Genetics & molecular biology

◉ Sheet ○ Slide

Number:	26
Done by:	Yusser Raja & Rahaf Oduibat
Corrected by:	Dara Suleiman
Doctor:	Diala Abu-Hassan

As mentioned in the previous lecture, the rough ER is the site where modification of proteins occurs (N-linked glycosylation, folding). The smooth ER, however, is the site of detoxification and lipid synthesis. What also happens in the smooth ER is the initiation of the formation of sphingolipids by the synthesis of its parent molecule, ceramide. Completion of this process and synthesis of sphingomyelin, as well as glycolipids, are the functions of our next station, the Golgi apparatus.

The Golgi Apparatus

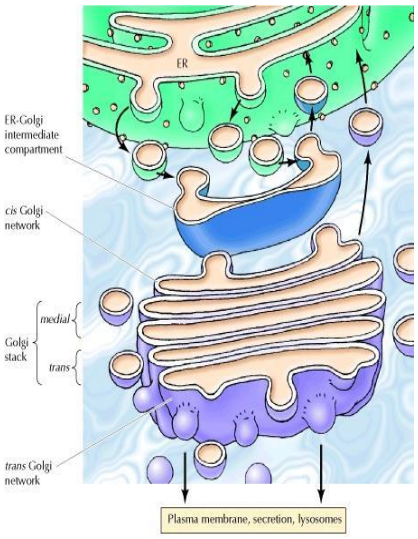
Overview of Functions of the Golgi:

- After undergoing some modification in the ER, proteins are **further processed** and modified in the Golgi.
- Protein are then **sorted and packaged for transport** to their eventual destination.
- In addition, as noted earlier, most glycolipids and sphingomyelin are synthesized within the Golgi.

After finishing the modification inside the ER, the vesicle moves from the transitional ER, through the ERGIC, and then fuses with the Golgi.

Structure of Golgi:

- Golgi is composed of flattened membrane-enclosed sacs (cisternae).
- These sacs are separated from each other.
- The Golgi is characterized by its structural and functional polarity;
  - Proteins enter at its ***cis* (entry face) Golgi network**, then are transported through the **Golgi stack** (which is composed of medial and *trans* compartments) to exit from the ***trans* (exit face) Golgi network.**

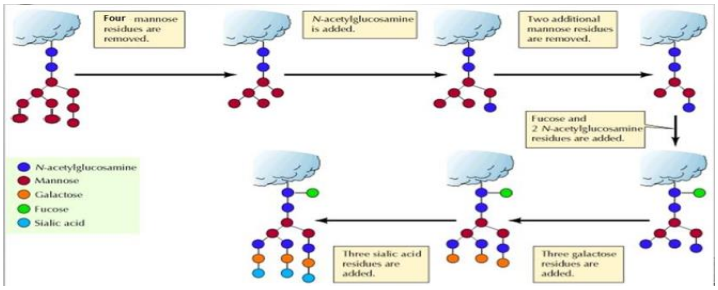


Movement of molecules within the Golgi apparatus does not happen by vesicles; instead, proteins are carried through compartments of the Golgi within the Golgi cisternae, which gradually mature and progressively move through the Golgi in the *cis* to *trans* direction.

Meaning that **each sac moves through every compartment**; *cis* matures to become medial, medial matures into *trans*, and so on, all while carrying the proteins within them.

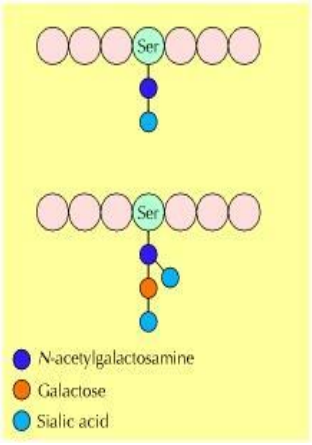
Protein Modification in Golgi:

- In the ER, proteins undergo N-linked glycosylation, a process in which oligosaccharides are added to asparagine in a specific polypeptide chain, in addition to some processing.
- These N-linked oligosaccharides received from ER are further modified in the Golgi by an ordered sequence of reactions involving the **removal and addition of other types of sugars**. (details about these reactions are not required)



