CYTOLOGY

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◉ Sheet ○ Slides

Number

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Protein Sorting (Golgi Apparatus)

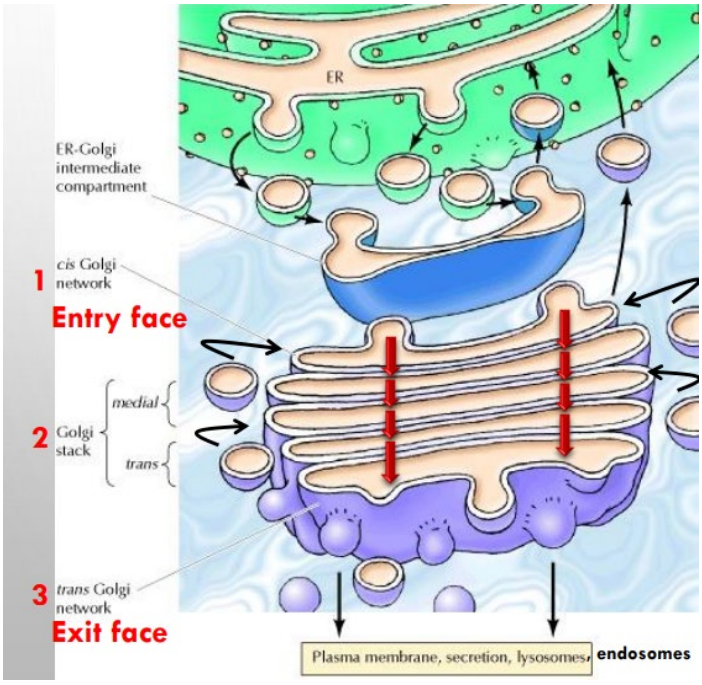
Structure and Functions of Golgi

Golgi Apparatus is the next stage in the secretory pathway
(ER → ERGIC → Golgi)

*Golgi differ in its structure from **ER** →(connected membranous sacs) but in case of **Golgi** → Composed of flattened membrane-unconnected sacs (cisternae) separated from each other and from the associated vesicles.

So, movement <u>through</u> **Golgi** –addition information-By two mechanisms; proteins are carried in the cis to trans direction: within the Golgi cisternae, which progressively mature and move through the Golgi apparatus or in transport vesicles.

While in **ER** molecules can move directly from the side close to the nucleus to the transition side without the need of vesicles.



• The Golgi apparatus is composed of cisternae that receive proteins from the ER, process them, and sort them to their eventual destinations.

• A striking feature of the Golgi apparatus is its distinct polarity in both structure and function.

<u>Note :</u>

1. The compartment that is close to ERGIC is called cis-Golgi network (CGN) or entry face.
2. In the middle region between cis and trans networks, there is a Golgi stack, and this region is split into 2 regions: medial and trans Golgi stacks.
3. The compartment that faces the plasma membrane and exports different types of proteins is called trans-Golgi network (TGN) or exit face.

Proteins from the ER-Golgi intermediate compartment enter the cis compartment of the Golgi apparatus where modification of proteins, lipids, and polysaccharides begins. After progress through the medial and trans compartments, where further modification takes place, they move to the trans-Golgi network (TGN), which acts as a sorting and distribution center, directing molecular traffic to endosomes, lysosomes, the plasma membrane, or the cell exterior. The mechanism by which proteins move.

*The direction of movement in Golgi→ from cis to trans, but sometimes some vesicles need to move in the opposite direction ☺ → from TGN to cis (such as a vesicle with a Golgi protein needs to be in the cis face)

*The red arrows in the picture above show the forward direction of movement of the whole sac with its content of proteins.

*The movement of sacs: after a certain sac finishes its processes, it moves from its position forwardly (for example from cis to be as a medial Golgi stacks) and a new stack will be formed to be as a cis stack, then the old one moves to be as a trans stack and the new one moves to be as a medial and so on.

