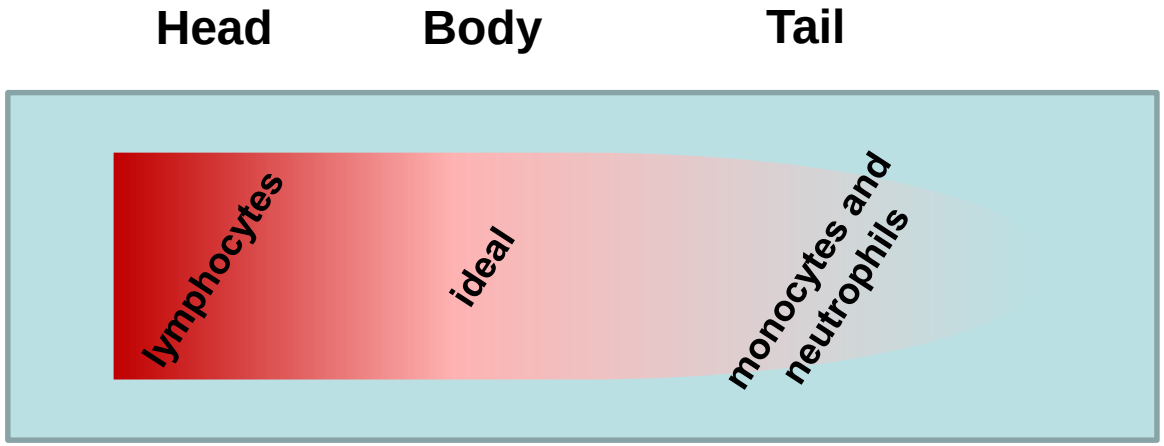


4.1-Human Blood (Giemsa)



4.2-Eel Blood (Giemsa)

Extensión de sangre periférica

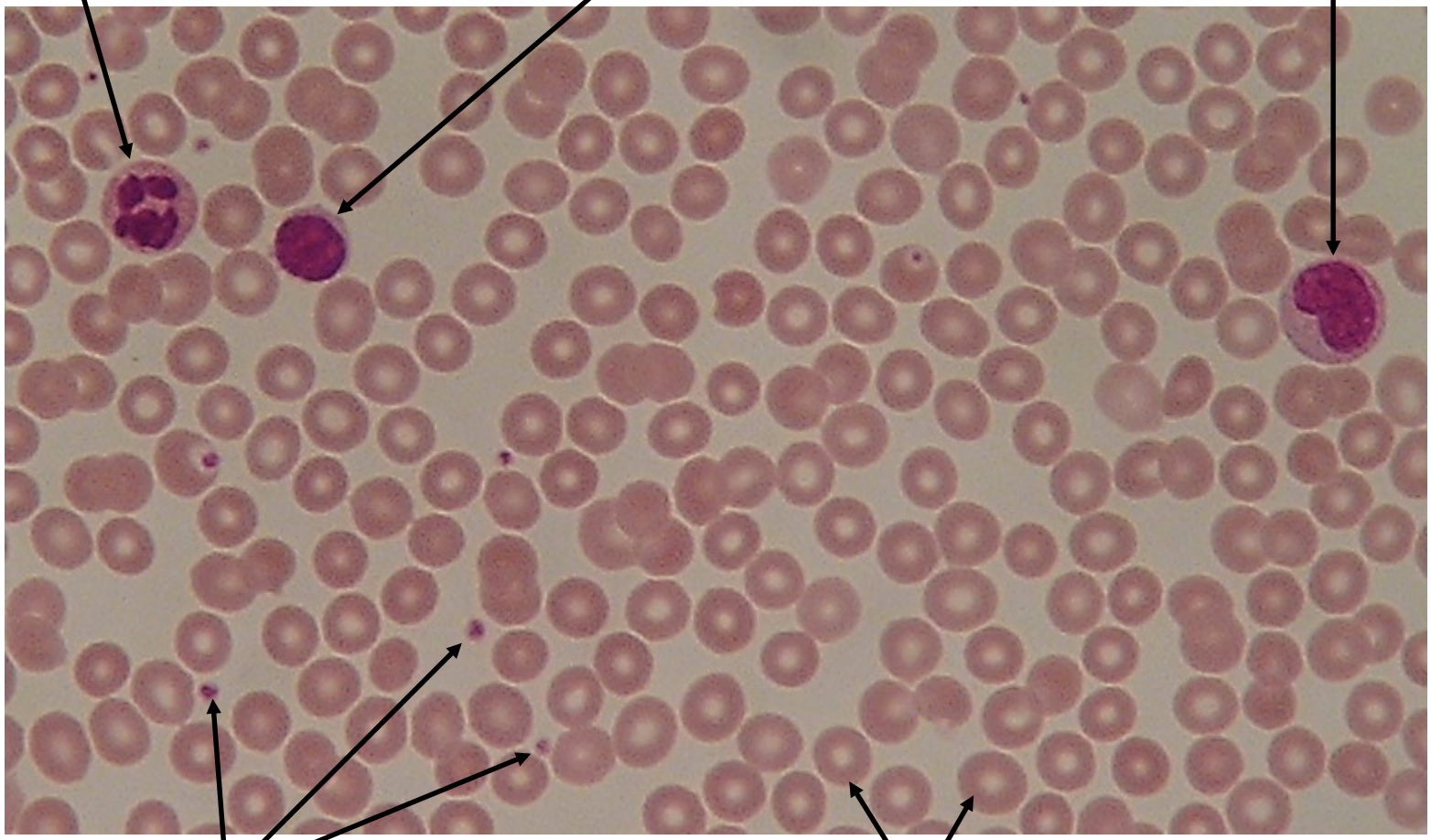


The area where the smear drop (‘head’) is too thick and cannot be properly assessed.

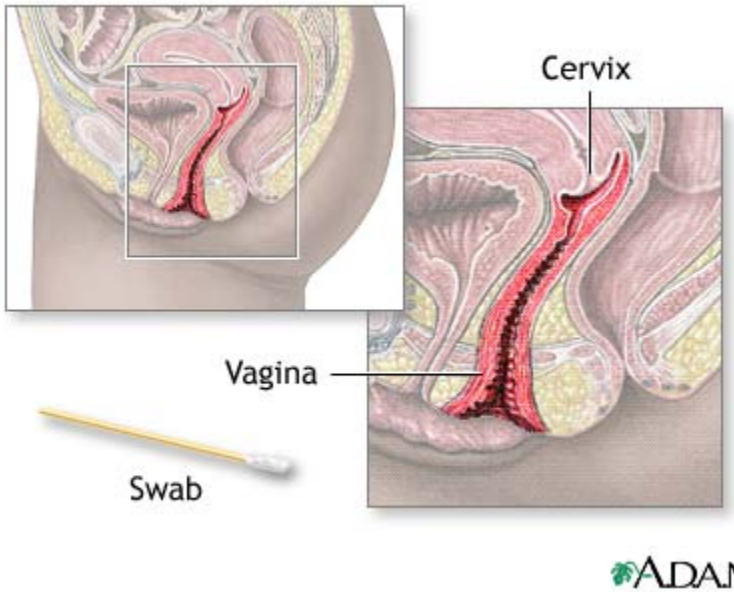
The ‘body’ is an area halfway of the smear where there is a balanced distribution of cells. This is the ideal region of interest.

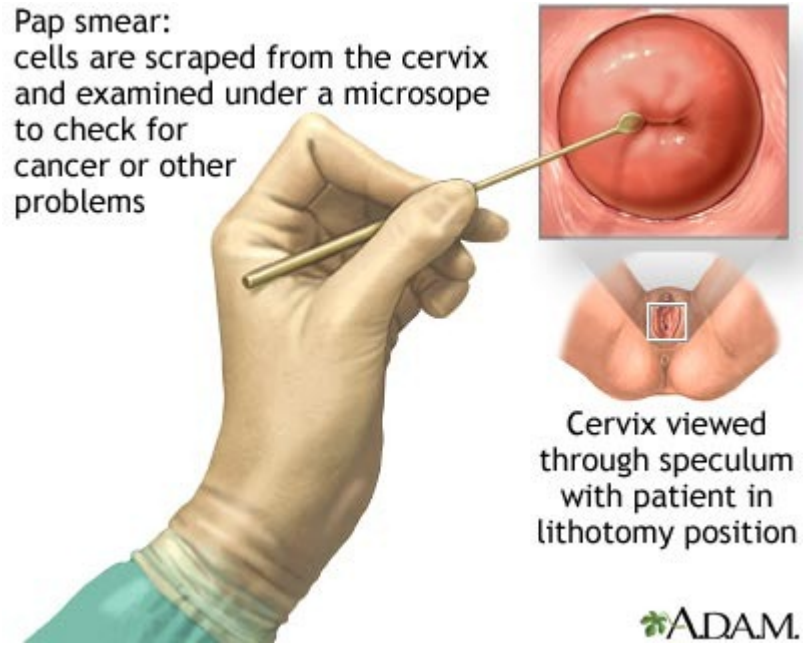
At the end of the smear, we can find the ‘tail’ where cells arrange forming rows.

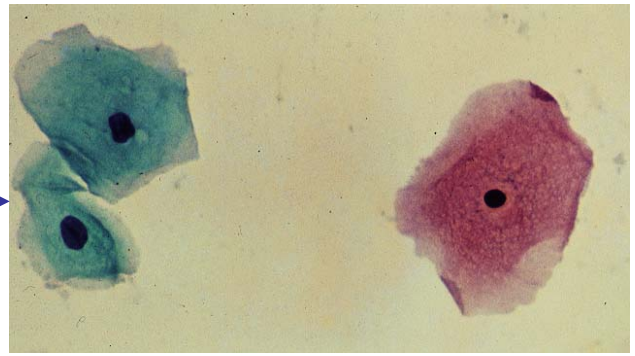
4.1-HUMAN BLOOD (Giemsa)



Pap smear

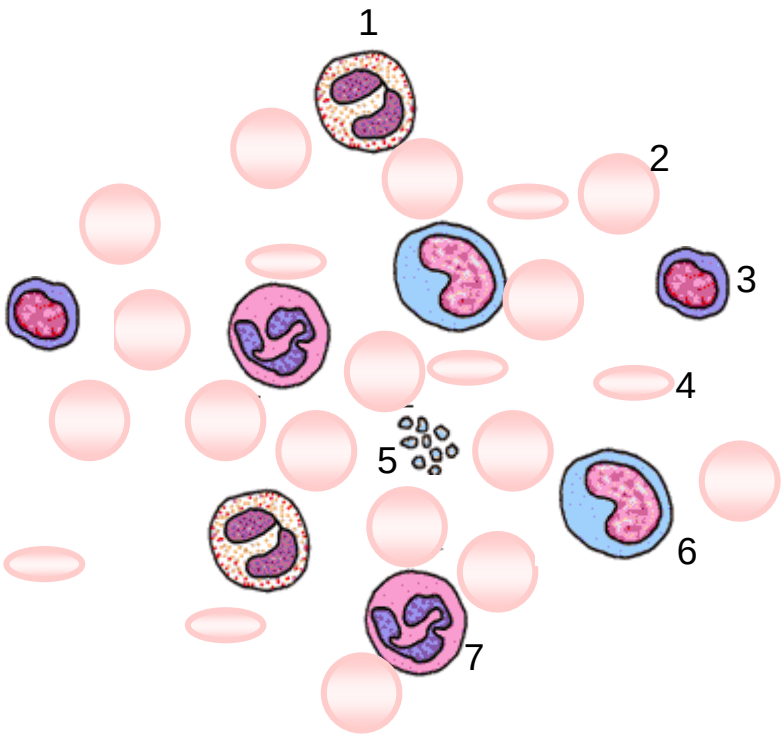

Cervix
Vagina
Swab
A.D.A.M.


Pap smear:
cells are scraped from the cervix and examined under a microsope to check for cancer or other problems
Cervix viewed through speculum with patient in lithotomy position
A.D.A.M.



PRACTICAL SESSION 4: CELL TYPES

Human blood: Identification of cell types



1……
2……
3……
4……
5……
6……
7……

ASSIGNATURA: BIOLOGIA (34446).
GRAU: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

ASIGNATURA: BIOLOGÍA (34446).
GRADO: MEDICINA.
DEPARTAMENT DE PATOLOGIA. UNIVERSITAT DE VALÈNCIA.

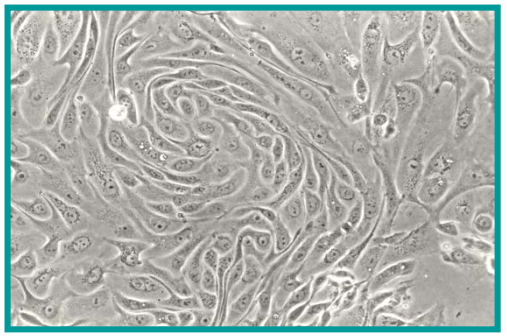
Department of Pathology
Biology Unit

PRACTICAL SESSION 5

CELL CULTURE

CELL CULTURE

1. Definition
2. Conditions for live cells maintenance
3. Techniques for obtaining cells to culture
4. Type of growth
5. Doubling time
6. Culture classification
7. Utility
8. Advantages vs. disadvantages



CELL CULTURE

Definition:

Maintenance of cells, tissues or organs outside the live organism for more than 24 hours.

Conditions for maintenance of live cells

- **Defined culture media**: MEM, RPMI-1640, F-12.

- **Sterility**: Manipulation
  Materials
  Media

- **Environmental conditions:**
  - ✓ Temperature: 37ºC
  - ✓ Controlled atmosphere: CO_2 in air
    Humidity
    Darkness

CELL CULTURE

Conditions for maintenance of live cells

- **Defined culture media**: MEM, RPMI-1640, F-12.

medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones…

Media Type	Examples	Uses
Balanced salt solutions	PBS, Hanks' BSS, Earle's salts DPBS HBSS EBSS	Form the basis of many complex media
Basal media	MEM	Primary and diploid culture
	DMEM	Modification of MEM containing increased level of amino acids and vitamins. Supports a wide range of cell types including hybridomas
	GMEM	Glasgows modified MEM was defined for BHK-21 cells
Complex media	RPMI 1640	Originally derived for human leukaemic cells. It supports a wide range of mammalian cells including hybridomas
	Iscoves DMEM	Further enriched modification of DMEM which supports high density growth
	Leibovitz L-15	Designed for CO_2 free environments
	TC 100 Graces Insect medium Schneider's Insect medium	Designed for culturing insect cells
Serum free media	CHO HEK293	For use in serum free applications
	Ham F10 and derivatives Ham F12 DMEM/F12	Note: these media must be supplemented with other factors such as insulin, transferrin and epidermal growth factor. These media are usually HEPES buffered

CELL CULTURE

Defined culture media

Serum

- Major source of various nutrients such as growth factors, adhesion factors, mineral, lipid….
- Promotes cell proliferation, cell attachment, regulates cell membrane permeability

+ Antibiotic

COMPONENTS	Molecular Weight	Concentration (mg/L)	Molarity (mM)
Amino Acids			
Glycine	75	30	0.400
L-Arginine hydrochloride	211	84	0.398
L-Cystine 2HCl	313	63	0.201
L-Glutamine	146	584	4.00
L-Histidine hydrochloride-H2O	210	42	0.200
L-Isoleucine	131	105	0.802
L-Leucine	131	105	0.802
L-Lysine hydrochloride	183	146	0.798
L-Methionine	149	30	0.201
L-Phenylalanine	165	66	0.400
L-Serine	105	42	0.400
L-Threonine	119	95	0.798
L-Tryptophan	204	16	0.0784
L-Tyrosine disodium salt dihydrate	261	104	0.398
L-Valine	117	94	0.803
Vitamins			
Choline chloride	140	4	0.0286
D-Calcium pantothenate	477	4	0.00839
Folic Acid	441	4	0.00907
i-Inositol	180	7.2	0.0400
Niacinamide	122	4	0.0328
Pyridoxine hydrochloride	205	4	0.0194
Riboflavin	375	0.4	0.00106
Thiamine hydrochloride	337	4	0.0119
Inorganic Salts			
Calcium Chloride (CaCl2) (anhyd.)	111	200	1.80
Ferric Nitrate (Fe(NO3)3"9H2O)	404	0.1	0.000248
Magnesium Sulfate (MgSO4) (anhyd.)	120	97.67	0.814
Potassium Chloride (KCl)	75	400	5.33
Sodium Bicarbonate (NaHCO3)	84	3700	44.05
Sodium Chloride (NaCl)	58	4750	81.90
Sodium Phosphate monobasic (NaH2PO4-H2O)	138	125	0.906
Other Components			
D-Glucose (Dextrose)	180	4500	25.00
HEPES	238	5958	25.03

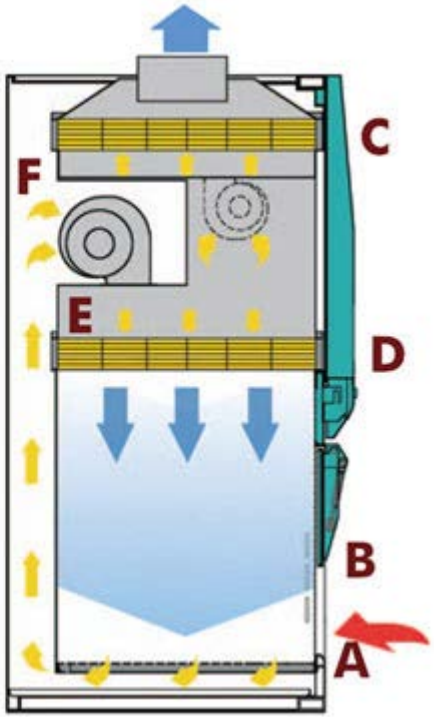
CELL CULTURE

Conditions for maintenance of live cells

- **Sterility**: Manipulation
  Materials
  Media

A: Apertura frontal.
B: Panel frontal.
C: Filtro HEPA de expulsión.
D: Filtro HEPA principal.
E: Plenum de presión positiva.
F: Plenum de presión negativa.

Aire ambiental.
Aire contaminado.
Aire purificado a través del filtro HEPA.



CELL CULTURE

Conditions for maintenance of live cells

- **Sterility**: Materials

PLASTICWARE

GLASSWARE

PREPARATION OF THE MATERIALS


GIBCO
Foetal Bovine Serum
EU Approved Origin
10270-106
GIBCO
RPMI 1640
1X
42401
+ HEPES
– L-Glutamine
PYREX
100 ml
pipetus-akku

Conditions for maintenance of live cells

- **Environmental conditions:**
  - ✓ Temperature: 37ºC
  - ✓ Controlled atmosphere:
    - CO2 in air
    - Humidity
    - Darkness

Conditions for maintenance of live cells

- **Environmental conditions:**

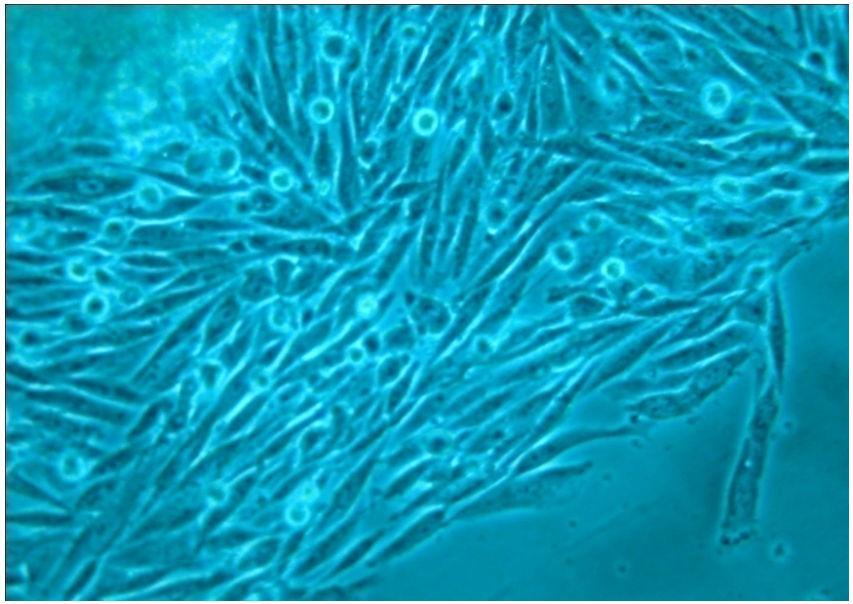
Observation of cultures: Phase-contrast microscopy

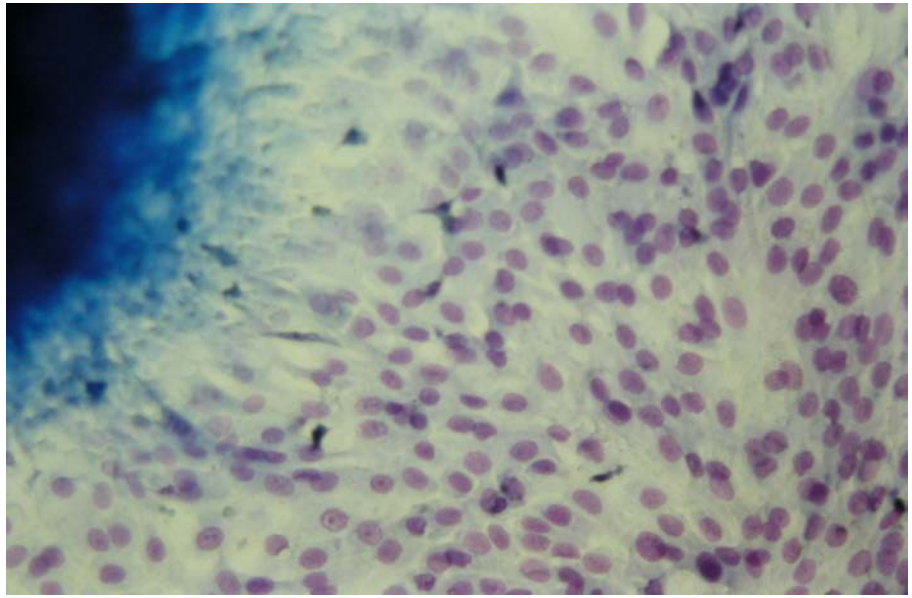
CELL CULTURE

TYPES OF CULTURES

Depending on the method of obtaining cells:

1. Explant cultures: a small fragment of tissue is seeded (1 mm^3), permitting any cell type to grow.





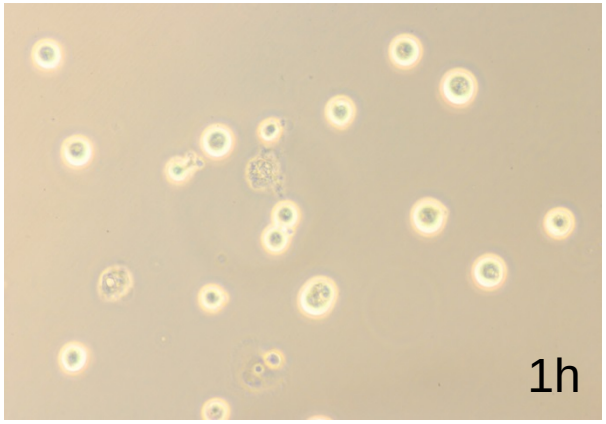
CELL CULTURE

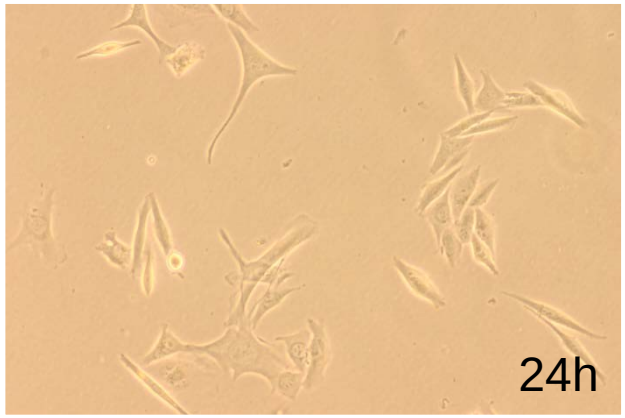
TYPES OF CULTURES

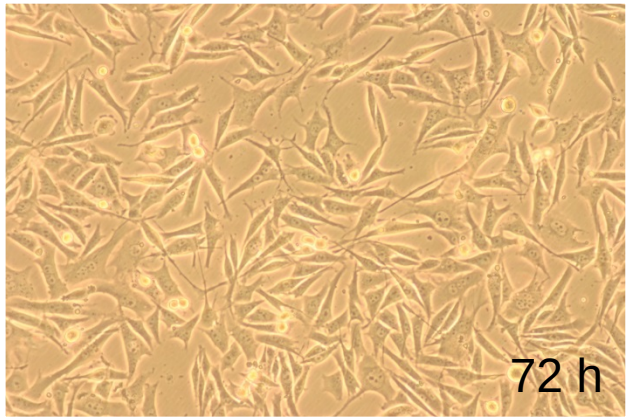
Depending on the method of obtaining cells:

2. Culture of isolated cells:

- ✓ Culture of single cells (blood, bone marrow).
- ✓ Mechanical disaggregation.
- ✓ Enzymatic disaggregation of tissues (with trypsin, collagenase, etc.)



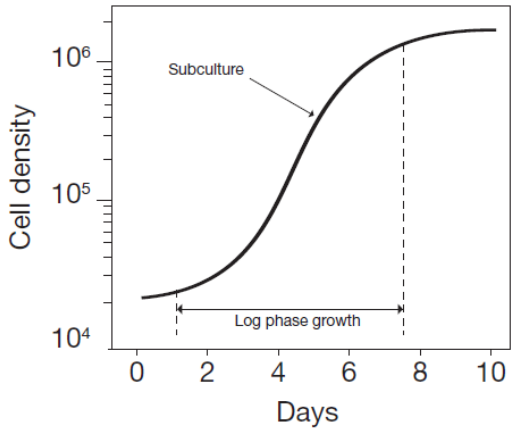




CELL CULTURE

CLASSIFICATION OF CULTURES

- **Primary**: obtained directly from the live being.
- **Secondary:** derived from a primary culture. Most cells die after a finite number of cell divisions.
- **Established cell lines:** cultures in which the cells have adapted to the conditions *in vitro* and can live indefinitely.



- ***Doubling time:*** *Time period in which a cell culture doubles the number of cells.*

CELL CULTURE

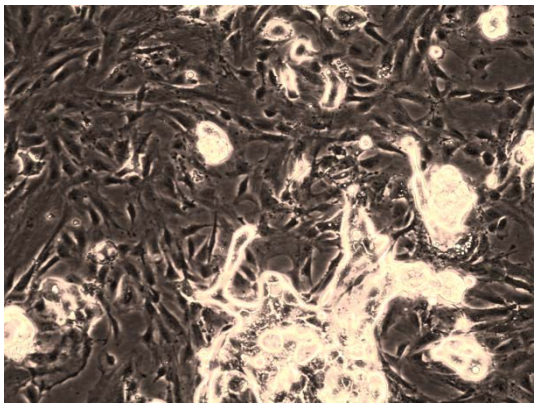
SUBCULTURE TECHNIQUE

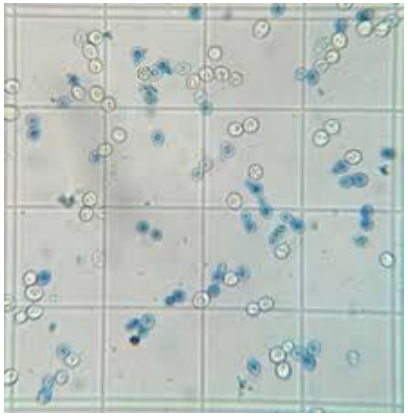
•The aim of a subculture (passage) is to maintain cells in conditions of normal duplication.

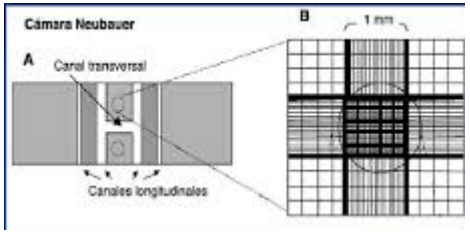
•In the subculture the culture medium is renewed and the relationship between cells/medium/support surface is taken to the initial values.

•Cells adhered to solid supports are detached and separated enzymatically (trypsin), resuspended, stained with a vital stain (Trypan Blue) and counted.

•If a suspension culture is used cells are counted directly.



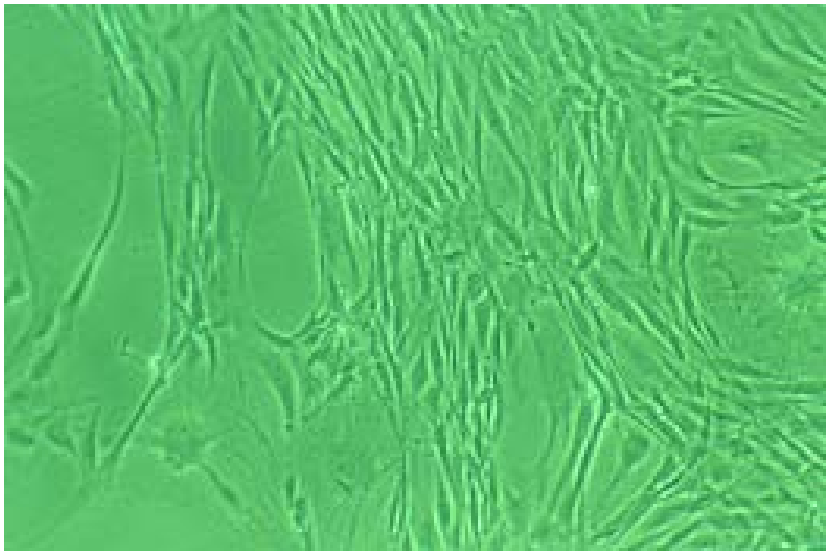




CELL CULTURE

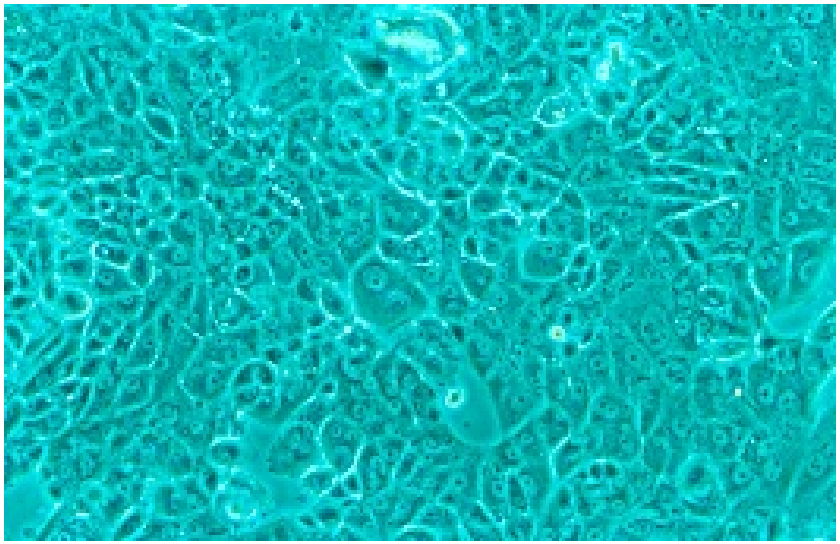
MORPHOLOGY OF CELL CULTURES

Fibroblastic pattern



cells are bipolar or multipolar and have elongated shapes.

