ELSEVIER

Biochimica et Biophysica Acta 1405 (1998) 147–154

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

The *PLC1* encoded phospholipase C in the yeast *Saccharomyces cerevisiae* is essential for glucose-induced phosphatidylinositol turnover and activation of plasma membrane H^+-ATPase

Paola Coccetti [a], Renata Tisi [a], Enzo Martegani [a,b], Leonardo Souza Teixeira [c], Rogelio Lopes Brandão [c,d], Ieso de Miranda Castro [c], Johan M. Thevelein [d,*]

[a] *Sezione di Biochimica Comparata, Dipartimento di Fisiologia e Biochimica Generali, Università degli Studi di Milano, via Celoria 26, I-20133 Milan, Italy*

[b] *III Facoltà di Scienze M.F. Naturali, Università di Milano, Via Ravasi 2, 21100 Varese, Italy*

[c] *Laboratório de Bioquímica e Fisiologia de Microrganismos, Escola de Farmácia, Universidade Federal de Ouro Preto, Rua Costa Sena 171, 35.400.00 Ouro Preto, MG, Brazil*

[d] *Laboratorium voor Moleculaire Celbiologie, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Leuven-Heverlee, Belgium*

Received 30 July 1998; accepted 10 August 1998

Abstract

Addition of glucose to glucose-deprived cells of the yeast *Saccharomyces cerevisiae* triggers rapid turnover of phosphatidylinositol, phosphatidylinositol-phosphate and phosphatidylinositol 4,5-bisphosphate. Glucose stimulation of PI turnover was measured both as an increase in the specific ratio of ^{32}P-labeling and as an increase in the level of diacylglycerol after addition of glucose. Glucose also causes rapid activation of plasma membrane H^+-ATPase. We show that in a mutant lacking the *PLC1* encoded phospholipase C, both processes were strongly reduced. Compound 48/80, a known inhibitor of mammalian phospholipase C, inhibits both processes. However, activation of the plasma membrane H^+-ATPase is only inhibited by concentrations of compound 48/80 that strongly inhibit phospholipid turnover. Growth was inhibited by even lower concentrations. Our data suggest that in yeast cells, glucose triggers through activation of the *PLC1* gene product a signaling pathway initiated by phosphatidylinositol turnover and involved in activation of the plasma membrane H^+-ATPase.

Keywords: Glucose induction; Signal transduction; Medium acidification; Compound 48/80; *Saccharomyces cerevisiae*

1. Introduction

The yeast *Saccharomyces cerevisiae* contains only one gene, *PLC1*, encoding a phospholipase C activity. The gene product is a supposedly phosphatidylinositol (PI)-specific phospholipase C with similar properties and most homology to the mammalian phospholipase C-δ. Deletion of this gene inhibits

Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; $Ins(1,4,5)P_3$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol

* Corresponding author. Fax: +32 (16) 321979; E-mail: johan.thevelein@bio.kuleuven.ac.be

0167-4889/98/$ – see front matter
PII: S0167-4889(98)00099-8

growth, in particular in minimal medium, at elevated temperatures and to a different extent depending on the genetic background of the cells [1–3]. This fits with a previous observation that phosphatidylinositol-4,5-bisphosphate (PIP_2) is essential for a normal growth rate in yeast cells [4]. The *PLC1* gene product shows phospholipase C activity with PI-containing phospholipids in vitro [1]. The growth defect caused by deletion of the gene is complemented by expression of mammalian phospholipase C-δ [3]. No further information is available on the physiological function of this phospholipase C and its possible connection with PI metabolism in vivo.

In mammalian cells, many compounds have been identified as stimulators of PI turnover [5]. In yeast, only the addition of glucose, the most-preferred carbon substrate, has been found to stimulate PI turnover [6–8]. It is unclear whether glucose-induced PI turnover in yeast triggers a similar signalling pathway as in mammalian cells. It has not been possible to detect a glucose-induced increase in the level of $Ins(1,4,5)P_3$ in yeast using a time resolution of 2 and 20 min, respectively [9,10]. This could be due to very short-lived increases in the level of second messengers, as is the case with cAMP in yeast, to intracellular compartmentation or to the particular growth conditions used [11]. Very short-lived increases in the level of $Ins(1,4,5)P_3$, i.e. with a maximum only 30 s after addition of agonist, have been observed in other systems (e.g. [12]).

