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Stationary-Phase Mitophagy in Respiring *Saccharomyces cerevisiae*

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Abstract

The clearance of malfunctioning mitochondria is an important housekeeping function in respiring eukaryotic cells and plays a role in physiological homeostasis as well as in the progression of late-onset diseases. This clearance is thought to occur by a specific form of autophagic degradation called mitophagy. Although the mechanism of nonspecific macroautophagy is relatively well established, the selective autophagic degradation of mitochondria has only recently begun to receive significant attention. An important step toward elucidating the mechanism by which defective mitochondria are selected and degraded is the establishment of conditions under which mitophagy is induced. This review covers our current understanding of mitophagy in the model organism *Saccharomyces cerevisiae* and its modes of activation, with a focus on stationary-phase mitophagy—a form of mitophagy that holds promise as a potential quality control mechanism. *Antioxid. Redox Signal.* 14, 2003–2011.

Mitochondria, Genetic Disease, and Aging

MITOCHONDRIA PERFORM A VARIETY of essential physiological functions in all eukaryotic cells. Clearly, the centerpiece in this panoply of roles is the production of ATP by oxidative phosphorylation. However, no less essential are the roles played by mitochondria in fatty acid oxidation and the biosynthesis of isoprenoids, heme, amino acids, and nucleotides, to mention a few. In fact, the capability of mitochondria to perform oxidative phosphorylation comes at a price. Breakdown of the chemical potential capacitor function of the mitochondrial outer membrane leads to nonproductive partial reduction of molecular oxygen to form reactive oxygen species (ROS) (2). Although ROS affect the cell as a whole, mitochondria, being in the line of fire, suffer a disproportionate level of oxidative damage. This damage is manifested in the mutagenesis of mitochondrial DNA as well as in oxidation reactions such as protein glycation and lipid oxidation. In addition, disruption of mitochondrial compartmentalization results in leakage of cytochrome *c* and other cytotoxic factors, and mitochondria with defective chemiosmotic coupling can cause an energy drain on the cell. Gradual accumulation of mitochondrial DNA mutations has been invoked as an explanation for aging phenomena. In addition, many maternally inherited forms of late-onset diseases, such as type II diabetes, deafness, mitochondrial encephalomyopathy, and optic neuropathy, to mention a few, have been linked to maternally inherited mutations in the mitochondrial genome (55, 56). These findings have led to a unified "mitochondrial theory of aging." Within the framework of this theory, a role has been proposed for a quality control mechanism that identifies malfunctioning mitochondria and prevents clonal takeover of the system by these defective cohorts (55).

Autophagy: Clearance and Recycling of Damaged or Unnecessary Cytosolic Components

From the earliest days of cell biology, investigators noted the occasional appearance in electron micrographs of mitochondria within other cellular compartments, later identified as lysosomes or lysosome-related organelles. It was also shown that the engulfment of mitochondria in lysosomal compartments could be regulated by glucagon treatment in liver slices (6, 7). These treatments also increased general protein degradation rates, leading investigators to call this process as autophagy or autophagocytosis. The 1990s saw an explosion of experimental data in the autophagy field. Two important initial developments were the emergence of yeast as a model system for studying autophagy (48) and the careful electron microscopy (EM) analysis of autophagosome formation and maturation in mammalian cells, which led to the putative identification of the ER as a membrane donor for autophagy (8, 9). Studies in yeast, pioneered independently and in parallel by Yoshinori Ohsumi and Michael Tumm, identified yeast genes required for yeast autophagy (48, 51). These genes were later found to largely overlap the cytoplasm to vacuole targeting (Cvt) genes, identified by the Klionsky lab as required for a selective form of autophagy known as the Cvt pathway (14, 15). The nonessential genes (*i.e.*, the genes, in a unicellular eukaryote such as *Saccharomyces cerevisiae*,

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without which the organism can live under a controlled laboratory environment) required for autophagy fall roughly under five categories: genes involved in Atg12 conjugation (34), genes involved in Atg8 conjugation, genes involved in the Atg1 kinase complex and its regulation (19), and gene products involved in an autophagy-specific PI3-kinase enzyme complex (26). An additional gene product, Atg9, is the only integral membrane protein that is absolutely required for general macroautophagy (36). The current state of knowledge regarding the function of these gene products in autophagy has been covered by many recent and excellent reviews (16, 58).

The physiological function of the induction of autophagy under starvation has been a matter of some debate in the past. The identification of the different yeast mutants pinpointed the fact that in the absence of macroautophagy, cells lose viability under starvation conditions, whereas wild-type cells survive. An elegant publication recently proved that this is due to recycling of amino acids through autophagy-dependent protein degradation, leading to a buffering of cytosolic levels of free amino acids (57).

Mitophagy as a Quality Control Mechanism for Culling Defective Mitochondria

The only known cellular mechanism that can actually mediate the degradation of entire organelles without spillage is, by definition, an autophagic mechanism. Indeed, for years, the identification of mitochondria in lysosomal and endosomal compartments was the only defining feature of autophagic processes. Until recently, however, there were no mechanistic studies of specific mitochondrial autophagy. The first studies came from the Lemasters lab, which also coined the term "mitophagy" (45–47). These studies suggested that MPT pore opening is a trigger for selective mitochondrial autophagy in mammalian cells. Indeed, a later study by the Shirihai group confirmed the hypothesis that malfunctioning mitochondria are selectively degraded by mitophagy in mammalian cells (52). This article was central in laying out the groundwork of a unified hypothesis for a mechanism of monitoring and culling of defective mitochondria. They found that (i) depolarized mitochondria are fusion defective; (ii) depolarized mitochondria are selectively formed during fission events (85% of fission events led to formation of one hyperpolarized compartment and one depolarized compartment); (iii) in the mitochondrial life cycle, the postfission state is the "resting state" and fusion was rapidly followed by fission; and finally, (iv) depolarized mitochondria, formed by selective fission, were then selectively autophagocytosed.