Epithelial pattern



cells are polygonal in shape with more regular dimensions.

CELL CULTURE

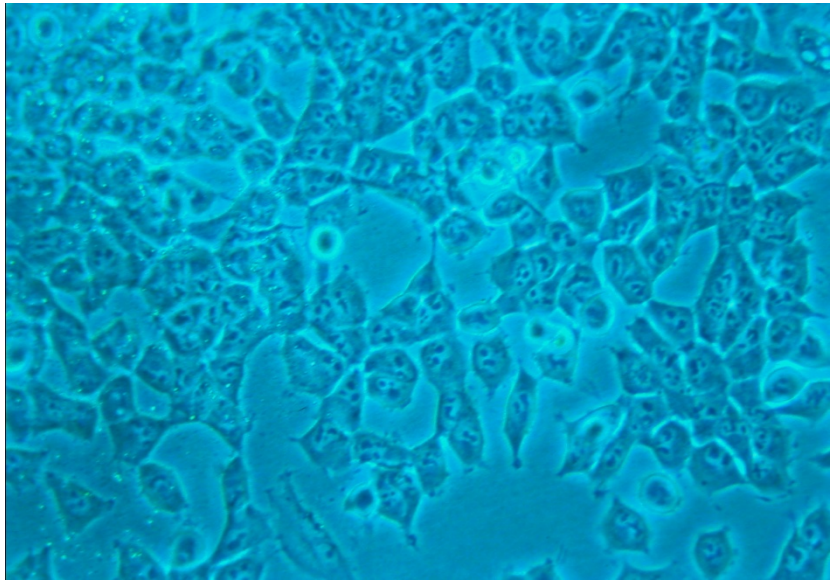
TYPES OF CELL CULTURES

By the form of growth:

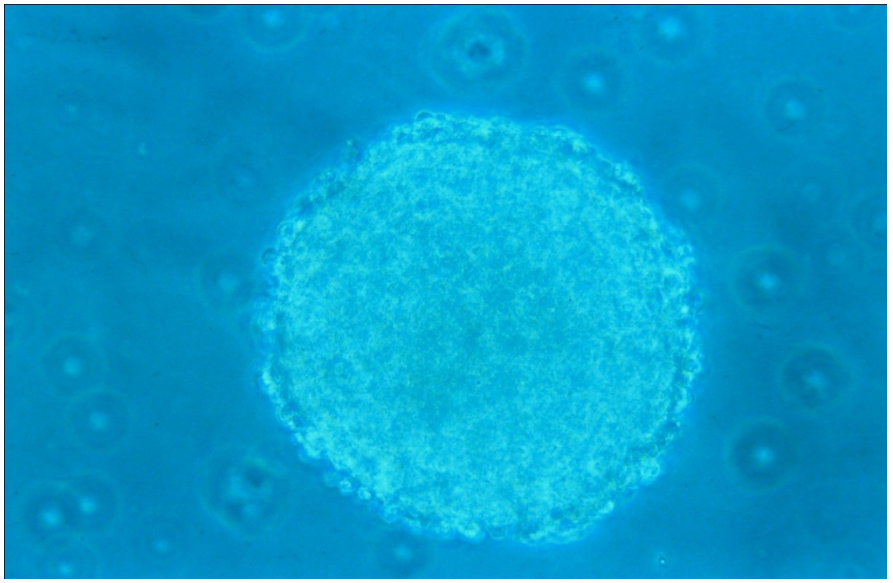
1. Monolayer cultures: A single layer of cells attached to a surface.

2. Suspension cultures: Cells multiply suspended in the cell media.

Monolayer culture



Suspension culture



CELL CULTURE

Advantages:

1. Cells grow in a controlled and defined medium and are directly accessible.
2. Cells usually form an homogeneous population (same type of cells).
3. A large number of cells are obtained.
4. Live cells are observed directly.

Disadvantages:

1. Adaptation to life *in vitro* means an important change in the natural microenvironment of the cell that can change its functions. They are not *exactly* the same as the cells *in vivo* from which they derived.
2. There is always a contamination danger (virus, mycoplasma, yeast, bacteria ...)

CELL CULTURE

Applications of cell cultures:

- Biochemistry.
- Molecular biology.
- Cytogenetics.
- Microbiology (support for culturing viruses) Vaccine production.
- Gynecology (fertilization *in vitro*).
- Gene therapy.
- Gene mapping.
- Pharmacology (drug testing, antibiotic resistance ...)
- Immunology (production of monoclonal antibodies from hybrid cells or hybridomas)
- Study of cell division (mitosis, meiosis)
- Treatment of skin burns

PRACTICAL SESSION 5

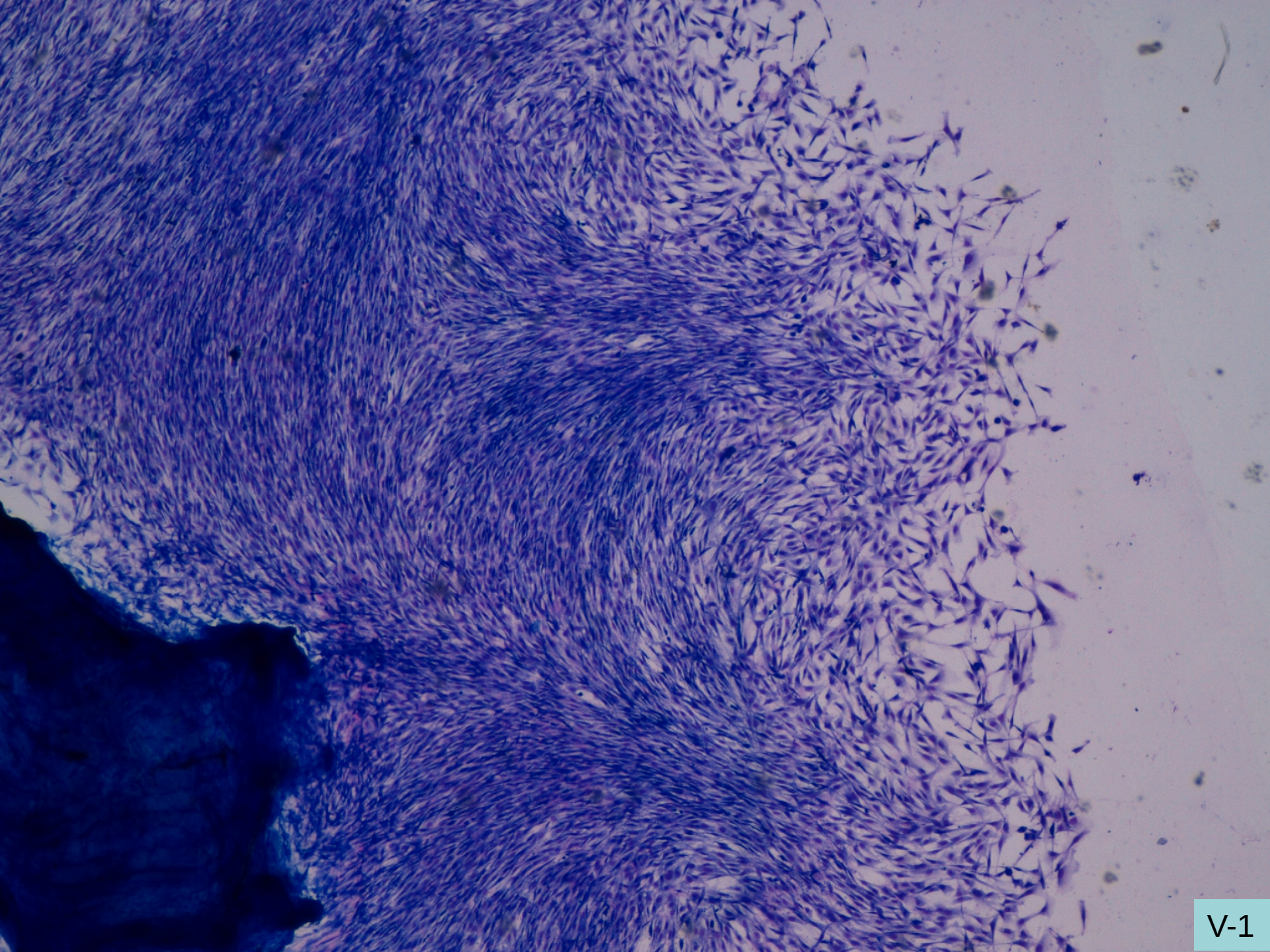
SLIDES

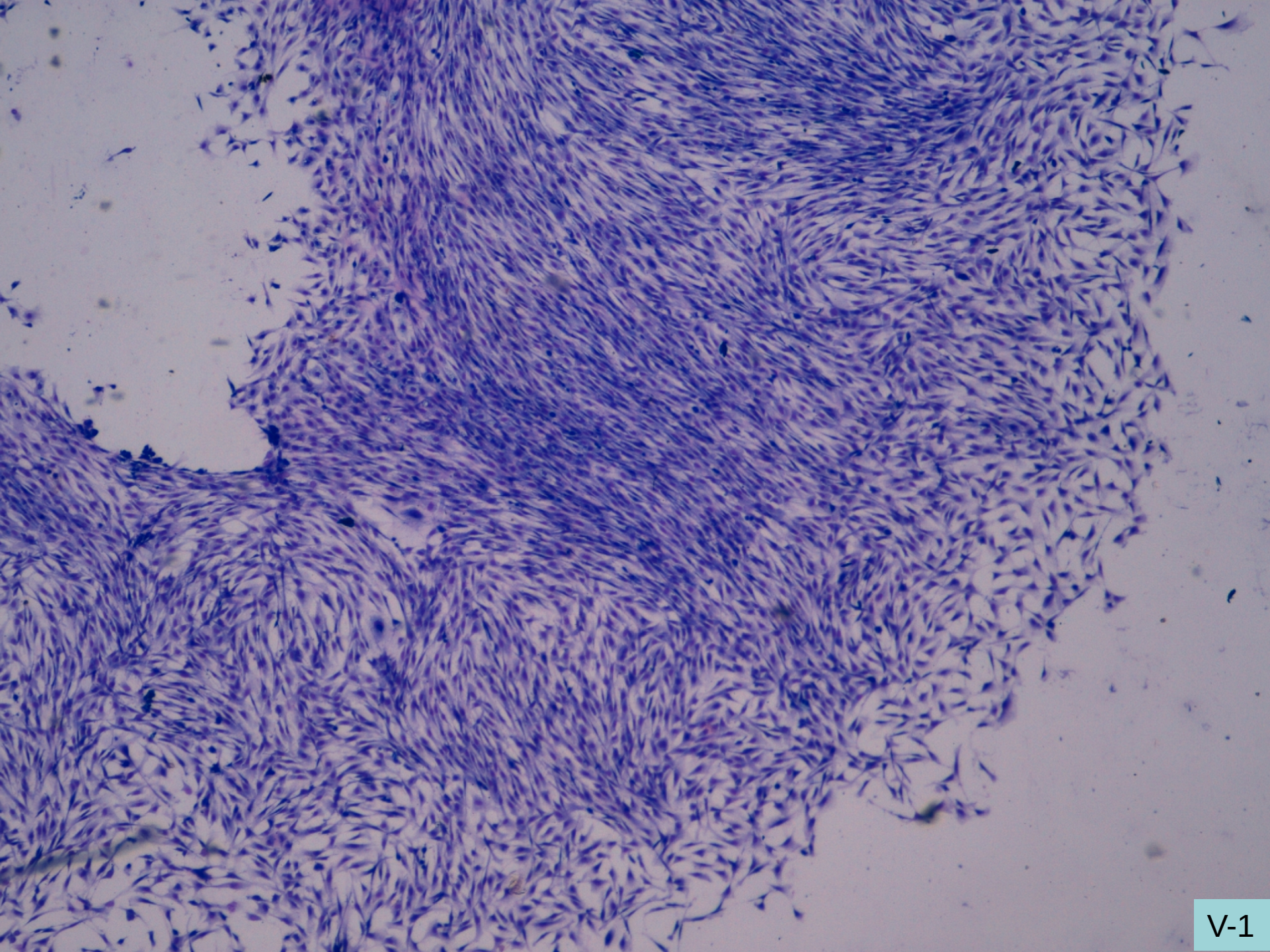
V-1 Primary explant culture. Giemsa

V-2 Secondary culture (fibroblasts). Giemsa.

V-3 Established cell line sarc-2. Giemsa.

V-4 Established cell line mel-1. Giemsa


V-1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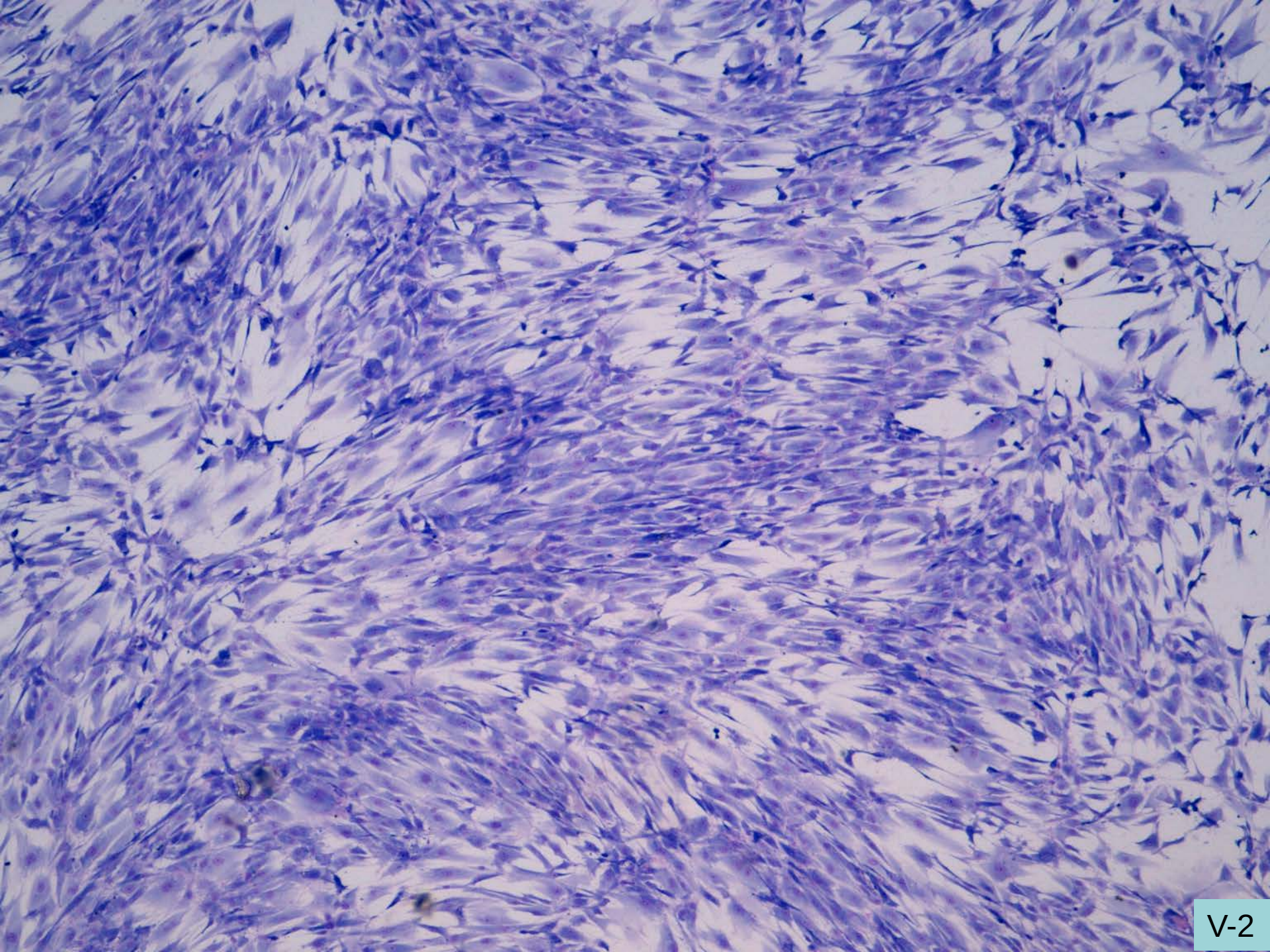
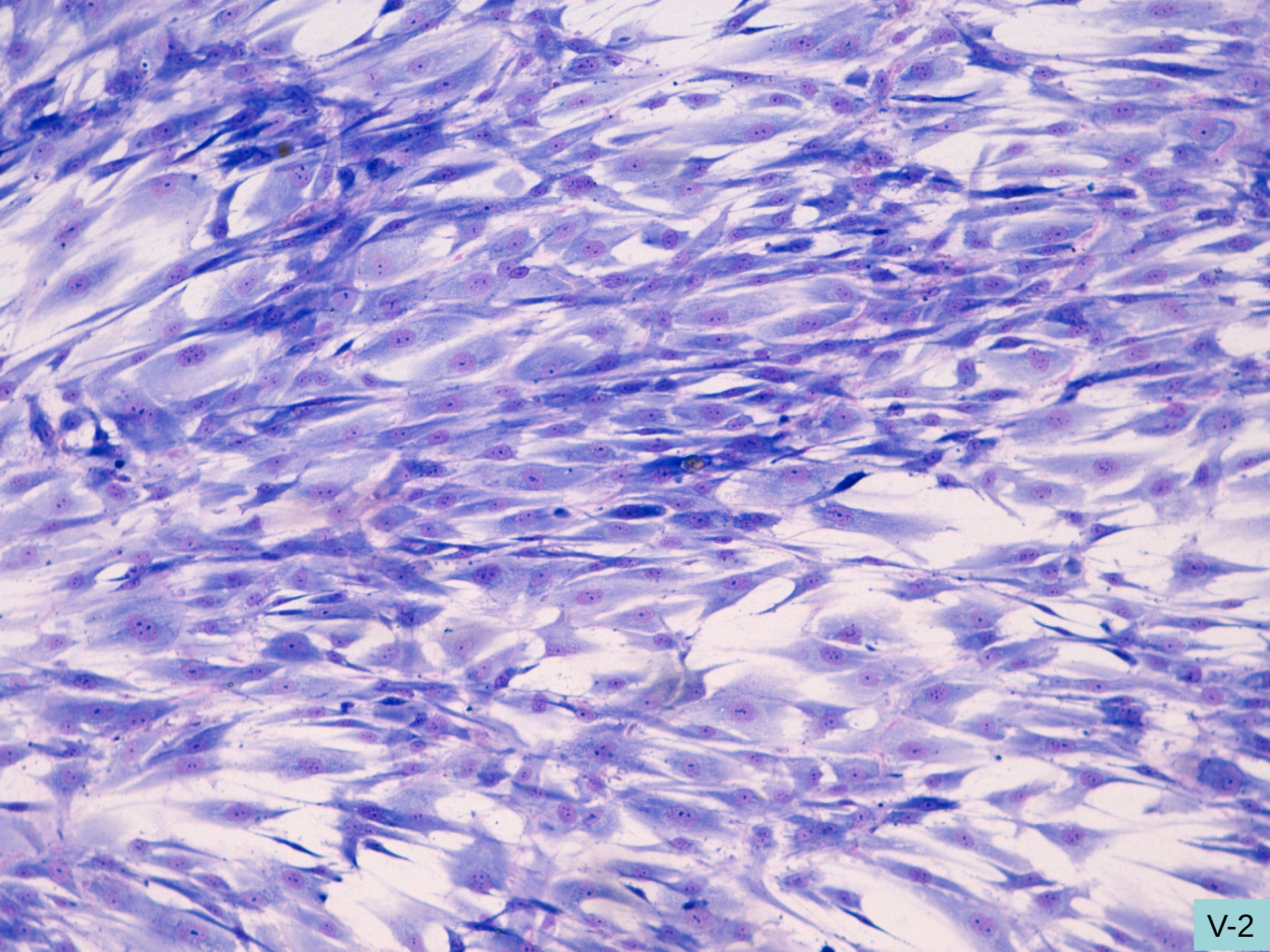
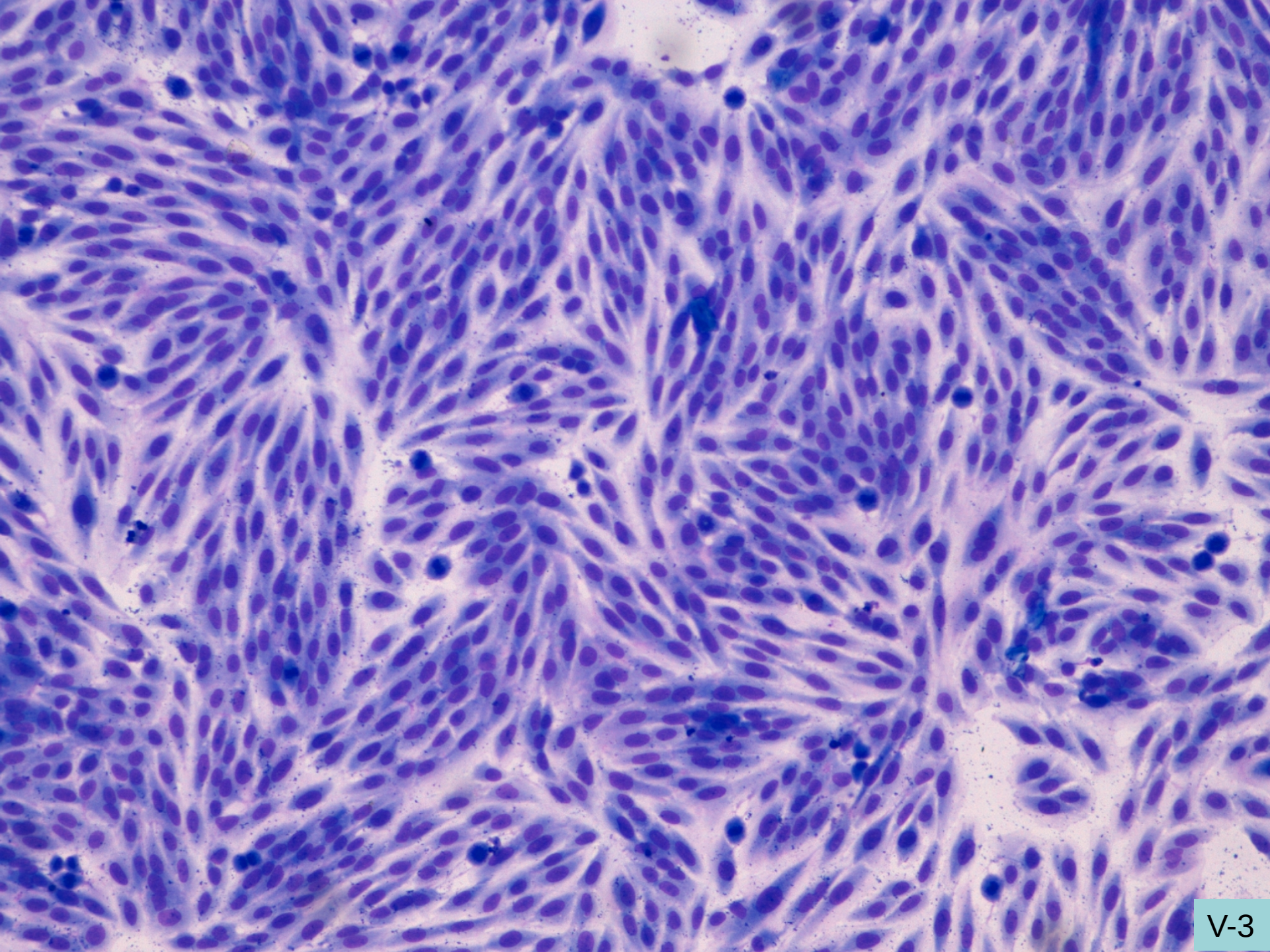


