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GENETICS & Molecular Biology

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Subject: Vesicular Transport & Lysosomes

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We finished golgi yesterday.
Let's move on to the next stage. Golgi packaged all proteins, all lipids in the vesicles and now they can be exported to the final destination.

VESICULAR TRANSPORT

What is the mechanism of vesicular transport? How is it directed to the lysosomes and so on? (Briefly)

At trans Golgi membrane, budding of the vesicles will happen. Membrane curvature starts to happen and it's going to induce the assembly of a coat for the vesicles. A protein coat will help in directing the vesicles to a certain destination. Then, it's going to walk on these microtubules inside the cytosol or cytoplasm. Once the vesicle is very close to its final destination, it will start to lose or disassemble these coat proteins.

We noticed that the vesicle was initially surrounded by a network, then this network is disassembled.
The vesicle can carry: carbo protein(Glycoprotein)(a membrane protein in the vesicular membrane) ,soluble protein (it is inside the lumen of the vesicles), a membrane lipid(part of the vesicular membrane)…etc., whatever the molecule is, it will be loaded by the vesicles.

Then the vesicle will fuse with the plasma membrane, and it will release its soluble contents that might be for example : hormone (insulin),and insert the membrane compounds(Proteins, lipids,…) inside the plasma membrane or in target membrane .

Now, we have coated protein. There are different types of coated protein depending on the:
1. Direction of movement (Are we going to move from golgi to plasma membrane (for example) or we need to retrieve some protein (from golgi to ER), is it going to be forward or back ward.
2. The budding location (Is it budding from the golgi? Is it budding from endosomes?...etc
3. The final destination (Direct vesicles to it's destination)

We have 3 types of coating proteins:
1. Clathrin
2. COP I
3. COP II

*COP - Co Protein

From ER to ERGIC to Golgi (forward direction) - **COP II**

From Golgi to ERGIC to ER (backward direction (retrieving protein) - **COP I**

*COP II moving FORWARD and COP I moving BACKWARD

Clathrin from trans Golgi network to the plasma membrane or to lysosomes.

For the protein to be inserted into the plasma membrane, there must be ***some factors*** that help directing the vesicles and its fusion with the target membrane.[That means if the vesicle is carrying a protein that is **not** a plasma membrane protein, it's not going to fuse because in this case these factors will not be present] . There are different types of these factors depending on the type of target membrane that these vesicles are directed to and which one is going to fuse with.

How does the formation of these clathrin coated vesicles happen?

Once we have the curvature, this will induce the assembly of the proteins, a collection of the proteins on the surface of the curvature. These proteins will interact with clathrin molecules to form a network or to form a net-shape coat around the vesicles. These molecules will interact until it makes a complete shell around the vesicles (Clathrin coated vesicles).

This process is mediated by GTP binding proteins. This type is specifically called ARF1 (a GTP binding protein). This protein is going to be activated at the first step by GEF (Guanosine exchange factor). It's going to remove GDP and attach GTP. And now, ARF 1 is active.

What is mean by ARF 1 is active?
It's mean that it can attract more proteins that will interact with the clathrin and brings it on the surface of the vesicles.

*the bluish label in the slide 6 is the ARF-GEF that converts the GDP to GTP

And now,ARF1-GTP will interacts with the cytoplasmic part of the membrane protein at the budding site. ARF 1 GTP is going to attract other molecules or proteins like GGA or AP 1 (Adaptor protein 1).
The membrane proteins from the inner side of the vesicles bind the substrate (might be the hormone or the protein we need to secrete).
We might also need to transfer these plasma membrane proteins. It is a dual reaction, transferring the receptor and secreting the soluble compounds or proteins to the outside of the cell like hormones.

Now, once we have all these complexes assembled on the receptor of the vesicular membrane, this will attract and also interact with clathrin molecules. Just imagine we will have so many of these receptors and each of them will interact with the clathrins and the clathrins interact with each to form the shell/coat around the vesicles.

Once we have the clathrins gathered/coating the vesicles completely, it's going to walk on the microtubules and then gradually starts to lose this coat when it approach its final destination.

How does it loses the clathrins coating?
Hydrolysis of GTP into GDP will inactivate the ARF 1 and start to disassemble these proteins from each other and also detach the clathrins that basically interact with the proteins.

Then, these vesicles can now fuse with the target membrane.

How does fusion of these vesicles happen on the molecular level?
Fusion site has specific type of protein called t-SNARES (target SNARES). And vesicle has some proteins called v-SNARES (vesicular SNARES).

