Genetics
& Cell biology

◉ Sheet ○ Slides

Number

Done by:

Corrected by:

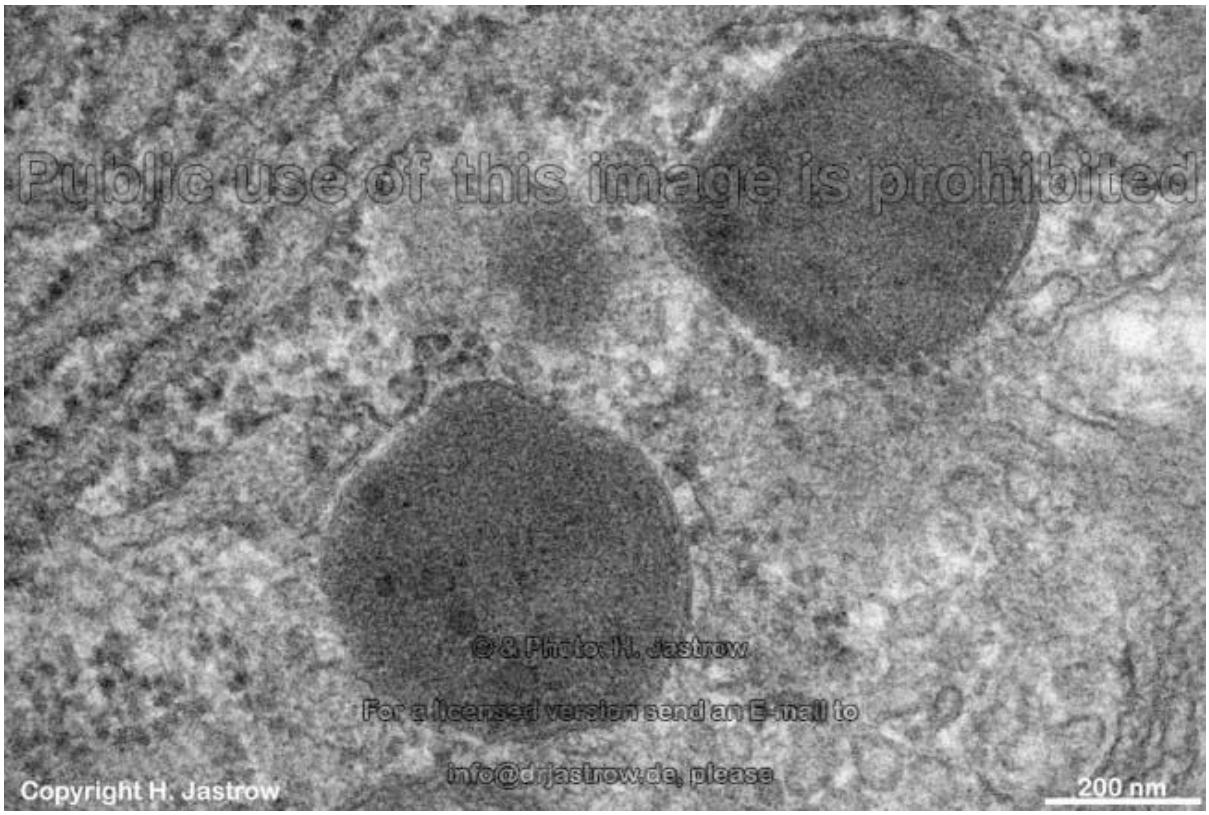
Doctor

Lysosomes:

Lysosomes are like the GI tract of the cell; they degrade all types of biological polymers including: proteins, carbohydrates and lipids.

Characteristics of lysosomes:

1. *No fixed shape or size*:
   Lysosomes, as you can see, are spherical structures that vary in shape and size. Unlike the mitochondria, lysosomes don't have a fixed shape. Also, their shape and size change according to the need of the cell.

