BHARATHIDASAN UNIVERSITY
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Programme: M.Sc., Biochemistry
Course Title : Cell biology
Course Code :BC105DCE

Unit-I
The cell

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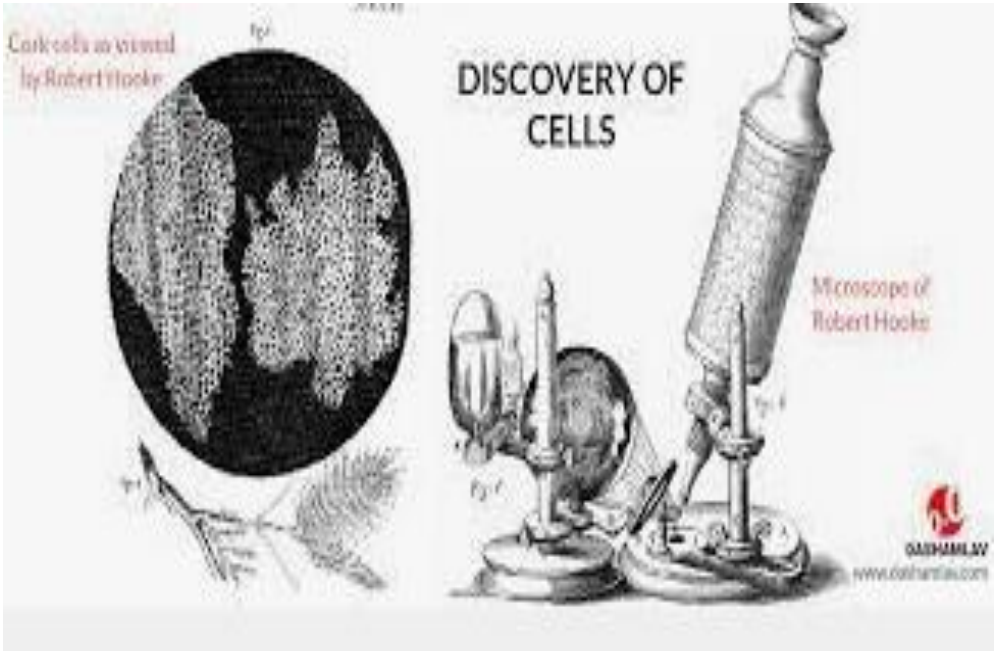
History of the Cell

- In the year of 1665, cells were first discovered by **Robert Hooke**, When he observed the cells in a cork slice with the help of first light microscope

- In 1674, **Leeuwenhoek** discovered the free living cells in pond water for the first time with the help of improved microscope (1 lens)

Robert Hooke

Leeuwenhoek



- In 1590, two Dutch lens makers by the name of **Hans and Zacharias Janssen** invented the first compound microscope when they put two of their lenses together in a tube.

- **Robert Brown** discovered the location of nucleus within the cell in 1831

Matthias Schleiden


Theodore Schwann


Robert Brown

Cell Theory

- In 1838 and 1839, **Matthias Schleiden** and **Theodore Schwann** viewed plants and animals under a microscope and discovered that plants and animals are both made of cells.
- In 1855, **Rudolph Virchow** collaborated his ideas with the other two scientists and they developed the Cell Theory.
- The Three main principles of cell theory.

1. All living organisms are made up of cells.
2. Cells are the most basic unit of life.
3. Cells only come from the division of pre-existing cells.

In other words, spontaneous generation of cells does not occur.

Rudolph Virchow

Protoplasm: It is defined as the organic and inorganic substances that constitute the living the nucleus, cytoplasm, plastids and mitochondria of the cell

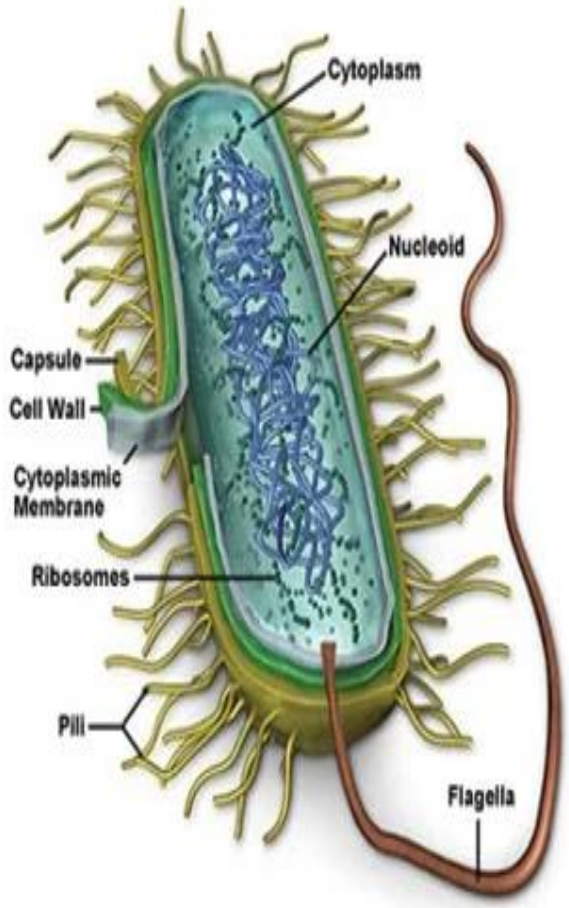
Components and Functions Of A Protoplasm

- The cytoplasm is the initial component of protoplasm, which is found between the cell membrane and the cell nucleus in a eukaryotic cell. In the cell, a cytoplasm plays a vital role in maintaining the cell environment, maintains the shape of cells and also stores substances required by the organelle.
- The nucleus is the second component of the protoplasm, which stores the genetic information of an organism. Ribosomes are also found in the nucleus, which is essential for the production of proteins in the cell. Prokaryotes contain a nucleoid instead of a nucleus where all the genetic information is found.
- Proteins, fats, enzymes, hormones, all make up the protoplasm. These are either dissolved or suspended in the water component of the protoplasm.

- **Organismal theory** was explained by several scientists such as Reichert a morphologist stated that an organism have structured plan; Strasberger a cytologist argues that cells in an organism are connected by cytoplasmic bridges, and Sherrington and Pavlov (neurophysiologists) says cells communicate with each other and co-ordinate with their actions.
- **Several postulates of organismal theory are as following:**
- Some organisms such as fungi are non-cellular and are unable to divide into cellular compartments. plant cells have cytoplasmic bridges between each other called plasmodesmata.
- some of the cells lack some basic components such as red blood cells and nucleus. Cytoplasm in unicellular organisms does not undergo subdivision into cells.
- If any cell is removed from the multicellular organisms, it would require complete life support to be alive.
- Homeostatic control or co-ordination between unicellular or multicellular organism is must to maintain the whole organism.

Broad classification of Cell types:

- Cell: The basic unit of a living organism, consisting of a quantity of protoplasm surrounded by a cell membrane, which is able to synthesize proteins and replicate itself.
- A living thing can be composed of either one cell or many cells.
- There are two broad categories of cells: prokaryotic and eukaryotic cells.
- Cells can be highly specialized with specific functions and characteristics.



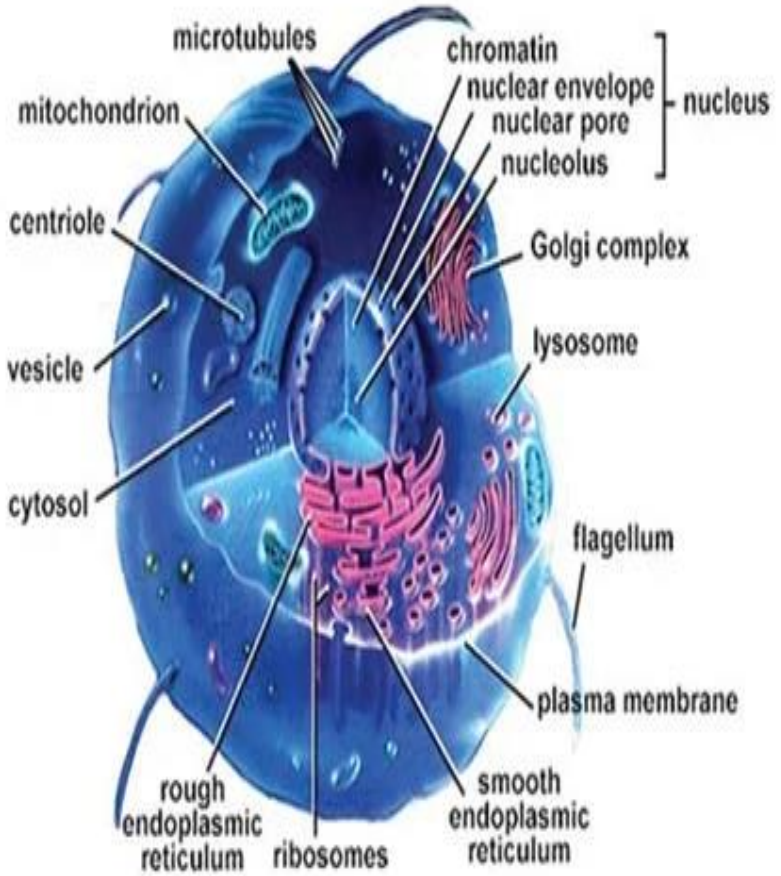


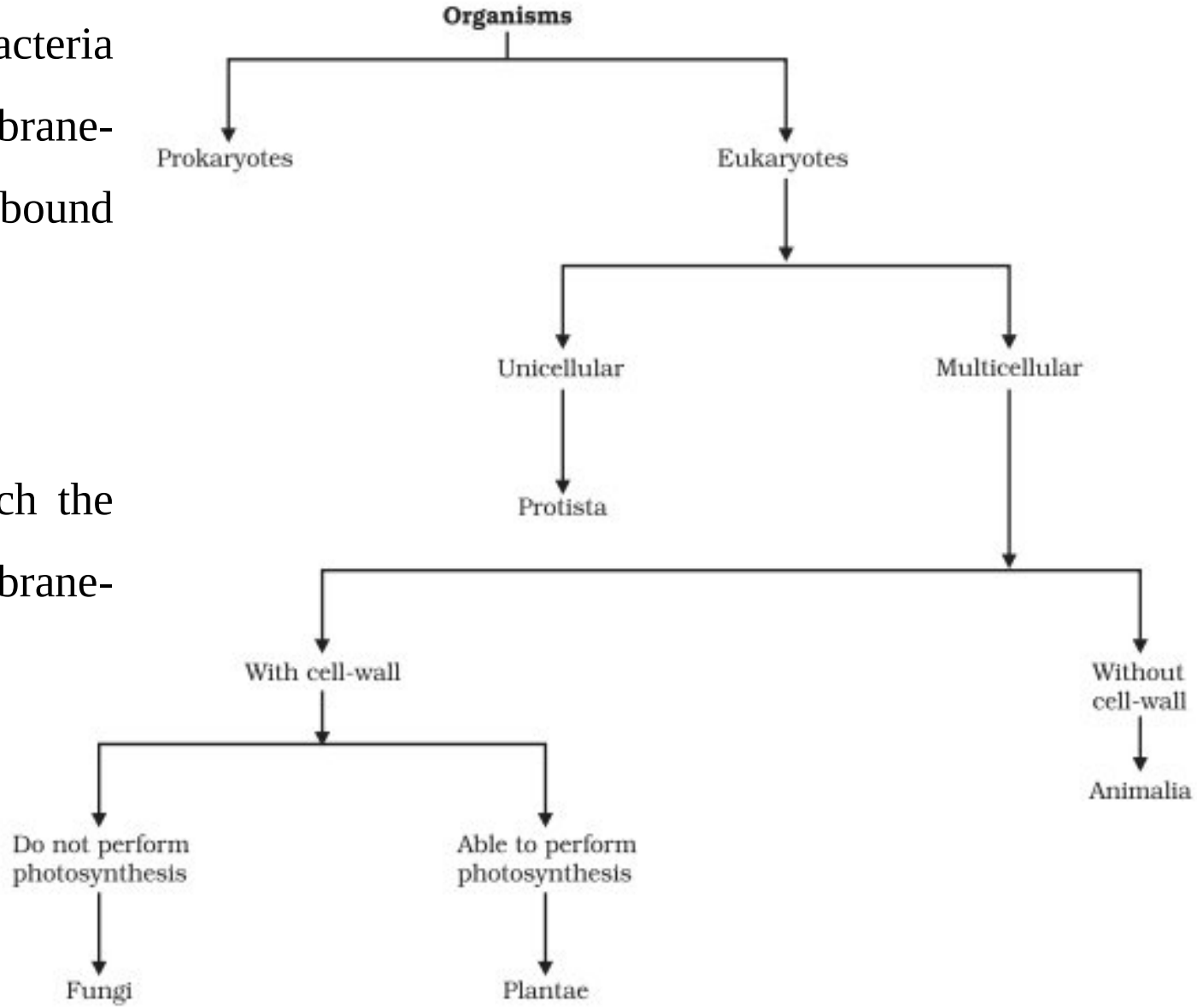
Image: Microbe notes

Comparison of Prokaryotic and Eukaryotic organisms

	Procaryotes	Eucaryotes
Organisms	bacteria and cyanobacteria	protists, fungi, plants, and animals
Cell size	generally 1 to 10 mm in linear dimension	generally 5 to 100 mm in linear dimension
Metabolism	anaerobic or aerobic	aerobic
Organelles	few or none	nucleus, mitochondria, chloroplasts, endoplasmic reticulum, etc.
DNA	circular DNA in cytoplasm	very long linear DNA molecules containing many noncoding regions; bounded by nuclear envelope
RNA and protein	RNA and protein synthesized in same compartment	RNA synthesized and processed in nucleus; proteins synthesized in cytoplasm
Cytoplasm	no cytoskeleton: cytoplasmic streaming, endocytosis, and exocytosis all absent	cytoskeleton composed of protein filaments; cytoplasmic streaming; endocytosis and exocytosis
Cell division	chromosomes pulled apart by attachments to plasma membrane	chromosomes pulled apart by cytoskeletal spindle apparatus
Cellular organization	mainly unicellular	mainly multicellular, with differentiation of many types

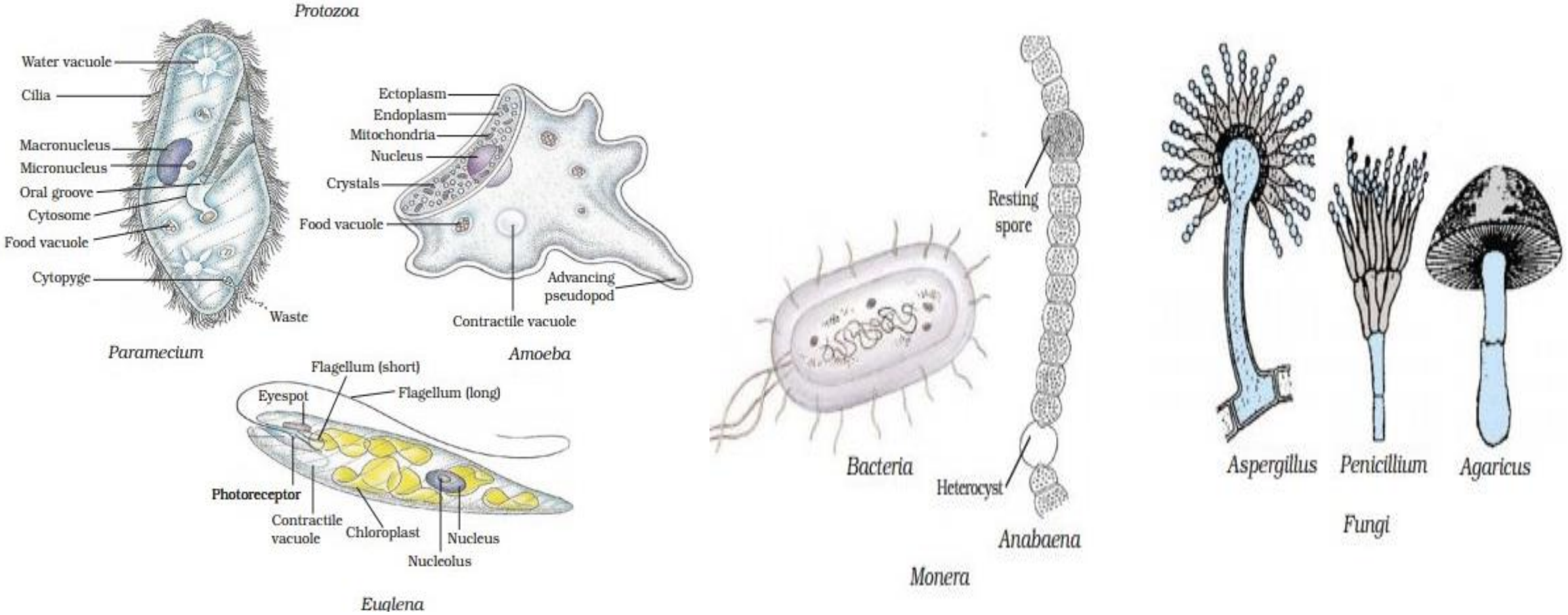
- Prokaryotic: Small cells in the domains Bacteria and Archaea that do not contain a membrane-bound nucleus or other membrane-bound organelles.

- Eukaryotic: Having complex cells in which the genetic material is contained within membrane-bound nuclei.

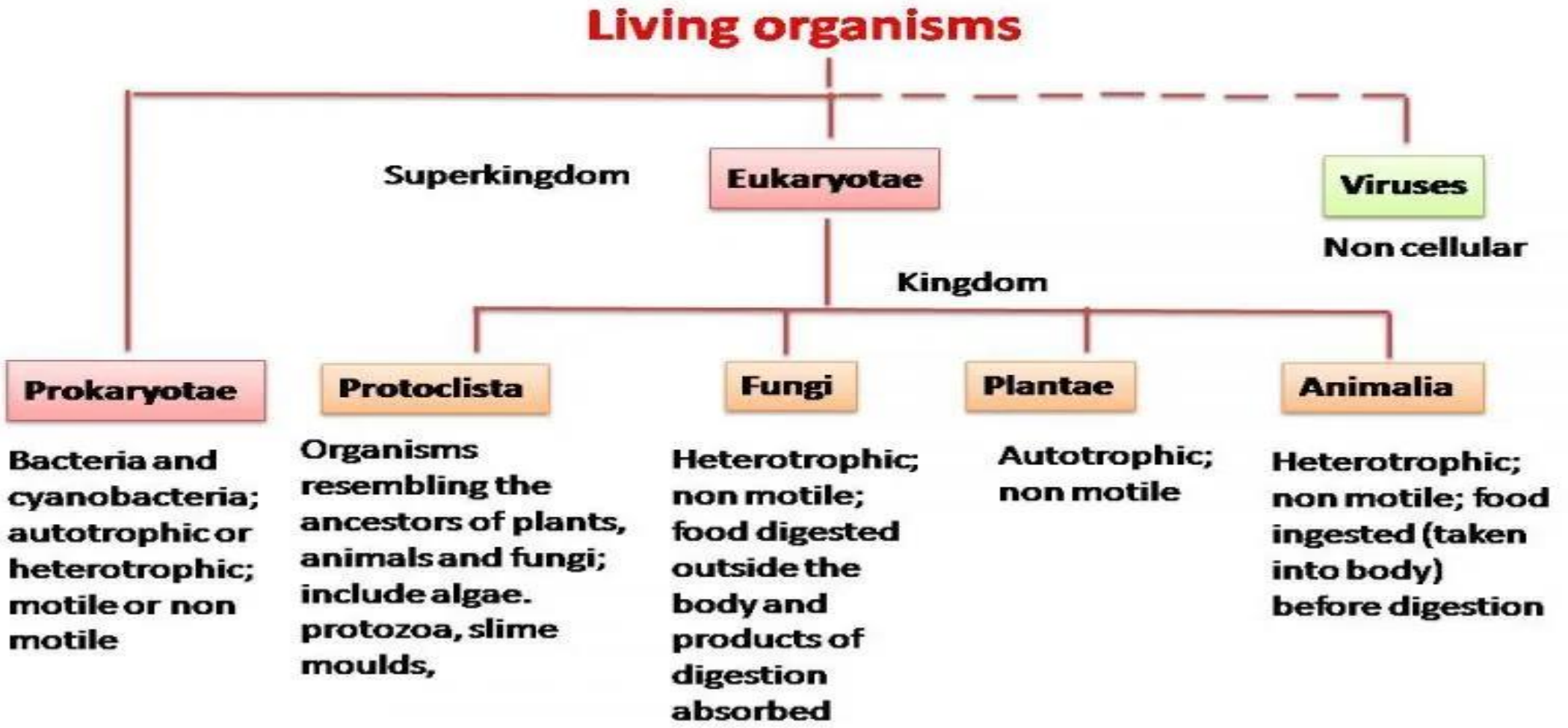


The Five Kingdom classification

- Eukaryotes is further classified into Unicellular and Multicellular
- Unicellular: Protoctista
- Multicellular: Plantae, Animalia, Fungi



The five kingdom classification of organisms (according to Margulis and Schwartz)



Universal features of cells

A. All Cells Store Their Hereditary Information in the Same Linear Chemical Code (DNA)

B. All Cells Replicate Their Hereditary Information by Templated Polymerization

C. All Cells Transcribe Portions of Their Hereditary Information into the Same Intermediary Form (RNA)

D. All Cells Use Proteins as Catalysts

E. All Cells Translate RNA into Protein in the Same Way

F. The Fragment of Genetic Information Corresponding to One Protein Is One Gene

G. Life Requires Free Energy

H. All Cells Function as Biochemical Factories Dealing with the Same Basic Molecular Building Blocks

I. All Cells Are Enclosed in a Plasma Membrane Across Which Nutrients and Waste Materials Must Pass

J. A Living Cell Can Exist with Fewer Than 500 Genes

The Diversity of Genomes and the Tree of Life

Cells Can Be Powered by a Variety of Free Energy Sources:

- Living organisms obtain their free energy in different ways. Some organisms get it by feeding on other living things or the organic chemicals they produce *organotrophic (trophe-* food*)*

  Eg. bacteria that live in the human gut

- energy directly from the nonliving world. These fall into two classes:

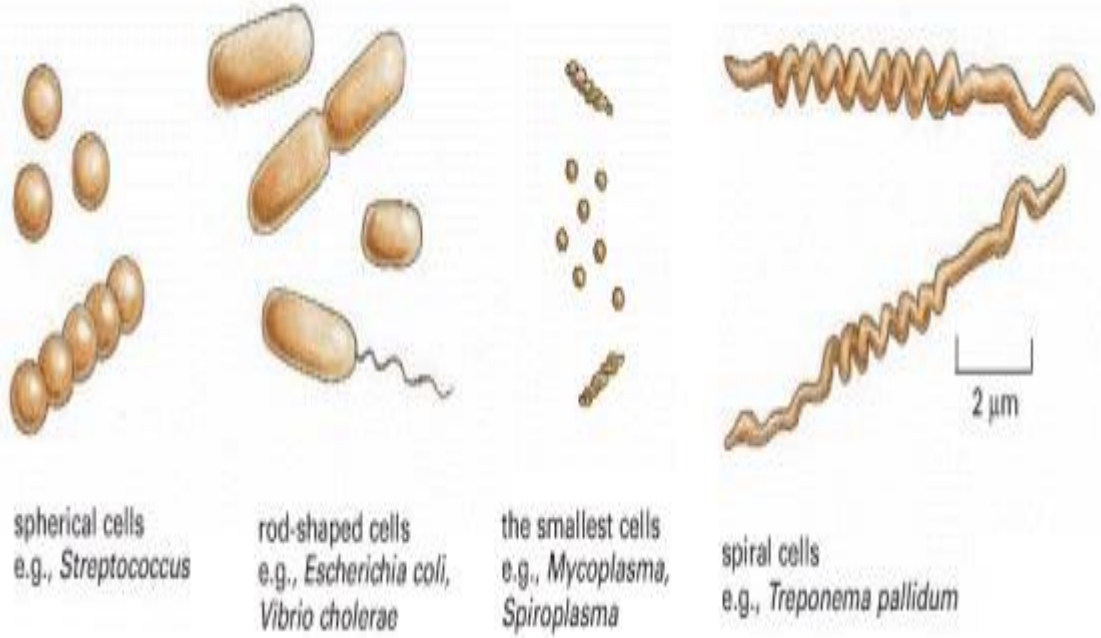
  -*phototrophic* (feeding on sunlight)

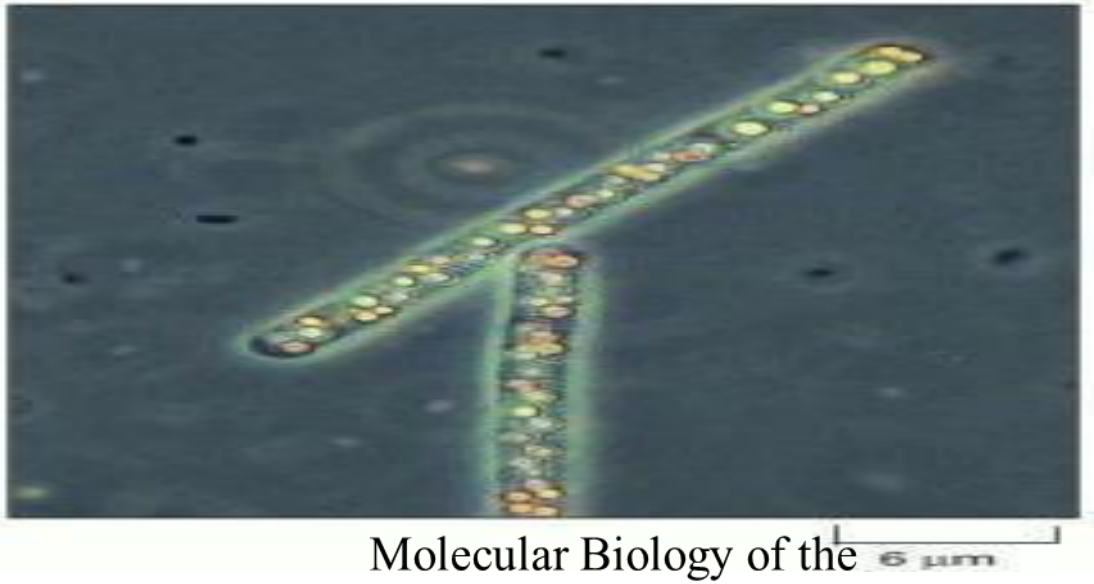
  Eg.Plants and algae

  -*lithotrophic* (feeding on rock)

- Microorganisms found in deep ocean, surface of earth crust, Volcano surface etc., get fueled from geochemical energy directly. They are aerobic and anaerobic in nature

- Some Cells Fix Nitrogen and Carbon Dioxide for Others. Atmospheric N2 and CO2, in particular, are extremely unreactive, and a large amount of free energy is required to drive the reactions that use these inorganic molecules to make the organic compounds needed for further biosynthesis—that is, to fix nitrogen and carbon dioxide, so as to make N and C available to living organisms.
- The Greatest Biochemical Diversity Is Seen Among Procaryotic Cells





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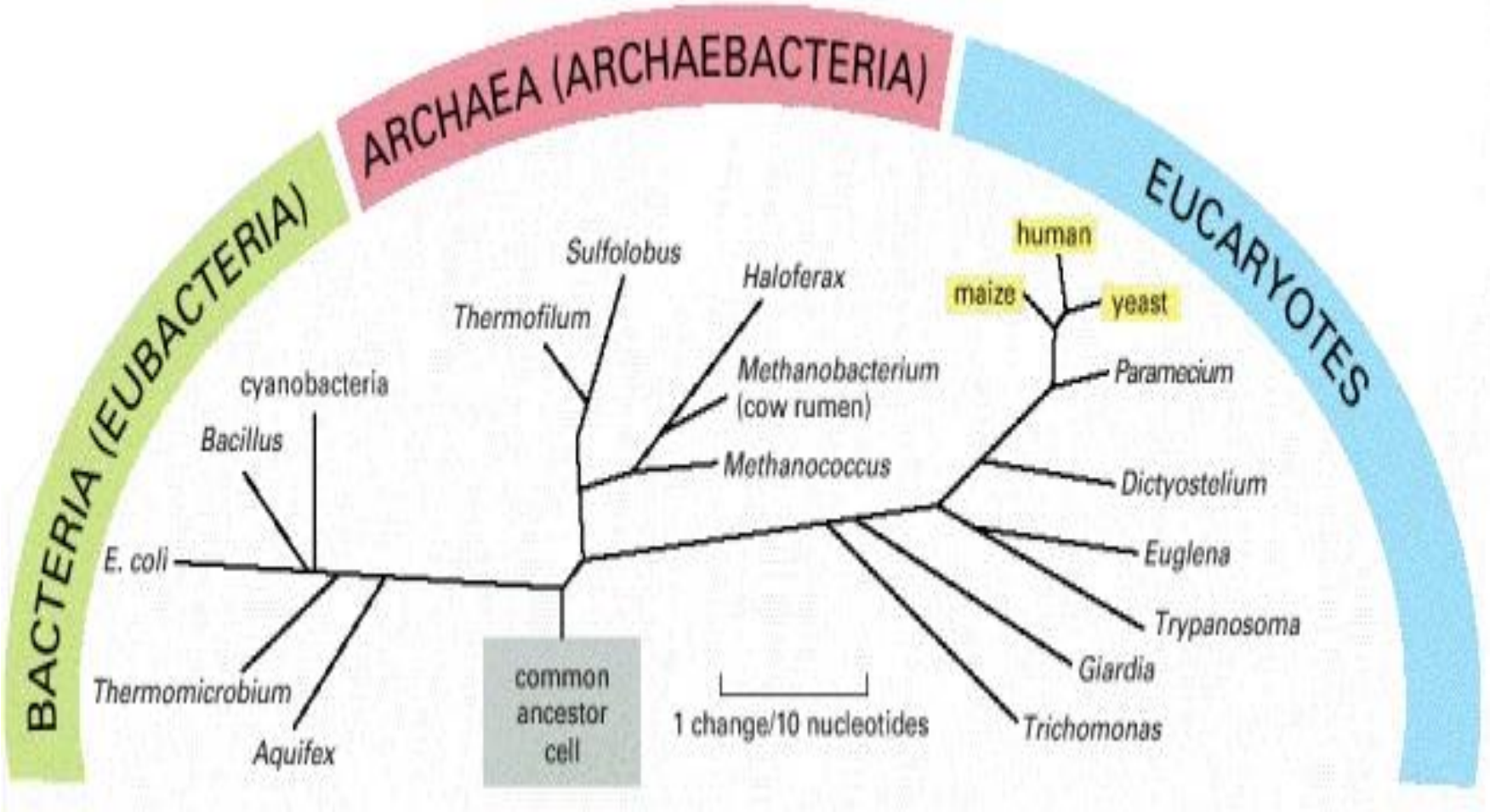
Tree of Life



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Key points in Tree of Life

- Some Genes Evolve Rapidly; Others Are Highly Conserved
- Most Bacteria and Archaea Have 1000–4000 Genes
- New Genes Are Generated from Preexisting Genes
- Gene Duplications Give Rise to Families of Related Genes Within a Single Cell
- Genes Can Be Transferred Between Organisms, Both in the Laboratory and in Nature
- Horizontal Exchanges of Genetic Information Within a Species Are Brought About by Sex
- The Function of a Gene Can Often Be Deduced from Its Sequence
- More Than 200 Gene Families Are Common to All Three Primary Branches of the Tree of Life
- Mutations Reveal the Functions of Genes
- Molecular Biologists Have Focused a Spotlight on *E. coli*

Cell Membranes

Basic Properties of Cell Membrane:

(1)Cell membranes are thin enclosures that form closed boundaries

(2) Cell membranes are made up of lipids, proteins and carbohydrates

(3) Cell membranes consists of a phospholipid bilayer

(4) Cell membranes are held together by non-covalent interactions

(5) Membranes are fluid-like structure

(6) Proteins diversify the functionality of cell membranes

(7) Membranes have polarity

(8) Membranes are asymmetrical structures

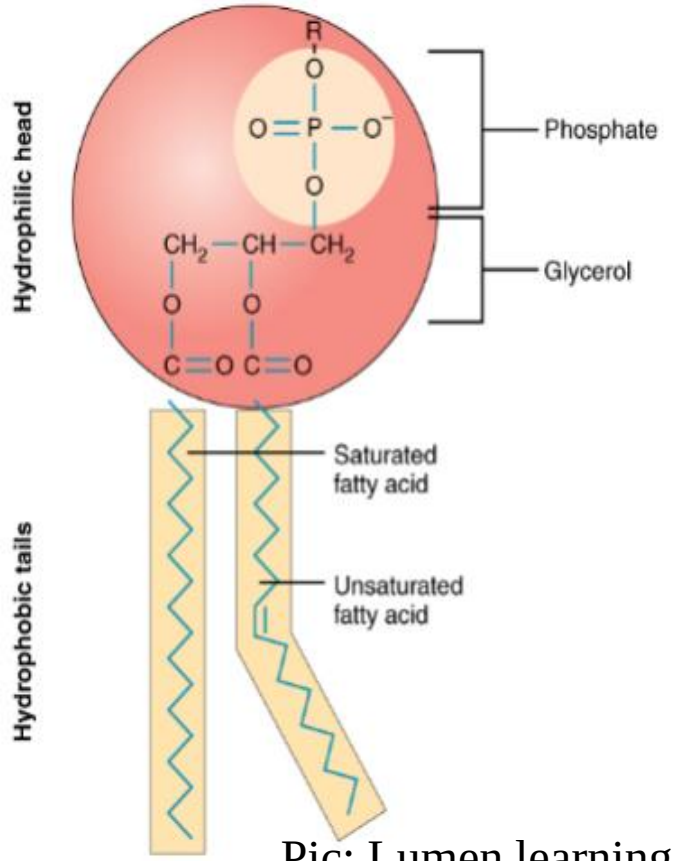
The lipid Bilayer

Composition and Structural Organization:

- Cell membrane is extremely flexible composed of back to back phospholipids
- Cholesterol is present which makes fluidity to the membrane
- A single phospholipid molecule has a phosphate group on one end, called the “head,” and two side-by-side chains of fatty acids that make up the lipid tails
- Phosphate group is negatively charged which makes it polar and hydrophilic head

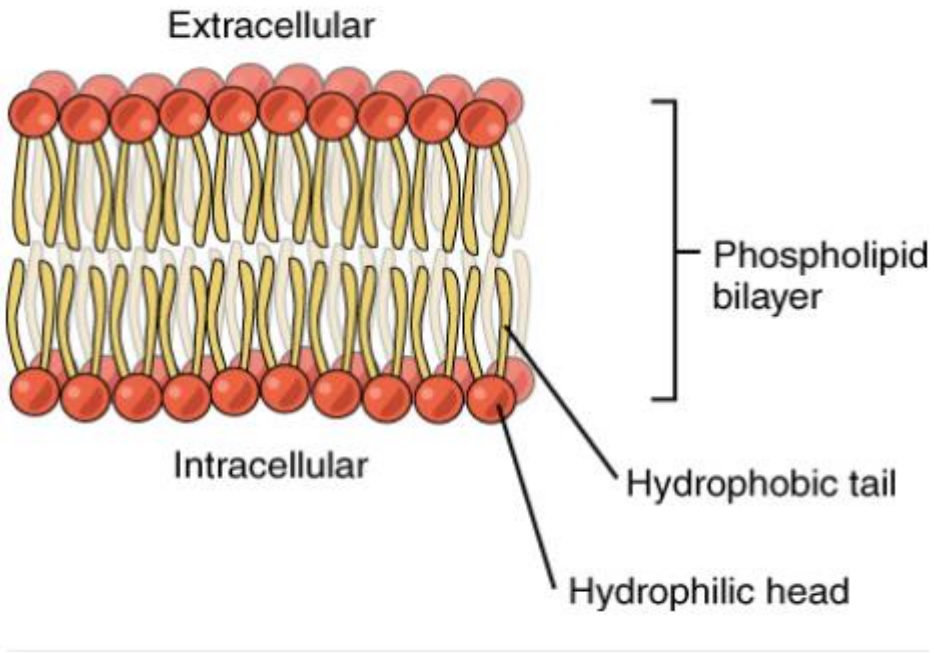
Hydrophobic:

- Molecules that repels water. Some lipids tails consists of Saturated and Un saturated fatty acids. This combinations adds fluidity to the tails and it will be in motion



Pic: Lumen learning

- An **amphipathic** molecule is one that contains both a hydrophilic and a hydrophobic region
- A **hydrophilic** molecule (or region of a molecule) is one that is attracted to water
- The cell membrane consists of two bilayer of phospholipids
- Lipid tail faces the other lipid tail and head of phospholipid face the interior and exterior surface of the cell
- Phosphate group of head attracts to intercellular fluid and Extracellular fluid
- **Interstitial fluid (IF)** is the term given to extracellular fluid not contained within blood vessels
- Because the lipid tails are hydrophobic, they meet in the inner region of the membrane, excluding watery intracellular and extracellular fluid from this space
- The cell membrane has many proteins, as well as other lipids (such as cholesterol), that are associated with the phospholipid bilayer
- An important feature of the membrane is that it remains fluid; the lipids and proteins in the cell membrane are not rigidly locked in place



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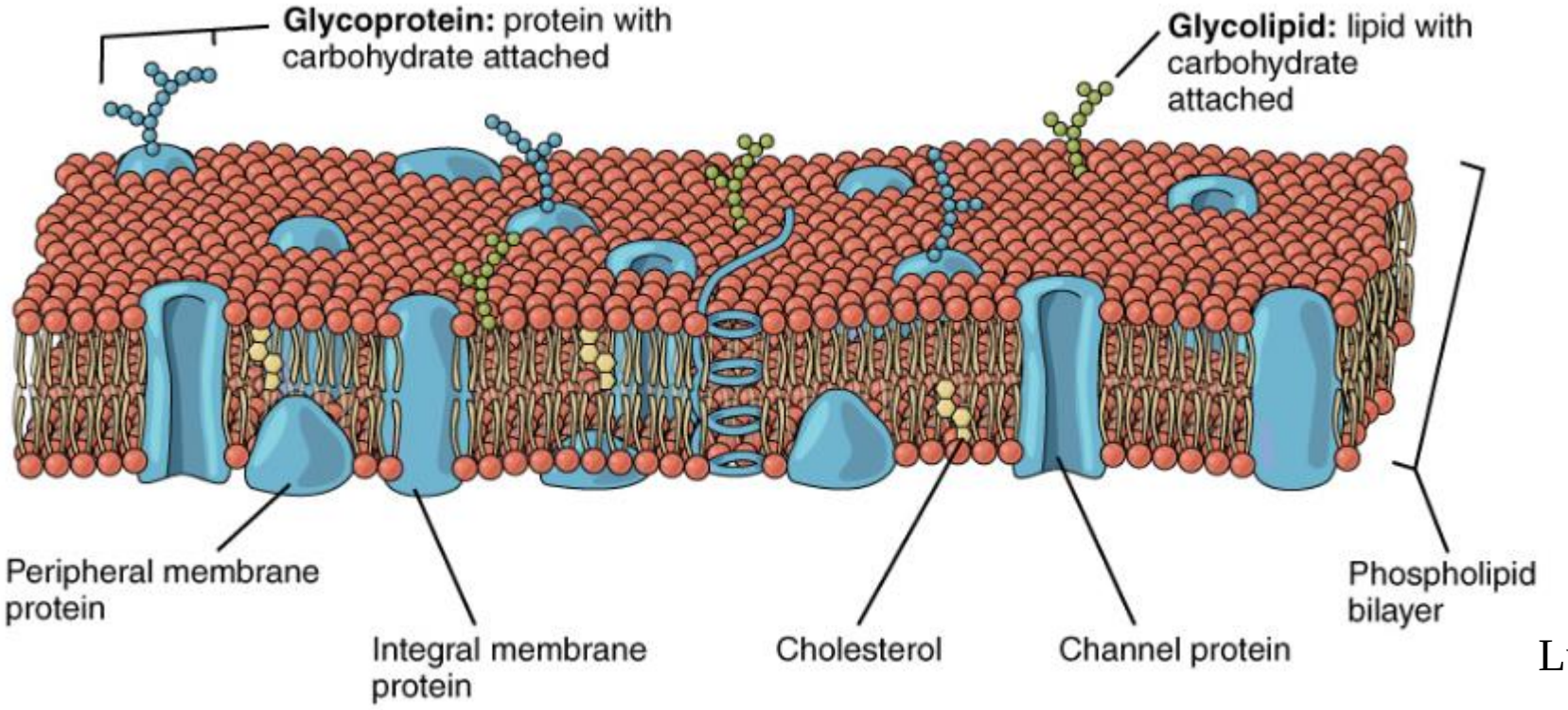
Membrane Proteins

- Lipid bilayer forms the cell membrane , but it is also contains various protein
- There are two types of Protein commonly associated with cell membrane (1) Integrin Proteins

(2) Peripheral Proteins

- Integral proteins are embedded in the cell membrane. Eg. Channel Protein which allows selective particles such as some ions



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- Other important integral protein is recognition protein, it marks an cell's identity, so that other cell recognize and binds
- A **receptor** is a type of recognition protein that can selectively bind a specific molecule outside the cell, and this binding induces a chemical reaction within the cell
- Ligand is a specific molecule which binds to receptor
- Example for Receptor – Ligand Interaction

When a dopamine molecule binds to a dopamine receptor protein, a channel within the transmembrane protein opens to allow certain ions to flow into the cell

- Some integral membrane proteins are glycoproteins
- **Glycoprotein** is a protein that has carbohydrate molecules attached, which extend into the extracellular matrix
- Carbohydrate attached in cells are aid in cell recognition
- Carbohydrate which extends from cell membrane and lipids from some membrane will collectively for **Glycocalyx**

- The **glycocalyx** is a fuzzy-appearing coating around the cell formed from glycoproteins and other carbohydrates attached to the cell membrane
- It has various roles . Example :it may have molecules that allow the cell to bind to another cell, it may contain receptors for hormones, or it might have enzymes to break down nutrients
- These are the products of person's genetic makeup
- This is the primary way that a person' immune cells refuse to attach its own cell
- It is also the reason for organ from other persons gets rejected

Peripheral proteins are typically found on the inner or outer surface of the lipid bilayer but can also be attached to the internal or external surface of an integral protein

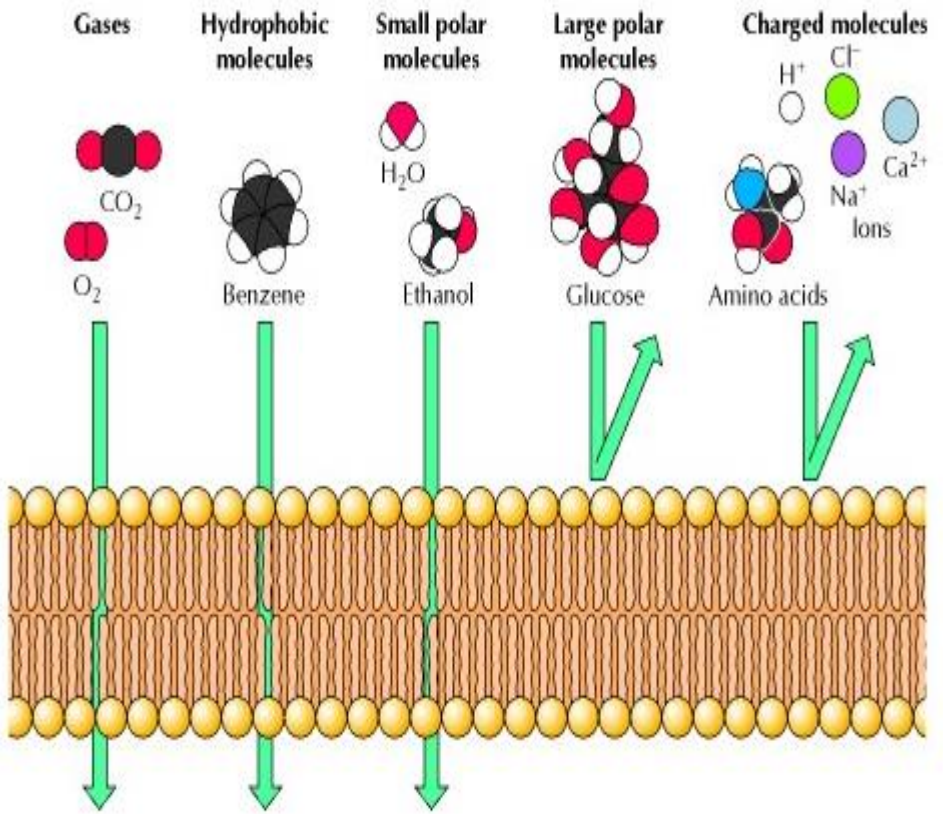
- Some peripheral proteins on the surface of intestinal cells, for example, act as digestive enzymes to break down nutrients to sizes that can pass through the cells and into the bloodstream.

Membrane transport of small molecules

- The internal composition of the cell is maintained because the plasma membrane is selectively permeable to small molecules
- Most biological molecules are unable to diffuse through the phospholipid bilayer, so the plasma membrane forms a barrier that blocks the free exchange of molecules between the cytoplasm and the external environment of the cell
- Specific transport proteins (carrier proteins and channel proteins) then mediate the selective passage of small molecules across the membrane, allowing the cell to control the composition of its cytoplasm.

Passive Diffusion:

- It is simplest mechanism in which any molecule can cross the plasma membrane
- Molecule simply dissolves in lipid bilayer and diffuse across it and dissolves in the aqueous solution of the other side of the membrane
- The net flow of molecules is always down their concentration gradient—from a compartment with a high concentration to one with a lower concentration of the molecule



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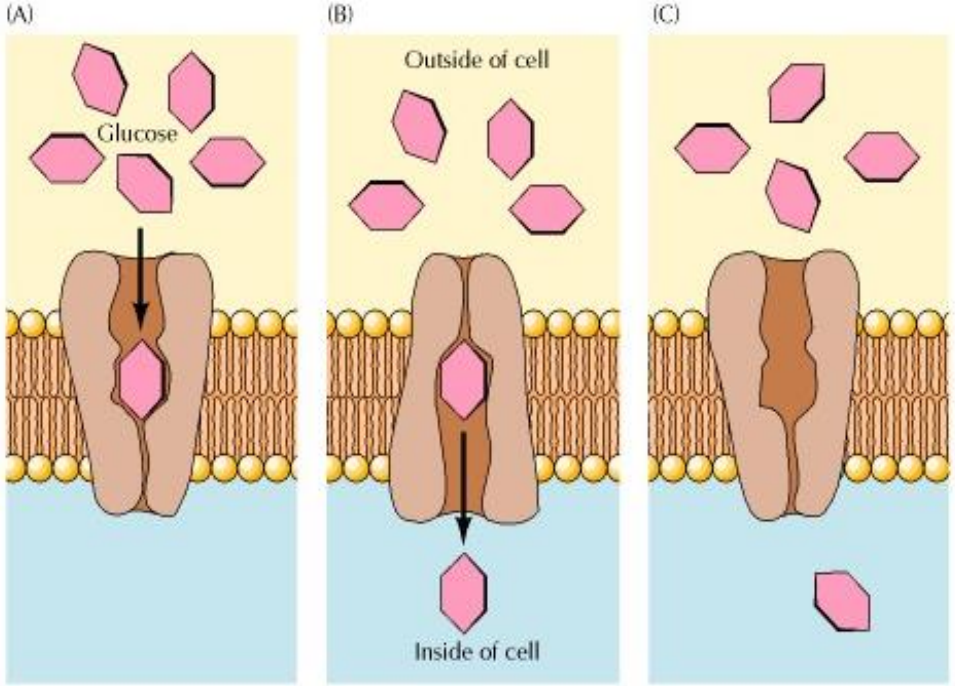
Facilitated Diffusion and Carrier Proteins

- It is like passive diffusion, the movement of the molecules is determined by their concentration inside and outside of the cell
- No external energy required, It differs from passive diffusion in that the transported molecules do not dissolve in the phospholipid bilayer
- Instead, their passage is mediated by proteins that enable the transported molecules to cross the membrane without directly interacting with its hydrophobic interior
- Facilitated diffusion therefore allows polar and charged molecules, such as carbohydrates, amino acids, nucleosides, and ions

- Two types of protein that mediates facilitate diffusion (1)Carrier proteins (2)Channel proteins
- Carrier proteins bind specific molecules to be transported on one side of the membrane
- They then undergo conformational changes that allow the molecule to pass through the membrane and be released on the other side
- In contrast, channel proteins form open pores through the membrane, allowing the free diffusion of any molecule of the appropriate size and charge



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Ion Channels

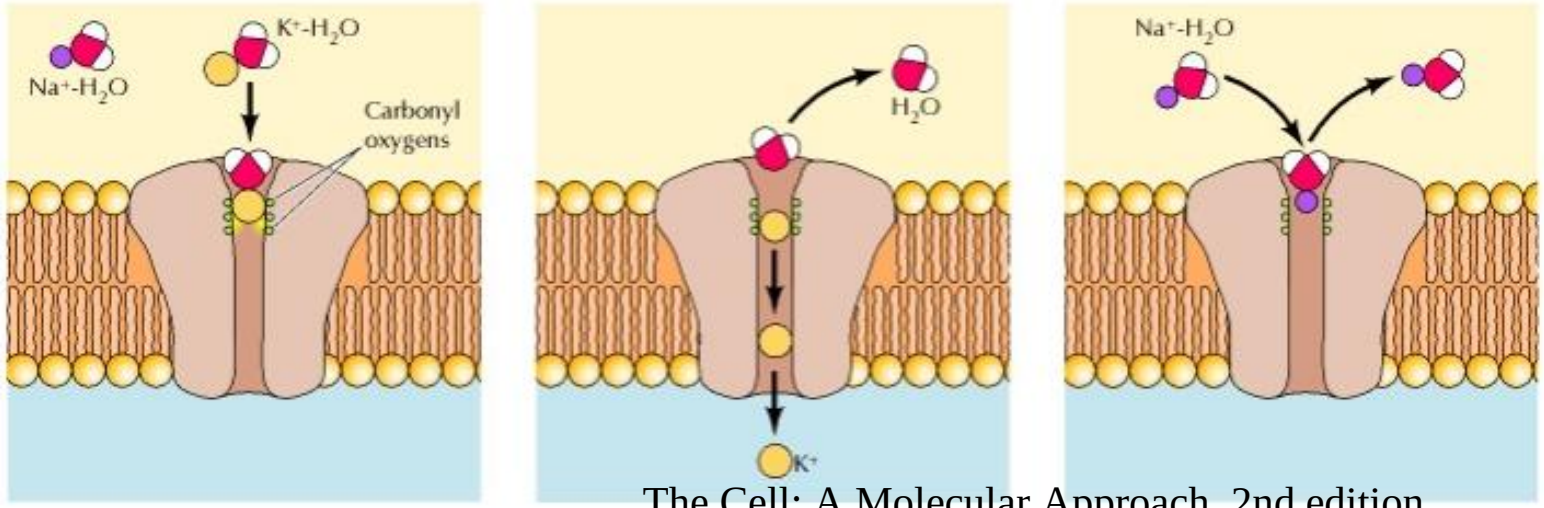
- Channel proteins simply form open pores in the membrane, allowing small molecules of the appropriate size and charge to pass freely through the lipid bilayer
- The plasma membranes of many cells also contain water channel proteins (aquaporins), through which water molecules are able to cross the membrane much more rapidly than they can diffuse through the phospholipid bilayer
- Three properties of ion channels

(1) Transport through channels is extremely rapid

(2) ion channels are highly selective

(3) most ion channels are not

permanently open



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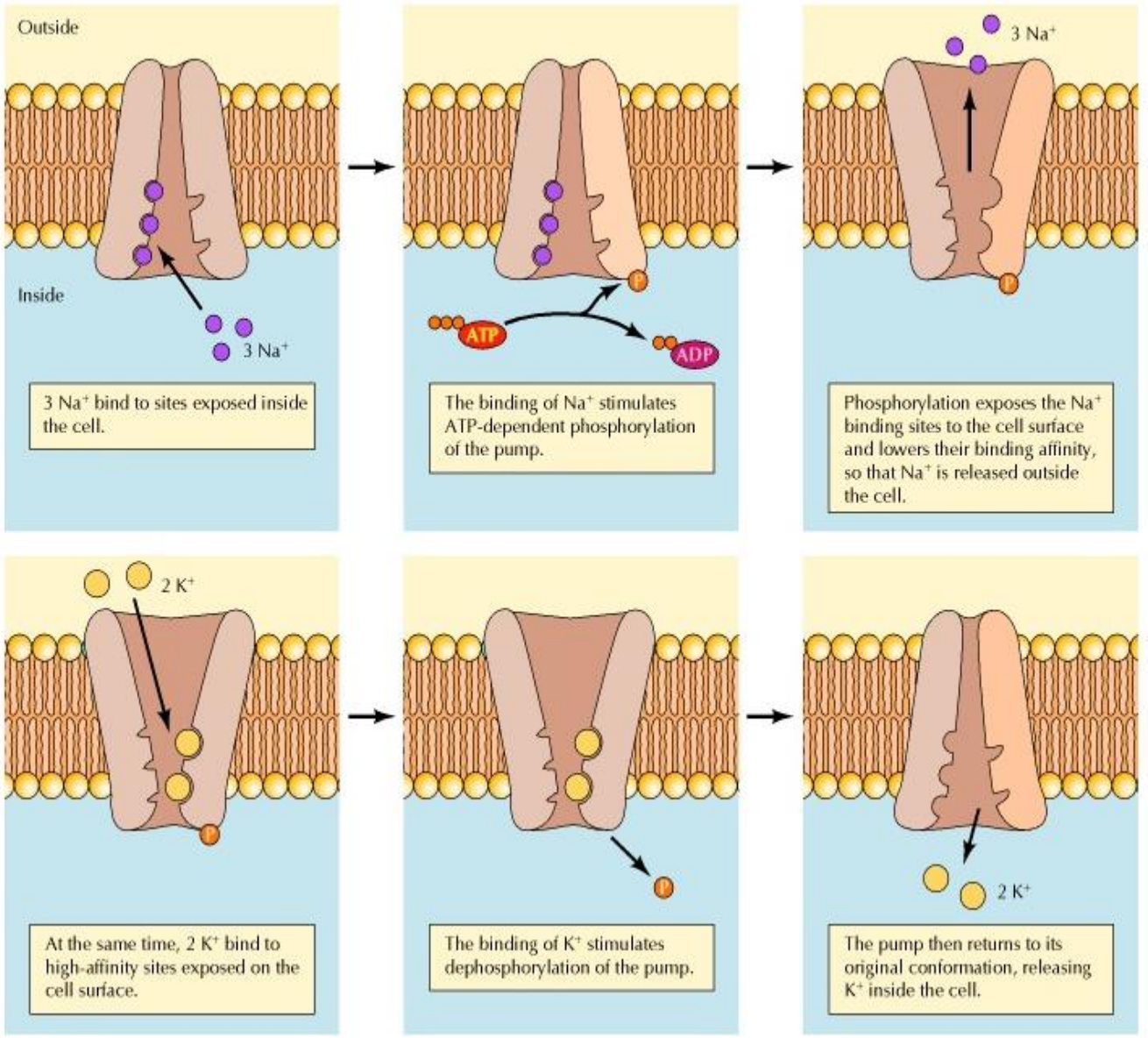
- the opening of ion channels is regulated by “gates” that transiently open in response to specific stimuli
- **ligand-gated channels**- open in response to the binding of neurotransmitters or other signaling molecules
- **voltage-gated channels**- open in response to changes in electric potential across the plasma membrane

