CYTOLOGY

بسم الله الرحمن الرحيم

MID – Lecture #

Lecture Title

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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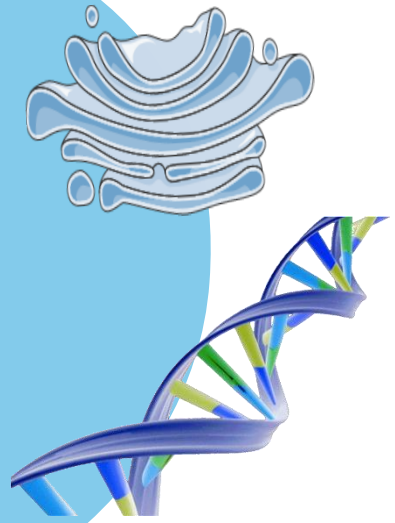


Lecture 1: Introduction & Endoplasmic Reticulum

Prof. Mamoun Ahram

School of Medicine

Second year, First semester, 2024-2025

Me!

- Prof. Mamoun Ahram
- Office location: first floor, School of Medicine, Main building
- Office hours: By appointment; Tuesday 12-2
- Come in groups

Course outline (1)

"Cell biology part "

Focus on diseases

- Introduction and biomembranes
- Endoplasmic reticulum and protein sorting
- Golgi apparatus
- Vesicular network
- Mitochondria and mitochondrial diseases
- Peroxisomes
- The nucleus
- Cytoskeletal networks
- The extracellular network
- Cell signaling, proliferation, differentiation, and death
- Cancer cells

Course outline (2)

“ Molecular biology part “

Focus on processes and techniques

- Introduction and the central dogma of molecular biology
- Gel electrophoresis, restriction endonucleases, recombinant DNA technology, DNA cloning, and RFLP
- The utilization of denaturation/renaturation concepts
  - Dot blotting and Southern blotting
- DNA replication
- PCR and DNA sequencing
- The human genome

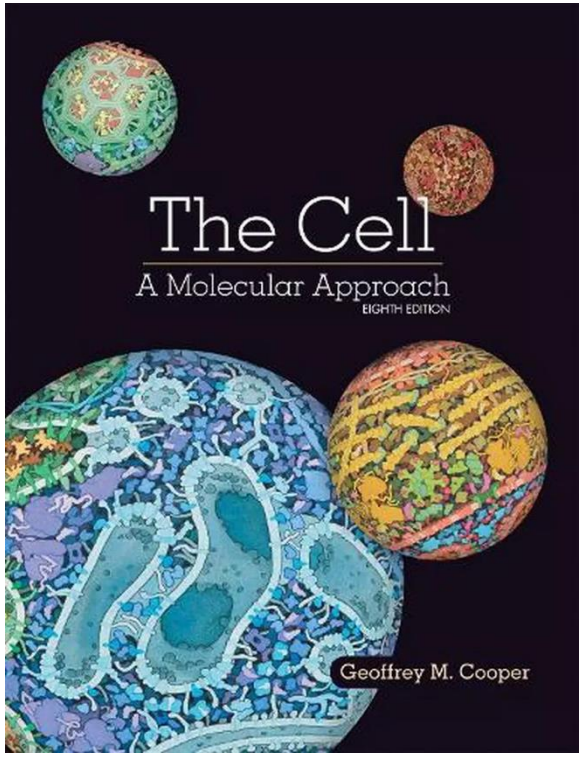
Important

- Transcription, **mechanisms** of regulation, and epigenetics
  - Coding and non-coding RNAs
- RNA detection, quantification, and detection
- Translation
- Yeast two-hybrid system
- DNA mutations
- DNA repair and CRISPR-Cas9

We get a result from tests (e.g PCR) and we should interpret and analyze the results

The textbook

- **The Cell: A Molecular Approach 8th Edition** by Geoffrey Cooper, Sinauer Associates is an imprint of Oxford University Press.



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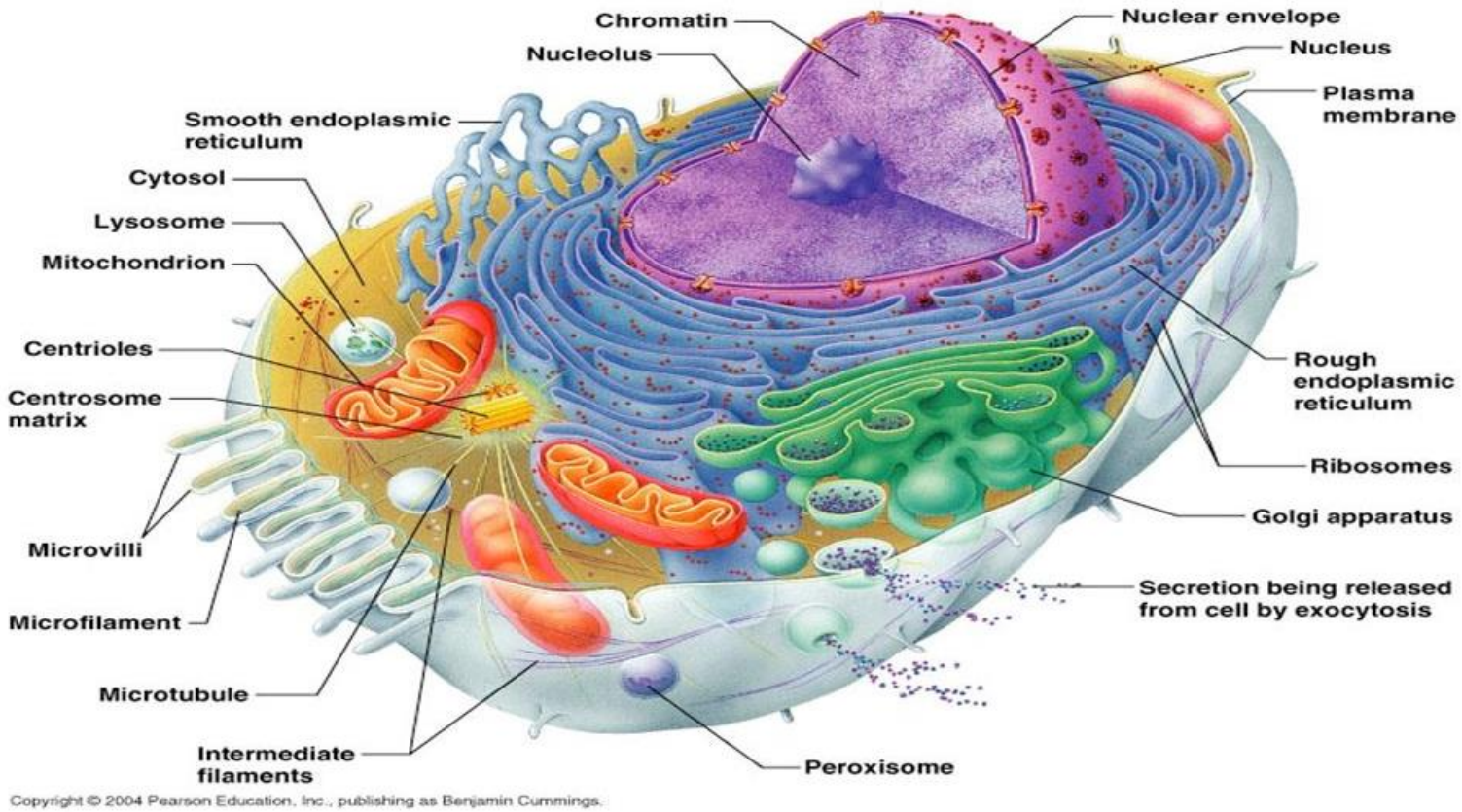
والله اكبر

