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بسم الله الرحمن الرحيم

GENETICS & Molecular Biology

Number: 19

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Subject: ER/Golgi

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Date:

**This sheet was written according to the records that belong to pack 1 and 2.

- the folding process that proteins undergo in the ER:
  - Formation of disulfide bonds (discussed in sheet 18)
  - N-linked glycosylation(discussed in sheet 18).
  - Addition of GPI anchors

Addition of GPI anchors:

Now, we have peripheral proteins and they need to be attached to the membrane via different types of anchors, ex: GPI, which is a glycolipid.

- First, proteins will be attached to the membrane via group of hydrophobic amino acids, then they will be cleaved so there is a new C-terminus which will interact with GPI's anchors by a covalent interaction between the NH2 of ethanolamine in GPI &the new C-terminus.
- Now, depending on what we took last lectures about orientation of membrane proteins, GPIs are located in the outer leaflet of the membrane which dictates that the proteins anchored to it are oriented to the outside surface of the plasma membrane.this orientation is determined at this stage of the pathway because GPI anchors are oriented to the luminal side of the ER and any protein anchored to it in the ER will be considered luminal.
  In conclusion :any luminal protein in the ER will be oriented to the luminal surface of the target organelle butthis doesn't apply to the plasma membrane because any luminal protein in the ER will be oriented to the exterior surface of the plasma membrane.

Note: Anchors facilitate the movement of proteins within the membrane and facilitate their action within the signalling pathway, ex:

- RAS >>it is attached to the membrane via frensylation , and when there is an activation of signalling pathway, it may need to move and dissociate from membrane by cleavage of the interaction between it and the frensyl group. so this make its job easier. { this example mentioned on section 1 only }

GPI : (Glycosyl Phpsphatidyl Inositol)

Which is a type of glycolipid (phospholipids), it is a part of the inner membrane of ER. It has 2 fatty acid chains, an oligosaccharides portion consisting of inositol and other sugars, and ethanol amine.so the carbohydrate portion interacts indirectly with the protein through it's attachment to ethanolamine.

❖ Quality control in ER:

ER is determined as quality control station inside the cell, what does this mean?!

- ER is responsible is responsible for the correct folding of proteins, and assists through its chaperons in the folding of the proteins, but after all these processes, mistakes will happen (even after the proofreading of chaperons.).
- These mistakes which will result in improper folding, so we have to check whether folding is correct or not**before** transferring it to golgi:
  - If it is correct: keep this protein and do whatever you want with it :P
  - If it is not correct: unfold it, try to refold again and if it stays misfolded just degrade it. This process is known as : ER-associated degradation.

- A famous example of the outcome of misfolded proteins is sickle cell anaemia in which we have a mutation in one amino acid (glutamic acid (polar) but is replaced by valine (non polar)), so normally polar amino acids tend to be in exterior of the protein, but in case of this mutation; the valine (non

polar) needs to cover itself from the aqueous environment, so it starts to aggregate itself with other valine amino acids from another protein molecules.

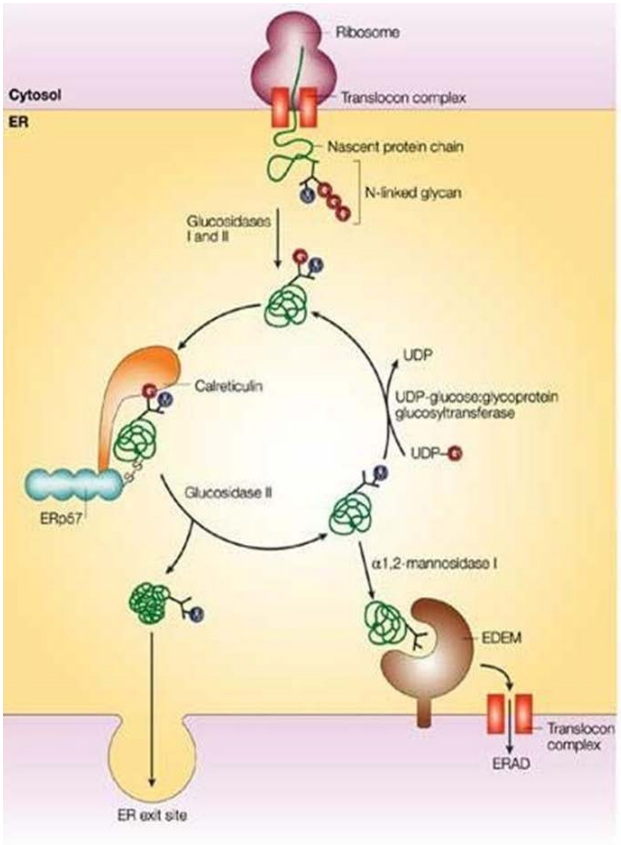
❖ How we will check the folding of these proteins? Or how does the ER senses the correct folding of proteins??

