University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 16

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Subject: Nucleus and Cell Membrane

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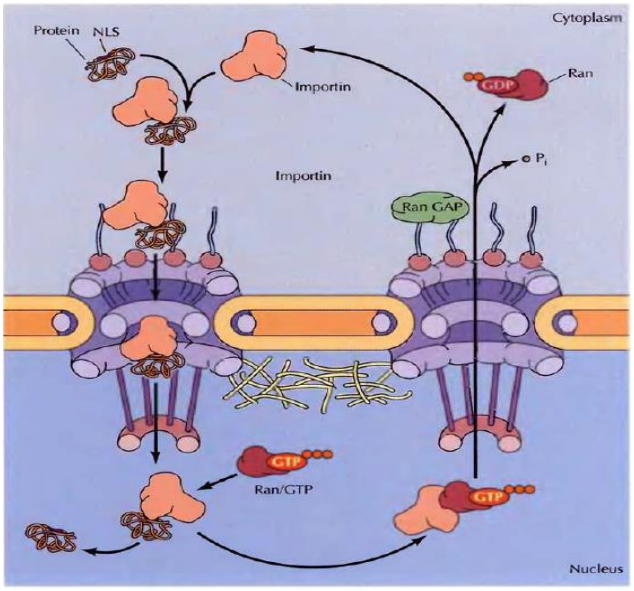
Price:

Date:

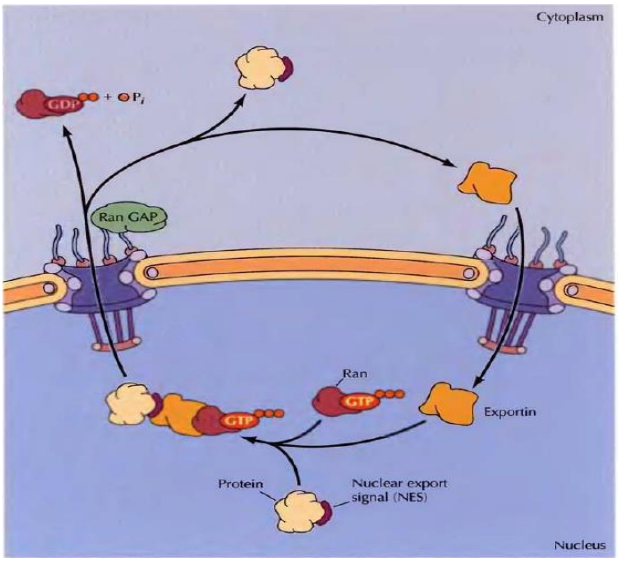
The first five pages were mentioned in the previous sheet, but the doctor explained them again.

Quick revision

- The nucleus has an envelope which is composed of two membranes (outer and inner membranes). These two membranes have an opening called the **nuclear pore complex** that allows the movement of molecules from the nucleus to the cytoplasm, or from the cytoplasm to the nucleus.
- Proteins, RNA, and other molecules can move to the nucleus (to the inside) or to the cytoplasm (to the outside).
- Movement of molecules to the nucleus is referred to as an **import**, and this is mediated by the action of a carrier protein called **importin**.
- **Mechanism of importing molecules**: a protein (the cargo) has a sequence called the **Nuclear Localization Sequence (NLS)** and this protein is transported to the nucleus through the following steps (as shown in the diagram below):
  1. Protein binds to the **importin protein**.
  2. The complex is carried through the nuclear pore complex.
  3. Once the complex is in the nucleus, the protein (that was transferred to perform a certain function such as acting as a transcription factor which binds to the DNA or facilitating gene expression) detaches from importin.
  4. This separation is mediated by **RAN/GTP molecule** to which the complex binds (it's the molecule that regulates the directionality of movement). After RAN/GTP binds to importin, **conformational changes** allow the dissociation of the cargo.
  5. The **complex** (importin + RNA/GTP) goes through the nuclear pore complex to do this cycle again in the cytoplasm.
  6. In the cytoplasm, dissociation happens by **RAN GAP** which dephosphorylates the RAN/GTP into RAN/GDP and Pi. This induces conformational changes again, so the importin is released in the cytoplasm where it can bind to other proteins and this cycle occurs again and again.

