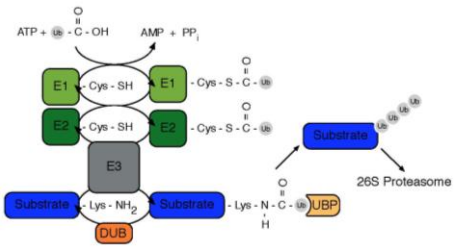
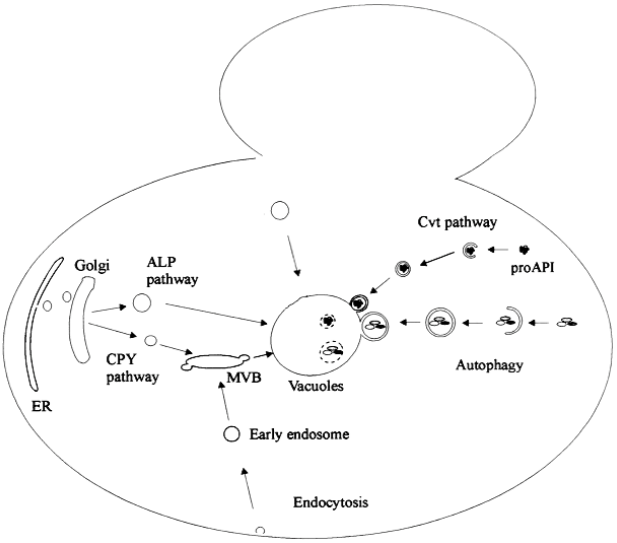
Protein Degradation

Regulated protein degradation can happen through



- Ubiquitin-dependent degradation: 26S proteasome, for single proteins, involved in many cellular processes (cell cycle progression, signal transduction)
- Selective autophagy: Vacuolar/lysosomal proteases, responsible for whole complexes, aggregates and organelles (everything that is too big for the proteasome)

Most prtoeins reach the vacuole by transiting through the secretory pathway. Proteins are sorted because of different informations and receptors. The main pathway used is the cpy pathway. Proteinase A also takes this pathway.



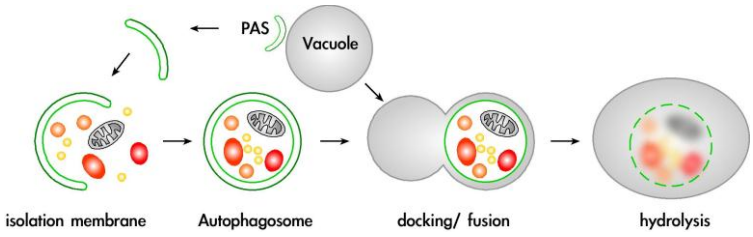
Multi vesicular bodies (MVB) are prevacuolar structures (vps mutant are defective in proper MVB sorting, e.g. ESCRT complex, ESCRT1 binds ubiquitin-cargo). The cpy and alp pathway converge at the vacuolar fusion and share many proteins there (alp otherwise is mush simpler, most vps mutants specific for cpy).

Clathrin-coated vesicles from the Golgi go to the MVB, under rich conditions, there is an alternative pathway, called cvt (aminopeptidase 1 (vacuole-resident hydrolase) and alpha-mannosidase). Ape1, vacuolar hydrolase, propeptides are cleaved in the vacuole, vesicular process, screening

Many cvt mutants are also defective in autophagy.

Vid pathway: FBPase (fructose 1-6 biphosphatase) degraded under high glucose, transported to vacuole via vesicles.

To summarize, in the vacuole, degradation happens and the products are stored and released when needed (not only destructive, but also a storage facility within the cell as it stores building blocks for new proteins).



Autophagy

Autophagy is a process in which cytosol and organelles are sequestered within double-membrane vesicles that deliver the

contents to the lysosome/vacuole for degradation and recycling of the resulting macromolecules. Autophagy plays an important role in the cellular response to stress (such as starvation, so everything that is not needed can be degraded and building blocks are available again), resistance to pathogens, developmental pathways and tumor suppression.

It seems that the PAS (phagosome assembly site), a very poorly understood structure, seeds a double-membrane which then encloses or engulfs aggregates, then fusion with the lysosome occurs and finally hydrolysis of the outer membrane.

Mitophagy and Pexophagy are two specialized selective organelle autophagies (for mitochondria and peroxisomes).

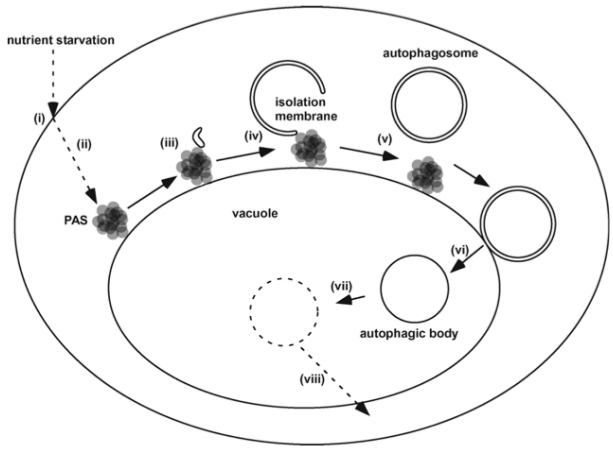
Very interesting is the connection of autophagy and neurodegeneration, as mice deficient for autophagy show enhanced aggregate formation and an early onset of Alzheimer's Disease phenotype (Atg5 flox mice). These mice accumulate aggregates in the brain and do not show any protective behaviour anymore, neurons die. Autophagy therefore is essential for the digestion of such aggregates. Moreover, some bacteria and viruses are cleared by autophagy (part of the innate immune system).

