Selective autophagy in budding yeast

Kuninori Suzuki*

Autophagy is a bulk degradation system, widely conserved in eukaryotes. Upon starvation, autophagosomes enclose a portion of the cytoplasm and ultimately fuse with the vacuole. The contents of autophagosomes are degraded in the vacuole, and recycled to maintain the intracellular amino-acid pool required for protein synthesis and survival under starvation conditions. Previously, autophagy was thought to be an essentially nonselective pathway, but recent evidence suggests that autophagosomes carry selected cargoes. These studies have identified two categories of selective autophagy – one highly selective and dependent on autophagy-related 11 (Atg11); another, less selective, that is, independent of Atg11. The former, selective category comprises the Cvt pathway, mitophagy, pexophagy and piecemeal microautophagy of the nucleus; acetaldehyde dehydrogenase 6 degradation and ribophagy belong to the latter, less selective category. In this review, I focus on the mechanisms and the physiological roles of these selective types of autophagy.

Facts

- Autophagy is a bulk degradation system widely conserved in eukaryotes and especially induced upon starvation.
- Under starvation conditions, autophagosomes enclose a portion of the cytoplasm and the contents of autophagosomes are degraded in the vacuole.
- Autophagy is essentially a nonselective pathway but carries a number of selective cargoes.
- There are two categories of selective autophagy – one highly selective and dependent on autophagy-related 11 (Atg11); another, less selective, that is independent of Atg11.
- Atg11 functions by connecting cargo – receptor complexes and organelles, with Atg proteins essential for autophagosomal membrane biogenesis.

Open questions

- Physiological roles of selective autophagy are not fully understood.
- The mechanism of how each type of selective autophagy is induced is mostly unknown.
- The molecular mechanisms underlying Atg11-independent selective autophagy remain to be addressed.

Autophagy is a bulk degradation system widely conserved in eukaryotes. Under starvation conditions, autophagosomes enclose a portion of the cytoplasm and the contents of autophagosomes are degraded in the vacuole. Autophagy is essentially a nonselective pathway but carries a number of selective cargoes.

The first selective autophagy cargo to be identified was the vacuolar aminopeptidase I (Ape1). Ape1 is synthesized in precursor form (prApe1) and subsequently processed in the vacuole to its mature form (mApe1). This biosynthetic pathway, which occurs under nutrient-rich conditions, was named the cytoplasm-to-vacuole targeting (Cvt) pathway. Mutants defective in the maturation of Ape1 were screened to obtain cvt mutants. Around the same time, other groups identified mutants defective in starvation-induced autophagy, termed apg (autophagy) and aut (autophagocytosis). apg and aut mutants have a phenotype similar to that of the cvt mutants, suggesting that the Cvt and autophagy pathways share some common machinery. Electron microscopy revealed that in the Cvt pathway, prApe1 is exclusively enclosed in double-membrane-bound organelles called Cvt vesicles, which are topologically similar to autophagosomes. However, the two compartments are of different sizes, ~150 nm for Cvt vesicles and ~500 nm for autophagosomes.

Peroxisome degradation mediated by autophagy has been described by several groups, and a number of the genes involved were named independently, for example, GSA, PAZ, and PDD. This list includes genes essential for peroxisome degradation as well as some required for bulk autophagy. To avoid confusion, the nomenclature was later consolidated; the genes are now collectively referred to as ATG genes.

Atg11 as a Scaffold Protein for Selective Autophagy

The pre-autophagosomal structure (PAS) mediates the membrane biogenesis of Cvt vesicles/autophagosomes. In atg11Δ cells, the core Atg proteins, a subgroup of ATG genes, autophagy-related genes; Ape1, aminopeptidase I; prApe1, Ape1 precursor form; mApe1, mature Ape1; Cvt, cytoplasm-to-vacuole targeting; PAS, pre-autophagosomal structure; VLP, virus-like particles; ROS, reactive oxygen species; MIPA, micropexophagic membrane apparatus; PMN, piecemeal microautophagy of the nucleus; NVJ, nucleus—vacuole junction

Received 02.3.12; revised 23.4.12; accepted 24.4.12; Edited by M Piacentini; published online 15.6.12