By chaperons, There are many types of Chaperons, ex: Chaperon hsp70, Hsp40,& calreticulin.

Note: chaperons are proteins concerned in providing the appropriate environment for the correct folding & sensing wether these proteins are correctly folded or not)

An example of the role of chaperons is Calreticulin:

- In the picture below, there's ribosome and the structure inside it is the primary polypeptide chain, then it will be transferred by translocon to the ER lumen .During this process the protein (poly peptide) is glycosylated so it becomes a glycoprotein.



Now it is inside the ER lumen ready for modification and folding.

- As the glycoprotein exits the translocon, 2 glucose residues are removed, allowing calreticulin to bind and assist in folding.

Removal of another glucose residue terminates the inter action with calreticulin releasing the glycoprotein.

- A protein(folding sensor)then assess the extent of folding of the glycoprotein, now there is 2 pathways depending on the extent of correctfolding:
    1. If correctly folded the glycoprotein is passed on to the transitional ER and then move it to the next station in Golgi apparatus.
    2. If incorrectly folded: the folding sensor will add glucose to the glycoprotein, allowing it to re-enter the calreticulin again in order to unfold and then refold it.
    3. If the protein is completely misfolded and it's very hard to re-fold it again; it will be degradedby ubiquitin proteosomal system.

❖ Unfolded Protein Response:

<< You have to return to the book page (391-392) for the details....it's required>>>

- What is the difference between UPR and ERAD ?!
    - ERAD: (ER- Associated Degradation), checks if the protein is folded probably or not, tries to rectify the misfolded proteins, and if the proteins are severely misfolded sends them to degradation via the ubiquitin – proteosomal system.
    - UPR (unfolded protein response) is a system activated when we have an overload of misfolded proteins { large number of proteins that are not folded probably ans will exhaust the ER with the energy consuming (fold- unfold – refold)process that it has to perform before it sends them to Golgi.

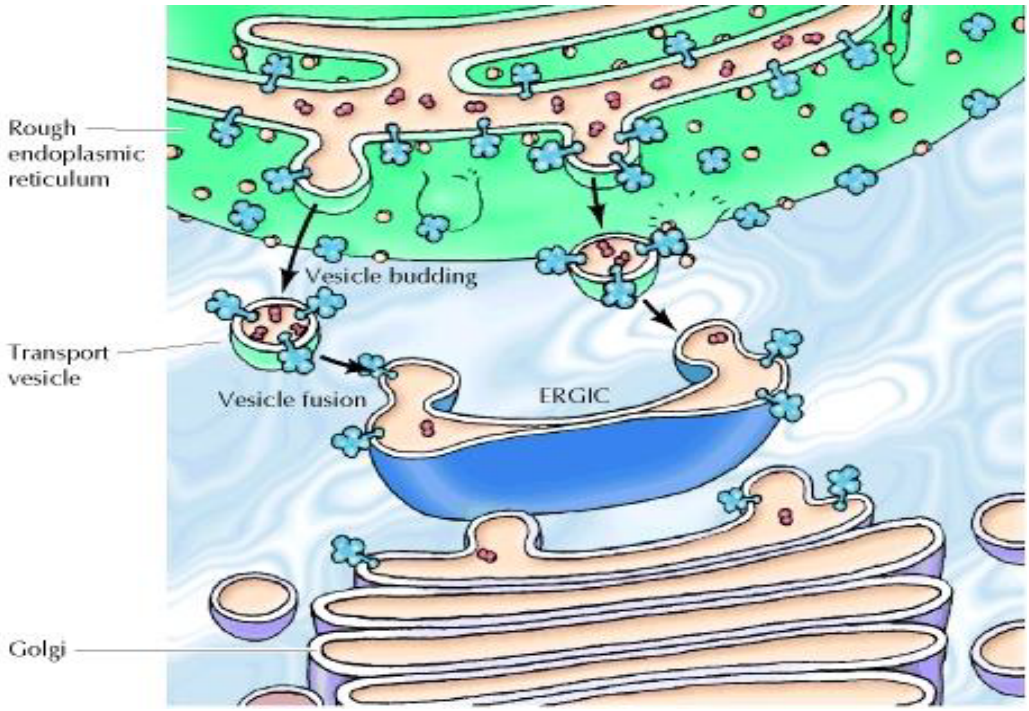
Activation of the UPR leads to :

