7

Cytology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

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This sheet is discussing concepts (8.8) & (8.9), pages (334-337) of the book, enjoy ☺

8.8 The Red Blood Cell: An Example of Plasma Membrane Structure

***The Characteristics of normal red blood cells (RBC)(also known as erythrocytes):**

-biconcave

- deformable (Capable of being reshaped under applied stress)

-flexible

-their diameter is less than 10 micrometers (µm). Notice that the inner diameter of the capillary is around 10 µm that's why the RBCs move through the capillaries one by one meaning that each RBC crosses the capillary first followed by the next one and so on, for example, they can't cross the capillary two at a time.

The plasma membrane of the human erythrocyte (red blood cell) (see figure 1↓) is the most studied and best understood, **due to many reasons including:**

1-The cells are inexpensive to obtain

2-they are readily available in huge numbers from whole blood

3- They are already present as single cells and need not be dissociated from a complex tissue

4- They are simple by comparison with other cell types since they lack nuclear and cytoplasmic membranes that contaminate (make the preparations more difficult) plasma membrane preparations from other cells.

*** So how can the plasma membrane of these cells be obtained and studied simply?**

In order to study the plasma membrane of the RBCs we have to rupture the cells, how can we do that? Simply by placing the cell in a dilute hypotonic salt solution, the cells respond to this osmotic shock by taking up water and swelling (this is called **hemolysis**) so as the surface of the cell increases the cell becomes leaky (the contents which composed of dissolved hemoglobin will flow out of the cell) and that leaves behind a plasma membrane ghost (figure 2 ↓).

Once erythrocyte plasma membranes are isolated, we can extract the membrane proteins using specific detergents (such as SDS) in order to study the diversity of

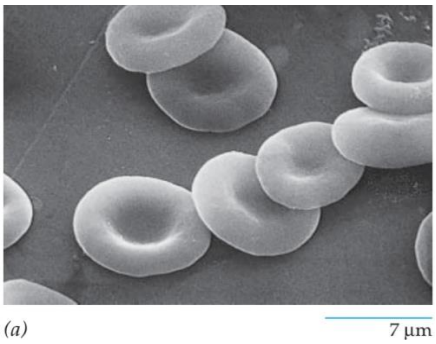
proteins within the membrane, the proteins then can be solubilized and separated from one another (fractionated) using polyacrylamide gel electrophoresis (PAGE) in the presence of the ionic detergent sodium dodecyl sulfate (SDS).

***How are proteins separated using the SDS-PAGE technique?**

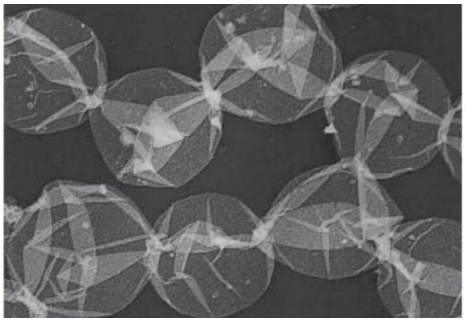
-The SDS keeps the integral proteins soluble and, in addition, adds a large number of negative charges to the proteins with which it associates (since SDS is negatively charged, it binds to the proteins and coat them in negative charge).

- Because the number of charged SDS molecules per unit weight of protein tends to be relatively constant, the molecules separate from one another according to their molecular weight.→ The largest proteins move most slowly through the molecular sieve of the gel, so they move shorter distances than the small proteins and their position will be at the top of the gel.

- The major proteins of the erythrocyte membrane are separated into about 12 bands by SDS–PAGE (Figure 3↓)(some of these bands are given either a number according to its position or a name). Among the proteins are a variety of enzymes (including glyceraldehyde 3-phosphate dehydrogenase, one of the enzymes of glycolysis), transport proteins (for ions and sugars), and skeletal proteins (e.g., spectrin).



(Figure 1)Scanning electron micrograph of human erythrocytes



(Figure 2) Micrograph showing plasma membrane ghosts, which were isolated by allowing erythrocytes to swell and hemolyze as described above

