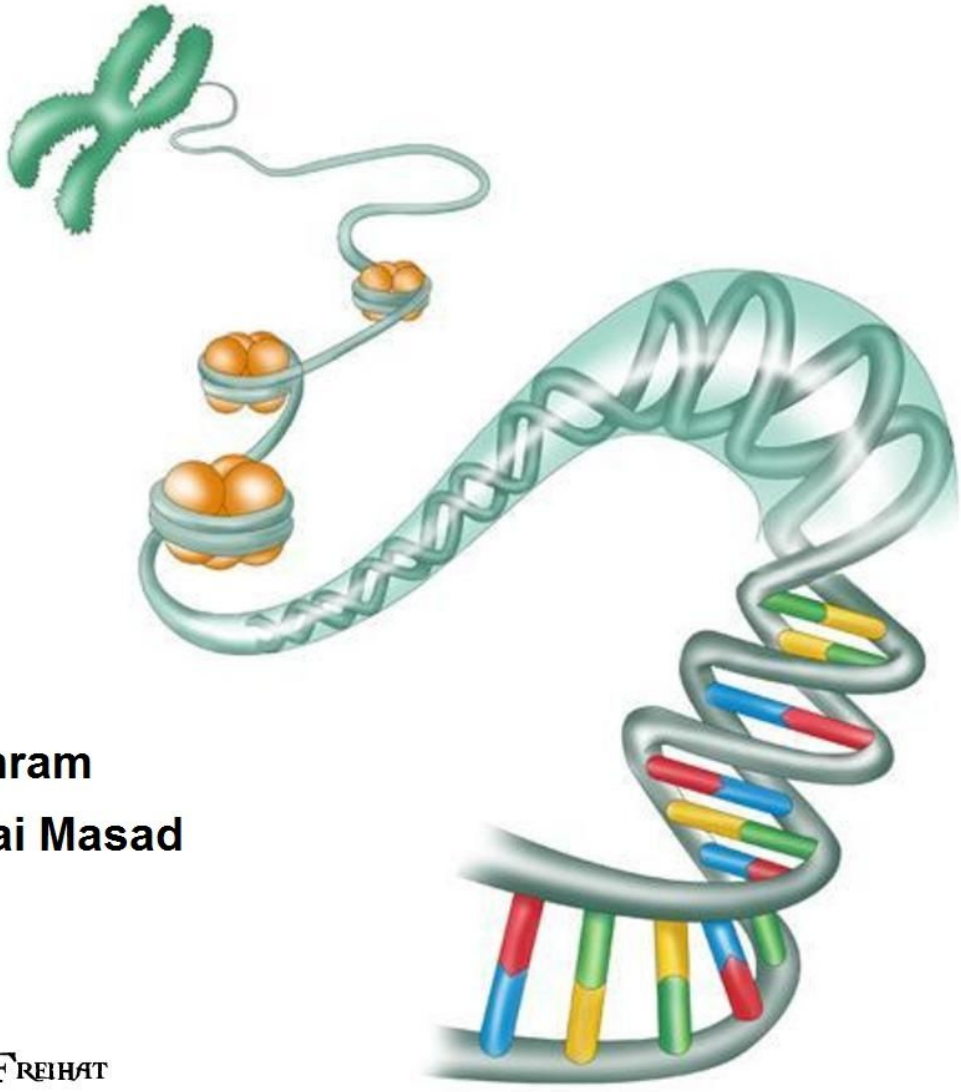
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Lecture # 4
Title:
Dr. Mamoun Ahram
Done By: Qusai Masad
Date:
Price:

Designed by Nadeen Al-Freihat

PROTEIN SORTING -2-

THE EASIEST LECTURE SO FAR , TRUST ME :p

هاي الجملة حطيتها قبل ما اكتب الشيت , رح تزهقوا حالكم ترست مي☺

*Dr said that the sheets are fine but not complete , if you depend on them you'll lose a few points , so you better take notes during the lecture.

