FEBS Letters 587 (2013) 1787–1792

FEBS Letters

journal homepage: www.FEBSLetters.org

Mitochondrial degradation during starvation is selective and temporally distinct from bulk autophagy in yeast

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ARTICLE INFO

Article history:
Received 8 March 2013
Revised 19 April 2013
Accepted 21 April 2013
Available online 7 May 2013

Edited by Noboru Mizushima

Keywords:
Mitochondria
Autophagy
Starvation
Respiration
Yeast

ABSTRACT

Selective degradation of mitochondria is a fundamental process that depends on formation of autophagy-related double-membrane vesicles exclusive to mitochondria, and is thus termed mitophagy. In yeast, mitophagy is induced by a shift from respiration to starvation, or prolonged respiratory growth. Here we show that mitochondrial degradation in yeast also occurs selectively under starvation conditions even without respiration. Induction of mitophagy takes place much later than that of bulk autophagy, requiring Atg11 and Atg32 essential for mitophagy as well as Atg17, Atg29, and Atg31 specific for bulk autophagy. We propose that these two discrete protein complexes cooperatively activate starvation-induced mitophagy.

1. Introduction

Mitochondria are essential organelles that serve as multitasking factories for energy conversion and various metabolic reactions, and control a broad spectrum of signaling events including cell death, innate immune response, and calcium homeostasis [1]. Proper management of mitochondrial quality and quantity is important for these processes, as damage accumulation and a surplus or deficit in the organellar volumes leads to dysregulation of mitochondrial functions [2]. To overcome this problem, cells employ a set of proteins to mediate selective turnover of mitochondria via macroautophagy (hereinafter referred to as autophagy), a catabolic system that sequesters cytosolic constituents into newly formed double-membrane vesicles called autophagosomes, and delivers them to lytic compartments such as lysosomes for degradation and recycling [3,4]. This process, termed mitophagy, requires specific receptors that recruit autophagy-related (Atg) proteins essential for autophagosome formation to the surface of mitochondria. Recent studies reveal that mitophagy is highly conserved from yeast to humans [5], and that defects in mitophagy are associated with mitochondrial genome instability, aberrant cell differentiation, and neurodegeneration [6–8].

Diverse cues and physiological changes induce mitophagy in different cell types, which is independent of nutrient deprivation, a primary stress to activate non-selective autophagy. In the budding yeast *Saccharomyces cerevisiae*, degradation of mitochondria is promoted by a shift of culture condition from respiration to nitrogen starvation, or long-term respiratory growth [9–11]. Mitophagy in the former case occurs almost concomitantly with autophagy during starvation. In the latter case, mitochondrial degradation is facilitated upon entry into stationary phase. Similarly, respiring cells treated with rapamycin, an autophagy inducer, exhibit mitophagy [12]. Respiration is thought to be a common prerequisite for degradation of mitochondria under these conditions, possibly in order to generate oxidative stress, which in turn increases the levels of Atg32, a membrane-anchored receptor essential for mitophagy [11,13]. Consistent with this idea, an antioxidant treatment can partially suppress Atg32 expression and mitochondrial degradation [11]. How Atg32 is induced to activate the mitophagy pathway remains largely unknown.

Mitochondria can also be degraded thorough non-selective autophagy. For example, starved yeast cells lacking protease activity in the vacuole, a lytic compartment, accumulate numerous intravacuolar membrane vesicles derived from autophagosomes. These structures, called autophagic bodies, contain cytosolic constituents such as ribosomes and occasionally even mitochondria [14,15]. Although mitochondrial appearance in autophagic bodies

Abbreviations: Atg, autophagy-related; Cvt, cytoplasm-to-vacuole targeting; GFP, green fluorescent protein; HA, hemaagglutinin; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; DHFR, dihydrofolate reductase

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.04.030

is quite rare, it can occur in an Atg32-independent manner, indicating that the process is random and non-selective (Eiyama and Okamoto, unpublished observations). Whether mitophagy occurs upon starvation without respiratory culture and, if so, when/how it is facilitated has not been addressed.

In this study, we demonstrate that yeast mitochondria are exclusively sequestered and transported into the vacuole during nitrogen deprivation, which is induced 9–12 h after bulk autophagy. Strikingly, respiration-deficient cells also exhibit degradation of mitochondria under the same conditions. This type of mitophagy requires Atg32 and Atg11, a scaffold protein specific for selective autophagy-related pathways [10,11,16], although the expression of Atg32 is not drastically upregulated. Despite the selective features of this catabolic process, mitochondrial degradation during starvation is strongly impaired in cells lacking Atg17, Atg29, or Atg31, three proteins forming a platform critical for non-selective autophagy [17–19]. Together, our data suggest that starvation triggers mitophagy in a manner temporally distinct from bulk autophagy and physiologically independent of respiration, but functionally dependent on the non-selective autophagy machinery.