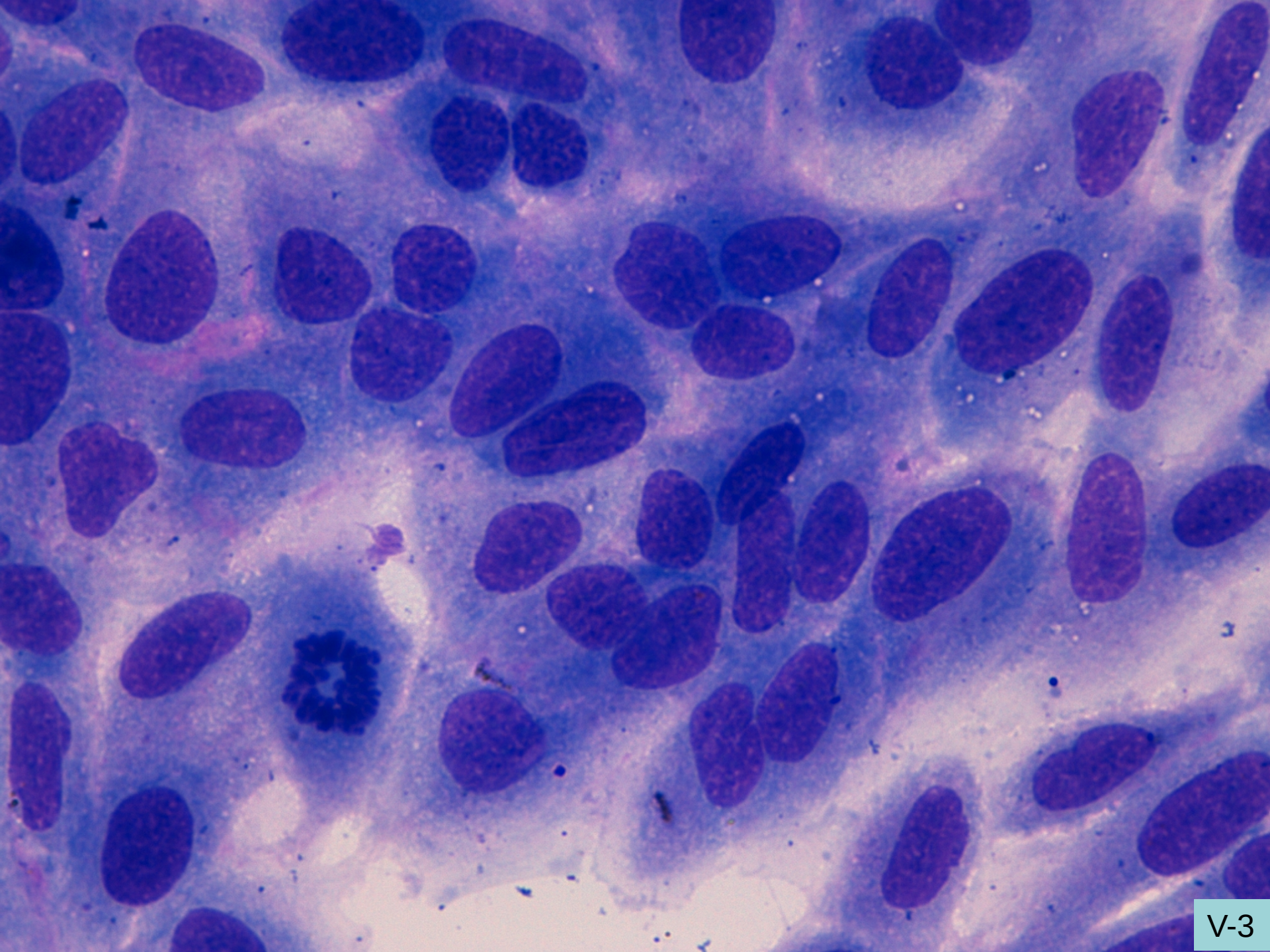
V-2

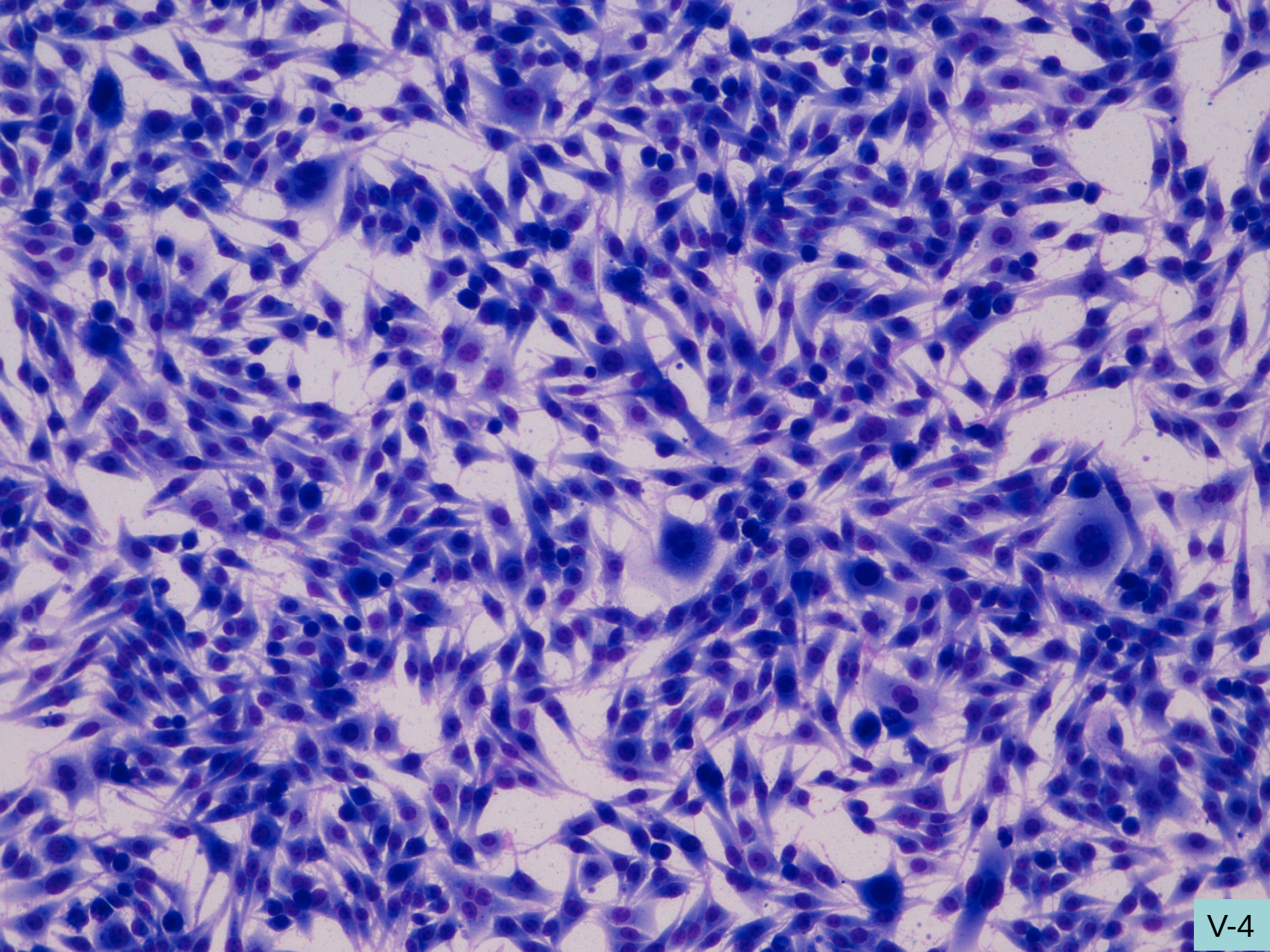


V-2

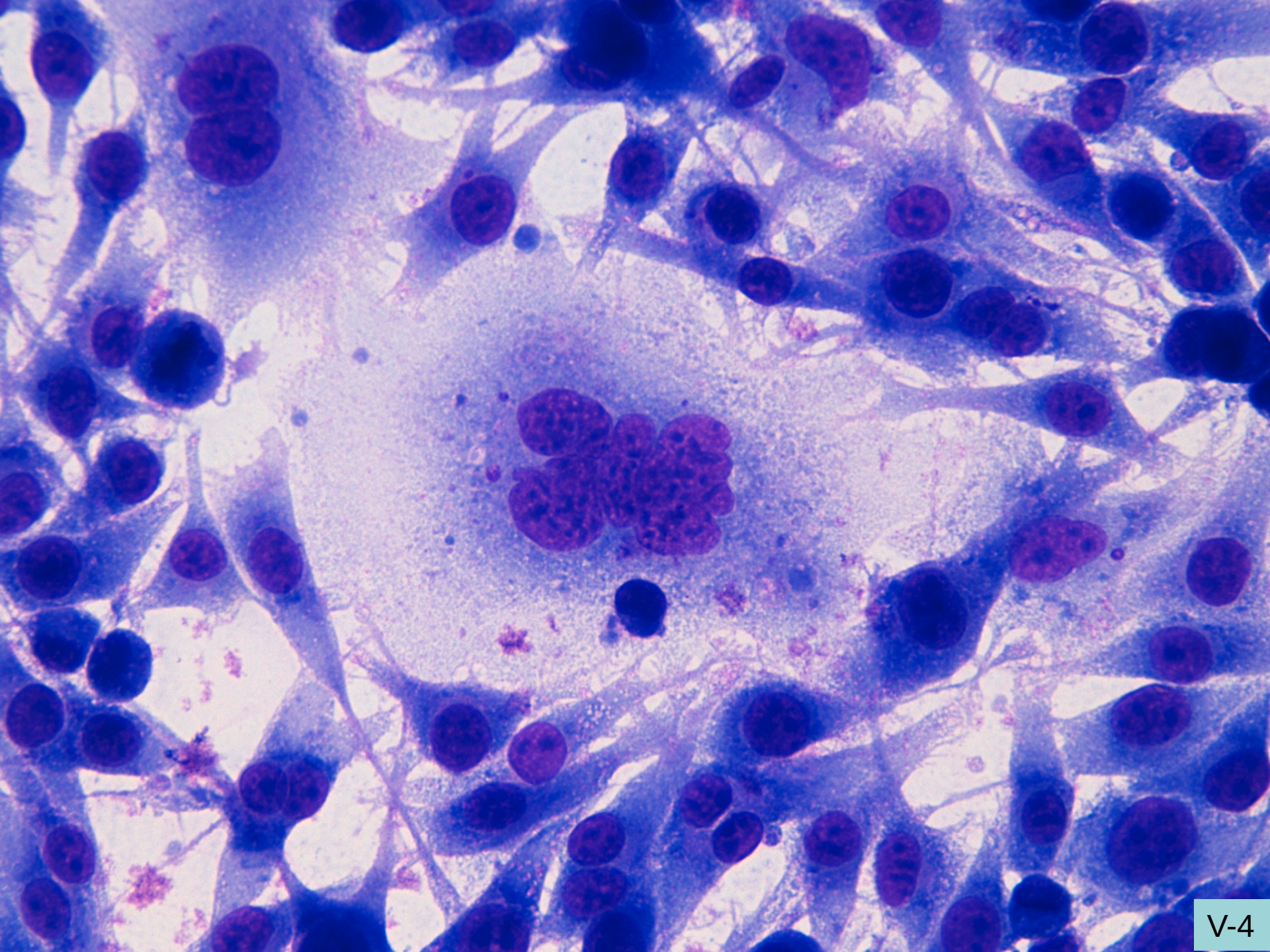


V-3


V-3



V-4



V-4

QUESTIONS

In what conditions do we have to maintain live cells in culture?

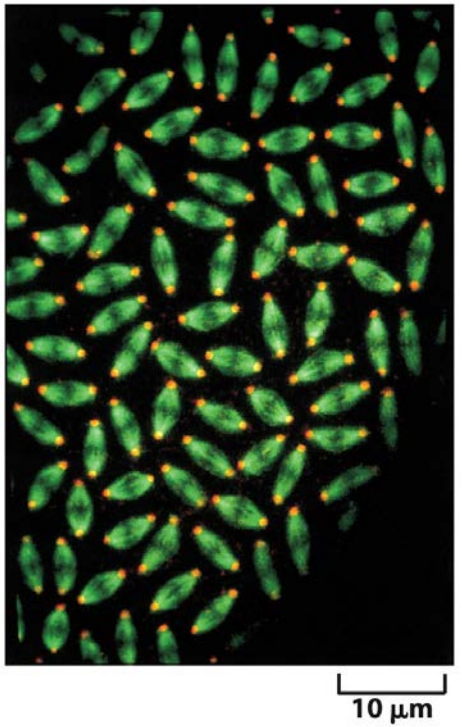
What is an explant culture? Is it a primary or secondary culture?

With what type of microscope can we observe live cells in a culture flask?

What do we do when cells with monolayer growth reach a high degree of confluency? How do we perform this process?

What advantages does the use of cell cultures have?

PRACTICAL SESSION 8
MITOSIS AND CHROMOSOMES

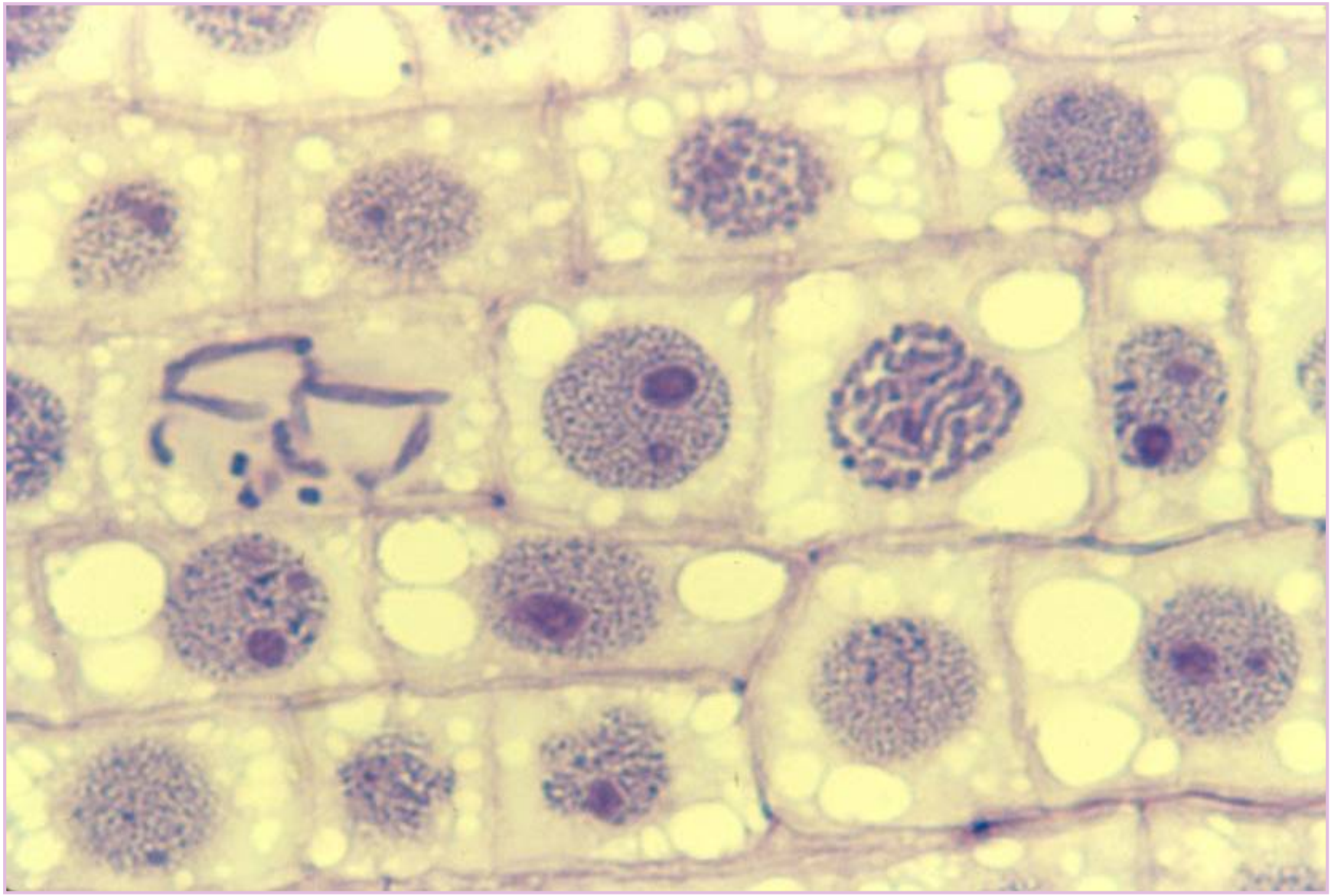


Department of Pathology
Cell Biology Unit

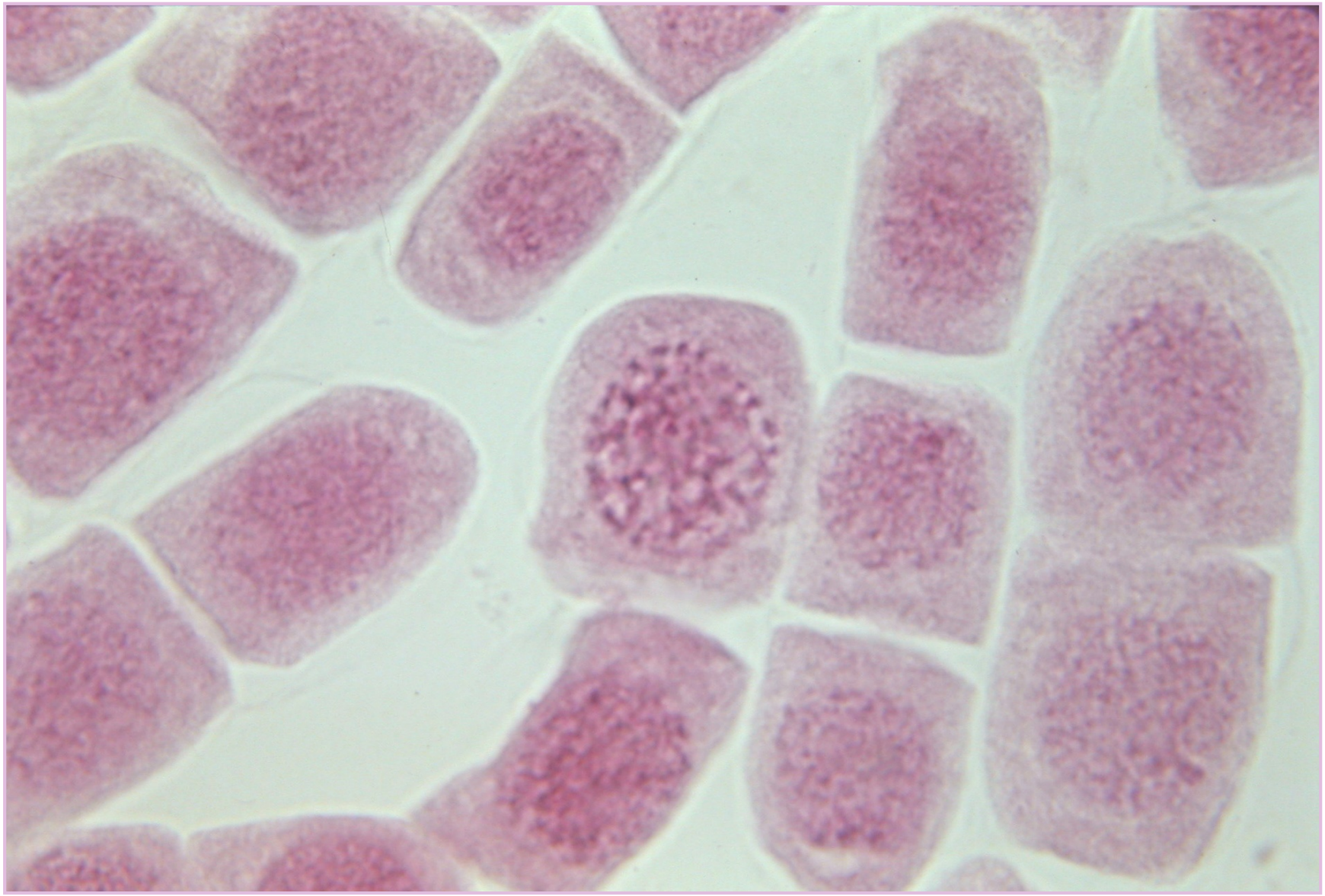
OBJECTIVES

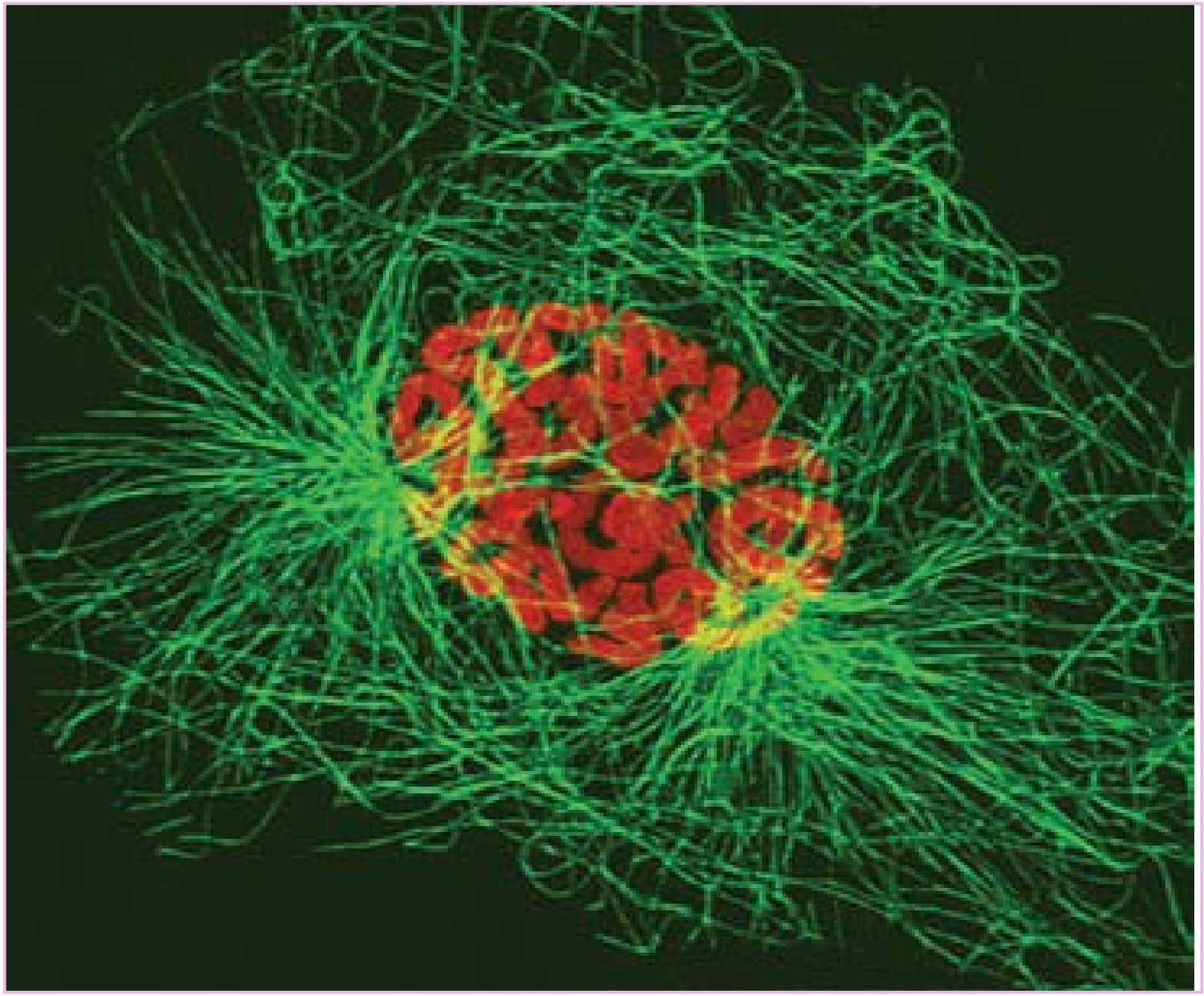
- Identify the different phases of mitosis.
- Analyze the morphology of human chromosomes.
- Analyze the chromosomes of an experimental rat tumor, observing their variability in number and morphology.

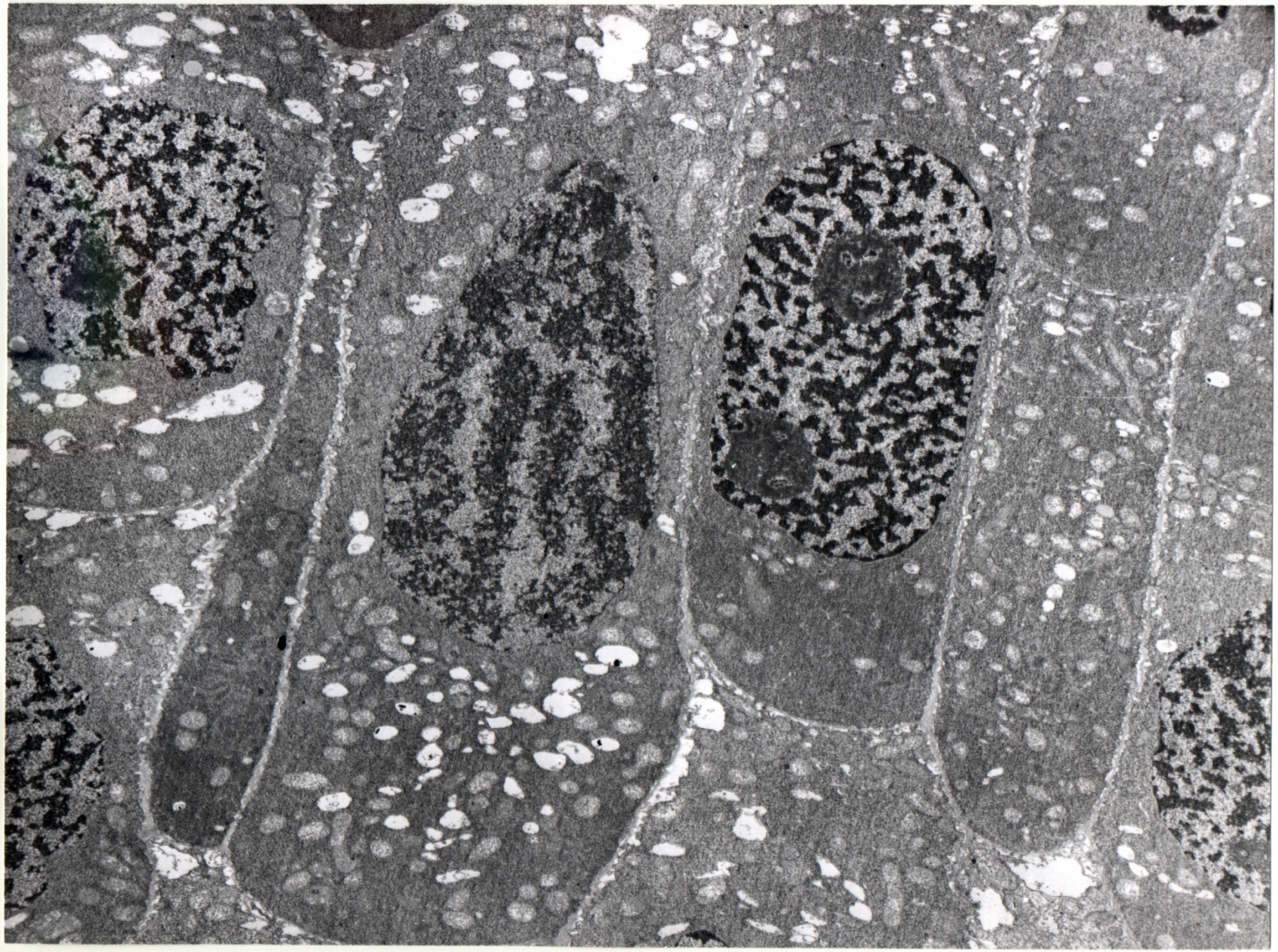
INTERPHASE

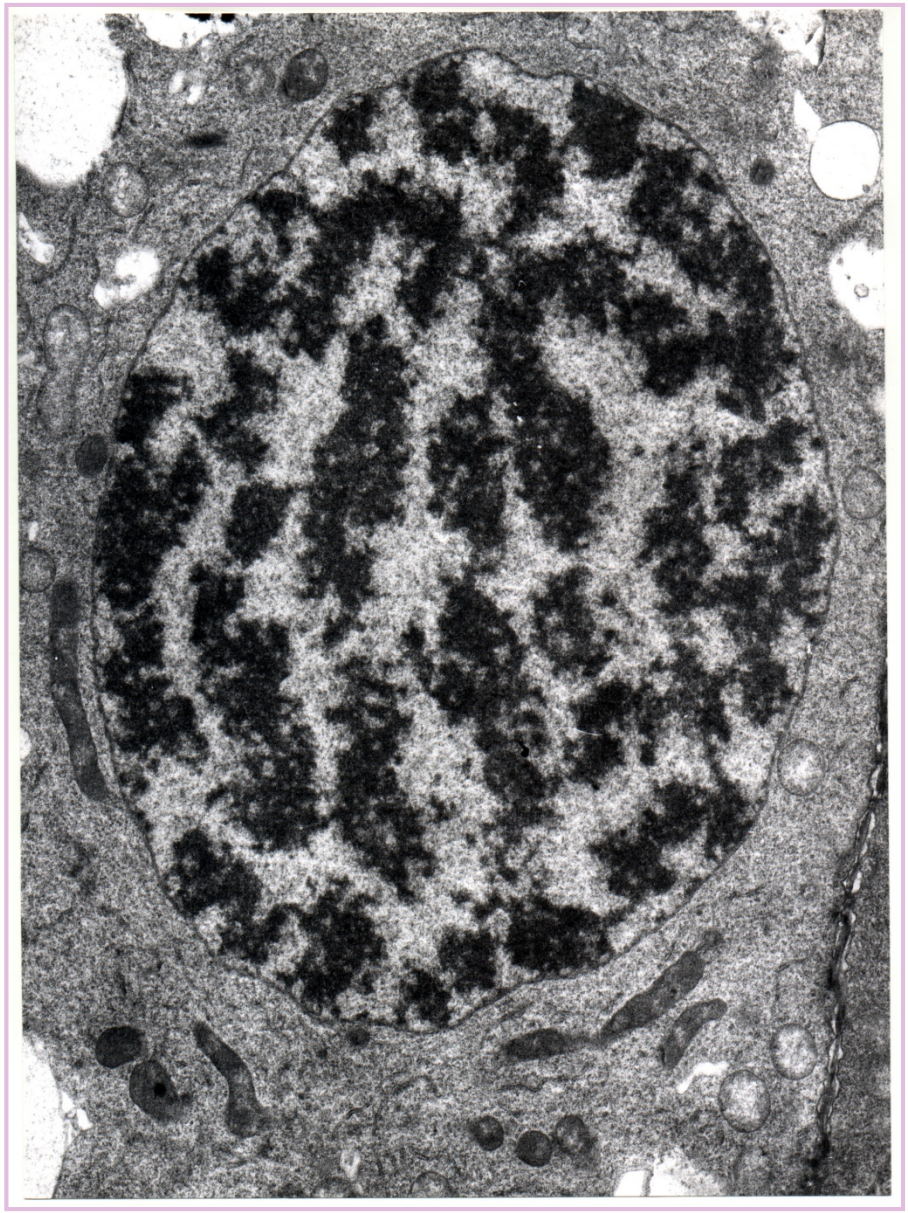


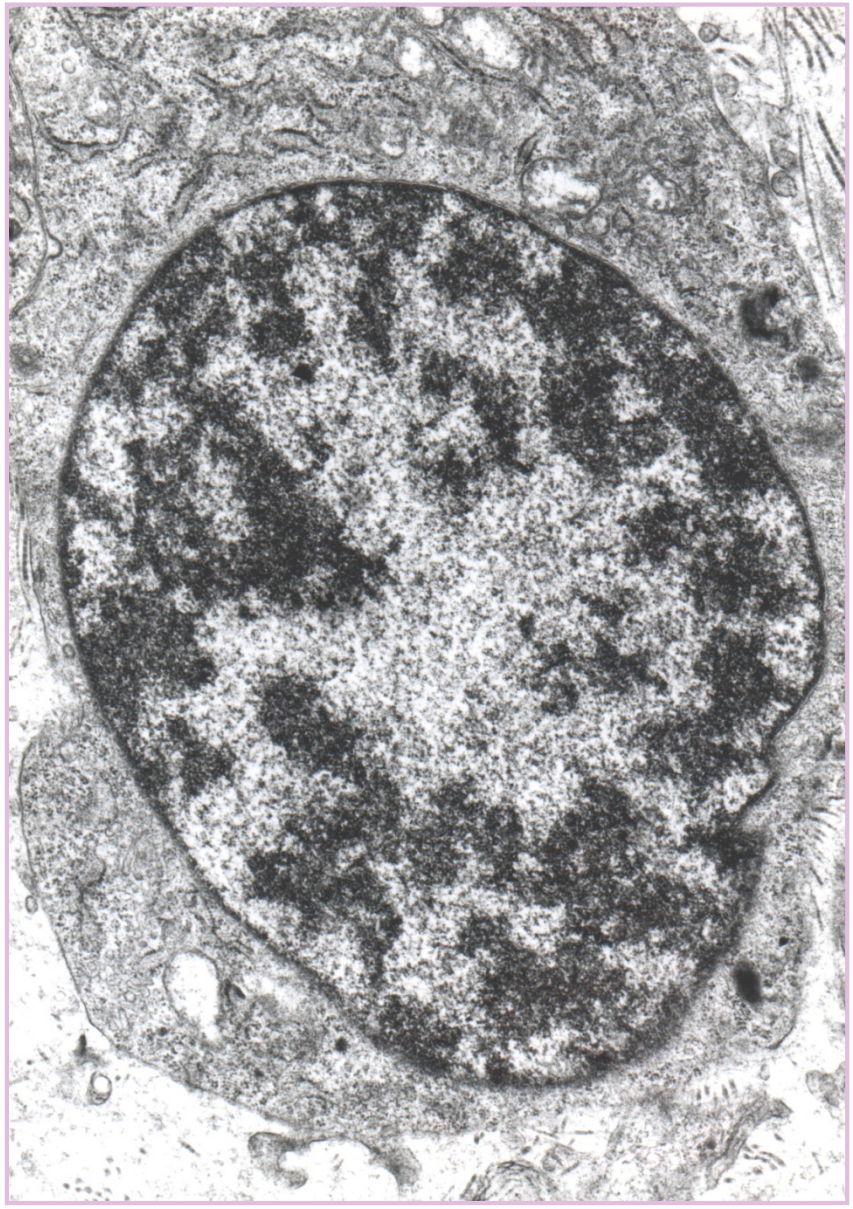
PROPHASE











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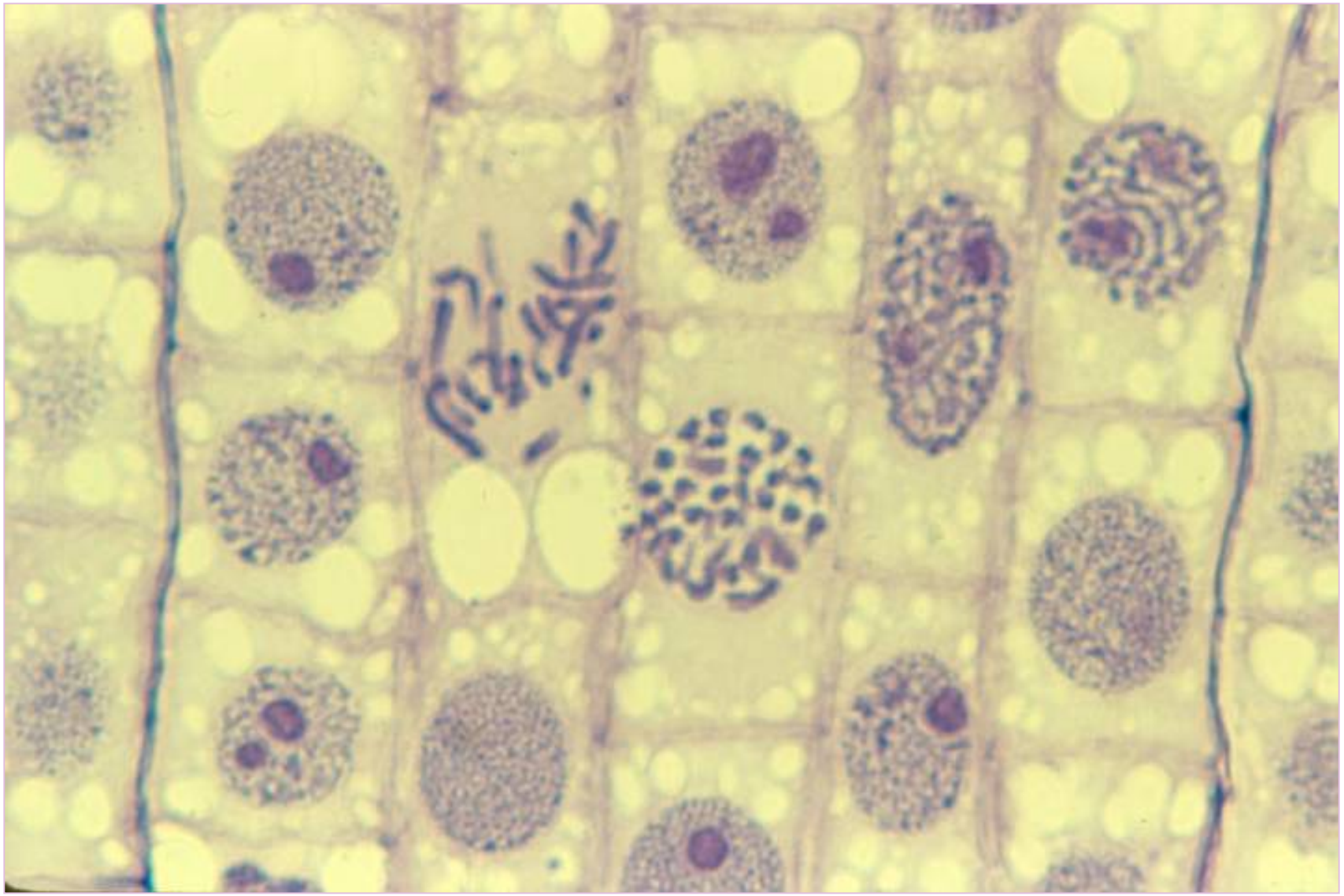