O-Linked Glycosylation:

- Proteins can also be modified by the addition of carbohydrates to the side chains of acceptor serine and threonine residues within specific sequences of amino acids.
- This connection, unlike N-liked glycosylation, is made through the **oxygen** of serine and threonine, which is the reason this process is called *O*-linked glycosylation.
- The serine or threonine is usually linked directly to N-acetylgalactosamine, to which other sugars can then be added, such as galactose and sialic acid (recall: modified sugar that shared among all gangliosides, present in ganglia)
- Some of these added sugars are further modified by the addition of sulfate groups in order to make them more polar, also in the Golgi.



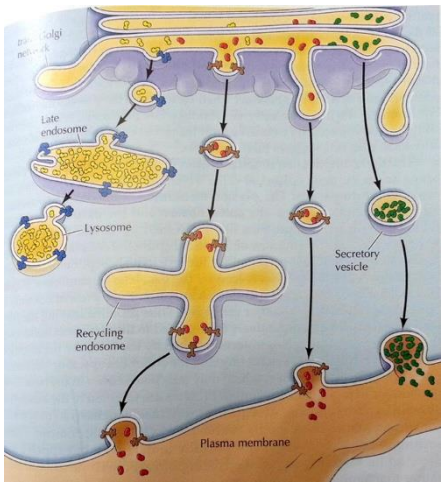
Lipid and Polysaccharide Metabolism in the Golgi:

- As mentioed earlier, glycerol phospholipids, cholesterol, and **ceramide** are synthesized in the ER.

- Sphingomyelin and glycolipids are then synthesized form ceramide in the Golgi apparatus.
- Sphingomyelin is synthesized by the transfer of a **phosphorylcholine** group from phosphatidylcholine to ceramide. This process occurs on the **luminal surface** of the Golgi.
- Alternatively, the addition of *sugar residues* to ceramide can yield a variety of different **glycolipids**.
- Unlike the synthesis of sphingomyelin, the addition of glucose to ceramide occurs in the **cytosolic side** of the membrane, producing glucosylceramide.
- This glucosylceramide then flips, and additional carbohydrates are added on the luminal side of the membrane.

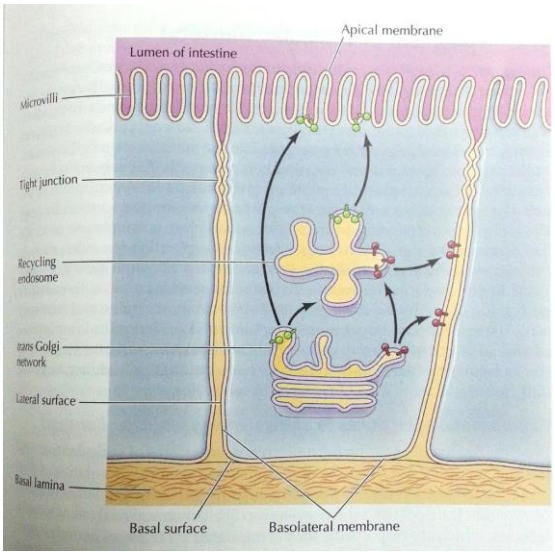
<u>Sorting and Export from the Golgi Apparatus:</u>

- Proteins as well as lipids are transported from the Golgi apparatus to their final destination through **transport vesicles**.
- Once the proteins reach the trans Golgi network, proteins that **share a common destination** gather at a specific area of the membrane to form a vesicular body.
- So proteins are sorted into different kinds of transport vesicles, which bud from the trans Golgi network and deliver their contents to their appropriate cellular locations.
- Some proteins are carried from the Golgi to the plasma membrane, either directly or via **recycling endosomes**; this is where the contents of the vesicles undergo some additional modification before reaching the plasma membrane.
- Alternatively, proteins can targeted to late endosome, which develop into **lysosomes**.