Active Transport Driven by ATP Hydrolysis

- The largest family of membrane transporters consists of the ABC transporters, so called because they are characterized by highly conserved ATP-binding domains or *A*TP-*b*inding *c*assettes
- In bacteria, ABC transporters utilize energy derived from ATP hydrolysis to transport a wide range of molecules, including ions, sugars, and amino acids
- In eukaryotic cells, the first ABC transporter was discovered as the product of a gene (called the multi drug resistance, or *mdr*, gene) that makes cancer cells resistant to a variety of drugs used in chemotherapy



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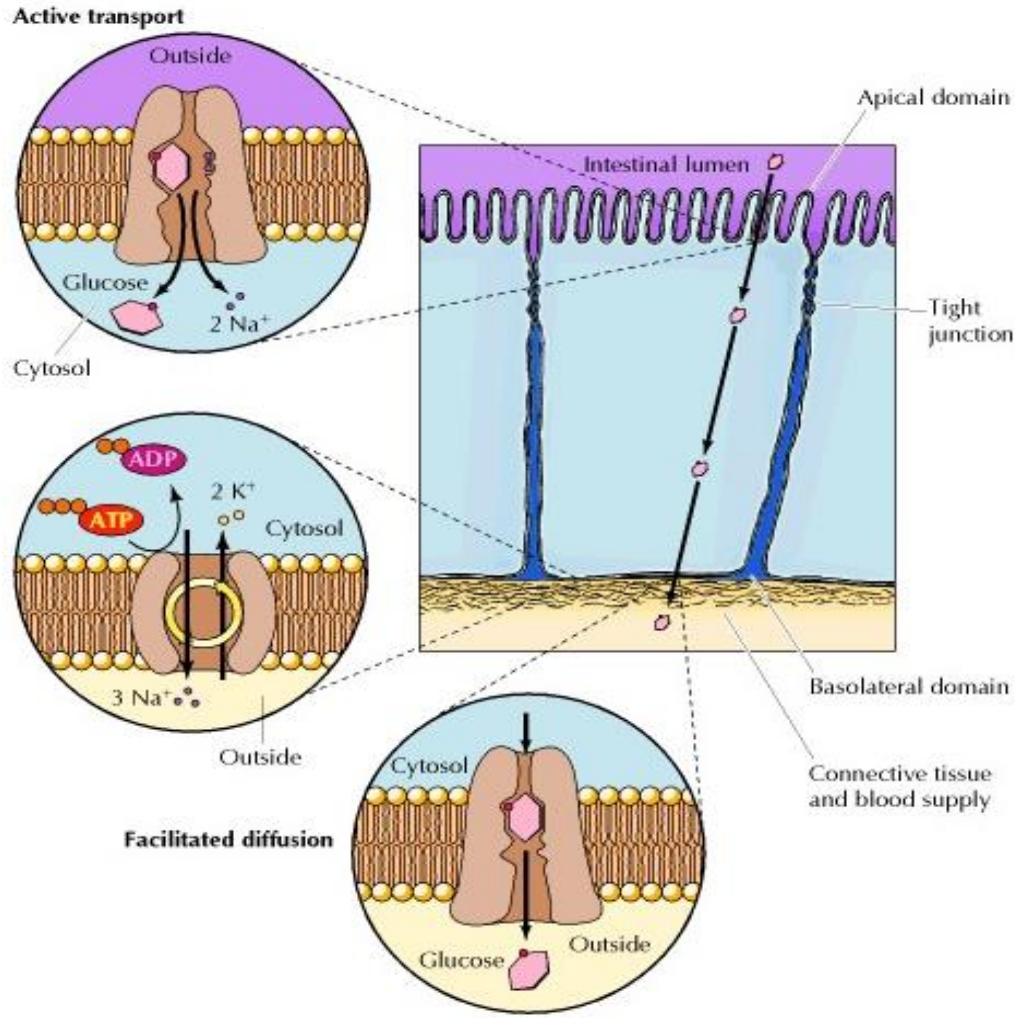
- The ion pumps and ABC transporters utilize energy derived directly from ATP hydrolysis to transport molecules against their electrochemical gradients
- molecules are transported against their concentration gradients using energy derived not from ATP hydrolysis but from the coupled transport of a second molecule in the energetically favorable direction



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Electrical Properties of Membranes

- Channel proteins form hydrophilic pores across the cell membrane
- Some channel proteins form gap junction between two adjacent cells, Each plasma membrane

equally contributes which connects the cytoplasm of each cell

Two properties of ion channel which distinguish it from aqueous protein

(1)Ion Channels Are Ion-Selective and Fluctuate Between Open and Closed States

The permeating ions have to shed most or all of their associated water molecules to pass, often in single file, through the narrowest part of the channel, which is called the *selectivity filter*

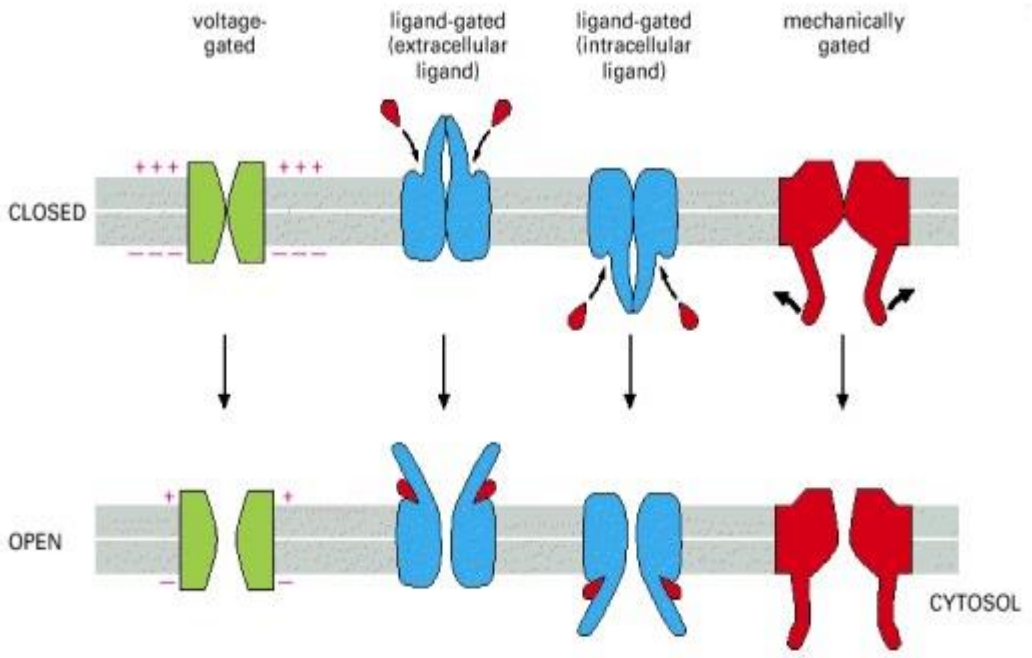
(2) ion channels are not continuously open, they are *gated*, which allows them to open briefly and then close again

- In most cases, the gate opens in response to a specific stimulus

  (1) voltage-gated channels- Open when there is change in voltage

  (2) ligand-gated channels- Opens when ligand binds intracellular or extracellular

  (3) Mechanically gated



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Endocytosis and Exocytosis

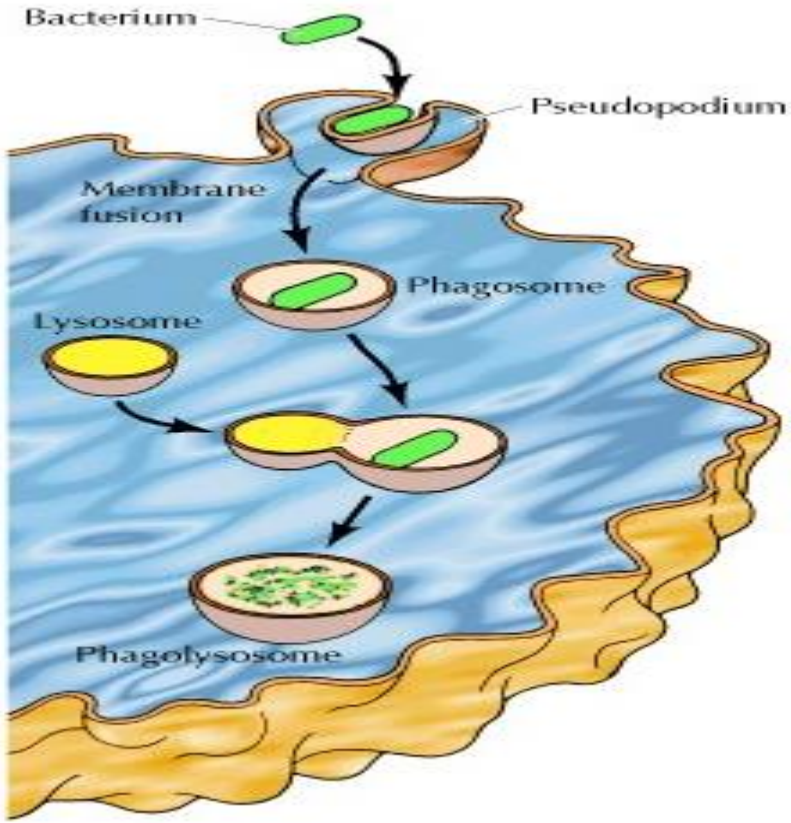
- Eukaryotic cells are also able to take up macromolecules and particles from the surrounding medium by a distinct process called **endocytosis**
- Phagocytosis(cell eating)

(1) The pseudopodia eventually surround the particle and their membranes fuse to form a large intracellular vesicle (>0.25 μm in diameter) called a phagosome

(2) The phagosomes then fuse with lysosomes, producing phagolysosomes in which the ingested material is digested by the action of lysosomal acid hydrolases

- pinocytosis (cell drinking)

The uptake of fluids or molecules into a cell by small vesicles



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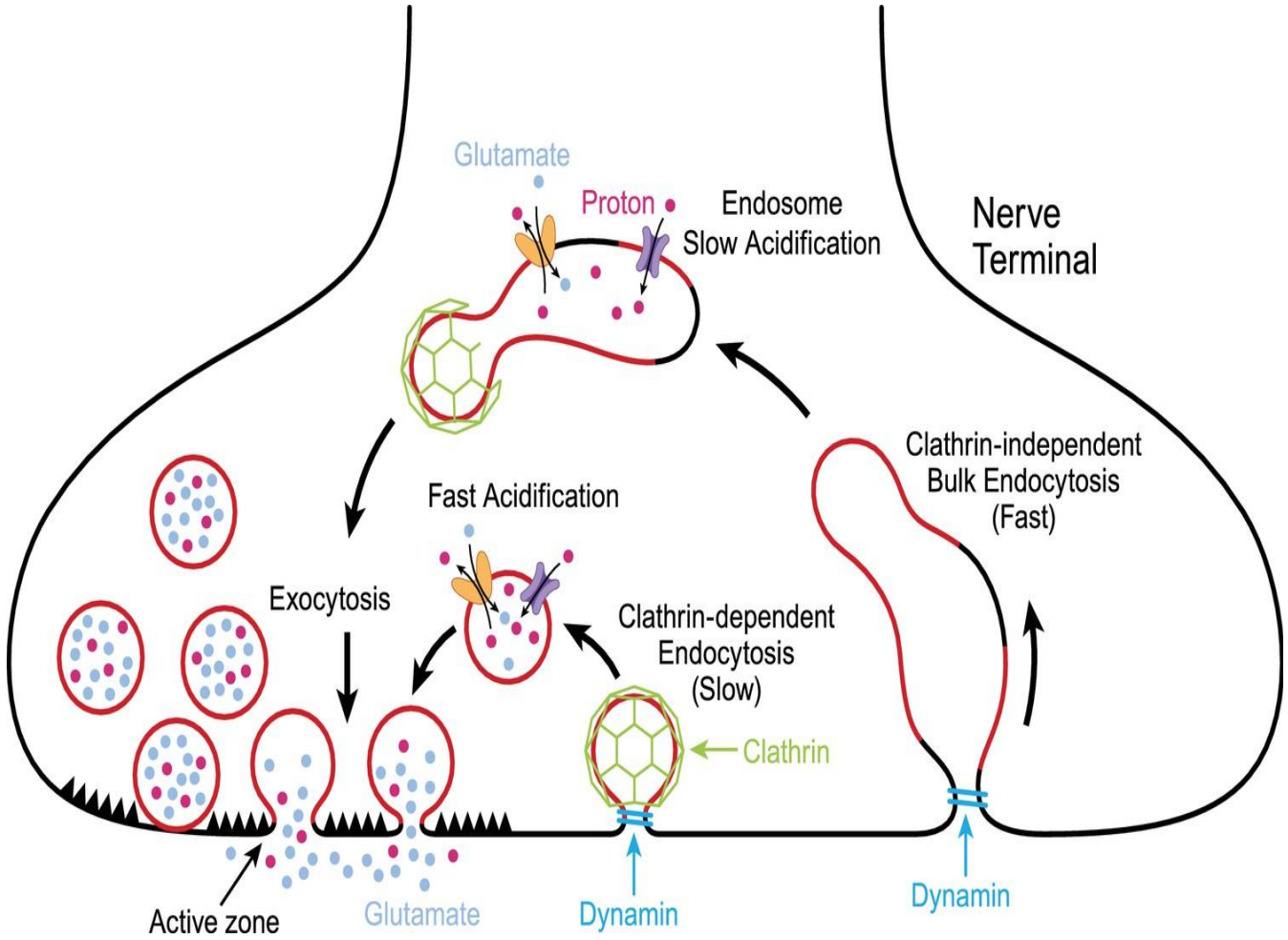
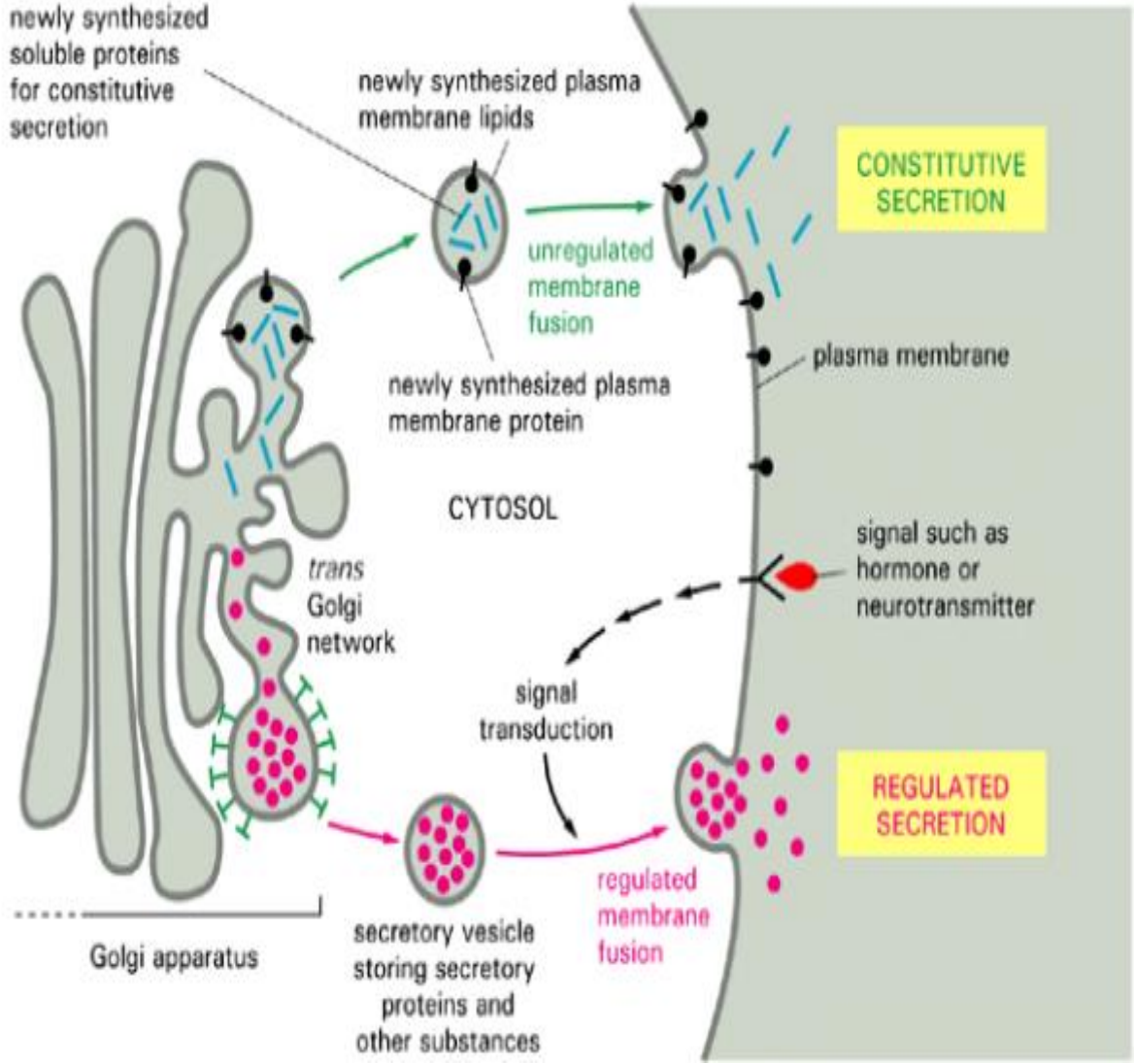
Exocytosis





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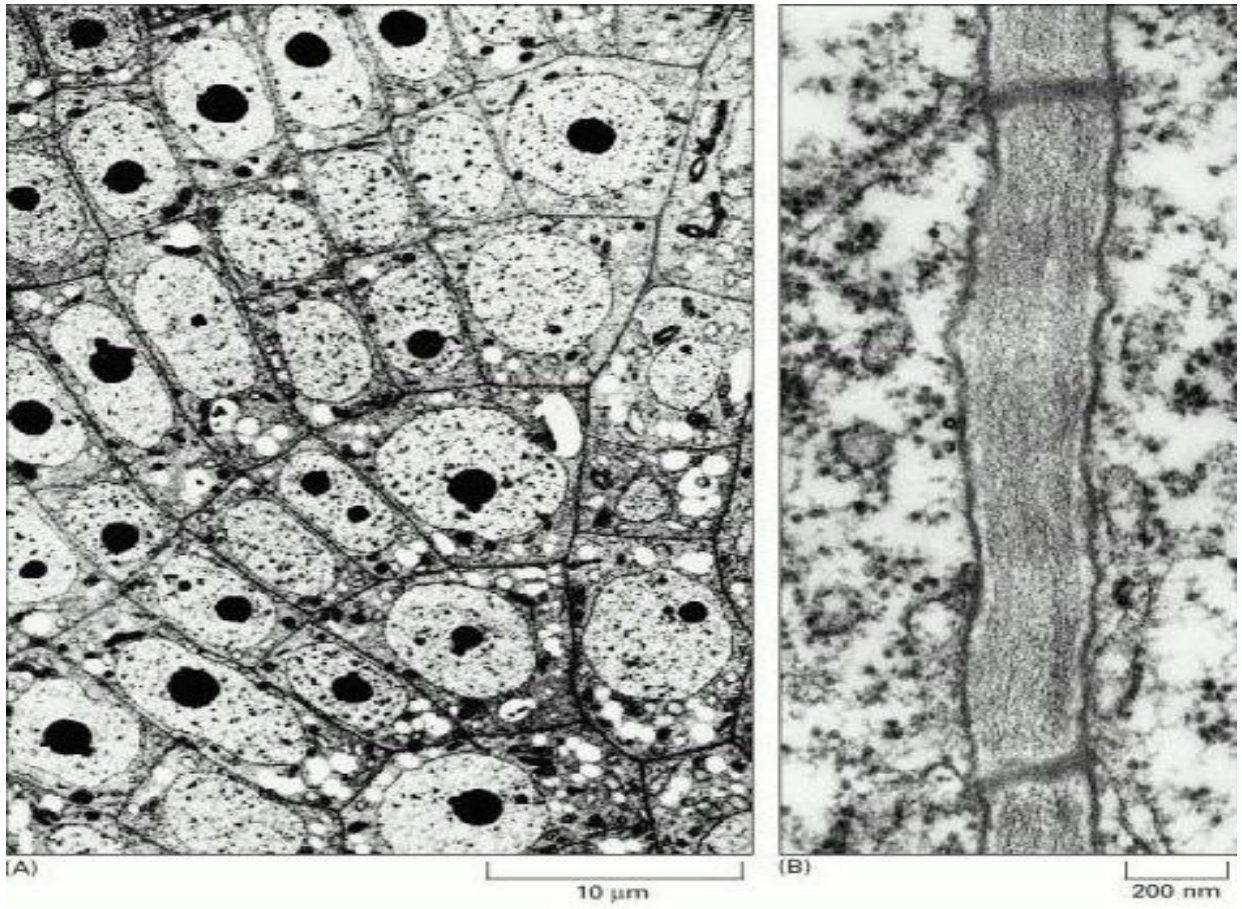
The Plant Cell Wall

- It is an elaborate extracellular matrix that encloses each cell in a plant. It was the thick cell walls of cork, visible in a primitive microscope, that in 1663 enabled Robert Hooke to distinguish and name cells for the first time



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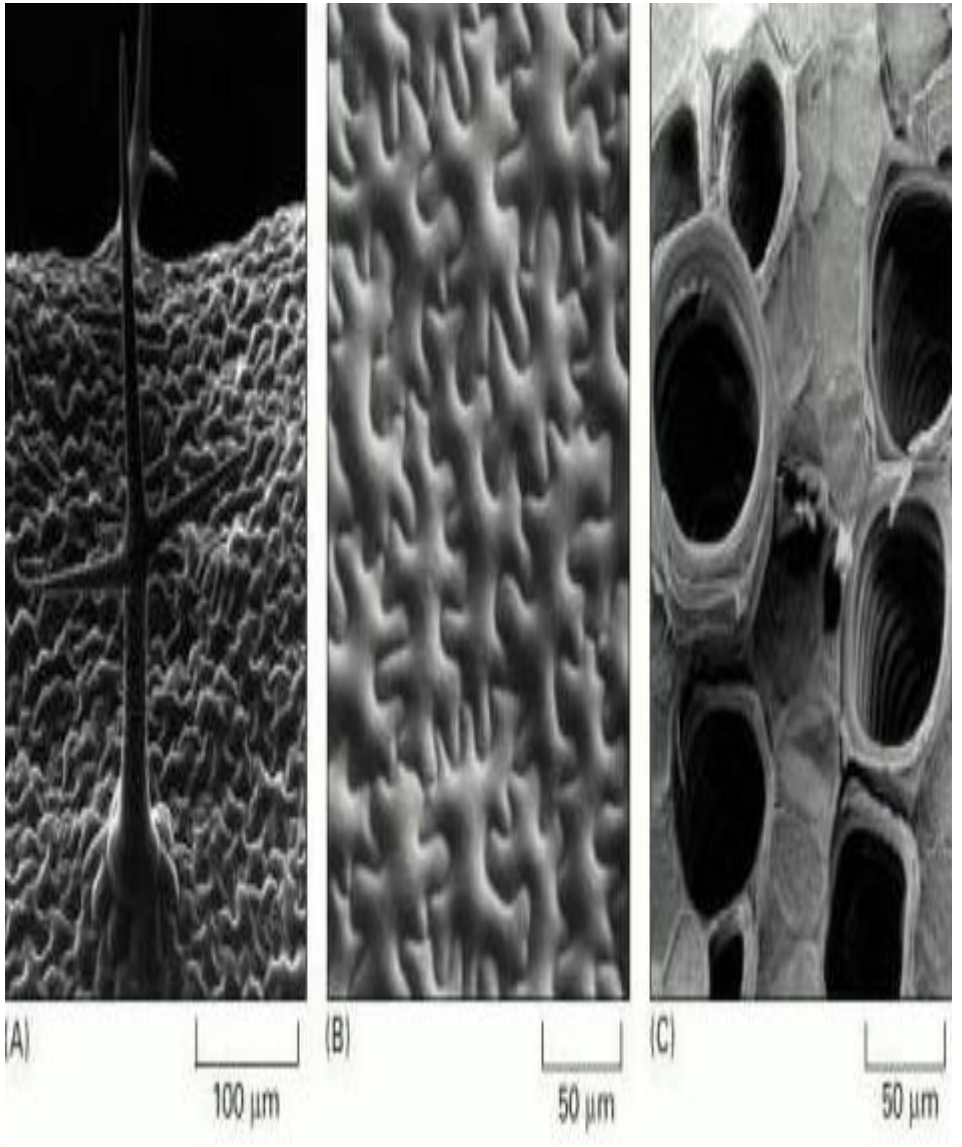
Figures A) Electron micrograph of the root tip of a rush, showing the organized pattern of cells that results from an ordered sequence of cell divisions in cells with relatively rigid cell walls. In this growing tissue, the cell walls are still relatively thin, appearing as fine black lines between the cells in the micrograph.

(B) Section of a typical cell wall separating two adjacent plant cells.

The Composition of the Cell Wall Depends on the Cell Type

- All cell walls in plants have their origin in dividing cells, as the cell plate forms during cytokinesis to create a new partition wall between the daughter cells
- The new cells are usually produced in special regions called *meristems*
- To accommodate subsequent cell growth, their walls, called **primary cell walls**, are thin and extensible, although tough
- Once growth stops, the wall no longer needs to be extensible: sometimes the primary wall is retained without major modification, but, more commonly, a rigid, **secondary cell wall** is produced by depositing new layers inside the old ones
- polymer in secondary walls is **lignin**, a complex network of phenolic compounds found in the walls of the xylem vessels and fiber cells of woody tissues

- (A) A trichome, or hair, on the upper surface of an *Arabidopsis* leaf. This spiky, protective single cell is shaped by the local deposition of a tough, cellulose-rich wall.
- (B) Surface view of tomato leaf epidermal cells. The cells fit together snugly like the pieces of a jigsaw puzzle, providing a strong outer covering for the leaf. The outer cell wall is reinforced with a cuticle and waxes that waterproof the leaf and help defend it against pathogens. (C) This view into young xylem elements shows the thick, lignified, hoop-reinforced secondary cell wall that creates robust tubes for the transport of water throughout the plant



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- The Cell: A Molecular Approach , Geoffrey M. Cooper
- Lumen learning

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Programme: M.Sc., Biochemistry
Course Title : Cell biology
Course Code :BC105DCE

Unit-2
CELL ORGANELLES

Dr. M. Anusuyadevi Jayachandran
Professor
Department of Biochemistry

Content

- Nucleus
- Mitochondria
- Microbodies
- Peroxisomes
- lysosomes
- Endoplasmic Reticulum
- Golgi apparatus
- Plastids
- Chloroplast
- Vacuoles
- Centrosomes
- Ribosomes

Organelles bounded by Double membrane Envelopes

Nucleus:

- Nucleus was discovered by Scottish botanist and paleobotanist Robert Brown in 1831
- Discovery of the Nucleus, a lecture-demonstration published in Linnean Society, 5 November
- The cell nucleus is a membrane-bound structure that contains the cell's hereditary information and controls the cell's growth and reproduction
- It is the command center of a eukaryotic cell and is commonly the most prominent organelle in a cell accounting for about 10 percent of the cell's volume
- In general, a eukaryotic cell has only one nucleus. However, some eukaryotic cells are enucleated cells (without a nucleus), for example, red blood cells (RBCs); whereas, some are multinucleate (consists of two or more nuclei), for example, slime molds

- The nucleus is separated from the rest of the cell or the cytoplasm by a nuclear membrane
- As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell
- nuclear envelope composed of two membranes: inner and outer nuclear membranes
- They are separated by a perinuclear space measuring about 20–40 nm across
- outer nuclear membrane is continuous with the endoplasmic reticulum, making the perinuclear space continuous with the lumen of the ER

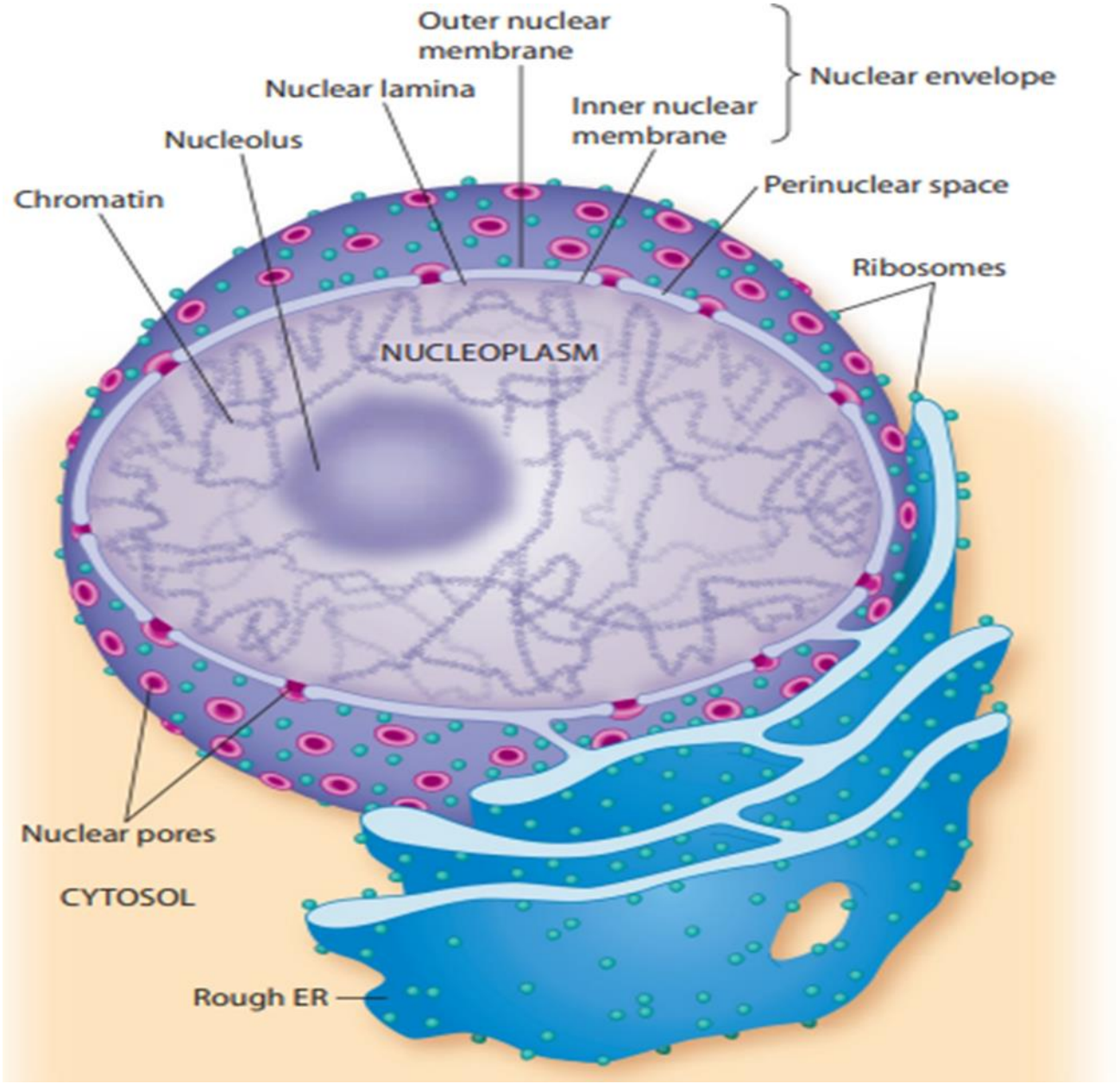


Figure:Becker's World of the Cell NINTH EDITION

- outer membrane of ER is studded on its outer surface with ribosomes engaged in protein synthesis
- Nuclear pore are specialized channels present in the nucleus envelop
- **Figure:Negative staining of an oocyte nuclear envelope reveals the octagonal pattern of the nuclear pore complexes**
- mammalian nucleus has about 3000–4000 pores, or about 10–20 pores per square micrometer of membrane surface area
- At each pore, the inner and outer membranes of the nuclear envelope are fused together, forming a channel that is lined with an intricate protein structure called the nuclear pore complex (NPC)

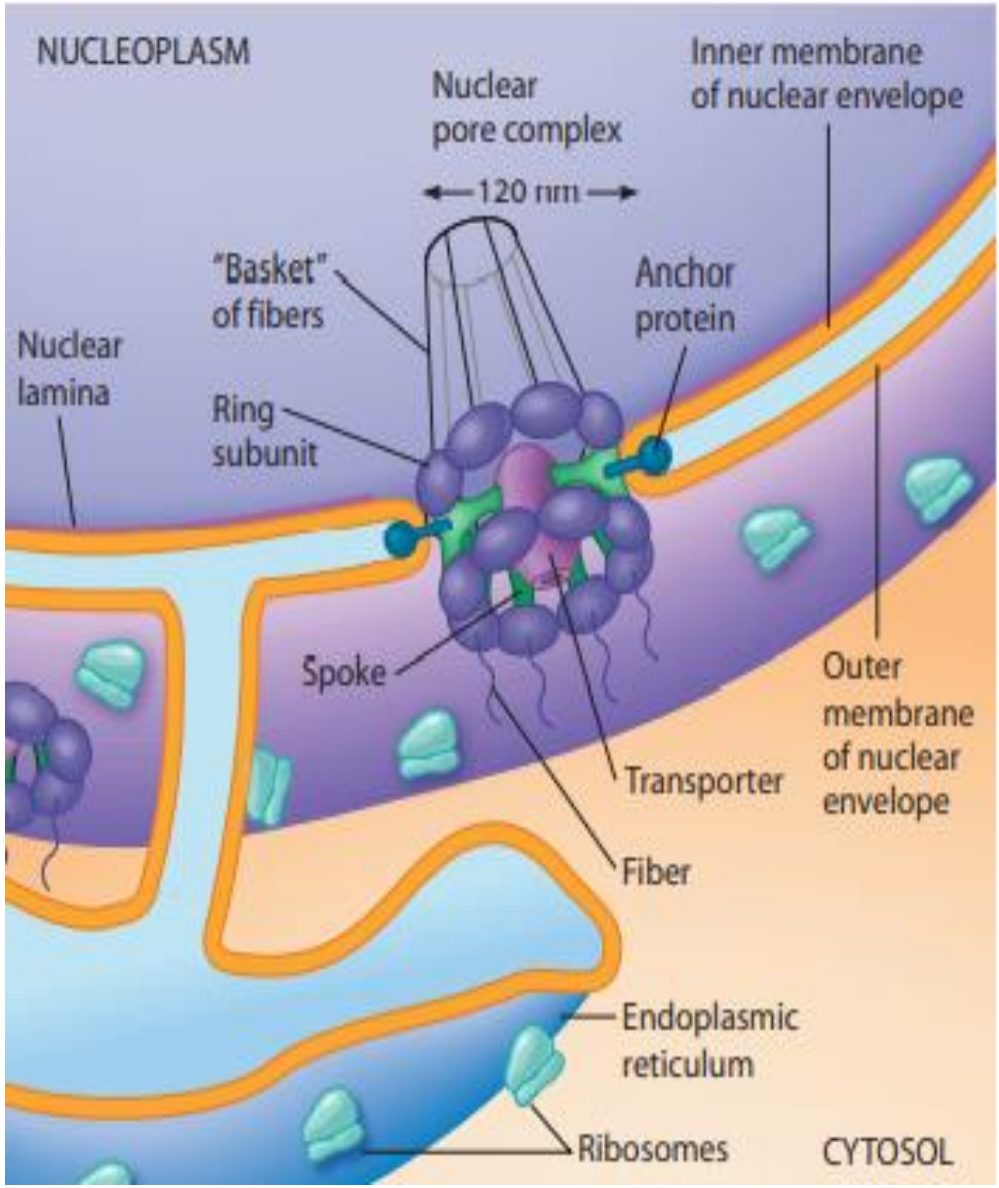


Figure:Becker's World of the Cell NINTH EDITION

Molecules Enter and Exit the Nucleus Through Nuclear Pores

- All the enzymes and other proteins required for chromosome replication and transcription of DNA in the nucleus must be imported from the cytosol,
- all the RNA molecules and partially assembled ribosomes needed for protein synthesis in the cytosol must be obtained from the nucleus
- An actively growing mammalian cell can easily be synthesizing 20,000 ribosomal subunits/min
- cell has about 3000–4000 nuclear pores, so ribosomal subunits must be transported to the cytosol at a rate of about five to six subunits per minute per pore

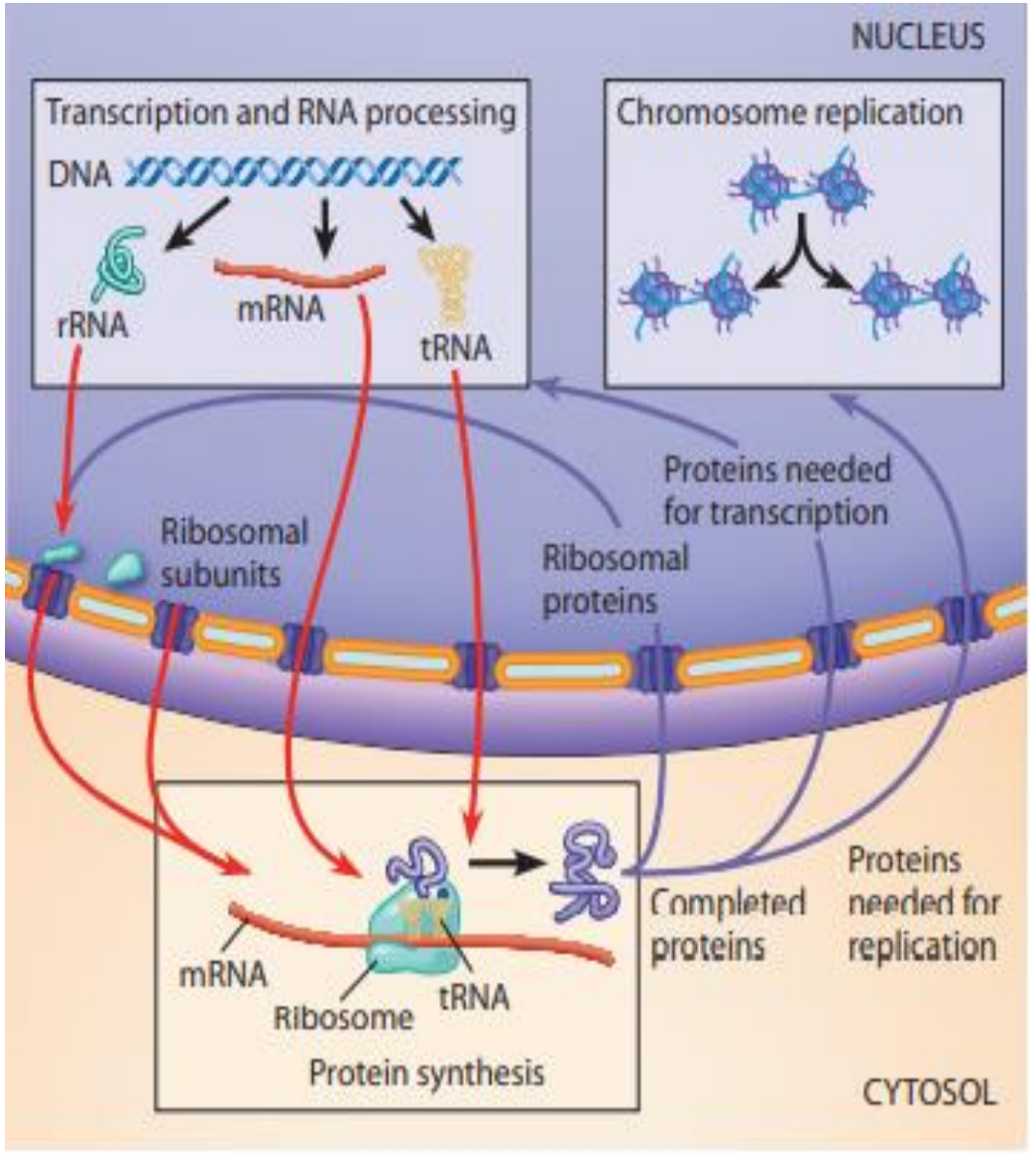


Figure:Becker's World of the Cell NINTH EDITION

- When chromosomes are being replicated, histones are needed at the rate of about 300,000 molecules per minute
- The rate of inward movement must be about 100 histone molecules per minute per pore
- In addition to all this macromolecular traffic, the pores mediate the transport of smaller particles, molecules, and ions

Other transport mechanisms:

1. Simple Diffusion of Small Molecules Through Nuclear Pores

- Maximum diameter 9 nm for simple diffusion

1. Active Transport of Large Proteins and RNA Through Nuclear Pores

- active transport through nuclear pores requires energy and involves specific binding of the transported substance to membrane proteins (Maximum diameter is 26 nm)

- molecular mechanism is best understood for Proteins are actively transported from the cytosol into the nucleus
- they possess one or more nuclear localization signals (NLS), which are amino acid sequences that enable the protein to be recognized by FG nucleoporins and then transported through the nuclear pore
- An NLS is usually 8–30 amino acids in length and often contains proline as well as the positively charged (basic) amino acids lysine and arginine

Nuclear Import via the Ran/Importin Pathway

- NLS-containing proteins are imported into the nucleus via a special receptor protein called an importin
- Mechanism is explained in the following figure

Transport Through the Nuclear Pore Complex

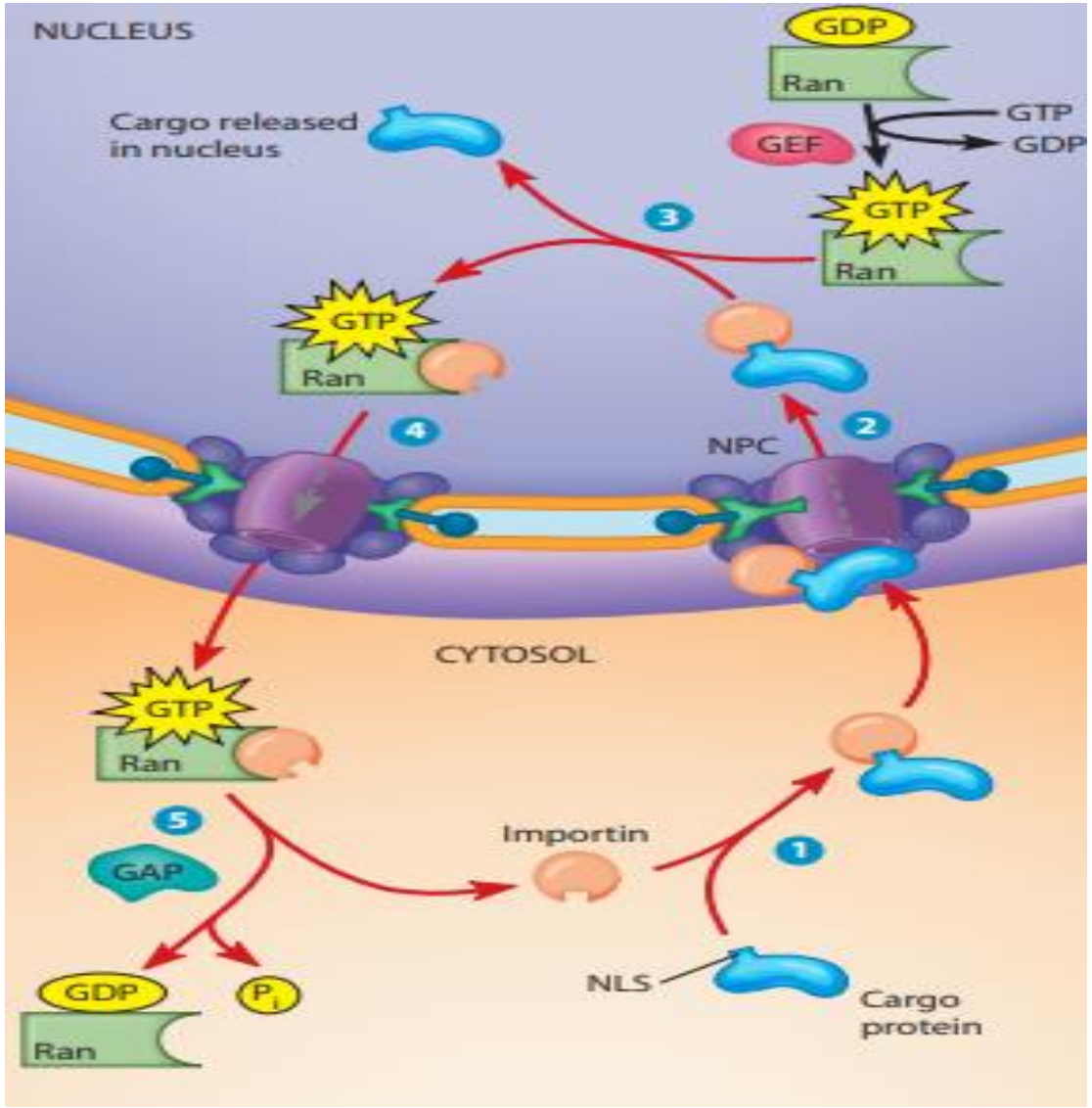


Figure:Becker's World of the Cell NINTH EDITION

Proteins made in the cytosol and destined for use in the nucleus contain a nuclear localization sequence (NLS) that targets them as "cargo" for transport through the nuclear pore complex.

1. An NLS containing cargo protein binds to importin
2. the importin-cargo complex is then transported through the nuclear pore complex
3. Nuclear Ran-GTP binds to importin, triggering the release of the cargo protein in the nucleus
4. The Ran-GTP-importin complex is transported back to the cytosol
5. the hydrolysis of GTP to GDP is accompanied by the release of importin

Nucleoplasm is the gelatinous substance within the nuclear envelope

- Also called karyoplasm, this semi-aqueous material is similar to the cytoplasm and is composed mainly of water with dissolved salts, enzymes, and organic molecules suspended within
- The nucleolus and chromosomes are surrounded by nucleoplasm, which functions to cushion and protect the contents of the nucleus
- Nucleoplasm also supports the nucleus by helping to maintain its shape. Additionally, nucleoplasm provides a medium by which materials, such as enzymes and nucleotides (DNA and RNA subunits), can be transported throughout the nucleus. Substances are exchanged between the cytoplasm and nucleoplasm through nuclear pores

Nucleolus: Contained within the nucleus is a dense, membrane-less structure composed of RNA and proteins called the nucleolus

- Some of the eukaryotic organisms have a nucleus that contains up to four nucleoli

- The 45S precursor rRNA is packaged in a large ribonucleoprotein particle containing many ribosomal proteins imported from the cytoplasm
- While this particle remains at the nucleolus, selected components are added and others discarded as it is processed into immature large and small ribosomal subunits
- The two ribosomal subunits attain their final functional form only after each is individually transported through the nuclear pores into the cytoplasm
- Other ribonucleoprotein complexes, including telomerase shown here, are also assembled in the nucleolus

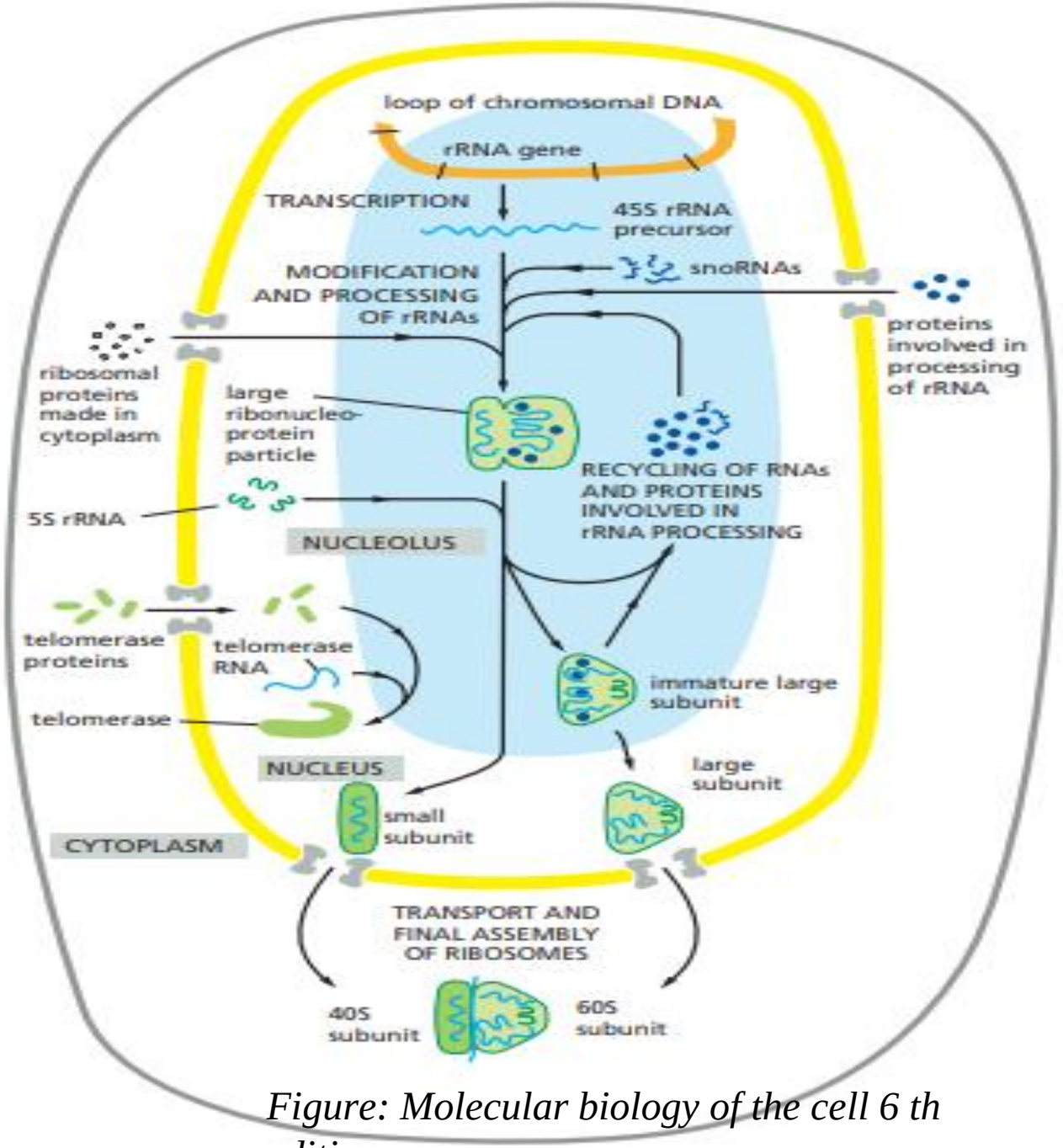


Figure: Molecular biology of the cell 6 th edition

- The nucleolus contains nucleolar organizers, which are parts of chromosomes with the genes for ribosome synthesis on them. The nucleolus helps to synthesize ribosomes by transcribing and assembling ribosomal RNA subunits. These subunits join together to form a ribosome during protein synthesis
- The nucleolus disappears when a cell undergoes division and is reformed after the completion of cell division

Chromatin

- The nucleus is the organelle that houses chromosomes
- Chromosomes consist of DNA, which contains heredity information and instructions for cell growth, development, and reproduction

- Chromosomes are present in the form of strings of DNA and histones (protein molecules) called chromatin
- When a cell is “resting” i.e. not dividing, the chromosomes are organized into long entangled structures called chromatin
- The chromatin is further classified into heterochromatin and euchromatin based on the functions. The former type is a highly condensed, transcriptionally inactive form, mostly present adjacent to the nuclear membrane. On the other hand, euchromatin is a delicate, less condensed organization of chromatin, which is found abundantly in a transcribing cell
- Besides the nucleolus, the nucleus contains a number of other non-membrane-delineated bodies. These include Cajal bodies, Gemini of coiled bodies, polymorphic interphase karyosome association (PIKA), promyelocytic leukemia (PML) bodies, paraspeckles, and splicing speckles
- Nuclear Structure and Dynamics | Basicmedical Key

Table 14-1 MAJOR NUCLEAR SUBDOMAINS

Structure	Comments
Cajal bodies	Formerly known as coiled bodies. About 0.2 to 1 μm in diameter, Cajal bodies have a coiled fibrous substructure. First identified by electron microscopy, up to 10 of these structures are seen per cell. They contain a characteristic protein called p80-coilin. They may be involved in snRNP and snoRNP assembly.
GEMs	GEMs are usually found paired with Cajal bodies, which they may overlap. They contain the survival of motor neurons (SMN) protein, which is encoded by the gene mutated in spinal muscular atrophy, a severe, inherited, human, muscular wasting disease. SMN and its cofactors appear to play an essential role in the assembly and maturation of snRNPs (see Chapter 16).
Nuclear bodies	Function unknown. 5 to 20 spots within the nucleus. Originally observed in electron micrographs of cells following hormonal treatments. However it is not clear that all nuclear bodies described in various cell types are structurally or functionally homologous. A marker antigen for some types of nuclear bodies (called PBC 95K—M_r 95 kD) is recognized by autoantibodies from patients with primary biliary cirrhosis. Some may correspond to PML bodies.
Nucleolus	The nucleolus (typically 1 to 5 structures of 0.5 to 5 μm diameter in mammalian cell nuclei) is the site of rRNA transcription and processing, as well as of preribosomal assembly. It is also the site of processing of several other noncoding RNAs, including the RNA component of the signal recognition particle (SRP—Chapter 20).
PIKA	The polymorphic interphase karyosomal association (PIKA) was later rediscovered and termed the OPT domain. The PIKA may be up to 5 μm in diameter during G_1 phase, but its morphology and number vary across the cell cycle. It appears to correspond to sites of sensing or repair of DNA damage as well as concentrations of certain transcription factors.
PML bodies	Also known as PODs and ND10, 10 to 30 of these structures are scattered throughout the nucleus. They are have links with human disease, and in some cases appear to be targeted during viral infections. Fusion of the marker protein PML to the α-retinoic acid receptor is often found in acute promyelocytic leukemia (hence the name PML), in which the PML bodies appear highly fragmented. The link with cancer appears significant, as treatments that are effective against PML appear to restore the normal morphology of PML bodies (see text).
Speckles	Speckles are concentrations of components involved in RNA processing. They often correspond to clusters of interchromatin granules seen by electron microscopy. They may serve as storage depots of splicing factors, or they may play a more active role in splicing factor modification and/or assembly.

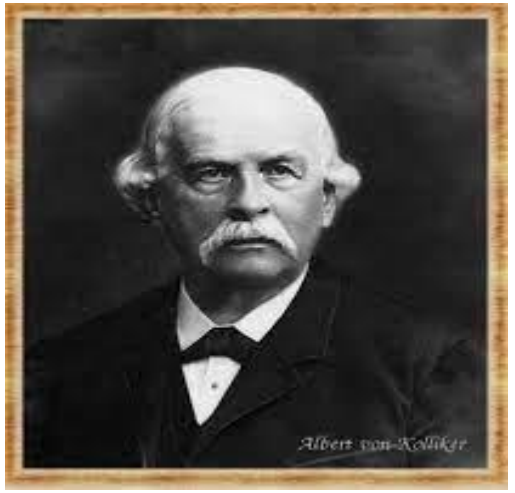
Functions of Nucleus

- The nucleus provides a site for genetic transcription that is segregated from the location of translation in the cytoplasm, allowing levels of gene regulation that are not available to prokaryotes. The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle.
- It controls the hereditary characteristics of an organism.
- The organelle is also responsible for protein synthesis, cell division, growth, and differentiation.
- Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatin.
- Storage of proteins and RNA (ribonucleic acid) in the nucleolus.

- The nucleus is a site for transcription in which messenger RNA (mRNA) are produced for protein synthesis.
- During the cell division, chromatins are arranged into chromosomes in the nucleus.
- Production of ribosomes (protein factories) in the nucleolus.
- Selective transportation of regulatory factors and energy molecules through nuclear pores.

