**Lesson: Lysosome**
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## Chapter: Lysosome

## Introduction

Nobel Prize laureate Christian de Duve and his co-workers discovered a new microbody in animal cells in late 1950s, which was named lysosome. Experiments to identify the localization of the two enzymes glucose-6-phosphatase and acid phosphatase in liver tissue homogenates by differential centrifugation clearly indicated that acid phosphatase enzyme was located in a new class of particles never reported before. In addition to acid phosphatase these new organelles contained several other hydrolytic enzymes including proteases, lipases,β-glucoronidases, ribonucleases, deoxyribonucleases all of which have an apparent role in cellular lysis. C. de Duve named this organelle lysosome for their role in lysis. The organelle contains approximately 50 different types of hydrolyzing enzymes.

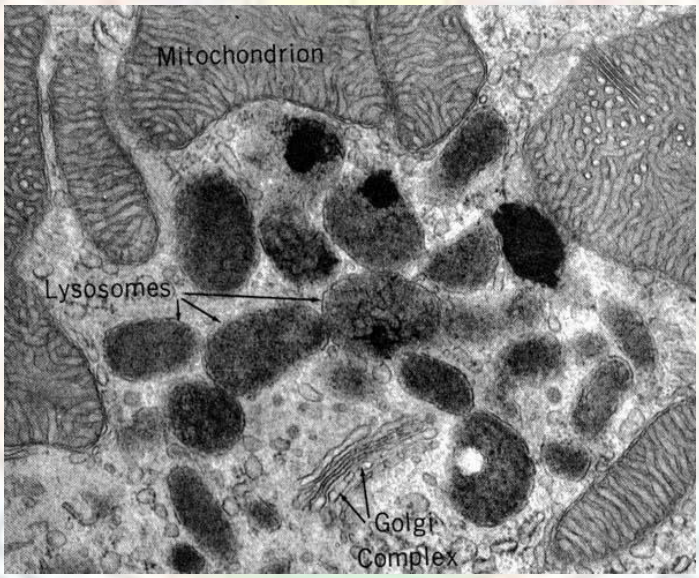



**Figure:**Under electron microscope, lysosomes are found to be of the size of mitochondrion, are small electron dense, nearly circular, single membrane structure with the diameter varies from 0.1-0.8 μm.

Source:http://www.dematice.org/ressources/PCEM1/Histologie/P1_histo_009/Web/res/figure18.jpg

To distinguish lysosome from other small, single membrane electron dense particles within the cells, in 1952 G.Gomori introduced modified cytochemical procedures and identified the lysosome based on high acid phosphatase content, which is the **marker enzyme** for the organelle.

## Structure

The lysosomes are bound by a single membrane. The lysosomal membrane separates the hydrolytic enzymes from the rest of the cell thus protecting the cell from these enzymes. Highly glycosylated integral proteins present in the lysosome membrane protect the membrane from the attack by the enclosed enzymes.

The lysosomal enzymes are active at low pH and have a requirement of an acidic pH (4.5 or less) for their optimal activity. Hence, unlike the cytosolic and most other organelle enzymes which require a near neutral pH, all the lysosomal enzymes are **acidhydrolases. The internal compartment of the lysosome providesthe low pH.**The unique property of the lysosome membrane, is to maintain its internal acidic pH by actively accumulating $H^+$ ions (protons) with the help of a V-type proton pump. The high internal proton concentration is regulated by a proton transporter ($H^+$-ATPase) located in the organelles boundary membrane, which actively transports protons from the cytosol there by maintaining the near neutral pH of the cytoplasm and a highly acidic pH within the organelle.

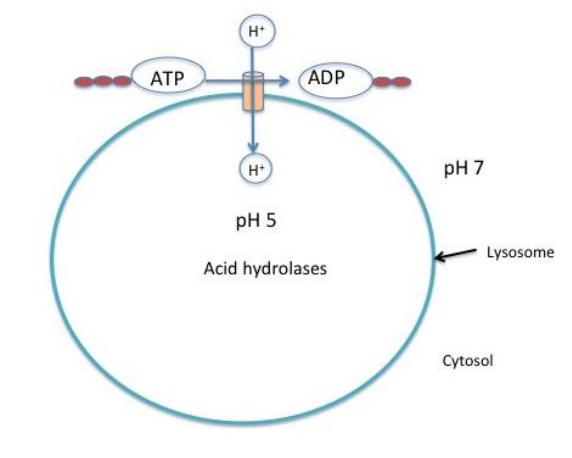



**Figure:** The lysosome has an acidic pH because of the pumping of protons by a membrane bound proton pump that imports protons from the cytosol. The enzymes that are present in the lysosome are active at low pH.