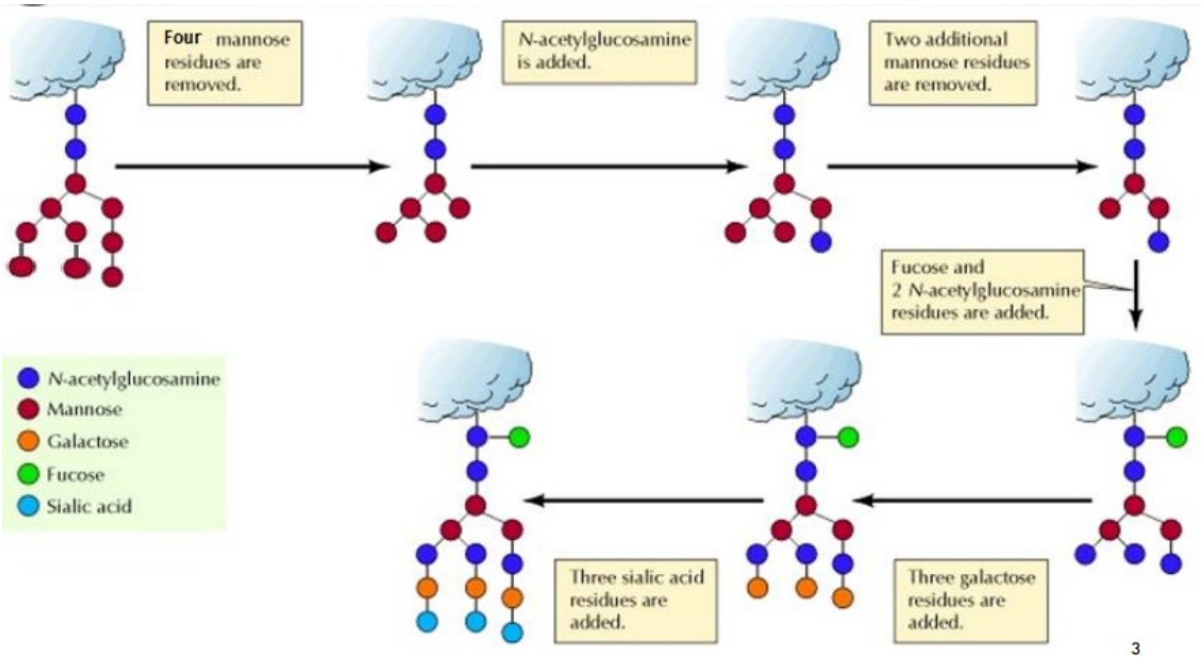
*The backward direction of movement carried out only by vesicles.

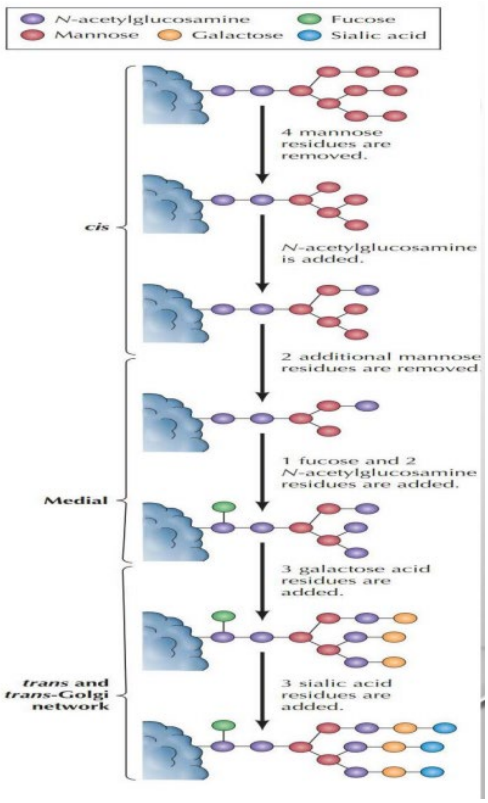
ϖ What happens inside Golgi Apparatus ?

- Further protein processing and modification
- Protein sorting and distribution(distinguish the final destination of each protein so being well sorted when packaging them)
- Synthesis of glycolipids and sphingomyelin
- Processing of N- linked glycosylation

* before budding, all the proteins that will transfer to a certain destination (for example mitochondria) will be gathered in a certain part of the sac membrane thus form a separate vesicle when bud, so separate vesicle are formed according to their final destination.