Many different diseases are related to autophagy, e.g. neurodegenerative diseases (such as Parkinson, Alzheimer's, Huntington's), infectious diseases, muscle disorders and cancers (link to cell death and apoptosis). Important for autophagy is the fact that both too much and too little can be dangerous.

Degradation can be either non-selective (anything that is needed is digested) or highly selective (first excess ribosomes, then ER and so on). How can targets be selected?

Selection signals can come from:

- Hormones (erythrocytes, do not possess mitochondria)
- Homeostatic signals
- Starvation signal (Tor-pathway, new building blocks)
- Specific signals to remove defective organelles (quality control)



I. Starvation signal sensing
II. Transmission of these signals to the autophagosome-generating machine (PAS, phagosome assembly site)
III. Generation of an isolation membrane (IM) from the PAS
IV. Expansion of the IM
V. Fusion of the leading edges of

the IM to complete autophagosome formation

VI. Fusion of the outer membrane of the autophagosome with the vacuolar membrane and subsequent release of autophagic bodies

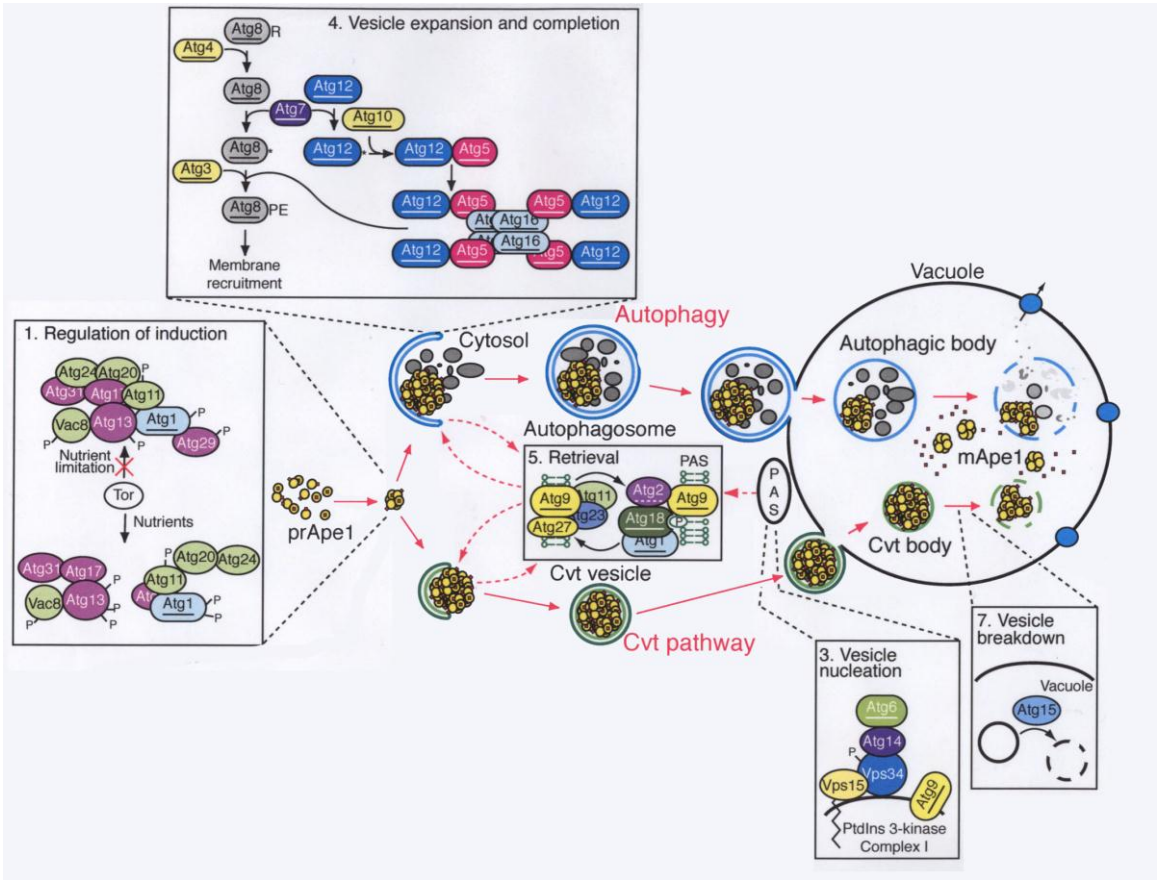
VII. Disintegration of the autophagic body and degradation of its contents by vacuolar hydrolases

