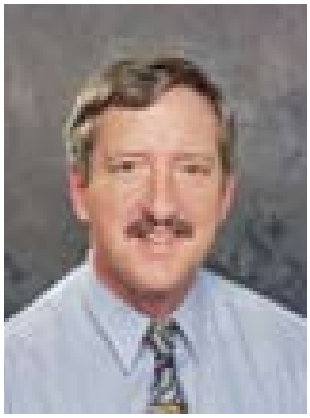
Organelle turnover by autophagy



Kristina N Turcic, Dalibor Mijaljica, Carlos Rosado, Mark Prescott & Rodney J Devenish

Department of Biochemistry & Molecular Biology, and ARC Centre for Excellence in Structural and Functional Microbial Genomics, Monash University, Clayton Campus
Melbourne VIC 3800 Australia
Tel: +61 3 9905 3782
Fax: +61 3 9905 3726
Email: rod.devenish@med.monash.edu.au

All eukaryotic cells turn over (degrade) parts of their internal structure, including organelles, by autophagy ('self eating'), a process that utilises a specialised compartment of cells; the vacuole in yeast and the lysosome in mammals. Defects in autophagy are increasingly being linked to pathological conditions, including neurodegenerative and muscle diseases and some forms of cancer. In addition, several studies report a role for autophagy as a mechanism for the removal of invading bacteria (e.g. *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, *Shigella flexneri*, *Listeria monocytogenes*) and viruses (e.g. Epstein-Barr virus and *herpes simplex* virus) from mammalian cells [1,2]. Organelle turnover is an essential process required for normal cellular homeostasis, growth and development. Recent studies show that organelle turnover can be a selective process involving new genes not yet fully characterised. Amongst these is OTP1, a gene specifically required for mitochondrial turnover in yeast.

The molecular mechanism of organelle turnover by autophagy is only now being characterised in detail. Autophagy is a conserved process in eukaryotes and is believed to be the only protein degradation pathway capable of degrading entire organelles [3]. Yeast *(S. cerevisiae)* has served as a model organism by which genetic analysis of the process has been greatly facilitated. In yeast two different types of autophagy, microautophagy and macroautophagy, occur (Figure 1). Microautophagy involves the direct sequestration of cytosol and cytosolic constituents by invagination of the vacuolar membrane [4]. Macroautophagy, is induced by stress or starvation and involves the sequestration of portions of the cytosol within a double membrane vesicle, (termed the autophagosome), which is then delivered to the vacuole where the sequestered material is degraded and recycled [4]. This process is regulated by Au*T*opha*G*y related genes (ATG genes).

To date, twenty-nine ATG genes have been identified as being specifically involved in and essential for macroautophagy and related processes. In many cases the requirement for these genes was determined by the lack of the characteristic accumulation of autophagic bodies in the vacuole (as observed using light microscopy), in mutant strains upon nutrient deprivation [5]. However, this (and other) means of characterisation did not specifically look at organelle turnover. As a result it was assumed that organelles, along with portions of the cytosol, were randomly sequestered into autophagic vesicles. However, evidence now suggests that in some circumstances the sequestration of organelles for degradation by autophagy is not random and that there must be genes that are required for the selective turnover of organelles.

There has been an increasing number of reports on the selective turnover of various organelles including peroxisomes, the nucleus, the endoplasmic reticulum (ER) and mitochondria.