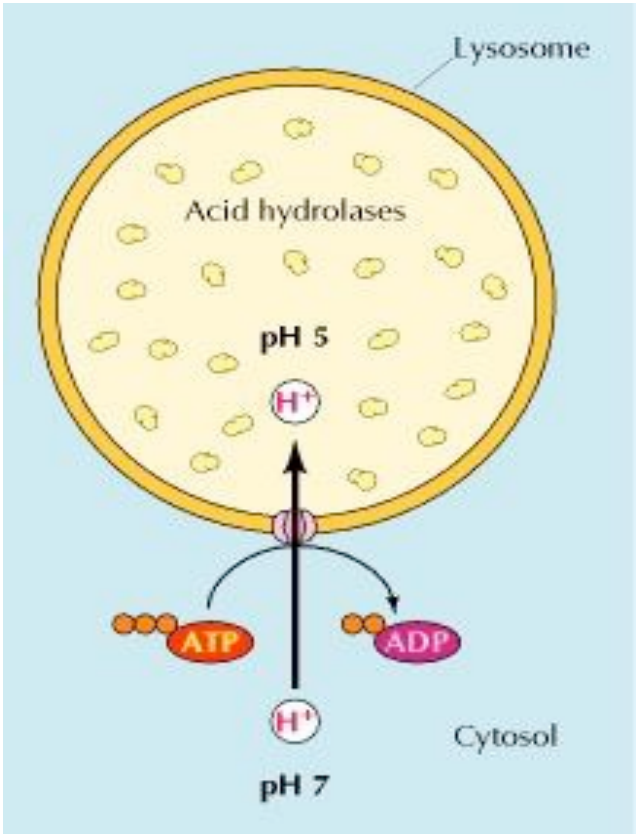


For example, they may get bigger or smaller depending on the amount of proteins, lipids or carbohydrates in the cell that needs to be degraded. (The more biological molecules that need to be degraded ➔ the larger the lysosome becomes). Think of it as the stomach of the cell, the more food you have in your stomach the larger it gets.

2. The most important structure in the Lysosome is its membrane (Lysosomes are *membrane enclosed organelles*). The function of the lysosomal membrane is to separate the lysosome's environment from the cytosolic one.

3. *Acidic environment*: the lysosomal environment is special since it's an acidic environment (pH of around 5)

*The pH of the cytosol is 6.9-7



There is a difference of 2 units of pH between the cytosol and the lysosome. Meaning, around a 100 fold more protons are inside the lysosome in comparison to the cytosol.

What brings those protons inside the lysosome? *Proton pumps* found on the lysosome membrane maintain the pH of the lysosome by pumping protons to the inside of the lysosome.

4. *Acid hydrolases:* Those enzymes are active at the acidic pH (about 5) that is maintained within the lysosome. Those enzymes are synthesized in the bound ribosomes and follow the pathway that was mentioned in the previous lectures (Lecture 2): Synthesized in the bound ribosome ➔ enter the ER ➔ get modified

(maybe get glycosylated, or get a sequence of amino acids that enable them to be inserted in the membrane) ➔ move to Golgi ➔ more modifications ➔ exported to the lysosome.

Those enzymes must stay inactive until they reach the lysosomes in order not to degrade the cellular contents. This is the why the environment of the lysosomes is acidic.

How do those inactive proteins become active?

1) Inactive Proteins (enzymes) ----> 2) affected by protons in the lysosome

3) Change in protonation status in the proteins

4) Change in conformation of the proteins leading to activation.

How do we protect the cell from these enzymes once they are active inside the lysosomes?

1- The lysosomal membrane: It acts as a barrier that prevents the enzymes from leaking out to the cytosol.

2- The defensive mechanism of the Acidic Media: The enzymes become deactivated once thy enter the cytosol since they're only active in acidic media.

There are two types of protein in the lysosome:

1- Lumenal lysosomal proteins (soluble).

2- Membrane lysosomal proteins.

How are those enzymes targeted to the lysosome?