الدكتور بدأ المحاضرة بأغلاق جميع الابواب , من بعد الي صار بمحاضرة البيوستات

يعني واحد انزنق و بده يطلع بالعقل ما فيها مشكلة , اما 40 واحد يطلعوا مع بعض لا كثير هيك

In the previous lecture we talked about "protein translocation" and there are two ways in which proteins are translocated into the ER:

1) **Co-translational translocation:** where proteins are synthesized during translocation.
2) **Posttranslational translocation:** where you have complete synthesis of the protein in the cytosol and, then they are carried by carriers or chaperons and translocated into the ER via the **translocon** with the help of **Bip** (which is a chaperon).

- Proteins in both mechanisms are targeted to the endoplasmic reticulum by a signal sequence at the amino terminus of the growing polypeptide chain.

For membrane proteins you have to take in consideration some few points, one of them whether they have single or multiple transmembrane domains and the orientation of the C-terminus and the N-terminus and this depends on the way they are translocated to the ER.

The lumen of the ER is topologically equivalent to the exterior of the cell, so the domains of plasma membrane proteins that are exposed on the cell surface correspond to the regions of the polypeptide chains that are translocated to the ER lumen. Mostly the C-terminus will be exposed to the cytosol.

Stop transfer sequence:

For example, a protein has a signal sequence. The signal sequence is cleaved as the polypeptide chain crosses the membrane so the N-terminus is exposed to the ER lumen. However, translocation is halted by a transmembrane **stop transfer sequence** that closes the translocon. Continued translation results in membrane spanning

protein with its C-terminus on the cytosolic side.
Proteins can also be anchored in the ER membrane by internal signal sequences that are not cleaved by signal peptidase. Because they are not cleaved by signal peptidase, however, these signal sequences act as transmembrane alpha helices that exit the translocon and anchor the protein in the ER membrane. Therefore, depending on the orientation of the signal sequence, proteins inserted into the membrane by this mechanism can have their amino or carboxy terminus exposed to the cytosol.

- The stop transfer domain or the signal sequence in both mechanisms are the transmembrane domains.

Proteins that span the membrane multiple times are thought to be inserted as an alternating series of internal signal sequences and transmembrane stop transfer sequences.

For example, an internal signal sequence can result in membrane insertion of a polypeptide chain with its amino terminus on the cytosolic side. If a stop transfer sequence is then encountered, the polypeptide will form a loop in the ER lumen, and the protein synthesis will continue on the cytosolic side of the membrane. If a second signal sequence is encountered, the growing polypeptide chain will again be inserted into the ER forming another looped domain on the cytosolic side. So, an alternating series of signal and stop transfer sequences can result in the insertion of proteins that span the membrane multiple times, with looped domains exposed on both luminal and cytosolic sides.

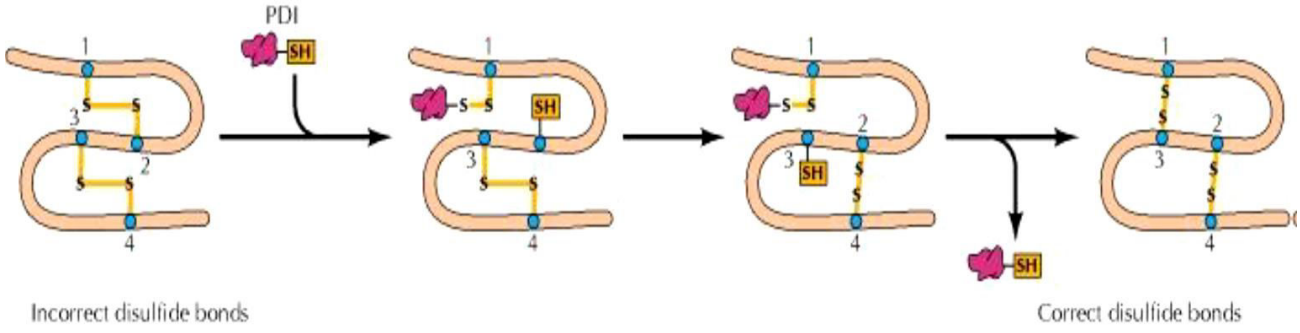
So during the translocation of these proteins, the channels (translocon) play an important role in stopping the transfer, or continuing the transfer. If there is a stop transfer sequence, the channel closes and continues the synthesis outside, and then there is another stop transfer sequence --> the translocon opens up and the synthesis continues inside and so on and forth.