Keywords: selective autophagy; autophagosome; ATG genes; yeast; Atg11

Abbreviations: ATG genes, autophagy-related genes; Ape1, aminopeptidase I; prApe1, Ape1 precursor form; mApe1, mature Ape1; Cvt, cytoplasm-to-vacuole targeting; PAS, pre-autophagosomal structure; VLP, virus-like particles; ROS, reactive oxygen species; MIPA, micropexophagic membrane apparatus; PMN, piecemeal microautophagy of the nucleus; NVJ, nucleus—vacuole junction
Atg proteins responsible for membrane biogenesis of Cvt vesicles/autophagosomes, are not targeted to the PAS under nutrient-rich conditions, leading to a defect in the Cvt pathway. As PAS localization of the core Atg proteins is largely abolished in atg17Δ cells under starvation conditions, Atg17 is thought to function as a scaffold protein for bulk autophagy. In atg17Δ cells, Ape1 transport to the vacuole is normal under nutrient-rich conditions. During starvation, Ape1 maturation is partially defective in atg11Δ and atg17Δ cells, indicating that Ape1 maturation depends on both Atg11 and Atg17 during autophagy. When Atg11 and Atg17 are both deleted, PAS formation is completely abolished, resulting in a total block in Ape1 maturation. Thus, Atg11 is involved in Ape1 maturation under both nutrient-rich and -starvation conditions, via its role in organization of the PAS. Moreover, Atg11 is important for other types of selective autophagy, such as mitophagy (selective degradation of mitochondria by autophagy), pexophagy (selective degradation of peroxisomes by autophagy) and piecemeal microautophagy of the nucleus. Cargo degraded by Atg11-independent selective autophagy have also been reported.

**Atg11-dependent Selective Autophagy**

Atg11 is required for various types of selective autophagy. It functions by connecting cargo – receptor complexes and organelles with core Atg proteins essential for autophagosomal membrane biogenesis. In the following section, I provide an overview of Atg11-dependent selective autophagy.

**The Cvt pathway.** The Cvt pathway, a constitutive biosynthetic pathway mediated by Cvt vesicles, is responsible for the transport of the Cvt complex under nutrient-rich conditions. Similarly, under starvation conditions, autophagy facilitates transport of the Cvt complex. prApe1, the major component of the Cvt complex, is synthesized, oligomerized to a dodecamer, and assembled to form the Ape1 complex, which constitutes the core structure of the Cvt complex. The Cvt complex is morphologically defined by microscopy (Figure 1). In addition to the Ape1 complex, the Cvt complex contains vacuolar \(\alpha\)-mannosidase (Ams1) and Ty1 virus-like particles (VLPs), which are produced by Ty1 retrotranposons in the yeast genome, can be observed by electron microscopy as particles surrounding the Ape1 complex. Ape1 and Atp34 receptors are also required for organization of the Cvt complex. In the absence of Ape1, the Ape1 complex and the Ty1 VLPs are both localized to the cytoplasm, but are not associated with one another, preventing their selective delivery to the vacuole. Ape1 is peripherally localized to the Ape1 complex, and may promote the association of Ty1 VLPs with the Ape1 complex as well as target the Cvt complex to the PAS. During starvation, either Ape1 or Atp34 is sufficient to target Ams1 to the Cvt complex. Thus, the Cvt complex is composed of cargo (prApe1, Ams1 and Ty1 VLP) and receptors (Ape1 and Atp34). The physiological role of Cvt complex transport to the vacuole is not well understood. The vacuolar enzymes may have a role in protein turnover. It has been reported that selective Ty1 VLP degradation is involved in maintaining genome integrity during starvation by decreasing the frequency of Ty1 transposition. Ape4 (aspartyl aminopeptidase) and Lap3 (leucine aminopeptidase) are also delivered to the vacuole by selective autophagy through association with the Cvt complex.