In mammalian cells, some of the mechanistic details of these steps have since been fleshed out. Several labs have shown that PTEN-induced putative kinase 1 (PINK1), a membrane-anchored mitochondrial protein kinase, phosphorylates and activates Parkin, a ubiquitin-conjugating E3-type enzyme subunit (3, 4, 25, 35, 50, 54). Parkin then directs the ubiquitination of Mfn1 and Mfn2, mitofusins that are required for mitochondrial fusion. Studies show that PINK1 is recruited to all mitochondria, but is degraded in a ubiquitin- and proteasome-dependent manner in energized mitochondria (35). Somewhat confusingly, however, some labs have reported that loss of PINK1 also leads to the induction of mitophagy (3, 4). Although these results account for some of the observations of Twig *et al.*, several important questions remain. First, as 85% of fission events lead to segregation between depolarized and hyperpolarized mitochondria, active segregation of functional and defective components must be taking place, and this segregation would likely be coupled with the PINK1 load of the outer mitochondrial membrane. Second, the precise mechanism of coupling between mitochondrial depolarization and inhibition of PINK1 degradation is unclear.

Mitophagy in *S. cerevisiae*

The initial report on autophagy in *S. cerevisiae* (48) demonstrated, by EM, the presence of mitochondria in nitrogen starvation-induced autophagic bodies, as was shown three decades earlier for mammalian cells (6). These pictures, however, are anecdotal: very few mitochondria are actually engulfed by autophagosomes during nitrogen starvation of *S. cerevisiae*. In fact, despite numerous attempts, the standard nitrogen starvation protocol, which is used to assay macroautophagy in yeast, does not yield any discernable biochemical or structural manifestations of mitophagy (see below).

The first report of general mitophagy occurring *en masse* in *S. cerevisiae* (as opposed to anecdotal EM pictures) came from studies by Kissova *et al.* (27). They showed that yeast cells that are grown in lactate medium and transferred to nitrogen starvation on glucose display mitophagy (as measured by transfer of a mitochondrially targeted green-fluorescent protein [GFP] to the vacuolar lumen) within 12 h. However, the procedure used is unlikely to reflect a quality control event (see discussion below).

Priault *et al.* (43) published intriguing data from experiments employing *fmc1Δ* mutants. These cells fail to properly assemble the F_1F_0 ATPase at high temperatures. The study showed that under anaerobic conditions and after 24 h at nonpermissive temperature, the cells accumulate what appear to be autophagosomes. In parallel, levels of ATPase subunits specifically decline, as do, to a lesser extent, the levels of mitochondrial porins. The authors suggested that these data point to selective autophagy being induced as a result of depolarization, but did not demonstrate, either morphologically or biochemically, that mitochondrial material was actually being degraded in the vacuole under these conditions. In a somewhat similar study, Nowikowsky *et al.* later showed that mitophagy occurs as an early event in response to induced depletion of $K+/H+$ antiporter activity. This uptake of mitochondria into vacuoles is preceded by osmotic swelling and is mediated by a microautophagic mechanism, in which vacuoles directly engulf mitochondria, thus generating mitochondria-containing autophagic bodies without forming cytosolic autophagosomes. They also demonstrated that Dnm1, a dynamin-like protein that is required for mitochondrial fission, is required for this type of mitophagy. These two reports share a common theme: introduction of a genetic lesion causes a physiological defect in mitochondrial function that leads to mitochondrial degradation in the vacuole, *via* mitophagy. These results uphold the original hypothesis, that is, mitophagy acts as a quality control mechanism for maintaining a healthy mitochondrial cohort. What they did not demonstrate was "what conditions are required for normal induction of mitophagy in wild-type cells." Wild-type *S. cerevisiae* do not show vacuolar localization of mitochond-

rially targeted GFP during growth on glucose, and even upon nitrogen starvation (in constant carbon source) they do not show any significant translocation of a mitochondrial GFP to the vacuole under conditions in which full-scale macroautophagy is known to occur.

As mentioned earlier, Kissova *et al.* (27) used wild-type cells under conditions in which both nitrogen source and nitrogen availability are simultaneously changed. Tal *et al.* (49) first showed that without outside intervention, wild-type yeast cells incubated into stationary phase under respiratory conditions (using lactate as carbon source) display massive mitophagy at very long time points, beyond 3 days of incubation.

The data by Tal *et al.* and Kissova *et al.* (27, 49) were valuable in two ways. First, they provided an experimental framework that allows us to ask mechanistic questions, by defining specific conditions under which mitophagy is induced in the absence of mutation or lesion. Second, both reports identified mutations that affected the process (also see below). The Kissova's paper implicated Uth1, a SUN domain protein that is required for mitophagy under their conditions, and the work by Tal *et al.* showed that Aup1, a novel protein phosphatase homolog, is required for efficient stationary-phase mitophagy. It is important to stress the difference between the two protocols. In the protocol of Kissova *et al.*, cells are subjected to two simultaneous changes, both of which are required to observe mitophagy: they are transferred from lactate-based medium to a glucose-based medium, and at the same time, they are subjected to nitrogen starvation. This change in carbon source is crucial: *S. cerevisiae* does not perform oxidative phosphorylation in the presence of substantial (>0.1%) concentrations of glucose, and therefore, this experimental setup is switching the cells from conditions where large numbers of mitochondria are required to conditions where most of the mitochondrial biomass is dispensable. As the cell is simultaneously switched from nitrogen-replete to nitrogen-depleted conditions, mitochondrial biomass becomes redundant, and at the same time, the cell requires wholesale recycling of nonessential components. Hence, it is unlikely that this protocol reports on a quality control function of mitophagy. It is important to note that glucose-grown cells do not perform significant mitophagy under nitrogen starvation conditions, even when assayed for a whole week after transfer to nitrogen starvation medium (H. Abeliovich, unpublished data). In the protocol published by Tal *et al.*, however, no medium change occurs and the cells are incubated under conditions were oxidative phosphorylation is active throughout. As a result, these conditions were thought likely to represent a situation in which mitochondrial culling may be occurring and would therefore represent a quality control mechanism. Indeed, Journo *et al.* (18) showed that general autophagy is constantly ongoing during incubation in lactate medium, even in the first day of the incubation, whereas mitophagy is only observed from the second or third day of incubation on lactate. This result strongly suggests that stationary-phase mitophagy is part of a more general developmental process that occurs in the culture, under long-term incubation in conditions of respiratory metabolism.