2. Materials and methods

2.1. Yeast strains and media

Yeast strains and plasmids used in this study are described in Supplemental Tables S1 and S2 online, respectively. Standard genetic and molecular biology methods were used for yeast and bacterial strains. Yeast cultures were incubated in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose), synthetic medium (0.17% yeast nitrogen base without amino acids and ammonium sulfate, 0.5% ammonium sulfate) with 0.5% casamino acids containing 2% dextrose (SDCA) or 0.1% dextrose plus 3% glycerol (SDGlyCA), supplemented with necessary amino acids. For starvation-induced mitophagy, cells grown to mid-log phase in YPD were transferred to starvation medium (SD−N; 0.17% yeast nitrogen base without amino acids and ammonium sulfate, 2% dextrose, 10% potassium acetate) and incubated at 30 °C. For stationary-phase mitophagy under respiratory conditions, cells grown to mid-log phase in SDCA were transferred to SDGlyCA and incubated at 30 °C.

2.2. Microscopy

Cells were observed using an inverted microscope (Axio Observer. Z1; Carl Zeiss) equipped with differential interference contrast optics, epifluorescence capabilities, a 100× objective lens (αPlan-APOCHROMAT 100×, NA: 1.46; Carl Zeiss), a monochrome CCD camera (AxioCam MRm; Carl Zeiss), and filter sets for green fluorescent protein (GFP) and mCherry (13 and 20, respectively; Carl Zeiss). Images were captured using acquisition and analysis software (Axio Vision 4.6; Carl Zeiss).

2.3. Electron microscopy

Ultrastructual analysis of yeast cells was performed by Tokai-EMA Inc. as described previously [11].

2.4. Immunoblotting

Samples corresponding to 0.1 OD_{600} units of cells were separated by SDS–PAGE followed by Western blotting and immunodecoration. Pgk1 was monitored as a loading control. After treatment with enhanced chemiluminescence reagents, proteins were detected using an image analyzer (LAS-4000 mini; GE Healthcare).

3. Results

3.1. Starvation-induced mitophagy is temporally distinct from autophagy

To investigate whether mitochondria are selectively degraded during nutrient starvation without respiratory culture, we visualized mitochondria and vacuoles using a mitochondrial matrix-targeted GFP (mito-GFP) and mCherry fused at the C-terminus of Vph1, a membrane-integrated subunit of vacuolar ATPase (Vph1-mCherry), respectively, in cells lacking Prb1, a vacuole-localized serine protease. In this degradation-deficient cell, membrane-bound vesicles and proteins transported into the vacuole can be accumulated as degradation intermediates. Cells were grown to mid-log phase in rich medium (YPD) and then subjected to nitrogen deprivation (SD−N). In these dextrose-containing media, yeast cells can generate ATP mostly by glycolysis, and mitochondrial respiration is not essential for viability. As reported previously [11], cells grown in YPD (–N, 0 h) contained tubular mitochondria that were not overlapped with vacuoles (Fig. 1A). Similarly, we hardly detected obvious overlaps between the two organellar markers in cells starved with nitrogen for 6 h (Fig. 1A). It is well established that numerous autophagic bodies are accumulated in vacuolar degradation-deficient cells at this time point [14]. Thus, bulk autophagy does not seem to promote degradation of mitochondria during starvation. Strikingly, at 24 h after starvation, we found several mito-GFP dots colocalized with Vph1-mCherry patterns in a manner dependent on Atg7, a protein essential for all autophagy-related processes (Fig. 1A). These observations raised the possibility that mitochondrial fragments were transported to the vacuole via bulk autophagy or mitophagy.

Next, we performed electron microscopy in order to distinguish between these two possibilities. As expected, autophagic bodies containing cytosolic ribosomes were detected in vacuolar degradation-deficient cells starved with nitrogen for 24 h (Fig. 1B). In addition, we found membrane-bound vesicles lacking ribosomal particles (major cargoes of bulk autophagy) that were hardly seen in the absence of Atg32 (Fig. 1B). Hence, it is likely that mitophagy occurs to generate vesicles exclusively containing mitochondria during starvation.

To verify our microscopic observations, we monitored mitochondrial degradation using mito-dihydrofolate reductase (DHFR)-mCherry, a reporter located in the matrix of mitochondria [20]. Upon transport of mitochondria to the vacuole, this fusion protein is processed to generate free mCherry that is considerably protease-resistant, thereby indicating degradation of mitochondria. We found that, at 24 h after starvation, free mCherry was accumulated in wild-type cells, which depended on Atg32 and Atg11, two proteins essential for mitophagy (Fig. 1C and D). Similar results were obtained from cells grown under respiratory conditions (Supplemental Fig. S1A and B) [10,11,13]. Together, these data exclude the possibility that mitochondria and/or their resident markers are transported into the vacuole via bulk autophagy or conventional membrane trafficking pathways, and rather support the idea that mitochondria are selectively degraded during starvation independently of autophagy.