Source: ILLL Inhouse

**Table** Enzymes present in lysosome

Source: Author

| Enzymes | Substrate |
|---|---|
| **Proteases and peptidases** | |
| • Cathepsin A, B, C, D, E | Various proteins and peptides |
| • Collagenase | Collagen |
| • Arylamidase | Amino acid arylamides |
| • Peptidase | Peptides |
| **Nucleases** | |
| • Acid ribonuclease | RNA |
| • Acid deoxyribonuclease | DNA |
| Phosphatases | |
| • Acid phosphatases | Phosphate monoesters |
| • Phosphodiesterase | Oligonucleotides, Phosphodiesters |
| • Phosphatidic acid phosphatase | Phosphatidic acids |
| **Enzymes acting on carbohydrate chains of glycoproteins and glycolipids** | |
| • βGalactosidase | βGalactosides |
| • Acetylhexosaminidase | Acetylhexosaminides, heparin sulfate |
| • βGlucosidase | βGalactosides |
| • αGlucosidase | Glycogen |
| • αMannosidase | αMannosides |
| • Sialidase | Sialic acid derivatives |
| **Enzymes acting on glycosaminoglycans** | |
| • Lysozymes | Mucopolysaccharides, bacterial cell wall |

| | |
|---|---|
| • Hyalouronidase | Hyaluronic acid, chondroitin sulfates |
| • βGlucuronidase | Polysaccharides, mucopolysaccharides |
| • Arylsulfatase A and B | Arylsulfates, cereboside sulfates, chondroitin sulfate |
| **Enzymes acting on lipids and phospholipase** | |
| • Phospholipase | Lecithin, phosphatidyl ethanolamine |
| • Esterase | Fatty acid esters |
| • Sphingomyelinase | Sphingomyelin |

## Role

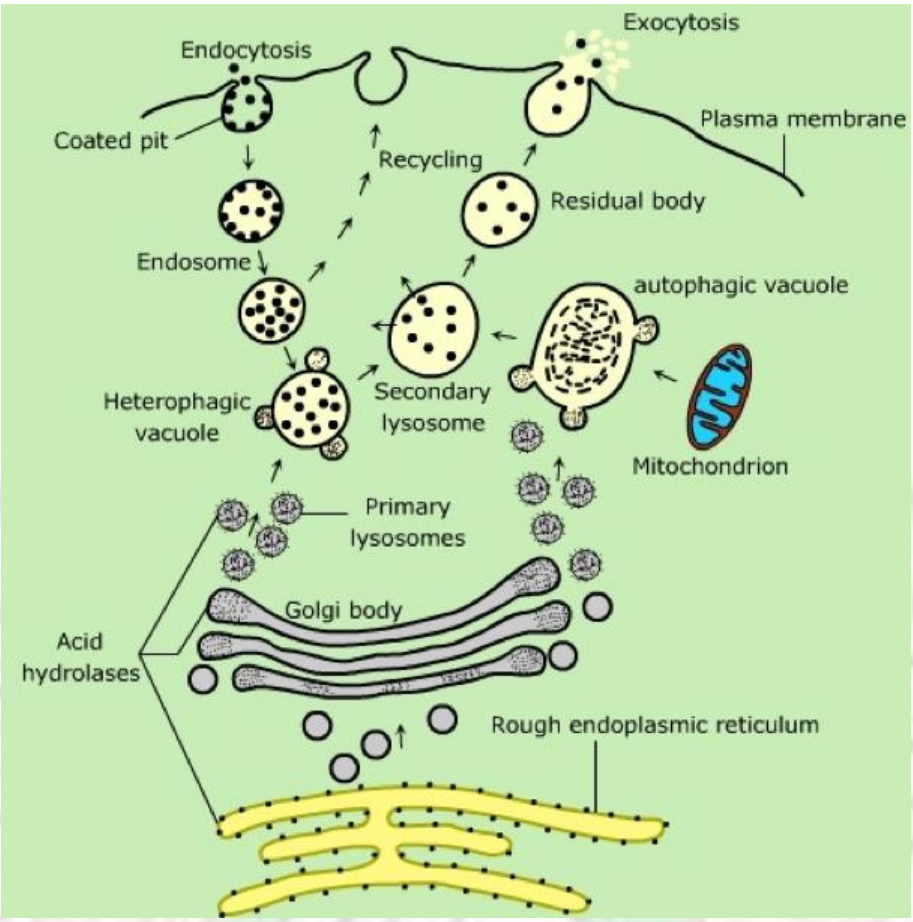



**Figure:**Formation and function of lysosomes

Source: ILLL Inhouse

**Animation:** http://highered.mcgraw-hill.com/olc/dl/120067/bio01.swf

The lysosomes have various functions like:

- Providing nutrition by – for example the digestive role in protozoans, the cellular autophagy during unfavourable conditions
- Defense aginst invading microorganisms by circulating macrophages.
- Recycling of cellular wastes and scavenging of aged and non functional organelles.
- Secretion of hydrolases by sperm for penetration into the egg.
- Digestion of yolk during the development of embryo
- Dissolution of blood clots and thrombi

Lysosomes are polymorphic. They vary in size, shape and function even within the same cell. Three major types of lysosome are: (a) Primary lysosome (b) Secondary lysosome (c) Residual bodies.

(a) **Primary lysosome** are newly formed and still virgin that is these have not entered into any hydrolytic pathway. Primary lysosomes originate in the Golgi complex and contain the hydrolytic enzymes.

(b) **Secondary lysosomes** are classified into two types namely (i) Heterophagic vacuole are also called heterosome or phagolysosome. Heterophagic vacoules are formed by the fusion of the cytoplasmic vacuoles containing the extracellular substances,which the cell receives through endocytosis (Phagocytosis)(refer lesson on Membrane: properties and selective perameability for details).The fusion, of the primary lysosomes with the heterosome, triggers the release of hydrolytic enzymes into the heterophagic vacuoles leading to the digestion of the ingested materials.The lysosomal enzymes are released into the vacoule and are now called as endosomes(ii) Autophagic vacuoles (also called Autosomes), contain aged and non functional cellular organelles that are no longer require by the cell. These organelles are digested and the contents relaesed (Autophagy).

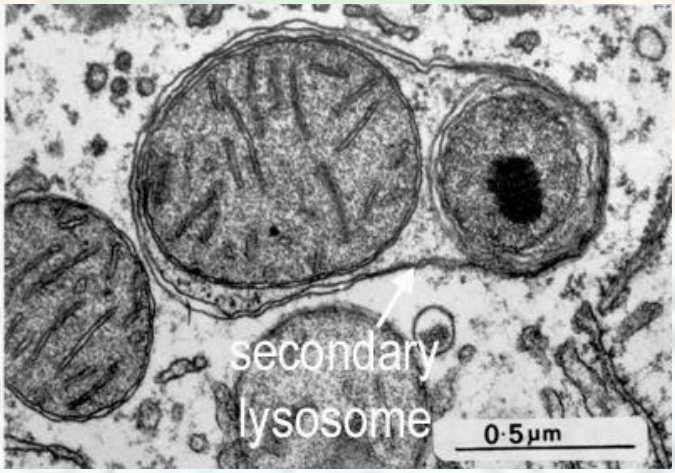



**Figure**: Electron micrograph showing autophagy. A mitochondrion and a peroxisome enclosed in a double membrane- autophagosome.

Source:http://www.histology.leeds.ac.uk/cell/assets/secondary_lys.gif

(c) **Residual bodies** – the lysosomes containing undigested endocytosed material or parts of the cell that are not completely degarded in the secondary lysosomes but

are later released outside are called as residual bodies. These are electron dense and usually do not contain the hydrolytic enzyme activity.

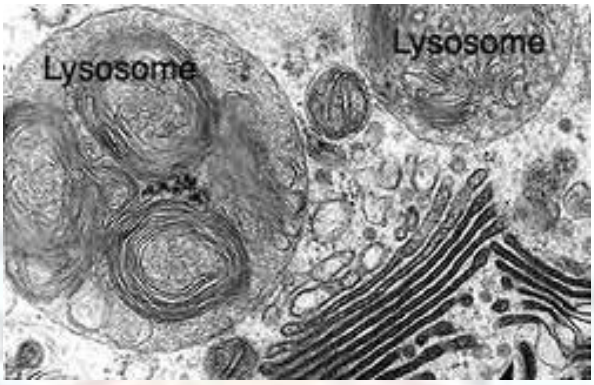



**Figure:** Residual bodies –lysosomes containing undigested material
Source:http://www.dematice.org/ressources/PCEM1/Histologie/P1_histo_009/Web/res/figure22_2.png