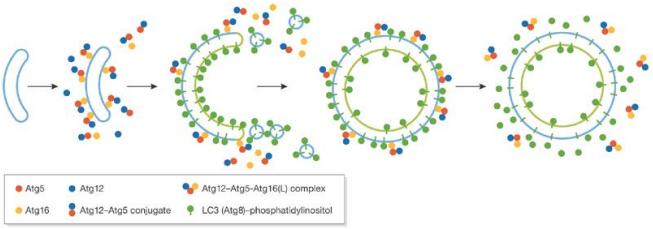
VIII. Transport of the resulting amino acids and lipids to the cytoplasm for recycling

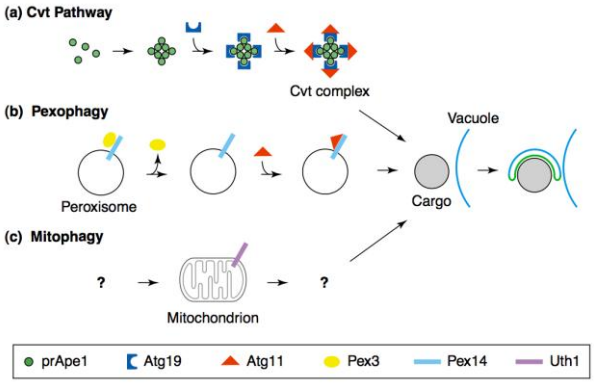
It is still not exactly known, where the autophagosome gets its double membrane from, some claim from the ER, others from the Golgi and others from the mitochondria; the issue is still controversial.

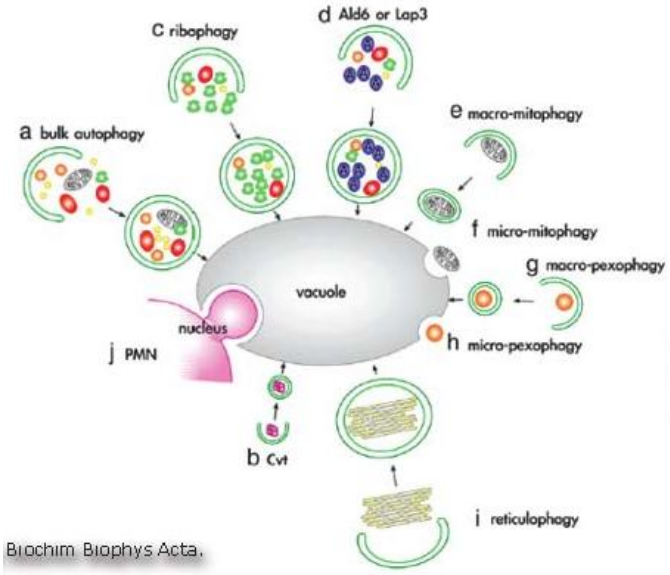
Finding components involved in autophagy: Do genetic screens, which genes are required for getting cargo into autophagosome etc. Biochemical approaches can then look at what is associated with cargo. Mass spectronomy is an important tool as well, what is in autophagosomes (problem here is that there are no means available to purify autophagosomes). GFP-tags can be used for localization experiments, and deletion of genes can be used for epistatic analysis. So far, 29 genes have been identified (ATG genes, only essential for starvation, so the genes are not used in other pathways but very specific for autophagy).

Many Atg-genes are essential for the induction of autophagy (Atg1 kinase complex), others for vesicle nucleation (Vps34, autophagy specific PI3K complex) and others for the expansion and completion of vesicles (2 ubiquitin-like systems important for expansion).





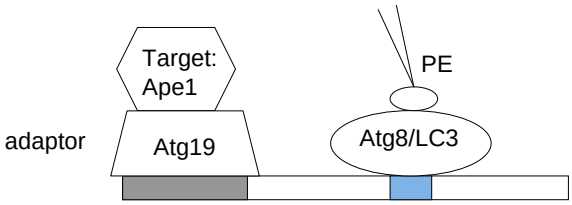




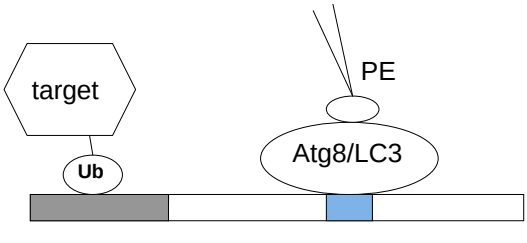
Target selection: Most pathways are highly selective (no bulk!). Atg11 e.g. is a specific protein which is not used in bulk autophagy, and ubiquitin seems to play a role as well.

Atg11 has an Atg19 binding site, and binds Atg8/LC3. The blue motif is also found on other proteins (PE=Phosphatidyl-ethanolamine).

p62 e.g. is involved in neurodegenerative diseases, protein aggregates are ubiquitinated (parkin is an E3 ubiquitin ligase that is required for mitophagy).



Atg11



p62

Regulated protein degradation

Ubiquitin-dependent degradation

26S proteasome

-single proteins

-> many cellular processes

Ub

Selective autophagy

Vacuolar/lysosomal proteases

- complexes/aggregates
- organelles

-> quality control
-> starvation