Addition of glucose causes a wide variety of regulatory effects in yeast cells, both at the post-translational and transcriptional level (for review see [11]). Glucose-induced activation of the Ras-adenylate cyclase pathway was shown not to be involved in glucose-induced stimulation of PI turnover [8]. Up to now, no glucose-induced regulatory effect has been linked to glucose stimulation of PI turnover, except for glucose-induced activation of plasma membrane H^+-ATPase [13]. This enzyme is activated within minutes after addition of glucose by means of protein phosphorylation [14–16]. As for glucose-induced PI turnover, glucose stimulation of the Ras-adenylate cyclase pathway is not involved in this effect [17,18]. We have previously shown that glucose-induced activation of the plasma membrane H^+-ATPase, as assayed in isolated plasma membranes, as well as glucose-induced H^+-extrusion from the cells, as measured in vivo, is blocked by several well-known inhibitors of the mammalian PI pathway and its downstream protein kinase targets. These inhibitors include neomycin, compound 48/80, and a combination of staurosporine and calmidazolium. In addition, both the activation of the plasma membrane H^+-ATPase and the stimulation of proton efflux from the cells can be mimicked by addition to intact cells of activators of the same pathway, including diacylglycerol, arachidonic and oleic acid [13,19,20]. In the present report, we show that compound 48/80 also inhibits glucose-induced PI turnover and that the *PLC1* encoded phospholipase C is essential both for glucose-induced PI turnover and activation of plasma membrane H^+-ATPase.

2. Materials and methods

2.1. Yeast strains and growth conditions

The following yeast strains were kindly provided by M. Fitzgerald-Hayes (Amherst). Wild-type strain WPY260 (*MATα ura3-52 his3-11,15 trp1-Δ901 ade2-101*) and phospholipase C-deficient strain WPY300 (*MATa ura3-52 trp1-Δ901 ade2-101 plc1Δ::URA3*) [2].

Yeast cells were grown in minimal medium containing 2% (w/v) glucose and 0.67% yeast nitrogen base without amino acids (Difco Laboratory, Detroit, MI, USA) at 24°C supplemented with the required amino acids (50 mg/l). Strain WPY300 was pre-grown in rich medium (2% glucose, 2% tryptone and 1% yeast extract) (see below). The cultures were grown in flasks in a shaking incubator. The cell density was determined after sonication and appropriate dilution with Isoton (Coulter Electronics, UK) and counted with a Coulter Counter ZBI, equipped with a 70-μm orifice. Cell volume distributions were determined with a Coulter Channelyzer C-1000, connected to a PC-XT computer. The fraction of budded cells was determined by microscopic counting of at least 400 cells, fixed in 4% formalin and mildly sonicated [8].

2.2. Plasmids

Plasmid pWP101, carrying the 4.1-kb *PLC1* cod-

ing sequence in the multicopy vector YEp351, was also provided by M. Fitzgerald-Hayes [2]. Yeast transformation was performed by a modified lithium acetate transformation protocol [21]; after a 20-h preincubation in liquid rich medium at 24°C transformants were selected for rescue of growth on minimal medium.