a) Increase the number of chaperons
b) Inhibition of translation
c) Inhibition of translocation
d) Expansion of ER, so it can accumulate more proteins.

The aim of the previously mentioned mechanisms is to adjust protein folding in the ER to a Normal level.
Note: sustained activity of the unfolded protein response leads to programmed cell death (apoptosis).

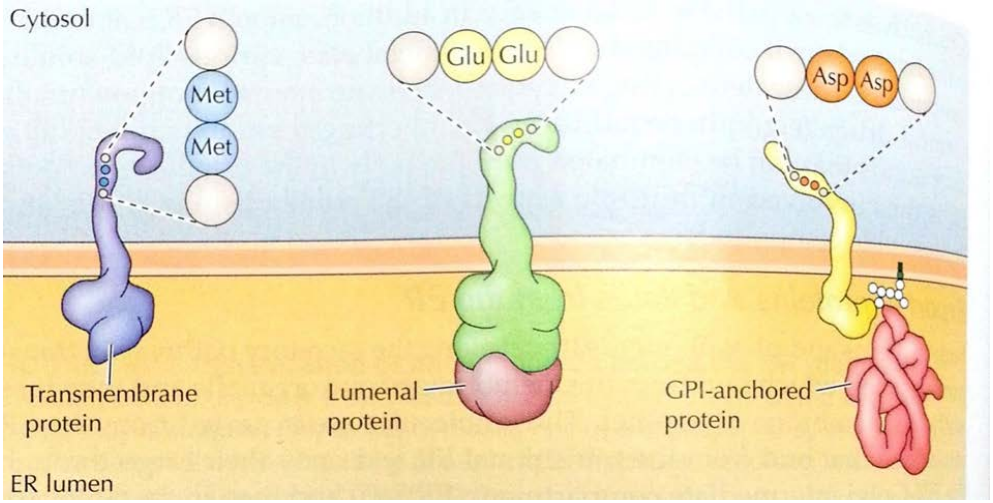
❖ ER-Golgi intermediate compartment (ERGIC)

- After the protein is modified in the ER, some proteins need more modifications which will occur in in Golgi apparatus, and transferring proteins from ER to Golgi occurs through a compartment known as ERGIC.
- Vesicle buds from ER → fuses with ERGIC → go to Golgi.
- The most important thing in the **picture below** is to notice the topological orientation.
- Orientation of membrane protein is kept through all this secretory pathway, which means that the membrane proteins which were in the outer leaflet in ER, will stay in the outer leaflet inERGIC and in Golgi, and the proteins which were in the lumen will also kept in the lumen of ERGIC and in Golgi.



❖ Protein Sorting and Retention

- We have some proteins which are specific to ER, but also they need some modifications (like : O- glycosylation which hhappens only in Golgi) so we transfer them to Golgi where we modify them and then return them back to ER through signal sequences like KDEL, KKXX.
    1. KDEL sequence (one letter abbreviation of Lys – Asp – Glu – Leu), and this sequence is situated next to the C-terminus of the protein.
    2. KKXX means 2 Lys residues and 2 any other amino acid.The retention of **transmembrane proteins** is mediated by this signal.

  Note:
    1. If this sequence is deleted; the protein is transported to Golgi and then secreted from the cell.
    2. Addition of the sequence causes a protein to be retained to the ER.
- There is other sequences rather than KDEL and KKXX , such as di-hydrophobic or di-acidic amino acids .which function as carriers of **GPI- anchored and lumenal proteins**.
- 

❖ Synthesis of phospholipids in SER

- SER is free of ribosomes, so it doesn't deal with protein modification or synthesis, but rather; it plays a major role in:
  1. Membrane lipid synthesis.
  2. Detoxification of chemicals.

