Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 338 (2005) 1884–1889
BBRC
www.elsevier.com/locate/ybbrc

Characterization of a novel autophagy-specific gene, *ATG29* ☆

Tomoko Kawamata [a,b], Yoshiaki Kamada [b,c], Kuninori Suzuki [b,c], Norihiro Kuboshima [d], Hiroshi Akimatsu [d], Shinichi Ota [d], Mariko Ohsumi [d], Yoshinori Ohsumi [b,c,*]

[a] *Department of Biology, Graduate School of Science and Technology, Kobe University, Kobe 657-8501, Japan*
[b] *Division of Molecular Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan*
[c] *School of Life Science, The Graduate University for Advance Studies, Okazaki 444-8585, Japan*
[d] *Department of Bioscience, Teikyo University of Science and Technology, Yamanashi 409-0193, Japan*

Received 13 October 2005
Available online 11 November 2005

Abstract

Autophagy is a process whereby cytoplasmic proteins and organelles are sequestered for bulk degradation in the vacuole/lysosome. At present, 16 *ATG* genes have been found that are essential for autophagosome formation in the yeast *Saccharomyces cerevisiae*. Most of these genes are also involved in the cytoplasm to vacuole transport pathway, which shares machinery with autophagy. Most Atg proteins are colocalized at the pre-autophagosomal structure (PAS), from which the autophagosome is thought to originate, but the precise mechanism of autophagy remains poorly understood. During a genetic screen aimed to obtain novel gene(s) required for autophagy, we identified a novel ORF, *ATG29*/*YPL166w*. *atg29Δ* cells were sensitive to starvation and induction of autophagy was severely retarded. However, the Cvt pathway operated normally. Therefore, *ATG29* is an *ATG* gene specifically required for autophagy. Additionally, an Atg29-GFP fusion protein was observed to localize to the PAS. From these results, we propose that Atg29 functions in autophagosome formation at the PAS in collaboration with other Atg proteins.

Keywords: Autophagy; ATG; Atg29; PAS; Cvt; Yeast

Proper cellular homeostasis requires that a strict balance be kept between protein synthesis and degradation. Within cells, autophagy is the major pathway by which long-lived proteins and cytoplasmic organelles are degraded [1,2]. During autophagy, cytoplasmic components are enwrapped into an autophagosome and delivered to the vacuole for degradation [3]. From genetic analyses using budding yeast, at least 16 autophagy-related genes (*ATG*) have been identified as essential for autophagosome formation [2,4]. Recently, it has been demonstrated that most Atg proteins are colocalized at the pre-autophagosomal structure (PAS) [5]. Therefore, the PAS is implicated to be the center of autophagosome formation. However, how these Atg proteins function cooperatively at the PAS to create the autophagosome still remains to be elucidated.

In *Saccharomyces cerevisiae*, autophagy overlaps with another pathway, the cytoplasm to vacuole targeting (Cvt) pathway. This pathway is responsible for the biosynthetic delivery of vacuolar hydrolase aminopeptidase I (API) from cytoplasm to vacuole [2,6]. The Cvt pathway takes place in cells grown in nutrient-rich conditions, while autophagy is induced by starvation [1]. Furthermore, most of the Atg proteins required for autophagy are also needed for the Cvt pathway [2,4]. Although the Cvt and autophagy pathways are mechanistically similar, the double-membrane vesicles produced by these pathways are of different sizes [7]. Cvt vesicles are approximately 140–160 nm in diameter and exclude cytosol, whereas autophagosomes are 300–900 nm in diameter and include cytosol and

☆ *Abbreviations:* PAS, pre-autophagosomal structure; *ATG*, autophagy-related genes; Cvt, cytoplasm to vacuole targeting; API, aminopeptidase I; GFP, green fluorescent protein; Vps, vacuolar protein-sorting; PE, phosphatidylethanolamine.

* Corresponding author. Fax: +81 564 55 7516.
E-mail address: yohsumi@nibb.ac.jp (Y. Ohsumi).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.10.163

occasionally organelles. To date, a set of proteins specifically required for the Cvt pathway has been identified, while *ATG17* is the only gene known to function specifically in autophagy [8]. Clearly, the identification of additional molecule(s) required specifically for this pathway is crucial for a more thorough understanding of this process.

In this study, we searched for mutants that showed defects in autophagy and identified a novel gene, *YPL166w*/*ATG29*.