The molecules that are taken in from outside the cell are carried into the cellby the endocytic vesicles that fuse with the early endosomes. Endosomes are the intermediaries between the secretorypathways (involved in processing and transport of proteins in the GERL) and the endocytic pathways ( involved in intake of material inside the cell from the exterior). There are three types of endosomes-

- Early endosome
- Late endosome
- Recycling endosome

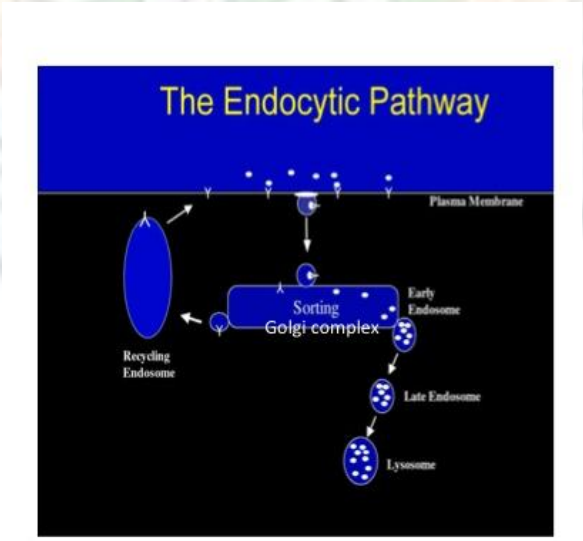


**Figure:** The endocytic pathway involves the three types of endosomes- the **early endosomes** that carry the material from cell exterior to cell interior, **the late endosome** that fuses with or mature into lysosomes and the **recycling endosome** that carries the molecules to be recycled back to the membrane (including the membrane receptors).

Source: http://www.wormbook.org/chapters/www_intracellulartrafficking/Itrafig1.jpg

The early endosomes differ from late endosomes in their protein composition. As the endosomes mature the contents become more and more acidic. The early endosomes provide a mild acidic environment in which the internalized proteins receptors change their conformation and release their ligands. The receptors are then recycled back to the plasma membrane in the recycling endosomes.

The early endosomes gradually mature into late endosomes either by fusing with each other or by fusing with already existing late endosomes. The late endosomes no longer send vesicles to the plasma membrane. The late endosomes fuse with existing lysosomes to for endolysosomes. When the majority of the contents of the endolysosomes is digested these mature into lysosomes. These are small, dense, round and can enter back into the cycle by fusing with the late endosomes or the endolysosomes.

