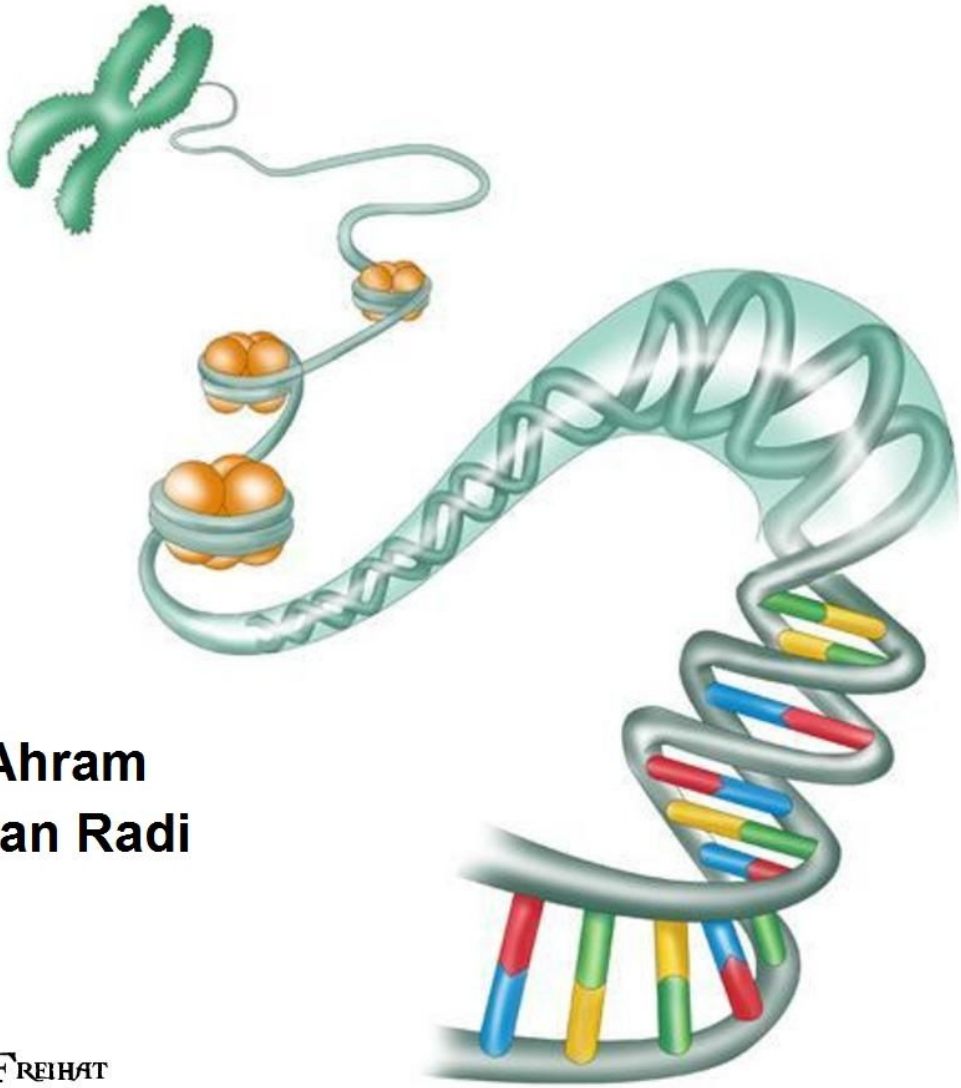
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Lecture # 5
Title:
Dr. Mamoun Ahram
Done By: Rakan Radi
Date:
Price:

Designed by Nadeen Al-Freihat

Protein sorting

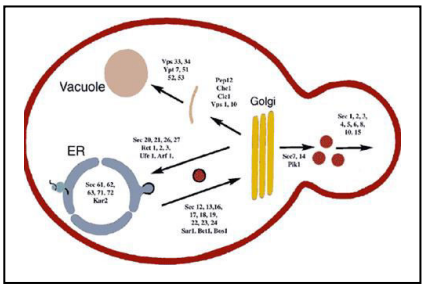
(Golgi apparatus and vesicular transport)

Al- Salam Aleikom. At first, I'd like to dedicate this sheet to The Teacher of Teachers, our prophet Mohammad –PBUH-. So صلوا على النبي and let's start.

As we have explained before, one mechanism by which luminal ER proteins are transported is by binding to transmembrane proteins, even GPI-anchored proteins can bind to these receptors, and then the receptors target these proteins to Golgi apparatus.