Materials and methods

Yeast strains and media. Growth media, culture conditions, and genetic manipulations were all as previously described [9]. The yeast strains used in this study are listed in Table 1. A deletion construct to disrupt *ATG29* with kanMX was amplified by PCR from BY4741-based *atg29* deletion mutants [10]. The resultant cassette was transformed into SEY6210, BJ2169, and KVY55 strains to make TMK4, TMK368, or TMK17, respectively. TMK372 was made by replacing *ATG8* gene with the *LEU2* gene [11]. TMK105 was made by replacing *ATG17* gene with the *URA3* genes in the strain TMK4. STY1133 was created as described previously [12]. TMK73 and TMK190 were created according to the one-step gene replacement method described previously [13].

Plasmid construction. A 1.7 kb fragment including the entire *ATG29* was cloned from yeast genomic DNA into pRS316 [14] to yield YCp*ATG29*. Plasmid YCp*ATG29-GFP* was generated as follows. The DNA fragment containing *ATG29-GFP*, which was PCR-amplified from TMK190, was inserted into pRS314 [14]. The plasmid pRS316 CFP-*ATG8* used in this study has been described previously [5].

Fluorescence microscopy. Intracellular localization was examined using a DeltaVision microscope system (Applied Precision, Issaque, WA) or an inverted fluorescence microscope (IX-71, Olympus) as described previously [5].

Other procedures. Isolation of mutants defective in autophagy was performed as described previously [15]. For measurement of autophagic activity, the ALP assay was performed as described previously [16]. Immunoblot analyses and in vitro protein kinase assays were performed as described previously [8,11].

Table 1
Yeast strains used in this study

Strain	Genotype	Source
BY4741	*MAT**a** his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	[10]
BYS7	BY4741 *atg7Δ::kanMX*	[10]
BYS16	BY4741 *atg16Δ::kanMX*	[10]
TMK181	BY4741 *atg29Δ::kanMX*	[10]
BJ2168	*MAT**a** ura3 leu2 trp1 ura3 pep4-3 prb1–1122 prc1–407*	Yeast Genetic Stock Center, Berkeley
TMK372	BJ2168 *atg8Δ::LEU2*	This study
TMK368	BJ2168 *atg29Δ::kanMX*	This study
SEY6210	*MATα ura3 leu2 his3 trp1 lys2 suc2*	[25]
KVY116	SEY6210 *atg13Δ::kanMX*	[5]
KVY117	SEY6210 *atg16Δ::LEU2*	[5]
JOY617	SEY6210 *atg17Δ::HIS3*	[26]
TMK4	SEY6210 *atg29Δ::kanMX*	This study
TMK105	SEY6210 *atg17Δ::URA3 atg29Δ::kanMX*	This study
KVY55	SEY6210 *pho8::pho8Δ60*	[11]
JOY687	KVY55 *atg7Δ::HIS3*	[27]
YYK382	KVY55 *atg17Δ::kanMX*	[21]
TMK17	KVY55 *atg29Δ::kanMX*	This study
STY1133	SEY6210 *RFP-APE1::LEU2*	This study
TMK190	SEY6210 *ATG29::ATG29-GFP-kanMX*	This study
TMK73	SEY6210 *ATG29::ATG29-YFP-kanMX*	This study

Results

Identification of ATG29/YPL166w

All previously discovered *atg* mutants, which are defective in autophagy, show a loss of viability under starvation conditions. Thus, sensitivity to starvation would seem to be a good indicator of autophagy defects [15]. To search for novel genes involved in autophagy, we performed a genome-wide screen using a collection of roughly 4500 yeast single-gene deletion mutants in the BY4741 background [10]. Each strain was first screened for the reduced viability under starvation conditions and over 250 strains were selected from this first screening. Strains that showed defects in the Cvt pathway were removed from the analysis. We next examined the autophagic activity of these candidates by measuring the accumulation of autophagic bodies. Of these strains, one of the most striking candidates was a disruptant of *YPL166w*. Here, we have named the ORF *YPL166w* as *ATG29*.

Atg29 is a previously unidentified protein of 213 amino acid residues with no predicted transmembrane domain, signal sequence, or other known motif. Coils 2.1 software [17] yielded a weak prediction of a coiled-coil in the region between amino acids 65 and 95 (Fig. 1). Atg29 has orthologues in other *Saccharomyces* species, but is not conserved in higher eukaryotes.

Vacuolar transport pathways are not impaired in the atg29Δ mutant

Autophagic degradation requires proper transport of vacuolar hydrolases. Thus we examined the processing of three kinds of vacuolar proteases, Pep4(PrA), PrB, and CPY, in *atg29Δ* to investigate whether the transport of these proteins was affected in the mutant. Immunoblot analyses of *atg29Δ* showed the presence of mature forms of these enzymes in similar amounts as wild-type cells, irrespective of growth conditions (data not shown). The fact that CPY processing was unaffected in *atg29Δ* indicates that *atg29Δ* is not a vacuolar protein-sorting (*vps*) mutant. Vacuolar acidification in *atg29Δ* was normal as judged by staining with quinacrine (data not shown). Furthermore, *atg29Δ* cells did not show sensitivity to 300 mM $CaCl_2$ (data not shown), indicating that *atg29Δ* cells have functional vacuoles.

