Trafficking: endocytosis

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Endocytosis function

- Intake of nutrients (iron, triglycerides, vitamin carriers...)
- Regulation of hormone signaling (desensitization)
- turnover of plasma membrane
- recycling of secretory cargo adaptors

pathological situations

- virus, bacteria and toxin entry
- phagocytosis of pathogens (macrophages)
    - processing of antigens for antigen presentation

General concepts

- Constitutive endocytosis
- Receptor-mediated endocytosis
    - both hormone and nutrients
- Specialized endocytosis
    - phagocytosis
- Transcytosis
    - vesicular transport across epithelia (endocytosis → exocytosis)
- Recycling
    - endocytosed material is brought back to the PM
- Degradation
    - endocytosed material is send further to the lysosome

Vesicle budding from plasma membranes

- Cargo on the PM is recognized by adaptor proteins
    - often long and unfolded → fishing line for clathrin
    - Epsin, AP-2, GGA
    - recruited through binding to $PI_{4,5}P_2$
- Clathrin assembles from individual triskelion structures
    - leads to the formation of a clathrin-coated pit (CCP)
    - many accessory proteins are recruited (including synaptojanin, HSP70-type ATPases,..., which will help in coat disassembly later on)
- Deeply invaginated CCP are pinched off by Dynamin

- GTPase
- assembles as contractile ring

- takes 20 sec to 2 min
- Clathrin disassembles from vesicle.

https://www.youtube.com/watch?v=o_EUHu4OJus

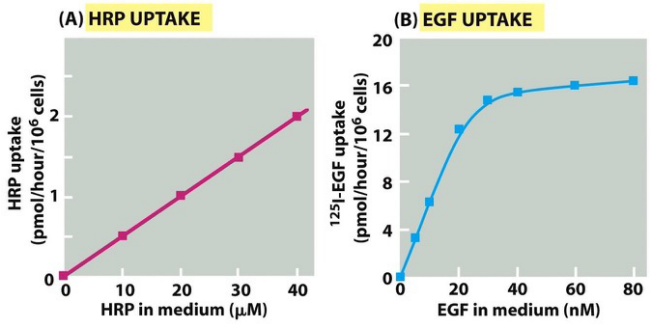
Note 1.

Many other clathrin-independent mechanism exist

- caveolae-mediated entry
- phagocytosis
- macropinocytosis

Fluid-phase vs. receptor mediated endocytosis

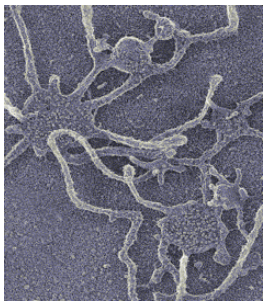
Figure 1: Difference between fluid intake (HRP) and receptor-mediated uptake (EGF). EGF is internalized through interaction with a receptor. When all molecules of the receptor are occupied, uptake speed levels up.



- Fluid-phase
  - non-saturable
  - require high concentration of molecule
- receptor-mediated
  - saturable
  - very efficient at concentrating low-abundant molecules

The early endosome

Figure 2: morphology of an early endosome



- Sorting compartment
  - recycling of receptor to PM
  - internalizing of Cargo
  - internalization of some receptors (desensitization)
- Rich in PI_3P
- Marked by Rab5

- pH slightly acidic (6-6.5)
  - leads to uncoupling of cargo and receptors
- Located at cell periphery
- sorting by geometry
  - large vesicles
    - low surface/volume → more soluble content, less membrane content
  - thin tubules
    - high surface/volume ratio → more membrane content, less soluble content
- tubules are separated to recycle
  - to the PM (via clathrin-dependent mechanisms)
  - to the Golgi apparatus (via retromer-dependent mechanism)

Note 2.