The mechanism of vesicular transport

Basically, it's the movement of vesicles from Golgi to endosomes, lysosome, secretory vesicles, plasma membrane or outside the cell.



We have learnt this mechanism by studying the yeast model system because it's easy to manipulate and create genetic mutations, so they started *mutating its genes* until they mutated a gene at which yeast could no longer use these vesicles, and by that knowing that this gene is responsible for a protein that is responsible for transporting vesicles from Golgi to endosomes for example. The mutants they created are called *sec-mutants*, because it's a group of proteins which are located on the vesicular membrane. We have many types of sec like sec10, sec61 and so on.. sec61 is responsible for translocation

channel which is responsible of transferring proteins during synthesis into the ER, and they noticed that these genes are exactly the same ones as those found in human cells. This is an extra evidence for the theory of evolution.

Another mechanism is *reconstitution of vesicular transport in cell-free systems*, this was a paper that was published in the 90s; what they did basically is that they isolated the Golgi apparatus and the vesicles, and put them in a test tube. (i.e. cell-free system) and they noticed that these vesicles can fuse with Golgi and transport their contents into Golgi.

Another method is *biochemical analysis of synaptic vesicles* by using synaptic vesicles, because neurons have a lot of these vesicles, they were able to isolate these vesicles thus being able to isolate and analyze many proteins.

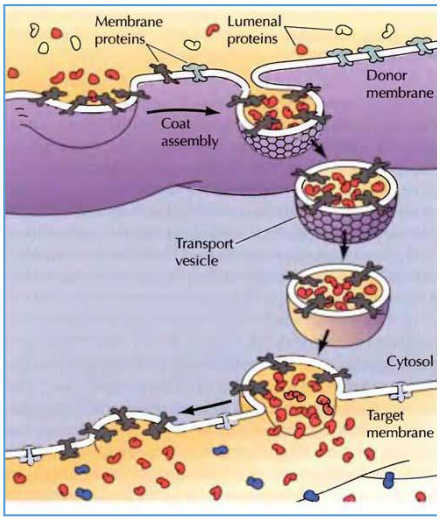
Another mechanism is by using the *green fluorescent fusion proteins*, like the green mice we have discussed earlier, that by isolating particular genes, they were able to make the mice fluoresce. What they did here is really smart which is fusing the gene they wanted to study with the green fluorescent protein gene together producing one gene, so when the cell wants to synthesize this gene, it synthesized a larger protein, fusion protein, which constitutes of the protein of interest plus the green fluorescent protein enabling them to study and detect the protein easily thus being able to study its cycle from the very start by being translated from the mRNA to the cytosol to synthesizing the "c" that goes into the ER to being packaged in vesicles to being sent to Golgi apparatus and then to lysosomes and so on.

Long story short, they tracked the protein cycle live under the microscope just by making it fluorescent. So protein by protein, they were able to study most if not all proteins function and life cycle.

So they were able to recognize the movement of proteins specifically; by fusing the green fluorescent protein with proteins X,Y,Z for example, they were able to see how these proteins move from one place to another in cells.

Another strategy is *proteomics analysis*. Remember that we said that proteomics is the study of large number of proteins, and one of the techniques used is mass spectrometry in which proteins are injected into the mass spectrometer which ionizes the peptides then they move into the mass analyzer and so on. So what they did is that they isolated these synaptic vesicles to purity, then isolated the proteins from these vesicles and identified them, being able to detect exactly which proteins are located in Golgi.

Coating the vesicles:



They have noticed something by studying cells under the *electronic microscope*, they were able to see that these vesicles bud off from the donor membrane whether it is the Golgi apparatus or the plasma membrane as when they start to pinch off, they are coated with certain proteins. And then by looking at these vesicles, the coat is released, then the vesicle moves and fuses with the target membrane releasing its contents.

-So what is the molecular mechanism by which the vesicles are coated, de-coated and fused to the target membrane?

By studying yeast and other eukaryotic cells, they found out that there are three coating proteins, and the vesicles are known as *COP1 coated vesicles*, *COP II coated vesicles* and *clathrin coated vesicles*.

And they have noticed, that when you have the COPII coated vesicles move these vesicles from the ER to the intermediate compartment to Golgi .