V-SNARES is going to interact with the t-SNARES and these proteins (both of them) have a structure called coiled coils (one helix and another helix that have a hinge like movement that can be compressed and released).

The first step is basically the interaction between the coiled coil of the vesicle and the target(refer to slide 7) at the fusion site. This interaction also needs the help of another GTP binding protein (Rabs). Once it is in the active form which is Rabs GTP, it can bind to these complexes on both (the target membrane and also the vesicular membrane). And there are so many Rabs molecules (50-60) and this also will specify the final destination and affect the site of the fusion of the vesicles.

Also, we need some other effector molecules or effector proteins in the middle connecting all of these components and bringing the vesicles in closer proximity to the target membrane. Once we have these two membranes in a very close proximity, this induces some sort of membrane instability in both membranes and cause fusion of these membranes. Notice that the coiled coils now are compressed and stretched to bring the membranes very close to each other so that they can fuse. Other proteins affect the fusion process which are the NSF and SNAPs. They gather at the very last step. Once the fusion starts to happen, they will help in the fusion of the membrane.

Then, we need these complexes (Rabs and Effector proteins) to dissociate and again under the hydrolysis reaction of GTP into GDP. (This will lead to disassembly of the complexes).

Now ,we will have now the v-SNARES inserted into the target membrane.

This is the fusion of the vesicles if they are coming from different compartments(golgi / lysosomes …)

How about the other steps if we bring the vesicles into the plasma membrane and then we need it to exit as a vesicle and release as a component (example : synapsis)?

We need another process that we call **exocytosis** for these vesicles to come out of the cell. Again, this process is mediated by other proteins (Rabs specifically).

NOTE: you will notice that in the figure in slide 9 there's also ARF, but Dr.Diala said that this figure is not accurate and it has been modified in the next edition of the book she took the photo from .

How does this process (exocytosis) happen?
The vesicle is coated by Rabs bounded to GTP,and some other effector molecules that we call altogether as exocysts (a collection of proteins that are specific for exocytosis). Some of them on the target membrane and some of them on the vesicles. Interaction of these proteins or exocysts is going to induce membrane fusion and release the contents and the transfer of the molecules.
Exocytosis is a very similar process except the effector molecules we have are called exocyst (specific proteins). Some of them found on the specific target membrane and some of them found on the vesicular membrane and they will interact with each other and mediate the fusion process.

In addition, we have another sets or another type of Rabs that mediate this process because we need GTP for activation (and hydrolysis of GTP into GDP to stop this process) .

Clinical Application : Disease related to the vesicular transport (Griscelli Syndrome -GS)

There are so many mutations that cause the disease but the end product is the same. For example is melanocyte.
Melanocyte is the cell that synthesis melanin pigment. If you remember when we were talking about the special products that are synthesized from the amino acid, we talked about melanin that's synthesized from tyrosine .

Synthesis of melanin takes place in melanocyte in a small compartments called melanosomes and there are two types:
Eumelanosommes and pheomelanosommes

So, once the melanin is synthesized by melanosomes, it needs to move to the keratinocytes in hair cells (for example) to give the color. They need to be transported as vesicles with the contents of the melanin The problem now is the mutations in MYO5A, RAB27A and MLPH that encode the MyoVA-RAB27a-Mlph protein that function in transporting melanosomes to keratinocyes. If we have the mutation that interferes with these proteins, it will affect the melanin transfers. No melanin transfer and accumulation will occur in the melanocytes and keratinocytes will lack of it. That's why I will expect

that the patients will have hypopigmentation. They have white or greyish colour of hair.

When we look under the microscope, we can see melanin clumps inside the shaft of the hair. (accumulate inside melanocytes)

LYSOSOMES

Lysosomes is one of the next station that the vesicles can move to. The main function of lysosomes is degradation of protein but we are not talking about its function now. We are talking about proteins that has to be localized or transported into the lysosomes to perform its function. That means the functional components or the proteins inside the lysosomes whether they are membrane proteins or soluble proteins as an acid hydrolyses inside the lysosomes.

The structure of the lysosomes
Lysosomes basically is an organelle that is very variable in its structure in terms of shapes and sizes depending what is inside them. I will expect to find them under microscope in very variable shapes and sizes and for sure there are membrane enclosed because of their specify function in degradation of protein. So, we need to have some sort of compartmentalization to avoid drastic effect if the hormones is released into the cytosol.