The stationary-phase protocol has the additional benefit of being nonlabor intensive: no change in medium is required. This makes it ideal for high-throughput screening, and indeed, two groups, the Klionsky lab and the Ohsumi lab, took advantage of this fact to carry out large-scale screening of the yeast knockout collection for strains that lost the ability to transfer mitochondrial GFP to the vacuole under these conditions. These screens identified a battery of interesting genes, as detailed below and in Table 1.

Aspects of Mitophagy: Targeting, Segregation, Engulfment

Not all mitochondria are degraded during the induction of mitophagy. At the very least, this is due to the fact that mitochondria are essential for cell survival. In addition, however, the study by Twig *et al.* (52) as well as Narendra *et al.* (35) suggests that depolarized mitochondria are generated specifically during fission events and that these depolarized units are specifically targeted for mitophagy. Again, the fact that 85% of mitochondrial fission events were shown to result in one significantly depolarized and one significantly hyperpolarized daughter must imply that a nonrandom segregation process is taking place. Such segregation, coupled with fission, will ultimately distill defective mitochondria out of the dynamic network. Combined with the conclusions in refs. (59, 60), which show that depolarization of mitochondria leads to ubiquitination of mitofusins, one can hypothesize that once segregation and fission have taken place, a dedicated mechanism generates a signature on the depolarized mitochondrion to identify the compartment as defective. A corresponding cytoplasmic mechanism would then identify this signal and recruit the autophagic machinery to carry out mitophagy. At this point, studies in yeast have not yet identified any of these functions, which were mostly derived from studies in mammalian cells and in Drosophila. However, given the fact that a screening system exists in yeast, and a battery of genes has already been identified, it is probably a matter of time before the pieces fall into place, and one would expect that genes involved in mitophagy would fall into theses categories. Surprisingly, however, none of the novel autophagy-dedicated genes identified by these screens has any clear mammalian homolog, suggesting that further exploration may still be required to identify conserved functions.

Methods for Monitoring Mitophagy in *S. cerevisiae* and Their Significance

A number of assays have been developed for assessing mitophagy in yeast. Initially, assays for mitophagy followed the levels of specific mitochondrial proteins. Specific reduction in the levels of mitochondrial proteins, in a vacuole-dependent fashion, was interpreted as indicating mitophagy. This type of assay was used, for example, in implicating Uth1 and Aup1 as affecting mitophagy and in showing that deletion of Fmc1 caused induction of mitophagy.

A second type of assay involves generating a construct in which a mitochondrial leader is fused to GFP. The resulting protein localizes to mitochondria, and induction of mitophagy leads to the appearance of green fluorescence in the vacuolar lumen. When mitophagy is induced, one expects to observe a fluorescent signal in the vacuole. The third and final assay involves an extension of the GFP assay. If, instead of generating a mitochondrial GFP in the fusion, one generates a tagged GFP fusion chimera involving a mitochondrial protein, then when using a fluorescence microscope one observes

Table 1. A Compendium of Yeast Genes Identified by Genomic Screening for Mutants Defective for Stationary-Phase Mitophagy in the BY4741/4742 Background

Gene name	*ORF*	*Putative function*	*Remarks*	*References*
ATG32		A putative integral membrane adaptor protein that links mitochondria with the autophagic factors Atg8 and Atg11. Downregulated by *N*-acetyl-L-cysteine and glucose		23, 38
ATG33		An integral membrane protein that may signal quality control regulation of mitophagy	Required for stationary phase mitophagy but not absolutely required for mitophagy upon transfer from synthetic lactate medium to starvation on glucose	22
FMC1		Protein required for the assembly of the F1 sector of the mitochondrial H^+-ATPase	Deletion of FMC1 causes induction of mitophagy at elevated temperatures. Puzzlingly, deletion abolishes stationary-phase mitophagy	22, 31, 43
DNM1		A dynamin-like GTPase required for mitochondrial fission	Other fission factors, such as Fis1, were not found in the screen	22, 39
AIM28		Inner-membrane protein required for assembly of the mitochondrial H^+-ATPase		13, 22
AIM26		Unknown	Mutant shows altered inheritance of mitochondria	22
MFM1	YPL060w	Inner-membrane magnesium transporter	Shows a decrease in respiratory growth rate	(13, 22)
	YPR146c	Dubious reading frame		22
RPL13B	*YMR142c*	Large ribosomal subunit protein		22, 41
RPL14A	YKL006w	Large ribosomal subunit protein	N-terminally actylated protein	22, 41
RPL15B	YMR121c	Component of large ribosomal subunit		22, 41
ICY2	YPL250c	Unknown	Associates with polysomes upon shift from fermentable to nonfermentable carbon source	22, 29
	YOR019W	Unknown	May interact with ribosomes	22
EGD1	YPL037c	β1 subunit nascent polypeptide-associated complex	Initiates protein targeting to mitochondria	12, 22
ARG82	YDR173c	Inositol polyphosphate multikinase		22, 44
ARO2	YGL148w	Bifunctional chorismate synthase/flavin reductase		17, 22
BCK1	YJL095w	MAP kinase kinase kinase	Known substrates, Mkk1 and Mkk2, were not identified in the screen	22, 30
BUB1	YGR188c	Protein kinase	Functions in anaphase spindle checkpoint; centromere-binding protein	22, 28
MAK10	YEL053c	Noncatalytic subunit of N-terminal acetyltransferase	Glucose-repressible expression; required for replication of dsRNA viruses	22, 42
NFT1	YKR103w/YKR104w	Putative multidrug resistance-associated transporter		22
PMR1	YGL167c	High-affinity Ca^{2+}/Mn^{2+} P-type ATPase	Required for Ca^{2+} transport into the Golgi; involved in Ca^{2+}-dependent protein sorting. ORF overlaps with PMR1	10, 22
HUR1	YGL168	Unknown	ORF overlaps with PMR1	22
	YIL165C	Putative nitrilase		22