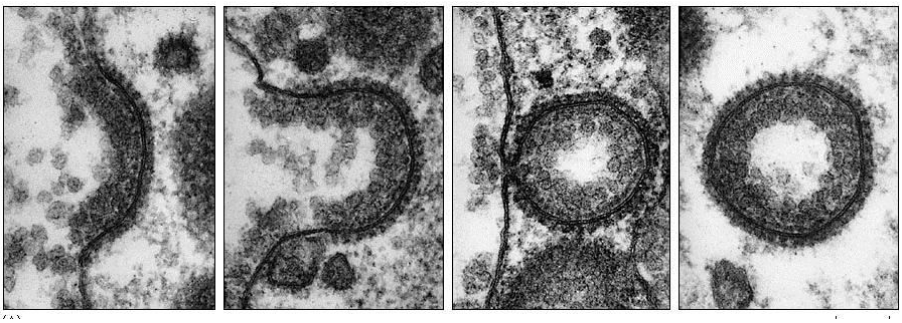
They have also noticed that the vesicles that move backwards (i.e. from Golgi back to the ER) are coated with COP I.

And the clathrin coated vesicles are the ones that move from Golgi to the plasma membrane and from the plasma membrane to Golgi via *endosomes* or directly.

Type of vesicles	Origin	Target
COP II	ER	to the intermediate compartment then to cis Golgi
COP I	Golgi	To the intermediate compartment then to ER
Clathrin (via endosomes)	1- Golgi 2- Plasma membrane	1- Plasma membrane 2- Golgi

Formation of clathrin-coated vesicles

Now let's take a look at this beautiful electron microscope image of a clathrin-coated vesicle.



Note how the plasma membrane is coated, how it starts to form, how it pinches off and then is released. Then they followed this vesicle and noticed that the coating is removed.

So they noticed that first you have the budding off of the vesicle, then coating of the membrane, and then you have the vesicle formed with clathrin totally coating it, then the clarthrin protein is released from these vesicles.

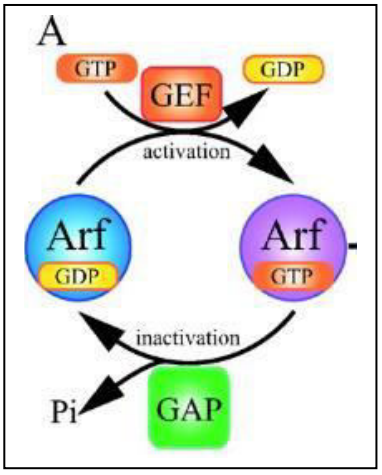
The molecular mechanism:

They noticed that this is controlled by a protein called *ARF1* (مش قرف), this protein is a GTP binding protein (just like the RAS protein) (i.e. that this class of proteins is regulated by GTP; they are active when bound to GTP and inactive when the GTP is hydrolyzed to GDP.)

So, there is a cycle between GTP and GDP which is regulated by two classes of proteins:

1- GEFs (GTP Exchange Factors)
2- GAPs (GTPase Activating Proteins)

The mechanism:



1. These GTP binding proteins are bound to GDP (so they are inactive)
2. GEFs come in and release the GDP allowing GTP to bind.
3. Now, the proteins are active.
4. Then the GAPs come in and activate the enzymatic activity of the GTPase (that is hydrolyzing the GTP into GDP), so this step inactivates the protein.
5. And the cycle goes on.

So ARF1 is activated by GEFs which are present on the cell membrane. Once it's activated, it recruits a protein, this protein is an adaptor protein, what it does is connecting the ARF1 to the receptor that is to be transported (the carrier receptor that's going to bind to the cargo).

Once you have this complex formed of the ARF1, the adaptor and the receptor, another adaptor protein comes in and binds to the receptor, and this new adaptor protein connects the clathrin protein to the region that will become vesicular (form the vesicle).

So, what happens is that when ARF1 is activated and you have recruitment of the clathrin protein, you have a formation of a clathrin coat; you have a large number of clathrin proteins coming together forming a lattice, coating the vesicle causing the budding off of the vesicle, now the vesicles move from the donor membrane, then the GTP that is bound to ARF1 is hydrolyzed into GDP making it inactive and the clathrin proteins are disassembled, so we have the uncoating of the vesicle and then it moves to the target membrane where it can fuse with it and become part of the membrane or the contents are released outside.

Vesicle fusion

How do vesicles fuse with the target membrane?

Again by isolating the synaptic vesicles and studying the proteins found within, and by also studying the yeast mutants, they were able to identify proteins known as *SNAREs* which are helical proteins that exist on both the vesicle membrane and the target membrane.