The cell

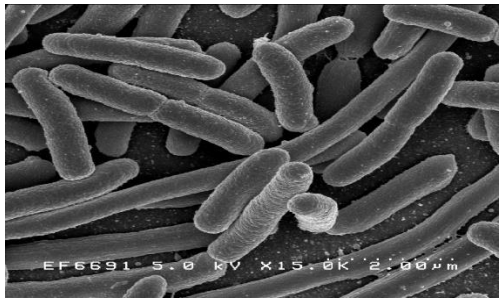
- ✓ The cell is the essential unit of life, enclosed by a lipid bilayer.
- ✓ Inside the prokaryotic cell we have machinery as ribosomes, DNA, RNA.
- ✓ Eukaryotes contain organelles (= structures surrounded by membrane) with essential and specific functions and organized in specific places.
- ✓ Each organelle depends on surrounding environment so they all function in harmony.



Any mutation affects the organelles in their functions (either increases or decreases)

What organisms do we use to study cells?

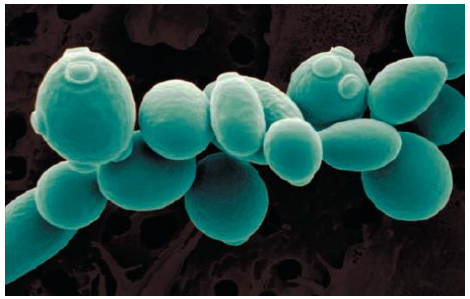
- Escherichia coli (E. coli)



Studying with models helps in understanding the functions of the cell.

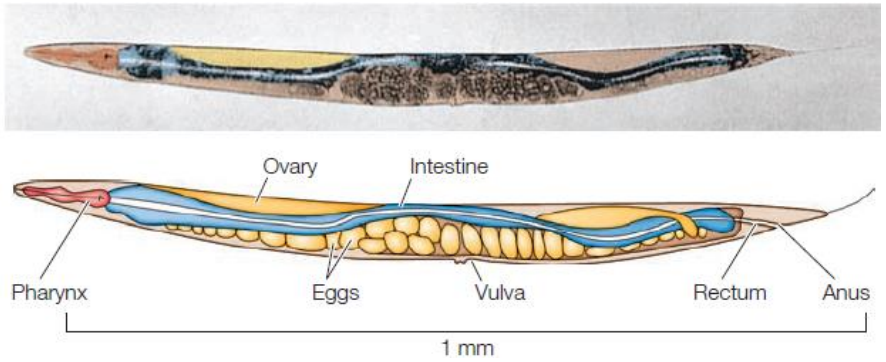
- ✓ **First one to study**
- ✓ **The simplest organism in prokaryotes**
- ✓ **Contains 4000 genes each of them makes proteins.**

- Yeast (Saccharomyces cerevisiae)



- ✓ **More complex model**
- ✓ **Eukaryote**
- ✓ **Grows really fast & single-celled.**

- Caenorhabditis elegans (C –elegans)



- ✓ **Microscopic worm**
- ✓ **Used to study cell death and cell differentiation (how the cell differentiates to specific tissue as nervous tissue).**
- ✓ **The immature C-elegans contains 1059 cells when they mature they become 959 cell..**

What organisms do we use to study cells?

- Drosophila melanogaster (ذبابة الفاكهة)

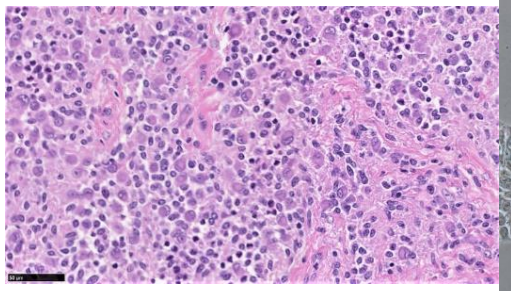
**clinical trials : experiments on human beings

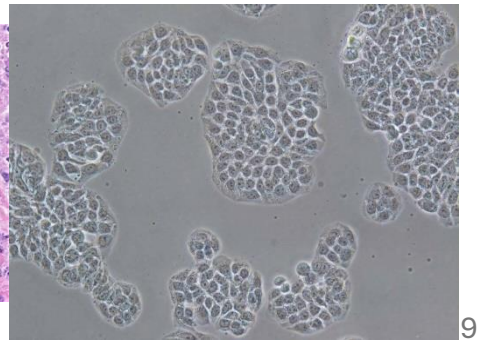
✓ **Used to understand cell differentiation.**

