

Mitochondria Autophagy in Yeast

Tomotake Kanki,¹ Daniel J. Klionsky,² and Koji Okamoto³

Abstract

The mitochondrion is an organelle that carries out a number of important metabolic processes such as fatty acid oxidation, the citric acid cycle, and oxidative phosphorylation. However, this multitasking organelle also generates reactive oxygen species (ROS), which can cause oxidative stress resulting in self-damage. This type of mitochondrial damage can lead to the further production of ROS and a resulting downward spiral with regard to mitochondrial capability. This is extremely problematic because the accumulation of dysfunctional mitochondria is related to aging, cancer, and neurodegenerative diseases. Accordingly, appropriate quality control of this organelle is important to maintain proper cellular homeostasis. It has been thought that selective mitochondria autophagy (mitophagy) contributes to the maintenance of mitochondrial quality by eliminating damaged or excess mitochondria, although little is known about the mechanism. Recent studies in yeast identified several mitophagy-related proteins, which have been characterized with regard to their function and regulation. In this article, we review recent advances in the physiology and molecular mechanism of mitophagy and discuss the similarities and differences of this degradation process between yeast and mammalian cells. *Antioxid. Redox Signal.* 14, 1989–2001.

Introduction

IN RESPONSE TO VARIOUS TYPES of cellular stress, such as nutrient starvation, cytosolic double-membrane vesicles emerge and sequester cytoplasmic components as cargoes, and then the vesicles deliver those cargoes into the lysosome/vacuole. The delivered components are degraded by the resident hydrolytic enzymes into small molecules, which are reused by the cell for survival. This catalytic process is highly conserved among eukaryotes and is called “macroautophagy.” In addition to its function as a cellular stress response that is observed in most eukaryotes, autophagy plays diverse roles in cellular development, immune response, aging, and tumor suppression and in the prevention of many diseases such as cancer, infection, diabetes, neurodegenerative diseases, gastrointestinal disorders, and cardiomyopathy in mammalian cells (51).

In addition to macroautophagy, there are two morphologically different types of autophagy: microautophagy and chaperone-mediated autophagy (CMA). Microautophagy sequesters cytoplasmic components by direct invagination or protrusion/septation of the lysosomal or vacuolar membrane. In contrast, CMA is a chaperone-dependent process relying on both cytosolic and lysosomal hsc70 to move substrates into the lysosome lumen and on hsp90 to stabilize the membrane-bound LAMP-2A receptor (41). The substrate proteins that

have the consensus amino acid sequence KFERQ or a similar motif are unfolded and directly translocated across the lysosomal membrane. CMA has been identified in higher eukaryotes, but not in yeast.

All of the cytoplasmic components, including proteins, nucleic acid, and organelles, can be sequestered by macroautophagy as a cargo. Actually, the presence of organelles, such as mitochondria, in the lysosome or vacuole was frequently found in both mammalian cells and yeast (8, 84). However, it has long been unclear whether these organelles are nonselectively, preferentially, or selectively degraded by autophagy. Recent studies, particularly in yeast, revealed that some organelles or proteins are selectively degraded by an autophagic process. Now it is known that mitochondria, peroxisomes, ribosomes, endoplasmic reticulum, protein aggregates, and invasive microbes are selectively degraded by autophagy, and these selective autophagic processes are called mitophagy, pexophagy, ribophagy, reticulophagy, aggrephagy, and xenophagy, respectively (4, 39, 42, 45, 79, 89). In addition to these specific types of autophagy, yeast cells have adapted the autophagic process for a biosynthetic purpose in the form of the cytoplasm-to-vacuole targeting (Cvt) pathway (40). This pathway selectively delivers at least two resident hydrolases, aminopeptidase I (Ape1) and α -mannosidase (Ams1), to the vacuole using most of the same

¹Department of Clinical Chemistry and Laboratory Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan.

²Life Sciences Institute, University of Michigan, Ann Arbor, Michigan.

³Laboratory of Mitochondrial Dynamics, Graduate School of Frontier Biosciences, Osaka University, Suita, Japan.

autophagic machinery that is used for nonselective autophagy. Yeast leucine aminopeptidase III (Lap3) has been recently reported to be selectively degraded through a Cvt pathway-related process, but only under starvation conditions when the protein was overexpressed (24). Although macropexophagy and the Cvt pathway are morphologically macroautophagy-like processes, the word "macroautophagy" is conventionally used to represent bulk (*i.e.*, nonspecific) autophagy essentially as an antonym to selective autophagy. Hereafter, we use "macroautophagy" when referring to bulk autophagy.

The mitochondrion is an organelle that is integrally involved in cellular energetics, carrying out various catabolic processes. In particular, mitochondrial oxidative phosphorylation supplies a large amount of energy that contributes to a range of cellular activities. However, this process also generates reactive oxygen species (ROS) that can damage the organelle. Damaged proteins and DNA in mitochondria cause further production of ROS, and the accumulation of mitochondrial damage is related to aging, cancer, and neurodegenerative diseases (92). Accordingly, the cell has devised specific mechanisms to ensure proper quality control of this organelle. Mitochondria have their own quality control systems including a protein degradation system (72), DNA repair enzymes (5, 43), and phospholipid hydroperoxide glutathione peroxidase (1). Further, recent evidence suggests that mitophagy eliminates mitochondria that contain excess damage beyond the capacity of the above quality control systems to effect repair. In mammals, mitophagy is closely related to cellular physiology, whereas mitochondrial dysfunction is associated with certain diseases. For example, during erythroid cell maturation, the mitochondrial outer-membrane protein Nix mediates mitochondrial elimination (74, 76). Loss-of-function mutations of the *PARK2* and *PARK6* genes, which encode Parkin and PTEN-induced putative kinase 1 (PINK1), respectively, cause Parkinson disease; PINK1 can stably localize on the outer membrane of impaired mitochondria and recruit Parkin from the cytosol to the mitochondrial membrane, thus promoting mitophagic degradation (14, 48, 54, 55, 91). However, the further mechanism for engulfing mitochondria has not been elucidated in mammalian cells. As with the autophagy-related (Atg) proteins, which were first characterized in yeast (38, 53), studies in this unicellular eukaryote recently uncovered some of the first mitophagy-related factors. This article will review recent studies from the physiology to the molecular mechanism of mitophagy in yeast.

Evidence for Selective Mitochondria Autophagy (Mitophagy)

The presence of mitochondria within the yeast vacuole was first reported in 1992 (84). Initially, this phenomenon was thought to be the result of nonselective engulfment of mitochondria by macroautophagy. However, recently, several lines of evidence suggest that impaired mitochondria are selectively degraded by autophagy in yeast. In the *fmc1* null mutant, the F_0F_1 ATPase subunits aggregate, and the mitochondrial membrane potential is impaired at 37°C. Under anaerobic conditions at 37°C, this mutant strain induces autophagy and preferentially removes the dysfunctional mitochondria. Importantly, the *fmc1* mutant has an increase in cellular ATP, confirming that autophagy in this setting is not

induced by ATP depletion. This case is, to our knowledge, the first report demonstrating the elimination of impaired mitochondria by autophagy in yeast (71).

There are two more studies suggesting that dysfunctional mitochondria are eliminated by autophagy. The first concerns Mdm38, a mitochondrial inner membrane protein with K^+ / H^+ exchange activity. Depletion of Mdm38 causes loss of the inner membrane potential, mitochondrial swelling, and fragmentation; eventually, these abnormal mitochondria are eliminated by autophagy (64, 65). In the second study, Zhang *et al.* altered mitochondria by expressing a temperature-sensitive, mutant mitochondrial DNA (mtDNA) polymerase (*mip1ts*). By culturing this mutant strain at the nonpermissive temperature, they blocked mtDNA replication and observed rapid degradation of mtDNA *via* autophagy. As mtDNA encodes some of the electron transport chain components, depletion of mtDNA affects the mitochondrial membrane potential. Thus, induction of mitochondrial degradation in this strain is likely due to a deficiency in the electron transport chain (103). Additional studies suggest that mitophagy is regulated independently of nonselective macroautophagy. Kanki and Klionsky found that mitophagy is blocked under strong macroautophagy-inducing nitrogen starvation conditions, if the carbon source makes mitochondria essential for metabolism (26). The Camougrand group found that *N*-acetylcysteine (NAC), a compound that increases the cellular reduced glutathione (GSH) pool, prevents mitophagy induced by nitrogen starvation or rapamycin, presumably because the cellular redox imbalance affects mitophagy induction but has no effect on nonspecific macroautophagy (9, 36). Taken together, these findings reveal that mitochondria are selectively degraded by autophagy in yeast. During the same period, accumulating evidence suggests selective mitochondria autophagy in mammalian cells (10, 73, 90). More recently, two groups simultaneously performed screens for mitophagy-deficient mutants, and both groups identified the same gene, *ATG32*, as encoding a mitophagy-specific factor (29, 68). This finding finally confirmed mitophagy as a selective process. The function of Atg32 is discussed below in detail.