1) **Membrane lipid synthesis** : SER is responsible of synthesizing:
   1. Phospholipids which are mainly synthesized in the liver
   2. Cholesterol which is mainly synthesized in the testis and ovary.
   3. Ceramide which will be further modified into glycolipids or sphingomylein in the Golgi apparatus.

Phospholipids synthesis :

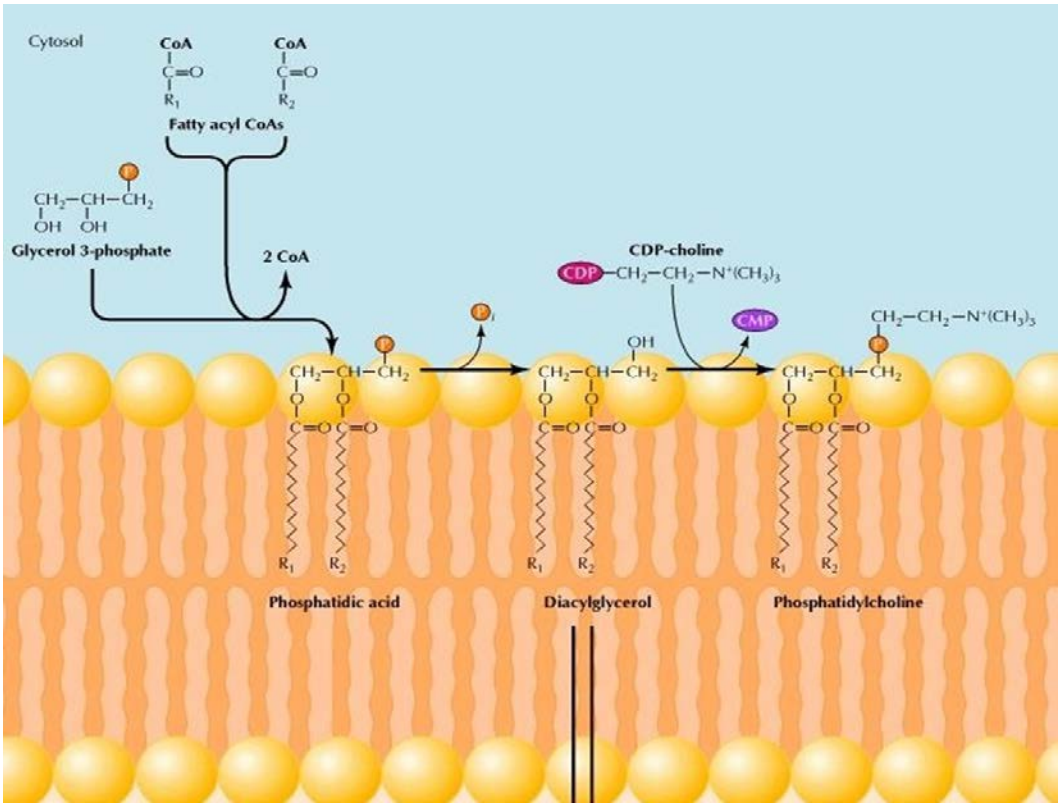
- Phospholipids consist of polar head and a hydrophobic tail. there are two types of molecules that are considered aphospholipid : sphingolipids like sphingomylein which are derived from a sphingosine back bone, and glycero – phospholipid like phosphatydil choline which are derived from a glycerol back bone.
- In order to synthesize a glycero-phospholipid, we want:
  - Glycerol, in its active form (phosphorylated in carbon number 3)
  - 2 fatty acids, in their active form (connected to CoA)
  - Different polar heads (phosphate, amine, serine..etc).
  -

** when we synthesize a phospholipid, we add 2 fatty acids to carbon 1,2 of glycerol, and by this we produce the tails which will be inserted directly to the membrane, because they're hydrophobic.

** and also we will have a polar head, which is a phosphorylated carbon.

The combination of the hydrophobic tail with the phosphorylated carbon yields a compound known as phosphatidic acid,which it the precursor to produce different types of phospholipids.

- Next step, removing phosphate with phosphatise enzyme, so we can add different types of polar heads, the resulted molecule known as Diacylglecerol.
- The addition of a polar alcohol head to Diacylglecerol yields a glycero- phospholipid.
- Examples of alcohols :
    - Inositol yeilding Phosphatidylinositol
    - Ethanolamine yeilding, Phosphatidyl ethanoamine.
    - Serine yielding , Phosphatidyl serine



    - Choline yielding phophatydil choline:
      phosphate group +choline group on it → in the form of CDP-choline (CDP is a carrier for choline) → PhosphatidylCholine.

