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Turnover of organelles by autophagy in yeast

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Efficient detection and removal of superfluous or damaged organelles are crucial to maintain cellular homeostasis and to assure cell survival. Growing evidence shows that organelles or parts of them can be removed by selective subtypes of otherwise unselective macroautophagy and microautophagy. This requires both the adaptation of the core autophagic machinery and sophisticated mechanisms to recognize organelles destined for turnover. We review the current knowledge on autophagic removal of peroxisomes, mitochondria, ER and parts of the nucleus with an emphasis on yeasts as a model eukaryote.

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Introduction

Autophagy encompasses all processes delivering cytosol and organelles to the lysosome for macromolecule turnover and recycling of building blocks. This definition includes non-vesicular cargo-delivery modes, such as 'chaperone-mediated autophagy (CMA)', and vesicular subtypes, such as macroautophagy and microautophagy [1]. While microautophagy sequesters cytosol into lysosomal invaginations, macroautophagy requires the formation of double membrane transport vesicles (autophagosomes) whose fusion with the lysosome releases autophagic bodies into the lysosome for degradation. The importance of autophagy during neurodegeneration, cancer, programmed cell death, ageing and removal of intracellular bacteria has drawn exponentially growing interest to these processes [1,2]. Starvation induced macroautophagy is generally considered an unselective process. In growing *S. cerevisiae* cells, the cytosol-to-vacuole transport (Cvt) pathway, which morphologically and genetically resembles macroautophagy, selectively targets aminopeptidase I precursor (prApe1) and α-mannosidase to the vacuole [3]. Here we discuss the selective autophagic removal of superfluous or damaged organelles focusing on yeasts.

Macroautophagy in *S. cerevisiae*

Since the isolation of autophagy mutants [4,5] 31 *ATG* (AuTophaGy) genes are known [6]. About half of the Atg proteins form the core machinery, essential for the biogenesis of both autophagosomes and Cvt vesicles. Other Atg proteins adapt this core machinery to the needs of autophagic subtypes.

Autophagosome biogenesis starts with the formation of a double-membrane structure termed the isolation membrane or phagophore, from a perivacuolar phagophore assembly site (PAS) (Figure 1). After elongation of the isolation membrane, its edges are sealed. Remarkably, this fusion step does not require yeast t-SNAREs or the yeast NSF Sec18 [7,8] suggesting that Atg, and perhaps other, proteins mediate these functions. In *S. cerevisiae* the core Atg proteins colocalize at the PAS, the site of autophagosome biogenesis [9–11], and form functional complexes, which assemble hierarchically.

The Atg1–kinase complex

The serine/threonine protein kinase, Atg1, and its regulatory factors Atg13, Atg17, Atg29 and Atg31 form a basic scaffold for PAS assembly during starvation [9–11,12•]. Atg1 and Atg13 are core Atg proteins, while Atg17, Atg29 and Atg31 are dispensable for the Cvt pathway. In rich media, Atg13 is phosphorylated depending on the rapamycin-sensitive TORC1. Upon induction of macroautophagy, Atg13 is dephosphorylated, and together with Atg17, Atg29, and Atg31, interacts with Atg1. This stimulates Atg1 kinase activity and modulates the autophagic response [3,10,13]. The kinase activity of Atg1 is essential for the Cvt pathway and macroautophagy, but dispensable for PAS assembly during starvation [3,9,10,13]. Probably, this kinase activity mediates dissociation of factors from the PAS.

Atg9 and its cycling system

The integral membrane protein, Atg9, is also recruited to the PAS early during its assembly. Atg9 additionally localizes to a peripheral compartment (PC) (at the ER or mitochondria) dependent on Atg1 (but not its kinase activity), Atg13, Atg2 and Atg18 [14••,15]. The absence of Atg23 or Atg27 leads to Atg9 accumulation at the PC [3].
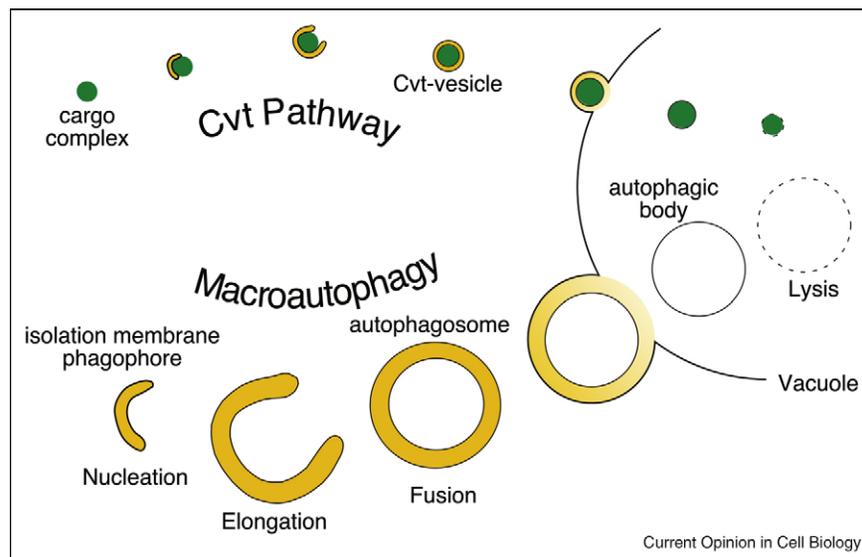
Morphology of macroautophagy and the Cvt pathway in *S. cerevisiae*. Starvation-induced macroautophagy starts with the formation of a double-layered isolation membrane or phagophore (nucleation). After elongation of this structure, fusion of the edges yields an autophagosome. The fusion of the autophagosomal outer membrane with the vacuole eventually releases an autophagic body into the vacuole for degradation. The morphologically similar Cvt pathway uses smaller transport vesicles, whose biogenesis starts at a cargo complex containing prApe1.

These findings led to a model wherein Atg9 cycles between its PC and the PAS [3] in vesicles that deliver membranes between these compartments. Since Atg9 is present at the PAS, but not on mature autophagosomes, this model postulates retrograde retrieval of Atg9 from the PAS. Atg23 and Atg27, proposed to act in Atg9 transport to the PAS, are specific for the Cvt pathway leading to the hypothesis that they only enhance Atg9 transport efficiency [3]. Self-association of Atg9 is also needed for efficient PAS targeting and for its function during the Cvt pathway and macroautophagy [16].