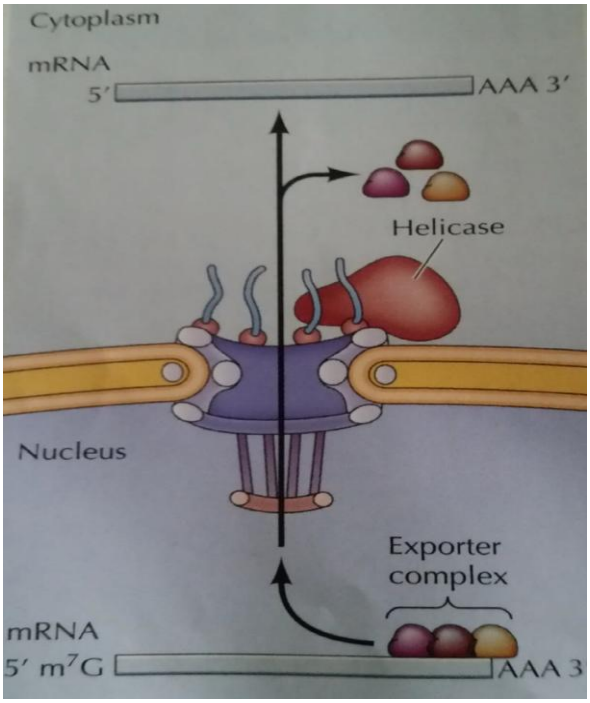


- The opposite process is called **export**. In order to move the protein from the nucleus to the cytoplasm, the protein must have a sequence (signal) that can be understood by exportin and this sequence is called **Nuclear Export Signal (NES).**
- **Mechanism of exporting molecules:**
  1. The protein binds to the exportin and RAN/GTP to form a complex (composed of 3 proteins) which moves through a nuclear pore complex to the cytoplasm.
  2. Dissociation occurs by **RAN GAP**, so **RAN/GTP** becomes **RAN/GDP** +**Pi** which induces **conformational change**. As a result, the protein is free now and can do its function, while the **exportin** returns to the nucleus where it can bind to other proteins to repeat the cycle.



- How can these movements across the membrane be regulated (other than directionality)? In other words, if there is a transcription factor, when should it move to the nucleus?
  Movement is regulated by structural changes (that show or hide the NLS). For example, if this transcription factor is **phosphorylated**, this phosphate group induces conformational changes (these changes can also occur by dissociation of the protein complex) that result in hiding NLS so it will not move. On the other hand, if this protein is to be transported as part of a **protein complex** and this binding hides NLS, the dissociation of this complex will result in exposing the sequence, so now the exportin or importin can bind to this sequence and transport the protein. Therefore, the target is the sequence. If it's **covered → no binding → no transport**. If it's **exposed → binding happens → transport happens**.

- How is mRNA transported across the nuclear membrane after transcription and splicing (converting pre-mRNA to mRNA) to bind to ribosomes so as to begin translation?
  1. mRNA is carried by molecules different from those which carry proteins.
  2. mRNA can't be transported as a nucleic molecule by itself; it has to bind to a complex protein called **Exporter Complex**.
  3. Then, this complex can leave as RNP (Ribonucleoprotein complex) through the pore to the cytosol.
  4. After it's out , we need to dissociate this complex to make mRNA free and this is mediated by **RNA helicase** that remove the **exporter complex** from the mRNA.
  5. Thus, mRNA can bind to a ribosome and start translation .

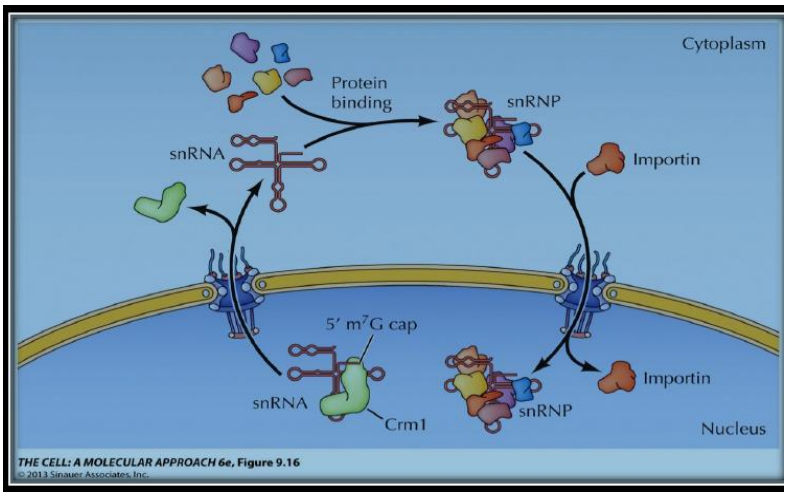


Important Notes:

- The exporter complex binds to mRNA before the processing of mRNA is done. Therefore, the exporter complex binds to the pre-mRNA (NOT MATURE) and then processing happens to form mature mRNA then remove through the complex.
- In transporting proteins, the RNA/GTP affects the directionality of movement. However, in transporting mRNA, the RNA helicase affects the directionality of movement because mRNA itself can't pass through the membrane; once it binds to the complex it can pass.