Mitophagy Requires Most of the Atg Proteins

Although the morphology of autophagy was first studied in mammalian cells, most of the molecular components were initially identified in yeast (37). Studies in *Saccharomyces cerevisiae* and other fungi have allowed the isolation of 34 *ATG* genes. At least 15 of these genes are essential for both macroautophagy and selective autophagy, and are categorized as part of the core autophagic machinery (53). Other genes have roles in certain types of autophagy. For example, Atg19, a receptor protein for the Cvt pathway, binds the Ape1 complex composed of precursor Ape1 (prApe1) and Ams1 to form the Cvt complex. Atg11, an adaptor protein for selective autophagy, interacts with Atg19 and recruits the Cvt complex to the phagophore assembly site (PAS), where the phagophores, the initial sequestering membrane structure, are generated (80). Similarly, during pexophagy in *Pichia pastoris*, Atg30 localizes to peroxisomes, where it subsequently binds Atg11, allowing recruitment of peroxisomes to the PAS (13).

In 2004, Kissova *et al.* reported that *ATG5* is required for mitophagy in yeast (34). Since then, the requirement of several *ATG* genes for mitophagy has been reported by various

groups (26, 35, 85, 103), and recently, all of *ATG* genes have been surveyed for mitophagy (28, 68). Table 1 summarizes the requirement of *ATG* genes for nonselective autophagy (macroautophagy), the Cvt pathway, pexophagy, and mitophagy in *S. cerevisiae*. *ATG* genes encoding the core machinery for autophagic membrane formation, such as the Atg1 protein kinase, the phosphatidylinositol 3-kinase complex I that is required for vesicle nucleation (including *ATG6* and *ATG14*), the ubiquitin-like Atg8–phosphatidylethanolamine (PE) conjugation machinery (Atg3, Atg8, Atg4, and Atg7), the ubiquitin-like Atg12–Atg5 conjugation machinery (Atg5, Atg7, Atg10, and Atg16), and components that are involved in supplying lipids to the phagophore (Atg2, Atg9, and Atg18), are essential for all types of autophagy, suggesting that both nonselective and selective autophagy processes fundamentally rely on the same membrane formation machineries. Interestingly, *ATG17*, *ATG29*, and *ATG31*, which encode components of the Atg1 kinase complex and regulate Atg1 activity (53), are completely or partially required for pexophagy, mitophagy, and macroautophagy, but not the Cvt pathway. These findings led us to speculate that the Atg1 kinase regulators are critical for degradation of organelles that require a generally large autophagosome to enwrap the cargo

(e.g., 500–1000 nm for mitophagy), but are not required for small protein complexes (e.g., a 150-nm vesicle is used for the Cvt pathway).

In contrast, *ATG11*, *ATG20*, and *ATG24*, which are required for both the Cvt pathway and pexophagy but not for macroautophagy (60), are also required for mitophagy, further suggesting that mitophagy is a type of selective autophagy. In particular, because Atg11 is an adaptor protein that recognizes and interacts with cargo-specific receptor proteins for the Cvt pathway and pexophagy, the requirement of Atg11 for mitophagy strongly suggests that mitochondria are degraded by an autophagic process that involves a receptor–adaptor interaction.

Genomic Screen for Yeast Mutants Defective in Mitophagy

To identify molecules acting in mitophagy, two groups used nonessential gene deletion strains and performed a genome-wide screen for mutants that were defective in selective mitochondria degradation (28, 68). Okamoto *et al.* visualized mitochondria by expressing green fluorescent protein (GFP) with a mitochondrial targeting signal fused to

TABLE 1. REQUIREMENT OF AUTOPHAGY-RELATED GENES FOR MACROAUTOPHAGY, THE CYTOPLASM TO VACUOLE TARGETING PATHWAY, PEXOPHAGY, AND MITOPHAGY

<i>ATG genes</i>	<i>Macroautophagy</i>	<i>Cvt pathway</i>	<i>Pexophagy</i>	<i>Mitophagy</i> ^a	<i>Reference</i>
<i>ATG1</i>	–	–	–	–	(18, 78, 85, 87, 103)
<i>ATG2</i>	–	–	–	–	(68, 78, 87, 93)
<i>ATG3</i>	–	–	ND	–	(16, 68, 78, 87)
<i>ATG4</i>	–	–	ND	–	(16, 68, 78, 87)
<i>ATG5</i>	–	–	–	–	(18, 34, 78, 87)
<i>ATG6</i>	–	–	ND	–	(78, 87, 103)
<i>ATG7</i>	–	–	–	–	(17, 18, 32, 68, 87)
<i>ATG8</i>	–	–	–	–	(17, 18, 87, 103)
<i>ATG9</i>	–	–	–	–	(17, 26, 35, 87, 88)
<i>ATG10</i>	–	–	–	–	(18, 68, 78, 87)
<i>ATG11</i>	++	–	–	–	(16, 26, 33)
<i>ATG12</i>	–	–	ND	–	(78, 87, 103)
<i>ATG13</i>	–	–	ND	–/+	(28, 68, 78, 87)
<i>ATG14</i>	–	–	–	–	(16, 18, 68, 78, 87)
<i>ATG15</i> ^b	–	–	–	–	(11, 12, 68, 86)
<i>ATG16</i>	–	–	ND	–	(16, 68, 78, 87)
<i>ATG17</i>	–	++	+	–/+	(7, 22, 25, 26, 28)
<i>ATG18</i>	–	–	–	–	(2, 15, 68)
<i>ATG19</i>	++	–	++	++	(26, 77)
<i>ATG20</i>	++	–	+	–/+	(26, 60, 68)
<i>ATG21</i>	++	–	ND	+/++	(3, 28, 68)
<i>ATG22</i> ^c	++	++	ND	++	(68, 98)
<i>ATG23</i>	+	–	++	+	(68, 88)
<i>ATG24</i>	++	–	+	–/+	(26, 28, 60, 68)
<i>ATG26</i>	++	++	++	++	(6, 68)
<i>ATG27</i>	+	+	+	+	(68, 96, 99)
<i>ATG29</i>	–	++	+	+/++	(26, 30, 68)
<i>ATG31</i>	–	++	–	+/++	(23, 26, 68)
<i>ATG32</i>	++	++	++	–	(29, 68)
<i>ATG33</i>	++	++	++	+	(28)

Phenotypes of the indicated gene knockout strain: ++, no defect; +, partial defect; –, severe defect.

^aPhenotypes are shown based on Refs. (68) and (28).

^b*ATG15* encodes a putative lipase required for intravacuolar lysis of autophagic and Cvt bodies.

^c*ATG22* encodes a vacuolar membrane protein required for efflux of amino acids during autophagic body breakdown in the vacuole.

ATG, autophagy-related; Cvt, cytoplasm to vacuole targeting; ND, not determined.

its N terminus (mito-GFP); mitochondrial transport into the vacuole occurs when mitophagy is induced by culturing cells to the postlog phase in a medium containing a nonfermentable carbon source. They examined 5150 knockout strains and found 36 mutants that impaired mitophagy, excluding existing *ATG* gene null strains (68). Kanki *et al.* generated a different chimera by tagging the C terminus of the mitochondrial outer-membrane protein Om45 with GFP (Om45-GFP); GFP accumulates in the vacuole as seen by fluorescence microscopy, and free GFP is generated by vacuolar processing of Om45-GFP as observed by western blotting, when mitophagy is induced by culturing cells to the postlog phase in a medium containing a nonfermentable carbon source or under conditions of nitrogen starvation. In this case they monitored 4667 knockout strains and found 32 mutants with impaired mitophagy, again excluding the existing *ATG* gene null mutants (28). Genes identified from both screens are summarized in Table 2, which has been updated based on recently obtained results (28, 68; unpublished data). Although both screens were based in part on the observed accumulation of a mitochondrially targeted GFP chimera in the vacuole, only 35% genes were common between the two screens (16 among the 45 total genes). One reason for this relative lack of overlap may be due to the fact that Kanki *et al.* did not screen strains that cannot grow well in nonfermentable medium. Another possibility may simply reflect different criteria used by the individuals carrying out the screen in characterizing mitophagy-positive and -negative strains. Notably, >40% of the genes identified from both screens are membrane trafficking-related genes (20 among the 45 total genes). The requirement of most of these genes for macroautophagy and/or the Cvt pathway has been previously reported, and it is widely thought that defects in membrane trafficking pathways affect the lipid supply that is needed for extension of the phagophore, the initial sequestering compartment that generates the autophagosome (21, 31, 49, 50, 57, 59, 66, 75, 81, 94, 95, 97, 100). Accordingly, it is reasonable—and partly a verification of the success of the screens—that both groups identified a certain number of membrane trafficking-related genes.

Interestingly, one screen identified *DNM1*, which encodes a mitochondrial dynamin-related GTPase required for mitochondrial fission (28). This finding is in agreement with previous reports that the fragmentation of mitochondria is a prerequisite for mitophagy in mammalian cells (90), and the *dnm1Δ* strain inhibits the mitophagy induced by *mdm38* conditional knockout in yeast (65). Although it remains unclear whether mitochondrial fusion and fission affect mitophagy, because of the limitation of the size of the autophagosome it may be essential to split mitochondria by fission to generate a sequestering vesicle of the size appropriate for mitophagy.