- Mice

✓ **Have same gene number of humans genome.**

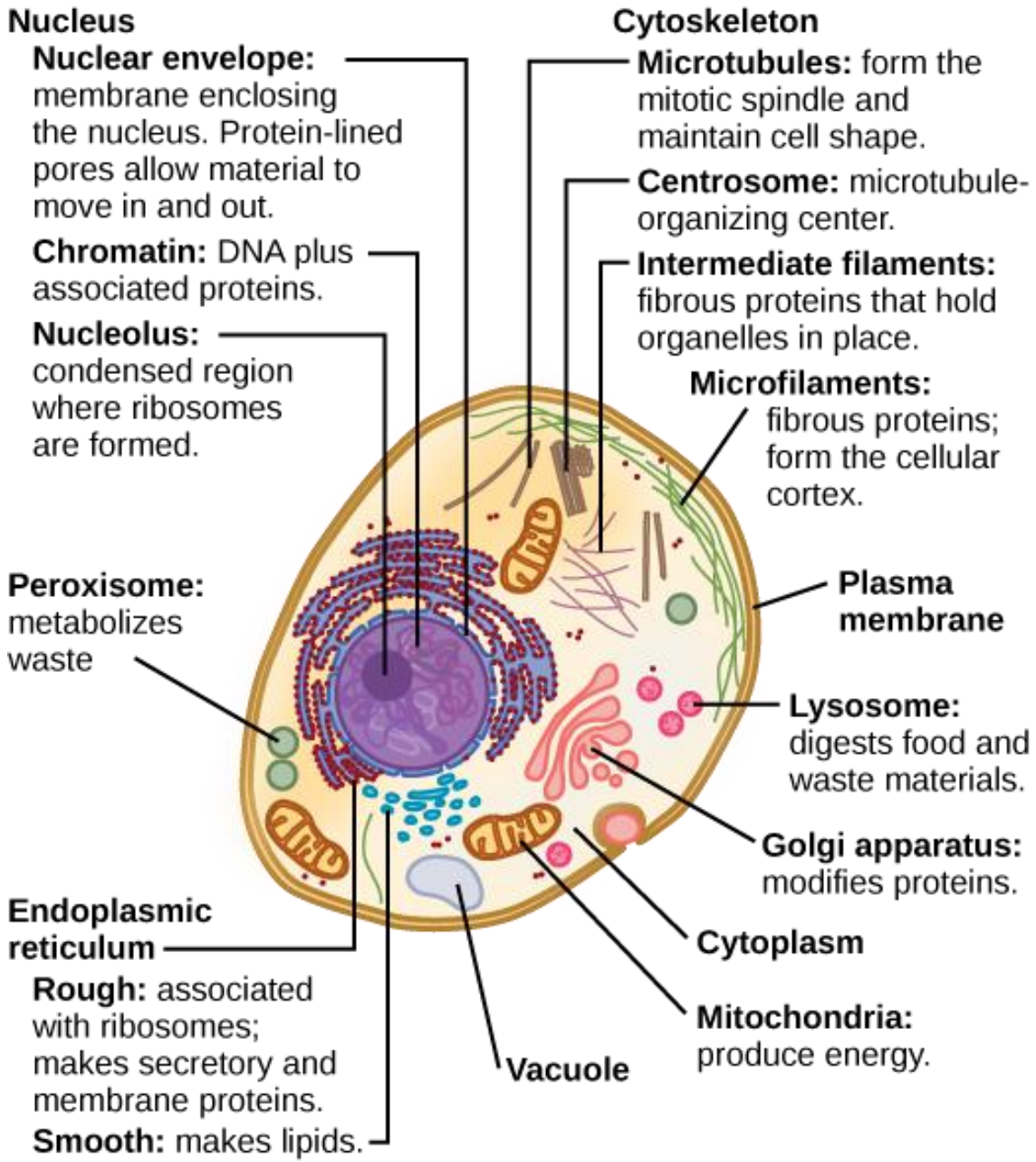
- Cultured cells and tissues





✓ **Proliferation of human cell in the laboratory**
✓ **Used to understand how it works in its environment.**

You should know the function of each :)



Organelles

You should know the overall function of each :)

TABLE 4-2 *Organelles*

Organelle	Function
Mitochondrion	transfers energy from organic compounds to ATP
Ribosome	organizes the synthesis of proteins
Endoplasmic reticulum (ER)	prepares proteins for export (rough ER); synthesizes steroids, regulates calcium levels, breaks down toxic substances (smooth ER)
Golgi apparatus	processes and packages substances produced by the cell
Lysosome	digests molecules, old organelles, and foreign substances
Microfilaments and microtubules	contribute to the support, movement, and division of cells
Cilia and flagella	propel cells through the environment; move materials over the cell surface
Nucleus	stores hereditary information in DNA; synthesizes RNA and ribosomes
Cell wall*	supports and protects the cell
Vacuole*	stores enzymes and waste products
Plastid*	stores food or pigments; one type (chloroplast) transfers energy from light to organic compounds

*Cell walls, large vacuoles, and plastids are found in the cells of plants and some other eukaryotes, but not in the cells of animals.

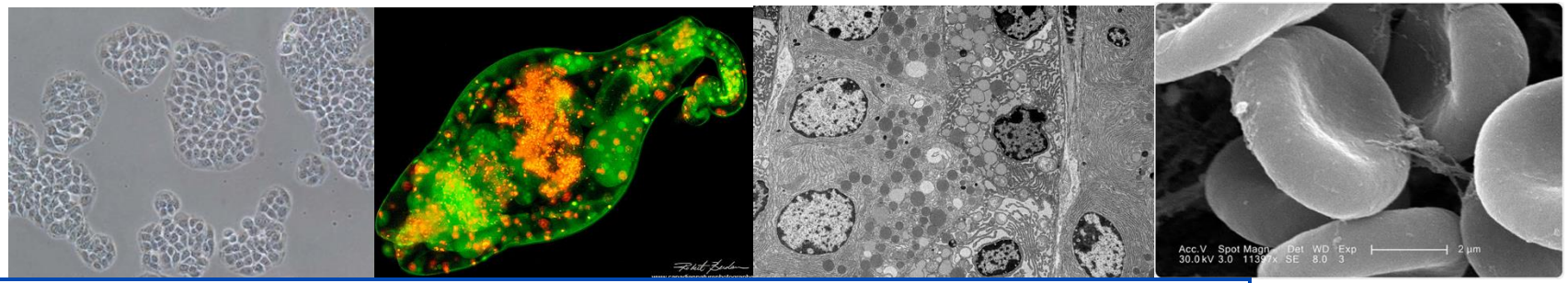
Major molecular components of cells

- Nucleic acids
- Carbohydrates
- Proteins
- Lipids (50% of mass of plasma membranes, 30% of mitochondrial membranes)
- Molecules function by interacting with each non-covalently.

Which governs the biochemical life of the cell.
Ex: interaction between ligand and its receptor is non covalent interaction (electrostatic , hydrophobic, Hydrogen bond ..)

How do we study cell components? Cell and protein detection

- Microscopy
  - Light, fluorescence (immunofluorescence), electron, scanning electron



Light microscope: we can see the cell with different magnifications

Fluorescence: labeling molecules with specific colors (lipid , sugar , DNA)
The molecule is exposed to light in a specific wavelength (green light for example) which activates the electrons and goes to higher orbit then they come back to their orbit and release the energy (as form of light , light = another wavelength (yellow light)).

Immunohistochemistry: targets the tissue section with antibody , this antibody binds to specific protein in thee cell in tissue section , so the protein is showed with specific color maybe fluorescence (so you determine that the protein is present).

Immunofluorescence: antibodies (have specific tag molecule which makes the antibody fluorescent) that target specific proteins inside the cell so the protein lights up (this light comes from antibodies not proteins)

Electron: zooming into specific organelles (peroxisomes, mitochondria..) with a high magnification.

Scanning electron: 3D image that determine the cell and organelles shapes

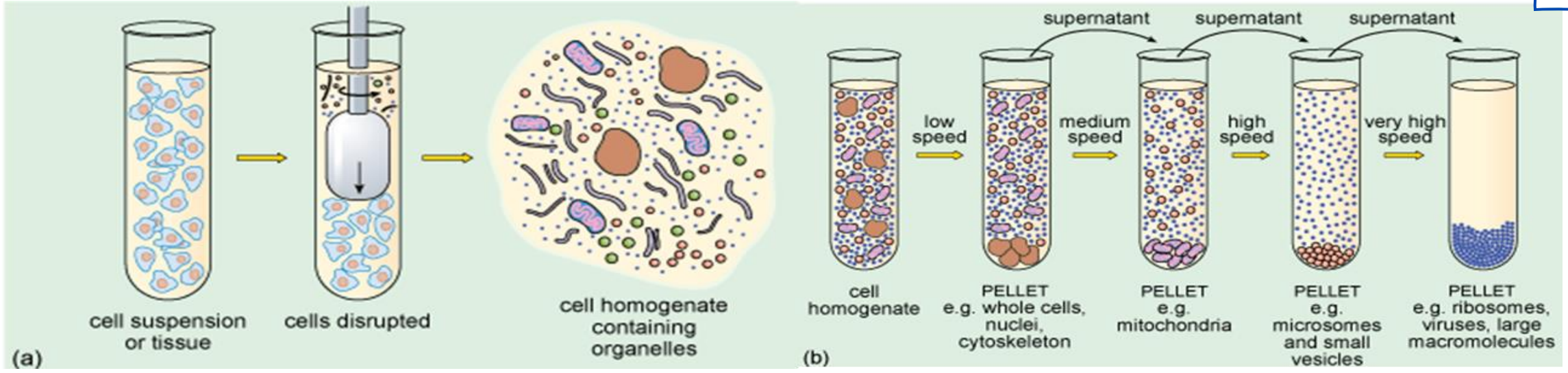
How do we study cell components? Cell and protein detection

- Cell fractionation

cell fractionation allows separation of different organelles inside the cell depending on the density and speed needed to separate the organelle.

