Biochemical and Biophysical Research Communications 491 (2017) 693–700

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Pah1p negatively regulates the expression of V-ATPase genes as well as vacuolar acidification

Goldie Libby Sherr [a, b], Nicole LaMassa [a], Erxin Li [a], Greg Phillips [a], Chang-Hui Shen [a, b, c, *]

[a] Department of Biology, College of Staten Island, City University of New York, 2800 Victory Blvd, Staten Island, NY 10314, United States
[b] PhD Program in Biology, The Graduate Center, City University of New York, 365 Fifth Avenue, New York 10016, United States
[c] Institute for Macromolecular Assemblies, City University of New York, 160 Convent Avenue, New York, NY 10031, United States

ARTICLE INFO

Article history:
Received 9 July 2017
Accepted 22 July 2017
Available online 26 July 2017

Keywords:
PAH1
Vacuole
V-ATPase
Vacuolar fusion
Gene expression
Vacuolar acidification

ABSTRACT

In yeast, *PAH1* plays an important role in cell homeostasis and lipid biosynthesis. *PAH1* encodes for the PA phosphatase, Pah1p, which is responsible for *de novo* TAG and phospholipid synthesis. It has been suggested that the lack of Pah1p causes irregular vacuolar morphology and dysfunctional V-ATPase pump activity. However, the molecular connection between Pah1p and V-ATPase activity has remained unclear. Through real-time PCR, we have shown that *PAH1* is maximally induced at the stationary stage in the presence of inositol. We also found that vacuoles were less fragmented when *PAH1* is maximally expressed. Subsequently, we observed that vacuoles from *pah1Δ* cells were more acidic than those in WT cells. Furthermore, V-ATPase genes were upregulated in the absence of Pah1p. These results suggest that Pah1p plays an important role in vacuolar activity by negatively regulating the expression of V-ATPase genes. As such, we provide evidence to show the role of Pah1p in vacuolar acidification and fragmentation.

1. Introduction

Phosphatidic acid phosphatase (PAP) enzymes are responsible for catalyzing the reaction that dephosphorylates phosphatidic acid (PA), which in turn produces diacylglycerol (DAG) and a phosphate group during phospholipid regulation (Fig. 1A) [1]. Subsequently, DAG can be used to either generate triacylglycerol (TAG), which stores energy and fatty acids in lipid droplets, or can be used to generate phosphatidylethanolamine (PE) and phosphotidylcholine (PC) via the Kennedy pathway [2]. Therefore, concentrations of PA and DAG are critical in maintaining the production of phospholipid levels, as well as determining the types of phospholipids that are synthesized [3].

Because of their role in regulating PA and DAG levels, PAP enzymes play a critical role in regulating the lipid biosynthetic pathway. Although four PAP enzymes have been identified, only *PAH1* is responsible for *de novo* TAG and phospholipid synthesis [4,5]. *PAH1* encodes for the PA phosphatase, Pah1p, which is part of a subfamily of PA phosphatases. Pah1p's activity depends on the presence of Mg^{2+} and is not only found in yeast, but also in higher organisms as well [4,6–8].

The physiological relevance of Pah1p has been studied intensively [4,9–13]. Since Pah1p is necessary for catalyzing the key step of dephosphorylating PA to form DAG, the absence of Pah1p leads to elevated levels of PA. The abnormal elevation of PA not only causes the hyperproliferation of the nuclear ER membrane, but also leads to reduced amounts of DAG and TAG [5,14,15]. Furthermore, the reduced levels of DAG observed in *pah1Δ* cells hampers TAG synthesis and the ability to cope with excess exogenous fatty acids, thus inducing lipotoxicity [1,4,10,11].

It has been shown that Pah1p is responsible for the recruitment of the phosphatidylinositol (PI) 3-kinase, Vps34p, to the vacuoles, which then produces PI3P there (Fig. 1A) [12]. The lack of Pah1p results in the absence of Vps34p at the vacuole and thus causes the defect of vacuolar fusion, suggesting that V-ATPase pump activity is implicated in *pah1Δ* cells. This is because irregular vacuolar morphology has been associated with dysfunctional V-ATPase pump activity [16]. However, recent studies have shown that de-acidification of the vacuole actually induces vacuolar fusion and that mutated vacuoles that had retained their internal acidic pH

* Corresponding author. Department of Biology, College of Staten Island, 2800 Victory Blvd, Staten Island, NY 10314, United States.
E-mail address: changhui.shen@csi.cuny.edu (C.-H. Shen).