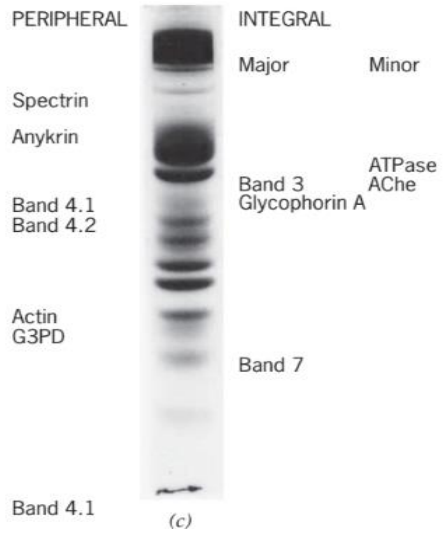


Figure (3): The results of SDS–polyacrylamide gel electrophoresis (SDS–PAGE) used to fractionate the proteins of the erythrocyte membrane, which are identified at the sides of the gel

**integral proteins of the erythrocyte membrane:

Through the use of transmission electron microscope and scanning electron microscope we find that there are integral proteins span the phospholipid bilayer, we'll focus on the most abundant two integral proteins of this membrane, they're both carbohydrate-containing, membrane-spanning proteins and they're called: **Glycophorin A**, and **Band 3** .

*Band 3 protein

-structure: Band 3, which gets its name from its position in an electrophoretic gel (Figure 3↑), is present as a dimer composed of two identical subunits (a homodimer). Each subunit spans the membrane at least 12 times and contains a relatively small amount of carbohydrate (6–8 percent of the molecule's weight). (See figure 4↓)

-function: Band 3 protein serves as a channel for the passive exchange of anions across the membrane. As blood circulates through the tissues, carbon dioxide becomes dissolved in the fluid of the bloodstream (the plasma) and undergoes the following reaction:

$$H_2O + CO_2 \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

So, as known, CO_2 is a product from cellular respiration, and as seen here, bicarbonate ions (HCO_3^-) are a product from CO_2 when it reacts with water.

Note: hemoglobin, HCO_3^- , and O_2 are carried by RBC

After that, (HCO3-) enters the erythrocyte in exchange for chloride ions, which leave the cell in the tissues. In the lungs, where carbon dioxide is released, the reaction is reversed and bicarbonate ions leave the erythrocyte in exchange for chloride ions.

→ The reciprocal movement of HCO_3^- and Cl^- occurs through a channel (hydrophilic channel) in the center of each Band 3 dimer.

Note: after bicarbonate ions leave the erythrocyte in the lungs, they will bind again with H+ to form H_2CO_3 which will decompose to H_2O AND CO_2 ,CO_2 then will leave the body through exhalation. And O_2 will enter the cell instead. RBC carries O_2 from the lungs to supply the tissues.

*Glycophorin A protein:

glycophorin A was the first membrane protein to have its amino acid sequence determined. (Other related glycophorins, B, C, D, and E, are also present in the membrane at much lower concentrations.)

-structure: Like band 3, glycophorin A is also present in the membrane as a dimer(composed of 2 subunits without a gap between them, it's a structural protein). Unlike band 3, each glycophorin A subunit(the hydrophobic domain) spans the membrane only once, another domain exists on the cell surface and it contains a dense carbohydrate cover consisting of 16 oligosaccharide chains that together make up about 60 percent of the molecule's weight(that's why it's called Glycophorin as GLYCO→ means sugar, and PHORIN→ means carrier). There's also a third domain; the cytosolic domain.

-function: the function of Glycophorin A is preventing the cells from clumping as they circulate through the body's tiny vessels. HOW? This protein contains a large number of negative charges carried on sialic acid(the sugar residue at the end of each carbohydrate chain, sialic acid will be ionized and will lose H+ to the environment so it becomes negatively charged). Because of these charges, red blood cells repel each other, which prevents the cells from colliding and clustering in the vessels.

notice that the phospholipid bilayer of the membrane is normally negatively charged inside and positively charged outside. But HERE we're talking about the net charge on the outermost surface of RBC which is negative because the negative outside domain is larger than the other domain so we get a net negative charge on the surface.

BOOK NOTES: persons who lack both glycophorin A and B in their red blood cells show no ill effects from their absence. At the same time, the band 3 proteins in these individuals are more heavily glycosylated, which apparently compensates for the otherwise missing negative charges required to prevent cell–cell interaction

BOOK NOTES: Glycophorin also happens to be the receptor utilized by the protozoan that causes malaria, providing a path for entry into the blood cell. Consequently, individuals whose erythrocytes lack glycophorin A and B are thought to be .protected from acquiring malaria

