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A genomic screen for yeast mutants defective in mitophagy

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Abstract

Mitochondria autophagy (mitophagy) is the process of selective degradation of mitochondria that has an important role in mitochondrial quality control. To gain insight into the molecular mechanism of mitophagy, we screened a yeast knockout library for strains that are defective in mitophagy. We found 32 strains that showed a complete or partial block of mitophagy. One of the genes identified, *YLR356W*, is required for mitophagy, but not for macroautophagy or other types of selective autophagy. The deletion of *YLR356W* partially inhibits mitophagy during starvation, whereas there is almost complete inhibition at post-log phase. Accordingly, we hypothesize that Ylr356w is required to detect or present aged or dysfunctional mitochondria when cells reach the post-log phase.

Keywords

autophagy; genetic screen; mitochondria; mitophagy; vacuole; yeast

The first report demonstrating the presence of mitochondria within an autophagosome was made in 1957. Since then, it has been thought that mitochondria are nonselectively engulfed by autophagosomes. Recent studies, however, reveal that autophagic degradation of mitochondria (mitophagy) is a selective pathway. Mitochondria are the organelle that carry out a number of important metabolic processes such as fatty acid oxidation, the citric acid cycle and oxidative phosphorylation. Mitochondrial oxidative phosphorylation supplies a large amount of energy that contributes to a range of cellular activities. On the other hand, mitochondria are a major source of reactive oxygen species that cause oxidative damage to mitochondrial DNA, protein and lipid. Accordingly, proper repair of damaged molecules or elimination of dysfunctional mitochondria is important to maintain optimal cellular homeostasis. Recent evidence from yeast to mammal suggests that mitophagy plays an important role in mitochondria quality control. However, the molecular mechanism of mitophagy is poorly understood.

To figure out the molecular mechanism of mitophagy, we recently established the Om45-GFP processing assay to monitor this process. Om45 is a mitochondrial outer membrane protein. Following the uptake of mitochondria into the vacuole, Om45-GFP is degraded, resulting in the release of the intact form of GFP, which is detected by immunoblotting, or the appearance of green fluorescence that accumulates in the vacuole lumen. With this method, we screened a yeast knockout library for strains that are deficient in mitophagy. Among 4,667 strains, we find 32 strains that show a complete or partial block of mitophagy, in addition to the *ATG* gene

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knockout strains. Among 32 genes for which the corresponding knockout strain shows defective mitophagy, nine of them are related to membrane trafficking pathways. It is widely thought that defects in membrane trafficking affect the lipid supply that is needed for extension of the phagophore, the initial sequestering compartment that generates the autophagosome. Indeed, the knockout strains of these nine genes display blocks in the Cvt pathway and macroautophagy. Accordingly, we further focused on the remaining 23 genes. We observed the Cvt pathway and macroautophagy in each gene knockout strain and observed the GFP tagged gene product localization. All of the remaining 23 genes are not required for the Cvt pathway. Ten of them are not required for macroautophagy, suggesting that these are candidates for mitophagy-specific genes, although 13 of them are partially required for macroautophagy. These gene products' function, localization and requirement for each type of autophagy are summarized in Table 1. The functions of these proteins are involved in diverse cellular processes; in most cases it is difficult to explain their involvement in mitophagy based on their reported functions. Atg32 and Dnm1, however, have functions that provide a known or reasonable explanation for their role in mitophagy. Atg32 is recently identified as a mitophagy-specific protein that is a mitochondrial receptor required for mitochondria recognition as a cargo. Dnm1 is a mitochondrial dynamin-related GTPase required for mitochondrial fission. The requirement of Dnm1 for mitophagy is in agreement with previous reports that the fragmentation of mitochondria is a prerequisite for mitophagy in mammalian cells, and the *dnm1*Δ strain inhibits the mitophagy induced by *mdm38* conditional depletion in yeast.

The mitophagy-related genes that we found from the screen include eight genes of unknown function. Among them, we decided to initially focus on *YLR356W*, because the gene product, Ylr356w, is reported to localize on mitochondria. Using a biochemical approach, we confirmed that Ylr356w predominantly localizes to the mitochondrial outer membrane. Although macroautophagy, the Cvt pathway and pexophagy are normal in the *ylr356w*Δ strain, mitophagy is substantially defective. Accordingly, we conclude that *YLR356W* is a mitophagy-specific gene and named this gene as *ATG33*. Interestingly, deletion of *ATG33* partially inhibits (36% decrease compared with wild type) mitophagy during starvation, whereas there is almost complete inhibition (more that 80% decrease compared with wild type) at post-log phase in YPL medium (with lactic acid as the primary carbon source). The specific requirement of Atg33 for mitophagy primarily at the post-log phase suggests that there is some difference(s) between the pathways of mitophagy induced during starvation versus post-log phase growth. Because only a fraction of the total mitochondria pool is degraded by mitophagy at post-log phase, it is reasonable to think that aged or dysfunctional mitochondria are selected for degradation. Based on this idea, we hypothesize that Atg33 contributes to the detection or presentation of aged or dysfunctional mitochondria for mitophagy when cells reach the post-log phase.

