Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The ER membrane insertase Get1/2 is required for efficient mitophagy in yeast

Mashun Onishi, Sachiyo Nagumo, Shohei Iwashita, Koji Okamoto*

Laboratory of Mitochondrial Dynamics, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 10 April 2018
Accepted 14 April 2018
Available online xxx

Keywords:
Mitochondria
Autophagy
ER membrane insertase
Yeast

ABSTRACT

Mitophagy is an evolutionarily conserved autophagy pathway that selectively eliminates mitochondria to control mitochondrial quality and quantity. Although mitophagy is thought to be crucial for cellular homeostasis, how this catabolic process is regulated remains largely unknown. Here we demonstrate that mitophagy during prolonged respiratory growth is strongly impaired in yeast cells lacking Get1/2, a transmembrane complex mediating insertion of tail-anchored (TA) proteins into the endoplasmic reticulum (ER) membrane. Under the same conditions, loss of Get1/2 caused only slight defects in other types of selective and bulk autophagy. In addition, mitophagy and other autophagy-related processes are mostly normal in cells lacking Get3, a cytosolic ATP-driven chaperone that promotes delivery of TA proteins to the Get1/2 complex. We also found that Get1/2-deficient cells exhibited wildtype-like induction and mitochondrial localization of Atg32, a protein essential for mitophagy. Notably, Get1/2 is important for Atg32-independent, ectopically promoted mitophagy. Together, we propose that Get1/2-dependent TA protein(s) and/or the Get1/2 complex itself may act specifically in mitophagy.

1. Introduction

Mitochondria are essential power plants that produce ATP as energy currency for cells. However, they accumulate their own byproducts, reactive oxygen species (ROS), generated during oxidative phosphorylation [1]. ROS accumulation eventually causes mitochondrial dysfunction that negatively affects cellular integrity, leading to diverse pathological consequences. In addition, cells need to flexibly adjust mitochondrial quantity to maintain a suitable balance between ATP production and consumption [2]. Therefore, degradation of dysfunctional and excess mitochondria is critical for cellular homeostasis. This catabolic process is defined as mitochondria-specific autophagy termed mitophagy, an intracellular membrane trafficking pathway through which mitochondria are enclosed by double-membrane vesicles called autophagosomes and transported to the lysosome (vacuole in yeast), a digestive compartment for degradation [3,4]. Mitophagy is a fundamental process conserved from yeast to humans [5] whose defects are associated with various pathologies including aging, cancer, heart failure, and neurodegeneration, highlighting its physiological significance [6].

In the budding yeast *Saccharomyces cerevisiae*, the mitochondria-anchored protein Atg32 is induced in response to oxidative stress and localized on the surface of mitochondria [7,8]. Atg32 interacts with Atg8, a ubiquitin-like protein conjugated to the phosphatidylethanolamine (PE) and localized to the autophagosome. Atg32 also interacts with Atg11, a selective autophagy-specific scaffold protein acting in assembly of core Atg proteins essential for autophagosome formation [7,8]. Accordingly, these Atg proteins recruited to the mitochondrial surface promote formation of autophagosomes enclosing mitochondria. Recent studies using yeast have also suggested that mitochondria-containing autophagosomes (mitophagosomes) are generated at membrane contact sites between mitochondria and the endoplasmic reticulum (ER) [9,10], raising the possibility that ER membranes and/or proteins may participate in degradation of structurally and functionally distinct organelles. Although it is apparent that mitophagy involves highly dynamic membrane biogenesis and deformation, molecular mechanisms underlying this intricate event remain largely unknown.

We show here that Get1 and Get2, ER membrane proteins of the GET (guided entry of tail-anchored proteins) pathway [11], are required for efficient degradation of mitochondria in yeast. In this ER protein biogenesis pathway, newly synthesized tail-anchored

* Corresponding author.
E-mail address: kokamoto@fbs.osaka-u.ac.jp (K. Okamoto).

https://doi.org/10.1016/j.bbrc.2018.04.114
0006-291X/

(TA) proteins are associated with a homodimer of Get3, a cytosolic ATPase chaperone, and delivered to Get1/2, a 2:2 tetrameric complex that drives insertion of TA proteins into the ER membrane [12–16]. Despite the strong defects in mitophagy, other selective and bulk autophagy processes are only slightly affected in cells lacking Get1/2. In contrast to this transmembrane complex, Get3 is mostly dispensable for all autophagy-related events. In addition, we found that Atg32 is normally induced and localized to mitochondria in *get1/2*-null cells. When ectopically promoted by the pexophagy (peroxisome-specific autophagy) protein Atg36 [19], mitophagy was also suppressed in the absence of Get1/2. Collectively, our data raise the possibility that Get1/2-dependent TA protein(s) and/or the Get1/2 complex itself may regulate mitophagy in a manner independent of Atg32.

2. Materials and methods

2.1. Yeast strains and growth conditions

Yeast strains and plasmids used in this study are described in Supplementary Tables 1 and 2. Standard genetic and molecular biology methods were used for *S. cerevisiae* strains. Yeast cells 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 (SDGCA), supplemented with necessary amino acids. For mitophagy, pexophagy, ER-phagy, the Cvt pathway, and autophagy assays under respiratory conditions, cells grown to mid-log phase in SDCA were transferred to SDGCA and incubated at 30 °C.