Regulation in export and transport:

- Now, let's suppose that the substances getting transported are **hormones**. In this case, the exporting process cannot be continuous. It requires a distinct **regulated** secretory pathway in which the hormones are **only secreted in response to certain stimulus**. This way, if a stimulus is present, secretion is activated; if there is no stimulus, it's inactive. Thus the hormones are stored until specific signals direct their transport and fusion with the plasma membrane.
- In addition, majority of the proteins in the Golgi are **membrane proteins**, and they have a **specific transmembrane sequence** (that is considered their signal sequence) that holds them in, or retrieves them back to the Golgi. This is in contrast to protein sorting in the ER, whose proteins head back to the ER through KKXX and KDEL signal sequences.
- A further complication in the transport of proteins to the plasma membrane arises in **polarized cells** – in other words, cells that are divided into two separate regions; an **apical domain** and a **basolateral domain**. Each region contains specific proteins that are related to their particular function – proteins that work in the apical domain will not work in the basolateral domain, and vice versa. Thus proteins leaving the Golgi must have a signal sequence that leads them to these distinct domains:
    i. For proteins heading towards the **basolateral domain**, the signal sequence are **small peptides**.
    ii. For proteins heading towards the apical domain, the signal sequence is a **sugar modification**.



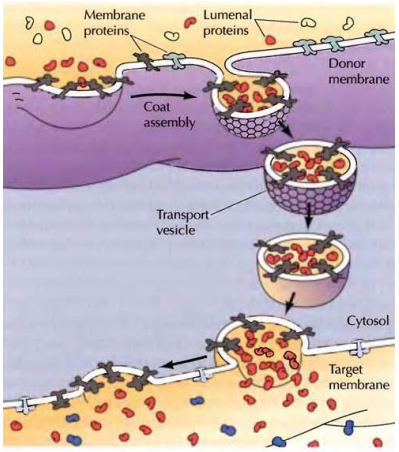
- The last example of signal sequences used in regulation is the **signal patch**. It is made up of amino acid residues that are distant from one another in the primary sequence but come close to each other in the tertiary structure of the folded protein, and this is how they act as a patch.

Vesicular Transport

Transport vesicles play a central role in the trafficking of molecules between membrane-enclosed compartments of the secretory pathway. As we've seen in previous topics, they are the transport method of choice between the ER, the Golgi apparatus, and other cellular locations.

General Scheme of Formation and Fusion of a Transport Vesicle:

- Membrane proteins and luminal secretory proteins are collected into selected regions of a donor membrane.
- Budding of a transport vesicle.
- Assembly of a coat protein (works as a shell) around the vesicle, which will direct the vesicle to its final destination.
- The vesicle is transported by motor proteins along microtubules to its target.
- Once the vesicle is close to its target, the coat is disassembled in the cytosol, which aids in fusion because the two membranes need to be very close to one another.

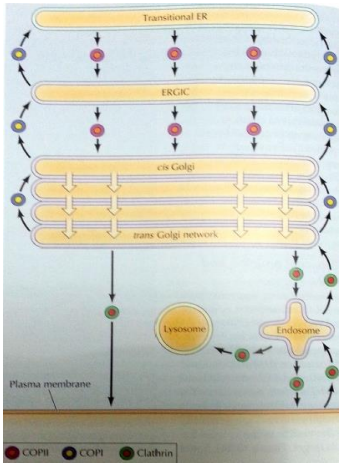


- The transport vesicle then docks at its target membrane, creating membrane instability, which will lead to fusion of the vesicle with its target.
  - Vesicular docking describe the process of the membranes drawing near to each other, similar to a ship docking.

Coat Proteins:

Three types of coat protein have been characterized: **clathrin**, **COPI**, and **COPII**.