Our results described above are consistent with the notion that mitophagy under starvation is promoted much later than bulk autophagy. Indeed, a more detailed time course assay revealed that free mCherry generated from the mitophagy marker was detected at 12–15 h after nitrogen deprivation (Fig. 1E), whereas autophagic bodies are known to accumulate in cells starved with nitrogen only for 3–6 h [14]. Moreover, we monitored processing of GFP-Atg8, an indicator of bulk autophagy, under the same conditions, and confirmed that free GFP was clearly detected at 6 h after nitrogen deprivation (Fig. 1F). In conclusion, we establish that yeast cells

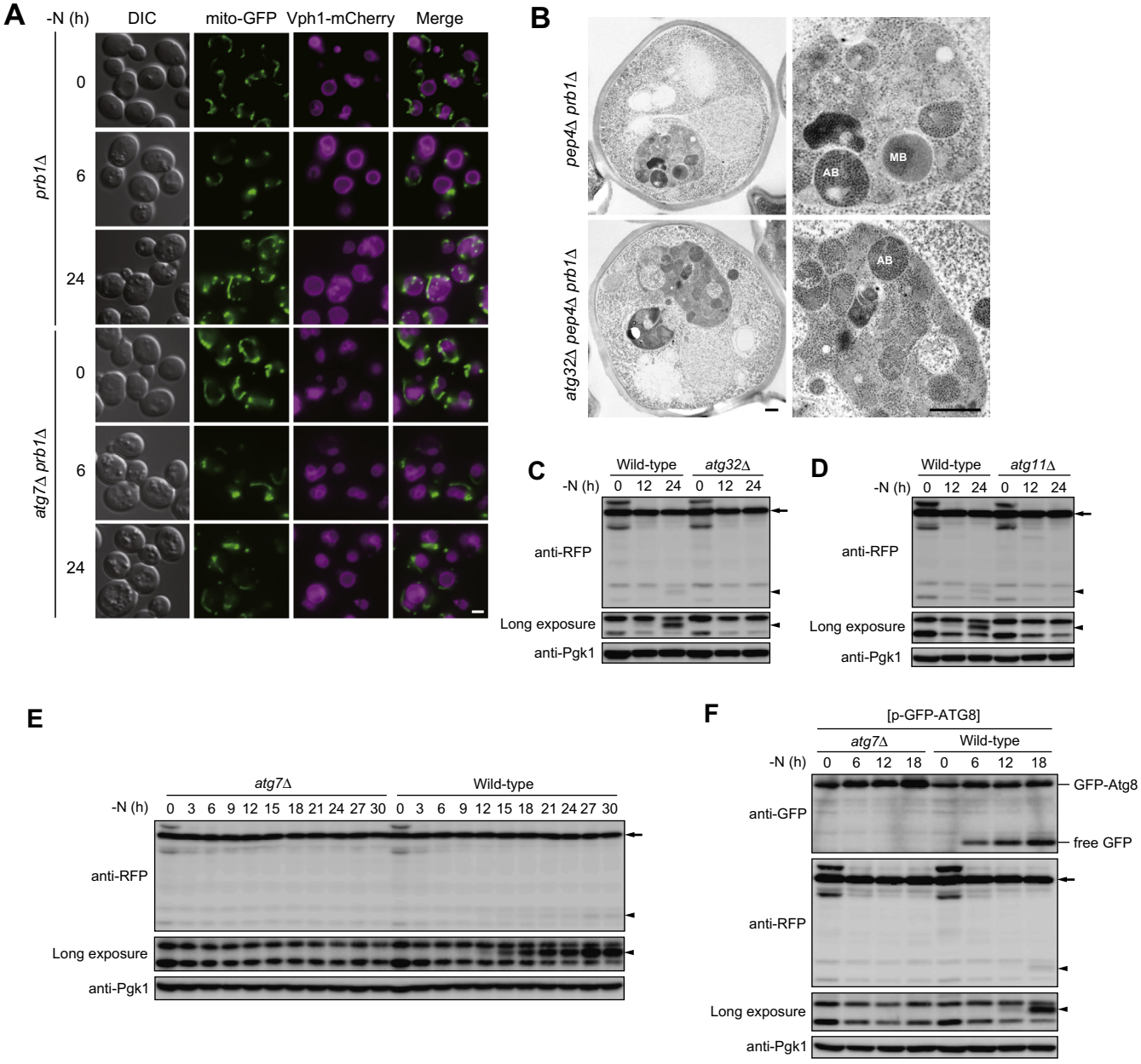

Fig. 1. Mitochondria are selectively degraded after bulk autophagy during nitrogen starvation. (A) Mitochondrial GFP- and vacuolar Vph1-mCherry-expressing *prb1Δ* and *atg7Δ prb1Δ* cells grown to mid-log phase in YPD were shifted to nitrogen starvation (–N) for 6 and 24 h, and investigated using fluorescence microscopy. Scale bar, 2 μm. (B) *pep4Δ prb1Δ* and *atg32Δ pep4Δ prb1Δ* cells were grown as in (A), exposed to starvation for 24 h, and examined by transmission electron microscopy. AB, autophagic body; MB, mitophagic body. Scale bars, 500 nm. (C) Mitochondrial matrix-targeted DHFR-mCherry-expressing (mito-DHFR-mCherry, depicted by arrow) wild-type and *atg32Δ* cells were grown to mid-log phase in YPD, incubated for the indicated time points in starvation medium (–N), and subjected to Western blotting. Generation of free mCherry (depicted by arrowheads) indicates transport of mitochondria to the vacuole. (D and E) Mito-DHFR-mCherry-expressing wild-type, *atg11Δ*, and *atg7Δ* cells were analyzed as in (C). (F) Cells used in (E) were transformed with a plasmid encoding GFP-Atg8 and analyzed as in (C). Generation of free GFP indicates progression of bulk autophagy.

during starvation undergo both autophagy and mitophagy in a mechanistically distinct fashion.

3.2. Respiration is dispensable for starvation-induced mitophagy

We have previously shown that the Atg32 levels are elevated 10- to 20-fold in respiring cells at mid-log phase, which is critical for efficient degradation of mitochondria during prolonged respiratory growth [11]. To ask if Atg32 is also upregulated during starvation, hemaagglutinin (HA)-tagged Atg32 was expressed from the endogenous locus and monitored by Western blotting. Vacuolar degradation-deficient cells were used to prevent Atg32 from mitophagy-dependent turnover. In contrast to the strong induction under respiratory conditions, Atg32 was not drastically increased in cells starved with nitrogen for 0–30 h (Fig. 2A), while the degree of starvation-induced mitophagy was about 50% compared to that of stationary-phase mitophagy under respiratory conditions (Supplemental Fig. S2A).