ATG29 is essential for autophagy, but not for the Cvt pathway

atg29Δ cells displayed reduced viability under starvation conditions compared to wild-type cells (Fig. 2A). The autophagic activity of *atg29Δ* was assayed by two different approaches. First, we observed autophagic bodies by light

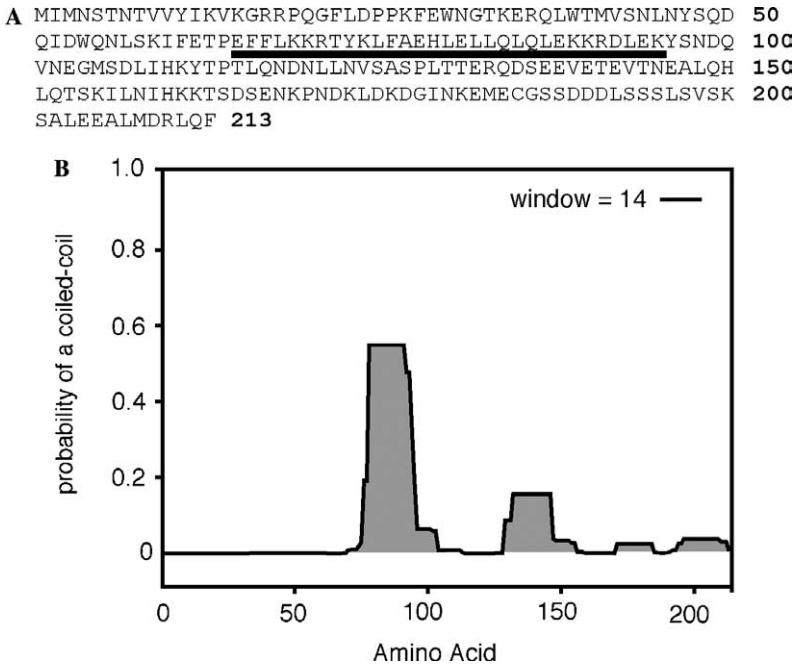


Fig. 1. Structure of Atg29 protein. (A) Amino acid sequence of Atg29. The putative coiled-coil region (amino acids 65 and 95) is underlined. (B) Coiled-coil prediction for Atg29. The amino acid sequence was analyzed using the Coils 2.1 program in the 14 residue window setting.

microscopy. Cells grown to late-log phase in YEPD were transferred to SD(-N) medium containing the protease inhibitor PMSF, and were observed after 6–8 h. In the case of vacuolar protease deficient strains such as BJ2168, autophagic bodies are visible without PMSF. Wild-type cells accumulated autophagic bodies in their vacuoles, whereas the *atg29Δ* mutant, either did not show accumulation or did so to a significantly lesser extent (Fig. 2B). As a control, we tested the autophagy mutant *atg16Δ*, which did not accumulate any autophagic bodies. This indicates that autophagy is severely compromised in the *atg29Δ* mutant. The autophagy phenotype of *atg29Δ* was significantly affected by genetic background (Fig. 2B). In the BJ2168 or BY4741 backgrounds, the latter of which was used for our systematic screening, the phenotype of *atg29Δ* was much more severe than that in the SEY6210 background.

Second, we examined the time course of autophagic activity by monitoring autophagy-dependent processing of the cytosolic form of alkaline phosphatase, Pho8Δ60 [16]. Four hours post-transfer into SD (-N) medium, the normally observed increase in ALP activity was significantly attenuated in the *atg29Δ* mutant (Fig. 2C). After 10 h, ALP activity was still significantly reduced in *atg29Δ* cells. In contrast, typical *atg* mutants such as *atg7Δ* did not show ALP activity even after 10 h starvation (Fig. 2C). From this result, it appeared that *atg29Δ* was severely defective for autophagy.