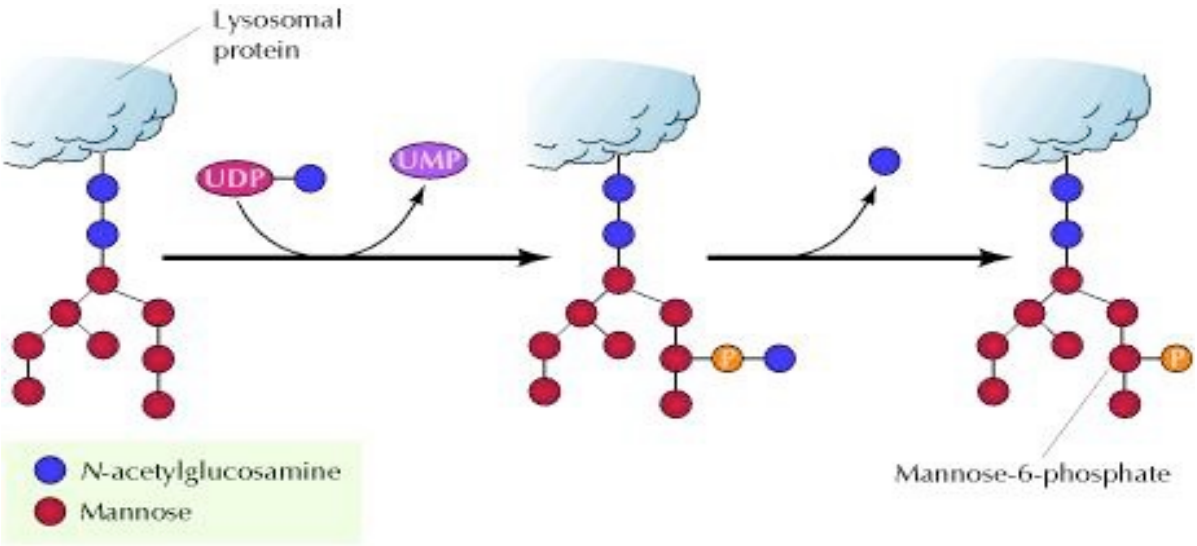
The lysosomal proteins are tagged by the sugar moiety added to them through (N-linked glycosylation process that happens in the ER, lecture 3):

A. We add 2 N-acetylglucosamine, 9 mannose, and 3 glucose residues.
B. We remove 3 glucose and 1 mannose.

Now, many proteins go through the same process mentioned above, but how are the lysosomal ones different?

For Lumenal proteins:

We add Phospho N-aceytlglucosamine (N-acteylglucosamine Phosphate). After it's added, N-acetylglucosamine is removed leaving Mannose-6-phosphate. This specific area where the mannose-6-phosphate is found will enable the protein to be recognized by mannose-6- phosphate receptor on a transport vesicle. Thus, the vesicle will attach to the lysosomal proteins from the mannose-6- phosphate side. Once the vesicle fuses with lysosomal membrane of *late endosome*, it releases the protein inside the late endosome, which matures into lysosome while the receptor is recycled.



What about membrane lysosomal proteins?

They have a tag (signal sequence) in their cytosolic part (cytoplasmic tails), which is the first to be recognized by the lysosomal membrane. Also, Receptors found in their cytoplasmic tails rather than Mannose-6-phosphate receptors.

Some applications and diseases associated with lysosomes:

What happens if there is a deficiency / deactivation of one of the lysosomal hydrolases (degradation proteins)?

1- Accumulation of proteins, lipids, and carbohydrates will occur. They will be transported to the lysosome, but they won't be degraded.

2- Accumulation leads to the enlargement of the lysosome and increase in their number. Thus, if we looked at a cell of a patient affected with such a disease under a microscope, we will find that the lysosomes are VERY large and with a foam-like appearance (they look like bubbles inside the cytosol.)

Diseases associated with deficiency or deactivation of lysosomal hydrolases are called "**Lysosomal Storage Diseases**" because they result from accumulation (storage) of some molecules inside the lysosomes.

Lysosomal storage diseases:

1- Glycolipidoses (Sphingolipids)

2-Oligosaccharidoses

3-Mucopolysaccharidoses: deficiencies in lysosomal hydrolases of GAGs (heparan, keratan, dermatan sulfates and chondroitin sulfates).

** 0-10 minutes

➔ Those diseases are chronic progressively debilitating disorders that lead to serve psychomotor retardation and premature death.

