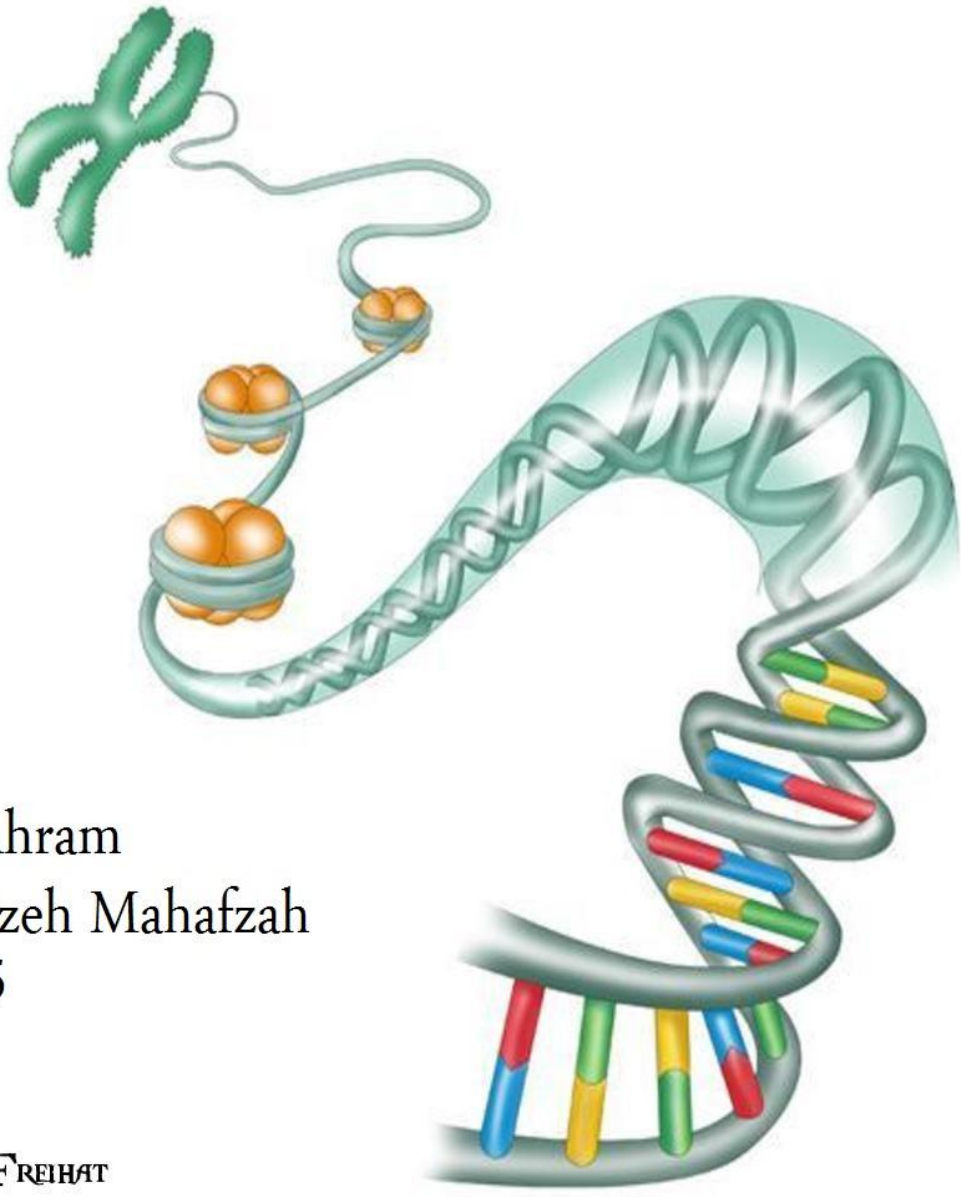
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Lecture # 2
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Date: 5/2/2015
Price:

Designed by Nadeen Al-Freihat

Major components of cells

We have said before that the cell is the basic unit of life . We mentioned also that cells' membranes are composed of macromolecules , three of them are polymers (proteins , carbohydrates and nucleic acids) , but the forth is not (which is lipids) .

For any cell to exist it has to be enclosed (coated) by a membrane , and this membrane is composed of lipids, proteins and carbohydrates . Mainly , cellular membranes are composed of lipids and protein as carbohydrates form the smallest portion of membranes' components .