Mitochondria:

- Powerhouse of the cell and double membrane bound organelle in cytoplasm
- It was discovered by German pathologist Richard Altman in 1894
- The word mitochondria was coined by German microbiologist Carl Benda in 1898
- Mito-Thread, Chondrion- granule (little granule) meaning in Greek
- Important role is to generate metabolic energy in eukaryotic cells by breaking down the glucose and fatty acids (Aerobic respiration)
- Mitochondrial DNA is maternally inherited in most animals
- Mother will have both genes and cytoplasm in their eggs
- Hence ,mt DNA is called maternal inheritance



Richard Altman

Carl Benda

Morphology

- Size: 0.05- 1.0µm
- Length- 1-10 µm long
- Bean shaped ,it is elongated thread like structure
- Numbers –It depends on the size, type and functional state of the cell
- Eg. An average liver cell contains 1500 mitochondria

Functions of Mitochondria

- The most important function of mitochondria is to produce energy. Mitochondria produce the molecule adenosine triphosphate (ATP), one of the cell's energy currencies that provide the energy to drive a host of cellular reactions and mechanisms
- The simpler molecules of nutrition are sent to the mitochondria to be processed and to produce charged molecules. These charged molecules combine with oxygen and produce ATP molecules. This process is known as oxidative phosphorylation
- Mitochondria may also produce heat (brown fat), and accumulate iron-containing pigments (Heme ferritin), ions of Ca2+ and HPO_4^{2-} (or phosphate; e.g., osteoblasts of bones or yolk proteins)

- Mitochondria help the cells to maintain the proper concentration of calcium ions within the compartments of the cell
- The mitochondria also help in building certain parts of blood and hormones like testosterone and estrogen
- The liver cell’s mitochondria have enzymes that detoxify ammonia
- The mitochondria also play an important role in the process of apoptosis or programmed cell death
- Abnormal death of cells due to the dysfunction of mitochondria can affect the function of an organ

Genetic system of Mitochondria

- Mitochondria contain own genetic system, which is unique from nuclear genome of the cell
- Most mitochondrial proteins are translated on free cytosolic ribosomes and imported into the organelle by specific targeting signals
- In addition, mitochondria are unique among the cytoplasmic organelles they contain their own DNA, which encodes tRNAs, rRNAs, and some mitochondrial proteins
- It thought to be evolved from the bacteria that developed a symbiotic relationship in which it lived in larger cell
- This hypothesis was proposed by Lynn Margulis in 1960
- the results of DNA sequence analysis, showed similarities between the genomes of mitochondria and of the bacterium *Rickettsia prowazekii* (Parasite)
- They may share common ancestor, mitochondrial has circular genome like bacteria

Endosymbiotic Theory –Origin of Mitochondria

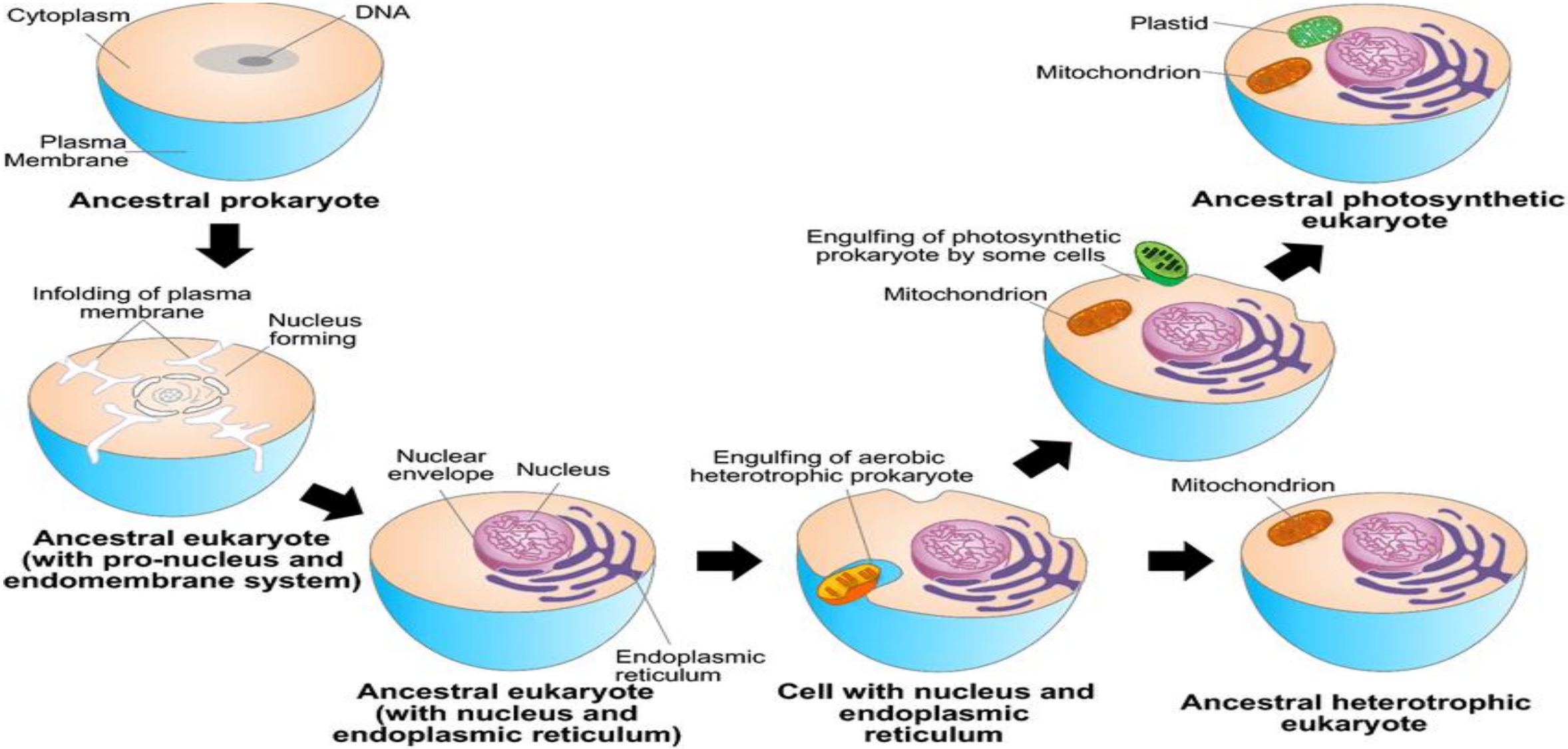


Figure: Cellular and Molecular Life Sciences

- Size of genome is 16 kb in human and most related animals, Plants – (<200 kb)
- Yeast -80 kb ,A.thaliana has 367kb were most of it are non coding.

Biogenesis:

- Human, Mitochonria genome encodes for 13 proteins involved in electron transport and oxidative phosphorylation are designated as components of respiratory complexes I, III, IV, or V (figure)
- The region of the genome designated “D loop” contains an origin of DNA replication and transcriptional promoter sequences
- the genome contains genes for 12S and 16S rRNAs and for 22 tRNAs, which are designated by the one-letter code for the corresponding amino acid

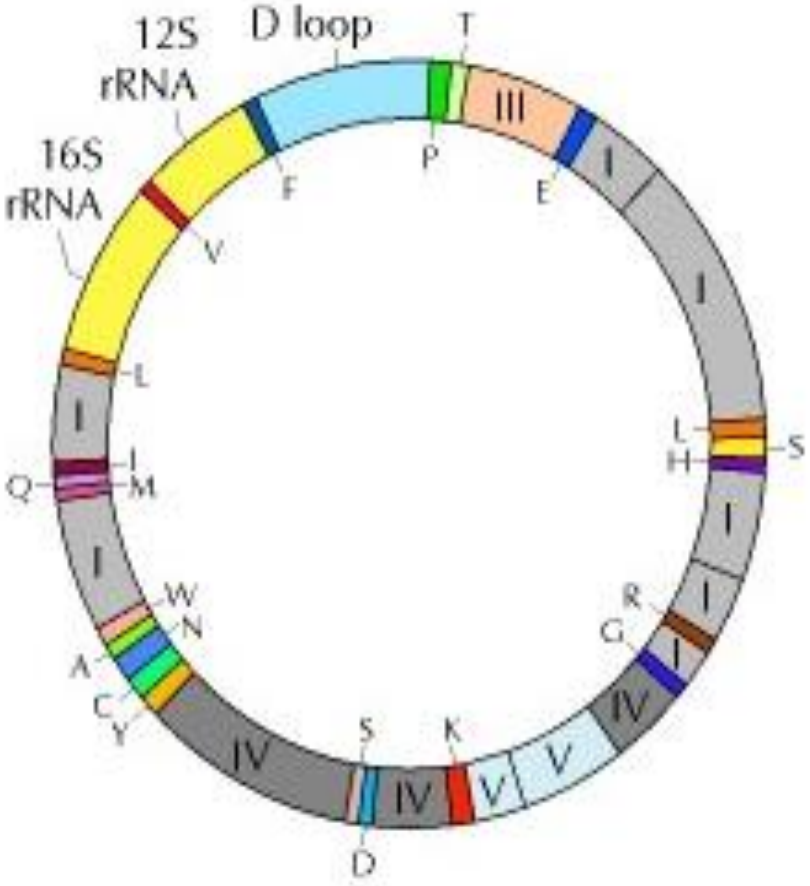


Figure: The Cell: A Molecular Approach. 2nd edition.

- Like other cells Mitochondria also divide and fuse to maintain their number of cells
- Mitochondrial biogenesis is required to compensate for decreased mitochondrial biomass resulting from mitochondrial degradation
- imbalance between mitochondrial fusion, fission, biogenesis and degradation events could cause substantial changes in mitochondrial number, biomass, shape and function
- P indicates a phagophore by which targeted mitochondria are engulfed during the sequestering process required for mitophagy.

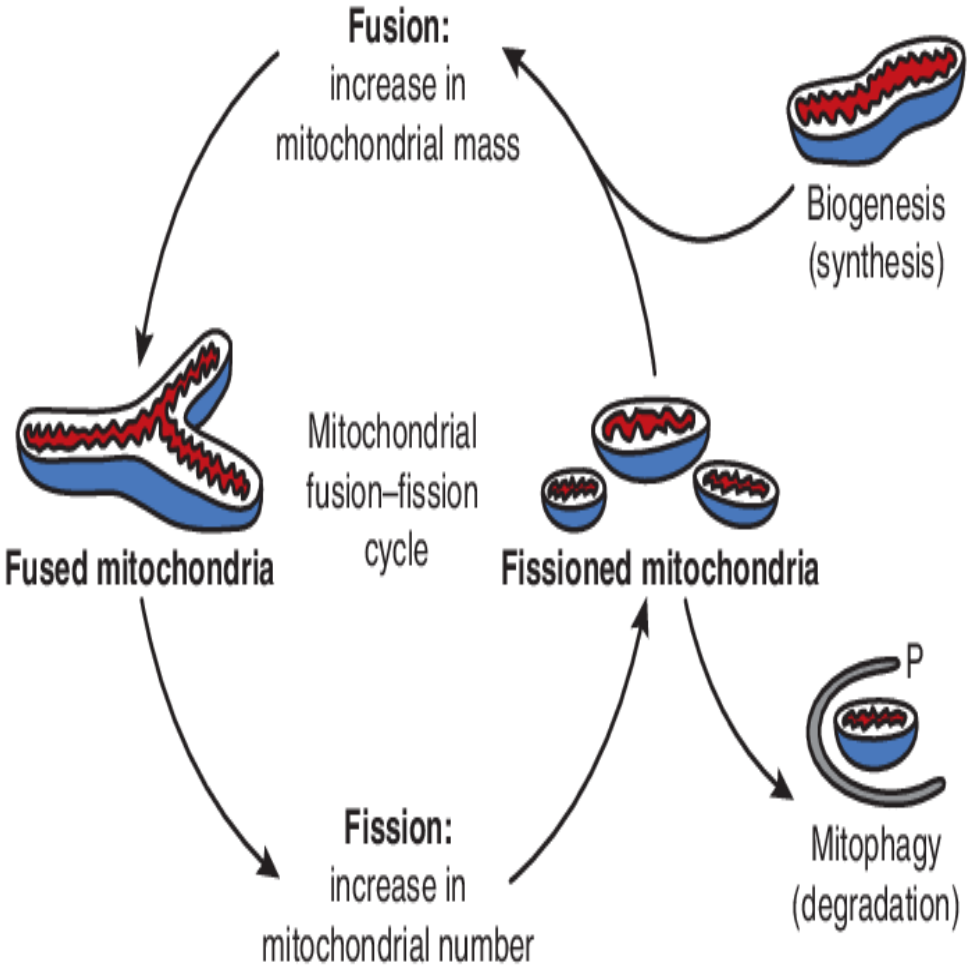


Figure:Journal of cell science

- Mitochondria are surrounded by a double-membrane system, consisting of inner and outer mitochondrial membranes separated by an inter-membrane space
- The inner membrane forms numerous folds (**cristae**), which extend into the interior (or matrix) of the organelle
- Each of these components plays distinct functional roles, with the matrix and inner membrane representing the major working compartments of mitochondria
- Matrix contains genetic information and enzymes responsible for oxidative mechanism.
- Initial stage , Glycolysis takes place in cytosol
- Pyruvate will transport to mitochondria to undergo central pathway of oxidative metabolism

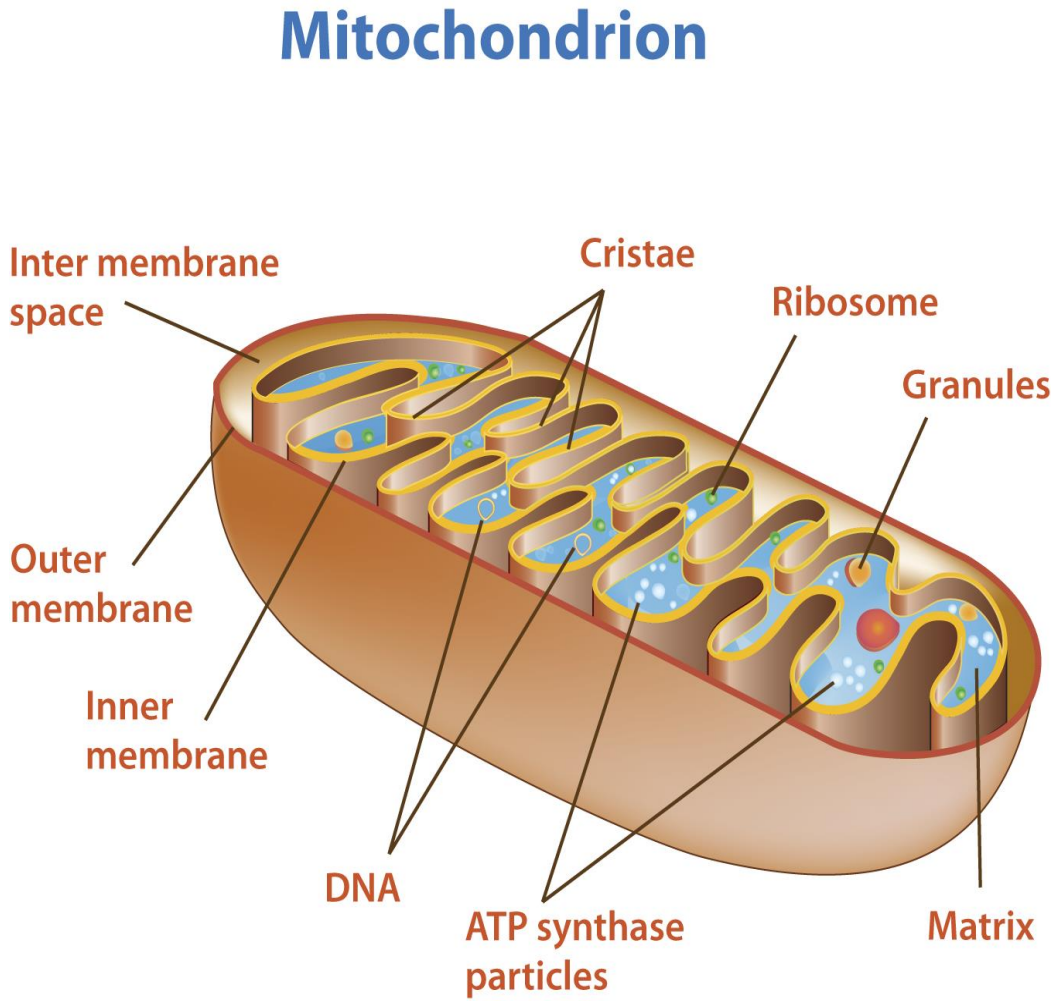


Figure: The Cell: A Molecular Approach. 2nd edition.

- Matrix will have enzymes ,DNA genome, ribosomes, tRNA,granules,fibrils and tubules
- Inner membrane : Oxidative phosphorylation will take place after oxidative mechanism
- high-energy electrons from NADH and $FADH_2$ are transferred through a series of carriers in the membrane to molecular oxygen
- energy derived is converted to potential energy stored in a proton gradient across the membrane
- It is the principal site of ATP generation
- Only permeable to O2,H2O,CO2
- Several antiport system exist in order exchange the anions between the cytosol and mitochondria

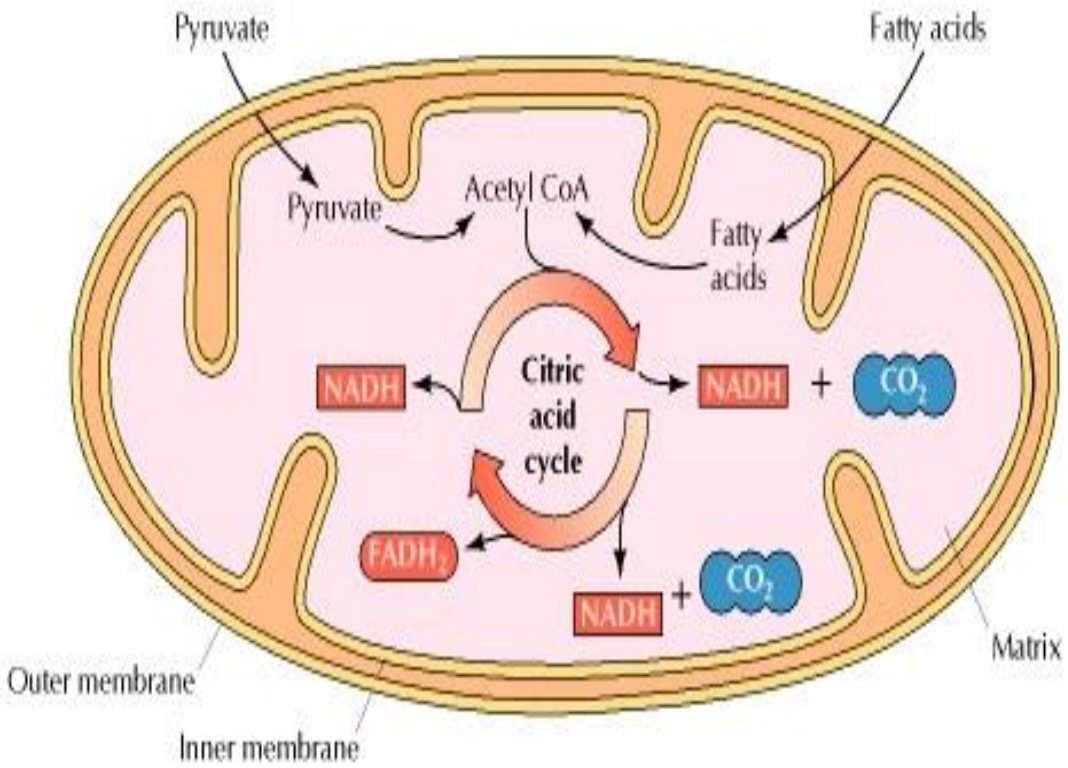


Figure: The Cell: A Molecular Approach. 2nd edition.

- It contains an unusually high percentage (greater than 70%) of proteins, which are involved in oxidative phosphorylation as well as in the transport of metabolites
- It is impermeable to most ions and small molecules—a property critical to maintaining the proton gradient that drives oxidative phosphorylation

Cristae: These are the fold of inner membrane , expand its surface area enhancing its ability to produce ATP

outer mitochondrial membrane : freely permeable to small molecules. Because, it contains proteins called **porins**

- Porins form channels that allow the free diffusion of molecules smaller than about 6000 daltons
- The composition of the intermembrane space is therefore similar to the cytosol with respect to ions and small molecules
- It is the functional barrier to the passage of small molecules between the cytosol and the matrix and maintains the proton gradient

Vacuoles

- Vacuole, in biology, a space within a cell that is empty of cytoplasm, lined with a membrane, and filled with fluid
- Especially in protozoa (single-celled eukaryotic organisms), vacuoles are essential cytoplasmic organs (organelles), performing functions such as storage, ingestion, digestion, excretion, and expulsion of excess water
- The large central vacuoles often found in plant cells enable them to attain a large size without accumulating the bulk that would make metabolism difficult
- Potent secondary metabolites, such as tannins or various biological pigments, are also sequestered in the vacuoles in plants, fungi, algae, and certain other organisms to protect the cell from self-toxicity

- Endocytosis and exocytosis are fundamental to the process of intracellular digestion
- Food particles are taken into the cell via endocytosis into a vacuole
- Lysosomes attach to the vacuole and release digestive enzymes to extract nutrients
- The leftover waste products of digestion are carried to the plasma membrane by the vacuole and eliminated through the process of exocytosis

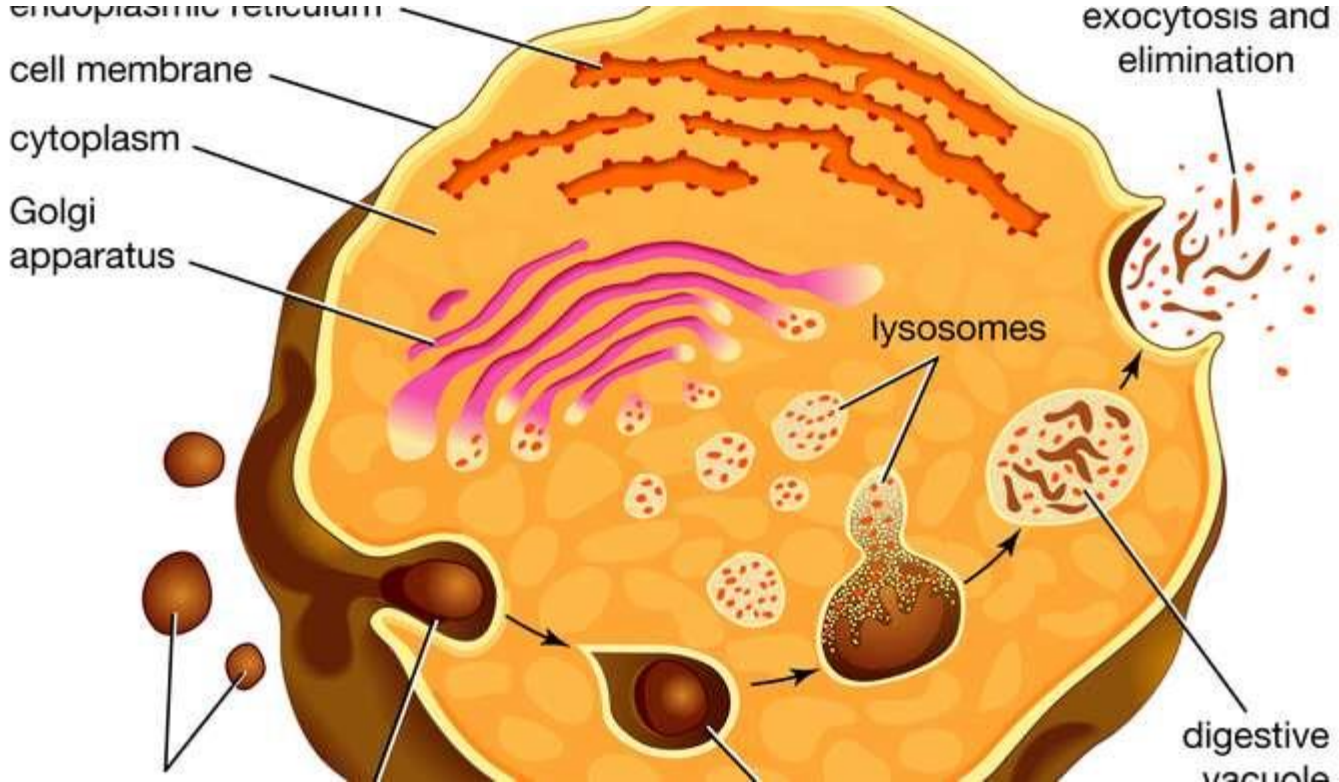


Figure:Britannica

Microbodies

- It contains variety of enzyme bearing, membrane bounded vesicle
- Present in plants, animals, protists and in fungi
- Distribution of enzymes into microbodies is one of the principle ways in which eukaryotic cells organize their metabolism
- While lysosomes bud from the endomembrane system, microbodies grow by incorporating lipids and protein, then replicate by dividing
- In plants, a special microbody called glyoxysome that contains enzymes that convert fats into acetyl-CoA for the glyoxylate bypass. They are temporary organelle present in germinating seeds
- Peroxisome, contains enzymes that catalyze the removal of electrons and associated hydrogen atoms (catalase and Urate oxidase) - Involve in beta-oxidation of fattyacids to acetyl-coA
- Essential biosynthetic function of animal peroxisomes - catalyse first reactions of formation of plasmologens - a abundant class of phospholipids in myelin

- Lysosomes and peroxisomes are vesicles that contain digestive and detoxifying enzymes
- The isolation of these enzymes in vesicles protects the rest of the cell from inappropriate digestive activity
- If these enzymes are not present in microbodies, they will tend to short-circuit the metabolism of the cytoplasm
- They will involve in the process of adding hydrogen atoms to oxygen
- Peroxisomes refers to the hydrogen peroxide produced as a by product of the process of activity of Oxidative enzyme
- Hydrogen peroxides are dangerous because of its violent chemical reactivity
- These enzymes will break down Hydrogen peroxides into water and oxygen

Organelles bounded by single membrane

Peroxisomes

- Peroxisomes are small, membrane-enclosed cellular organelles containing oxidative enzymes that are involved in a variety of metabolic reactions, including several aspects of energy metabolism
- They are considered as an important type of microbody found in both plants and animal cells

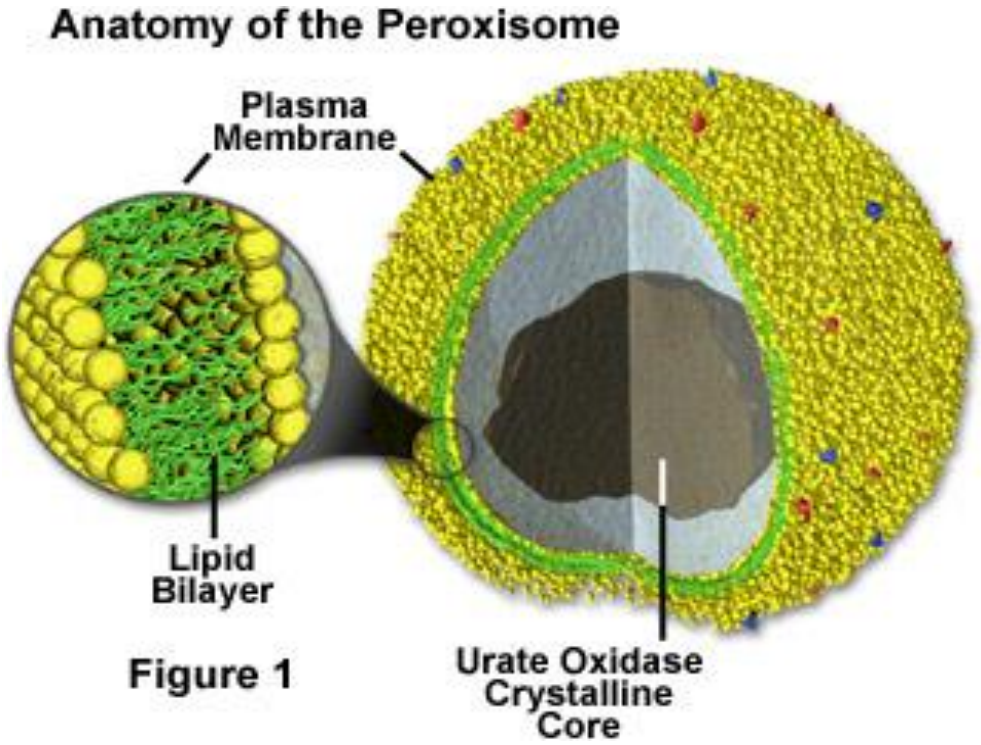


Figure: Molecular Expression

- They were identified as organelles by Belgian cytologist Christian de Duve in 1967
- First peroxisomes to be discovered were isolated from leaf homogenate of spinach
- They are most abundantly found in detoxifying organs such as the liver and kidney cells
- However, they can be induced to proliferate in response to metabolic needs
- They are membrane-bound spherical bodies of 0.2 to 1.5 μm in diameter found in all eukaryotic organisms including both plants and animal cells
- They are found floating freely in the cytoplasm in close association of ER, mitochondria or chloroplast within the cell
- They are among the simplest of eukaryotic organelles
- They exist either in the form of a network of interconnected tubules called peroxisome reticulum or as individual microperoxisomes

- They are variable in size and shape according to the cell and usually circular in cross-section.
- They range from 0.2 -1.5 μm in diameter
- It consists of a single limiting membrane of lipid and protein molecules enclosing the granular matrix
- The matrix consists of fibrils or a crystalloid structure containing enzymes

Peroxisomal Enzymes

- Approximately 60 known enzymes are present in the matrix of peroxisomes.
- They are responsible to carry out oxidation reactions leading to the production of hydrogen peroxide.
- The main groups of enzymes include:
- Urate oxidase
- D-amino acid oxidase
- Catalase

Functions of Peroxisomes

1. Hydrogen Peroxide Metabolism:

2. Fatty acid oxidation:

- Oxidation of fatty acids, in animal cells, occurs in both peroxisomes and mitochondria, but in yeasts and plants, only limited to peroxisomes
- Oxidation is accompanied by the production of H_20_2 which is decomposed by catalase enzyme. This provides a major source of metabolic energy

3. Lipid biosynthesis

- Synthesis of cholesterol and dolichol occurs in both ER and peroxisomes. Bile acid synthesis takes place from cholesterol in the liver
- Peroxisomes contain enzymes to synthesize plasmalogens, a family of phospholipids which are important membrane components of tissues of the heart and brain

4. Germination of seeds

- Peroxisomes in seeds responsible for the conversion of stored fatty acids to carbohydrates, critical to providing energy and raw materials for the growth of germinating plants
- Peroxisomes seen in tomato seeds in near germination (around 4 days) storing fat molecules

5. Photorespiration

- Peroxisomes in leaves particularly in the green ones carry out the photorespiration process along with chloroplasts

6. Degradation of purines

- Carry out the catabolism of purines, polyamines and amino acids especially by uric acid oxidase

7. Bioluminescence

- Luciferase enzyme found in the peroxisomes of fireflies help in bioluminescence and thus aid the flies in finding a mate or its meal

8. Importance of import process in peroxisomes was explained using inherited human disease - zellweger syndrome, in which a defect in importing proteins into peroxisomes leads to profound peroxisomal deficiency. Individuals with cells containing empty peroxisomes have severe abnormalities in their brain, liver and kidneys and they die soon.

Lysosomes

- Lysosomes were discovered by the Belgian cytologist Christian René de Duve in the 1950
- It is membrane-enclosed organelles contains array of enzymes capable of breaking down all types of biological polymers -proteins, nucleic acids, carbohydrates, and lipids
- Function as digestive system of cell, degrade material taken from outside the cell and to digest obsolete components of the cell itself
- They are dense spherical in shape but the shape and size as a result of difference in the size of the material taken up for digestion

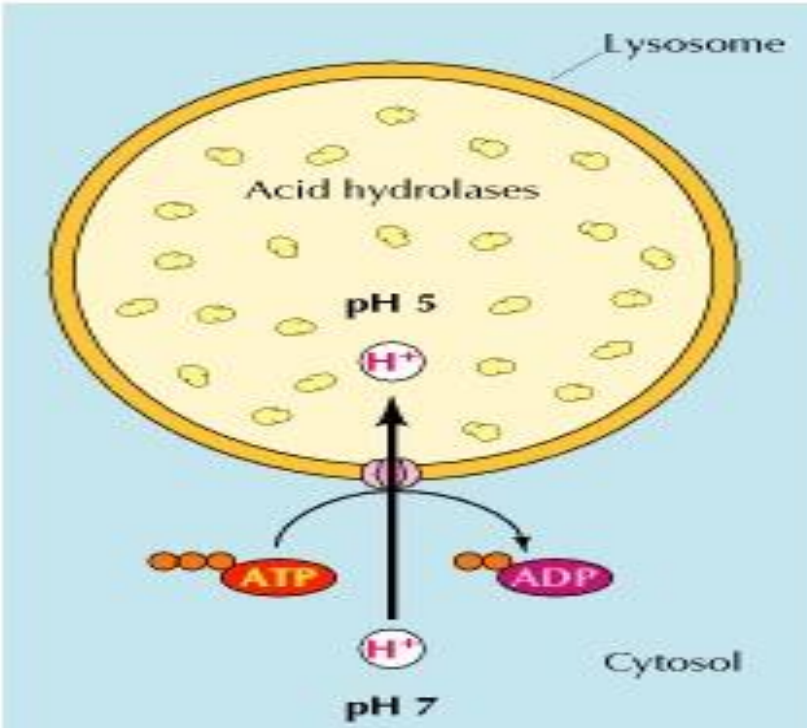


Figure: The Cell: A Molecular Approach. 2nd e

<u>Lysosomal Acid Hydrolases</u>

- It contains more than 50 different degrading enzymes and they are active at 5 pH (inside the lysosomes)
- These will hydrolyze the DNA,RNA,proteins, lipids and Polysaccharides

- All of the lysosomal enzymes are acid hydrolases, the neutral pH (about 7.2) characteristic of the rest of the cytoplasm
- even if the lysosomal membrane were to break down, the released acid hydrolases would be inactive at the neutral pH of the cytosol
- To maintain their acidic internal pH, lysosomes must actively concentrate H^+ ions (protons)
- This pumping requires expenditure of energy in the form of ATP hydrolysis, since it maintains approximately a hundred fold higher H^+ concentration inside the lysosome.
- Mutations in the genes that encode these enzymes are responsible for more than 30 different human genetic diseases, which are called lysosomal storage diseases
- Most of these diseases result from deficiencies in single lysosomal enzymes
- For example, Gaucher’s disease (the most common of these disorders) results from a mutation in the gene that encodes a lysosomal enzyme required for the breakdown of glycolipids

I-cell disease mechanism vs normal

(a) Normal trafficking of lysosomal enzymes depends on addition of mannose to hydrolases in the rough ER. Mannose is then phosphorylated to mannose-6-phosphate (mannose-6-P) in the Golgi

- Mannose-6-P-tagged enzymes move through the Golgi, eventually fuse with endosomes, and are ultimately incorporated into lysosome

(b)In cells of I-cell patients, the enzyme in the Golgi that adds a phosphoryl group to mannose is absent, so the enzymes are misrouted to the plasma membrane.

- Ultimately, lysosomes become swollen in such cell

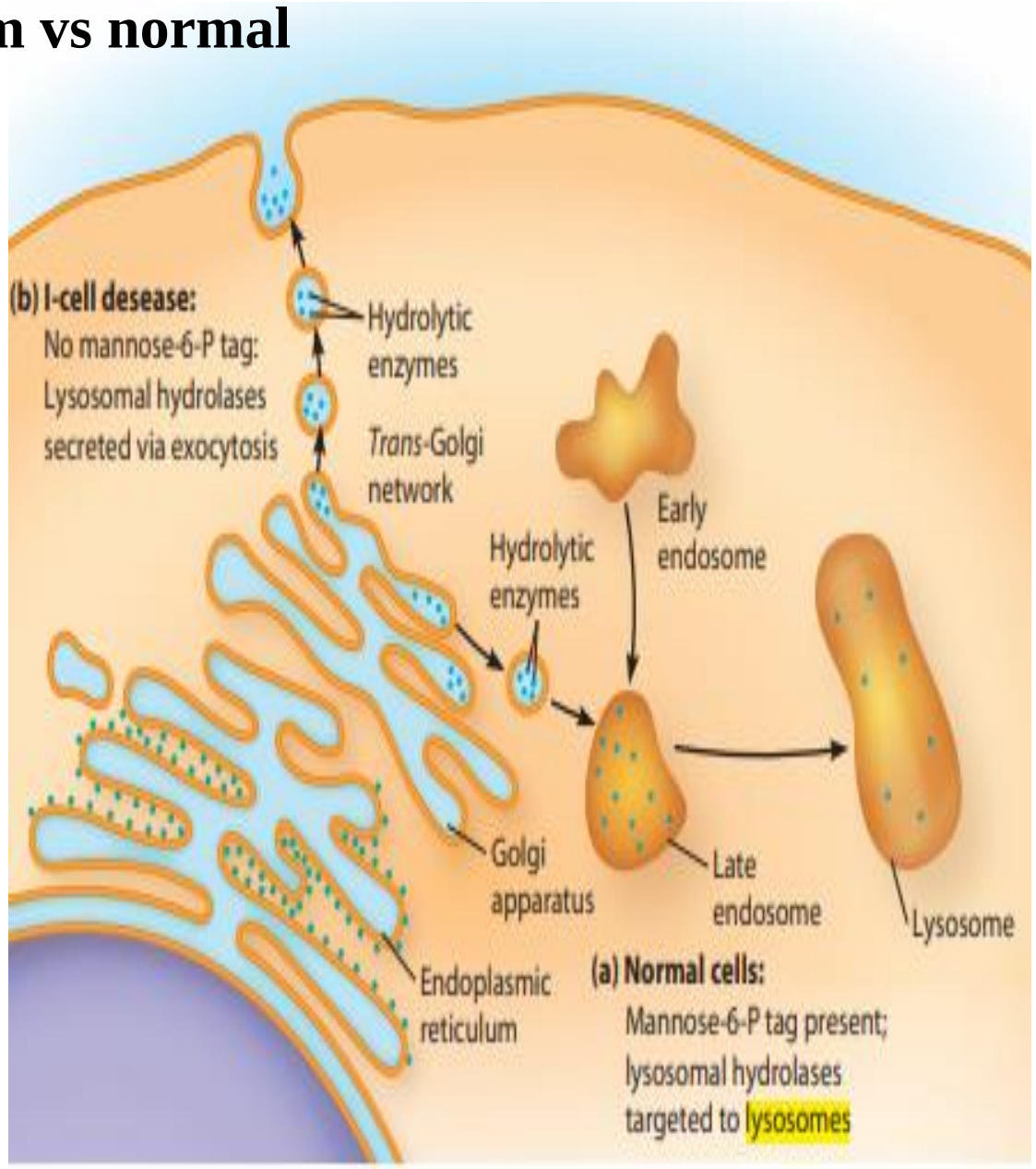


Figure:Becker's World of the Cell NINTH EDITION

- lysosomes digest material derived from two other routes: phagocytosis and autophagy
- In phagocytosis, specialized cells, such as macrophages, take up and degrade large particles, including bacteria, cell debris, and aged are taken up in phagocytic vacuoles (**phagosomes**), which then fuse with lysosomes, resulting in digestion of their contents.
- The lysosomes formed in this way (**phagolysosomes**)
- can be quite large and heterogeneous, since their size and shape is determined by the content of material that is being digested Lysosomes
- for autophagy, the gradual turnover of the cell's own components. The first step of autophagy appears to be the enclosure of an organelle (e.g., a mitochondrion) in membrane derived from the ER.
- The resulting vesicle (an **autophagosome**) then fuses with a lysosome, and its contents are digested

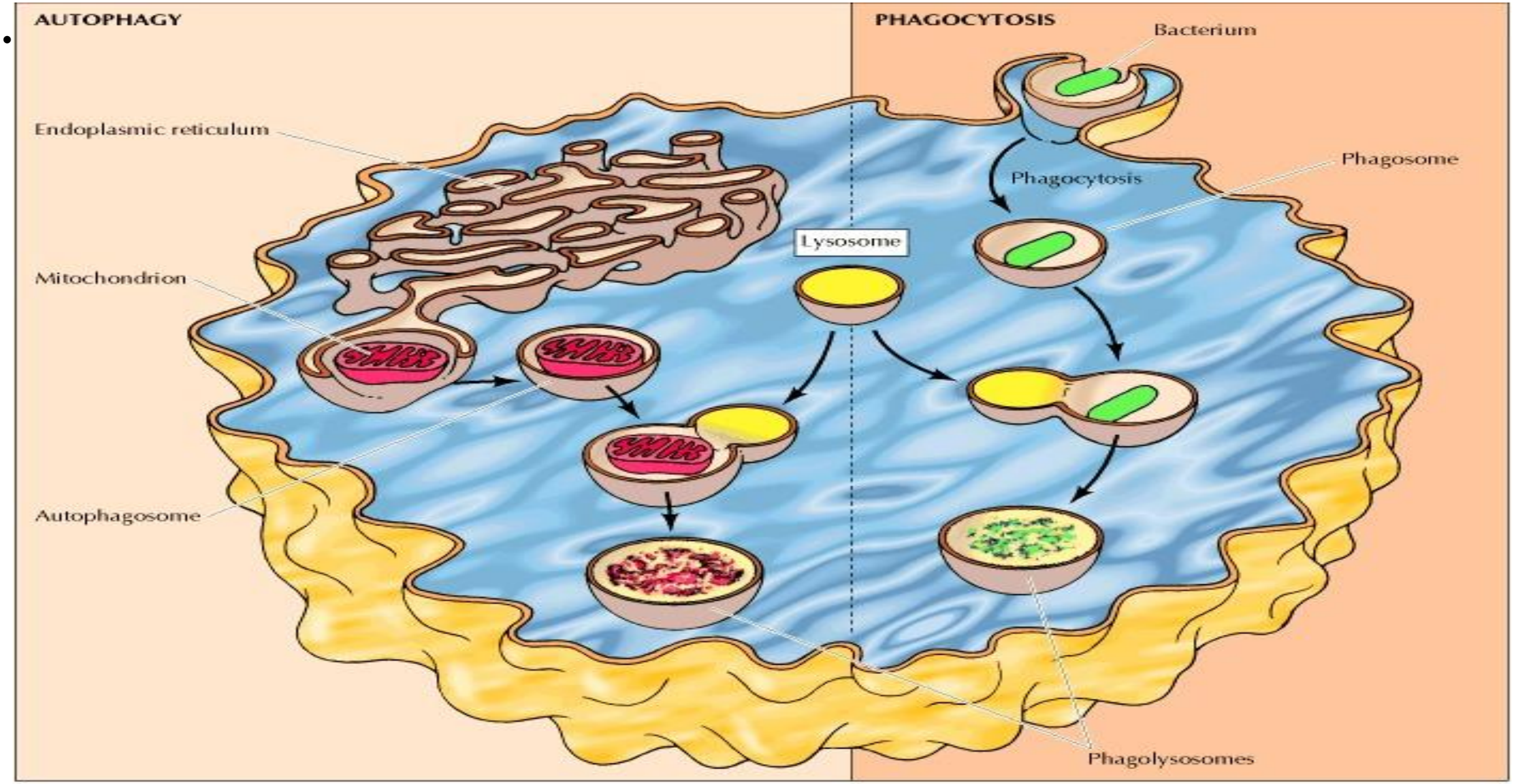


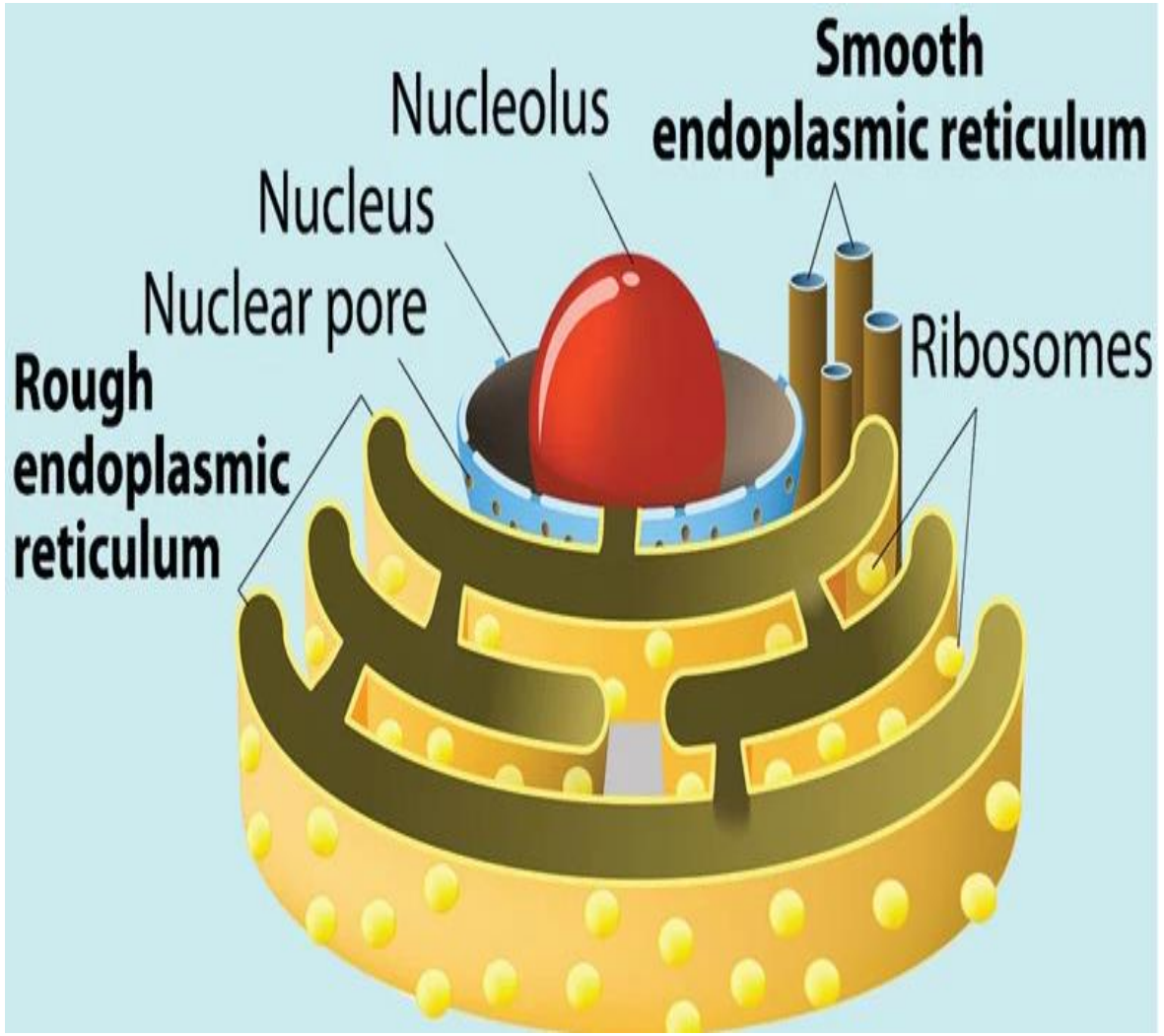
Figure: The Cell: A Molecular Approach. 2nd edition.

Endoplasmic Reticulum (ER)

- It was discovered independently by **Porter (1945) and Thompson (1945)**.
- The name was given by Porter in 1953
- Endoplasmic reticulum is a 3-dimensional, complicated and interconnected syncretic of membrane-lined channels that run through the cytoplasm.

Albert Claude

Keith Porter



- Within the cytoplasm of most animal cells is an extensive network (reticulum) of membrane-limited channels, collectively called the endoplasmic reticulum (or ER)
- The endoplasmic reticulum is a name derived from the fact that in the light microscope it looks like a “net in the cytoplasm”
- The endoplasmic reticulum is only present in the eukaryotic cells. However, the occurrence of the endoplasmic reticulum varies from cell to cell
- For example, the erythrocytes (RBC), egg and embryonic cells lack in the endoplasmic reticulum
- Some portion of ER membranes remains continuous with the plasma membrane and the nuclear envelope
- ER may be rough or smooth. The outer surface of rough ER has attached ribosomes, whereas smooth ER does not have attached ribosomes
- The endoplasmic reticulum acts as secretory, storage, circulatory and nervous system for the cell. It is also the site of the biogenesis of cellular membranes

Structure :

- The membrane of the endoplasmic reticulum is 50 to 60 Aº thickness and fluid-mosaic like the unit membrane of the plasma membrane.
- The membranes of the endoplasmic reticulum are found to contain many kinds of enzymes that are needed for various important synthetic activities. The most important enzymes are the stearases, NADH-cytochrome C reductase, NADH diaphorase, glucose-6-phosphatase, and Mg++ activated ATPase.
- The membrane of endoplasmic reticulum remains continuous with the membranes of the plasma membrane, nuclear membrane, and Golgi apparatus.
- The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products.

The endoplasmic reticulum may occur in the following three forms:

- Lamellar form or cisternae
- Vesicular form or vesicle and
- Tubular form or tubules
- **The Cisternae**
- RER usually exists as cisternae that occur in those cells which have synthetic roles as the cells of the pancreas, notochord, and brain.
- The cisternae are long, flattened, sac-like, unbranched tubules having a diameter of 40 to 50 μm.
- They remain arranged parallelly in bundles or stakes.

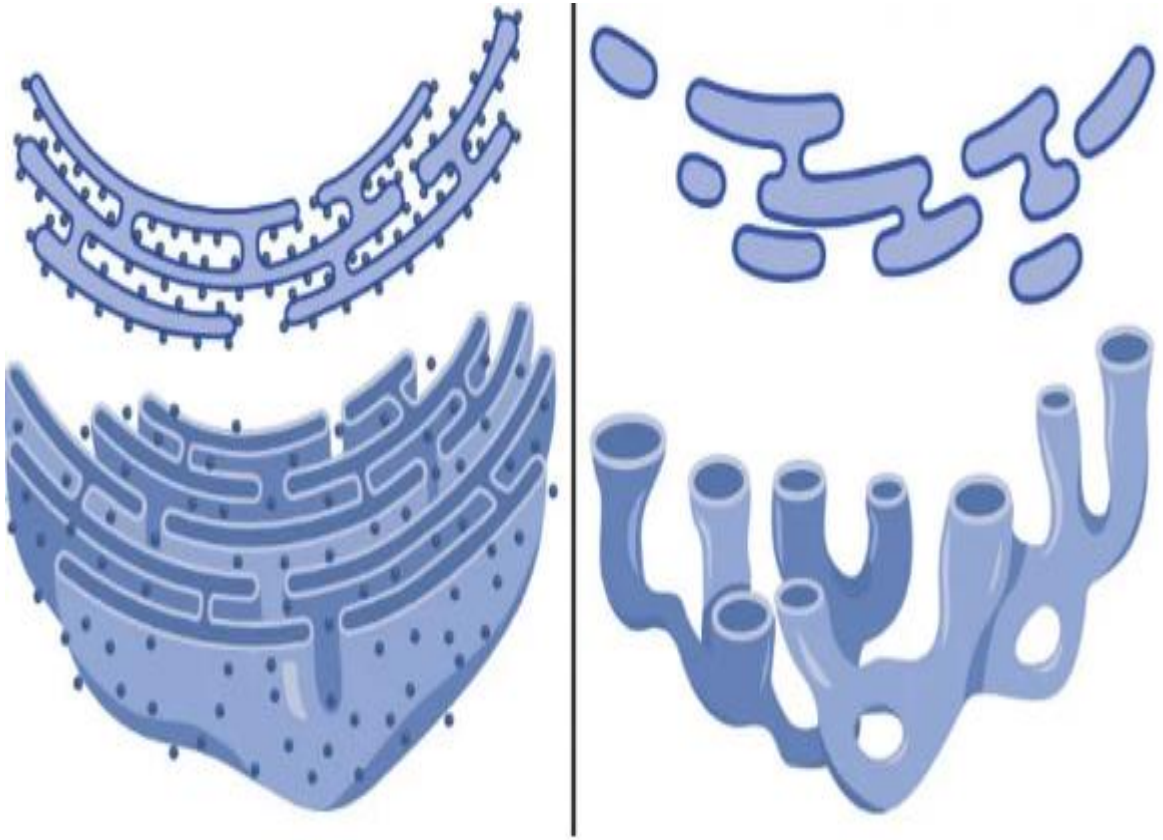
- **The Vesicles**
- The vesicles are oval; membrane-bound vacuolar structures having a diameter of 25 to 500 μm
- They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER
- **The Tubules**
- The tubules are branched structures forming the reticular system along with the cisternae and vesicles
- They usually have a diameter from 50 to 190 μm and occur almost in all the cells
- Tubular form of ER is often found in SER and is dynamic in nature, i.e., it is associated with membrane movements, fission and fusion between membranes of cytocavity network

Types of Endoplasmic Reticulum (ER)

- **1. Smooth Endoplasmic Reticulum**
- They are also called as the agranular endoplasmic reticulum.
- This type of endoplasmic reticulum possesses smooth walls because the ribosomes are not attached to its membranes.
- The smooth type of endoplasmic reticulum occurs mostly in those cells, which are involved in the metabolism of lipids (including steroids) and glycogen. Eg. adipose cells, interstitial cells, glycogen storing cells of the liver, conduction fibers of heart, spermatocytes, and leucocytes.

2. Rough Endoplasmic Reticulum

- It possesses rough walls because the ribosomes remain attached to its membranes.
- On their membranes, rough ER (RER) contains certain ribosome specific, transmembrane glycoproteins, called ribophorin I and II, to which are attached the ribosomes while engaged in polypeptide synthesis.
- The rough type of endoplasmic reticulum is found abundantly in those cells which are active in protein synthesis such as pancreatic cells, plasma cells, goblet cells, and liver cells.



Basis for comparison	Rough endoplasmic reticulum (RER)	Smooth endoplasmic reticulum (SER)
Definition	The rough endoplasmic reticulum is a type of endoplasmic reticulum consisting of flattened sacs, studded with protein-synthesizing particles termed ribosomes on the outer surface.	Smooth endoplasmic reticulum (SER) is a type of endoplasmic reticulum consisting of tubular vesicles that lack ribosomes on the outer surface and is involved in the synthesis and storage of lipids.
Ribosomes	Rough ER has ribosomes on the outer surface.	Smooth ER doesn' thave ribosomes on the outer surface.
Location	The rough endoplasmic reticulum is mostly found around the nuclear membrane.	The smooth endoplasmic reticulum is mostly found near the cell membrane.
Origin	Rough ER is formed from the nuclear membrane.	Smooth ER is formed after the shedding of ribosomes from rough ER.

Structure	Rough ER is mainly composed of cisternae with few tubules.	Smooth ER is mainly composed of a network of tubules with few cisternae.
	Rough ER posses narrow pores below the ribosomes that allow the passage of newly synthesized polypeptides to the cytosol.	No such pores are present on the surface of the smooth ER.
Ribophorins	Ribophorins are present on the surface of the rough ER.	Ribophorins are absent on the surface of the smooth ER.
Involved in	Rough ERs are involved in the formation of lysosomes.	Smooth ERs are involved in the formation of spherosomes or oleosomes.
Found in	Numerous rough ER is found in lipid synthesizing cells.	Numerous smooth ER is found in protein synthesizing cells.
Type of cell	RER is mostly found in cells of glands and protein-producing organs.	SER is mostly found in cells like muscle cells and nerve cells.
Golgi apparatus	Rough ER provides proteins and lipids for the Golgi apparatus.	Smooth ER provides vesicles for the cis-face of the Golgi apparatus.