The precise role of the PAS during biogenesis of autophagosomes is still poorly understood. In a model that probably best fits the data, the core autophagic machinery first mediates assembly of the isolation membrane, then coordinates the incoming lipid flux and finally mediates sealing of the phagophore. Since components such as Atg9 are absent from mature autophagosomes, their retrograde retrieval must take place. In this model the PAS is a site where the autophagic machinery and the developing autophagosome converge.

The PtdIns3P-generating complex
In *S. cerevisiae* the sole PtdIns 3-kinase, Vps34, is present in two complexes. Complex I, consisting of Vps34, Vps15, Vps30/Atg6 and Atg14, localizes to the PAS and is required for autophagy. Complex II contains Vps38 instead of Atg14, acts in the vacuolar protein sorting pathway and localizes to endosomes and the vacuolar membrane [17]. A major function of PtdIns3P at the PAS is to recruit proteins such as Atg18. The *S. cerevisiae* genome contains Atg21 and Ygr223c, two Atg18 homologues. While Atg18 is a core Atg protein, Atg21 is Cvt-specific [18,19] and Ygr223c is needed for efficient micronucleophagy [20]. All three proteins bind PtdIns3P and PtdIns(3,5)P_2 [19,21,22]. Atg18 forms a complex with Atg2 and binds via PtdIns3P to the PAS [14$^{\bullet\bullet}$,23] and to endosomes [23,24]. PtdIns(3,5)P_2 is not needed for macroautophagy or the Cvt pathway [21,25]. Accordingly, Atg18, recruited to the vacuolar membrane via PtdIns(3,5)P_2, carries out non-autophagic functions such as the retrograde transport to the late endosome [21,26], a function that fits with its proposed role in Atg9 cycling.

The ubiquitin-like conjugation systems
Atg12 is covalently conjugated to Atg5 by a ubiquitin-related conjugation system consisting of Atg7 as an E1-like, and Atg10 as an E2-like, enzyme (Figure 2) [13,27$^{\bullet\bullet}$,28]. The Atg12–Atg5 conjugate then interacts with Atg16, whose self-interaction leads to oligomerization. This complex primarily decorates the outer membrane of the isolation membrane, but is absent from mature autophagosomes. This behaviour is reminiscent of coat complexes in vesicular trafficking in the secretory pathway, however a recent study suggests that the Atg16 level at the PAS is too low for such a function [29].

A second ubiquitin-like conjugation (Ublc) system using Atg7 as E1-enzyme and Atg3 as an E2-enzyme conjugates Atg8 to the membrane lipid phosphatidylethanolamine (PE) [13,28,30$^{\bullet\bullet}$]. To allow lipidation, the carboxyterminus

Figure 2
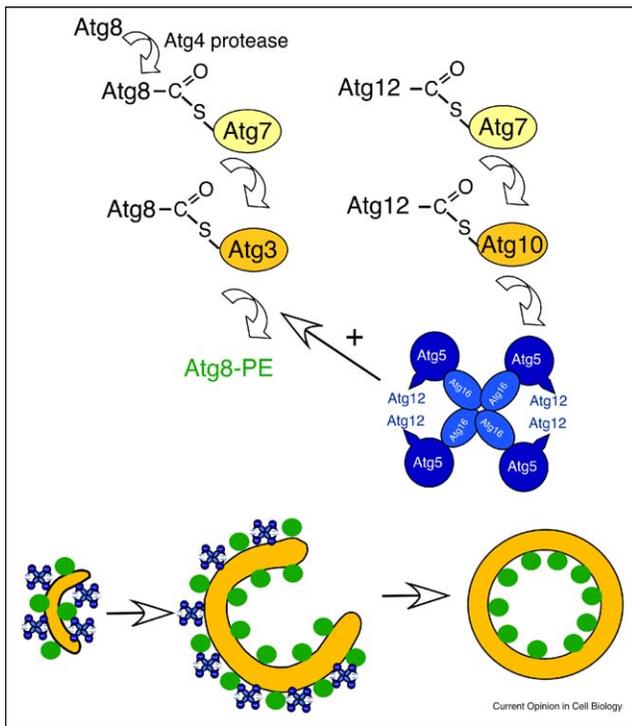
Scheme of the two ubiquitin-like conjugation systems involved in macroautophagy. After proteolytic processing of its carboxyterminus by the Atg4 protease, Atg8 is coupled to the membrane lipid phosphatidylethanolamine (PE). A second conjugation system attaches Atg12 to Atg5. The Atg12–Atg5 conjugate then forms an oligomer with Atg16 and stimulates the formation of Atg8-PE. As indicated, after elongation of the phagophore the Atg12–Atg5/Atg16 oligomer predominantly localizes to the outer membrane. The release of Atg8 from the mature autophagosome requires Atg4. Green circles: Atg8-PE.

of Atg8 must first be processed by the cysteine protease Atg4. Efficient macroautophagy additionally requires Atg4 for deconjugation of Atg8, which is found at the isolation membrane and at autophagosomes. Despite the deconjugation activity of Atg4, part of Atg8 is enclosed into autophagosomes and finally degraded, opening a way to monitor autophagy. Atg8 mediates membrane tethering and hemifusion and its expression level correlates with the size of autophagosomes [31[••],32[•]]. Atg8 hypothetically affects fusion of incoming vesicles with the isolation membrane during elongation and the final sealing of the phagophore.

The Atg12–Atg5 conjugate interacts with Atg3 and stimulates its activity similar to an E3 enzyme [33]. Work on a mammalian Atg16 homologue further suggests that it determines the cellular site of Atg8 lipidation [34].

Source of lipids for membrane expansion at the PAS
This is largely unknown. There are hints for an involvement of mitochondria [15], the trans-Golgi network and the endocytic system [35]. Additionally, the requirement

of early secretory proteins for macroautophagy was shown, suggesting a role for the ER or Golgi [7,8].

Intravacuolar events
As a final step of macroautophagy the autophagic bodies are lysed inside the vacuole dependent on the lipase-like Atg15 and vacuolar proteinases [25].

The Cvt pathway
In the Cvt pathway, the cargo pApeI is important for PAS assembly and Cvt vesicle biogenesis (Figure 1) [3]. prApe1 assembles into a large complex, which recruits its cargo receptor Atg19. This receptor further interacts with Atg8 and the Cvt-specific protein Atg11, which functions as a basic scaffold in assembling the Cvt-specific PAS by interacting with itself and a multitude of proteins such as Atg1, Atg9 and Atg17 [3]. This scaffolding function of Atg11 in rich medium resembles the function of the Atg17 complex during starvation. The levels of Atg11 and Atg17 do not change during the switch from the Cvt pathway to macroautophagy [29]. However, the interaction of Atg1 and Atg17 is strengthened upon starvation [10], suggesting a role of protein complex formation in switching the PAS from the Cvt pathway to macroautophagy.