Different types of cells and organelles as well may differ in the percentages of these components in their membranes : for example, RBC's membranes have a ratio of lipids / proteins equals to 40% / 52% . But if we look to myelin sheath, we'll find that its membrane is mainly composed of lipids . In contrast, if we look to mitochondria, we'll find that their membranes are mainly composed of proteins, because their function requires more proteins.

We have different classes of lipids, the most common one in membranes is phospholipids . phospholipids are further divided into many types (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol … etc). If we look at these components, we'll find that they are distributed differently in the membranes of different types of cells and organelles. For example, mitochondrial membrane is mainly composed of phosphatidylethanolamine and phosphatidylcholine. In contrast, phosphatidylethanolamine does not exist much in the ER membrane, but we have a lot of phosphatidylcholine.

We can find other differentiations by comparing the different classes of lipids, for example myelin sheath has a unique large amount of glycolipids in its membrane.

NOTE: the doctor said that you need to know the names and what is abundant, but you do not necessarily need to memorize the percentages.

In animal plasma membranes, there are small amounts of cholesterol. This cholesterol exists in animal cells, NOT in bacterial cells NOR in plant cells (plant cells rather have other types of sterols). So, cholesterol is a feature of animal cells.

What is also important about membrane lipids is that they are dynamic (motile), they are not static. In other words, they move, they change place horizontally or vertically, they can flip. ** we will talk about these movements later when we talk about the ER**.

Table 8.18. Mass % Biochemical Composition of Cell and Organelle Membranes[531,939,996,997]

Type of Membrane Molecule	Liver Cell Plasma Membrane	Red Cell Plasma Membrane	Myelin Sheath	Mitochondrion Inner/Outer Membranes	Endoplasmic Reticulum Membrane	*E.coli* (Bacterial Membrane)
Lipid	--	40%	~81%	~24%/~48%	--	--
Protein	~50%	52%	~19%	~76%/~52%	~50%	~50%
Carbohydrate	--	8%	--	--	--	--
Lipid Class:						
Cholesterol	17%	23%	22%	3%	6%	0%
Phospholipids						
Phosphatidylethanolamine	7%	18%	15%	35%	17%	70%
Phosphatidylserine	4%	7%	9%	2%	5%	trace
Phosphatidylcholine	24%	17%	10%	39%	40%	0%
Sphingomyelin	19%	18%	8%	0%	5%	0%
Glycolipids	7%	3%	28%	trace	trace	0%
Other lipids	22%	13%	8%	21%	27%	30%

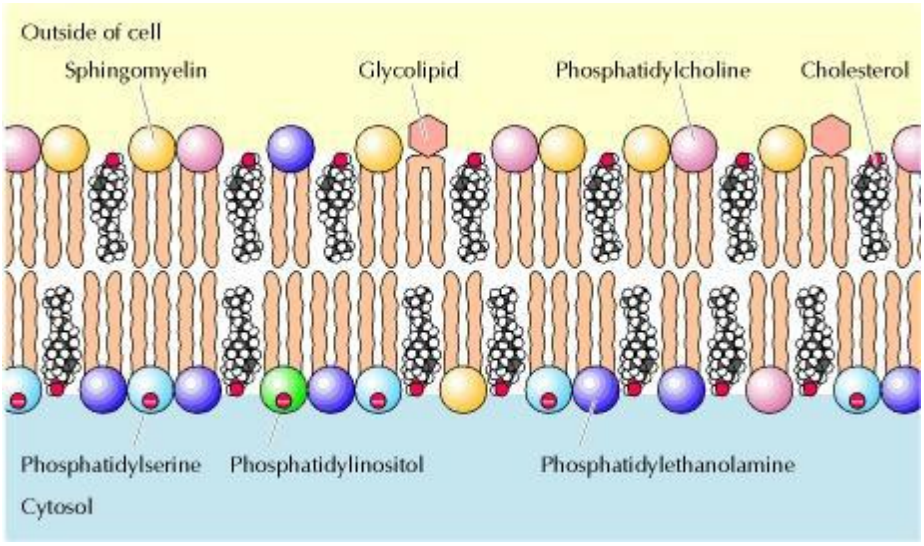
A plasma membrane has two leaflets of phospholipids: an outer leaflet (which is exposed to the outside of the cell) and an inner leaflet (which is exposed to the inside of the cell). If we look at the phospholipids of each leaflet, we will find that they are also not evenly distributed between the inner and outer leaflets of the same membrane:

In the Outer leaflet → there is a lot of phophatidylcholine and sphingomyelin.