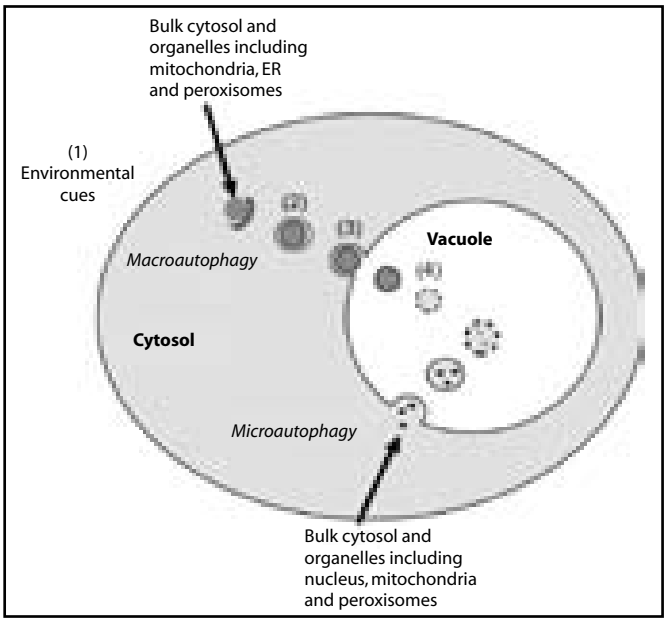


Figure 1. Macroautophagy and microautophagy in yeast. Macroautophagy can be divided into four distinct steps: 1) induction by environmental cues such as starvation or stress; 2) formation and completion of the double membrane vesicle (the autophagosome) and sequestration of cytosol and/or organelles; 3) docking and fusion of the vesicle onto the vacuolar membrane; 4) breakdown and recycling of the sequestered cargo. In contrast, microautophagy involves the invagination of the vacuolar membrane and direct sequestration of cytosol and/or organelles.

The selective turnover of peroxisomes, termed 'pexophagy' (best described in the methylotrophic yeast *Hansenula polymorpha*, *Pichia pastoris* and *Pichia methanolica* [6]), is seen when yeast are shifted from growth medium containing methanol or oleic acid as the carbon source (a condition under which peroxisomes proliferate), to growth medium containing glucose or ethanol as the carbon source. This leads to the selective sequestration and degradation of excess peroxisomes by either macroautophagy or microautophagy (dependent on the carbon source) [7]. Another selective type of microautophagy is piecemeal microautophagy of the nucleus (PMN). Here, regions of the nucleus are pinched off and released into the vacuole for degradation, allowing for the removal of non-essential components of the nucleus [8]. Similarly, the ER is selectively turned over by macroautophagy, which occurs in response to ER stress [9]. Lastly, the selective turnover of mitochondria, termed mitophagy, has been observed in yeast [10]. Two genes, *UTH1* and *AUP1,* in addition to the *ATG* genes have been recently identified as being involved in mitochondrial turnover. Cells lacking the expression of these genes show reduced mitochondrial turnover. However, neither strain completely arrests mitochondrial turnover [11,12], suggesting the likelihood of other genes being involved in this process.

To follow organelle turnover by autophagy, we developed a red/green fluorescent biosensor. The biosensor is comprised of a pH stable red fluorescent protein fused to a pH (acid) sensitive green fluorescent protein, which can be expressed in the cytosol or specifically targeted to various organelles. For example, when expressed in the mitochondrion (~ pH 8.2) both red and green fluorescence are detected, but when delivered to the vacuole (~ pH 5.5) green fluorescence is lost and only red fluorescence can be detected. Therefore, we can assay for the uptake of mitochondria into the vacuole based on the accumulation of red fluorescence there.

Using this technology, we looked at the turnover of organelles in yeast and found that two genes, *ATG5* and *ATG12* required for the bulk degradation of the cytosol, are not required for the degradation of mitochondria or the ER. Atg5p and Atg12p are involved in one of two ubiquitin-like conjugation systems required for autophagy. Atg5p, Atg12p and Atg16p form a complex, embedded into the pre-autophagosomal membrane, which is thought to be essential for autophagosome elongation [13,14]. However, because Atg5p or Atg12p are not required for turnover of mitochondria, or of the ER, we hypothesised that there may be homologous proteins that compensate for their loss; or alternatively, that turnover of these organelles may occur via an alternative pathway. Using a bioinformatics approach, we identified a hypothetical open reading frame, OTP1, as sharing 22% amino acid sequence homology to Atg5p.