the same phenomenon as with the simple mitochondrial GFP. However, this latter setup has the added bonus that upon induction of mitophagy, the molecular weight of the fusion protein will be reduced to the molecular weight of GFP ($\sim$27 kDa) because vacuolar proteases will degrade the non-GFP moiety, as GFP itself is highly resistant to the proteases in the yeast vacuole. This allows an immunoblot-based assay for mitophagy, using anti-GFP antibody (18, 20, 21).

These assays, all measuring mitophagy in different ways, can have different interpretations. Most prominently, the linkage between mitophagy and segregation of intramitochondrial material into damaged and undamaged

subcompartments is represented in different ways in these assays. For example, analysis of Pep4-dependent (*i.e.*, vacuole dependent) degradation of aconitase shows a mitophagic defect in *aup1Δ* cells, whereas analysis of a mitochondrial GFP construct shows only a weak effect (Journo and Abeliovich, unpublished data). Aconitase is a protein that is highly susceptible to oxidation because of its iron–sulfur cluster (11), and our interpretation is that Aup1 is required in these assays for proper segregation of oxidized aconitase (and presumably other proteins as well) into the depolarized compartment, but not for the actual targeting and engulfment process.

Genes Involved in Regulating Mitophagy: Factors Identified in Targeted Studies

As mentioned earlier, targeted investigations initially suggested that deletion of Fmc1 induces mitophagy, whereas deletion of Aup1, Rtg3, and Uth1 causes a reduction of mitophagy. These results are briefly summarized below.

Fmc

Deletion of FMC1, encoding a mitochondrial matrix protein required for proper assembly of the mitochondrial H^+-ATPase, leads to a temperature-sensitive collapse of the mitochondrial electrochemical gradient under anaerobic conditions. It was found that mitophagy is induced under these conditions. Curiously, later studies claimed that deletion of FMC1 abolishes mitophagy (Table 1).

Uth1

This factor was originally identified in a screen for oxidative stress-response genes. Mutants lacking the gene display enhanced survival under starvation and increased lifespan (Austriaco, 1996). Uth1 is a SUN domain protein that shows strong homology to glucanases. Uth1 was reported to have a dual localization to the cell wall and the mitochondrial outer membrane (53). The work by Kissova *et al.* showed that transfer of yeast from a nitrogen-replete respiratory medium (lactate as carbon source) to a glucose-based nitrogen starvation medium leads to induction of mitophagy (although these conditions may not reflect quality control culling of defective mitochondria, as discussed above). They also showed that deletion of *UTH1* leads to a delay in transport of mitochondrial GFP to the vacuolar lumen and a defect in Pep4-dependent degradation of porins, under these conditions.

Aup1

The *AUP1* gene was identified in a screen for phosphatase homologs having a synthetic phenotype in combination with a partial loss-of-function mutant of the autophagy-specific protein kinase, Atg1. Aup1 is part of a novel subfamily of the protein phosphatase 2C protein phosphatase superfamily. It has been localized, by different authors, to either the mitochondrial intermembrane space or the mitochondrial matrix. This protein is distinct among other mitophagy-related *S. cerevisiae* genes in having putative mammalian orthologs. In stationary-phase mitophagy assays, the *aup1Δ* mutant shows an inability to degrade aconitase in a *PEP4*-dependent fashion while having little or no effect on mitochondrial GFP transport into the vacuole. These results may suggest that Aup1 plays a role in segregating defective mitochondrial components. In support of this hypothesis, *aup1Δ* cells accumulate oxidized proteins more rapidly than wild-type cells under stationary-phase mitophagy conditions (18).

Rtg3

In the 1980s, the late Ron Butow's lab identified genes that are upregulated in ρ^- cells lacking functional mitochondria (40). By screening for mutants defective in this upregulation, they identified the *RTG* genes, which include *RTG1*, *RTG2*, and *RTG3* (32) and are putatively involved in retrograde mitochondria-to-nucleus signaling. Rtg1 and Rtg3 are transcription factors, whereas Rtg2 is a regulatory protein that inhibits a negative regulator of Mks1, which itself is a negative regulator of Rtg1/3 nuclear translocation (33). Recently published data suggest that Rtg3 is translocated into the nucleus under stationary-phase mitophagy and that this correlates with the upregulation of RTG client genes such as Cit2. In fact, the translocation of Rtg3 under mitophagy conditions appears to depend on Aup1, and mutants in Rtg3 are defective in stationary-phase mitophagy (18). At this point, it is unclear whether this reflects a function of the classical RTG pathway in mitophagy or a novel function that is specific to Rtg3.