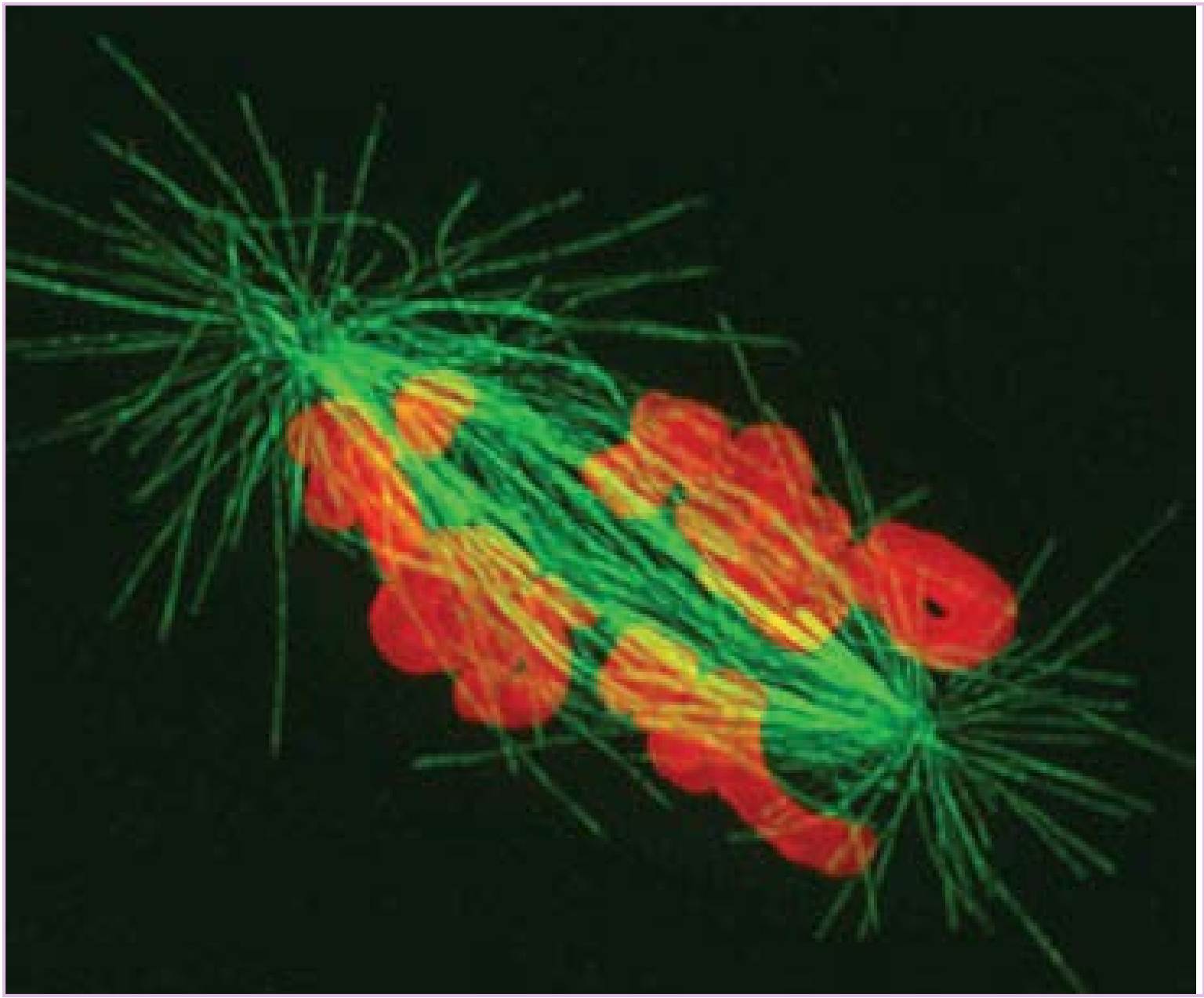

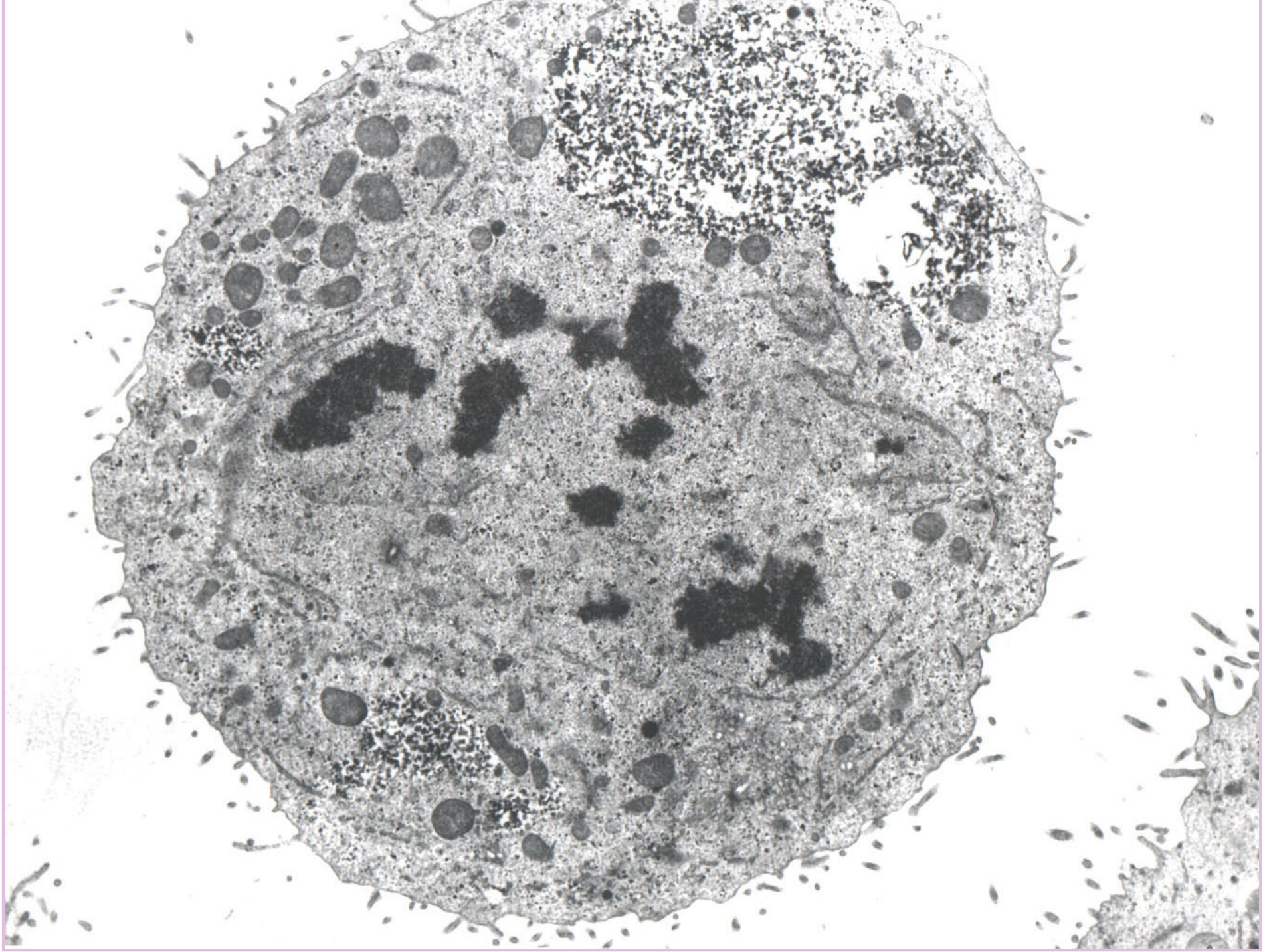

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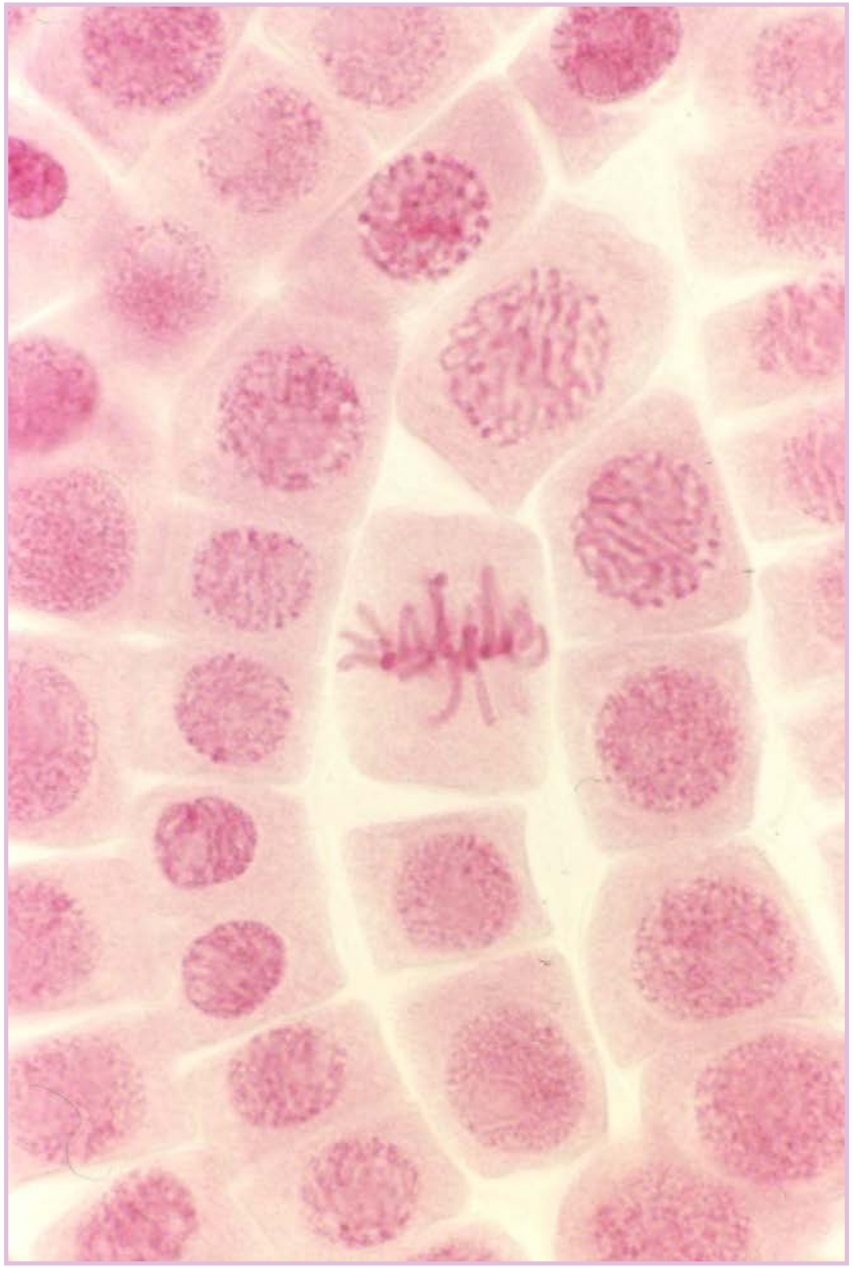