To test whether the amount of Atg32 is the rate-limiting factor of mitophagy during starvation, we introduced two low-copy plasmids encoding HA-tagged Atg32 into cells expressing a chromosomal version of HA-tagged Atg32, and monitored processing of mito-DHFR-mCherry. When starved with nitrogen, cells containing an ectopically increased level of Atg32 exhibited more efficient mitophagy (Fig. 2B). It should be noted that these cells grown to mid-log phase in rich medium (–N, 0 h) contain 5- to 10-fold

higher amount of Atg32 compared to wild-type cells, but do not promote mitochondrial degradation, raising the possibility that additional mechanism(s) may be needed to activate Atg32-mediated mitophagy.

The fact that Atg32 is not strongly upregulated during nitrogen deprivation led us to ask if mitochondrial respiration is required for starvation-induced mitophagy. Strikingly, degradation of mitochondria was almost at wild-type levels in respiration-deficient cells lacking Cyt1, a component of the cytochrome bc1 complex, or Cox7, a subunit of cytochrome c oxidase (Fig. 2C). Cells treated with antimycin A, an antibiotic that inhibits the cytochrome bc1 complex, also promoted mitophagy at levels equivalent to mock-treated cells (Supplemental Fig. S2B). Thus, these data suggest that degradation of mitochondria during starvation can be promoted in a manner independent of respiration.

3.3. Mitophagy during starvation requires Atg13, Atg17, Atg29, and Atg31

Our previous study reveals that Atg13, a core Atg protein required for activation of the Atg1 protein kinase [21], is not essential for mitophagy in stationary phase under respiratory conditions [11]. In addition, Atg17, Atg29, and Atg31, three proteins forming a scaffold specific for bulk autophagy, are dispensable for degradation of mitochondria during respiratory growth [11]. Hence, we sought to investigate whether these Atg proteins are also unnecessary for starvation-induced mitophagy. When grown under respiratory conditions, cells lacking Atg13, Atg17, Atg29, or Atg31 exhibited partial defects in mitochondrial degradation as well as the cytoplasm-to-vacuole targeting (Cvt) pathway, a selective Atg process that mediates transport of some vacuolar proteins including Ape1, an aminopeptidase, from the cytosol to the vacuole (Supplemental Fig. S3A–C). To the contrary, we found that mitophagy during starvation was almost completely blocked in cells lacking these Atg proteins (Fig. 3A and C). Maturation of Ape1 was partially impaired in these mutant cells under the same conditions, indicating that Atg11 was functional, at least for the Cvt pathway (Fig. 3C). Finally, we monitored processing of GFP-Atg8 under the same conditions, and confirmed that autophagy was induced only in wild-type cells (Fig. 3B and D). Therefore, despite the fact that mitophagy in starved cells is selective and temporally distinct from bulk degradation, it seems to depend on the autophagy-specific machinery.