Each of these proteins leads the vesicle to a different location:

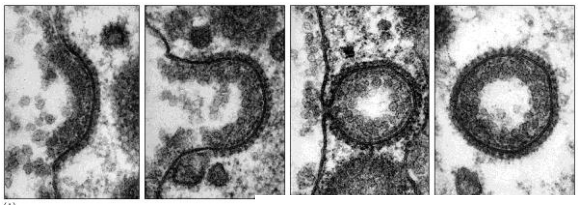


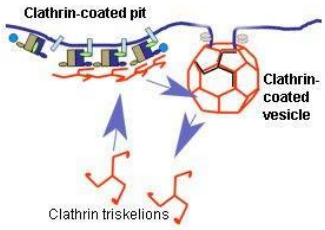
- Clathrin is responsible for carrying vesicles in both directions between the trans Golgi network, endosome, lysosome, and plasma membranes.
- COPI is responsible for the **retriever pathway**; from the *trans* Golgi network to the *cis* Golgi network, or from the Golgi to the ER.
- COPII is responsible for the transport of the vesicle from the transitional ER to the ERGC to the Golgi apparatus.

Formation of Coated Vesicles:

We will focus on the formation and regulation of clathrin-coated vesicles.

- **Formation:** As seen in the figure below, the vesicle starts to bud, resulting in membrane curvature and evagination. There will then be constriction of the membrane until the vesicle and membrane detach completely. The free vesicle gets coated by a coat protein (ex. Clathrin) which forms a honeycomb or a shell-like structure around the membrane.





- Clathrin has a triskellion shape.

The Role of Arf1 in Clathrin-Coated Vesicle Formation and Regulation:

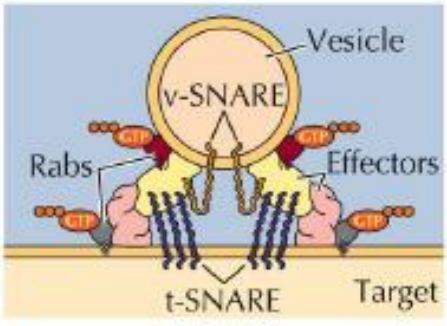
The formation of coated vesicle is regulated by small proteins that bind GTP, one of them being Arf1.

- The purpose of this binding is not energy, it is activation through conformational changes. Arf1/GDP is inactive, Arf1/GTP is the active form.

- Arf **GEF (GTP exchange factor)** is another protein involved in this process. It is responsible for the activation of Arf1 by the exchange of the GDP molecule bound to the inactive Arf1 with a GTP.
- *However, note that this process does not involve phosphorylation. It is merely an exchange. This is because phosphorylation requires energy, and exchange does not, which makes exchange more energetically-favored.*
- Now that we have an active Arf/GTP, it will recruit several proteins, such as adaptor protein AP1, GGA , etc. which will all aggregate next to the cargo-complex (soluble proteins) and serve as a binding site for the assembly of a clathrin coat. This will lead to curvature of the membrane and formation of a vesicle.

Vesicular Fusion:

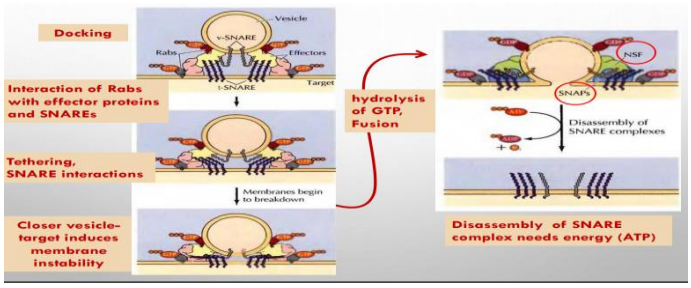
- Now the vesicle is carried by **motor proteins** and is transported to an area that is close to the target membrane; thus the protein coat is removed and the vesicle is now ready for docking process.
- The main point in fusion is to **make the vesicle really close to the target membrane to create membrane instability**; this occurs by interaction of membrane proteins of vesicles with the membrane proteins of target membranes.
- Both membranes combine together and are attracted towards each other, thus the distance shortens. The attraction is not the only thing that affects the ditance but also some conformational changes - bending of certain molecules - play a major role. By those molecules we mean specifically two membrane proteins that will interact: **v-SNARES** (of the vesicles) and **t-SNARES** (of the target membrane). T-SNARES have a hinge that allows them to bend allowing conformational changes to occur (interact).