In the inner leaflet → there is phophatidylserine, phophatidylethanolamine and phophatidylinositol.

Phophatidylinositol is a very minor component in plasma membranes (in amount), but it plays a very important role functionally. So it is not only a structural lipid, rather it also has a function by sending signals from outside to inside the cell. So it can be extracted and multiplied, as a result the signal can be sent to components inside the cell.

Phophatidylinositol and Phophatidylserine are negatively charged, and that makes the overall charge of the cytoplasmic face of the plasma membrane negative as well.



In the past they said hypothetically that the plasma membrane has a fluid mosaic structure because there is an overall distribution of lipids, proteins and carbohydrates in the membrane. But now they notice that the structure of the plasma membrane does not exactly look like a mosaic (a repetition of structures), rather it is like a "painting" [in a painting, there are areas with special contents] as it contains areas that are specialized; one of these areas is known as **Lipid Rafts.**

So again lipid rafts are: areas in the plasma membrane that differ from the rest of the surface of the plasma membrane, because when they looked to these areas they noticed that they are clusters of specialized certain types of lipids like sphingomyelin as well as glycolipids.

The idea is that sphingolipids provide an ordered environment for lipids as they (sphingolipids) are recruited by moving to form clusters (lipid rafts).

Lipid rafts are rich in proteins that are anchored to the cell membrane via a molecule known as GPI (glycosylPhophatidylinositol). These proteins are important in **cell signaling** inside the cell as well as **trafficking**.

Trafficking is: the regulation of membrane (or vesicular) movements within the cell; in other words when there is endocytosis (when a vesicle pinches off the membrane to the inside) or exocytosis (when a vesicle fuses with the membrane) in the cell, the movement of the formed vesicles from one part of the cell to another part must be regulated (because a vesicle cannot decide where to go by itself) and this is what we mean by "trafficking". That's why they noticed that lipid rafts are active regions of membrane movement (endocytosis and exocytosis).

Lipid rafts have importance in the field of diseases and their treatment. For example, HIV (which causes AIDS) ,after its replication inside the cell, it is released from the cell (by budding), in fact it is released from the lipid rafts. So it seems that the virus attaches to certain specific components that exist in the lipid raft. As a treatment, we should know what these components are then we can make a bulk that competes the virus on these components.

Also lipid rafts are important for influenza virus to get into the cells, because it actually binds to components in the lipid rafts.

Another example of this is prion disease. prion is a protein that has an altered structure, its alpha-helicals all changed into beta-sheets and this causes cross links and aggregation with other proteins. What is important to know here is that prion protein is converted to the abnormal protein (which has beta-sheets) in the lipid rafts.

Membrane proteins:

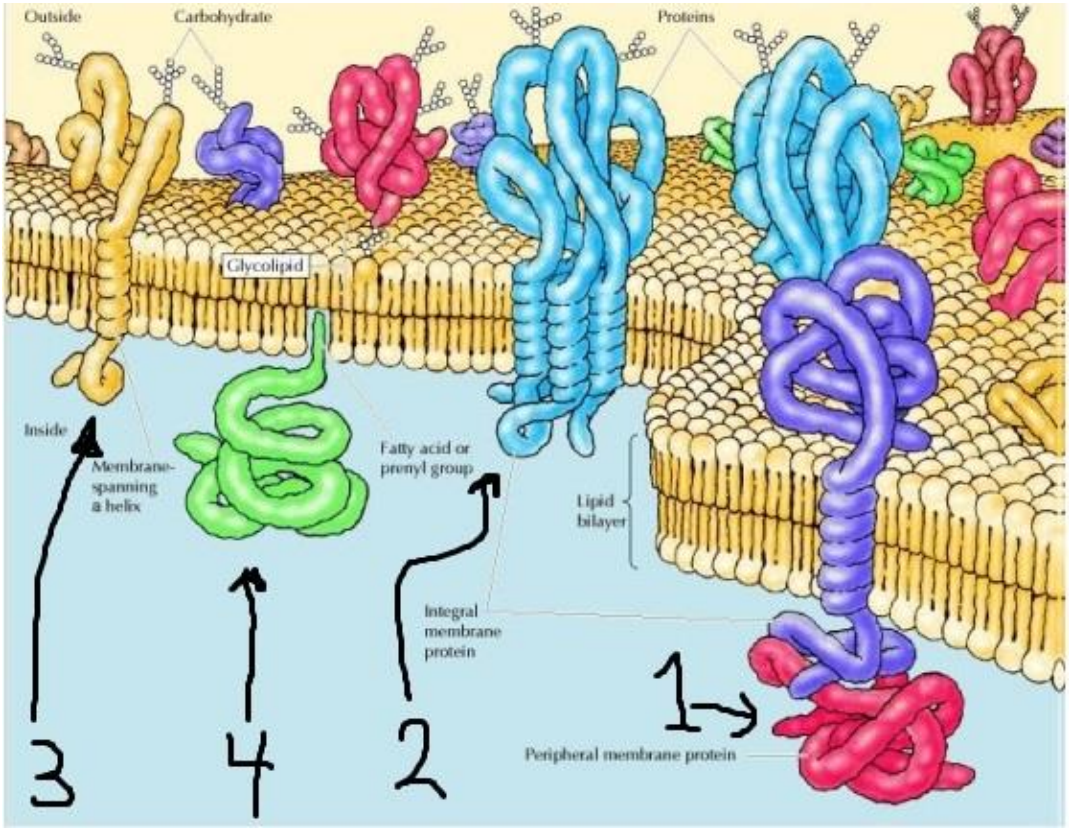
Now we will talk about membrane proteins. Generally, proteins form 50% of plasma membrane components. there are many ways by which proteins can attach to the membrane.

