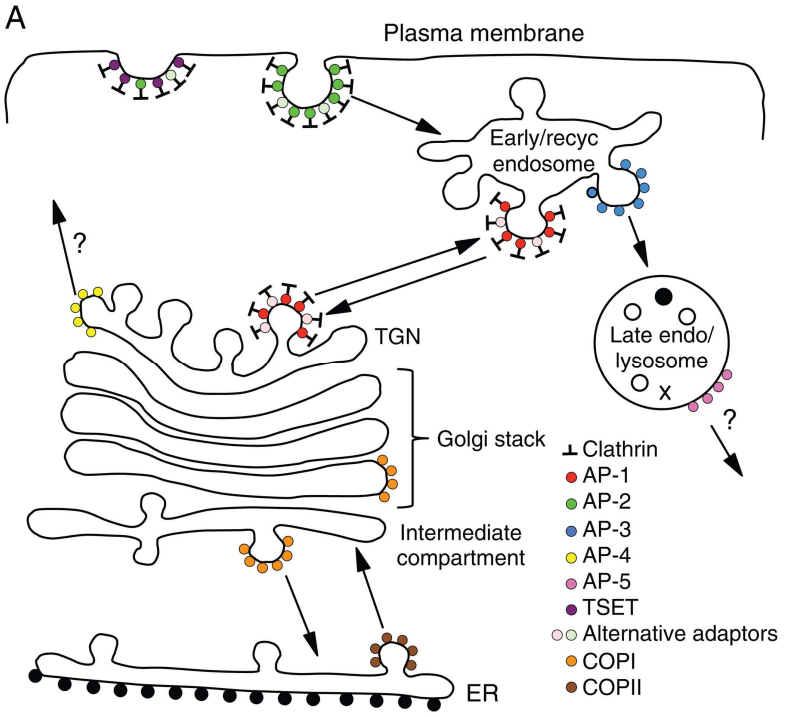
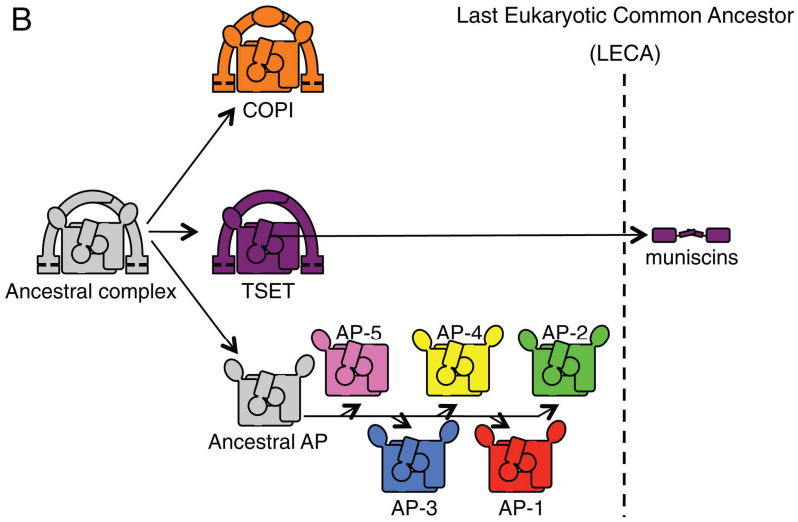
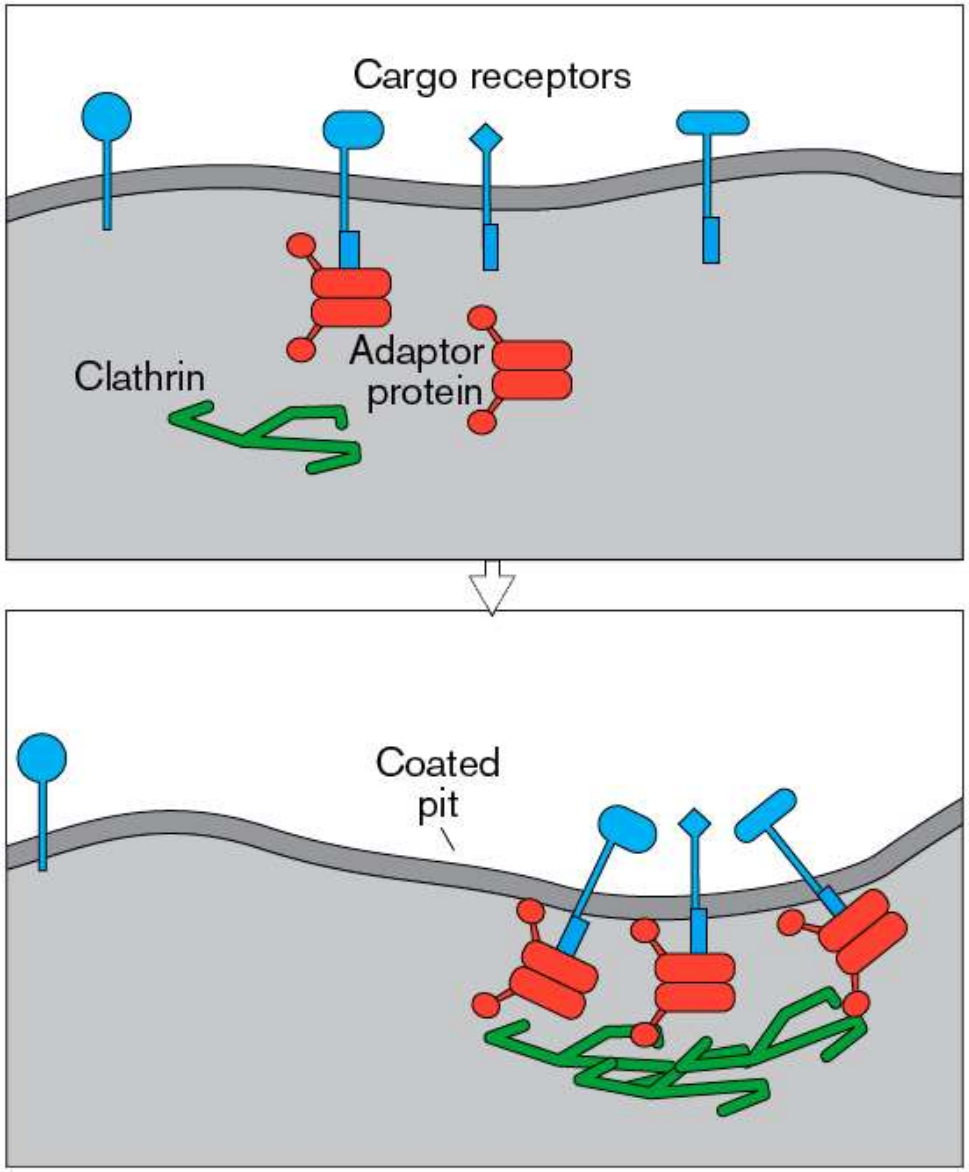
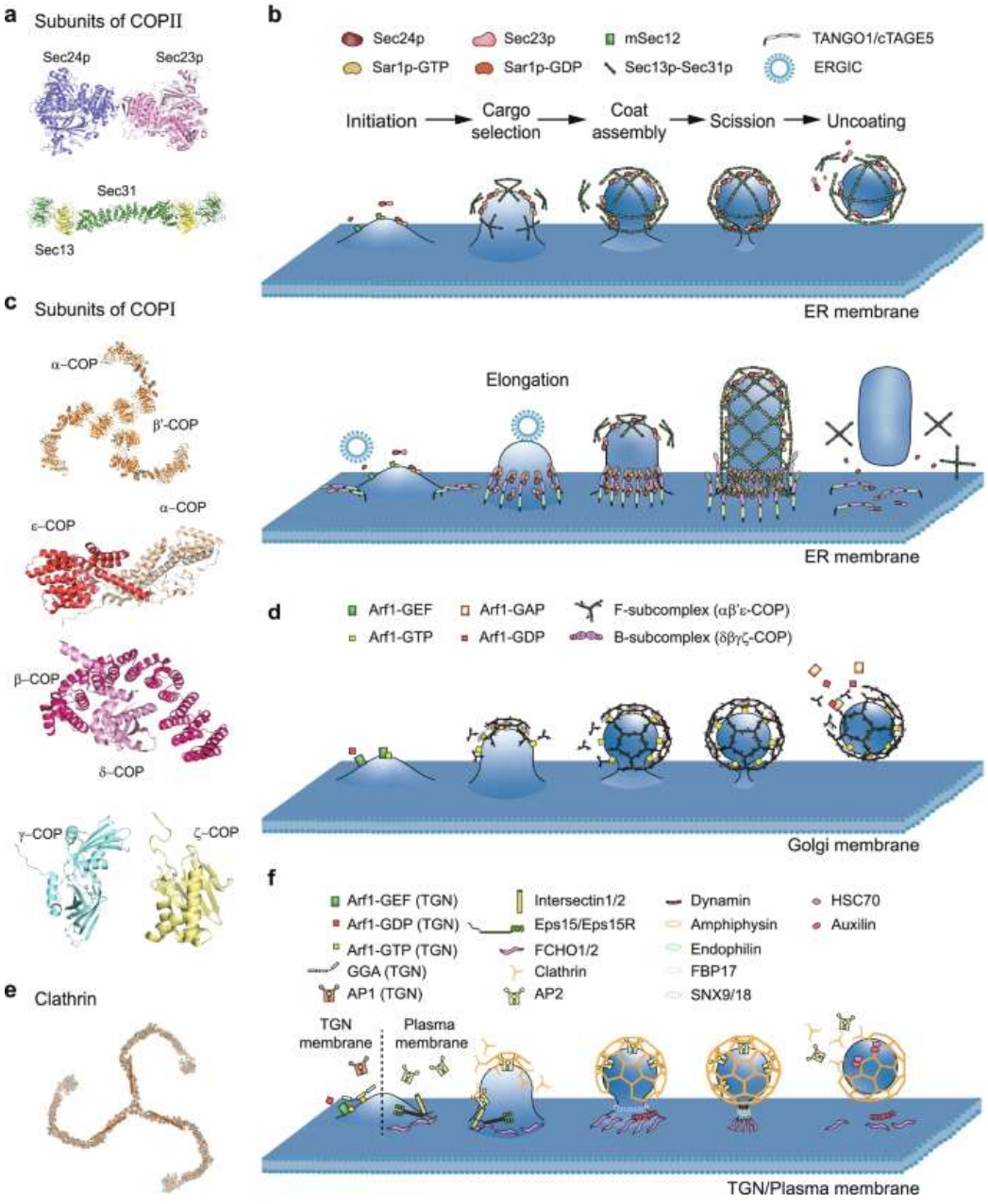

A
Plasma membrane
Early/recyc endosome
?
TGN
Late endo/ lysosome
x
?
Golgi stack
Intermediate compartment
ER
Clathrin
AP-1
AP-2
AP-3
AP-4
AP-5
TSET
Alternative adaptors
COPI
COPII