Pexophagy
Peroxisome number and size can change rapidly in response to environmental and/or physiological cues. In yeast, conditions in which peroxisome metabolism is required will stimulate the biogenesis of these organelles. Different inducers can induce different types of peroxisomes. For example, most yeasts metabolize oleate and this medium induces small non-clustered peroxisomes, but in addition, methylotrophic yeasts (e.g. *P. pastoris*, *H. polymorpha*) metabolize methanol, which induces giant, clustered peroxisomes. Independent of the type of peroxisomes induced, growth of these yeast cells in a carbon source where the peroxisome metabolism is not required will induce their degradation by a selective type of autophagy, referred to as pexophagy.

Two modes of pexophagy – macropexophagy and micropexophagy – analogous to macroautophagy and microautophagy, occur in *P. pastoris* and *H. polymorpha*, however pexophagy exists in all species [36–39] (Figure 3). In macropexophagy, the isolation membrane engulfs one peroxisome at a time, forming a double-membrane vesicle called a pexophagosome, whose outer membrane fuses with the vacuolar membrane to deliver the pexophagic body and the peroxisome into the vacuolar lumen for degradation. By contrast, during micropexophagy, the vacuole membrane invaginates to engulf the peroxisome cluster. This membrane also protrudes along the peroxisome surface. This protrusion, denoted as the vacuolar sequestering membrane (VSM), often septates. In addition, a cup-shaped double-membrane structure, the

Figure 3
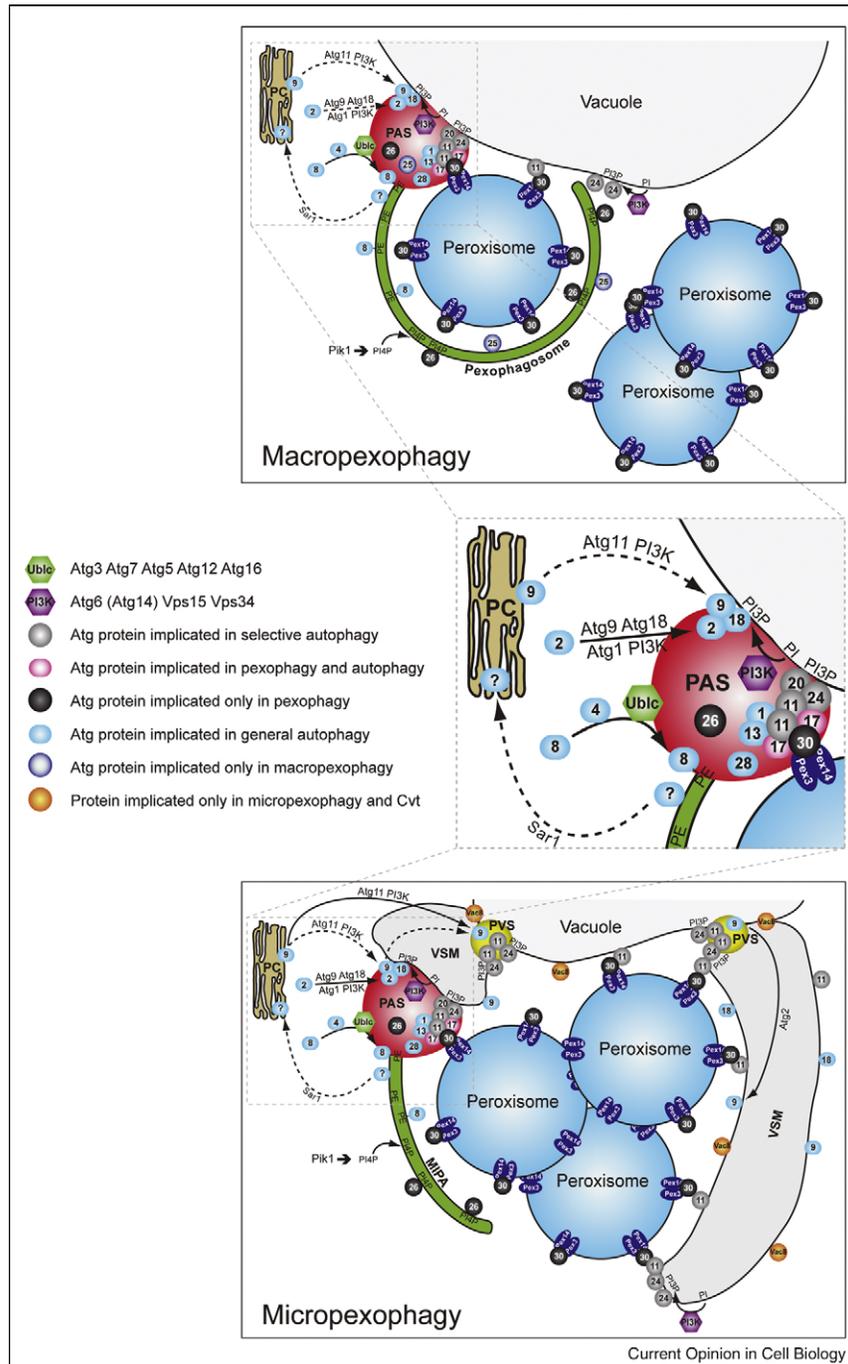
Macropexophagy and micropexophagy model. A late stage of pexophagy is represented in the figure, which shows the PAS, PC, PVS, VSM, MIPA and pexophagosome, and proteins localized to these structures. The figure summarizes the Atg protein requirements for pexophagy of several yeasts. The diagram depicts a steady state, meaning that not all these proteins are in these structures, and not all of them interact, simultaneously. Almost all the localization studies during pexophagy conditions have been done in methylotrophic yeasts and most of the interaction studies come from *S. cerevisiae*, during growing or starvation conditions. Atg14 has not been found in methylotrophic yeasts and probably is not involved in PtdIns3P formation at the vertex ring of the vacuolar membranes. Dashed arrow indicates speculative trafficking. Ublc: ubiquitin-like conjugation system, Pik1: PtdIns 4–kinase, PE: Phosphatidylethanolamine, PI3K: PtdIns 3–kinase complex, PI: PtdIns, PI3P: PtdIns3P, PI4P: PtdIns4P.

micropexophagic membrane apparatus (MIPA), is formed on the peroxisome surface and mediates fusion between the VSM tips engulfing the peroxisomes [40••].