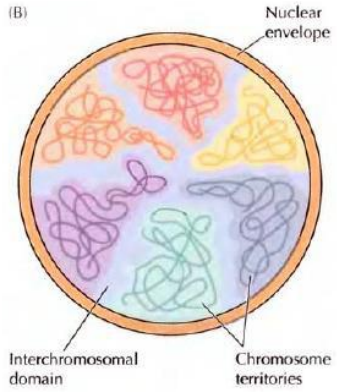
- How is **snRNA (Small nuclear RNA)** transported to the cytosol?
  The mechanism is very easy and it's just like the mechanism of mRNA transport:
  1. The **snRNA** in the nucleus **can't** be transported as a single molecule, so it must bind to a protein complex (here, we called it ***Crm1***) .
  2. The **RNP complex (composed of snRNA and Crm1)** goes through the pore to the cytosol.
  3. The **snRNA** is a part of the RNA processing machinery.
     Even though the processing of RNA occurs in the nucleus, snRNA is transported to the cytoplasm because we need to bind the snRNA to special proteins that exist in the cytoplasm to form snRNP complex (protein + snRNA). As a result, this complex can process the RNA.

- ***Remember***:
  1. Crm1 is separated from snRNA once it reaches the cytosol so snRNA is free to bind to the proteins.
  2. The snRNP binds to importin to return to nucleus.



Chromosome Organization

- How are the chromosomes organized in different phases of cell cycle in the nucleus?
  Chromosomes **do not randomly** wind around one another. They occupy discrete **territories** within the nucleus.
- Every chromosome takes a place in a special region (**territories**). Within these regions; depending on the gene expression status, the chromosomes in their regions move in the territories.
- There are spaces between these territories called **interchromosomal domain**.
- If one gene in the chromosome is active right now, the chromosome will **rotate** to make this gene directed towards the interchromosomal domain to start the transcription.



Chromosome Organization and Gene Expression:

- There are two types of **chromatin** (chromatin = DNA + histone proteins):

Heterochromatin	Euchromatin
Highly condensed	Decondensed
Transcriptionally **inactive**	Transcriptionally **active**
Includes **non-transcriptional** DNA sequences such as telomeres and centromeres	Contains **transcriptional** DNA regions
Located close to the nuclear envelope and around the nucleolus and binds to lamins and proteins to the inner nuclear membrane	Localized to the periphery of chromosome territories adjacent to channels between chromosomes

Notes for the table above:

- Euchromatin is the activated form of chromatin.
- In the interphase (before mitotic divisions), there are both heterochromatin and euchromatin.
- Once mitosis starts, there is only heterochromatin (the inactive form), because we concentrate on cell division not transcription.
- It's important to know the location of each type: Euchromatin (active DNA) is located towards the interchromosomal domain, while heterochromatin in the periphery, center, and around nucleolus and it binds to lamins.

The Functional Internal Organization of the Nucleus

- How are the chromosomes and proteins organized in the term of function inside the nucleus?
  There are sub-compartments or regions within the nucleus in which distinct nuclear processes occur and these compartments are:
  1. ***Replication factories:***
     Remember that there are many replication forks in one DNA molecule once replication starts. To make this process more efficient, you have to activate different molecules at the same time; we call them "**Replication Factories**".
     Replication factories are different from replication forks because replication forks have several locations in the same DNA molecule, while replication factories start at different sites of DNA. Each factory has several forks so the factory is larger than a fork.
     Replication factories are basically specialized for DNA replication.

  2. ***Nuclear bodies:***
     They are nuclear organelles that compartmentalize the nucleus and concentrate proteins and RNAs that function in specific nuclear processes (Replication, repair, editing, etc...)
     Examples on nuclear bodies:
     - **Cajal body** → snRNP complex assembly.
     - **Clastosome** → proteasomal proteolysis; protein degradation inside the nucleus.
     - **Histone locus body** →activation of histone gene (transcription and processing of histone pre-mRNAs).
     - **Nuclear speckle** → storage of pre-mRNA splicing factors.
     - **Nuclear stress body** → response to stress (oxidative stress, mechanical stress, …..) and deal with it efficiently.