There are many types of SNAREs such as

- v-SNAREs (vesicular SNAREs) which are located on the membrane of the vesicles.
- t-SNAREs (target SNAREs) which are located on the target's membrane whether we are talking about Golgi or plasma membranes.

There are three interactions that take place:

1. **Recognition** which is carried out by the *SNAREs* and effector proteins.

2. **Binding** which is mediated by *effector proteins* and SNAREs

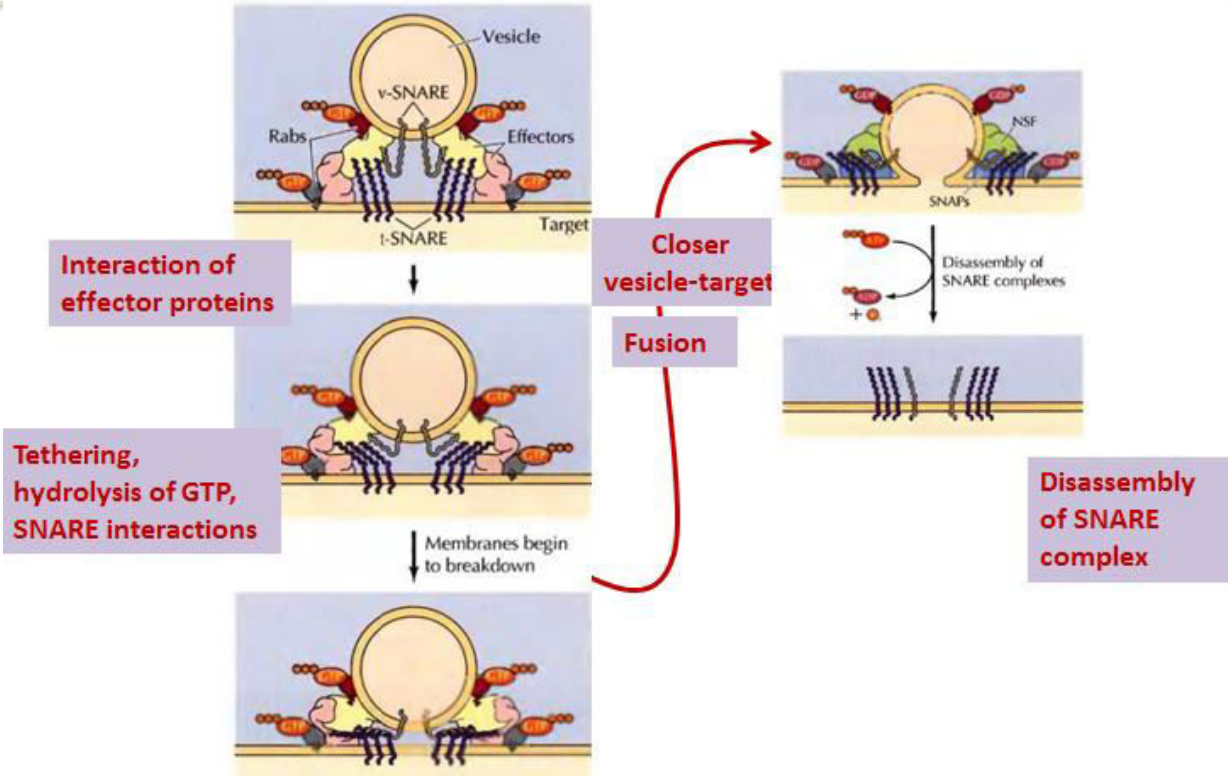
3. **Fusion** with the plasma membrane which is controlled by *Rabs* (also a GTP binding protein)

We have Rabs on Golgi, endosomes, lysosomes and on the plasma membrane controlling the fusion of the vesicles with the target membranes.

We also have a mechanism called *tethering* following the interaction which is basically forming of a rope, so the v-SNARE with the t-SNARE tether together forming one complex by intertwining with very strong interactions between them, causing the hydrolysis of GTP as well as SNARE interactions getting the vesicle close to the target membrane, then this is followed by dissolution of the vesicular membrane and becomes part if the targets membrane, followed by disassembly of the SNARE complex because the vesicles are now part of the plasma membrane.