The signaling events are not known but pexophagy can be induced by different media and the mode of pexophagy can vary [37,38,41]. Moreover, the same medium can induce different modes of pexophagy in different yeasts. Starvation can also induce pexophagy, together with autophagy, nevertheless in *P. pastoris*, pexophagy is a selective process and in the absence of the peroxisome receptor (Atg30), pexophagy does not occur [42••]. In *P. pastoris*, ATP levels determine the mode of pexophagy; high ATP levels induce micropexophagy and lower levels induce macropexophagy [43]. The higher ATP demand of micropexophagy could be associated with an ATP-dependent actin rearrangement for invagination of the vacuolar membrane.

Macropexophagy involves a multi-step sequestration of peroxisomes from the cytosol into pexophagosomes. These steps include the induction of the pathway, cargo selection, nucleation of the isolation membrane, expansion of this membrane, pexophagosome completion, retrieval of autophagic machinery components, fusion of pexophago-somes with the vacuole, breakdown of the pexophagic body and peroxisomes and recycling of the free amino acids and lipids to the cytosol. Because most of these steps are common to all autophagy-related pathways, it is not surprising that the same core Atg proteins (see macroau-tophagy section) are shared. Pexophagy adapts the autophagic machinery to become selective. A few selective factors have been discovered: one is Atg11, also involved in the delivery of the cargo complex to the PAS and the organization of this structure in the Cvt pathway, and a similar role has been postulated for pexophagy [44]; another factor is Atg30, the peroxisome receptor for pexophagy and the final target of a signaling cascade resulting in a phosphorylation event that induces the pathway [42••]. Atg30 localizes at the peroxisome membrane, interacting with two peroxins, Pex3 and Pex14. During pexophagy it interacts with the autophagy machinery, through Atg17 and Atg11, the latter interaction being dependent on Atg30 phosphorylation. Pex3 and Pex14 also play an important role in pexophagy in *H. polymorpha* [39].

Most morphological studies on pexophagy have been done in *P. pastoris* and *H. polymorpha*, taking advantage of their giant methanol-induced peroxisome clusters. Because of this giant cargo, the isolation membrane (pexophagosome and the MIPA) are larger and can be visualized distinctly from the other perivacuolar struc-tures. As in *S. cerevisiae*, Atg8 localizes to these isolation membranes [40••]. During peroxisome proliferation, the total Atg8 level increases, localizing mostly to the cytosol. However, upon a shift to pexophagy conditions, the Atg8 level remains the same but the lipidated Atg8 (Atg8-PE)

levels double and its localization changes to a perivacuolar dot, the PAS, the site where the MIPA and pexophago-some will form. Most of the autophagic machinery loca-lizes soon thereafter at the PAS, where proteins such as Atg5, Atg8, Atg9, Atg11, Atg16, Atg17, Atg24, Atg25, Atg26, Atg28 and Atg30 have been localized during peroxisome proliferation or pexophagy conditions [42••,45–48,49•]. The assembly of the MIPA from the PAS requires at least Atg1, Atg2, Atg3, Atg4, Atg7, Atg8, Atg9, Atg11, Atg26, Atg30 and Vps15 [39,42••,47], (R. Mathewson, personal communication). In summary, MIPA and probably pexophagosome formation, require the complete set of core Atg proteins and the pexophagy-specific factors Atg11, Atg26 and Atg30. In *H. polymorpha*, pexophagosome formation also requires Atg25, another protein that localizes to this isolation membrane [46].

Atg26, a UDP–glucose:sterol glucosyltransferase, whose function has not been elucidated, is required exclusively for pexophagy of large, methanol-induced peroxisomes [41]. Atg26 binds phosphatidylinositol 4-phosphate (PtdIns4P), a lipid that is required for its localization at the MIPA and pexophagosome [49•,50]. Therefore the formation of PtdIns4P by a phosphatidylinositol-4-OH kinase (Pik1) is essential for MIPA (and probably pexophagosome) formation, as well as for pexophagy in methylotrophic yeasts.

The signaling event that induces the formation of VSMs during micropexophagy is independent of Atg30 phos-phorylation and even the presence of peroxisomes. Sev-eral mutants do not form the complete VSMs, such as Δatg2, Δatg9, Δatg11, Δatg18, Δatg28, Δvac8 and Δvps15 [39,44,51•,52,53]. Interestingly, Atg11 and Atg9 coloca-lize to perivacuolar dot-like structures (PVSs) distinct from the PAS, and located at the base of the VSMs [52]. This PVS is supposed to be important for VSM formation. Atg9 shuttles from a peripheral compartment (PC) [somewhere in the ER in *P. pastoris* or near mito-chondria in *S. cerevisiae*] to the PVS, in an Atg11-de-pendent and Vps15-dependent manner, but it is unclear if it goes directly or through the PAS [52]. The movement of Atg9 from the PVS to the VSM requires Atg2 [52], which shuttles from the cytosol to a dot-like structure juxtaposed to the vacuole, probably the PAS, and this trafficking requires the function of Atg1, Atg9, Atg18 and Vps15. It is possible that Atg2 and/or Atg9 trafficking contribute to deliver membrane to the PAS and/or PVS for the elongation of isolation membranes and/or VSMs, respectively. After the VSMs form, Atg11 localizes mainly on the VSM and this localization depends on Vac8, which is also localized at the VSM [44,53]. Potentially, Atg11 at the VSM could interact with Atg30, to help peroxisome recognition by the VSMs.

Similar to the Cvt and autophagy pathways, the isolation membrane is absent in most of the autophagy mutants,

suggesting the presence of retrograde pathways. A recent study suggests that the Ras-like small GTPase, Sar1, could play a role in recycling ER components from the pexophagosome/MIPA back to the ER, and this event could be a prerequisite for isolation membrane formation and maturation [54•].

Finally, closure of the isolation membrane proceeds via the fusion of the MIPA with the VSMs, or by fusion of the pexophagosome with the vacuole membrane, for micro-pexophagy and macropexophagy, respectively. These fusion events require Atg24 [45], a protein that binds phosphatidylinositol 3-phosphate (PtdIns3P) and that accumulates at the vertex ring of the contact area be-tween: (1) septated vacuoles, (2) the MIPA and tips of the VSMs, and, (3) the pexophagosome and vacuole mem-brane. Some Atg24 localizes close to or at the PVS, but no colocalization data are available. Vam7, a component of a t-SNARE complex, is also involved in a late stage of pexophagy, probably in the fusion between the pexopha-gosome/MIPA and the vacuolar membranes. Vac8 is required for vacuolar homotypic fusion and it has been suggested that this function is also required for a late event of micropexophagy [53].

