Molecules and Cells

Minireview

Mechanisms and Physiological Roles of Mitophagy in Yeast

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Mitochondria are responsible for supplying of most of the cell's energy via oxidative phosphorylation. However, mitochondria also can be deleterious for a cell because they are the primary source of reactive oxygen species, which are generated as a byproduct of respiration. Accumulation of mitochondrial and cellular oxidative damage leads to diverse pathologies. Thus, it is important to maintain a population of healthy and functional mitochondria for normal cellular metabolism. Eukaryotes have developed defense mechanisms to cope with aberrant mitochondria. Mitochondria autophagy (known as mitophagy) is thought to be one such process that selectively sequesters dysfunctional or excess mitochondria within double-membrane autophagosomes and carries them into lysosomes/vacuoles for degradation. The power of genetics and conservation of fundamental cellular processes among eukaryotes make yeast an excellent model for understanding the general mechanisms, regulation, and function of mitophagy. In budding yeast, a mitochondrial surface protein, Atg32, serves as a mitochondrial receptor for selective autophagy that interacts with Atg11, an adaptor protein for selective types of autophagy, and Atg8, a ubiquitin-like protein localized to the isolation membrane. Atg32 is regulated transcriptionally and post-translationally to control mitophagy. Moreover, because Atg32 is a mitophagy-specific protein, analysis of its deficient mutant enables investigation of the physiological roles of mitophagy. Here, we review recent progress in the understanding of the molecular mechanisms and functional importance of mitophagy in yeast at multiple levels.

Keywords: Atg32, autophagy, mitochondria, mitophagy, yeast

INTRODUCTION

Mitochondria, double-membrane-enclosed organelles in eukaryotic cells, are involved in a wide range of cell signaling that controls cell death, immune responses, and calcium homeostasis. Mitochondria also play various roles in numerous metabolic pathways, such as amino acid metabolism, the TCA cycle, oxidative phosphorylation, fatty acid oxidation, and biosynthesis of heme and iron-sulfur clusters. In particular, oxidative phosphorylation in mitochondria produces a large amount of the energy required for cellular activities. In contrast, mitochondria generate reactive oxygen species (ROS) as a byproduct of the respiratory chain, which can cause severe oxidative damage to mitochondrial materials, such as proteins, nucleic acids, and lipids, leading to the production of dysfunctional mitochondria. Mitochondrial dysfunction has been associated with aging and various human diseases, such as neurodegeneration, metabolic disorders, diabetes, and cancer (Wallace, 2005). Thus, eliminating excess or dysfunctional mitochondria is crucial for protecting cells from the potential harm associated with disordered mitochondrial metabolism. One important pathway that contributes to mitochondrial quantity and quality control is the selective removal of mitochondria by autophagy, which is called mitophagy (Lemasters, 2005). In humans, mitophagy deficiency is associated with mitochondrial dysfunction

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and disorders, such as neuronal degeneration (Youle and Narendra, 2011).

Autophagy is a ubiquitous catabolic process that is highly conserved among eukaryotes (Fig. 1A). Double-membranous structures called isolation membranes emerge at the preautophagosomal structure or phagophore assembly site (PAS), and they extend to sequester cytoplasmic constituents as cargos into double-membrane vesicles called autophagosomes (Wen and Klionsky, 2016). Autophagosomes are then transported and fused to the lysosome/vacuole where the cargos are digested by hydrolytic enzymes for recycling. Studies of autophagy using the budding yeast *Saccharomyces cerevisiae* as a model have identified and characterized many proteins involved in this process, which are termed the Atg proteins (Klionsky et al., 2003). Although autophagy initially was recognized as a bulk, nonselective process, accumulating evidence has revealed that specific cellular materials are degraded selectively by the autophagy pathway (Farre and Subramani, 2016). Thus, in addition to bulk autophagy, there are several selective types of autophagy, including the cytoplasm-to-vacuole targeting (Cvt) pathway in *S. cerevisiae*, which delivers specific proteins (precursors of the aminopeptidase I Ape1 and α-mannosidase Ams1) to the vacuole, as well as different forms of organelle autophagy that are known as mitophagy (mitochondria), pexophagy (peroxisomes), ribophagy (ribosomes), and ER-phagy or reticulophagy (endoplasmic reticulum (ER)). Molecular understandings of mitophagy have been advanced through studies using yeast as a model. This review outlines the molecular details of yeast mitophagy and predicts its physiological roles.

ATG32 SERVES AS A RECEPTOR FOR MITOPHAGY IN YEAST

Important breakthroughs in the understating of mitophagy in yeast have been provided by identification of genes involved in mitophagy. These have made it possible to study whether mitochondria are selectively delivered to the vacuole, how induction of mitophagy is regulated, and how mitochondria are targeted to the autophagy machinery.