2.3. PI turnover

For measurement of the stimulation of PI turnover, yeast cells were pregrown in YNB-glucose at 24°C in the presence of 1 μCi/ml of [^{3}H]*myo*-inositol (Amersham). WPY300 is not able to grow in minimal medium, so it was pregrown in rich medium, collected and then incubated in minimal medium in the presence of [^{3}H]*myo*-inositol. After incubation, cells were recovered by filtration, washed with sterile water and resuspended in 0.1 M 2-(*N*-morpholino) ethanesulfonic acid (MES)/Tris, pH 6.5 (MES buffer) at a density of about 10^8 cells/ml. Cells were incubated for 1 h at 24°C and then 100 μCi/ml of carrier-free ^{32}P-orthophosphate (Amersham) was added. After an additional 1-h incubation, the cells were stimulated by addition of 100 mM glucose (final concentration). Incubations were stopped by addition of cold trichloroacetic acid (10% final concentration) and phospholipids were extracted according to Roldan and Harrison [22]. Thin-layer chromatography was done on silica gel plates (0.25 mm thick, Merck) pretreated with 1% potassium oxalate, activated at 110°C for 15 min and developed with chloroform/methanol/acetic acid/acetone/water (43:13:12:15:8 v/v). Appropriate standards (PI, PIP, PIP_2, PE, PC) were added and the phospholipids were detected after spraying with 8-anilino, 2-naphthalene sulfonic acid (ANS) (1 mM in water) under a long-wave UV light. Multiple spots reflect different fatty acid compositions. After autoradiography the phosphoinositides spots were scraped and counted for ^{3}H- and ^{32}P-radioactivity in a Packard Prias beta-counter. The PI turnover was calculated as (^{32}P/^{3}H)stimulated/(^{32}P/^{3}H)unstimulated. Inhibitors were added 15 min before glucose. All the experiments were done in triplicate. The differences of measured PI turnover between repeated experiments were less than 15%.

2.4. Quantitative assay for total cellular diacylglycerol (DAG)

DAG cellular content was determined as in Marini et al. [23]. Briefly, $\sim 5\times10^7$ cells for each assay were harvested, washed, resuspended in 0.1 M MES/Tris (pH 6.5) and incubated for 2 h at room temperature. Stimulation was conducted with 100 mM glucose for 15 min at room temperature. Cells were then collected and extracted as in Marini et al. [23]. Phospholipids were dried under vacuum and stored at −20°C. DAG content was determined as in Schomerus and Küntzel [9] using the DAG kinase linked assay of Preiss et al. [24]. Reactions were conducted using 0.05 U per assay of *Escherichia coli* DAG kinase (Calbiochem). The amount of DAG in each sample was determined using pure *sn*-1,2-diacylglyerol (Sigma) as a standard.

2.5. Assay for generation of [^{3}H]inositol phosphates

Yeast cells were grown in [^{3}H]inositol as described by Hawkins et al. [10] (except that the concentration of [^{3}H]inositol was 2 μCi/ml), harvested, washed and resuspended in MES buffer. The radioactive cells (0.25 ml, 10^8 cells) were stimulated with glucose as described above and the reaction was terminated as described by Hawkins et al. [10]. Separation of water-soluble [^{3}H]inositol-labeled metabolites was effected by chromatography on small (1 ml) Dowex-1 X8 (Sigma) formate-form resin columns and eluted according to Hawkins et al. [10]. Standard [^{3}H]IP_3 (Amersham) was used to calibrate the column. According to the literature data the IP_3 was eluted in the E4 fraction (1 M ammonium formate, 0.1 M formic acid).

2.6. H^+-ATPase activity

Cells were grown in YP glycerol (3%, v/v) until the end of exponential phase, harvested and resuspended in MES buffer (25 mM, pH 6.5) for 20 min after which glucose (100 mM) was added. At different time points, samples were taken, cell extracts made, plasma membranes isolated and H^+-ATPase activity measured as described previously [18].

Table 1
Glucose-induced stimulation of PI turnover

Strain	Relevant genotype	Stimulation of PI turnover		
		PI	PIP	PIP_2
WPY260	wild-type	15 ± 5	10 ± 2	6 ± 2
WPY300	*plc1Δ*	0.8 ± 0.2	1 ± 0.3	1 ± 0.3
WPY300[pWP101]	*plc1Δ[PLC1]*	20 ± 4	10 ± 2	7 ± 2

2.7. Medium acidification

Initial H^+-efflux from the cells after the addition of glucose was measured for 5 min at 30°C in cells grown in the same way as specified for the measurement of H^+-ATPase activity. The cells were resuspended in medium containing 100 mM KCl and 10 mM MES buffer adjusted to pH 4.5. A 50-mg (wet wt.) amount of cells was used in a total volume of 2.5 ml in a water-jacketed vessel. The evolution of the pH of the suspension was recorded before and after the addition of different glucose concentrations. For calibration, pulses of 1000 nmol HCl were used. The maximal rate of proton pumping was calculated from the slope of the line indicating the pH evolution in the medium.