- The interaction between t-SNARES and v-SNARES will allow attraction of effector proteins - mostly **RABs** (group of proteins that differ from one vesicle to another; they are GTP-binding proteins for conformational changes).

- **RABs allow the bending of t-SNARES and v-SNARES making the vesicle much closer to the membrane, allowing fusion.**
- After fusion, the SNARE complex disassembles with the help of a collection of proteins in addition to exchange of GTP for GDP (which leads to conformational changes). *This process requires energy in the form of ATP.* V- SNARES and t-SNARES are not part of the target membrane.

Here's a figure to help you visualize the process better:

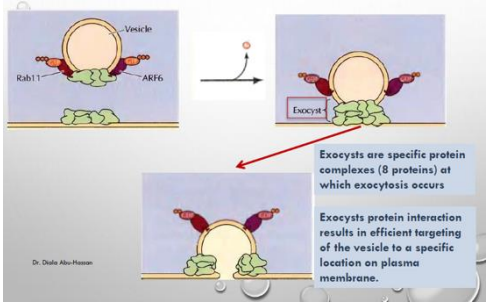


That's the general scheme of fusion for a vesicle.

How to differentiate between types of vesicles?

1. By the type of vesicular components which are: RABs, ARFs, type of protein coats, and other proteins such as exocysts.
2. When is the vesicle formed: endocytosis, exocytosis, or during transportation of molecules.

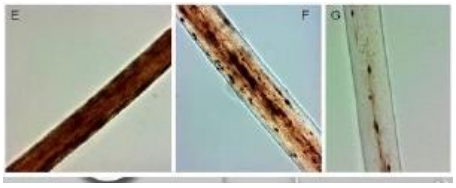
- **Exocysts:** group of 8 proteins that play a major role in exocytosis (*recall*: exocytosis is a form of active transport and bulk transport in which a cell transports molecules out of the cell by secreting them). They are illustrated by the green molecules in the following figure.
- The presence of exocysts allows us to know that this vesicle is responsible for exocytosis. Exocysts may exist on target membranes and on vesicles making it easier for the vesicle to fuse.



Clinical Application: *Griscelli Syndrome*

- A hereditary disease; rare genetic condition.

- Normally, melanin is formed in melanocytes specifically in melanosomes, then it's carried throughout the entire cell to pigment it by the help of vesicles.
- If there is a mutation in a specific RAB; **RAB 27A** or in **myosin** (here it acts as a motor molecule) it will result in the inability of melanosomes to leave melanocytes.
- Clinical presentation: Patients will have diluted skin (light in color) and grey hair regardless of the patient's age.
- If a hair specimen is examined under the microscope, melanosomes are found to be accumulated.



Lysosomes

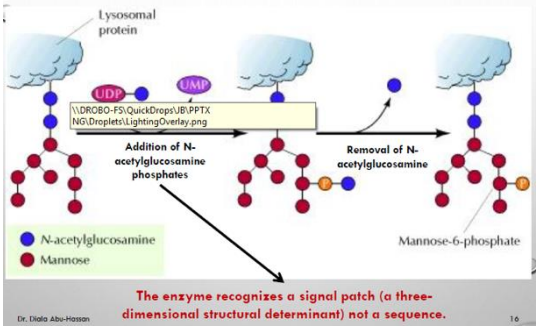
- Lysosomes are organelles made of two *leaflets*: inner and outer, they are **spherical in shape**, and **similar in action to the GIT**; so we call them the digestive system of cell.
- They achieve this by **acid hydrolases**; which are found in lysosome with a pH of 5 in contrast to cytosol's pH of 7 (100 folds more H+ in lysosomes than cytosol)
- Lysosomes are not only responsible for protein degradation, but also for nucleic acid, as well as polysaccharide degradation, and so on.
- The acidic environment must be established and obtained not only for the enzymes to function in an optimum manner, but also to **prevent the enzyme to work in an unusual place,** and to activate the enzyme; as it is secreted as a zymogen (a mechanism for protecting the surrounding organelles that's why **they are contained in a membrane**).
- Acid hydrolases can be either membrane or luminal proteins.