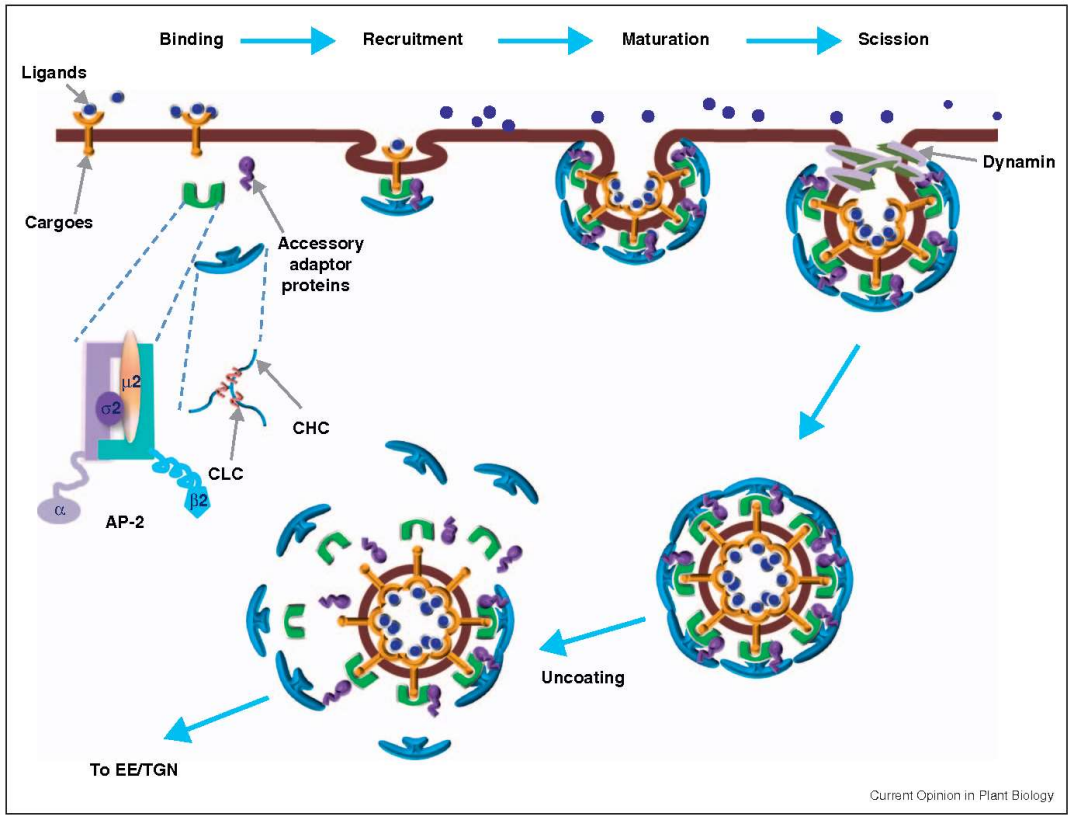

B
Last Eukaryotic Common Ancestor
(LECA)
COPI
Ancestral complex
TSET
muniscins
AP-5
AP-4
AP-2
Ancestral AP
AP-3
AP-1


Cargo receptors
Clathrin
Adaptor protein
Coated pit

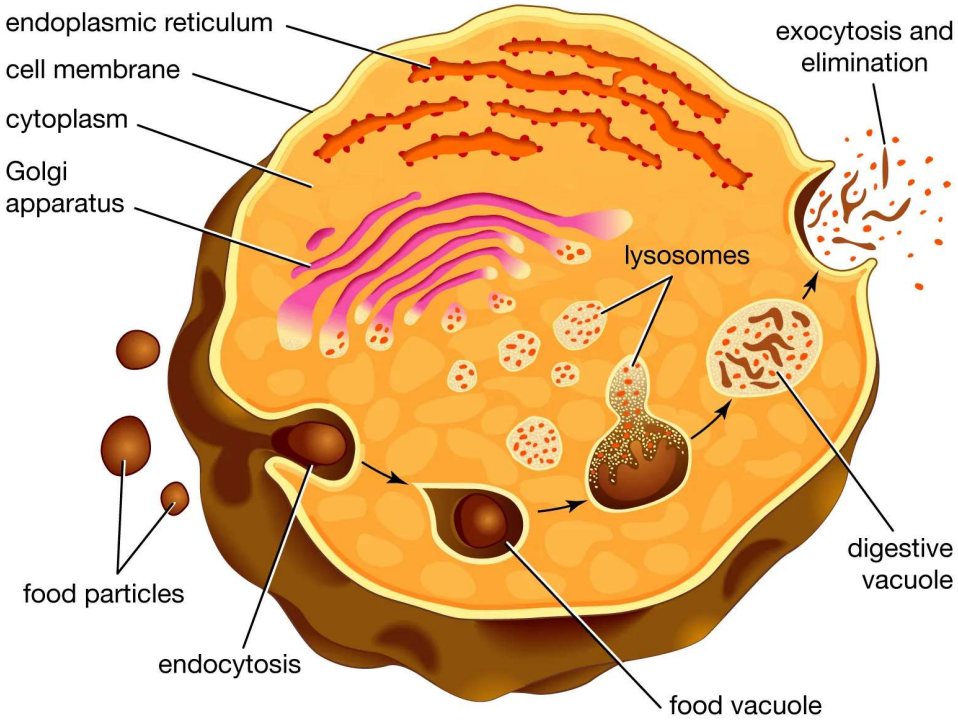
Proteínas de revestimento

Clathrin





Lysosomes

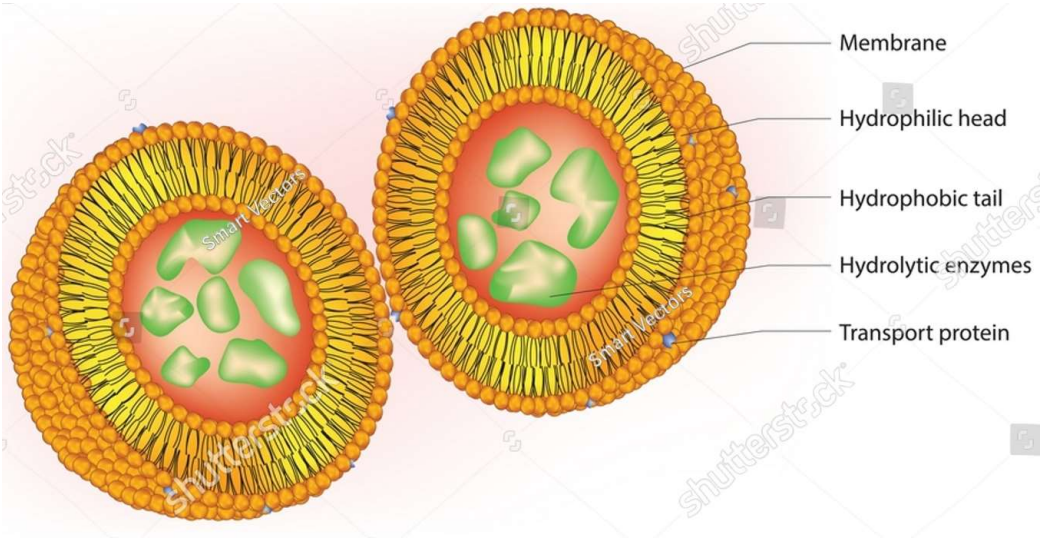


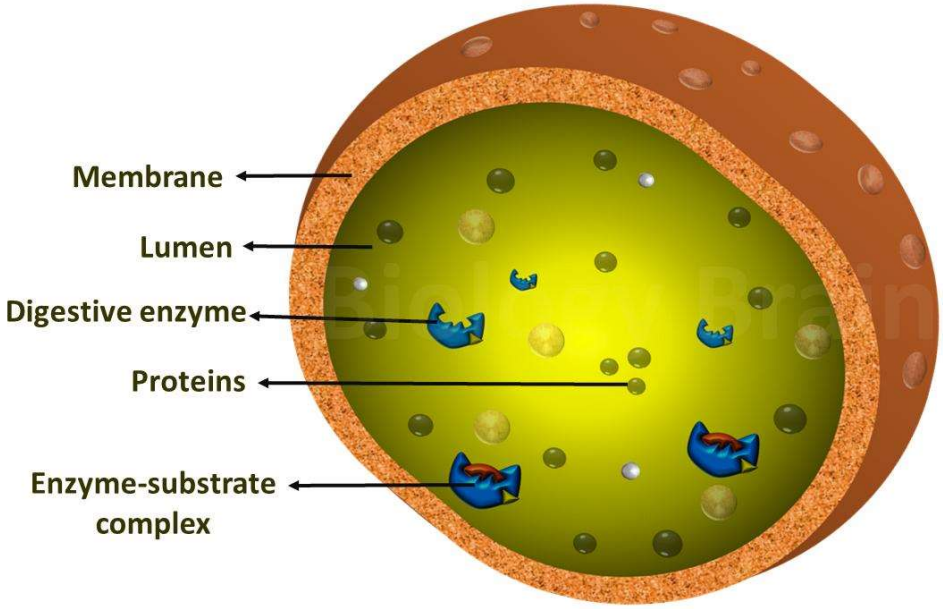
Importance:

- Main **proteolytic** compartments of mammalian cells comprising of a battery of hydrolases.
- Lysosomes dispose and **recycle** extracellular or intracellular macromolecules by fusing with endosomes or autophagosomes.
- The digestion of products are exported and reused as building blocks to maintain cellular homeostasis.
- Lysosome dysfunction causes a wide variety of human disorders such as lysosomal storage diseases (LSDs) and neurodegenerative diseases.

- Implicated in **nutrient sensing**, immune cell **signaling**, **metabolism**, and **membrane repair**.
- Lysosomes **interact** with other intracellular organelles (e.g., mitochondria, ER) for mutual homeostatic regulation.

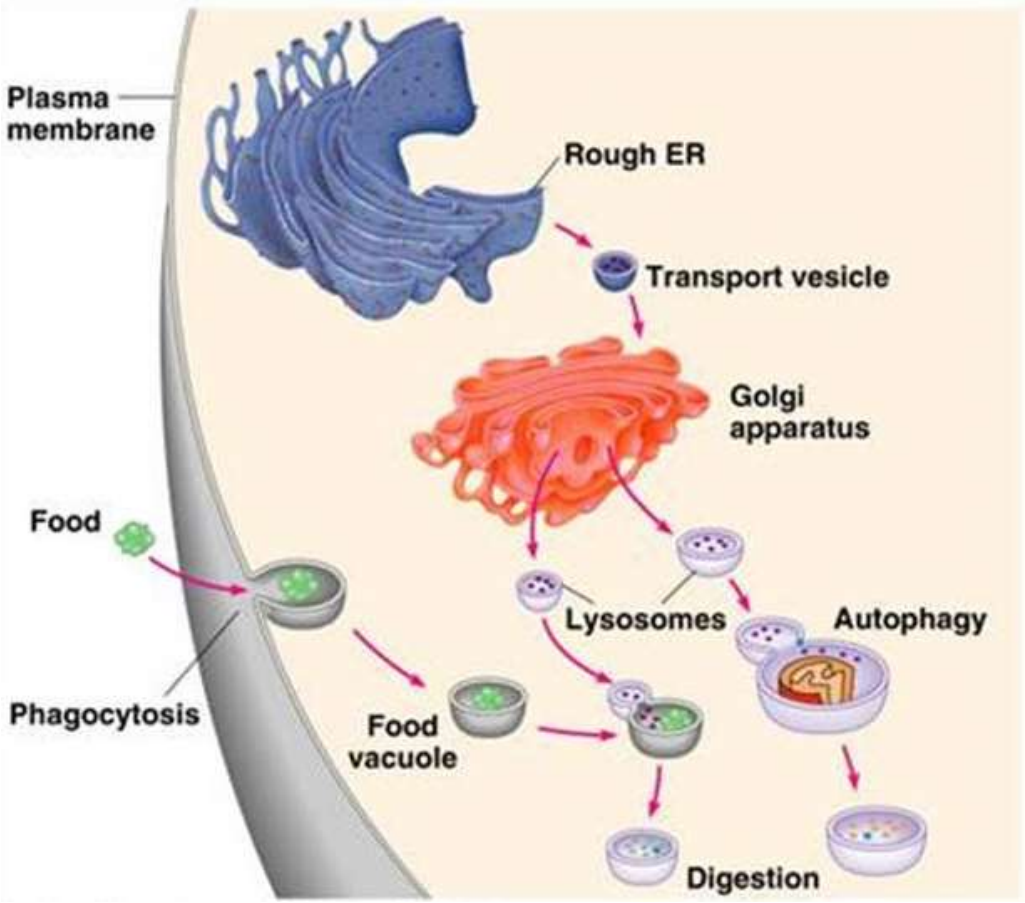
Lysosome structure





- **External phospholipid-bilayer comprises high carbohydrate content due to heavily glycosylated lysosomal membrane proteins.**
- **Protects the membrane from lytic enzymes found in the lysosome.**
- **Lysosomes contain over 60 types of hydrolytic enzymes.**