Lysosomal enzymes (slide 13)
This is the lysosomal structures with the acid hydrolases inside. This proteins are functional with maximum activity at the lower pH than the pH of the cytosol or cytoplasm. Their optimum pH is around 5 while the cytosol is around 7. There are 100 fold more of hydrogen in the lysosomes than the cytosol. For 2 unit of pH, that's mean I have a 100 fold more protons inside the lysosomes compared to the cytosol.

That's why we need to pump protons from the cytosol to the lumen of the lysosomes to guarantee in preparing and create the acidic environment. The importance of the acidic environment is to activate the enzyme in the first place, to unfold the proteins to be degraded.). If the lysosome has ruptured and released its enzymes, they will be inactivated in the basic environment inside the cytosol and this prevent the degradation of the cellular component.

Proteins that will be transported from trans golgi network to the lysosomes are divided into two types:
1. Membrane proteins
2. Luminal proteins (soluble)

Luminal lysosomal proteins are going to be modified inside the golgi in a specific certain way to shuttle them from trans golgi network to lysosomes only. Once we have N-linked glycosylation that we talked about it before with (2 N-acetyl glucosamine, 9 mannoses and then 3 glucoses. Then, they will be cut of three glucose residues and a cut at another mannose). So, I will have 8

mannose. Now, I have 10 sugar residues, 2 N-acetyl glucosamine and 8 mannoses as any proteins.

What happen to specify these proteins to the lysosomes is the addition of one N-acetylglucosamine phosphate. Once this molecule is added , it will be followed by cut or cleavage of the N-acetylglucosamine part. The aim of the reaction is to phosphorylate one of the mannose residues. The mannose-6-phosphate (the phosphorylated mannose) is going to be identified by specific receptors for mannose-6-phosphate and this modification only happen to the lysosomal proteins.

Firstly, I identify the proteins, bind it with the receptors on the vesicular membrane, budding of the vesicles and now it moves and only can be used with the lysosomal membrane.

How can we transport the lysosomal enzymes/proteins? (luminal & membrane proteins)

1. Luminal lysosomal proteins are identified by the mannose-6-phosphate. They have specific receptor that can identify them. Once it is identified, it is either fuse with the structure or organelles called late endosomes or go directly to lysosomes. (Late endosomes is going to mature into lysosomes).
2. Membrane lysosomal proteins have specific sequences in their cytoplasmic tails.

APPLICATION : Lysosomal Storage Diseases

Lysosomes is important structure in many diseases that we call Lysosomal Storage Diseases. If there is a mutation in one or more of these acid hydrolases we will end up with the accumulation of the molecules (lipid / proteins / DNA / anything) because degradation of these molecules will not happen inside the lysosomes. (There are different types of enzymes that degrades these components of molecules. If the enzyme responsible for the degradation is missing, they will accumulate inside the lysosomes and lysosomes will become bigger in size). Example of the enzymes that are mutated in the lysosomes that will lead to diseases are:

1. Glycolipidoses
2. Oligosaccharidoses
3. Mucopolysaccharidoses

*different types with different severities

Glucocerebroside

- This is not a disease. It is a molecule that will accumulate.
- It is a glycolipids (just one glucose molecule + ceramide)
- The enzyme, glucocerebrosidase is going to degrade the glucocerebroside into ceramide and glucose. Remove glucose and the rest is ceramide. If the enzyme is missing, this result in Gaucher Disease and it may have several severities. It is specifically is common in Jews.
- The accumulation of the glucocerebroside will enlarge the lysosomes inside the cytosol and results in some neural symptoms or another symptoms depending on the patients. This is the most common for the lysosomal storage disease.

Oligosaccharidoses - Pompe Disease

- This disease is basically due to lacking of a-1,4 glucosidase (degradation glycogen to glucose) and accumulation of glycogen inside the lysosomes.

I-cell Disease

- A very severe disease. Patient usually die earlier.
- The problem is usually the phosphorylation of the mannose to target proteins into the lumen of the lysosomes. So, I will expect that all of the lysosomal enzymes that are luminal proteins to be missing and not going to be in the lysosomes.
- That's why the symptoms are more severe in this situation because not just one enzyme is missing.
- Mutation occurs in the enzyme that modify and targets lysosomal luminal proteins that result in this type of disease.

Sorry for any mistake :)

"I have not failed. I've just found 10,000 ways that won't work."
- Thomas Edison-

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