Characterization of the Mitochondrial Receptor Atg32

Among >30 genes identified from the above screen, both Okamoto *et al.* and Kanki *et al.* paid careful attention to *ECM37/YIL146C* as a mitophagy-specific gene and designated it as *ATG32* (29, 68). The deletion of *ATG32* does not affect nonselective autophagy, the Cvt pathway, and pexophagy, but completely inhibits mitophagy. Atg32, a protein composed of 529 amino acids, is predicted to have a single transmembrane domain. A proteinase sensitivity assay conducted on crude

mitochondrial fractions suggested that Atg32 is located in the mitochondrial outer membrane with its N- and C-terminal domains oriented toward the cytosol and the intermembrane space, respectively (68). Yeast two-hybrid and immunoprecipitation experiments revealed that Atg32 can bind Atg11 and Atg8. Because Atg11 is an adaptor protein that interacts with cargo-specific receptor proteins for selective autophagy, Atg32 is thought to be a mitochondrial receptor for mitophagy. Notably, the Atg11–Atg32 interaction dramatically increases under conditions of nitrogen starvation that can induce mitophagy. Thus, it is thought that the Atg11–Atg32 interaction is the first physical step for mitochondria degradation *via* autophagy. Atg8 is a component of the autophagosome, and the PE-conjugated form of Atg8 (Atg8-PE) is involved in phagophore expansion (19, 52). Atg8 and LC3, a mammalian homolog of Atg8, bind Atg19 and p62, respectively. Interestingly, the cytosolic domain of Atg32 has a WXXI/L/V sequence, which is identified as an Atg8 binding motif present in Atg19 and p62 (20, 62, 70). In fact, the WXXI/L/V motif of Atg32 is critical for Atg8–Atg32 interaction and, to some extent, contributes to mitophagy (67, 68). The reason for this partial contribution might be that Atg32 also interacts with Atg11, a primary factor for linking the autophagy machinery with mitochondria at the early stage of mitophagy, whereas Atg32 interacts with Atg8 for assisting efficient formation of phagophores surrounding mitochondria at a late stage of mitophagy. Alternatively, there might be other binding sites for Atg8–Atg32 interaction *in vivo*, so that disruption of the WXXI/L/V motif only results in partial defects in mitophagy.

The Cvt Pathway, Pexophagy, and Mitophagy Depend on a Similar Molecular Process

The identification and characterization of Atg32 provided some insight into the process of selecting and delivering mitochondria to the vacuole in yeast. As shown in Figure 1, this process resembles those of the Cvt pathway and pexophagy. Different from other types of autophagy, the Cvt pathway constitutively (*i.e.*, regardless of nutrient conditions or other types of cellular stress) delivers the cytosolic precursor form of Ape1 (prApe1) and Ams1 to the vacuole. PrApe1 is synthesized in the cytosol, forms dodecamers, and is further assembled into an Ape1 complex composed of multiple dodecamers. The Ape1 complex is recognized and bound by the receptor protein Atg19, which independently recruits Ams1 and forms the prApe1–Atg19–Ams1 complex (the Cvt complex) (77). The adaptor protein Atg11 recognizes and binds Atg19 in the context of the Cvt complex and transports the complex to the PAS, where most of the Atg proteins accumulate and where the initial sequestering membrane structure (*i.e.*, the phagophore) is generated (80, 83). When the Cvt complex reaches the PAS, the Cvt complex binds the phagophore membrane through an interaction between Atg19 and the lipid-conjugated Atg8-PE. The phagophore membrane expands around the Cvt complex, excluding bulk cytoplasm and forming the Cvt vesicle. Subsequently, the Cvt outer membrane fuses with the vacuole, releasing the inner vesicle, which is now termed a Cvt body, into the vacuole lumen. The membrane of the Cvt body is subsequently broken down, and the Cvt complex becomes matured; the propeptide of prApe1 is proteolytically removed and the Ape1 complex dissociates into dodecamers (46, 101) (Fig. 1).

TABLE 2. GENES IDENTIFIED FROM YEAST GENOME-WIDE SCREEN FOR MITOPHAGY-DEFECTIVE MUTANTS

	<i>Okamoto et al.</i> (initial screen)	<i>Kanki et al.</i> (initial screen)	<i>Function</i>
Membrane trafficking			
VPS41	ND1 (-)	-(-)	Vacuolar protein sorting
TRS85	-(-)	-(-)	Endoplasmic reticulum-to-Golgi transport
YPT7	-(-)	-(-)	Endocytosis, vacuole fusion
VAM7	ND1 (-)	-(-)	Vacuolar assembly
CCZ1	ND1 (-)	-(-)	Vacuolar assembly
MON1	ND1 (-)	-(-)	Vacuolar assembly
VPS15	ND1 (-)	ND1	Vacuolar protein sorting
VPS34	ND1 (-)	ND1	Vacuolar protein sorting
VPS18	ND1 (-)	ND1	Vacuolar protein sorting
VPS33	ND1 (-)	ND1	Vacuolar protein sorting
VAM3	ND1 (-)	ND2 (++)	Vacuolar assembly
VPS16	ND1 (-)	ND3 (-)	Vacuolar protein sorting
VPS39	ND1 (-)	ND3 (-)	Vacuolar protein sorting
VPS2	+(+)	ND1	MVB sorting
COG1	ND1 (+)	ND1	Golgi transport
SNF7	+(+)	ND2 (++)	MVB sorting
VPS4	+(+)	ND2 (++)	MVB sorting
VPS24	+(+)	ND2 (++)	MVB sorting
VPS36	ND2 (++)	+(-)	Vacuolar protein sorting
PEP12	ND1 (+)	-(-)	Endosome-to-vacuole transport
Mitochondrial			
ATG32	-(-)	-(-)	Mitophagy receptor
AIM26	ND2 (++)	+(-)	Unknown
AIM28/FCJ1	ND2 (++)	-(-)	Formation of mitochondrial cristae junction
DNM1	ND2 (++)	-(-)	Mitochondrial fission
FMC1	ND2 (++)	-(-)	Assembly of mitochondrial F ₁ F _o ATP synthase
MEM1/LPE10	ND2 (++)	+(-)	Mitochondrial magnesium transporter
YLR356W/ATG33	+(+)	+(-)	Unknown
YPR146C	ND2 (++)	+(-)	Unknown
Others			
EGD1	+(+)	+(-)	Protein targeting
ARD1	+(+)	ND2 (++)	N-terminal protein acetylation
OPI3	+(+)	ND2 (++)	Phosphatidylcholine biosynthesis
RPL13B	ND1 (+)	+(-)	Ribosomal subunit
RPL14A	ND1 (+)	-(-)	Ribosomal subunit
RPL15B	ND2 (++)	-(-)	Ribosomal subunit
ARG82	ND2 (++)	+(-)	Inositol polyphosphate multikinase
ARO2	ND1 (+)	-(-)	Chorismate synthase/flavin reductase
BCK1	ND1 (+)	+(-)	MAP kinase kinase kinase
BUB1	ND1 (+)	+(-)	Protein kinase
ICY2	ND2 (++)	-(-)	Unknown
MAK10	ND1 (+)	+(-)	Noncatalytic subunit of N-terminal acetyltransferase of the NatC type
NFT1	ND2 (++)	+(-)	Unknown
PMR1 ^a	ND2 (++)	-(-)	Ca ²⁺ /Mn ²⁺ P-type ATPase
HUR1 ^a	ND2 (++)	+(-)	Unknown
YIL165C	ND2 (++)	-(-)	Unknown
YOR019W	ND2 (++)	+(-)	Unknown

^aPMR1 and HUR1 partially overlap.

MVB, multivesicular body; ND1, not determined; ND2, not determined because the initial screen was negative; ND3, not determined because of slow growth of the knockout strain in lactate medium; ++, mitophagy is normal in the knockout strain; +, mitophagy is partially defective in the knockout strain; -, mitophagy is completely defective in the knockout strain.

Contrary to macroautophagy and the Cvt pathway, which are well characterized in *S. cerevisiae*, pexophagy is extensively studied in methylotrophic yeast, such as *P. pastoris* and *Hansenula polymorpha*. When no longer required, superfluous peroxisomes are degraded by a macroautophagic- or microautophagic-like process, called macro-

pexophagy and micropexophagy, respectively. In *P. pastoris*, adaptation from methanol to glucose medium induces micropexophagy, whereas a switch from methanol to ethanol medium induces macropexophagy (56, 58, 89). During peroxisome proliferation in *P. pastoris*, PpAtg30 is induced and binds the peroxisomal proteins PpPex3 and PpPex14.