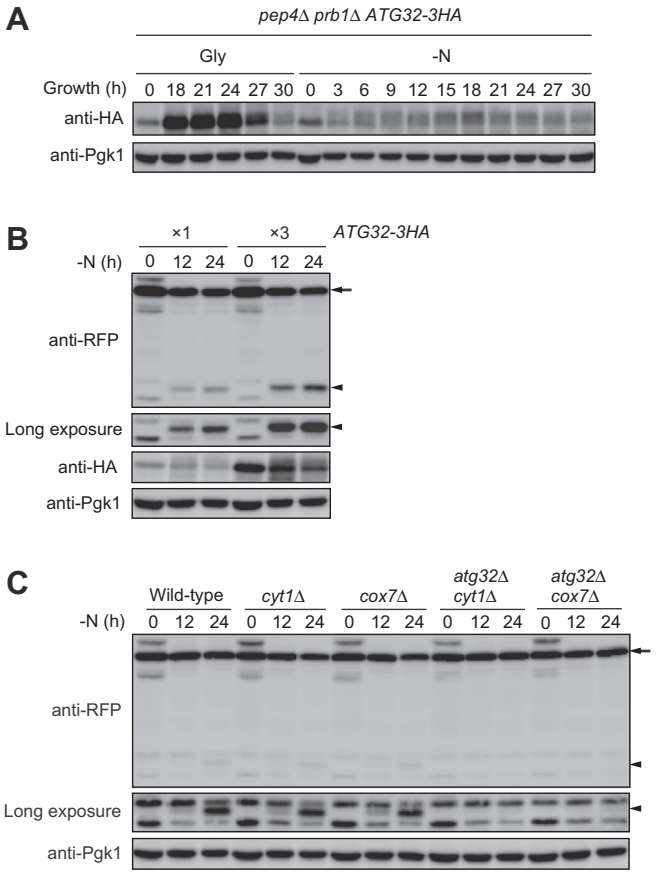

Fig. 2. Mitophagy occurs even without strong Atg32 induction and mitochondrial respiration. (A) Atg32-3HA-expressing wild-type cells grown to mid-log phase in SDCA and YPD were transferred to respiration medium (Gly) and nitrogen-free medium (–N), respectively, collected at the indicated time points, and subjected to Western blotting. (B) Atg32-3HA- and mito-DHFR-mCherry expressing cells were transformed with empty vectors or two plasmids encoding Atg32-3HA, grown to mid-log phase in YPD, shifted to nitrogen starvation (–N), collected at the indicated time points, and subjected to Western blotting. Generation of free mCherry (depicted by arrowheads) indicates transport of mitochondria to the vacuole. (C) Mito-DHFR-mCherry-expressing wild-type, *cyt1Δ*, *cox7Δ*, *cyt1Δ atg32Δ*, and *cox7Δ atg32Δ* cells were analyzed as in (B).

4. Discussion

In this study, we demonstrate that yeast cells starved with nitrogen undergo Atg11- and Atg32-dependent selective degradation of mitochondria 9–12 h after autophagy (Fig. 1A–E), suggesting that mitophagy during starvation is mechanistically distinct from bulk degradation. This type of mitochondrial turnover proceeds under culture conditions containing fermentable carbon source such as dextrose, and is likely to be independent of respiration. In support of this idea, strong expression of Atg32, which occurs in respiring cells [11], is not detected during starvation (Fig. 2A). Furthermore, respiration-deficient starved cells can promote mitophagy (Fig. 2C and Supplemental Fig. S2B). Mitochondrial DNA (mtDNA) is also dispensable for degradation of mitochondria during starvation (Eiyama and Okamoto, unpublished results). Finally, cells lacking mtDNA do not accumulate reactive oxygen species (ROS) under starvation conditions [22]. Thus, it seems possible that starvation-induced mitophagy does not require mitochondrial respiration and ROS generation. As Atg32 appears to be phosphorylated during nitrogen starvation (Fig. 2A), we speculate that a different type of phosphorylation could qualitatively hyperactivate Atg32 in order to overcome the shortage of this mitophagy receptor.