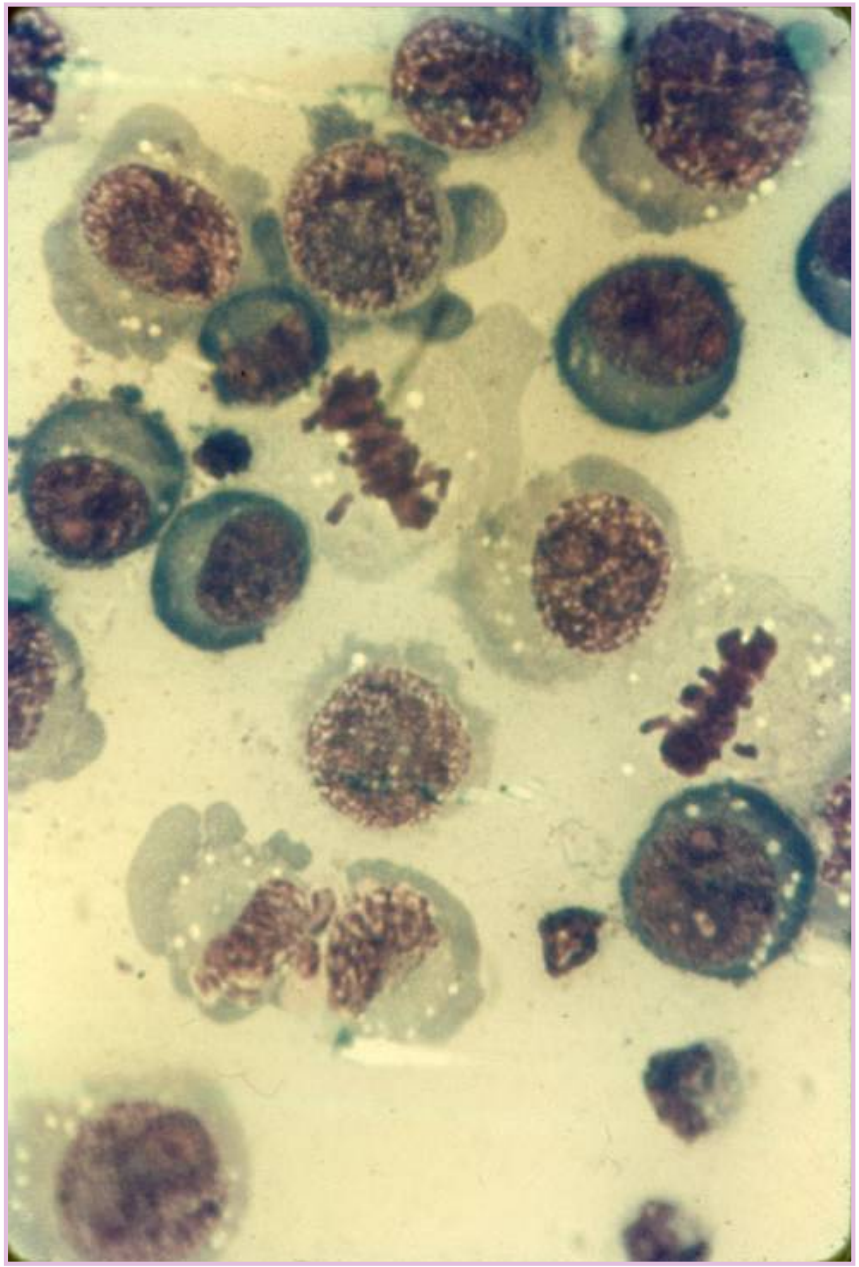

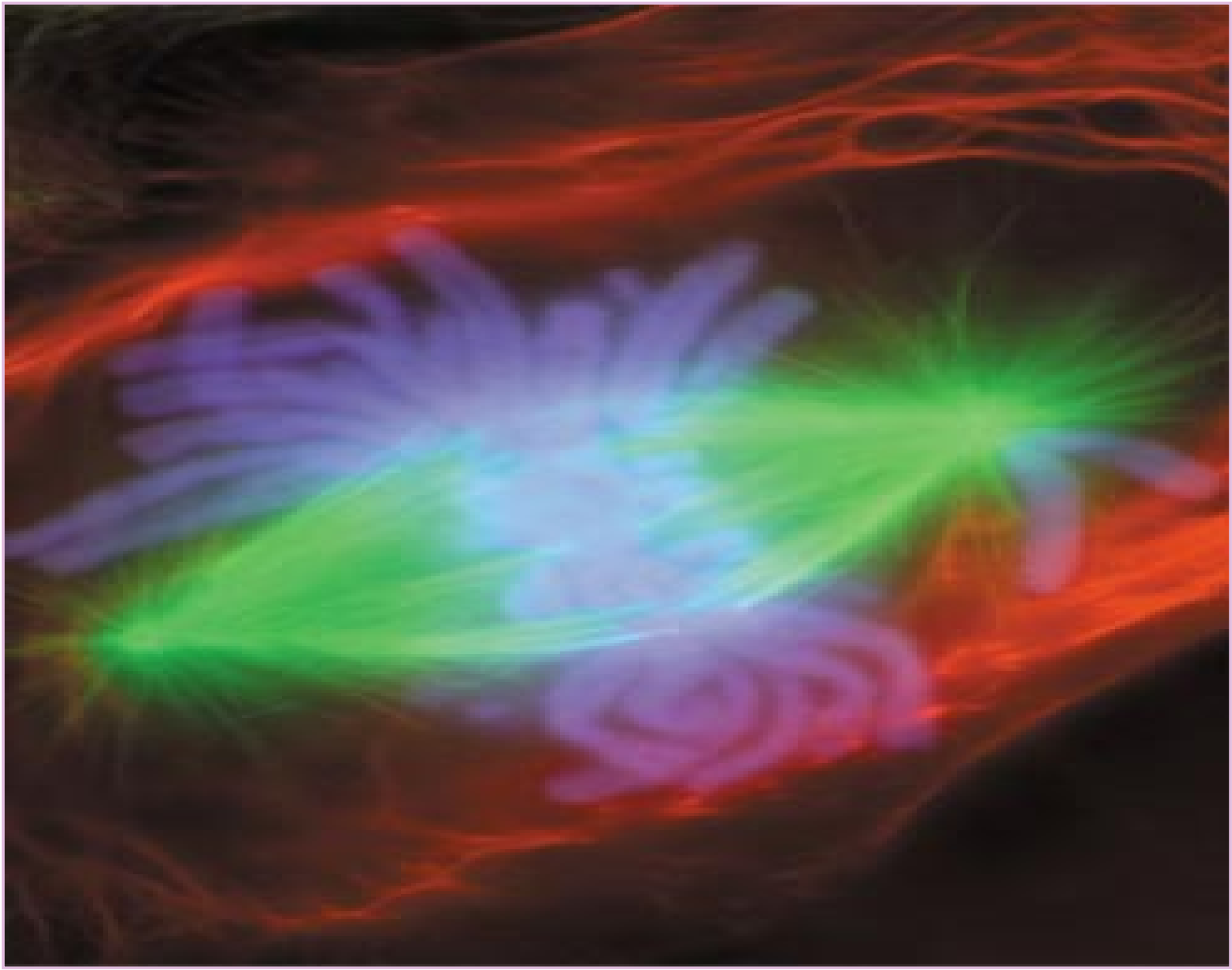

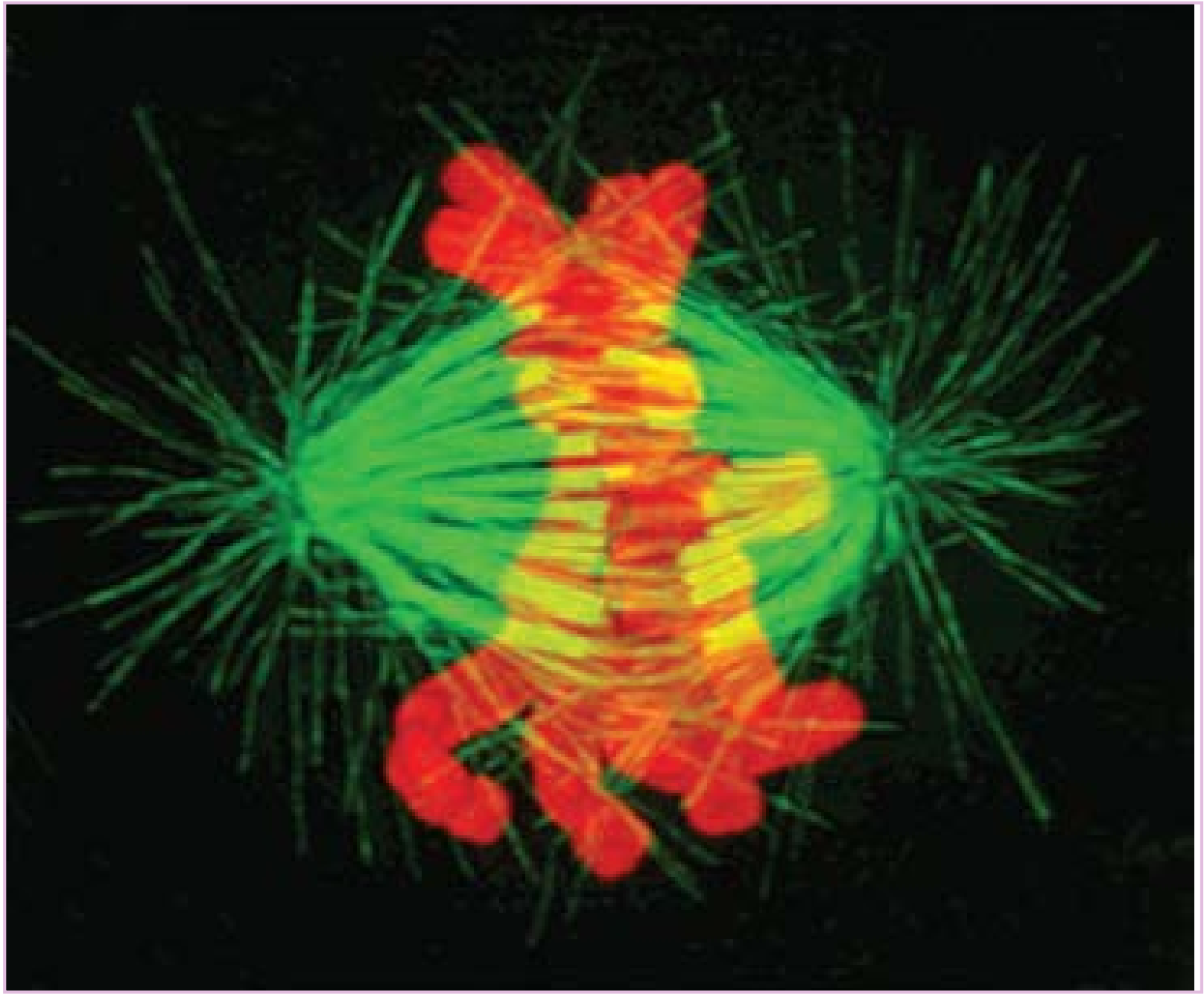

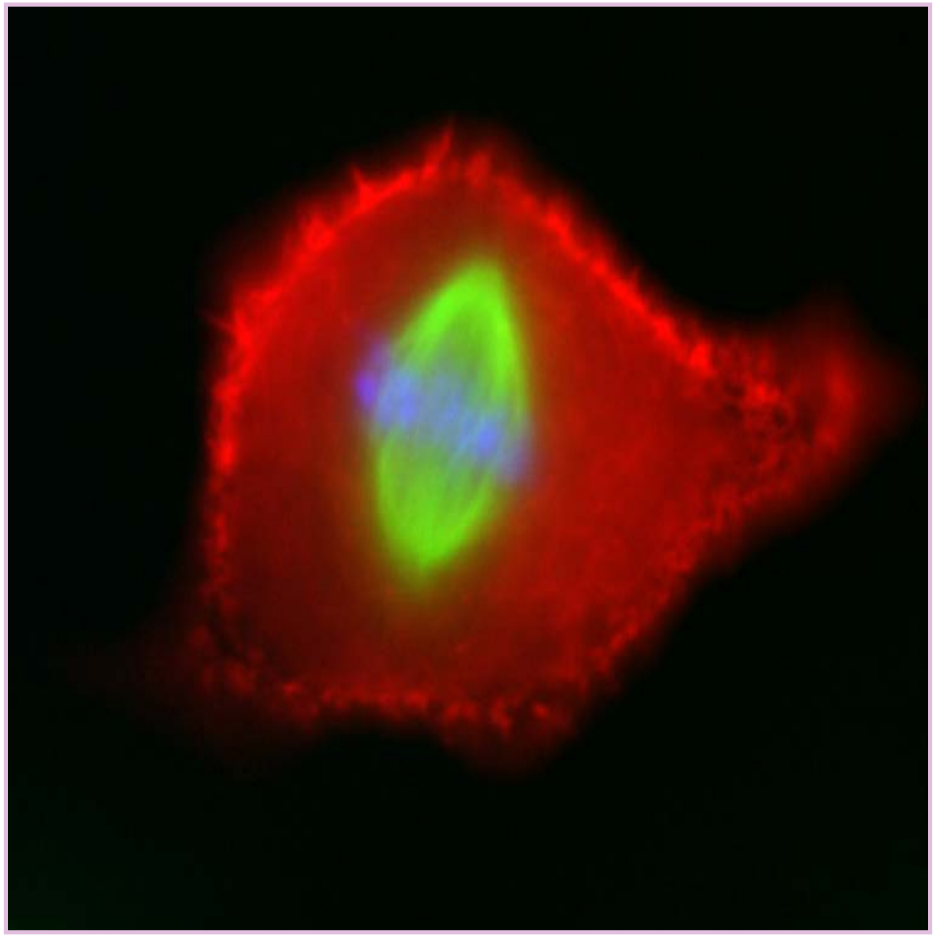

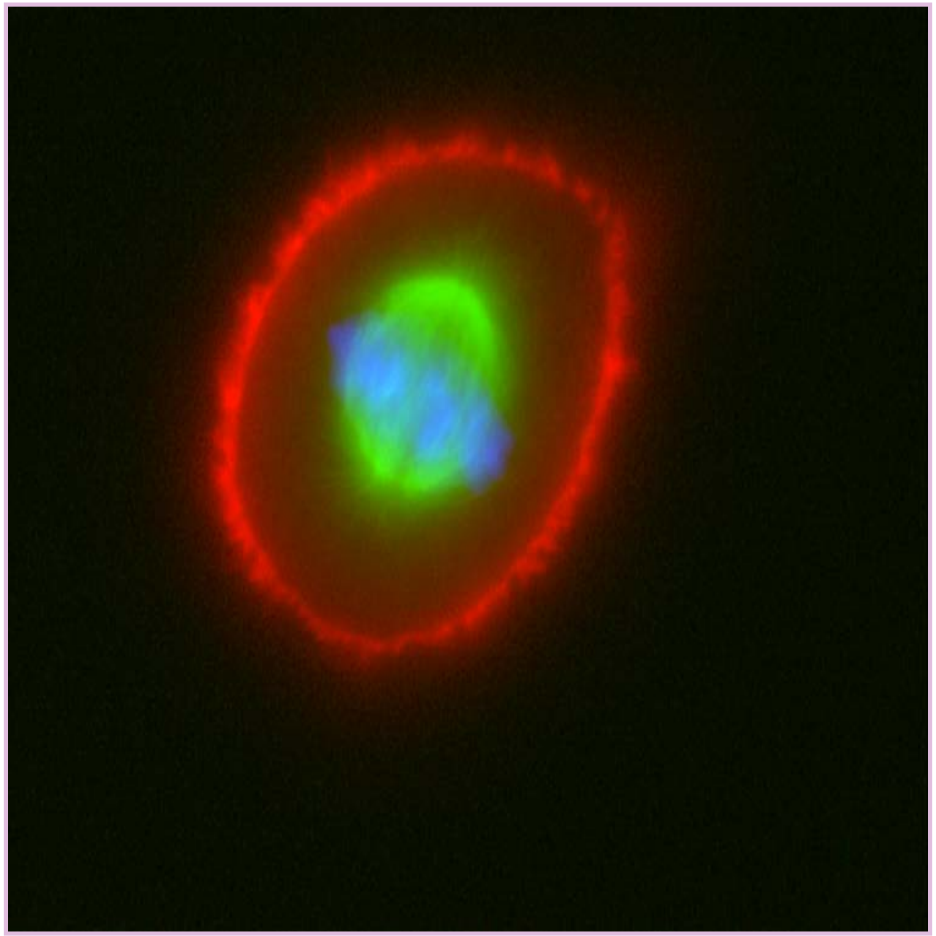

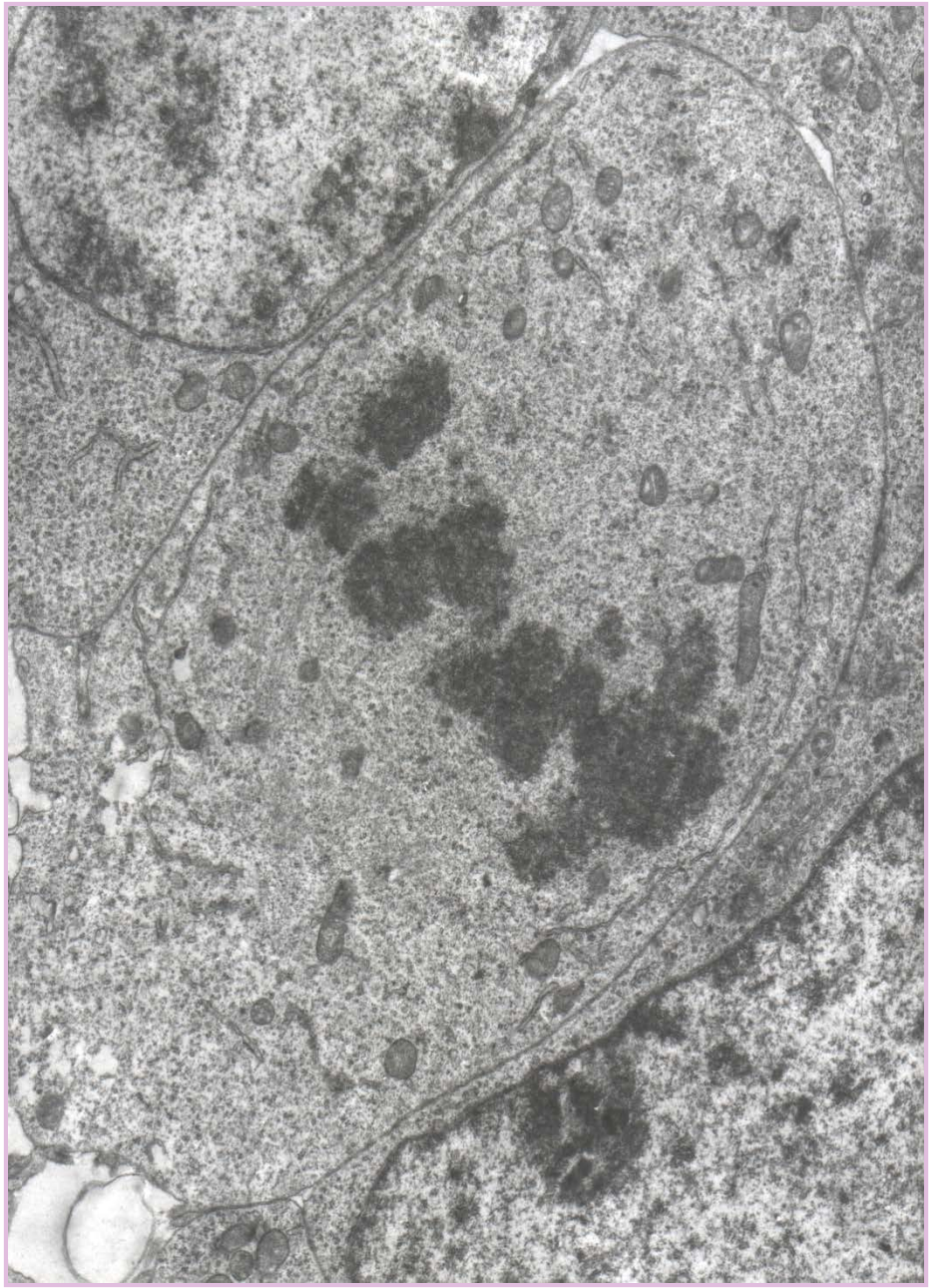

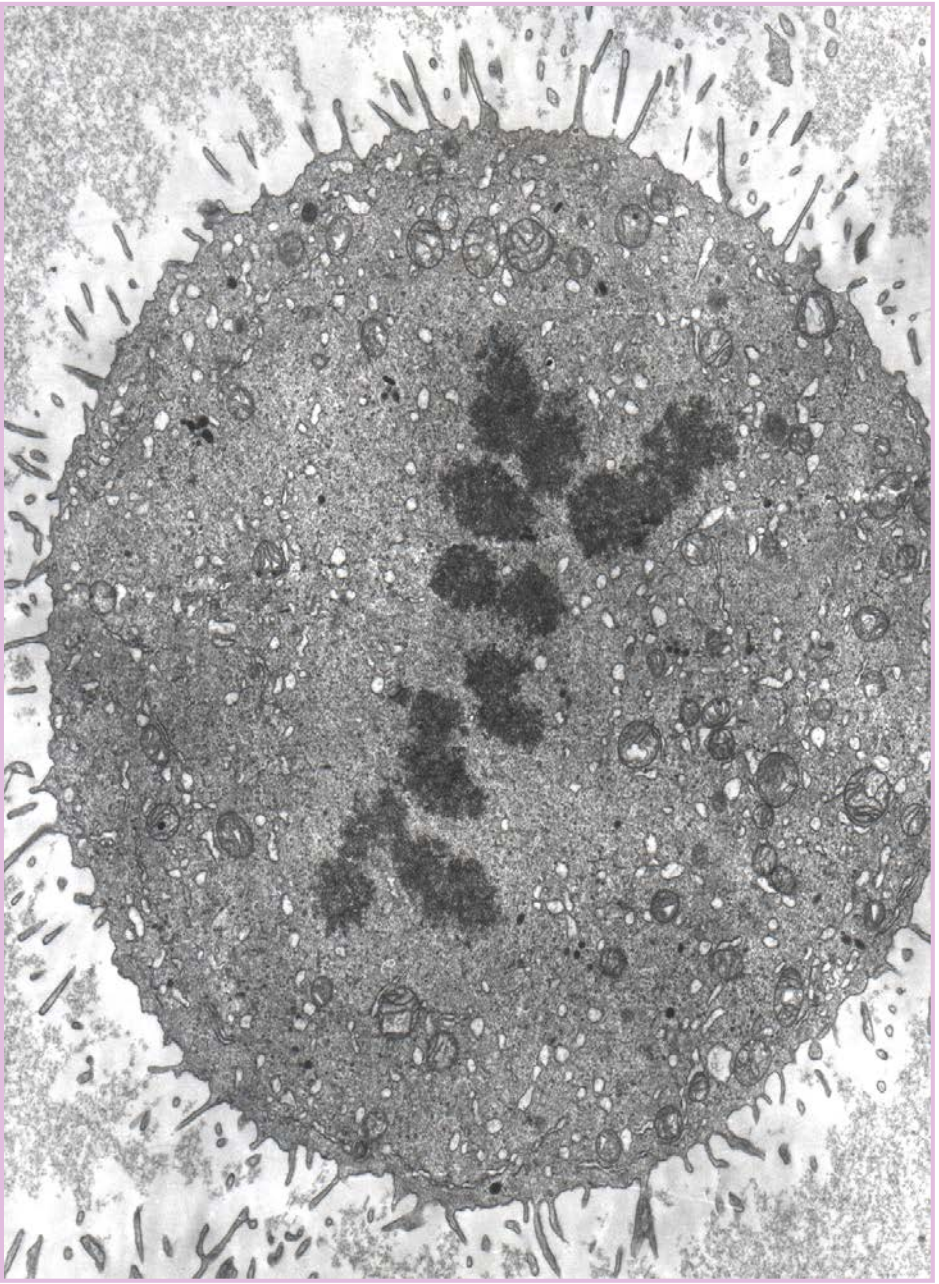

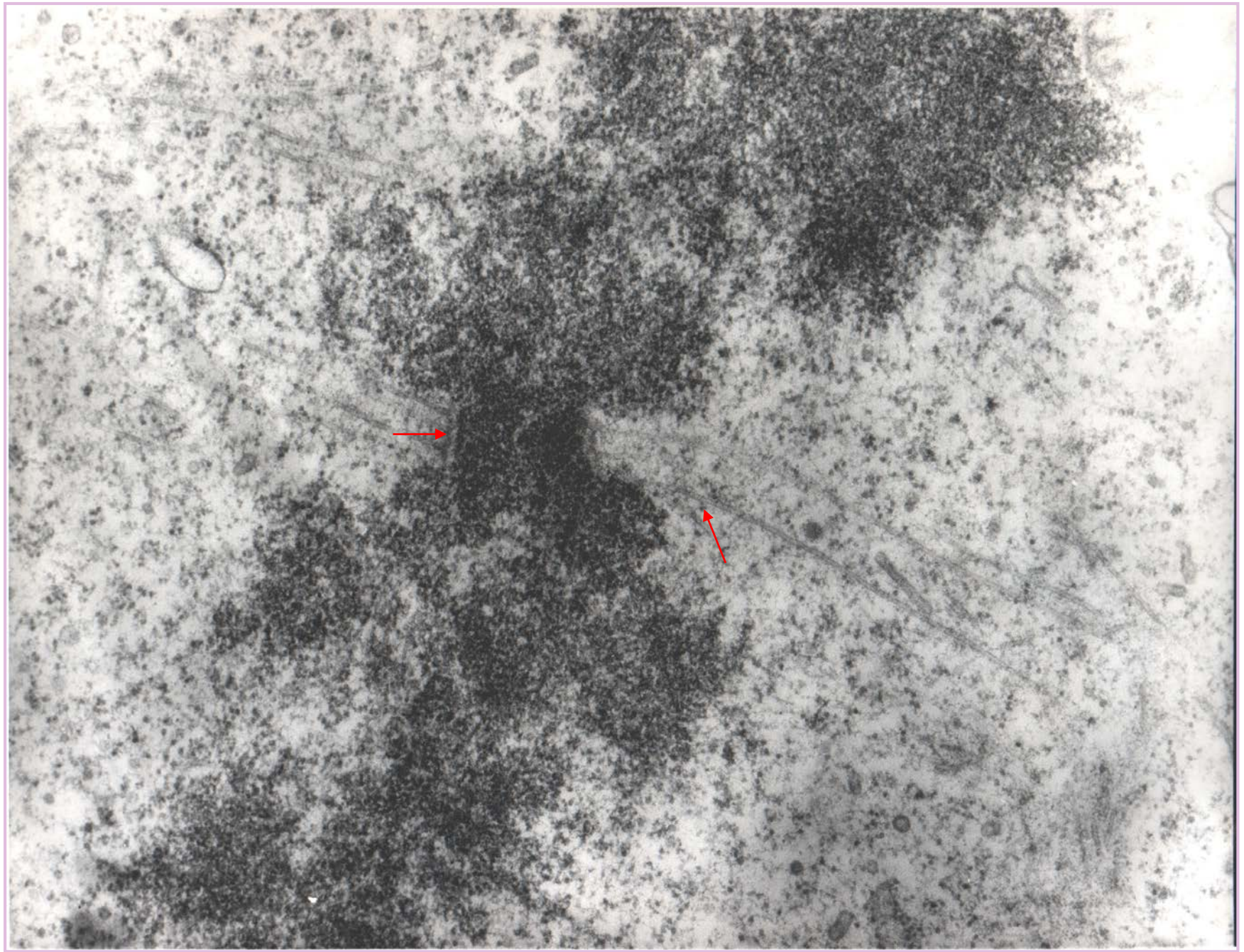

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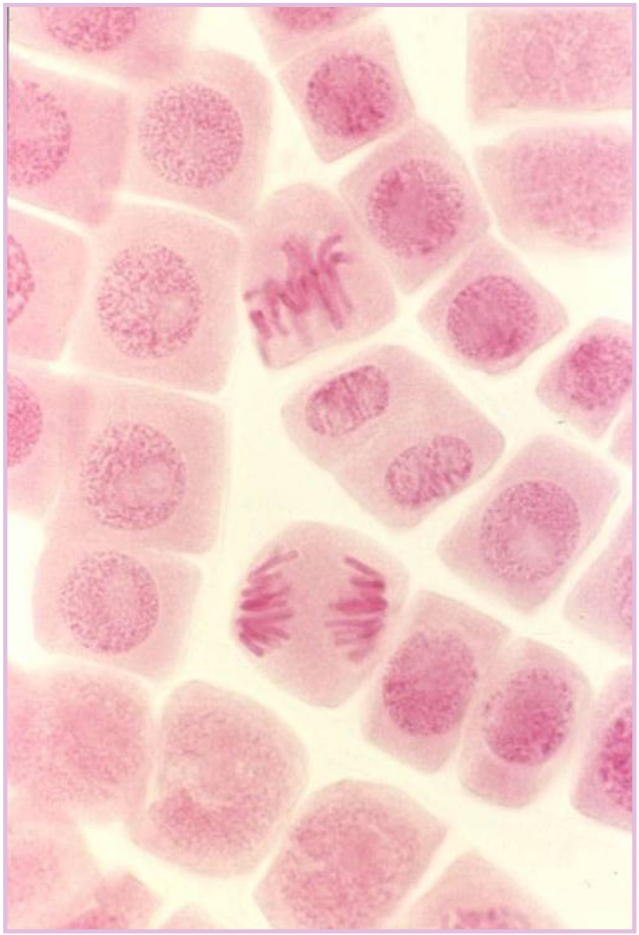