Mitophagy in yeast is a selective type of autophagy

In early studies, mitophagy was detected by electron microscopy as mitochondrial fragments in the vacuole (Kissova et al., 2007; Takeshige et al., 1992). Methods to monitor mitophagy have been developed using GFP-tagged mitochondrial proteins, whose localization to the vacuole, degradation, and release of free GFP indicate the occurrence of mitophagy (Kanki and Klionsky, 2008; Kissova et al., 2004). Mitophagy can be induced by long-term cultivation in respiratory medium that contains a nonfermentable carbon source, such as lactate and glycerol, or by a shift from respiratory medium to nitrogen-free fermentation medium that contains glucose (Kanki and Klionsky, 2008; Kissova et al., 2004; Tal et al., 2007). These methods have revealed that most of the basic Atg proteins are essential for mitophagy (Kanki and Klionsky, 2008; Kanki et al., 2009b; Okamoto et al., 2009). Moreover, mitophagy requires Atg11, Atg20, and At24, which are important for selective autophagy but are dispensable for bulk autophagy (Kanki and Klionsky, 2008; Okamoto et al., 2009), indicating that mitophagy is a selective type of autophagy. Atg11 is a scaffold for assembling the PAS and serves as an adaptor protein for selective autophagy to recruit cargos to the PAS (Kim et al., 2001). The Atg17-Atg29-Atg31 protein complex, another autophagy scaffold specific to bulk autophagy (Kawamata et al., 2008), is dispensable for mitophagy, supporting the notion that mitophagy proceeds as a selective autophagy (Kanki and Klionsky, 2008; Okamoto et al., 2009). A precise role for Atg20 and Atg24 in selective autophagy remains unclear (Nice et al., 2002). Recently, Atg20 and Atg24 have been shown to be involved in autophagic degradation of mitochondria in the fission yeast *Schizosaccharomyces pombe*, which is highly diverged from *S. cerevisiae*, suggesting that mitophagy occurs in a selective manner in this organism (Zhao et al., 2016).

Identification of Atg32 as a mitophagy-specific receptor

Selective autophagy requires specific receptor proteins that tether cargos to the site of autophagosome formation. In yeast, specific receptors have been described for the Cvt pathway (Atg19 and Atg34), pexophagy (Atg30 in the methylotrophic yeast *Pichia pastoris* and Atg36 in *S. cerevisiae*), and ER-phagy (Atg39 and Atg40) (Farre et al., 2008; Mochida et al., 2015; Motley et al., 2012; Scott et al., 2001; Shintani et al., 2002; Suzuki et al., 2010). Atg32, a mitophagy receptor, has been identified in two independent comprehensive screens for yeast mutants defective in mitophagy (Kanki et al., 2009b; Okamoto et al., 2009). Atg32 is a 59-kDa single-spanning mitochondrial outer membrane protein that has its N- and C-termini exposed to the cytoplasm and mitochondrial intermembrane space, respectively. Atg32 is a mitophagy-specific protein; mitophagy is completely blocked in cells lacking Atg32, but Atg32 is dispensable for bulk autophagy, the Cvt pathway, or pexophagy (Kanki et al., 2009b; Okamoto et al., 2009). At its cytosolic N-terminus, Atg32 can bind to Atg8 and Atg11 (Fig. 1B) (Aoki et al., 2011; Kanki et al., 2009b; Okamoto et al., 2009). Atg8 is a ubiquitin-like protein that is conjugated to lipid phosphatidylethanolamine and required for autophagosome formation (Kirisako et al., 1999). Atg32 contains an Atg8-family-interacting motif/LC3-interacting region (AIM/LIR), whose consensus is (W/F/Y)XX(L/I/V), conserved among autophagic receptor proteins in yeast as well as in mammals (Fig. 1C) (Noda et al., 2010). Atg32 binds Atg8 through the AIM, and this binding is required for efficient mitophagy (Kondo-Okamoto et al., 2012; Okamoto et al., 2009). The Atg11-binding region of Atg32 contains (I/V)LS, a sequence shared among Atg11-interacting regions of selective autophagy receptors (Fig. 1D) (Aoki et al., 2011; Farre et al., 2013; Mochida et al., 2015). Through the interactions with Atg8 and Atg11, Atg32 recruits mitochondria to the PAS at the vacuole surface, where the autophagy machinery assembles to generate the mitochondrial autophagosome (mitophagosome). Notably, artificial relocation of the Atg32 cytosol domain to the surface of peroxisomes causes autophagic degradation of peroxisomes (Kondo-Okamoto et al., 2012),