- All these processes occur in the **outer surface**of the ER membrane, because we get the precursors (glycerol, polar heads) from the cytosol.
- These phospholipids will insert directly to the membrane because they are amphipathic molecule, and they will be more stable if stay in an amphipathic environment.
- After synthesizing these molecules, we add them to **the outer leaflet of membrane**, and there are many phospholipids in the outer leaflet of the membrane in comparison with the inner leaflet .so to make stable number of phospholipids in the inner leaflet and outer leaflet→ we can use flip-flop but it is energitically unfavoured.
  **we use an enzyme called Flippase to reduce the amount of required energy for this process and put these phospholipids in the inner leaflet and return of stability between the two leaflets.

Synthesis of cholestrol and its derivatives:

Steroid hormones are synthesized from cholestrolin the ER, so we expect the presence of large amount of SER (we mean here bigger SER not more in number,because we are dealing with sacs not viscles) in steroid producing cells, like ovaries and testes.

Synthesis of Ceramide

Ceramide is another type of lipids, and it is like the fatty acyl glycerol; but instead of glycerol there's sphingosine.

Synthesis of it happens inside SER; to use it as a precursor in synthesis of sphingomylien and glycolipids.

2) Detoxification of chemicals :

There are enzymes for metabolizing or detoxifing lipid soluble compounds(chemicals) inside some cells like liver cells; ex:

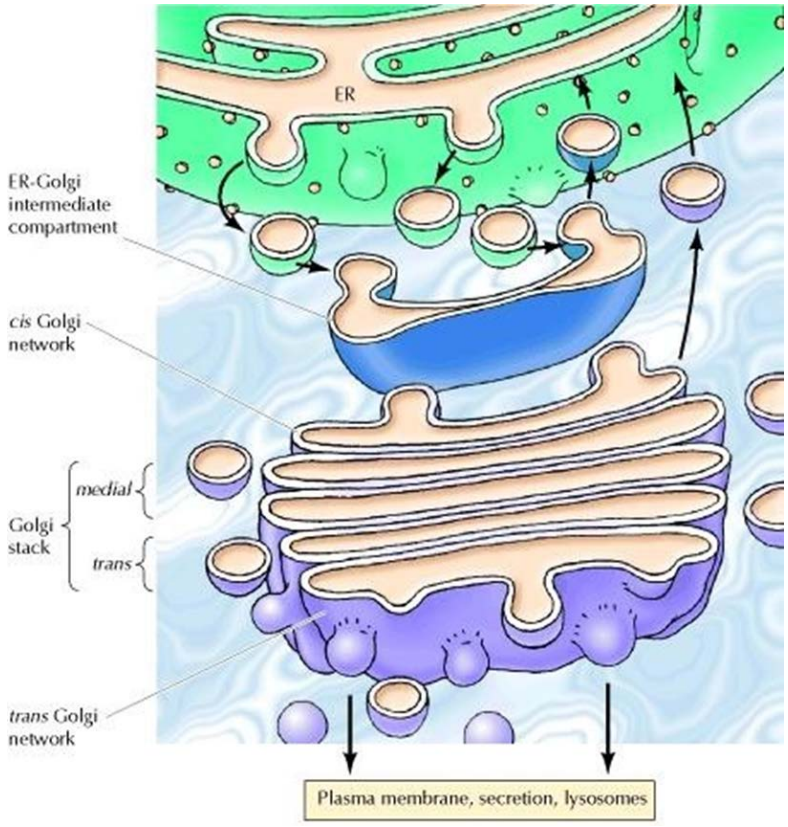
Detoxification of drugsand any other material always happen in the liver, and that's why liver cells have large size of SER; because SER contains enzyms that metabolize various lipid-soluble compounds.

The detoxifying enzymes inactivate a number of potenially harmful drugs (ex: Phenobrabital used in epilepsy), by converting them to water soluble compounds that can be eliminated from the body in the urine.