- **Paraspeckle** →convert Adonesine to Inosine RNA editing , this type of editing happens to some RNA molecules .
  Biochemistry link: in the nucleotide synthesis (specifically purines), there is a branching point that starts from IMP; the process can produce either AMP or GMP. After the conversion of adenosine to inosine, inosine can be read as guanosine. This results in the translation of the protein later on and causes diseases (the mechanism is not fully clear).
- **PML body** → transcription regulation & DNA repair (proofreading).
- **Polycomb body** → gene silencing.

The Nucleolus is a Nuclear Body

- The nucleolus has no surrounding membrane (Not separated from the nucleus).
- Main function: **synthesis and assembly of ribosomal subunits**.
- Remember: **ribosomes** are made of 60% **rRNA** and 40% **proteins**. Ribosomes have two subunits (1 small and 1 large subunit).
- The nucleolus has the genes for most of the ribosomal subunits for rRNA. The gene codes for RNA NOT for protein).
- Each rRNA Gene is a single transcription unit containing the 5.8S, 18S, 28S rRNAs → transcribed **as a *single* unit** by **"polymerase I**" in the nucleolus. (Note: 5s not present here in the same gene).

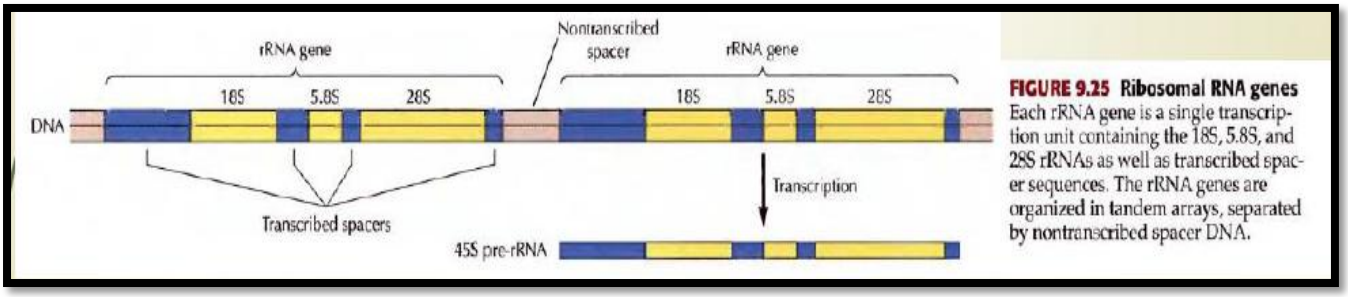
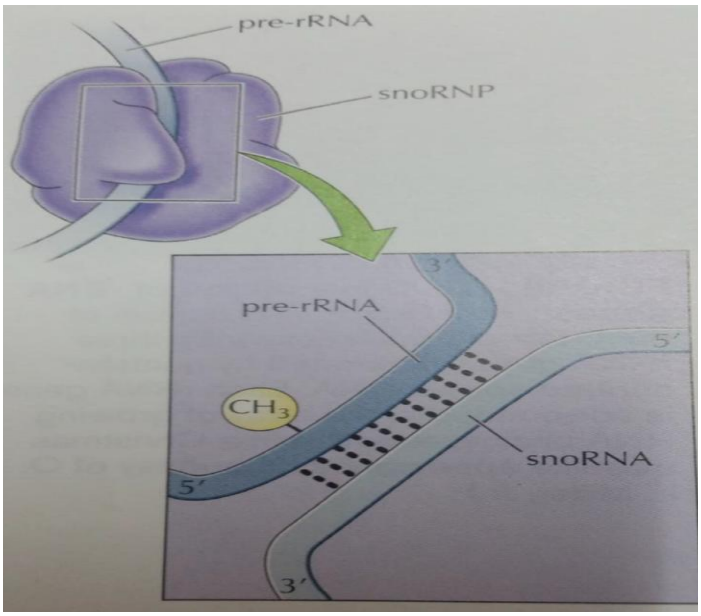


FIGURE 9.25 Ribosomal RNA genes Each rRNA gene is a single transcription unit containing the 18S, 5.8S, and 28S rRNAs as well as transcribed spacer sequences. The rRNA genes are organized in tandem arrays, separated by nontranscribed spacer DNA.