Micronucleophagy (piecemeal microautophagy of the nucleus, PMN)
In *S. cerevisiae* the interaction of the ER membrane protein, Nvj1, and vacuolar Vac8 generates contacts between the nuclear envelope/ER and the vacuole. Upon starvation, in a process termed micronucleophagy or PMN, these junctions bulge into invaginations of the vacuole (Figure 4). Subsequent fission of the nuclear ER membranes and fusion of the vacuolar membrane then releases vesicles containing nuclear material into the vacuole for degradation [55]. Consequently, the PMN vesicles are limited by three membranes, the outermost derived from the vacuole, the other two from the nuclear ER.

Nvj1 further recruits the enoyl–CoA reductase Tsc13 and Osh1, a homologue of a mammalian oxysterol-binding protein, to the nucleus–vacuole junctions [56]. Following the breakdown of these markers therefore permits monitoring of micronucleophagy, which requires, besides the core Atg proteins, a set of subtype-specific Atg proteins, including Atg11 and Atg17 [20]. Efficient micronu-cleophagy also depends on the Atg18 homologue, Ygr223c [24]. Typical components of the homotypic vacuole fusion machinery are not needed for micronu-cleophagy [20]. On the basis of this and electron micro-scopic data, it has been speculated that the Atg machinery might mediate the formation of the vacuolar protrusions and their final fusion functions that are similar to those used during micropexophagy.

Mitophagy
Deterioration of mitochondrial function might lead to the production of excess reactive oxygen species or the untimely release of pro-apoptotic proteins causing cell death. Accordingly, as a quality control system, the controlled removal of mitochondria via autophagy is expected. Indeed, in autophagy-deficient *S. cerevisiae* cells, reduced mitochondrial functionality was detected, compatible with an accumulation of damaged mitochon-dria [57]. On the contrary, the lack of a mitochondrial membrane potential ($\Delta\Psi$) [58] and osmotic swelling caused by the depletion of Mdm38 [59] induced autop-hagic degradation of mitochondria. Also the lack of mitochondrial ATP-dependent metalloproteinase, Yme1, on non-fermentable carbon sources increased vacuolar degradation of mitochondria. Remarkably, these conditions did not increase vacuolar turnover of cytosol indicating selective mitophagy [60].

Figure 4
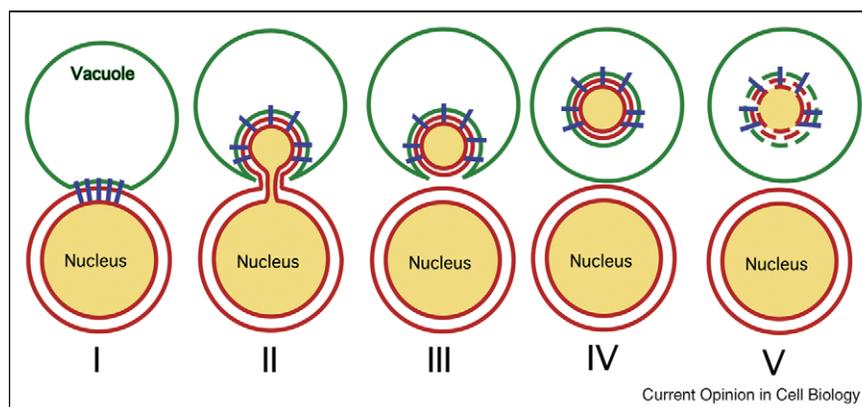
Different stages of micronucleophagy. In *S. cerevisiae* the vacuolar membrane and the nuclear envelope/ER form contact sites by the interaction of the ER membrane protein, Nvj1, and vacuolar Vac8 (I). During starvation these junctions bulge into invaginations of the vacuole (II). Then a micronucleus buds off (III) and after fusion of the vacuolar extensions (IV), a vesicle limited by three membranes is released for degradation (V) into the vacuole. Figure adapted from [20].

So far in *S. cerevisiae* the highest mitochondrial turnover was not measured by compromising mitochondrial function, but after shifting wild-type cells from a medium containing the non-fermentable carbon source, lactate, to nitrogen-free glucose-medium [61]. Under these conditions electron microscopy suggests the occurrence of two distinct types of micromitophagy. In subtype I, mitochondria are engulfed by vacuolar indentations together with cytosolic material, while in the variant II only mitochondria are sequestered [62]. Both mitochondrial uptake processes depend on almost the complete set of Atg proteins, including the Cvt-specific, Atg11, and the macroautophagy-specific Atg17 and Atg29, but not on the prApe1-receptor, Atg19 [61,62]. The mitochondrial outer membrane protein, Uth1, is required only for micromitophagy subtype II [62], while the mitochondrial protein phosphatase homologue, Aup1, is generally implicated in mitophagy [63].

Mitochondria dynamically undergo fission and fusion events. Inhibition/deletion of the dynamin-like GTPase Drp1 (in yeast Dnm1) interfered with mitophagy in both mammalian and *S. cerevisiae* cells, showing the importance of mitochondrial fission for mitophagy [59,64]. In mammalian cells the occurrence of a macromitophagic process was suggested, which might be due to the size of lysosomes [65•,66]. As part of its housekeeping function, starvation-induced unselective macroautophagy also can randomly sequester mitochondria [3,13].