How degradation of mitochondria occurs much later than bulk degradation is not entirely clear. Although mitophagy during starvation depends on the autophagy-specific proteins, Atg17, Atg29, and Atg31 (Fig. 3A and B), it does not require vacuolar lytic activities (Fig. 1A and B). These results imply that nutrient recycling is not a trigger and/or prerequisite for starvation-induced mitochondrial turnover. It has been previously reported that a shift from respiration to starvation promotes mitophagy concomitantly with autophagy [13]. In this case, Atg32 is upregulated upon respiratory growth, which may be a key to quickly facilitate mitochondrial degradation. Similarly, introducing extra copies of the *ATG32* gene can accelerate starvation-induced mitophagy (Fig. 2B). Hence, the Atg32 levels are likely to be a factor that determines the timing of mitochondrial clearance. The other determinant might be organelle size: mitochondria in starved cells seem to elongate as tubules that are too large to be surrounded by autophagosome-like membranes (Fig. 1A). In analogy to our observations, mitochondrial fragmentation is suppressed upon nutrient deprivation in mammalian cells, reducing the risk of mitochondrial turnover by autophagy [23,24]. Further studies are also needed to clarify whether starvation activates Atg32 through mechanisms including phosphorylation similar to, or different from respiration.

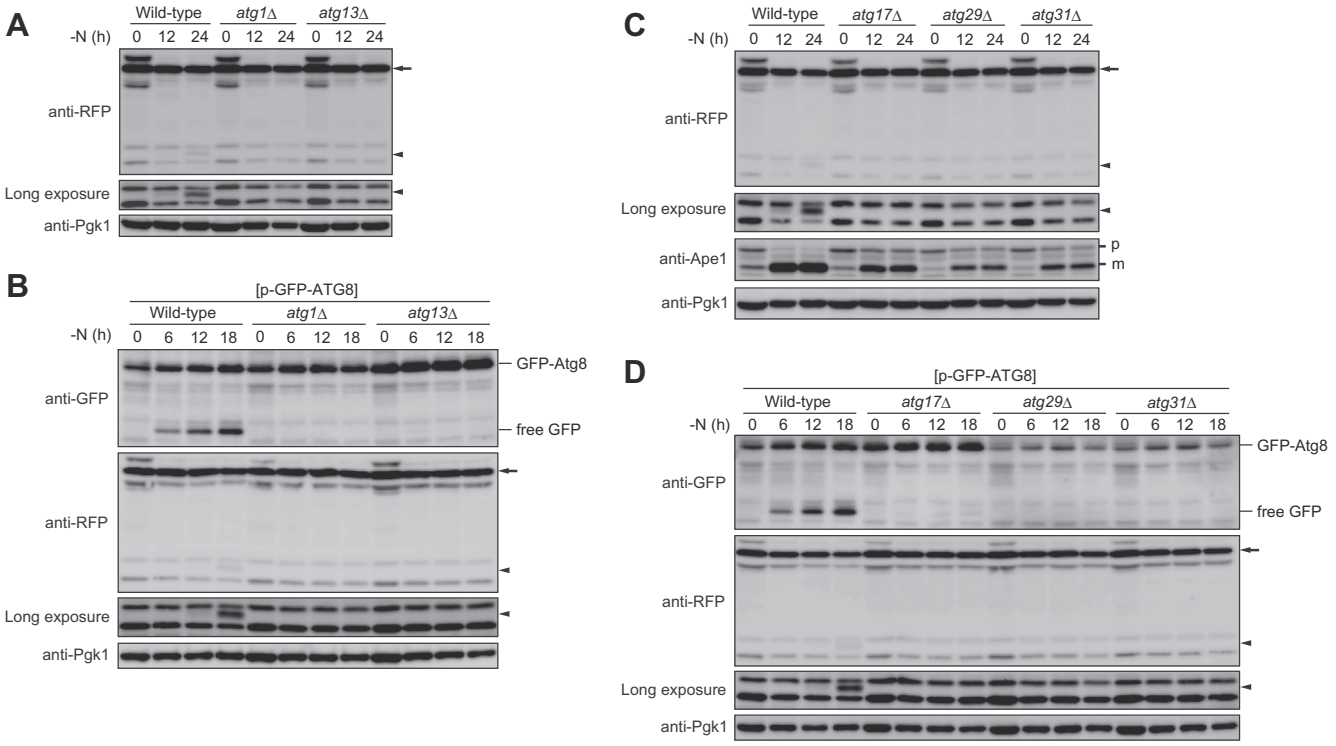

Fig. 3. Cells lacking Atg13, Atg17, Atg29, or Atg31 are defective in starvation-induced mitophagy. (A) Mito-DHFR-mCherry-expressing wild-type, *atg1Δ*, and *atg13Δ* cells grown to mid-log phase in YPD were shifted to nitrogen starvation (–N), collected at the indicated time points, and subjected to Western blotting. Generation of free mCherry (depicted by arrowheads) indicates transport of mitochondria to the vacuole. (B) Cells used in (A) were transformed with a plasmid encoding GFP-Atg8 and analyzed as in (A). Generation of free GFP indicates progression of bulk autophagy. (C) Mito-DHFR-mCherry-expressing wild-type, *atg17Δ*, *atg29Δ*, and *atg31Δ* cells were analyzed as in (A). Precursor form (p) of Ape1 is transported to the vacuole via the Cvt pathway, and processed to be a mature form (m). (D) Cells used in (C) were transformed with a plasmid encoding GFP-Atg8 and analyzed as in (A).