Protein glycosylation within Golgi Processing of N-linked Oligosaccharides in Golgi





- **Processing of N- linked oligosaccharides in the Golgi** → The N-linked oligosaccharides of glycoproteins transported from the ER are further modified (addition and removing) by an ordered sequence of reactions catalyzed by enzymes in different compartments of the Golgi.

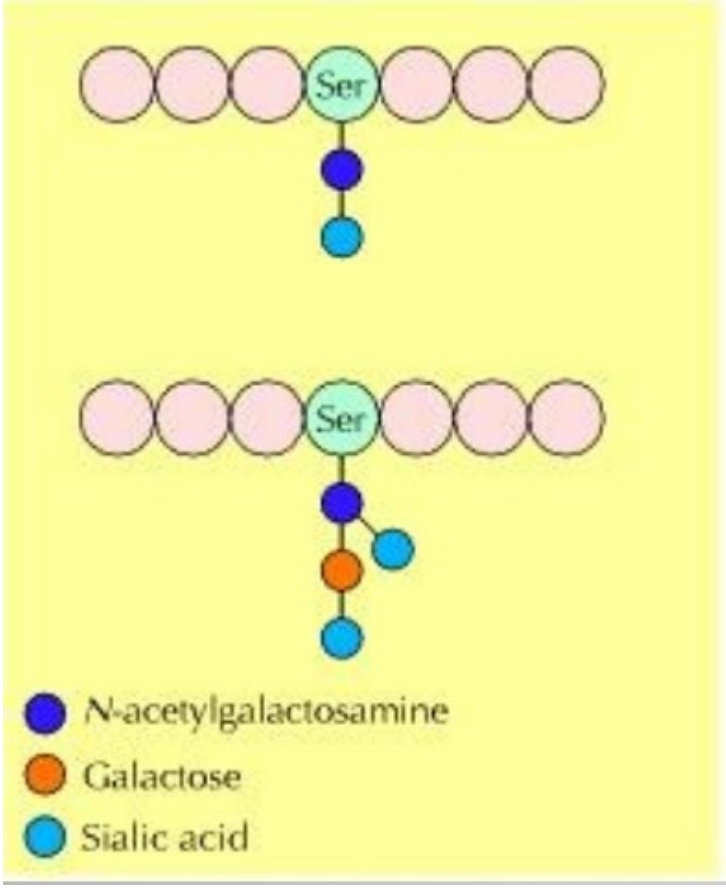
* Remove an OH group from galactose (reduction) produce **Fucose.**
* **Sialic acid** is a modified sugar.

* Different glycoproteins are modified in different ways during their passage through the Golgi, depending on both the structure of the protein and on the processing enzymes present in the Golgi complexes of each cell type. Consequently, proteins can emerge from the Golgi with a variety of different N-linked oligosaccharides.

*The basic units for proteins that have been reached in the ER are not enough to finish the process of modifying them so they transfer to Golgi where the enzymes that catalyze there further modifications are found rather than in the ER.

O-linked Glycosylation

• Happens completely from the beginning in Golgi.
• Carbohydrates are added to the side chains of acceptor serine and threonine residues.
• Amino acids (serine and threonine) to which these sugars are added have OH group in their side chain (R group).
• The serine or threonine is usually linked directly to N-acetylgalactosamine, to which other sugars can then be added.
N-acetylgalactosamine is added as a first step directly to serine and threonine in a certain protein then sugars from other types (here Galactose + Sialic acid) are added.
• Some of the added sugars are further modified by the addition of sulfate groups(sulfation).



ADDITIONAL NOTES

protoglycane : a compound consisting of a protein bonded to polysaccharide groups such as Glycosaminoglycans.

Glycosaminoglycans :mostly contain modified sugars such as N-acetylglucosamine or N-acetylgalactosamine and some has a sulfur group that give them a negative charge so they are highly polar molecules work as cushions in the ECM because they attract water molecules (act as shock absorptions).

"العالم كله يتنحى جانبا ليفسح المجال للشخص الذي يعرف تماما إلى أين يتَّجِه" !!