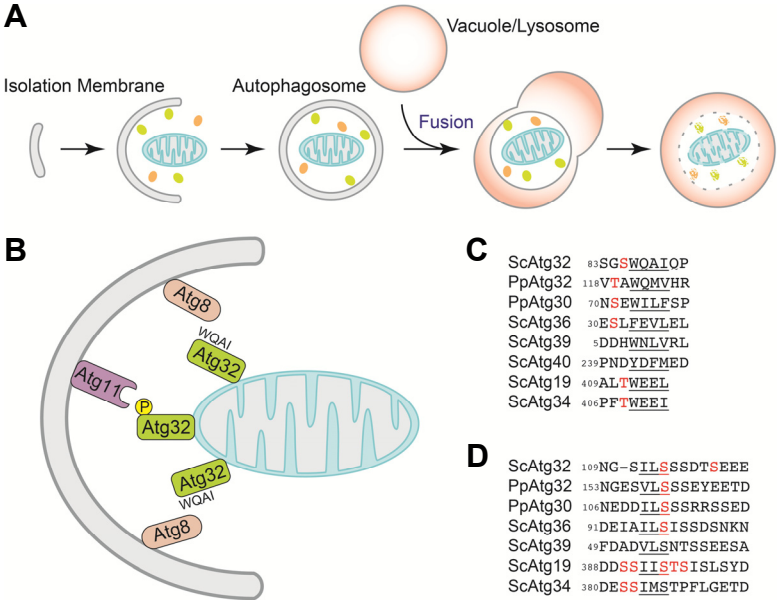

Fig. 1. Mitophagy in yeast. (A) Schematic representation of autophagy. When autophagy is induced, isolation membranes nucleate at the preautophagosomal structure/phagophore assembly site (PAS). They grow and engulf cytoplasmic cargos to form a double-membrane vesicle called an autophagosome. Autophagosomes subsequently fuse with the lysosome/ vacuole and release the cargo for degradation. (B) Atg32-mediated mitophagy in yeast. Atg32 acts as a mitophagy receptor that interacts with the adaptor protein Atg11 and the ubiquitin-like protein Atg8 to recruit mitochondria to the PAS. The Atg32-Atg11 interaction depends on the phosphorylation of Atg32 at Ser^{114} by CK2. (C, D) Sequence alignments of the Atg8-interacting (C) and Atg11-interacting (D) regions in selective autophagy receptor proteins. Atg8-family-interacting motif and (I/V)LS motif, which are well conserved among receptor proteins, are underlined. Red letters indicate residues subjected to phosphorylation (Farre and Subramani, 2016).

corroborating the role of Atg32 as a selective autophagy receptor.

TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATION OF ATG32

Expression of Atg32 is induced upon mitophagy induction. When Atg32 is analyzed by immunoblotting, multiple sizes of Atg32 are detected, suggesting that Atg32 undergoes post-translational modifications. Phosphorylation of Atg32 by casein kinase 2 (CK2) is one of the well-studied modifications of Atg32. Modifications other than phosphorylation also have been revealed.

Transcriptional regulation of ATG32

Efficient induction of mitophagy in *S. cerevisiae* requires prior respiration in media containing a nonfermentable carbon source, which upregulates Atg32 (Kanki and Klionsky, 2008; Kanki et al., 2009b; Okamoto et al., 2009). In the methylotrophic budding yeast *P. pastris*, which uses most of the pyruvate generated from glycolysis for oxidative phosphorylation, mitophagy can be induced by nitrogen starvation without prior growth in nonfermentable medium (Aihara et al., 2014). Expression of *P. pastris* Atg32 (PpAtg32) is highly suppressed in nutrient-rich media, and it is rapidly induced upon starvation (Aihara et al., 2014). This starvation-responsive induction of PpAtg32 is regulated at the transcriptional level. The DNA-binding protein Ume6 and the histone deacetylase complex Sin3-Rpd3 directly interact with the promoter region of the gene encoding PpAtg32 to repress its transcription (Aihara et al., 2014). In the absence of these proteins, PpAtg32 is expressed even in a nitrogen-rich condition. Treatment of cells with rapamycin, a specific inhibitor of target of rapamycin (TOR), also can cause derepression of Atg32. The transcriptional repression by Ume6-Sin3-Rpd3 and TOR is conserved in *S. cerevisiae*; Atg32 is de-repressed either by the absence of Ume6-Sin3-Rpd3 or by the addition of rapamycin during growth in fermentable medium. Thus, both in *S. cerevisiae* and *P. pastoris*, Ume6-Sin3-Rpd3 and TOR negatively regulate Atg32 expression to suppress mitophagy.