To study a specific protein that exists in mitochondria we need to extract it, by taking a collection of cells with all components as they are and putting them in a centrifuge جهاز الطرد المركزي (this device spins the solution every time with different speeds). And since they differ in size and density, structures are precipitated depending on the speed (slow speed -> dense & heavy structures are precipitated (pellet) and light & less dense structures are still in the solution (supernatant). And then we put the supernatant in the centrifuge and so on until we get the structure we wanted to get.

Know them , nuclei heavier than ribosome (going from left to right)

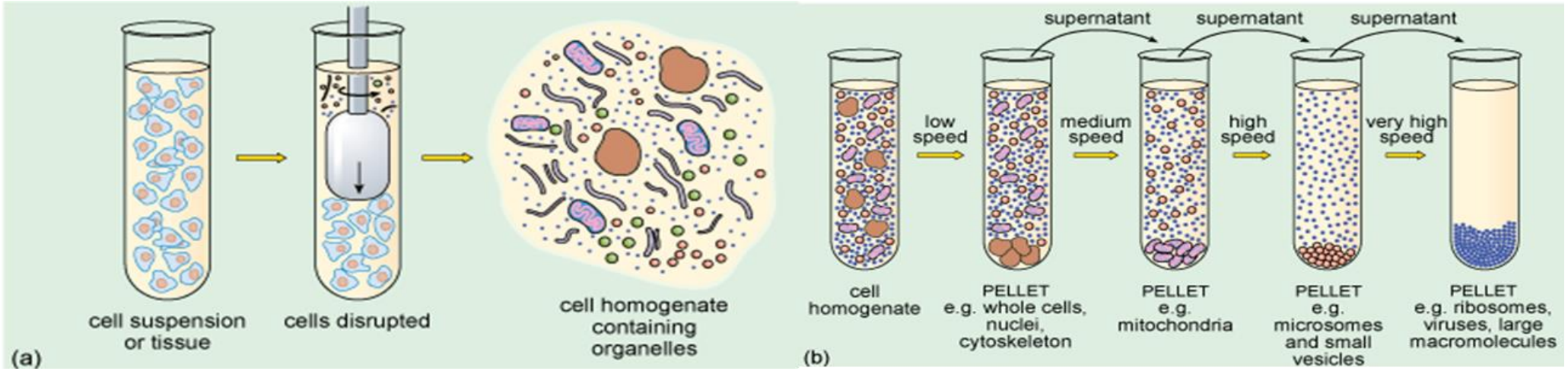


How do we study cell components? Cell and protein detection

Explained again:

- ✓ **In cell fractionation, the process of separating cellular components is often done through centrifugation. When you centrifuge a sample, heavier cellular components sediment to the bottom of the tube due to the force exerted by the spinning motion. The liquid above the sedimented material is known as the supernatant.**

- ✓ **When you transfer the supernatant to another tube and increase the centrifugation velocity, you're effectively separating out lighter cellular components that remain in the supernatant. Each successive centrifugation at higher velocities allows you to isolate different cellular organelles or components based on their size and density.**



Biochemical composition of plasma membranes

The membrane is composed of two layers of phospholipids, the fatty acids are facing each other and the phosphate groups are facing the outside and the inside of the cell.

The plasma membrane has 45% proteins and 45% lipids and 10% carbohydrates

Do NOT memorize the numbers but know which structure has the most protein,lipid...

Membrane	Protein (%)	Lipid (%)	Carbohydrate (%)	Weight fraction of protein	Ratio of protein to lipid
Plasma membranes					
Myelin	18	79	3	0.18	0.23
Blood platelets	33—42	51—58	7.5	0.4	0.7
Mouse liver cells	46	54	2—4	0.46	0.85
Human erythrocytes	49	43	8	0.49	1.1
Amoeba	54	42	4	0.54	1.3
Rat liver cells	58	42	(5—10)*	0.58	1.4
HeLa cells	60	40	2.4	0.6	1.5
Nuclear envelope of rat liver cells	59	35	2.9	0.59	1.6
Retinal rods, bovine	51	49	4	0.51	1.0
Mitochondrial outer membrane	52	48	(2—4)*	0.52	1.1
Sarcoplasmic reticulum	67	33	—	0.67	2.0
Chloroplast lamellae, spinach	70	30	(6)*	0.7	2.3
Mitochondrial inner membrane	76	24	(1—2)*	0.76	32
Gram-positive bacteria	75	25	(10)*	0.75	3.0
Halobacterium purple membrane	75	25		0.75	3.0

However, myelin (nerve cell coating) has 80% lipids which has the highest amount of lipids.

Every organelle has different composition depending on the function, e.g the inner mitochondrial membrane has a lot of proteins because it is the site of electron transport chain.

Lipid composition of organelles

Table 1: *Head group composition of the membranes of some mammalian liver cells, erythrocytes , and nerve cells in weight percent. Adapted from Jamieson and Robinson (1977). Abbreviations: PC = phosphatidylcholines, PE = phosphatidylethanolamines, PS = phosphatidylserines, PI = phosphatidylinositols, SM = sphingomyelin, CL = cardiolipin.*

Membrane	PC	PE	PS	PI	SM	CL	Glycolipid	Cholesterol	Others
Erythrocyte (human)	20	18	7	3	18	–	3	20	11
Plasma (rat liver)	18	12	7	3	12	–	8	19	21
ER	48	19	4	8	5	–	tr	6	10
Golgi	25	9	3	5	7	–	0	8	43
Lysosome	23	13	–	6	23	≈ 5	–	14	16
Nuclear membrane	44	17	4	6	3	1	tr	10	15
Mitochondria	38	29	0	3	0	14	tr	3	13
Neurons	48	21	5	7	4	–	3	11	1
Myelin	11	17	9	1	8	–	20	28	6

- **Myelin a high amount of lipids (especially glycolipids for cell interaction & cholesterol for rigidity)**

Do NOT memorize the numbers

Cholesterol is an essential component of animal plasma membranes.
It is not present in bacteria and plant cells, but the latter cells contain sterols.