2.2. Fluorescence microscopy

Yeast living 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) derived from the jellyfish *Aequorea victoria* and mCherry, a monomeric version of the red fluorescent protein DsRed derived from the reef corals *Discosoma* sp. (13 and 20, respectively; Carl Zeiss). Cell images were captured using acquisition and analysis software (Axio Vision 4.6; Carl Zeiss).

2.3. Western blotting

Samples corresponding to 0.1 OD_{600} units of cells were separated by SDS-PAGE followed by western blotting and immunodecoration with primary antibodies raised against mCherry (1:2,000, Abcam ab125096), Pgk1 (1:10,000, Abcam ab113687), Ape1 and Atg8 (1:5,000, gift from Dr. Hitoshi Nakatogawa, Tokyo Institute of Technology, Japan), Por1 (1:2,000, Invitrogen, 459,500), GFP (1:1,000, Invitrogen, A11122), and HA (1:5,000, Sigma, A2095). After treatment with the secondary antibodies, horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (H + L) for mCherry, Pgk1, Por1, and GFP and goat anti-rabbit IgG (H + L) for Ape1 and Atg8 (1:10,000, Jackson ImmunoResearch 315-035-003 and 111-035-003, respectively) followed by the enhanced chemiluminescence reagent Western Lightning Plus-ECL (NEL103001EA, PerkinElmer), proteins were detected using a luminescent image analyzer (LAS-4000 mini; GE Healthcare). Quantification of the signals was performed using ImageQuant TL (GE Healthcare).

3. Results

3.1. Mitophagy is strongly suppressed in cells lacking Get1/2

We have previously performed a genome-wide visual screen for non-essential, single gene deletion strains exhibiting mitophagy defects [7]. Our candidates included mutants lacking Get1 and Get2, key components of the GET pathway [11]. To elucidate the role of Get1/2 in mitophagy, we observed transport of mitochondria to the vacuole, a lytic compartment in yeast. Mitochondria and vacuoles were visualized using mito-dihydrofolate reductase (DHFR)-mCherry, a reporter located in the matrix of mitochondria and GFP fused at the C terminus of Vph1, a membrane-integrated subunit of the vacuolar ATPase (Vph1-GFP), respectively [20]. When cells are grown in non-fermentable glycerol medium (Gly), mitochondria become active in respiration and are eventually degraded by mitophagy. Wild-type cells grown in Gly for 72 h contained mitochondrial mCherry signals co-localized with Vph1-GFP in an Atg32-dependent manner (Fig. 1A). We hardly detected mito-DHFR-mCherry signals localized to the vacuole in cells lacking Get1 or Get2 under the same conditions (Fig. 1A). Notably, *get3*-null cells exhibited mitochondrial mCherry signals co-localized with Vph1-GFP, indicating that Get3 is dispensable for mitophagy (Fig. 1A).

We next quantified mitochondrial degradation in cells lacking Get proteins using the mito-DHFR-mCherry probe. Upon mitophagy, DHFR-mCherry is processed to generate free mCherry in the vacuole that is appreciably protease-resistant, thereby semi-quantitatively detecting degradation of mitochondria [20]. We found that accumulation of free mCherry was greatly decreased to 32% and 40% in cells lacking Get1 and Get2, respectively, compared to wild-type cells at the 72 h time point (Fig. 1B). In addition, we investigated the effect of Get1/2 depletion on mitophagy by monitoring the levels of Por1, an endogenous mitochondrial outer membrane protein. In wild-type cells the Por1 levels were degreased during prolonged respiratory growth, indicating that Por1 is degraded via mitophagy (Fig. 1B). Consistent with our microscopic analysis, we hardly detected a decrease in the expression levels of Por1 in *get1*-or *get2*-null cells under the same conditions (6.6 and 5.8 a.u. in *get1*-and *get2*-null cells, respectively, compared to 3.2 a.u. in wild-type cells at the 72h time point), suggesting that mitophagy is strongly suppressed in the absence of Get1/2 (Fig. 1B and C). By contrast, our western blot analysis revealed that degradation of mitochondria is only slightly affected in the absence of Get3 at the 72 h time point (for generation of free mCherry, 87% compared to wild-type cells; for Por1, 4.3 a.u. in *get3*-null cells compared to 3.2 a.u. in wild-type cells) (Fig. 1B and C). Together, these results suggest that the Get1/2 complex is required for efficient mitophagy.

3.2. Loss of Get1/2 has slight effects on other types of selective autophagy and bulk autophagy

We further sought to clarify whether loss of Get1/2 affects the other autophagy-related events. To this end, we examined the cytoplasm-to-vacuole targeting (Cvt) pathway, a selective autophagy-related process that mediates transport of vacuolar enzymes such as Ape1, an amino peptidase, from the cytosol to the vacuole under mitophagy-inducing respiratory conditions. As described previously, Ape1 was constitutively synthesized as a precursor form in the cytosol, transported to the vacuole in a manner dependent on Atg19, a protein crucial for the Cvt pathway, and processed to become a mature form in the vacuolar lumen [17]

Please cite this article in press as: M. Onishi, et al., The ER membrane insertase Get1/2 is required for efficient mitophagy in yeast, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.114