Function	The rough endoplasmic reticulum is mostly associated with the production, modification, and transfer of proteins.	The smooth endoplasmic reticulum is mostly associated with the production of lipids and the storage of calcium ions.
Diseases	Disease like spondyloepimetaphyseal dysplasia is attributed due to the accumulation of misfolded collagen proteins in the RER.	Prolonged SER stress might result in the development and progression of many diseases, including neurodegeneration, atherosclerosis, type 2 diabetes, liver disease, and even cancer.

Functions

- Functions of smooth ER include lipid metabolism (both catabolism and anabolism; they synthesize a variety of phospholipids, cholesterol, and steroids).
- Glycogenolysis (degradation of glycogen; glycogen being polymerized in the cytosol).
- Drug detoxification (by the help of the cytochrome P-450).
- The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic matrix.
- The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of the endoplasmic reticulum.
- The endoplasmic reticulum is the main component of the endomembrane system, also called the cytoplasmic vacuolar system or cytocavity network.

- The endoplasmic membranes contain many enzymes that perform various synthetic and metabolic activities. Further, the endoplasmic reticulum provides an increased surface for various enzymatic reactions
- The endoplasmic reticulum acts as an intracellular circulatory or transporting system
- As a growing secretory polypeptide emerges from the ribosome, it passes through the RER membrane and gets accumulated in the lumen of RER
- Here, the polypeptide chains undergo tailoring, maturation, and molecular folding to form functional secondary or tertiary protein molecules
- RER pinches off certain tiny protein-filled vesicles which ultimately get fused to cis Golgi

- The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibers
- The ER membranes form the new nuclear envelope after each nuclear division
- The SER contains several key enzymes that catalyze the synthesis of cholesterol which is also a precursor substance for the biosynthesis of two types of compounds— the steroid hormones and bile acids
- RER also synthesize membrane proteins and glycoproteins which are cotranslationally inserted into the rough ER membranes. Thus, the endoplasmic reticulum is the site of the biogenesis of cellular membranes

The Golgi Apparatus

- The Golgi stain and the cytological studies performed by Camillo Golgi were destined, however, to exert a great impact not only on the neurosciences, but also on cell biology
- Golgi made his observations leading to the discovery of the intracellular organelle in the last years of the nineteenth century
- Functions as a factory process and sort the protein (from ER) transport to their destination
- Glycolipids and shingomyelin are synthesized
- In Plants, complex polysaccharides of the cell wall are synthesized
- It involve in the processing of broad range of cellular constituents that travels along the secretory pathways

Organization of the Golgi

- the Golgi is composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles
- distinct polarity in both structure and function
- Proteins from the ER enter at its *cis* face (entry face), which is convex and usually oriented toward the nucleus
- They are then transported through the Golgi and exit from its concave *trans* face (exit face)
- As they pass through the Golgi, proteins are modified and sorted for transport to their eventual destinations within the cell
- It is also known as **dictyosomes**

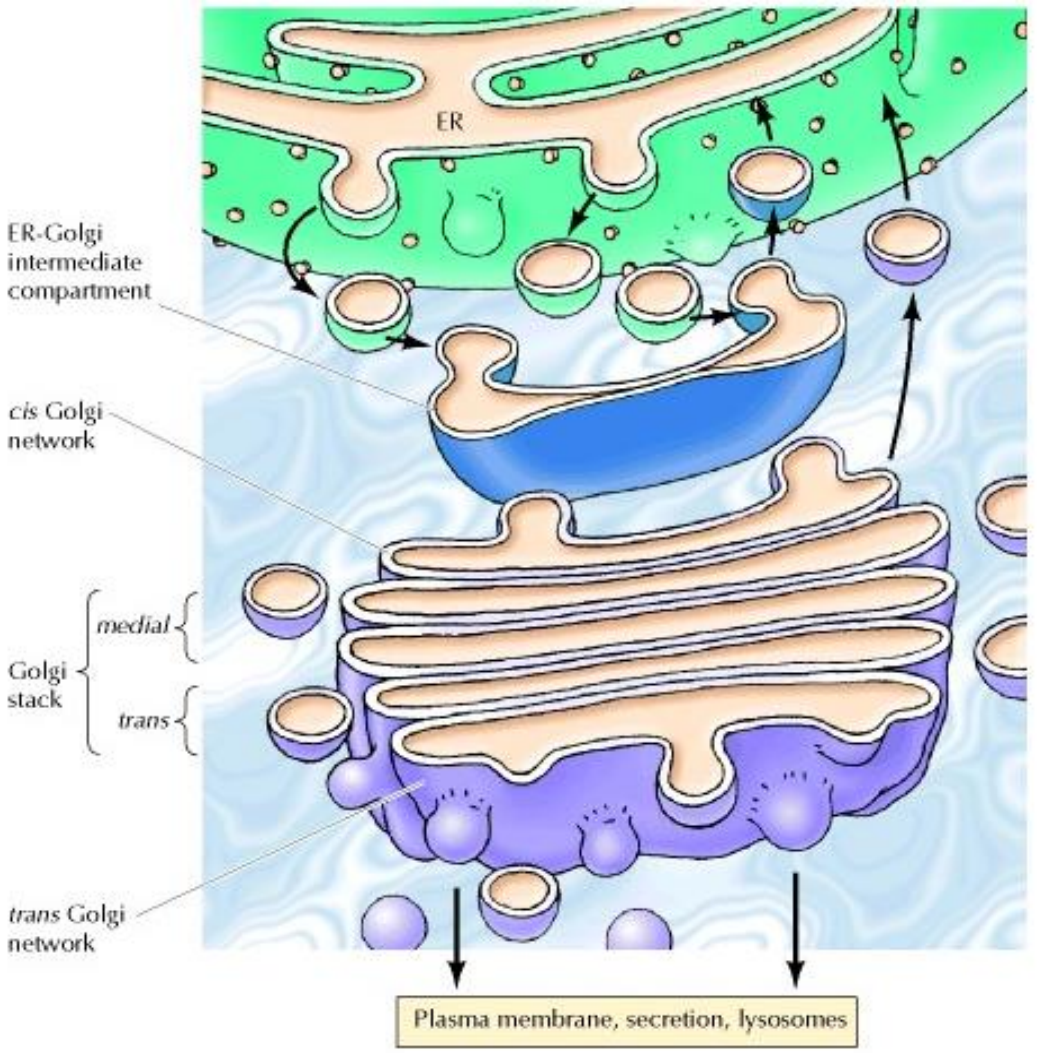


Figure: The Cell: A Molecular Approach. 2nd edition

- Functional region :cisGolgi network, the Golgi stack (which is divided into the medial and trans subcompartments), and the transGolgi network
- Proteins from the ER are transported to the ER-Golgi intermediate compartment and then enter the Golgi apparatus at the *cis* Golgi network

area of controversy among cell biologists:

Two possibilities are

1. transport vesicles carry proteins between the cisternae of the Golgi compartments
2. proteins are simply carried through compartments of the Golgi within the Golgi cisternae, which gradually mature and progressively move through the Golgi in the *cis* to *trans* direction

Protein Glycosylation within the Golgi

- proteins are modified within the ER by the addition of an oligosaccharide consisting of 14 sugar residues
- Three glucose residues and one mannose are then removed while the polypeptides are still in the ER
- Following transport to the Golgi apparatus, the *N*-linked oligosaccharides of these glycoproteins
- Image description: Processing of *N*-linked oligosaccharides in the Golgi. The *N*-linked oligosaccharides of glycoproteins transported from the ER are further modified by an ordered sequence of reactions in the Golgi

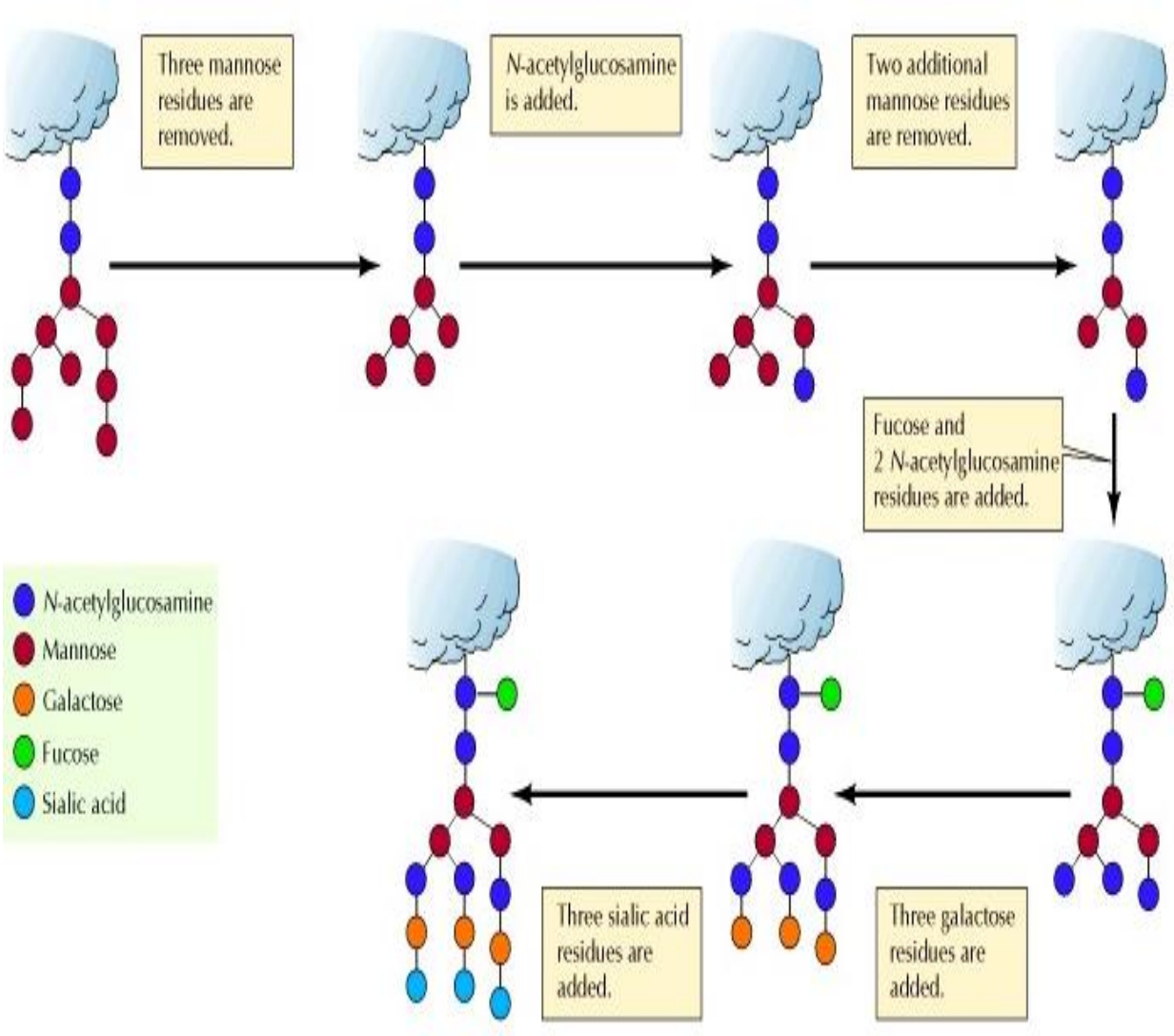


Figure: The Cell: A Molecular Approach. 2nd edition.

- different glycoproteins are modified to different extents during their passage through the Golgi, depending on both the protein structure and on the amount of processing enzymes that are present within the Golgi complexes of different types of cells
- proteins can emerge from the Golgi with a variety of different *N*-linked oligosaccharides
- The processing of the *N*-linked oligosaccharide of lysosomal proteins differs from that of secreted and plasma membrane proteins
- In the first step of this reaction, *N*-acetylglucosamine phosphates are added to specific mannose residues, probably while the protein is still in the *cis* Golgi network

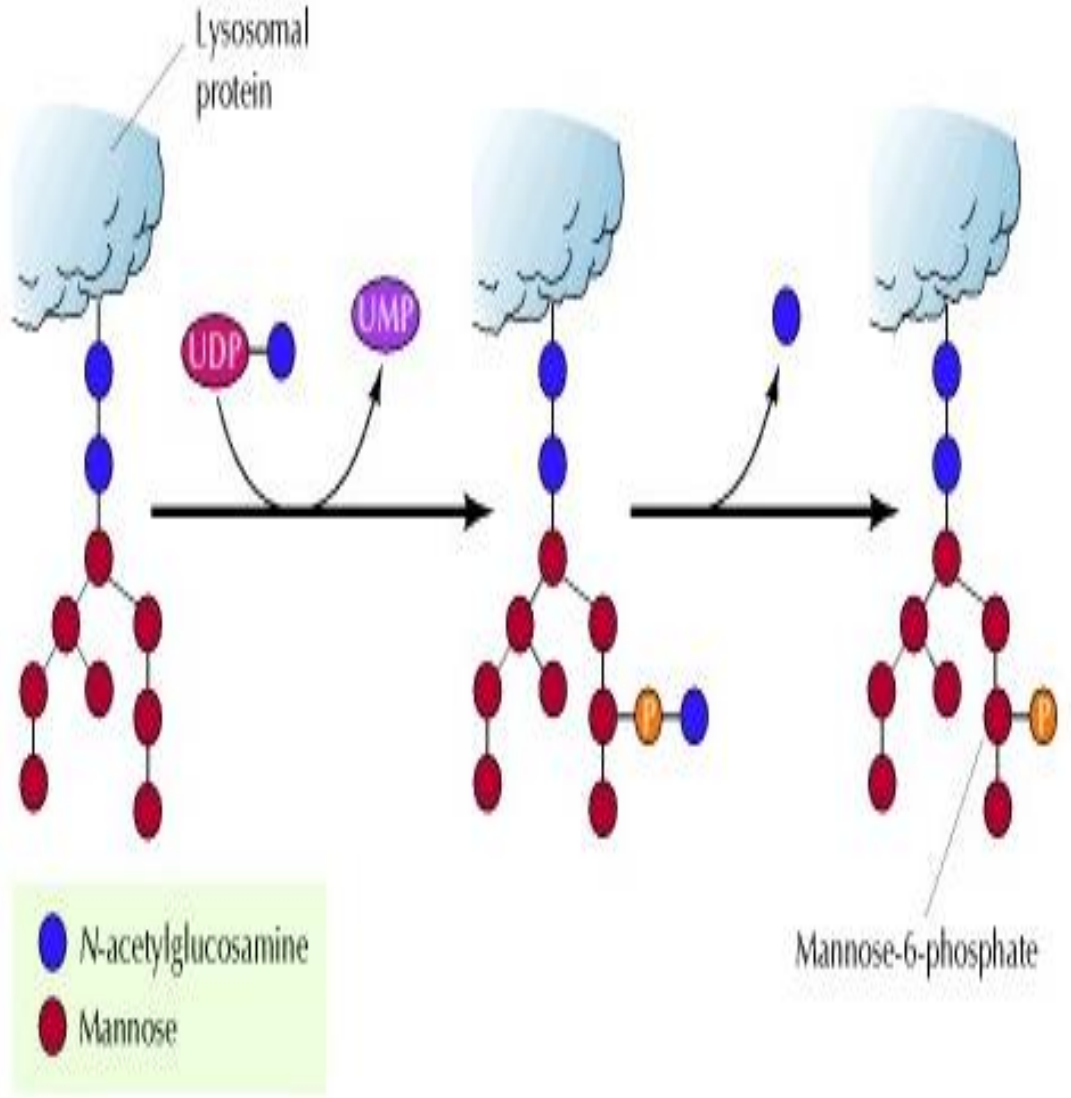


Figure: The Cell: A Molecular Approach. 2nd edition.

- removal of the *N*-acetylglucosamine group, leaving **mannose-6-phosphate** residues on the *N*-linked oligosaccharide
- these residues are not removed during further processing
- These are specifically recognized by a mannose-6-phosphate receptor in the *trans* Golgi network, which directs the transport of these proteins to lysosomes
- Phosphorylation is the crucial step in sorting the lysosomes
- the selective addition of *N*-acetylglucosamine phosphates to lysosomal proteins act as structure determinant which is not present in secretion and plasma membrane proteins
- Recognition determinants are not simply in sequence, but it formed in the folded protein by the juxtaposition of amino acids
- **signal patches :**the recognition determinant that leads to mannose phosphorylation, and targets proteins to lysosomes, depends on the 3D conformation of the folded protein

- Protein modification by the addition of carbohydrates to the side chains of acceptor serine and threonine residues within specific sequences of amino acids (*O*-linked glycosylation)
- serine or threonine is usually linked directly to *N*-acetylgalactosamine, to which other sugars can then be added

Lipid and Polysaccharide Metabolism in the Golgi

- the Golgi apparatus functions in lipid metabolism—in particular, in the synthesis of glycolipids and sphingomyelin
- glycerol phospholipids, cholesterol, and ceramide are synthesized in the ER
- Sphingomyelin - only nonglycerol phospholipid in cell membranes
- It is synthesized by the transfer of a phosphorylcholine group from phosphatidylcholine to ceramide
- Addition of carbohydrates to ceramide can yield a variety of different glycolipids

- It is synthesized on the lumenal surface of the Golgi, glucose is added to ceramide on the cytosolic side
- Glucosylceramide flips, additional carbohydrates are added on the lumenal side of the membrane
- sphingomyelin, glycolipids are not able to translocate across the Golgi membrane, they are found only in the lumenal half of the Golgi bilayer
- Following vesicular transport, they are localized to the exterior half of the plasma membrane, with their polar head groups exposed on the cell surface
- oligosaccharide portions of glycolipids are important surface markers in cell-cell recognition

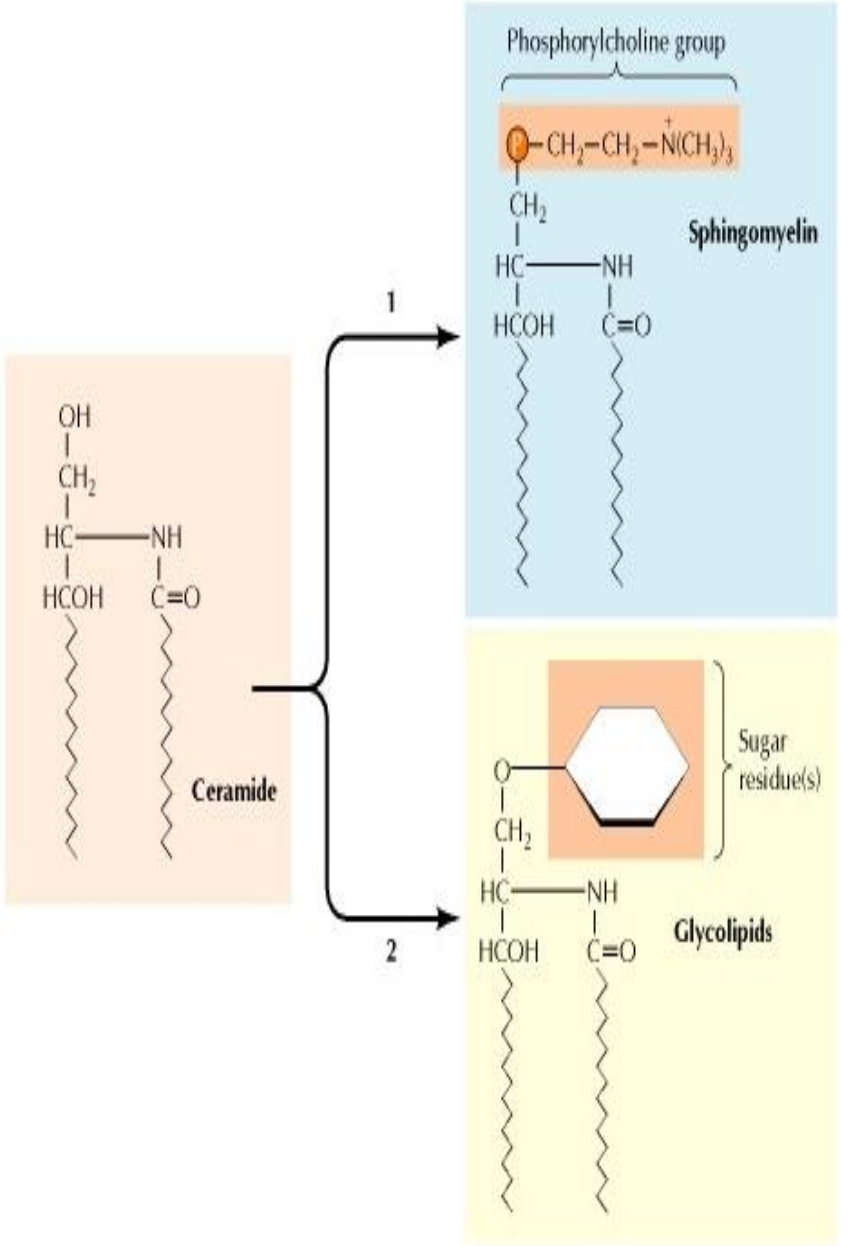


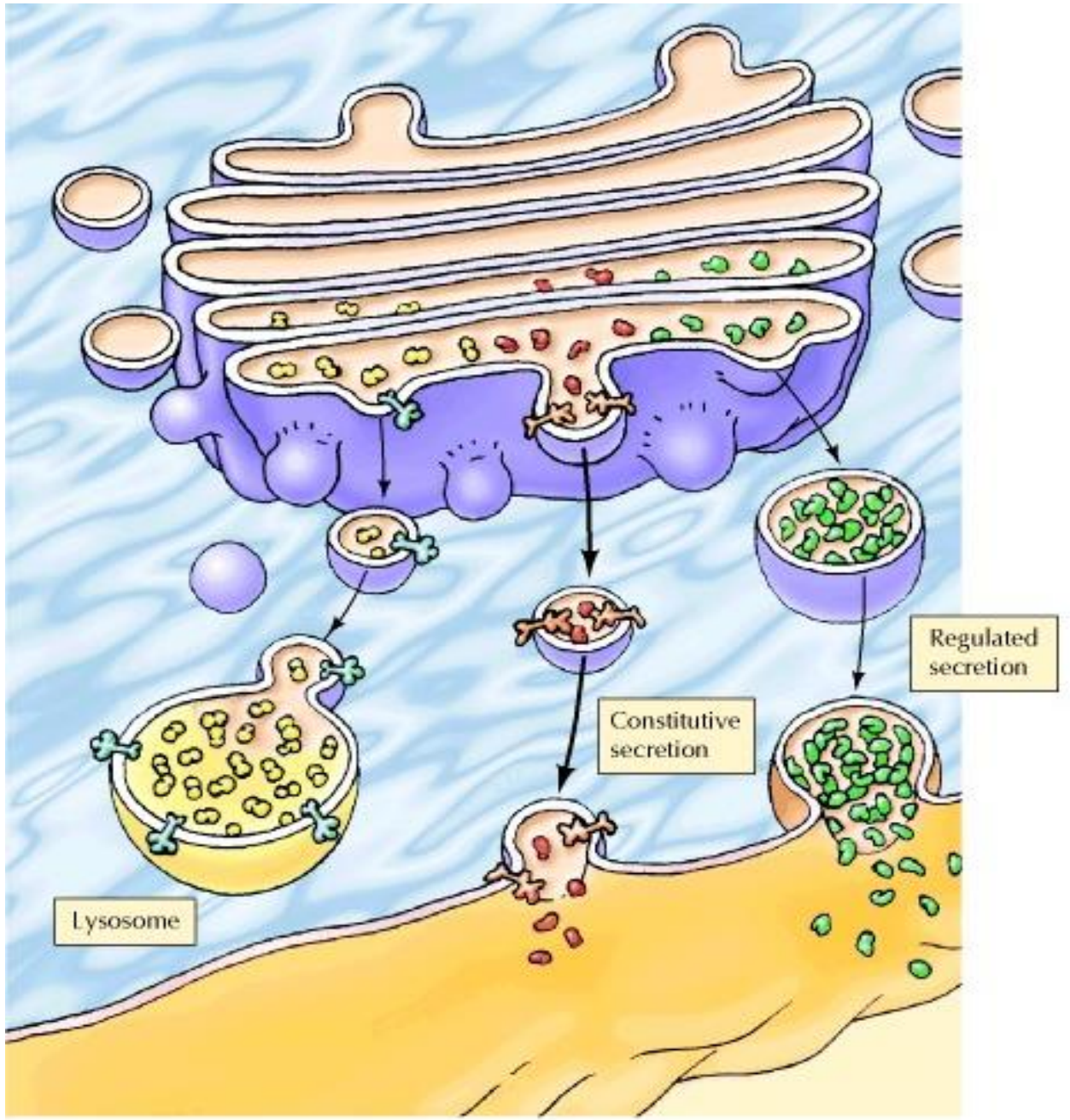
Figure: The Cell: A Molecular Approach. 2nd edition.

- In plants, cell wall polysaccharides (hemicelluloses and pectins), complex branched chain molecules are synthesized in the Golgi apparatus
- then transported in vesicles to the cell surface
- The synthesis of these cell wall polysaccharides is a major cellular function, and as much as 80% of the metabolic activity of the Golgi apparatus in plant cells may be devoted to polysaccharide synthesis

Protein Sorting and Export from the Golgi Apparatus

- Proteins are sorted into different transport vesicles, which bud from the *trans* Golgi network and deliver their contents to the appropriate cellular locations
- Proteins are transport to plasma membrane by consecutive secretory pathway

- Functional proteins of GA are retained, signals from cytoplasmic tails of some Golgi proteins will help these proteins from packing in transport vesciles
- some cells also possess a distinct regulated secretory pathway in which specific proteins are secreted in response to environmental signals
- Examples: release of hormones from endocrine cells, the release of neurotransmitters from neurons, and the release of digestive enzymes from the pancreatic acinar cells
- secretory vesicles larger than other transport vesicles, store their contents until specific signals direct their fusion with the plasma membrane



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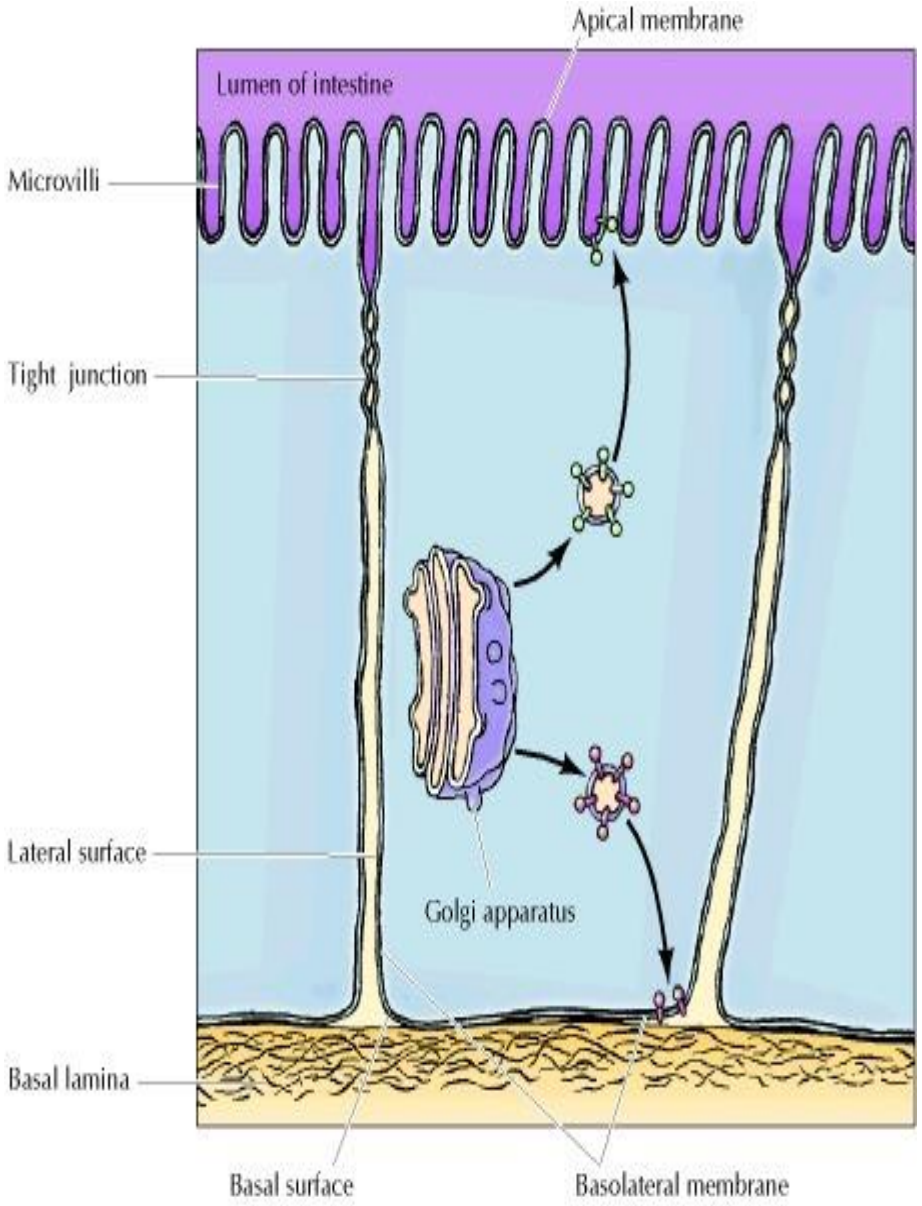


- Complication of transport arises in many epithelial region is they are polarized when organized into tissues
- The plasma membrane is divided into two separate regions, the apical domain and the basolateral domain, contains proteins related to their particular functions
- Examples: apical membrane of intestinal epithelial cells faces the lumen of the intestine for the efficient absorption of nutrients
- the remainder of the cell is covered by the basolateral membrane
- Distinct domains are present in all types of cell
- the constitutive secretory pathway must selectively transport proteins into at least two types of constitutive secretory vesicles that leave the *trans* Golgi network targeted specifically for the apical or basolateral plasma membrane domains of the cell

Figure: The Cell: A Molecular Approach. 2nd edition.

Plastids

- All plastids contain the same genome as chloroplasts, but they differ in both structure and function
- All develop from **proplastids**, small (0.5 to 1 μm in diameter)
- **Chloroplasts** are so named because they contain chlorophyll
- **Chromoplasts** lack chlorophyll but contain carotenoids; they are responsible for the yellow, orange, and red colors of some flowers and fruits, although their precise function in cell metabolism is not clear
- **Leucoplasts** are non-pigmented **plastids**, which store a variety of energy sources in non-photosynthetic tissues
- **Amyloplasts** and **elaioplasts** are examples of leucoplasts that store starch and lipids, respectively

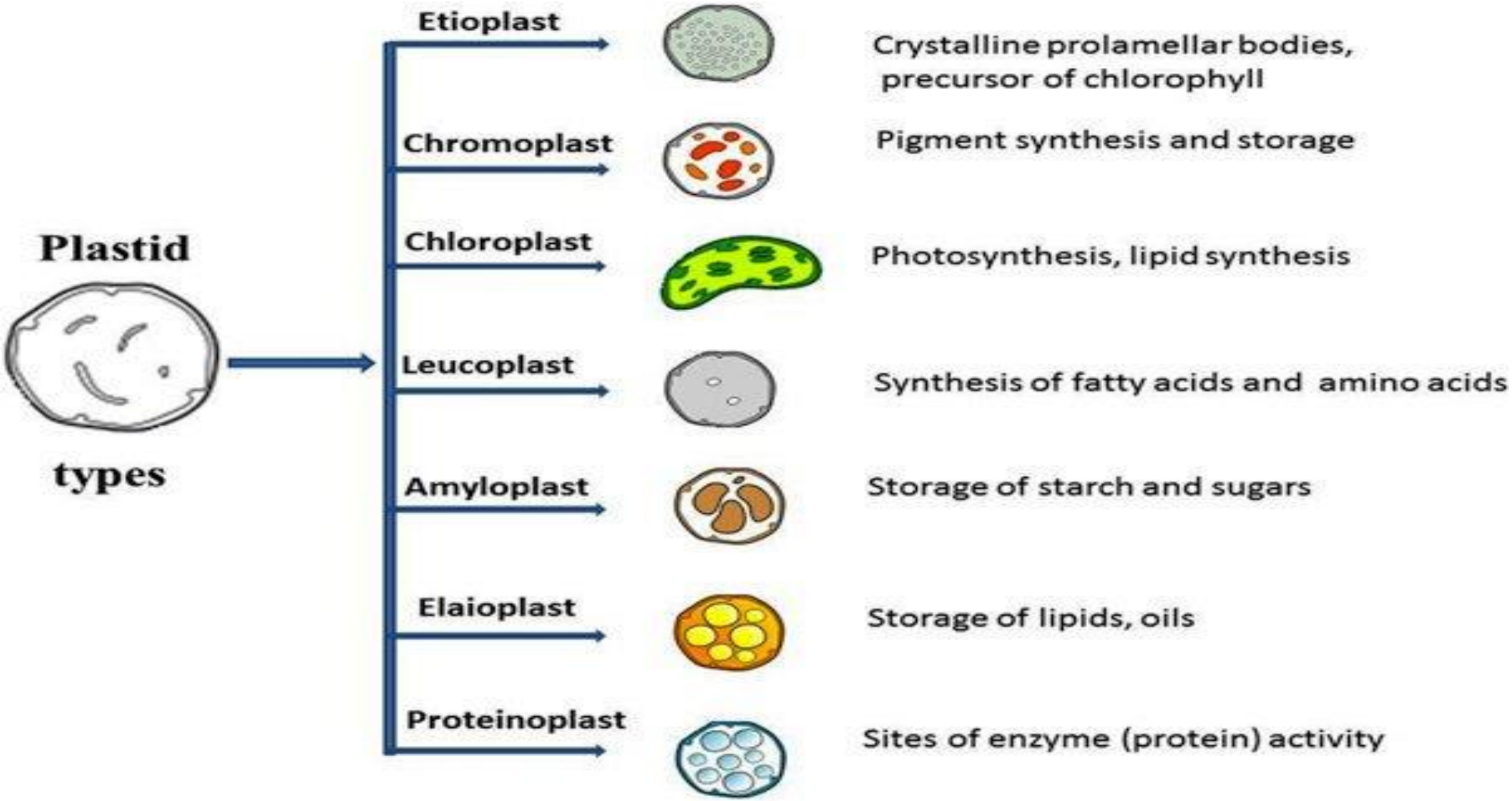


Figure:Microbenotes.com

- If plants are kept in the dark, the development of proplastids in leaves is arrested at an intermediate stage (called **etioplasts**), in which a semicrystalline array of tubular internal membranes has formed but chlorophyll has not been synthesized
- Chromoplasts develop from chloroplasts, for example, during the ripening of fruit (e.g., tomatoes). During this process, chlorophyll and the thylakoid membranes break down, while new types of carotenoids are synthesized
- dual control of **plastid** development involves the coordinated expression of genes within both the **plastid** and nuclear genomes

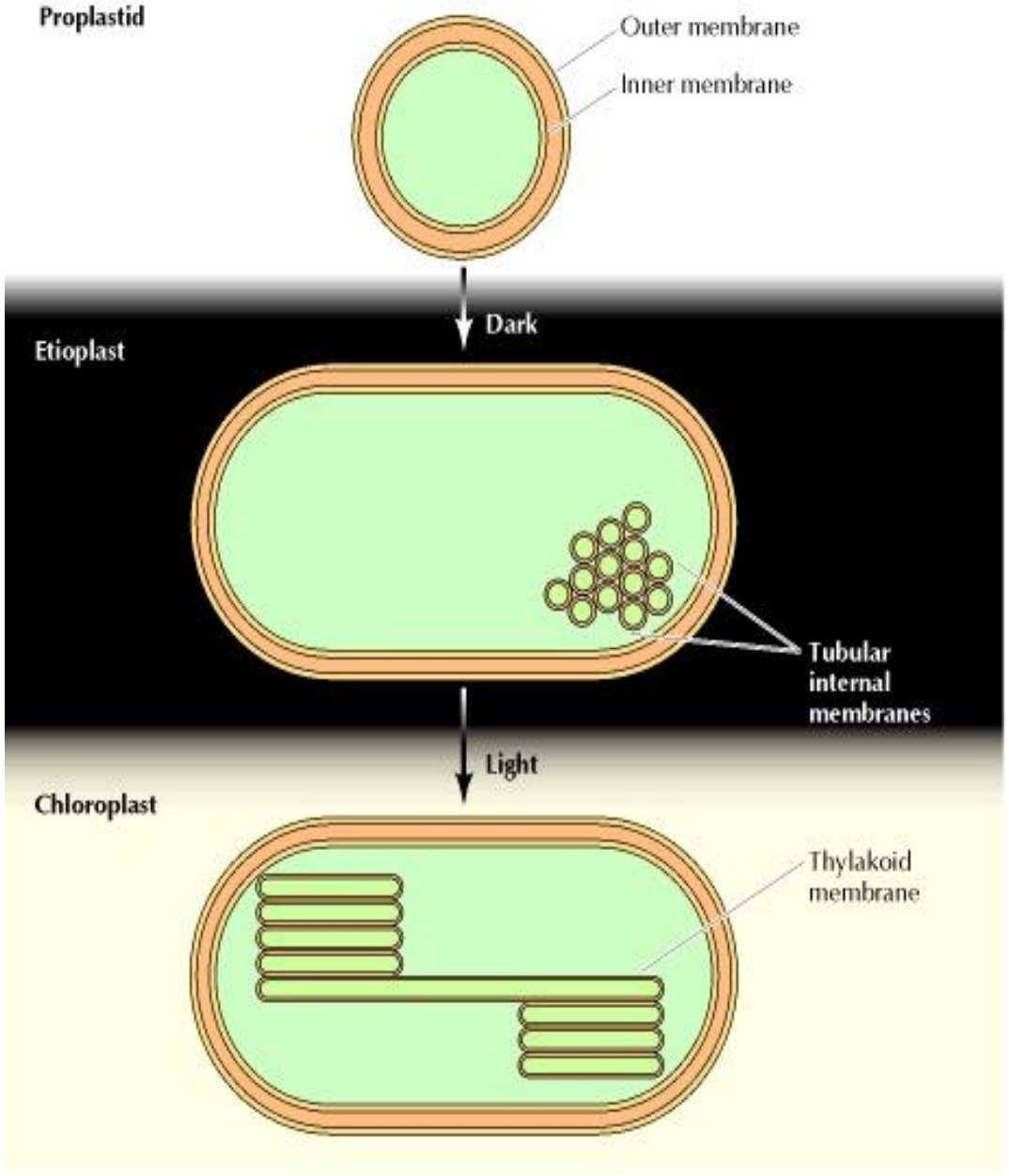


Figure: The Cell: A Molecular Approach. 2nd edition.

Chloroplast

- The word *chloroplast is* derived from the Greek words *chloros*, which means green, and *plastes*, which means “the one who forms”.
- Chloroplasts are a type of membrane-bound plastids that contain a network of membranes embedded into a liquid matrix and harbor the photosynthetic pigment called chlorophyll.
- It is this pigment that imparts a green color to plant parts and serves to capture light energy.
- Chloroplasts can be found in the cells of the mesophyll in plant leaves.
- There are usually 30-40 per mesophyll cells.
- These organelles have an even more complex structure than mitochondria

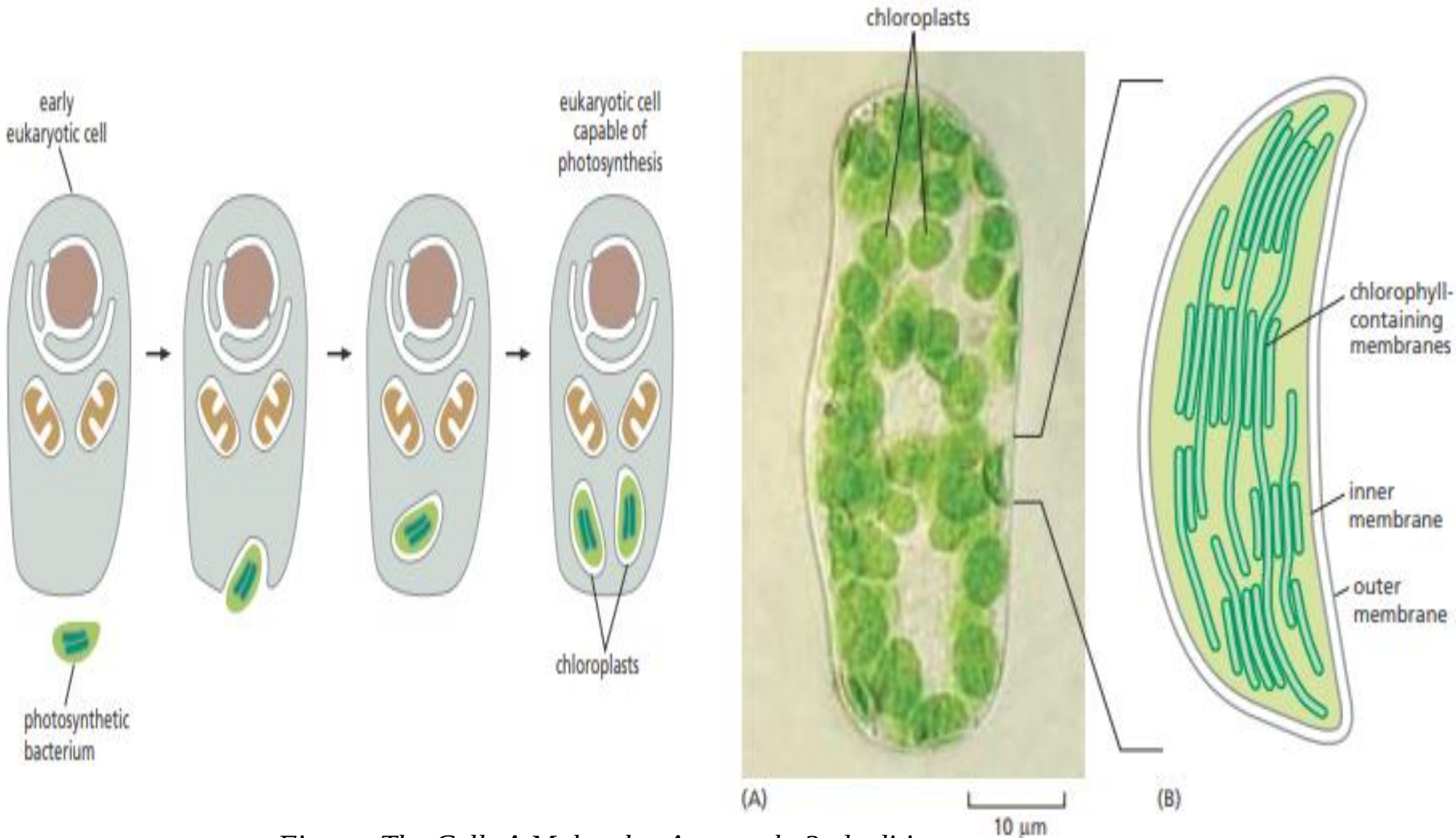


Figure: The Cell: A Molecular Approach. 2nd edition.

Structure of Chloroplasts

- Chloroplasts found in higher plants are generally biconvex or planoconvex shaped.
- In different plants, however, chloroplasts may have different shapes, varying from spheroid, filamentous saucer-shaped, discoid or ovoid-shaped.
- They can be found in the cells of the mesophyll in plant leaves. They are vesicular and have a colorless center.
- The average size of the chloroplast is 4-6 Âµ in diameter and 1-3 Âµ in thickness.
- The chloroplast has an inner and outer membrane with an empty intermediate space in between. Inside the chloroplast are stacks of thylakoids, called grana, as well as stroma, the dense fluid inside of the chloroplast
- These thylakoids contain the chlorophyll that is necessary for the plant to go through photosynthesis. The space the chlorophyll fills is called the thylakoid space.

- A chloroplast thus has the following parts:

Envelope (Outer membrane)

- It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.

Intermembrane Space

- It is usually a thin inter-membrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast

Inner membrane

- The inner membrane of the chloroplast forms a border to the stroma. It regulates the passage of materials in and out of the chloroplast. In addition to regulation activity, fatty acids, lipids, and carotenoids are synthesized in the inner chloroplast membrane

Stroma

- Stroma is an alkaline, aqueous fluid that is protein-rich and is present within the inner membrane of the chloroplast
- The space outside the thylakoid space is called the stroma
- The chloroplast DNA chloroplast ribosomes and the thylakoid system, starch granules and many proteins are found floating around the stroma.

Thylakoid System

- The thylakoid system is suspended in the stroma
- The thylakoid system is a collection of membranous sacs called thylakoids
- The chlorophyll is found in the thylakoids and is the sight for the process of light reactions of photosynthesis to happen

- The thylakoids are arranged in stacks known as grana
- Each granum contains around 10-20 thylakoids.

Peripheral Reticulum

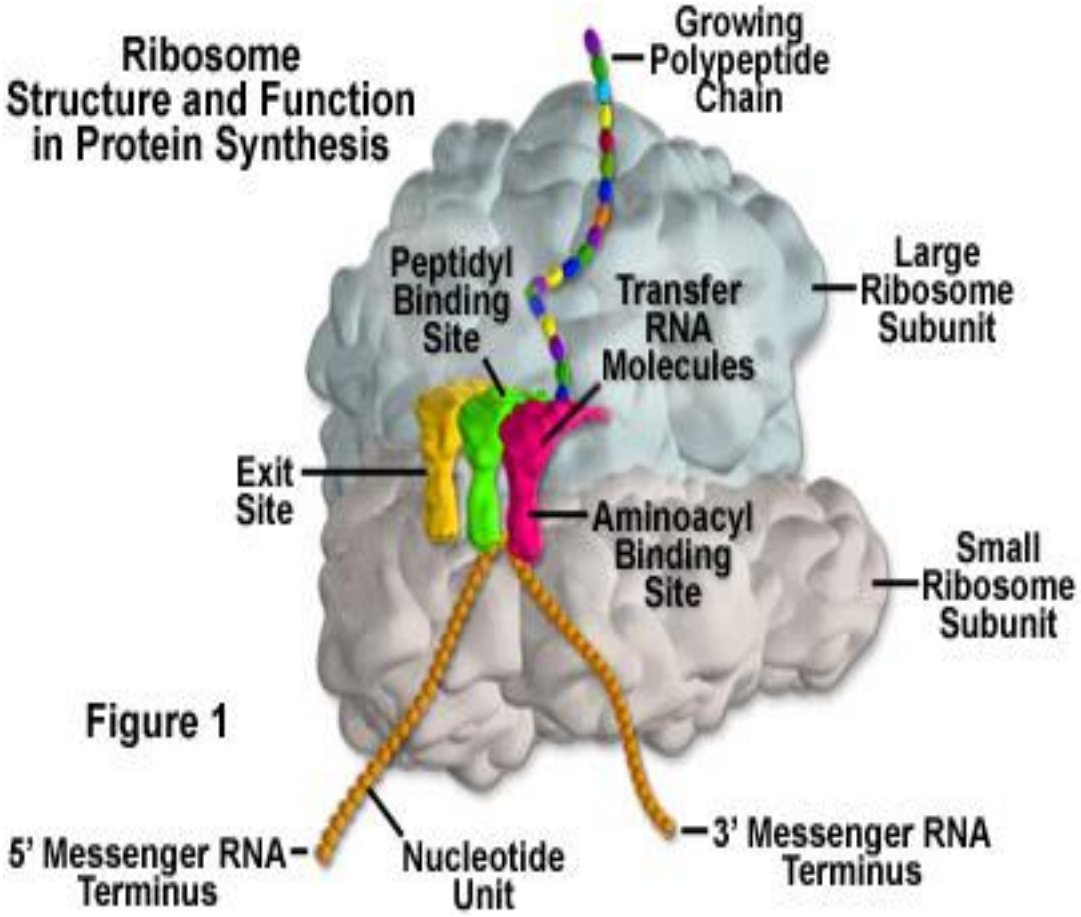
- The chloroplasts of certain plants contain an additional set of membranous tubules called peripheral reticulum that originates from the inner membrane of the envelope
- Tiny vesicles bud off from the inner membrane of the chloroplast and assemble to form the tubules of the peripheral reticulum.

Functions of Chloroplasts

- Chloroplasts are the sites for photosynthesis, which comprises a set of light-dependent and light-independent reactions to harness solar energy and convert it into chemical energy
- The components of chloroplast participate in several regulatory functions of the cell as well as in photorespiration
- Chloroplasts also provide diverse metabolic activities for plant cells, including the synthesis of fatty acids, membrane lipids, isoprenoids, tetrapyrroles, starch, and hormones
- Plants lack specialized immune cells—all plant cells participate in the plant response
- The chloroplasts with the **nucleus** and cell membrane and **ER** are the key organelles of pathogen defense
- Chloroplasts can serve as cellular sensors

Ribosomes

- In 1955, George E. Palade discovered ribosomes and described them as small particles in the cytoplasm that preferentially associated with the endoplasmic reticulum membrane
- Along with other scientists, Palade discovered that ribosomes performed protein synthesis in cells, and he was awarded the Nobel Prize in 1974 for his work



- the RNA components of the ribosome are final gene products
- synthesize approximately 10 million copies of each type of ribosomal RNA in each cell generation to construct its 10 million ribosomes
- The cell can produce adequate quantities of ribosomal RNAs only because it contains multiple copies of the rRNA genes that code for ribosomal RNAs (rRNAs)
- There are four types of eukaryotic rRNAs, each present in one copy per ribosome
- Three of the four rRNAs (18S, 5.8S, and 28S) are made by chemically modifying and cleaving a single large precursor rRNA (Figure)
- the fourth (5S RNA) is synthesized from a separate cluster of genes by a different polymerase, RNA polymerase III, and does not require chemical modification

- chemical modifications occur in the 13,000-nucleotide-long precursor rRNA before the rRNAs are cleaved out of it and assembled into ribosomes
- These include about 100 methylations of the 2′-OH positions on nucleotide sugars and 100 isomerizations of uridine nucleotides to pseudouridine

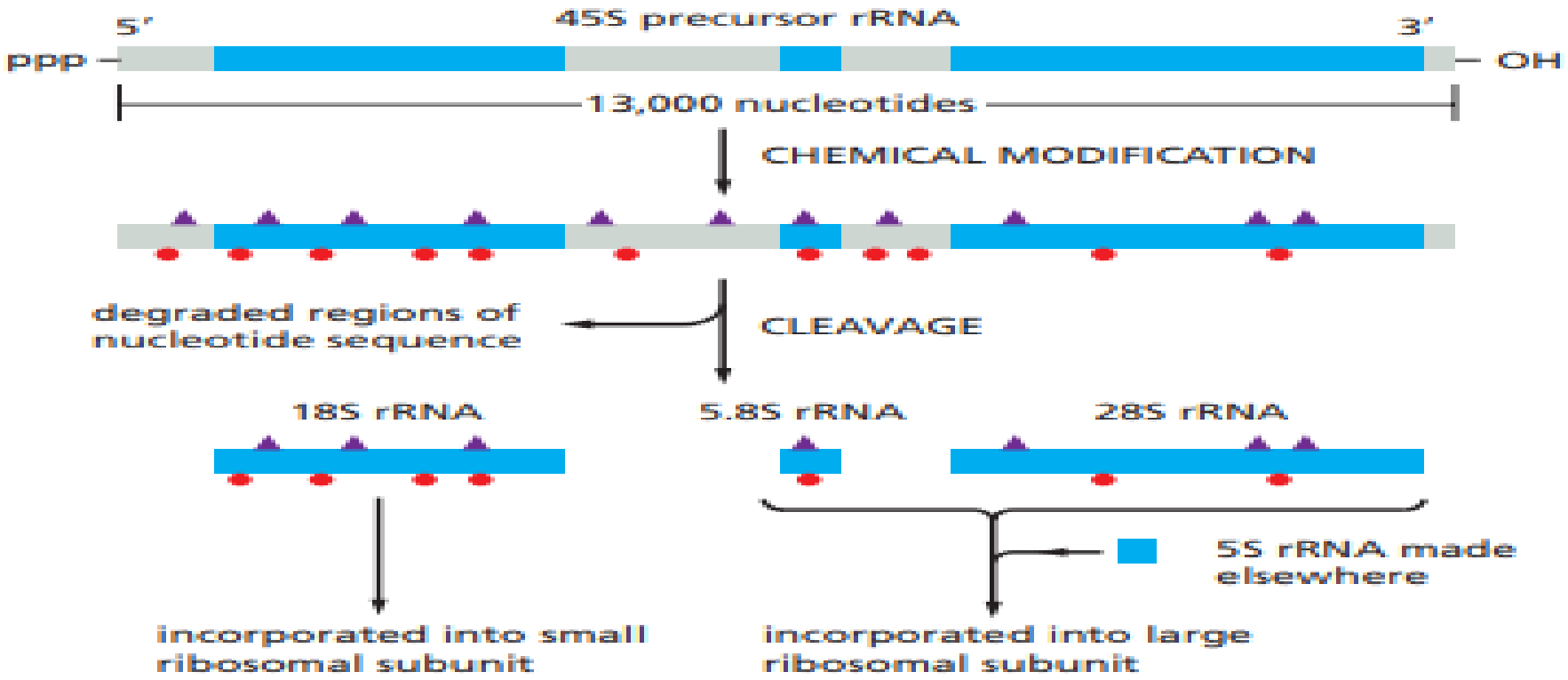
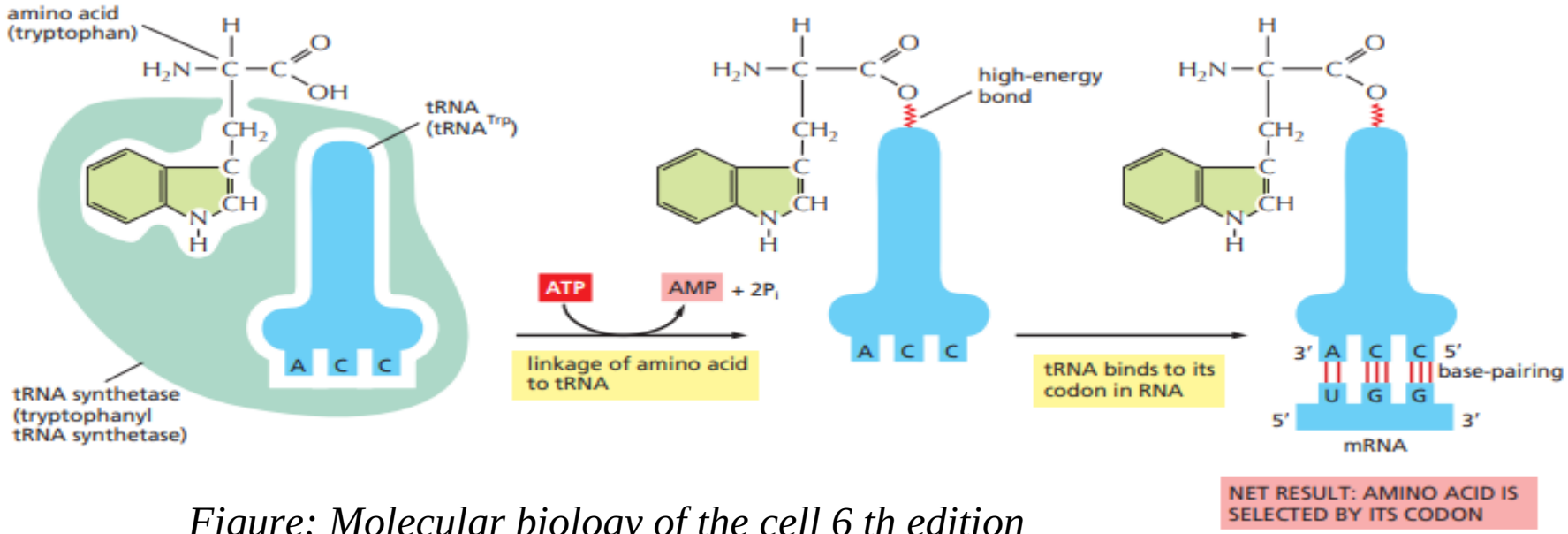


Figure: Molecular biology of the cell 6 th edition

- Figure. The chemical modification and nucleolytic processing of a eukaryotic 45S precursor rRNA molecule into three separate ribosomal RNAs
- Two types of chemical modifications are made to the precursor rRNA before it is cleaved.
- Nearly half of the nucleotide sequences in this precursor rRNA are discarded and degraded in the nucleus by the exosome
- The rRNAs are named according to their “S” values, which refer to their rate of sedimentation in an ultracentrifuge
- The larger the S value, the larger the rRNA

Editing by tRNA Synthetases Ensures Accuracy

- Several mechanisms working together ensure that an aminoacyl-tRNA synthetase links the correct amino acid to each tRNA
- Most synthetase enzymes select the correct amino acid by a two-step mechanism
- The correct amino acid has the highest affinity for the active-site pocket of its synthetase and is therefore favored over the other 19; in particular, amino acids larger than the correct one are excluded from the active site



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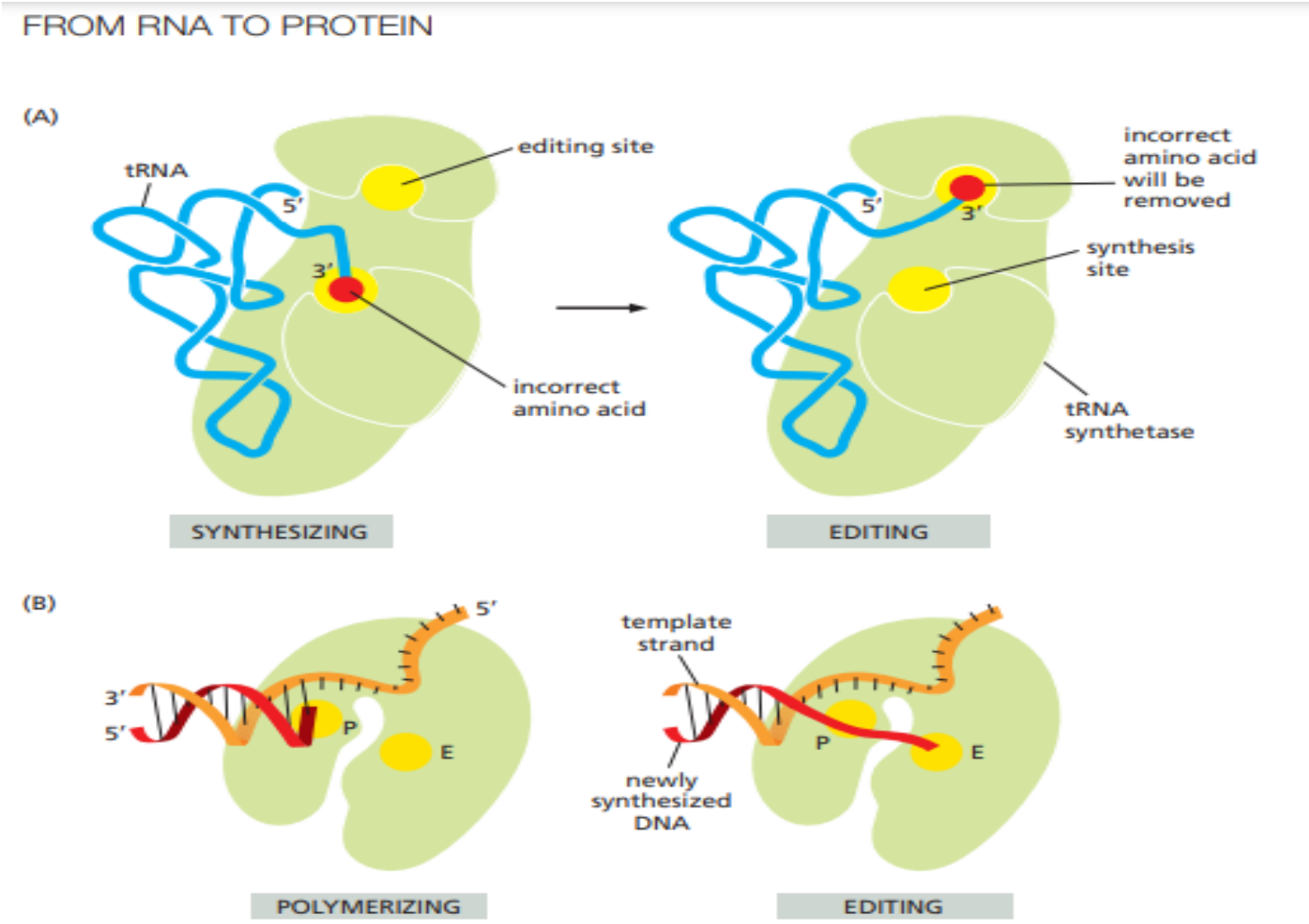

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Hydrolytic editing.