Protein folding and processing in the ER:

NOW ONCE THE PROTEIN IS INSIDE, few things can happen:
- If the protein is part of a quaternary structure that is composed of other additional polypeptide chains, this takes place in the ER, with the help of chaperons.
- ER is also the site of protein folding by chaperons.

Proteins are translocated across the ER membrane as unfolded polypeptide chains while their translation is still in progress. These polypeptides, therefore, fold into their three-dimensional conformations assisted by molecular chaperons that facilitate their folding. Chaperons prevent the clustering of hydrophobic regions within the protein and thus mediate proper protein folding within the ER.

-Disulfide bond formation: Protein disulfide isomerase (PDI), which is an enzyme found in the ER lumen that facilitate disulfide bond formation between the side chains of cysteine residues during protein folding and assembly and what helps is that the environment in the ER is oxidizing environment relative to the reducing environment outside in the cytosol. Reducing environment maintain the cysteine residues in their reduced states while oxidizing environment promote disulfide bond formation.



-Some proteins are attached to the plasma membrane by glycolipids rather than membrane spanning regions. Because these membrane anchoring glycolipids contain phosphatidylinositol, they are called **glycosylphosphatidylinositol anchors (GPI).** GPI anchors contain two fatty acid chains, an oligosaccharide portion consisting of inositol and other sugars and ethanolamine. The GPI anchors are assembled in the ER and added to polypeptides anchored in the membrane by carboxy-terminal spanning regions. The membrane-spanning region is cleaved and the new carboxy-terminus is joined to the NH_2 group of ethanolamine immediately after translation is completed, leaving the protein attached to the membrane by GPI anchor.

-The other thing is that proteins can also be glycosylated so sugar molecules can be added to the protein.

Remember that proteins are glycosylated via:

1- Linking the sugar to Asparagine (N-linked glycosylation).

2- Linking sugar to Serine or Threonine and other amino acids. (O-linked glycosylation)

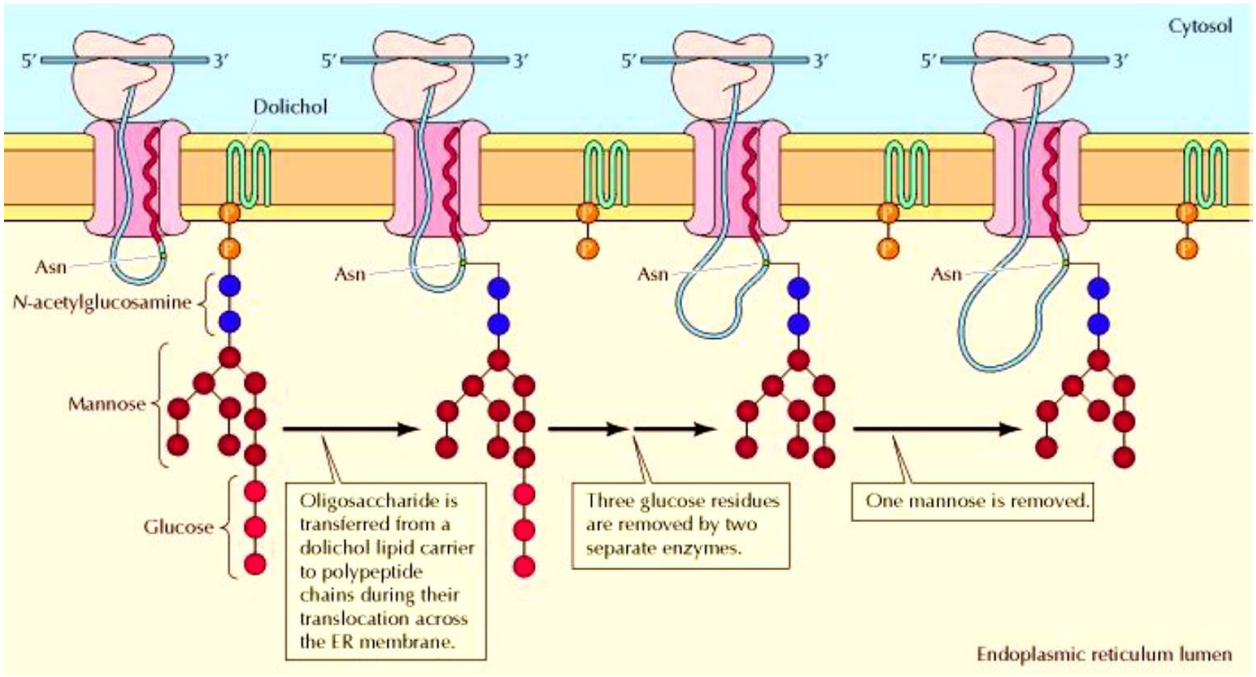
*Acceptor Asparagine residues are found in a signal sequence:

Asn-X-Ser/Thr

X: any amino acid. And this sequence is the signal that tells that the protein is glycosylated.

Similar to the addition of GPI anchors, oligosaccharide units are added to acceptor asparagine residues of growing polypeptide chains as they are translocated into the ER. The oligosaccharide is synthesized on a **lipid (dolichol)** carrier anchored in the ER membrane.

*Notice that the first sugar that is linked to the asparagine is an N-acetylglucosamine followed by additional sugar molecule.



The purpose of these sugar molecules is:
- To prevent protein aggregation in the ER.
- Help in further protein modification and sorting, and this is important when we start

talking about lysosomal proteins, because lysosomal proteins are modified via **phosphomannose**, and this is the signal that tells the cells that this is a lysosomal protein. Phosphomannose= mannose-6-phosphate.

Fate of glycoproteins:

So what happens to this glycoprotein exactly??

- Many proteins synthesized in the ER are rapidly degraded, primarily because they fail to fold correctly; others reside in the ER for several hours while they are properly folded. Thus an important role of the ER is to identify misfolded proteins, mark them and divert them to a degradation pathway. Chaperons and protein processing enzymes in the ER lumen act as sensors of misfolded proteins.

-Glycoprotein chaperons (Calreticulin and calnexin) bind sugar residues on the partially folded glycoproteins before translocation is complete and assist the glycoprotein in folding correctly. Two glucose residues from the last three glucose residues are removed and the presence of the terminal glucose residue allows the protein to bind to Calreticulin (or calnexin).

-After folding by Calreticulin, the glycoprotein is released and the remaining glucose is removed. Then, the glycoprotein binds to a folding sensor to check if properly folded.

NOW if the glycoprotein is:

-Folded properly, the protein is released from the sensor and it leaves the ER in a transport vesicle.

-If the glycoprotein is not folded properly, there are 2 things that can happen:

1. If the glycoprotein is misfolded, a glucose molecule is transferred to the glycoprotein, allowing it to undergo additional cycles of folding by Calreticulin.
2. If the glycoprotein cannot fold properly and the final product is damaged and there is no way to fix it, then the protein is **degraded**.