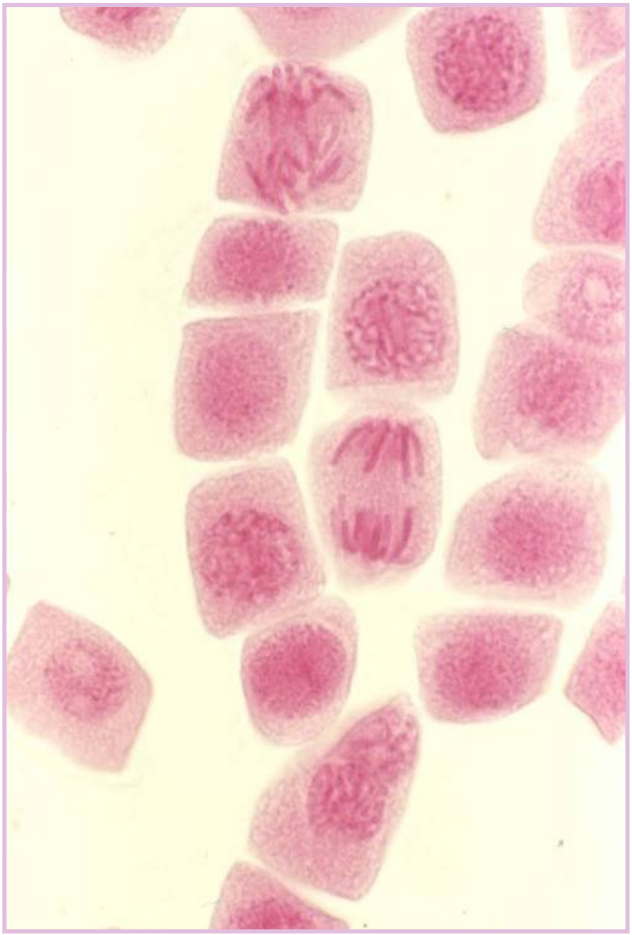

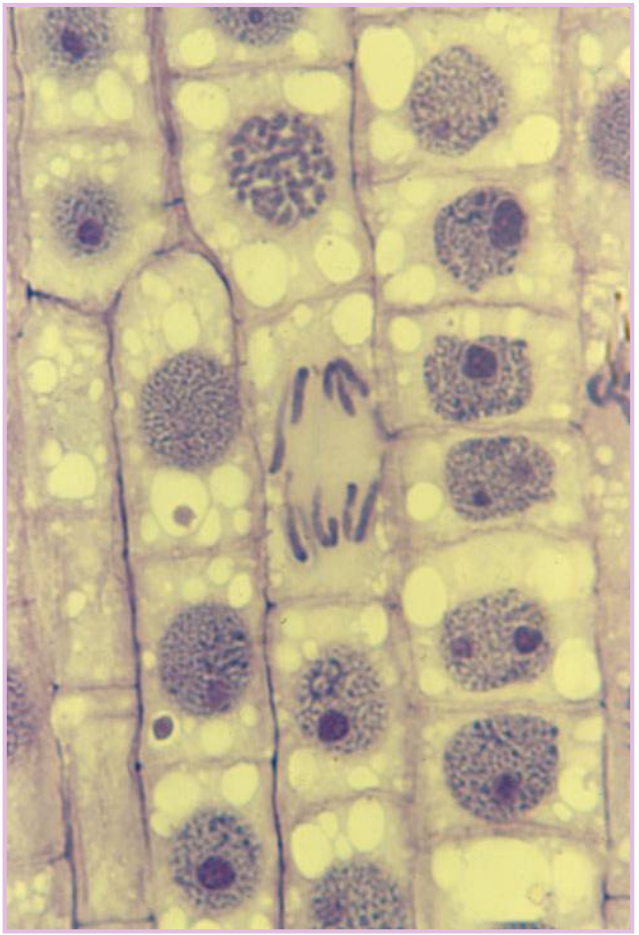

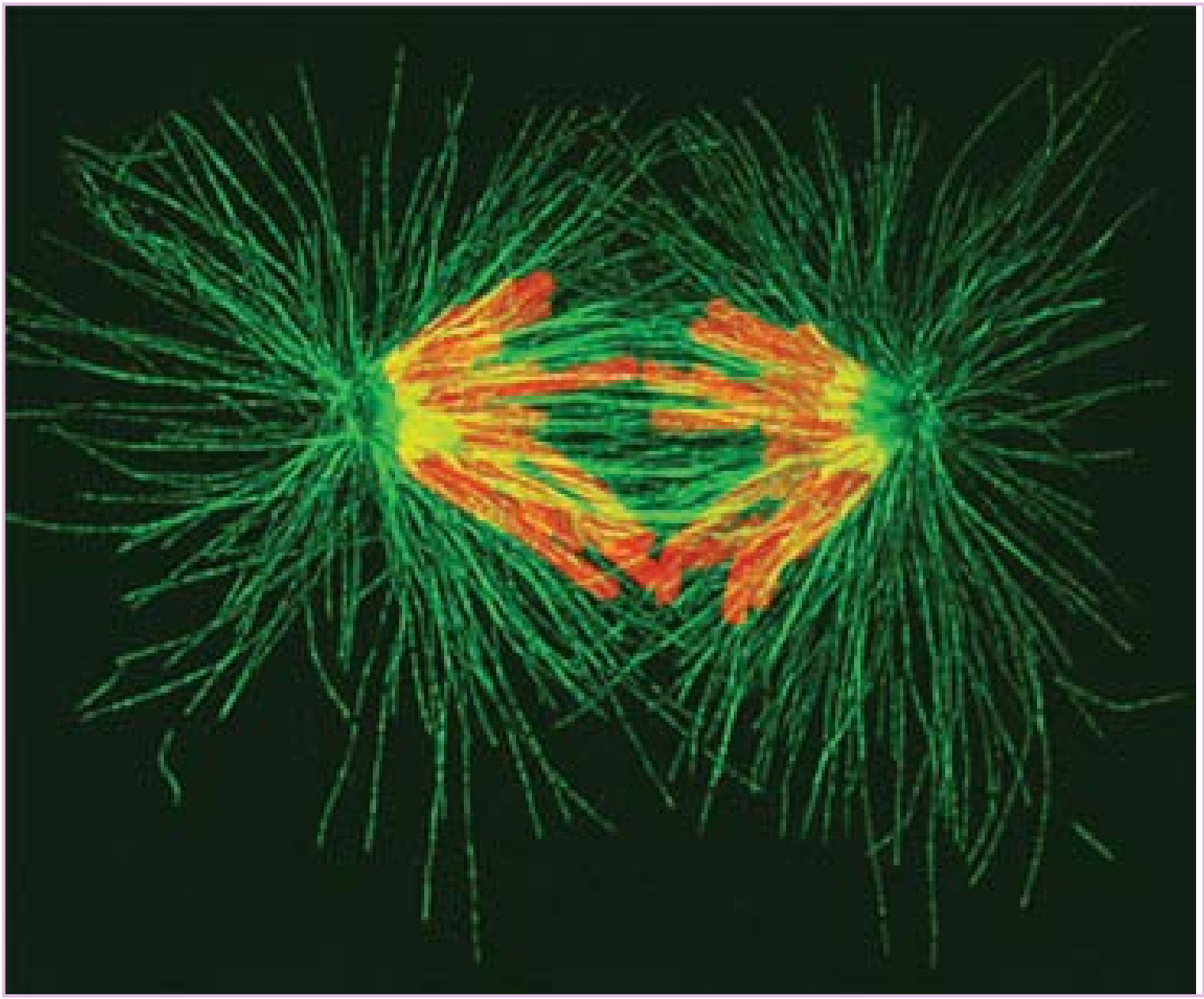

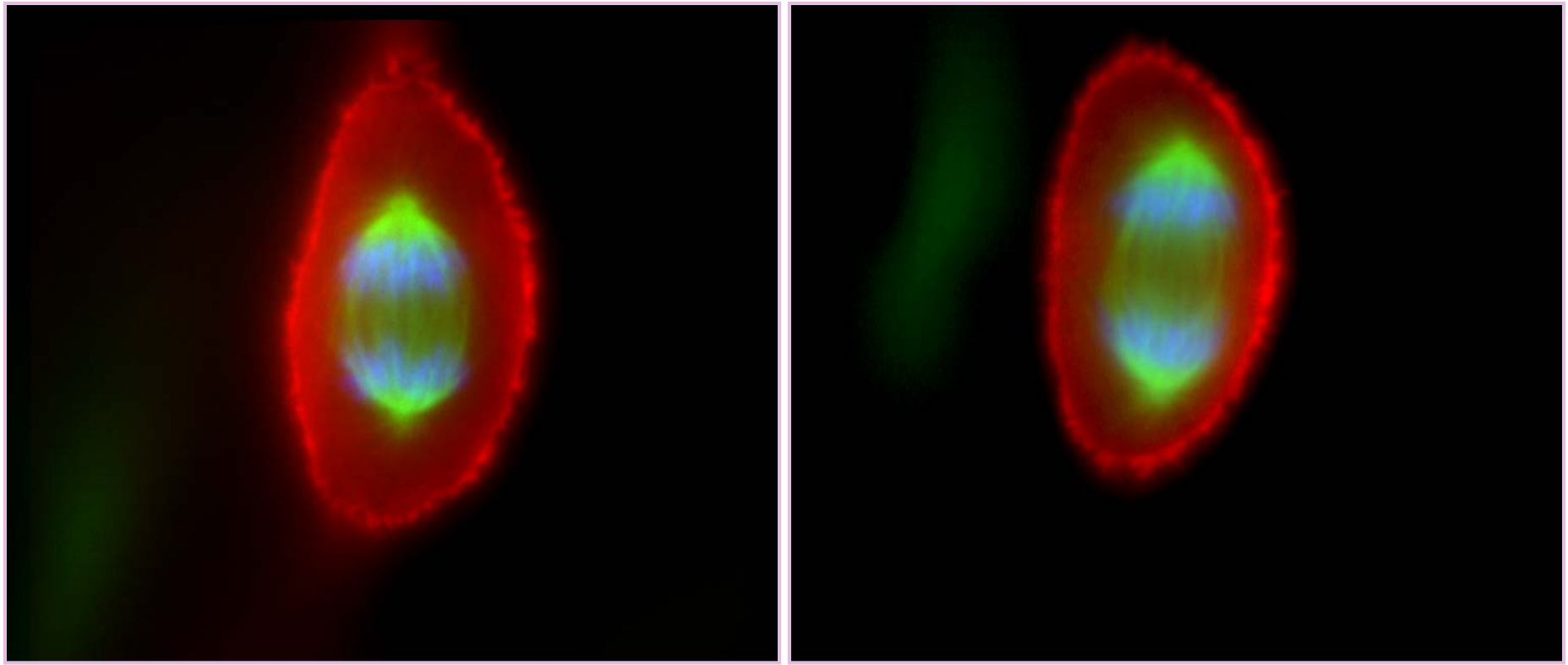

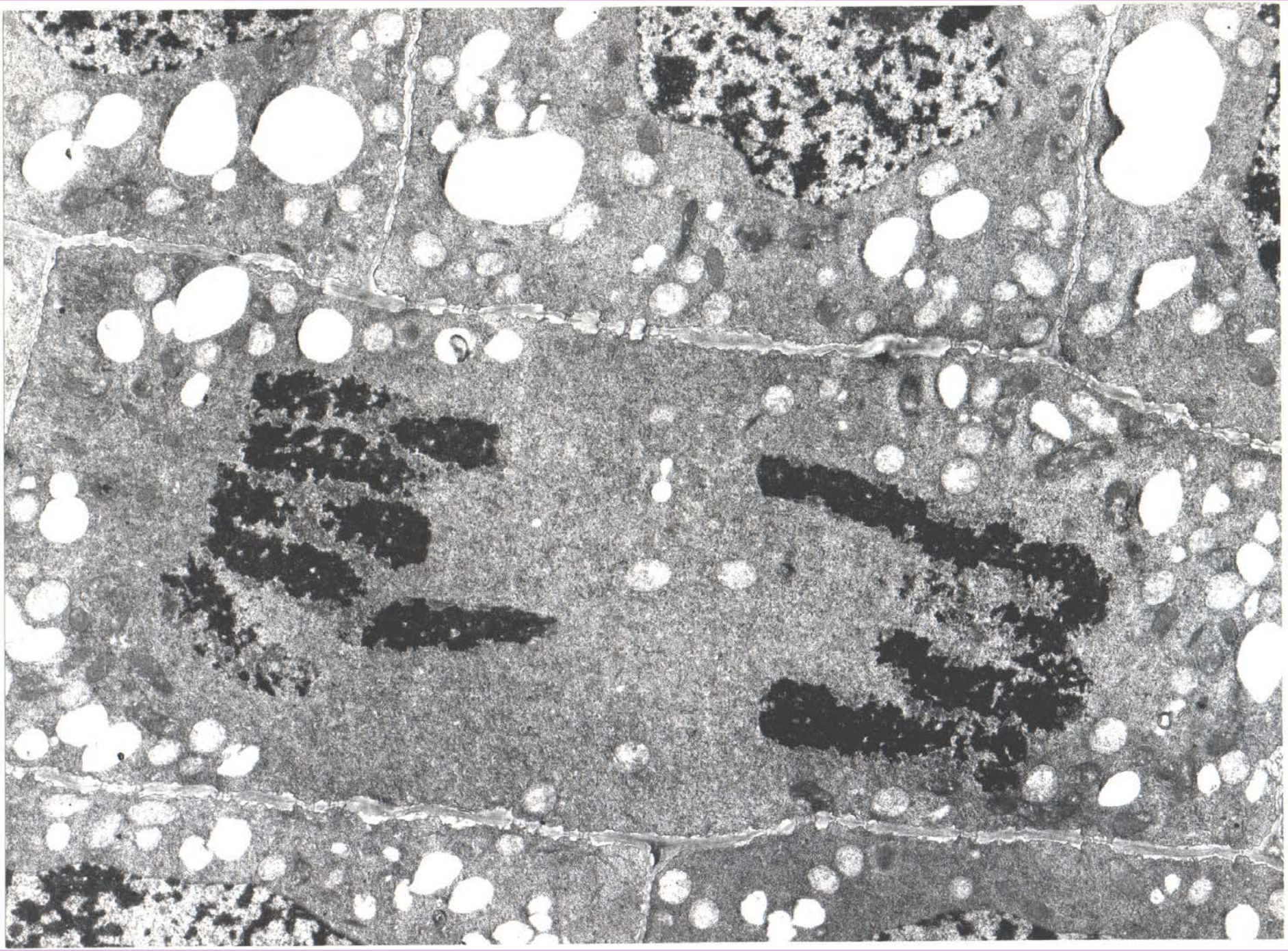

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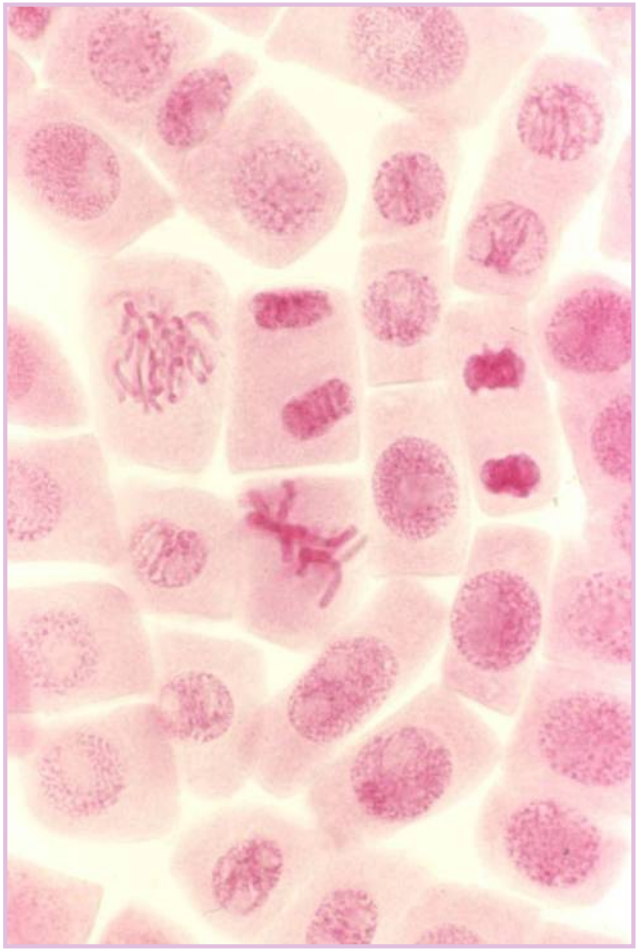
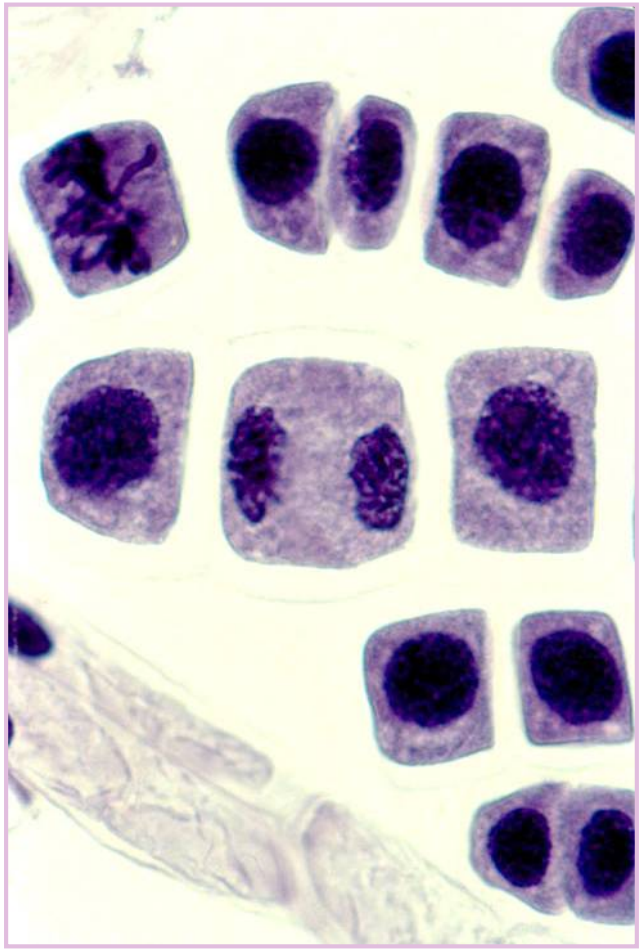
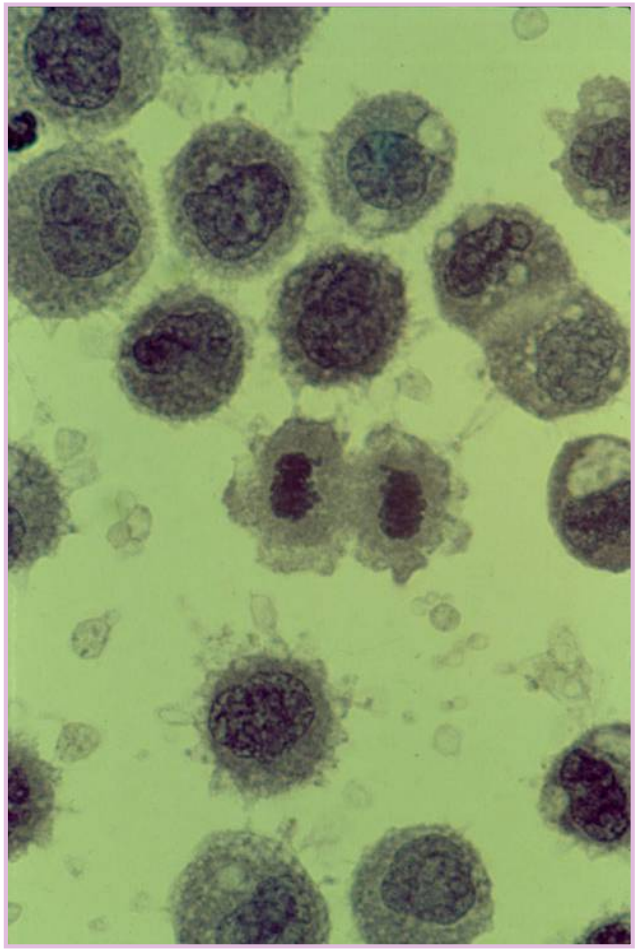
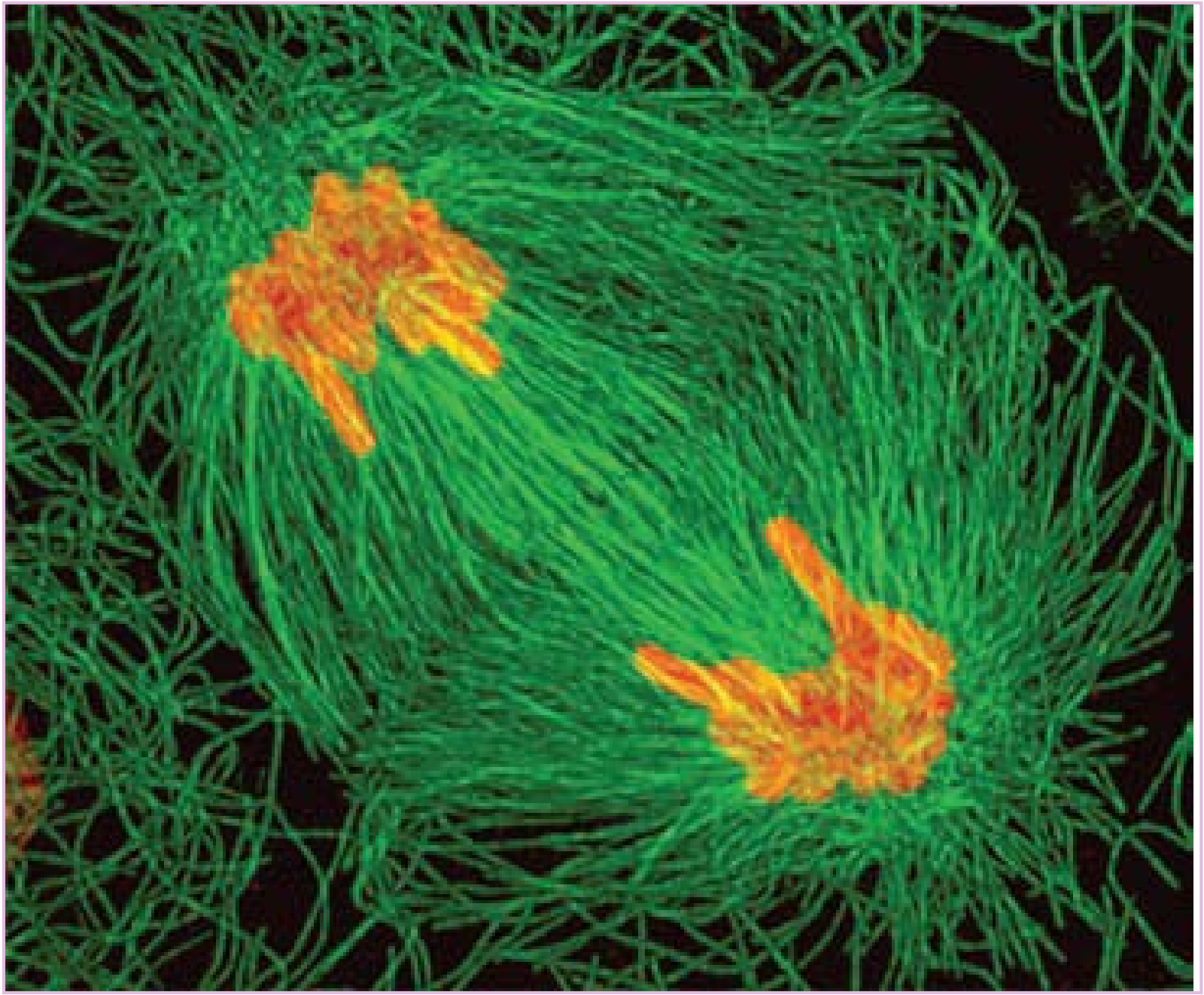
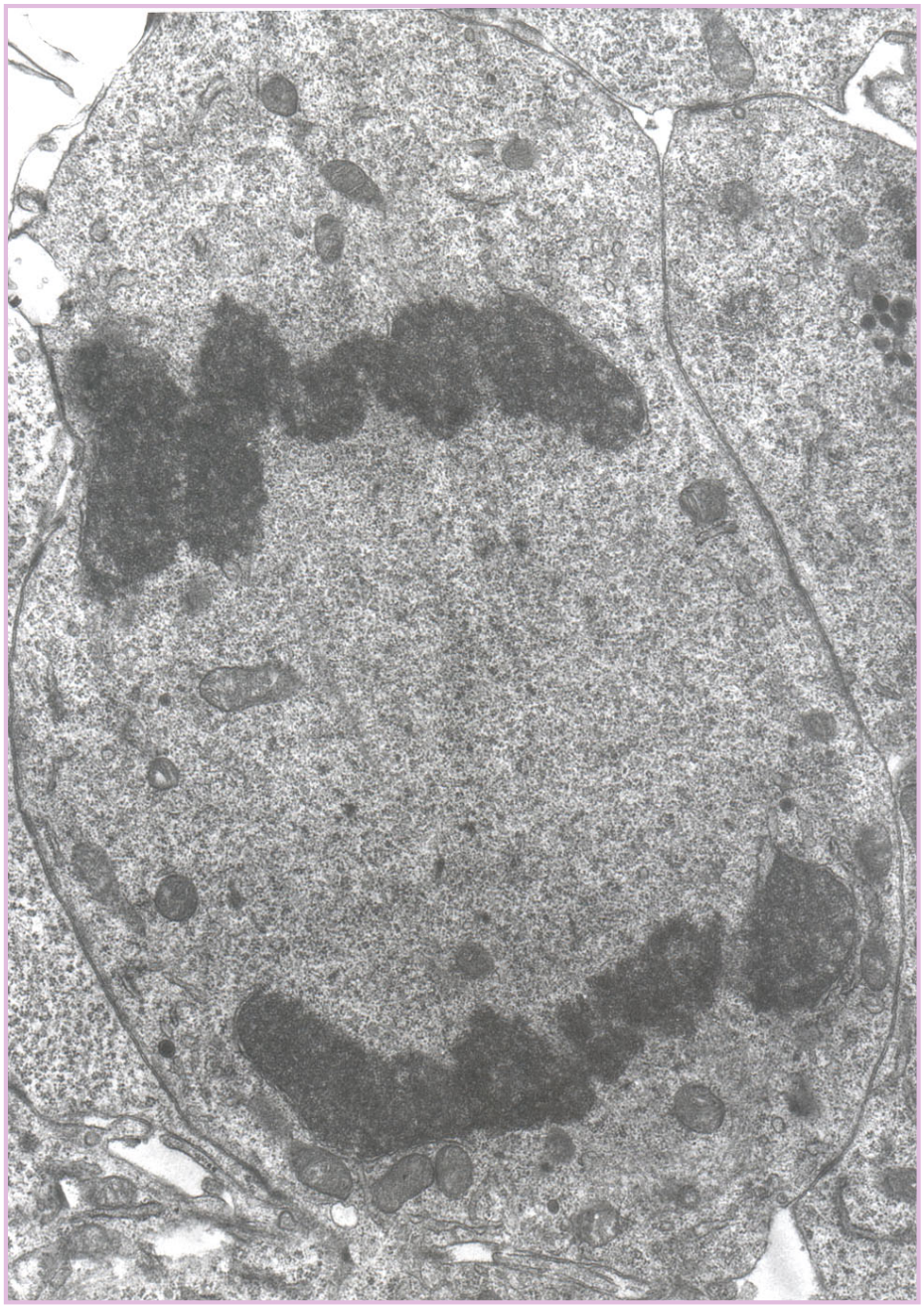
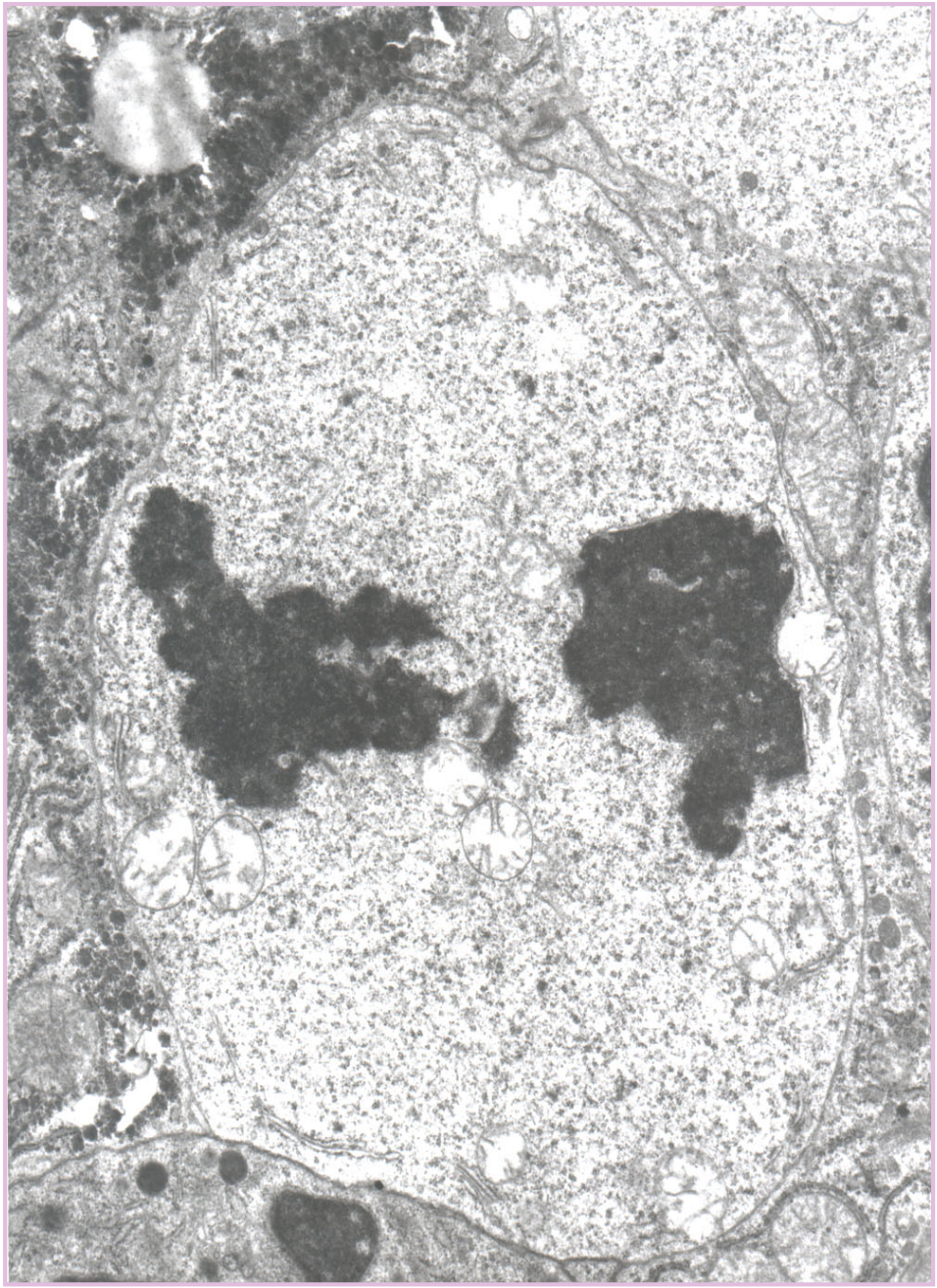