Origin of Lysosomes



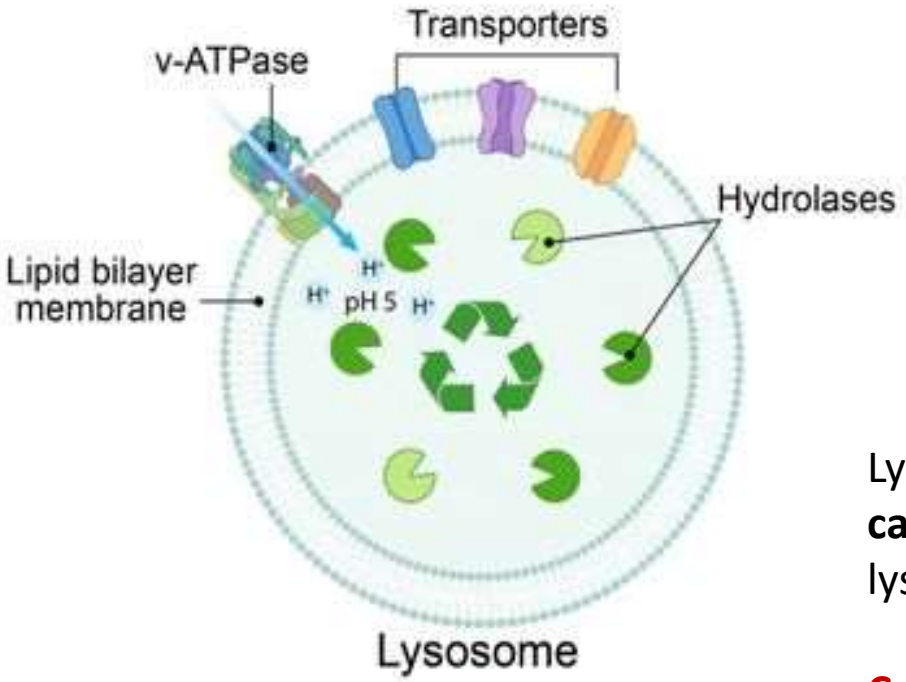
Lysosomes originate by budding off from the membrane of the trans-Golgi network, a region of the Golgi complex responsible for sorting newly synthesized proteins.

Reform from digestive lysosomes - Lysosome recycling.

The lysosomes then fuse with membrane vesicles that derive from one of three pathways:

- **Endocytosis**
- **Autophagocytosis**
- **Phagocytosis**.

Lysosome structure



Hydrolases require an acidic pH of **~5.0**, which is established by an ATP-driven proton pump, the vacuolar H+-ATPase (**V-ATPase**), in cooperation with ion channels.

Lysosomes concentrate metal ions such as **zinc**, **iron**, **copper** and **calcium** within their lumen; iron and copper storage within the lysosome prevents their harmful accumulation in the cytoplasm.

Controlled released of calcium from the lysosomal lumen regulates fusion with other membrane compartments.

lysosome structure

LAMPs:

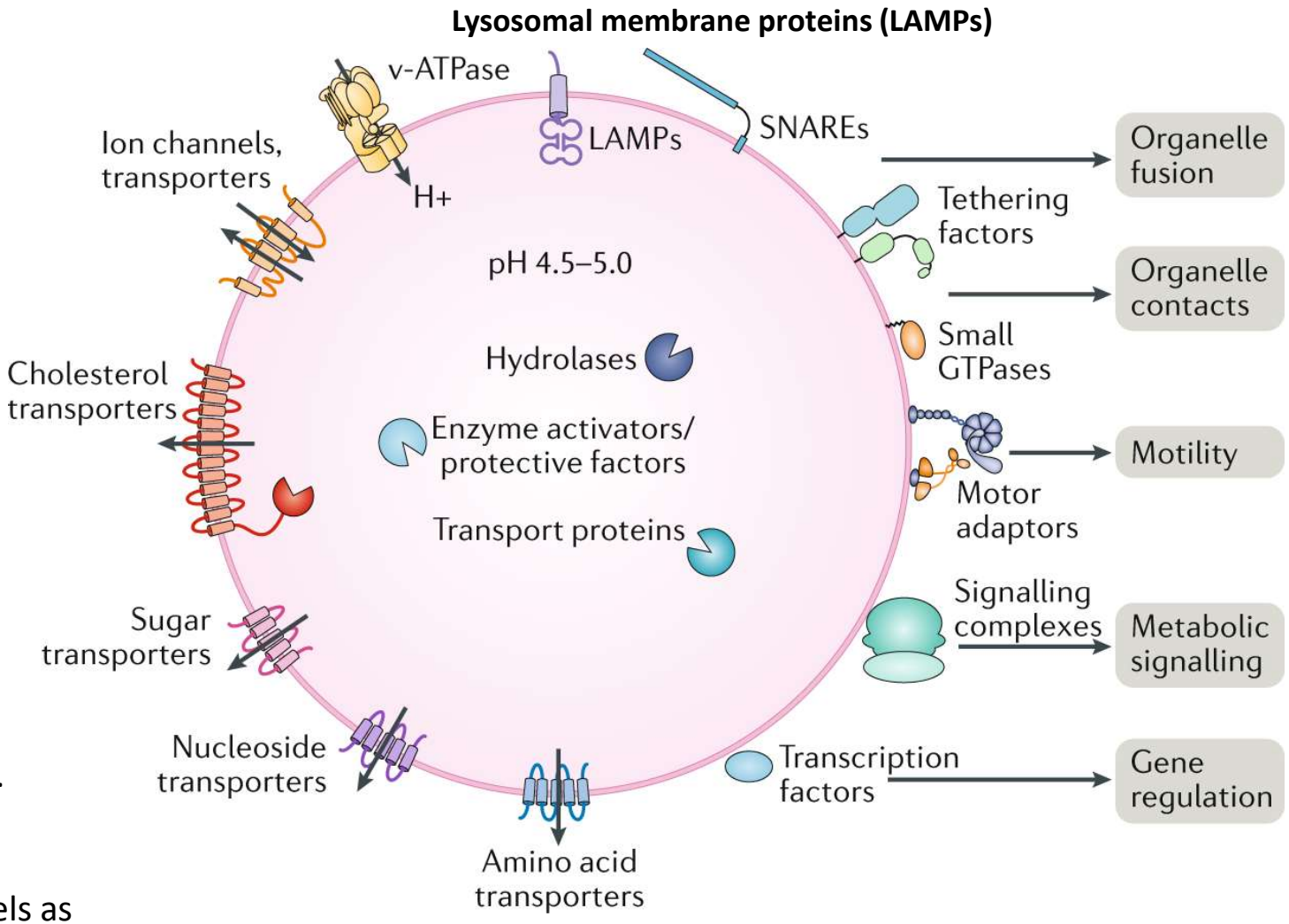
Ion channels, exert an indispensable role in regulating lysosomal pH and function

Signaling - The transmembrane segments of **LAMPs** are generally implicated in transport events across the membrane while the short cytosolic part of lysosomal membrane proteins mediates interactions with cytosolic proteins and/or proteins present on other organelles.

Transporters and exchangers

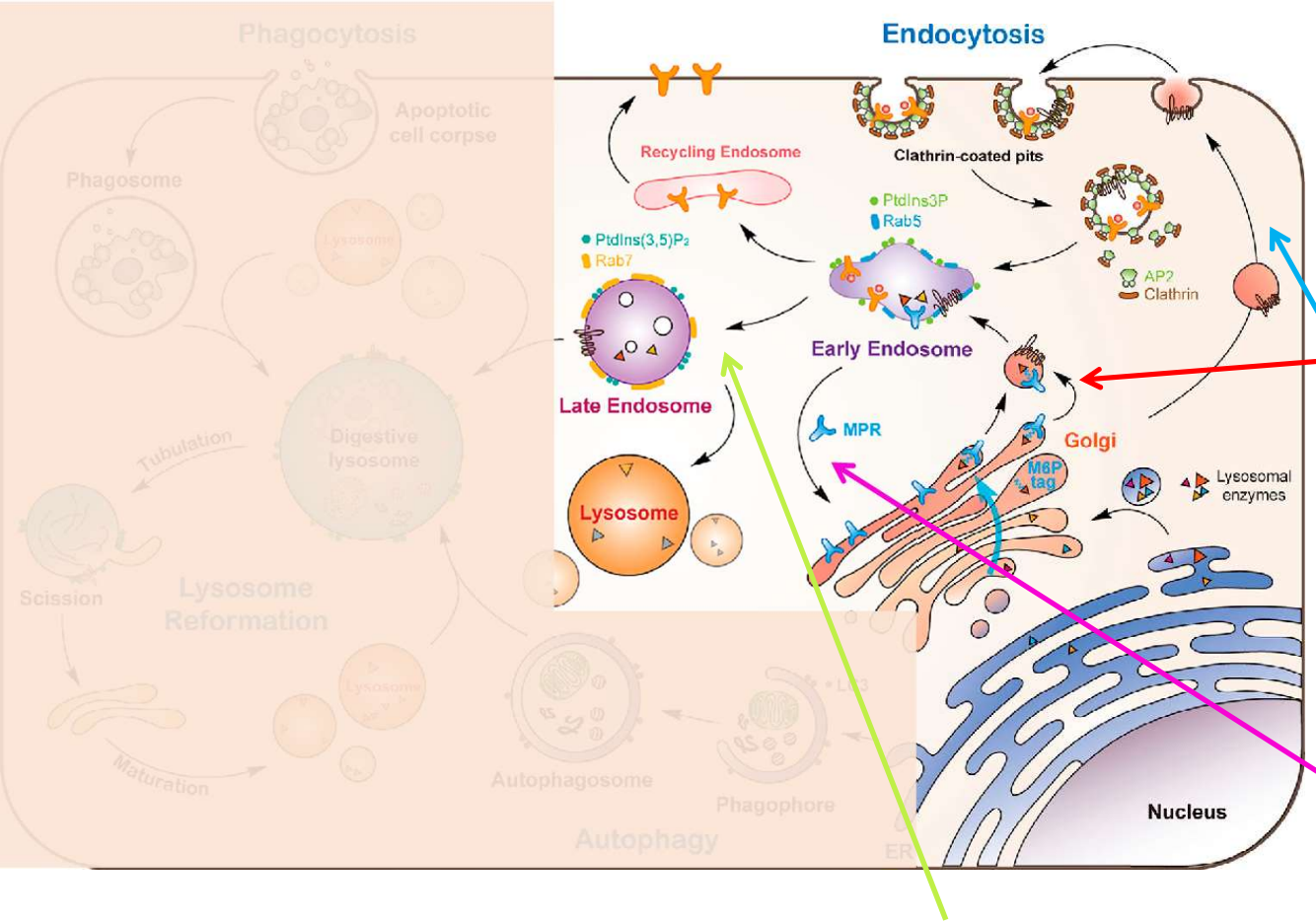
- cholesterol
- Sugar channels including spindling (SPIN).
- AA transporters
- Ion channels

They all assure the regulation of calcium levels as well as stores of AA, sugars and ions (Cu^{+}, Fe^{+})



Lysosome Physiology

Lysosomes are initially formed in the TGN but….