Genes Involved in Regulating Mitophagy: Factors Identified in Whole-Genome Screens

Once fluorescence-based high-throughput assays using the yeast deletion collection became feasible for analyzing stationary-phase mitophagy, a clearer picture began to emerge (Table 1). It was found that many genes involved in macroautophagy and the Cvt pathway are also required for stationary-phase mitophagy in nonfermentable media. Strikingly, mitophagy is normal or near normal in mutants that specifically affect macroautophagy but do not affect the Cvt pathway, such as *atg17Δ*, *atg19Δ*, and *atg31Δ*. Conversely, genes that are essential for the Cvt pathway and not required for macroautophagy are essential for mitophagy. There are some notable exceptions, namely *atg23Δ* and, to a lesser degree, *atg24Δ*. Broadly, these results suggest that stationary-phase mitophagy may represent a function of the long-orphaned Cvt pathway. Although this trafficking pathway, which brings aminopeptidase I (Ape1) to the vacuole in nutrient-rich medium, depends to a large extent on the same basic set of gene products as does macroautophagy and has been known for quite some time, its physiological function remained unclear (not least because the role of Ape1 is unclear). The implication is that the Cvt pathway, or more properly the Cvt pathway machinery, is required for certain types of selective autophagy.

In addition to the analysis of these previously known autophagy genes, a set of factors that are specifically involved in mitophagy were also identified (Table 1). Many of these are novel, previously unannotated reading frames or ubiquitous proteins whose specific link to mitophagy is presently obscure, such as cytosolic ribosomal subunits. A comprehensive list is provided in Table 1. A caveat to these studies is that the strain background used, BY4742/4741, is not well suited for mitochondrial studies. In fact, unlike the SEY6210 or W303 genetic backgrounds wherein mitophagy occurs after 3 days in respiratory medium, BY4741 and BY4742 strains require 6 days for the occurrence of mitophagy (H. Abeliovich, unpublished

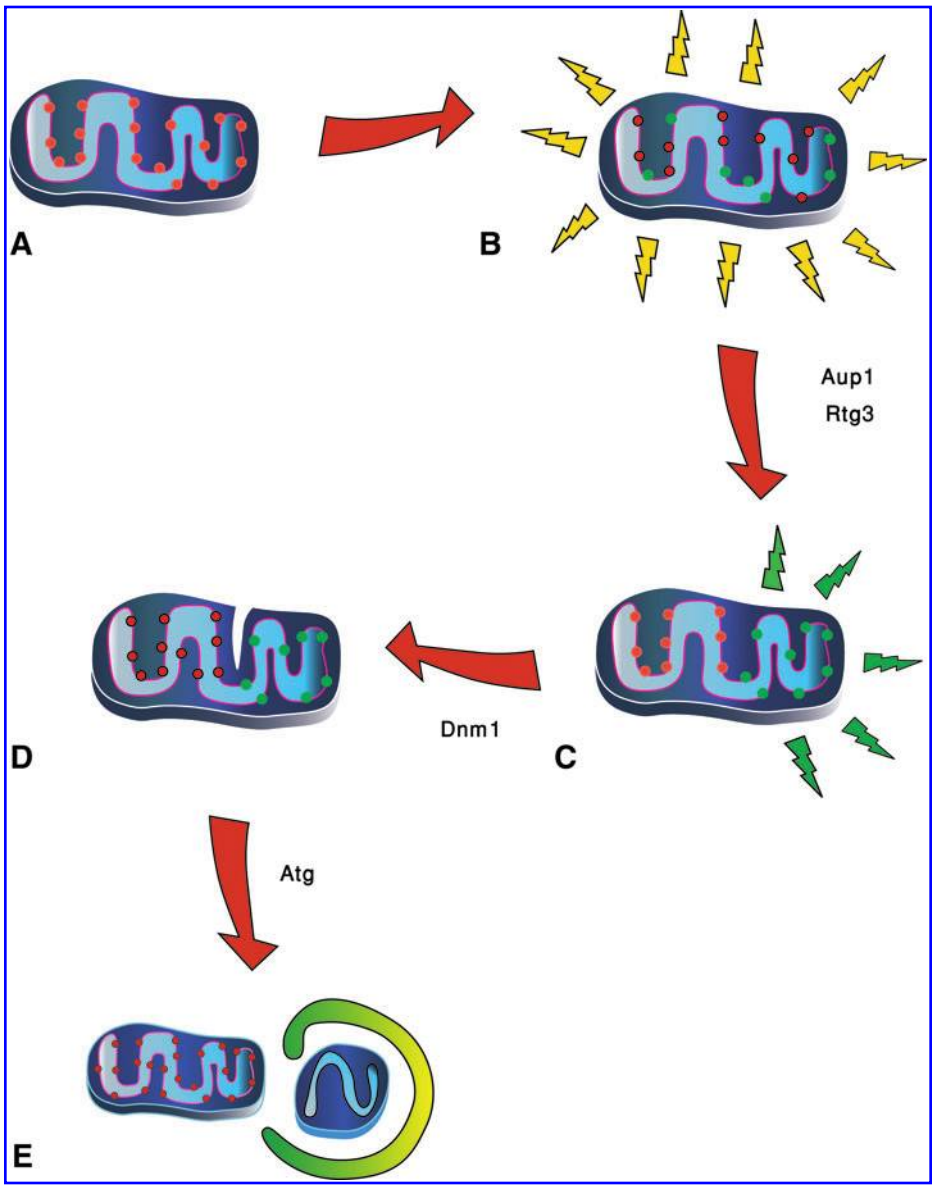

FIG. 1. Hypothetical steps in stationary-phase mitophagy, and the genes implicated in these steps. We propose that current data point to a sequence of steps that lead to the engulfment of mitochondria. **(A)** A functioning, healthy mitochondrion. **(B)** Upon prolonged respiration, inactive mitochondrial components appear (green). **(C)** The cumulative metabolic load due to this accumulation leads to a signal that depends on Aup1 and Rtg3. This signal is global and does not implicate specific mitochondria for degradation. This signal leads to segregation of defective components within the compartment. **(D)** The segregation of defective components leads to recruitment of fission factors, bringing about a topological segregation of the defective components on a separate mitochondrial compartment that, by analogy with findings in mammalian systems, are fusion defective. **(E)** Fusion-defective, malfunctioning mitochondrial compartments are selectively degraded by autophagy in an Atg32, Atg33, and general Atg machinery-dependent fashion. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