Some proteins are peripheral, means that they exist outside the membrane, means that they do not insert into the membrane, but they also can be named "membrane proteins" because they exist at the surface of the membrane (red to the right, #1).

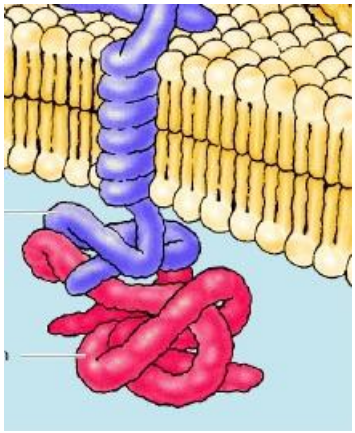
Other proteins are called integral proteins because they insert themselves into the membrane. They might exist in the both sides of the cell, Such as the one that is blue (#2), or they may exist in one side of it. We can also notice that they can insert themselves in the membrane one time like the yellow one (#3),

or many times like the blue one (#2). (We will talk about mechanisms by which proteins integrate into the plasma membrane).

Also we have anchored proteins like the green one (#4) at the left.

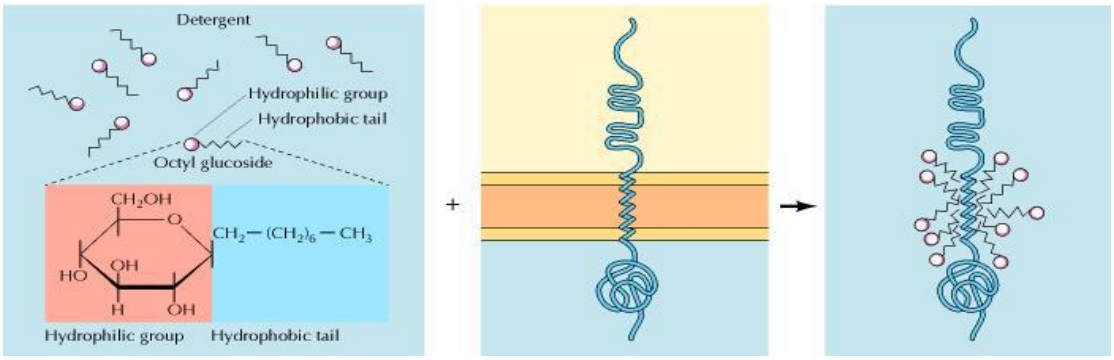


Peripheral membrane proteins: they are loosely attached, as they bind other membrane proteins by ionic or electrostatic interactions. This means that they can be removed easily with a "gentle" treatment. For example, by changing the PH making it very high or very low, or by changing the



salt concentration. These changes will disrupt (break) the interactions between the peripheral (red) protein with the one it is bound to (the purple one). We can then release this protein without disrupting the membrane, and this is the way by which we can study peripheral proteins.