Factors modulating Atg32 expression

The induction of Atg32 is affected by N-terminal acetyltransferase A (NatA), which co-translationally catalyzes acetylation of nascent peptide chains (Eiyama and Okamoto, 2015; Polevoda and Sherman, 2003). The enzymatic activity and ribosomal association of NatA are important for yeast mitophagy (Eiyama and Okamoto, 2015). Transcription of *ATG32* is reduced in NatA-deficient mutants, and overexpression of Atg32 partially suppresses mitophagy defects of the mutants. It is possible that the nascent polypeptides of proteins, which are involved in Atg32 expression, are a target of NatA-mediated acetylation. Induction of Atg32 and mitophagy also is linked to phospholipid methylation. Although the precise mechanism remains unknown, loss of Opi3, a phospholipid methyltransferase involved in the biosynthesis of phosphatidylcholine from phosphatidylethanolamine, causes impaired expression of Atg32 (Sakakibara et al., 2015).

Phosphorylation of Atg32

Under mitophagy-inducing conditions, Atg32 is phosphorylated at Ser^{114} and Ser^{119} (Fig. 1D) (Aoki et al., 2011). Specifically, phosphorylation of Ser^{114} is crucial for mitophagy and for the Atg32-Atg11 interaction, while phosphorylation at Ser^{114} and Ser^{119} are dispensable for Atg32-Atg8 binding (Aoki et al., 2011; Kondo-Okamoto et al., 2012). CK2 was

identified as a kinase that catalyzes the phosphorylation of Atg32 at Ser^{114} and Ser^{119} (Kanki et al., 2013). CK2-deficient cells exhibit a reduction in Atg32 phosphorylation, Atg32-Atg11 interaction, and mitophagy. Since CK2 is active in nutrient-rich conditions, it remains unclear how CK2-dependent phosphorylation takes place and promotes mitophagy during starvation. In addition to the phosphorylation required for Atg11 binding, Thr^{119} near the AIM of PpAtg32 in *P. pastoris* is likely to be phosphorylated and contribute to Atg8 binding and efficient mitophagy (Fig. 1C) (Farre et al., 2013).

Modification of Atg32 not due to phosphorylation

Although the N-terminal cytosolic region of Atg32 is phosphorylated and important for mitophagy, its C-terminus in the intermembrane space is dispensable for mitophagy (Aoki et al., 2011; Kondo-Okamoto et al., 2012). Atg32 was found to be proteolytically processed at its C-terminal intermembrane space domain upon induction of mitophagy (Wang et al., 2013). This proteolytic processing is mediated by the inner membrane i-AAA (ATPases associated with various cellular activities) protease Yme1. Blocking the processing by C-terminal tagging of Atg32 or by the *yme1Δ* mutation causes a defect in mitophagy. Moreover, the interaction between Atg32 and Atg11 is reduced in the *yme1Δ* mutant, suggesting that Yme1 contributes to recruitment of mitochondria to the PAS. However, it should be noted that a role of Yme1 in mitophagy is controversial (Campbell and Thorsness, 1998; Gaspard and McMaster, 2015; Thorsness et al., 1993; Wang et al., 2013; Welter et al., 2013).

Post-translational modification of Atg32 that increases the molecular weight of Atg32 by approximately 20 kDa has been detected (Levchenko et al., 2016). This modification takes place after mitophagy induction upon starvation or rapamycin treatment, and it is detectable only in the absence of the vacuolar proteinase Pep4, suggesting that the modified form of Atg32 is efficiently degraded in vacuoles. The modification requires the autophagy machinery, including the Atg1-Atg13 complex, Atg8 and its conjugation proteins, and Atg11. A role for this modification remains to be elucidated, but exploring the temporal order and interrelationship among this modification, Yme1-mediated C-terminal processing, and CK2-mediated N-terminal phosphorylation would be an interesting future direction to pursue.

INDUCTION OF MITOPHAGY

Although Atg32 is induced by mitophagy-inducing conditions, its ectopic expression under nutrient-rich conditions does not cause mitophagy, indicating that other factors are necessary for mitophagy induction. Several factors have been identified, but the molecular mechanisms of mitophagy induction are still unknown.

Oxidative stress

Treatment of cells with *N*-acetylcysteine (NAC), an antioxidant, can suppress mitophagy (Deffieu et al., 2009; Okamoto et al., 2009), suggesting that oxidative stress facilitates mitophagy induction. However, it also was shown that suppression of mitophagy by NAC does not occur by its ROS scavenging properties but by its effect on glutathione metabolism (Deffieu et al., 2009). Therefore, cellular redox status is one of the key factors that govern mitophagy induction.