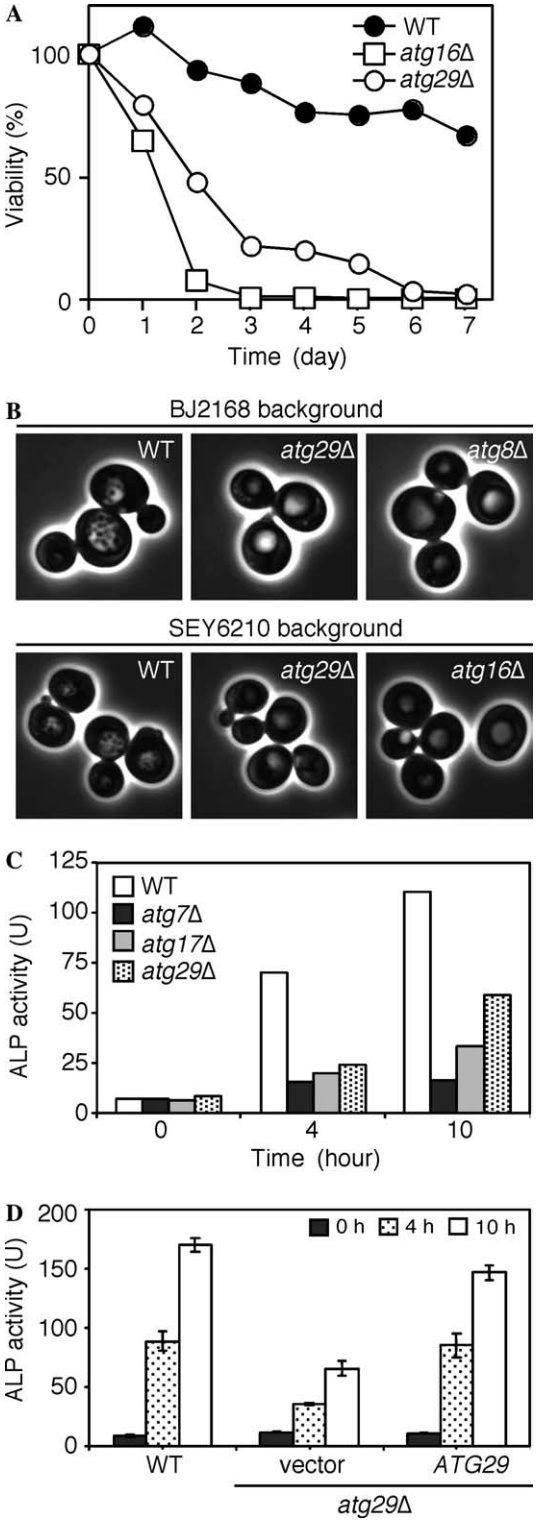
We next clarified whether *ATG29* itself complements the autophagy defect observed in *atg29Δ* cells. *ATG29/YPL166w* is located on the Watson strand in chromosome XVI, where it shares a small stretch of overlap with a Crick strand ORF, *SET6/YPL165c*. However, when a low-copy plasmid carrying only the *ATG29* ORF was introduced into *atg29Δ* cells, the autophagy defect was fully restored (Fig. 2D). Therefore, *ATG29/YPL166w* is indeed the gene responsible for the autophagy defect of the mutant.

API is synthesized in the cytosol as a 61 kDa precursor (proAPI), and is transported to the vacuole through the Cvt and autophagy pathways under growth and starvation conditions, respectively, where it is processed into its 55 kDa mature form (mAPI) [2]. All Atg proteins except for Atg17 are required for the Cvt pathway, so we sought to determine whether *ATG29* was also involved in the Cvt pathway. Maturation of proAPI in *atg29Δ* cells was observed to be normal under both growth and starvation conditions (Fig. 3A). We examined the activity of the Cvt pathway in *atg17Δatg29Δ* double disruptant and observed that the processing of proAPI was normal in the *atg17-Δatg29Δ* cells (Fig. 3B). This result suggested that Atg29 is not functionally redundant with Atg17 in the Cvt pathway. Therefore, *ATG29* is dispensable for the Cvt pathway. Though the autophagic activity of *atg29Δ* was influenced by strain background (Fig. 2B), the Cvt activity of *atg29Δ* was always normal regardless of strain (Figs. 3A and B). Based on the result, we concluded that*ATG29* is a novel *ATG* gene specifically required for autophagy, but not for the Cvt pathway.

Atg29 localization to the PAS

Most of the proteins involved in the Cvt and autophagy pathways are localized to the PAS [5]. We constructed cells expressing Atg29-GFP fusion protein, with GFP linked to the C-terminus of Atg29, and determined its localization

under growth and starvation conditions. We confirmed that Atg29-GFP is functionally active by measuring autophagic activity (data not shown). Atg29-GFP was detected as a bright dot(s) proximal to the vacuole. Next, using Atg8 and API as markers for the PAS, we examined the colocalization of Atg29 with the PAS. Atg8-CFP and API-RFP were found to colocalize with Atg29-YFP and Atg29-GFP, respectively (Fig. 4). From these observations, we concluded that Atg29 is also localized to the PAS and plays a role in autophagosome formation in concert with other Atg proteins.