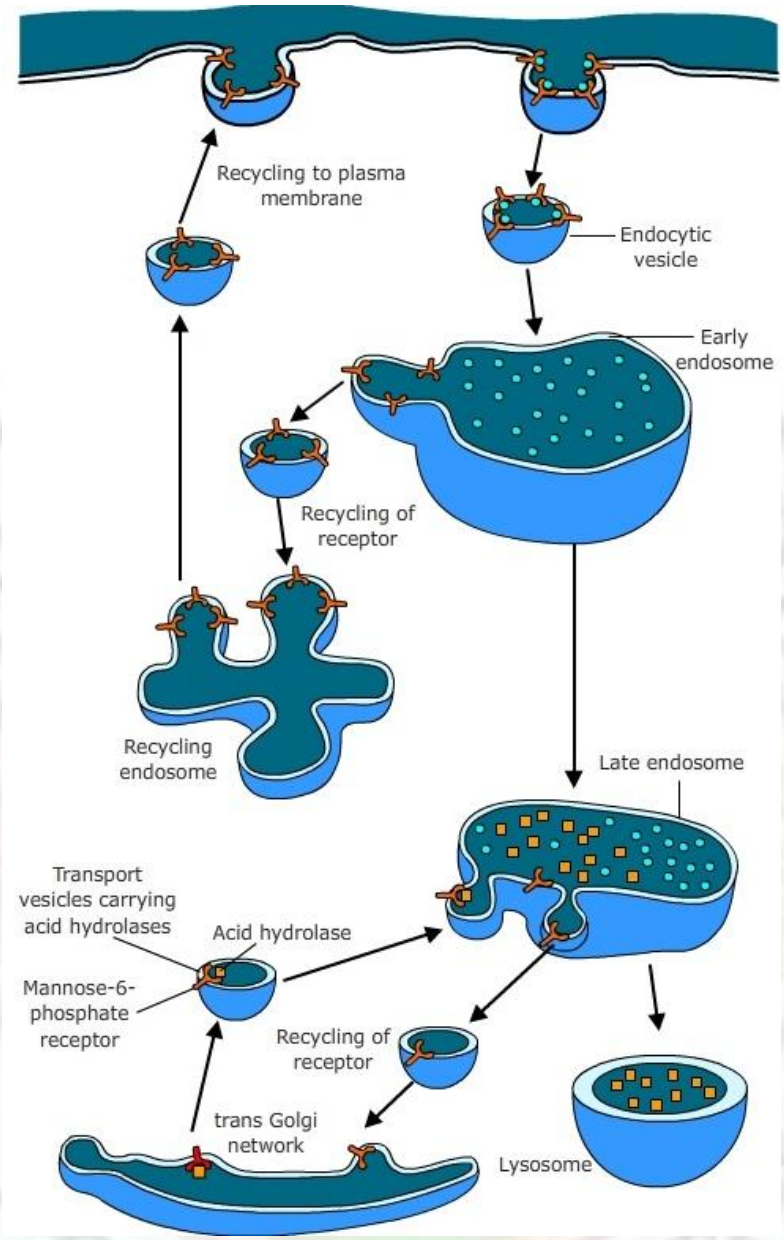



**Figure:** Endocytosis – The intake of material from outside in endocytic vesicles that fuse with the early endosomes. The membrane receptors are then recycled back to the plasma membrane by the recycling endosomes. Early endosomes mature into late endosomes that fuse with transport vesicles carrying the hydrolytic enzymes from the *trans* Golgi network. The acid hydrolases separate from the mannose-6-phospahte receptor in the late endosomes that now mature into lysosomes. The receptors are recycled back to the *trans* Golgi network.

Source: ILLL Inhouse

## Phagocytosis

A well-studied example of heterophagic vacuoles is the phagocytosis of food particles by unicellular organisms like *Amoeba*, where food vacuoles get fused with the lysosomes and the soluble nutrients then diffuse across the lysosomal membrane into the cytosol. In higher organisms like mammals, phagocytotic cells like macrophages and neutrophiles present in WBC act as scavengers for the ingested debris such as micro -organisms. Micro-organisms once ingested as phagosome fuse with the macrophages which, are then inactivated by the low pH and get digested by the hydrolytic enzymes. Thus, lysosomes play an important role in animal defence mechanism. Not all micro-organisms can be destroyed by phagocytic cells. The bacteria that cause some of the dangerous disease in human body like *Mycobacteriumtuberculosis*, which cause tuberculosis form phagosome but fail to fuse with the lysosome. Another bacteria like *Coxiellaburnetii* where the engulfed organism fail to fuse with the lysosome, rather multiply within the phagosome. Phagosome fuses with the lysosomes to form a **phagolysosome.** Besides destroying the unwanted ingested materials within the cell, an unusual **extra cellular digestion** by the lysosome is seen in animals during fertilization. The lysosome enzymes present within the acrosome of sperm cells, digest the outer covering of the egg cell so that the sperm nucleus can fuse with the egg nucleus.

## Autolysis

Autosomes also play an important role in the turnover of cellular organelles, where the cells own cytoplasmic organelles like mitochondria, chloroplast, ER etc fuse with the lysosome for **autodigestion**. Autodigestion is a normal event during the cellular growth, repair and ageing. It has been calculated that one mitochondrion gets digested in every 10 mins.

**Autolysis** is a process of cellular self-destruction or self-digestion, which plays a crucial role during the development of particular structure and organ system by which the unwanted cells are destroyed. Creation of individual digits (fingers and toes) from the initially webbed hands and feet of human embryo by selective removal of individual cells is an example of such **programmed cell death** during development process. Progressive loss of tail, as a tadpole matures is mediated by lysosomal **autolysis.** The lysosomes are also called the **"suicidal bag"** of the cell due to its deliberate release of digestive enzymes during programmed death of the cell or tissue.

Lysosomes degrade cytoplasmic organelles in ageing cell, autolysis of cell or tissues during developmental process and destroy the unwanted endosomes containing materials from external environment. However, lysosomes are not involved in destroying abnormal and unused (no longer required for any function) proteins in the cells. A multiplex protein complex called **proteasomes** present in the cell destroy the unwanted proteins.