3. Results and discussion

When glucose is added to glucose-deprived yeast cells of a wild-type strain stimulation of the turnover of phosphatidylinositol is observed [7,8]. In yeast, it is very difficult to measure IP_3 in a reliable way; by using the standard Dowex-1 X8 ion-exchange chromatography, very few ^3H-cpm can be recovered in the E4 fraction, and the small difference observed between unstimulated and stimulated wild-type cells were not significant (not shown). Therefore we used a double labeling procedure to measure turnover as described by Kaibuchi et al. [7], with the modification indicated in Frascotti et al. [8]. The effect of glucose on PI turnover was calculated as the ratio ($^{32}P/^3H$) stimulated/($^{32}P/^3H$) unstimulated. In a wild-type strain a value between 10- and 20-fold was usually observed for PI and 4–6-fold for PIP and PIP_2 [8]. In a yeast strain deficient in the *PLC1* encoded phospholipase C, glucose-induced stimulation of PI turnover was completely absent (Table 1), the turnover was restored by reintroducing the *PLC1* gene on a multicopy plasmid (pWP101). As a further support we measured the diacylglycerol levels in the same conditions (Fig. 1). After glucose addition the DAG level increases by 1.7-fold in wild-type cells, while in the *plc1Δ* strain the increase was significantly reduced. This indicates that *PLC1* encoded phospholipase C significantly contributes to DAG production likely through polyphosphoinositide hydrolysis. These observations link the Plc1 phospholipase C to PI metabolism and suggest that it is a target of the glucose signal responsible for the stimulation of PI turnover. It also suggests that the Plc1 phospholipase C is the only enzyme able to trigger phosphatidylinositol turnover, at least upon glucose stimulation. The pattern of phospholipids, detected by TLC, is not deeply affected by the *plc1Δ* mutation, suggest-

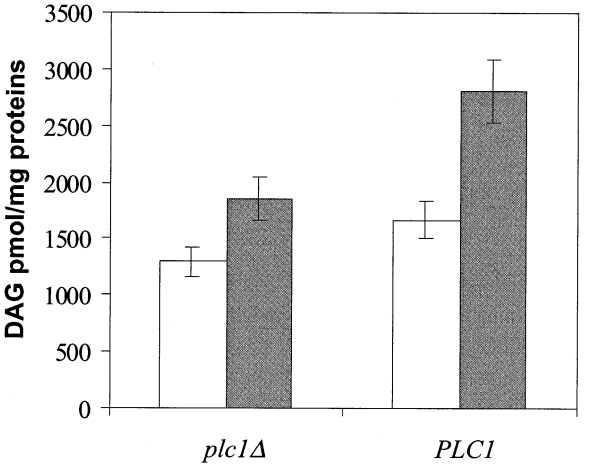

Fig. 1. Induction of DAG production by stimulation with glucose. Cells were grown in minimal medium, collected and starved as described in Section 2. Open bars refer to cells without stimulation, closed bars refer to the DAG level in cells stimulated for 15 min with a glucose pulse. DAG levels are determined as described in Section 2. DAG level stimulation-fold is 1.7 in the wild-type strain, while it is 1.4 in the *plc1Δ* strain.

ing that phosphoinositides biosynthesis was not impaired in these cells, at least during growth in rich medium (results not shown). Since the complete sequence of the yeast genome has recently become available in public databases, we have performed a database screen with the *PLC1* gene and with several of the conserved regions of the phospholipase C family as a probe. We have been unable to detect another member of the phospholipase C family in the yeast genome.