We demonstrated a mitophagy defect in the majority of the *ATG* gene knockout strains independent of the genome-wide screen discussed above. *ATG* genes that play a fundamental role in autophagy (*ATG1* to *10*, *12* to *16* and *18*) are essential for mitophagy, suggesting that the core autophagic machinery is used in common between mitophagy and other types of autophagy. Notably, *ATG11*, *ATG20* and *ATG24*, which are required for both the Cvt pathway and pexophagy but not for macroautophagy, are required for mitophagy, suggesting that selective autophagy uses certain components in common that are in part different from those used in macroautophagy, although the function of *ATG20* and *ATG24* is poorly understood. In addition to these genes encoding selective components, *ATG17*, *ATG29* and *ATG31*, which are required for macroautophagy but not for the Cvt pathway, are also required for mitophagy.

In this screen, we identified 23 mitophagy-related genes. However, the requirement of these genes for mitophagy is poorly understood. So far, only three genes are identified, *ATG11*, *ATG32* and *ATG33*, which are specifically required as part of the mitochondria degradation machinery. Unfortunately, none of these genes have homologs that are identified in higher

eukaryotes. The identification of homologs or counterparts of these genes should be the next step that can allow us to understand the physiological role of mitophagy.

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Table 1

Genes required for mitophagy

Gene name	Mitophagy[1]	Macroautophagy[2]	Cvt pathway	Function[3]	Localization[4]
AIM26	++	++	No defect	Unknown	Mitochondria
AIM28/FCJ1	−	No defect	No defect	Formation of Mitochondrial crista junction	Mitochondria
DNM1	−	No defect	No defect	Mitochondrial fission	Mitochondria
ATG32	−	No defect	No defect	Mitophagy	Mitochondria
FMC1	−	++	No defect	Assembly of Mitochondrial F_1F_o ATP synthase	Mitochondria
LPE10	+	No defect	No defect	Mitochondrial magnesium transporter	Mitochondria
YLR356W/ATG33	++	No defect	No defect	Unknown	Mitochondria
YPR146C	+	No defect	No defect	Unknown	Mitochondria
RPL13B	++	No defect	No defect	Ribosomal subunit	Cytoplasm
RPL14A	−	+/++	No defect	Ribosomal subunit	Cytoplasm
RPL15B	−	++/No defect	No defect	Ribosomal subunit	Nucleus
ARG82	+	++/No defect	No defect	Inositol polyphosphate multikinase	Nucleus, Cytoplasm
ARO2	−	No defect	No defect	Chorismate synthase/Flavin reductase	Cytoplasm
BCK1	+	++/No defect	No defect	MAP kinase kinase kinase	Cytoplasm
BUB1	+	+/++	No defect	Protein kinase	Nucleus
EGD1	+	++/No defect	No defect	Subunit β1 of the nascent polypeptide-associated complex	Cytoplasm
ICY2	−	+	No defect	Unknown	Vacuole
MAK10	++	No defect	No defect	Non-catalytic subunit of N-terminal acetyltransferase of the NatC type	Cytoplasm
NFT1	+	++/No defect	No defect	Unknown	Cytoplasm
*PMR1**	−	++/No defect	No defect	Ca^{2+}/Mn^{2+} P-type ATPase	ER
*HUR1**	+	++/No defect	No defect	Unknown	Unknown
YIL165C	−	++	No defect	Unknown	Unknown
YOR019W	+	No defect	No defect	Unknown	Unknown

++, slight defect; +, severe defect; −, complete defect;

[1] Om45-GFP processing assay (mitophagy is induced by nitrogen starvation);

[2] Pho8Δ60 assay, both the SEY6210 and BY4742 strains were used;

[3] Based on the SGD database, http://www.yeastgenome.org/;

[4] Based on Huh, et al., Nature 425, 686–691 (2003) and our data;

* *PMR1* and *HUR1* partially overlap.

This table was modified from data presented in Kanki T, Wang K, Baba M, Bartholomew CR , Lynch-Day MA, Du Z, Geng J, Mao K, Yang Z, Yen W-L, Klionsky DJ. A genomic screen for yeast mutants defective in selective Mitochondria autophagy. Mol Biol Cell 2009; 20:4730–8, which are reproduced by permission of the American Society for Cell Biology, copyright 2009.