Lysosomes receive proteins and cargos from multiple pathways

Newly synthesized lysosomal membrane proteins are sorted at the TGN and delivered to endosomes (**direct pathway**)

First delivered to the plasma membrane and then endocytosed to reach early endosomes (**indirect pathway**).

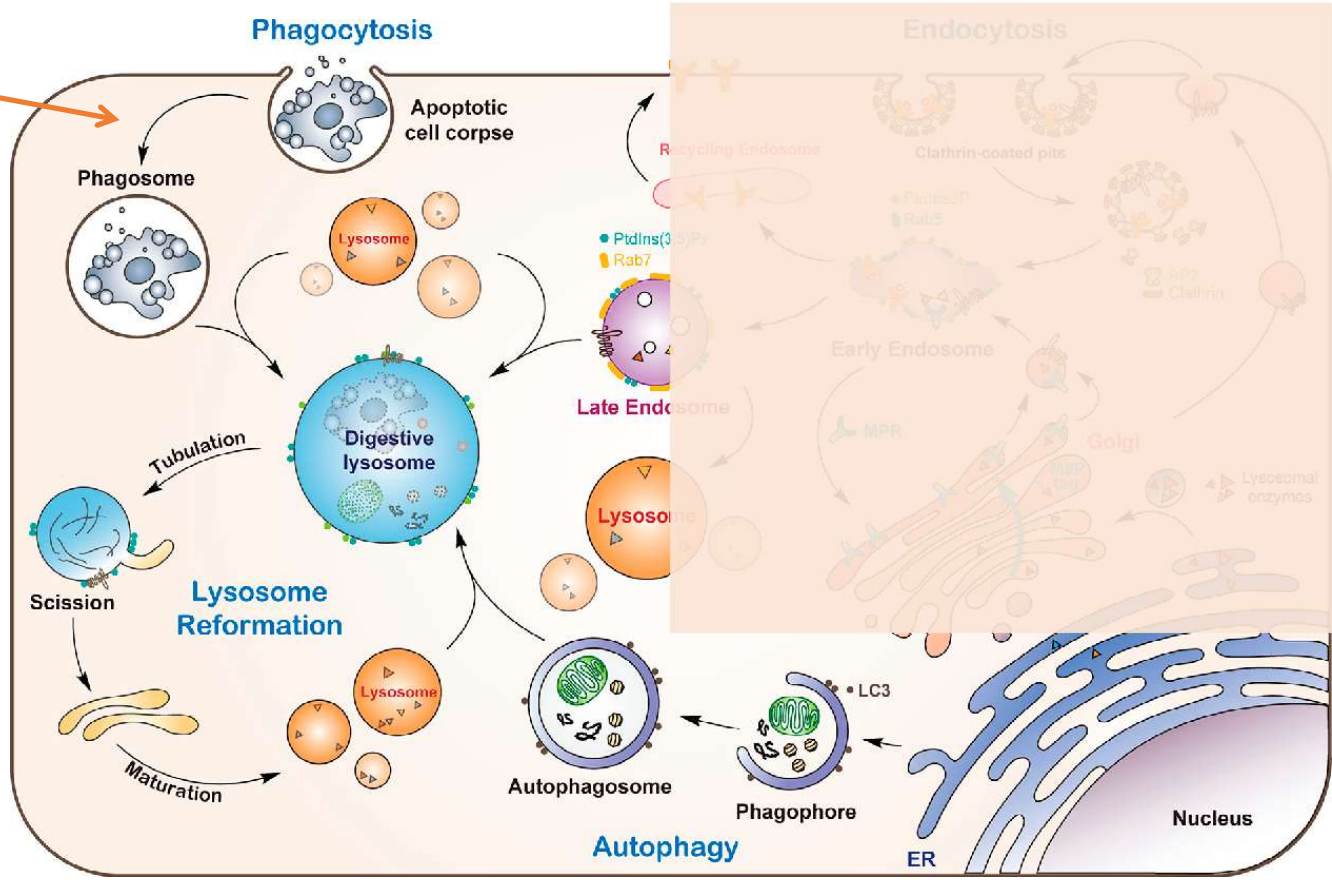
Receptors not destined for lysosomes are recycled back to the plasma membrane or Golgi.

Early endosomes undergo a conversion to late endosomes, which then fuse with lysosomes.

Lysosome Physiology

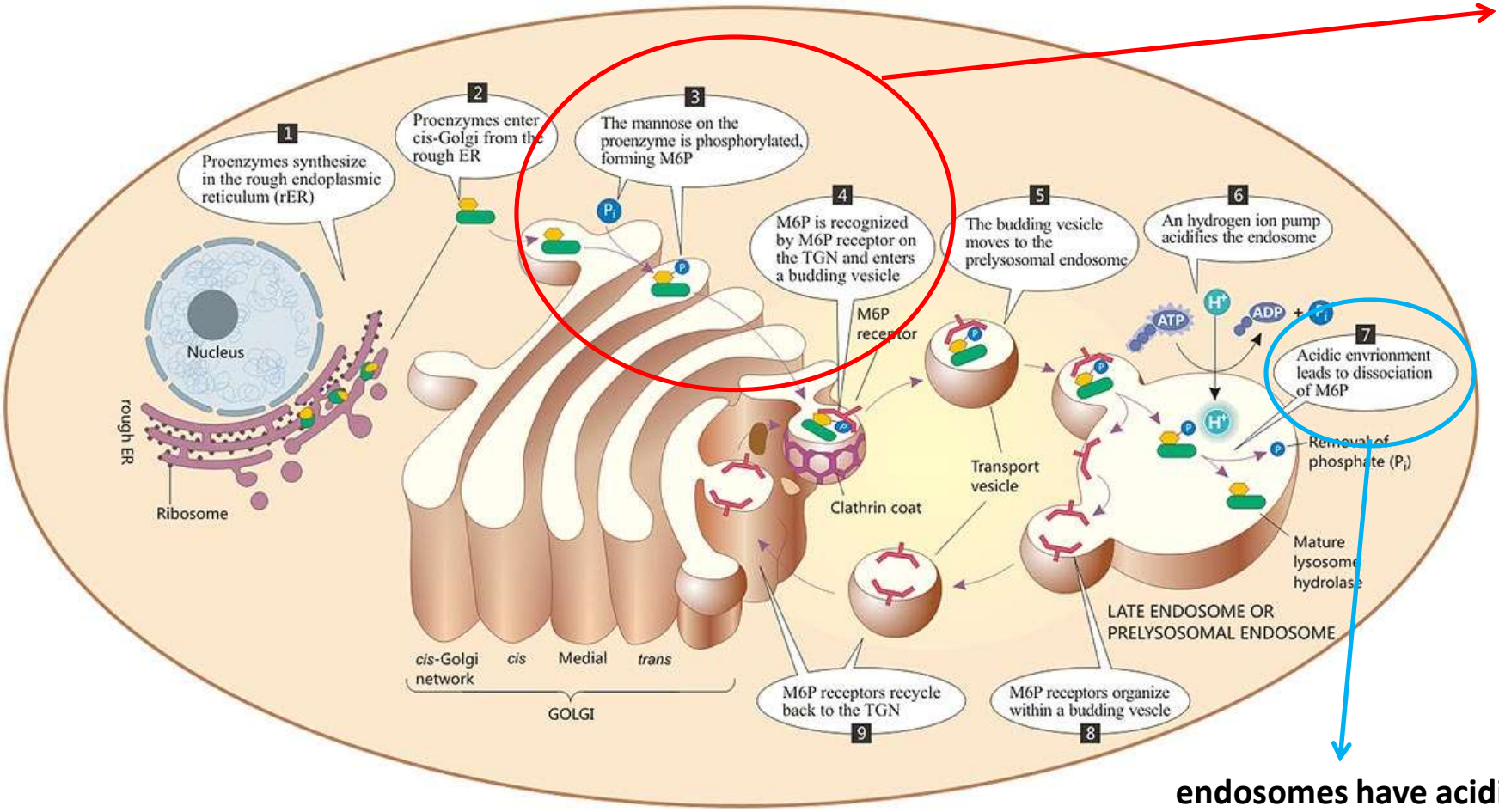
Phagocytosed cargos are enclosed in phagosomes, which undergo a maturation process and fuse with lysosomes.

Lysosomes reform from digestive lysosomes (endo-, phago-, and autolysosomes) by tubulation and scission to form protolysosomes, which mature into functional lysosomes.



Autophagic cargos are delivered to lysosomes by fusion of autophagosomes with lysosomes.

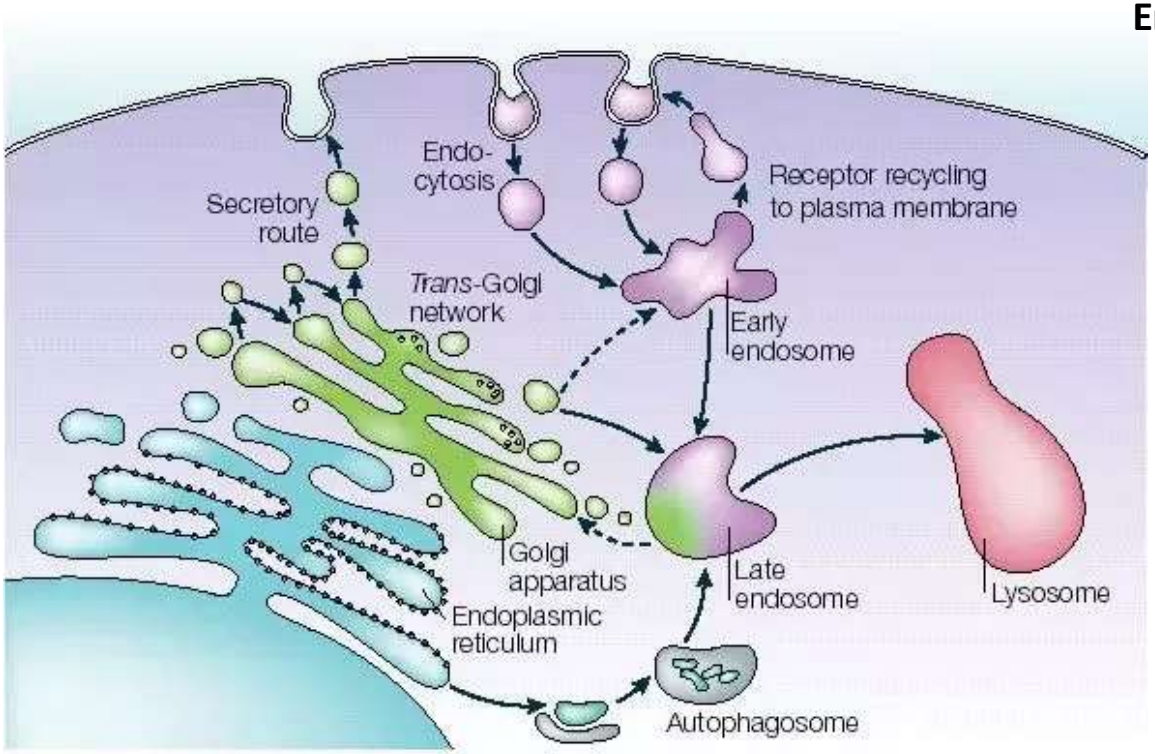
Lysosomal Membrane Proteins



enzymes are initially tagged with mannose-6-phosphate residues, targeting them for specific binding to the mannose-6-phosphate receptors (M6PRs) in the trans-Golgi network (TGN). Subsequently, enzymes tagged with M6PRs are packed into plasma membrane localized clathrin-coated vesicles (CCVs) for biosynthetic transport to LEs either directly or indirectly via EEs

endosomes have acidic intraluminal pH, facilitating uncoupling of ligands from M6PRs.

