Chapter 6: ENDOMEMBRANE SYSTEM

A. ENDOPLASMIC RETICULUM

I. DEFINITION

It is a set of membranes delimiting cavities under shape of cisterns or tubules. II can be deprived of ribosomes, it is the case of the smooth endoplasmic reticulum (SER); or carrier of ribosomes it is the granular endoplasmic reticulum (GER) which is related to the nuclear envelope.

II. ULTRASTRUCTURE OF THE MEMBRANE

In TEM: the membrane of the ER is trilamellar with more reduced thickness (6nm) than the plasmic membrane. Observed under BEM: there is a presence of globular particles intramembrane.

III. CHEMICAL COMPOSITION

1. Isolation

The ER is isolated from the third base of the DUC in the shape of microsomes (small vesicles) rough or smooth. A sucrose gradient centrifugation, separate the rough microsomes from the smooth ones.

- The rough microsomes centrifugation in presence of a detergent permits to detach ribosomes coupled on membranes.
- A smooth microsomes centrifugation in a gradient of sucrose concentration permits to separate them according to their density and their origin (SER, apparatus of Golgi and plasma membrane.

2. Results of the analysis

2.1. Membrane

It contains about 70% of proteins (glycosyltransferase, P450 cytochrome, glucose 6-phosphatase); 30% of lipids, the phospholipids constitute a bilayer with an important percentage of unsaturated fatty acids (high fluidity). The cholesterol is present in small amounts. Carbohydrates in small percentage are attached to proteins and lipids. They are toward the luminal side. The membrane molecular architecture is an asymmetric fluid mosaic.

2.2. Content cavity

It is different from a cell to another one: as examples, the cavity of the GER from the exocrine pancreas cell contains enzymatic proteins, therefore the luteal cell SER cavity contains steroid hormones and the cell cavity of the sarcoplasmic reticulum contains Ca^{++}.

IV. Endoplasmic reticulum functions

1. GER functions

Highlighted between1960-1964 by Palade experiment (**figure 1**) which demonstrated that a cell is able to synthesize and transport proteins into its cytoplasm by vesicles. The experimenter uses a pulse-hunt marking technique and exploits results in autoradiography. He provides to the pancreatic cells a precursor of proteins (leucine radioactive), labeled with tritium during a short time "pulse" then he provides the same precursor, non-radioactive, more concentrate "hunt". The first proteins are labeled the following are not. We can follow fate and the path of labeled proteins which be identified by autoradiography.

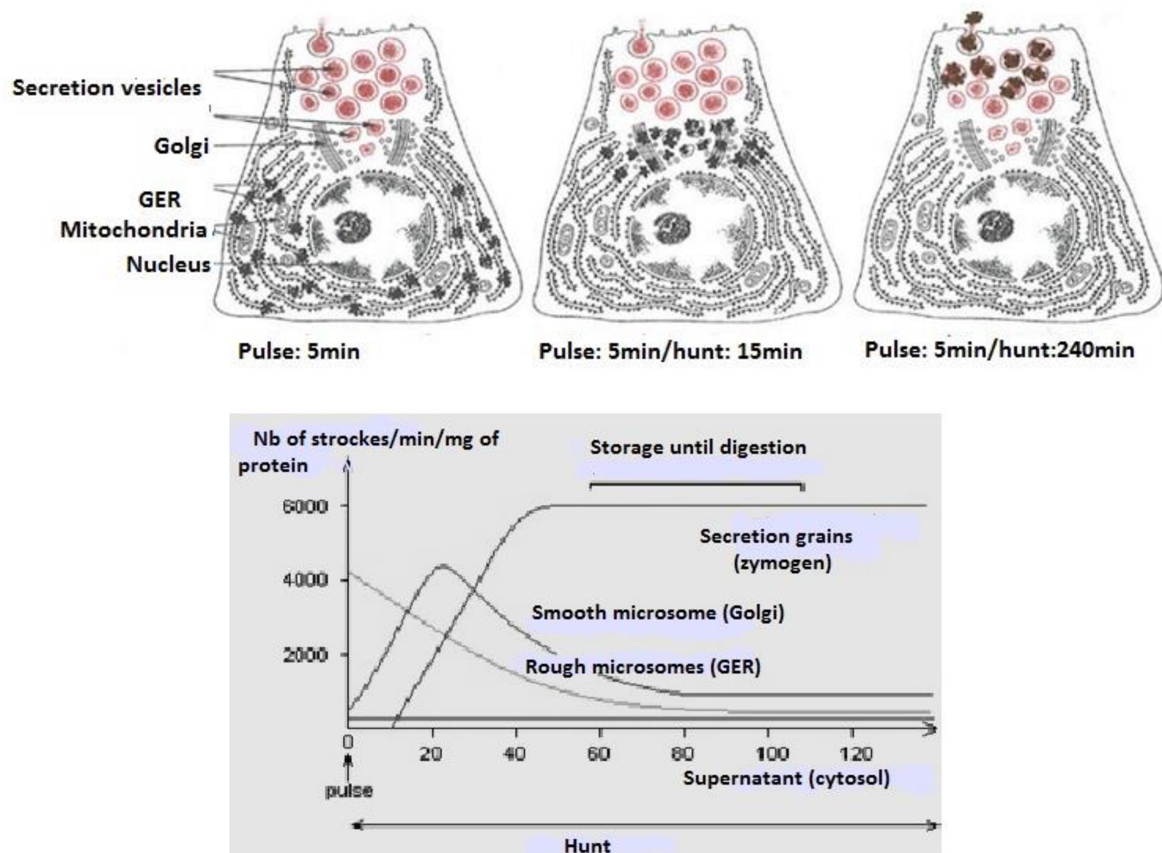


Figure 1: Experiment of Palade: highlighting the fate of secreted protein.

Note: This experiment highlighting the path followed by the proteins exported to outside of the cell (here pancreatic enzyme): GER surface, GER lumen, lumen of the Golgi saccule, Golgi vesicles and exocytosis (outside of the cell).

1.1. Synthesis and translocation of proteins

The synthesis of proteins always begins in the cytosol with the initiation and beginning of elongation. Two types of proteins can be synthesized at GER the level, the soluble proteins or luminal and the hydrophobic proteins that will be inserted in the membrane.