http://dx.doi.org/10.1016/j.bbrc.2017.07.127
0006-291X/

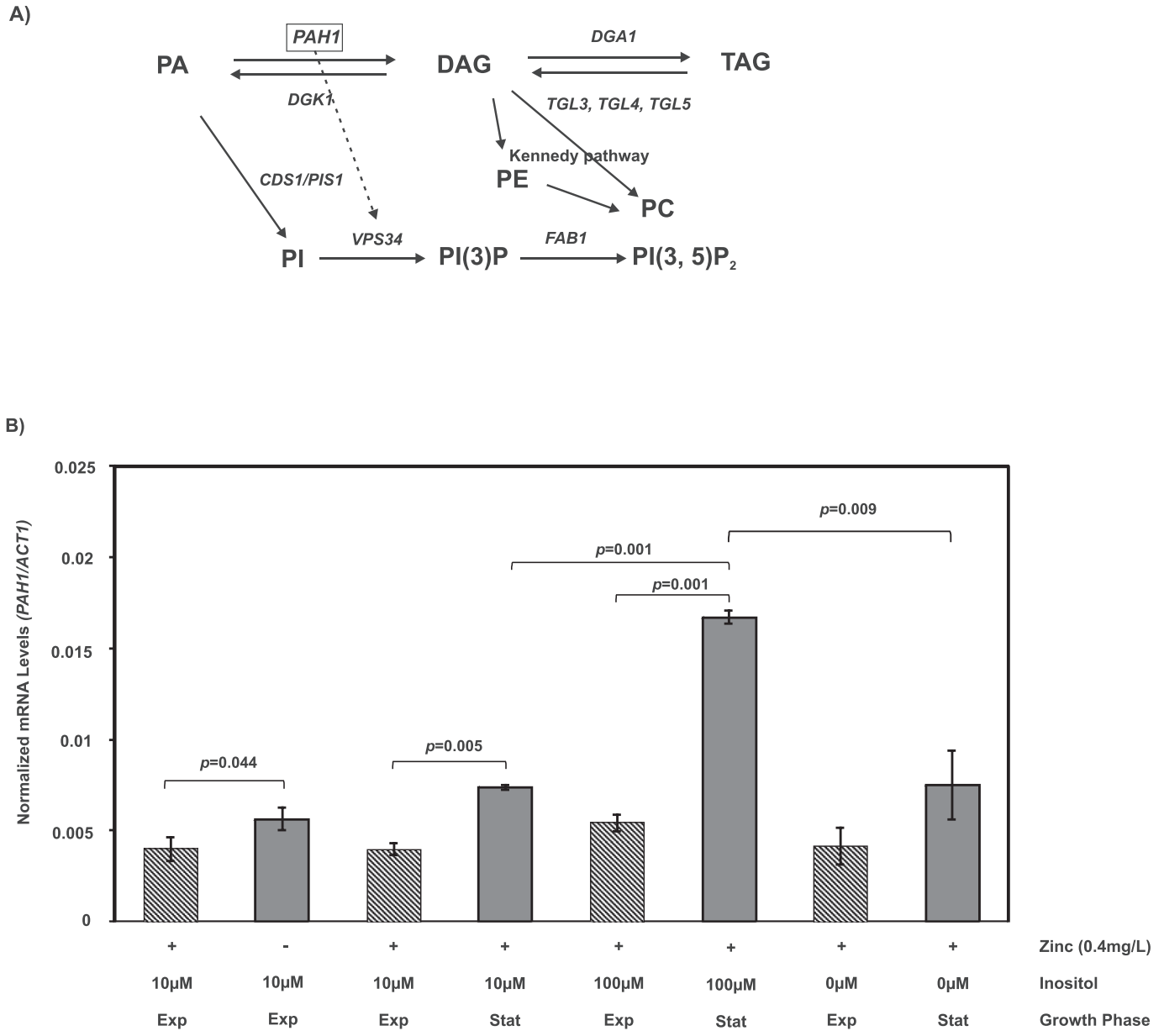

Fig. 1. *PAH1* is maximally induced during the stationary phase of growth with excess inositol in the medium. (A) Pah1p catalyzes the reaction that dephosphorylates phosphatidic acid (PA) and converts it into diacylglycerol (DAG), which can be used to produce triacylglycerol (TAG), or can be used to generate the phospholipids, phosphatidylethanolamine (PE), and phosphotidylcholine (PC) via the Kennedy pathway. Pah1p is responsible for the recruitment of the phosphatidylinositol (PI) 3-kinase, Vps34p, to the vacuoles, which then produces phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol 3, 5-bisphosphate (PI(3, 5)P_2). (B) *PAH1* mRNA was examined by qRT-PCR analysis to determine the effect of different growth conditions. WT cells were grown to mid logarithmic phase (A_{600} 1.0 ± 0.2) in 10 μM inositol SC media and were subsequently washed and grown in respective media. The expression levels of *PAH1* mRNA were normalized to the housekeeping gene *ACT1*. The expression ratio is graphed as a mean ± standard deviation. All experiments were repeated at least twice, and in each experiment the PCR reactions were done in triplicates.

might have a blockage of this fusion [17]. These results suggest that the lack of pump activity might actually favor vacuolar fusion *in vivo*. As such, it is uncertain whether irregular vacuolar morphology, caused by the lack of Pah1p, can be associated with the V-ATPase activity.

Although Pah1p plays an important role in lipid synthesis and in vacuolar activity and morphology, it is still unclear how Pah1p regulates V-ATPase activity. Previous research has shown that *PAH1* negatively regulates UAS_{INO} containing genes, including *INO1*, *INO2* and *OPI3* [9]. Although many V-ATPase genes are also UAS_{INO} containing genes, it is still unknown whether Pah1p regulates the expression of V-ATPase genes. Here, we performed biochemical analysis to understand how Pah1p affects vacuolar morphology and its activity. We first examined the inducing conditions for maximum *PAH1* expression through qRT-PCR analysis. An electron microscopy (EM) study was performed to examine whether the lack of Pah1p influences vacuolar morphology. Subsequently, a growth sensitivity assay and a vacuolar pH assay were employed to study how Pah1p affects vacuolar function. We also examined how Pah1p regulates the expression of V-ATPase genes through qRT-PCR analysis.

2. Materials and methods

2.1. Yeast strains and growth conditions

WT (BY4741; *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), *vma3Δ* (*MATa his3Δ200 leu2Δ0 met15Δ0 ura3Δ0 Δvma3*), and *pah1Δ* (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δpah1*) were used in this study. Yeast cells were normally grown at 30 °C in SC media (synthetic complete media) containing 2% glucose (wt/vol), 10 μM myo-inositol and 0.4 mg/L Zn.

2.2. Total mRNA preparation and qRT-PCR analysis

The total RNA preparation and qRT-PCR analysis was performed as described previously and is described in the supplementary materials [18]. The real-time PCR primers are described in the supplementary materials. All experiments were repeated twice, and in each experiment, the PCR reactions were done in triplicates in a 7500 sequence detection system (Applied Biosystems).

