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FORUM REVIEW ARTICLE

Mitochondria Autophagy in Yeast

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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

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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_oF_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 mitochondria 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

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

Phenotypes of the indicated gene knockout strain: + +, no defect; +, partial defect; −, severe defect.
[a]Phenotypes 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

	Okamoto et al. *(initial screen)*	*Kanki* et al. *(initial screen)*	*Function*
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_1F_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

[a] *PMR1* 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 methylotropic yeast, such as *P. pastoris* and *Hansenula polymorpha*. When no longer required, superfluous peroxisomes are degraded by a macroautophagic- or microautophagic-like process, called macropexophagy 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.

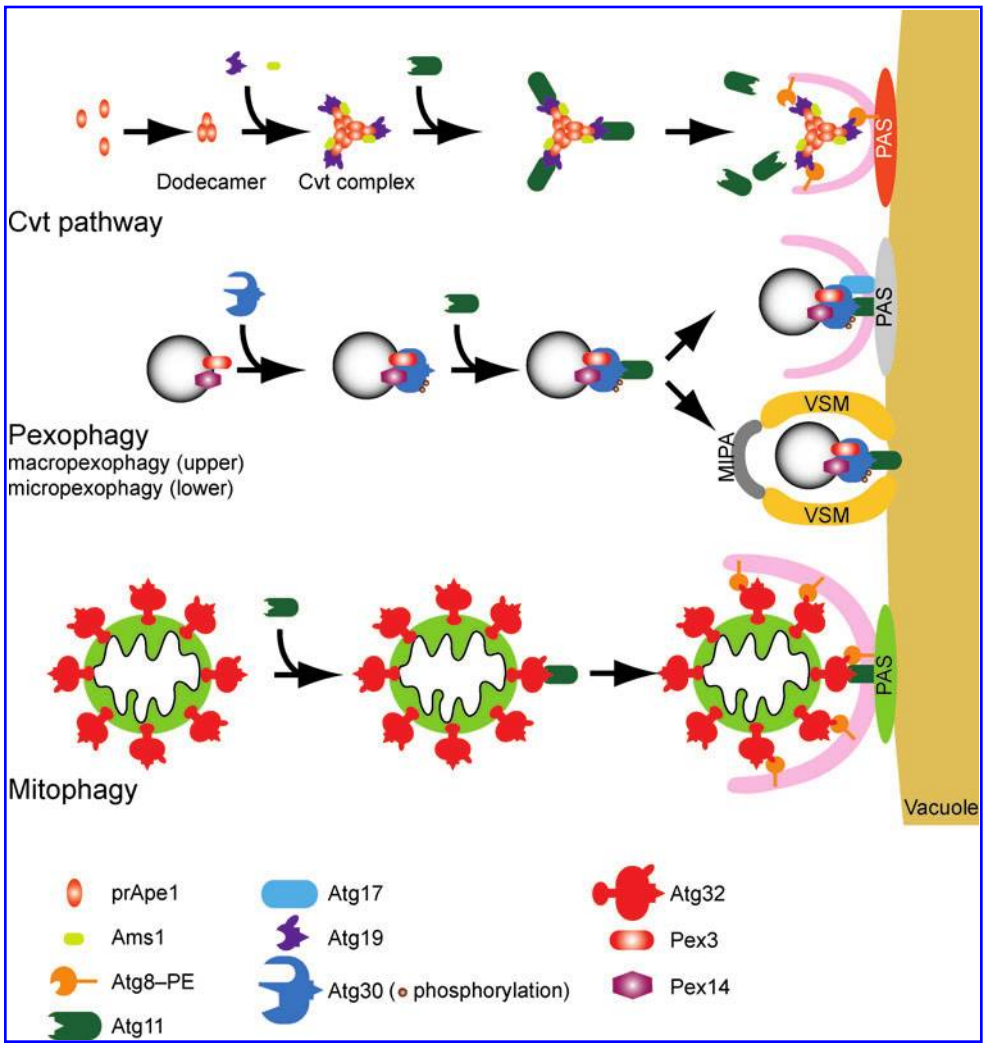


FIG. 1. Molecular processes of selective autophagy in yeast. The cytoplasm-to-vacuole targeting (Cvt) pathway: Precursor aminopeptidase I (prApe1) is synthesized in the cytosol, forms dodecamers, and is further assembled into an Ape1 complex composed of multiple dodecamers. The prApe1 complex is recognized 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 microsocopy, some researchers 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_oF_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).