OTP1 is largely uncharacterised. The deletion strain is viable and has been reported to have an accumulation of glycogen in the cytosol [15, 16]. Otp1p appears to be localised to punctuate structures in the cytosol, but a definitive localisation remains to be established. Based on amino acid sequence, it has between six and eight predicted transmembrane domains, no mitochondrial or ER targeting sequences and no motifs related to its potential function.

Using the mitochondrially targeted fluorescent biosensor, we have compared mitochondrial turnover in wild-type cells and in cells lacking the expression of OTP1p (Otp1 null). We observed

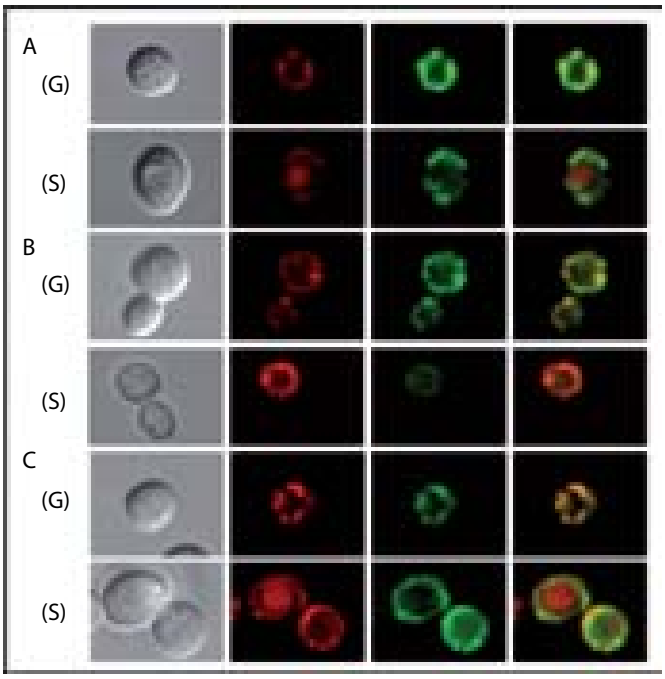


Figure 2. Delivery to the vacuole of mitochondria in cells expressing the mitochondrially targeted fluorescent biosensor. Cells were inoculated into medium containing essential salts and amino acids with 2% ethanol as carbon source, and grown for 48 hours at 28 °C. Cells were then washed, transferred into either non-starvation 'growth' [G] medium (0.67% yeast nitrogen base without amino acids or ammonium sulphate, essential amino acids and 2% ethanol), or 'starvation [S] medium (containing only 0.17% yeast nitrogen base without amino acids or ammonium sulphate and 2% ethanol). After incubation for six hours, cells were viewed using confocal microscopy to observe mitochondrial turnover. (A) Wildtype cells. Red fluorescence accumulates in the vacuole of cells subjected to starvation conditions. (B) *OTP1* null cells. By contrast to wildtype cells, red fluorescence does not accumulate in the vacuole when cells are subjected to starvation conditions. (C) *OTP1* null cells expressing the native the *OTP1* gene. Red fluorescence accumulates in the vacuole when cells are subjected to starvation conditions.

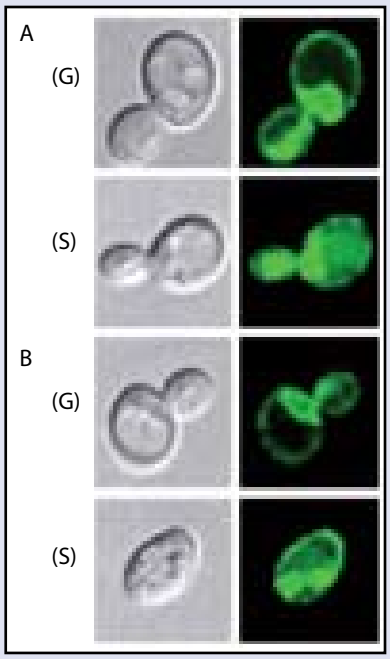


Figure 3. Delivery to the vacuole of ER in cells expressing Sec63-GFP. Cells were inoculated into medium containing essential salts and amino acids with 2% glucose as carbon source, and grown for 24 hours at 28°C. Cells were washed and transferred into growth (G) or starvation (S) medium (as for the experiment depicted in Figure 2, except the media contained 2% glucose). After incubation for 9 hours cells were viewed using confocal microscopy to observe turnover of the ER. A = wild-type cells, B = *OTP1* null cells. Green fluorescence accumulates in the vacuole of both wildtype and *OTP1* null cells when subjected to starvation conditions.