2.3. Electron microscopy study, growth sensitivity assay and vacuolar pH analysis

The Electron Microscopy analysis, growth sensitivity assay, and vacuolar pH analysis were performed as described in the supplementary materials.

3. Results

3.1. Pah1p is expressed maximally at the stationary phase of growth in the presence of inositol

Previous studies have shown that the *PAH1* promoter region includes both UAS_{ZRE} sequences and a UAS_{INO}, indicating that both zinc and inositol influence the induction of *PAH1* [15,18]. Although a P_{PAH1}-*lacZ* reporter gene system has been used to understand *PAH1* expression, it is still not clear how *PAH1* is expressed at its maximum levels, as an *in vivo* study has yet to be performed. Thus, we first wanted to examine the expression of *PAH1 in vivo*.

RNA analysis was performed to examine *PAH1* expression. Under normal growth media which includes 0.4 mg/L Zinc and 10 μM inositol, *PAH1* was minimally expressed in WT cells (Fig. 1B). When the zinc was removed from the media, *PAH1* was significantly upregulated. *PAH1* was not induced at the exponential phase, but it was significantly upregulated at the stationary phase when 100 μM inositol was added. Furthermore, the level of *PAH1* expression reached maximum levels in the presence of 100 μM inositol at the stationary phase, in which the expression level was significantly higher than cells grown in media without zinc (0.0170 ± 0.0004 and 0.0056 ± 0.0006; $p = 0.023$). This demonstrated that while *PAH1* can be upregulated in the absence of zinc, it was highly induced when cells entered the stationary phase in the presence of 100 μM inositol.

3.2. The absence of Pah1p leads to vacuolar fragmentation at the stationary phase of growth

Previously, it has been shown that the deletion of *PAH1* blocked the recruitment of the phosphatidylinositol (PI) 3-kinase, Vps34p, and its product, PI3P, to the vacuoles [12]. This result suggested that Pah1p is required for the maturation of vacuoles through the import of PI3P, and that the lack of Pah1p may lead to the vacuolar fragmentation. Since we have observed that Pah1p is expressed maximally at the stationary phase, electron microscopy was employed to examine the effect of Pah1p on vacuolar morphology at different growth stages. We observed that most of the WT cells contained fragmented vacuoles at the exponential stage, but later at the stationary stage most WT cells contained a single large vacuole (Fig. 2A). Fig. 2B showed that 74.2% of WT cells had fragmented vacuoles during the exponential phase, but then dropped to 49.9% during the stationary phase. These observations suggest that fragmented vacuoles fuse to a single large vacuole in more than 50% of WT cells, and that the fusion occurred at the stationary phase in which Pah1p is maximally expressed.

On the other hand, we found that almost all vacuoles in the *pah1Δ* cells were fragmented in both exponential and stationary stages (Fig. 2A). In fact, it showed that 96.4% of the *pah1Δ* cells had fragmented vacuoles at the exponential phase and subsequently remained at 96.9% during the stationary phase (Fig. 2B). Therefore, the number of fragmented vacuoles between the WT and *pah1Δ* cells differed significantly in both the exponential phase and the stationary phase. The fact that the number of fragmented vacuoles was even significantly higher in the *pah1Δ* cells than in the WT cells at the exponential stage strongly suggests that the presence of Pah1p is required for vacuolar fusion.

3.3. The absence of Pah1p can sustain growth in neutral environments

We observed that the deletion of Pah1p leads to vacuolar fragmentation (Fig. 2). Since vacuolar fragmentation is a hallmark of defective vacuole homeostasis, we wanted to see if vacuolar functions were impaired in the absence of Pah1p. One important aspect of vacuolar function is to examine pH homeostasis. If the vacuolar function is normal, yeast cells can survive in a neutral pH environment. On the other hand, if the vacuolar function is defective, yeast cells are unable to survive in the neutral conditions. We therefore decided to perform a growth sensitivity serial dilution analysis to determine if the *pah1Δ* strain was able to withstand neutral growth conditions.

At pH 5.5, all WT, *vma3Δ*, and *pah1Δ* cells grew well, with all strains reaching the 10^{-6} dilution, and WT extending a little further and reaching the 10^{-8} dilution (Fig. 3A). However, results differed vastly for the pH 7 conditions. While the WT and *pah1Δ* cells still grew relatively the same in the pH 7 medium as they did in the pH 5.5 medium, the *vma3Δ* mutant was unable to grow at all in the pH 7 environment and exhibited no growth at the lowest dilution of 10^{-2}. The *vma3Δ* strain was used as the negative control, since it does not contain a functional V-ATPase pump and thus cannot grow well in a neutral pH. To further confirm this finding, we performed a growth analysis. Results showed that all strains grew well in the pH 5.5 medium. At pH7, the *vma3Δ* cells did not grow well (Fig. 2B). On the other hand, both the WT and *pah1Δ* cells still grew well. Furthermore, the doubling time of the *pah1Δ* cells was significantly better than that of the WT cells. These results indicated that cells adapt well to the neutral conditions in the absence of Pah1p.