**Mitophagy.** When yeast cells are cultured in media containing a nonfermentable carbon source, such as glycerol or lactate, they shift from anaerobic to aerobic respiration. As the latter condition places oxidative stress on the mitochondria, stress-related damage can occur. As a quality control step to eliminate damaged mitochondria in post-log phase cells, mitochondria-specific autophagy (mitophagy) is induced under these conditions. Mitophagy also occurs when cells grown under aerobic respiration conditions are transferred to nitrogen-starvation medium. In addition to the core Atg proteins, Atg11 and Atg32 are required for mitophagy (Figure 2). Atg32 is anchored to the outer mitochondrial membrane, and confers selectivity for mitochondrial sequestration by recruiting autophagic machinery through interactions with Atg8 and Atg11. When mitophagy is induced, Atg32 is phosphorylated; this modified form of Atg32 is able to bind Atg11. Hog1 and Pbs2, kinases involved in the osmoregulatory signal transduction cascade, have a role in Atg32 phosphorylation. Moreover, Atg33, another mitophagy-specific protein, has an important role in mitophagy in post-log phase cells. Mitophagy maintains mitochondrial quality by eliminating damaged mitochondria. This physiological role of mitophagy was confirmed using atg11Δ and atg32Δ cells. When these mitophagy-deficient cells are faced with nutrient starvation, mitochondria damaged by exposure to reactive oxygen species (ROS) are not degraded. These damaged mitochondria lose their mitochondrial DNA, and host cells exhibit the ‘petite’ phenotype, an indication that aerobic respiration has been compromised. Bulk autophagy is also important for maintenance of mitochondrial quality. When cells grown in fermentable medium with glucose as the sole carbon source are transferred to nitrogen-starvation medium, autophagy-defective cells mostly die within 5 days. In nitrogen-starvation medium that has been buffered at neutral pH, autophagy-defective cells can survive, but the majority demonstrate the ‘petite’ phenotype. This may occur because autophagy-defective cells cannot scavenge ROS accumulated in the

![Figure 1](image_url) Schematic of the Cvt complex. The Cvt complex is localized near the vacuole in *S. cerevisiae* (left panel). Ty1 VLPs (gray) are associated with the Ape1 complex, the main component of the Cvt complex (dark gray). These two structures can be observed by electron microscopy.
mitochondria. Autophagy-defective cells cannot produce ROS-scavenging enzymes, likely due to a shortage of free amino acids for de novo protein synthesis. In contrast, atg32Δ cells do not show the petite phenotype. Together, these different types of autophagy may have complementary roles in the maintenance of mitochondrial quality, for example, with bulk autophagy serving as a preventive measure to preserve mitochondrial activity by allowing synthesis of ROS-scavenging enzymes, while mitophagy eliminates damaged mitochondria to prevent their harmful effects.

Pexophagy. Peroxisome degradation by selective autophagy, called pexophagy, has been studied in several methylotrophic yeasts, such as Pichia pastoris, Hansenula polymorpha and Yarrowia lipolytica. When these cells are grown in a methanol medium, they synthesize peroxisomes, which form clusters. Subsequent transfer of the cells to glucose medium induces a process called micropexophagy (Figure 3): first, the vacuolar membrane begins to enwrap the cluster; next, a cup-shaped membrane structure termed the micropexophagic membrane apparatus (MIPA) emerges on the cluster’s open surface. Enclosure of the peroxisome cluster is completed by fusion between the MIPA and the vacuolar membrane, resulting in transport of the peroxisomes into the vacuole. Conversely, when cells grown in methanol are transferred to an ethanol medium, individual peroxisomes are enclosed in special autophagosomes, termed pexophagosomes, and delivered to the vacuole one by one. This type of pexophagy is termed macropexophagy (Figure 3). Core Atg proteins are required for both micro- and macropexophagy. Several other factors are specifically required for both types of pexophagy, including PpAtg26, a sterol glucosyltransferase; PpAtg28, a coiled-coil protein; and PpAtg30, a receptor protein. Moreover, PpAtg35 and HpAtg25 are specifically required for micropexophagy and macropexophagy, respectively.