Golgi Apparatus

- It is a collection of sacs, membrane closed cisternae.
- The main characteristic in Golgi that it has rows of different sacs:
    - The compartment that is close to ER and GERC is called **CIS Golgi** or **entry face**.
    - The compartment that faces plasma membrane and export different types of proteins is called **TRANS Golgi** or **exit face.**
    - In the middle region between cis and trans networks; there is a **Golgi stack**, and this region is splited into 2 regions →**medial** and **trans** golgi stacks.

  Note: There is a difference between Trans Golgi network (located near plasma membrane), and Trans Golgi stack (part of middle compartment).



Vesicles move through Golgi by this pathway:

Vesicles from ER →ERGIC then enter Golgi sac through cis (entry) face → medial stack → trans stack → trans (exit) network → budding of vesicle and its content → transfer to their destination.

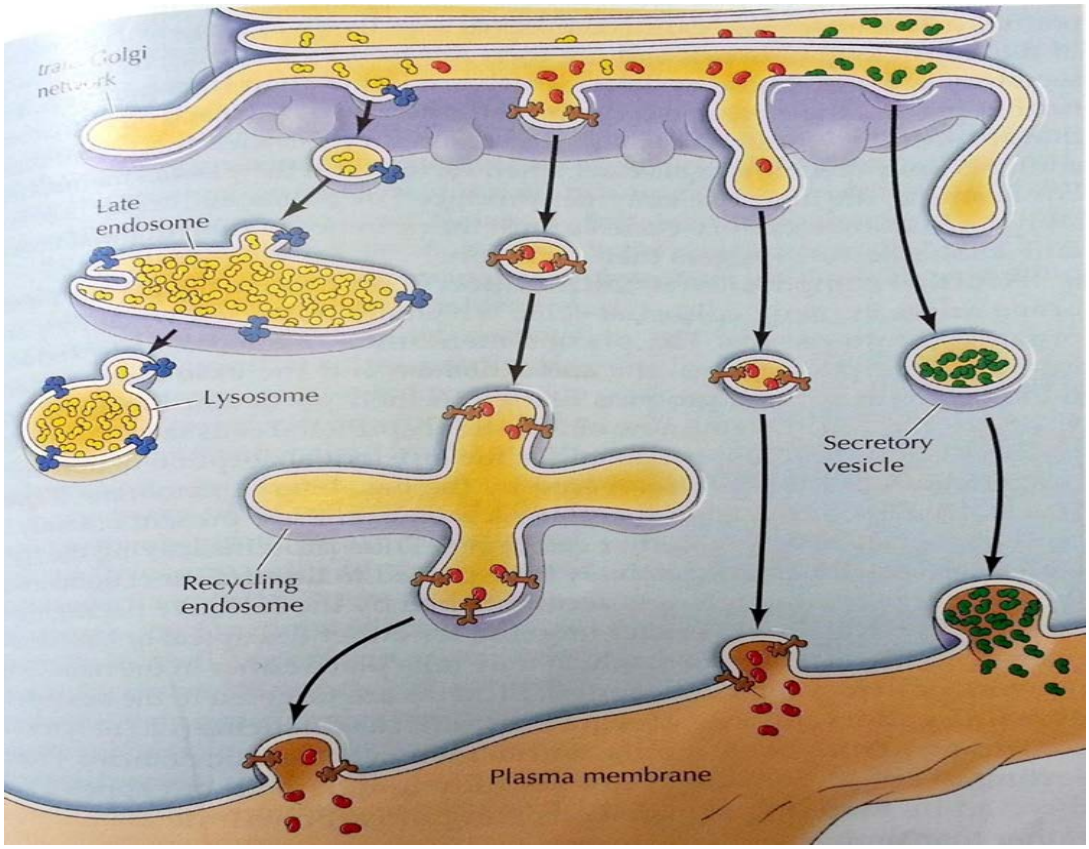
Vesicles won't fuse with the entry face and release their content into the sac, rather; the whole sac will move from one compartment to other one . imagine that vesicle enter the first sac which is now in the entry face , then this sac which contains the vesicles and their conents will move to the other compartment which is the media stack , then also will go to the trans stack , and finally to exit face , and during this journey all modification will happen . (I try my best to explan it ..if you still doesn't get it..try youtube)

- then it will be packaged and exit as budding vesicle from the exit face to their final destination.
- signal batches: regions of amino acids in the protein to be exported outside of Golgi that identify the final destination of

the protein.these batches are composed of different regions of the primary sequence of the protein.in addition , these signal batches are produced in golgi.