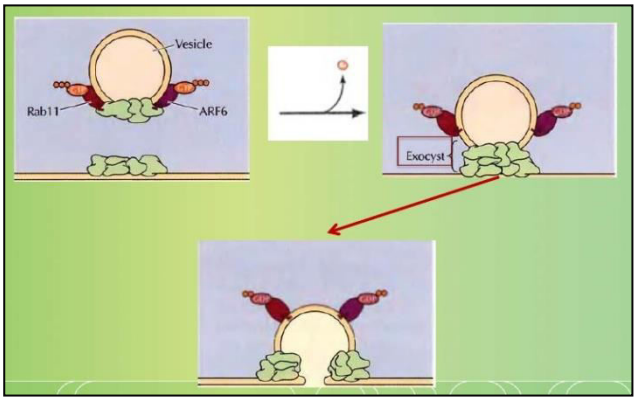
RECAP:

1. Interaction between different effector proteins
2. Tethering of the SNAREs
3. Hydrolysis of the GTP to GDP
4. The snares interact with each other
5. The vesicle membrane gets closer to the plasma membrane and then it dissolves by fusing into it.
6. The SNARE complex is disassembled
7. The vesicle becomes part of the membrane.



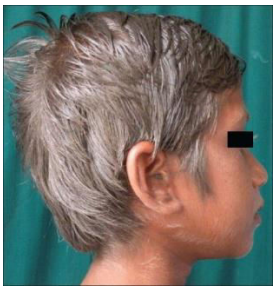
Exocytosis

They noticed that fusion is also controlled by *exocyst* which is a complex of 8 different proteins and they are specialized for certain vesicles and fusion mechanisms.



What happens is that we have the ARF(a different ARF), formation of the exocyst between the vesicle and the membrane, followed by fusion of the vesicle into the membrane, so basically exocyst is the formation of this 8 protein complex where by the vesicle can recognise and interact with a specific spot on the plasma membrane at which it will fuse.

Griscelli syndrome (GS)



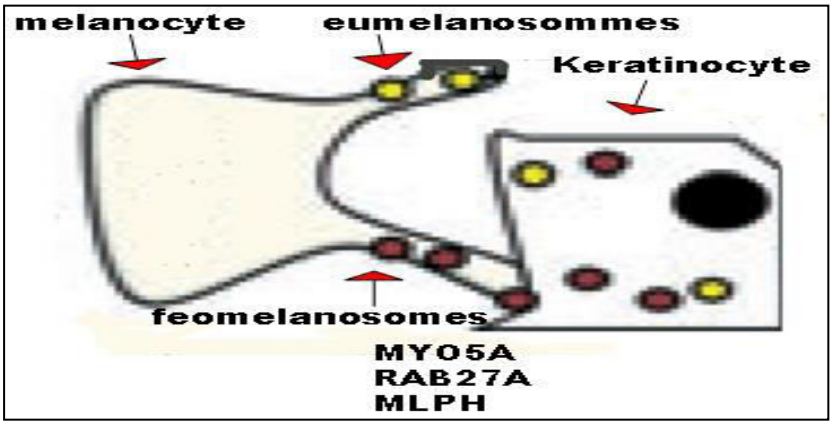
It is a rare genetic condition. They are different types depending on the mutated gene. One of them is a Rab protein and another one is a mysoin protein which is an actin binding protein.

These three proteins interact with each other forming a protein complex helping in the movement of vesicles known as *Melanosomes* which exist in our skin cells (keratinocytes).

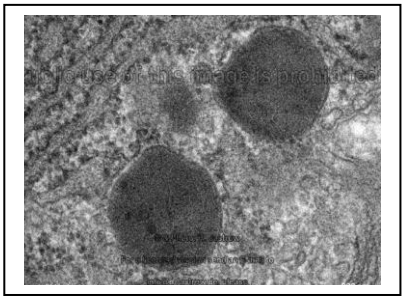
In our skin we have melanocytes and keratinocytes, the melanocytes are responsible of forming the melanin which is the molecule that gives our skin its color, so africans have a high portion of it while scandinavians almost have none. Melanin plays a crucial rule in protectiong our skin from the UV light of the sun.

The melanosomes are transferred from melanocytes to keratinocytes.

They noticed that individuals with Griscelli syndrome have these patches of color in their hair and skin which apper to be pale, because they lack this pathway (the movement of melanosomes from melanocytes to keratinocytes) so we have accumulation of melanosomes in melanocyte.



Lysosomes



Now, let's talk about lysosomes in this case.