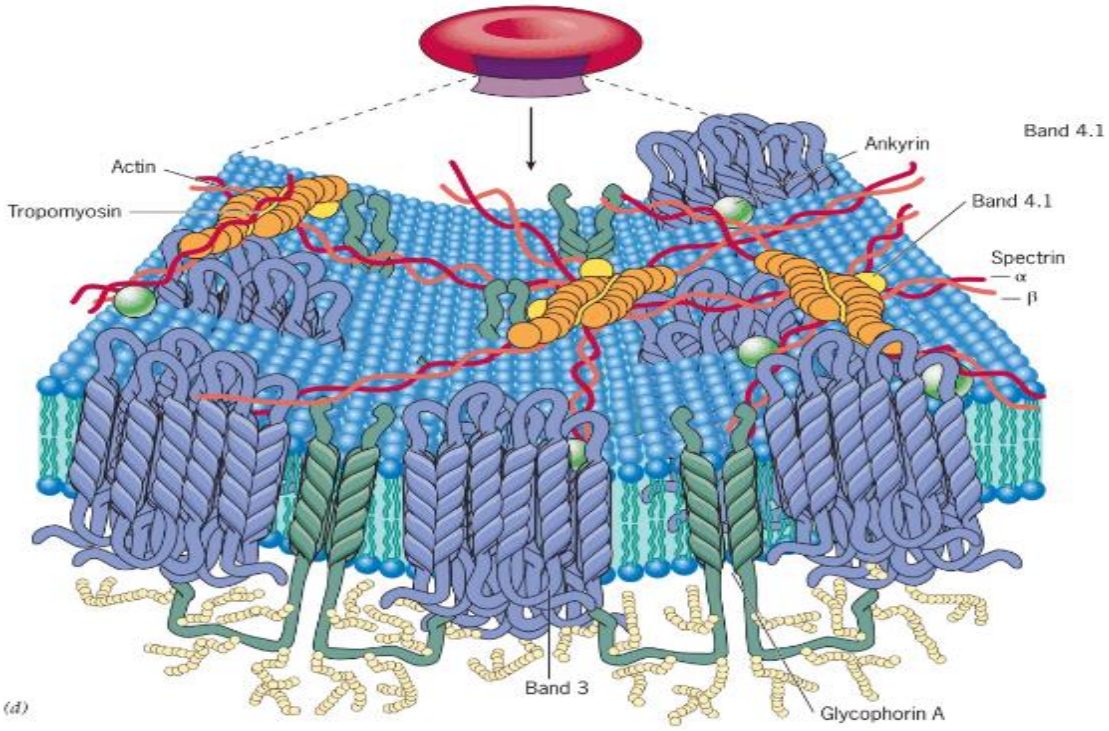


Figure (4) : A model of the erythrocyte plasma membrane as viewed from the internal surface, showing the integral proteins embedded in the lipid bilayer and the arrangement of peripheral proteins that make up the membrane's internal skeleton. The band 3 dimer shown here is simplified. The band 4.1 protein stabilizes actin–spectrin complexes

** The Erythrocyte Membrane Skeleton :

The erythrocyte plasma membrane is supported by a fibrillar skeleton composed of peripheral membrane proteins that play a major role in determining the biconcave shape of the erythrocyte, the most important proteins(on the cytosolic side) are:

1-spectrin

The major component of the skeleton is **spectrin**, which is an elongated fibrous protein. It's a heterodimer approximately 100 nm long, consisting of an **A** and **B** subunit that curl around one another. Two such dimeric molecules are linked at their head ends to form a 200-nm-long filament that is both flexible and elastic.

Spectrin is attached to the internal surface of the membrane by means of noncovalent bonds to another peripheral protein*, ankyrin*

2-ankyrin:

This peripheral protein is linked noncovalently to the cytoplasmic domain of a band 3 molecule. (you can see it as green spheres in Figure 4↑)

3-Band 4.1:

It gets its name from its position on the electrophoretic gel.

The main function of these 3 peripheral proteins (which are directly connected to either the heads of phospholipid bilayer or the hydrophilic domain of Band3 and glycophorin A) is to anchor the *actin* filaments to band3 and glycophorin since a network of Actin filaments is needed to support the fluid plasma membrane.

→spectrin filaments are organized into hexagonal or pentagonal arrays. This two-dimensional network is constructed by linking both ends of each spectrin filament to a cluster of proteins that include a short filament of *actin* and *tropomyosin* (proteins typically involved in contractile activities)

→ The spectrin– actin network gives the cell the strength, elasticity, and pliability necessary to carry out its demanding function, while it circulates around the body it's squeezed under pressure through microscopic capillaries whose diameter is less than that of the erythrocytes themselves, so in order to traverse these narrow passageways,

and to do so day after day, the red blood cell must be highly deformable, durable, and capable of withstanding shearing forces that tend to pull it apart.