(A) Aminoacyl tRNA synthetases correct their own coupling errors through hydrolytic editing of incorrectly attached amino acids. the correct amino acid is rejected by the editin

(B) The error-correction process performed by DNA polymerase has similarities; however, it differs because the removal process depends strongly on a mispairing with the template

(P, polymerization site; E, editing site.)

Translating an mRNA molecule

- Each amino acid added to the growing end of a polypeptide chain is selected by complementary basepairing between the anticodon on its attached tRNA molecule
- the next codon on the mRNA chain
- Because only one of the many types of tRNA molecules in a cell can base-pair with each codon, the codon determines the specific amino acid to be added to the growing polypeptide chain
- The four-step cycle shown is repeated over and over during the synthesis of a protein
- A site- site for Aminoacyl binding site for charged t-RNA molecules

during protein synthesis

- P site –Polymerisation site
- E site-editing site

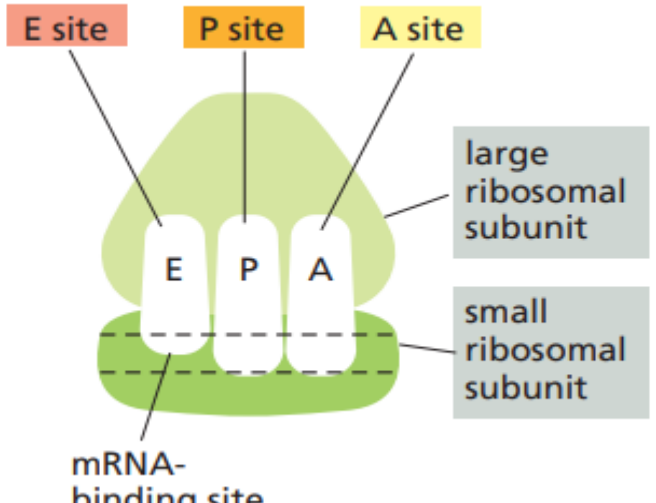


Figure: Molecular biology of the cell 6 th edition

The incorporation of an amino acid into a protein

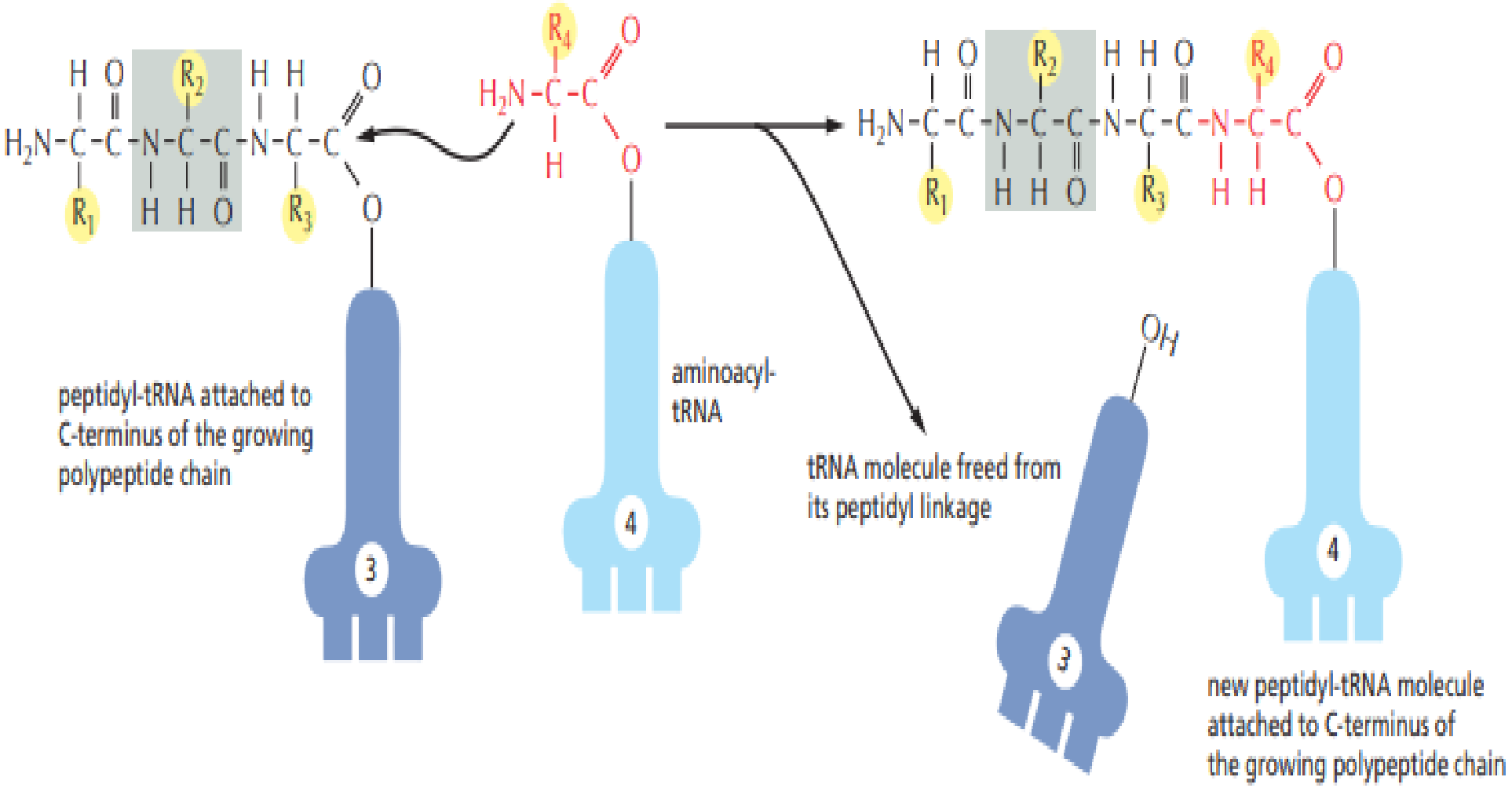


Figure: Molecular biology of the cell 6 th edition

- step 1, an aminoacyl-tRNA molecule binds to a vacant A site on the ribosome
- step 2, a new peptide bond is formed
- step 3, the large subunit translocates relative to the small subunit, leaving the two tRNAs in hybrid sites: P on the large subunit and A on the small, for one; E on the large subunit and P on the small, for the other
- step 4, the small subunit translocates carrying its mRNA a distance of three nucleotides through the ribosome. This “resets” the ribosome with a fully empty A site, ready for the next aminoacyl-tRNA molecule to bind
- As indicated, the mRNA is translated in the 5’-to-3’ direction, and the N-terminal end of a protein is made first, with each cycle adding one amino acid to the C-terminus of the polypeptide chain

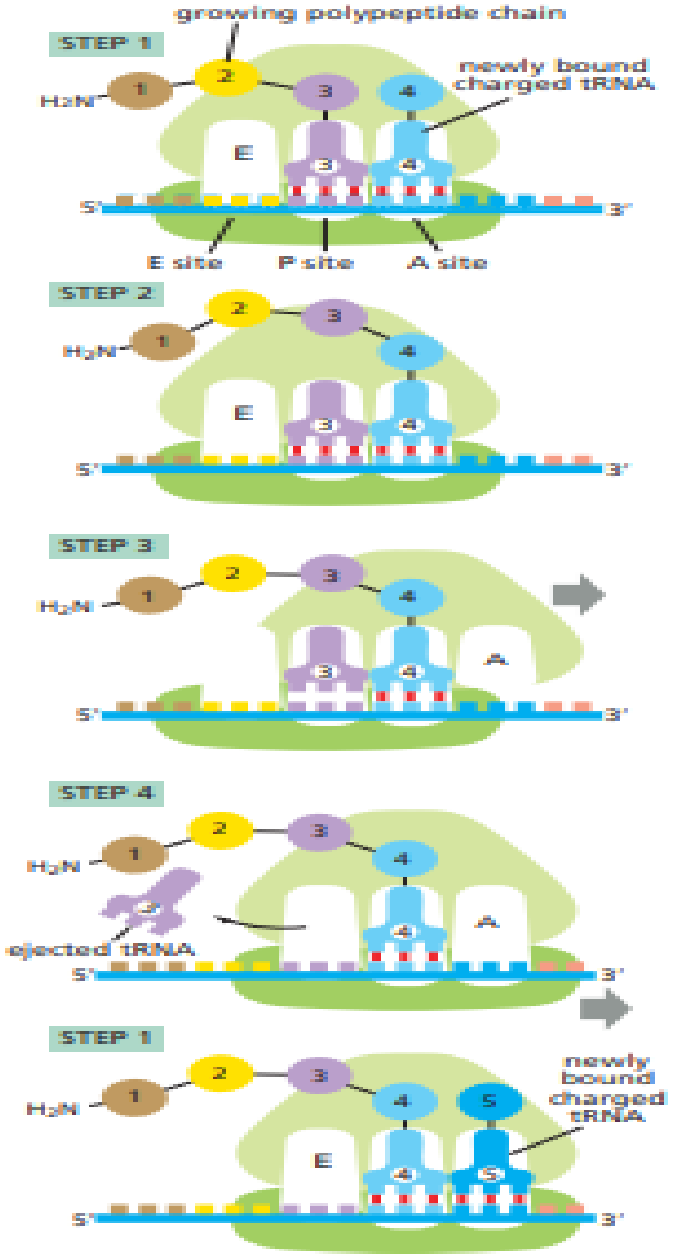
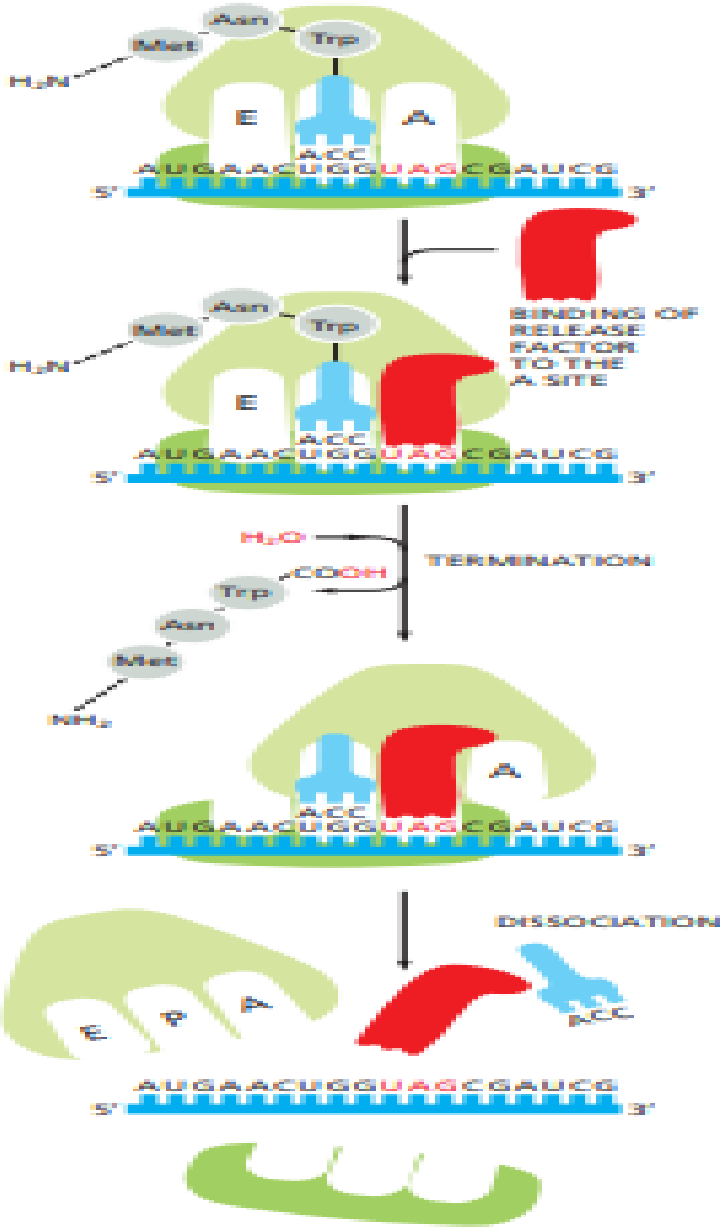


Figure: Molecular biology of the cell 6 th edition

The final phase of protein synthesis.

- The binding of a release factor to an A site bearing a stop codon terminates translation
- The completed polypeptide is released and, in a series of reactions that requires additional proteins and GTP hydrolysis (not shown), the ribosome dissociates into its two separate subunits



Centrosomes

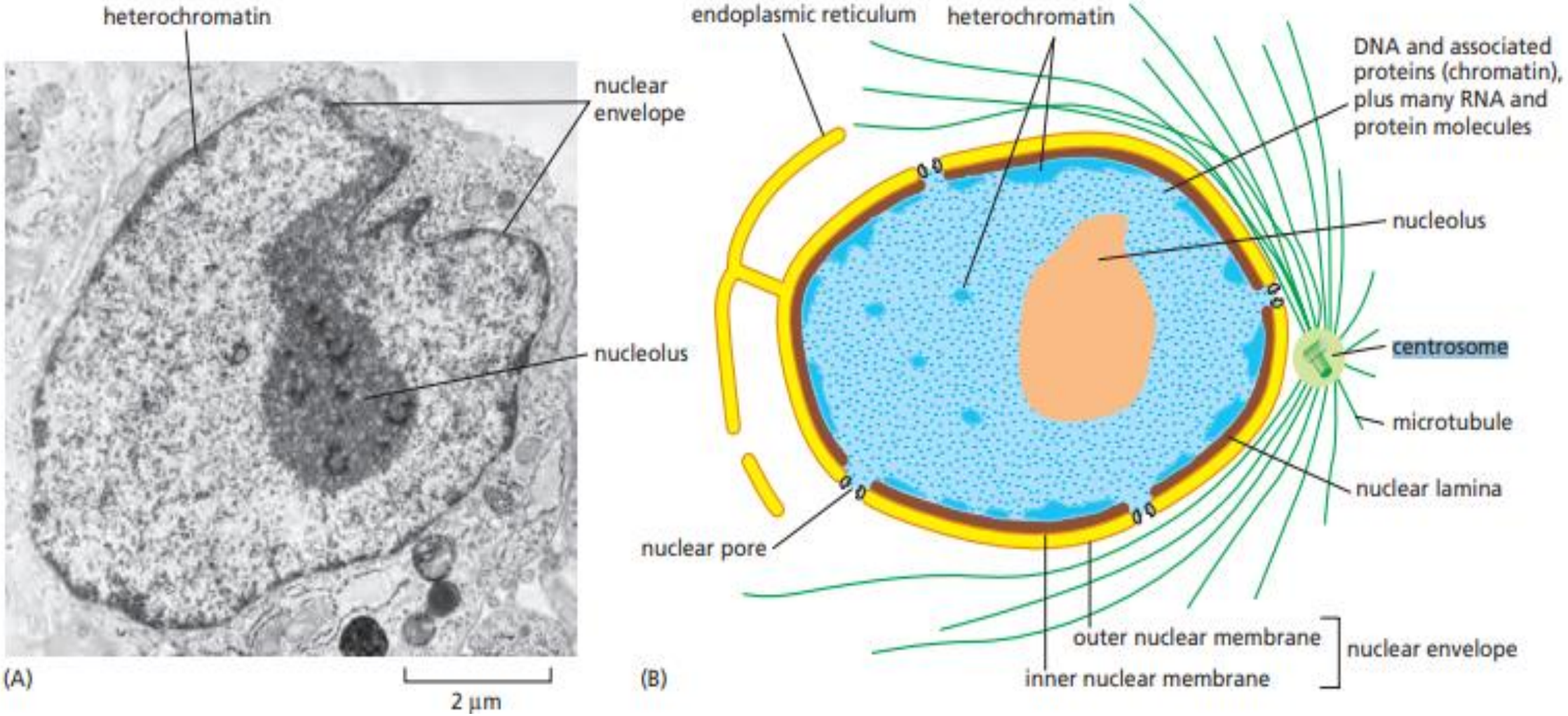


Figure: Molecular biology of the cell 6 th edition

- The centrosome is considered to be the main microtubule-organizing center (MTOC) therefore regulating cell adhesion, motility, and polarity
- It also promotes the spindle pole organization in an animal cell during mitotic replication
- Defects in the function of spindle-organization are present in many cancers and may be attributed to genomic instability
- Irrregular or extra centrosomes may contribute to abnormal cell division.

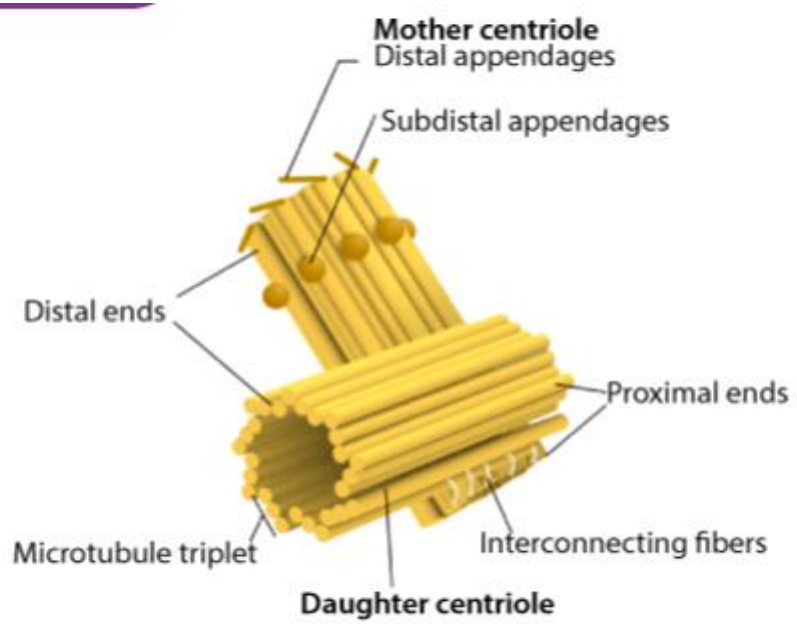
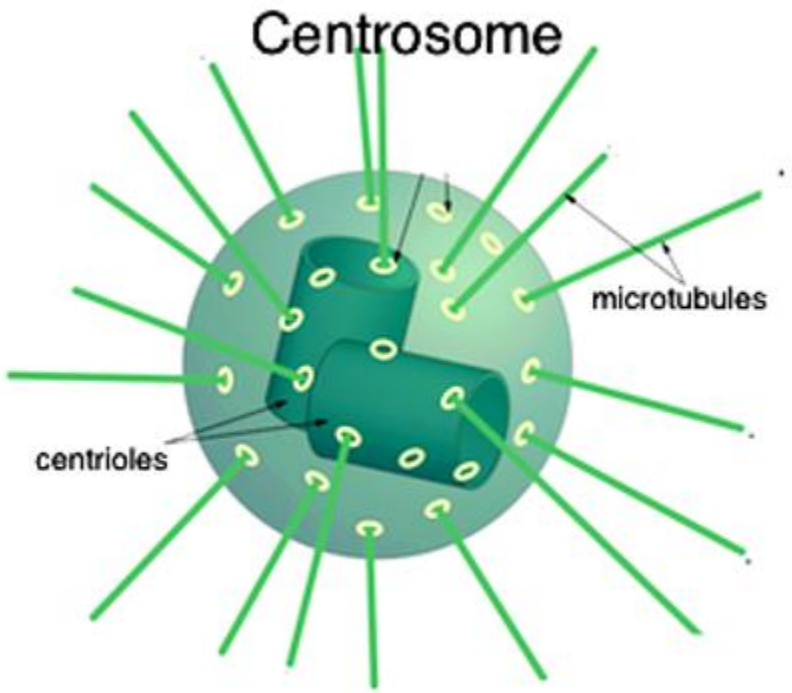


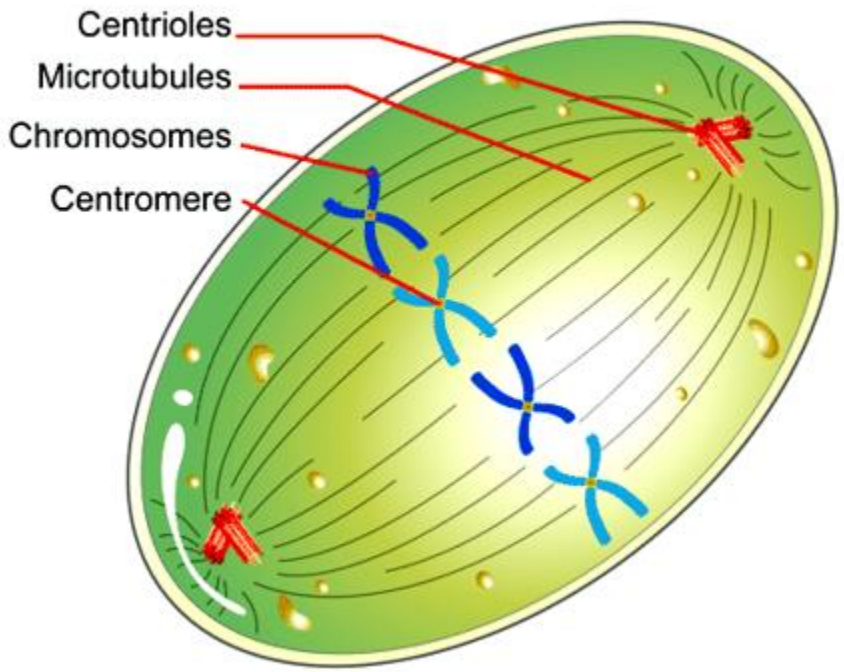
Figure:BYJU's

- The centrosome is made up of two perpendicular centrioles, a daughter centriole, and a mother centriole, linked together by interconnecting fibres
- It consists of a complex of proteins that helps in the formation of additional microtubules
- An amorphous pericentriolar matrix surrounds the centrioles
- It is involved in the nucleation and anchoring of cytoplasmic microtubules
- Centrosome in the animal cells is very much like DNA. During cell division, one centrosome from the parent cell is transferred to each daughter cell
- In proliferating cells, the centrosome starts dividing before the S-phase begins
- The newly formed centrosomes participate in organizing the mitotic spindles
- During Interphase, the centrosome organizes an astral ray of microtubules that help in intracellular trafficking, cell adhesion, cell polarity, etc

The centrosome cycle consists of four phases:

- G1 phase where the duplication of centrosome takes place
- G2 phase where the centrosome maturation takes place
- The mitotic phase where the centrosome separation takes place
- A late mitotic phase where the chromosome disorientation takes place





Centrosome Function

- The major functions of centrosome are listed below:
- The centrosomes help in cell division
- They maintain the chromosome number during cell division
- They also stimulate the changes in the shape of the cell membrane by phagocytosis
- In mitosis, it helps in organizing the microtubules ensuring that the centrosomes are distributed to each daughter cell
- They regulate the movement of microtubules and cytoskeletal structures, thereby, facilitating changes in the shapes of the membranes of the animal cell

Reference

- The Cell: A Molecular Approach. 2nd edition
- Marks’ basic medical biochemistry: A clinical approach
- Cell Biology genetics and Molecular Biology
- https://www.biologyonline.com/dictionary/centrosome

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Programme: M.Sc., Biochemistry
Course Title : Cell biology
Course Code :BC105DCE

Unit-3
INTERNAL ORGANIZATION OF THE CELL

Dr. M. Anusuyadevi Jayachandran
Professor
Department of Biochemistry

Cytoskeleton

- All cells have to be correctly shaped, physically robust, properly structured internally
- They have to be able to rearrange their internal components as they grow, divide and adapt to changing circumstances
- These spatial and mechanical functions depends on a system of filaments called cytoskeleton

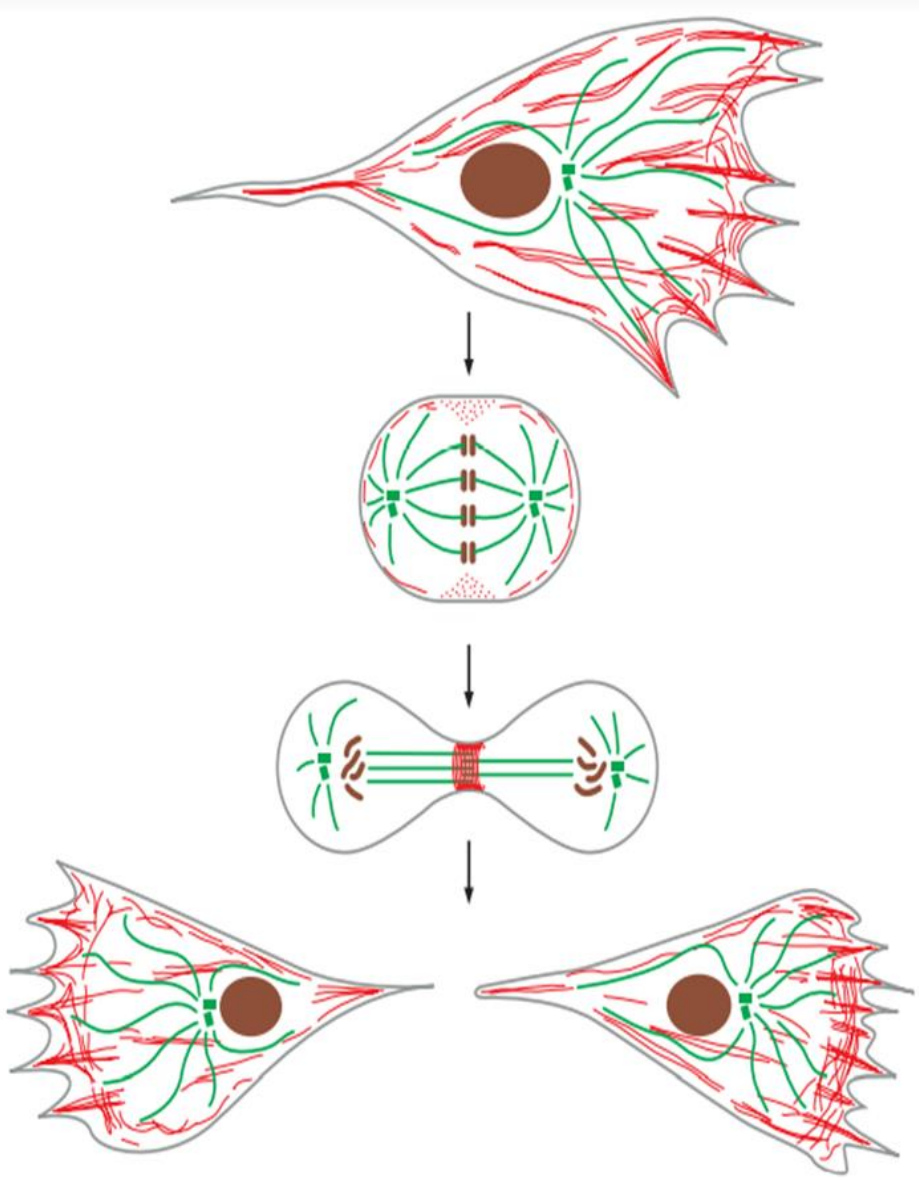
Three families of protein filaments

- Actin filament
- Microtubules
- Intermediate filaments

Cytoskeletal Filaments Adapt to Form Dynamic or Stable Structures

- a fibroblast growing in a tissue-culture dish
- After the chromosomes have replicated, the interphase microtubule array that spreads throughout the cytoplasm is reconfigured into the
- bipolar mitotic spindle, which segregates the two copies of each chromosome into daughter nuclei
- At the same time, the specialized actin structures that enable the fibroblast to crawl across the surface of the dish rearrange so that the cell stops moving and assumes a more spherical shape
- Actin and its associated motor protein myosin then form a belt around the middle of the cell, the contractile ring, which constricts like a tiny muscle to pinch the cell in two

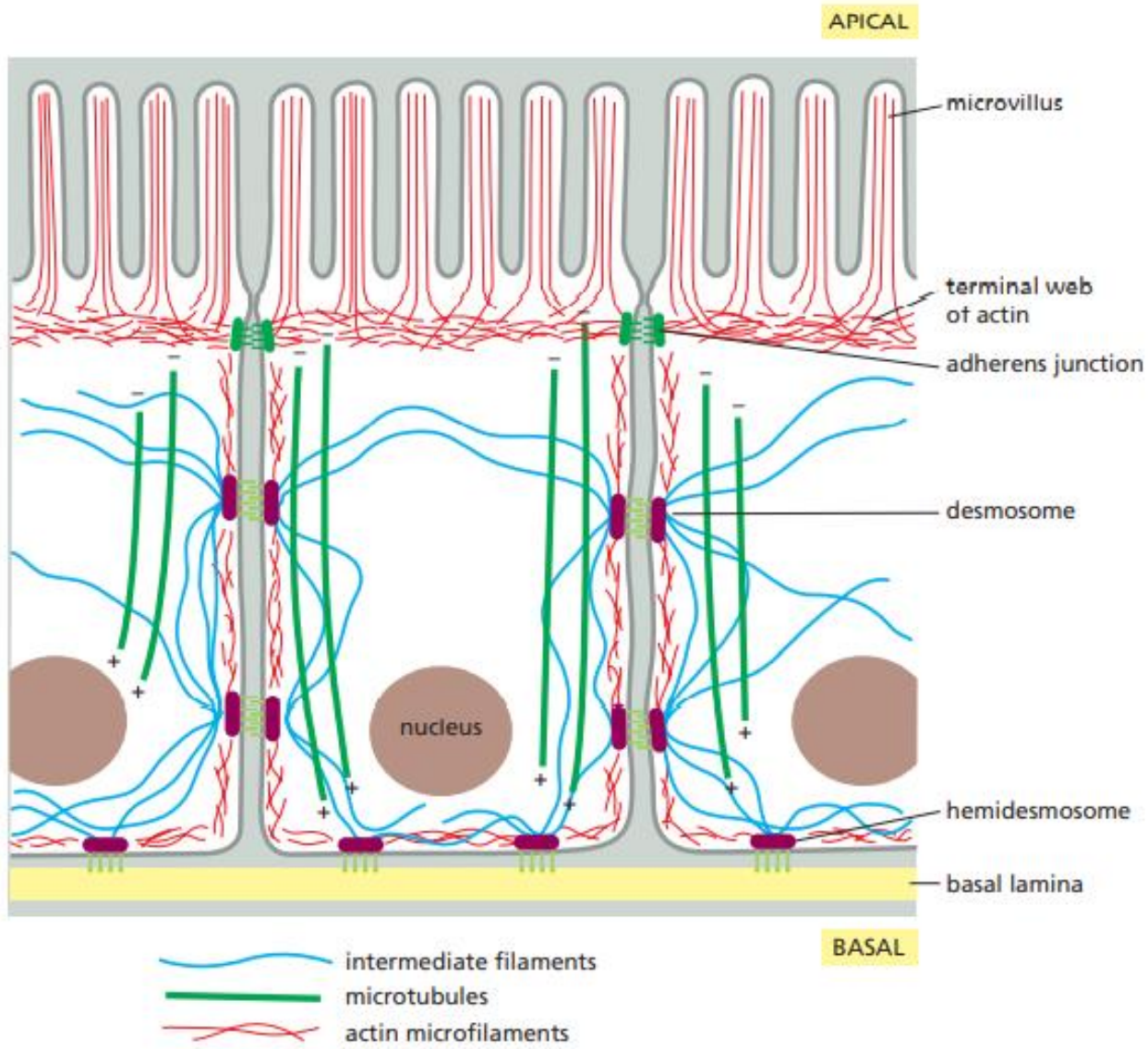
- When division is complete, the cytoskeletons of the two daughter fibroblasts reassemble into their interphase structures to convert the two rounded-up daughter cells into smaller versions of the flattened, crawling mother cell
- actin cytoskeleton – Red
- microtubule cytoskeleton – Green
- segregating the duplicated chromosomes – Brown
- Many cells require rapid cytoskeletal rearrangements for their normal functioning during interphase as well
- neutrophil, a type of white blood cell, chases and engulfs bacterial and fungal cells that accidentally gain access to the normally sterile parts of the body, as through a cut in the skin



- Like most crawling cells, neutrophils advance by extending a protrusive structure filled with newly polymerized actin filaments
- When the elusive bacterial prey moves in a different direction, the neutrophil is poised to reorganize its polarized protrusive structures within seconds
- On the intestine and the lung, cytoskeletal-based cell-surface protrusions including microvilli and cilia are able to maintain a constant location, length, and diameter over the entire lifetime of the cell
- Polarized epithelial cells use organized arrays of microtubules, actin filaments, and intermediate filaments to maintain the critical differences between the apical surface and the basolateral surface
- They also must maintain strong adhesive contacts with one another to enable this single layer of cells to serve as an effective physical barrier

Filaments Assemble from Protein Subunits That Impart Specific Physical and Dynamic Properties

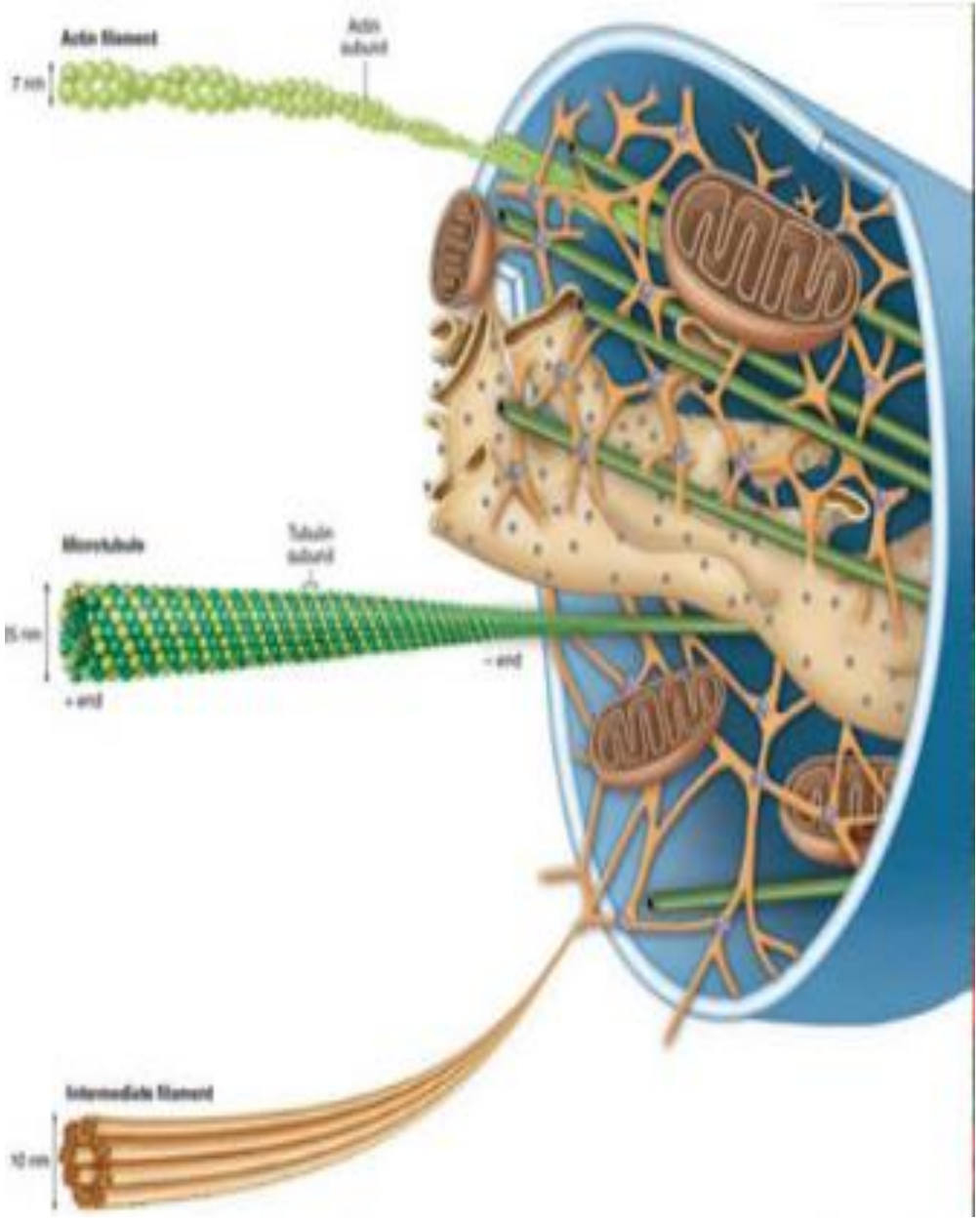
- Below the microvilli, a circumferential band of actin filaments is connected to cell–cell adherens junctions that anchor the cells to each other
- Intermediate filaments (blue)are anchored to other kinds of adhesive structures, including desmosomes and hemidesmosomes, that connect the epithelial cells into a sturdy sheet and attach them to the underlying extracellular matrix
- Microtubules (green) run vertically from the top of the cell to the bottom and provide a global coordinate system that enables the cell to direct newly synthesized components to their proper locations



- Actin filaments determine the shape of the cell’s surface and are necessary for whole-cell locomotion; they also drive the pinching of one cell into two
- Microtubules determine the positions of membrane-enclosed organelles, direct intracellular transport, and form the mitotic spindle that segregates chromosomes during cell division
- Intermediate filaments provide mechanical strength
- The accessory proteins are essential for the controlled assembly of the cytoskeletal filaments in particular locations, and they include the motor proteins
- molecular machines that convert the energy of ATP hydrolysis into mechanical force that can either move organelles along the filaments or move the filaments themselves

Actin Filament:

- The thinnest are the microfilaments (7 nm in diameter) which are solid and are principally made of two intertwined strands of a globular protein called actin. For this reason, microfilaments are also known as actin filaments
- Actin is powered by ATP to assemble its filamentous form, which serves as a track for the movement of a motor protein called myosin
- This enables actin to engage in cellular events requiring motion such as cell division in animal cells and cytoplasmic streaming, which is the circular movement of the cell cytoplasm in plant cells



Basic functions of Actin filaments

- Actin filaments underlie the plasma membrane of animal cells, providing strength and shape to its thin lipid bilayer
- They form many types of cell projections
- Dynamic structures such as *lamellipodia* and *filopodia* used for locomotion and to explore the territory
- More stable arrays allow cells to brace themselves against an underlying substratum and enable muscle to contract
- regular bundles of *stereocilia* on the surface of hair cells in the inner ear contain stable bundles of actin filaments that tilt as rigid rods in response to sound, and similarly organized microvilli on the surface of intestinal epithelial cells vastly increase the apical cell-surface area to enhance nutrient absorption
- In plants, they drive rapid streaming of plasma inside cells

Microtubules

- The thickest are the microtubules (20 nm in diameter) which consist primarily of the tubulin protein.
- Each tubulin subunit is made up of one alpha and one beta-tubulin that are attached to each other, so technically tubulin is a heterodimer, not a monomer. Since it looks like a tube, it is named as microtubule.
- In a microtubule structure, tubulin monomers are linked both at their ends and along their sides (laterally). This means that microtubules are quite stable along their lengths.
- Since the tubulin subunits are always linked in the same direction, microtubules have two distinct ends, called the plus (+) and minus (-) ends.
- On the minus end, alpha-tubulin is exposed, and on the plus end, beta-tubulin is exposed.
- Microtubules preferentially assemble and disassemble at their plus ends.

Intermediate Filament:

- The fibers of the middle-order are called the intermediate filaments (IFs) having a diameter of 10 nm.
- They are composed of a family of related proteins sharing common structural and sequence features.
- They having been classified according to their constituent protein such as desmin filaments, keratin filaments, neurofilaments, vimentin, and glial filaments

Microtrabecular Lattice

- Recently, cytoplasm has been found to be filled with a three-dimensional network of interlinked filaments of cytoskeletal fibers, called a micro-trabecular lattice
- Various cellular organelles such as ribosomes, lysosomes, etc., are found anchored to this lattice
- The micro-trabecular lattice being flexible changes its shape and results in the change of cell shape during cell movement.

Basic functions of Microtubules

- Microtubules, which are frequently found in a cytoplasmic array that extends to the cell periphery, can quickly rearrange themselves to form a bipolar mitotic spindle during cell division
- They also form cilia which act as motile whips or sensory devices on the surface of the cell
- Tightly align and serve as tracts for the transport of materials down long neuronal axons
- In plants, organized arrays of microtubules help to direct the pattern of cell wall synthesis
- In many protozoans they form the framework upon which the entire cell is built

Basic functions of Intermediate filament:

- Intermediate filaments line the inner face of the nuclear envelope, forming a protective cage for the cell’s DNA
- In cytosol, they are twisted into strong cables that can hold epithelial cell sheets together
- help nerve cells to extend long and robust axons
- they allow us to form tough appendages such as hair and fingernails

Intermediary filaments are symmetrical in nature and therefore donot form polarized filaments with two different ends.
Intermediate filaments donot catalyse the hydrolysis of nucleotides

Cell Junction

- Occur at points of cell-cell and cell-matrix contact in all tissues and they are particularly plentiful in epithelia
- Cell junctions are best visualized using either conventional or freeze-fracture electron microscopy
- reveals that the interacting plasma membranes are highly specialized in these regions

Cell junctions can be classified into three functional groups:

1.Occluding junctions seal cells together in an epithelium in a way that prevents even small molecules from leaking from one side of the sheet to the other

2.Anchoring junctions mechanically attach cells to their neighbors or to the extracellular matrix

3.Communicating junctions mediate the passage of chemical or electrical signals from one interacting cell to its partner

- cell junctions and the extracellular matrix is critical for every aspect of the organization, function, and dynamics of multicellular Structures
- Defects in this apparatus underlie an enormous variety of diseases
- two broad categories of tissues that are found in all animals:

1. Connective tissues -such as bone or tendon

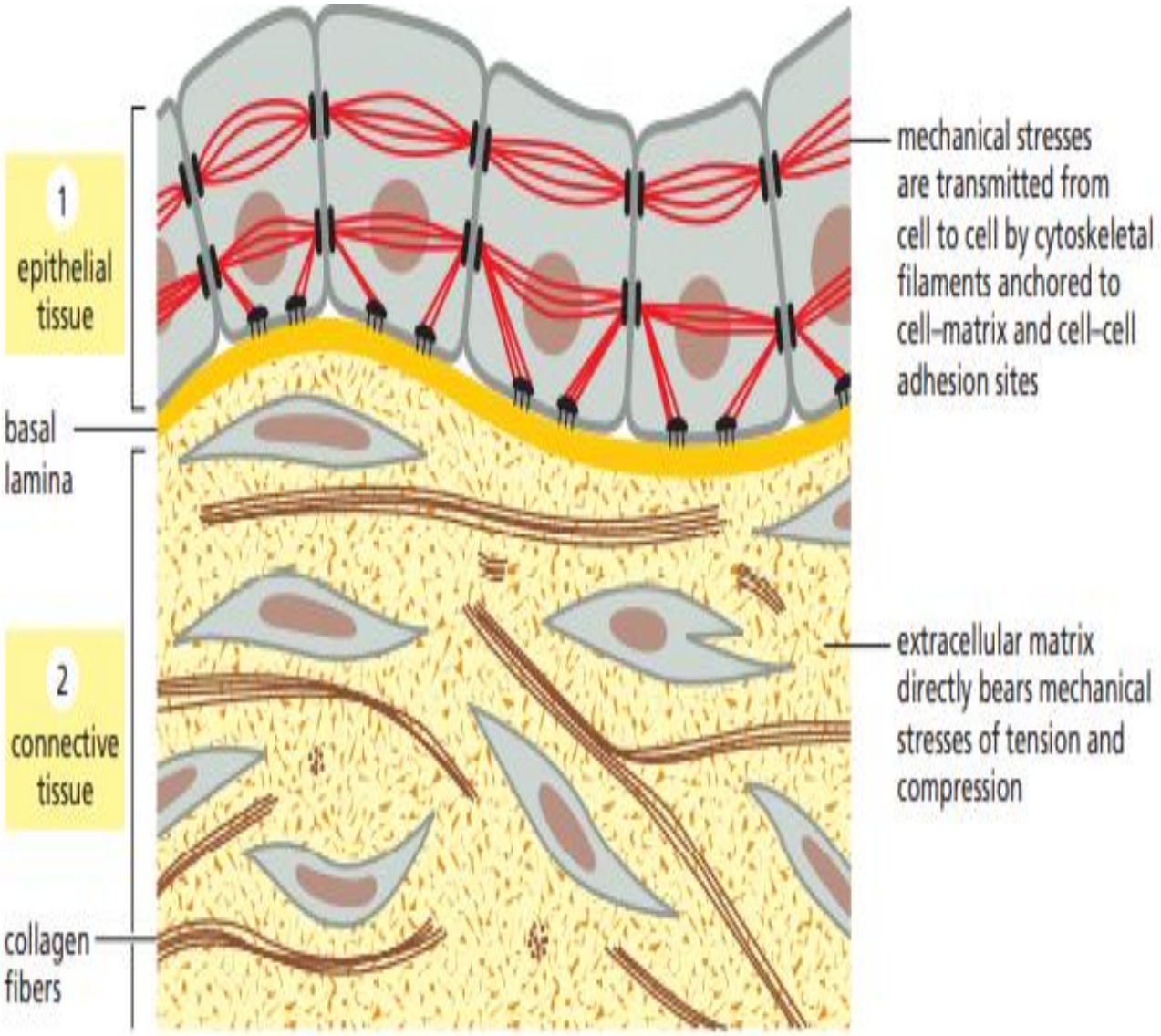
- It is formed from an extracellular matrix produced by cells distributed in the matrix
- cell–matrix junctions link the cytoskeleton to the matrix, allowing the cells to move through the matrix and monitor changes in its mechanical properties

2. epithelial tissues

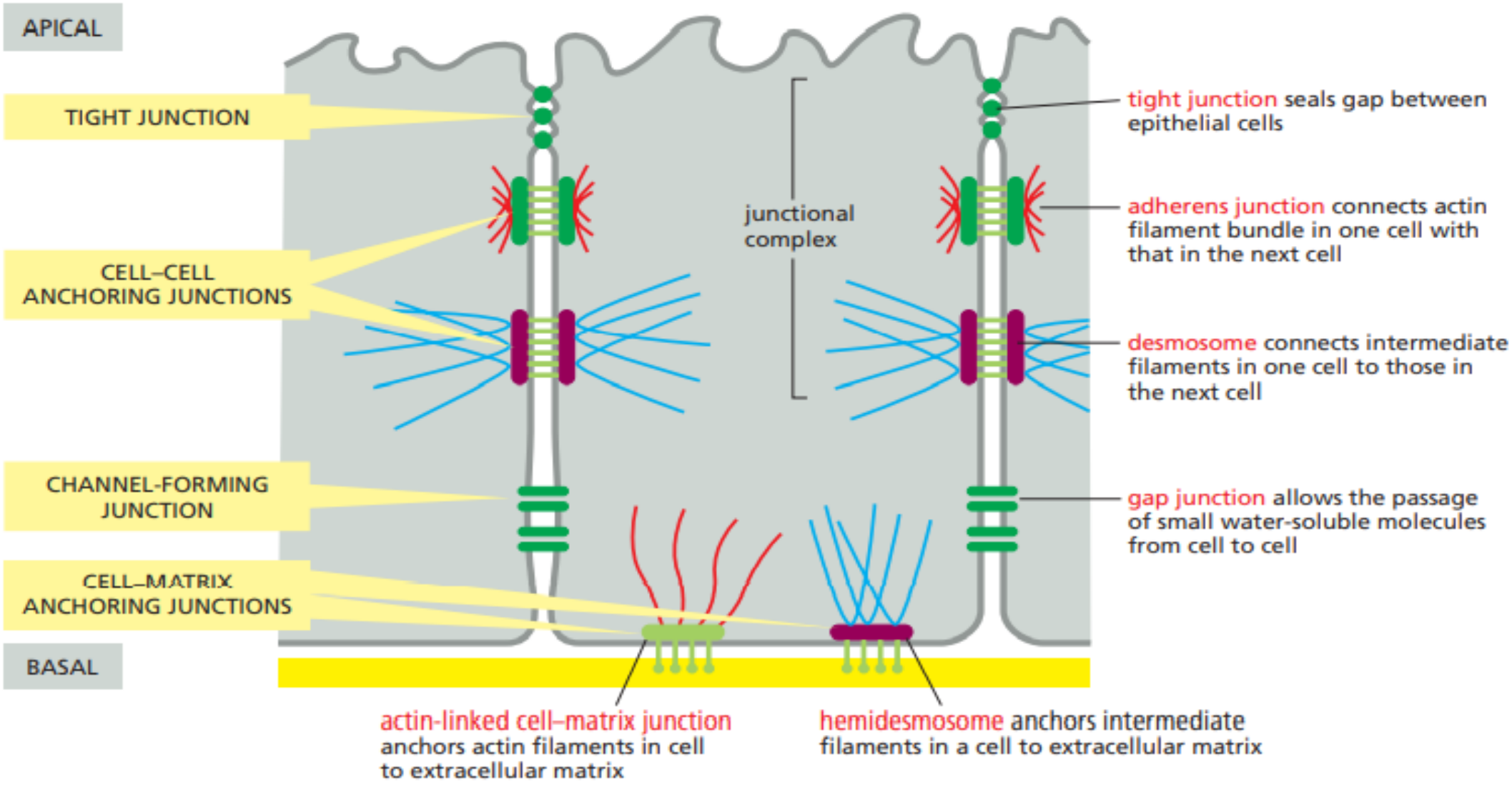
- cells are tightly bound together into sheets called epithelia

- the epithelium, cells are attached to each other directly by cell-cell junctions, where cytoskeletal filaments are anchored
- Two types of anchoring junctions link the cytoskeletons of adjacent cells: adherens junctions are anchorage sites for actin filaments; desmosomes are anchorage sites for intermediate filaments

Two main ways in which animal cells are bound together



cell junctions in a vertebrate epithelial cell, classified according to their primary functions



- four major anchoring junction types depends on transmembrane adhesion proteins that span the plasma membrane
- They fall into two protein superfamily based on the external attachment

1. Cadherin superfamilies mediates cell to cell attachment
2. Integrin superfamilies mediates cell to matrix attachment

- some cadherins link to actin and form adherens junctions
- while others link to intermediate filaments form desmosomes
- some integrins link to actin and form actin linked cell–matrix junctions
- while others link to intermediate filaments and form hemidesmosomes

Figure : Transmembrane adhesion proteins link the cytoskeleton to extracellular structures

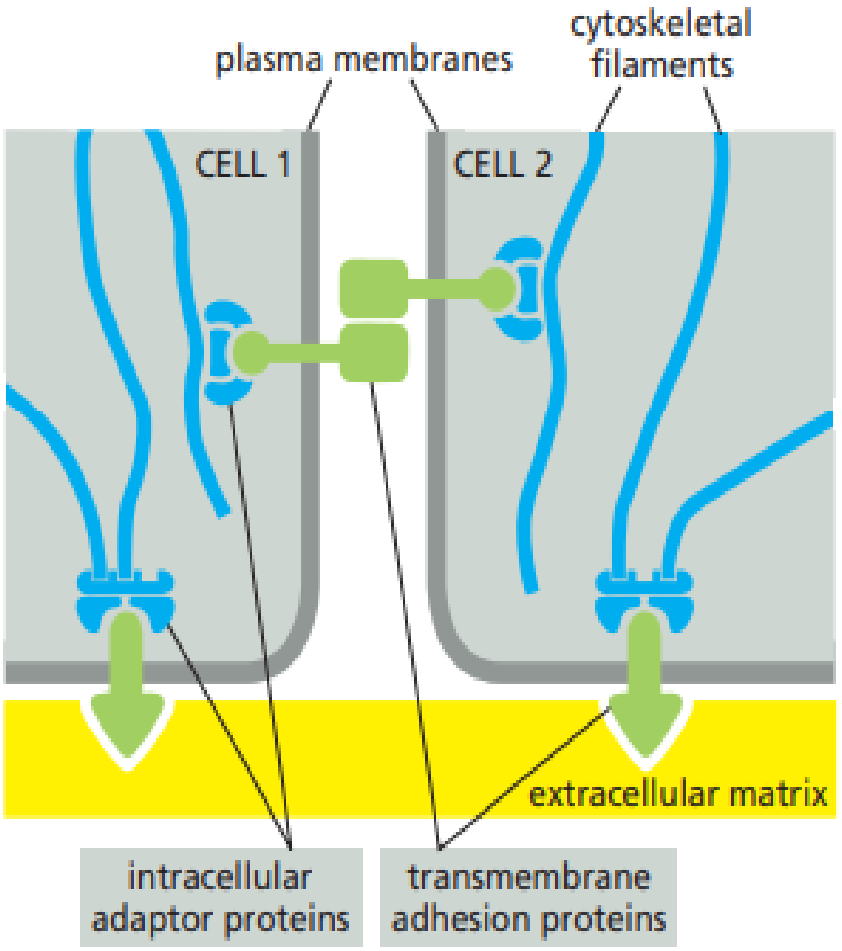


TABLE 19–1 Anchoring Junctions

Junction	Transmembrane adhesion protein	Extracellular ligand	Intracellular cytoskeletal attachment	Intracellular adaptor proteins
Cell–Cell				
Adherens junction	Classical cadherins	Classical cadherin on neighboring cell	Actin filaments	α-Catenin, β-catenin, plakoglobin (γ-catenin), p120-catenin, vinculin
Desmosome	Nonclassical cadherins (desmoglein, desmocollin)	Desmoglein and desmocollin on neighboring cell	Intermediate filaments	Plakoglobin (γ-catenin), plakophilin, desmoplakin
Cell–Matrix				
Actin-linked cell–matrix junction	Integrin	Extracellular matrix proteins	Actin filaments	Talin, kindlin, vinculin, paxillin, focal adhesion kinase (FAK), numerous others
Hemidesmosome	$\alpha_6\beta_4$ Integrin, type XVII collagen	Extracellular matrix proteins	Intermediate filaments	Plectin, BP230

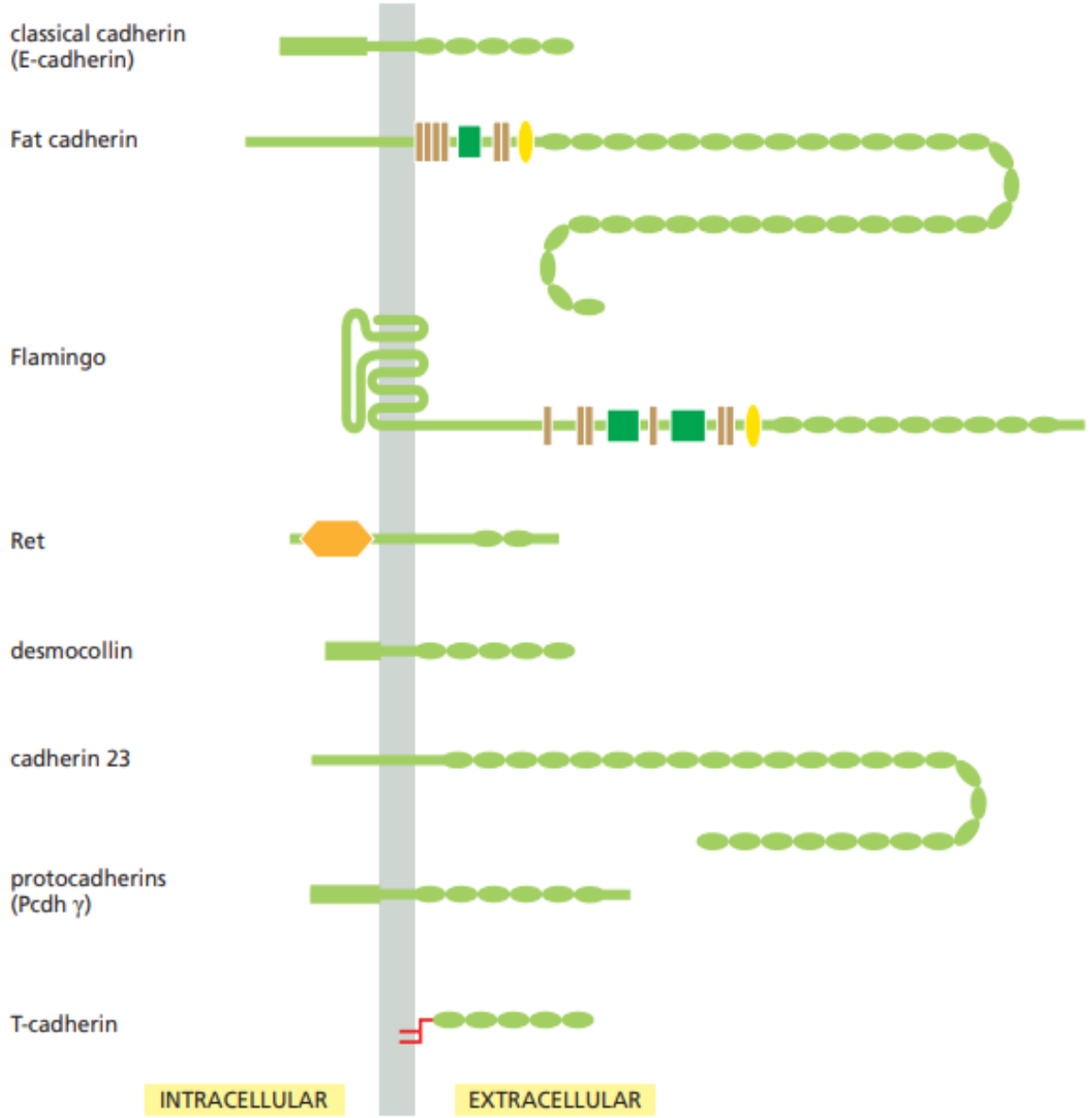
CELL - CELL JUNCTIONS

Cell - cell anchoring junctions - employ cadherins that link cytoskeleton of one cell to another

Its primary function is to resist the external forces that pull cells apart.