Addition of glucose to glucose-deprived yeast cells also causes a rapid stimulation of the plasma membrane H^+-ATPase, as measured in isolated membranes, and a concomitant stimulation of proton efflux from the cells in vivo. In the strain with a deletion of the *PLC1* gene glucose-induced stimulation of plasma membrane H^+-ATPase activity was absent (Fig. 2). Glucose-induced H^+-efflux from the cells was reduced from 0.44 ± 0.02 in the wild-type strain to 0.32 ± 0.02 mmol H^+ h^{-1} g^{-1} dry weight in the *plc1Δ* strain. This indicates that the basal activity of the plasma membrane H^+-ATPase can support a large fraction of the H^+-efflux during the initiation of glucose catabolism. However, glucose-activation of the enzyme also provides a significant contribution. These observations support the tentative conclusion from our previous studies on glucose-induced activation of the H^+-ATPase with inhibitors of the mammalian PI pathway [13] that glucose might stimulate a similar pathway in yeast cells with the plasma membrane H^+-ATPase as one of the targets.

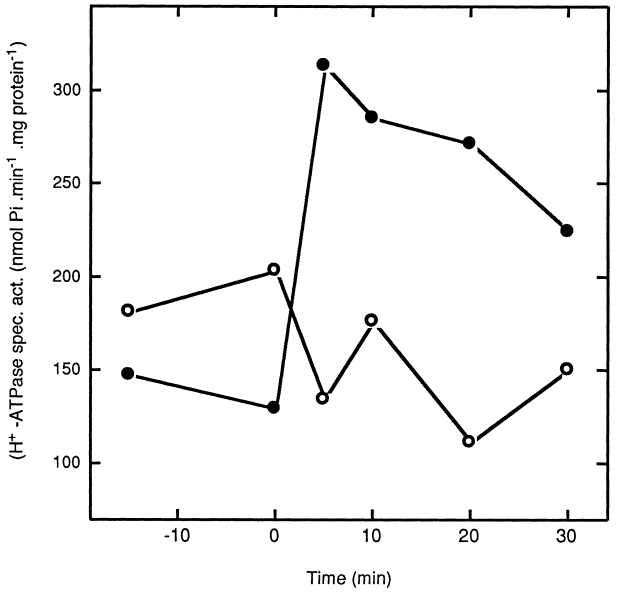

Fig. 2. Absence of glucose-induced activation of plasma membrane H^+-ATPase in the *plc1Δ* strain. Corresponding wild-type strain (●), *plc1Δ* strain (○). Glucose (100 mM) was added to cells resuspended in MES buffer for 20 min. Plasma membranes were isolated and ATPase activity determined as described in Section 2.

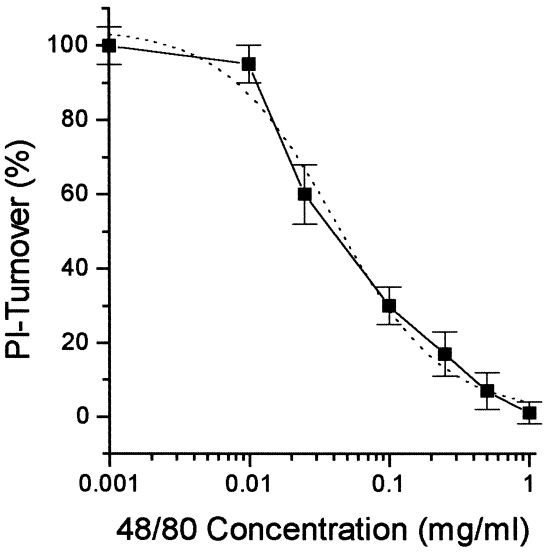

Fig. 3. Inhibition of glucose-induced PI turnover by compound 48/80. The PI turnover was measured by double-labeling procedure as described in Section 2. The inhibitor 48/80 was added to glucose starved cells, 15 min before addition of 100 mM glucose (final concentration) and phospholipids were extracted 15 min later. The PI turnover as a function of 48/80 concentrations is reported as % of the value obtained for the control (without inhibitor). All the experiments were done in triplicate.