Mitochondrial dysfunction

Accumulation of dysfunctional mitochondria can trigger mitophagy. In mammals, mitophagy can be induced by drugs that impair mitochondrial oxidative phosphorylation, including the uncoupler carbonyl cyanide *m*-chlorophenyl-hydrazone (CCCP), which depolarizes mitochondria, the mitochondrial ATP synthase inhibitor oligomycin, and antimycin A, which inhibits the electron transport chain (Lazarou et al., 2015; Narendra et al., 2008). In yeast, autophagic degradation of mitochondria is induced by the impaired mitochondrial ATP synthase biogenesis or by the inactivation of mitochondrial K^+/H^+ exchange (Nowikovsky et al., 2007; Priault et al., 2005). In contrast, neither CCCP nor oligomycin treatment effectively induces mitophagy in yeast (Kanki and Klionsky, 2008; Kissova et al., 2004; Mendl et al., 2011). Interestingly, treating yeast cells with antimycin A in a respiratory condition induces bulk autophagy in a manner dependent on Atg32 and Atg11 (Deffieu et al., 2013). How Atg32 contributes to bulk autophagy remains to be elucidated.

FACTORS MODULATING MITOPHAGY

In addition to Atg32, multiple factors have been shown to be involved in mitophagy. In most cases, their roles are specifically crucial for mitophagy but not for other types of autophagy, suggesting that mitophagy is facilitated and regulated by these specific mechanisms.

Aup1, Atg33, and Whi2

The mitochondrial protein phosphatase homolog Aup1 is required for mitophagy during long-term cultivation in respiration medium (Tal et al., 2007). Aup1 appears to regulate mitophagy through the transcription factor Rtg3 by an unknown mechanism (Journo et al., 2009).

The mitochondrial outer membrane protein Atg33 was identified through a genome-wide screen for mitophagy-deficient mutants (Kanki et al., 2009a). Atg33 facilitates mitophagy, but its role remains unknown. Whi2, which functions in the general stress response and Ras-protein kinase A (PKA) signaling pathway, is required for efficient mitophagy induction by rapamycin treatment (Mendl et al., 2011). In contrast, Whi2 is dispensable for mitophagy induction by nitrogen starvation (Mao et al., 2013). Cells lacking Whi2 exhibit aberrant mitochondrial morphology, mitochondrial dysfunction, and ROS accumulation, raising the possibility that damaged mitochondria are not eliminated effectively (Leadsham et al., 2009). Whi2 might regulate mitophagy through nutrient signaling pathways that involve the stress response transcription factor Msn2 and/or Ras-PKA (Muller and Reichert, 2011).

ER-mitochondria encounter structure

In mammalian cells, autophagosomes are generated at ER-mitochondria contact sites where lipids were supplied for

growth of isolation membrane (Hamasaki et al., 2013). In yeast, ER-mitochondria contact is mediated by the ER-mitochondria encounter structure (ERMES) complex (Klecker et al., 2014). Lack of ERMES subunits causes severe mitophagy defects (Bockler and Westermann, 2014). Artificial tethering between mitochondria and ER in the ERMES mutants suppresses mitophagy defects, indicating that mitophagy is promoted by the contact between mitochondria and the ER *per se*. During starvation, ERMES is present at sites of isolation membrane expansion, suggesting that mitophagosomes are formed at ER-mitochondria contact sites. In ERMES mutants, immature structures of isolation membrane, which are labeled by Atg5, are accumulated, suggesting that ERMES is required for the expansion of isolation membranes. Conceivably, ERMES facilitates the proximity of expanding mitophagosomes to the ER, so that sufficient membrane materials are supplied from the ER to engulf a large cargo. Two ERMES components (Mdm34 and Mdm12) are ubiquitinated by the E3 ligase Rsp5, contributing to efficient mitophagy (Belgareh-Touze et al., 2017).

Lipids

Sphingolipids are involved in the regulation of mitochondrial function. In yeast, Isc1, which generates ceramides from complex sphingolipids, translocates to the mitochondria when respiration is induced (Vaena de Avalos et al., 2004). Yeast cells lacking Isc1 exhibit mitochondrial dysfunction and hyperactivation of mitophagy (Teixeira et al., 2015). Mitophagy deficiency exacerbates the growth defect and shortened chronological lifespan of the *isc1*Δmutant. How sphingolipid signals modulate mitophagy remains to be elucidated.