data). This suggests that this strain background is already partly defective in the regulation of mitophagy and that additional factors exist outside of those identified in the published screens. A small subset of these genes has been analyzed more closely and is discussed below in more detail.

Atg32

Atg32 is an integral protein of the outer mitochondrial membrane that interacts with Atg11 (a Cvt-pathway specific factor) as well as Atg8. Hence, it is suspected to be a cargo adaptor module that links the mitochondrion with the autophagic machinery, and its interaction with this machinery must therefore be under strict regulation. Consistent with this hypothesis, expression of *ATG32* is repressed during growth on a fermentable carbon source as well as in response to *N*-acetylcysteine (38). There is no known homolog of Atg32 in animals.

Atg33

Atg33 is a polytopic mitochondrial outer membrane protein. It was identified by Kanki *et al.* (22) as a factor required for stationary-phase mitophagy. Interestingly, loss of this gene has only a minor effect on mitophagy induced by transfer of lactate-grown cells to nitrogen starvation on glucose. This result suggests that Atg33 may play a specific role in quality-control signaling that identifies dysfunctional mitochondria. Like Atg32, Atg33 is specific to fungi and is not found in mammalian cells.

Dnm1

Dnm1 is a dynamin-like protein that is required for mitochondrial fission. In agreement with models postulating a role for mitochondrial fission in segregating normal mitochondria from dysfunctional mitochondria (1), *dnm1Δ* mutants are defective in stationary-phase mitophagy (Journo and Abe-

liovich, unpublished data) and are also found to be defective in mitophagy because of transfer from respiratory growth to glucose-based nitrogen starvation (24) and under stress conditions due to mutation (37).

Redox Stress, Mitophagy, and Mitochondrial Homeostasis

At the end of the day, molecular science has to be able to connect the dots and explain the biological role of the process that is being studied. Clearly, in the context of the mitochondrial theory of aging, one would be able to demonstrate that mitophagy plays a role in alleviating a potentially dangerous situation for eukaryotic cells. Hypothetically, nonfunctional mitochondria could either hamper the cell by being a metabolic burden or by generating harmful substances such as reactive oxygen species. Paradoxically, some studies found no increases in the levels of ROS under conditions in which mitophagy was blocked (23). However, Twig *et al.* found an increase in oxidized proteins in COS7 cells expressing FIS1 RNAi or a dominant negative DRP1 mutant. This result is corroborated in yeast by Journo *et al.*, who found that *aup1Δ* cells accumulate more oxidized proteins than wild-type cells under stationary-phase mitophagy conditions. Perhaps the most interesting results come from a study by Deffleu *et al.* (5) They showed that the antioxidant *N*-acetyl-L-cysteine (NAC) can inhibit mitophagy in yeast, but not general nitrogen-starvation induced macroautophagy. The effect on mitophagy was not due to changes in ROS levels, but to an increase in glutathione levels. Indeed, mutants in glutathione synthetase show a significantly higher level of mitophagy that is not inhibited by NAC. These results may suggest that accumulation of oxidized species (possibly proteins) in the cell may trigger mitophagy and relates also to the fact that NAC inhibits the expression of *ATG32* (38).

Concluding Remarks

Although mitophagy as a phenomenon has been known for several decades, we have only recently begun its mechanistic analysis at the molecular level. The discovery of stationary-phase mitophagy in respiring yeast cells has opened the door to high-throughput genomic screens and has allowed the identification of a number of factors required for this process. It is clear that we are only at the very beginning of the molecular elucidation of the mechanism(s) of mitophagy. We are beginning to gain an understanding of the outline of the process, but much more work is required before we can say that we have actual molecular insight. Most workers would agree that there must be a signaling mechanism that identifies defective mitochondria. However, this signaling mechanism must also be coupled to an intramitochondrial mechanism that sorts defective, perhaps oxidized, factors. It is possible that this signal activates and uses the mitochondrial fission and fusion machinery to "distill" defective components out of the general mitochondrial milieu. One can, therefore, also postulate at least two signaling events, not one: the original overall physiological burden as sensed by the cells due to accumulation of unsorted mitochondrial damage may induce the "sorting" process, and the segregation of defective mitochondrial compartments could induce a specific "eat me" signal mediated by Atg32 and perhaps Atg33 (Fig. 1). Within this hypothetical framework, different classes of genes, having different effects on the various stages described, would be predicted to contribute to the overall process. Much more work, both scientific and theoretical, will be needed to test these hypotheses and to identify the molecular machinery behind them.