Integral membrane proteins: for isolating them, we do need a harsh treatment that disrupts (breaks) the plasma membrane. This is done by using detergents such as SDS (Sodium Dodecyl Sulfate) because it is an amphipathic molecule that has an ionic part and a hydrophobic part.



The hydrophobic part does two things:

1. It inserts into the membrane easily, it then interacts and disrupts the hydrophobic interactions between the fatty acids of membrane lipids, so that we can break the structure of the plasma membrane.
2. It interacts with the **transmembrane domain** of the integral protein (the exact domain that is inserted in the membrane) as it is hydrophobic.

By these two activities we achieve two goals: we disrupt the plasma membrane, and the protein is dissociated from the plasma membrane.

The question now is : what is special about the transmembrane domain ??

a. It is hydrophobic.
b. It has a special structure:

In eukaryotic cells, mainly the transmembrane domain is actually alpha-helical, and it is made of 20 to 25 hydrophobic amino acids.

On the other hand, **in prokaryotic** cells (like bacterial cells) and in mitochondrion (because it used to be a bacterial cell … as they think that the origin of the mitochondria is bacterial cells), the transmembrane domain is actually composed of beta-sheets. It also can be complex (like beta-barrel): an example of this is a protein called porin. It is found in E.coli, mitochondria and chloroplasts and it forms a channel by which ions and other molecules can go into or out of bacterial or mitochondrial cells. (note: the R groups of the amino acids that are exposed to the lumen of the channel are hydrophilic and those of amino acids that are exposed to the outside are hydrophobic).

- We have a third type of proteins that are anchored to the plasma membrane by adding a lipid to these proteins (they are attached to the membrane indirectly by binding a lipid).

There are different types of attachment:

1. Myristoylation : which means that we have a fatty acid myristic acid (myristate), a 14-carbon fatty acid, attached to a **terminal** Glycine of a protein.
2. Palmitoylation: the addition of palmitic acid, a 16-carbon fatty acid, to an **internal** cysteine of a protein

(internal means that it is does not exist in the C terminus nor in the N terminus of the protein).

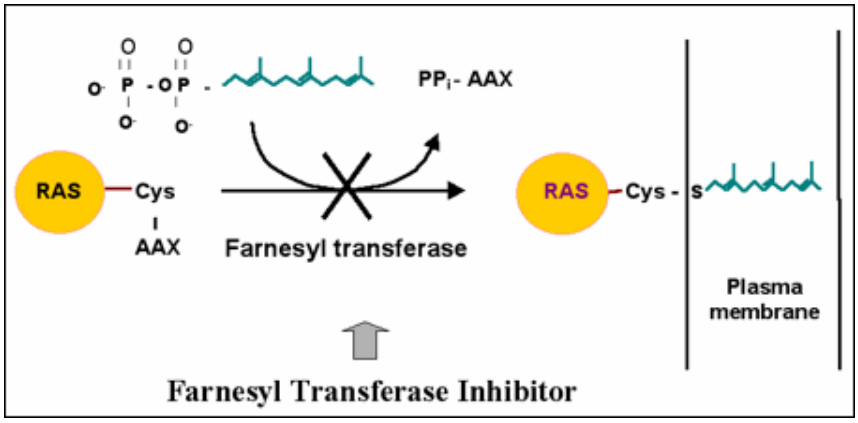
3. Prenylation: adding a repetitive structure of lipid to a terminal cysteine of a protein that exists at the C-terminus of a protein. (fernyselation is a type of prenylation).
4. GPI (glycosylphosphatidylinositol): here we have a fatty acid usually it is of a phospholipid (usually it is ethanolamine), that has a number of sugars and these sugars are attached to proteins. [so the sugars are mediating the attachment between the protein and the fatty acid].

There is an oncogene (a gene that causes cancer by synthesizing oncoproteins), so (from the name) we expect that it stimulates cells to grow. One of these oncoproteins is Ras. Ras is a farnesylated protein, which means that by adding a moiety of a molecule called Farnesyl to it, it can integrate to the membrane and stimulate cells to grow and may cause cancer. The most common cancer caused by a mutation of Ras gene is pancreatic cancer, in fact 95% of pancreatic cancers are caused by Ras mutations.

There is an enzyme called farnesyltrasferase which helps in the farnesylation of Ras protein. The strong question is: why did not we inhibit farnesyltrasferase to pervent Ras from stimulating cellular growth??