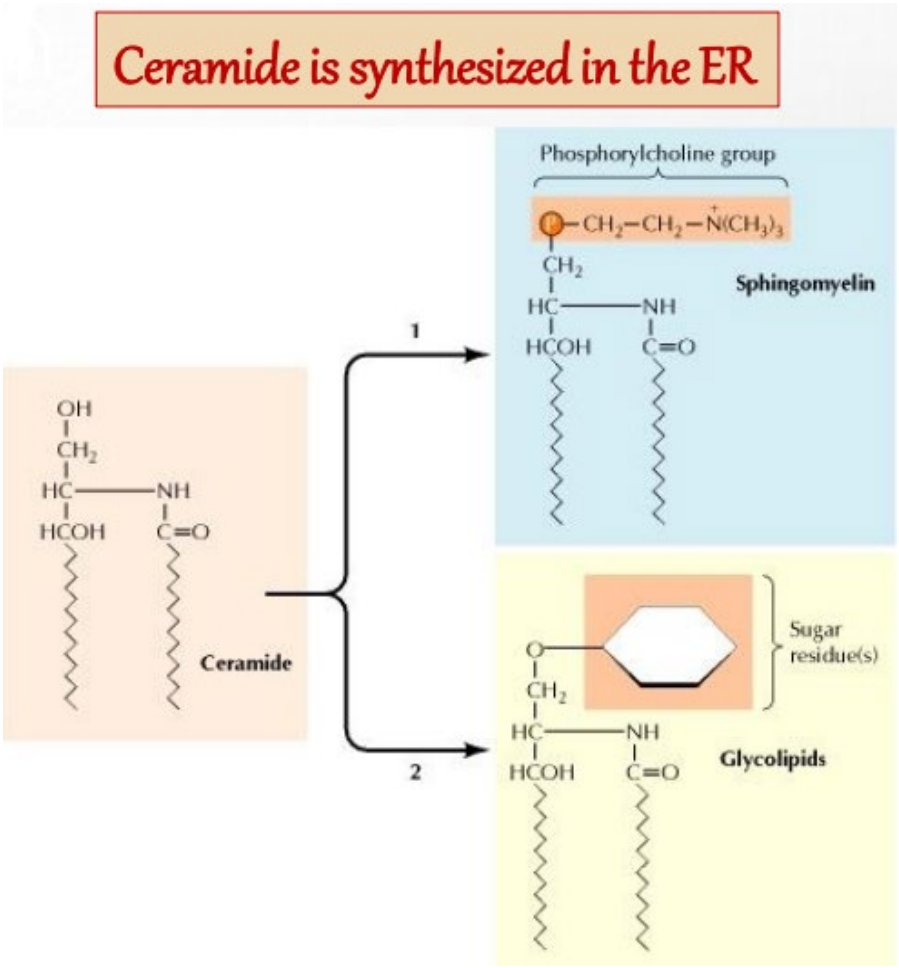
Lipid and Polysaccharide Metabolism in the Golgi

* In addition to its activities in processing and sorting glycoproteins, the Golgi apparatus functions in lipid metabolism-in particular, in the synthesis of glycolipids and sphingomyelin.

* The glycerol phospholipids, cholesterol, and ceramide are synthesized in the ER.

* Sphingomyelin and glycolipids are then synthesized from ceramide in the Golgi apparatus.

* Sphingomyelin is the only nonglycerol phospholipid in cell membranes.

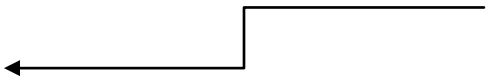


Synthesis of sphingomyelin and glycolipids

Ceramide, which is synthesized in the ER, is converted either to sphingomyelin (a phospholipid) or to glycolipids in the Golgi apparatus.
In the first reaction, a phosphorylcholine group is transferred from phosphatidylcholine to ceramide which produce sphingomyelin. Alternatively, a variety of different glycolipids can be synthesized by the addition of one or more sugar residues (e.g., glucose).

* Phospholipids are made in the cytosolic side (outside the ER) then flip to the inside of it.

***AT FIRST:** Sphingomyelin ⟶ is synthesized in the lumenal surface of the Golgi. but glycolipid (glucosylceramide) ⟶ is synthesized – by adding **glucose to ceramide – in the cytosolic surface .
THEN: glucosylceramide apparently ***flips and additional carbohydrates; oligosaccharide or another sugar are added on the lumenal side of the membrane,
Why?