How is the acidic environment established?

⇨ By **proton pumps** that allow high concentrations of H+ to enter the lysosome.

Processing of Luminal Proteins:

- Luminal proteins are targeted to the lysosome by specific signalling sequences; related to N-linked glycosylation.
- After N-linked glycosylation, *phosphate is added to mannose #6 by a specific enzyme*; next N-acetylglucosamine is removed leaving mannose-6-phosphate. *Mannose-6-phosphate will act as a signal indicating that this protein will go to the lumen of the lysosome.*



Transport of Lysosomal Proteins:

- Mannose-6-phosphate on luminal proteins binds to the receptor on lysosome. The complexes are packaged into transport vesicles destined for late endosomes, which mature into lysosomes. Lysosomal membrane proteins are targeted by sequences in their cytoplasmic tails, rather than by mannose-6-phosphates.

Lysosomal Storage Disease

- If one of the previous enzymes is missed due to a genetic mutation, the reaction won't proceed, leading to **accumulation of molecules.** Thus the lysosome will enlarge, giving us a bubble-like shape under the microscope, resulting in the patient developing a lysosomal storage disease.
- The effect and severity of the disease depend on the enzyme missing and the number of reactions that are altered.
- The following disease are examples of lysosomal storage diseases: a) **glycolipidosis**, b) **oligosaccharidosis**, c) **mucopolysaccharidoses** (that works on GAGs).

The normal scenario:

- Glucocerebroside is a glycolipid ending with a glucose molecule.

- Inside the lysosome, an enzyme known as *glucocerebrosidase*, separates glucose from glucocerebroside, yielding *glucose and ceremide.*
- Some patients may lack this enzyme, causing the reaction to stop and develop a lysosomal storage disease. The disease is of 3 types, noting that type 1 is the most common, and least in severity; while types 2 and 3 are more severe and rarer.

Gaucher Disease

- Most common lysosomal storage disease.
- Not common in the Middle East but is seen more often in the western countries, epecially in Jewish people.
- Failure of lysosomes to degrade substances that they normally break down.
- The accumulation of non-degraded compounds leads to an increase in the size and number of lysosomes within the cell.

Oligosaccharidoses-Pompe Disease (type II)

- Related to α-1,4-glucosidase enzyme that is important in glycogen degradation (recall from biochem: glycogen degradation occurs in cytosol, but in this case it is affecting the degradation in lysosome).
- Glycogen structure is normal, but its amount is excessive.

I-Cell Disease

- Most dangerous type of lysosomal storage diseases.
- The enzyme missing here is the one responsible for phosphorylating mannose, so no lysosomal lumen protein will be targeted.
- So, we conclude that it is a disease of multiple enzyme deficiencies; this is what makes it the most dangerous.
- It has severe phenotypes; psychomotor retardation, and patients don't usually live long - they die by 5 to 8 years of age.

Treatment for lysosomal storage diseases:

1. Replacing the enzyme deprived; *enzyme replacement therapy*.

2. Activating enzyme synthesis - only if the enzyme is synthesized in the body.
3. Gene therapy; occurs by replacing the abnormal gene with a normal one since those disease are hereditary (not a common tx).
4. Giving the end product of the enzyme.

Chloroquine: Application

- Anti-malarial drug (malaria is caused by a parasite that enters RBCs).
- Inside the parasite's vacuole, heme and protein are separated from each other and are then degraded; heme is degraded by heme polymerase.
- Thus the function and conformation of RBCs will be altered.
- If heme inside the parasite's vacuole is not degraded, it will accumulate, causing toxicity inside the parasitic cell; thus the parasite will be killed.
- So, chloroquine functions by crossing the membrane of parasite, entering the vacuole, and inhibiting heme polymerase.

What does this have to do with lysosomes?

⇨ Chloroquine is a weak base, so when it enters the lysosome, it is going to be protonated - activating it, so it can be secreted by a vesicle.

So we conclude that lysosomes activate chloroquine by protonating it.

exocytosis and phagocytosis are covered in the next lecture

Good luck