ER-Phagy
The ER is a major centre for protein folding and post-translational modifications. A network of quality control systems monitors the fidelity of these processes. During ER-associated degradation (ERAD) misfolded proteins are transported from the ER to the cytosol and degraded by the ubiquitin–proteasome system. ER stress, caused for example by dithiothreitol (DTT), interfering with the formation of disulfide bonds, or the use of tunicamycin, an inhibitor of glycosylation, causes the accumulation of unfolded proteins and induces the unfolded protein response (UPR). The presence of unfolded proteins in the ER-lumen is transmitted to the cytosol by activating the endoribonuclease activity of the transmembrane ER protein, Ire1. Splicing of the HAC1 mRNA then leads to translation of an active Hac1 transcription factor. Active Hac1 induces the transcription of a large set of genes increasing the ER-folding capacity and stimulating ERAD. To restore ER homeostasis, the autophagic machinery is additionally activated, when either the capacity of the proteasomal system is exceeded or when the misfolded proteins aggregate, impairing their transport to the cytosol [67–69]. Indeed, DTT-treated *S. cerevisiae* cells showed increased Atg8 levels and lipidation [67,69]. Under these conditions the Atg8-dependent formation of autophagosome-like structures containing tightly packed stacks of ER reverses the stress-induced proliferation of ER [67]. Remarkably, the outer membrane of these structures was continuous with the ER and studded with ribosomes, suggesting a role of the ER as a membrane source for at least these specialized type of autophagosomes. The expression of active Hac1 raised the Atg8 levels, but was unable to induce formation of ER-containing autophagosomes. This suggests that induction of ER-Phagy requires also an Ire1 and Hac1-independent signal [67], Astonishingly, the sequestration, but not the vacuolar degradation of the proliferated ER, seems to be sufficient to restore viability [67]. At the moment it is unclear how ER membranes are marked for selective uptake into autophagosomes. One possibility is that misfolded proteins or protein aggregates are concentrated in specialized areas of the ER destined for ER-Phagy.

Conclusions
Selective and unselective forms of autophagy play crucial roles in organelle homeostasis. However, many molecular details, for example the recognition of damaged organelles by the autophagic machinery, are hardly understood. Most probably, related mechanisms are used in recognition of intracellularly replicating bacteria [1,2]. We therefore expect that deeper understanding of selective subtypes of autophagy will also provide us with new tools to fight diseases.

Acknowledgements

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Mizushima N, Levine B, Cuervo AM, Klionsky DJ: **Autophagy fights disease through cellular self-digestion**. *Nature* 2008, **451**:1069-1075.

2. Levine B, Kroemer G: **Autophagy in the pathogenesis of disease**. *Cell* 2008, **132**:27-42.

3. Xie Z, Klionsky DJ: **Autophagosome formation: core machinery and adaptations**. *Nat Cell Biol* 2007, **9**:1102-1109.

4. Tsukada M, Ohsumi Y: **Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae***. *FEBS Lett* 1993, **333**:169-174.

5. Thumm M, Egner R, Koch B, Schlumpberger M, Straub M, Veenhuis M, Wolf DH: **Isolation of autophagocytosis mutants of *Saccharomyces cerevisiae***. *FEBS Lett* 1994, **349**:275-280.

6. Klionsky DJ, Cregg JM, Dunn WA, Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M et al.: **A unified nomenclature for yeast autophagy-related genes**. *Dev Cell* 2003, **5**:539-545.

7. Ishihara N, Hamasaki M, Yokota S, Suzuki K, Kamada Y, Kihara A, Yoshimori T, Noda T, Ohsumi Y: **Autophagosome requires specific early Sec proteins for its formation and NSF/SNARE for vacuolar fusion**. *Mol Biol Cell* 2001, **12**:3690-3702.

8. Reggiori F, Wang CW, Nair U, Shintani T, Abeliovich H, Klionsky DJ: **Early stages of the secretory pathway, but not**

endosomes, are required for Cvt vesicle and autophagosome assembly in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2004, **15**:2189-2204.

9. Cheong H, Nair U, Geng J, Klionsky DJ: **The Atg1 kinase complex is involved in the regulation of protein recruitment to initiate sequestering vesicle formation for nonspecific autophagy in** *Saccharomyces cerevisiae*. *Mol Biol Cell* 2008, **19**:668-681.

10. Kawamata T, Kamada Y, Kabeya Y, Sekito T, Ohsumi Y: **Organization of the pre-autophagosomal structure responsible for autophagosome formation**. *Mol Biol Cell* 2008, **19**:2039-2050.

11. Suzuki K, Kubota Y, Sekito T, Ohsumi Y: **Hierarchy of Atg proteins in pre-autophagosomal structure organization**. *Genes Cells* 2007, **12**:209-218.

12. Cao Y, Cheong H, Song H, Klionsky DJ: *In vivo* **reconstitution of**
• **autophagy in** *Saccharomyces cerevisiae*. *J Cell Biol* 2008, **182**:703-713.
In a very elegant approach, 24 *ATG*-genes were consecutively deleted. This multiple knockout strain allowed the study of the assembly of defined Atg proteins at the PAS in the absence of the others.

13. Suzuki K, Ohsumi Y: **Molecular machinery of autophagosome formation in yeast,** *Saccharomyces cerevisiae*. *FEBS Lett* 2007, **581**:2156-2161.

14. Reggiori F, Tucker KA, Stromhaug PE, Klionsky DJ: **The Atg1-Atg13**
•• **complex regulates Atg9 and Atg23 retrieval transport from the pre-autophagosomal structure**. *Dev Cell* 2004, **6**:79-90.
The authors present for the first time the idea of a retrograde retrieval of the membrane protein, Atg9, from the PAS.

15. Reggiori F, Shintani T, Nair U, Klionsky DJ: **Atg9 cycles between mitochondria and the pre-autophagosomal structure in yeasts**. *Autophagy* 2005, **1**:101-109.

16. He C, Baba M, Cao Y, Klionsky DJ: **Self-interaction is critical for Atg9 transport and function at the phagophore assembly site during autophagy**. *Mol Biol Cell* 2008, **19**:5506-5516.

17. Obara K, Sekito T, Ohsumi Y: **Assortment of phosphatidylinositol 3−kinase complexes—Atg14p directs association of complex I to the pre-autophagosomal structure in** *Saccharomyces cerevisiae*. *Mol Biol Cell* 2006, **17**:1527-1539.

18. Meiling-Wesse K, Barth H, Voss C, Eskelinen EL, Epple UD, Thumm M: **Atg21 is required for effective recruitment of Atg8 to the preautophagosomal structure during the Cvt pathway**. *J Biol Chem* 2004, **279**:37741-37750.

19. Stromhaug PE, Reggiori F, Guan J, Wang CW, Klionsky DJ: **Atg21 is a phosphoinositide binding protein required for efficient lipidation and localization of Atg8 during uptake of aminopeptidase I by selective autophagy**. *Mol Biol Cell* 2004, **15**:3553-3566.

20. Krick R, Muehe Y, Prick T, Bremer S, Schlotterhose P, Eskelinen EL, Millen JI, Goldfarb DS, Thumm M: **Piecemeal microautophagy of the nucleus requires the core macroautophagy genes**. *Mol Biol Cell* 2008, **19**:4492-4505.