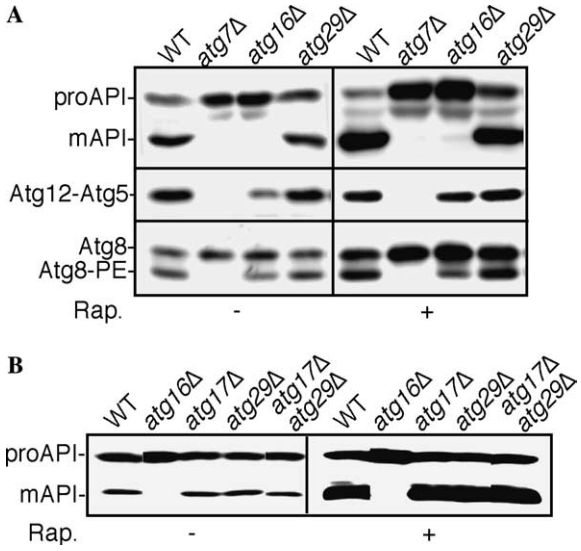


Fig. 3. *ATG29* is specifically required for autophagy, but not for the Cvt pathway. (A) Wild-type (BY4741), *atg7Δ* (BYS7), *atg16Δ* (BYS16), or *atg29Δ* (TMK181) cells were grown in YEPD (Rap.−) or treated with rapamycin for 3 h (Rap.+). Total cell extracts were prepared by glass beads disruption and subjected to immunoblotting with anti-API (top), anti-Atg12 (middle) antibodies. For Atg8-PE detection, total cell lysates were separated by SDS–PAGE containing 6 M urea and subjected to immunoblotting with anti-Atg8 antibodies (bottom). (B) Wild-type (SEY6210), *atg16Δ* (KVY117), *atg17Δ* (JOY617), atg29Δ (TMK4), or *atg17Δ atg29Δ* (TMK105) cells were grown in YEPD (Rap.−) or treated with rapamycin for 3 h (Rap.+). Protein extracts from these cells were analyzed by immunoblotting with anti-API antibodies as in (A).

Some Atg proteins, such as Atg8 and Atg1, are known to be sequestered into the autophagosome and transported into the vacuole under starvation conditions [5]. Upon induction of autophagy by rapamycin, which induces the starvation response even under nutrient-rich conditions [18], the vacuole was not stained by Atg29-GFP (Fig. 4),

Fig. 2. *ATG29* is essential for autophagy. (A) The *atg29Δ* strain is sensitive to starvation. Wild-type (BY4741; closed circles), *atg16Δ* (BYS16; open squares), or *atg29Δ* (TMK181; open circles) grown to late-log phase in YEPD were transferred to SD (-N) medium for 7 days. At the indicated time points, cells were diluted and plated on YEPD plates. Viability (%) was scored as the percentage of viable cells that were able to form colonies. (B) *atg29Δ* cells do not accumulate autophagic bodies in their vacuoles during starvation. Wild-type (BJ2168, SEY6210), *atg8Δ* (TMK372), *atg16Δ* (KVY117), and *atg29Δ* (TMK368, TMK4) cells were grown to late-log phase in YEPD and then transferred to SD (-N) medium containing 1 mM PMSF. After 6–8 h incubation, cells were observed by phase-contrast microscopy. (C) Autophagic activity is severely decreased in *atg29Δ*. Wild-type (KVY55), *atg7Δ* (JOY687), *atg17Δ* (YYK382), or *atg29Δ* (TMK17) cells were grown to late-log phase in YEPD. After incubation in SD (-N) medium for 0, 4, or 10 h, lysates were prepared from these cells and subjected to the ALP assay. (D) *ATG29* is essential for autophagy. Wild-type (KVY55) or TMK17 cells harboring empty vector (pRS316) or *ATG29* were subjected to the ALP assay as in (C). The bars represent the SD of three independent experiments.