Q: what happens If the peripheral proteins are removed from erythrocyte ghosts?

A: the membrane becomes fragmented into small vesicles, indicating that the inner protein network is required to maintain the integrity of the membrane.

A number of genetic diseases (hemolytic anemias) characterized by fragile, abnormally shaped erythrocytes have been traced to mutations in ankyrin or spectrin.

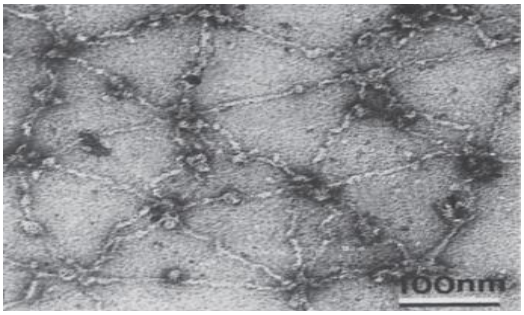


Figure5: Electron micrograph showing the arrangement of the proteins of the inner membrane skeleton.

*Scientists used to think that the membrane skeleton of the erythrocyte is a unique structure suited to the unique shape and mechanical needs of this cell type. However, as other cells were examined, similar types of membrane skeletons containing members of the spectrin and ankyrin families have been revealed, indicating that inner membrane skeletons are widespread. (read the example below)

EXAMPLE(from the book): Dystrophin,(a member of the spectrin family that is found in the membrane skeleton of muscle cells). Mutations in dystrophin are responsible for causing muscular dystrophy,(a disease that cripples and kills children). The most weakening mutations are ones that lead to a complete absence of the protein in the cell. The plasma membranes of muscle cells lacking dystrophin are apparently destroyed as a consequence of the mechanical stress exerted on them as the muscle contracts. As a result, the muscle cells die and eventually are no longer replaced.

8.9 The Movement Of Substances Across Cell Membranes

Now, we'll move to the most important function of the plasma membrane; transport of substances and controlling the separation and exchange of molecules into and out of the cell due to its selective permeability.

Because the contents of the cell are completely surrounded by its plasma membrane, all communication between the cell and the extracellular medium must be mediated by this barrier which consists of phospholipid bilayer and proteins, the tails of these phospholipids are hydrophobic but most of the molecules that the cell needs are polar and they can't cross the lipid bilayer that's why we have integral protein in the membrane.

The plasma membrane is a barrier that retains(keeps) the materials of the cell so that they do not simply leak out into the environment. The lipid bilayer of the membrane prevents the loss of charged and polar solutes from a cell.

Consequently, to allow the movement of nutrients, ions, waste products, and other compounds in and out of the cell, there are two means for the movement of substances through a membrane: passive transport and active transport, Both types of movements lead to the net flux of a particular ion or compound. (The term net flux indicates that the movement of the substance into the cell (influx) and out of the cell (efflux) is not balanced, but that one exceeds the other. In other words, net flux is the difference between influx and efflux of a substance.)

1-passive transport:

By this mechanism, molecules cross the membrane from high to low concentration (down the gradient, whether it's chemical or electrochemical gradient)and regardless the way this molecule crosses the membrane (whether through the lipid bilayer or through a channel like ions), passive transport is a spontaneous process, it's **exergonic** (ΔG is negative)

→There are basically 3 types of passive transport:

1- simple diffusion through the lipid bilayer;

In general, Diffusion is a spontaneous process, simple diffusion is nonmediated transport, some molecules diffuse through the lipid bilayer such as oxygen, water, and some hydrocarbons. However, some ions cross the membrane through channels(the book considered this ion transport as simple diffusion not facilitated since the protein isn't really involved, it just acts as a pore in the membrane)

2- simple diffusion through an aqueous, protein-lined channel;

as we know, water can cross the lipid bilayer at a slow rate (because it's a very small molecule), but sometimes the cell needs to excrete large amounts of water, so here we have a channel protein specifically for water called Aquaporin.

In our bodies, we have aquaporin mainly in the kidney tubules.(that's why when we don't drink enough water, the urine will become more concentrated because the kidney retakes water through aquaporins in order to minimize the amount of water lost as urine with the help of ADH, which is a hormone secreted from the posterior pituitary gland, it reduces sodium concentration by increasing water reabsorption in the kidneys which maintain pH in blood)
In addition, aquaporins play important role in plant cells, they're found in the root hairs of plants since plants need to absorb large amounts of water.

Lecture Note: cells are self-regulating.HOW?through membrane fluidity control,tepreture control, nervous and hormones activiteies.