Other mechanism exist for recycling of components to PM

Late endosomes

- Mature from early endosomes
  - there may be more intermediates in the maturation pathway
- Rab7-positive
- Do not recycle to PM nor to Golgi anymore
- pH more acidic still (5-6)
- contain cargo for degradation
- contain future lysosomal content
- more centrally localized in the cell

Multivesicular bodies

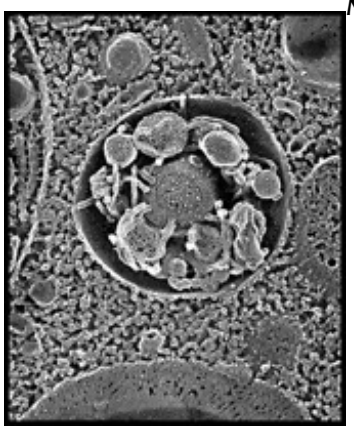


Figure 3: Scanning Electron micrograph of a multivesicular body

- Alternative name for late endosomes
  - from the fact that they are filled with internal (intralumenal) vesicles
  - vesicle formation starts at early endosome stage
  - caused by recruitment of ESCRT complex (same complex for cytokinesis abscission and virus budding).
  - Allows to quench the signaling from TM receptor kinases (EGF-receptor)
  - Allows efficient subsequent degradation of PM transmembrane proteins in lysosome

Lysosomes

- Degradative organelles
  - full of nucleases, proteases, lipases, phospholipases
- acidic pH (4.5-5.5)
- centrally located in human cells
- called vacuoles in plant and yeast cells
- terminal organelle.
  - No vesicle buds off lysosomes
  - No recycling of lysosomal content

Lysosomes fuse with late endosomes/multivesicular bodies to generate an **endolysosome**. More than one lysosome can fuse with endolysosome. After digestion of the content, the endolysosome matures into a lysosomes, that can, again, fuse with a late endosome → the lysosome cycle.

Lysosomes also fuse with autophagosomes (double-membraned organelles product of autophagy) to form **autolysosomes**

Safeguarding mechanisms

- All lysosomal content starts in the ER → Golgi → early endosome → late endosome before reaching lysosome.
  - Proteases, phospholipases etc. must be kept inactive during trafficking
  - activation by pH
    - e.g. acid phsophatase, acid hydrolases
  - activation by removal of pro-domain
    - proteases made as an inactive longer precursor. It is activated by proteolytic cleavage in the lysosome
    - e.g. carboxypeptidase Y (enzyme that cleaves proteins from the carboxy-terminus).

Phospholipases in lysosomes can degrade the membranes of the intralumenal vesicles. They should not however attack the limiting membrane of the lysosome

- The lipid composition of the internal leaflet of the lysosome is protected from phosphlipases by a thick and dense **glycocalyx** (high density of of heavily glycosylated proteins, and glycolipids)

Lysosomal acidification

Mediated by the vacuolar H^+-ATPase (v-type ATPase)

- rotary proton pump
- similar to f0-f1 ATPase, but works in opposite direction

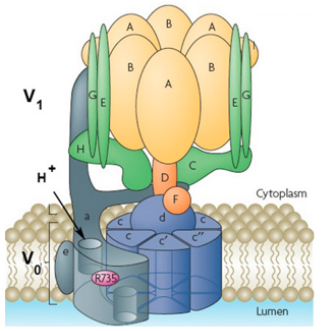


Figure 3. The structure of the V-ATPase complex is shown. Taken from Forgac, 2007.

Figure 4: Structure of the vacuolar ATPase

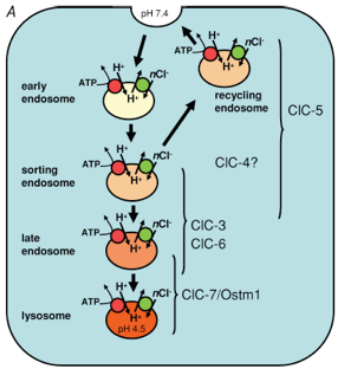


Figure 5: various CLCs in various compartments lead to different pHs

- Electrogenic: each proton pumped adds one + charge into compartment → build-up of + charges limits the acidification

Chloride channels (CLCs)

- Cl^-/H^+ exhcangers
- neutralize the charge of H^+ → allow further drop of pH.
- Different compartments have different CLCs, to adjust the pH