Cardiolipin, a unique dimeric phospholipid, is synthesized and localized in the mitochondrial inner membrane. Yeast cells lacking the cardiolipin synthase Crd1 exhibit a mitophagy defect (Shen et al., 2017). In mammalian neuronal cells, cardiolipin directly regulates mitophagy. Upon mitophagy induction, cardiolipin is redistributed to the outer from the inner mitochondrial membrane (Chu et al., 2013). LC3, a homolog of Atg8, can bind to cardiolipin, and this binding is important for mitophagy.

Maturation of cardiolipin requires the cardiolipin remodeling enzyme, tafazzin (TAZ). Mutations in *Taz* cause Barth syndrome, which is characterized by perturbation of cardiolipin metabolism, leading to mitochondria dysfunction, cardio/skeletal myopathy, neutropenia, and growth retardation (Barth et al., 1983). TAZ deficiency in primary mouse embryonic fibroblasts causes a defect in mitophagosome formation (Hsu et al., 2015). Yeast cells deficient in Taz1, an ortholog of TAZ, displays a growth defect and reduction in mitophagy when lacking the i-AAA protease Yme1 (Gaspard and McMaster, 2015). Thus, the involvement of mitochondrial lipid metabolism that regulates mitophagy is of interest for further study.

Ubiquitin

The roles of ubiquitination in mitophagy have been revealed, especially in mammals. The E3 ubiquitin ligase Parkin translocates to the mitochondria upon induction of mitophagy and promotes ubiquitination of mitochondrial proteins (Yamano et al., 2016; Youle and Narendra, 2011). Reichert and coworkers developed a method that enables a biochemical high-throughput screen for mitophagy regulators in a genome-wide manner (Muller et al., 2015). They identified 86 positive and 10 negative regulators of mitophagy. Among them, the Ubp3-Bre5 deubiquitination complex, which translocates to mitochondria upon rapamycin-induced starvation, suppresses mitophagy, whereas it promotes other types of selective autophagy, such as ribophagy and the Cvt pathway (Kraft et al., 2008; Muller et al., 2015). Identifying the targets of Upb3-Bre5 and the mechanism of opposing effects of Ubp3-Bre5 on mitophagy and ribophagy/Cvt would be intriguing.

Mitochondrial dynamics

Mitochondrial fusion and fission determine the morphology and size of mitochondria in response to various intra- and extracellular stimuli (Okamoto and Shaw, 2005). As the size of mitochondria is much larger than that of autophagosomes, mitochondrial fission has been thought to facilitate mitophagy by dividing mitochondria into small fragments that can be engulfed by isolation membranes. Indeed, mitophagy is reduced in yeast cells lacking Dnm1, a dynamin-related GTPase that assembles at fission sites and promotes mitochondrial fission (Abeliovich et al., 2013; Kanki et al., 2009a; Mao et al., 2013). Moreover, when mitophagy is induced, Dnm1 is recruited to the sites of the mitochondria that are destined for degradation (Mao et al., 2013). Dnm1 can bind to Atg11, and this binding is required for the recruitment of Dnm1 to degrading mitochondria and efficient mitophagy. It is thus proposed that Atg11 recruits the fission machinery to the sites of mitophagosome formation.

Although mitophagy is reduced to some extent, substantial mitophagy takes place in the absence of the fission machinery (Abeliovich et al., 2013; Bernhardt et al., 2015; Mao et al., 2013; Mendl et al., 2011; Yamashita et al., 2016). Moreover, mitophagy can be induced in mammalian cells by hypoxia or the iron-chelating drug DFP in Drp1 (mammalian homolog of Dnm1)-deficient cells (Yamashita et al., 2016). Mitophagosomes can be detected in both mammalian and yeast cells in the absence of Drp1/Dnm1 (Yamashita et al., 2016). These insights raise the possibility that Dnm1-independent mitochondrial division occurs during mitophagy. Indeed, a small fragment of mammalian mitochondria is divided and released from parental mitochondria during mitophagosome formation. This process is independent of Drp1 and depends on elongation of the isolation membrane. Molecular mechanisms of the Drp1/Dnm1-independent mitochondrial division during mitophagy are of particular interest for further study.

PHYSIOLOGICAL ROLES OF MITOPHAGY IN YEAST

In mammals, mitophagy is thought to be related to physiological events. Recently, the physiological importance of mitophagy in yeast has begun to be clarified. As Atg32 is a mitophagy-specific protein, its deficient mutant can be utilized to investigate the biological significance of mitophagy in yeast.