3- diffusion that is facilitated by a protein transporter;

As we know, glucose and amino acids can't cross the lipid bilayer, they need specific transporters (multimeric transmembrane proteins) that facilitate the transport of glucose from the blood to the cells so, here you need glucose transporter (glut1, glut2, glut3...)
since this is facilitated transport it's mediated by the presence of the transporters (Note: if these transporters don't work well, diabetes occurs)

Note: the difference between integral & transmembrane proteins;
Transmembrane→it completely spans the membrane, such as glycophorin A and band 3./ integral→it spans the membrane but not completely, like there are integral proteins embed into a single face of the membrane. and don't span the whole way across, we call this protein: monotopic.

2-active transport;

In active transport, molecules move from low concentration to high concentration(against the gradient), this type of transport requires energy, this energy is derived from ATP, electron transport chain, or from light, or energy stored in resulting gradient of another molecule.
As molecules are moving against their concentration gradients, active transport cannot occur without assistance. A carrier protein is always required in this process. Like facilitated diffusion, a protein in the membrane carries the molecules across the membrane through changing its conformation,(but in facilitated diffusion the process is spontaneous, here it's not).
Notice that active transport is **endergonic** (ΔG is positive)because it's energy-requiring (it's the ATP normally that is used to do the work)(see figure 6↓)

Note: the difference in concentration normally determines the rate of diffusion, and normally the diffusion is in both directions.

Note; the structure of a channel protein:

It is hydrophobic but the interior part of this protin is hydrophilic,so the hydrophobic amino acids will be arranged in a way by which they're in contact with phospholipid tails(R group is nonpolar), and the hydrophilic amino acids will be lining the channel in the middle(R group is polar) providing a hydrophilic passageway .also the part on the cell surface is hydrophilic. For example, the alpha helix will be arranged in this order(hydrophobic, hydrophilic, hydrophobic…)

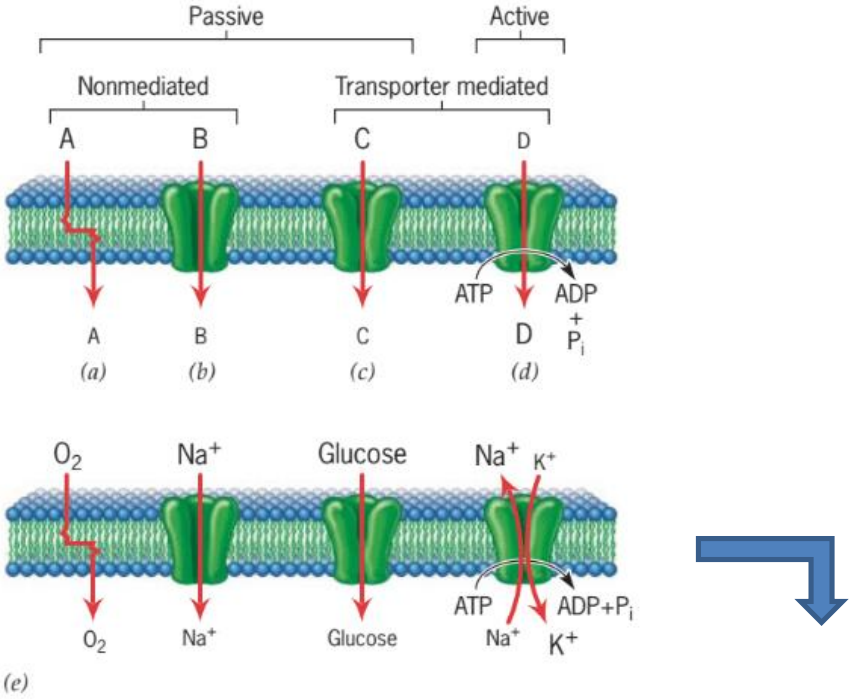


Figure6:Four basic mechanisms by which solute molecules move across membranes. The relative sizes of the letters indicate the directions of the concentration gradients. (a) Simple diffusion through the bilayer. (b) Simple diffusion through an aqueous channel formed within an integral membrane protein or a cluster of such proteins. As in a, movement is always down a concentration gradient. (c) Facilitated diffusion in which solute molecules bind specifically to a membrane protein carrier (a facilitative transporter). As in a and b, movement is always from high to low concentration. (d) Active transport by means of a protein transporter with a specific binding site that undergoes change in affinity driven with energy released by an exergonic process, such as ATP hydrolysis. (e) Examples of each type of mechanism as it .occurs in the membrane of an erythrocyte

THE END