Lysosome Physiology



Endocytic compartments such as EE mature to become LE which are only moderately acidic

endosomes

maturation ↓ **Moves from periphery to center of cell**

Late endosomes

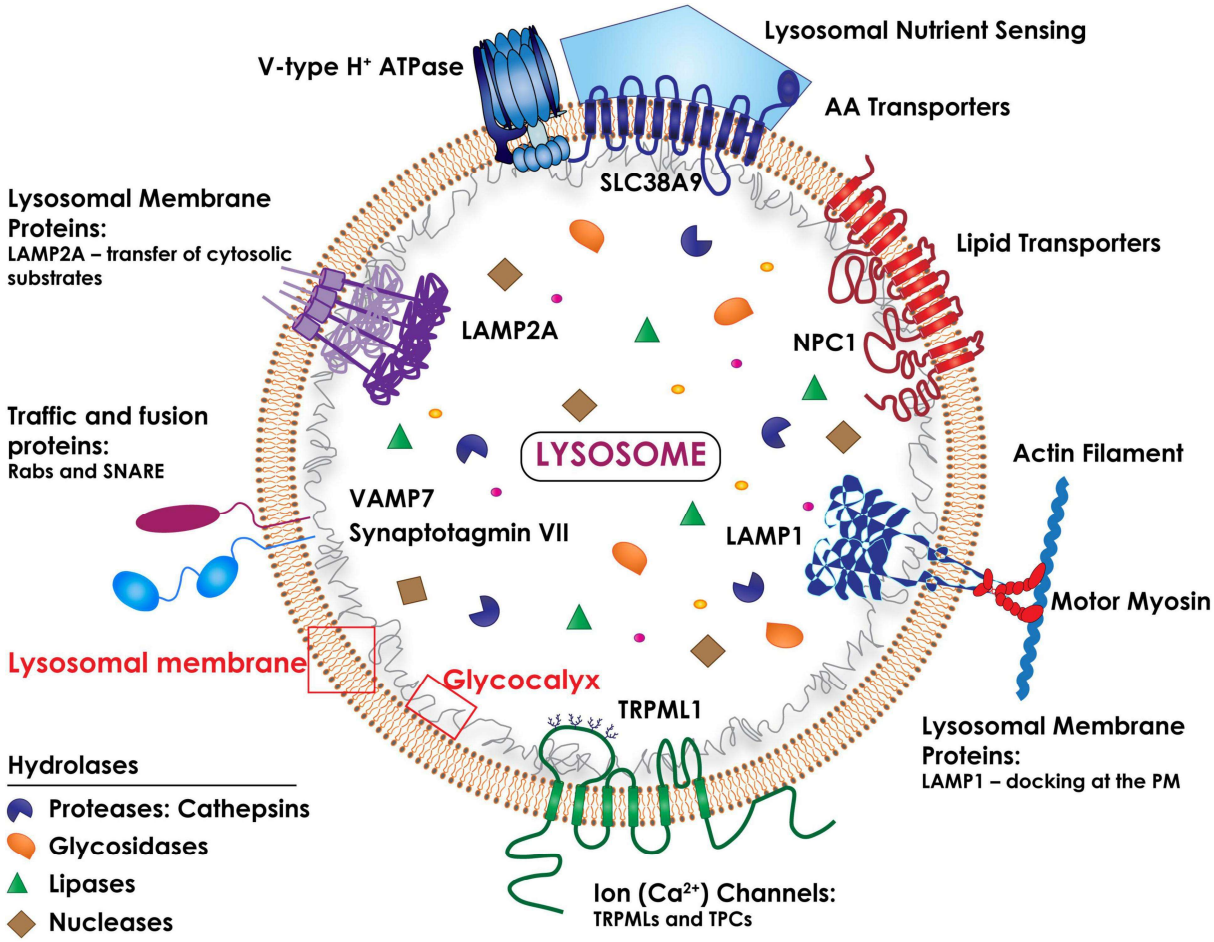
The fusion of LE and terminal storage lysosomes creates a hybrid organelle named the endolysosomes, the principal intracellular site of acid hydrolase activity

Lysosomes contain over 60 types of hydrolytic enzymes (proteases, lipases, nucleases and other hydrolytic enzymes) that break down complex macromolecules into their constituent building blocks.

pH, Acidification, Ion Flux and Transporters

While the V-ATPase assures an electrogenic proton translocation in endolysosomes, a flux of ions other than protons are needed as a compensatory flow of charge.

Two additional ions that seem to play crucial roles in endocytic processes and acidification are chloride (Cl^-) and calcium (Ca^{2+}).

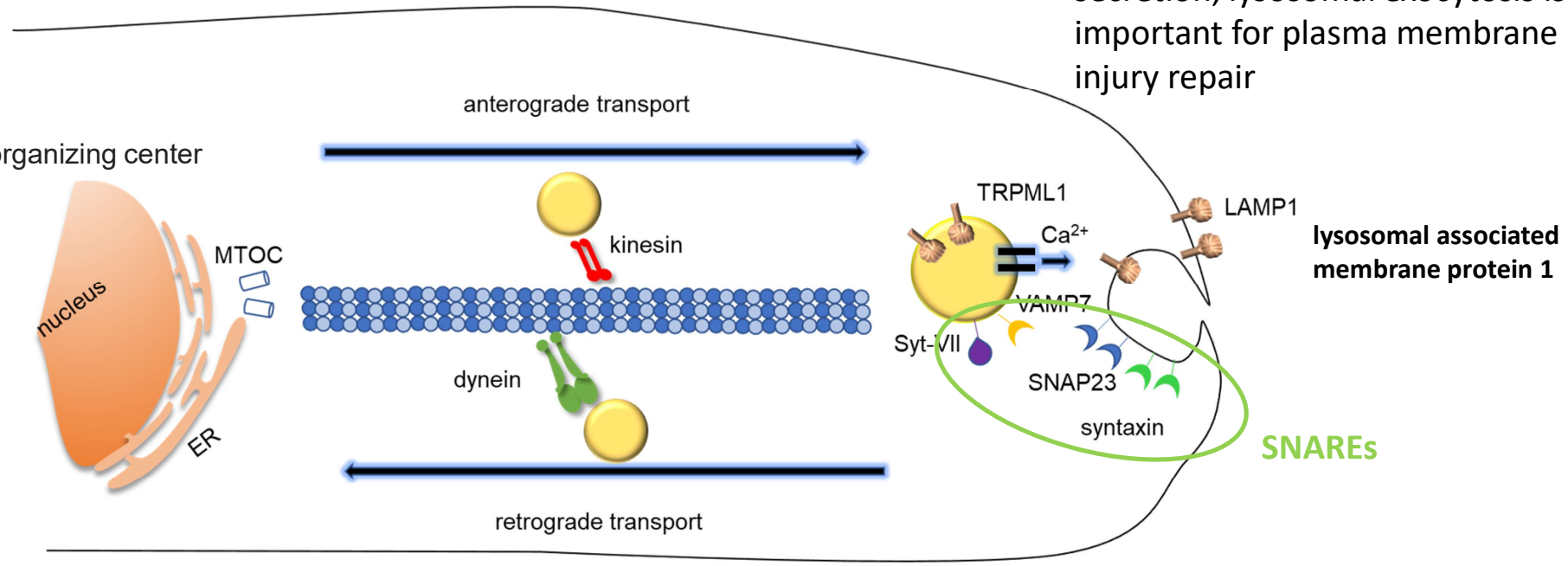


Glycocalyx- glycoprotein + glycolipid covering

Lysosomal Functions: Exocytosis

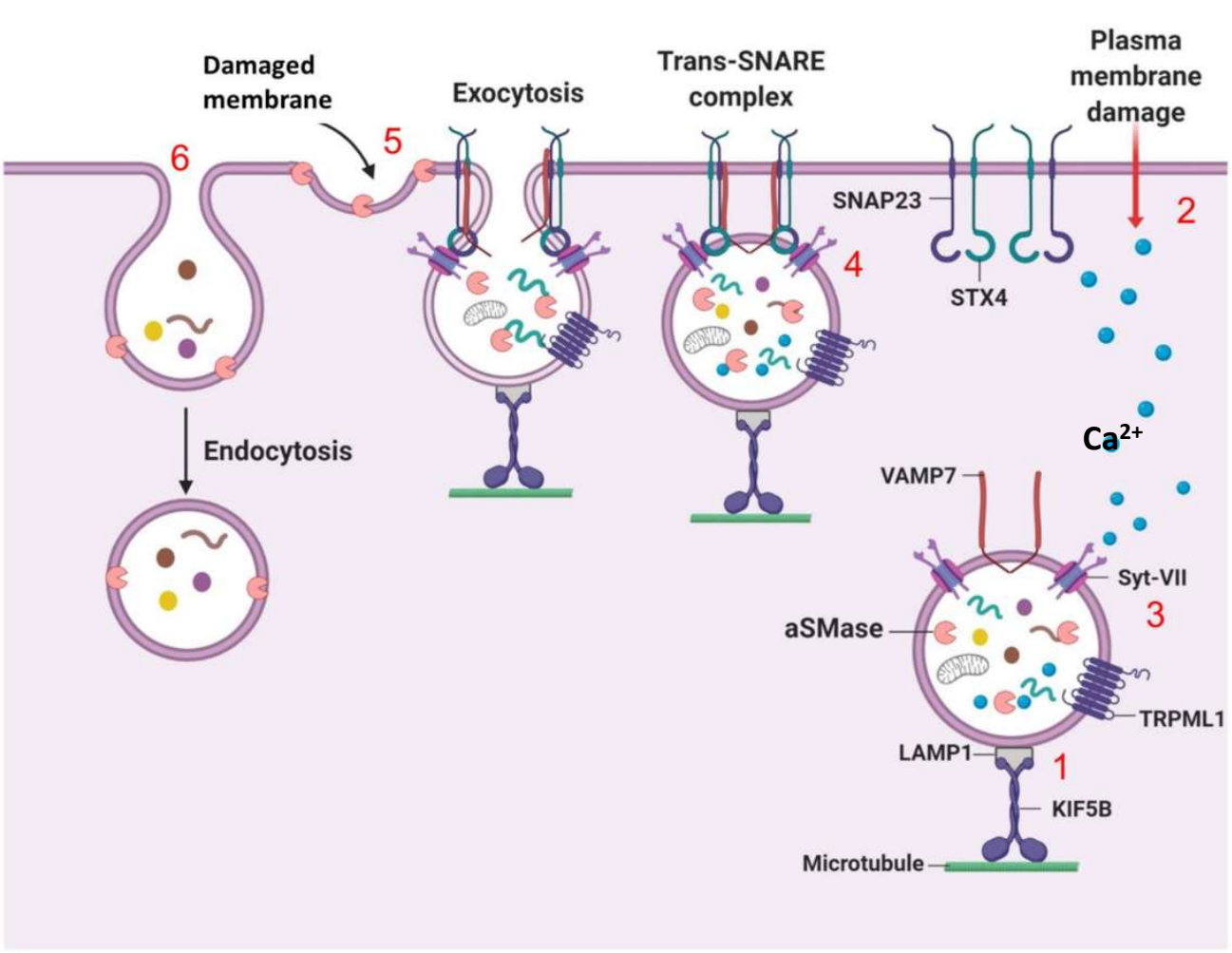
Aside from lysosomal content secretion, lysosomal exocytosis is important for plasma membrane injury repair