Lysosomes are membrane closed organelles

that contain acid hydrolases (digestive enzymes)

that degrade molecules such as carbohydrates, proteins and lipids thus simplifying them. So as we said, the enviroment of the lysosomes is very acidic (pH=5) compared to the cytosol (pH=7.2-7.4) and that is for two reasons:

1. The acid hydrolases are only active in acidic pH
2. It's a protective mechanism, so that in case if the lysosomal membrane was disrupted, the hydrolases won't ruin the cell structure as they won't be active due to the different pH.

Lysosomes are acidic due to the proton pumps found on their membrane, which pump protons from the cytosol to the lysosome using ATP.

Lysosomal storage diseases:

They are associated to the lysosomes due to different mutations that may occur there. And there are different classes of diseases, such as:

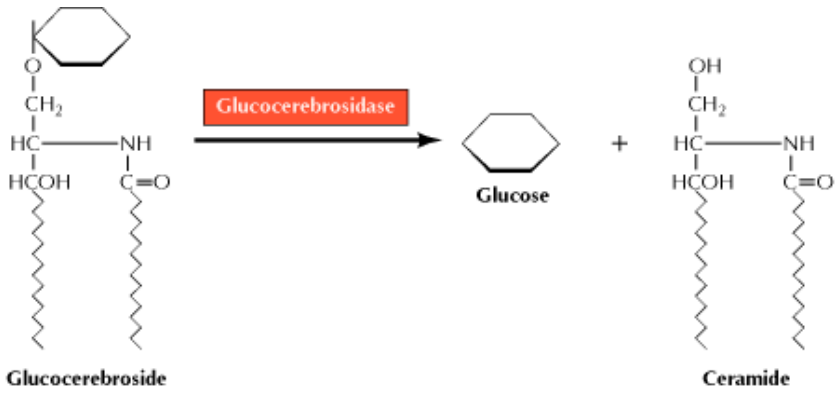
- Glycolipidoses (sphingolipidoses): which is due to a mutation (defect) in the metabolism of glycosphingolipids.
- Oligosaccharidoses which is inability to break down carbohydrates.
- Mucopolysaccharidoses: deficiencies in lysosomal hydrolases

These all are chronic progressive diseases that can cause death.

Glucocerebroside

It is a glycosphingolipid which is a byproduct of normal recycling of RBCs which are phagocytosed by macrophages inside which they are degraded into the components of lipids and sugars. And they noticed that there are different types of this condition according to its severity. We have type 1 which is less severe and more common an also type 2 and 3 which are more severe and they're rare (an example of this is *Gaucher* disease.)

So normally, what happens is that this glucocerebroside is degraded by glucocerbrocidases into glucose and ceramide molecules. And in type1 condition, there is a deficiency of this enzyme so there is an accumulation of the substrate (glucocerebroside) and an enlargement of lysosomes (that's why it's called lysosomal storage disease) especially in macrophages. If the enzyme is completely defecient (100%), it could be fatal.



Another case is *carbohydrate metabolism*, like when glycogen is degraded into glucose, the release of glucose takes place in lysosome by an enzyme called glucosidase.

There are also different oligosaccharidoses (diseases and defeciencies) what we want to focus on is *Pompe disease* which results from defect in glucosidase which releases one glucose monomer from the glycogen. All organs are going to be affected, and there is a massive increase in the

amount of glycogen which might cause death before the age of two. In this case, glycogen's structure is normal but its amount is abundant.

Another disease is the *I-cell disease* which is lack of targeting of lysosomal enzymes from Golgi, so the lysosomes don't have any acid hydrolases that cause retardation and eventually death. The main reason for that is that the enzyme that's responsible of adding phosphate to the mannose is defecient, remember that this enzyme doesn't recognize a sequence but a signal that's known as the signal patch, which is a 3D region of the protein. So the signal patch is a region in lysosomal protein that adds phosphate to the mannose.

Treatment is variable and depends on the severity, mainly it's done by enzyme replacing therapy where the patient is given this enzyme, gene therapy is still under investigation.

And that's it. Don't forget to take a look at the slides as they may contain extra information.

Shout out for Ali Khresat, Hamzeh Mahafzah and Omar Arman.

قَالَ رَسُولِ اللَّهِ -صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ-: (مَنْ سَلَكَ طَرِيقًا يَلْتَمِسُ فِيهِ عِلْمًا سَهَّلَ اللَّهُ لَهُ طَرِيقًا إِلَى الْجَنَّةِ.)