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References

1. Abeliovich H. Mitophagy: the life-or-death dichotomy includes yeast. *Autophagy* 3: 275–277, 2007.
2. Balaban RS, Nemoto S, and Finkel T. Mitochondria, oxidants, and aging. *Cell* 120: 483–495, 2005.
3. Cherra SJr, Dagda RK, Tandon A, and Chu CT Mitochondrial autophagy as a compensatory response to PINK1 deficiency. *Autophagy* 5: 1213–1214, 2009.
4. Dagda RK, Cherra SJr, Kulich SM, Tandon A, Park D, and Chu CT. Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. *J Biol Chem* 284: 13843–13855, 2009.
5. Deffieu M, Bhatia-Kissova I, Salin B, Galinier A, Manon S, and Camougrand N. Glutathione participates in the regulation of mitophagy in yeast. *J Biol Chem* 284: 14828–14837, 2009.
6. Deter RL, Baudhuin P, and De Duve C. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J Cell Biol* 35: C11–C16, 1967.
7. Deter RL and De Duve C. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *J Cell Biol* 33: 437–449, 1967.
8. Dunn WAJ. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. *J Cell Biol* 110: 1923–1933, 1990.
9. Dunn WAJ. Studies on the mechanisms of autophagy: maturation of the autophagic vacuole. *J Cell Biol* 110: 1935–1945, 1990.
10. Durr G, Strayle J, Plemper R, Elbs S, Klee SK, Catty P, Wolf DH, and Rudolph HK. The medial-Golgi ion pump Pmr1 supplies the yeast secretory pathway with Ca2+ and Mn2+ required for glycosylation, sorting, and endoplasmic reticulum-associated protein degradation. *Mol Biol Cell* 9: 1149–1162, 1998.
11. Flint DH, Tuminello JF, and Emptage MH. The inactivation of Fe-S cluster containing hydro-lyases by superoxide. *J Biol Chem* 268: 22369–22376, 1993.
12. George R, Beddoe T, Landl K, and Lithgow T. The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria *in vivo*. *Proc Natl Acad Sci U S A* 95: 2296–2301, 1998.
13. Gregan J, Bui DM, Pillich R, Fink M, Zsurka G, and Schweyen RJ. The mitochondrial inner membrane protein Lpe10p, a homologue of Mrs2p, is essential for magnesium homeostasis and group II intron splicing in yeast. *Mol Gen Genet* 264: 773–781, 2001.
14. Harding TM, Hefner-Gravink A, Thumm M, and Klionsky DJ. Genetic and phenotypic overlap between autophagy and the cytoplasm to vacuole protein targeting pathway. *J Biol Chem* 271: 17621–17624, 1996.
15. Harding TM, Morano KA, Scott SV, and Klionsky DJ. Isolation and characterization of yeast mutants in the cyto-