3.4. The absence of Pah1p leads to a more acidic vacuole and an upregulation of V-ATPase gene expression in the stationary phase of growth

We demonstrated that the *pah1Δ* cells can grow better than the WT cells in neutral media (Fig. 2B). This may suggest that the vacuoles of the *pah1Δ* cells function well in neutral media, which means that their ability to acidify the vacuole is not negatively implicated by the deletion of the *PAH1* gene, despite the fact that morphology is altered (Fig. 2). Thus, we next wanted to examine whether the acidic vacuolar pH could be maintained in the absence of Pah1p in the neutral conditions, and then subsequently confirm that the deletion of Pah1p enhanced the vacuolar activity. To this

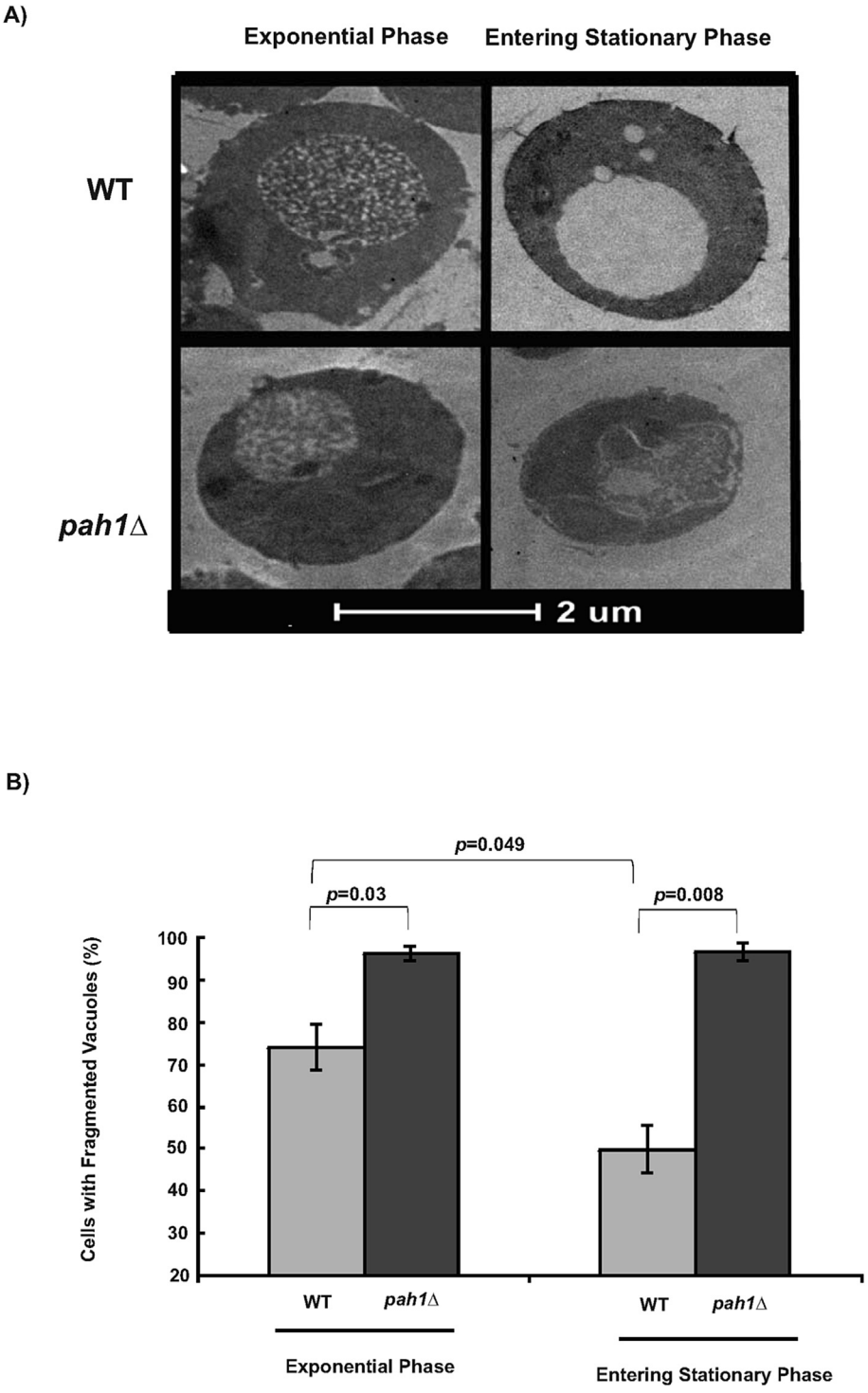

Fig. 2. The absence of Pah1p leads to vacuolar fragmentation at the stationary phase. (A) Electron microscopy images of the WT and *pah1Δ* cells in the exponential and stationary phases. Cells were grown in SC media and subsequently washed, collected and fixed at the exponential phase and the stationary phase, respectively. (B) Quantification of the cells with fragmented vacuoles in the WT and *pah1Δ* cells in both the exponential and stationary phases of growth. 100 cells per condition were counted and cells containing five or more vacuoles were scored as fragmented as previously described [24]. The average of fragmented cells is graphed as a mean ± standard deviation. All experiments were repeated three times.

end, the vacuolar pH assay was conducted with media that was neutral (pH 7.0), and while the cells were entering the stationary phase (with 100 μM inositol). Results showed that the WT cells that were grown in media buffered to pH 7.0 maintained the acidic pH of their vacuoles, which was 6.00 ± 0.04 (Fig. 4A). This was within the normal physiological pH range of ~6.0 [19]. The pH of the vacuoles of the *vma3Δ* cells, however had an average vacuolar pH of 6.72 ± 0.11, confirming that these cells were unable to maintain the acidity required, and thus were defective in vacuolar acidification at pH 7. The vacuolar pH measured in the *pah1Δ* cells, on the other