Why does mitophagy during starvation strongly depend on Atg17, Atg29, and Atg31? These three Atg proteins form a scaffold to recruit the Atg1–Atg13 protein kinase complex to the site for autophagosome formation [19]. One possibility could be that the autophagy-specific scaffold and the mitophagy platform (Atg11–Atg32) collaborate to mediate formation of autophagosome-like membranes surrounding mitochondria. Supporting this idea, Atg11 has been suggested to interact with Atg17 and Atg29 [25–27]. Such interaction may help specializing the Atg1–Atg13 protein kinase complex for degradation of mitochondria. In addition, Atg13, an Atg1 activator, is not essential for mitophagy during prolonged respiratory growth [11], whereas it is indispensable for mitophagy under nitrogen starvation (Fig. 3A). Thus, targeting of strongly activated Atg1 to mitochondria might be critical to compensate for the low levels of Atg32 expression in starved cells, thereby ensuring mitophagy to occur.

The biological significance of late-induced mitophagy during starvation remains to be elucidated. In addition to massive degradation and recycling, cell structure and function must be remodeled and adapted properly to nutrient deprivation. These events may require mitochondrial metabolic activities, and therefore mitochondria should avoid clearance by bulk autophagy. Upon completion of the adaptation processes, cells may promote mitophagy to degrade surplus mitochondria, which further provides recycled biomolecules and reduces overall cellular energy demand. Moreover, late-induced mitophagy may also contribute to mitochondrial quality control under starvation conditions. In accordance with this idea, cells lacking Atg32 exhibit defects in mitochondrial respiration during long-term starvation [6] (Kondo-Okamoto and Okamoto, unpublished data). Future studies will address the issue of how nutrient-starved cells target the autophagy-specific machinery to mitochondria and regulate the timing of mitophagy.

Acknowledgments

We are grateful to Toshiya Endo (Nagoya University, Nagoya, Japan), Hayashi Yamamoto (Tokyo Institute of Technology, Yokohama, Japan), Kaori Sakakibara and Ayako Hashimoto (Osaka University, Osaka, Japan) for kind gifts of antibodies, plasmids, and yeast strains. We also thank Chika Kondo-Kakuta, Sho Suzuki, and Yoshinori Ohsumi (Tokyo Institute of Technology, Yokohama, Japan) for technical support and valuable discussions. This work was supported in part by the Ministry of Education, Culture, Science, and Technology of Japan, Grant-in-Aid for Young Scientists (B) (22770124) (to N. Kondo-Okamoto), Scientific Research on Innovative Areas (23113717), Challenging Exploratory Research Grant (23657090 and 25650067), Scientific Research (B) (25291045), special coordination funds from the Osaka University Life Science Young Independent Researcher Support Program to Disseminate Tenure Tracking System, and by Grants from the Ono Medical Research Foundation (to K. Okamoto).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.04.030.

References

[1] Nunnari, J. and Suomalainen, A. (2012) Mitochondria: in sickness and in health. J. Cell Biol. 148, 1145–1159.
[2] Okamoto, K. and Kondo-Okamoto, N. (2012) Mitochondria and autophagy: critical interplay between the two homeostats. Biochim. Biophys. Acta 1820, 595–600.