1.1.1. Case of luminal proteins: they occur in several steps (figures 2 and 3A).

a. Step1: the signal sequence is constituted by the first hydrophobic amino acids synthesized in the cytosol located at the N-Terminal extremity of the protein. It permits adressage of the synthesized protein. While it emerges from the ribosome, this sequence is recognized by ribonucleoproteic complex present in the cytosol called Signal Recognition Particle "SRP" which fixed on the sequence signal and provoke a temporary stop of the translation. This stop favored the linked of the SRF on his receptor located on cytosolic face of GER membrane.

b. Step2: this receptor captures the ribosome-mARN-SRP complex, encouraging its interaction with the translocon (translocation protein complex). The large sub unit of the ribosome is fixed on the GER membrane at its receptor. The translocon then opens up by a hydrophilic channel permitting the translocation of the protein being synthesized towards the light. Thus, the translation can resume. The SRP then is separates from its receptor and the sequence signal.

c. Step 3: The protein continues its elongation and enters into the translocon channel.

d. Step 4: when the protein releases in the light (cavity) of the GER, a peptidase of the signal located on the luminal face of GER membrane cuts the signal sequence.

e. Step 5: as the protein appears in light, it is taken in charge by chaperon proteins, which play a role in translocation and post-translational modification. The protein released in the lumen of GER is a luminal protein.

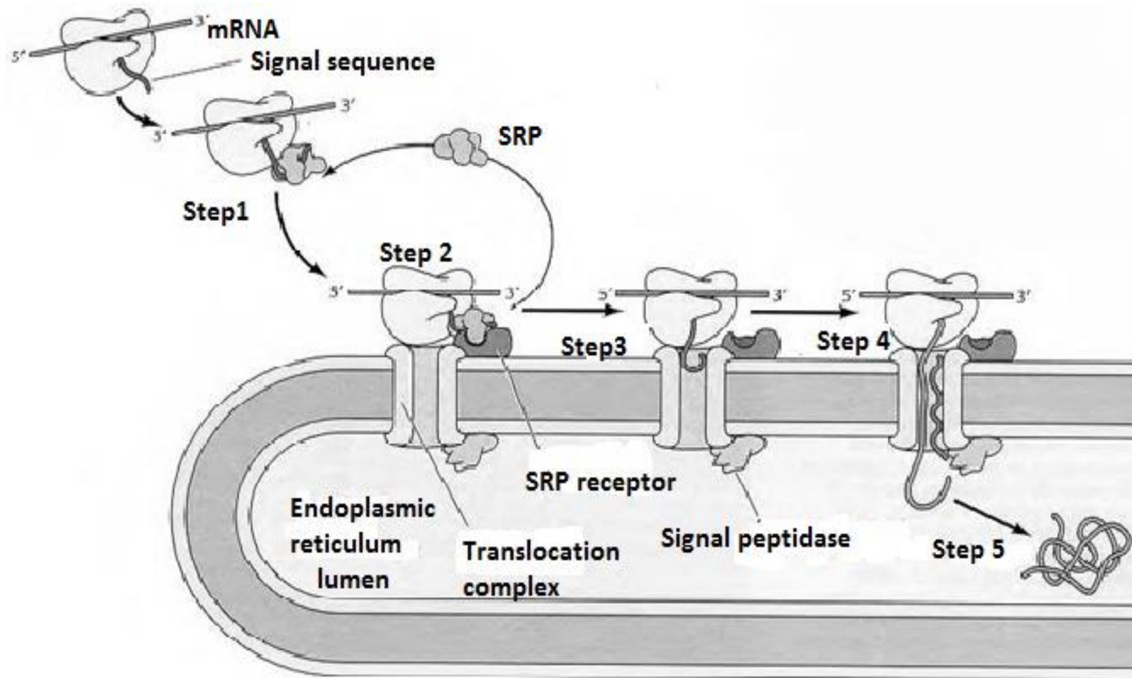


Figure 2: Proteosynthesis and translocation of a luminal protein.

1.1.2. Case of membrane proteins: proteins intended to membranes also undergo an adressage by the signal sequence; but the translocation of these proteins is blocked by a very hydrophobic sequence that destabilizes the translocon, thus, the protein remains inserted in the GER membrane: it is a membrane protein (**figures 3B** and **3C**).

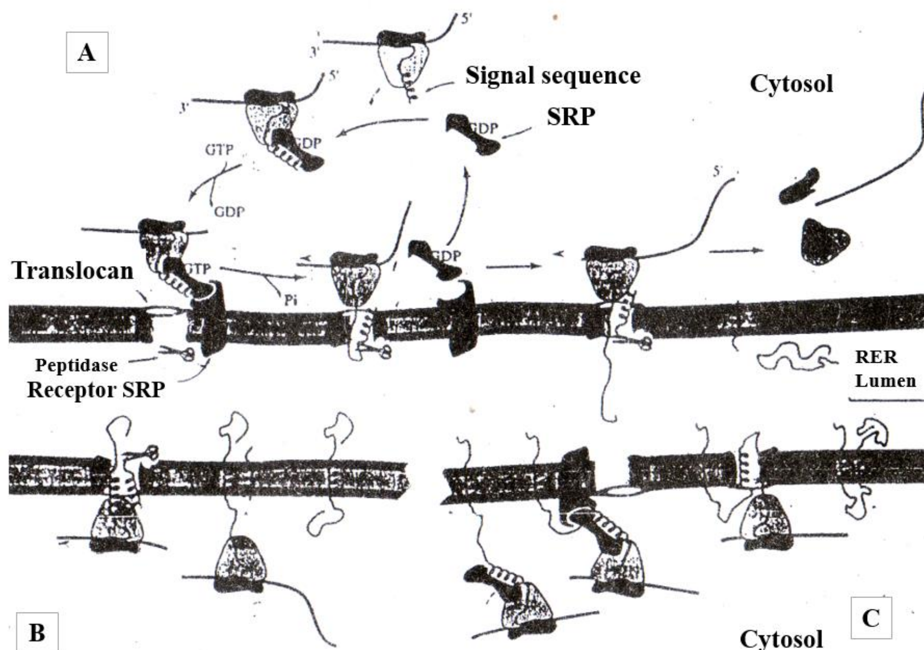


Figure 3: Translation at the level of the GER: (**A**) translocation of a luminal protein during its synthesis, (**B**) insertion of membrane protein and (**C**) insertion of a second transmembrane domain.