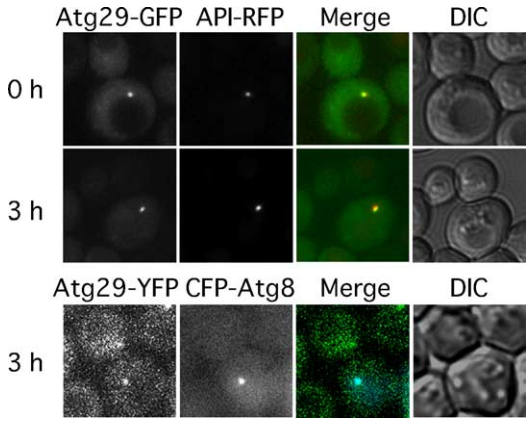


Fig. 4. Localization of Atg29 at the PAS. STY1133 cells harboring *ATG29-GFP* (YCp*ATG29-GFP*) were grown in SD medium containing casamino acid supplemented with adenine and uracil and then treated with 0.2 μg/ml rapamycin for 3 h. Growth (0 h) and rapamycin treated (3 h) cells were observed by fluorescence microscopy (IX-71, top). TMK73 cells carrying *CFP-ATG8* were grown in SD medium containing casamino acid supplemented with adenine and tryptophan, and observed under a fluorescence microscope (Deltavision) after treatment with 0.2 μg/ml rapamycin for 3 h (bottom).

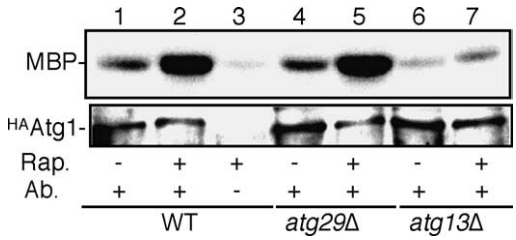


Fig. 5. Atg1 kinase activity is not downregulated in *atg29Δ*. Wild-type (SEY6210), *atg13Δ* (KVY116), and *atg29Δ* (TMK4) cells harboring a plasmid coding for HA-tagged *ATG1* were grown in YPD to late-log phase and treated with 0.2 μg/ml rapamycin for 1 h. ^{HA}Atg1 was immunoprecipitated from extracts of rapamycin-treated or -untreated cells and subjected to in vitro kinase assay. Immunoprecipitated ^{HA}Atg1 was incubated with [γ-^{32}P]ATP and myelin basic protein (MBP, 4 μg) at 30 °C for 20 min. The reaction products were separated by SDS–PAGE and detected by autoradiography (top). Immunoprecipitated ^{HA}Atg1 protein was monitored by immunoblotting with anti-HA antibody (bottom).

indicating that Atg29 is not transported to the vacuole coordinately with the progression of autophagy.

Atg29 is not required for either Atg12–Atg5 conjugation or lipidation of Atg8

Atg12 and Atg8 are ubiquitin like proteins which undergo conjugation to Atg5 and phosphatidylethanolamine (PE), respectively [19,20]. These conjugation reactions are essential for autophagosome formation. Yet, both reactions proceeded normally in *atg29Δ* mutant cells (Fig. 3A), suggesting that the autophagy-related activity of *ATG29* is separate from Atg12–Atg5 and Atg8–PE conjugation.

Atg29 does not regulate Atg1 kinase activity

Atg1 is a protein kinase which interacts with Atg13 and Atg17 [8]. Atg1 kinase activity is essential for autophagy [8,21] and is highly elevated under starvation conditions. Its activation is largely impaired in *atg13Δ* and *atg17Δ* mutant cells, demonstrating that Atg13 and Atg17 act upstream of the Atg1 kinase [8,21]. To examine whether Atg29 also affects Atg1 kinase activity, we measured Atg1 kinase activity in *atg29Δ* cells using MBP as substrate. Atg1 kinase activity was normal in *atg29Δ* cells under growth and starvation conditions (Fig. 5). Therefore, Atg29 has no effect on Atg1 kinase activity, in contrast to Atg17, the only other protein known to be specific for autophagy.

Discussion

The yeast, *S. cerevisiae*, represents an excellent system for studying the molecular mechanisms of autophagy. In this organism, the machinery used in autophagy is similar to that used by the Cvt pathway. Among the 27 *ATG* genes involved in the Cvt pathway and/or autophagy, *ATG17* is the only gene known to function specifically in autophagy. Recently, novel *ATG* genes required only for the Cvt pathway were isolated by screening for defects in proAPI maturation [12,22]. In contrast, it is more difficult to identify novel genes required specifically for autophagy. We carried out systematic screening to search for mutants defective in autophagy but not the Cvt pathway. In this study, we have characterized one such gene, *ATG29*.

As per the screening, disruption of *ATG29* exhibited the starvation sensitivity phenotype common to all *atgΔ* genes required for autophagy. *ATG29* was a previously uncharacterized ORF, but recent large-scale analyses showed that *ypl166wΔ/atg29Δ* cells exhibited a defect in spore formation [23,24]. This is not surprising, because all *atgΔ* mutants involved in autophagy display defects in sporulation.