- Golgi functions:

1. ***<u>sorting and exporting modified proteins to the final destination(main function.)</u>***



- There are many ways for secretion, one of them is called **Direct-Simple secretion**which happens by budding of vesicls and their contents depending on the type of protein; whether it is membrane protein binding to ligand, or soluble protein packaged in vesicles and bind to plasma membrane and this type of secretion is continuous but unregulated and

unspecific.this way of transport is continuous and unregulated.

- Other type of secretion called **transport via recycling endosomes:**

  Once we have these budding vesicles from trans Golgi network, it is going to fuse with structure called endosome (vesicular structure), and endosomes can fuse with plasma membrane, and this way of transport is continuous and unregulated.

- The third type of secretion is **called regulated secretion after signalling:**

  Specific ligand is going to bind cell surface receptor and activate series of events and signal transduction pathways that will ultimately lead to the syntheses and exportof proteins (such as hormones binding to their receptors).

  Golgi takes part in the export process of the signal transduction pathway via the third type of secretion.

  Example:When glucose level in blood is raised, this will signal the release of insulin, and this hormone will activate mechanisms to digest and get rid of this high amount of glucose. The secretion of insulin which is a protein is activated by a ligand binding to it's receptor in the pancreatic cells. Golgi takes part in the secretion of insulin by exporting it to the plasma membrane where it will be exported to the extra cellular matrix.

Polarized cells like intestinal epithelial cells have 2 surfaces : apical and basolateral , each one of them contain different groups of proteins depending on the function whether absorption(apical) or release of absorbed molecules to the portal circulation(basolateral), so we need to direct these proteins in golgi either to basolateral or apical surfaces.

So a Golgi packages an :

apical protein with targeting signals consisting of carbohydrates

basolateral protein with short amino acid sequences such as dileucine or tyrosine.

2. ***Lipid metabolism:***

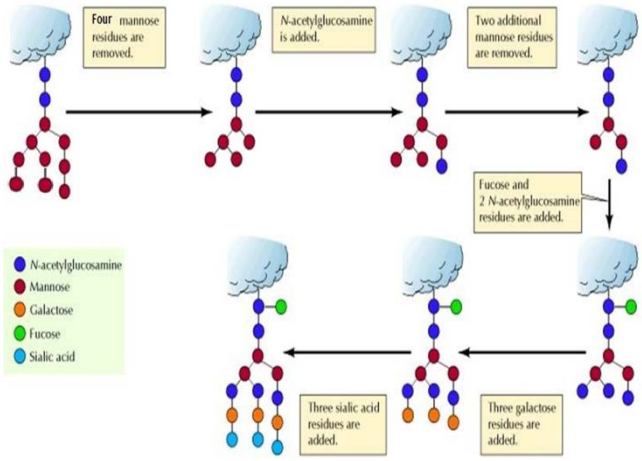
Lipids: After we have a ceramide from SER; we'll add different types of polar heads so we'll produce **sphingomylein and glycolipid**.

Sphingomyelin is synthesized in luminal face of golgi, but once it fuse with the membrane it will go out to outer leaflet.that makes sense, since we know that glycolipids are restricted to the outer surface.

Glycolipids, first we add sugars to ceramide in cytosolic face to produce glycosyl ceramide then there's a flip because also glycolipids are found in the outer leaflet of plasma membrane.

3. ***Protein glycosylation by:***

- Modification of the N-linked glycoprotein: Golgi already receive proteins that's N-linked, but they need further modification.



For example; the N-linked oligosaccharides of glycoprotein transported from the ER &distended to the lysosomes are modified by:

before the modification in golgi it composed of 2 N-acetyl glucose amine +9 residues of mannose

in golgi it's modified by removing 6 mannose residues and adding (3 N-acetyl glucose amine +fucose+3 galactose)

** so as a conclusion we add and delete different types of sugars for further modification.

It may need to be cut and change the sugar moiety of the integral membrane protein or any membrane protein because simply these proteins are different from each other in the number of sugar residues they contain .

** any other details are not required, only understand the cocept of N-linked glycosylated protein need to be further modified (addition or deletion) of the protein itself.

- Synthesis of O – linked glycoprotein.

  (because the interaction happens through oxygen and here the environment is rich with it), the interaction happens through serine or threonine because they have OH group.)

The sheet is over.