1.2. Post- translational modifications of proteins

1.2.1. N-Glycosylation: during their synthesis, proteins can undergo a glycosylation, a "oligosaccharide tree" rich in mannose (ose with 6 carbons) is transferred in block to the protein during translation by an oligosaccharidyl transferase enzyme on the amine group of the asparagine (amino acid with two amine function).

1.2.2. Changes in sugars: oligosaccharide tree is immediately modified by GER enzymes.

1.2.3. Establishment of disulfides bridges: bonds are formed between sulfur amino acids.

1.2.4. Association between proteins and membrane lipids: case of integrated proteins anchored into the outer or the inner lipidic layer (figure 1 chapter 2 Plasma membrane).

1.2.5. Exit from the GER by a vesicular transport: there are vesicles which come from the budding of GER and merge with proximal saccule (cis face) of a dictyosome (Golgi apparatus). These vesicles have a membrane derived from the GER and can transport luminal proteins and integrated membrane protein (**figure 4**).

The intermediate compartment between GER and device of Golgi apparatus is called "Endoplasmic Reticulum-Golgi Intermediate Compartment" or ERGIC. According to certain sequences, transmembrane or luminal proteins called "residents of the GER" (specific to GER) cannot leave the GER. If they have been transported by error in ERGIC, their return to the GER is assured.

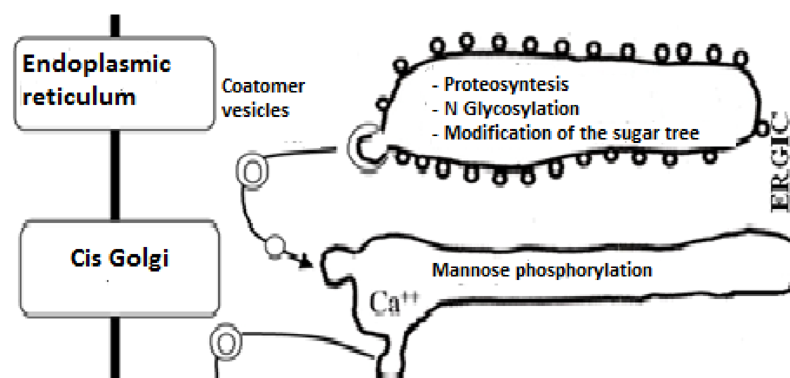


Figure 4: Vesicular transport of GER proteins to the cis Golgi.

2. SER functions smooth endoplasmic reticulum: SER

2.1. Synthesis of phospholipids

The SER is the main supplier of phospholipids for the cell membranes. They are synthesized using transmembrane enzymes whose active site is oriented toward the cytosol, then distributed in bilayer by flipases. The latter integrated into the membrane of the SER; make them switch from the cytosolic hemi-membrane to luminal hemi-membrane.

2.2. Synthesis of hormones steroids

The SER synthesizes hormones steroids with the help of the P450 cytochrome whose active site is located on cytosolic face of its membrane. It uses the Pregnenolone as precursor produced in the mitochondria with crests tubular by the P450 cytochrome.

2.3. Detoxification

SER P450 cytochrome is involved in hydroxylation of toxic products. These products become hydrosolubles; they will be easily transported and eliminated by the organism.

2.4. Accumulation and release of Ca^{++}

The Ca^{++} crosses the SER membrane using an ATPase (pump-Ca^{++}). It is stored in the cavity by a binding protein (Calsequestrine) then released into the cytosol during the muscle contraction, by the calcic channels.

B. GOLGI APPARATUS

I. DEFINITION

This is an organelle situated near the nucleus, close to the pericentrosomal material. It consists of stacks of flattened saccules associated with numerous vesicles.

II. STRUCTURE AND ULTRASTRUCTURE

1. Structure

Under a light microscope, after impregnation with silver nitrate, the Golgi apparatus appears as small scales near the nucleus.

2. Ultrastructure

2.1. Organisation

In the TEM, a stack of saccules and small vesicles. Each stack of 4 to 8 saccules is a dictyosome (on average 20 dictyosomes per cell). Each dictyosome comprises cis saccules (close to the ER); the input side supplied by the ER, median saccules and trans saccules (output side) in continuity with a network of canaliculi called the Trans-Golgi Network (TGN).

2.2 Golgi membranes

In the TEM, the membrane is tripartite; in the SEM, integrated globular particles are present. The thickness of the membranes is variable and intermediate between those of the GER (or RER) and the plasma membrane (cis saccule: 6nm and trans saccule: 7,5nm).

III. CHEMICAL COMPOSITION

1. Isolation technique

- Rough grinding of an organ fragment + DUC give microsomes in the third pellet.
- Gentle grinding + DUC gives stacked saccules in the third pellet.

2. Analysis results

2.1. Membranes

2.1.1. Lipids: lipid content, fatty acid chain length, degree of saturation and therefore fluidity are intermediate between ER and plasma membranes.

2.1.2. Proteins: levels are intermediate between ER and plasma membrane proteins. A distinction is made between proteins common to the membranes of the endoplasmic reticulum and the Golgi apparatus (Glycosyl-transferase, Cytochrome b5-reductase) and proteins specific to the Golgi membranes (Sulpho-transferase, Phosphatase).

2.1.3. Carbohydrates: the quantity of sugars is negligible; they are located on the luminal surface.
Molecular architecture of the membranes: **these are asymmetric fluid mosaics.**

2.2 Cavity contents: Same products as in the ER cavities, but in different concentrations.

IV. FUNCTIONS (figure 5)

1. Transport
ERGIC delivers proteins, glycoproteins, lipids and glycolipids to the Golgi-cis, which are then transported to the Golgi-medium and then to the Golgi-trans to reach the trans-Golgi network (TGN). Vesicles from the different compartments carry out this transport.

2. Definitive protein glycosylation

2.1. Sugar modifications: during protein transport from the cis saccules to the medial and then trans saccules, the glycoproteins undergo modifications to their N-linked oligosaccharide trees (N-Glycosylated).

2.2 O-Glycosylation: O-linked oligosaccharides (O-glycosylated) are built up in the medial and trans saccules. These oligosaccharides are built up ose by ose on the protein at the free hydroxyl group (OH) of serine or threonine (amino acids with two OH groups), thanks to glycosyl transferases (specific Golgi enzymes).

