Genetics
& Cell biology

◉ Sheet ○ Slides

Number

4

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Recap
Points to know from last lecture:
-The Golgi apparatus has cis and trans faces
-Its cisternae moves
-Not connected
-Molecules that undergo modification in the Golgi aren't transported by vesicles between Golgi sacs, instead, Golgi sacs themselves mature and develop as they modify molecules (cis sac →middle → trans)
-O-linked glycosylation and completion of N-linked glycosylation occurs there
-Sphingolipids: Ceramide is synthesized in the sER then it is modified in the Golgi by addition of polar groups (phosphocholine from phosphotidylcholine, or sugars)
**Student's question:
Q: When sphingolipids are synthesized, where are the head groups added?
A: Depends, Sphingomyelin is synthesized on the *luminal side*, and glycolipid on the *cytoplasmic side.*
Note: Use the terms "luminal" and "cytosolic" so as not to mix "outer" and "inner" sides for different organelles.
-In contrast to the ER, most of the proteins retained within the Golgi complex are associated with the Golgi membrane (transmembrane proteins) rather than being soluble proteins within the lumen.
Note: Please have a look at slide 6 and 7, the doctor didn't talk about them in the record.(lecture 3 slides)
End of recap

Protein Sorting and Export

-To target different vesicles that are exported from the Golgi to different destinations, tagging of the proteins is done by a *special signal sequence* (content wise), and by *coat proteins* that surround the vesicle right after budding, which gives the vesicle mechanical support and prevents it from being loose because of the cage like structure surrounding it.

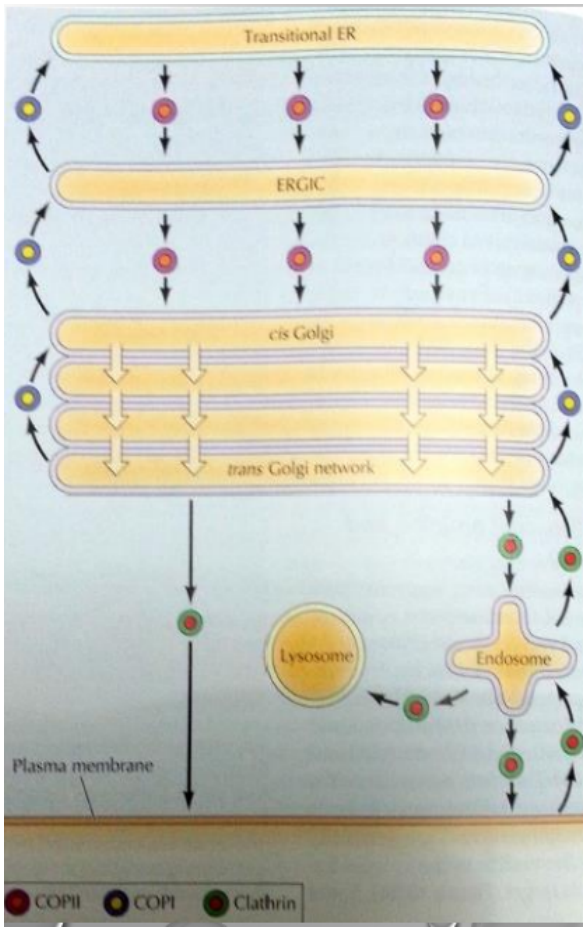
-We have different kinds of coat proteins and each lead the vesicle to different destinations and directions of movement, some move it forward, some backwards, etc. (In third year when we take more about the cytoskeleton, which is the road that these vesicles/organelles move on, we'll know that microtubules have carrier molecules that carry the vesicles and moves them, and those carrier molecules and microtubules also affect the vesicle's direction of movement.)

-Depending on the direction of movement, budding location and final destination, we have 3 types of coating proteins:

1- COPII (coat protein 2): exports proteins in the forward direction in the early steps of secretory pathway E.g. From ER to Transitional ER to Cis face of Golgi.

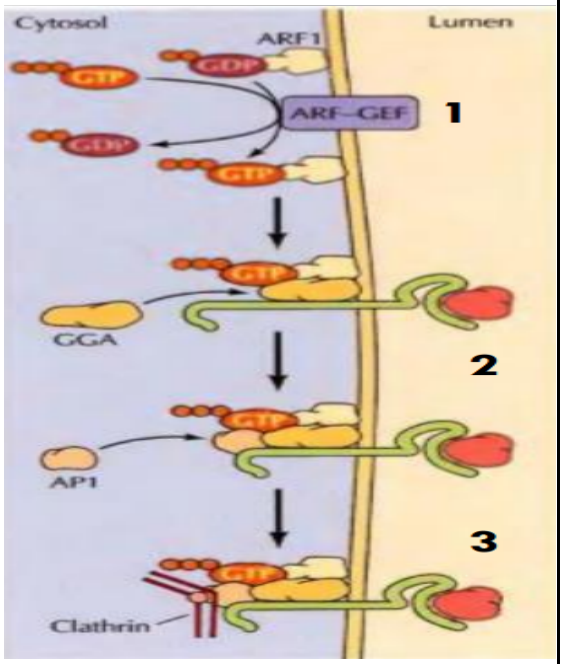
2- COPI (coat protein 1): Retrieval of vesicles in the opposite direction of the secretory pathway E.g. from Trans Golgi to cis Golgi. Or from Golgi to ER.