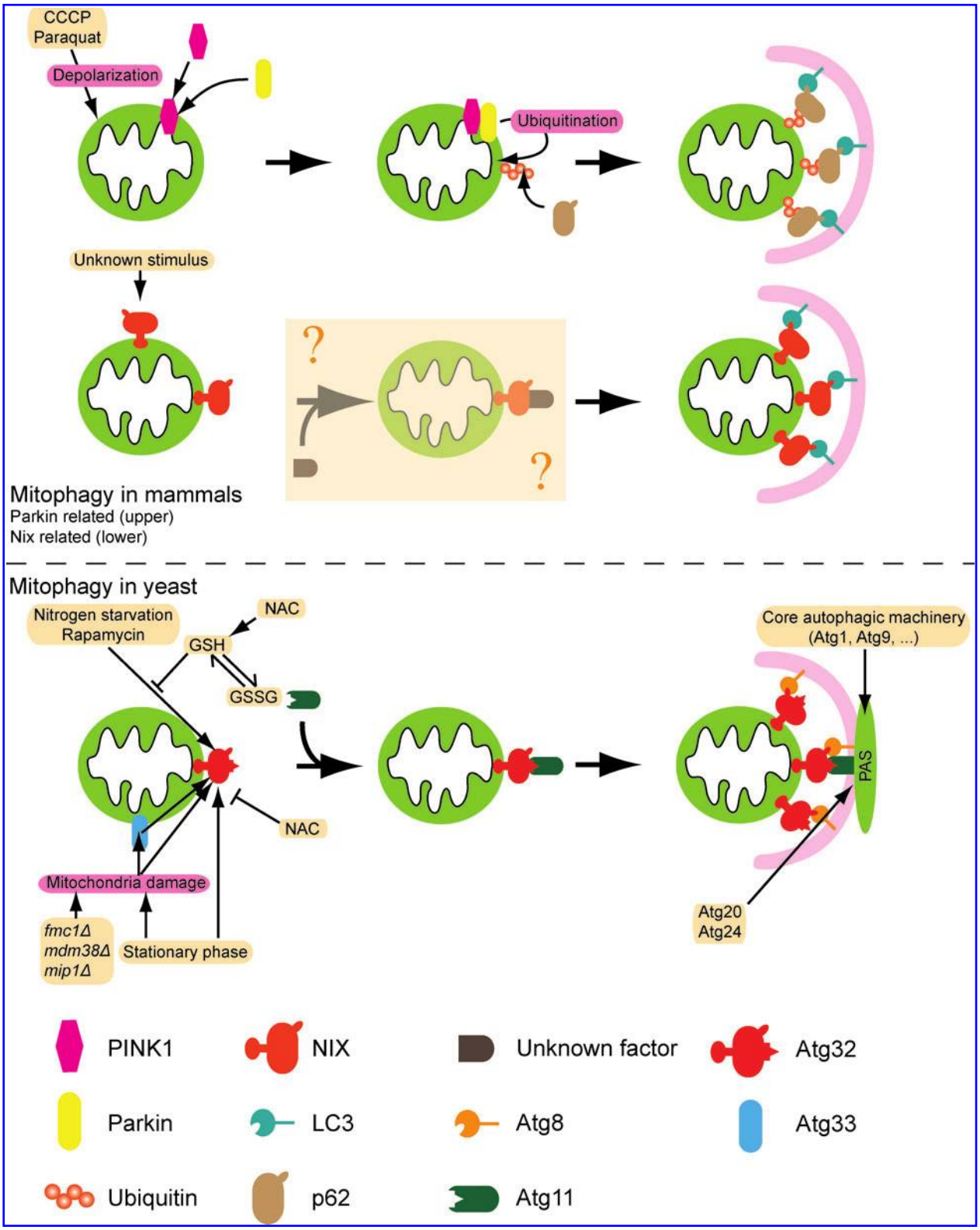
In a similar way, mitophagy in yeast is thought to have a role in mitochondrial quality control and elimination of excess mitochondria. As described in the foregoing section, there are several lines of evidence supporting the idea that impaired mitochondria are selected and degraded by mitophagy. However, 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 multisignaling 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/HADC6–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.

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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

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