3. Mannose phosphorylation
The mannoses of the sugar tree are phosphorylated to mannose 6-P in the cis cisternae. Only glycoproteins (acid hydrolases) destined for lysosomes undergo this change.

4. Sulphation
Addition of an SO_4^{2-} group by sulpho-transferases in the cisternae of the Golgi-trans.

5. Modification of lipids
Certain lipids assembled at the SER are modified to form glycolipids.

6. Calcium storage and release

7. Formation of macroautophagy vacuoles
The membrane cisterna encircling the cellular organelle to be destroyed during macroautophagy comes from a cisterna originating from the TGN (see D: Lysosomes).

8. Sorting and maturation
These two phenomena take place at the same time. Sorting takes place in the TGN. The vesicles being formed have a cytoplasmic coat formed by proteins and are called coated vesicles. This coat recognizes the signals carried by the substance to be transported and enables it to be concentrated in a restricted area (sorting). It also allows the membrane to bud into vesicles (**figures 6** and **7**). The vesicles can follow two ways:

8.1. Secretory pathways

8.1.1. Constitutive secretion pathway: this allows the permanent release of proteins by exocytosis into the extracellular environment (e.g. secretion of proteins making up the extracellular matrix). A coat of coatomers covers these vesicles.

8.1.2. Regulated secretion pathway: the secretory material, whether hormonal (e.g. insulin) or exocrine (e.g. pancreatic enzymes), undergoes maturation during transport in vesicles covered by a coat of clathrin and adaptation proteins. The vesicles increase in size to form mature secretion grains. This type of secretion takes place after a signal, as required.

8.2. Endosomal and lysosomal pathways

This involves proteins with mannose-6P (M6P), which has been phosphorylated at the cis-saccule. These luminal proteins recognize a membrane receptor and the whole is sent to the lysosomes via an intermediate endosomal compartment characterized by a relatively acid pH (5 to 6). The vesicles, destabilized by the acidic pH, lose their mannose-6P receptor, which is recycled to the TGN. The result is a vesicle with no M6P receptor, containing acid hydrolases and with an acidic pH lumen. Whichever route the vesicles take, they lose their coat before reaching their destination.

V. Conclusion: Biogenesis of the plasma membrane (handout p.107).

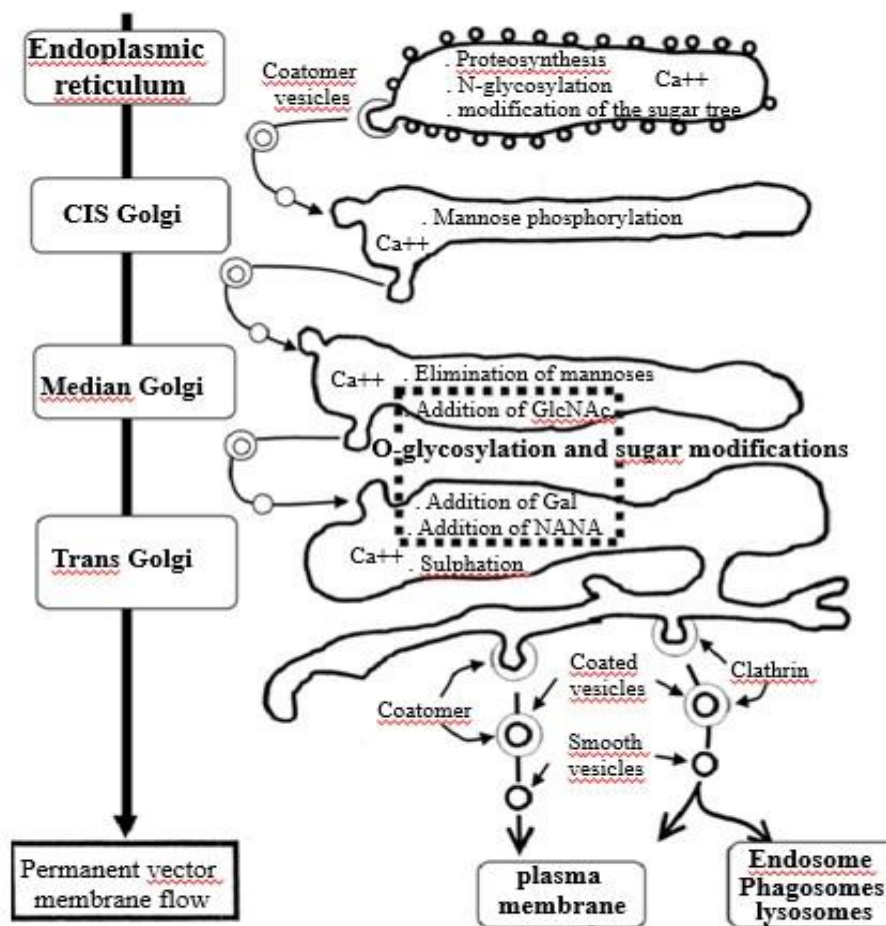


Figure 5: Functional specializations of golgian cisternae.

Note: **Mannose**: ose, **GlcNAc**: N-acetyl-glucosamine, **Gal**: galactose, **NANA**: N-acetyl neuraminic acid.

C. ENDOSOMAL COMPARTMENT

The endosomal compartment, studied in part in the exchanges with the deformation (figure 4, Chapter 2: Plasma membrane), is part of the endomembrane system. The first endosomes formed just after endocytosis, close to the plasma membrane, are called early endosomes (**figure 9**).

Their function is to sort ingested molecules and, in the case of receptor-mediated endocytosis, they also play a role in recycling receptors to the plasma membrane. The endosomes containing the sorted molecule, brought closer to the nucleus, are called late endosomes (**figure 9**). They evolve into lysosomes.