## Biogenesis

<iframe width="420" height="315" src="//www.youtube.com/embed/u38LjCOvDZU" frameborder="0" allowfullscreen></iframe>

**Animation:** Synthesis of lysosomal proteins and their transport

Source:http://www.youtube.com/watch?v=u38LjCOvDZU

Enzymes that are destined for the lysosomes are synthesized by the rER, following which the core glycosylation takes place within the rER lumen (for details see lesson on ER), the proteins are transported to the Golgi complex for further modification. Once within the cis cisternae of the Golgi, these glycoproteins get a mannose-6-phosphate (M6P) tag (see lesson on Golgi for details), which acts as a **signal patch** for all the lysosomal proteins to recognize and fuse with their specific target that is the lysosomes. Signal patches act as a recognition determinant formed by the three-dimensional folding of a polypeptide chain. Cytosolic coat proteins also help in the transport pathway and the final vesicle fusion. Hence, all the lysosomal enzymes are glycosylated, being synthesized by the bound ribosomes and not by free cytosolic ribosomes. Transmembrane M6P receptor proteins present in the trans Golgi network (having pH 6.5-6.7) recognize the M6P groups bind to the lysosomal hydrolases on the lumenal side and the assembling clathrin coats on the cytosolic side. Thus packaging the enzymes-hydrolases into clathrin coated vesicles. These vesicles fuse with the late endosomes where the receptor disassociates from the acid hydrolases at the lower pH 6 present inside the late endosomes. The receptors are recycled back to the plasma membrane.

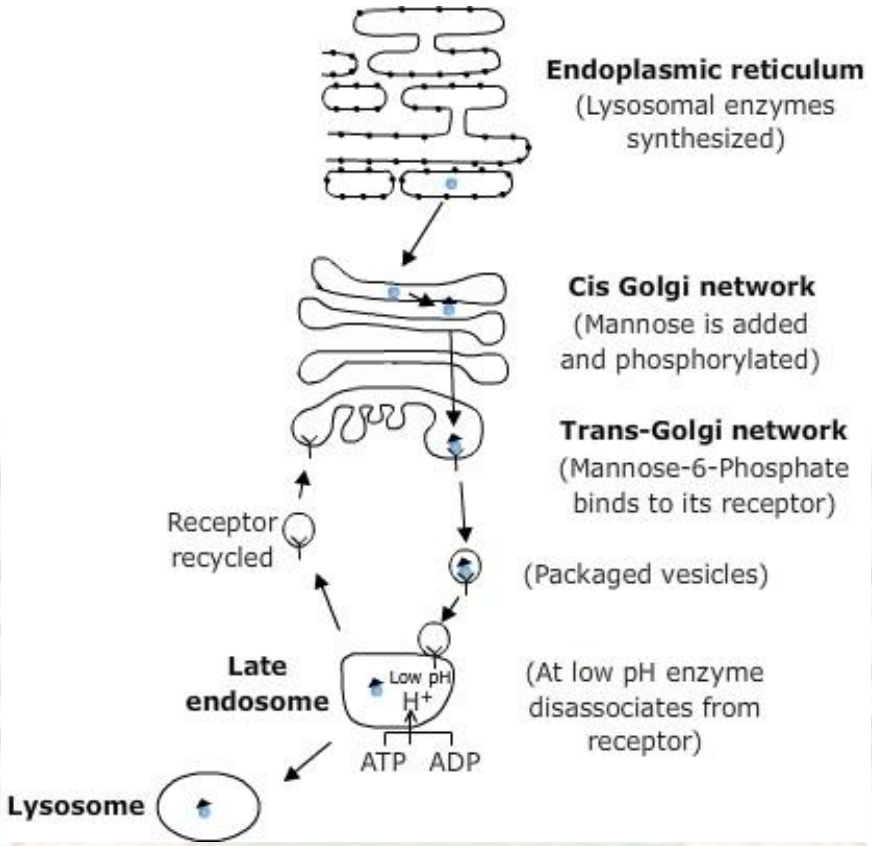



**Figure:** The lysosomal enzymes are synthesized in the ER and then carried by the vesicles to the Golgi where mannose is added and phosphorylated. The binding of the mannose to its receptor in the trans Golgi is followed by its packaging in the vesicles and transport and fusion with late endosomes and maturation into lysosomes.