that mitochondrial turnover was almost completely stopped in Otp1 null cells. Expression of the native *OTP1* gene in these cells, resulted in recovery of mitochondrial turnover (Figure 2), confirming a role for Otp1p in mitochondrial turnover. By contrast, use of the ER protein Sec63 (a component of the Sec complex involved in post-translational protein translocation across the ER membrane [17]) fused to GFP, indicated turnover of the ER could still be observed in Otp1 null cells (Figure 3).

We have identified the *OTP1* gene as being specifically involved in mitochondrial turnover. It is likely that other genes remain to be identified and characterised to fully understand the mechanisms of organelle turnover. A better understanding of the selective nature of organelle turnover in yeast will surely contribute to revealing the mechanisms underlying diseases involving defects in autophagy.

References

1. Deretic V. Autophagy as an immune defense mechanism. *Curr Op Immun* 2006; 18:375-82.
2. Swanson MS. Autophagy: eating for good health. *J Immun* 2006; 177:4945-51.
3. Wang CW & Klionsky DJ. The molecular mechanism of autophagy. *Mol Med* 2003; 9:65-76.
4. Reggiori F & Klionsky DJ. Autophagy in the eukaryotic cell. *Eukaryotic Cell* 2002; 1:11-21.
5. Tsukada M & Oshumi Y. Isolation and characterisation of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Letts* 1993; 333:169-74.
6. Monastyrska I & Klionsky DJ. Autophagy in organelle homeostasis: Peroxisome turnover. *Mol Aspects Med* 2006; 27:483-94.
7. Kim J & Klionsky DJ. Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells. *Ann Rev Biochem* 2000; 69:303-42.
8. 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; 14:129-41.
9. Bernales S, McDonald KL & Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. *PLoS Biology* 2006; 4:2311-24.
10. Rodriguez-Enriquez S, He L & Lemasters JJ. Role of mitochondrial permeability transition pores in mitochondrial autophagy. *Int J Biochem Cell Biol* 2004; 36:2463-72.
11. Kissova I, Deffieu M, Manon S & Camougrand N. Uth1p is involved in the autophagic degradation of mitochondria. *J Biol Chem* 2004; 279:39068-74.
12. 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; 282:5617-24.
13. Oshumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. *Nature Rev Mol Cell Biol* 2001; 2:211-6.
14. Kuma A, Mizushima N, Ishihara N & Oshumi Y. Formation of the ~350-kDa Apg12-Apg5·Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. *J Biol Chem* 2002; 277:18619-25.
15. Giaever G, Chu AM, Ni L, *et al.* Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 2002; 418:387-91.
16. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, *et al.* Global analysis of protein localization in budding yeast. *Nature* 2003; 425:686-91.
17. Feldheim D, Rothblat J & Schekman R. Topology and functional domains of Sec63p, an endoplasmic reticulum membrane protein required for secretory protein translocation. *Mol Cell Biol* 1992; 12:3288-96.

Of further interest to readers of Microbiology Australia *are two recent reports implicating autophagy as a key process in fungal developmental biology:*

Veneault-Fourrey C, Barooah M, Egan M, Wakley G & Talbot NJ. Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science* 2006;312:580–3.

Kikuma T, Ohneda M, Arioka M & Kitamoto K. Functional analysis of the ATG8 homologue Aoatg8 and role of autophagy in differentiation and germination in *Aspergillus oryzae*. *Eukaryotic Cell* 2006;5:1328–36.