MTOC - microtubule-organizing center



Lysosomes migrates from the perinuclear region to plasma membrane (PM) proximity and fuse with the PM, releasing their content extracellularly. Most lysosomes are localized around the nucleus, but a pre-requisite for lysosomal exocytosis is their transport along microtubules via kinesin motor proteins. The dynamics of lysosomes within cytoplasm also includes the retrograde transport from periphery to nucleus along microtubules, mediated by dynein motor proteins. Vesicle-associated membrane protein 7 (**VAMP7**) is present on the surface of lysosomes and interacts with syntaxin-4 and with synaptosome-associated protein of 23 kDa (**SNAP23**) on the plasma membrane. The lysosomal Ca^{2+} channel **TRPML1** provides Ca^{2+} for lysosomal membrane fusion, which is sensed by **Syt-VI.**

Lysosomal Functions: Exocytosis

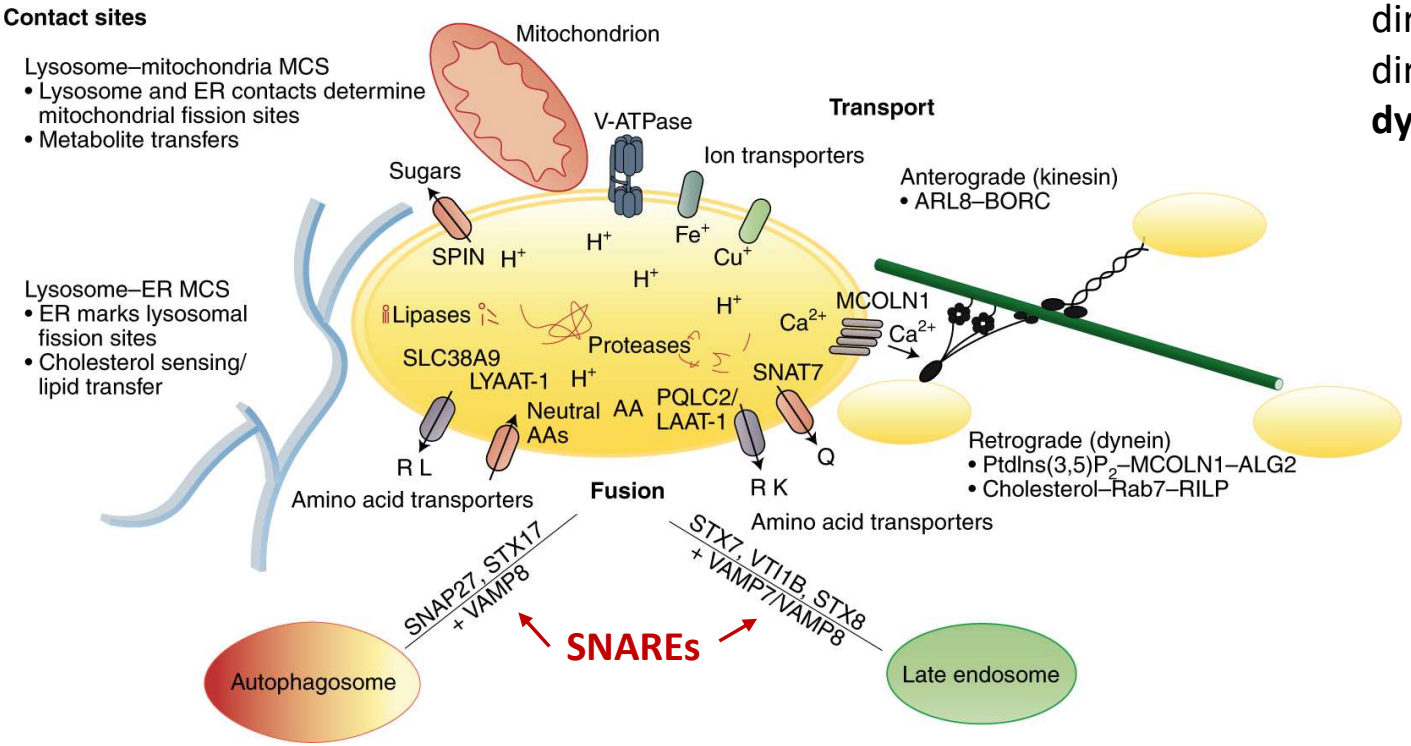


Exocytosis of the lysosome involves two main steps: (1) lysosomal movement to the cell periphery for plasma membrane (PM) fusion, and (2) exocytosis of the luminal content of the lysosome into the extracellular milieu. Lysosomal exocytosis is paramount for various cellular physiological processes such as **PM repair** (PMR), **immune response**, bone resorption and **cell signaling.**

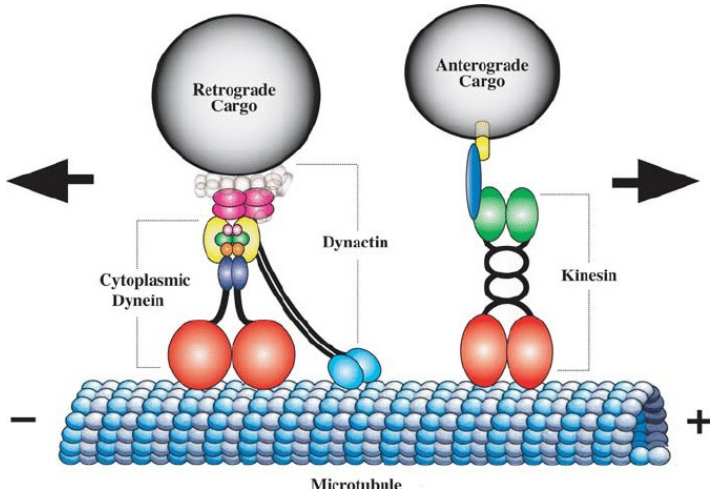
Exocytosis is initiated by the lysosomal membrane localized VAMP7 (vesicle-associated membrane protein 7) that forms a Trans-SNARE complex with syntaxin-4 and SNAP23 (synaptosome-associated protein 23 kDa) on the PM. Trans-SNARE complex brings the lysosome in a close proximity to the PM to initiate the fusion of lysosome and PM.

Lysosome trafficking and crosstalk with other organelles

Lysosomes develop membrane contact sites with other organelles to transfer signaling information, to share metabolites and to facilitate ionic homeostasis.

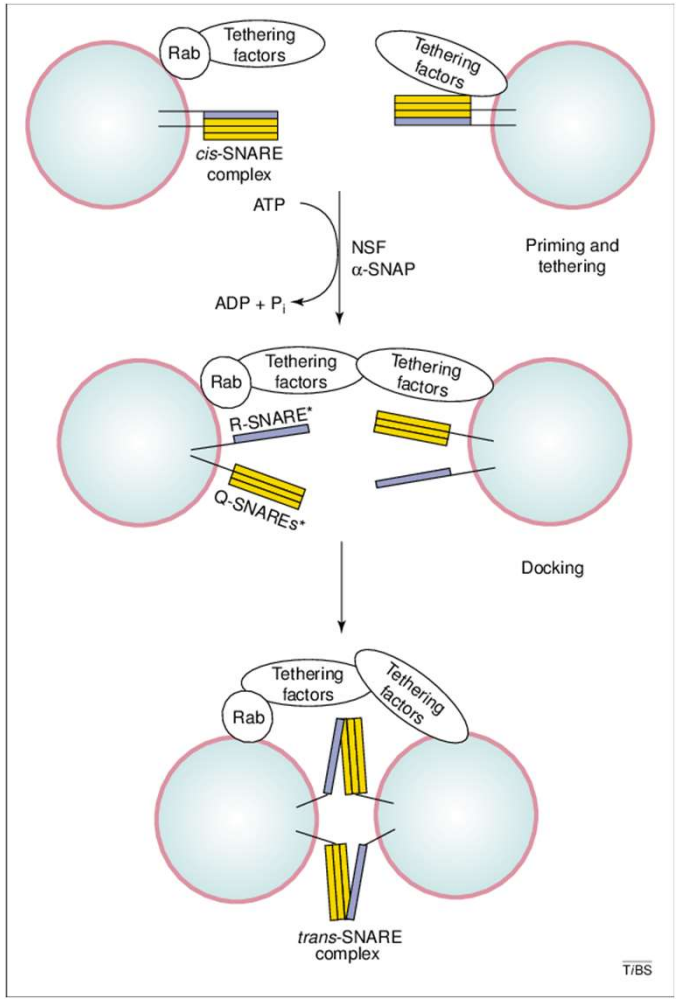


Lysosomes move along microtubules and change direction by rapidly switching between plus-end directed **kinesin** motors and minus-end-directed **dynein**–dynactin motor complexes

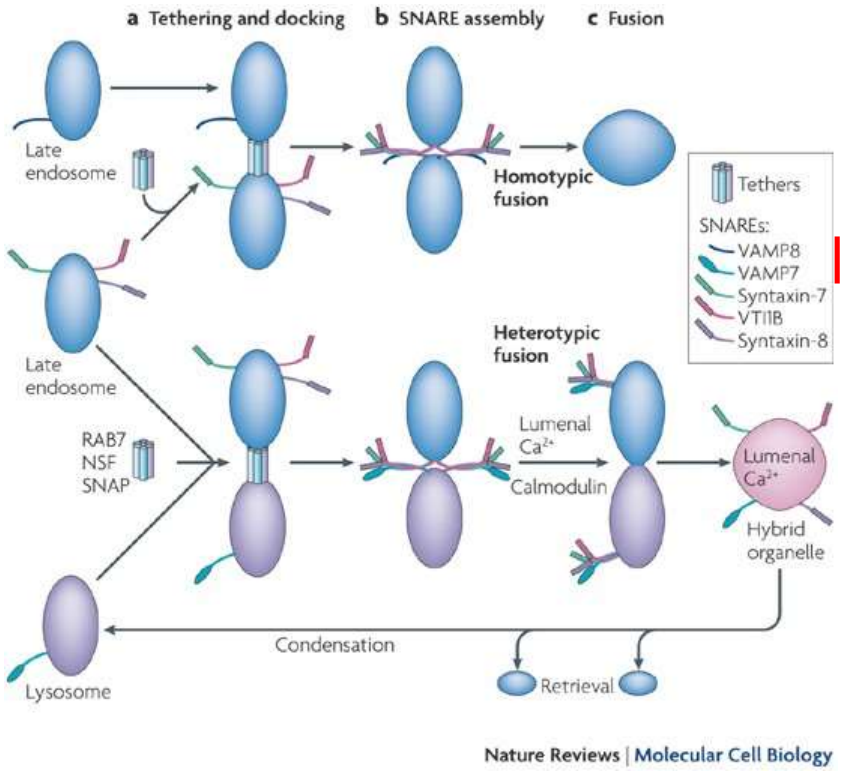


Lysosomal association with dynein–dynactin is promoted by calcium released from the lysosomal lumen through the mucolipin 1 (**MCOLN1**) channel.