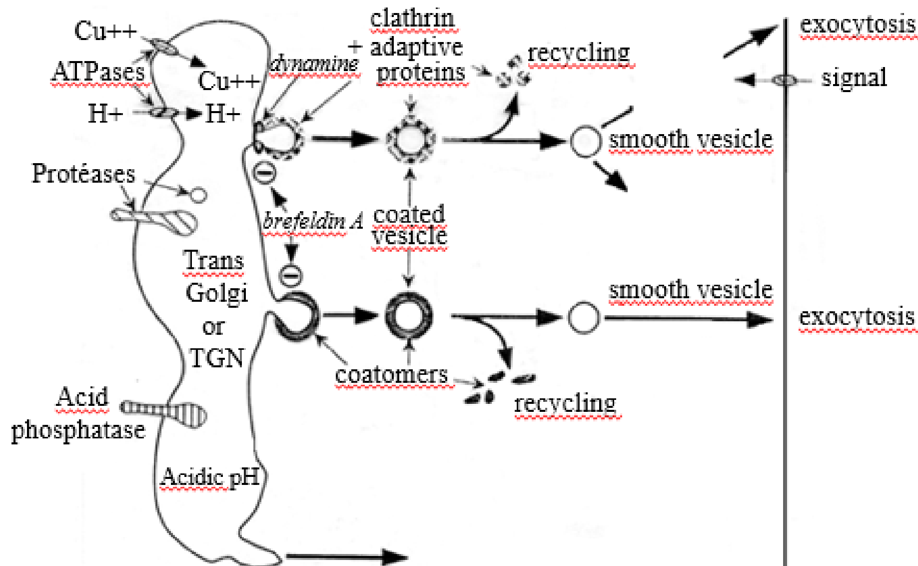


Figure 6: Coated vesicles involved in permanent membrane vectorial flow.

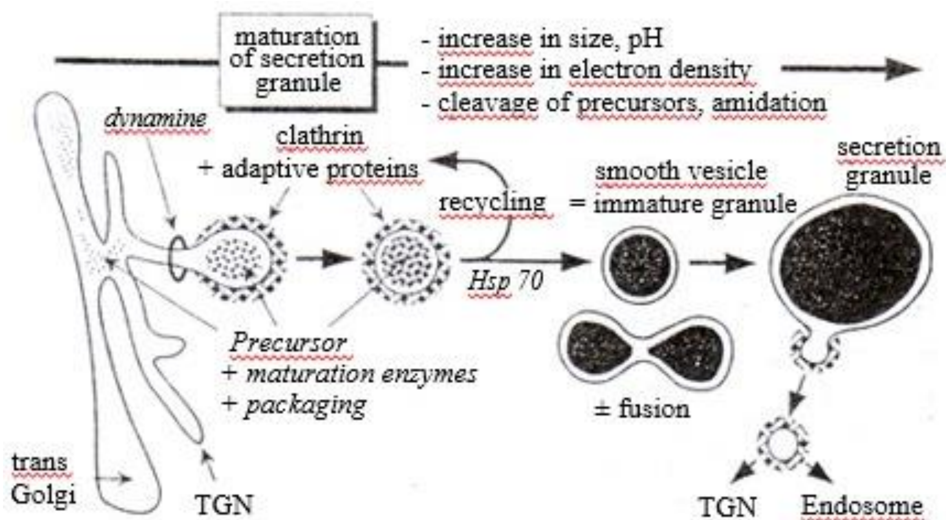


Figure 7: Maturation of regulated secretion granules.

D. LYSOSOMAL COMPARTMENT

I. Definition

Discovered for the first time by De Duve through to their function, they are compartments of variable shape, they contain hydrolytic enzymes (acid hydrolases) allowing the digestion of ingested or intracellular particles or molecules of small soluble sizes and cellular organelles old or unnecessary.

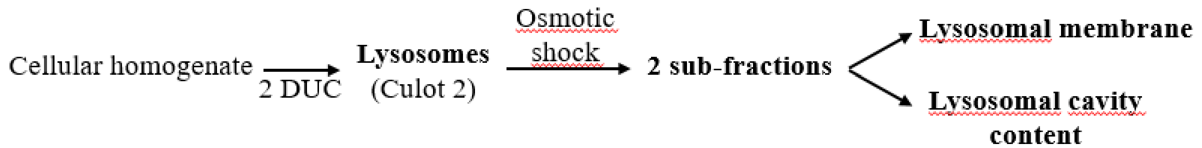
II.ULTRASTRUCTURE

They appear as vesicles of varying shapes and sizes from 0,05 to 0,5 µm in diameter depending on the material ingested. They can be identified by cytochemical techniques using an enzyme

present in their membrane, acid phosphatase, which forms a precipitate of electron-dense lead phosphate (see TP).

III. CHEMICAL COMPOSITION

1. Isolation



2. Analysis results

2.1. Membrane

It is a special membrane because most of these proteins are unusually highly glycosylated on the luminal side to protect it from acid hydrolases contained in the cavity. They are of different types (**figure 8**):

- Enzymatic (e.g. acid phosphatase) and non-enzymatic proteins, permeases, transporters of small cytosolic materials to be degraded and end products of the digestion of macromolecules (e.g. amino acids, nucleotides, simple sugars, etc.).
- Proton (H^+) ATPases, which pump H^+ towards the cavity and maintain a very acidic pH in the lysosome.

2.2. Cavity contents

It contains approximately 40 types of acid hydrolases, digestive hydrolytic enzymes, which degrade proteins, nucleic acids, oligosaccharides and phospholipids. Their activity is optimal at pH 5.

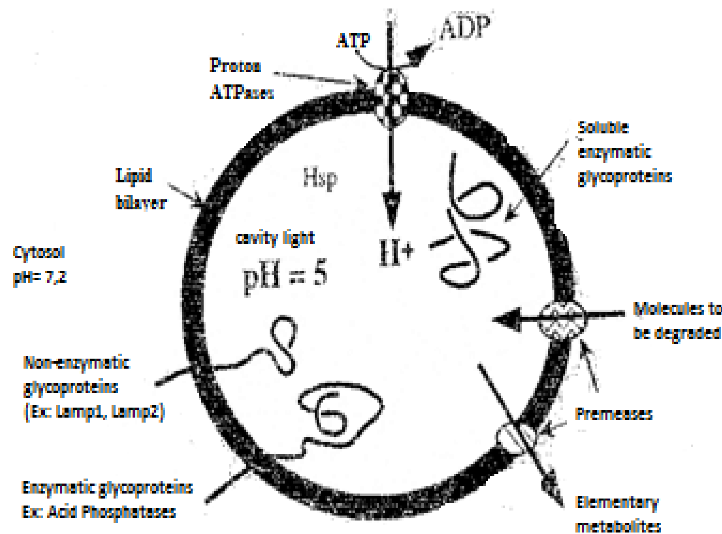


Figure 8: the constituents of a lysosome.