Let's talk about each one of them in more details:

1- Glucocerebroside

Glucocerebroside is related to the metabolism of sphingolipids and specifically glycolipid. For example, if we want to degrade glucocerebroside, which is composed of ceramide with glucose as a head group, logically it will give us glucose and ceramide. This reaction is done by a lysosomal enzyme called **glucocerebrosidase**.

So, if this enzyme is missing for some reason, like for example a genetic mutation, it will lead to a disease called "**Gaucher disease.**" Gaucher is very common in Jews, and it's **the most common** lysosomal storage disease.

Gaucher disease is classified according to its degree of deficiency and severity to **3 types**:

Type 1:
(Least severe, most common) the nervous system is not involved; spleen and liver enlargement, development of bone lesions. In this type, we will notice the features mentioned above: Glucocerebroside will accumulate inside the lysosomes enlarging the size of the lysosomes giving that foamy appearance of the cell.

Types 2 and 3:
(More severe, much rarer): the only cells affected in Gaucher'sdisease are macrophages.

*Macrophages eliminate aged and damaged cells by phagocytosis that involves continuous ingestion of large amounts of lipids in lysosomes for degradation.

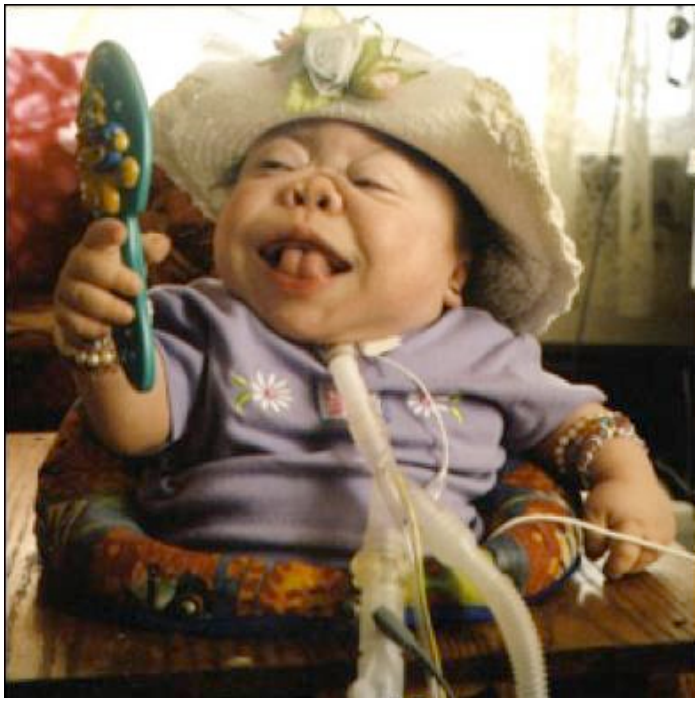
2- Oligosaccharidoses

A)Pompedisease (type II)

Glycogen is broken down inside the lysosome to produce glucose using the enzyme α-1,4-glucosidase in a none-metabolic process. [1]
So any deficiency in this enzyme will lead to the accumulation of glycogen inside the lysosomes because the enzyme that was supposed to break it down the glycogen to glucose is missing/non-functional. In this disease, Glycogen *structure is normal* but its *amount is excessive*.

Note[1] : *Please remember that the degradation and digestion processes that happen inside the lysosome are NOT metabolic processes. We are not breaking down the molecules to a final form. We are just simplifying it, but it is not the final form.*

B) I-Cell disease



A very severe phenotype disease: very small size, specific facial features are affected. Usually it results in the death of most patients (children) due to a deficiency in the targeting of lumenal lysosomal proteins to the lumen. Since we have quite a lot of lumenal lysosomal enzymes this disease will affect plenty of enzymes leading to severe problems.

Features: severe psychomotor retardation that rapidly progresses leading to death between 5 and 8 years of age.