Cadherins Superfamilies

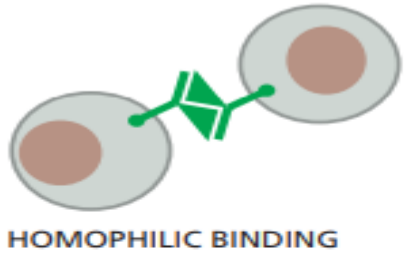
- The cadherins take their name from their dependence on Ca2+ ions: removing Ca2+ from the extracellular medium causes adhesions mediated by cadherins to come apart
- First three cadherins discovered earlier
- E-cadherin is present on many types of epithelial cells
- N-cadherin on nerve, muscle, and lens cells
- P-cadherin on cells in the placenta and epidermis
- These and other classical cadherins are closely related in sequence
- Non-classical cadherins are more distantly related in sequence, with more than 50 expressed in the brain

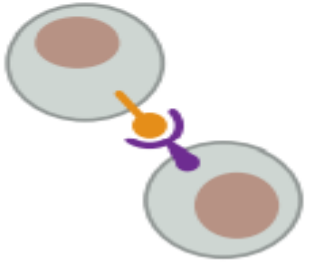


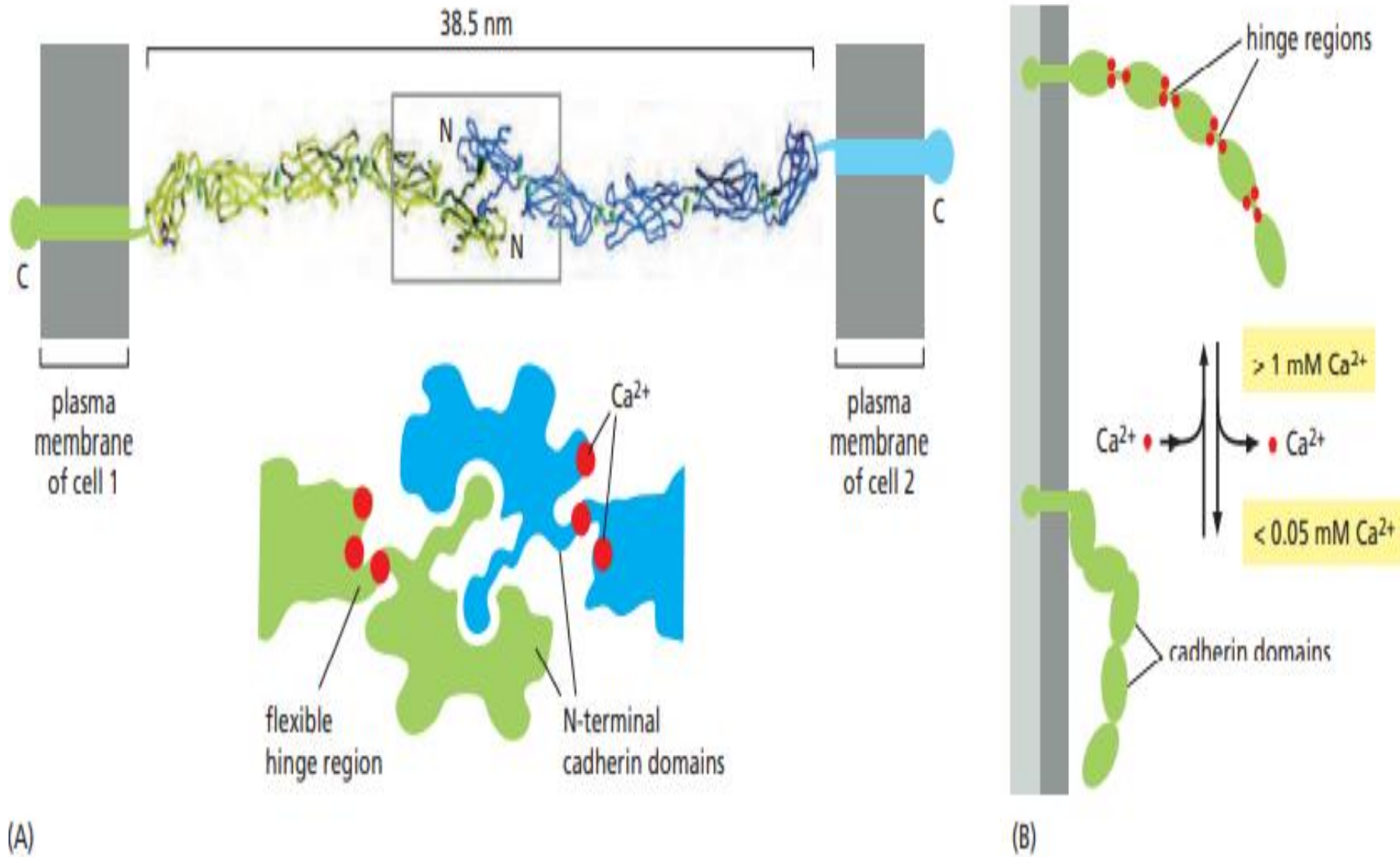
- These proteins all have extracellular portions containing multiple copies of the extracellular cadherin domain (green ovals)
- In the classical cadherins of vertebrates there are 5 of these domains, and in desmogleins and desmocollins there are 4 or 5, but some non-classical cadherins have more than 30
- The intracellular portions are more varied, reflecting interactions with a wide variety of intracellular ligands, including signaling molecules and adaptor proteins that connect the cadherin to the cytoskeleton
- In some cases, such as T-cadherin, a transmembrane domain is not present and the protein is attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor
- The differently colored motifs in Fat, Flamingo, and Ret represent conserved domains that are also found in other protein families

Cadherins Mediate Homophilic Adhesion

- the binding between cadherins is generally hemophilic
- It occurs at the N-terminal tips of the cadherin molecules—the cadherin domains that lie farthest from the membrane
- These terminal domains each form a knob and a nearby pocket, and the cadherin molecules protruding from opposite cell membranes bind by insertion of the knob of one domain into the other
- Cadherin domains joined to the next cadherin domain by a hinge (figure a)
- Ca2+ ions bind to sites near each hinge and prevent it from flexing, so that the whole string of cadherin domains behaves as a rigid and slightly curved rod
- When Ca2+ is removed, the hinges can flex, and the structure becomes floppy (figure b)



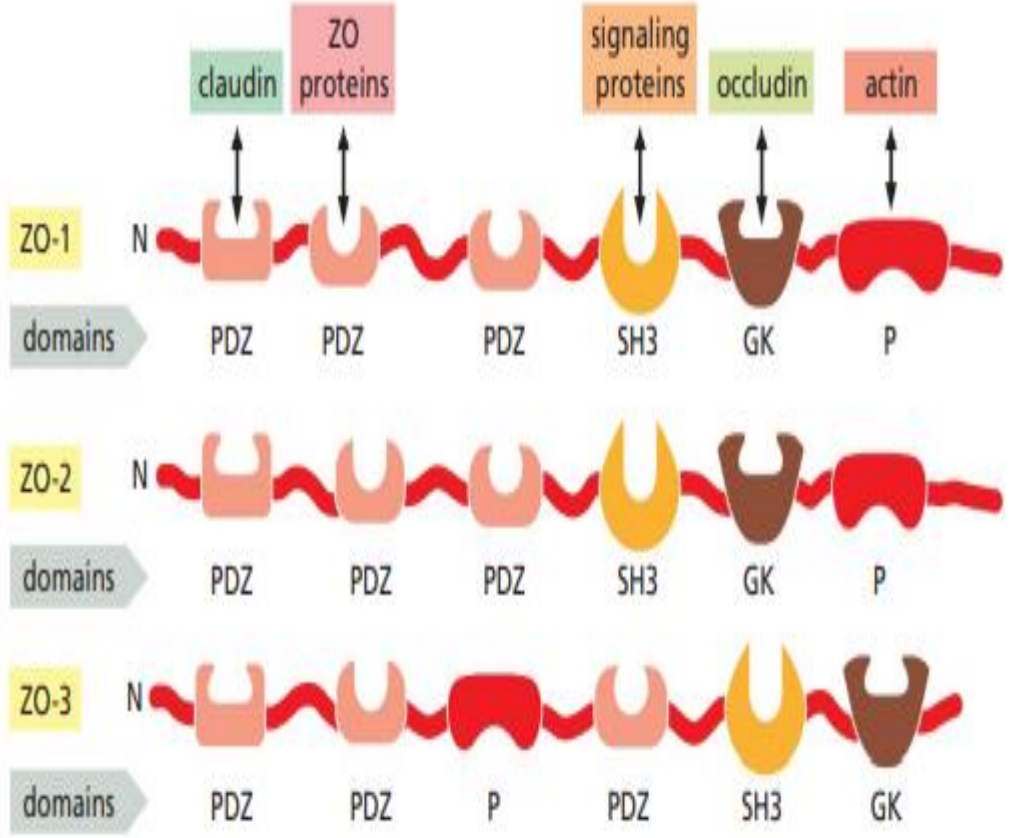



38.5 nm
N
N
C
C
plasma membrane of cell 1
plasma membrane of cell 2
Ca2+
flexible hinge region
N-terminal cadherin domains
(A)
hinge regions
> 1 mM Ca2+
Ca2+
Ca2+
< 0.05 mM Ca2+
cadherin domains
(B)

Scaffold Proteins Organize Junctional Protein Complexes

- In adherens junctions, the organization of adhesion proteins in a tight junction depends on additional proteins that bind the cytoplasmic side of the adhesion proteins
- key organizational proteins at tight junctions are the zonula occludens (ZO) protein
- Three major members of the ZO family—ZO-1, ZO-2, and ZO-3—are large scaffold proteins that provide a structural support on which the tight junction is built
- intracellular molecules consist of strings of protein-binding domains, typically including several PDZ domains—segments about 80 amino acids long that can recognize and bind the C-terminal tails of specific partner proteins
- One domain of these scaffold proteins can attach to a claudin protein, while others can attach to occludin or the actin cytoskeleton
- In this way, the cell can assemble a mat of intracellular proteins that organizes and positions the sealing strands of the tight junction

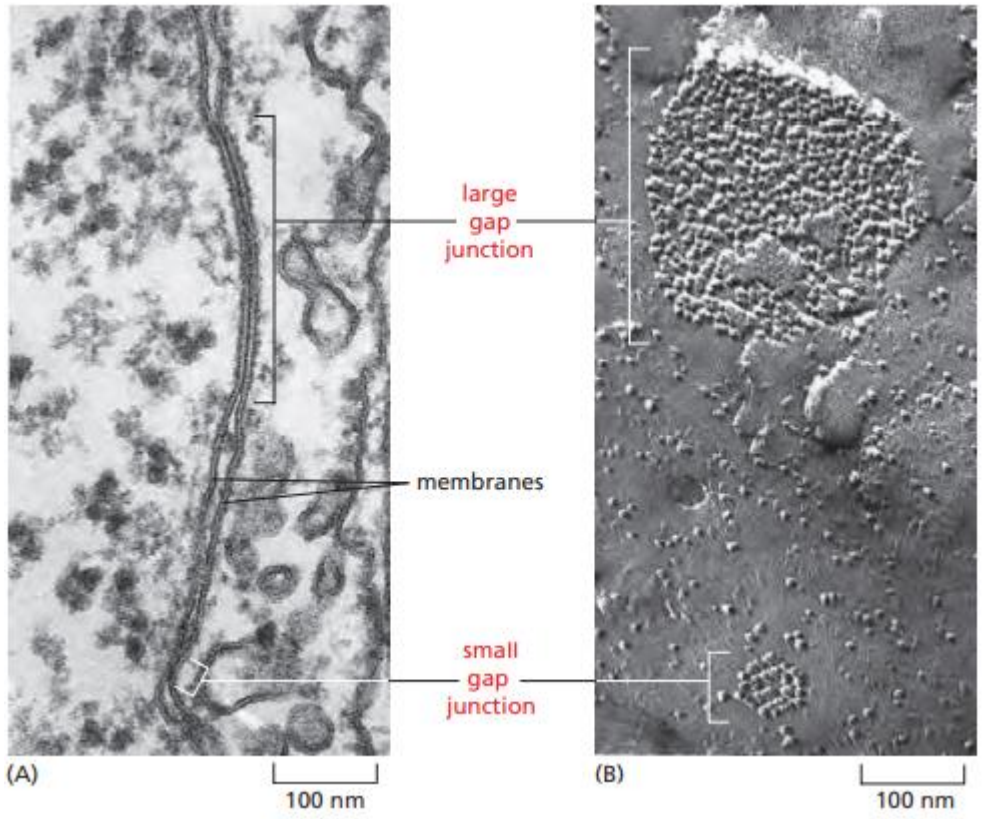
- The scaffold proteins ZO-1, ZO-2, and ZO-3 are concentrated beneath the plasma membrane at tight junctions.
- Each of the proteins contains multiple protein-binding domains, including three PDZ domains, an SH3 domain, and a GK domain, linked together like beads on a flexible string
- These domains enable the proteins to interact with each other and with numerous other partners, as indicated here, to generate a tightly woven protein network that organizes the sealing strands of the tight junction and links them to the actin cytoskeleton
- Scaffold proteins with similar structure help organize other *junctional complexes*, including those at neural synapses



Gap Junctions Couple Cells Both Electrically and Metabolically

junctional structure has a radically different function:

- it bridges gaps between adjacent cells so as to create direct channels from the cytoplasm of one to that of the other ,**Gap junctions**
- They are present in most animal tissues, including connective tissues as well as epithelia and heart muscle
- Each gap junction appears in conventional electron micrographs as a patch where the membranes of two adjacent cells are separated by a uniform narrow gap of about 2–4 nm



- Gap junctions also occur in many tissues whose cells are not electrically excitable
- The sharing of small metabolites and ions provides a mechanism for coordinating the activities of individual cells in such tissues and for smoothing out random fluctuations in small-molecule concentrations in different cells
- Figure: When fluorescent molecules of various sizes are

injected into one of two cells coupled by gap junctions,

molecules with a molecular weight (MW) of less than

about 1000 daltons can pass into the other cell,

but larger molecules cannot

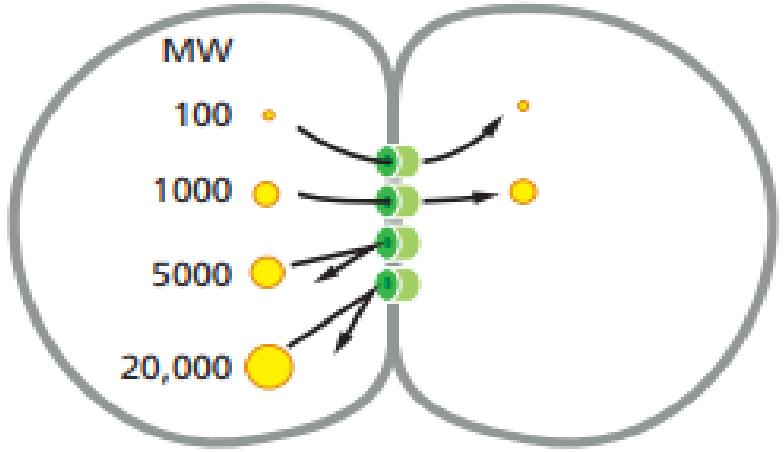


Figure: Determining the size of a gap-junction channel

GAP JUNCTIONS

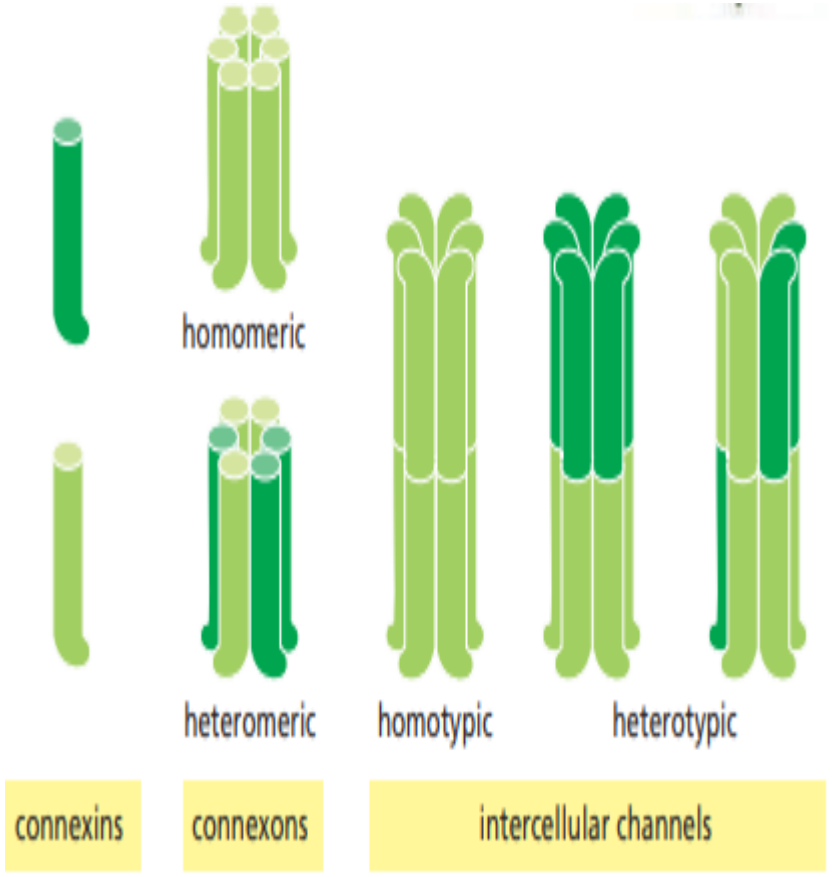
The gap is spanned by channel-forming proteins, of which there are two distinct families

1. connexins
2. Innexins

- Connexins are the predominant gap-junction proteins in vertebrates, with 21 isoforms in humans
- Innexins are found in the gap junctions of invertebrates
- Gap junctions have a pore size of about 1.4 nm, which allows the exchange of inorganic ions and other small water-soluble molecules, but not of macromolecules such as proteins or nucleic acids
- An electric current injected into one cell through a microelectrode causes an electrical disturbance in the neighboring cell, due to the flow of ions carrying electric charge through gap junctions
- electrical coupling through gap junctions synchronizes the contractions of heart muscle cells as well as those of the smooth muscle cells responsible for the peristaltic movements of the intestine

A Gap-Junction Connexon Is Made of Six Transmembrane Connexin Subunits

- Connexins are four-pass transmembrane proteins, six of which assemble to form a hemichannel, or connexon
- When the connexons in the plasma membranes of two cells in contact are aligned, they form a continuous aqueous channel that connects the two cell interiors
- A gap junction consists of many such connexon pairs in parallel, forming a sort of molecular sieve
- Not only does this sieve provide a communication channel between cells, but it also provides a form of cell–cell adhesion that supplements the cadherin- and claudin-mediated adhesions



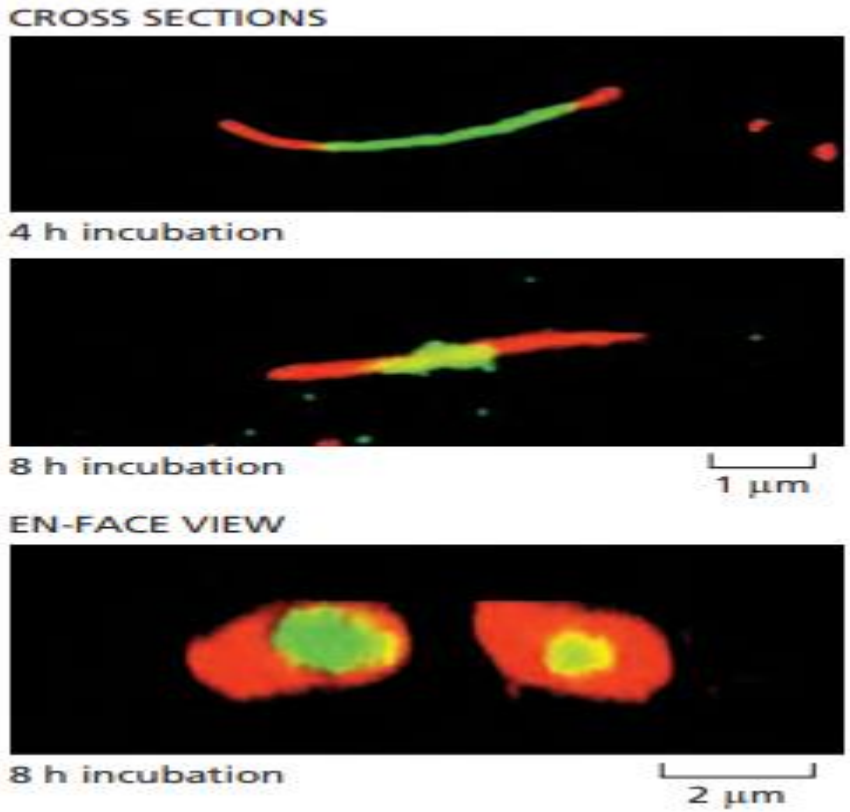
- Most cell types express more than one type of connexin, and two different connexin proteins can assemble into a heteromeric connexon, with its own distinct properties
- individual gap junction channels do not remain open all the time; instead, they flip between open and closed states
- These changes are triggered by a variety of stimuli including

1. the voltage difference between the two connected cells
2. the membrane potential of each cell
3. various chemical properties of the cytoplasm
4. including the pH and concentration of free Ca2+
5. Some subtypes of gap junctions can also be regulated by extracellular signals such as neurotransmitters

- Each gap-junctional plaque is a dynamic structure that can readily assemble, disassemble, or be remodeled, and it can contain a cluster of a few to many thousands of connexons
- Studies with fluorescently labeled connexins in living cells show that new connexons are continually added around the periphery of an existing junctional plaque, while old connexons are removed from the middle of it and destroyed
- This turnover is rapid: the connexin molecules have a half-life of only a few hours

Figure:

- coding for a connexin with a short amino acid tag containing four cysteines in the sequence Cys-Cys-X-XCys-Cys (where X denotes an arbitrary amino acid)
- The fluorescence images show gap junctions between pairs of cells treated in this way
- The central part of the gap-junction plaque is green, indicating that it consists of old connexin molecules
- the periphery is red, indicating that it consists of connexins synthesized during the previous 4 or 8 hours



In Plants, Plasmodesmata Perform Many of the Same Functions as Gap Junctions

- tissues of a plant are organized on different principles from those of an animal
- because plant cells are imprisoned within tough cell walls
- composed of an extracellular matrix rich in cellulose and other polysaccharides
- cell walls of adjacent cells are firmly cemented to those of their neighbors, which eliminates the need for anchoring junctions to hold the cells in place
- But a need for direct cell–cell communication remains
- In Plants,only one class of intercellular junctions present, plasmodesmata
- the cell wall between a typical pair of adjacent cells is at least 0.1 μm thick, and so a structure very different from a gap junction is required to mediate communication across it,Plasmodesmata helps to solve the problem

- plasma membrane of one cell is continuous with that of its neighbor at each plasmodesma, which connects the cytoplasms of the two cells by a roughly cylindrical channel with a diameter of 20–40 nm
- Running through the center of the channel in most plasmodesmata is a narrower cylindrical structure, the desmotubule, which is continuous with elements of the smooth endoplasmic reticulum (ER) in each of the connected cells
- Between the outside of the desmotubule and the inner face of the cylindrical channel formed by plasma membrane is an annulus of cytosol through which small molecules can pass from cell to cell
- passage of molecules with a molecular weight < 800

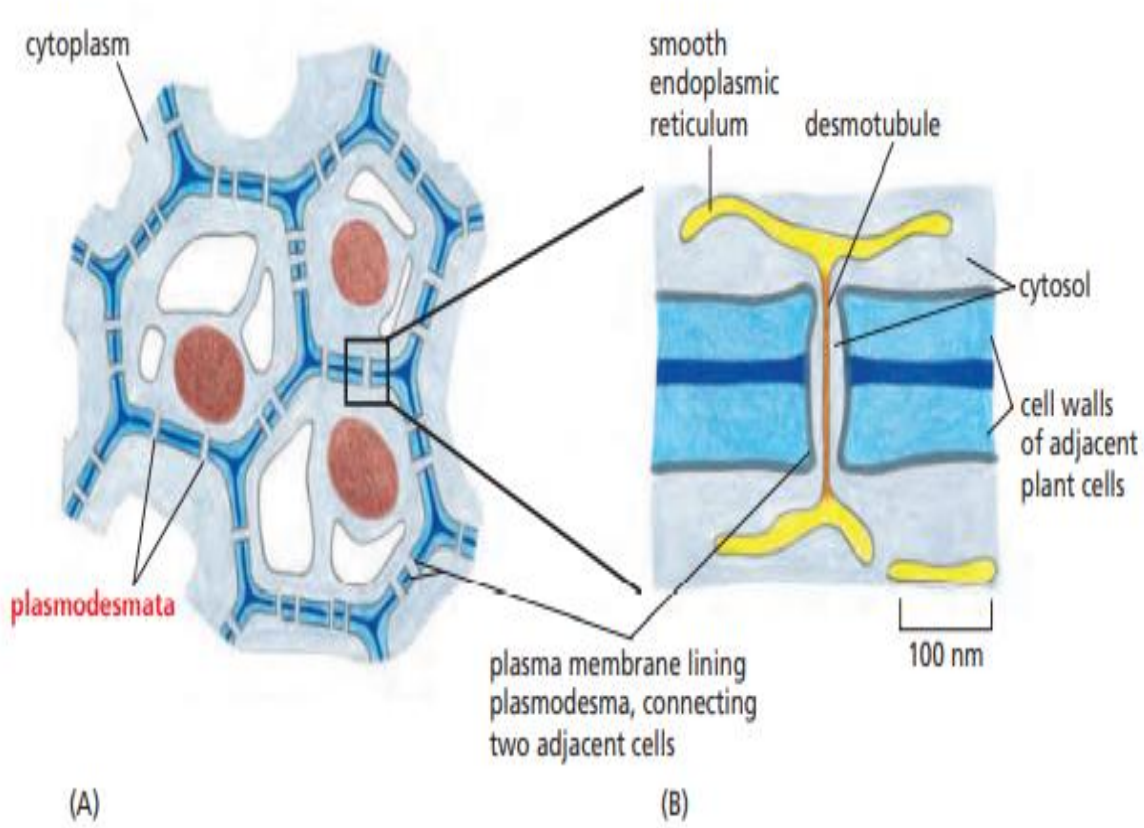


Figure: The cytoplasmic channels of plasmodesmata pierce the plant cell wall and connect cells in a plant together

(B) Each plasmodesma lines the two connected cells

Selectins Mediate Transient Cell–Cell Adhesions in the Bloodstream

- Cell cell adhesion protein families:
- integrins, selectins,and the adhesive immunoglobulin (Ig) superfamily members
- Selectins, like cadherins and integrins, require Ca2+ for their adhesive function; Ig superfamily members do not
- They are cell-surface carbohydrate-binding proteins (lectins) that mediate a variety of transient cell–cell adhesion interactions in the bloodstream
- main role, in vertebrates at least, is in governing the traffic of white blood cells into normal lymphoid organs and any inflamed tissues
- selectins control the binding of white blood cells to the endothelial cells lining blood vessels, thereby enabling the blood cells to migrate out of the bloodstream into a tissue

- They are transmembrane protein with a conserved lectin domain that binds to a specific oligosaccharide on another cell
- three types:
- L-selectin on white blood cells
- P-selectin on blood platelets and on endothelial cells that have been locally activated by an inflammatory response
- E-selectin on activated endothelial cells
- In Lymph node or the spleen, the endothelial cells express oligosaccharides that are recognized by L-selectin on lymphocytes, causing the lymphocytes to loiter and become trapped
- At sites of inflammation, the roles are reversed: the endothelial cells switch on expression of selectins that recognize the oligosaccharides on white blood cells and platelets, flagging the cells down to help deal with the local emergency

- adhesions mediated by both selectins and integrins are heterophilic
- selectins bind to specific oligosaccharides on glycoproteins and glycolipids, while integrins bind to specific Ig-family proteins
- The selectins mediate a weak adhesion because the binding of the lectin domain of the selectin to its carbohydrate ligand is of low affinity
- This allows the white blood cell to adhere weakly and reversibly to the endothelium, rolling along the surface of the blood vessel, propelled by the flow of blood
- The rolling continues until the blood cell activates its integrins
- these transmembrane molecules can be switched into an adhesive conformation that enables them to latch onto specific macromolecules external to the cell—in the present case, proteins on the surfaces of the endothelial cells
- Once it has attached in this way, the white blood cell escapes from the bloodstream into the tissue by crawling out of the blood vessel between adjacent endothelial cells.

The structure and function of selectins

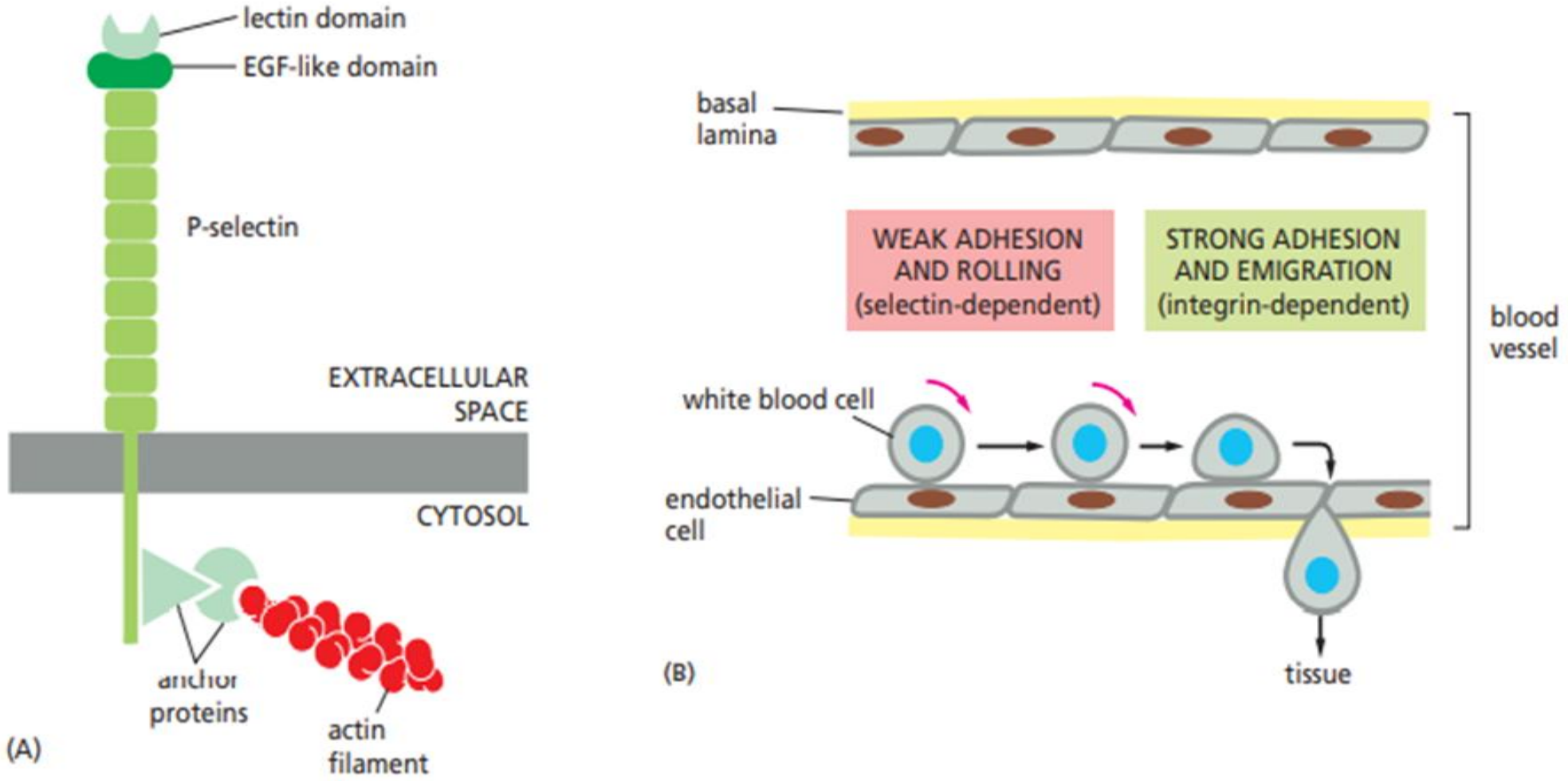


Figure: (A) The structure of P-selectin. The selectin attaches to the actin cytoskeleton through adaptor proteins that are still poorly characterized.

- (B) How selectins and integrins mediate the cell–cell adhesions required for a white blood cell to migrate out of the bloodstream into a tissue
- First, selectins on endothelial cells bind to oligosaccharides on the white blood cell, so that it becomes loosely attached and rolls along the vessel wall
- Then the white blood cell activates a cell surface integrin called LFA1, which binds to a protein called ICAM1 (belonging to the Ig superfamily) on the membrane of the endothelial cell
- The white blood cell adheres to the vessel wall and then crawls out of the vessel by a process that requires another immunoglobulin superfamily member called PECAM1 (or CD31). EGF, epidermal growth factor

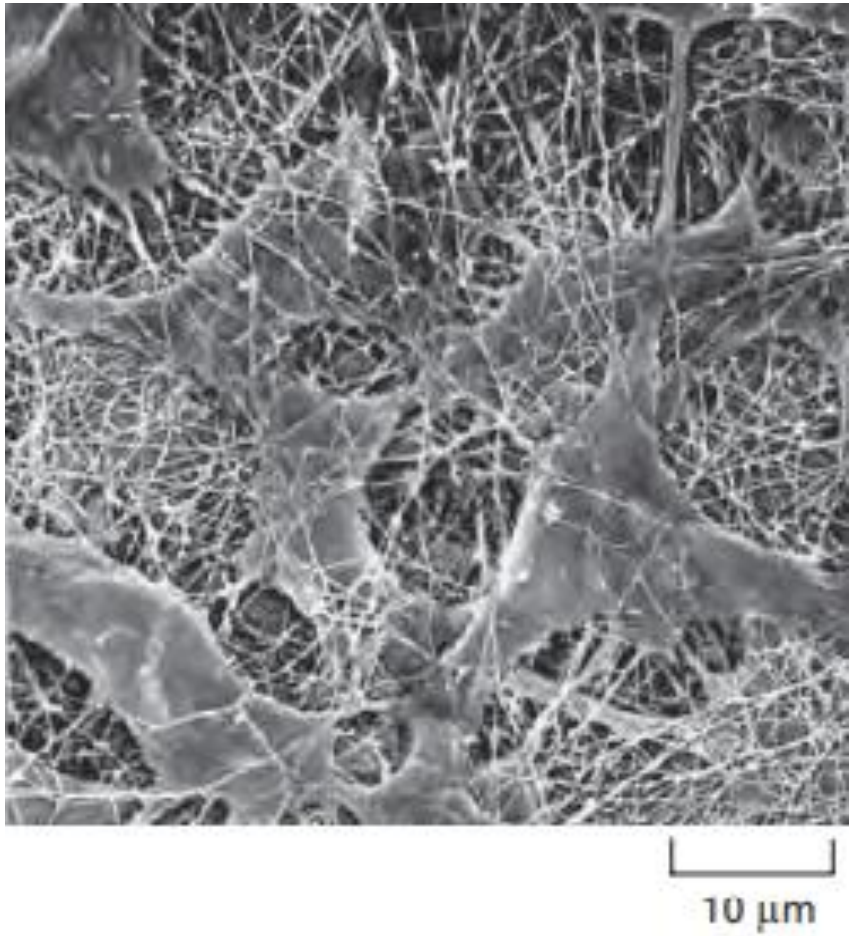
Extracellular Matrix of Animals

- extracellular matrix: Tissues are not only made up of cells, but it has complex and intricate network of macromolecules
- Matrix can become to either calcified form of rock hard structure (bone,teeth), or can form transparent subtances (cornea), or can adopt rope like structures (tendons)
- It is more than a passive scaffold to provide physical support
- It has an active and complex role in regulating the behavior of the cells and influencing their survival, development, migration, proliferation, shape, and function
- basal lamina, the thin layer of specialized extracellular matrix that lies beneath all epithelial cells

The Extracellular Matrix Is Made and Oriented by the Cells Within It

- the orientation of the cytoskeleton inside the cell can control the orientation of the matrix produced outside
- most connective tissues, the matrix macromolecules are secreted by cells called **fibroblasts**
- specialized types of connective tissues, such as chondroblasts, for example, form cartilage, and osteoblasts form bone
- The extracellular matrix is constructed from three major classes of macromolecules: (1) glycosaminoglycans (GAGs), which are large and highly charged polysaccharides that are usually covalently linked to protein in the form of proteoglycans

(2) fibrous proteins, which are primarily members of the collagen family



Fibroblasts in connective tissue

(3) a large class of non collagen glycoproteins, which carry conventional asparagine-linked oligosaccharides

- They are in different shapes and size
- Mammals will have 300 matrix proteins, including about 36 proteoglycans, 40 collagens, and 200 glycoproteins. They contain multiple sub domains self associate to form multimers
- matrix-associated proteins and enzymes that can modify matrix behavior by cross-linking, degradation, or other mechanisms
- Each tissue contains its own unique blend of matrix components, resulting in an extracellular matrix that is specialized for the needs of that tissue
- proteoglycan molecules in connective tissue typically form a highly hydrated, gel-like “ground substance” in which collagens and glycoproteins are embedded

- polysaccharide gel resists compressive forces on the matrix while permitting the rapid diffusion of nutrients, metabolites, and hormones between the blood and the tissue cells
- The collagen fibers strengthen and help organize the matrix, while other fibrous proteins, such as the rubberlike elastin, give it resilience
- many matrix glycoproteins help cells migrate, settle, and differentiate in the appropriate locations

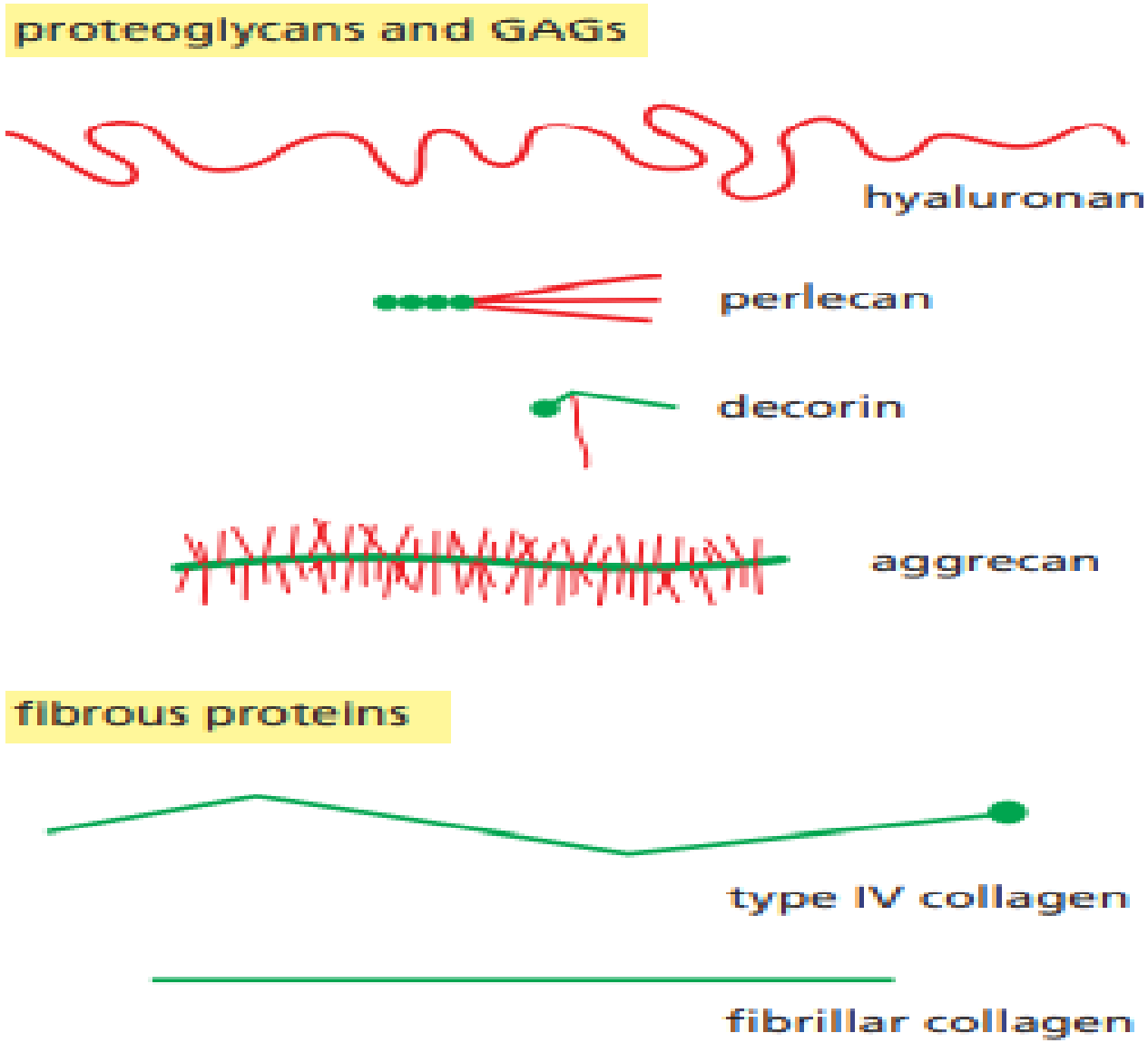
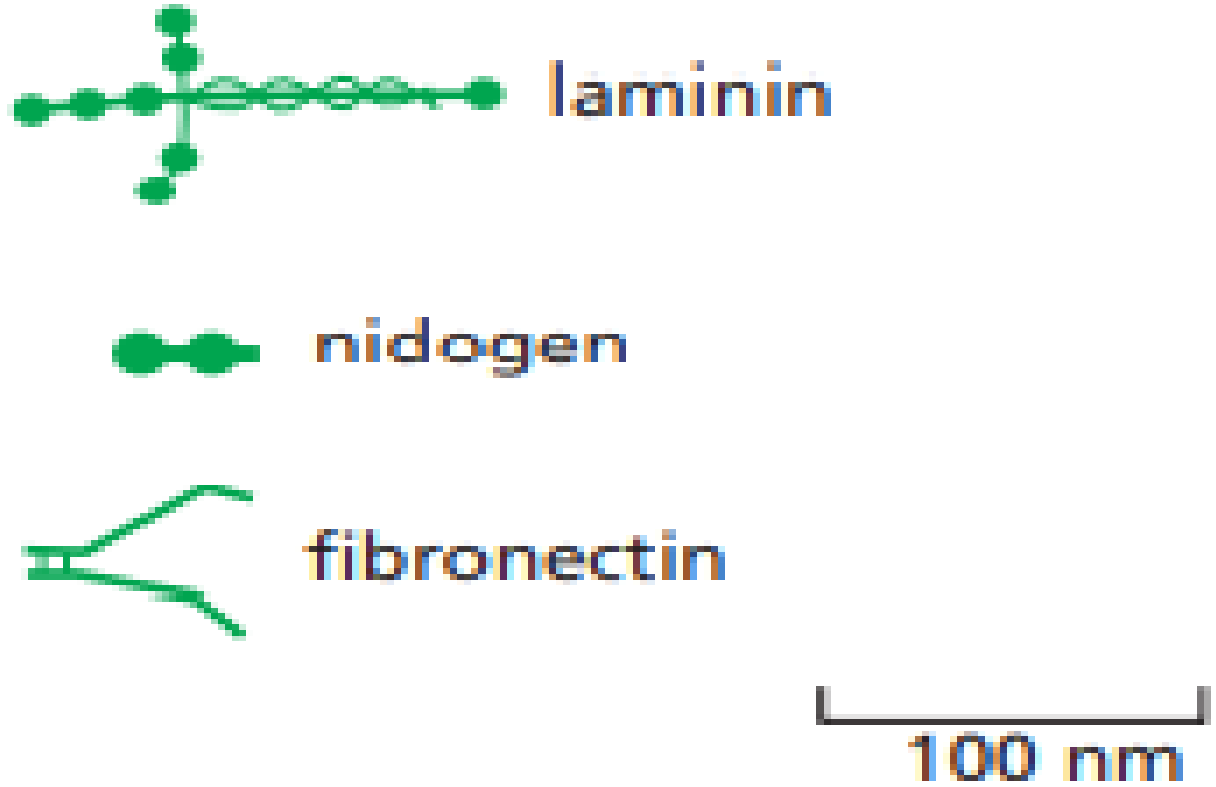


Fig:The comparative shapes and sizes of some of the major extracellular matrix macromolecules. Protein is shown in green, and glycosaminoglycan (GAG) in red

glycoproteins



Glycosaminoglycan (GAG) Chains Occupy Large Amounts of Space and Form Hydrated Gels

- **unbranched polysaccharide** chains composed of repeating disaccharide units (NAG-Uronic)
- Two sugars are N-acetylglucosamine or N-acetylgalactosamine –mostly sulphated, another sugar is uronic acid (glucuronic or iduronic)
- sulfate or carboxyl groups on most of their sugars, GAGs are highly negatively charged and therefore the most anionic molecules produced by animal cells
- Four main groups of GAGs are distinguished by their sugars, the type of linkage between the sugars, and the number and location of sulfate groups: (1) hyaluronan
- (2) chondroitin sulfate and dermatan sulfate
- (3) heparan sulfate
- (4) keratan sulfate

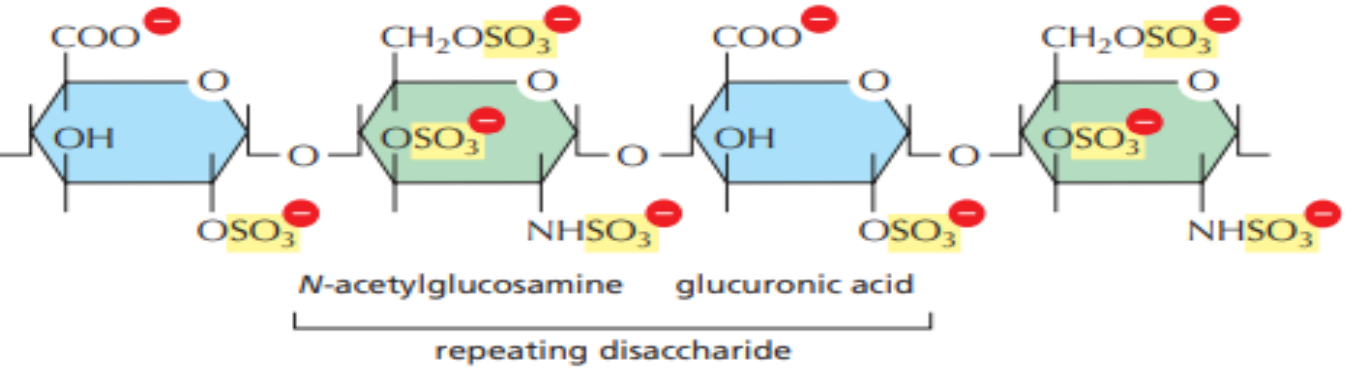


Fig : The repeating disaccharide sequence of a heparan sulfate glycosaminoglycan (GAG) chain

- Polysaccharide chains are too stiff to fold into compact globular structures, and strongly hydrophilic
- GAGs occupy a huge volume relative to their mass and they form hydrated gels even at very low concentrations
- Their high density of negative charges attracts a cloud of cations, especially Na+, that are osmotically active, causing large amounts of water to be sucked into the matrix
- This creates a swelling pressure that enables the matrix to withstand compressive forces (in contrast to collagen fibrils, which resist stretching forces)

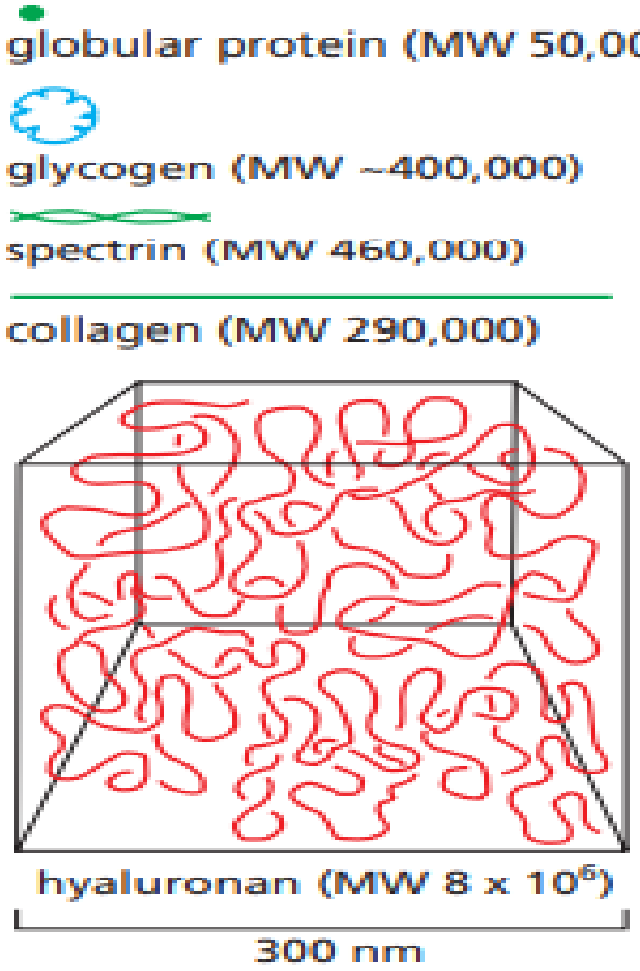


Fig. The relative dimensions and volumes occupied by various macromolecules

- The cartilage matrix that lines the knee joint, for example, can support pressures of hundreds of atmospheres in this way
- Defects in the production of GAGs can affect many different body systems
- for example, if there is a severe deficiency in the synthesis of dermatan sulfate disaccharide, the affected individuals have a short stature, a prematurely aged appearance, and generalized defects in their skin, joints, muscles, and bones

Hyaluronan Acts as a Space Filler During Tissue Morphogenesis and Repair

- simplest form of GAGs, no sulphonate group and have repeating sequence of up to 25,000 dissacharide units not linked covalently
- It has important role as a space filler during embryonic development,where it force a change in the shape of the structure
- Small quantity expands to occupy large volume
- Resisting compressive forces in tissues and joints
- Other GAGs synthesized inside the cell and released by exocytosis
- But Hyaluronan spun out directly from the cell surface by enzymes in the plasma membrane
- It will be synthesized from the basal side, can deform the epithelium by creating the free space beneath it, cells to migrate. After cell migration hyalurone will be degraded by hyaluronidase
- Eg. It forms valves and septa in heart. It will produced in large quantities during wound healing

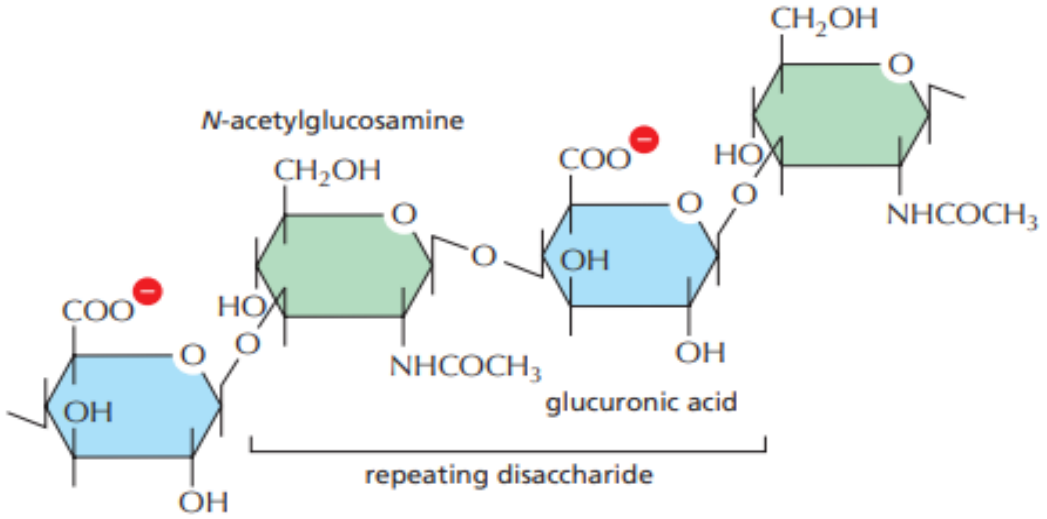


Fig. The repeating disaccharide sequence in hyaluronan, a relatively simple GAG

Proteoglycans Are Composed of GAG Chains Covalently Linked to a Core Protein

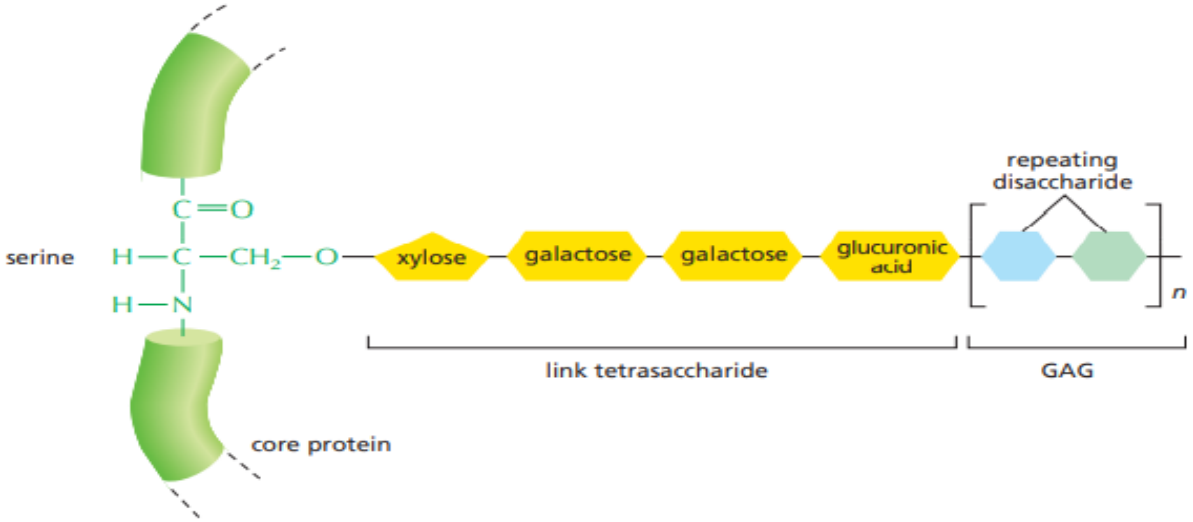


Fig. The linkage between a GAG chain and its core protein in a proteoglycan molecule.