***Glycolipids will be extracellular.
So, if the oligosaccharide-which is a very large and huge molecule- is added cytosolic (without flipping to the luminal surface in the Golgi membrane), then the flipping process (that should be occur in the plasma membrane) will be very hard and energy consuming.
**sugar (glucose) must be added to the molecule to know that this is going to be glycolipid not sphingomyelin. then it enters and further modifications are done.

Glycolipids are not able to translocate across the Golgi membrane, so they are found only in the lumenal half of the Golgi bilayer as is most sphingomyelin. Following vesicular transport they are correspondingly localized to the exterior half of the plasma membrane, with their polar head groups exposed on the cell surface.

Protein Sorting and Export

• Proteins as well as lipids and polysaccharides are transported from the Golgi apparatus to their final destinations through the secretory pathway.
• The proteins are sorted into different kinds of transport vesicles(according to the final destination), which bud from the trans-Golgi network and deliver their contents to the appropriate cellular locations.

• Some proteins are carried from the Golgi to the plasma membrane, either directly or via endosomes as an intermediate compartment. In addition, proteins can be sorted into distinct secretory granules for regulated secretion. Alternatively, proteins can be targeted to late endosomes, which develop into lysosomes.

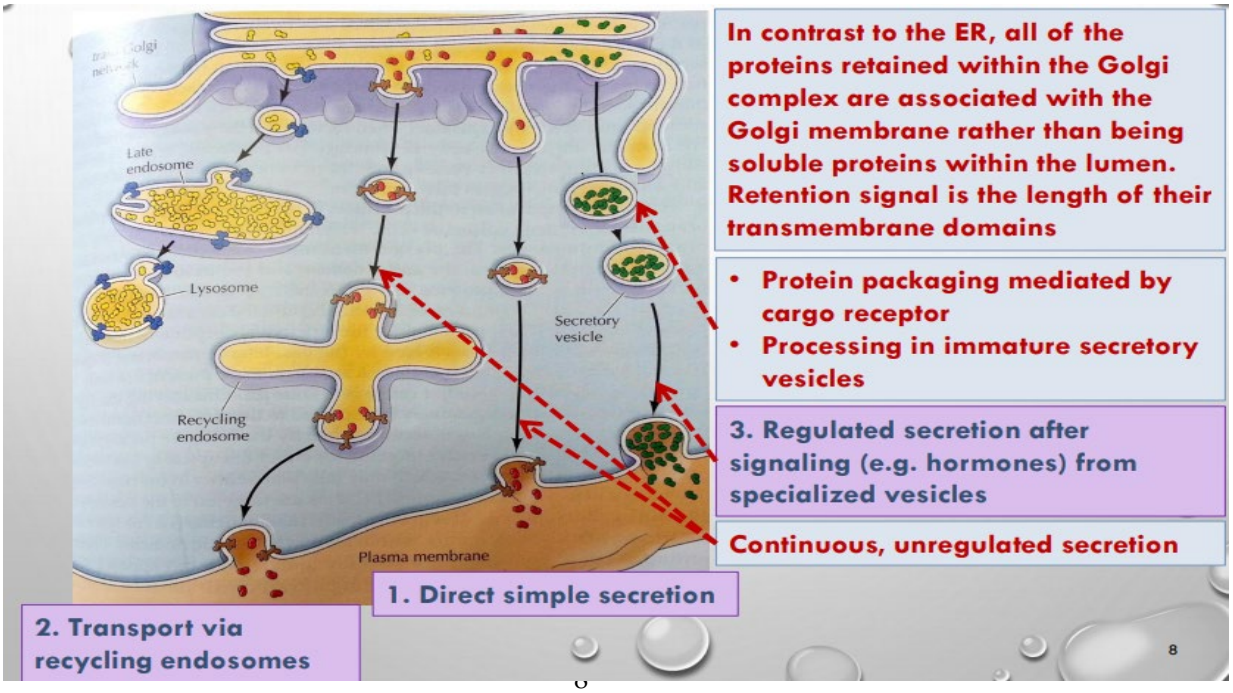
• Transport from the Golgi apparatus to the cell surface can occur by at least three routes:

1. *Direct transport* from the trans-Golgi network to the plasma membrane, which leads to the continuous secretion (unregulated secretion) of soluble proteins from the cell as well as the incorporation of membrane proteins and lipids into the plasma membrane.