Peroxisome biogenesis is necessary for the pathogenicity of the plant fungus Colletotrichum orbiculare, which causes disease in cucumber plants. A recent study showed that pexophagy has an important role in pathogenicity (Figure 4). An insertional mutation library screen identified the CoATG26 gene as critical for pathogenicity. To invade host plants, C. orbiculare develops an infection structure termed the appressorium. In Coatg26 mutant cells, the biogenesis of peroxisomes is normal, but these structures accumulate in the appressoria, indicating that pexophagy is defective. Furthermore, Coatg26 mutants exhibit a specific defect in invasion. Upon infection, ring- or cup-shaped structures labeled with GFP-CoAtg8 are detected along peroxisomes, suggesting that macropexophagy has been induced in the appressoria. Domain and localization analyses of CoAtg26 show that both the phosphoinositide binding and sterol
glucosyltransferase activities displayed by this enzyme are required for infection-related pexophagy. By contrast, normal appressoria do not differentiate in Coatg8 mutant cells, which are defective in both bulk autophagy and pexophagy. Together, these different autophagic pathways have complementary roles in the pathogenicity of the fungus: bulk autophagy is required for the early stages of infection-related C. orbiculare morphogenesis, and pexophagy is needed for later stages of infection occurring after development of appressoria.

Piecemeal microautophagy of the nucleus. When yeast cells are faced with nutrient limitations, a dispensable portion of the nucleus is protruded into the vacuolar lumen as a teardrop-shaped bleb and subsequently pinched off. The resultant vesicles are sequestered by three lipid bilayers and degraded by vacuolar hydrolases. This process is termed piecemeal microautophagy of the nucleus (PMN). PMN occurs at the nucleus–vacuole junction (NVJ) formed by interactions between Nvj1, localized to the outer nuclear membrane, and Vac8, localized to the vacuolar membrane. Nvj1 and Vac8 are required for PMN. Moreover, an electrochemical gradient across the vacuolar membrane and lipid-modifying enzymes are necessary for PMN. Because Nvj1 is degraded in a PMN-dependent manner, progression of this phenomenon can be monitored by observing the degradation of Nvj1-GFP. As with mitophagy and pexophagy, Atg11 and core Atg proteins are essential for PMN. PMN also requires Atg17, Atg29, and Atg31, which are essential for starvation-induced autophagosome formation, but not for other types of selective autophagy. An understanding of the physiological roles of PMN in eukaryotic cells will hopefully emerge from future studies.

Atg11-independent Selective Autophagy

Previously, it was believed that abundant cytoplasmic components are enclosed in autophagosomes nonselectively. However, it has recently been reported that cytosolic acetaldehyde dehydrogenase (Ald6) and ribosomes are
The molecular mechanisms underlying this preferential degradation of preferential autophagy than Atg11-dependent selective autophagy, in an Atg11-independent manner.

Acetaldehyde dehydrogenase 6. Ald6, a soluble cytoplasmic enzyme, was identified in a systematic differential proteomic screen of wild-type and autophagy-deficient yeast cells as a protein that is degraded by autophagy after nitrogen starvation for 24h. This degradation depends on core Atg proteins and active vacuolar proteases, but not on known selective autophagic factors such as Atg11. Ald6 is preferentially enclosed in autophagosomes, then delivered to the vacuole for degradation. When active Ald6 accumulates in the cytosol, the viability of nitrogen-starved cells decreases. Accumulation of inactive Ald6 has little effect on viability; therefore, it may be that the enzymatic activity of Ald6 negatively impacts survival under starvation conditions, leading to rapid death of autophagy-defective cells. The molecular mechanisms underlying this preferential autophagy remain to be addressed.

Ribophagy. The observation of ribosomal degradation by autophagy was considered to support the hypothesis that autophagy is nonselective. Nevertheless, a recent report indicates that ribosomes are preferentially degraded by autophagy. Upon nutrient starvation, ribosomes are degraded along with other cytoplasmic components. In Saccharomyces cerevisiae, this degradation involves a novel type of selective autophagy termed ribophagy. As ribophagy is independent of Atg19, it is unlikely that Atg11 is important for this pathway. A genome-wide screen of a set of nonessential gene disruptants demonstrated that the Ubp3/Br65 deubiquitination complex is involved in ribophagy. Moreover, this complex interacts with the Cdc48/Ufd3 complex, which has an important role in the ubiquitin-proteasome system. However, defects in proteasomal degradation do not greatly impact ribophagy. In cells defective in ribophagy, autophagic pathways other than ribophagy appear to be normal. A functional relationship between ubiquitination and ribophagy may exist, but the molecular mechanisms of ribophagy remain unknown.