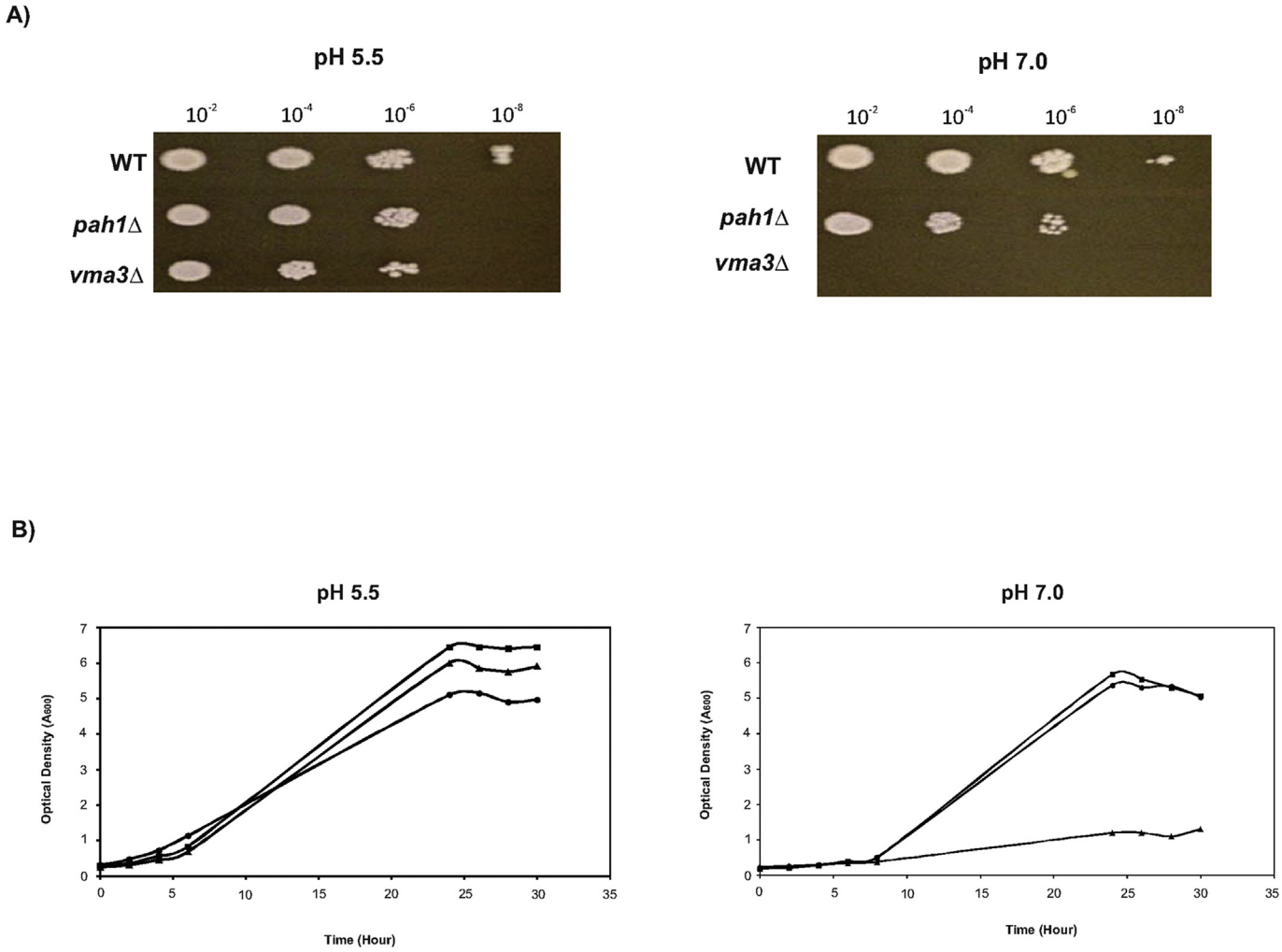

Fig. 3. Cells without Pah1p can sustain growth in neutral environments. (A) pH sensitivity assay on WT, *pah1Δ* and *vma3Δ* cells. Individual colonies of WT, *vma3Δ*, and *pah1Δ* were incubated overnight in SC media with 100 μM inositol until mid-log phase. Each strain's OD was then adjusted to 1 A_{600} per ml. Serial dilution was performed for every 100-fold dilution in SC medium, and 5 μl of each diluted cells were plated on YPD plates buffered to pH 5.5 and pH 7.0, respectively. Plates were incubated at 30 °C for three days before they were analyzed. (B) Growth Curve analysis of WT, *pah1Δ* and *vma3Δ* cells in pH 5.5 and pH 7.0 media. Cells from overnight cultures were adjusted to an optical density of A_{600} of around 0.2–0.3 and grown in SC media with 100 μM inositol buffered to either pH5.5 or pH 7.0 until the stationary phase. WT: (●), *pah1Δ* (■), *vma3Δ* (▲).

hand, was 5.89 ± 0.01 (Fig. 4A). As such, the vacuolar pH was significantly lower than that of the WT cells in the absence of Pah1p, suggesting that the *pah1Δ* cells are better at acidifying their vacuoles than the WT. This observation confirms that the *pah1Δ* cells can grow better in the neutral conditions (Fig. 2B).

Since *pah1Δ* cells can acidify their vacuoles better than the WT cells, our findings suggest that V-ATPase pump activity is upregulated in the *pah1Δ* cells. This is due to the fact that vacuolar acidification is maintained by V-ATPase pump activity. As such, we wanted to know whether this upregulated V-ATPase activity resulted from the upregulation of genes that encode for the V-ATPase pump. RNA analysis was performed on the genes that encode for the components that comprise the V-ATPase pump. Our results showed that all genes that encode for proteins comprising the peripherally associated catalytic V_1 subcomplex of the V-ATPase were upregulated in the *pah1Δ* cells compared to the WT cells. These genes included *VMA1*, *VMA2*, *VMA4*, *VMA5*, *VMA7*, *VMA8*, *VMA10*, and *VMA13* (Fig. 4B). Furthermore, genes encoding the V_0 domain of V-ATPase, including *VMA3*, *VMA6* and *VMA16*, were also upregulated in the *pah1Δ* cells. Both *VMA3* and *VMA16* are involved in acidification and *VMA6* is required for the V1 domain assembly on the vacuolar membrane. As such, all genes that are involved in the acidification process have been upregulated in the absence of Pah1p.