3- Clathrin: exports protein in the forward direction in the last steps of secretory pathway E.g. from trans Golgi network to Plasma Membrane, Lysosome, etc. It looks triskelion which helps in the making of the cage-like structure surrounding the vesicle



-Proteins moving from Cis face of Golgi to Trans face of Golgi (Forward direction) do not need vesicles to move, as the cisterna of Golgi itself that makes the cis face moves to become the Golgi stack, which then moves again to become the Trans face. But Proteins moving from Trans Golgi to Cis Golgi (Reverse direction) need vesicles to move.
Coat proteins accumulate right after evagination occurs.
After modification is done, vesicles are going to be formed and transported.

-How do vesicles form?
Evagination and membrane curvature is a very complicated process, so there should be an accumulation of certain proteins at the site of evagination to stimulate vesicle forming. One of these proteins is the ARF1, which is a GTP binding protein, so the presence of GTP is going to either activate or inactivate this protein (Conformational changes occur due to presence of GTP/GDP which will affect the vesicle formation).



10 Minutes

-In the inactive form ARF1 is bound to GDP, to be activated, a GTP exchange factor (GEF) will replace GDP with GTP, which will turn ARF1 to the active form so now it can interact with other proteins

that are involved in the vesicular formation and gathers them, like GGA, and AP1, which then in turn interact with one of the membrane receptors/proteins that will be a part of this vesicle.

-The presence of these proteins together in this arrangement is important to bring in clathrin. Notice how the proteins, including clathrin started accumulating before complete separation of the vesicle. In case of coat proteins other than clathrin, proteins other than ARF1, AP1, and GGA accumulate. (Large number of proteins, and large number of isoform for each protein which affect targeting and making different types of vesicles)

Vesicular Fusion

-Now the vesicle is made and coated and has been transported to its target membrane, once the vesicle is very close to the target membrane, disassembly of the coat occurs to facilitate fusion.

-Fusion needs another batch of proteins to occur, these can be membrane proteins or soluble proteins, such as:

1- RABs (GTP binding proteins): different combinations of RAB proteins mark different organelles and transport vesicles.
2- Effector Proteins: facilitate the interaction between target membrane and vesicular membrane.
3- SNAREs: membrane proteins can be present on the target membrane and is called t-SNARE or on the vesicular membrane and is called v-SNARE.

-When the vesicle is approaching the target membrane it already has the v-SNARE built in its structure, and the target membrane has the t-SNARE built in its structure. Notice how the molecules are slightly bent in the original inactive form.

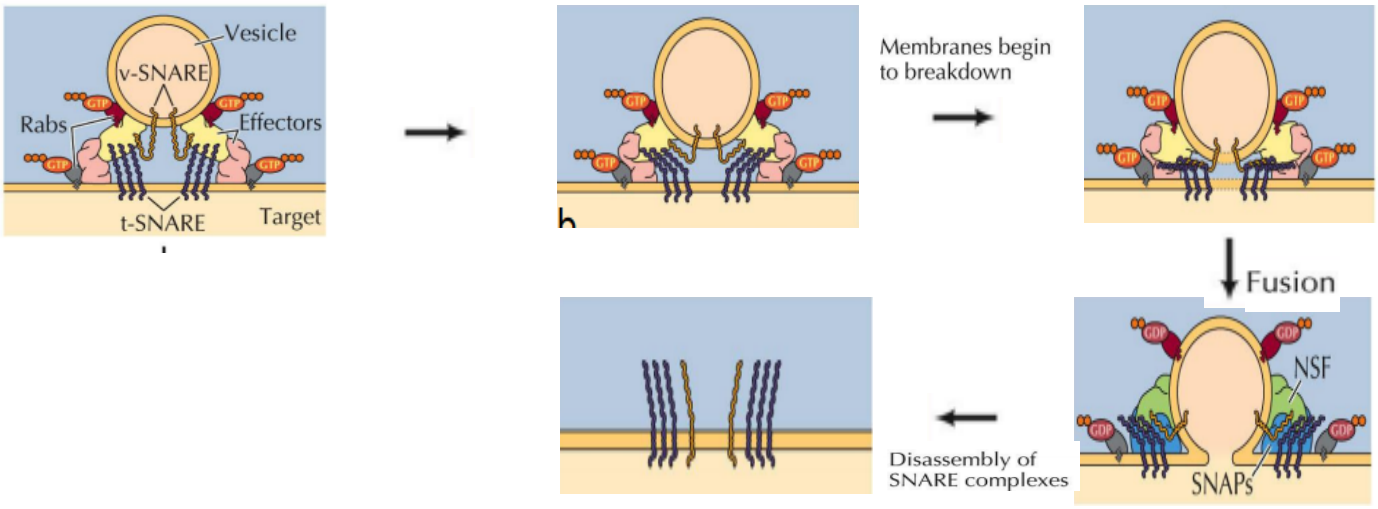
-Fusion starts by docking and accumulation of RABs in the active form (bound to GTP), and effector proteins which connect between the RABs and proteins present of target membrane with the RABs and proteins present on vesicular membrane. This induces a conformational change in the present proteins, the most important change is the increase of bending of the t-SNARE and v-SNARE which brings the two membranes in closer proximity to each other, and that creates an instability in the membrane, so they either fuse or separate, and since we have proteins holding those membranes together this activates fusion.

