[Autophagy 3:3, 263-265; May/June 2007]; ©2007 Landes Bioscience

Addendum

Autophagy-Related Pathways and Specific Role of Sterol Glucoside in Yeasts

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Original manuscript submitted: 01/15/07
Manuscript accepted: 01/23/07

Previously published online as an *Autophagy* E-publication:
http://www.landesbioscience.com/journals/autophagy/abstract.php?id=3907

KEY WORDS

peroxisome, pexophagy, Cvt pathway, autophagy, Atg26, sterol glucoside, yeast

ACKNOWLEDGEMENTS

This work was supported by NIH grants DK41737 and GM069373 to S.S.

Addendum to:

The Requirement of Sterol Glucoside for Pexophagy in Yeast Is Dependent on the Species and Nature of Peroxisome Inducers

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Mol Biol Cell 2007; 18:106-18

ABSTRACT

Recently, we showed that the requirement of sterol glucoside (SG) during pexophagy in yeasts is dependent on the species and the nature of peroxisome inducers. Atg26, the enzyme that converts sterol to SG, is essential for degradation of very large methanol-induced peroxisomes, but only partly required for degradation of smaller-sized oleate- and amine-induced peroxisomes in *Pichia pastoris*. Moreover, oleate- and amine-induced peroxisomes of another yeast, *Yarrowia lipolytica*, are degraded by an Atg26-independent mechanism. The same is true for degradation of oleate-induced peroxisomes in *Saccharomyces cerevisiae*. Here, we review our findings on the specificity of Atg26 function in pexophagy and extend our observations to the role of SG in the cytoplasm to vacuole targeting (Cvt) pathway and bulk autophagy. The results presented here and elsewhere indicate that Atg26 might increase the efficacy of all autophagy-related pathways in *P. pastoris*, but not in other yeasts. Recently, it was shown that *P. pastoris* Atg26 (PpAtg26) is required for elongation of the pre-autophagosomal structure (PAS) into the micropexophagic membrane apparatus (MIPA) during micropexophagy. Therefore, we speculate that SG might facilitate elongation of any double membrane from the PAS and this enhancer function of SG becomes essential when extremely large double membranes are formed.

INTRODUCTION

Atg26 is a UDP-glucose:sterol glucosyltransferase that converts sterol to SG.[1,2] Atg26 is essential for degradation of methanol-induced peroxisomes (both macro- and micropexophagy), but not for bulk autophagy, in the methylotrophic yeast *P. pastoris*.[3,4] However, the function of sterol glucosyltransferase in pexophagy in other yeasts was questioned in 2003[4] and this issue has remained open until now. Additionally, we could not address the role of PpAtg26 in another selective mode of autophagy, the Cvt pathway, until its existence was demonstrated in *P. pastoris*.[5] In our recent study we showed that the role of Atg26 in pexophagy depends on the yeast species and also the nature of the peroxisome inducers.[6] Here, we summarize our pexophagy findings and present some unpublished results on the role of Atg26 in the Cvt and general autophagy pathways. Finally, we discuss our current view on the role of SG in autophagy-related pathways in yeasts.