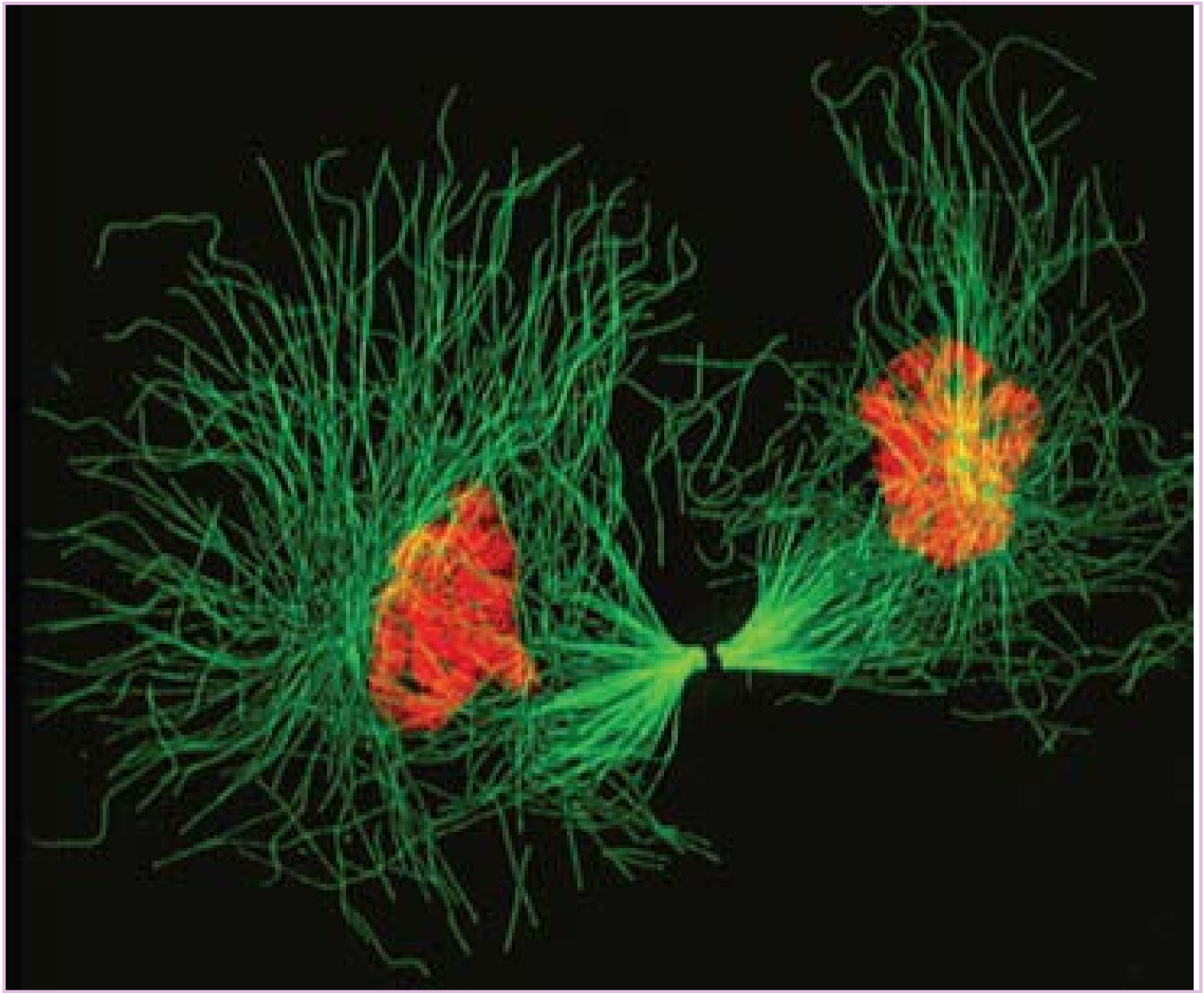


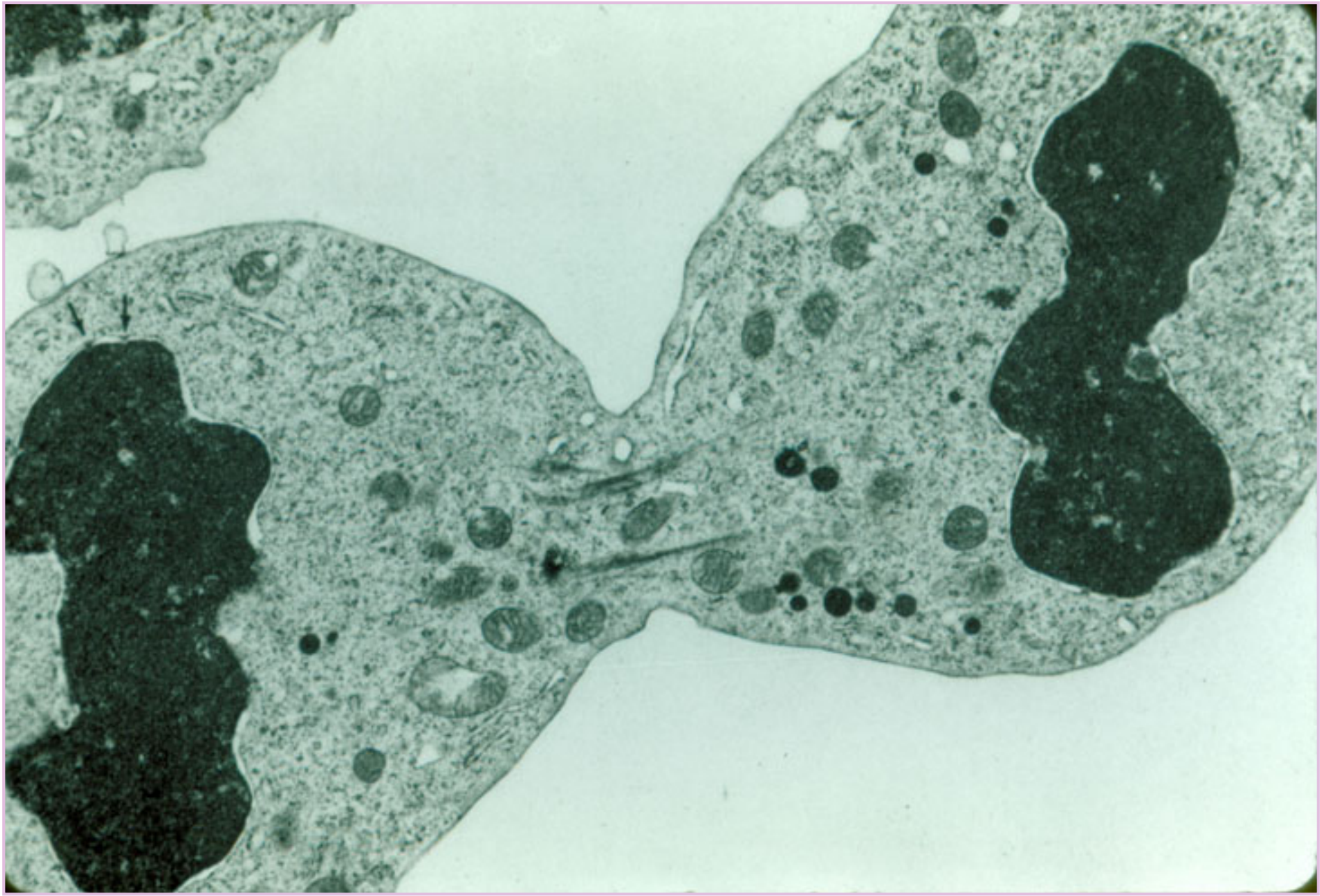


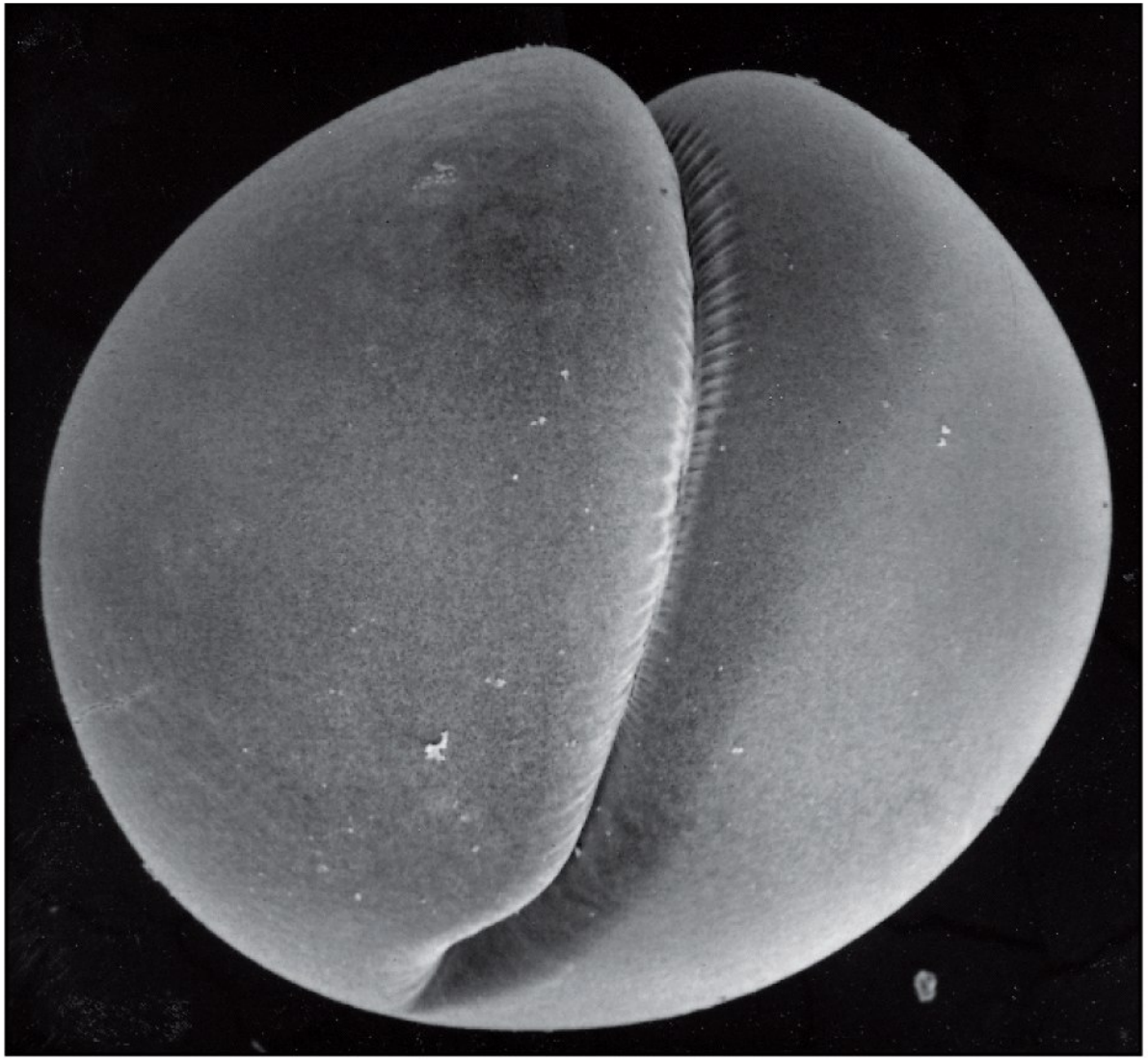


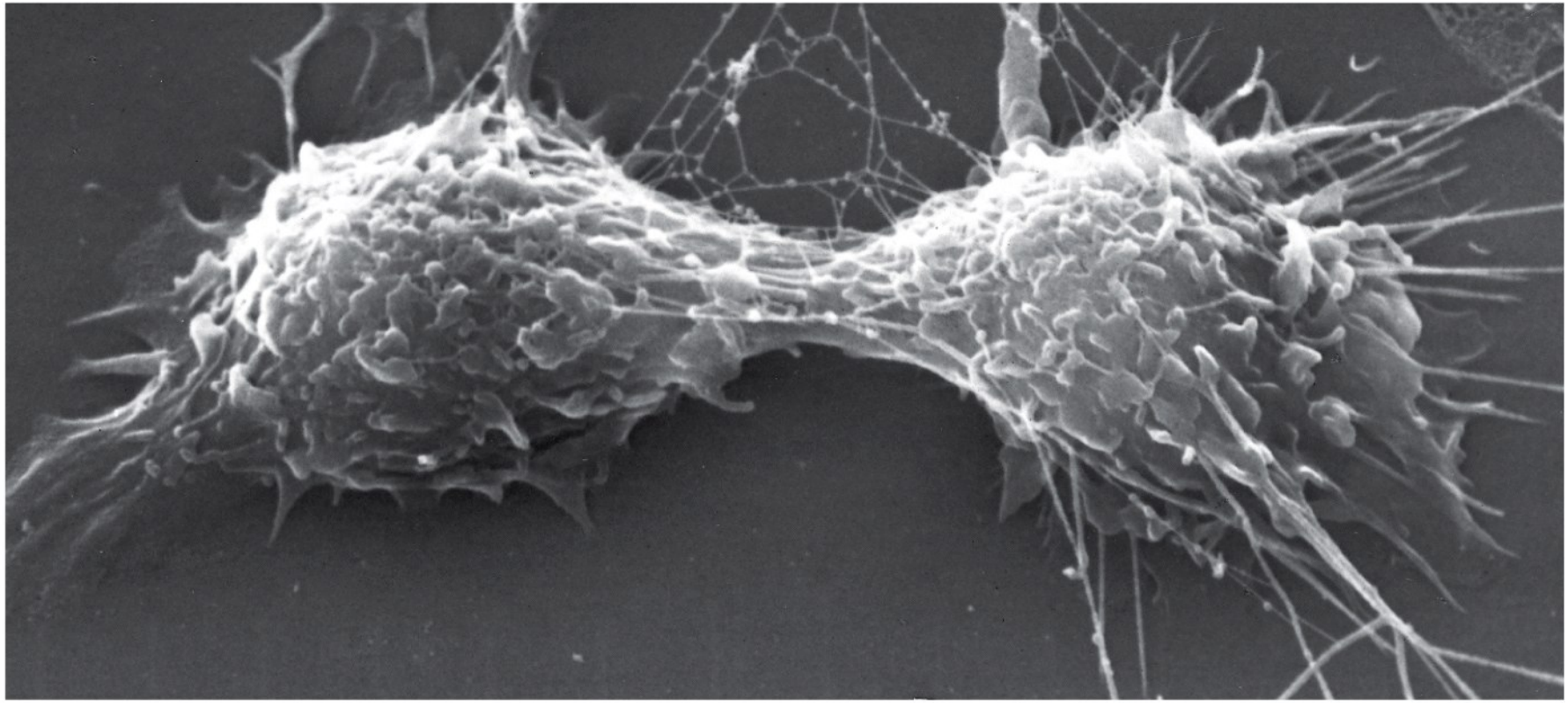


CYTOKINESIS






200 µm


10 μm

prophase

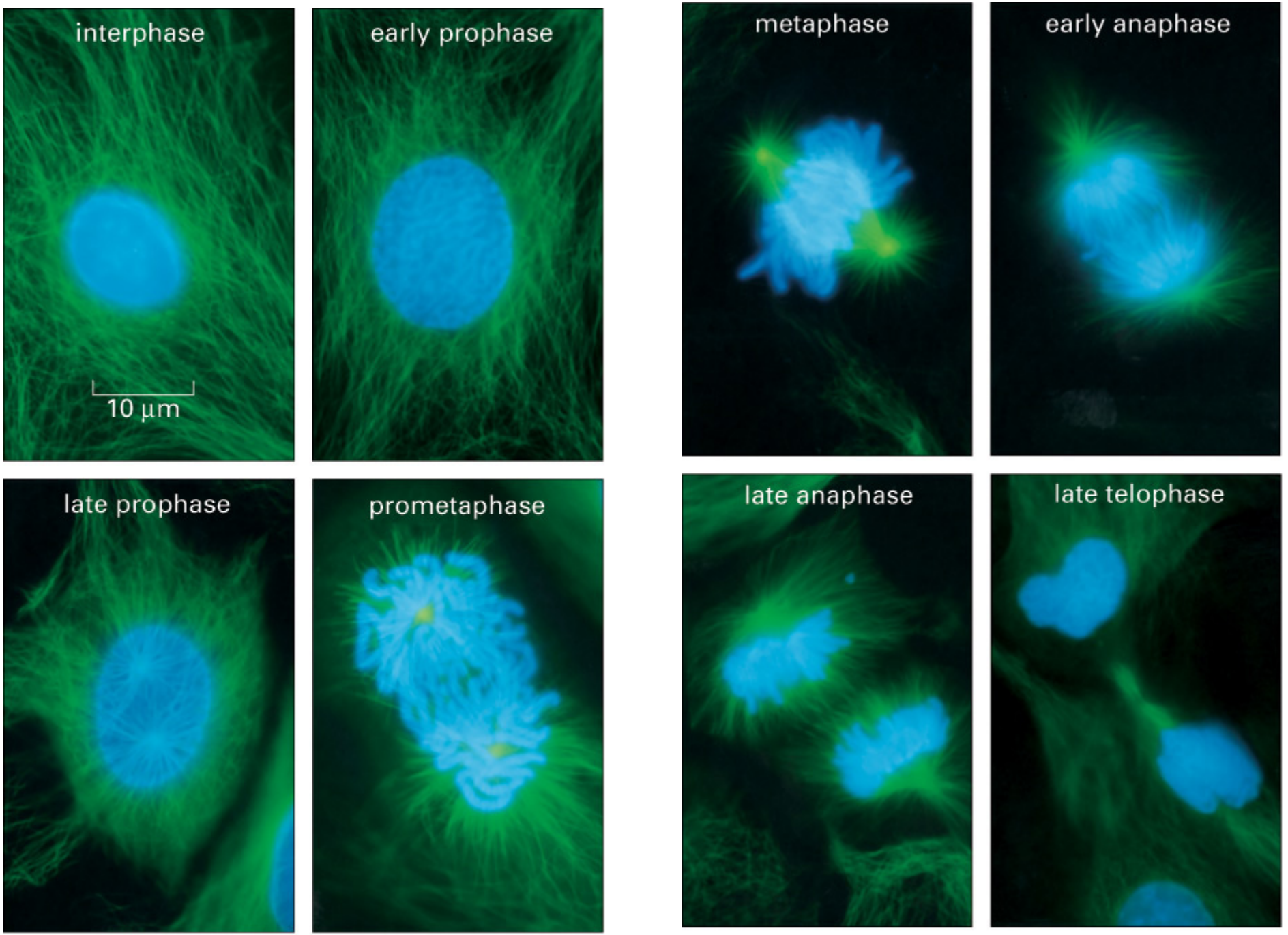
prometaphase

metaphase

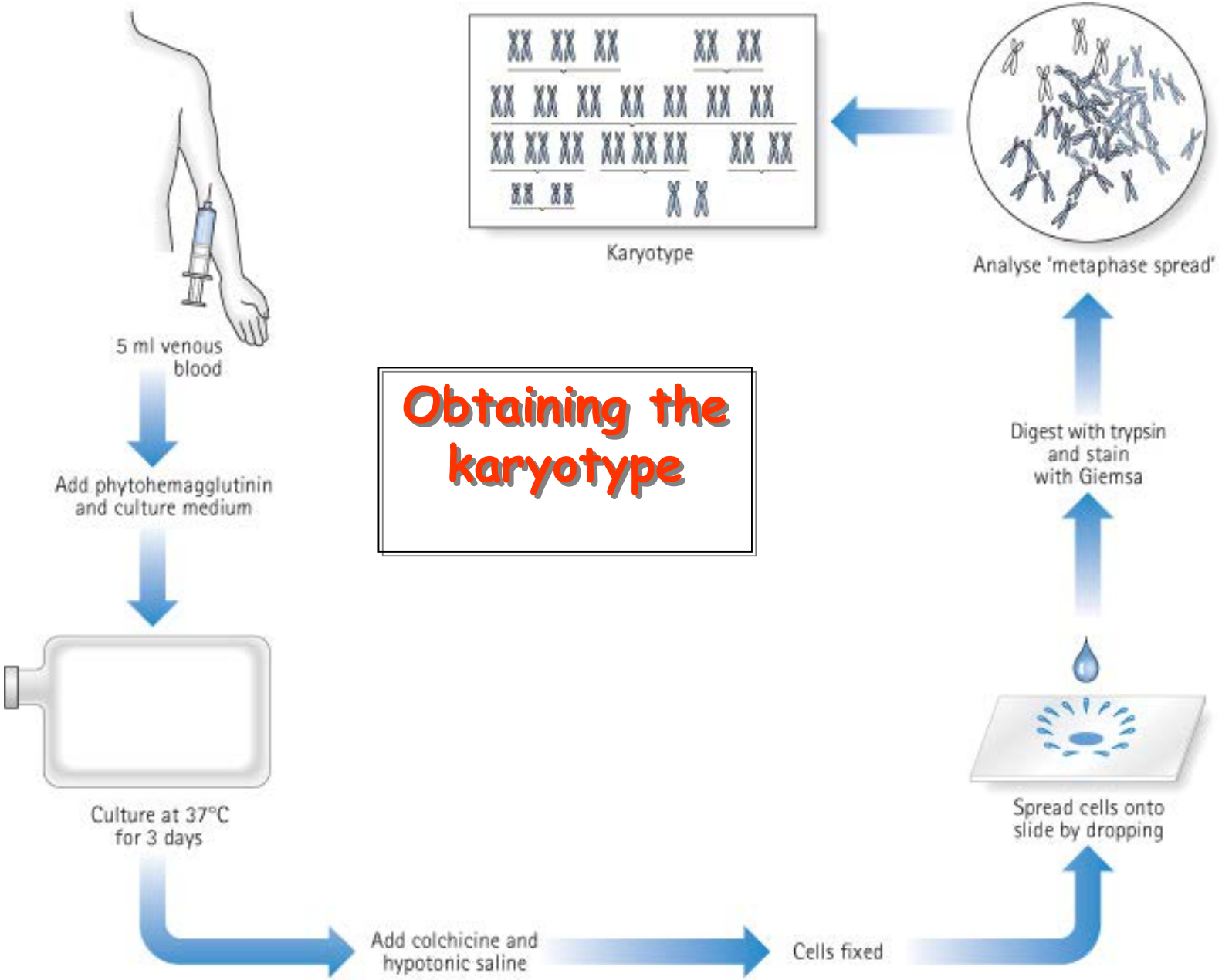
anaphase

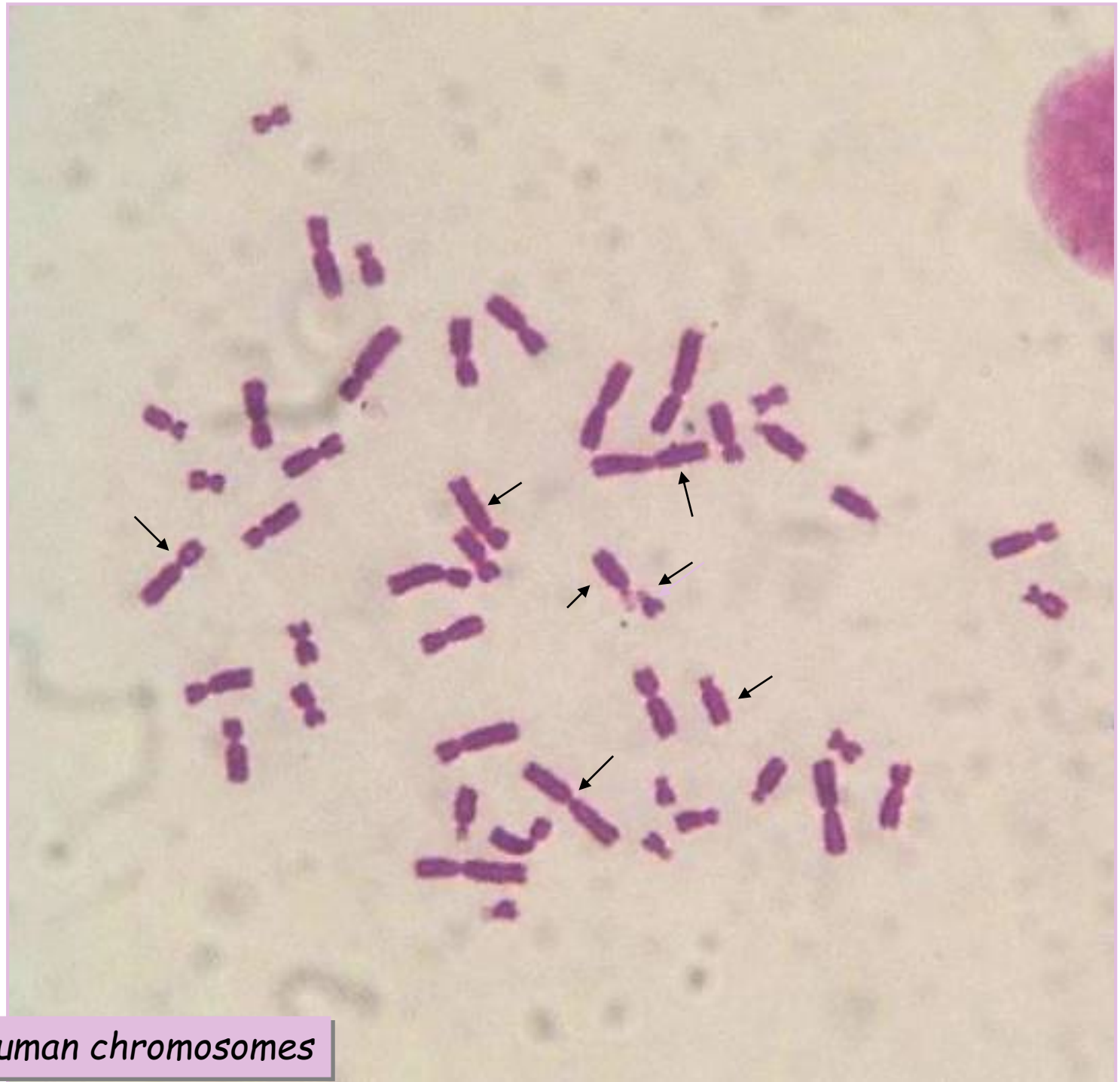
telophase

cytokinesis

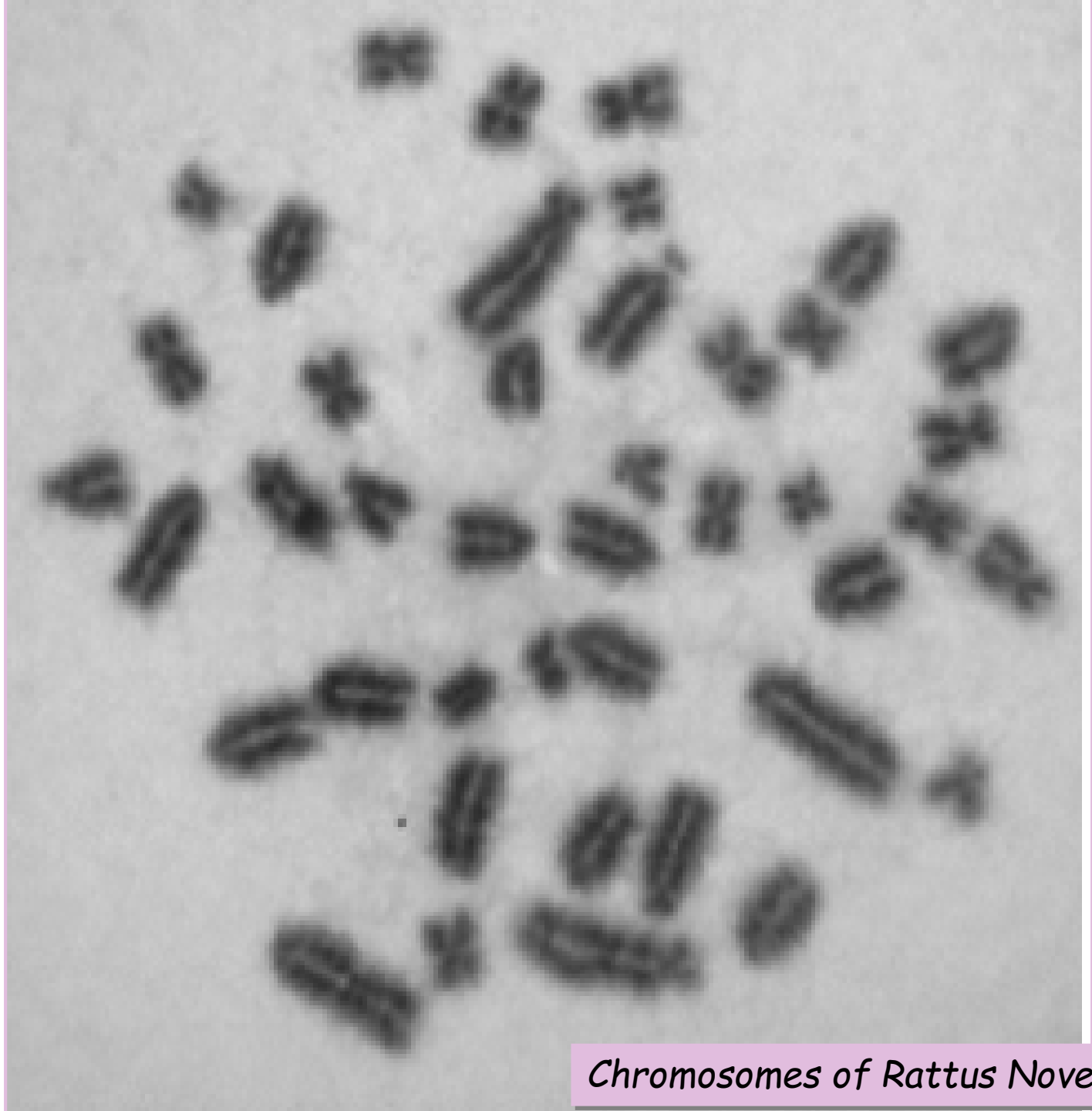

interphase
10 µm
early prophase
metaphase
early anaphase
late prophase
prometaphase
late anaphase
late telophase