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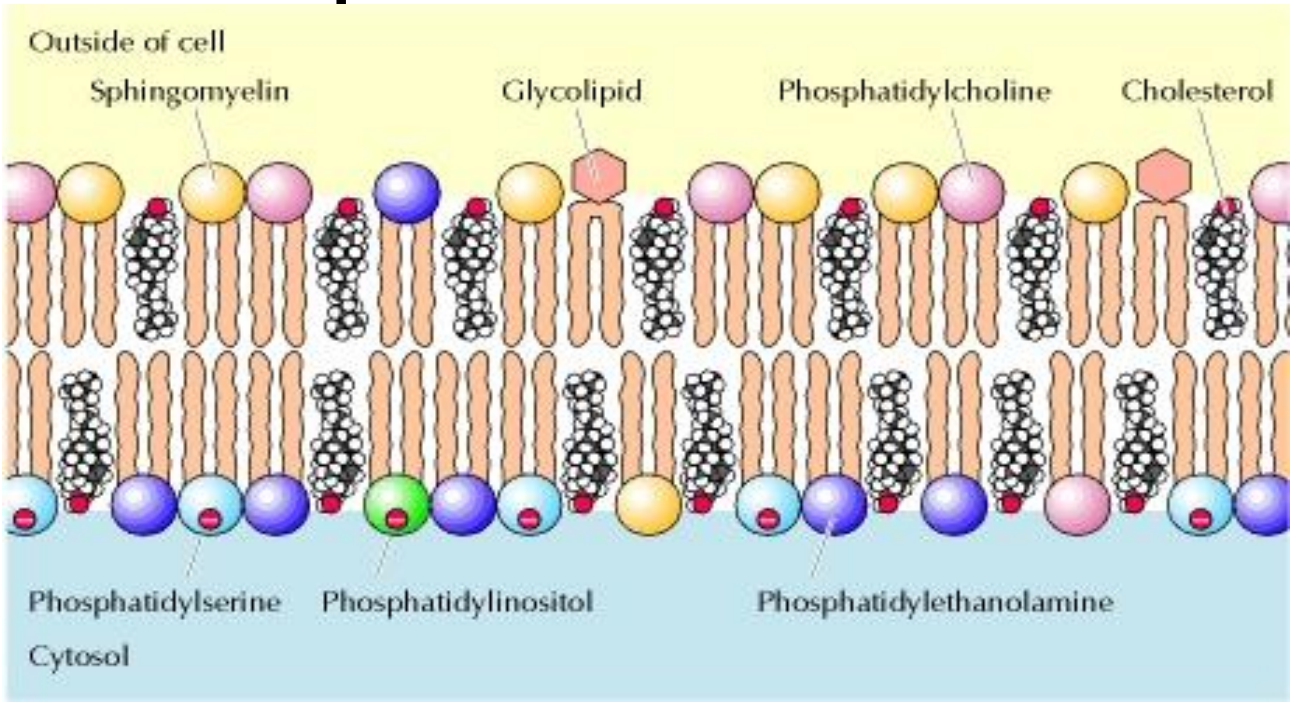
Composition and properties of plasma membranes

- The phospholipids are asymmetrically distributed between the two halves of the membrane bilayer.
  - The outer leaflet: Ⓟcholine, sphingomyelin
  - The inner leaflet: Ⓟethanolamine, Ⓟserine, Ⓟinositol (minor)
    - Ⓟinositol has a role in cell signaling.

sending signals from outside the cell to the inside of the cell.
(that's why it's in the inner leaflet)

  - Glycolipids are found exclusively on the outer membrane.



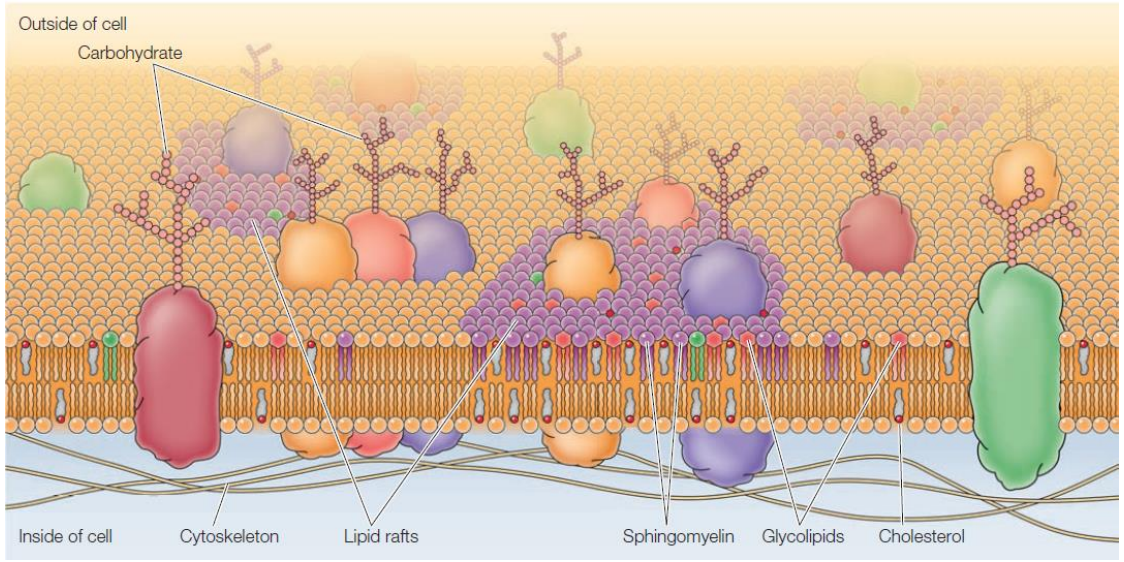
The plasma membrane follows the fluid mosaic model, therefore the distribution of structures is NOT random, they are present as clusters in certain regions of the cell. e.g different types of phospholipids are distributed depending on the function

Lipid rafts

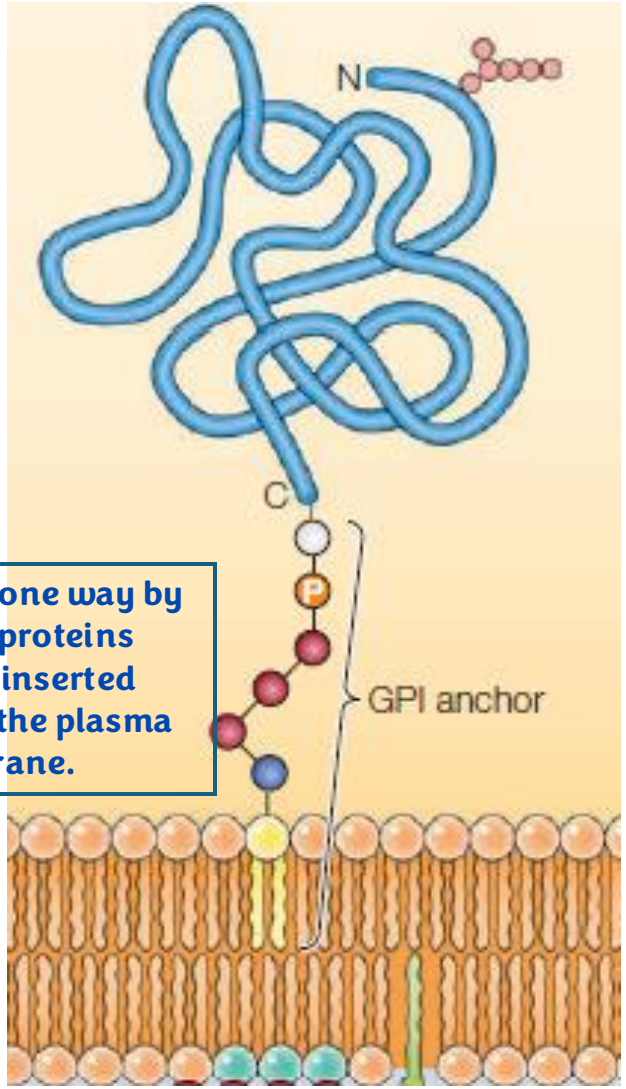
- Specialized membrane regions with clusters of cholesterol and the sphingolipids (sphingomyelin and glycolipids).

- Rafts are enriched in glycosylphosphatidylinositol (GPI)-anchored proteins, and proteins involved in signal transduction and intracellular vesicular trafficking (transport).

And enriched in cholesterol (more than any other region in the plasma membrane)



✓ **It has proteins inserted with glycosylphosphatidylinositol (GPI)**
GPI has two parts, a sugar part and a lipid part which is integrated inside the plasma membrane



✓ **This is one way by which proteins can be inserted inside the plasma membrane.**

Caveolae
(Latin for “little caves”)

- They are a subset of lipid rafts that require cholesterol for their formation.
- They are formed the membrane protein caveolin, which interacts with cholesterol and the cytoplasmic protein cavin.
- They are important for several cellular activities, including endocytosis, cell signaling, regulation of lipid transport, and protection of the plasma membrane against mechanical stress.