4. Discussion

In yeast, *PAH1* is important for cell homeostasis and lipid biosynthesis. Here, we have demonstrated that *PAH1* transcript levels are at a minimum at the exponential phase, and that even in the presence of inositol, *PAH1* expression is still low at the exponential phase. However, *PAH1* is greatly induced at the stationary phase and reaches the maximum expression level in the presence of 100 μM inositol compared to other inducing conditions (Fig. 1). The maximum induction of *PAH1* at the stationary phase is more than two folds of other inducing conditions and four folds of repressing conditions. Therefore, the expression of *PAH1* was induced as cells progressed from the exponential to the stationary phases of growth. Furthermore, the growth phase-mediated induction of *PAH1* was further stimulated by inositol supplementation in stationary phase cells.

Previously, using a P_{PAH1}-*lacZ* reporter gene system, it has been

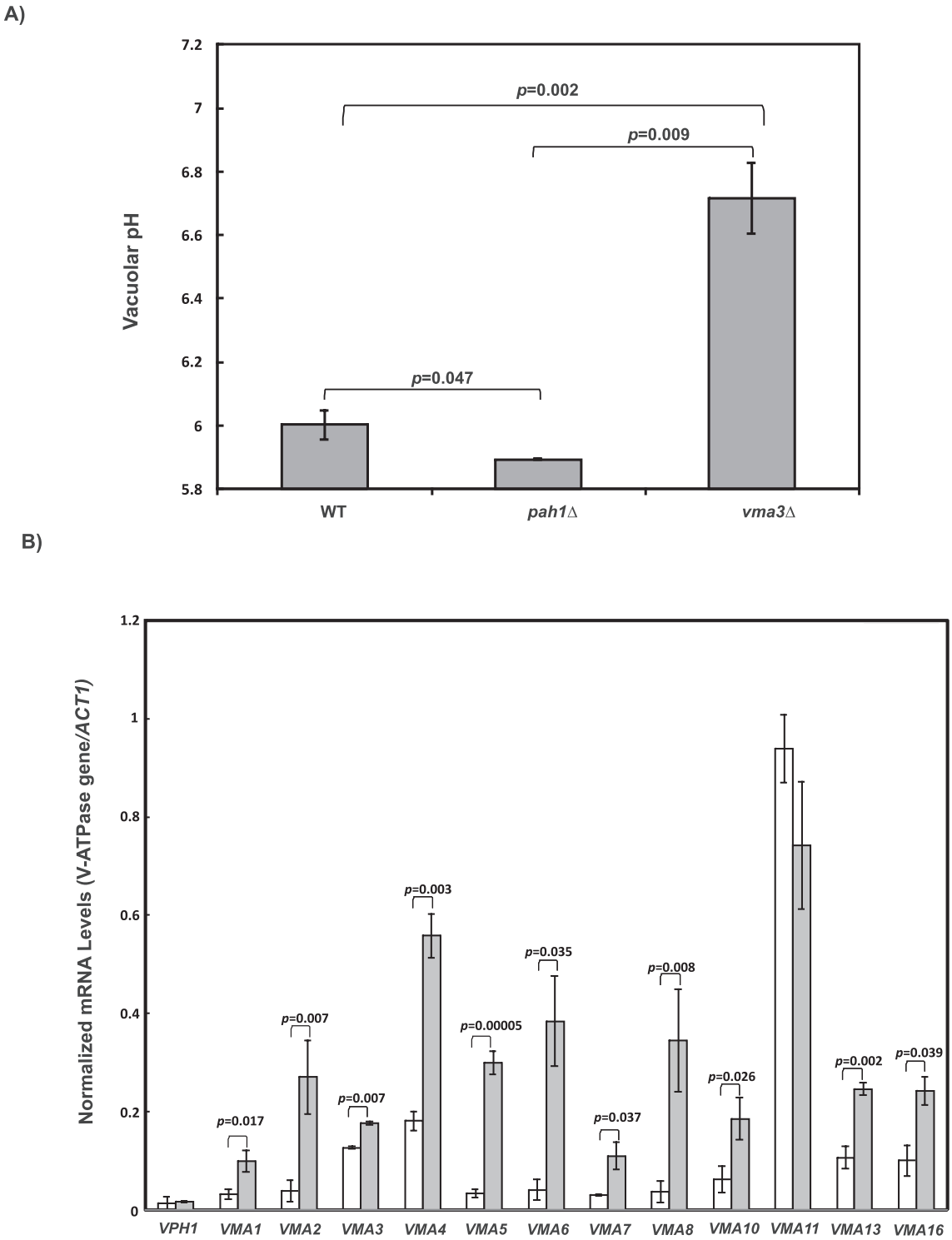

Fig. 4. Cells without Pah1p maintain a more acidic vacuolar pH than WT cells through upregulated V-ATPase gene expression during stationary phase. (A) WT, *pah1Δ* and, *vma3Δ* cells were grown in SC with 100 μM inositol (SC+) media that was buffered to pH 7 until stationary phase. Cells were then pelleted for internal vacuolar pH analysis. (B) The expression of V-ATPase genes was examined through their mRNA levels by qRT-PCR analysis. Expression levels of mRNA were normalized to the housekeeping gene *ACT1*. Data was graphed as a mean ± standard deviation. WT: () and *pah1Δ:* (▭). Annotation was as described in the legend of Fig. 1.