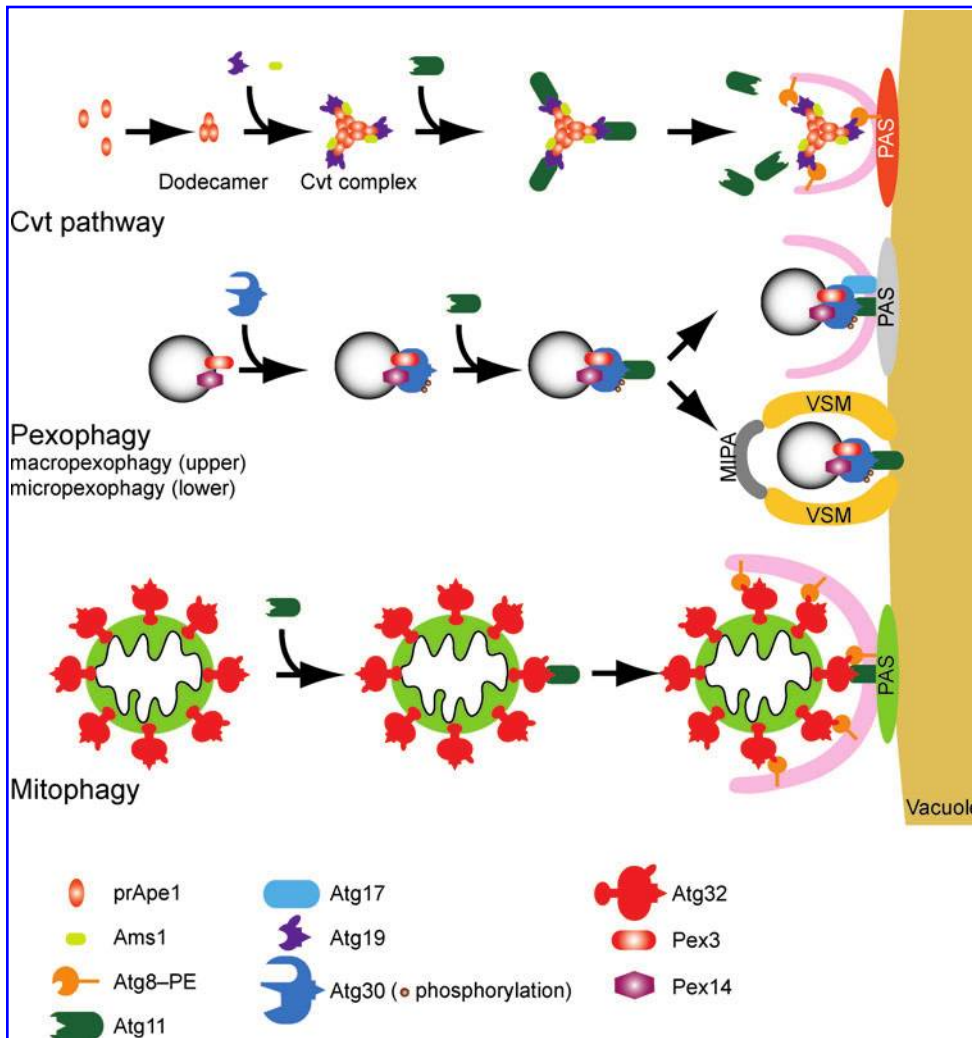


FIG. 1. Molecular processes of selective autophagy in yeast. The cytoplasm-to-vacuole targeting (Cvt) pathway: Precursor aminopeptidase I (prApe1) is synthesized in the cytosol, forms dodecamers, and is further assembled into an Ape1 complex composed of multiple dodecamers. The prApe1 complex is recognized and bound by the receptor protein autophagy-related 19 (Atg19), which independently recruits α -mannosidase (Ams1) and forms the prApe1-Atg19-Ams1 complex (the Cvt complex). The adaptor protein Atg11 recognizes and binds Atg19 in the Cvt complex and transports the complex to the phagophore assembly site (PAS). When the Cvt complex reaches the PAS, the complex binds the phagophore membrane through an interaction between Atg19 and the lipid-conjugated Atg8-phosphatidylethanolamine (PE). The phagophore membrane expands around the Cvt complex, excluding bulk cytoplasm and forming the Cvt vesicle. Pexophagy in *Pichia pastoris*: During peroxisome proliferation in *P. pastoris*, PpAtg30 is induced and

binds the peroxisomal proteins PpPex3 and PpPex14. Following the induction of pexophagy, PpAtg30 is phosphorylated and interacts with PpAtg11 and PpAtg17, allowing recruitment of the peroxisome to the PAS for both macropexophagy and micropexophagy. At the PAS, peroxisomes are sequestered by the phagophore membrane during macropexophagy or by the vacuolar sequestering membrane (VSM) and cup-shaped micropexophagy apparatus (MIPA) during micropexophagy. Mitophagy: When mitophagy is induced, Atg11 binds the mitochondrial resident protein Atg32. The Atg11-Atg32 complex recruits mitochondria to the PAS. When mitochondria reach the PAS, mitochondria are surrounded by the phagophore membrane through an interaction between Atg32 and Atg8-PE. The last step of the mitophagy pathway is controversial. In this figure, we show a macroautophagy-like process; however, some researchers observed a microautophagy-like process.

Following the induction of pexophagy, PpAtg30 is phosphorylated and interacts with PpAtg11 and PpAtg17, allowing recruitment of the peroxisome to the PAS for both macro- and micropexophagy (13). At the PAS, peroxisomes are sequestered by the phagophore membrane during macropexophagy or are sequestered by the vacuolar sequestering membrane and cup-shaped micropexophagy apparatus during micropexophagy (13, 47) (Fig. 1).

Similar to the Cvt pathway and pexophagy, and as discussed above, mitophagy requires Atg11 as an adaptor protein. When mitophagy is induced, Atg11 binds the mitochondrial resident protein Atg32. The Atg11-Atg32 interaction is needed to recruit mitochondria to the PAS. When mitochondria reach the PAS, the phagophore membrane begins to sequester the organelle; this depends on the interaction between Atg32 and Atg8-PE (67, 68). The last step of the mitophagy pathway is controversial. Based on electron microscopy, some research-

ers found that mitochondria are sequestered through a microautophagy-like process at the vacuole limiting membrane (9, 35, 65), whereas others observed macroautophagy-like mitophagosomes in the cytosol (28, 29, 68). The specific process may vary depending on the mitophagy triggers, similar to the situation with pexophagy.

In yeast, the PAS is usually formed next to the vacuole surface and most of the Atg proteins accumulate at the PAS during selective and nonselective autophagy (83). In the Cvt pathway, pexophagy and mitophagy, cargoes are delivered to the PAS *via* their interaction with Atg11 before they are sequestered within vesicles. In an *atg1Δ* strain, cargoes accumulate at the PAS; when different cargoes are labeled with fluorescent proteins, and cargo localization at the PAS is observed in an *atg1Δ* strain, CFP-Ape1 (Cvt pathway marker) and GFP-Atg32 (mitophagy marker) accumulate at different sites near the vacuole surface (29). This finding suggests that

the Cvt pathway and mitophagy use a different site for biogenesis of the PAS, although it is thought that macroautophagy and the Cvt pathway use the same site (27, 29). Similarly, pexophagy utilizes a pexophagy-specific PAS (47). These findings fit with the concept that cargoes are selectively sequestered during specific types of autophagy, and different cargo molecules or bulk cytoplasmic components are excluded from the resulting vesicles.

Mitophagy Induction and Regulation

There are several lines of evidence suggesting that damaged mitochondria are eliminated by mitophagy in yeast. For example, as discussed above, interference with F_0F_1 -ATPase biogenesis in a temperature-sensitive *fmc1* deletion mutant (71), or osmotic swelling of mitochondria caused by depletion of the mitochondrial K^+/H^+ exchanger Mdm38 (65), induces mitophagy. On the other hand, mitochondrial depolarization caused by an uncoupler such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) does not induce mitophagy in yeast (29, 34). At present, we know of very few conditions that can induce mitophagy in wild-type yeast cells. Mitophagy can be induced in wild-type yeast cells by nitrogen starvation or treating with the target of rapamycin (TOR) kinase inhibitor rapamycin after preculturing yeast in a nonfermentable medium that facilitates the proliferation of mitochondria (*e.g.*, where lactate or glycerol is the sole carbon source), or it can be induced at stationary phase when yeast cells are cultured in a nonfermentable medium (26, 29, 34, 68, 85). Although macroautophagy is also activated under these mitophagy-inducing conditions, mitochondria are specifically selected and degraded by mitophagy *via* the Atg11–Atg32 interaction (29, 68). Because a certain amount of mitochondria, but not the majority, are degraded by mitophagy, there is presumably some mechanism that can distinguish mitochondria that need to be degraded from those that are functioning normally. Two lines of evidence support this idea. Deffieu *et al.* and Okamoto *et al.* reported that NAC, a compound that increases the cellular GSH pool, prevents mitophagy induction (9, 68). This finding suggests that the mitochondrial redox status or ROS production level is one of the factors used to distinguish between healthy and unhealthy mitochondria. Atg33 is a mitochondrial outer-membrane protein identified as a mitophagy-related protein. Kanki *et al.* found that the deletion of *ATG33* blocks mitophagy to half the level of the wild type when induced by starvation, but it blocks mitophagy almost completely when mitophagy is induced at stationary phase (28). This finding raises the possibility that Atg33 is required to detect or present aged mitochondria for mitophagy when cells have reached the stationary phase. Taken together, Atg33 and another unknown factor(s) may serve to detect damaged, aged, or redox status-compromised mitochondria and promote their degradation by mitophagy in yeast.

Physiological Role of Mitophagy

In mammalian cells, two important roles of mitophagy have been reported. One is mitochondrial quality control and the other is mitochondria elimination during development as occurs during erythropoiesis. Studies on Parkinson disease reveal PINK1–Parkin-mediated degradation of depolarized mitochondria by autophagy (14, 48, 54, 55, 91). Studies on erythrocyte maturation demonstrate that the elimination of

mitochondria from reticulocytes is the result of Nix-mediated mitophagy (74, 76).