In our previous study on glucose-induced activation of plasma membrane H^+-ATPase we have made use of compound 48/80, a well-known inhibitor of mammalian PI-specific phospholipase C [25]. Inhibition of glucose-induced activation of plasma membrane H^+-ATPase was observed between 0.2 and 1 mg/ml ([13]; unpublished results). A significant retardation of glucose-induced proton efflux in vivo was observed with concentrations starting at about 50 μg/ml [13]. Compound 48/80 inhibited glucose-induced PI turnover with an ID_{50} of 47 μg/ml (Fig. 3). Complete inhibition required about 1 mg/ml (Fig. 3). The labeling pattern of the PI phospholipids resembles strongly that observed in the *plc1Δ* strain (results not shown). These results suggest that complete in-

hibition of the putative PI signaling pathway is required for abolishment of the activation of the plasma membrane H^+-ATPase, although an unspecific effect of the inhibitor cannot be excluded.

We have also investigated the inhibition of growth by different concentrations of compound 48/80 to check whether the effect on PI turnover or on the activation of the H^+-ATPase could be responsible for the growth inhibition. However, growth was much more sensitive to compound 48/80 than PI turnover or H^+-ATPase activation. Inhibition of growth occurred in a very sharp concentration interval (Fig. 4): 10 μg/ml had no significant effect on the growth rate, although it decreased the fraction of budded cells (Fig. 5), while a final concentration of 15 μg/ml or higher caused complete inhibition of growth (Fig. 4). The growth inhibition was accompanied by a significant decrease of the fraction of budded cells (Fig. 5) and by a dramatic reduction of cell volume (Fig. 6). In addition, after 2–3 h of treatment with the inhibitor, most of the cells were eosine positive, suggesting that cell death occurs. Although it cannot be excluded from these data alone that the slightest inhibition of PI turnover is lethal, it appears more likely that compound 48/80 inhibits another essential target in yeast cells. One possibility is a calmodulin-dependent process since compound 48/80 is a known calmodulin antagonist in mammalian cells [26]. Moreover, recent work has indicated that calmodulin antagonists inhibit the yeast plasma membrane H^+-ATPase through an as yet unidentified calmodulin-dependent membrane protein [27].

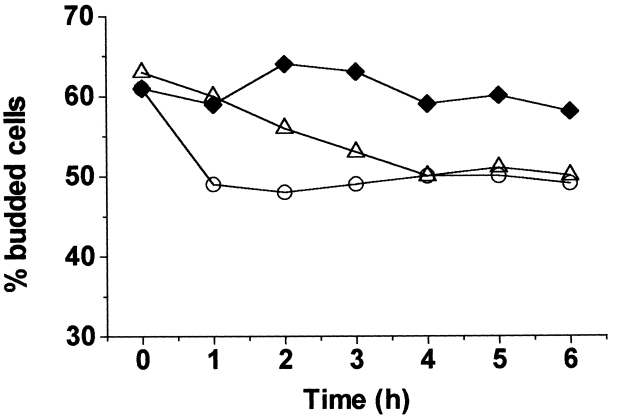

Fig. 5. Effect of compound 48/80 on the fraction of budded cells. Compound 48/80 was added at zero time to exponentially growing culture of WPY 260 cells in YNB-glucose medium. ♦, control; ○, 10 μg/ml; △, 25 μg/ml.

Our results suggest that the *PLC1* encoded phospholipase C is the target for glucose stimulation of PI turnover in yeast. Apparently no other phospholipase-C enzymes are present that are able to trigger rapidly PI turnover in response to glucose. In the experiments of Hawkins et al. [10], evidence was obtained for glucose-stimulated PI turnover by the action of a phospholipase B, but the time period used was much longer than in our experiments. However, the observation that glucose stimulates a fast DAG

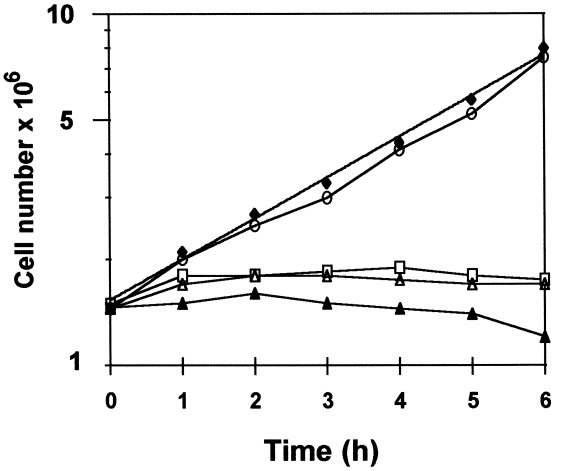

Fig. 4. Inhibition of growth by compound 48/80. Compound 48/80 was added at zero time to an exponential growing culture of WPY260 cells in YNB-glucose medium. ♦, control; ○, 10 μg/ml; □, 15 μg/ml; △, 25 μg/ml; ▲, 100 μg/ml.