-Now that RABs and effector proteins are no longer needed, the Disassembly of the RABs and effector proteins from target and vesicular membranes and transfer of

vesicular content occur next. This is facilitated by two new proteins which are SNAPs and NSF. Exchange of GTP bound to RABs with GDP, inactivates RABs thus also facilitates disassembly.
Note: disassembly of SNARE complex needs energy.

-Proteins of the vesicular membrane (v-SNARE and other proteins) are inserted into the target membrane releasing the content of the vesicle.

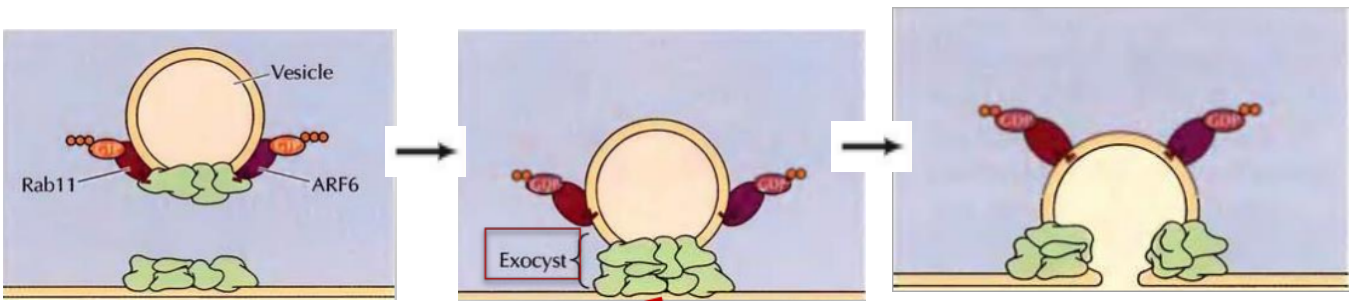
Note: the table in slide 15 is not included, it is just to show how complicated of a process fusion is, and how types of protein change according to direction of movement, which adds specialization to the different types of vesicles.



Exocytosis

-The process of releasing content to the outside of the cell.

-How is it different from export?
Exocytosis releases content to the outside of the cell while export transfers content from one organelle to the other inside the cell.



-Proteins that are needed in exocytosis:
1- RAB11
2- ARF6
3- Exocysts: 8 proteins, some accumulate on the vesicular membrane, and some accumulate on the plasma membrane. Their interaction results in efficient targeting of the vesicle to a specific location on plasma membrane.

-Again, the accumulation of these proteins on the surface at the interaction site between the two membranes is what facilitates the fusion of this special type of vesicles.

-Exocysts are still not 100% understood, and there aren't many details about how they work, but what is known is that there is an interaction.

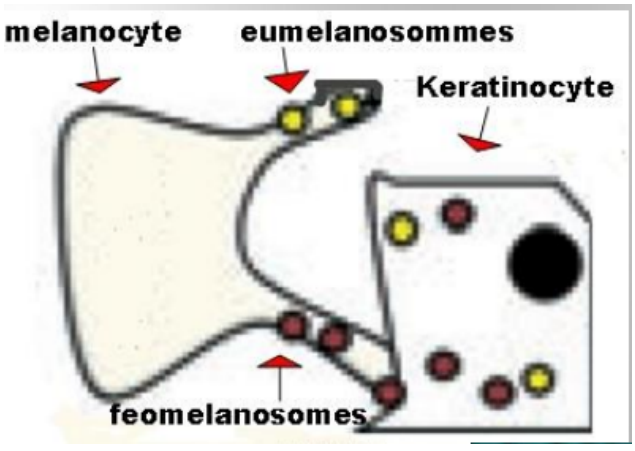
20 minutes

Clinical application

-Applications of vesicle secretions include the transfer of neurotransmitters, hormones, etc. Other applications include the transfer of pigments in the vesicles, such as Melanin (made from tyrosin)

-Melanin is made in melanosomes (small compartments within melanocytes), but is effective in keratinocytes in the skin. This calls for the melanin to be packaged and transported from melanocytes to keratinocytes by vesicles.

-Griscelli Syndrome is a rare syndrome that affects people with mutations in one of the proteins which are involved in vesicular movement and fusion. Those proteins can be RABs (RAB27A), Myosin (MYO5A), and MLPH.



-Symptoms: silver gray hair, melanin clumps in hair shaft because mature melanosomes accumulate in the center of melanocytes, pigmentary dilution of the skin.

-Other applications: Why do we get tanned in the sun?
Because the accumulation of proteins responsible for the melanin transport to keratinocytes increases with light, and the vesicles transporting the melanin are stimulated with light.

25 minutes

The End, Sorry for any mistakes.