PEXOPHAGY AND THE SPECIFIC ROLE OF Atg26

First, we studied the role of Atg26 in macropexophagy of oleate- and amine-induced peroxisomes in two model yeasts, *Y. lipolytica* and *P. pastoris*. Surprisingly, both biochemical and fluorescence microscopy evidence suggest that in *Y. lipolytica* the biosynthesis of SG is not required for degradation of peroxisomes that were induced by C_1 (methylamine), C_2 (ethylamine) or C_{18} (oleate) substrates.[6] Similar results, observed with oleate-induced peroxisomes of *S. cerevisiae* using biochemical and fluorescence microscopy experiments, also prove that ScAtg26 is not required for pexophagy.[7] At the same time, peroxisomes induced by oleate or primary amines were degraded less efficiently in the absence of SG in *P. pastoris*. However, comparison of macropexophagy of methanol-, oleate- and amine-induced peroxisomes clearly demonstrates that the *P. pastoris ATG26* gene is not essential for degradation of peroxisomes induced by oleate and primary amines.[6] In contrast, degradation of methanol-induced peroxisomes was completely dependent on the conversion of sterol to SG during both glucose and ethanol adaptation of this methylotrophic yeast. Therefore, we conclude that the requirement of SG for pexophagy in yeasts is dependent on the species and the nature of the peroxisome inducers.[6] In other words, SG is specifically essential for degradation of methanol-induced peroxisomes in *P. pastoris*, but it also increases the efficiency of degradation of *P. pastoris* peroxisomes induced by other substrates. These observations suggest that *P. pastoris* acquired the Atg26-dependent mechanism of pexophagy enhancement essentially as an adaptation of cells to the switch from methylotrophic to non-methylotrophic growth conditions during evolution. Based on this assumption we can predict that a similar requirement of SG for pexophagy might also exist in other methylotrophic yeast species. However, why SG is specifically essential in *P. pastoris* for degradation of only methanol-induced peroxisomes is not clear at present and will be discussed below.

THE ROLE OF STEROL GLUCOSIDE IN THE CVT PATHWAY

The Cvt pathway is another selective autophagy-related pathway that transports the precursors of vacuolar resident hydrolases, Ape1 and Ams1, to the vacuole for processing and function in *S. cerevisiae*.[8] Recently, it was reported that *S. cerevisiae* Atg26 is not required for the Cvt pathway, since maturation of Ape1 and targeting of GFP-Ape1 to the vacuole were not affected in a *S. cerevisiae* Δ*atg26* strain.[7] Since the precursor of *P. pastoris* Ape1 is also delivered to the vacuole via the Cvt pathway,[5] we extended our studies on Atg26 to the role of this protein in the Cvt pathway of *P. pastoris*. Here, we show that in rich medium the targeting and maturation of PpApe1-CFP is completely abolished in the *P. pastoris* Δ*atg8* Δ*atg11* double mutant, as previously described.[5] In contrast, PpApe1-CFP is substantially processed and localized to the vacuole in the *P. pastoris* wild-type strain, but to a much lesser extent in the Δ*atg26* mutant (Fig. 1A and B). The *P. pastoris* mutant lacking SG exhibited an intermediate phenotype suggesting that Atg26 is not essential for the *P. pastoris* Cvt pathway, but increases its efficacy. Therefore, as in pexophagy, the function of SG in the Cvt pathway is species-specific in yeasts in that it is partly required for the Cvt pathway in *P. pastoris*, but not in *S. cerevisiae*.