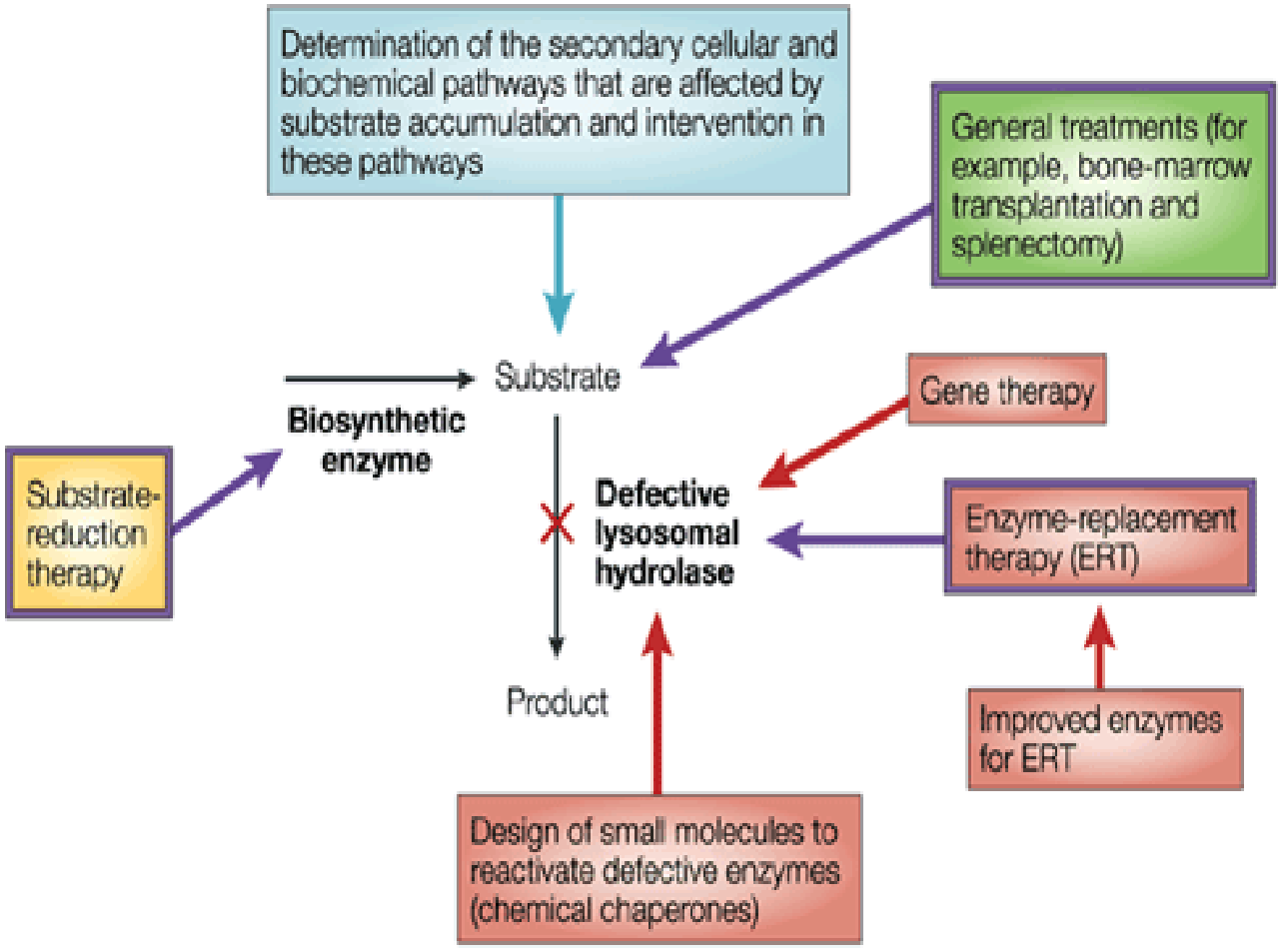
Treatment of lysosomal storage diseases:

1. Dietary restrictions
2. Replacement therapy: if the enzyme is missing, we synthesize the enzyme and administrate it to the patient. i.e Insulin for diabetic patient.
3. Genetic therapy: recent approach, there is no disease that has been successfully treated with gene therapy. The idea of gene therapy is replacing the abnormal gene with a normal one. How can we do this? By loading the normal DNA sequence inside a viral particle. This particle is going to be targeted to a certain type of cells only. The problem with this method:

A. Targeting is not easy. There is no a specific marker for each type of cells (we didn't discover them yet)
B. To use the viral particle, we remove the harmful gene of it, yet it can still be recognize as a foreign body and get deactivated by the liver. Therefore, this approach is now mainly used for avascular tissues like those found in the eyes: lens, cornea…etc.