The mannose residues will be removed and the protein is retro-translocated to the cytosol, ubiquinated and degraded in the proteosome. This whole process is called **ER protein quality control.**

Unfolded protein response (UPR):

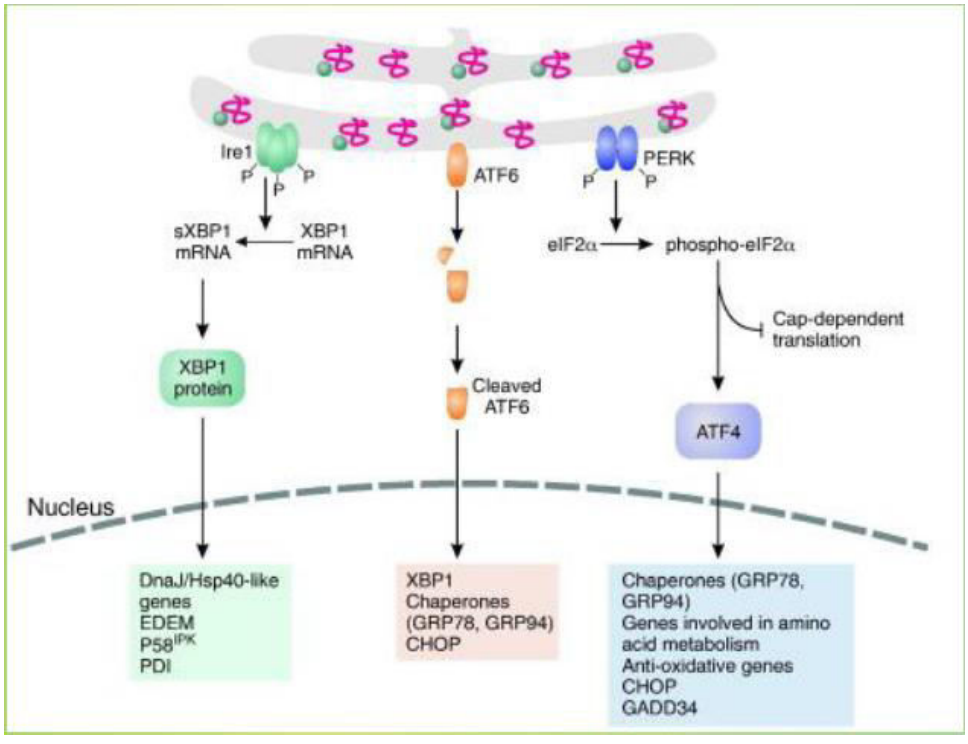
If an excess of misfolded proteins accumulates, signaling via Bip initiates a process known as the unfolded protein response. It includes general inhibition of protein synthesis, increased expression of chaperones and an increase in the activity of proteosomes.

There are 3 molecular mechanisms, in which this occurs:

1- The misfolded protein binds to a receptor that will be activated and cleaves mRNA and stimulates translation from this mRNA, producing a protein (**XBP1)** which is a transcription factor that activates UPR (Unfolded Response genes) such as additional chaperons.

2-There is another receptor that when bound to a misfolded protein it's activated (cleaved) and released and it also acts as a transcription factor that goes to the nucleus. (**ATF6)**

3- The last mechanism is for emergency cases, when this receptor is activated it causes phosphorylation of a translation-initiation factor, when this factor is phosphorylated, it stops translation of all proteins until the problem is fixed, except a single protein which is **ATF4**. When ATF4 is activated it activates UPR target genes. So, there are different levels of controlling protein folding.



What happens then?

-Properly folded proteins are transferred from the ER into the Golgi apparatus in transport vesicles that bud from the membrane of the transitional ER, fuse to form the vesicles and tubules of the **ER- Golgi intermediate compartment (ERGIC)** and are then carried to the Golgi. Membrane proteins maintain the same orientation in the Golgi as in the ER. And luminal ER proteins are taken up by the vesicles and released in the lumen of the Golgi.

-Some proteins are destined to be in the ER and these proteins must always exist in the ER, just like the Bip Chaperons and protein disulfide isomerase, but
Does that necessarily mean that these proteins will not be packaged inside vesicles and moved on to the Golgi apparatus?
NO, it doesn't mean that.
Actually, ER proteins can be targeted to Golgi by mistake but then they are recycled back to ER, thanks to a special targeting sequence known as the **KDEL sequence**; the single letter code of these amino acids--> (Lys-Asp-Glu-Leu). And this sequence exists at the C-terminus.