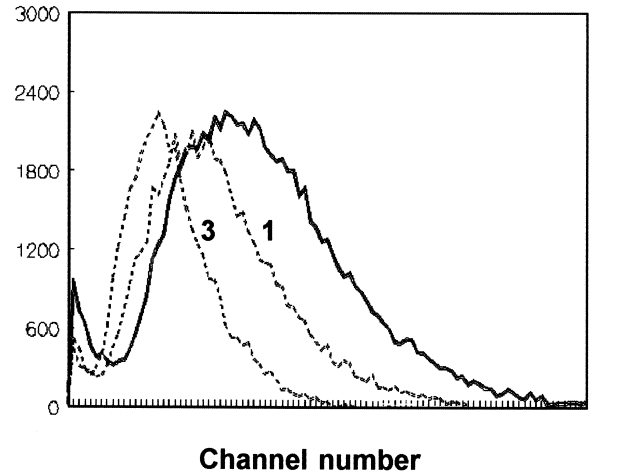

Fig. 6. Cell volume distribution of WPY260 cells. ——, cells exponentially growing in YNB-glucose; - - - -, cells 1 and 3 h after the addition of 25 μg/ml of compound 48/80.

production and that a residual stimulation was observed also in *plc1Δ* cells, indicates that other phospholipases could be present in yeast cells and are able to respond to glucose; for instance, evidence was reported for a start transition dependent DAG level increase involving a phosphatidylcholine-phospholipase C [23].

Apparently, yeast cells might contain a similar PI pathway as present in mammalian cells, functioning in signalling nutrient availability. The plasma membrane H^+-ATPase is an important target of glucose regulation [14]. This enzyme is essential for pumping protons out of the cell which are generated by the metabolism of glucose. Our previous results [13], together with the results presented in this paper, suggest that glucose activation of plasma membrane H^+-ATPase is a consequence of glucose stimulation of PI turnover. Since glucose activation of the H^+-ATPase is caused by phosphorylation of the enzyme [15,16], it is tempting to speculate that PI turnover triggers a signaling pathway causing activation of a protein kinase that phosphorylates the H^+-ATPase.

Yeast cells display a variety of glucose-induced regulatory effects, which are apparently mediated by different signaling pathways [11]. Inhibitors of PI turnover, for instance, have no effect on glucose stimulation of the Ras-cAMP pathway [13]. Activation of distinct signaling pathways by glucose might be essential for adaptation to glucose under different conditions. When yeast cells are starved on a glucose-containing medium for another essential nutrient, like nitrogen, they continue to ferment glucose. Hence, stimulation of the H^+-ATPase with concomitant pumping out of the cell of the protons generated by glucose metabolism continues to be important. Interestingly, starvation of a *plc1Δ* strain on glucose medium for nitrogen causes accelerated loss of viability compared to a wild-type strain [1]. On the other hand, glucose stimulation of the Ras-cAMP pathway under such conditions has to be shut off to allow proper entrance of the cells into the stationary phase G0 [28]. Usage of distinct glucose-triggered signaling pathways each controlling a subset of glucose-regulated targets could allow proper adaptation of yeast metabolism to glucose under different conditions.

Acknowledgements

We are grateful to M. Fitzgerald-Hayes (Amherst) for the provision of yeast strains and to M.J. Tropia and W. Verheyden for excellent technical assistance. This work was supported by grants from the European Commission (International Scientific Cooperation ERBCI1*CT940101 and Human Capital and Mobility CHRX-CT93-0265) to J.M.T., R.B. and E.M., a fellowship and a grant from the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (Brazil) to R.B., the Belgian National Fund for Scientific Research (FGWO), the Research Fund of the Katholieke Universiteit Leuven and the Belgian Office for Cooperation with Developing Countries (ABOS) to J.M.T.