Lipid rafts have Cavin proteins that help in the formation of a protrusion (نتوء) outside to the inside of the cell by clustering proteins to surround signaling molecules and proteins in a specific region.

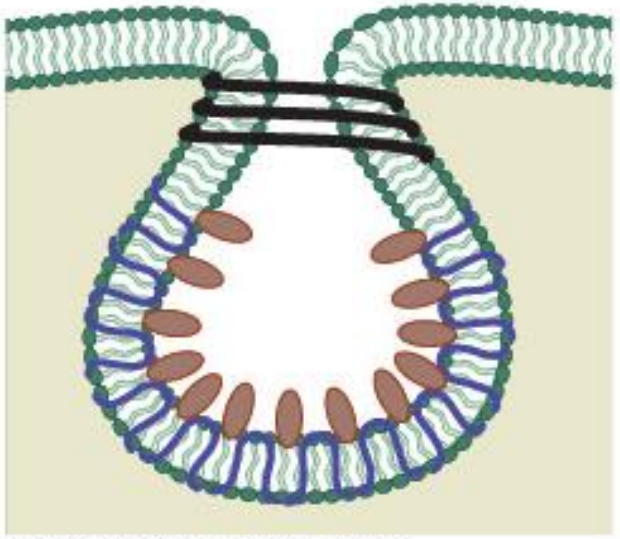
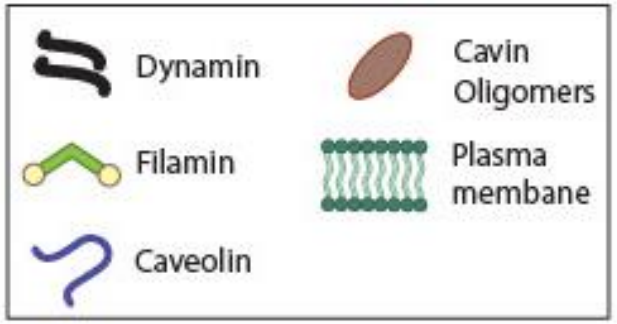


Fig. Simplified model of the Caveolae



Note: Endocytosis is the process of engulfing substances from the outside of the cell to the inside

caveolae

Electron microscope

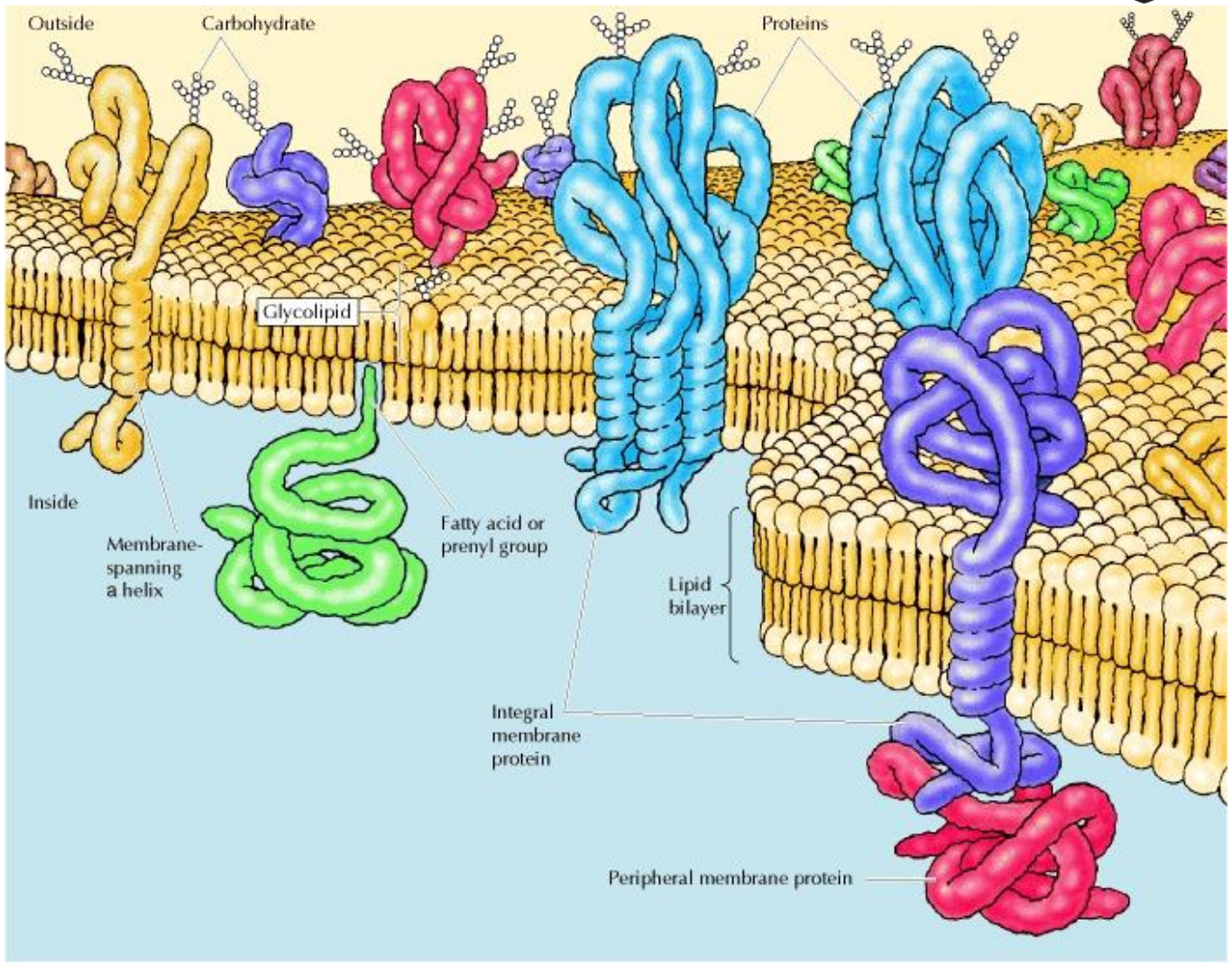
0.2 μm

Membrane proteins

Proteins are associated to the membrane in different ways/mechanisms

- **They can be integrated inside the membrane by a single region we call it a “single integral protein” (just like the yellow structure) the transmembrane domain of this protein is alpha helical and hydrophobic and has two facing sides (one to the outside and one to the inside)**

- **Proteins can be integrated via multiple transmembrane domains (e.g the blue structure)**