- The functions of the nucleolus:
  1. rRNA synthesis
  2. Ribosome production
  3. RNA modification and assembly of ribonucleoprotein particles
  4. Small RNA production such as tRNA, snRNA, RNase P RNA (the catalytic part of the tRNA processing enzyme), and SRP (targets proteins to ER).
  5. Cell division and response to stress, by expression of specific genes.

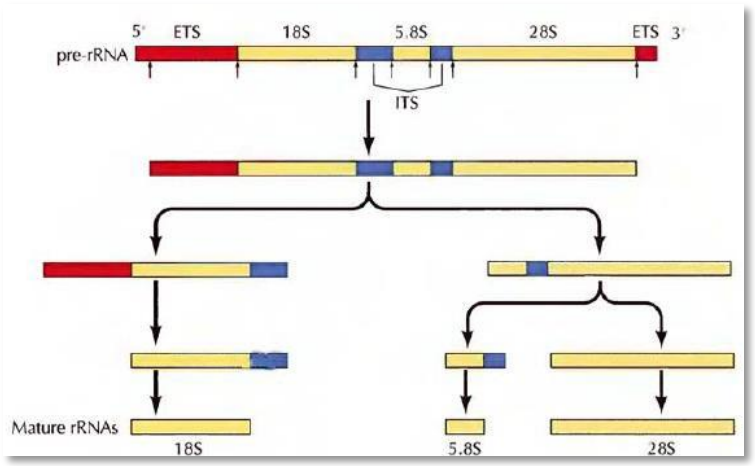
- **snoRNAs (small nucleolar RNA):**

1. They are localized RNAs related to the nucleolus.
2. Function: They contribute to the processing of **pre-rRNA** (because rRNA is produced as immature molecule).
3. snoRNAs produced → exit to the cytosol → bind to the proteins and become snoRNPs → return back to the nucleus → process pre-rRNA.



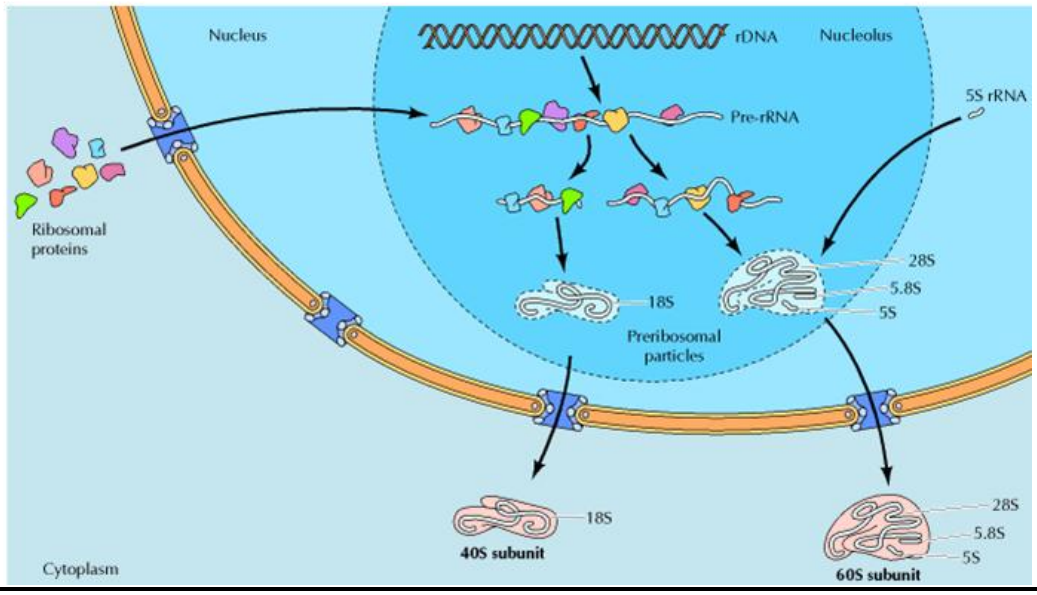
- **How is pre-rRNA processed?**
  4. snoRNA binds to the pre-rRNA by making base pairing (complementary to each other) .
  5. This binding will induce the function of the proteins and enzymes to split the unwanted regions of this molecule (catalyze base modification).