Actually, we did. scientists have created farnesyltrasferase inhibitors (FTIs). They tried it in cultured cells and it worked nicely. They also tried it on mice and it worked

beautifully. They tried it on humans and spent billions of dollars, but it did not work!!



- There are two reasons explain why it did not work:

1. There are other Ras isoforms known as N-Ras,K-Ras. These isoforms can also cause cancer but they are not inhibited by FTIs.
2. There are other proteins that have important roles in cell growth but they are not inhibited somehow by FTIs.

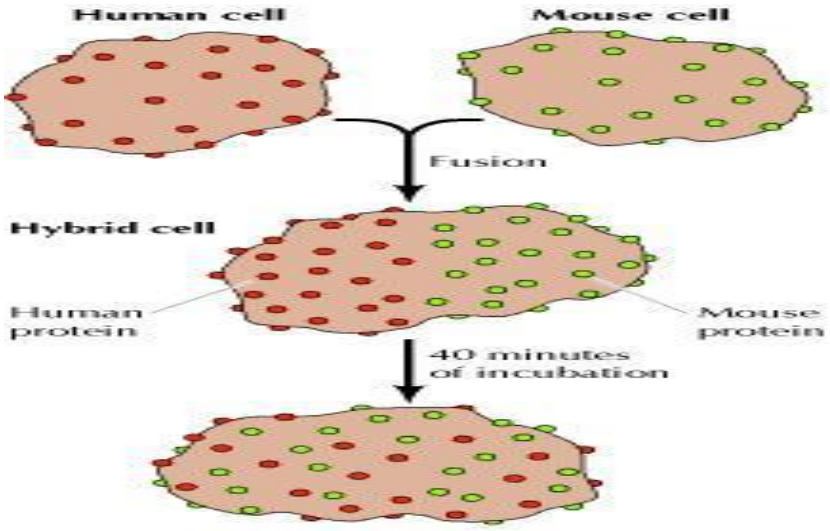
We have another protein that is farnesylated called lamin A, when it is mutated, it causes a disease called Progeria. This condition hopefully can be treated by FTIs.

The doctor said a story about an oncologist who has taught him: that he has invented a drug called AZF to treat cancer, but it did not work, so he put it on the shaft. In somewhere else, they create exactly the same drug and it was used against HIV to treat AIDS, so they made millions of dollars that are lost by that unlucky oncologist.

Membrane proteins, like membrane lipids , are not static, they can move within the membrane. There was an experiment that proved proteins' movement' the whole story is this:

They grew two cells → they stain the membrane proteins of one of them with a green color and those of the other

cell with a red color → they allowed the cells to fuse →after few hours, they noticed that the membrane had an equal mix of green and red colors, meaning that membrane proteins keep moving.



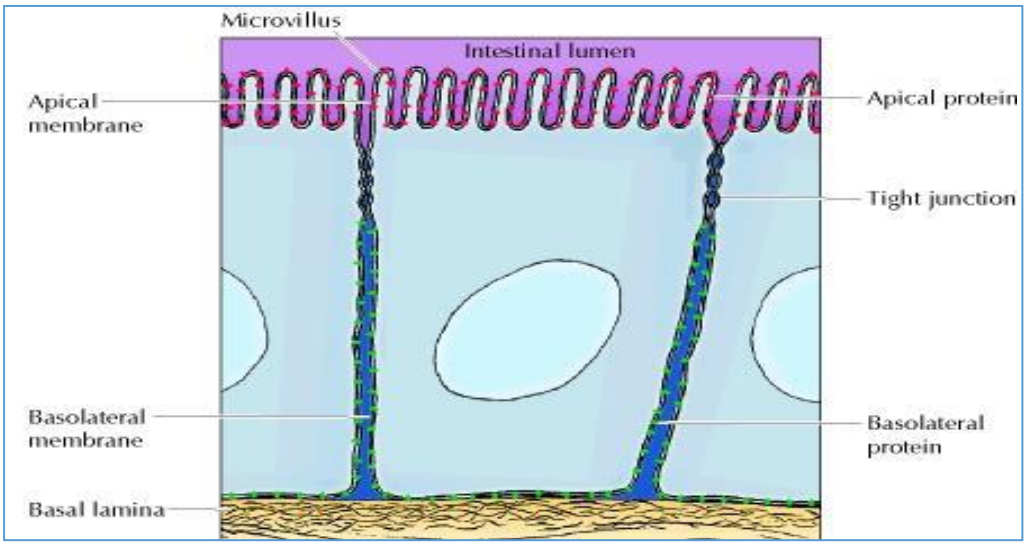
Some cells have specialized membranes, like intestinal cells: they have on one part (the basolateral side) proteins that could absorb nutrients. On the other side (the apical side), there are proteins that can transport nutrients outside to the blood vessels. So they have to have certain proteins at the apical side and certain proteins at the basolateral side.