Conclusion and Perspectives

Selective autophagy provides cells with multiple means to protect themselves against severe environmental conditions. Atg11 is a protein that functions as a scaffold for a group of selective autophagy pathways, including the Cvt pathway, mitophagy, pexophagy and PMN. Atg11 seems to be involved in the selective transport of protein aggregates and organelles. During Atg11-dependent selective autophagy, targets are associated with Atg11, which recruits autophagic machinery to the cargo by interacting with a variety of receptor proteins, for example, Atg19 and Atg34 (Cvt pathway); Atg32 (mitophagy); and PpAtg30 (pexophagy). These receptor proteins confer selectivity on the Atg11-dependent targets. By contrast, Ald6 and ribosomes are selectively degraded by Atg11-independent mechanisms, which exhibit weaker selectivity than Atg11-dependent mechanisms. Receptors for Ald6 and ribosomes have not yet been identified. Other mechanisms may confer selectivity to these cargos. For instance, Ald6 and ribosomes might interact with the inner surface of the isolation membrane, or form a loose complex at the PAS.

Recently, autophagosome-associated proteins have been identified from human breast cancer cells. Future analysis of autophagosomal cargoes in yeast cells will hopefully elucidate the mechanisms of selective autophagy in eukaryotic cells.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements. I thank Dr. Yoshihori Ohsumi for helpful comments on this manuscript. This work was supported by the Hamaguchi Foundation for the Advancement of Biochemistry, the NOVARTIS Foundation (Japan) for the Promotion of Science and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
30. Watanabe Y, Noda NN, Kumeta H, Suzuki K, Ohsumi Y, Inagaki F. Selective transport of the mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. Mol Biol Cell 2009; 20: 365–376.

31. Suzuki K, Kondo C, Morimoto M, Ohsumi Y. Selective transport of a large oligomeric protein by the autophagic pathways: identification of a novel receptor, Atg34p. Mol Biol Cell 2004; 15: 209–218.

32. Roberts P, Moshitch-Moshkovitz S, Kvam E, O’Ttoole E, Winey M, Goldfarb DS. Piecemeal membrane structure. Mol Biol Cell 2003; 14: 4173–4183.

33. Krick R, Muehe Y, Prick T, Brewer S, Schlottnerase E, Eskelinen EL et al. Piecemeal microautophagy of the nucleus coincides with a vacuolar diffusion barrier at nuclear-vacuolar junctions. Autophagy 2011; 7: 58–70.

34. Kageyama T, Suzuki K, Ohsumi Y. Lap3 is a selective target of autophagy in yeast, Saccharomyces cerevisiae. Biochim Biophys Acta 2003; 1657: 551–557.

35. Watanabe Y, Noda NN, Kumeta H, Suzuki K, Ohsumi Y, Inagaki F. Selective transport of αmannosidase by autophagic pathways: structural basis for cargo recognition by ATG19 and ATG34. J Biol Chem 2010; 285: 30026–30033.

36. Okamoto K, Kondo-Okamoto N, Ohsumi Y. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev Cell 2009; 17: 87–97.

37. Kondo-Okamoto N, Noda NN, Suzuki SW, Nakatogawa H, Takahashi I, Matsumoto M et al. Autophagy-related protein 32 acts as autophagic degron and directly initiates mitophagy. J Biol Chem 2012; 287: 10631–10638.

38. Asakura M, Ninomiya S, Sugimoto M, Oku M, Yamashita S, Okuno T et al. Mitochondrial transport: a selective mechanism. Autophagy 2005; 1: 92–100.

39. Monastyrska I, Kiel JA, Komduur JA, Veenhuis M, van der Klei IJ. The Cvt19p vesicle budding complex in the cytoplasm-to-vacuole targeting pathway. Mol Biol Cell 2012; 23: 129–141.