We presented several lines of evidence suggesting *ATG29* is a novel *ATG* gene. In the *atg29Δ* mutant, induction of autophagy was retarded, and the final level of autophagy activity was also lower. To investigate the function of Atg29, we investigated the relationship between known Atg subgroups. So far we have been unable to find any previously described defects, such as defects in Atg12–Atg5 conjugation and Atg8–PE formation, in the *atg29Δ* mutant.

Previous studies demonstrated that *atg17Δ* cells form only a small number of small autophagosomes, suggesting that Atg17 functions in the expansion of autophagosomes to their normal size [21]. Similar results were also seen in *atg29Δ*, albeit with a weaker phenotype than that of *atg17Δ* (Fig. 2C). Interestingly, Atg17 regulates Atg1 kinase activity, while Atg29 does not (Fig. 5). This would seem to suggest that Atg29 functions downstream of the Atg1 kinase, through Atg17 and Atg13. We obtained several results demonstrating that Atg17 and Atg29 physically interact

with each other. Therefore, further exploration of the relationship between Atg17 and Atg29 may uncover a switching mechanism between the Cvt pathway and autophagy (Kawamata et al., manuscript in preparation).

Proper autophagosome formation requires the concerted functioning of at least 16 Atg proteins. The original screening used to derive *apg*(*atg*) mutants are nearly saturated, and we do not expect so many unidentified classical and typical *apg* mutants. However, it is also possible that additional genes are required for proper progression of autophagy, some of which may be essential for processes other than autophagy. Alternatively some genes, like *atg29Δ*, may show leaky defects in autophagy. To fully understand the mechanism of autophagosome formation, it is critical to identify these genes. The Cvt pathway may be a specific pathway for *S. cerevisiae*, since it is not conserved in all eukaryotes. However, the identification of novel autophagy-related genes will provide crucial clues to understand membrane dynamics during this complex process.

Acknowledgments

We thank the members of the Ohsumi laboratory for helpful discussion, and the National Institute for Basic Biology Center for Analytical Instruments for technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] K. Takeshige, M. Baba, S. Tsuboi, T. Noda, Y. Ohsumi, Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction, J. Cell Biol. 119 (1992) 301–311.

[2] D.J. Klionsky, Y. Ohsumi, Vacuolar import of proteins and organelles from the cytoplasm, Annu. Rev. Cell Dev. Biol. 15 (1999) 1–32.

[3] M. Baba, K. Takeshige, N. Baba, Y. Ohsumi, Ultrastructural analysis of the autophagic process in yeast: detection of autophagosomes and their characterization, J. Cell Biol. 124 (1994) 903–913.

[4] D.J. Klionsky, J.M. Cregg, W.A. Dunn Jr., S.D. Emr, Y. Sakai, I.V. Sandoval, A. Sibirny, S. Subramani, M. Thumm, M. Veenhuis, Y. Ohsumi, A unified nomenclature for yeast autophagy-related genes, Dev. Cell 5 (2003) 539–545.

[5] K. Suzuki, T. Kirisako, Y. Kamada, N. Mizushima, T. Noda, Y. Ohsumi, The pre-autophagosomal structure organized by concerted functions of *APG* genes is essential for autophagosome formation, Embo J. 20 (2001) 5971–5981.

[6] D.J. Klionsky, R. Cueva, D.S. Yaver, Aminopeptidase I of*Saccharomyces cerevisiae* is localized to the vacuole independent of the secretory pathway, J. Cell Biol. 119 (1992) 287–299.

[7] M. Baba, M. Osumi, S.V. Scott, D.J. Klionsky, Y. Ohsumi, Two distinct pathways for targeting proteins from the cytoplasm to the vacuole/lysosome, J. Cell Biol. 139 (1997) 1687–1695.

[8] Y. Kamada, T. Funakoshi, T. Shintani, K. Nagano, M. Ohsumi, Y. Ohsumi, Tor-mediated induction of autophagy via an Apg1 protein kinase complex, J. Cell Biol. 150 (2000) 1507–1513.

[9] C. Kaiser, S. Michaelis, A. Mitchell, Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994.