2. *Indirect transport* from the Golgi to the plasma membrane via an intermediate of recycling endosomes, which are one of three types of endosomes in animal cells.(→budding and formation of a vesicle which contain soluble and membrane proteins, types of sphingolipids, phospholipids and other molecules → the vesicle fuse with the recycling endosome→ then form a vesicle again and move toward the plasma membrane).

3. Some cells also possess a distinct *regulated secretory* pathway in which specific proteins are secreted in response to environmental signals (e.g. release of hormones from endocrine cells, the release of neurotransmitters from neurons). In another words, if the protein that I want to be secreted is a hormone -such as Insulin- the process should not be as simple as mentioned before because I don't want the hormone to be continuously secreted but under certain stimulation (someone ate a meal full of sugar→ secrete Insulin to reduce sugar amount in blood) so the process should be more regulated to avoid losing control [a stimulus activate signaling pathway which cause the secretion of the hormone].

- In this case the vesicle contain only soluble proteins =secretory proteins
- not all hormones are proteins.



Transport to the plasma membrane of polarized cells

• The plasma membrane of polarized cells is divided into two separate regions, the **Apical domain** and the **Basolateral domain**

• the **Apical** membrane of intestinal epithelial cells faces the lumen of the intestine and is specialized for the efficient absorption of nutrients , the remainder of the cell is covered by the **Basolateral** membrane.

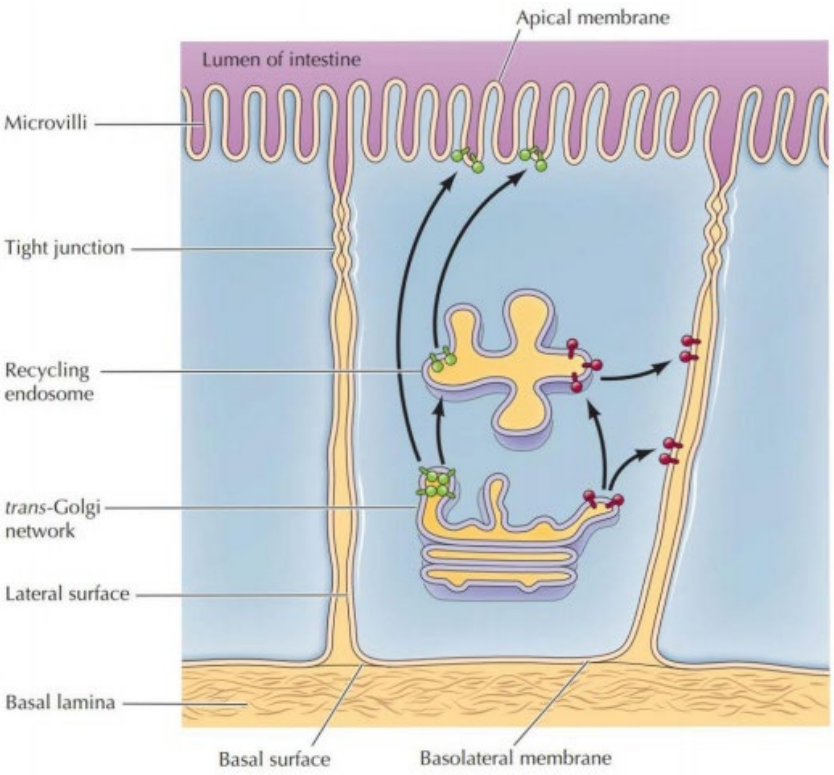
•The transport of some proteins to specific parts of the plasma membrane especially in polarized cells (in which some proteins need to be targeted toward the **Basolateral** surface of the membrane and some toward the **Apical** surface because they can't move in response of the tight junctions) should be correctly targeted to the correct final destination of the plasma membrane.

ϖ How does this process happen?

This distinguishing happens by the selective packaging in which the vesicle that will transfer (from the trans Golgi or recycling endosomes) to the **Apical** surface differ with its content from the one targeted to the **Basolateral**.

*If the protein will transfer to the **Basolateral** surface then a special amino acid sequence (signal sequence) will guide it to this area.

*If the protein will transfer to the **Apical** surface then targeting is determined by sugar modification.



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