Obtaining the karyotype

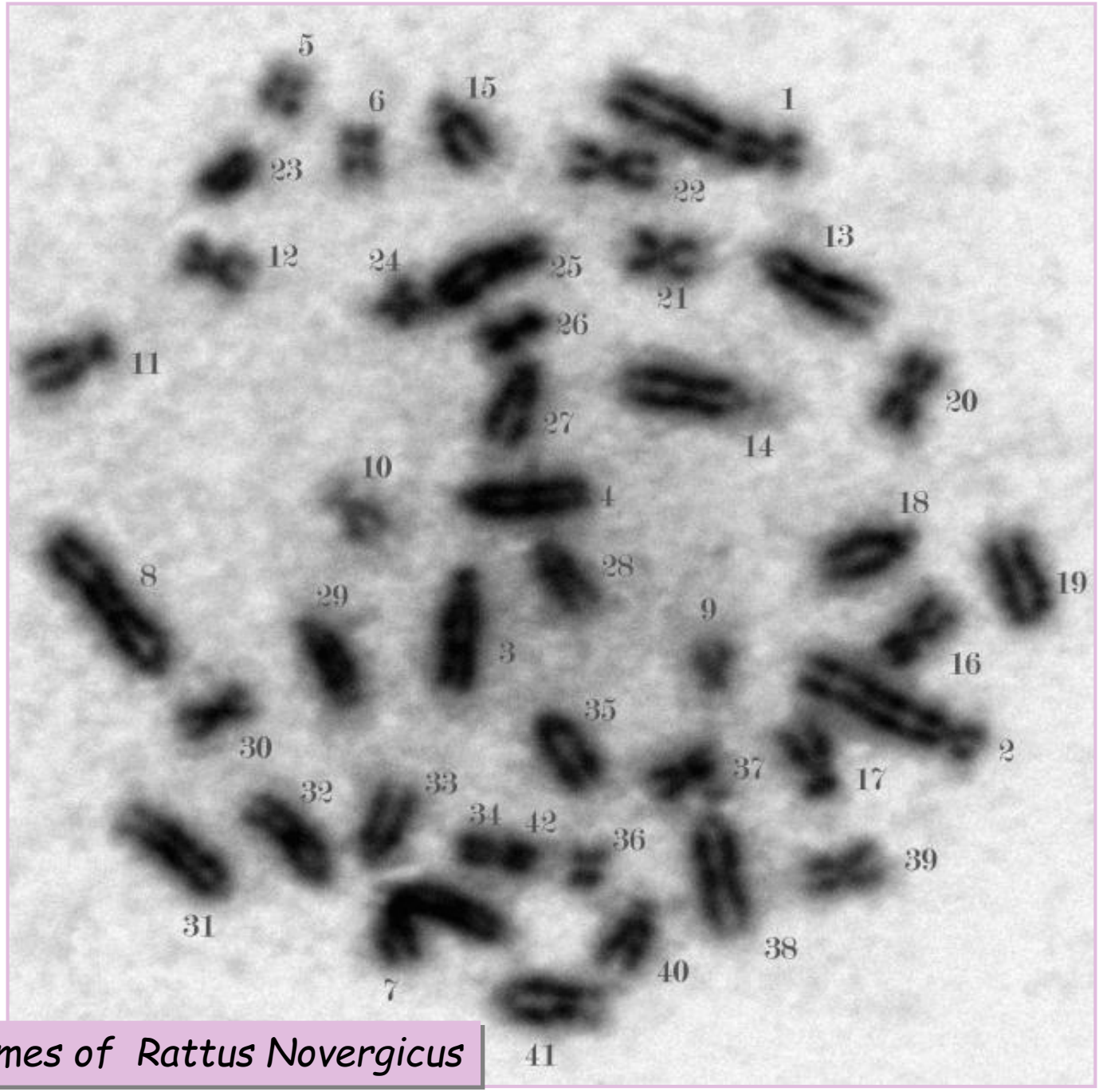




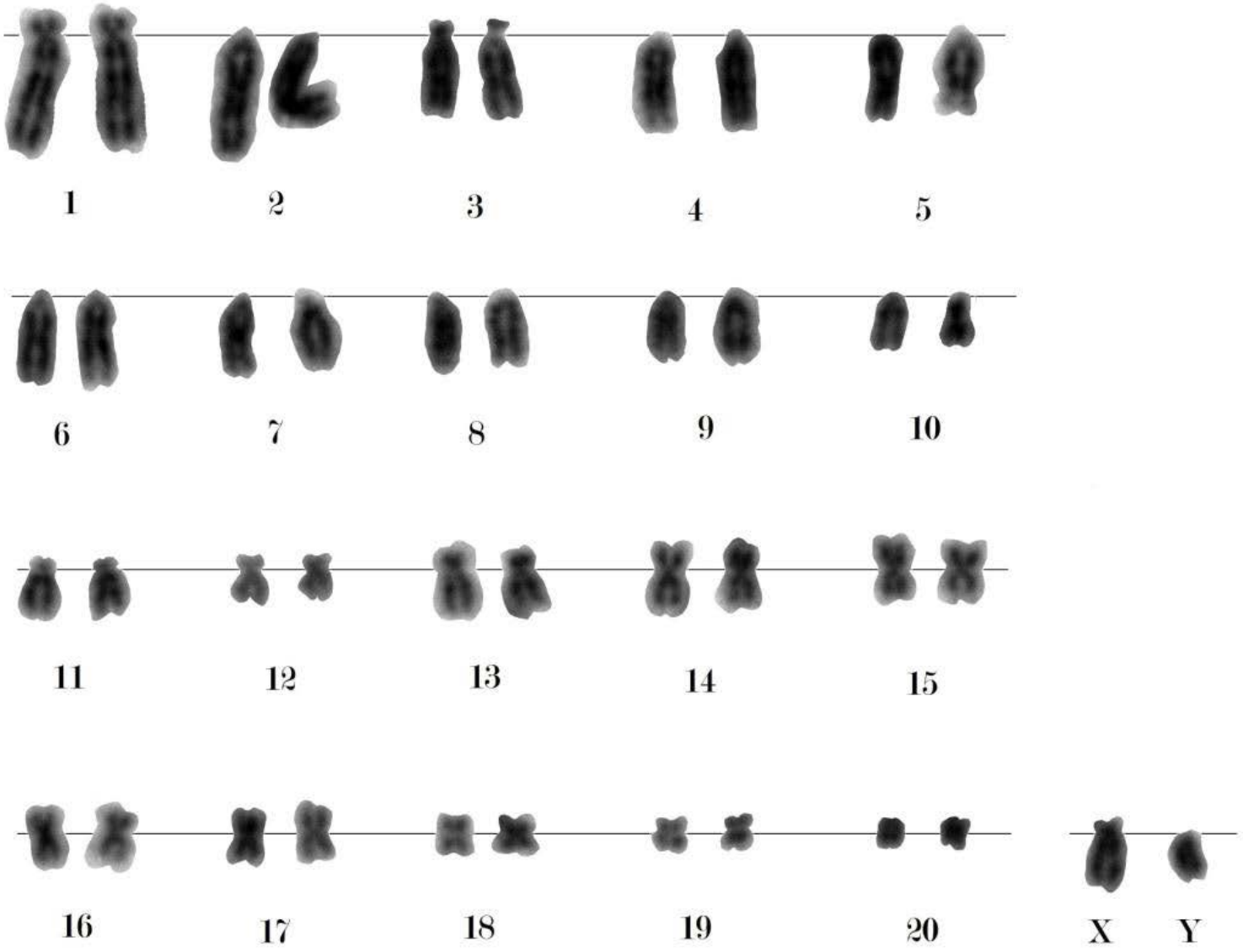
Human chromosomes



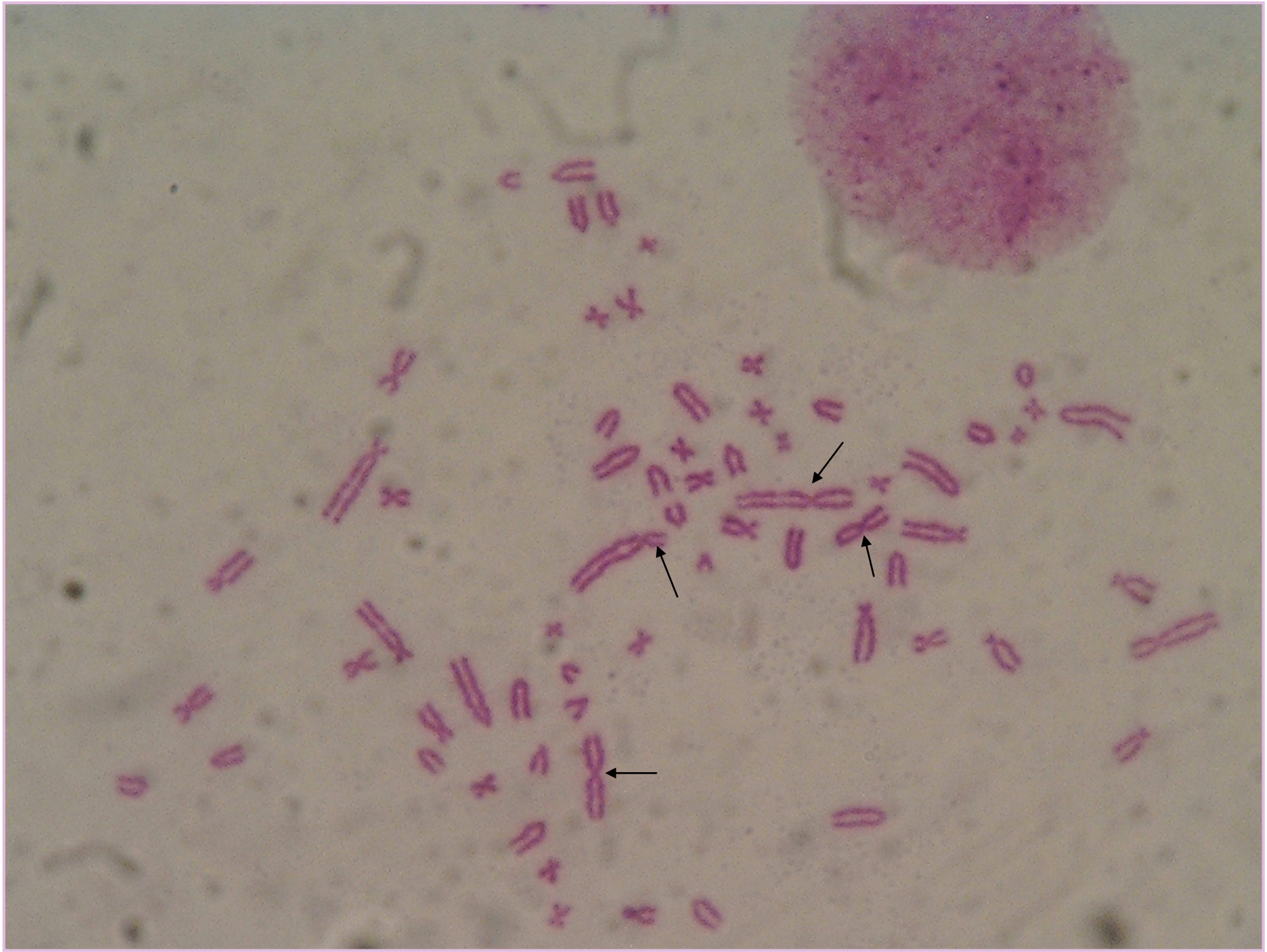
Chromosomes of Rattus Novergicus



Chromosomes of Rattus Novergicus

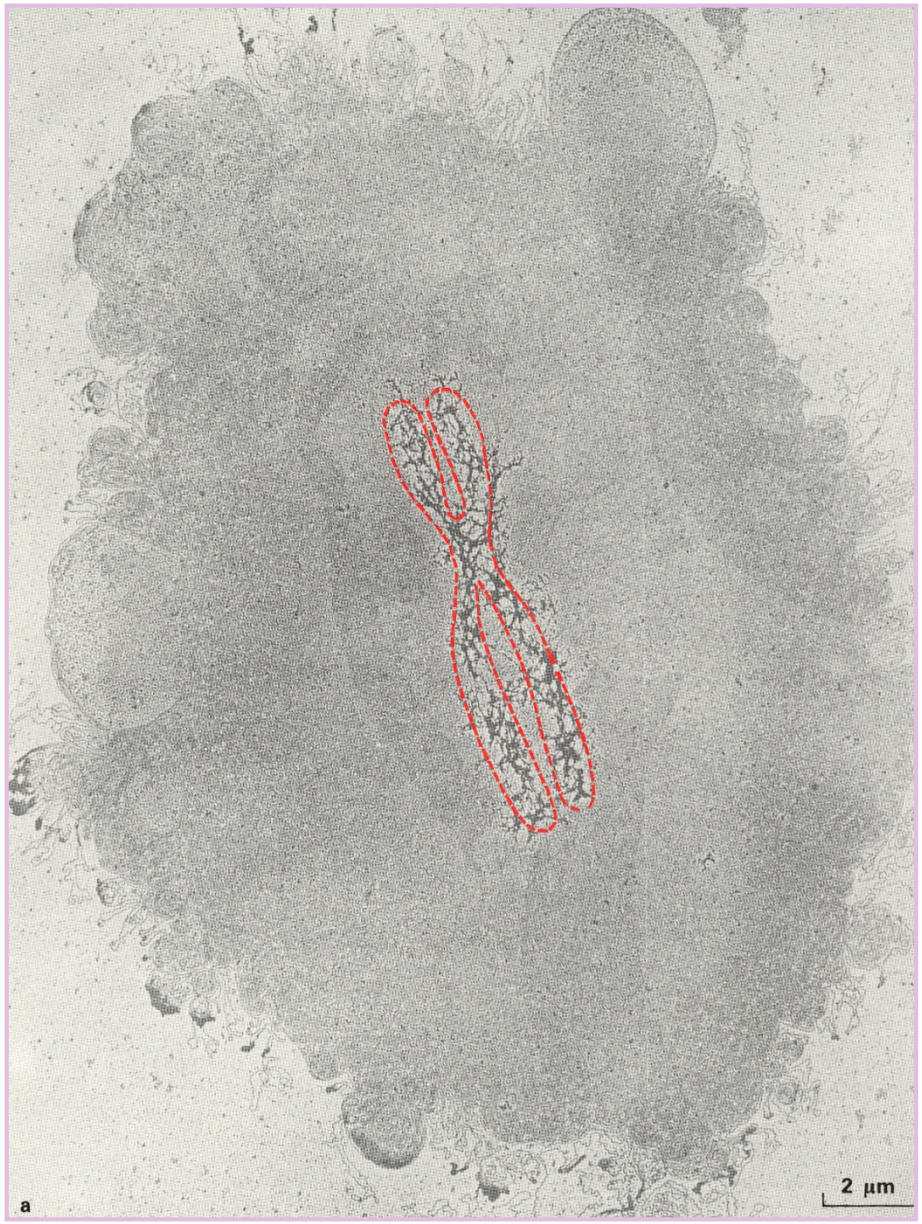


Karyotype of Rattus Novergicus

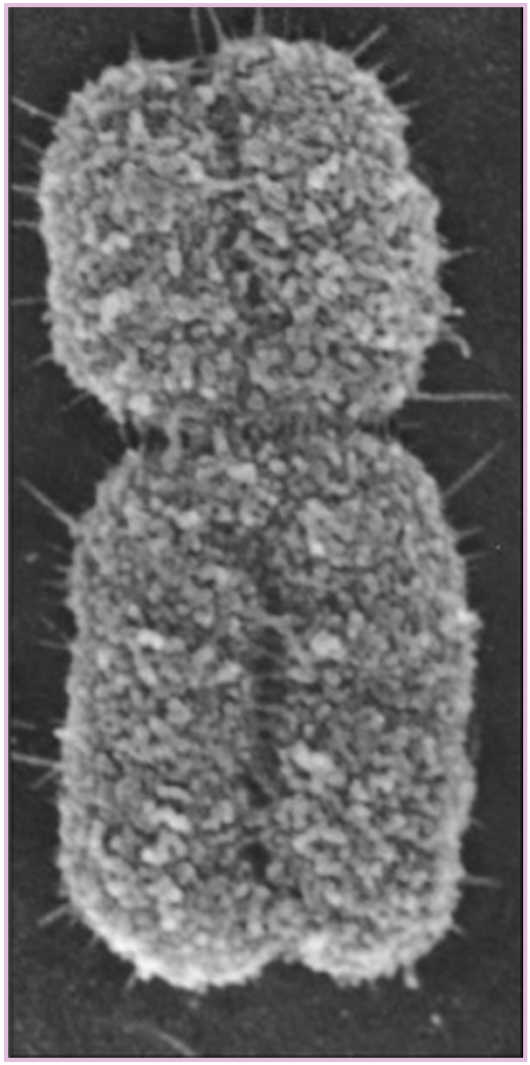


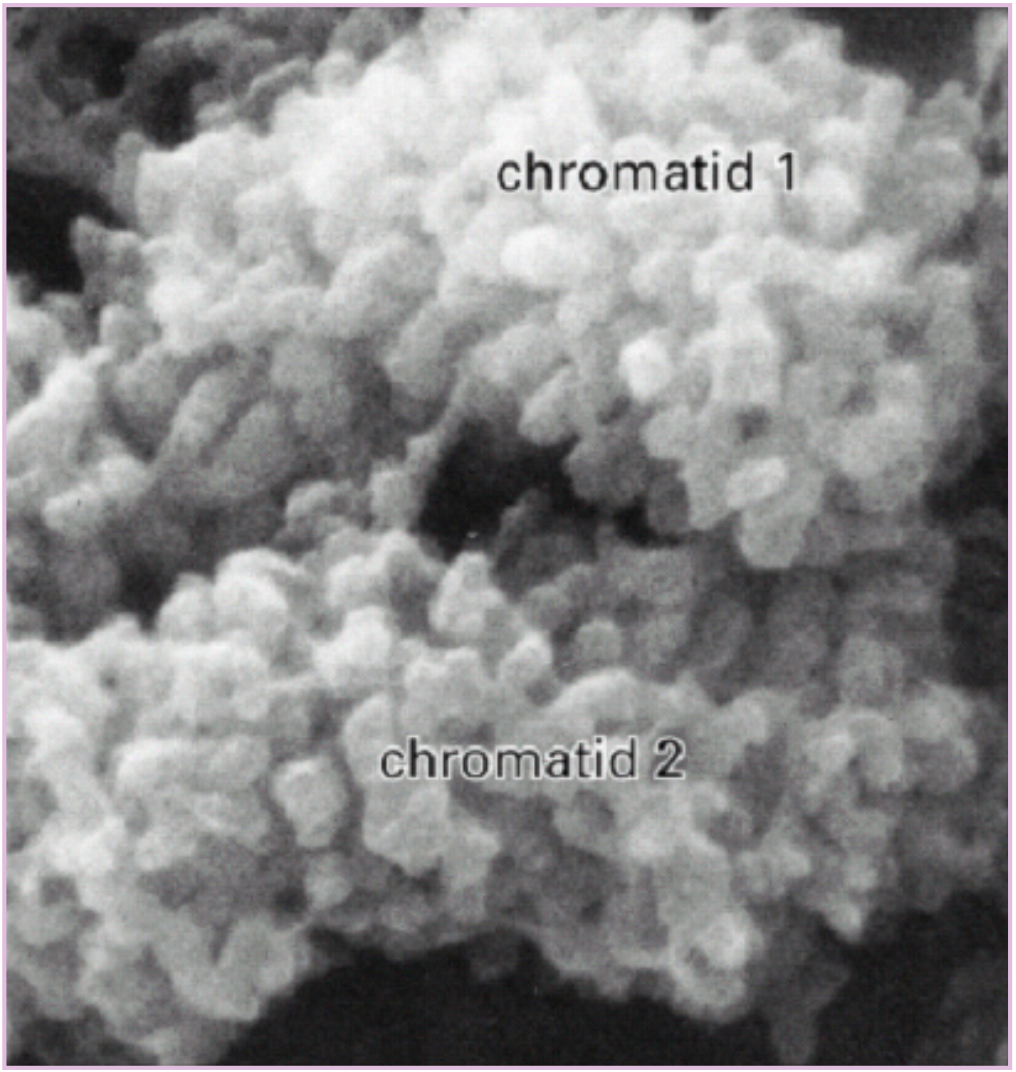
Chromosome by T.E.M., Dupraw's technique

Chromosomes by T.E.M,. Technique of Paulson y Laemmli

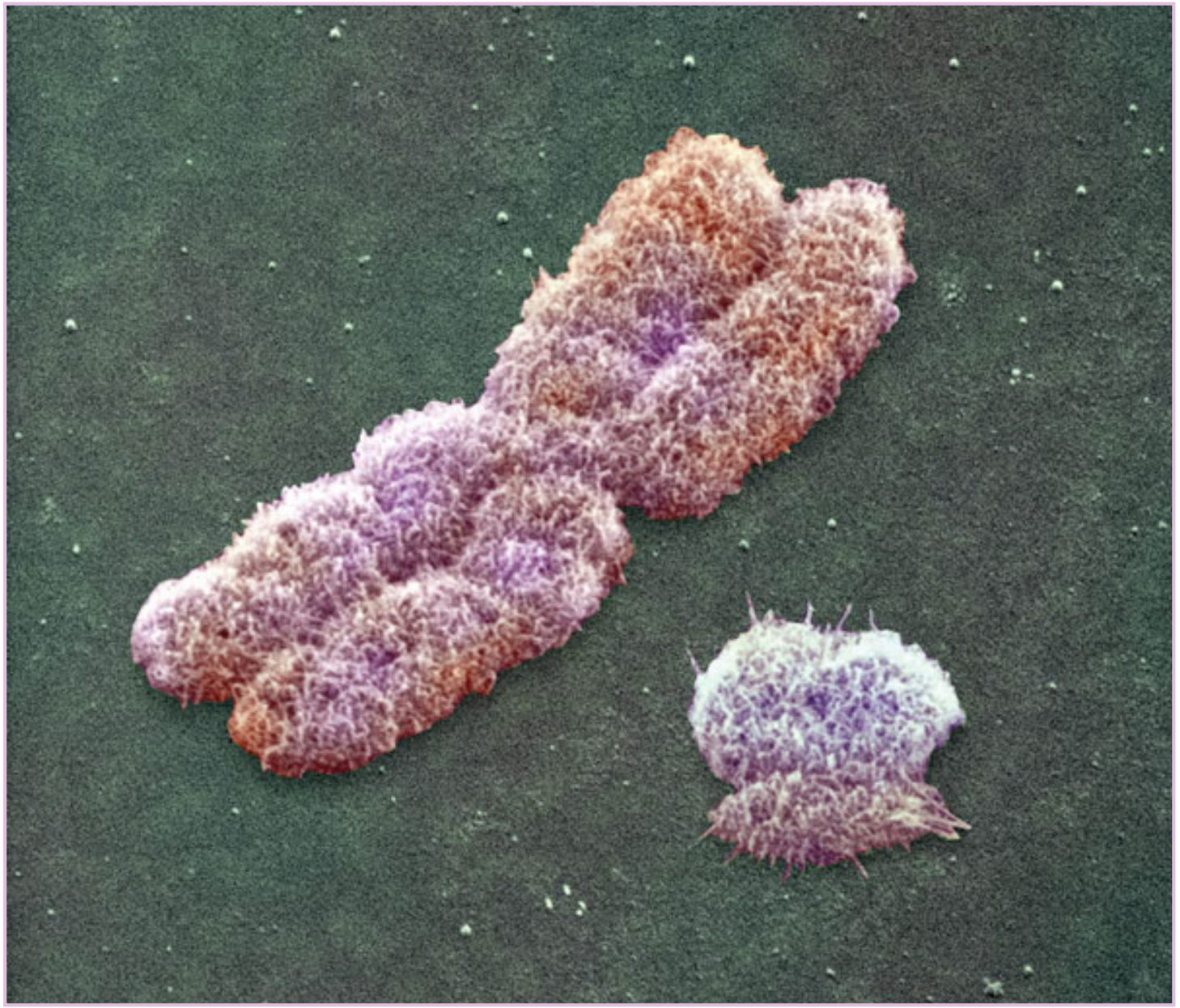


Chromosome by S.E.M.



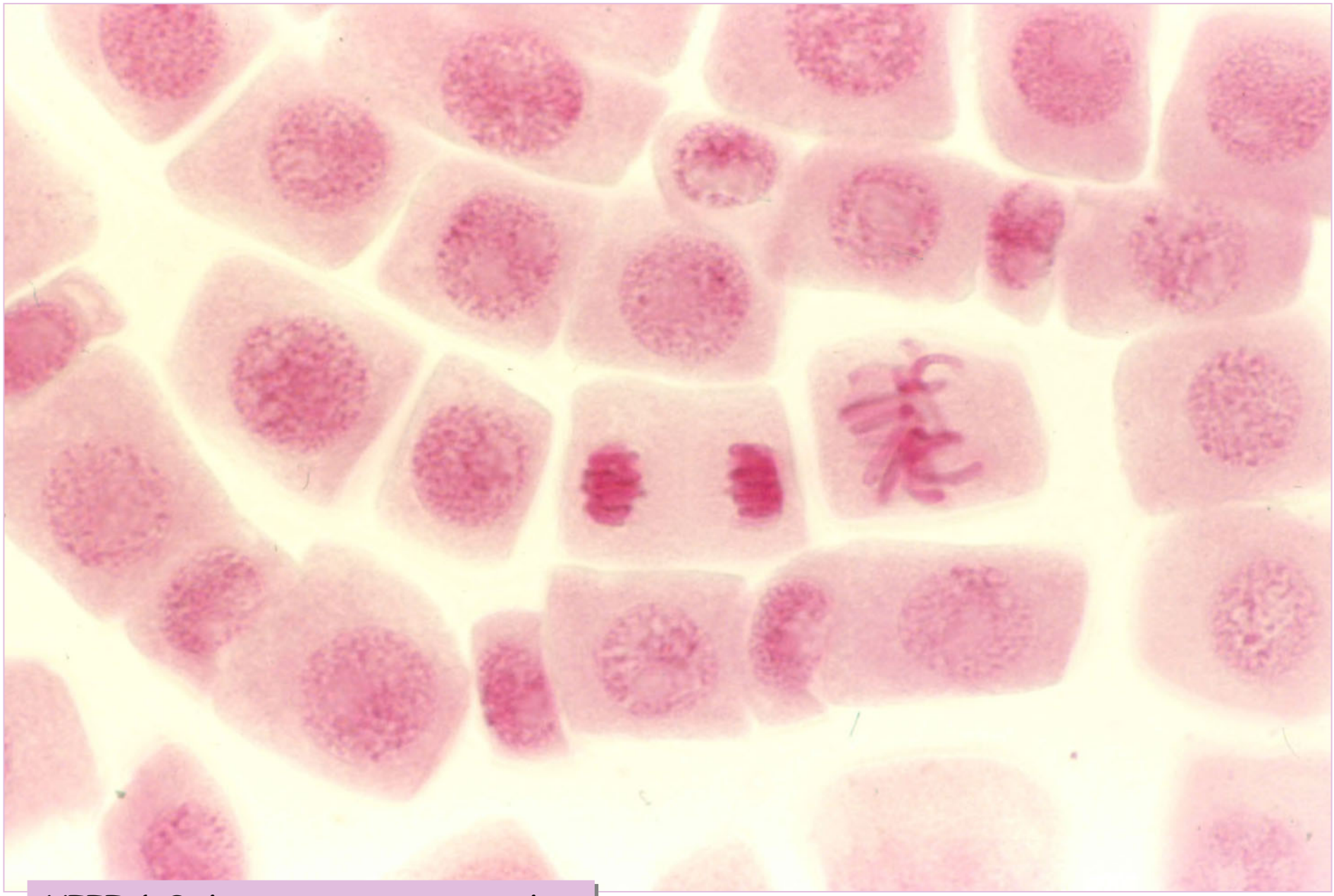


Chromosome by S.E.M.

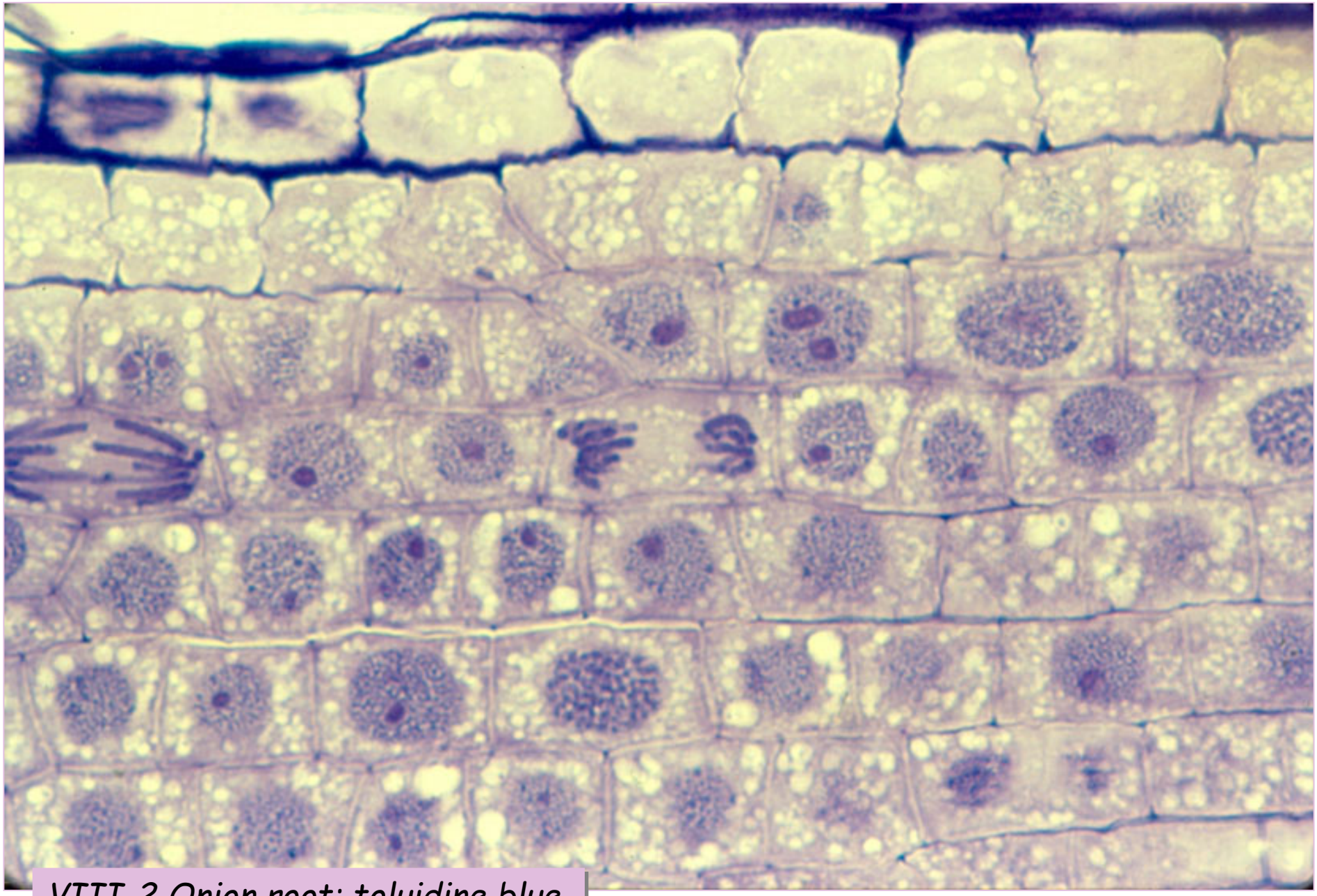


SLIDES

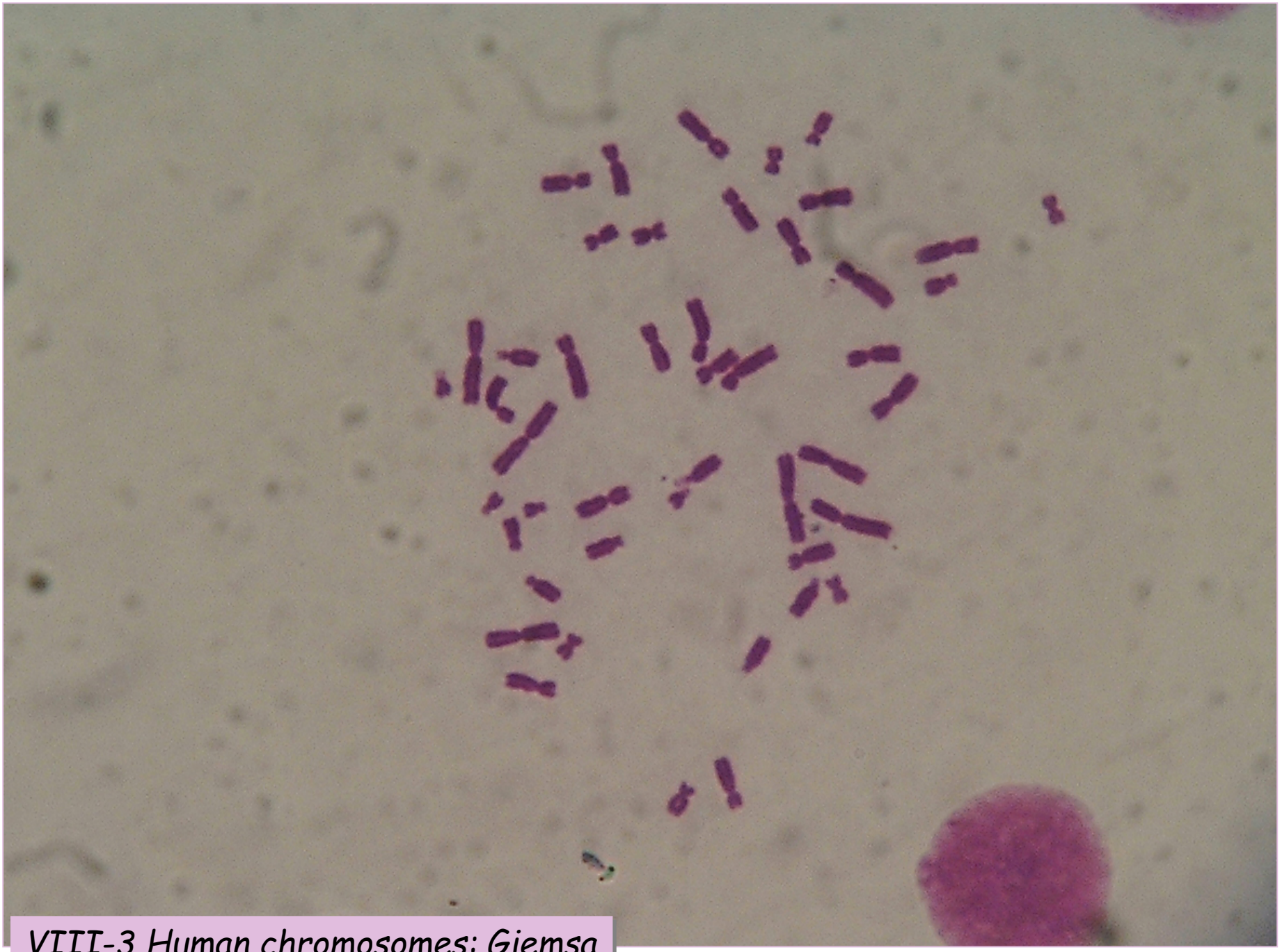
- VIII-1 Onion root: aceto-carmine.
- VIII-2 Onion root: semi-thin section, toluidine blue.
- VIII-3 Human chromosomes: Giemsa.
- VIII-4 Chromosomes of an experimental tumor: Giemsa



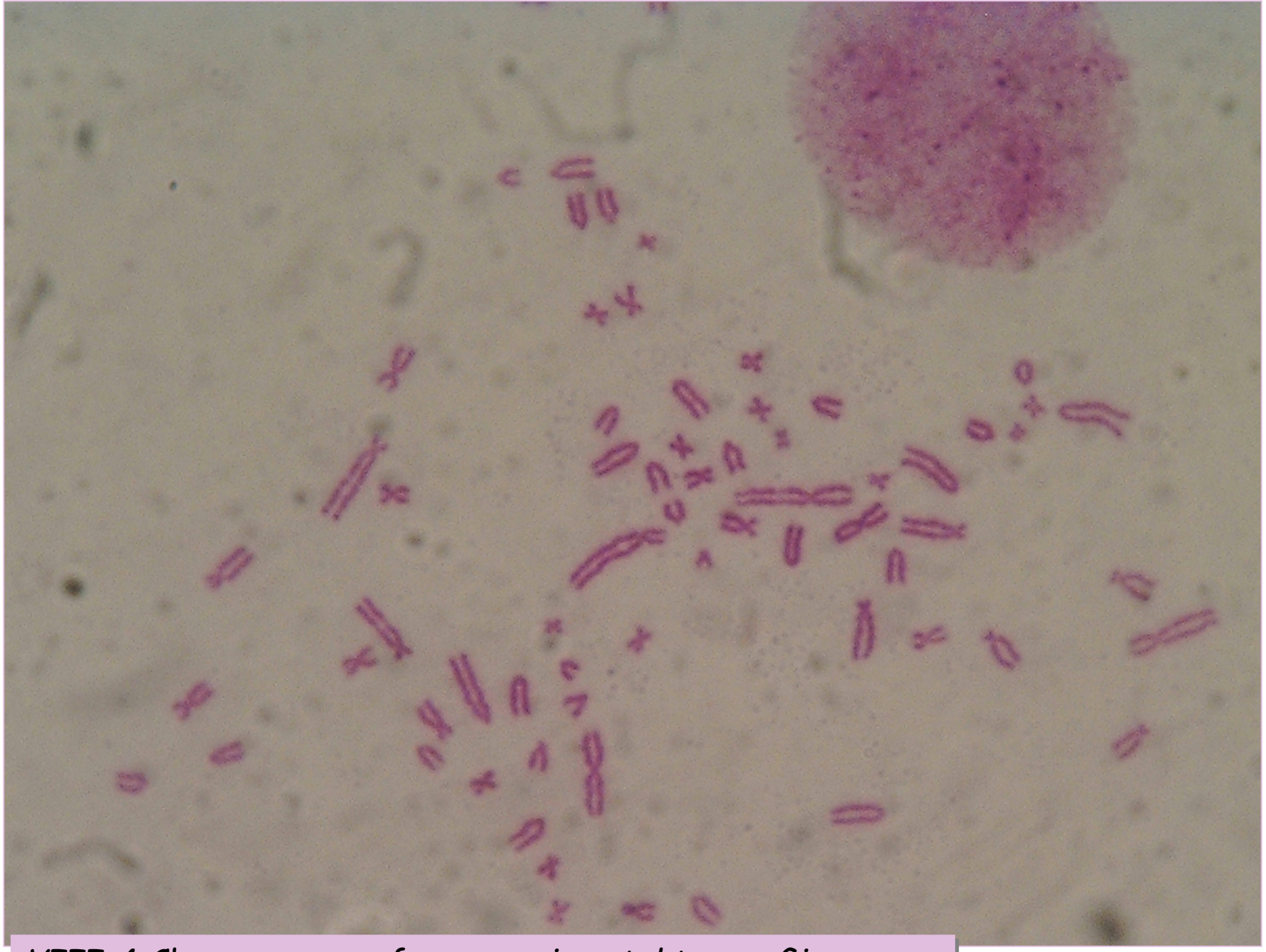
VIII-1 Onion root: aceto-carmine.



VIII-2 Onion root: toluidine blue.



VIII-3 Human chromosomes: Giemsa

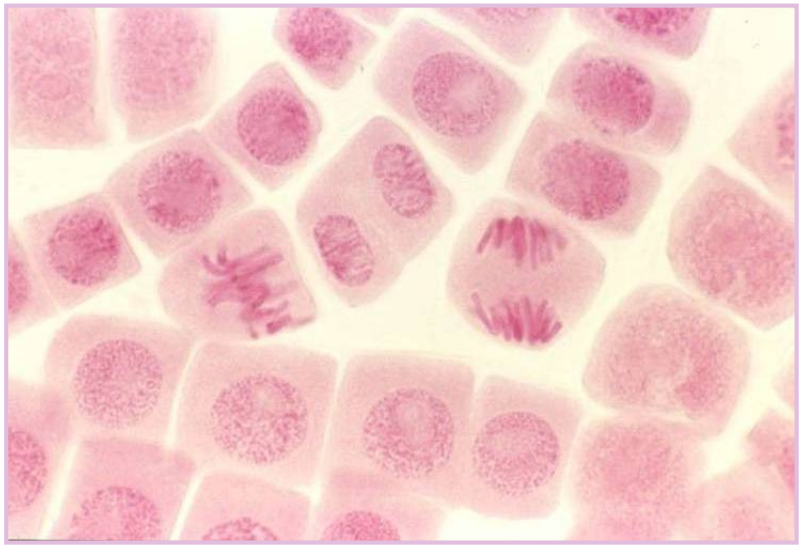


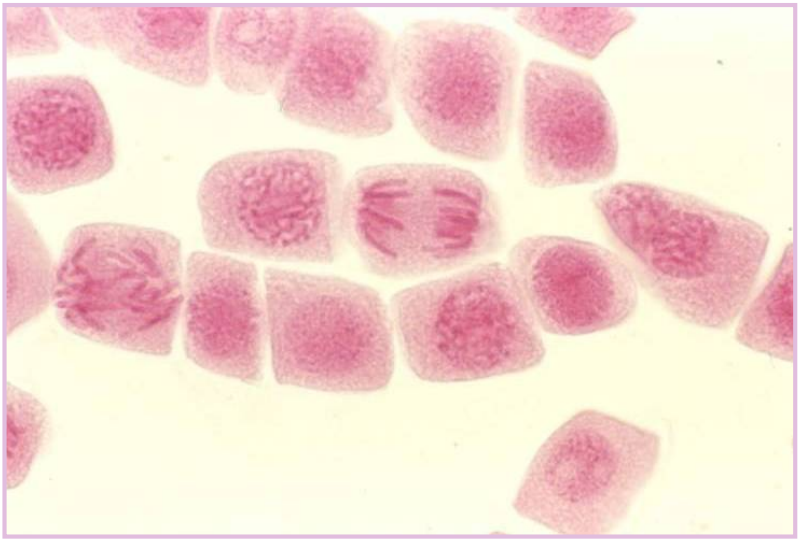
VIII-4 Chromosomes of an experimental tumor:Giemsa

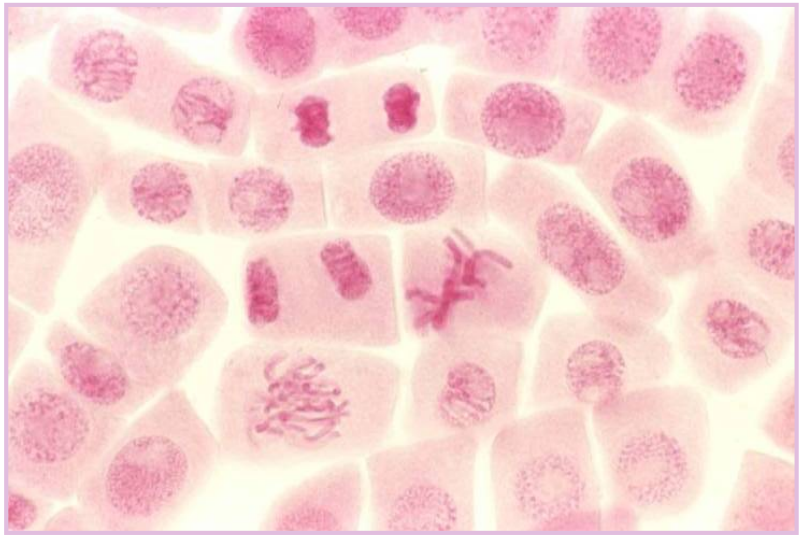
QUESTIONS

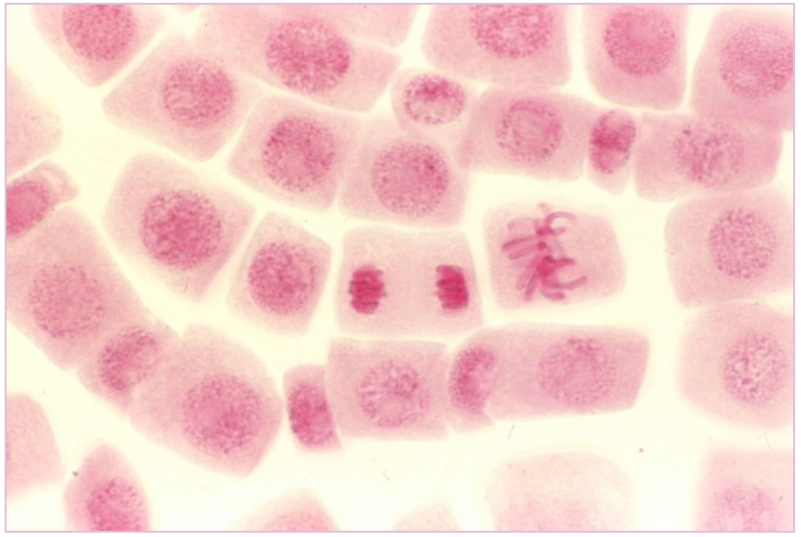
- What type of microscopes would you use to observe the entire process of mitosis and why?.
- What utility does transmission electron microscopy have for studying cells in mitosis?.
- With what type of microscope can you analyze alterations of the mitotic spindle during cell division?.
- Explain what a marker chromosome is and what is the utility of it's study.

Find in the following images three cells in prophase, three in metaphase, three in anaphase and three in telophase.









Find five metacentric , five submetacentric and five acrocentric (three large and two small) chromosomes, and also chromosome Y