Viability during starvation

It has been shown that autophagy-deficient yeast cells accumulate ROS and dysfunctional mitochondria during starvation (Suzuki et al., 2011; Zhang et al., 2007). In *S. pombe*, autophagy-dependent mitochondrial degradation takes place during nitrogen starvation upon proteasome inactivation (Takeda et al., 2010). Dysfunctional proteasomes result in ROS accumulation in mitochondria during starvation, and degradation of mitochondria by autophagy seems to be important for ROS elimination and cell survival. As for *S. cerevisiae*, mitophagy-defective cells, such as the *atg32Δ* and *atg11Δ* mutants, suffer from mitochondrial genome instability during starvation after respiratory growth (Kurihara et al., 2012). This is due to ROS production from the mitochondria, since elimination of ROS by NAC suppresses the phenotype. Conceivably, yeast cells control the amount of mitochondria by mitochondrial proliferation and mitophagy to keep the balance of energy production and suppression of ROS.

Loss of Atg32 shortens the chronological lifespan of cells grown under a caloric restriction condition (Richard et al., 2013). Under the condition, the *atg32Δ* mutant exhibits increased size and number of mitochondria and altered mitochondrial morphology. Moreover, mitochondrial composition and function are severely altered in the *atg32Δ* mutant. These support the notion that mitophagy contributes to the maintenance of a healthy population of mitochondria under longevity-extending conditions.

mtDNA inheritance

Yeast cells usually carry multiple copies of identical mtDNA (homoplasmy), but cells also can contain mtDNA with different sequences (heteroplasmy) derived from replication error. The fraction of mtDNA variants changes among generations due to competition (Stewart and Chinnery, 2015). Yeast zygotes inherit mtDNA from both gametes by mating, producing heteroplasmic diploids. Mutant mtDNA with large deletions (*rho*$^-$), which are respiration defective, can replicate faster than wild-type, full-length mtDNA (*rho*$^+$), frequently producing homoplasmic *rho*$^-$ diploid descendants. It has been shown that mitophagy is induced in heteroplasmic zygotes and is further activated by decreasing the mitochondrial transmembrane potential using uncouplers (Karavaeva et al., 2017). Moreover, adding an uncoupler decelerates clonal expansion of the *rho*$^-$ variants. The advantage for the wild-type mtDNA is decreased in mitophagy-deficient cells, suggesting that heteroplasmic zygotes preferentially degrade mitochondria with mutated mtDNA. Thus, yeast cells appear to have developed a mechanism that upregulates mitophagy to avoid the inheritance of mutated mtDNA.

Virulence

The budding yeast *Candida glabrata* is a human opportunistic pathogen, which causes systemic infections. During infection, the amount of free iron is likely to be limited due to iron-chelating proteins, such as transferrin. Under iron-depleted conditions, *C. glabrata* Atg32 is upregulated and contributes to longevity under iron deficiency (Nagi et al., 2016). In a mouse model of disseminated infection, *C. glabrata* cells lacking Atg32 show a significant reduction in virulence, suggesting that mitophagy is induced during infection and plays a role in survival inside host organs where free iron ions are depleted.

Industrial applications

Mitophagy is proposed to be an important target for improving ethanol fermentation. During alcohol brewing, mitophagy is induced (Shiroma et al., 2014). Production of CO_2 and ethanol is enhanced in mitophagy-deficient yeast cells that lack Atg32. Interestingly, fermentation is reduced in the *atg11Δ* and *atg8Δ* mutants, suggesting that mitophagy defects specifically enhance fermentation. Thus, mitophagy-deficient strains could be useful for industrial application, such as brewing and bioethanol production.

MITOPHAGY IN MAMMALIAN CELLS

Mitophagy receptors in mammals

The molecular mechanism of mitophagy in mammals and yeasts is greatly different. The mammalian homolog of yeast Atg32 has not been identified, whereas several functional counterparts of Atg32, such as BCL2/adenovirus E1B 19-kDa-interacting protein 3 (BNIP3), BNIP3L/Nix, BCL2-like 13 (BCL2L13), and FUN14 domain-containing protein 1 (FUNDC1), have been reported as mitophagy receptors in mammals (Murakawa et al., 2015; Novak et al., 2010; Sandoval et al., 2008; Schweers et al., 2007; Thomas et al., 2011). All of these receptors are integrated into the outer mitochondrial membrane and have LIRs, which can interact with LC3, a mammalian homolog of Atg8. Thus, the interaction between the LIR of mitophagy receptors and LC3 is thought to be an important step in selecting mitochondria as cargo.

BNIP3 and its homolog BNIP3L/NIX are BH3-only proteins and members of the pro-apoptotic BCL2 family. BNIP3 and BNIP3L/NIX have one transmembrane region and an LIR on its N-terminal region exposed to the cytoplasm. BNIP3L/Nix-related mitophagy is reported to be important for the elimination of mitochondria during the maturation of reticulocytes to erythrocytes. Thus, the peripheral blood of BNIP3L/NIX knockout mice has been reported to show decreased mature erythrocytes and increased reticulocytes (Sandoval et al., 2008; Schweers et al., 2007). The interaction between BNIP3 and LC3 is affected by the phosphorylation status of BNIP3. When Ser^{17} and Ser^{24} adjacent to the LIR are phosphorylated, the interaction between BNIP3 and LC3 is enhanced; however, the regulation of the phosphorylation of BNIP3 remains unclear (Zhu et al., 2013).