- **The transcription of rRNA:**



1. Once the pre-rRNA transcript is present as a single gene (with the three subunits**: 18S, 5.8S, 28S**), the subunits are separated and then reassembled to form the ribosome.
2. Pre-rRNA has the 3 subunits and between them there are unwanted sequences called: **ETS (external transcribed spacers)** and **ITS (internal transcribed spacers)**.
3. Cleavage of the first segment results in the formation of **18S**.
4. The remaining segment contains 2 subunits. They are split into two smaller segments to form **5.8S and 28S**.
5. Result: 3 subunits; 18S, 28S and 5.8 S. They are called **mature rRNAs**.
6. Note that there are no 5S segments produced.

- **Ribosome assembly:** (as shown in the diagram below)
  1. The ribosomal proteins are transcribed outside the nucleolus by **polymerase II** and translated in the cytosol by ribosomes. Then, they are transported again to the nucleus through the pores and finally bind to rRNA in the nucleolus.
  2. Some of these proteins bind to the **18S only**, so they produce the small subunit of ribosome (**40S subunit**).
  3. Some of these proteins bind to the 3 subunits (**28S, 5.8S and 5S**) → so produce the large subunit **(60S subunit**).
  4. **5S** segment is produced separately **outside** the nucleolus by **polymerase III** from a different gene.
  5. The 60S subunit and 40S subunits move through the pores to the cytosol to from the mature ribosomes.



This is the end of slide 1.
Please refer to the table in the slides that displays the different organelles inside the cell.

Cell Membranes

- The major components in any cell are: **lipids**, **proteins**, **carbohydrates** and **nucleic acids**.
- For example, the proteins are part of the cytosol, part of the organelles and part of the membrane. Proteins form about 75% of the inner mitochondrial membrane. The inner mitochondrial membrane has many enzymes and is involved in several pathways so it has a high percentage of proteins to support its function.
- On the other hand, the plasma membrane comprises of 50% protein and 50% lipid.
- The percentages and distribution of the components (lipids and proteins) that form different membranes (membranes of cells, mitochondria, lysosomes, & ER) are **NOT** the same.
- The table, in slide 2, shows different types of molecules (proteins, carbohydrates, lipids, etc...) and different types of membranes in cells. (You should know the highest percentages).
- For example, **myelin sheath** has high amount of **sphingomyelin** (sphingomyelin is a membrane lipid) which is about 28%. On the contrary, other membranes have low percentages of sphingomyelin.
- **Phosphatidylserine** is found in high amounts in the **inner mitochondrial membrane** and **endoplasmic reticulum**.
- High amount of **proteins,** about 76%, are found in the **inner mitochondrial membrane**.
- The basic structure of any membrane is: **two layers of phospholipids mainly (not the only type), proteins, cholesterol (lipid molecule),** and **sphingolipids.**
- The phospholipid can move in 3 ways. It can move within the same leaflet (**lateral**), **rotates around itself** or between the two layers of the phospholipid bilayer occasionally (**flip flop**). Flip flop process needs enzymes and energy.
- **Cholesterol** is inserted between the **tails** of these phospholipids.
- Remember: the structure of cholesterol encompasses 4 fused planar rigid rings.
- Higher concentration of cholesterol in the membrane is going to **reduce** the **fluidity** and **increase** the **rigidity**.
- Cholesterol also works as a buffer to maintain the membrane in the **gel-like** state by reducing the hydrophobic interaction between the long hydrocarbon chains of fatty acids in the tails of phospholipids. It is the reason why membranes don't freeze at very low temperatures.
- The main lipid in the membrane is the **phospholipid**, but there are other types of lipids like sphingolipids.
- **Phospholipids** and **sphingolipids** are different types of lipids and have different distribution.

- Remember: the size of the polar head, the type of fatty acid (saturated or unsaturated) and the length of the fatty acid affect the position in outer or inner leaflet.
- Although these lipids are found in both leaflets, their concentration in each leaflet differs.
- The only lipid that is found in the outer leaflet is the glycolipid which is related to the function of carbohydrates like signalling and protection against infection.
- In the **outer membrane: phosphatidylcholine, sphingomyelin** and **glycolipids** are found in high concentrations.
- In the **inner membrane**: **phosphatidylethanolamine, phosphatidylserine** and **phosphatidylinositol** are found in high concentrations (for specific function).
- **Phosphatidylethanolamine** and **phosphatidylserine** have **negative charges**. Therefore, they give the **inner** membrane a **negative charge**.

The sheet is over
Good Luck Everyone

Revised and Edited by: Amer Sawalha