- All GAGs are covalently attached to proteins as proteoglycans
- Ribosomes make the polypeptide chain or core protein of proteoglycan
- Then threaded into the lumen of the endoplasmic reticulum , polypeptide chain assembled in GA
- a special linkage tetrasaccharide is attached to a serine side chain on the core protein to serve as a primer for polysaccharide growth; then, one sugar at a time is added by specific glycosyl transfer
- many of the polymerized sugars are covalently modified by a sequential and coordinated series of reactions
- It can be ditinguished from other glycoproteins by the nature, quantity and arrangement of their sugar side chains, it must have atleast one GAG

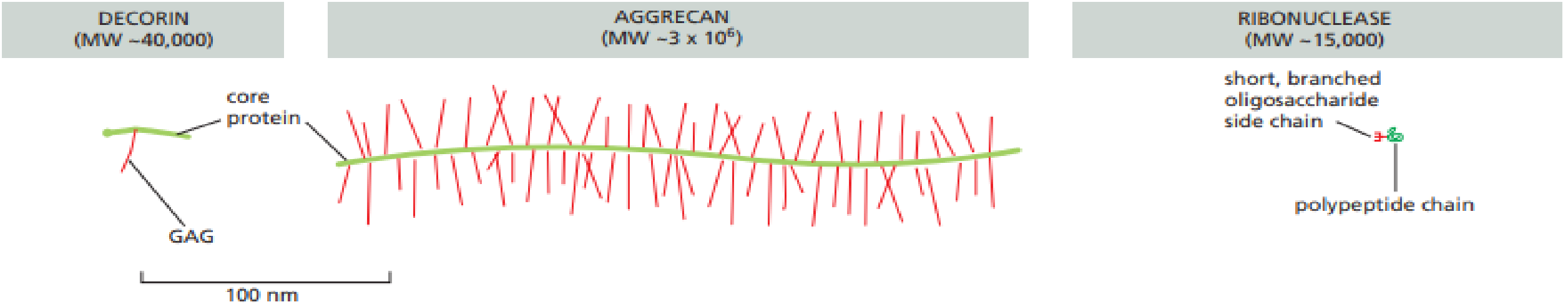
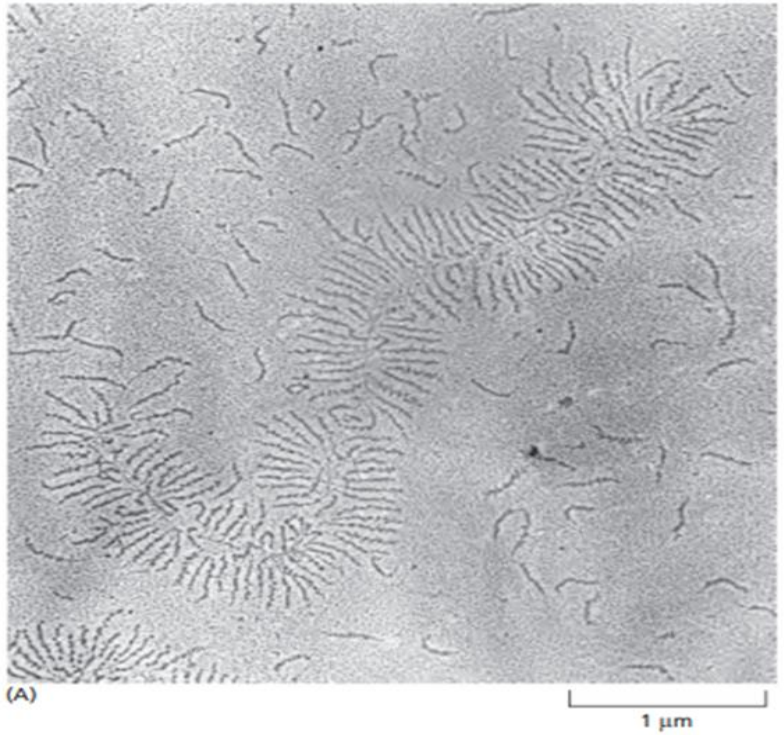


Fig. decorin and aggrecan

proteoglycan found in the extracellular matrix.

The figure compares these two proteoglycans with a typical secreted glycoprotein molecule, pancreatic ribonuclease B

- Aggrecan typically consists of about 100 chondroitin sulfate chains and about 30 keratan sulfate chains linked to a serine-rich core protein of almost 3000 amino acids
- Decorin “decorates” the surface of collagen fibrils



A) An electron micrograph of an aggrecan aggregate shadowed with platinum

- Many free aggrecan molecules are also visible.

(B) A drawing of the giant aggrecan aggregate shown in (A).

It consists of about 100 aggrecan monomers noncovalently

bound through the N-terminal domain of the core protein to a single hyaluronan Chain A link protein binds both to the core protein of the proteoglycan and to the hyaluronan chain, thereby stabilizing the aggregate

- The link proteins are members of a family of hyaluronan-binding proteins, some of which are cell-surface proteins
- The molecular mass of such a complex can be 108 daltons or more, and it occupies a volume equivalent to that of a bacterium, which is about 2 × 10–12 cm3

- Proteoglycans have the potential, limitless heterogeneity
- Many core protein share characteristic domain such as LINK domain , involved in binding to GAGs
- Aggrecan –huge proteoglycan
- It is a major component of cartilage, 3 × 106 daltons with over 100 GAG chains
- Decorin –small which have only 1-10 GAG chains
- Secreted by fibroblast and has single GAG
- Decorin binds to collagen fibrils and regulates fibril assembly and fibril diameter; mice that cannot make decorin have fragile skin that has reduced tensile strength
- GAGs and proteoglycans of these various types can associate to form even larger polymeric complexes in the extracellular matrix
- Molecules of aggrecan, for example, assemble with hyaluronan in cartilage matrix to form aggregates that are as big as a bacterium

- Besides associating with one another, GAGs and proteoglycans associate with fibrous matrix proteins such as collagen and with protein meshworks such as the basal lamina, creating extremely complex composites

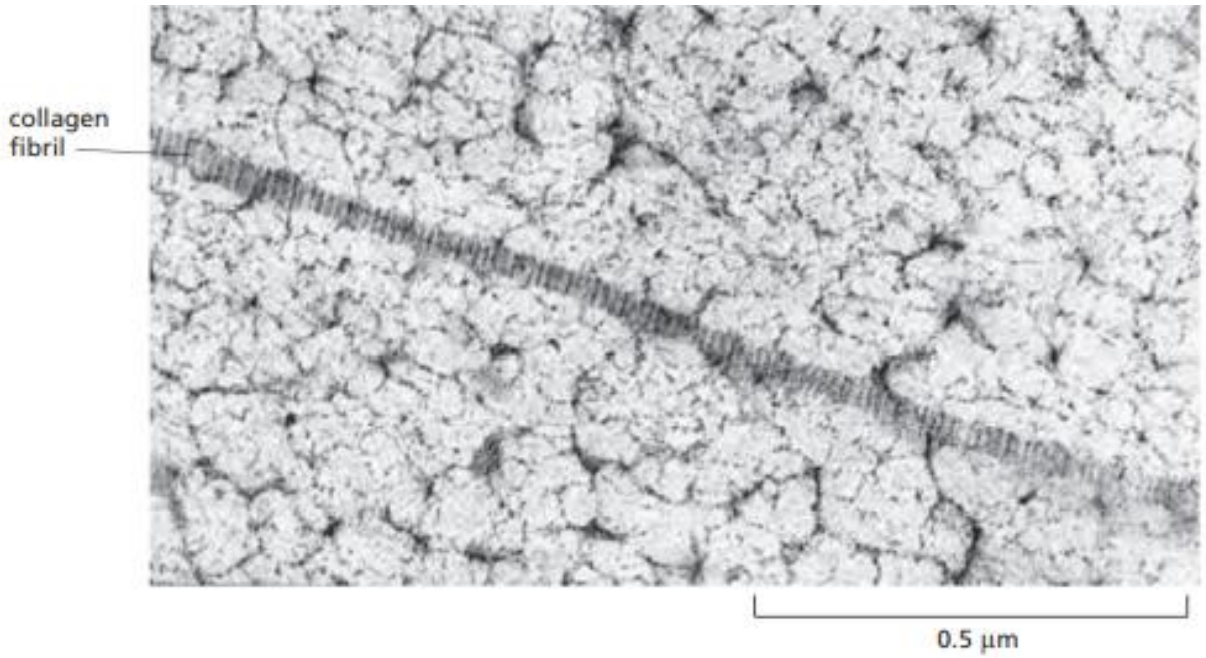


Fig. Proteoglycans in the extracellular matrix of rat cartilage

- Tissues was fixed using freeze substitution method
- the proteoglycan molecules are seen to form a fine filamentous network in which a single striated collagen fibril is embedded

- All proteoglycans are not secreted components of EM
- Their core protein either inserted across the lipid bilayer or attached to the lipid bilayer by a glycosylphosphatidylinositol (GPI) anchor
- Among the best-characterized plasma membrane proteoglycans are the syndecans, which have a membrane-spanning core protein whose intracellular domain is thought to interact with the actin cytoskeleton and with signaling molecules in the cell cortex

- syndecans can be found in cell–matrix adhesions where they modulate integrin function by interacting with fibronectin on the cell surface and with cytoskeletal and signaling proteins inside the cell
- syndecan and other proteoglycans also interact with soluble peptide growth factors, influencing their effects on cell growth and proliferation

Collagens Are the Major Proteins of the Extracellular Matrix

- The collagens are a family of fibrous proteins found in all multicellular animals.
- secreted in large quantities by connective-tissue cells, major component of skin and bone
- 25% of the total protein mass
- Primary features is long, stiff, triple stranded helix structure, in which three polypeptide chain are called as α chains
- Rich in Proline , Glycine which is important in helix formation

- 42 distinct genes codes for different collagen alpha chain
- Various combination using 42 α chains are formed - only a limited number of triple helical combinations are possible
- 40 types of collagens were found to exhibit various combinations based on the need
- Type I –most common , principal collegen of skin and bone ,belongs to fibrillar collagens or fibril-forming collagen
- After secreted into EM , they will assemble into higher order polymers called collagen fibrils- thin structure (10-300nm)
- They will aggregate into large cable lie structre which can be visible in light microscope as collagen fibres
- Collagen types IX and XII are called fibril-associated collagens, as they decorates the surface of the collegen fibrils

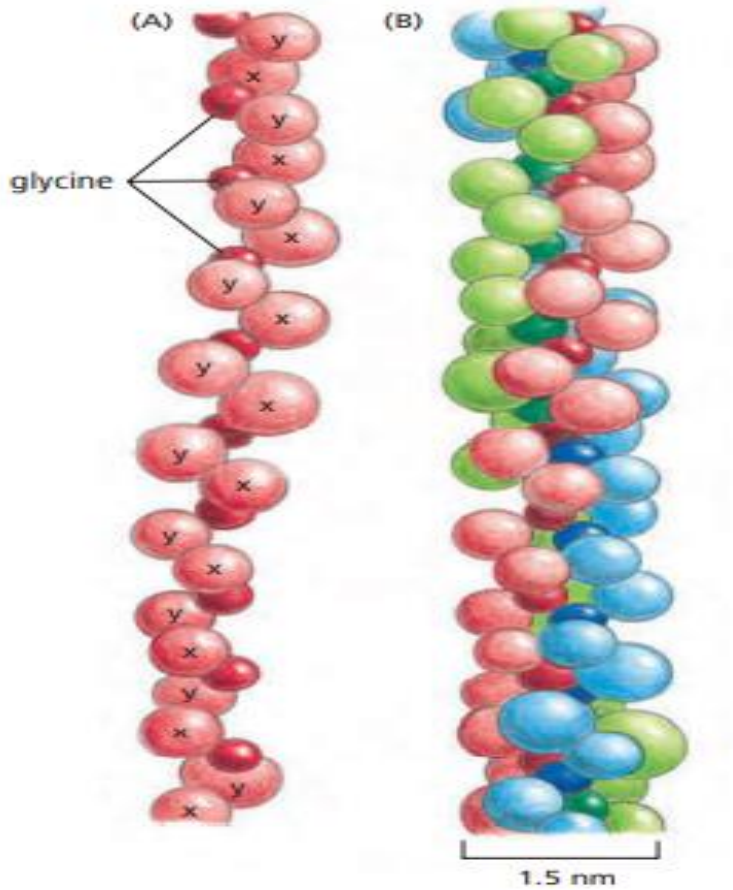


Fig. The structure of a typical collagen molecule

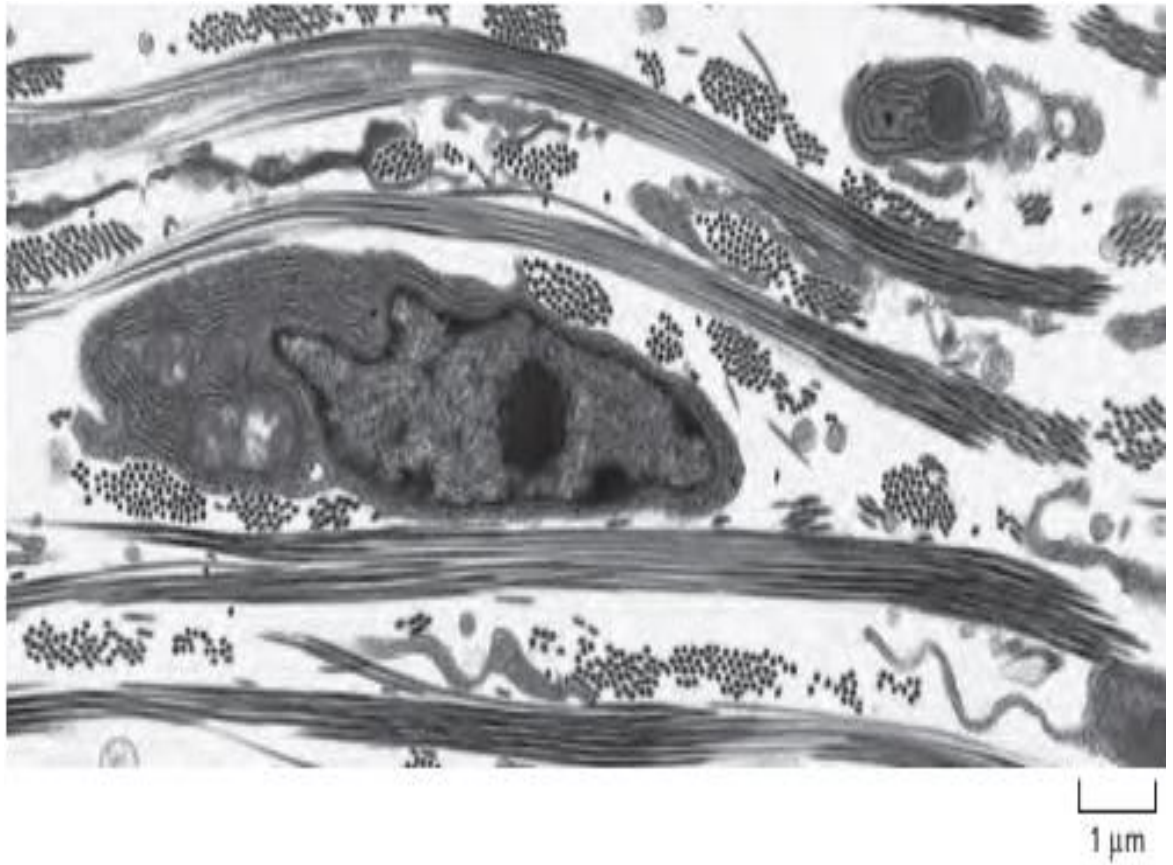


Fig. A fibroblast surrounded by collagen fibrils in the connective tissue of embryonic chick skin.

- They are thought to link these fibrils to one another and to other components in the extracellular matrix
- Type IV is a network-forming collagen, forming a major part of basal laminae
- type VII molecules form dimers that assemble into specialized structures called anchoring fibrils
- Anchoring fibrils help attach the basal lamina of multilayered epithelia to the underlying connective tissue and therefore are especially abundant in the skin
- There are also a number of “collagen-like” proteins containing short collagen-like segments. These include collagen type XVII, which has a transmembrane domain and is found in hemidesmosomes, and type XVIII, the core protein of a proteoglycan in basal laminae
- exons are 54, or multiples of 54, nucleotides long, suggesting that these collagens originated through multiple duplications of a primordial gene containing 54 nucleotides and encoding exactly six Gly-X-Y repeats

TABLE 19–2 Some Types of Collagen and Their Properties

	Type	Polymerized form	Tissue distribution	Mutant phenotype
Fibril-forming (fibrillar)	I	Fibril	Bone, skin, tendons, ligaments, cornea, internal organs (accounts for 90% of body collagen)	Severe bone defects, fractures (*osteogenesis imperfecta*)
	II	Fibril	Cartilage, intervertebral disc, notochord, vitreous humor of the eye	Cartilage deficiency, dwarfism (*chondrodysplasia*)
	III	Fibril	Skin, blood vessels, internal organs	Fragile skin, loose joints, blood vessels prone to rupture (*Ehlers–Danlos syndrome*)
	V	Fibril (with type I)	As for type I	Fragile skin, loose joints, blood vessels prone to rupture
	XI	Fibril (with type II)	As for type II	Myopia, blindness
Fibril-associated	IX	Lateral association with type II fibrils	Cartilage	Osteoarthritis
Network-forming	IV	Sheetlike network	Basal lamina	Kidney disease (glomerulonephritis), deafness
	VII	Anchoring fibrils	Beneath stratified squamous epithelia	Skin blistering
Transmembrane	XVII	Nonfibrillar	Hemidesmosomes	Skin blistering
Proteoglycan core protein	XVIII	Nonfibrillar	Basal lamina	Myopia, detached retina, hydrocephalus

Note that types I, IV, V, IX, and XI are each composed of two or three types of α chains (distinct, nonoverlapping sets in each case), whereas types II, III, VII, XVII, and XVIII are composed of only one type of α chain each.

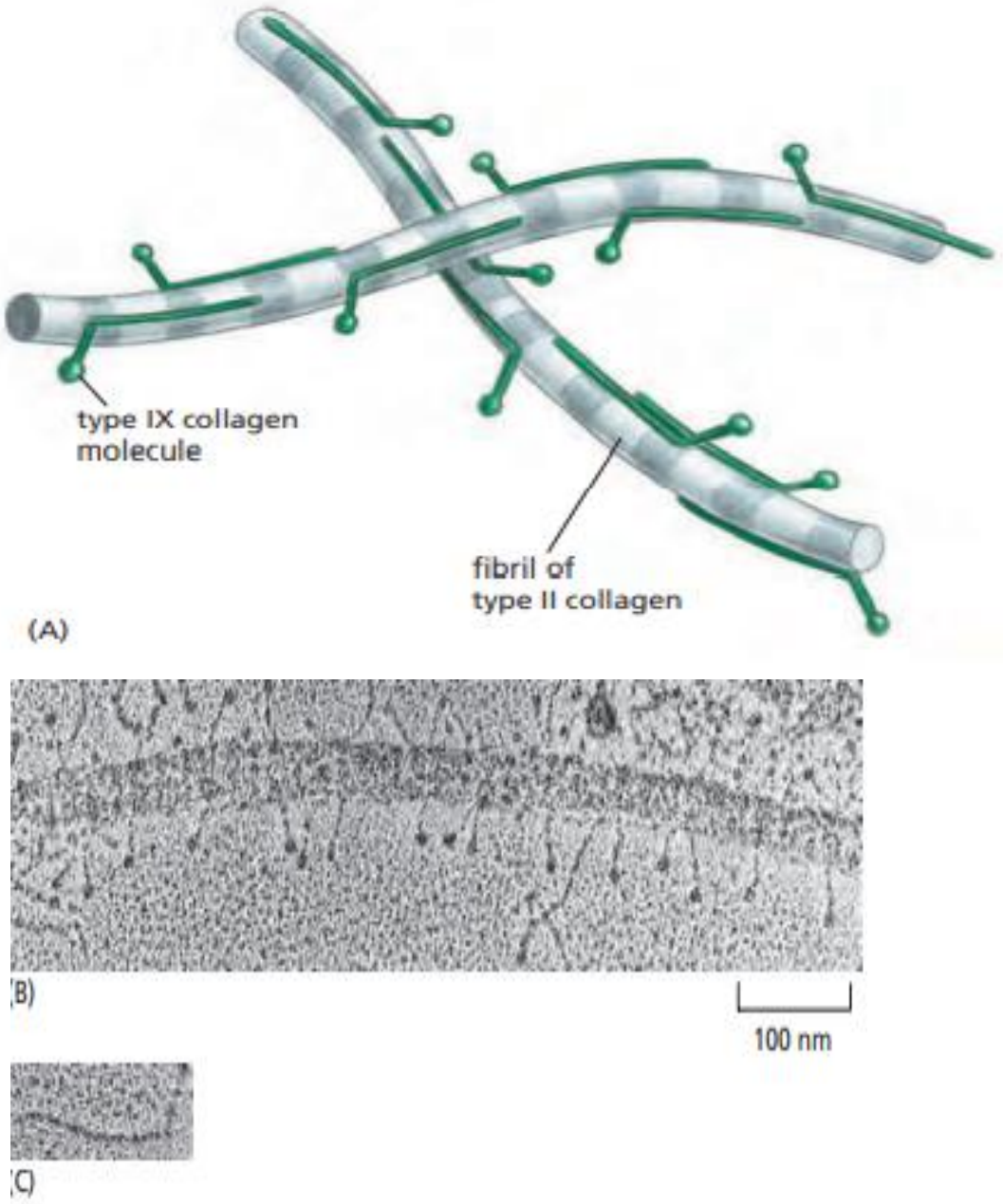


Figure. Type IX collagen.

(A) Type IX collagen molecules binding

in a periodic pattern to the surface of a fibril containing type II collagen

(B) Electron micrograph of a rotary-shadowed type-II-collagen-containing

fibril in cartilage, decorated by type IX collagen molecules

(C) An individual type IX collagen molecule.

Cells Help Organize the Collagen Fibrils They Secrete by Exerting Tension on the Matrix

- If two small pieces of embryonic tissue containing fibroblasts are placed far apart on a collagen gel, the intervening collagen becomes organized into a compact band of aligned fibers that connect the two explants
- The fibroblasts subsequently migrate out from the explants along the aligned collagen fibers. Thus, the fibroblasts influence the alignment of the collagen fibers, and the collagen fibers in turn affect the distribution of the fibroblasts

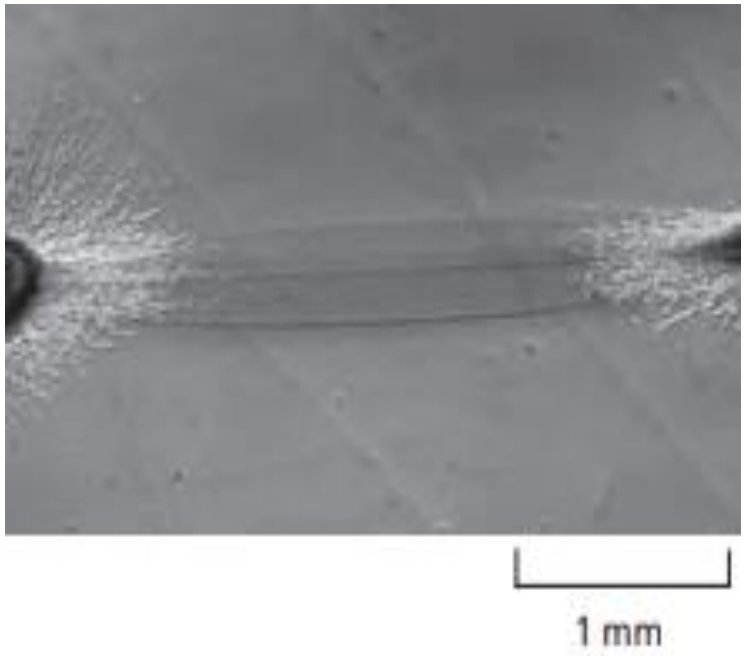


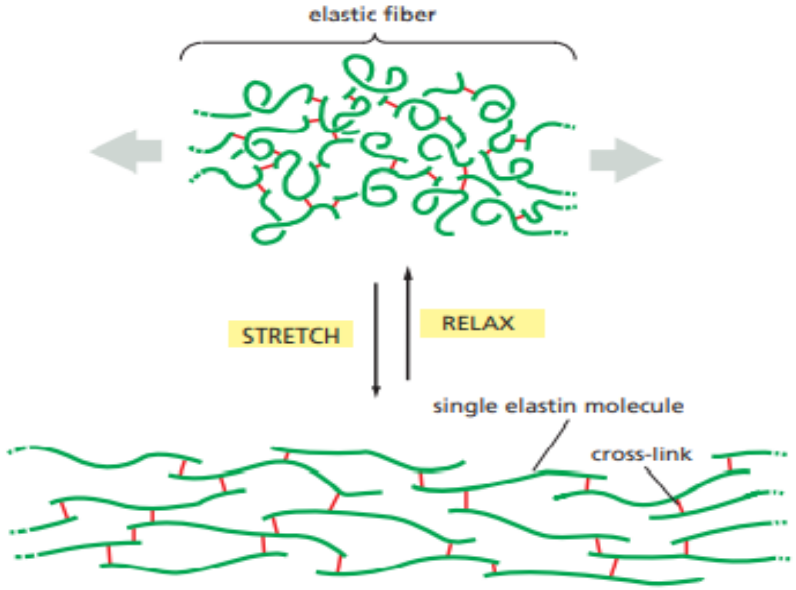
Fig. The shaping of the extracellular matrix by cells

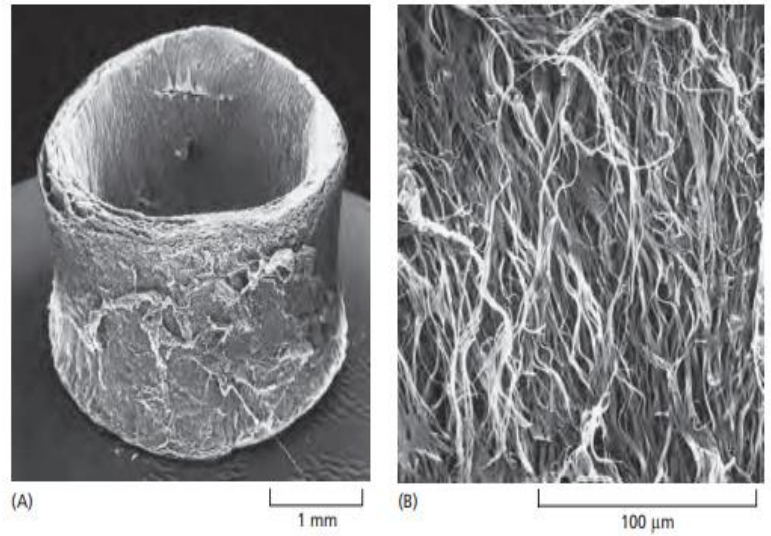
- Fibroblasts may have a similar role in organizing the extracellular matrix inside the body
- First they synthesize the collagen fibrils and deposit them in the correct orientation
- Then they work on the matrix they have secreted, crawling over it and tugging on it so as to create tendons and ligaments and the tough, dense layers of connective tissue that surround and bind together most organs
- All the other components have been digested away with enzymes and formic acid

Elastin Gives Tissues Their Elasticity

- In mammals, skin, blood vessels, and lungs, need to be both strong and elastic in order to function
- A network of elastic fibers in the extracellular matrix of these tissues gives them the resilience to recoil after transient stretch
- inelastic collagen fibrils are interwoven with the elastic fibers to limit the extent of stretching and prevent the tissue from tearing
- Major component is elastin, a highly hydrophobic protein about 750 amino acids long
- Soluble tropoelastin (the biosynthetic precursor of elastin) is secreted into the extracellular space and assembled into elastic fibers close to the plasma membrane
- After secretion, they will form highly cross linked to form network of elastin fibres and sheets
- Elastin protein have two short segment-hydrophobic segments, which are responsible for the elastic properties of the molecule

- Other one is alanine- and lysine-rich α-helical segments, which are cross-linked to adjacent molecules by covalent attachment of lysine residues
- Each segment is encoded by a separate exon
- Fig 1. parts of the elastin polypeptide chain, like the polymer chains in ordinary rubber, adopt a loose “random coil” conformation
- it is the random coil nature of the component molecules cross-linked into the elastic fiber network that allows the network to stretch and recoil like a rubber band
- Fig. 2 Elastin is the dominant extracellular matrix protein in arteries, comprising 50% of the dry weight of the largest artery—the aorta





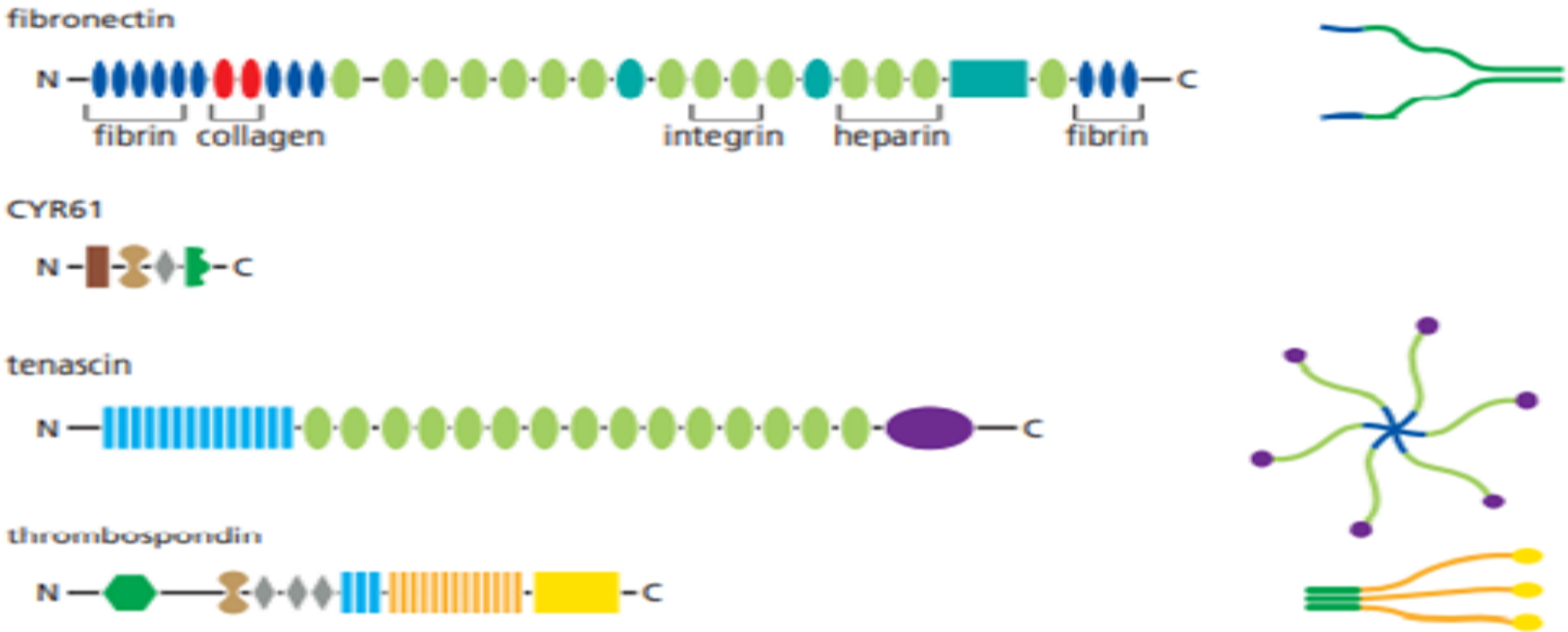
SEM image

- The elastin core is covered with a sheath of microfibrils, each of which has a diameter of about 10 nm
- microfibrils appear before elastin in developing tissues and seem to provide scaffolding to guide elastin deposition
- Arrays of microfibrils are elastic in their own right, and in some places they persist in the absence of elastin: they help to hold the lens in its place in the eye, for example
- Microfibrils are composed of a number of distinct glycoproteins, including the large glycoprotein fibrillin, which binds to elastin and is essential for the integrity of elastic fibers
- Marfan’s syndrome, a relatively common human disorder
- The aorta is prone to rupture; other common effects include displacement of the lens and abnormalities of the skeleton and joints
- Affected individuals are often unusually tall and lanky: Abraham Lincoln is suspected to have had the condition

Fibronectin and Other Multidomain Glycoproteins Help Organize the Matrix

- glycoproteins that typically have multiple domains, with specific binding sites for other matrix macromolecules and for receptors on the surface of cells
- organizing the matrix and helping cells attach to it
- they also guide cell movements in developing tissues by serving tracks along which cells can migrate or as repellents that keep cells out of forbidden areas
- They can also bind and thereby influence the function of peptide growth factors and other small molecules produced by nearby cells
- fibronectin, a large glycoprotein found in all vertebrates and important for many cell– matrix interactions

- Mutant mice that are unable to make fibronectin die early in embryogenesis because their endothelial cells fail to form proper blood vessels
- The defect is thought to result from abnormalities in the interactions of these cells with the surrounding extracellular matrix, which normally contains fibronectin
- Fibronectin is a dimer composed of two very large subunits joined by disulfide bonds at their C-terminal ends
- Fibronectin is a dimer composed of two very large subunits joined by disulfide bonds at their C-terminal ends
- major repeat domain in fibronectin is called the type III fibronectin repeat, which is about 90 amino acids long and occurs at least 15 times in each subunit. This repeat is among the most common of all protein domains in vertebrates



Complex glycoproteins of the extracellular matrix.

•Each domain is folded into a discrete globular structure, and many such domains are arrayed along the protein like beads on a string

•This diagram shows four representative proteins among the roughly 200 matrix glycoproteins that are found in mammals

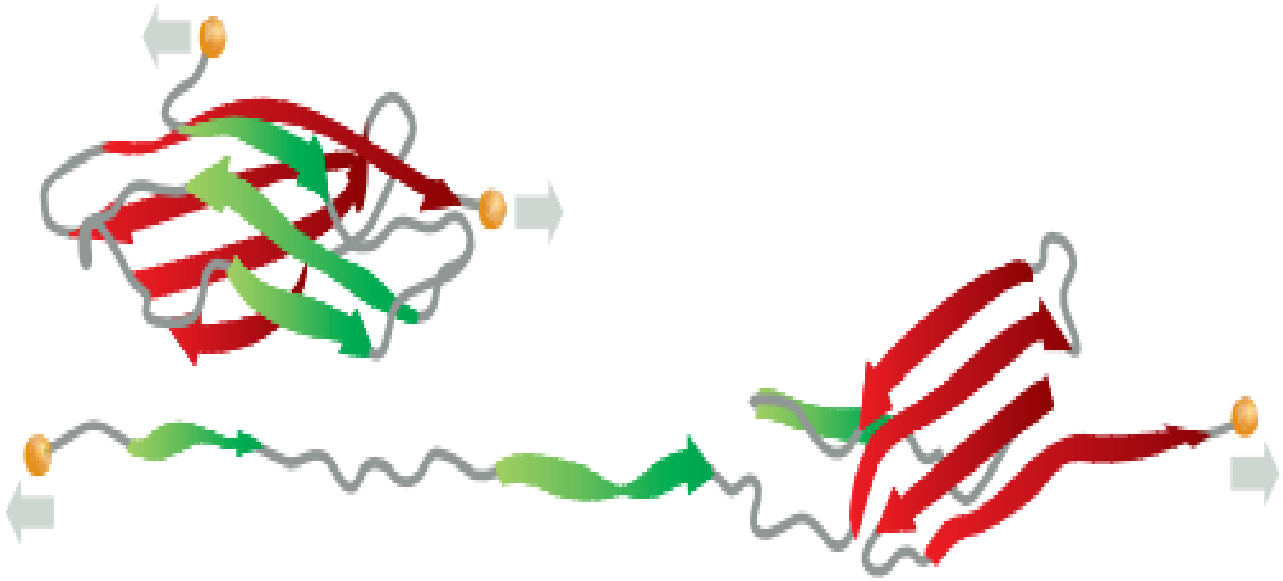
• •Two type III repeats near the C-terminus contain important binding sites for cell-surface integrins, whereas other FN repeats are involved in binding fibrin, collagen, and heparin, as indicated Other matrix proteins contain repeated sequences resembling those of epidermal growth factor (EGF), a major regulator of cell growth and proliferation; these repeats might serve a similar signaling function in matrix proteins.

TO ADD MORE STRUCTURAL DIVERSITY, MANY OF THESE PROTEINS ARE ENCODED BY RNA TRANSCRIPTS THAT CAN BE SPLICED IN DIFFRENT WAYS, ADDING OR REMOVING EXONS, SUCH AS THOSE IN FIBRONECTIN.

Fibronectin Binds to Integrins

- To analyze a complex multifunctional protein molecule such as fibronectin is to synthesize individual regions of the protein and test their ability to bind other proteins
- Other method is, one region of fibronectin binds to collagen, another to proteoglycans, and another to specific integrins on the surface of various types of cells
- binding depends on a specific tripeptide sequence (Arg-Gly-Asp, or RGD) that is found in one of the type III repeats
- very short peptides containing this RGD sequence can compete with fibronectin for the binding site on cells, thereby inhibiting the attachment of the cells to a fibronectin matrix
- Many of these proteins are components of the extracellular matrix, while others are involved in blood clotting

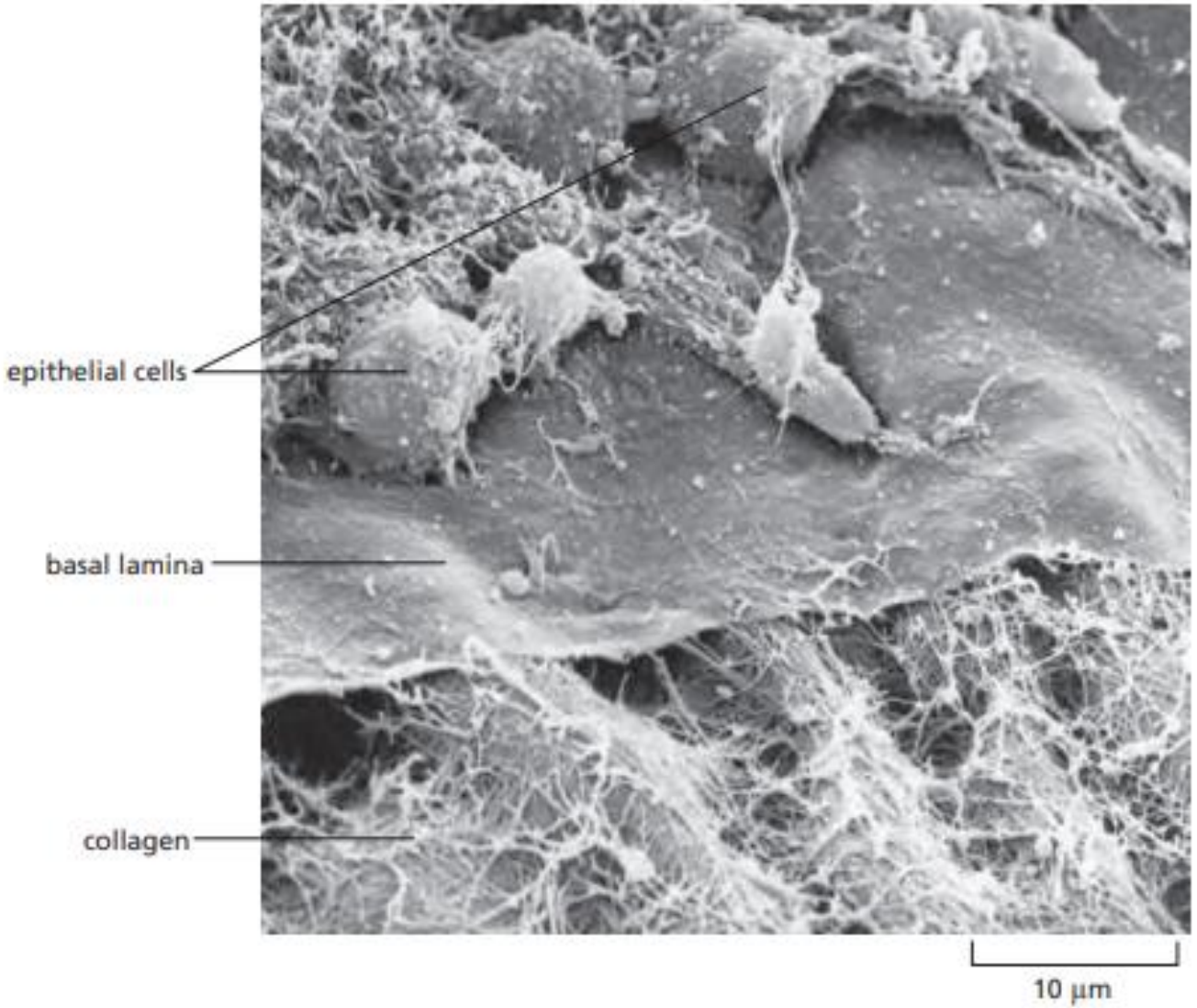
Tension Exerted by Cells Regulates the Assembly of Fibronectin Fibrils



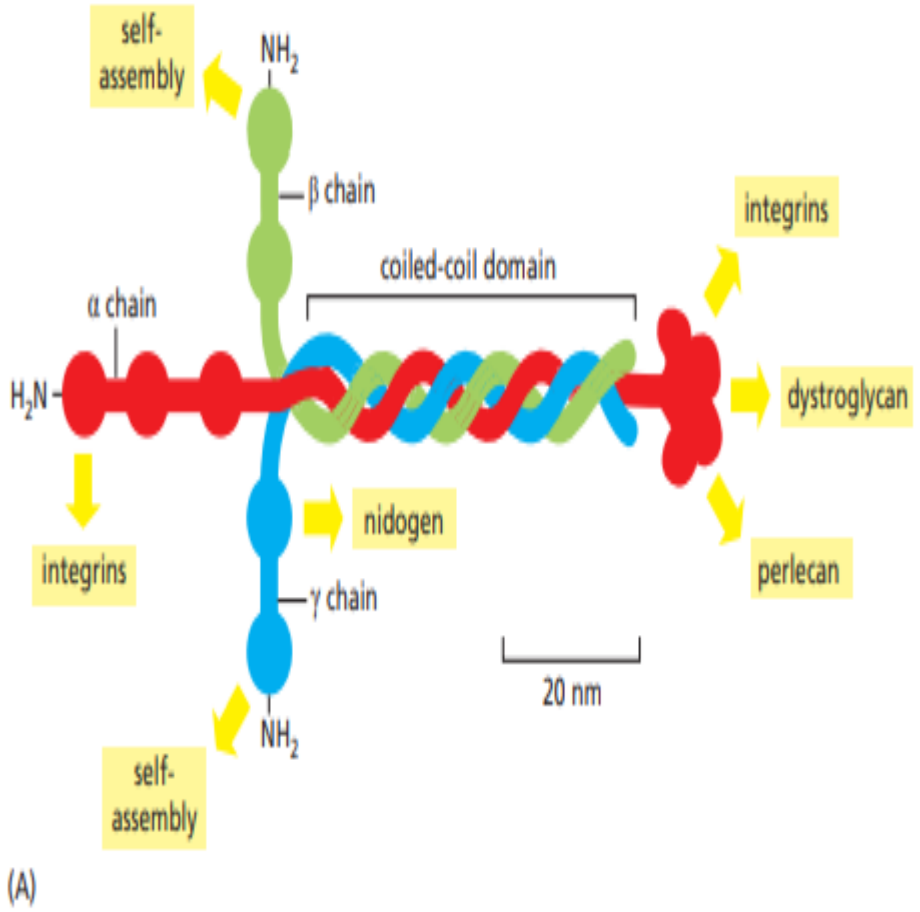
- Fibronectin can exist in soluble form, circulating in the blood and other body fluids
- Tension-sensing by fibronectin.
- Some type III fibronectin repeats are thought to unfold when fibronectin is stretched.
- The unfolding exposes cryptic binding sites that interact with other fibronectin molecules resulting in the formation of fibronectin filaments

The Basal Lamina Is a Specialized Form of Extracellular Matrix

- components are assembled into a specialized type of extracellular matrix called the basal lamina
- also known as the basement membrane
- This exceedingly thin, tough, flexible sheet of matrix molecules is an essential underpinning of all epithelia
- Like cadherin it is important in vertebrates in the construction
- major molecular components of the basal lamina are among the most ancient extracellular matrix macromolecules
- They are 40–120 nm thick
- A sheet of basal lamina not only lies beneath epithelial cells but also surrounds individual muscle cells, fat cells,and Schwann cells



SEM image of The basal lamina in the cornea of a chick embryo

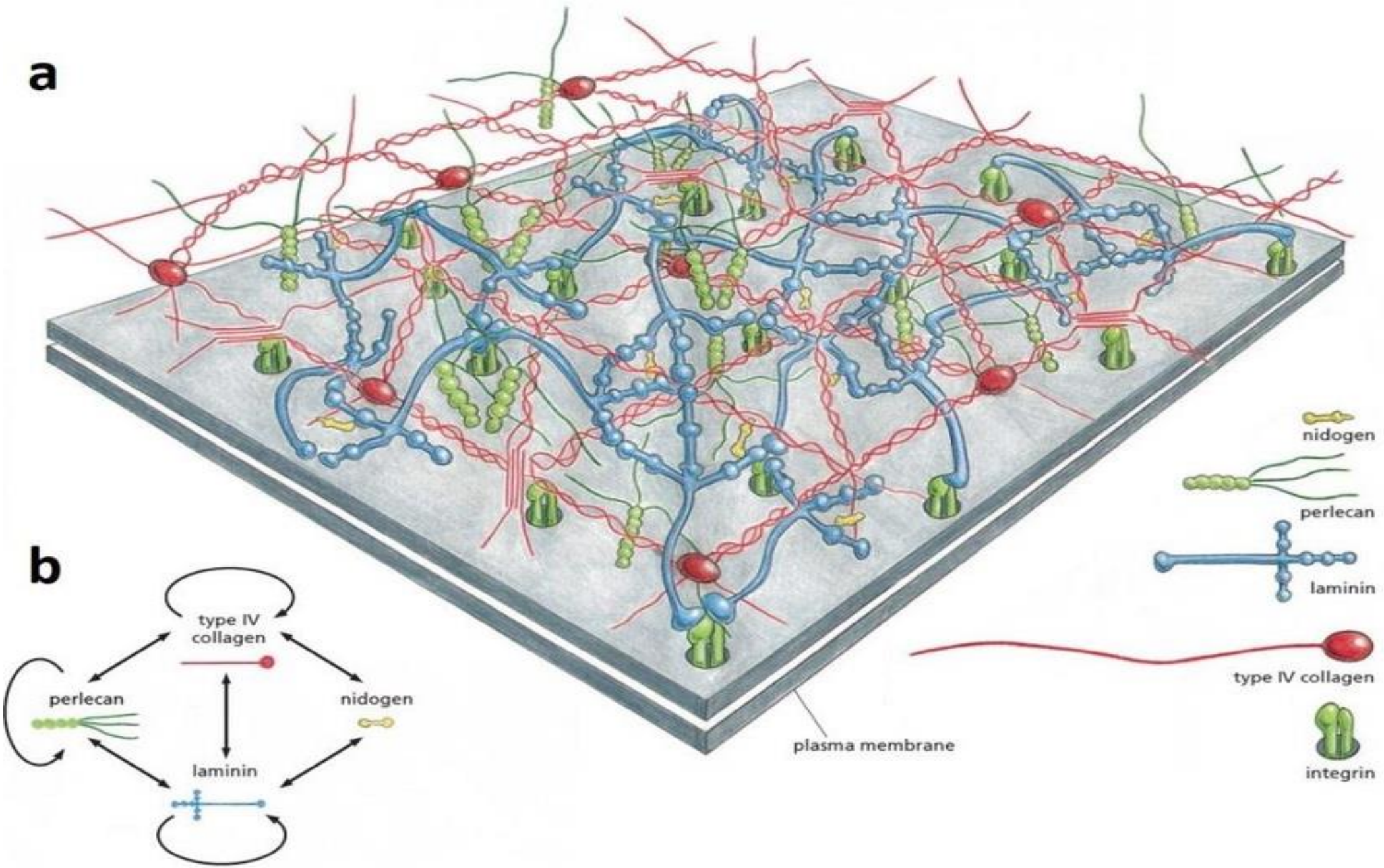


The structure of laminin

- Three ways in which basal laminae are organized. Basal laminae (yellow) surround certain cells (such as skeletal muscle cells), underlie epithelia, and are interposed between two cell sheets (as in the kidney glomerulus)
- Note that, in the kidney glomerulus, both cell sheets have gaps in them, and the basal lamina has a filtering as well as a supportive function, helping to determine which molecules will pass into the urine from the blood
- The filtration also depends on other protein-based structures, called slit diaphragms, that span the intercellular gaps in the epithelial sheet

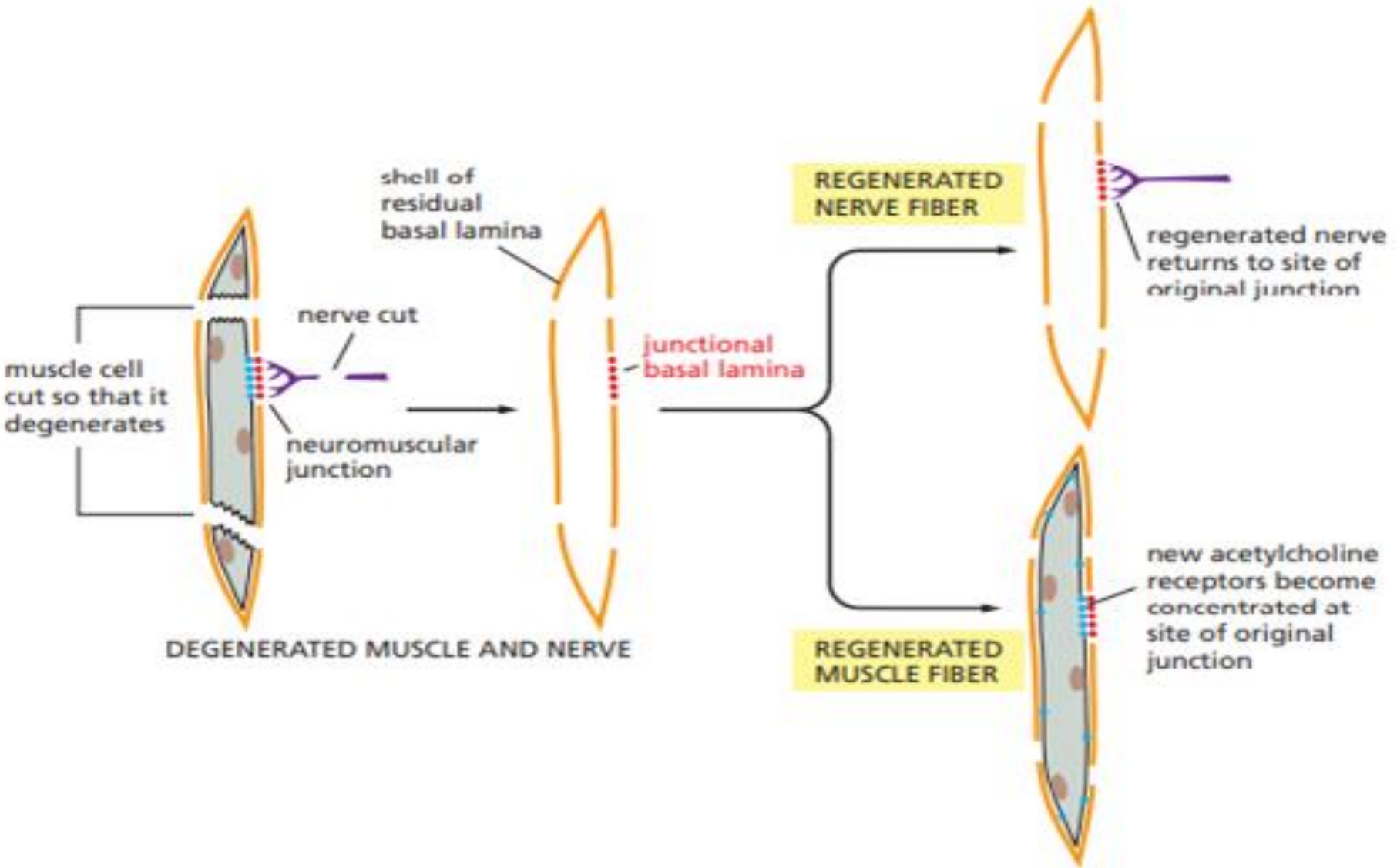
- They are able to determine cell polarity; influence cell metabolism; organize the proteins in adjacent plasma membranes; promote cell survival, proliferation, or differentiation; and serve as highways for cell migration
- The mechanical role is nevertheless essential. In the skin, for example, the epithelial outer layer—the epidermis—depends on the strength of the basal lamina to keep it attached to the underlying connective tissue—the dermis
- In people with genetic defects in certain basal lamina proteins or in a special type of collagen that anchors the basal lamina to the underlying connective tissue, the epidermis becomes detached from the dermis
- This causes a blistering disease called junctional epidermolysis bullosa, a severe and sometimes lethal condition

MODEL OF MOLECULAR STRUCTURE OF BASAL LAMINA



Laminin and Type IV Collagen Are Major Components of the Basal Lamina

- The basal lamina is synthesized by the cells on each side of it: the epithelial cells contribute one set of basal lamina components,while cells of the underlying bed of connective tissue
- most of them are glycoproteins laminin, type IV collagen, and nidogen, along with the proteoglycan perlecan
- Laminin is the primary organizer of the sheet structure, and, early in development, basal laminae consist mainly of laminin molecules
- laminin γ1 chain is, however, a component of most laminin heterotrimers; mice lacking it die during embryogenesis because they are unable to make basal laminae
- Type IV collagen is a second essential component of mature basal laminae
- Type IV collagen molecules interact via their terminal domains to assemble extracellularly into a flexible, feltlike network that gives the basal lamina tensile strength



Regeneration experiments in frog demonstrating the special character of the junctional basal lamina at a neuromuscular junction.