[3] Mizushima, N., Yoshimori, T. and Ohsumi, Y. (2011) The role of Atg proteins in autophagosome formation. Annu. Rev. Cell Dev. Biol. 27, 107–132.
[4] Mizushima, N. (2011) Autophagy in protein and organelle turnover. Cold Spring Harbor Symp. Quant. Biol. 76, 397–402.
[5] Youle, R.J. and Narendra, D.P. (2011) Mechanisms of mitophagy. Nat. Rev. Mol. Cell Biol. 12, 9–14.
[6] Kurihara, Y., Kanki, T., Aoki, Y., Hirota, Y., Saigusa, T., Uchiumi, T. and Kang, D. (2012) Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation of mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. J. Biol. Chem. 287, 3265–3272.
[7] Schweers, R.L., Zhang, J., Randall, M.S., Loyd, M.R., Li, W., Dorsey, F.C., Kundu, M., Opferman, J.T., Cleveland, J.L., Miller, J.L. and Ney, P.A. (2007) NIX is required for programmed mitochondrial clearance during reticulocyte maturation. Proc. Natl. Acad. Sci. USA 104, 19500–19505.
[8] Narendra, D., Tanaka, A., Suen, D.F. and Youle, R.J. (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. 183, 795–803.
[9] Tal, R., Winter, G., Ecker, N., Klionsky, D.J. and Abeliovich, H. (2007) Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival. J. Biol. Chem. 282, 5617–5624.
[10] Kanki, T. and Klionsky, D.J. (2008) Mitophagy in yeast occurs through a selective mechanism. J. Biol. Chem. 283, 32386–32393.
[11] Okamoto, K., Kondo-Okamoto, N. and Ohsumi, Y. (2009) Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev. Cell 17, 87–97.
[12] Mendl, N., Occhipinti, A., Muller, M., Wild, P., Dikic, I. and Reichert, A.S. (2011) Mitophagy in yeast is independent of mitochondrial fission and requires the stress response gene *WHI2*. J. Cell Sci. 124, 1339–1350.
[13] Kanki, T., Wang, K., Cao, Y., Baba, M. and Klionsky, D.J. (2009) Atg32 is a mitochondrial protein that confers selectivity during mitophagy. Dev. Cell 17, 98–109.
[14] Takeshige, K., Baba, M., Tsuboi, S., Noda, T. and Ohsumi, Y. (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. J. Cell Biol. 119, 301–311.
[15] Baba, M., Takeshige, K., Baba, N. and Ohsumi, Y. (1994) Ultrastructural analysis of the autophagic process in yeast: detection of autophagosomes and their characterization. J. Cell Biol. 124, 903–913.
[16] Kim, J., Kamada, Y., Stromhaug, P.E., Guan, J., Hefner-Gravink, A., Baba, M., Scott, S.V., Ohsumi, Y., Dunn Jr., W.A. and Klionsky, D.J. (2001) Cvt9/Gsa9 functions in sequestering selective cytosolic cargo destined for the vacuole. J. Cell Biol. 153, 381–396.
[17] Kabeya, Y., Noda, N.N., Fujioka, Y., Suzuki, K., Inagaki, F. and Ohsumi, Y. (2009) Characterization of the Atg17–Atg29–Atg31 complex specifically required for starvation-induced autophagy in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 389, 612–615.
[18] Kabeya, Y., Kamada, Y., Baba, M., Takikawa, H., Sasaki, M. and Ohsumi, Y. (2005) Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy. Mol. Biol. Cell 16, 2544–2553.
[19] Kawamata, T., Kamada, Y., Kabeya, Y., Sekito, T. and Ohsumi, Y. (2008) Organization of the pre-autophagosomal structure responsible for autophagosome formation. Mol. Biol. Cell 19, 2039–2050.
[20] Kondo-Okamoto, N., Noda, N.N., Suzuki, S.W., Nakatogawa, H., Takahashi, I., Matsunami, M., Hashimoto, A., Inagaki, F., Ohsumi, Y. and Okamoto, K. (2012) Autophagy-related protein 32 acts as autophagic degron and directly initiates mitophagy. J. Biol. Chem. 287, 10631–10638.
[21] Kamada, Y., Funakoshi, T., Shintani, T., Nagano, K., Ohsumi, M. and Ohsumi, Y. (2000) Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J. Cell Biol. 150, 1507–1513.
[22] Suzuki, S.W., Onodera, J. and Ohsumi, Y. (2011) Starvation induced cell death in autophagy-defective yeast mutants is caused by mitochondria dysfunction. PLoS One 6, e17412.
[23] Gomes, L.C., Di Benedetto, G. and Scorrano, L. (2011) During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat. Cell Biol. 13, 589–598.
[24] Rambold, A.S., Kostelecky, B., Elia, N. and Lippincott-Schwartz, J. (2011) Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. Proc. Natl. Acad. Sci. USA 108, 10190–10195.
[25] Yorimitsu, T. and Klionsky, D.J. (2005) Atg11 links cargo to the vesicle-forming machinery in the cytoplasm to vacuole targeting pathway. Mol. Biol. Cell 16, 1593–1605.
[26] Krogan, N.J., Cagney, G., Yu, H., Zhong, G., Guo, X., Ignatchenko, A., Li, J., Pu, S., Datta, N., Tikuisis, A.P., et al. (2006) Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. Nature 440, 637–643.
[27] Gavin, A.C., Aloy, P., Grandi, P., Krause, R., Boesche, M., Marzioch, M., Rau, C., Jensen, L.J., Bastuck, S., Dumpelfeld, B., et al. (2006) Proteome survey reveals modularity of the yeast cell machinery. Nature 440, 631–636.