40. Kurihara Y, Kanki T, Aoki Y, Hirota Y, Saigusa T, Uchiumi T et al. Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation mutagenesis by the Ty1 retrotransposon in Saccharomyces cerevisiae. Mol Cell Biol 2003; 23: 13704–13713.

41. Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z et al. Atg32 is a novel coiled-coil protein involved in autophagic degradation of peroxisomes in the methylophytic yeast Pichia pastoris. Autophagy 2006; 2: 30–38.

42. Kurihara Y, Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA. Atg32 is a microperoxisome-specific protein that regulates microperoxisomal apparatus formation in Pichia pastoris. Autophagy 2011; 7: 375–385.

43. Veenhuis M, van der Klei IJ, Titorenko V, Harder LM. HAUSP interacts with the cytoplasm-to-vacuole targeting determinant in aminopeptidase I. Autophagy 2008; 4: 732–743.

44. Dunn Jr WA, Cregg JM, Kiel JA, van der Klei U, Ohk U, Sakai Y et al. Pexophagy: the selective autophagy of peroxisomes. Autophagy 2005; 1: 75–83.

45. Mukaiyama H, Baba M, Osumi M, Aoyagi S, Kato N, Ohsumi Y et al. Modification of a ubiquitin-like protein Pazz conducted microperoxisome formation through a novel mitophagy mechanism. Mol Biol Cell 2003; 14: 4173–4183.

46. Ohk U, Warnecke D, Noda T, Muller F, Heinz E, Mukaiyama H et al. Peroxisome degradation requires catalytically active sterol glucosyltransferase with a GRAM domain. EMBO J 2003; 22: 3231–3241.

47. Stasny OV, Styak OG, Mathewson RD, Farre JC, Nazarko VY, Krasovskia OS et al. Atg32, a novel coiled-coil protein involved in autophagic degradation of peroxisomes in the methylotrophic yeast Pichia pastoris. Autophagy 2006; 2: 30–38.

48. Farre JC, Manjihaya R, Mathewson RD, Subramani S. PcpAtg30 tags peroxisomes for turnover by selective autophagy. Dev Cell 2008; 14: 365–376.

49. Nazarko VY, Nazarko TF, Farre JC, Styak OV, Warnecke D, Ulaszewski S et al. Atg35, a microperoxisome-specific protein that regulates microperoxisomal apparatus formation in Pichia pastoris. Autophagy 2011; 7: 375–385.

50. Kondo-Okamoto N, Suzuki K, Ohsumi Y, Inagaki F. Selective transport of αmannosidase by autophagic pathways: structural basis for cargo recognition by ATG19 and ATG34. J Biol Chem 2010; 285: 30026–30033.

51. Kondo-Okamoto N, Noda NN, Suzuki SW, Nakatogawa H, Takahashi I, Matsumoto M et al. Autophagy-related protein 32 acts as autophagic degron and directly initiates mitophagy. J Biol Chem 2012; 287: 10631–10638.

52. Asakura M, Ninomiya S, Sugimoto M, Oku M, Yamashita S, Okuno T et al. Mitochondrial transport: a selective mechanism. Autophagy 2005; 1: 92–100.

53. Monastyrska I, Kiel JA, Komduur JA, Veenhuis M, van der Klei U. The Hansenula polymorpha Atg25 gene encodes a novel coiled-coil protein that is required for macroperoxisome formation. Autophagy 2005; 1: 92–100.

54. Onodera J, Ohsumi Y. Autophagy is required for maintenance of amino acid levels and protein synthesis under nitrogen starvation. FEBS Lett 1992; 309: 393–403.

55. Scott SV, Guan J, Hutchins MU, Kim J, Klionsky DJ. Cvt19 is a receptor for the cytoplasm-to-vacuole targeting determinant in aminopeptidase I. Autophagy 2008; 4: 732–743.

56. Ohsumi Y. Initiation of autophagic pathways. Genes Cells 2007; 12: 155–172.

57. Kraft C, Deplazes A, Sohrmann M, Peter M. Mature ribosomes are selectively degraded by autophagy upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nat Cell Biol 2008; 10: 602–610.