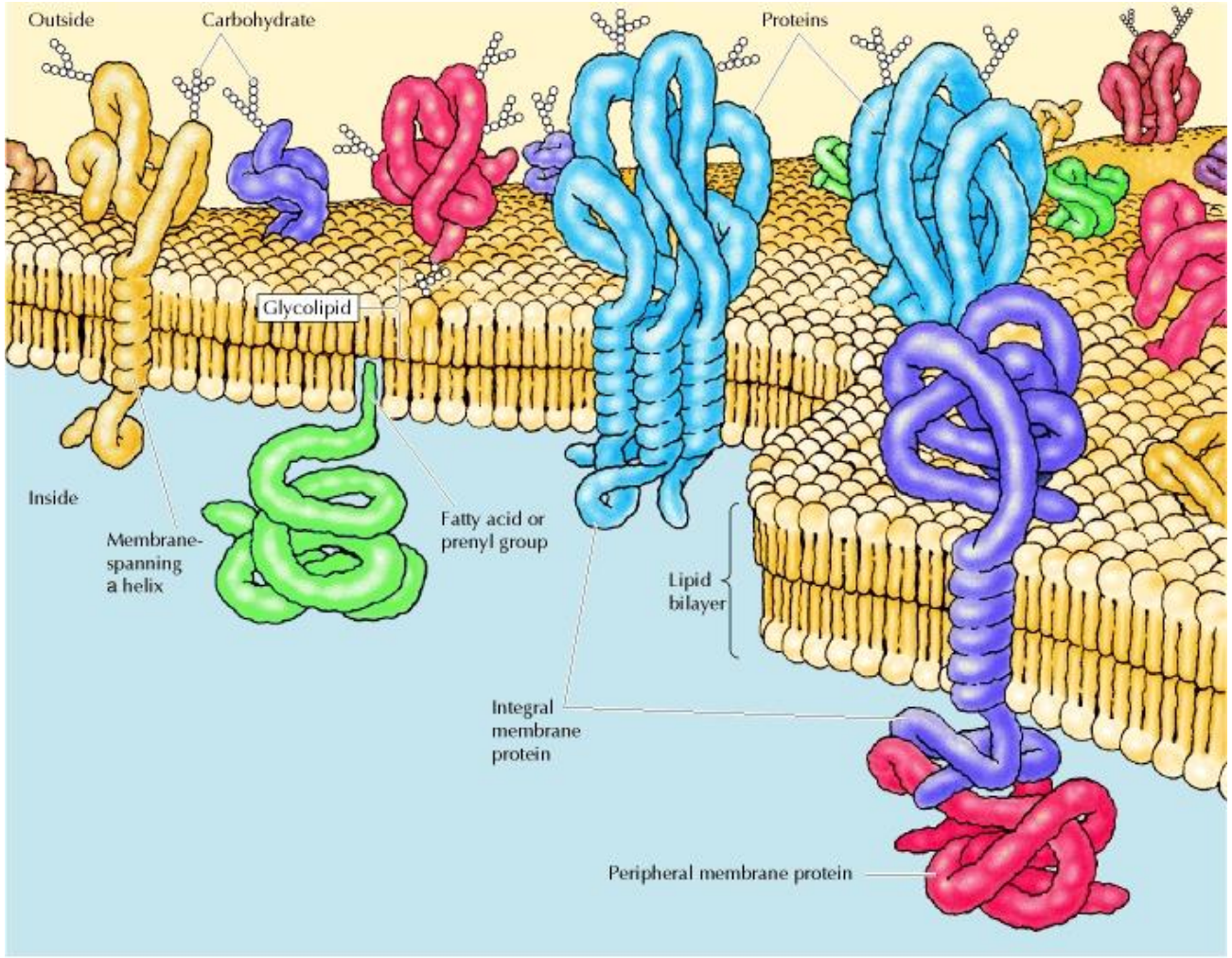


Membrane proteins

- **Proteins can be associated with the membrane via GPI (as mentioned earlier) lipid part is integrated inside membrane**

- **Proteins can be associated only with a fatty acid molecule that is inside of the membrane (e.g the green structure)**

- **Proteins also can be associated with the membrane by interacting electrostatically with the phosphate groups on the surface of the membrane**



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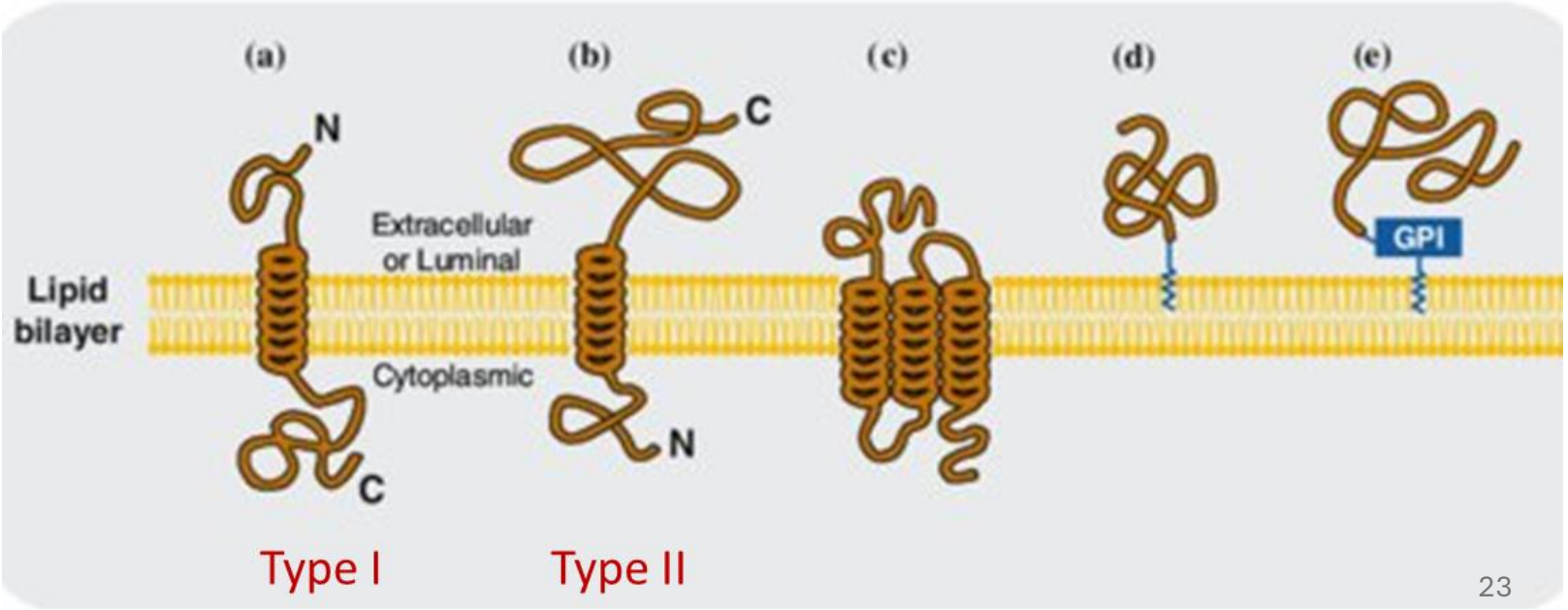
Types of membrane proteins

- Peripheral membrane proteins are indirectly and loosely associated with membranes through protein-protein interactions, mainly ionic bonds.
- Integral membrane proteins have some of their helical parts inserted into the lipid bilayer.
  - Single-pass (type I or II) or multi-pass proteins.
- Lipid-anchored membrane proteins (myristoylation, palmitoylation , glycosyl-phosphatidylinositol)

✓ **Even a protein with a single transmembrane domain differs in its organization (e.g the N terminus can be outside (type I) or inside (type II)) therefore, we have different mechanisms of inserting proteins inside the cell.**