-Experiments were done to study this sequence and they found out that if this sequence is removed from the ER proteins, then these proteins will move to the Golgi and will be secreted from the cell instead of going back to the ER. Conversely, addition of KDEL sequence to non ER proteins (normally secreted) blocks their secretion and traps them inside the ER.

-Some ER transmembrane proteins are similarly marked by short C-terminal sequences that contain two lysine residues. (KKXX sequence)

What actually happens is that these proteins with this sequence bind to a specific recycling receptor known as KDEL or KKXX RECEPTOR in the membrane of the Golgi, and the protein is recycled back to the ER.

-Many transmembrane proteins possess di-acidic (e.g. Asp-Asp or Glu-Glu) or di-hydrophobic (Met- Met) amino acid sequences in their cytosolic domains that function as ER export signals. They also function as carriers of other proteins that must go into the Golgi. (GPI anchored proteins and luminal proteins)

SO THERE ARE DIFFERENT PROTEINS THAT FUNTION IN DIFFERENT CIRCUMSTANCES

The smooth ER and lipid synthesis:

ER is the major site in which membrane lipids are synthesized.

-Synthesis of phospholipids:

Glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors. Two fatty acids linked to CoA are joined to glycerol-3-phosphate (which comes from glycolysis). This takes place on the cytosolic surface of the ER SO THE ENZYMES necessary for the synthesis of these phospholipids exist on the cytosolic surface of the ER membrane.

Phosphatidic acid will be formed, inserted into the membrane and converted to diacylglycerol. Diacylglycerol can be modified further by attaching to different polar head groups, and results in the formation of **phosphatidylcholine**, phosphatidylethanolamine, and phosphatidylinositol.

*The source of phosphatidylserine is actually phosphatidylethanolamine.

These phospholipids are synthesized on the cytosolic side of the ER membrane, so they will be added to the cytosolic half of the bilayer. Eventually, you will have more phospholipids on one leaflet compared to the other, and they must have equal distribution (in terms of number of phospholipids) on both sides. Later they are translocated across the membrane by phospholipid **flipases** resulting in even growth of both halves of the phospholipid bilayer.

*Because they are extremely hydrophobic membrane lipids are synthesized in association with already existing cellular membranes rather than in the aqueous environment of the cytosol.

-Synthesis of ceramide:

One example of a lipid that is synthesized in the smooth ER is **Ceramide** which is synthesized from the conjugation of serine and Palmitoyl Co-A or Palmitic acid. This ceramide can be modified further in the Golgi apparatus where the synthesis of glycosphingolipid and sphingomyelin takes place. If a cell has a lot of sphingomyelin or glycosphingolipid and the cell wants to get rid of these molecules then metabolism of these sphingolipids takes place in the lysosome, where sphingomyelin for example is converted back to ceramide.

*Smooth ER is abundant in cells that are particularly active in lipid metabolism. For example synthesis of steroid hormones such as the androgens; estrogen; progesterone can take place in the ER starting from cholesterol.

*In addition, ER is abundant in the hepatocytes (liver cells) where it contains enzymes that are necessary for the metabolism of lipid soluble compounds. These detoxifying enzymes inactivate a number of potentially harmful drugs and xenobiotics, (acetaminophen, Phenobarbitals) by converting them to water soluble compounds that can be eliminated through urine.