BCL2L13 has one transmembrane region and an LIR on its N-terminal region exposed to the cytoplasm. BCL2L13 was identified as one of the functional counterparts of Atg32 because the exogenous expression of BCL2L13 can partially recover a mitophagy defect in the *atg32Δ* yeast. Similar with the case of Atg32, the phosphorylation of Ser^{272} on BCL2L13 plays an important role in the interaction between BCL2L13 and LC3 (Murakawa et al., 2015).

FUNDC1 has three transmembrane regions and an LIR on its N-terminal region exposed to the cytoplasm. When cells

are cultured in hypoxic conditions or when cellular mitochondria are depolarized under stress conditions, FUNDC1 is dephosphorylated at its Ser^{13} and can interact with LC3 (Chen et al., 2014; Liu et al., 2012).

PINK1 and Parkin-mediated mitophagy

PTEN-induced putative kinase 1 (PINK1) and Parkin are causative genes of young onset familial Parkinson's disease. PINK1 and Parkin accumulate on depolarized mitochondria and induce selective autophagic degradation of mitochondria (Narendra et al., 2008; 2010). PINK1 has a mitochondrial targeting signal (MTS) and is constitutively transported into the mitochondrial inner membrane where mitochondrial processing peptidase (MPP) cleaves MTS from PINK1. The cleaved form of PINK1 is further cleaved by rhomboid protease presenilin-associated rhomboid-like (PARL) and then completely degraded by the ubiquitin proteasome system (Greene et al., 2012; Jin et al., 2010; Yamano and Youle, 2013). When mitochondria are depolarized, PINK1 cannot translocate to the mitochondrial inner membrane and is accumulated on the mitochondrial outer membrane. Then, the accumulated PINK1 recruits Parkin from the cytoplasm to the mitochondria, and the Parkin ubiquitinates mitochondrial proteins. Autophagy adaptor proteins, such as the neighbor of BRCA1 gene 1 (NBR1), optineurin (OPTN), calcium binding and coiled-coil domain 2 (CALCOCO2/NDP52), and TAX1-binding protein 1 (TAX1BP1), have a ubiquitin-binding domain and interact with ubiquitinated mitochondrial proteins (Lazarou et al., 2015). These autophagy adaptor proteins also have LIRs and recruit an isolation membrane via interaction with LC3 for the selective autophagic degradation of the mitochondria.

Although both mitophagy receptor-mediated mitophagy and PINK1/Parkin-mediated mitophagy have been well studied recently, it remains unknown how these two types of mitophagy differ in expression among different tissues or in output of mitochondrial degradation.

CONCLUSION

In eukaryotic cells, mitophagy degrades excess or dysfunctional mitochondria to ensure a healthy population of the multitasking organelles. Atg32 was identified as a mitophagy receptor in yeast, which interacts with Atg11 and Atg8, to recruit mitochondria to the site of mitophagosome formation. Atg32 is regulated at multiple steps, including transcription, post-translational modifications, and proteolysis. Several mitophagy receptors have been identified and analyzed in mammalian cells. Thus, Atg32 is a good example for understanding the complex regulatory network controlling mitophagy through receptors. Mechanisms that induce mitophagy or that prevent excessive mitophagy have remained obscure. Through the study of nutrient signaling pathways, ubiquitin systems, and lipid metabolisms, it will be revealed how various signaling cascades and molecules positively and negatively regulate mitophagy. Furthermore, dissecting the molecular steps for selective recognition and removal of dysfunctional mitochondria is an important issue that should be addressed.

Using the *atg32Δ* mutant as a model for mitophagy dysfunction, in combination with general autophagy-deficient mutants, the physiological functions of mitophagy and bulk autophagy can be explored in various conditions. Mitophagy-deficient yeast cells exhibit mitochondrial genome instability and defects in limiting the propagation advantage of selfish mtDNA mutants. mtDNA mutations and their clonal expansion are an important cause of human inherited disease. Moreover, mitophagy dysfunction has been implicated in neurodegeneration. Mitophagy is a fundamental cellular process that is highly conserved among eukaryotes. Therefore, studies using yeast as a model will continue to contribute significantly to understanding the molecular mechanisms and physiological roles of mitophagy in eukaryotic cells.

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