- example: role of the basal lamina in regeneration comes from studies of the neuromuscular junction, the site where the nerve terminals of a motor neuron form a chemical synapse with a skeletal muscle cell
- In vertebrates, the basal lamina that surrounds the muscle cell separates the nerve and muscle cell plasma membranes at the synapse, and the synaptic region of the lamina has a distinctive chemical character with special isoforms of type IV collagen and laminin and a proteoglycan called agrin
- After a nerve or muscle injury, the basal lamina at the synapse has a central role in reconstructing the synapse at the correct location
- Defects in components of the basal lamina at the synapse are responsible for some forms of muscular dystrophy, in which muscles develop normally but then degenerate later in life.

Reference

Albert Bruce 6th edition Molecular Biology of the cell

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Programme: M.Sc., Biochemistry
Course Title : Cell biology
Course Code :BC105DCE

Unit-4
CELL CYCLE

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Cell cycle control system governs progression at three major regulatory transitions:-

First - at the start in Late G1 (where cell commits to cell-cycle entry and chromosome duplication)

Second - at G2/M transition (Where control system triggers early mitotic events that lead to chromosome alignment on mitotic spindle in metaphase)

Third - at Metaphase - Anaphase transition (where the control system stimulates sister-chromatid separation leading to completion of mitosis and cytokinesis)

CYCLIN DEPENDENT KINASES

Cdks are family of protein kinases that rise and fall as the cell progresses through the cell cycle - leading to cyclical changes in the phosphorylation of intracellular proteins that initiate or regulate the major events of the cell cycle.

Ex: An increase in Cdk activity at G2/M transition, for ex: increases the phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown, spindle assembly and other events that occur in early mitosis.

Regulators of Cdks are the proteins known as cyclins.

The levels of Cdk proteins, by contrast, are constant. Cyclical changes in cyclin protein levels result in the cyclic assembly and activation of cyclin-Cdk complexes at specific stages of the cell cycle.

4 CLASSES OF CYCLINS

G1/S cyclins activate Cdks in late G1 and thereby help trigger progression to cell cycle entry. Their levels fall in S phase

S-Cyclins - bind Cdks soon after progression through start - help chromosome duplication. Their levels remain elevated until mitosis and also contribute to control some of early mitotic events.

M-Cyclins - activate Cdks that stimulate entry into mitosis at the G2/M transition. M-Cyclin levels fall in the mid-mitosis.

In most cells, a fourth class of cyclins, the G1-cyclins, help govern the activities of the G1/S cyclins, that control progression through start in the late G1.

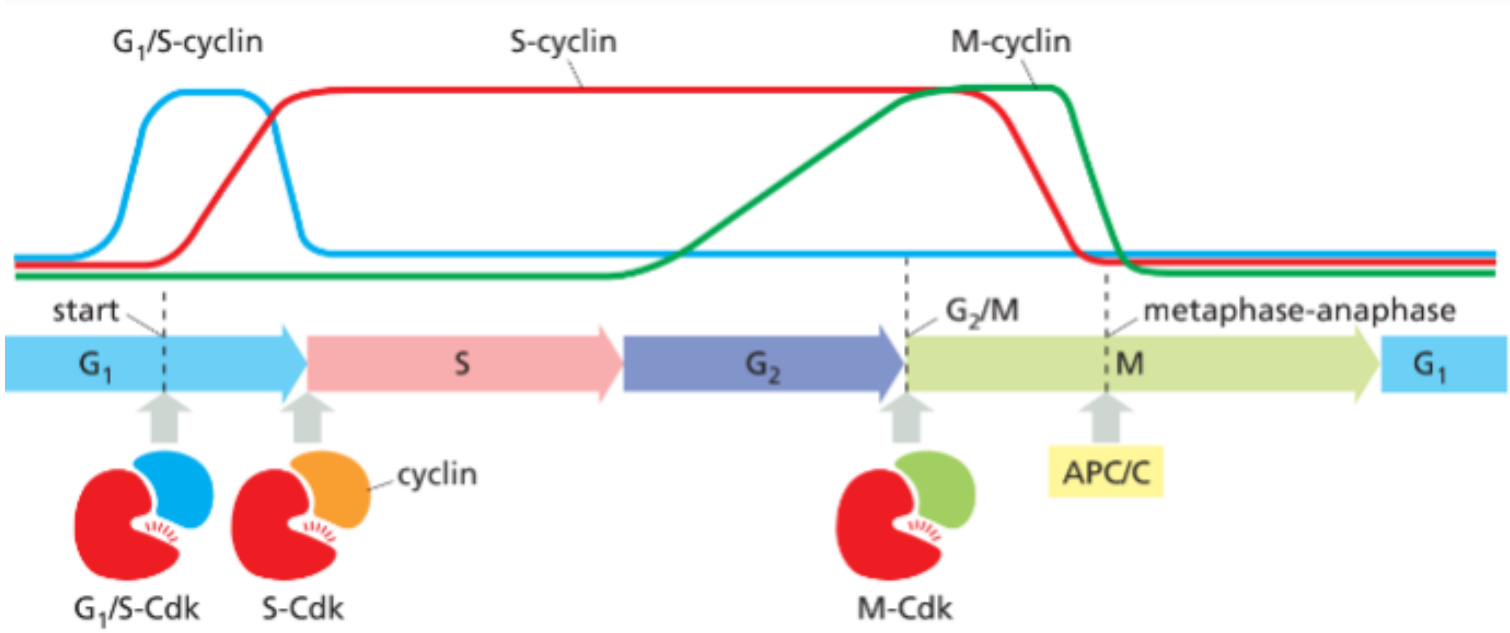
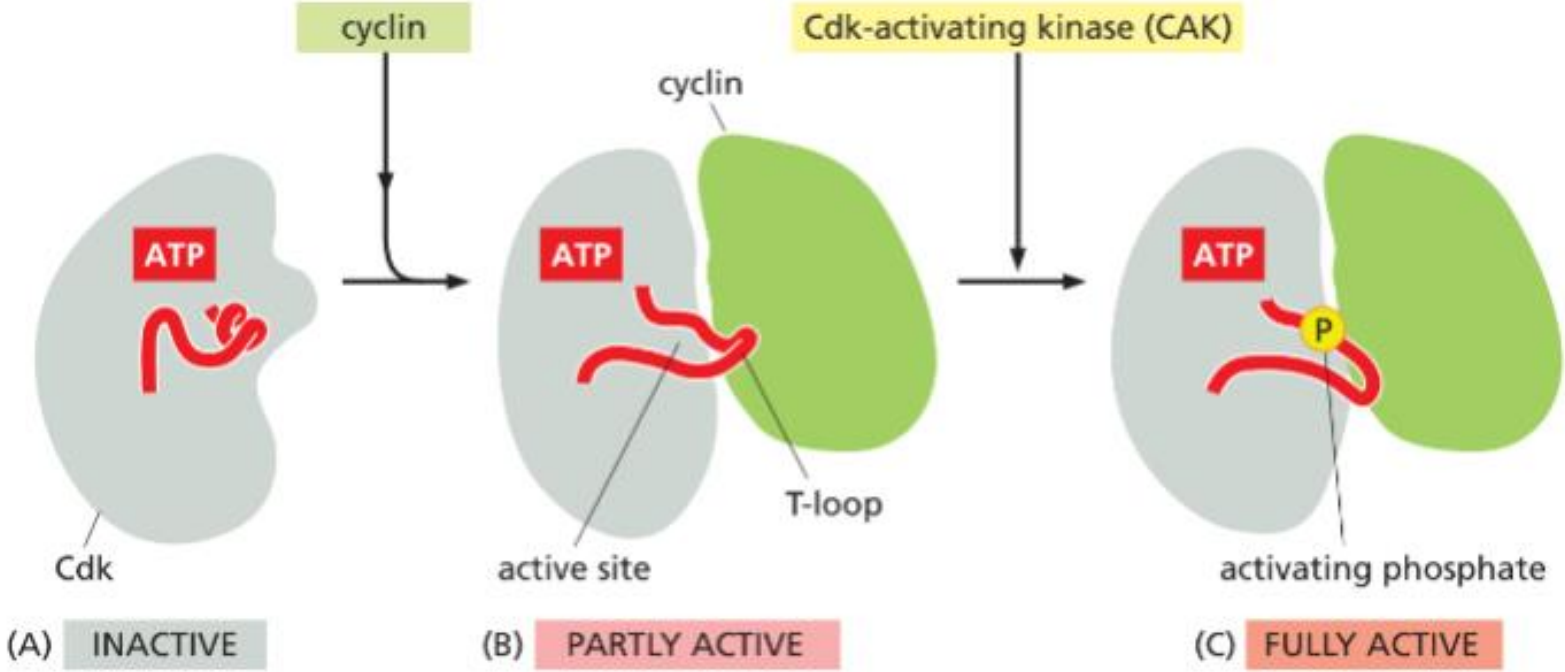

G1/S-cyclin
S-cyclin
M-cyclin
start
G2/M
metaphase-anaphase
G1
S
G2
M
G1
cyclin
APC/C
G1/S-Cdk
S-Cdk
M-Cdk

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

	Vertebrates		Budding yeast	
Cyclin–Cdk complex	Cyclin	Cdk partner	Cyclin	Cdk partner
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

Cdk activating kinase (CAK) - phosphorylates amino acid near the entrance of Cdk active site

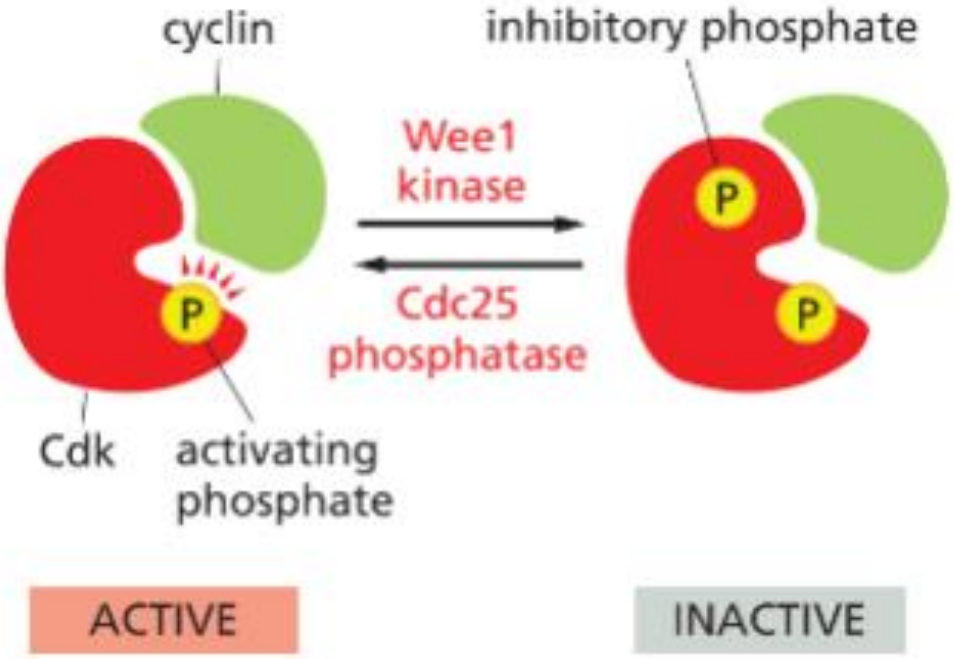


Cdk activity can be suppressed by inhibitory phosphorylation and Cdk inhibitor proteins (CKI)

The rise and fall of cyclin levels is the primary determinant of Cdk activity during the cell cycle.

Phosphorylation at amino acid of kinase active site inhibits the activity of Cyclin-Cdk complex. Ex: PO4 by Wee1 kinase inhibits Cdk while dephosphorylation by Cdc25 phosphatase increases its activity

Cdk INHIBITOR PROTEINS (CKI) - Wee1 phosphorylates two closely spaced sites above the active site



Other mechanism include Cdk inhibitor proteins (CKIs) inactivates cyclin-cdk complexes by stimulating large rearrangement in the structure of the Cdk active site, rendering it inactive

Cells use CKIs primarily to help govern the activities of G1/S - and S-Cdks early in the cell cycle.

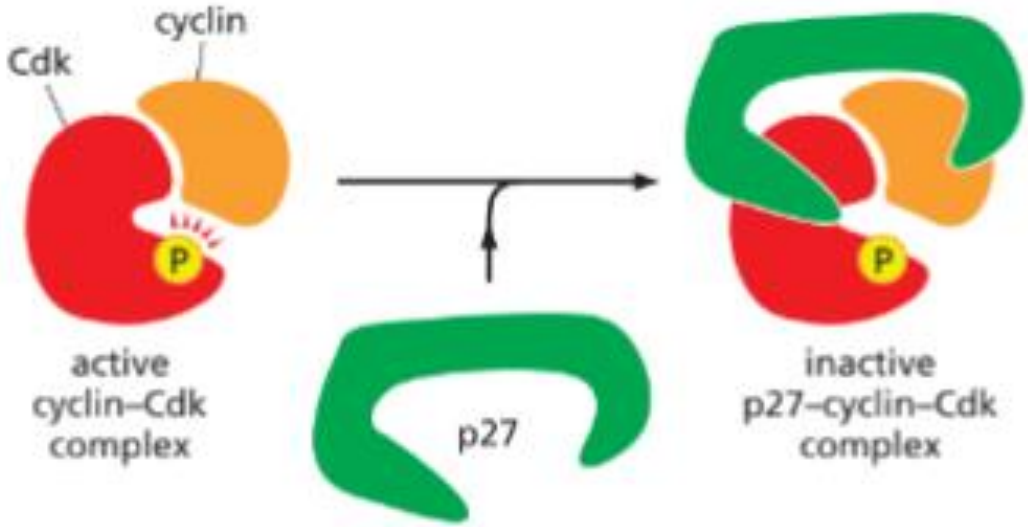


Figure 17–14 The inhibition of a cyclin–Cdk complex by a CKI. This drawing is based on the three-dimensional structure of the human cyclin A–Cdk2 complex bound to the CKI p27, as determined by x-ray crystallography. The p27 binds to both the cyclin and Cdk in the complex, distorting the active site of the Cdk. It also inserts into the ATP-binding site, further inhibiting the enzyme activity.

Regulated proteolysis triggers Metaphase - Anaphase transition - M-A phase is triggered not by phosphorylation, rather by protein destruction leading to final stages of cell division.

APC/C - Anaphase promoting complex / cyclosome - a member of ubiquitin ligase family of enzymes - that stimulates the proteolytic destruction of specific regulatory proteins.

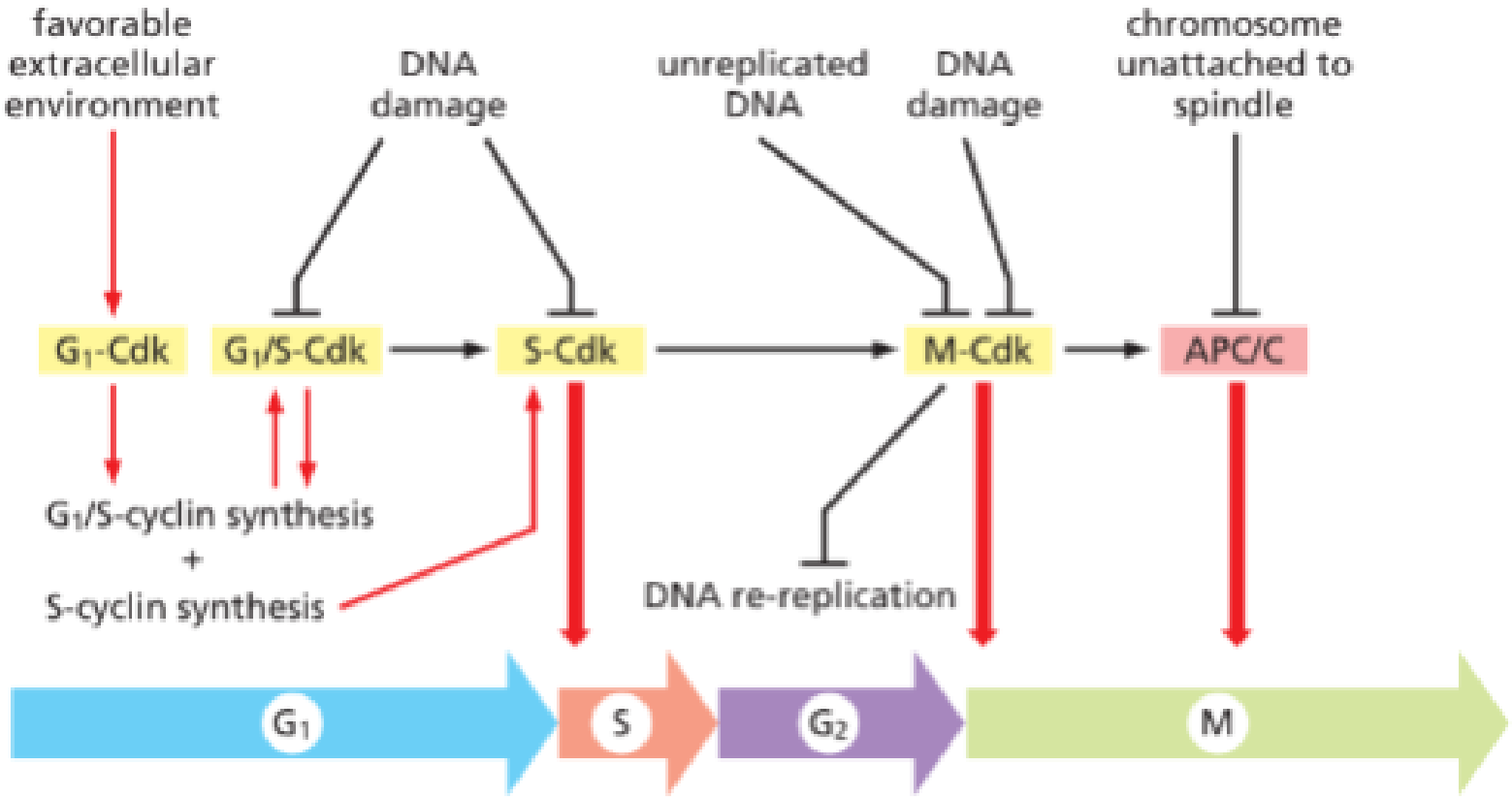
APC poly ubiquitinylate specific target proteins, and destructs in proteosomes.

APC/C catalyses the ubiquitylation of
i) securin - that protect protein linkages that hold sister-chromatid pairs together in early mitosis

Destruction of securin in metaphase activates a protease that separates the sisters and unleashes anaphase.
ii) S- and M - cyclins are the second major targets of APC/C - help completion of M phase, including the final steps in mitosis and then cytokinesis.

TABLE 17–2 Summary of the Major Cell Cycle Regulatory Proteins	
General name	Functions and comments
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	Phosphorylates an activating site in Cdks
Wee1 kinase	Phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	Removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	Suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	Suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	Suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	Suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
APC/C	Catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including securin and S- and M-cyclins; regulated by association with activating subunits Cdc20 or Cdh1
Cdc20	APC/C-activating subunit in all cells; triggers initial activation of APC/C at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Cdh1	APC/C-activating subunit that maintains APC/C activity after anaphase and throughout G_1; inhibited by Cdk activity
SCF	Catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including some CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity

An overview of the cell cycle control system



MODEL ORGANISM TO STUDY CELL CYCLE

The most commonly used model organisms are

1. Unicellular yeasts,
2. The early embryos of frogs and fruit flies and
3. Mammalian cells in cell culture

Yeast includes the models of great importance for genetics. Viz., Saccharomyces cerevisiae; Schizosacchromyces pombe; Neurospora crassa etc.,

The budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe are unicellular and considered to be among the best genetically tractable organisms for a comprehensive understanding of biology.

Budding yeast was the eukaryote whose genome was sequenced first in 1996 followed by the genome sequence of fission yeast, which was completed in 2002.

Allthough started later, S.pombe has been particularly influential in studies of cell cycle regulation, DNA damage/ repair mechanisms and chromosome dynamics, including RNA interference.

Furthermore, the fission yeast S.pombe harbors less genome duplication compared with other eukaryotes and holds the smallest sequenced eukaryotic genome, which led to its popularity as a eukaryotic model in the last decade.

Both budding yeast and fission yeast are able to exist in either a diploid or a haploid state.

Although most strains of yeast used in laboratory are haploid strains, in the wilderness, budding yeast tend to live in as diploid state, whereas fission yeast tend to stay in haploid state.

The proportion of the life cycle of budding yeast that they spend in the diploid state or haploid state varies, depending on the environment. When nutrient are plentiful, budding yeast proliferates as diploid cells. If starved, they undergo meiosis to form haploid spores.

In contrast, fission yeast typically proliferate as haploid cells. They fuse in response to starvation to form diploid cells and these diploid cells promptly undergo meiosis and sporulation.

Advantages of using yeast as model organism:

These two yeast system are non-pathogenic and are thus safe to handle

Their reproduction cycle are fast and easy to monitor with doubling times from 90 minutes for wild type cells in rich medium to a few hours for mutant cells

Distinct features of the cell cycles in S.cerevisiae and S. pombe

Both undergo closed mitosis - nuclear envelope doesnot breakdown

In contrast, most multicellular/ eukaryotic organism undergo open mitosis

Although both are yeasts, they exhibit distinct features for cell division.

Fission yeast undergo typical eukaryotic cell cycle with consecutive G1, S, G2 and M Phases. The G2/M phase is fuzzy, as spindle assembly starts to occur in S phase. Therefore the control of G2/M phase is more visible in fission yeast.

While G1/S transition start is the major control of cell cycle in budding yeast S. cerevisiae.

Cdc mutants in different models of yeast help learn cell cycle processes.

Using this genetic approach, researchers identified a cast of vital players in cell cycle control, including the genes encoding Cdc2, Wee1 protein kinases, as well as Cdc25 tyrosine phosphatase. The discoveries also provide the basis for the concept of checkpoint control of cell division

Early embryo of frog

Eggs of frog Xenopus laevis are a special type of cell very useful to study cell cycle.

The eggs of amphibians, marine invertebrates, and insects are large cells and they divide very rapidly following fertilization in early embryo development.

DISTINGUISHING feature of early embryonic cell cycle in frog - no cell growth occurs and each daughter cell produced from the cell division is half the size of the parent cell.

Therefore compared to the standard cell division, the duration of frog egg cycle is extraordinarily short, only consisting of alternating S phase and M phase without intervening G1 and G2 phase.

Because of the specialized rapid cell division of the frog embryo, they are very useful for studying the mechanisms of interphase–M phase transition.

The early development of the frog X. laevis embryo provides a particularly powerful system to analyze the factors that drive cells into mitosis.

Since the 1970s a lot of what we know about the control mechanisms of the mitotic cell cycle has been learned from the studies on the interphase–M phase transition in the frog egg system

A fully grown oocyte arrests in G2, when triggered by hormone, the oocyte matures into an egg and arrests in metaphase of the meiosis II. Fertilization releases the metaphase arrest, so that the egg completes its second meiotic division and enters the interphase of the first embryonic cell cycle. Since an immature oocyte arrests in meiotic G2, whereas a mature egg arrests in meiotic M phase, the abundant source of the cytoplasm can be extracted from these embryonic cells at defined stages of the cell cycle.

Moreover, because of their big size, they are amenable for injecting materials such as a molecule of interest or protein lysates into them, or for extracting out of the cytoplasm.

When M-phase cytoplasm from a mature egg is injected into a G 2-phase immature oocyte, the oocyte is driven into M phase and completes its maturation

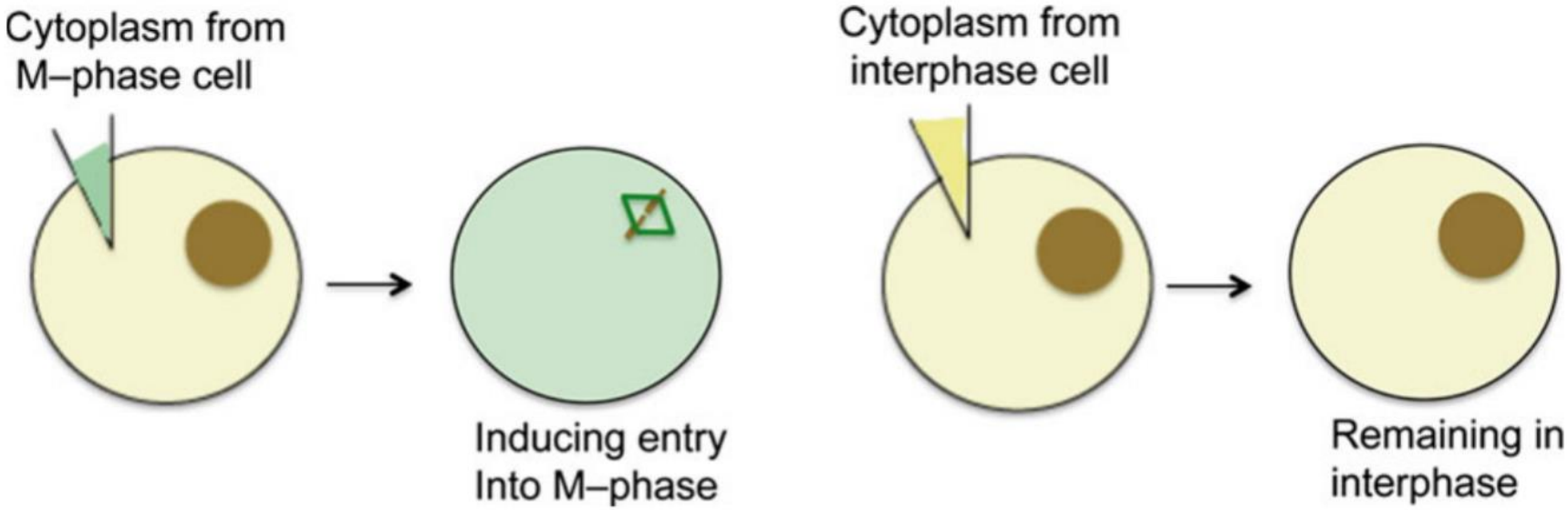


Fig. 13 Oocyte injection experiment. Injection of the cytoplasm from a mature egg in M phase into an immature oocyte in interphase induces the oocyte to become mature and enter M phase

CELL FUSION EXPERIMENT

Mammalian cells are generally not as large as frog oocytes; therefore, it is not as easy to use them for cytoplasmic injections. However, we can perform a logically equivalent test by fusing a mitotic cell with an interphase cell, so that the nucleus of the interphase cell is exposed to any active components present in the cytoplasm of the mitotic cell. In such experiments the interphase cell is directly driven into mitosis, no matter whether it is in G 1, S, or G 2, and whether it has replicated its DNA or not. This cytoplasmic activity is also named MPF—M-phase-promoting factor. It became clear several years later that MPF plays a general role in mitotic induction in somatic cells of all eukaryotic cells from yeasts to humans.

DISCOVERING CYCLINS

Protein synthesis was examined in sea urchin eggs by Tim Hunt. The fertilized eggs were incubated with water containing the radioactive amino acid, 35S-methionine. Samples were removed at allocated time points and analyzed by SDS-PAGE (polyacrylamide gel electrophoresis). The experiment revealed a novel class of proteins, appearing in a periodic fashion, although most proteins in sea urchin eggs accumulate continuously after fertilization. The family of oscillating proteins increases steadily during interphase until the metaphase–anaphase transition, at which they are suddenly abolished. The proteins are thus given the name of cyclin because of their characteristic cycling pattern during the cell cycle

It is this model organism that enables researchers to biochemically purify MPF and functionally identify the key regulators of the cell cycle including, Cdc2, later named as cyclin-dependent kinase1 (Cdk1), Cdc25 tyrosine phosphatase, and Wee1 tyrosine kinase.

FRUIT FLY - Drosophila melanogaster

Fruit Fly Drosophila melanogaster is a valuable model organism for cell cycle studies. The components of the cell cycle control system in Drosophila are structurally and functionally similar to humans.

The generation time of Drosophila is 2 weeks and it is relatively easy to grow and maintain them in a laboratory.

Drosophila has a genome size of about 14,000 genes, which is 2–3 times of yeasts and about half the number in humans.

An early Drosophila embryo undergoes rapid and synchronous nuclear divisions after the fusion of egg and sperm nuclei to give rise to a zygote nucleus. These divisions each last less than 10 min and proceed without gap phases, resulting in a syncytium, in which many nuclei share the same cytoplasm. The nuclei subsequently move to the surface of the embryo after nine divisions and start cytokinesis to form about 6000 cells at the end of the 13th division.

The synchronous progression of the nuclear division in early Drosophila embryos provides a good resource to isolate important regulators of the cell cycle

MAMMALIAN CELLS

Although the fundamental principles of cell cycle control are studied effi ciently in simpler systems including yeasts and Drosophila, it cannot supersede the research in the mammalian cell cycle. Only in mammalian cells can we ultimately decipher the complex circuits regulating the cell cycle.

However, in complex multicellular organisms such as humans, various cells divide at very different rates. The cells that line our intestine live only 3 days and must be constantly replaced by the division of precursor cells. On the other hand, the life span of liver cells is more than a year, thus cell division in this organ is rare.

The cell cycle in mammalian cells varies greatly. The variability in the length of the cell cycle of different cells occurs mainly in G 1 and G 2. It refl ects the ability of cells to exit from the cell cycle during either G 1 or G 2 phase

Many cells can withdraw from the cell cycle, entering G 0 or a stable G 2 arrest. Cells in G 0 have left the cycle after division but before the restriction point at the G 1–S transition. These cells account for most of the non-growing, non-proliferating cells in the human body. Some cells such as epidermal cells leave the cycle during G 2 and arrest without growth or proliferation

It is difficult to study cell proliferation in intact multicellular animals; therefore, most studies of cell cycle control are performed on cells proliferating in culture.

The tissue culture studies on the cell cycle contribute to our understanding of cancer development at a cellular level. Insights into the regulation of cell growth and proliferation in mammals have been provided by studies of cell lines and transformed cancer cells in cell culture.

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Programme: M.Sc., Biochemistry
Course Title : Cell Biology
Course Code :BC105DCE

Unit-5
STEM CELLS

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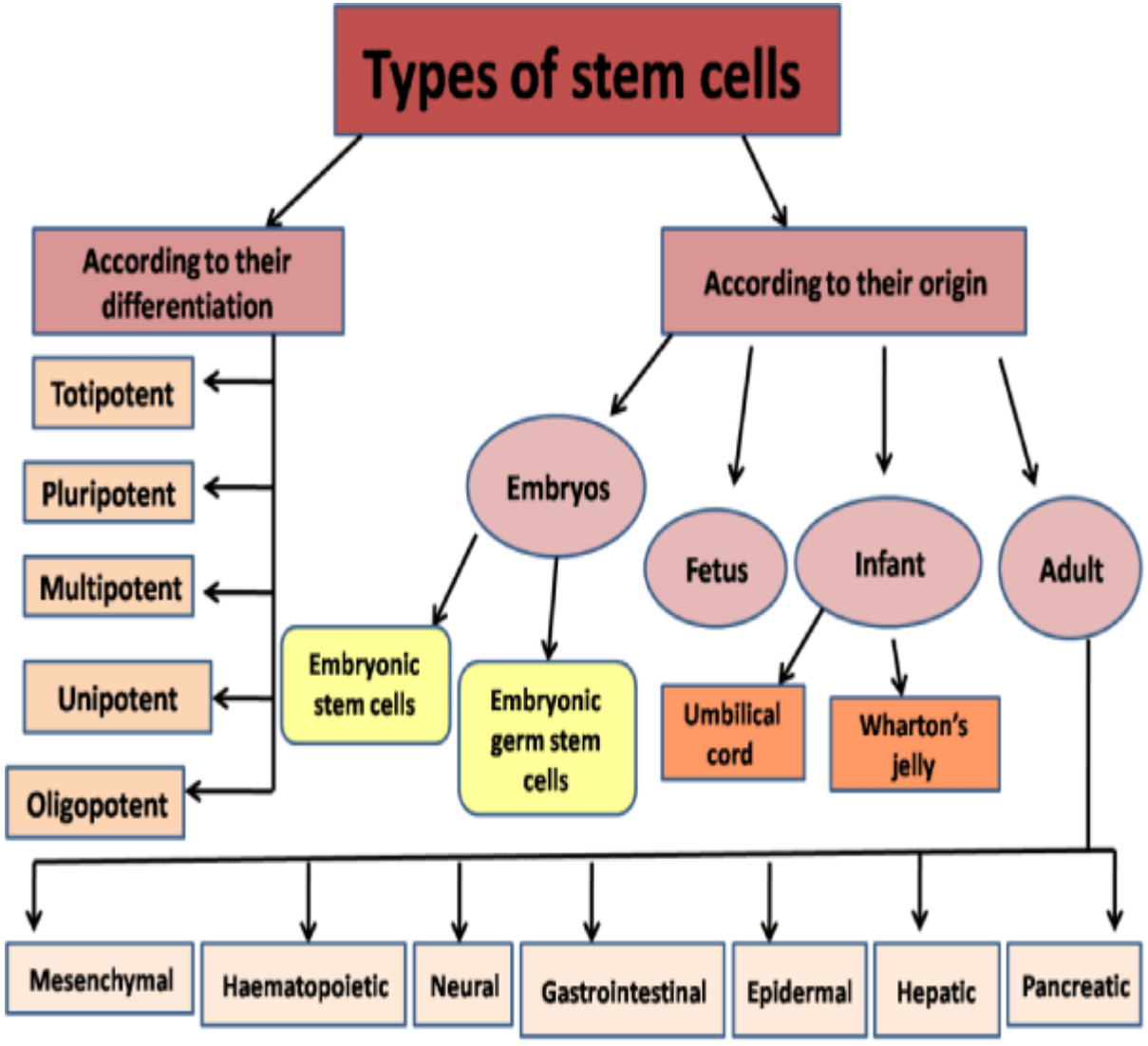
History

- The **history of stem cell therapy** and research began in the year 1902 when a Russian histologist Alexander M. Maximow referred to them (stem cells) as ‘polyblasts’ and ‘wandering cells at rest’
- From 1896 until 1902, Maximow authored documents, which identified his interest in the blood and connective tissues
- He confirmed the unitarian theory of hematopoiesis and proved that all blood cells mature from a precursor cell
- Since then, scientists from all over the world followed the study of stem cells, experimenting (on mice, plants and human patients) in search of a cure for various disorders, diseases, and injuries
- The history of stem cell therapy was successfully investigated in 1969 by *Edward Donnall Thomas*
- He performed the first bone marrow transplant with stem cells collected from another person

What are Stem Cells

- They are not specialized cell and have the ability of self replicating , differentiation according to suitable signals
- Stem cells can reproduce itself over and over again through asymmetric cell division, they can produce the newly produced offspring cells preserve the characteristics of the mother cell that has a different potency and lineage potential, such as a committed progenitor that transiently amplifies to make several offspring



Classification and Sources of Stem Cells:

Stem cells can be classified according to their origin into four broad types,

1. From Embryos
2. from the fetus
3. from the infants
4. from the adult.

Also, they can be classified according to their potency

Stem Cells Classification according to their Origin

Embryonic Stem Cells (ESCs)

- Embryonic stem cells are pluripotent, self-renewing cells that can be derived from both mouse or human blastocysts, they are taken from the very early stages of embryo development after 4-5 days after fertilization
- They can be stored in culture as undifferentiated cell lines and can be stimulated to differentiate into any cell line
- They can differentiate into endoderm, mesoderm, and ectoderm embryonic germ layers, and also any type of somatic cells
- They, therefore, hold a great capacity in tissue regeneration therapy

Embryonic Germ Stem Cells:

- Embryonic Germ (EG) cells are taken from the later stages of the embryo development cells. They are derived from Primordial Germline Cells (PGCs) in the early development. They are mainly isolated from the fetal tissue in narrow-window timing
- The PGC-derived cells were pluripotent, although, it was not possible to demonstrate pluripotency by generating the formation of teratomas in mice

Fetal stem cells:

- Fetal stem cells are primal cell types found in the organs of the fetuses. They are able to differentiate into two types of stem cells: pluripotent stem cells and hematopoietic stem cells
- Neural crest stem cells, fetal hematopoietic stem cells and pancreatic islet cells have been isolated in the fetuses
- Human fetal stem cells have been used by many people, children and adults that are suffering from many of mankind’s most devastating diseases

Infant stem cell

- Umbilical cord stem cells: Umbilical cord blood contains prevalent stem cells which differ from those of bone marrow and adult peripheral blood
- Cord blood stem cells have shown to be multipotent as it being able to differentiate into neurons and liver cells
- Wharton’s jelly: Wharton’s jelly, which is the umbilical cord matrix, is considered to be a source of mesenchymal stem cells
- These cells express typical stem cell markers, can be propagated for long times and can be induced to differentiate *in vitro* into neurons

Adult stem cell

- any stem cells taken from mature tissue; they are found in the tissues of a fully developed child (whole embryo) or adult and can only produce a limited number of cell types
- They have limited potential as compared to the stem cells that derived from embryos and fetuses because of the stage of development of these cells
- They play a vital role in tissue repair, regeneration; and they are referred to their tissue origin
- Bone marrow is an abundant source of adult stem cells
- Mesenchymal stem cells:
- Mesenchymal Stem Cells (MSCs) are a different population of cells with the potential to differentiate into various somatic lineages
- They were at first described as adherent cells with a fibroblast-like appearance that can differentiate into osteocytes, chondrocytes, adipocytes, tenocytes and myocytes

- MSCs can be isolated from the bone marrow and readily discreted from the hematopoietic stem cells due to their plastic adherence
- They are used in tissue engineering and regenerative medicine
- They are character by long-storage without major loss of their potency

Hematopoietic stem cells:

- cells having the self-renewing potential and the capacity to give rise to differentiated cells of all hematopoietic lineages
- Therefore, they transplanted for complete healing of hematologic disorders and after high-dose chemotherapy against malignant diseases

Neural Stem Cells:

- multipotent and self-replication cells, they are established in specialized molecular microenvironments in the adult mammalian brain
- They can display the potential role in cellular therapy of the brain

Gastrointestinal stem cells: The stem cells of the gastrointestinal tract reside in a “niche” in the intestinal crypts and gastric glands

- The mechanism and the direction of the diffusion of this converted clone in the gastrointestinal mucosa are hotly disputed, and the central to this case is the position and nature of the gastrointestinal stem cells

Epidermal stem cells:

- The mammalian epidermis is a rapidly rejuvenating tissue that consists of three types of keratinocytes with varying differentiation potential: epidermal stem cells, Transiently Amplified Cells (TA cells) and terminally differentiated cells
- The epidermal stem cells have free self-renewal power
- They are establishing in the basal layer and remarkable in maintaining homeostasis and cellular regeneration of normal skin; wound healing and neoplasm formation, whereas TA cells, progeny of the epidermal stem cells, undergo terminal differentiation after 3–5 divisions
- After division, TA cells leave the basal layer and move through the suprabasal layers to the tissue surface, where they are periodically shed as squames

Hepatic stem cells:

- The liver has a strong regenerative capacity, utilizing different modes of regeneration according to the type and extent of the injury. Mature liver cells can propagate to replace the damaged tissue permit the recovery of the parenchymal function
- Chronic liver injury gives rise to a potential stem cell compartment which is located in the smallest branches of the intrahepatic biliary tree being activated, which called oval cell ductular reaction
- These oval cells are derived from the canal of Hering, which amplifies this biliary populations prior to these cells differentiate into hepatocytes
- In the human liver, the organization of the biliary tree is different, with the canal of hering extending to the proximate third of the lobule and so apparently requiring a name change from oval cells to hepatic progenitor cells

Pancreatic stem cells:

- Insulin-producing cells previously generated from pluripotent stem cells.
- The generation of these cells would provide a novel cell source for drug discovery and cell transplantation therapy in people suffering from diabetes
- Insulin-producing beta-cells turnover every 40-50 days by processes of apoptosis and the propagation and differentiation of the newly islet cells from progenitor epithelial cells, which are located in the pancreatic ducts

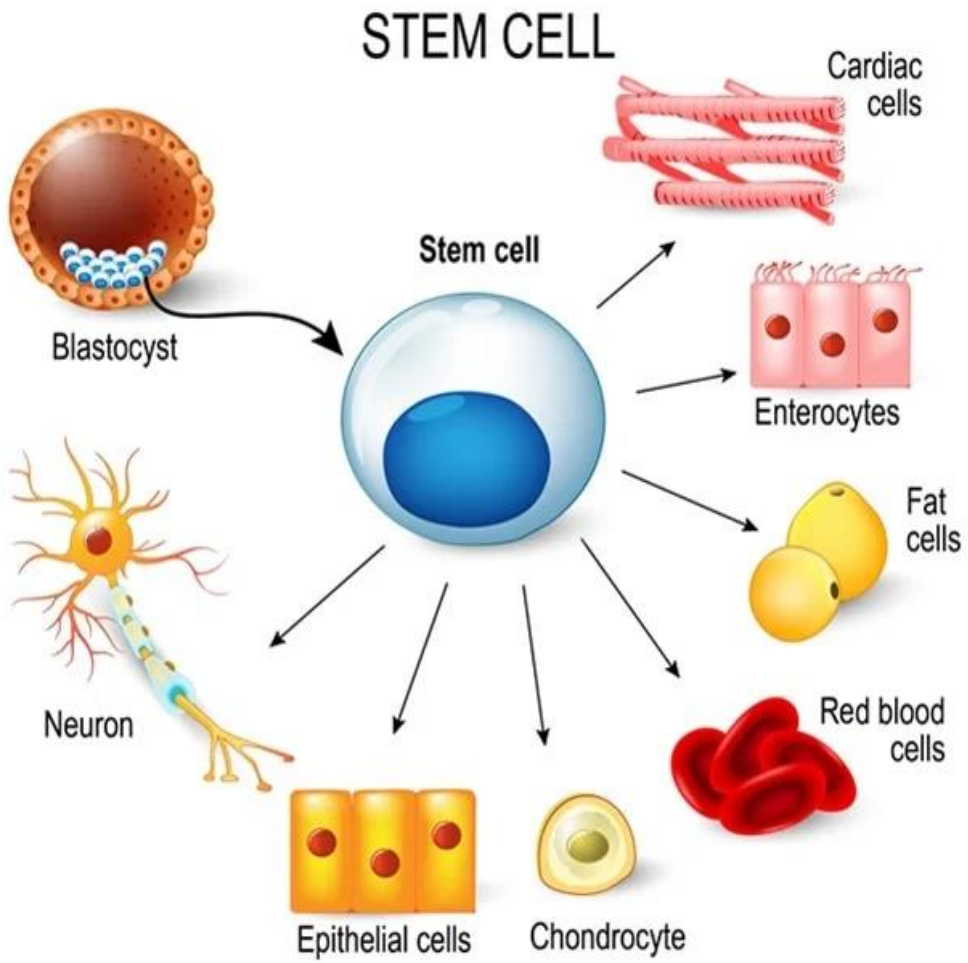


Fig. News Medical.net

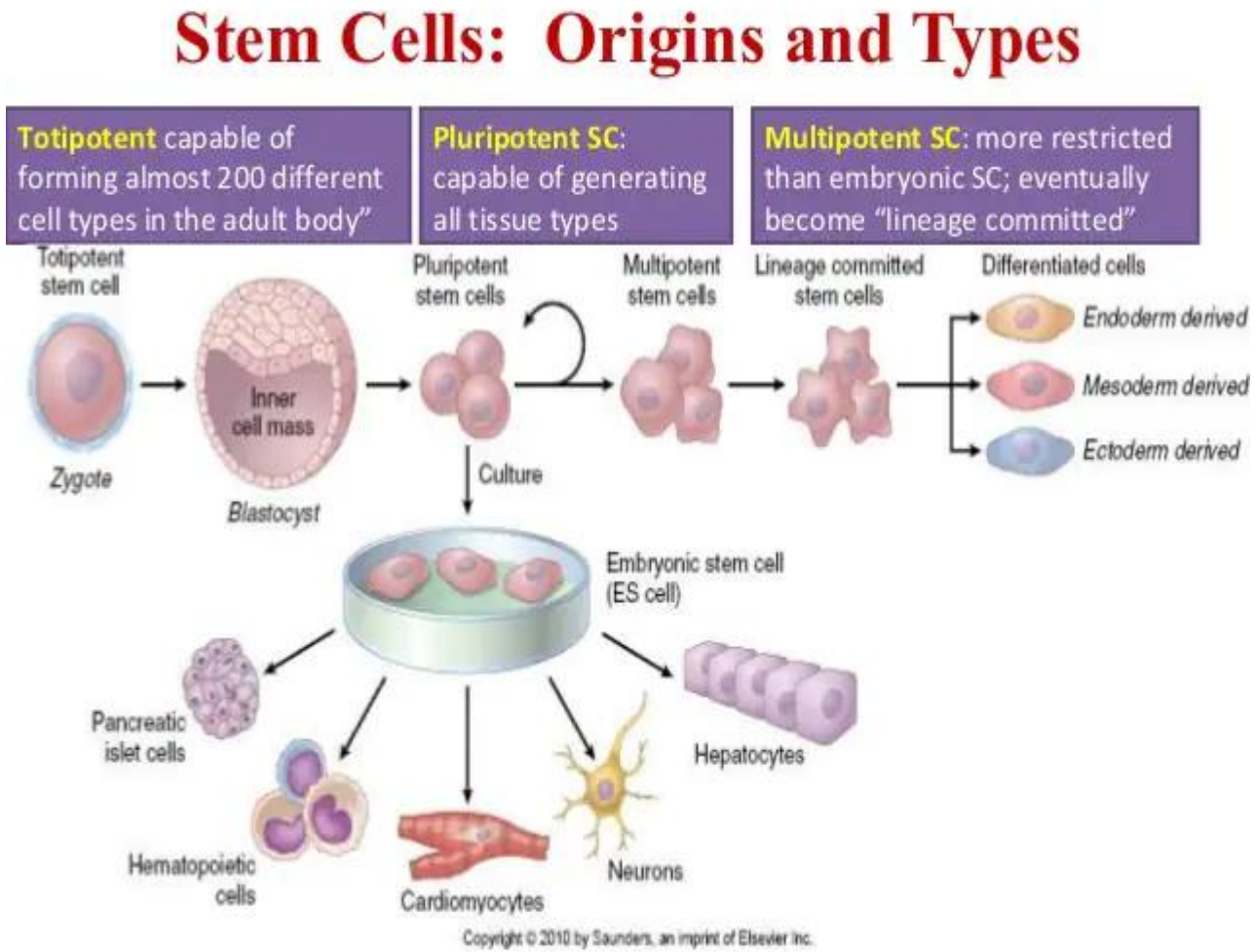


Fig. Elsevier

Types of stem cells according to their differentiation

- Stem cells can be classified according to their differentiation potential as a totipotent, pluripotent, multipotent, unipotent and oligopotent
- Totipotent stem cells: Totipotency means that it has the total potential to give rise to all types of cells
- Totipotent is the capacity of a single cell to divide and differentiate into all cell types in an organism and produce fertile offspring
- Oocytes and sperm are the best differentiated cells in our body and they are capable of forming any tissue in the body

Pluripotent stem cells:

- Pluripotency is the ability of the cells to produce any type of cells in the organism.
- They have been derived from the mouse embryo.

- All are capable of differentiating into cells representative of a variety of adult tissue types in various assays, including embryoid body, teratoma, and some can contribute to mouse development in chimeras.
- There are many differences being recognized among pluripotent stem cell types, such as their morphology, gene expression profiles and growth factor requirements

Multipotent stem cells:

- Multipotency means to those cells that can only give rise to cells of the tissue from which they are isolated

Unipotent stem cell:

- Adult stem cells are found in the tissues of the adults they produce a limited number of cell types and can repair damaged tissue by replacing specialized cells

- Because of their restricted lineage, they were thought to be either multipotent, with the ability to differentiate into a limited range of cells or unipotent, with the ability to produce only one cell type

Oligopotent stem cells:

- Oligopotency means to those cells that can differentiate into only a few cell types, like lymphoid or myeloid stem cells

Stem Cell Research

Scientists use different types of stem cells for this purpose:

- Neural stem cells (NSCs)
- Mesenchymal stem cells (MSCs)
- Embryonic stem cells (ESCs)
- Induced pluripotent stem cells (iPSCs)

Some of the clinical application of stem cells

1. Stem Cells and diabetes mellitus

- Stem cells have generated incredible interest for repairing failing tissues and organs
- Stem cell therapy has become a tantalizing idea to provide glucose-responsive insulin-producing cells to Type 1 diabetic patients as an alternative to islet transplantation
- Mesenchymal stem cells will grow and differentiate according to their environment
- When MSCs injected into the pancreas *in vivo*, it is expected that MSCs will differentiate into pancreatic cells that have both exocrine and endocrine functions.
- Thus, transplantation of MSCs from bone marrow stem cells can repair the pancreas in its role to provide paracrine effects and other cell differentiation effects

2. Parkinson’s Disease

- Parkinson’s disease (PD) is a widespread neurodegenerative disease that characterized by bradykinesia, rigidity, and tremor
- The pathological causes of PD are due to the Decrease of Nigrostriatal Dopamine (DA) neurons, but neuronal degeneration also occurs in non-DA-ergic systems
- MSCs are capable of differentiating into tyrosine hydroxylase-positive neurons and can ameliorate motor performance in mice Parkinson’s disease model
- Moreover, it has been demonstrated that cells with DA-ergic can be produced from both rat and human MSCs, and that transplantation of these cells showed an improvement of motor function in an animal model of PD

3. Heart Disease

- Cardiac transfer of stem and progenitor cells can have an adequate effect on tissue perfusion and contractile performance of the injured heart
- Stem cells have the potency to promote myocardial perfusion and contractile performance in patients who are suffering from acute myocardial infarction, advanced coronary artery disease, and chronic heart failure

4. Autoimmune diseases

- According to their ability to modulate immune responses, MSCs have also been proposed as a treatment for autoimmune diseases
- Patients who are suffering from severe autoimmune diseases do not respond to the standard therapy and often require autologous or allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

5. Liver diseases

- Liver failure and cirrhosis occur as a result of a variety of chronic hepatic injuries
- MSCs have the potential to be used for the treatment of liver diseases due to their regenerative potential and immunom-odulatory properties
- They display sequential and overlapping severe pathogenic processes that include severe inflammation, hepatocyte necrosis, and fibrosis/ cirrhosis, and carry a high mortality rate
- MSCs have been demonstrated to play an immune-modulatory role through producing inhibitory cytokines or inducing the development of regulatory T cells
- MSC therapy appears to be effective in regulating the immune response in tissue injury, transplantation, and autoimmunity in both animal models of liver disease and patients in clinical trials

6. Kidney disease

- Mesenchymal stem cells can migrate to deteriorate kidney tissue where they can generate an array of anti-inflammatory cytokines and chemokines that can alter the course of the injury
- Mesenchymal stem cells are thought to elicit repair through paracrine and/ or endocrine mechanisms that mend the immune response resulting in tissue repair and cellular replacement

Reference

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