[10] G. Giaever, A.M. Chu, L. Ni, C. Connelly, L. Riles, S. Veronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. Andre, A.P. Arkin, A. Astromoff, M. El-Bakkoury, R. Bangham, R. Benito, S. Brachat, S. Campanaro, M. Curtiss, K. Davis, A. Deutschbauer, K.D. Entian, P. Flaherty, F. Foury, D.J. Garfinkel, M. Gerstein, D. Gotte, U. Guldener, J.H. Hegemann, S. Hempel, Z. Herman, D.F. Jaramillo, D.E. Kelly, S.L. Kelly, P. Kotter, D. LaBonte, D.C. Lamb, N. Lan, H. Liang, H. Liao, L. Liu, C. Luo, M. Lussier, R. Mao, P. Menard, S.L. Ooi, J.L. Revuelta, C.J. Roberts, M. Rose, P. Ross-Macdonald, B. Scherens, G. Schimmack, B. Shafer, D.D. Shoemaker, S. Sookhai-Mahadeo, R.K. Storms, J.N. Strathern, G. Valle, M. Voet, G. Volckaert, C.Y. Wang, T.R. Ward, J. Wilhelmy, E.A. Winzeler, Y. Yang, G. Yen, E. Youngman, K. Yu, H. Bussey, J.D. Boeke, M. Snyder, P. Philippsen, R.W. Davis, M. Johnston, Functional profiling of the *Saccharomyces cerevisiae* genome, Nature 418 (2002) 387–391.

[11] T. Kirisako, M. Baba, N. Ishihara, K. Miyazawa, M. Ohsumi, T. Yoshimori, T. Noda, Y. Ohsumi, Formation process of autophagosome is traced with Apg8/Aut7p in yeast, J. Cell Biol. 147 (1999) 435–446.

[12] P.E. Stromhaug, F. Reggiori, J. Guan, C.W. Wang, D.J. Klionsky, Atg21 is a phosphoinositide binding protein required for efficient lipidation and localization of Atg8 during uptake of aminopeptidase I by selective autophagy, Mol. Biol. Cell 15 (2004) 3553–3566.

[13] M.S. Longtine, A. McKenzie 3rd, D.J. Demarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, J.R. Pringle, Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*, Yeast 14 (1998) 953–961.

[14] R.S. Sikorski, P. Hieter, A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*, Genetics 122 (1989) 19–27.

[15] M. Tsukada, Y. Ohsumi, Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*, FEBS Lett. 333 (1993) 169–174.

[16] T. Noda, A. Matsuura, Y. Wada, Y. Ohsumi, Novel system for monitoring autophagy in the yeast *Saccharomyces cerevisiae*, Biochem. Biophys. Res. Commun. 210 (1995) 126–132.

[17] A. Lupas, M. Van Dyke, J. Stock, Predicting coiled coils from protein sequences, Science 252 (1991) 1162–1164.

[18] T. Noda, Y. Ohsumi, Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast, J. Biol. Chem. 273 (1998) 3963–3966.

[19] N. Mizushima, T. Noda, T. Yoshimori, Y. Tanaka, T. Ishii, M.D. George, D.J. Klionsky, M. Ohsumi, Y. Ohsumi, A protein conjugation system essential for autophagy, Nature 395 (1998) 395–398.

[20] Y. Ichimura, T. Kirisako, T. Takao, Y. Satomi, Y. Shimonishi, N. Ishihara, N. Mizushima, I. Tanida, E. Kominami, M. Ohsumi, T. Noda, Y. Ohsumi, A ubiquitin-like system mediates protein lipidation, Nature 408 (2000) 488–492.

[21] Y. Kabeya, Y. Kamada, M. Baba, H. Takikawa, M. Sasaki, Y. Ohsumi, Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy, Mol. Biol. Cell 16 (2005) 2544–2553.

[22] H. Barth, K. Meiling-Wesse, U.D. Epple, M. Thumm, Mai1p is essential for maturation of proaminopeptidase I but not for autophagy, FEBS Lett. 512 (2002) 173–179.

[23] A.M. Deutschbauer, R.M. Williams, A.M. Chu, R.W. Davis, Parallel phenotypic analysis of sporulation and postgermination growth in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 99 (2002) 15530–15535.

[24] A.H. Enyenihi, W.S. Saunders, Large-scale functional genomic analysis of sporulation and meiosis in *Saccharomyces cerevisiae*, Genetics 163 (2003) 47–54.

[25] J.S. Robinson, D.J. Klionsky, L.M. Banta, S.D. Emr, Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases, Mol. Cell. Biol. 8 (1988) 4936–4948.

[26] J. Onodera, Y. Ohsumi, Ald6p is a preferred target for autophagy in yeast, *Saccharomyces cerevisiae*, J. Biol. Chem. 279 (2004) 16071–16076.

[27] K. Suzuki, Y. Kamada, Y. Ohsumi, Studies of cargo delivery to the vacuole mediated by autophagosomes in *Saccharomyces cerevisiae*, Dev. Cell 3 (2002) 815–824.