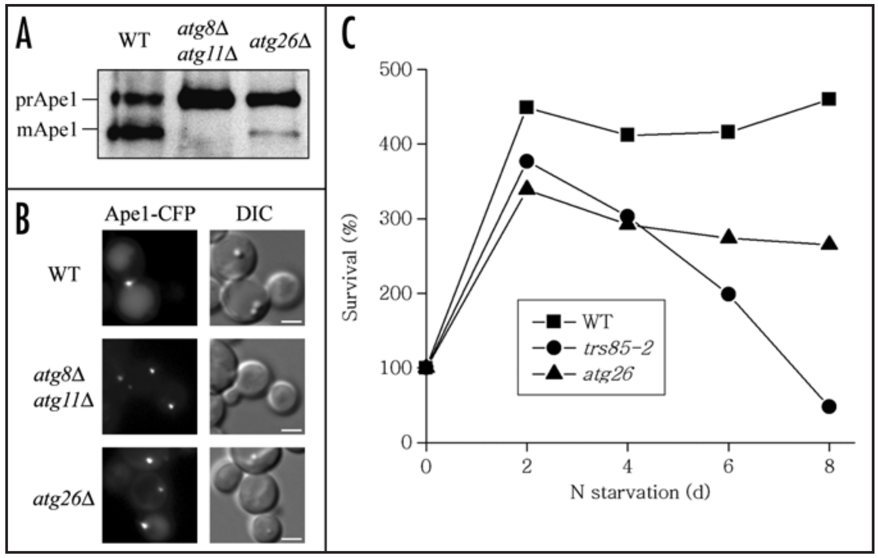

Figure 1. The role of Atg26 in the Cvt pathway and bulk autophagy. (A) Maturation and (B) localization of Ape1-CFP in the *P. pastoris* wild-type (SJCF483), Δ*atg8* Δ*atg11* (SJCF510) and Δ*atg26* (SJCF650) cells transformed with the plasmid pJCF239[5]. Bars, 2 μm. (C) Survival of the *Y. lipolytica* wild-type (H222), *trs85-2* (Ain19) and *atg26* (N155) cells under nitrogen starvation conditions. Cells were grown in glucose synthetic medium with nitrogen to log phase, and shifted to this same medium lacking nitrogen. Aliquots were removed at the indicated time points, spread on YPD plates and colonies were counted. The number of colonies at day zero was normalized to 100%.

Table 1 **Summary of the role of Atg26 in autophagy-related pathways in yeasts**

Protein	Degradation of Peroxisomes that were Induced by:			Cvt Pathway	Bulk Autophagy	Refs.
	Methanol	Oleate	Amines			
PpAtg26	essential	partly required	partly required	partly required	partly required	3, 4, 6, this study
YlAtg26	-	not required	not required	-	not required	4, 6, this study
ScAtg26	-	not required	-	not required	not required	7

BULK AUTOPHAGY AND THE POSSIBLE ROLE OF Atg26

To further explore the role of Atg26 in autophagy-related pathways, we studied the survival of the *Y. lipolytica* wild-type, *trs85-2* and *atg26* cells under nitrogen starvation conditions (Fig. 1C). As expected, the mutant *trs85-2,* affected in the 85 kDa subunit of the TRAPP complex, progressively lost viability in the medium lacking nitrogen indicating a defect in bulk autophagy, as described.[9] However, as was the case for the wild-type strain, the *Y. lipolytica atg26* mutant had essentially the same survival rates during 2–8 days of nitrogen limitation. Therefore, YlAtg26 is not required for general autophagy. The role of the Atg26 protein in bulk autophagy was also addressed in *S. cerevisiae* by an alkaline phosphatase assay that showed the same autophagic activities for the *S. cerevisiae* Δ*atg26* and wild-type strains.[7] Atg26 was also suggested to be dispensable for starvation-induced autophagy in *P. pastoris*. However, after 10 days in the medium without nitrogen, the *P. pastoris* Δ*atg26* mutant clearly exhibited an intermediate viability relative to the wild-type and *atg7* strains.[3] Thus, other quantitative studies are required to examine the efficacy of autophagy in the *P. pastoris* strain lacking SG. Altogether, the available data demonstrate that the Atg26 protein might also have a species-specific role in yeast autophagy. This will be substantiated if SG can indeed increase the efficacy of the process in *P. pastoris*.

HYPOTHESIS ON THE ROLE OF SG IN *P. pastoris*

The data published in our original paper,[6] in combination with those presented here (Fig. 1) and elsewhere[3,4,7] demonstrate that SG might play a *P. pastoris*-specific role not only in pexophagy, but also in the Cvt pathway and, probably, even in bulk autophagy (Table 1). Recently, it was reported that the synthesis of SG at the PAS is required for its maturation and elongation into the MIPA.[10]

However, the MIPA seems to be the structure homologous to the so-called "isolation membrane", an intermediate in the biogenesis of autophagosomes, pexophagosomes and Cvt vesicles. Therefore, we hypothesize that in *P. pastoris,* SG acquired a new function during evolution related to facilitation of the elongation of the double membranes from the PAS. The enhancer function of SG becomes essential when cells are challenged with elongation of the extremely large double membranes, i.e., during biogenesis of the MIPA or pexophagosome, around methanol-induced peroxisomes. We are currently testing this hypothesis in our lab.

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