Source: ILLL Inhouse

The close interrelationship in the function and synthesis of lysosomal proteins with the ER and Golgi makes the GERL complex. The hydrolytic enzymes only become active, when packaged within the secretory vesicles and are pinched off from the TGN. Any of these enzymes if by default, released from the earlier Golgi cisternae remain inactive. It is also to be noted, that these dangerous lytic enzymes, which can cause havoc in normal cell, do not come in direct contact with the cytoplasm. From their biogenesis within the rER lumen, through the form of shuttle vesicles transported to the various Golgi cisternae and finally through specific signal patches, recognize the final destination. Lysosomal enzymes remain inactive in the cytosol, if accidentally released due to the near neutral pH of the cytoplasm.

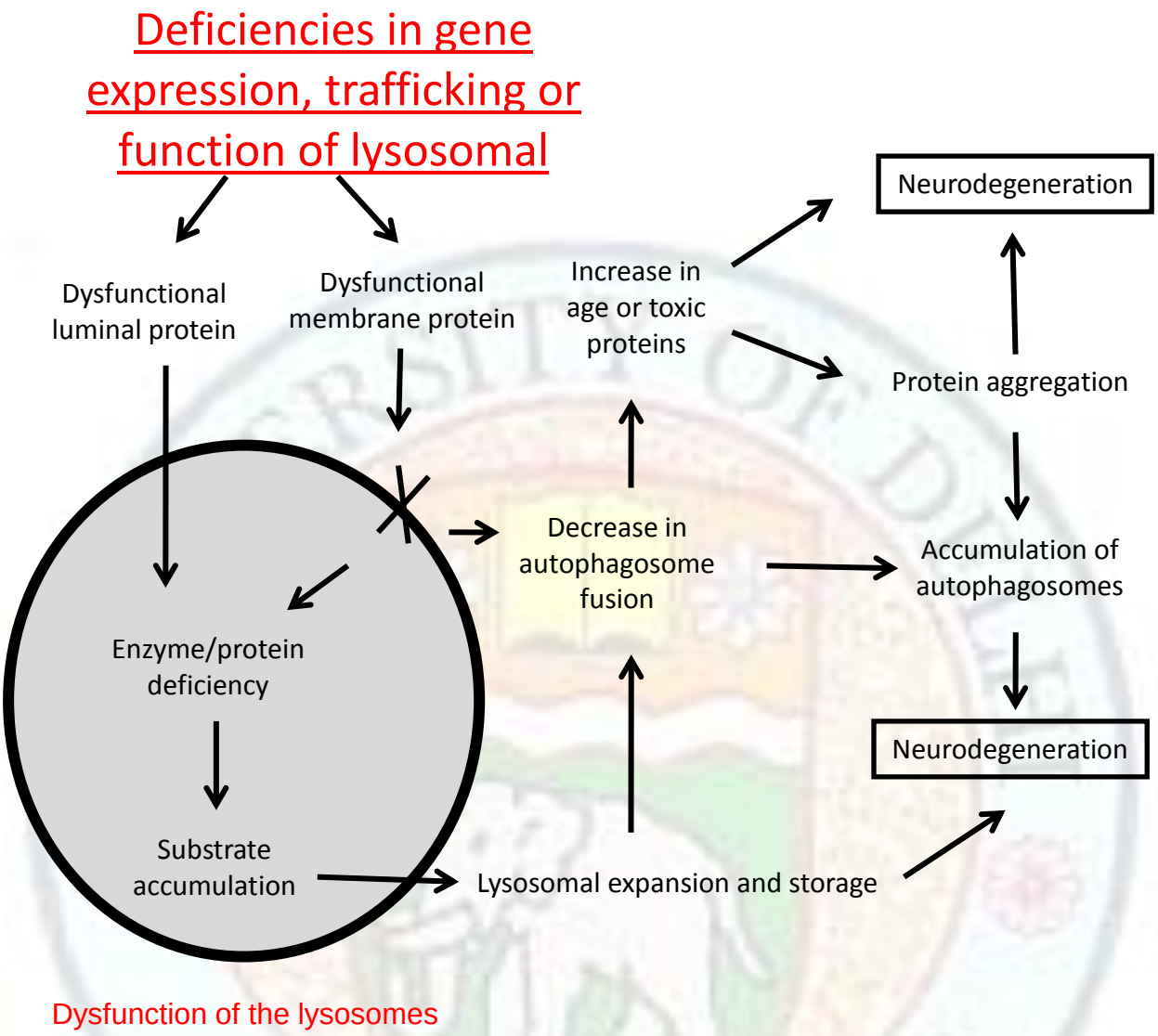
# Lysosomal storage diseases (LSD)