plasm to vacuole protein targeting pathway. *J Cell Biol* 131: 591–602, 1995.
16. He C and Klionsky DJ Regulation mechanisms and signaling pathways of autophagy. *Annu Rev Genet* 43: 67–93, 2009.
17. Henstrand JM, Schaller A, Braun M, Amrhein N, and Schmid J. *Saccharomyces cerevisiae* chorismate synthase has a flavin reductase activity. *Mol Microbiol* 22: 859–866, 1996.
18. Journo D, Mor A, and Abeliovich H. Aup1-mediated regulation of Rtg3 during mitophagy. *J Biol Chem* 284: 35885–35895, 2009.
19. Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, and Ohsumi Y. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J Cell Biol* 150: 1507–1513, 2000.
20. Kanki T, Kang D, and Klionsky DJ. Monitoring mitophagy in yeast: the Om45-GFP processing assay. *Autophagy* 5: 1186–1189, 2009.
21. Kanki T and Klionsky DJ. Mitophagy in yeast occurs through a selective mechanism. *J Biol Chem* 283: 32386–32393, 2008.
22. Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z, Geng J, Mao K, Yang Z, Yen WL, and Klionsky DJ. A genomic screen for yeast mutants defective in selective mitochondria autophagy. *Mol Biol Cell* 20: 4730–4738, 2009.
23. Kanki T, Wang K, Cao Y, Baba M, and Klionsky DJ. Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev Cell* 17: 98–109, 2009.
24. Kanki T, Wang K, and Klionsky DJ. A genomic screen for yeast mutants defective in mitophagy. *Autophagy* 6: 278–280 2010.
25. Kawajiri S, Saiki S, Sato S, Sato F, Hatano T, Eguchi H, and Hattori N. PINK1 is recruited to mitochondria with parkin and associates with LC3 in mitophagy. *FEBS Lett* 584: 1073–1079, 2010.
26. Kihara A, Noda T, Ishihara N, and Ohsumi Y. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in *Saccharomyces cerevisiae*. *J Cell Biol* 152: 519–530, 2001.
27. Kissova I, Deffieu M, Manon S, and Camougrand N. Uth1p is involved in the autophagic degradation of mitochondria. *J Biol Chem* 279: 39068–39074, 2004.
28. Kitagawa K, Abdulle R, Bansal PK, Cagney G, Fields S, and Hieter P. Requirement of Skp1-Bub1 interaction for kinetochore-mediated activation of the spindle checkpoint. *Mol Cell* 11: 1201–1213, 2003.
29. Kuhn KM, DeRisi JL, Brown PO, and Sarnow P. Global and specific translational regulation in the genomic response of *Saccharomyces cerevisiae* to a rapid transfer from a fermentable to a nonfermentable carbon source. *Mol Cell Biol* 21: 916–927, 2001.
30. Lee KS and Levin DE. Dominant mutations in a gene encoding a putative protein kinase (BCK1) bypass the requirement for a *Saccharomyces cerevisiae* protein kinase C homolog. *Mol Cell Biol* 12: 172–182, 1992.
31. Lefebvre-Legendre L, Vaillier J, Benabdelhak H, Velours J, Slonimski PP, and di Rago JP. Identification of a nuclear gene (FMC1) required for the assembly/stability of yeast mitochondrial F(1)-ATPase in heat stress conditions. *J Biol Chem* 276: 6789–6796, 2001.
32. Liu Z and Butow RA. Mitochondrial retrograde signaling. *Annu Rev Genet* 40: 159–185, 2006.
33. Liu Z, Sekito T, Spirek M, Thornton J, and Butow RA. Retrograde signaling is regulated by the dynamic interaction between Rtg2p and Mks1p. *Mol Cell* 12: 401–411, 2003.
34. Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, and Ohsumi Y. A protein conjugation system essential for autophagy. *Nature* 395: 395–398, 1998.
35. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, Cookson MR, and Youle RJ. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 8: e1000298, 2010.
36. Noda T, Kim J, Huang WP, Baba M, Tokunaga C, Ohsumi Y, and Klionsky DJ. Apg9p/Cvt7p is an integral membrane protein required for transport vesicle formation in the Cvt and autophagy pathways. *J Cell Biol* 148: 465–480, 2000.
37. Nowikovsky K, Reipert S, Devenish RJ, and Schweyen RJ. Mdm38 protein depletion causes loss of mitochondrial K+/H+ exchange activity, osmotic swelling and mitophagy. *Cell Death Differ* 14: 1647–1656, 2007.
38. Okamoto K, Kondo-Okamoto N, and Ohsumi Y. Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev Cell* 17: 87–97, 2009.
39. Otsuga D, Keegan BR, Brisch E, Thatcher JW, Hermann GJ, Bleazard W, and Shaw JM. The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. *J Cell Biol* 143: 333–349, 1998.
40. Parikh VS, Morgan MM, Scott R, Clements LS, and Butow RA. The mitochondrial genotype can influence nuclear gene expression in yeast. *Science* 235: 576–580, 1987.
41. Planta RJ and Mager WH. The list of cytoplasmic ribosomal proteins of *Saccharomyces cerevisiae*. *Yeast* 14: 471–477, 1998.
42. Polevoda B and Sherman F. NatC Nalpha-terminal acetyltransferase of yeast contains three subunits, Mak3p, Mak10p, and Mak31p. *J Biol Chem* 276: 20154–20159, 2001.
43. Priault M, Salin B, Schaeffer J, Vallette FM, di Rago JP, and Martinou JC. Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. *Cell Death Differ* 12: 1613–1621, 2005.
44. Resnick AC, Snowman AM, Kang BN, Hurt KJ, Snyder SH, and Saiardi A. Inositol polyphosphate multikinase is a nuclear PI3-kinase with transcriptional regulatory activity. *Proc Natl Acad Sci U S A* 102: 12783–12788, 2005.
45. Rodriguez-Enriquez S, He L, and Lemasters JJ. Role of mitochondrial permeability transition pores in mitochondrial autophagy. *Int J Biochem Cell Biol* 36: 2463–2472, 2004.
46. Rodriguez-Enriquez S, Kai Y, Maldonado E, Currin RT, and Lemasters JJ. Roles of mitophagy and the mitochondrial permeability transition in remodeling of cultured rat hepatocytes. *Autophagy* 5: 1099–1106, 2009.
47. Rodriguez-Enriquez S, Kim I, Currin RT, and Lemasters JJ. Tracker dyes to probe mitochondrial autophagy (mitophagy) in rat hepatocytes. *Autophagy* 2: 39–46, 2006.
48. Takeshige K, Baba M, Tsuboi S, Noda T, and Ohsumi Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J Cell Biol* 119: 301–311, 1992.
49. Tal R, Winter G, Ecker N, Klionsky DJ, and Abeliovich H. Aup1p, a yeast mitochondrial protein phosphatase homolog, is required for efficient stationary phase mitophagy and cell survival. *J Biol Chem* 282: 5617–5624, 2007.

50. Tanaka A. Parkin-mediated selective mitochondrial autophagy, mitophagy: Parkin purges damaged organelles from the vital mitochondrial network. *FEBS Lett* 584: 1386–1392, 2010.
51. Thumm M, Egner R, Koch B, Schlumpberger M, Straub M, Veenhuis M, and Wolf DH. Isolation of autophagocytosis mutants of *Saccharomyces cerevisiae*. *FEBS Lett* 349: 275–280, 1994.
52. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, and Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 27: 433–446, 2008.
53. Velours G, Boucheron C, Manon S, and Camougrand N. Dual cell wall/mitochondria localization of the "SUN" family proteins. *FEMS Microbiol Lett* 207: 165–172, 2002.
54. Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J, May J, Tocilescu MA, Liu W, Ko HS, Magrane J, Moore DJ, Dawson VL, Grailhe R, Dawson TM, Li C, Tieu K, and Przedborski S. PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci U S A* 107: 378–383, 2010.
55. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 39: 359–407, 2005.
56. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 283: 1482–1488, 1999.
57. Yang Z, Huang J, Geng J, Nair U, and Klionsky DJ. Atg22 recycles amino acids to link the degradative and recycling functions of autophagy. *Mol Biol Cell* 17: 5094–5104, 2006.
58. Yang Z and Klionsky DJ. An overview of the molecular mechanism of autophagy. *Curr Top Microbiol Immunol* 335: 1–32, 2009.
59. Ziviani E, Tao RN, and Whitworth AJ. Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci U S A* 107: 5018–5023, 2010.
60. Ziviani E and Whitworth AJ. How could Parkin-mediated ubiquitination of mitofusin promote mitophagy? *Autophagy* 6: 660–662, 2010.

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Abbreviations Used

Cvt = cytoplasm to vacuole targeting
EM = electron microscopy
GFP = green fluorescent protein
NAC = *N*-acetyl-L-cysteine
PINK1 = PTEN-induced putative kinase 1
ROS = reactive oxygen species