IV. ORIGIN OF MATERIALS TO BE DEGRADED AND LYSOSOME BIOGENESIS

The materials to be degraded follow different paths to the lysosome stage depending on their origin. They can have two origins (**figure 9** and handout p.119):

1. Extracellular: heterophagic pathway

In this case, the materials to be degraded are ingested by endocytosis (phagocytosis, pinocytosis or endocytosis by receptors); they are then contained in phagosomes for the first case or in early

endosomes at neutral pH 7,4 for the other two. However, their pH gradually decreases because of the H^+ ATPases provided by the fusion of Golgi vesicles of secretions, coming from the TGN and containing in their cavity some acid hydrolases. Early endosomes then transform into late endosomes at pH 6,5. Numerous Golgi vesicles flowing from the TGN continue to fuse with late endosomes, thereby increasing the number of H^+ ATPases and acid hydrolases, allowing the conversion of late endosomes into lysosomes at pH 5. Golgi vesicles with a membrane rich in H^+ ATPases and acid hydrolases in their cavity fuse with the phagosome, which is also converted, but directly into a lysosome at pH 5.

2. Intracellular: autophagic pathways

2.1. Microautophagy: direct entry (diffusion) into the lysosome of small soluble molecules from the cytosol (e.g. peptides) via permeases.

2.2. Macroautophagy: formation of an autophagic vacuole (macro-autophagosome) formed by the sequestration of spent organelle(s) and a small amount of cytosol by a membrane cistern of the TGN. This membrane cistern contains in its cavity some acid hydrolases, which become active after changing the intra-cavitary pH, apparently thanks to the presence of H^+ ATPases in the membrane of this cistern. Digestion therefore begins in the autophagosome and ends in the lysosome into which it is converted.

2.3. Crinophagy: it is a form of autophagy that concerns the elimination of secretion granules (regulated secretion) which are no longer used (e.g. prolactin).

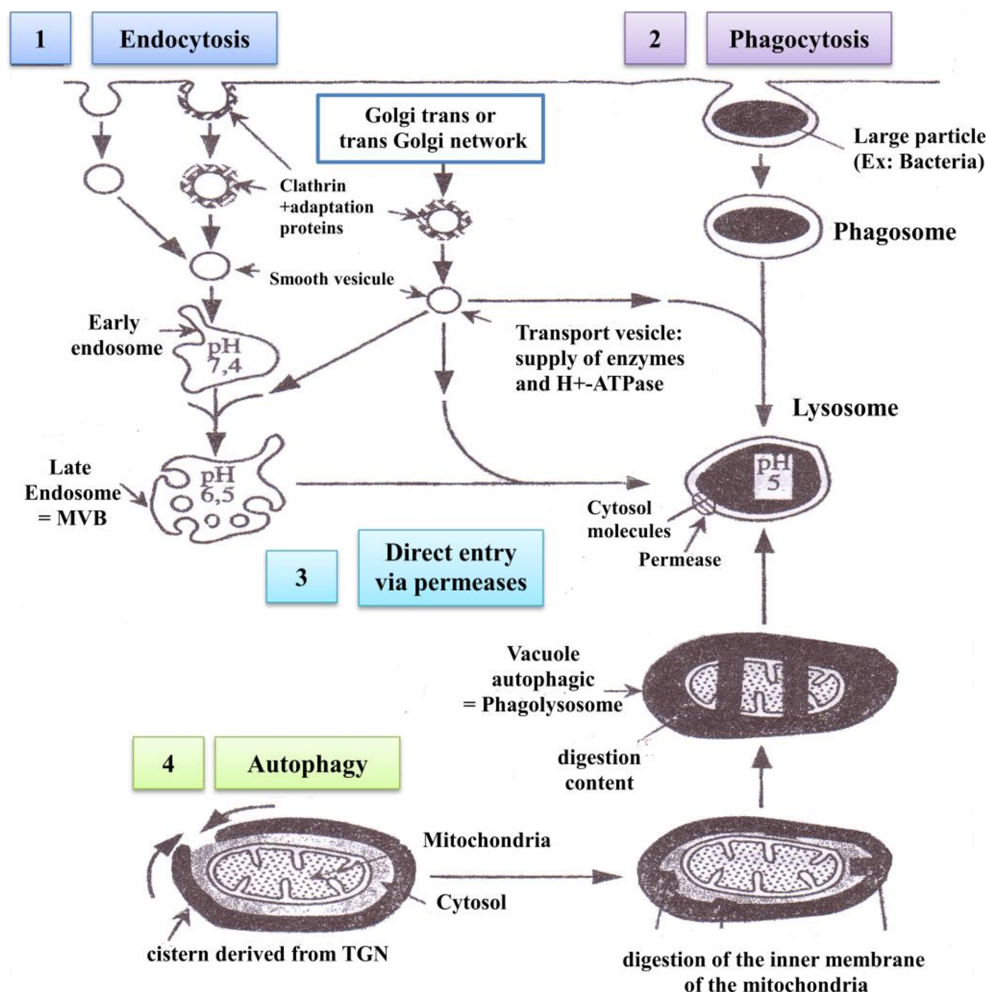


Figure 9: The four entry pathways into the lysosome of materials to be degraded.

V. BECOME LYSOSOMES

Aged lysosomes, lacking functional acid hydrolases, constitute residual bodies. Most often these residual bodies release their contents outside the cell by exocytosis: this is cellular defecation (**figure 10**); but can also persist in the cell all their life (handout p. 119).

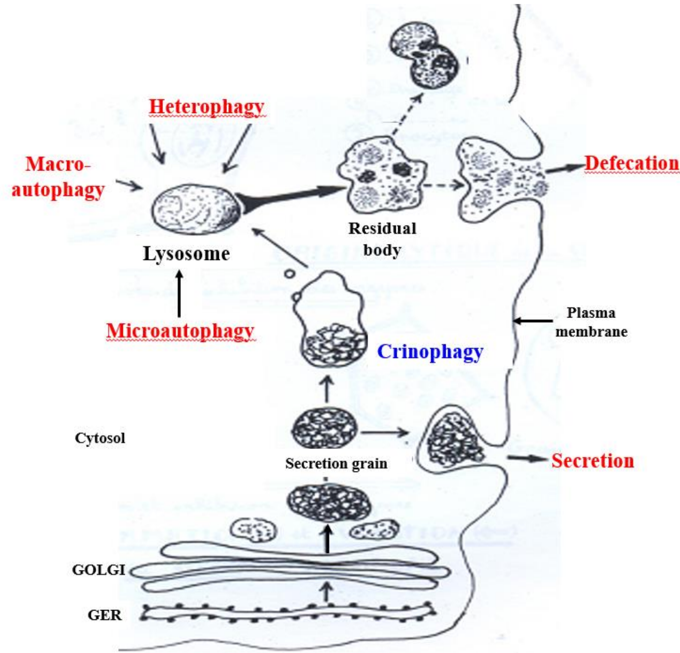


Figure 10: Become lysosomes.