يلا هانت هانت ضل شوي

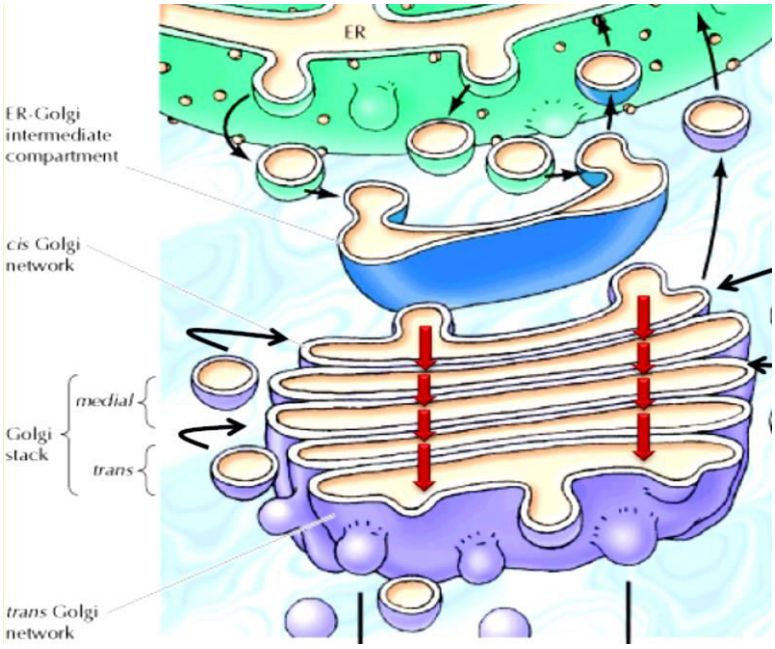
THE GOLGI APPARTUS:

So proteins move on from the ER to the Golgi, What happens in the **Golgi**? What is the function of the Golgi??

There are 3 main functions:

- Further processing and modification by glycosylation for example.
- Protein sorting (the Golgi looks at these proteins and says , this protein should go to the plasma membrane , and this protein should go into the lysosomes or secreted) :P
- Synthesis of glycolipids and sphingomyelin .

The structure of GOLGI:



Golgi is most commonly viewed as consisting of four functionally distinct regions:

1- The cis Golgi network –Proteins enter the Golgi at the cis Golgi network.

2- Golgi stack which is divided into medial and trans subcompartments- Most of the Golgi metabolic activities take place within these two compartments.

3- The trans Golgi network- Modified proteins move to the trans Golgi network which acts as a sorting and distribution center.

*Protein modification actually takes place in all three parts of the Golgi apparatus in the cis Golgi network, the Golgi stack as well as the trans Golgi network.

The mechanism by which proteins move through the Golgi apparatus has not yet been established. It was thought before that proteins actually moved from one part of the Golgi to the other via vesicles, but this turned out to be wrong and that 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.

Now finally.. ! Not finally :p lsa baadriiiiiii (3m b3mel 7ali zareef)

Protein glycosylation within the Golgi:

Initial modification of proteins by carbohydrates conjugation takes place in the ER but this continues further in the Golgi apparatus.

-N-linked oligosaccharides:

N-linked oligosaccharides are processed in an ordered sequence of reactions:

In most cases, the first modification of proteins is the removal of four mannose residues. This is followed by the addition of N-acetylglucosamine, the removal of two more mannoses and the addition of a fucose and two more N-acetylglucosamine. Finally, three galactose and three sialic acid residues are added.

If you remember in biochemistry the very last slide in the carbohydrate lecture (اشك) لو حدا متذكر is the Sialic acid and it said that sialic acid is a sugar -a modified sugar- that is added to proteins and glycoproteins and is always present at the terminal of the sugar complex.

*The N-linked sugars are added to asparagine in the ER, now in the Golgi you can have modification to these N-linked sugars as we said or the addition and modification of O-linked sugars- not direct modification.

-O-linked oligosaccharides:

Proteins can also be modified by the addition of carbohydrates to the side chains of acceptor serine and threonine residues. These modifications take place by the sequential addition of single sugar residues. The serine or threonine is usually linked directly to **N-acetylgalactosamine** to which other sugars can then be added.