In a similar way, mitophagy in yeast is thought to have a role in mitochondrial quality control and elimination of excess mitochondria. As described in the foregoing section, there are several lines of evidence supporting the idea that impaired mitochondria are selected and degraded by mitophagy. However, mitophagy-deficient yeast, such as the *atg32Δ* strain, do not show any phenotype resulting from the accumulation of impaired mitochondria. Cell growth on a nonfermentable carbon source, the production of ROS, mtDNA copy number, and the amount of electron transport chain complex proteins are indistinguishable between wild-type and the *atg32Δ* strains (29, 68). These findings are not easily reconciled with the idea that mitophagy plays a critical role in mitochondrial quality control in yeast, and further studies are required to clarify this point. Nonetheless, it is clear that mitophagy in yeast has a role in the elimination of excess mitochondria. Mitophagy is induced at the stationary phase in media containing a nonfermentable carbon source. At stationary phase, the cellular energy requirement is reduced, and accordingly, the requirement for mitochondria is decreased. It is energetically costly to maintain mitochondria that are not needed, and there is the potential for ROS production if these organelles are not maintained. As a result, mitophagy is induced to eliminate excess mitochondria. Similarly, mitophagy is induced when cells are cultured in medium containing lactate as the sole carbon source and then shifted to nitrogen starvation medium supplemented with glucose; however, mitophagy is blocked when cells are shifted to nitrogen starvation medium supplemented with lactate (26). In this case, even under severe starvation conditions, the mitochondria are essential for energy production, and mitophagy is not induced. In contrast, if mitochondria are present in excess in glucose medium, mitochondria degradation is activated.

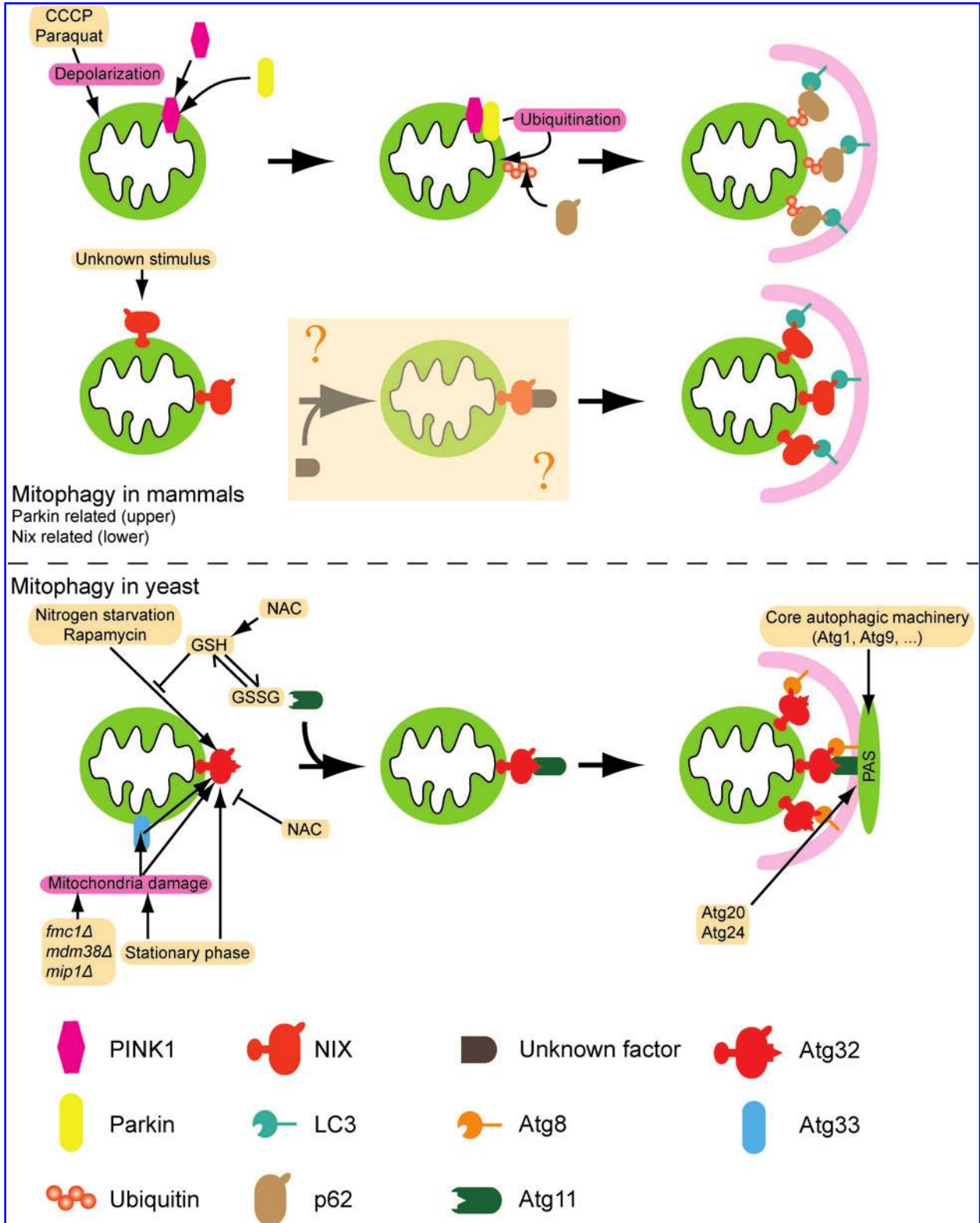
Mitophagy in Mammals and Yeast

Figure 2 summarizes current models of mitophagy in mammals and yeast. The mitochondrial kinase PINK1 has a mitochondrial targeting signal and is constitutively delivered to the mitochondrial outer membrane. PINK1 on the surface of mitochondria is, however, quickly cleaved by an unknown mechanism and is degraded by the proteasome (55). Only when mitochondria are depolarized, does PINK1 remain stably localized on the mitochondrial outer membrane. PINK1 then recruits Parkin, an E3 ubiquitin ligase, to the mitochondrial surface. Subsequently, Parkin ubiquitinates mitochondrial proteins (14, 48, 104). Although several substrates of Parkin, such as voltage-dependent anion channel or mitofusin, have been reported (14, 104), the specific substrate required for mitophagy remains unclear. Finally, the multi-signaling adaptor protein p62/SQSTM1 and/or the microtubule-associated histone deacetylase 6 (HDAC6) interact with the ubiquitinated mitochondrial proteins, and then autophagosomes enwrap the mitochondria *via* the p62/HDAC6–LC3 interaction (14, 44) (Fig. 2, upper panel). This step remains controversial, as it has been reported that *p62* knockout mouse embryonic fibroblasts show wild-type levels of mitophagy (69).

Nix, a BH3-only member of the Bcl-2 family, is a mitochondrial outer-membrane protein and has a WXXL

sequence that is identified as a yeast Atg8 and mammalian LC3 binding motif (62, 63). During terminal erythroid differentiation, an unknown stimulus triggers mitophagy. The next step remains controversial; mitochondrial depolarization may occur in a Nix-dependent manner (74), or mitochondrial depolarization may not occur at this time

(102). Nix then interacts with LC3, and this interaction contributes to the formation of selective autophagosomes (mitophagosomes) that surround mitochondria (63). Because Nix functions as a mitochondria tag, this BH3-only protein is considered to be a functional counterpart of Atg32. However, it is not known whether Nix requires an



adaptor protein corresponding to Atg11 in yeast (Fig. 2, middle panel, beige box).

Mitochondrial damage caused by mutations of *fmc1*, *mdm38*, or *mip1* induces mitophagy in yeast, which is detected and signaled to the mitophagy machinery through, at least in part, Atg33. Mitophagy is also induced at stationary phase when cells are grown in a nonfermentable medium. In the latter case, some, if not all, mitochondria are aged and damaged during the growing phase. These aged and damaged mitochondria are also detected and signaled to the mitophagy machinery through Atg33. The expression level of Atg32 is dramatically increased in nonfermentable medium, positively affecting the induction of mitophagy. NAC, a scavenger of free radicals, can suppress the expression of Atg32 and mitophagy. Thus, oxidative stress positively affects mitophagy induction (Fig. 2, lower panel). Nitrogen starvation or the TOR kinase inhibitor rapamycin also induces mitophagy in yeast pregrown on a nonfermentable medium. The cellular pool of GSH, which affects the mitochondrial redox status, negatively regulates mitophagy induced by nitrogen starvation or rapamycin. Once mitophagy is induced, mitochondrial Atg32 interacts with Atg11, followed by recruitment of the mitochondria to the PAS and mitophagy-specific uptake.

Perspectives

During the last few years, there has been significant progress in studies on mitophagy in yeast. In particular, the identification of Atg32 has provided substantial insight into the molecular aspects of mitophagy. There are, however, many questions still to be addressed. (i) It is clear that the specific interaction between Atg32 and Atg11 is an initial selection step of mitochondria as a cargo. However, how these proteins interact and what factors regulate this interaction are not known. In particular, whether the mitophagy induction signals are derived from mitochondria or the cytosol is an important issue. In other words, the question is whether mitochondria can dictate their own self-degradation by detecting internal damage or, instead, whether some cytosolic factors control mitochondria degradation by monitoring the impairment of mitochondria (the levels of mitochondrial ROS,

for example). (ii) The physiological role of mitophagy in yeast remains unclear. It is apparent that mitophagy in mammalian cells contributes to maintaining the quality of mitochondria. Thus, we think that there should be a similar role of mitophagy in yeast. However, deletion of the mitophagy-specific gene *ATG32* does not affect cell growth on nonfermentable medium or increase cellular ROS production (29, 68), which leaves open the question of how yeast mitophagy contributes to the quality control of mitochondria. Further studies focusing on a potential link between mitophagy and mitochondrial quality control in yeast are required. (iii) Unfortunately, mammalian homologs of the Atg proteins required for mitophagy (especially Atg11, 32, and 33) have not yet been identified. Considering the complexity of the mammalian macroautophagy processes including conventional Atg5- and Atg7-dependent macroautophagy and Atg5- and Atg7-independent macroautophagy (61), mitophagy might not be a simple process. If damaged mitochondria can be specifically degraded by mitophagy, it is reasonable to think that a yeast mitophagy-like receptor–adaptor interaction is present in mammalian cells, and Nix is a good candidate for the counterpart of Atg32.