** 10-20 minutes

C. Loading capacity: The viral particle is like a hollow ball that is going to be loaded with a normal DNA, so there is a little bit of space inside. The largest size of the DNA that can be loaded inside a viral particle is 5 thousand nucleotides. This is a relatively small sequence. Some genes are 18 thousand nucleotides, thus they can't fit inside the viral particle. The most common viral particle used in gene therapy is AAD, adeno- associated virus. Since it the least virulent and has the largest loading capacity among other viruses.



4. Stem cells.

Application: role of lysosomal enzymes in the effectiveness and mediation of some drugs

1. Chloroquine:

- Anti-malarial agent. There is no specific drug that can cure malaria 100%, but one of the agents used for the treatment of malaria is chloroquine. Normally, Malaria as a parasite targets the red-blood cells, and specifically the haemoglobin. The haemoglobin enters the parasite's lysosome called vacuole. Once it is inside the vacuole, haemoglobin gets digested and an enzyme called hemepolymerase affects the structure of the heme by modifying it. If heme is not modified, it becomes toxic to the parasite.

- Chloroquine crosses membranes into the malarial digestive vacuole and inhibits this enzyme by prevents its action. Thus, the accumulation of heme becomes toxic to the parasite and kills it.

- Chloroquine is a weak base that becomes protonated at acidic pH. Thus it takes advantage of the acidic environment in the vacuole and uses it to get activated.

Endocytosis: How lysosomes are formed

- Molecules are taken up from outside the cell in endocytic vesicles, which fuse with early endosomes.

- *Early endosomes* separate molecules targeted for recycling from those targeted for degradation.

- Membrane receptors are recycled via recycling endosomes.

- Early endosomes mature into *late endosomes*.

- Transport vesicles carrying acid hydrolases from the Golgi fuse with late endosomes, which mature into *lysosomes.*

- The acid hydrolases dissociate from the mannose-6-phosphate receptor and the receptors are recycled to the Golgi.

Phagocytosis and autophagy:

Phagocytosis: The up taking of a foreign body by invagination of the cell membrane, which is then fused with lysosome for digestion to degrade it down. It is a type of endocytosis. Process: the cell's plasma membrane starts to expand and surround the material that we want to ingest forming phagosomes. Those phagosomes fuse with lysosomes.

Autophagy (self-eating): It is the turnover of the cell organelles. Process: The up taking of an internal organelle that is surrounded by a membrane made from the ER forming a structure called autophagosome. Then the autophagosome fuses with the lysosome.

Autophagy can happen due to many reasons:

1. Embryonic development.
2. Apoptosis: Autophagy usually a process that precedes apoptosis. Before going through apoptosis, the cell activates autophagy so it can decrease its load. In specific, it decreases the number of mitochondria.
3. Autophagy can be used as a defensive mechanism when the cell is under stressful conditions

** 20-35 minutes

Introduction to Mitochondria:

1. Mitochondria are the energy factory inside the cell; highly active organelle inside the cell.
2. Double membrane structure with an outer-membrane (permeable) and inner (impermeable).
3. The inner membrane forms folds called cristae to increase its surface area.

4. Inside the inner space (matrix), we have a lot of enzymes proteins that are required for the function of Mitochondria.
5. It has its own DNA. It is the only organelle that has its own DNA other than the nucleus.
6. The intramembranous space is also a place where many other reactions occur. For example, changes of proteins structure.
7. What is the difference between inner and out membrane of mitochondria:
    A. Structure: Most of the enzymes are found in the inner membrane, that's why the inner membrane is rich in proteins compared to the outer membrane. In fact, around 75% of the inner mitochondrial membrane is made of proteins.
    B. Permeability

What is the function of the DNA found in Mitochondria?
It contains genes that encode some proteins that are involved in the function of mitochondria. A large number of proteins that are found in the mitochondria are actually encoded for in the nucleus.

"Some people choose to see the ugliness in this world, the disarray. I choose to see the beauty. To believe there is an order to our days. A purpose."