Lipid and polysaccharide metabolism in the Golgi:

Golgi apparatus can function in the synthesis of sphingomyelin and glycolipids. Glycerol phospholipids, ceramide and cholesterol are synthesized in the ER. Sphingomyelin and glycolipids are then synthesized from ceramide in the Golgi apparatus.

Sphingomyelin is synthesized on the lumenal side of the Golgi but glucose is added to ceramide on the cytosolic side. Glucosylceramide then apparently flips (via flipases) and additional carbohydrates are added on the luminal side of the membrane.

☹ملقت بعرف , والله اني زهقت اكثر منكم

Protein sorting and export from the Golgi apparatus:

So what does happen to proteins that go into the Golgi ?

-_- -_- -_- -_- -_-

Proteins as well as lipids and polysaccharides are transported from the Golgi apparatus to their final destination through the secretory pathway. These proteins are sorted to different parts of the cell, they can be secreted or they can be conjugated or attached to the membrane by having a transmembrane domain. Again remember that the topology and the protein orientation remain unchanged.

-There are different mechanisms in which proteins are secreted from the Golgi:

- **Constitutive secretory pathway**; unregulated continuous pathway which accounts for the incorporation of new proteins and lipids into the plasma membrane as well as

for the continuous secretion of proteins from the cell. The protein is packaged inside vesicles and these vesicles continually without any regulation fuse with the plasma membrane and the contents are released outside directly. It can also occur indirectly by first fusing with what is known as the "recycling endosomes" and then the vesicles bud off and are transported to the Golgi or they are secreted.

- **Regulated secretory pathway**; proteins are packed inside regulated secretory vesicles. Specific proteins are secreted in response to environmental signals. The secretory vesicles involved in this pathway are larger than transport vesicles and store their contents until specific signal direct their fusion with the plasma membrane.

-Other proteins are specifically targeted to other intracellular destinations such as lysosomes.

*In contrast to the ER, all of the proteins retained within the Golgi complex are associated with the Golgi membrane rather than being soluble proteins within the lumen.

<u>What if the cell has specialized membrane like intestinal epithelial cells that are polarized when organized into tissues?</u>

The plasma membrane of these cells is divided into an apical domain and basolateral domain that contain specific proteins related to their particular functions. Some proteins are packaged and destined to the apical portion of the membrane; others are destined to the basolateral portion. This distinguishing depends on signal molecules which are special sequences in the case of basolateral proteins or sugar modifications if the protein is apical.

- The best-characterized pathway of protein sorting in the Golgi is the selective transport of proteins to lysosomes. As we said, lumenal lysosomal proteins are marked by mannose-6-phosphates that are formed by the modification of their N-linked oligosaccharides after entering the Golgi. The modifying enzyme responsible for this recognizes the protein " signal patch" which is made up of amino acid residues that are distant to each other in the primary sequence but come close to each other in the tertiary structure and contain information to

send a given protein to the indicated location in the cell. A specific receptor in the membrane of the trans Golgi network then recognizes the mannose-6-phosphate residues. The resulting complex of receptor plus lysosomal enzyme are packaged into transport vesicles and destined to late endosomes which mature into lysosomes. Lysosomal membrane proteins are targeted by sequences in their cytoplasmic tails rather than by mannose -6-phosphates.

Thumbs up la 2rwa3 naas shokraan 3la kol al da3m :P
Ali Halabi,Qusai Sharif , Nour Hayek , Abd Tayar , Mohannad Ashhab, Marah Atari ,Maryan Abaza, Abu Alia , 3laa Shaban ,Sura Diaa , Eiad , Mustafa, Rashid Dahabreh , Sophia Haddadin ,Dina, Shurman , Tareq , w Lajneh w Hiba MIhyar 3l correction
اطول ديديكيشن بعرف p:

و احلى تحية لمحمد الزغول الي خرب الميكروفون

و لا تنسونا من دعائكم :*

Rephrased and modified by: Hiba Mihyar

*Please refer back to the slides or the book for the illustrating figures.

Written By: Qusai Masad