References

[1] J.S. Flick, J. Thorner, Mol. Cell. Biol. 13 (1993) 5861–5876.
[2] W.E. Payne, M. Fitzgerald-Hayes, Mol. Cell. Biol. 13 (1993) 4351–4364.
[3] T. Yoko-o, Y. Matsui, H. Yagisawa, H. Nojima, I. Uno, A. Toh-e, Proc. Natl. Acad. Sci. USA 90 (1993) 1804–1808.
[4] I. Uno, K. Fukami, H. Kato, T. Takenawa, T. Ishikawa, Nature 333 (1988) 188–190.
[5] M.J. Berridge, Annu. Rev. Biochem. 56 (1987) 159–193.
[6] R.T. Talwalkar, R.L. Lester, Biochim. Biophys. Acta 306 (1973) 412–421.
[7] K. Kaibuchi, A. Miyajima, K. Arai, K. Matsumoto, Proc. Natl. Acad. Sci. USA 83 (1986) 8172–8176.
[8] G. Frascotti, D. Baroni, E. Martegani, FEBS Lett. 274 (1990) 19–22.
[9] C. Schomerus, H. Küntzel, FEBS Lett. 307 (1992) 249–252.
[10] P.T. Hawkins, L.R. Stephens, J.R. Piggott, J. Biol. Chem. 268 (1993) 3374–3383.
[11] J.M. Thevelein, Yeast 10 (1994) 1753–1790.
[12] Y. Shibanaka, H. Hayashi, M. Takai, N. Fujita, Eur. J. Biochem. 211 (1993) 427–430.
[13] R.L. Brandão, N.M. de Magalhães-Rocha, R. Alijo, J. Ramos, J.M. Thevelein, Biochim. Biophys. Acta 1223 (1994) 117–124.
[14] R. Serrano, FEBS Lett. 156 (1983) 11–14.
[15] A. Chang, C.W. Slayman, J. Cell Biol. 115 (1991) 289–295.
[16] F. Portillo, P. Eraso, R. Serrano, FEBS Lett. 287 (1991) 71–74.
[17] M.J. Mazon, M. Behrens, F. Portillo, R. Piñon, J. Gen. Microbiol. 153 (1989) 1453–1460.

[18] J. Becher dos Passos, M. Vanhalewyn, R.L. Brandão, I.M. Castro, J.R. Nicoli, J.M. Thevelein, Biochim. Biophys. Acta 1136 (1992) 57–67.
[19] A. Kotyk, J. Membr. Biol. 138 (1994) 29–35.
[20] A. Kotyk, G. Georghiou, Bull. Mol. Biol. Med. 19 (1994) 127–134.
[21] R. Schiestl, R.D. Gietz, Curr. Genet. 16 (1989) 339–346.
[22] E.R.S. Roldan, R.A.P. Harrison, Biochem. J. 259 (1989) 397–406.
[23] N.J. Marini, E. Meldrum, B. Buehrer, A.V. Hubberstey, D.E. Stone, A. Traynor-Kaplan, S.I. Reed, EMBO J. 15 (1996) 3040–3052.
[24] J. Preiss, C.R. Loomis, W.R. Bishop, R. Stein, J.E. Niedel, R.M. Bell, J. Biol. Chem. 261 (1986) 8597–8600.
[25] C. Bronner, C. Wiggins, D. Monté, F. Märki, A. Capron, Y. Landry, R.C. Franson, Biochim. Biophys. Acta 920 (1989) 301–305.
[26] B.D. Gehm, R.M. Pinke, S. Laquerre, J.G. Chafouleas, D.A. Schultz, D.J. Pepperl, D.G. McConnell, Biochemistry 30 (1991) 11302–11306.
[27] I. Romero, A.M. Maldonado, P. Eraso, Biochem. J. 322 (1997) 823–828.
[28] F. Dumortier, J.-C. Argüelles, J.M. Thevelein, Microbiology 141 (1995) 1559–1566.