21. Dove SK, Piper RC, McEwen RK, Yu JW, King MC, Hughes DC, Thuring J, Holmes AB, Cooke FT, Michell RH *et al.*: **Svp1p defines a family of phosphatidylinositol 3,5-bisphosphate effectors**. *EMBO J* 2004, **23**:1922-1933.

22. Krick R, Tolstrup J, Appelles A, Henke S, Thumm M: **The relevance of the phosphatidylinositolphosphat-binding motif FRRGT of Atg18 and Atg21 for the Cvt pathway and autophagy**. *FEBS Lett* 2006, **580**:4632-4638.

23. Obara K, Sekito T, Niimi K, Ohsumi Y: **The ATG18-ATG2 complex is recruited to autophagic membranes via PtdIns(3)P and exerts an essential function**. *J Biol Chem* 2008, **282**:23972-23980.

24. Krick R, Henke S, Tolstrup J, Thumm M: **Dissecting the localization and function of Atg18, Atg21 and Ygr223c**. *Autophagy* 2008, **4**:896-905.

25. Epple UD, Eskelinen EL, Thumm M: **Intravacuolar membrane lysis in** *Saccharomyces cerevisiae*. **Does vacuolar targeting of Cvt17/Aut5p affect its function?** *J Biol Chem* 2003, **278**:7810-7821.

26. Efe JA, Botelho RJ, Emr SD: **Atg18 regulates organelle morphology and Fab1 kinase activity independent of its membrane recruitment by phosphatidylinositol 3,5-bisphosphate**. *Mol Biol Cell* 2007, **18**:4232-4244.

27. Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD,
•• Klionsky DJ, Ohsumi M, Ohsumi Y: **A protein conjugation system essential for autophagy**. *Nature* 1998, **395**:395-398.
This hallmark paper reports the formation of the Atg12−Atg5 conjugate by a ubiquitin-related system.

28. Geng J, Klionsky DJ: **The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. 'Protein modifications: beyond the usual suspects' review series**. *EMBO Rep* 2008, **9**:859-864.

29. Geng J, Baba M, Nair U, Klionsky DJ: **Quantitative analysis of autophagy-related protein stoichiometry by fluorescence microscopy**. *J Cell Biol* 2008, **182**:129-140.

30. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y,
•• Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M *et al.*: **A ubiquitin-like system mediates protein lipidation**. *Nature* 2000, **408**:488-492.
Here conjugation of Atg8 to phosphatidylethanolamine by a ubiquitin-related system is uncovered.

31. Nakatogawa H, Ichimura Y, Ohsumi Y: **Atg8, a ubiquitin-like**
•• **protein required for autophagosome formation, mediates membrane tethering and hemifusion**. *Cell* 2007, **130**:165-178.
In vitro reconstitution of Atg8 lipidation points to a function of the conjugate in membrane tethering and fusion.

32. Xie Z, Nair U, Klionsky DJ: **Atg8 controls phagophore expansion**
• **during autophagosome formation**. *Mol Biol Cell* 2008, **19**:3290-3298.
This paper describes a relationship between the size of autophagosomes and the expression level of Atg8.

33. Hanada T, Noda NN, Satomi Y, Ichimura Y, Fujioka Y, Takao T, Inagaki F, Ohsumi Y: **The Atg12−Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy**. *J Biol Chem* 2007, **282**:37298-37302.

34. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T: **The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy**. *Mol Biol Cell* 2008, **19**:2092-2100.

35. Young AR, Chan EY, Hu X, Kochl R, Crawshaw SG, High S, Hailey DW, Lippincott-Schwartz J, Tooze SA: **Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes**. *J Cell Sci* 2006, **119**:3888-3900.

36. Hutchins MU, Veenhuis M, Klionsky DJ: **Peroxisome degradation in** *Saccharomyces cerevisiae* **is dependent on machinery of macroautophagy and the Cvt pathway**. *J Cell Sci* 1999, **112**:4079-4087.

37. Sakai Y, Koller A, Rangell LK, Keller GA, Subramani S: **Peroxisome degradation by microautophagy in** *Pichia pastoris*: **identification of specific steps and morphological intermediates**. *J Cell Biol* 1998, **141**:625-636.

38. Veenhuis M, Douma A, Harder W, Osumi M: **Degradation and turnover of peroxisomes in the yeast** *Hansenula polymorpha* **induced by selective inactivation of peroxisomal enzymes**. *Arch Microbiol* 1983, **134**:193-203.

39. Dunn WA, Cregg JM, Kiel JA, van der Klei IJ, Oku M, Sakai Y, Sibirny AA, Stasyk OV, Veenhuis M: **Pexophagy: the selective autophagy of peroxisomes**. *Autophagy* 2005, **1**:75-83.

40. Mukaiyama H, Baba M, Osumi M, Aoyagi S, Kato N, Ohsumi Y,
•• Sakai Y: **Modification of a ubiquitin-like protein Paz2 conducted micropexophagy through formation of a novel membrane structure**. *Mol Biol Cell* 2004, **15**:58-70.
The authors detected by fluorescence microscopy the isolation membranes (MIPA and pexophagosome) during pexophagy, using Atg8 as a marker and studied its behaviour in this pathway.

41. Nazarko TY, Polupanov AS, Manjithaya RR, Subramani S, Sibirny AA: **The requirement of sterol glucoside for pexophagy in yeast is dependent on the species and nature of peroxisome inducers**. *Mol Biol Cell* 2007, **18**:106-118.

42. Farré JC, Manjithaya R, Mathewson RD, Subramani S: **PpAtg30**
•• **tags peroxisomes for turnover by selective autophagy**. *Developmental Cell* 2008, **14**:365-376.
Represents the first description, and mechanistic insight into the role, of an organelle-specific receptor that delivers an organelle, the peroxisome, to the autophagic machinery for turnover.

43. Ano Y, Hattori T, Kato N, Sakai Y: **Intracellular ATP correlates with mode of pexophagy in *Pichia pastoris***. *Biosci Biotechnol Biochem* 2005, **69**:1527-1533.

44. Kim J, Kamada Y, Stromhaug PE, Guan J, Hefner-Gravink A, Baba M, Scott SV, Ohsumi Y, Dunn WA, Klionsky DJ: **Cvt9/Gsa9 functions in sequestering selective cytosolic cargo destined for the vacuole**. *J Cell Biol* 2001, **153**:381-396.