The importance of lysosomes in cellular turnover, autolysis and various other hydrolytic processes results in LSD due to the deficiency of specific lysosomal enzymes. More than 40 such diseases have been identified, which are either due to excessive accumulation or incomplete digestion of specific undesirable substances. Some of the important symptoms of LSD are muscle weakness, skeletal deformation, organ deformities and mental retardation leading to fatal outcome. In each of these diseases, there is either a deficiency or absence of specific lysosomal enzymes. Two well known LSD are the Hurler syndrome and Hunter syndrome. In both the cases there is a defective hydrolytic pattern of glycosaminoglycan, which result in the accumulation of undesirable acid glycosaminoglycans.

**Table:**Some of the important LSD due to incomplete metabolism of glycolipids are tabulated below .

Source: Author

| Disease | Deficient enzyme | Symptoms |
|---|---|---|
| Tay- Sachs disease | Hexosaminidase A | Mental retardation, Blindness, death by the age of 3 |
| $G_{M1}$ Gangliosidosis | $G_{M1}$ β Galactosidase | Mental retardation, enlargement of the liver, dead by the age of 2 |
| Fabry's disease | αGalactosidase A | Rashes on skin, kidney failure and pain in lower body |
| Gaucher's disease | Glucerebrosidase | Liver and enlargement of spleen, bone degradation, mental retardation |
| Krabbe's disease | Galactocerebrosidase | Loss myelin, mental retardation, death by age 2 |




Source: Schneider and Zhang**. Molecular Neurodegeneration** 2010, 5:14.

**Figure: Lysosomal deficiencies that may lead to neurodegeneration**. Functions of lysosomes are regulated at multiple levels, including coordinated transcriptional regulation of lysosomal genes, trafficking of lysosomal proteins to the lysosomes, and proper function of the lysosomal membrane proteins and luminal acid hydrolases. Deficiencies of any of these processes may lead to deficiencies of reduced lysosomal degradation of aged or toxic proteins. The lysosomal substrate accumulation may in turn result in lysosomal expansion and storage and further disruption of its activities. Accumulation of aged, toxic or aggregated proteins and organelles, and accumulation of autophagosomes may lead to eventual neurodegeneration.

## Summary

Lysosomes are small, single membrane microbodies present in animal cells, which contain acid hydrolases. The most important and abundant enzyme of lysosome is the acid phosphatase besides around 50 other digestive enzymes. Lysosomes are responsible for the programmed death during embryogenesis and organ development, hence are referred the **Suicidal Bags** of the cell. All the lysosomal enzymes are glycosylated since they are synthesized within the rER lumen and processed in the Golgi cisternae before being dispatched to the target organelle. Hence, lysosome forms an important component of GERL complex. Depending on their digestive role, lysosomes can be phagosomes or autosomes. Lysosomes play important role in the defence mechanisms of animals and facilitate the fusion of the sperm and egg cells during fertilization. The deficiency or absence of any lysosome enzymes results into serious defects in organisms called the LSD, signifies the importance of lysosomes.

## Glossary

**Acid hydrolase:** Group of hydrolytic enzymes with the optimal activity at acidic pH

**Autophagy:** Self destruction or the degradation of cells own organelles

**Heterophagy:** Degradation of materials, that enter the cell through endocytosis.

**Proteamers:** Multi protein complex that destroy abnormal cellular proteins

**Autolysis:** Programmed cellular digestion

## Exercise

(1) Describe the structure and functions of the lysosomes.
(2) What do you understand by programmed death? Explain giving suitable examples.
(3) Enumerate the different types of lysosomes and elaborate upon their functions.
(4) Describe some unique features of lysomal enzymes and its boundary membrane.
(5) How lysosomes help in the process of fertilization in animals?
(6) Justify the statements:
    (a) Enzymes of lysosomes remains inactive in the cytosol
    (b) All animal cells carry safely a "suicidal bag" within.
    (c) Lysosomes play important role in bodies defence mechanism.

(d) In spite of the lytic role of lysosomes they  an important organelle

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## Links

1. http://www.uni-mainz.de/FB/Medizin/Anatomie/workshop/EM/EMpLysoE.html
2. http://en.wikibooks.org/wiki/Structural_Biochemistry/Cell_Organelles/Lysosome