VI. FUNCTION OF LYSOSOMES

All families of biological molecules are degraded into elementary metabolites that return to the cytosol thanks to transporter proteins of the lysosomal membrane. Thus lysosomes play an essential role in cellular digestion, the destruction of foreign bodies and therefore in cellular nutrition (**figure 11**).

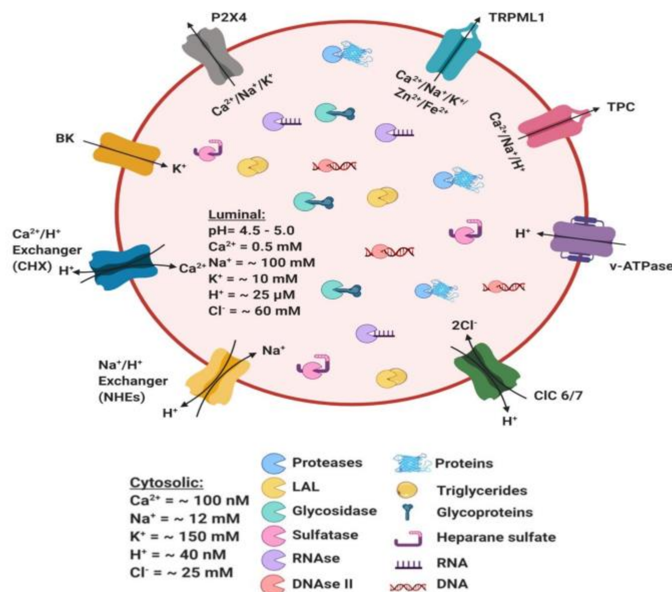


Figure 11: The different hydrolytic functions of lysosomes.

E. VACUOLE OR PHYTOLYSOSOME

I. DEFINITION

Most plant cells contain one or more fluid-filled vesicles called vacuoles. All the vacuoles in a cell make up the vacuolar apparatus or vacuome, which depending on the cell type; can occupy 30 to 90% of the cell volume.

II. STRUCTURE AND ULTRASTRUCTURE

1. Under a light microscope

A continuous membrane called the tonoplast bound the vacuole; this delimits an internal solution called the vacuolar juice (colored pink or other colors depending on pH or colorless).

2. In the transmission electron microscope (TEM)

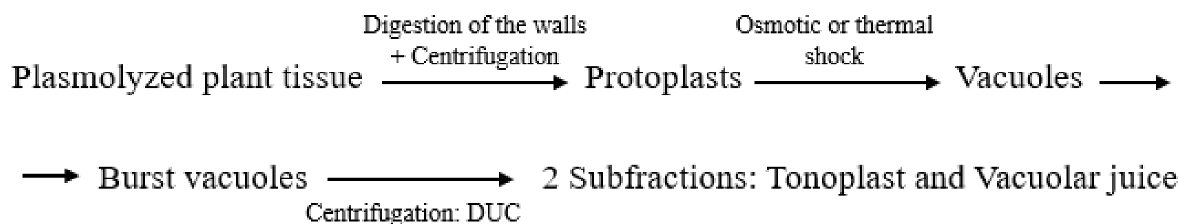
The tonoplast is a membrane with a trilamellar structure, asymmetrical; the dense cytosolic layer is thinner than the dense luminal layer (presence of a fibrous coating).

3. In the scanning electron microscope (SEM)

Presence of globular particles after cryostripping.

III. CHEMICAL COMPOSITION

1. Isolation



2. Results of the analysis

2.1. Tonoplast

Three types of molecules (lipids, proteins and carbohydrates) (**figure 12**):

- Lipids: phospholipids and sterols (stigmasterol and sitosterols).

- Proteins: great diversity (structural, enzymatic and transporters).

- Carbohydrates: fibrous coating (polysaccharide chains).

2.2. Vacuolar juice

Contains a wide variety of compounds, which form aqueous solutions with water, the concentration of which varies according to the plant species, function and physiological state.

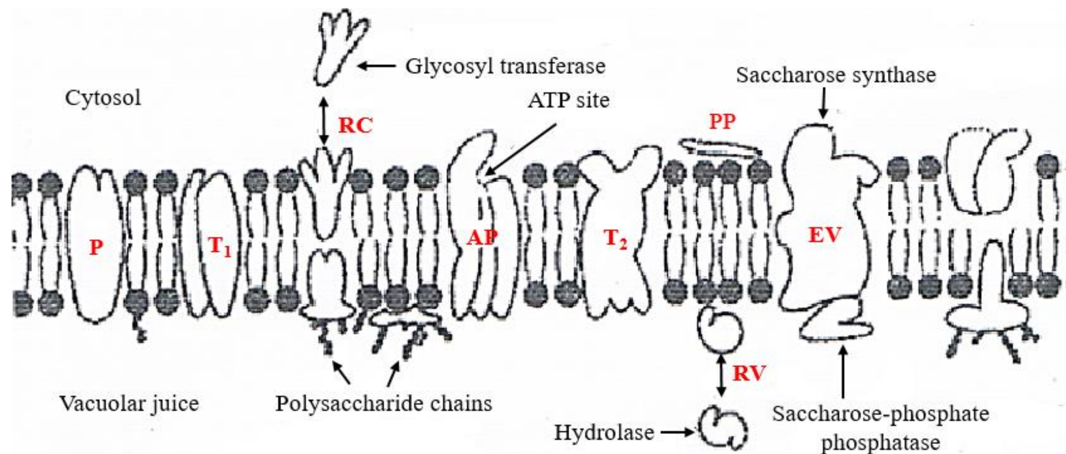


Figure 12: Constituents and molecular architecture of the tonoplast.