✓ **The order/organization is regulated**

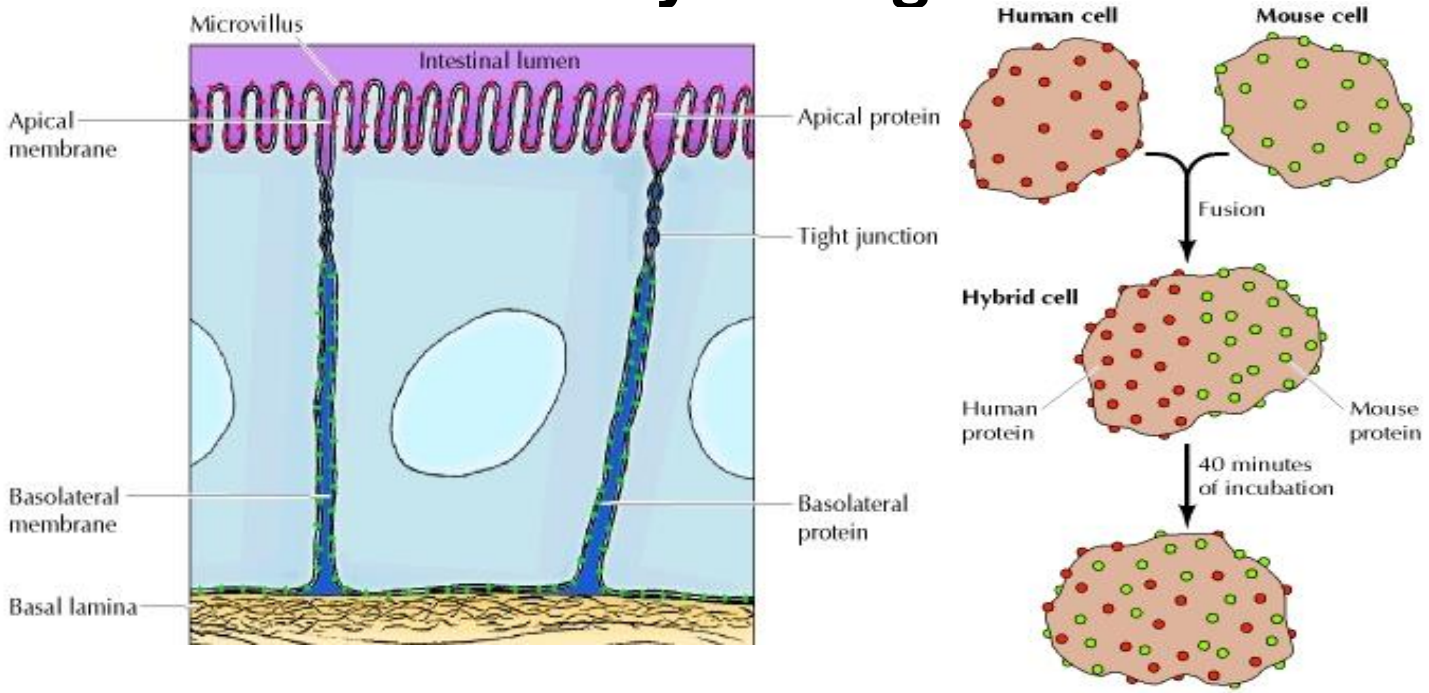


Protein mobility

The protein movement is important in determining the region of the receptors (e.g insulin receptors)

The proteins & lipids inside the cell are dynamic; they keep moving inside the plasma membrane.
In lipid rafts, the proteins will move and eventually will cluster in a specific region

- **Proteins and lipids are able to diffuse laterally through the membrane.**
- **The mobility of membrane proteins is restricted by**
  - **Their association with the cytoskeleton**
  - **Specific membrane domains, which maintain the specific distribution of apical and basolateral proteins**
  - **Specific lipid composition (e.g. lipid rafts).**



The plasma membrane of cells can be organized in different ways as well; for example the proteins of the apical portion (upper part) of epithelial cells are different from the basal portion (lower part)

This is a picture to show you the movement of proteins and lipids: two cells with different fluorescent-tagged-proteins are fused together, after incubation we can see the distribution of the two proteins on the surface of the cell

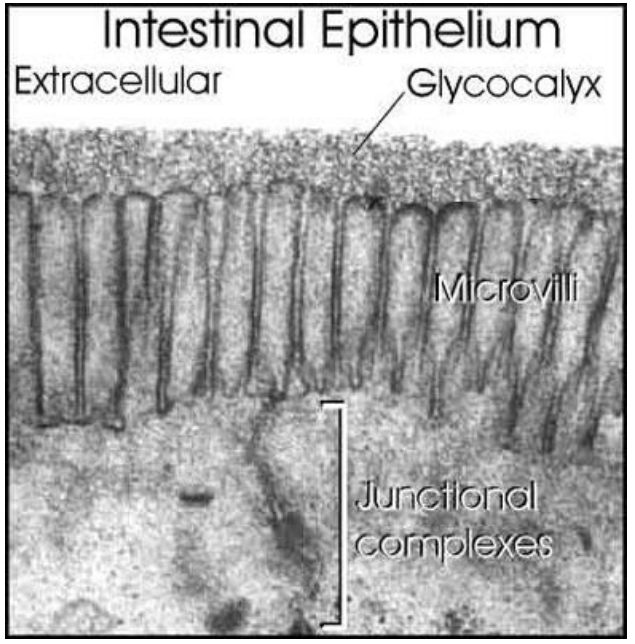
Glycocalyx

The surface of cells (plasma membrane), specifically on top of microvilli, can be coated by sugar molecules (and some of them are heavily coated) which is called glycocalyx.

- The surface of the cell is covered by a carbohydrate coat, known as the glycocalyx, formed by the oligosaccharides of glycolipids and glycoproteins.

Functions:

- Cell-cell interactions such as immune cells
- Protection of cell surface from ionic and mechanical stress
- Formation of a barrier for microorganisms



Bye-Bye

{فَاقْضِ مَا أَنتَ قَاضٍ ۖ إِنَّمَا تَقْضِي هَٰذِهِ الْحَيَاةَ الدُّنْيَا}

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide #20 Slide #14	Proteins can be associated with the membrane via GPI (e.g the violet structure) The membrane is composed of two bilayers of phospholipids	Proteins can be associated with the membrane via GPI The membrane is composed of two layers of phospholipids
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

هي صعبة صعبة، الشغل صعب، والتعامل مع الناس صعب، وإن الواحد يعمل فلوس صعب، و يصحى بكير صعب، وإن احنا نروح الشغل عشان دخل لا يكفى برضو صعب. بس سيدنا عمر بن عبد العزيز قال: لو أن الناس كلما استصعبوا أمرًا تركوه، ما قام للناس دنيا ولا دين ...فمينفعش نبطل نعمل حاجة أو نبطل نحاول عشان الحاجة دي صعبة، لأن هي دي الدنيا و مفروضة عليك فاصلِح العمل و {الآخرة خيرٌ و أبقى }.

اللهم وكلت أمري إليك، وألجأت ظهري إليك، وفوّضت أمري إليك، لا رادّ لفضلك، ولا مانع لعطائك، لا إله إلا أنت سبحانك إني كنت من الظالمين اللهم إنّي أسألك أن توفقنا ، وأن ترزقنا النجاح والتفوق يا كريم. اللهم هوّن عليّنا صعوبة الامتحانات، وأكرمنا بها في أكمل الدرجات، واهدنا إلى الإجابة الصحيحة.

اللهم ارزقني فهم النبيين وحفظ المرسلين وإلهام الملائكة المقربين

اللهم صلّ وسلّم وبارك على سيّدنا محمد

7 October