The question here is: What prevents the proteins on the basolateral side from moving all the way to the apical side and vice versa??

The answer is that this mobility is restricted by three mechanisms:

1. These proteins can interact with proteins in the cytoskeleton, and that's why they cannot move freely.

2. They have certain regions that make Tight junctions, and this cell-cell interaction forms a "belt", so if a protein moves it will hit this belt and it will not move further.
3. Restriction by Lipid rafts (specialized region of plasma membranes).



Some cells like intestinal cells have a sugar coating at the surface. You have a lipid or a protein, and attached to this lipid or protein you have carbohydrates This coat (carbohydrates) is known as glycocalyx. Its importance is the involvement in:

1. Cell-cell interaction: some cells can recognize other cells by knowing the sugars that are present on the cell surface.
2. Protection of the cell surface from mechanical stress. For example let's say that the cells of the airway have these sugars, so they will be protected for example when you breathe dust.
3. Forming a barrier for microorganisms, to prevent their penetration into the membrane.

How can we study proteins inside cells??

1. Centrifugation: a sample is added to a tube → go in a centrifuge → keeps on rotating → because of its weight, it goes to the palm of the tube.
2. Sedimentation: we talk about it in biochemistry and we said that it depends on four factors (the mass of the molecule, its density, its shape and the density of the solution itself).
3. Cell fractionation: we can study mitochondrial proteins by their simple isolation from mitochondria. We can study membrane proteins and cytosolic proteins by removing all other components and keep the protein itself by doing cell fractionation where we can centrifuge the sample at different speeds and one by one we isolate the protein.

In the old days, they used to study one protein or one gene at a time by the science of **Genetics**, but we need to look at a whole picture not a single molecule. So how can I study proteins in a whole system interacting with each other or with lipids??

4. This is studied by a group of sciences known as **omics** (as their names end with the suffix "omics").

It contains the science of **Genomics**, where we can study tens to hundreds to thousands of genes at the same time.

Then we had **transcriptomics**, where we study MRNA (gene expression).

Then there was **proteomics**, where we can study tens,hundreds and thousands of proteins at the same time.

And then we had a science that known as **metabolomics**, where we study metabolites (like ions, glucose … etc).

By collecting information from genomics, transcriptomics, proteomics and metabolomics we'll have a big picture and a new science known as **systems biology**, which studies and understands the system as a whole rather than just studying one cell or one molecule.

5. Two-dimensional gel electrophoresis, where we can separate proteins based on **charge and size** and then we can look at the profile (the different proteins that exist in a cell).

6. (((Note: this was mentioned only in sec.2 but in sec.1 the doctor stopped at point 5 above))) … **Basics of Tandem mass spectrometer**. This technique is a part of the science of proteomics .. It tries to identify a single protein in a sample by converting it into an ion.

basically the basic mass spectrometer has:

1) An ion source that ionizes the peptide (the protein).
2) The mass analyzer that makes the ion travel.
3) The detector that detects the ion.

Firstly, we take the protein and inject it in the mass spectrometer system → it is then converted into an ion → then the ion travels and hits a detector (so we generate a signal). Based on the ion's speed we can determine where will it hit (its speed is inversely related to its size: the larger the protein is, the slower the ion will travel).

- If we have a protein that has the same speed of the one in the sample, how can we know exactly what is the sample's protein??

This is done by adding another mass analyzer separated from the first one by another chamber (CID). What this chamber does is that it dissociates the peptide into smaller peptides (or even single amino acids), and these peptides then travel to the detector generating another profile (another spectrum). So it allows us to know the amino acid sequence of the peptide.

We can also use HPLC (high-performance liquid chromatography) to separate proteins, and the destination is the same (traveling and detection).

Finally, to identify the protein, we enter the collected data into a computer system.

Note: This sheet only includes what the doctor said in 43.57 mins :/ (mashallah 3leeh) , It did not include the whole details in the slides.

Done by: Hamzah Mahafzah.

Sorry for mistakes … Best of luck … o ed3ole ☺

Ma79'awi …