shown that the β-galactosidase activity was 9-fold higher in the stationary phase cells when compared with those of the exponential phase [15]. Furthermore, inositol supplementation did not have a major effect on the expression of β-galactosidase activity in exponential phase cells. However, in stationary phase cells, the addition of inositol to the growth medium resulted in a dose-dependent increase in reporter gene activity. As such, our findings, which were derived from the direct observation of the endogenous gene expression, along with previous observations which were derived from the episomal reporter plasmid, confirm the inducing conditions of the *PAH1* gene and the relationship between Pah1p PA phosphatase activity and TAG synthesis. This is

because Pah1p encoded PA phosphatase activity increases as cells progress from the exponential to stationary phases of growth. Furthermore, the increase in PA phosphatase activity correlates with the accumulation of TAG that occurs in the stationary phase [20]. Interestingly, the increased *PAH1* gene expression and activity, does not correlate with increased Pah1p protein levels, as has been shown by previous studies, and Pah1p is degraded via the proteasome to avoid the toxic effects of too much Pah1p buildup [21]. Our results also suggest that the Ino2p/Ino4p/Opi1p regulatory circuit is likely involved in the regulation of *PAH1* expression in response to growth phase and inositol supplementation since the addition of inositol causes a change in *PAH1* transcript level.

Through the EM studies, we have demonstrated that fragmented vacuoles decreased significantly when cells entered the stationary phase in WT cells (Fig. 2). On the other hand, fragmented vacuoles were significantly higher at both the exponential and stationary stages in the *pah1Δ* cells. Previously, it has been suggested that the lack of Pah1p causes irregular vacuolar morphology and dysfunctional V-ATPase pump activity [12,16]. Accordingly, it has also been proposed that the conversion from PA to DAG is a critical event leading to vacuolar fusion [12]. As the most important PAP enzyme, Pah1p regulates this conversion process. Therefore, our observations strongly suggest that Pah1p is required for vacuolar fusion. Furthermore, we observed that the *pah1Δ* cells have more fragmented vacuoles than the WT cells in both exponential and stationary stages. We observed that 74.2% of the WT cells and 96.4% of the *pah1Δ* cells had fragmented vacuoles in the exponential phase. At the stationary stage, 96.9% of the *pah1Δ* cells' vacuoles remained fragmented while the WT cells only had 49.9% of their vacuoles fragmented. The percentage of fragmented vacuoles in the *pah1Δ* cells remained the same while the percentage of fragmented vacuoles in the WT cells dropped significantly at the stationary phase. These observations strongly suggest that Pah1p plays an important role in the upstream of the regulatory pathway for vacuolar fusion.

Although our EM studies demonstrated that the *pah1Δ* cells had more fragmented vacuoles than the WT cells, the *pah1Δ* cells grew just as well in pH 7.0 as they had in pH 5.5, similar to, or even better, than the WT cells (Fig. 3). Furthermore, the *pah1Δ* cells exhibited an even more acidic vacuolar pH than the WT in alkaline conditions (Fig. 4). One would expect that abnormal vacuolar morphology is associated with abnormal vacuolar acidification due to dysfunctional V-ATPase pumps. However, this is not always the case. Mutants with some of the phenotypic characteristics of abnormal vacuoles, including fragmented vacuoles, seem to either have functional V-ATPases or are partially defective in the assembly of the V-ATPase [22,23]. Therefore, abnormal vacuolar morphology is not necessarily associated with abnormal vacuolar acidification.

While the *pah1Δ* cells had more fragmented vacuoles, the V-ATPase pump activity of the *pah1Δ* cells was more active than that of the WT cells because of their more acidic vacuoles (Fig. 4A). These results led us to believe that the genes involved in the vacuolar pump activity should be upregulated. Indeed, RNA analysis results showed that all the genes involved in the acidification process were upregulated in the absence of Pah1p compared to that of WT during the stationary phase. The two genes that were not upregulated, *VPH1* and *VMA11*, are not part of the acidification process required to acidify the vacuole. Thus, it is possible that upregulation of the genes involved in the acidification process is responsible for the acidic vacuolar pH found in the *pah1Δ* mutant. This result may also suggest that Pah1p has a negative effect on the expression of genes involved in the acidification process. Interestingly, quite a few of these genes contain a UAS_{INO}, which are types of genes *PAH1* can negatively regulate. For example, *VMA1, VMA5, VMA8, VMA13*, and *VMA16* all contain a UAS_{INO} in their promoter regions. It is likely that Pah1p can regulate these genes negatively by binding to their UAS_{INO} and controlling their expression. Thus, the molecular link between Pah1p and V-ATPase gene expressions has been established.

Recent studies have indicated that V-ATPase activity negatively regulates vacuolar fusion *in vivo* [17]. Since we have demonstrated that the *pah1Δ* cells have a more acidic vacuolar pH than the WT cells, and the genes involved in the pump activity are upregulated compared to WT, the *pah1Δ* cells' acidity could be a contributing factor to the fragmented vacuolar phenotype. As such, we provide evidence to show the role of Pah1p in the expression of V-ATPase genes, vacuole acidification and vacuolar fragmentation.

Acknowledgements

We are grateful to our laboratory colleagues for technical assistance and comments on the manuscript. This work was supported by an NSF Grant (MCB-0919218) and PSC-CUNY (69250-0047, 60246-0048, and NATO SPS G5266) awards to C.-H. S.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2017.07.127.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2017.07.127.