45. Ano Y, Hattori T, Oku M, Mukaiyama H, Baba M, Ohsumi Y, Kato N, Sakai Y: **A sorting nexin PpAtg24 regulates vacuolar membrane dynamics during pexophagy via binding to phosphatidylinositol-3-phosphate**. *Mol Biol Cell* 2005, **16**:446-457.

46. Monastyrska I, Kiel JA, Krikken AM, Komduur JA, Veenhuis M, van der Klei IJ: **The *Hansenula polymorpha ATG25* gene encodes a novel coiled-coil protein that is required for macropexophagy**. *Autophagy* 2005, **1**:92-100.

47. Schroder LA, Dunn WA: **PpAtg9 trafficking during micropexophagy in *Pichia pastoris***. *Autophagy* 2006, **2**:52-54.

48. Stasyk OV, Stasyk OG, Mathewson RD, Farre JC, Nazarko VY, Krasovska OS, Subramani S, Cregg JM, Sibirny AA: **Atg28, a novel coiled-coil protein involved in autophagic degradation of peroxisomes in the methylotrophic yeast *Pichia pastoris***. *Autophagy* 2006, **2**:30-38.

49. Yamashita S, Oku M, Wasada Y, Ano Y, Sakai Y: **PI4P-signaling**
• **pathway for the synthesis of a nascent membrane structure in selective autophagy**. *J Cell Biol* 2006, **173**:709-717.
Describes a novel role for PtdIns4P and Pik1 in pexophagy of methanol-induced peroxisomes in *P. pastoris*.

50. Oku M, Warnecke D, Noda T, Muller F, Heinz E, Mukaiyama H, Kato N, Sakai Y: **Peroxisome degradation requires catalytically active sterol glucosyltransferase with a GRAM domain**. *EMBO J* 2003, **22**:3231-3241.

51. Mukaiyama H, Oku M, Baba M, Samizo T, Hammond AT, Glick BS,
• Kato N, Sakai Y: **Paz2 and 13 other *PAZ* gene products regulate vacuolar engulfment of peroxisomes during micropexophagy**. *Genes Cells* 2002, **7**:75-90.
Together with reference [37], this study describes the morphological steps of two different forms of pexophagy: macropexophagy and micro-pexophagy and the first comprehensive analysis of genes required for pexophagy.

52. Chang T, Schroder LA, Thomson JM, Klocman AS, Tomasini AJ, Stromhaug PE, Dunn WA: **PpATG9 encodes a novel membrane protein that traffics to vacuolar membranes, which sequester peroxisomes during pexophagy in *Pichia pastoris***. *Mol Biol Cell* 2005, **16**:4941-4953.

53. Fry MR, Thomson JM, Tomasini AJ, Dunn WA: **Early and late molecular events of glucose-induced pexophagy in *Pichia pastoris* require Vac8**. *Autophagy* 2006, **2**:280-288.

54. Schroder LA, Ortiz MV, Dunn WA: **The membrane dynamics of**
• **pexophagy are influenced by Sar1p in *Pichia pastoris***. *Mol Biol Cell* 2008, **19**:4888-4899.
This study suggests retrograde trafficking from the pexophagosome to the ER and the participation of Sar1 in this process.

55. Roberts P, Moshitch-Moshkovitz S, Kvam E, O'Toole E, Winey M, Goldfarb DS: **Piecemeal microautophagy of nucleus in *Saccharomyces cerevisiae***. *Mol Biol Cell* 2003, **14**:129-141.

56. Kvam E, Goldfarb DS: **Nucleus–vacuole junctions and piecemeal microautophagy of the nucleus in *S. cerevisiae***. *Autophagy* 2007, **3**:85-92.

57. Zhang Y, Qi H, Taylor R, Xu W, Liu LF, Jin S: **The role of autophagy in mitochondria maintenance: characterization of mitochondrial functions in autophagy-deficient *S. cerevisiae* strains**. *Autophagy* 2007, **3**:337-346.

58. Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, Martinou JC: **Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast**. *Cell Death Differ* 2005, **12**:1613-1621.

59. Nowikovsky K, Reipert S, Devenish RJ, Schweyen RJ: **Mdm38 protein depletion causes loss of mitochondrial K+/H+ exchange activity, osmotic swelling and mitophagy**. *Cell Death Differ* 2007, **14**:1647-1656.

60. Campbell CL, Thorsness PE: **Escape of mitochondrial DNA to the nucleus in *yme1* yeast is mediated by vacuolar-dependent turnover of abnormal mitochondrial compartments**. *J Cell Sci* 1998, **111**:2455-2464.

61. Kanki T, Klionsky DJ: **Mitophagy in yeast occurs through a selective mechanism**. *J Biol Chem* 2008, **283**:32386-32393.

62. Kissova I, Salin B, Schaeffer J, Bhatia S, Manon S, Camougrand N: **Selective and non-selective autophagic degradation of mitochondria in yeast**. *Autophagy* 2007, **3**:329-336.

63. Tal R, Winter G, Ecker N, Klionsky DJ, Abeliovich H: **Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival**. *J Biol Chem* 2007, **282**:5617-5624.

64. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G et al.: **Fission and selective fusion govern mitochondrial segregation and elimination by autophagy**. *EMBO J* 2008, **27**:433-446.

65. Narendra D, Tanaka A, Suen DF, Youle RJ: **Parkin is recruited**
• **selectively to impaired mitochondria and promotes their autophagy**. *J Cell Biol* 2008, **183**:795-803.
Parkin is a mammalian ubiquitin ligase, whose defect leads to early onset of Parkinson's disease. After the addition of the mitochondrial uncoupler CCCP, Parkin was selectively recruited to mitochondria with low membrane potential. These mitochondria were subsequently degraded via mitophagy in an Atg5-dependent manner.

66. Kim I, Rodriguez-Enriquez S, Lemasters JJ: **Selective degradation of mitochondria by mitophagy**. *Arch Biochem Biophys* 2007, **462**:245-253.

67. Bernales S, McDonald KL, Walter P: **Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response**. *PLoS Biol* 2006, **4**:e423.

68. Kruse KB, Brodsky JL, McCracken AA: **Characterization of an ERAD gene as *VPS30/ATG6* reveals two alternative and functionally distinct protein quality control pathways: one for soluble Z variant of human alpha-1 proteinase inhibitor (A1PiZ) and another for aggregates of A1PiZ**. *Mol Biol Cell* 2006, **17**:203-212.

69. Yorimitsu T, Nair U, Yang Z, Klionsky DJ: **Endoplasmic reticulum stress triggers autophagy**. *J Biol Chem* 2006, **281**:30299-30304.