Role of tethering factors and SNARE proteins in fusion process



In addition to lysosomal transmembrane proteins, the lysosomal membrane accommodates several other proteins such as tethering factors and SNARE proteins which is crucial for fission and fusion events.



R-SNARE

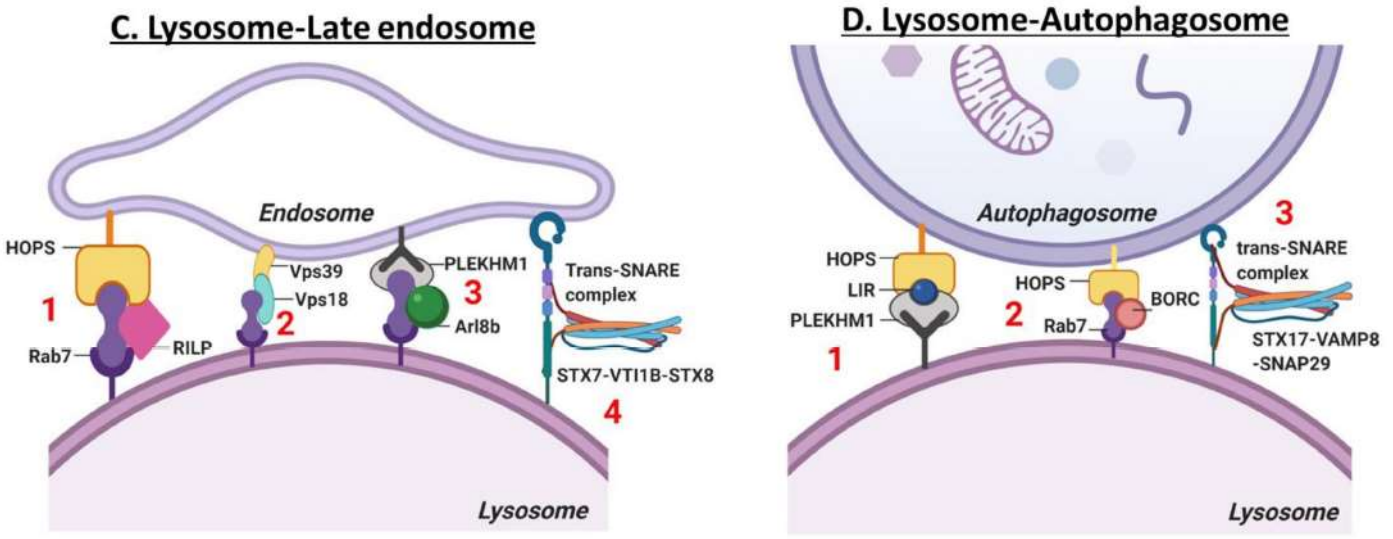
Q-SNARE

Fusion with late endosomes is mediated by the assembly between an R-SNARE (VAMP7 or VAMP8) located at the lysosomal membrane, and three Q-SNAREs (syntaxin 7, syntaxin 8, and VTI1B) located on LEs

Late Endosome-Lysosome-Autophagosome Fusion

Tethering of lysosomes and endosomes requires the small GTPase Rab7, which interacts with RILP (Rab7-interacting protein) and recruits **HOPS** (**Ho**motypic fusion and vacuole **P**rotein **S**orting) complex (Tethering complex)

Vacuolar protein sorting-associated protein 18 (Vsp18): important role in segregation of intracellular molecules into distinct organelles

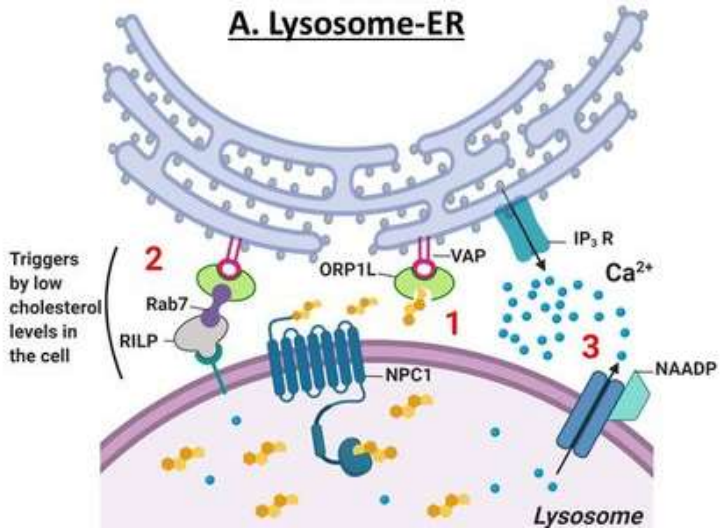


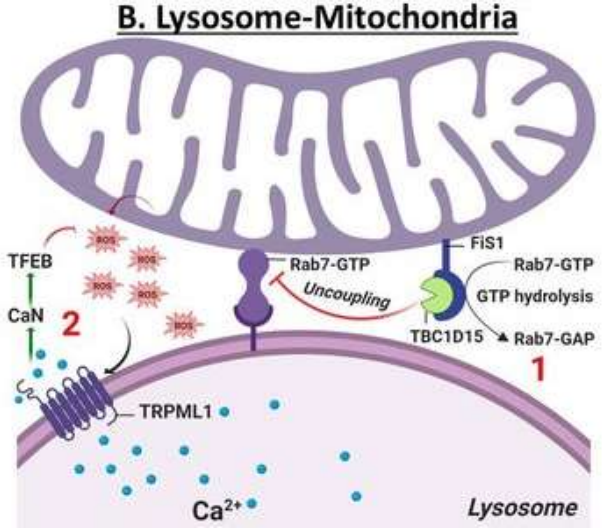
Fusion of lysosomes-LEs also requires Trans-SNARE (**S**oluble **N**-ethylmaleimide-sensitive factor **A**ttachment protein **Re**ceptor) assembly, composed of three clustered Q-SNARE (Qa, Qb and Qc) elements, which interact with R-SNARE through their N-terminal end of the SNARE motif.

Physical contacts with other organelles

Lysosomes engage in physical contacts with other organelles, including the endoplasmic reticulum and mitochondria, without undergoing membrane fusion. The bilayers of the respective organelles are held in close proximity (5–20 nm) by specialized tethering proteins.

Several tethering proteins harbour specialized lipid-binding domains that mediate the rapid transport of lipids, phospholipids and sterols.



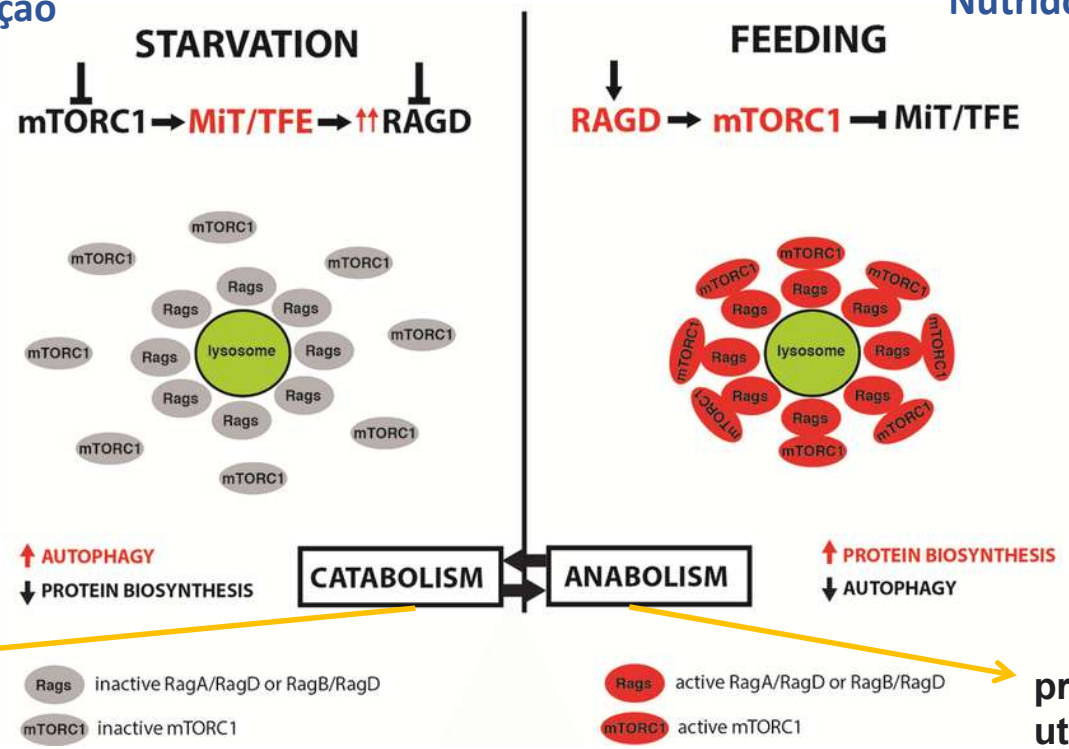


Play metabolic regulatory roles: assist efficient transfer of lysosome-derived metabolites into the mitochondrial matrix to fuel the tricarboxylic acid cycle

Lysosome-dependent regulation of cellular physiology in response to nutrients

Inanição

Nutrido



Promotes the maintenance of stem cell populations

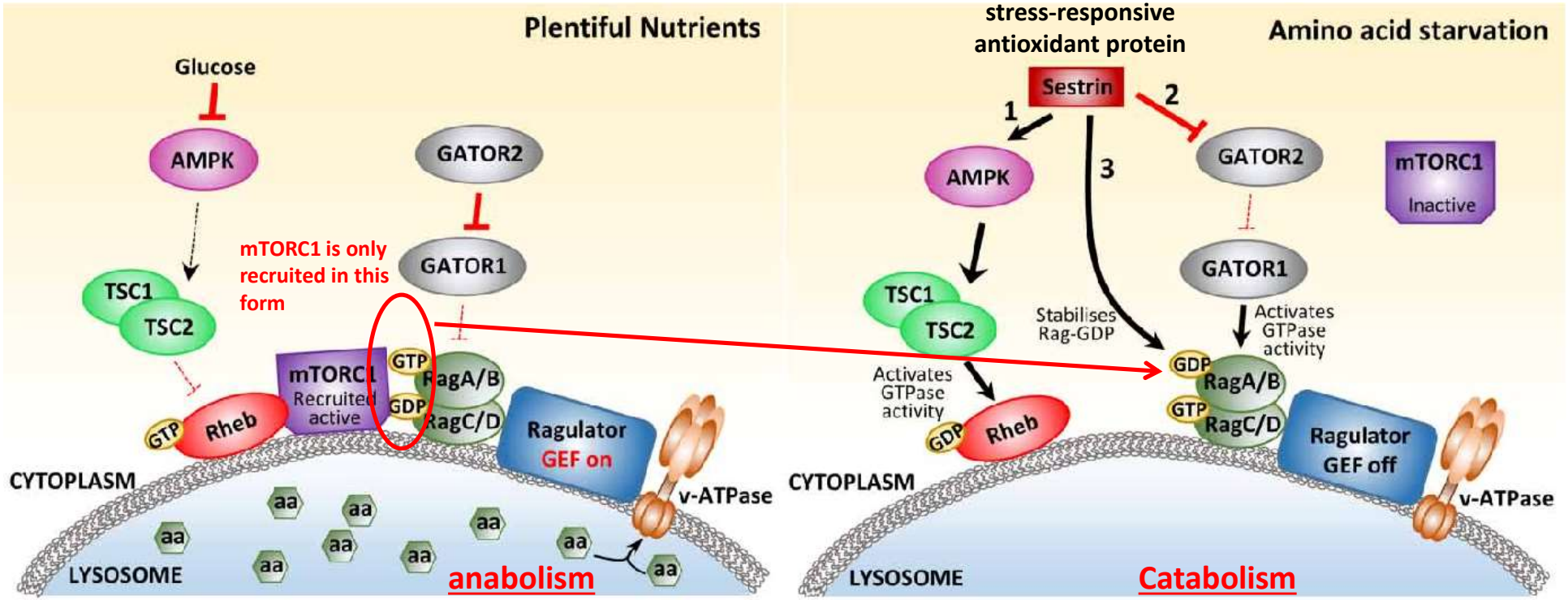
nutrient and energy inputs can direct cell metabolism towards growth or, alternatively, promote quiescence and repair

enzymatic reactions that breakdown organic or inorganic materials such as proteins, sugars, fatty acids, etc.