References

[1] G.S. Han, S. Siniossoglou, G.M. Carman, The cellular functions of the yeast lipin homolog PAH1p are dependent on its phosphatidate phosphatase activity, J. Biol. Chem. 282 (2007) 37026–37035.
[2] S. Siniossoglou, Lipins, lipids and nuclear envelope structure, Traffic 10 (2009) 1181–1187.
[3] F. Pascual, G.M. Carman, Phosphatidate phosphatase, a key regulator of lipid homeostasis, Biochim. Biophys. Acta 1831 (2013) 514–522.
[4] G.S. Han, W.I. Wu, G.M. Carman, The Saccharomyces cerevisiae Lipin homolog is a Mg2+-dependent phosphatidate phosphatase enzyme, J. Biol. Chem. 281 (2006) 9210–9218.
[5] L. O'Hara, G.S. Han, S. Peak-Chew, N. Grimsey, G.M. Carman, S. Siniossoglou, Control of phospholipid synthesis by phosphorylation of the yeast lipin Pah1p/Smp2p Mg2+-dependent phosphatidate phosphatase, J. Biol. Chem. 281 (2006) 34537–34548.
[6] A. Golden, J. Liu, O. Cohen-Fix, Inactivation of the C. elegans lipin homolog leads to ER disorganization and to defects in the breakdown and reassembly of the nuclear envelope, J. Cell Sci. 122 (2009) 1970–1978.
[7] G.S. Han, G.M. Carman, Characterization of the human LPIN1-encoded phosphatidate phosphatase isoforms, J. Biol. Chem. 285 (2010) 14628–14638.
[8] Z. Fang, S. Wang, X. Du, P. Shi, Z. Huang, Phosphatidate phosphatase-1 is functionally conserved in lipid synthesis and storage from human to yeast, Acta Biol. Hung 65 (2014) 481–492.
[9] H. Santos-Rosa, J. Leung, N. Grimsey, S. Peak-Chew, S. Siniossoglou, The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth, EMBO J. 24 (2005) 1931–1941.
[10] O. Adeyo, P.J. Horn, S. Lee, D.D. Binns, A. Chandrahas, K.D. Chapman, J.M. Goodman, The yeast lipin orthologue Pah1p is important for biogenesis of lipid droplets, J. Biol. Chem. 192 (2011) 1043–1055.
[11] S. Fakas, Y. Qiu, J.L. Dixon, G.S. Han, K.V. Ruggles, J. Garbarino, S.L. Sturley, G.M. Carman, Phosphatidate phosphatase activity plays key role in protection against fatty acid-induced toxicity in yeast, J. Biol. Chem. 286 (2011) 29074–29085.
[12] T. Sasser, Q.S. Qiu, S. Karunakaran, M. Padolina, A. Reyes, B. Flood, S. Smith, C. Gonzales, R.A. Fratti, Yeast lipin 1 orthologue Pah1p regulates vacuole homeostasis and membrane fusion, J. Biol. Chem. 287 (2012) 2221–2236.
[13] Y. Park, G.S. Han, E. Mileykovskaya, T.A. Garrett, G.M. Carman, Altered lipid synthesis by lack of yeast Pah1 phosphatidate phosphatase reduces chronological life span, J. Biol. Chem. 290 (2016) 25382–25394.
[14] G.S. Han, L. O'Hara, G.M. Carman, S. Siniossoglou, An unconventional diacylglycerol kinase that regulates phospholipid synthesis and nuclear membrane growth, J. Biol. Chem. 283 (2008) 20433–20442.
[15] F. Pascual, A. Soto-Cardalda, G.M. Carman, PAH1-encoded phosphatidate phosphatase plays a role in the growth phase-and inositol-mediated

regulation of lipid synthesis in Saccharomyces cerevisiae, J. Biol. Chem. 288 (2013) 35781–35792.
[16] B. Conradt, J. Shaw, T. Vida, S. Emr, W. Wickner, In vitro reactions of vacuole inheritance in Saccharomyces cerevisiae, J. Biol. Chem. 119 (1992) 1469–1479.
[17] Y. Desfougères, S. Vavassori, M. Rompf, R. Gerasimaite, A. Mayer, Organelle acidification negatively regulates vacuole membrane fusion *in vivo*, Sci. Rep. 6 (2016) 29045.
[18] R. Wimalarathna, C.H. Tsai, C.H. Shen, Transcriptional control of genes involved in yeast phospholipid biosynthesis, J. Microbiol. 49 (2011) 265–273.
[19] L.A. Graham, T.H. Stevens, Assembly of the yeast vacuolar proton-translocating ATPase, J. Bioenerg. Biomembr. 31 (1999) 39–47.
[20] T.T. Nguyen, A. Lewandowska, J.-Y. Choi, D.F. Markgraf, M. Junker, M. Bilgin, C.S. Ejsing, D.R. Voelker, T.A. Rapoport, J.M. Shaw, Gem1 and ERMES do not directly affect phosphatidylserine transport from ER to mitochondria or mitochondrial inheritance, Traffic 13 (2012) 880–890.
[21] F. Pascual, L.S. Hsieh, A. Soto-Cardalda, G.M. Carman, Yeast Pah1p phosphatidate phosphatase is regulated by proteasome-mediated degradation, J. Biol. Chem. 289 (14) (2014) 9811–9822.
[22] C.K. Raymond, I. Howald-Stevenson, C.A. Vater, T.H. Stevens, Morphological classification of the yeast vacuolar protein sorting mutants: evidence for a prevacuolar compartment in class E vps mutants, Mol. Cell Biol. 3 (1992) 1389–1402.
[23] L.S. Weisman, R. Bacallao, W. Wickner, Multiple methods of visualizing the yeast vacuole permit evaluation of its morphology and inheritance during the cell cycle, J. Biol. Chem. 105 (1987) 1539–1547.
[24] L. Michaillat, T.L. Baars, A. Mayer, Cell-free reconstitution of vacuole membrane fragmentation reveals regulation of vacuole size and number by TORC1, Mol. Biol. Cell 23 (2012) 881–895.