In mammals, mitophagy governs the elimination of damaged mitochondria. At present, except for Parkinson disease, there is little evidence that defects in mitophagy are associated with mitochondrial diseases due to the dysfunction of the respiratory chain. Mitochondrial disease is a collective designation including some types of neurodegenerative disease and diabetes mellitus and is caused by many etiologies, such as accumulation of mitochondria DNA mutations, nuclear DNA mutations encoding mitochondrial proteins, nucleotide pool imbalance, and so on. Notably, mitochondria in most mitochondrial disease cells are heteroplasmic (*e.g.*, functional and impaired mitochondria are present heterogeneously in the same cell). These types of mitochondrial diseases are potential targets for therapeutic treatment using mitophagy, which could selectively eliminate compromised mitochondria from a heterogeneous population (27). Recently, it has been reported that overexpression of Parkin can eliminate mitochondria with deleterious mtDNA mutations, but not those with wild-type mtDNA, in heteroplasmic cybrid cells (82).

FIG. 2. Schematic models of mitophagy in mammals and in yeast. Mitophagy in mammalian cells: Parkin-related process—When mitochondria are depolarized (*e.g.*, by carbonyl cyanide *m*-chlorophenylhydrazone [CCCP] or paraquat treatment), PINK1 can stably localize on the mitochondrial outer membrane. PINK1 recruits Parkin to the surface of mitochondria. Subsequently, Parkin ubiquitinates mitochondrial proteins, although the specific substrate required for mitophagy remains unclear. Finally, p62 and/or histone deacetylase 6 (HDAC6) interact with ubiquitinated mitochondrial proteins, and then autophagosomes enwrap the mitochondria by p62/HDAC6–LC3 interaction. Nix-related process—During terminal erythroid differentiation, an unknown stimulus triggers mitophagy. Nix interacts with LC3; this interaction contributes to the formation of autophagosomes surrounding mitochondria. It is not known whether Nix requires an adaptor protein corresponding to Atg11 in yeast (beige box). Mitophagy in yeast: Mitochondrial damage caused by mutations of *fmc1*, *mdm38*, or *mip1* induces mitophagy. Mitophagy is also induced at stationary phase when cells are grown in nonfermentable medium. Appropriate signals are delivered to the mitophagic machinery through, in part, Atg33. At stationary phase, the expression level of Atg32 is dramatically increased, positively affecting the induction of mitophagy. *N*-acetylcysteine (NAC), a scavenger of free radicals, can suppress the expression of Atg32. Nitrogen starvation or the target of rapamycin kinase inhibitor rapamycin also induces mitophagy. The cellular pool of reduced glutathione (GSH) negatively regulates mitophagy induced by nitrogen starvation or rapamycin. Once mitophagy is induced, mitochondrial Atg32 interacts with Atg11. Atg11 recruits mitochondria to the PAS, where mitophagy-specific uptake occurs. At the PAS, Atg32 interacts with Atg8 on the phagophore membrane and promotes formation of the autophagosome surrounding mitochondria (the mitophagosome). Atg20, Atg24, and most of the core autophagy machinery components form a complex at the PAS and cooperate to complete mitophagosome formation. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

This finding strongly supports the idea that mitophagy is a valid therapeutic target for mitochondrial diseases. However, further investigations are required prior to actual clinical application.

Acknowledgments

This work was supported in part by Grant-in-Aids for Scientific Research on Priority Areas (20059034, 22020012, and 22020028 to T.K. and K.O.) and Scientific Research (C) (20570144 to K.O.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT), The Uehara Memorial Foundation (T.K.), Takeda Science Foundation (T.K.), Osaka University Life Science Young Independent Researcher Support Program through the Special Coordination Funds for Promoting Science and Technology from the MEXT (to K.O.), and National Institutes of Health (Grant GM53396 to D.J.K.).

References

1. Arai M, Imai H, Koumura T, Yoshida M, Emoto K, Umeda M, Chiba N, and Nakagawa Y. Mitochondrial phospholipid hydroperoxide glutathione peroxidase plays a major role in preventing oxidative injury to cells. *J Biol Chem* 274: 4924–4933, 1999.
2. Barth H, Meiling-Wesse K, Epple UD, and Thumm M. Autophagy and the cytoplasm to vacuole targeting pathway both require Aut10p. *FEBS Lett* 508: 23–28, 2001.
3. Barth H, Meiling-Wesse K, Epple UD, and Thumm M. Mai1p is essential for maturation of proaminopeptidase I but not for autophagy. *FEBS Lett* 512: 173–179, 2002.
4. Bernales S, Schuck S, and Walter P. ER-phagy: selective autophagy of the endoplasmic reticulum. *Autophagy* 3: 285–287, 2007.
5. Bogenhagen DF. Repair of mtDNA in vertebrates. *Am J Hum Genet* 64: 1276–1281, 1999.
6. Cao Y and Klionsky DJ. Atg26 is not involved in autophagy-related pathways in *Saccharomyces cerevisiae*. *Autophagy* 3: 17–20, 2007.
7. Cheong H, Yorimitsu T, Reggiori F, Legakis JE, Wang C-W, and Klionsky DJ. Atg17 regulates the magnitude of the autophagic response. *Mol Biol Cell* 16: 3438–3453, 2005.
8. Clark SL Jr. Cellular differentiation in the kidneys of newborn mice studies with the electron microscope. *J Biochem Biochem Cytol* 3: 349–362, 1957.
9. Deffieu M, Bhatia-Kissova I, Salin B, Galinier A, Manon S, and Camougrand N. Glutathione participates in the regulation of mitophagy in yeast. *J Biol Chem* 284: 14828–14837, 2009.
10. Elmore SP, Qian T, Grissom SF, and Lemasters JJ. The mitochondrial permeability transition initiates autophagy in rat hepatocytes. *FASEB J* 15: 2286–2287, 2001.
11. Epple UD, Eskelinen E-L, and Thumm M. Intravacuolar membrane lysis in *Saccharomyces cerevisiae*. Does vacuolar targeting of Cvt17/Aut5p affect its function? *J Biol Chem* 278: 7810–7821, 2003.
12. Epple UD, Suriapranata I, Eskelinen E-L, and Thumm M. Aut5/Cvt17p, a putative lipase essential for disintegration of autophagic bodies inside the vacuole. *J Bacteriol* 183: 5942–5955, 2001.
13. Farre JC, Manjithaya R, Mathewson RD, and Subramani S. PpAtg30 tags peroxisomes for turnover by selective autophagy. *Dev Cell* 14: 365–376, 2008.
14. Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, and Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 12: 119–131, 2010.
15. Guan J, Stromhaug PE, George MD, Habibzadegah-Tari P, Bevan A, Dunn WA Jr., and Klionsky DJ. Cvt18/Gsa12 is required for cytoplasm-to-vacuole transport, pexophagy, and autophagy in *Saccharomyces cerevisiae* and *Pichia pastoris*. *Mol Biol Cell* 12: 3821–3838, 2001.
16. Harding TM, Hefner-Gravink A, Thumm M, and Klionsky DJ. Genetic and phenotypic overlap between autophagy and the cytoplasm to vacuole protein targeting pathway. *J Biol Chem* 271: 17621–17624, 1996.
17. Harding TM, Morano KA, Scott SV, and Klionsky DJ. Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. *J Cell Biol* 131: 591–602, 1995.
18. Hutchins MU, Veenhuis M, and Klionsky DJ. Peroxisome degradation in *Saccharomyces cerevisiae* is dependent on machinery of macroautophagy and the Cvt pathway. *J Cell Sci* 112: 4079–4087, 1999.
19. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M, Noda T, and Ohsumi Y. A ubiquitin-like system mediates protein lipidation. *Nature* 408: 488–492, 2000.
20. Ichimura Y, Kumanomidou T, Sou YS, Mizushima T, Ezaki J, Ueno T, Kominami E, Yamane T, Tanaka K, and Komatsu M. Structural basis for sorting mechanism of p62 in selective autophagy. *J Biol Chem* 283: 22847–22857, 2008.
21. Ishihara N, Hamasaki M, Yokota S, Suzuki K, Kamada Y, Kihara A, Yoshimori T, Noda T, and Ohsumi Y. Autophagosome requires specific early Sec proteins for its formation and NSF/SNARE for vacuolar fusion. *Mol Biol Cell* 12: 3690–3702, 2001.
22. Kabeya Y, Kamada Y, Baba M, Takikawa H, Sasaki M, and Ohsumi Y. Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy. *Mol Biol Cell* 16: 2544–2553, 2005.
23. Kabeya Y, Kawamata T, Suzuki K, and Ohsumi Y. Cis1/Atg31 is required for autophagosome formation in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 356: 405–410, 2007.
24. Kageyama T, Suzuki K, and Ohsumi Y. Lap3 is a selective target of autophagy in yeast, *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun* 378: 551–557, 2009.
25. Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, and Ohsumi Y. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J Cell Biol* 150: 1507–1513, 2000.
26. Kanki T and Klionsky DJ. Mitophagy in yeast occurs through a selective mechanism. *J Biol Chem* 283: 32386–32393, 2008.
27. Kanki T and Klionsky DJ. Atg32 is a tag for mitochondria degradation in yeast. *Autophagy* 5: 1201–1202, 2009.
28. Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z, Geng J, Mao K, Yang Z, Yen W-L, and Klionsky DJ. A genomic screen for yeast mutants defective in selective mitochondria autophagy. *Mol Biol Cell* 20: 4730–4738, 2009.
29. Kanki T, Wang K, Cao Y, Baba M, and Klionsky DJ. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev Cell* 17: 98–109, 2009.
30. Kawamata T, Kamada Y, Suzuki K, Kuboshima N, Aki-matsu H, Ota S, Ohsumi M, and Ohsumi Y. Characterization of a novel autophagy-specific gene, *ATG29*. *Biochem Biophys Res Commun* 338: 1884–1889, 2005.