process by which the body utilizes the energy released by catabolism to synthesize complex molecules

mTORC1 activity must be carefully balanced to ensure healthy organ development and homeostasis

The lysosome as a metabolic signaling center



- **mTORC1 is activated only in the presence of both growth factors and nutrients.**
- **Nutrient-dependent activation of the heterodimeric Rag GTPases recruits mTORC1 to the lysosomal surface and via growth factor-driven activation of the lysosome-bound GTPase Rheb, activates mTORC1 at the lysosome.**

A major mechanism for mTORC1 suppression during starvation is the promotion of GTP hydrolysis on RagA by the GATOR1 complex.

NOTES clathrin and APs

The vesicular transport mechanism involves two types of coated vesicles:

1. Clathrin-coated vesicles, transporting products from the Golgi apparatus to lysosomes and carrying products from the exterior of the cell to lysosomes (for example, cholesterol).

2. COP-coated vesicles (COP stands for **c**oat **p**rotein), transporting products between stacks of the Golgi apparatus (**COPI**-coated vesicles) and from the endoplasmic reticulum to the Golgi apparatus (**COPII**-coated vesicles).

AP2- clathrin vesicles - Cargo capturing by AP complexes and clathrin Adaptor protein complexes bind to cargo proteins via specific signal sequences. Clathrin binds to AP-1 and AP-2 complexes, which leads to further crosslinking of the adaptor proteins and concentration of cargo proteins in the forming vesicle.

The sorting of acid hydrolases and LMPs (Lysosomal Membrane Proteins) requires the heterotetrameric adaptor protein complex AP1, AP2, AP3 and AP4, each composed of four adaptin subunits. Specific localization of AP governs sorting of M6PRs and LMPs and thereby lysosome biogenesis. AP1 is localized to the TGN and endosomes and assists in the recycling of M6PRs within the TGN. AP2 and AP3 are located on the plasma membrane and endosomes respectively, aiding the transportation of LMPs to lysosomes.

NOTES

Lysosome is the cellular compartment for the degradation of biological macromolecules. Endocytic, autophagic and phagocytic pathways facilitate macromolecule degradation within the lysosome. Acid hydrolases and lysosomal membrane proteins (LMPs) dictate lysosomal function. The acidity of the lysosome stabilizes and mediates the activity of ~60 luminal hydrolytic enzymes. The lysosomal limiting membrane harbors ~25 LMPs, which include transporters, tracking/fusion machinery, ion channels and structural proteins.

The catabolic function of the lysosome is accomplished by an array of approximately 60 proteases, lipases, nucleases and other hydrolytic enzymes that break down complex macromolecules into their constituent building blocks . These hydrolases require an acidic pH of ~4.5, which is established by an ATP-driven proton pump, the vacuolar H+-ATPase (v-ATPase), in cooperation with ion channels. The basic metabolites generated by lysosomal degradation are eventually exported to the cytoplasm via dedicated permeases that span the lysosomal membrane.

Lysosomes move towards and away from the cell centre at high speeds (several micrometres per second), continuously fuse with each other and with other organelles including endosomes, phagosomes and autophagosomes, and reform by tubulating out of the resulting hybrid organelles.

Inter-organelle contacts play increasingly diverse roles. Endoplasmic reticulum–lysosome contacts mark lysosomal fission sites and seem to be directly involved in this process. Contacts between lysosomes and mitochondria were recently proposed to aid mitochondrial fission, probably in cooperation with the endoplasmic reticulum. Finally, lysosome–mitochondria contacts seem to play metabolic regulatory roles, as they may assist efficient transfer of lysosome-derived metabolites into the mitochondrial matrix to fuel the tricarboxylic acid cycle.

NOTES

In brief, endocytosis begins with the budding of an endocytic vesicle from the plasma membrane and fuses with an early endosome (EE). This internalized material will meet different fates by trafficking to various compartments: to late endosomes (LE), multivesicular bodies (MVB) and to lysosomes for degradation while proteins that need recycling will travel back to the plasma membrane through either a fast route involving EEs or a slower pathway involving recycling endosomes (RE)

As EEs progressively mature and acidify into LEs, they move from cell periphery to cell center. During maturation, EEs lose Rab5 on their membrane and gain Rab7, underscoring the importance of Rab proteins in the EE-to-LE maturation process. Additionally, maturation of EEs to LEs/lysosomes requires v-ATPase, a proton pump that acidifies LEs/lysosomes to a pH of 5.5/5.0. In addition to proton homeostasis, ionic Ca_{2+} balance within LEs and lysosomes is indispensable for endo-lysosomal functions including receptor-ligand uncoupling and lysosomal enzyme transport and activity.

Lysosomes are abundant in hydrolytic enzymes such as proteases, sulfatases, nucleases, lipases, phosphatases, glycosidases and nucleases, all of which degrade complex macromolecules. Lysosomal enzymes have optimal activity at pH 5.

Lysosomal hydrolases are first synthesized and modified by linkage with oligosaccharides in the ER. They are then transported to the Golgi apparatus, where mannose residues in the oligosaccharide chain on most lysosomal hydrolases are phosphorylated and recognized by the mannose 6-P receptor (MPR). The hydrolase–MPR complex is then enclosed in clathrin-coated vesicles at the TGN and delivered to early endosomes. Some hydrolases are sent to endosomes in an MPR independent manner.

Lysosomal delivery of newly synthesized lysosomal membrane proteins occurs through both direct and indirect pathways. In the direct pathway, lysosomal membrane proteins are sorted at the TGN and delivered directly to endosomes, while the indirect pathway involves delivery to the plasma membrane before undergoing endocytosis to reach early endosomes (**Slide 2**)

NOTES

Organelle fusion ensues via tethering processes when two organelles form a contact site over a distance of ~25 nm. Tethering of lysosomes and endosomes requires the small GTPase Rab7, which interacts with RILP (Rab7-interacting protein) and recruits HOPS (mammalian homotypic fusion and vacuole protein sorting) complex.

Proteins implicated in lysosome-endosome fusion events. SNAp REceptor (SNARE) proteins regulate fusion events between lysosomes and other organelles such as late-endosomes, autophagosomes, and amphisomes. VAMP7 and VAMP8 are R-SNAREs, a type of SNARE proteins that provides an arginine (R) residue in the assembly of the SNARE complex and are located at the lysosomal membrane. Syntaxin 7, Syntaxin 8 and VTI1B are Q-SNARES, providing a glutamine (Q) residue in the assembly of the SNARE complex, and localize to late endosomes (LE).

mTORC1 is activated only in the presence of both growth factors and nutrients. This mechanism is achieved by nutrient-dependent activation of the heterodimeric Rag GTPases that recruit mTORC1 to the lysosomal surface and via growth factor-driven activation of the lysosome-bound GTPase Rheb, which activates mTORC1 at the lysosome (**Slide 17**). The Rag GTPases are a heterodimer composed of the functionally equivalent RagA or RagB in complex with either RagC or RagD. The Rags recruit mTORC1 to the lysosome when RagA/B is bound to GTP and RagC/D is bound to GDP. When Rags are in the converse nucleotide state, they cannot bind to mTORC1, which remains inactive in the cytoplasm.

The MiT/TFE basic leucine zipper transcription factors, which include TFEB, TFE3, TFEC and MiTF, control the expression of many lysosomal target genes, including hydrolases, lysosomal membrane proteins and subunits of the v-ATPase by binding to CLEAR elements in the promoters of these genes (**Slide 18**). mTORC1 regulates MiT/TFE transcription factor activity through phosphorylation on key residues. mTORC1-dependent phosphorylation of TFEB at Ser 142 and Ser 211 causes binding of TFEB to 14-3-3 proteins, which mask a nuclear localization signal and prevent TFEB translocation into the nucleus. Conversely, TFEB dephosphorylation by the calcium-regulated phosphatase calcineurin drives nuclear translocation and activation of TFEB under low-nutrient conditions. When nutrient levels are low (particularly in the contexts of amino acid or glucose starvation), the CLEAR-dependent transcriptional network is activated, resulting in enhanced lysosomal biogenesis. The induction of CLEAR element driven genes also promotes autophagosome biogenesis, lysosomal exocytosis and alterations in lipid metabolism.

NOTES

The rate of autophagy, the process of recycling intracellular components via membrane-mediated inclusion and digestion, also profoundly influences lysosomal composition and function. TFEB drives the expression of many key autophagy pathway proteins. TFEB overexpression increases the number of autophagosomes and lysosomes, implicating TFEB as a critical regulator of cellular catabolism, in balance with mTORC1 at the lysosome.

Modulation of mTORC1 activity by its nutrient and energy inputs can direct cell metabolism towards growth or, alternatively, promote quiescence and repair. At the lysosome, mTORC1 phosphorylates and activates S6-kinase, which in turn promotes biosynthesis of lipids and nucleotides, as well as ribosome biogenesis and a switch to glucose metabolism. A major consequence of mTORC1 activation is the upregulation of protein synthesis, which occurs via phosphorylation of 4E-binding proteins 1 and 2.

Thus, the detachment of mTORC1 from the lysosome that occurs in nutrient-poor states strongly promotes autophagy initiation. At the lysosomal surface, mTORC1 phosphorylates and inhibits the MiT/TFE factors transcription factor EB (TFEB), TFE3, TFEC and microphthalmia-associated transcription factor (MiTF), which are master regulators of lysosomal and autophagic gene expression. Inhibition of mTORC1 pro-differentiation and proliferation activity is required for the maintenance of stem cell populations in the muscle, brain, haematopoietic system and skin of adult organisms. An age-dependent increase of mTORC1 signaling in these tissues is thought to underlie a progressive loss of stem cells that contributes to organ and tissue ageing.

Conversely, enhanced autophagic–lysosomal function is associated with maintenance of stem cell populations in the bone marrow and the brain. These observations indicate that mTORC1 activity must be carefully balanced to ensure healthy organ development and homeostasis.