AP: ATPase; **EV**: Vector enzyme group; **P**: Protein pore; T_1 and T_2: Specific carriers; **PP**: Peripheral proteins; **RC**: Release and cytosolic absorption; **RV**: Release and vacuolar absorption.

IV. FUNCTIONS

1. Transport: functions common to all biological membranes

The tonoplast is involved in exchanges with the cytosol (**figure 13**) through:

1.1. Passive transport

Simple diffusion through the lipid bilayer and facilitated diffusion of water (plasmolysis and turgidity) using aquaporins and ions through ion channels.

1.2 Active transport

Proton pump (ATPase-H^+, symport and antiport) and specific transport of sucrose which couples the passage of this compound to its synthesis from its two precursors using a vector transmembrane enzyme complex (Juice-vacuolar cytosol).

1.3. Transport with deformation

Example, intra-vacuolar pinocytosis.

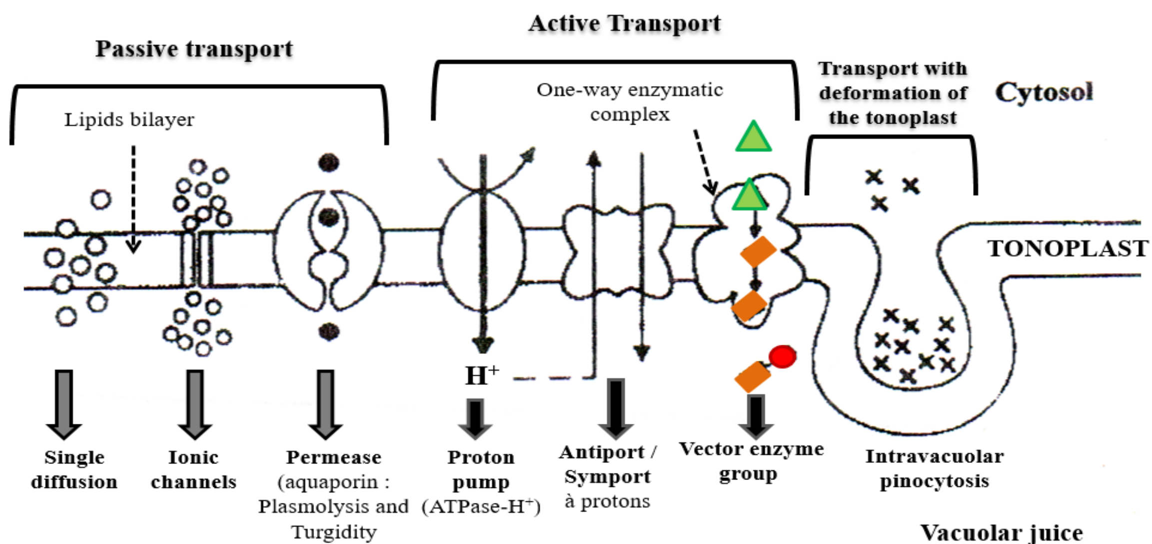


Figure 13: Transport mechanisms across the tonoplast.

2. Characteristic functions of the vacuole

2.1. Storage

The vacuole stores a wide variety of substances, both mineral and organic. Some of these substances can be reused by the cell (ions, amino acids, sugars, …), while others, which are toxic to the cell, are trapped. For example, unlike the animal cell, which excretes its toxic waste, the plant cell uses the vacuole to isolate its waste from the cytoplasm.

2.1.1. Diffusion

Reusable substances are transported by diffusion (**figures 13** and **14**).

2.1.2. Trapping

Toxic substances that have passed through the tonoplast undergo various types of modification that prevent them from re-emerging (**figure 14**), for example:

- Ionization of lipophilic molecules (as in the case of alkaloids).
- Crystallization (calcium oxalate macles, because Ca is present in the plant).
- Formation of bonds with other compounds and accumulation: mucilage, polyphosphate, Mg, polyphenols and tannins.

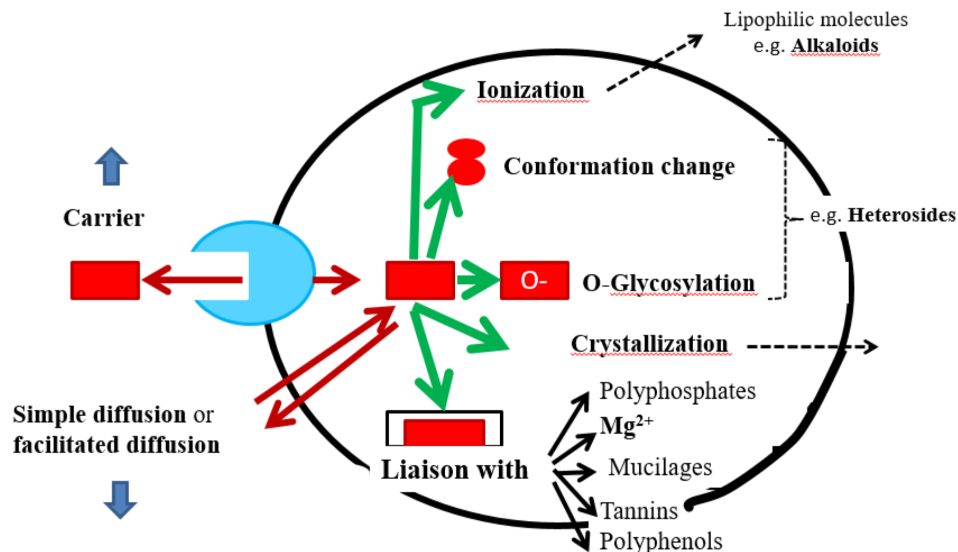


Figure 14: Mechanisms of intra-vacuolar storage and trapping.

2.2. Mechanical support

Thanks to its turgidity, the vacuole provides mechanical support for the soft tissues (upright support of herbaceous plants).

2.3. Cell growth

Cells grow in width and length thanks to the turgidity of the vacuole.

3. Functions common to the lysosome

The vacuole (phytolysosome) is assimilated to the lysosome (Handout p.121).

- Autophagy: the hydrolases of the vacuolar juice, similar to those of the lysosomes, are capable of degrading all substrates, including cytoplasmic contents (e.g., dead cells of the cork, wood,…. see plant histology course).
- Heterophagy: is a means of defense in the event of parasitic attack (bacteria, fungi,….).