31. Kihara A, Noda T, Ishihara N, and Ohsumi Y. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*. *J Cell Biol* 152: 519–530, 2001.
32. Kim J, Dalton VM, Eggerton KP, Scott SV, and Klionsky DJ. Apg7p/Cvt2p is required for the cytoplasm-to-vacuole targeting, macroautophagy, and peroxisome degradation pathways. *Mol Biol Cell* 10: 1337–1351, 1999.
33. Kim J, Kamada Y, Stromhaug PE, Guan J, Hefner-Gravink A, Baba M, Scott SV, Ohsumi Y, Dunn WA Jr., and Klionsky DJ. Cvt9/Gsa9 functions in sequestering selective cytosolic cargo destined for the vacuole. *J Cell Biol* 153: 381–396, 2001.
34. Kissova I, Deffieu M, Manon S, and Camougrand N. Uth1p is involved in the autophagic degradation of mitochondria. *J Biol Chem* 279: 39068–39074, 2004.
35. Kissova I, Salin B, Schaeffer J, Bhatia S, Manon S, and Camougrand N. Selective and non-selective autophagic degradation of mitochondria in yeast. *Autophagy* 3: 329–336, 2007.
36. Kissova IB and Camougrand N. Glutathione participates in the regulation of mitophagy in yeast. *Autophagy* 5: 872–873, 2009.
37. Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. *Nat Rev Mol Cell Biol* 8: 931–937, 2007.
38. Klionsky DJ, Cregg JM, Dunn WA Jr., Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, and Ohsumi Y. A unified nomenclature for yeast autophagy-related genes. *Dev Cell* 5: 539–545, 2003.
39. Klionsky DJ, Cuervo AM, Dunn WA Jr., Levine B, van der Klei I, and Seglen PO. How shall I eat thee? *Autophagy* 3: 413–416, 2007.
40. Klionsky DJ, Cueva R, and Yaver DS. Aminopeptidase I of *Saccharomyces cerevisiae* is localized to the vacuole independent of the secretory pathway. *J Cell Biol* 119: 287–299, 1992.
41. Kon M and Cuervo AM. Chaperone-mediated autophagy in health and disease. *FEBS Lett* 584: 1399–1404, 2010.
42. Kraft C, Deplazes A, Sohrmann M, and Peter M. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat Cell Biol* 10: 602–610, 2008.
43. Larsson NG, Garman JD, Oldfors A, Barsh GS, and Clayton DA. A single mouse gene encodes the mitochondrial transcription factor A and a testis-specific nuclear HMG-box protein. *Nat Genet* 13: 296–302, 1996.
44. Lee JY, Nagano Y, Taylor JP, Lim KL, and Yao TP. Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *J Cell Biol* 189: 671–679, 2010.
45. Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res* 8: 3–5, 2005.
46. Lynch-Day MA and Klionsky DJ. The Cvt pathway as a model for selective autophagy. *FEBS Lett* 584: 1359–1366, 2010.
47. Manjithaya R, Nazarko TY, Farre JC, and Subramani S. Molecular mechanism and physiological role of pexophagy. *FEBS Lett* 584: 1367–1373, 2010.
48. Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA, Sou YS, Saiki S, Kawajiri S, Sato F, Kimura M, Komatsu M, Hattori N, and Tanaka K. PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol* 189: 211–221, 2010.
49. Meiling-Wesse K, Barth H, and Thumm M. Ccz1p/Aut11p/Cvt16p is essential for autophagy and the cvt pathway. *FEBS Lett* 526: 71–76, 2002.
50. Meiling-Wesse K, Epple UD, Krick R, Barth H, Appelles A, Voss C, Eskelinen E-L, and Thumm M. Trs85 (Gsg1), a component of the TRAPP complexes, is required for the organization of the preautophagosomal structure during selective autophagy via the Cvt pathway. *J Biol Chem* 280: 33669–33678, 2005.
51. Mizushima N, Levine B, Cuervo AM, and Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 451: 1069–1075, 2008.
52. Nakatogawa H, Ichimura Y, and Ohsumi Y. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 130: 165–178, 2007.
53. Nakatogawa H, Suzuki K, Kamada Y, and Ohsumi Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat Rev Mol Cell Biol* 10: 458–467, 2009.
54. Narendra D, Tanaka A, Suen DF, and Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J Cell Biol* 183: 795–803, 2008.
55. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, and Youle RJ. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 8: e1000298, 2010.
56. Nazarko TY, Farre JC, and Subramani S. Peroxisome size provides insights into the function of autophagy-related proteins. *Mol Biol Cell* 20: 3828–3839, 2009.
57. Nazarko TY, Huang J, Nicaud JM, Klionsky DJ, and Sibirny AA. Trs85 is required for macroautophagy, pexophagy and cytoplasm to vacuole targeting in *Yarrowia lipolytica* and *Saccharomyces cerevisiae*. *Autophagy* 1: 37–45, 2005.
58. Nazarko TY, Polupanov AS, Manjithaya RR, Subramani S, and Sibirny AA. The requirement of sterol glucoside for pexophagy in yeast is dependent on the species and nature of peroxisome inducers. *Mol Biol Cell* 18: 106–118, 2007.
59. Nebauer R, Rosenberger S, and Daum G. Phosphatidylethanolamine, a limiting factor of autophagy in yeast strains bearing a defect in the carboxypeptidase Y pathway of vacuolar targeting. *J Biol Chem* 282: 16736–16743, 2007.
60. Nice DC, Sato TK, Stromhaug PE, Emr SD, and Klionsky DJ. Cooperative binding of the cytoplasm to vacuole targeting pathway proteins, Cvt13 and Cvt20, to phosphatidylinositol 3-phosphate at the pre-autophagosomal structure is required for selective autophagy. *J Biol Chem* 277: 30198–30207, 2002.
61. Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, Komatsu M, Otsu K, Tsujimoto Y, and Shimizu S. Discovery of Atg5/Atg7-independent alternative macroautophagy. *Nature* 461: 654–658, 2009.
62. Noda NN, Kumeta H, Nakatogawa H, Satoo K, Adachi W, Ishii J, Fujioka Y, Ohsumi Y, and Inagaki F. Structural basis of target recognition by Atg8/LC3 during selective autophagy. *Genes Cells* 13: 1211–1218, 2008.
63. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, Rogov V, Lohr F, Popovic D, Occhipinti A, Reichert AS, Terzic J, Dotsch V, Ney PA, and Dikic I. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep* 11: 45–51, 2010.

64. Nowikovsky K, Devenish RJ, Froschauer E, and Schweyen RJ. Determination of yeast mitochondrial KHE activity, osmotic swelling and mitophagy. *Methods Enzymol* 457: 305–317, 2009.
65. Nowikovsky K, Reipert S, Devenish RJ, and Schweyen RJ. Mdm38 protein depletion causes loss of mitochondrial K⁺/H⁺ exchange activity, osmotic swelling and mitophagy. *Cell Death Differ* 14: 1647–1656, 2007.
66. Obara K, Noda T, Niimi K, and Ohsumi Y. Transport of phosphatidylinositol 3-phosphate into the vacuole via autophagic membranes in *Saccharomyces cerevisiae*. *Genes Cells* 13: 537–547, 2008.
67. Okamoto K, Kondo-Okamoto N, and Ohsumi Y. A landmark protein essential for mitophagy: Atg32 recruits the autophagic machinery to mitochondria. *Autophagy* 5: 1203–1205, 2009.
68. Okamoto K, Kondo-Okamoto N, and Ohsumi Y. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev Cell* 17: 87–97, 2009.
69. Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, Sou YS, Kimura M, Sato S, Hattori N, Komatsu M, Tanaka K, and Matsuda N. p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes Cells* 15: 887–900, 2010.
70. Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, and Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J Biol Chem* 282: 24131–24145, 2007.
71. Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, and Martinou JC. Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. *Cell Death Differ* 12: 1613–1621, 2005.
72. Rep M and Grivell LA. The role of protein degradation in mitochondrial function and biogenesis. *Curr Genet* 30: 367–380, 1996.
73. Rodriguez-Enriquez S, He L, and Lemasters JJ. Role of mitochondrial permeability transition pores in mitochondrial autophagy. *Int J Biochem Cell Biol* 36: 2463–2472, 2004.
74. Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, and Wang J. Essential role for Nix in autophagic maturation of erythroid cells. *Nature* 454: 232–235, 2008.
75. Sato TK, Darsow T, and Emr SD. Vam7p, a SNAP-25-like molecule, and Vam3p, a syntaxin homolog, function together in yeast vacuolar protein trafficking. *Mol Cell Biol* 18: 5308–5319, 1998.
76. Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, Dorsey FC, Kundu M, Opferman JT, Cleveland JL, Miller JL, and Ney PA. NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc Natl Acad Sci U S A* 104: 19500–19505, 2007.
77. Scott SV, Guan J, Hutchins MU, Kim J, and Klionsky DJ. Cvt19 is a receptor for the cytoplasm-to-vacuole targeting pathway. *Mol Cell* 7: 1131–1141, 2001.
78. Scott SV, Hefner-Gravink A, Morano KA, Noda T, Ohsumi Y, and Klionsky DJ. Cytoplasm-to-vacuole targeting and autophagy employ the same machinery to deliver proteins to the yeast vacuole. *Proc Natl Acad Sci U S A* 93: 12304–12308, 1996.
79. Scott SV and Klionsky DJ. Delivery of proteins and organelles to the vacuole from the cytoplasm. *Curr Opin Cell Biol* 10: 523–529, 1998.
80. Shintani T, Huang W-P, Stromhaug PE, and Klionsky DJ. Mechanism of cargo selection in the cytoplasm to vacuole targeting pathway. *Dev Cell* 3: 825–837, 2002.
81. Shirahama K, Noda T, and Ohsumi Y. Mutational analysis of Csc1/Vps4p: involvement of endosome in regulation of autophagy in yeast. *Cell Struct Funct* 22: 501–509, 1997.
82. Suen DF, Narendra DP, Tanaka A, Manfredi G, and Youle RJ. Parkin overexpression selects against a deleterious mtDNA mutation in heteroplasmic cybrid cells. *Proc Natl Acad Sci U S A* 107: 11835–11840, 2010.
83. Suzuki K, Kubota Y, Sekito T, and Ohsumi Y. Hierarchy of Atg proteins in pre-autophagosomal structure organization. *Genes Cells* 12: 209–218, 2007.
84. Takeshige K, Baba M, Tsuboi S, Noda T, and Ohsumi Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 119: 301–311, 1992.
85. Tal R, Winter G, Ecker N, Klionsky DJ, and Abeliovich H. Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival. *J Biol Chem* 282: 5617–5624, 2007.
86. Teter SA, Eggerton KP, Scott SV, Kim J, Fischer AM, and Klionsky DJ. Degradation of lipid vesicles in the yeast vacuole requires function of Cvt17, a putative lipase. *J Biol Chem* 276: 2083–2087, 2001.
87. Tsukada M and Ohsumi Y. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 333: 169–174, 1993.
88. Tucker KA, Reggiori F, Dunn WA Jr., and Klionsky DJ. Atg23 is essential for the cytoplasm to vacuole targeting pathway and efficient autophagy but not pexophagy. *J Biol Chem* 278: 48445–48452, 2003.
89. Tuttle DL and Dunn WA Jr. Divergent modes of autophagy in the methylotrophic yeast *Pichia pastoris*. *J Cell Sci* 108: 25–35, 1995.
90. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, and Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 27: 433–446, 2008.
91. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, May J, Tocilescu MA, Liu W, Ko HS, Magrane J, Moore DJ, Dawson VL, Grailhe R, Dawson TM, Li C, Tieu K, and Przedborski S. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci U S A* 107: 378–383, 2010.
92. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 39: 359–407, 2005.
93. Wang C-W, Kim J, Huang W-P, Abeliovich H, Stromhaug PE, Dunn WA Jr., and Klionsky DJ. Apg2 is a novel protein required for the cytoplasm to vacuole targeting, autophagy, and pexophagy pathways. *J Biol Chem* 276: 30442–30451, 2001.
94. Wang C-W, Stromhaug PE, Kauffman EJ, Weisman LS, and Klionsky DJ. Yeast homotypic vacuole fusion requires the Ccz1-Mon1 complex during the tethering/docking stage. *J Cell Biol* 163: 973–985, 2003.
95. Wichmann H, Hengst L, and Gallwitz D. Endocytosis in yeast: evidence for the involvement of a small GTP-binding protein (Ypt7p). *Cell* 71: 1131–1142, 1992.
96. Wurmser AE and Emr SD. Novel PtdIns(3)P-binding protein Etf1 functions as an effector of the Vps34 PtdIns 3-kinase in autophagy. *J Cell Biol* 158: 761–772, 2002.

97. Wurmser AE, Sato TK, and Emr SD. New component of the vacuolar class C-Vps complex couples nucleotide exchange on the Ypt7 GTPase to SNARE-dependent docking and fusion. *J Cell Biol* 151: 551–562, 2000.
98. Yang Z, Huang J, Geng J, Nair U, and Klionsky DJ. Atg22 recycles amino acids to link the degradative and recycling functions of autophagy. *Mol Biol Cell* 17: 5094–5104, 2006.
99. Yen W-L, Legakis JE, Nair U, and Klionsky DJ. Atg27 is required for autophagy-dependent cycling of Atg9. *Mol Biol Cell* 18: 581–593, 2007.
100. Yen W-L, Shintani T, Nair U, Cao Y, Richardson BC, Li Z, Hughson FM, Baba M, and Klionsky DJ. The conserved oligomeric Golgi complex is involved in double-membrane vesicle formation during autophagy. *J Cell Biol* 188: 101–114, 2010.
101. Yorimitsu T and Klionsky DJ. Autophagy: molecular machinery for self-eating. *Cell Death Differ* 12: 1542–1552, 2005.
102. Zhang J, Randall MS, Loyd MR, Dorsey FC, Kundu M, Cleveland JL, and Ney PA. Mitochondrial clearance is regulated by Atg7-dependent and -independent mechanisms during reticulocyte maturation. *Blood* 114: 157–164, 2009.
103. Zhang Y, Qi H, Taylor R, Xu W, Liu LF, and Jin S. The role of autophagy in mitochondria maintenance: characterization of mitochondrial functions in autophagy-deficient *S. cerevisiae* strains. *Autophagy* 3: 337–346, 2007.
104. Ziviani E, Tao RN, and Whitworth AJ. *Drosophila* Parkin requires PINK1 for mitochondrial translocation and ubiquitinates Mitofusin. *Proc Natl Acad Sci U S A* 107: 5018–5023, 2010.

Address correspondence to:

Asst. Prof. Tomotake Kanki

Department of Clinical Chemistry and Laboratory Medicine
Kyushu University Graduate School of Medical Sciences
Fukuoka 812-8582
Japan

E-mail: kanki@cclm.med.kyushu-u.ac.jp

Assoc. Prof. Koji Okamoto
Laboratory of Mitochondrial Dynamics
Graduate School of Frontier Biosciences
Osaka University
Suita 565-0871
Japan

E-mail: kokamoto@fbs.osaka-u.ac.jp

Date of first submission to ARS Central, November 5, 2010;
date of final revised submission, December 14, 2010; date of
acceptance, January 1, 2011

Abbreviations Used

Ams1 = α -mannosidase
Ape1 = aminopeptidase I
ATG = autophagy-related
CCCP = carbonyl cyanide m-chlorophenylhydrazone
CMA = chaperone-mediated autophagy
Cvt = cytoplasm to vacuole targeting
GFP = green fluorescent protein
GSH = reduced glutathione
HDAC6 = histone deacetylase 6
MIPA = micropexophagy apparatus
mtDNA = mitochondrial DNA
MVB = multivesicular body
NAC = N-acetylcysteine
PAS = phagophore assembly site
PE = phosphatidylethanolamine
PINK1 = PTEN-induced putative kinase 1
prApe1 = precursor Ape1
ROS = reactive oxygen species
TOR = target of rapamycin
VSM = vacuolar sequestering membrane

This article has been cited by:

1. Maša Ždralovi#, Nicoletta Guaragnella, Lucia Antonacci, Ersilia Marra, Sergio Giannattasio. 2012. Yeast as a Tool to Study Signaling Pathways in Mitochondrial Stress Response and Cytoprotection. *The Scientific World Journal* **2012**, 1-10. [[CrossRef](#)]
2. Koji Okamoto, Noriko Kondo-Okamoto. 2011. Mitochondria and autophagy: Critical interplay between the two homeostats. *Biochimica et Biophysica Acta (BBA) - General Subjects* . [[CrossRef](#)]
3. Ignacio Vega-Naredo, Beatriz Caballero, Verónica Sierra, Marina García-Macia, David de Gonzalo-Calvo, Paulo J. Oliveira, María Josefa Rodríguez-Colunga, Ana Coto-Montes. 2011. Melatonin modulates autophagy through a redox-mediated action in female Syrian hamster Harderian gland controlling cell types and gland activity. *Journal of Pineal Research* no-no. [[CrossRef](#)]