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Biochimica et Biophysica Acta 1509 (2000) 189–194

BIOCHIMICA ET BIOPHYSICA ACTA

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The cell wall integrity/remodeling MAPK cascade is involved in glucose activation of the yeast plasma membrane H^+-ATPase

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Received 2 May 2000; received in revised form 14 July 2000; accepted 18 July 2000

Abstract

Glucose triggers transcriptional and post-transcriptional mechanisms that increase the amount and the activity of *Saccharomyces cerevisiae* plasma membrane H^+-ATPase. In a previous study, we found that a mutation in the Rsp5 ubiquitin–protein ligase enzyme affected the post-transcriptional activation of the enzyme by glucose. Mutations at the *RSP5* locus alter the glucose-triggered K_m decrease. In a genetic screening for multicopy suppressors of the *rsp5* mutation, we identified the *WSC2/YNL283c* gene. Deletion of the *WSC2* gene disturbs ATPase activation by glucose, abolishing the K_m decrease that occurs during this process. Wsc2 is a component of the PKC1-MPK1 mitogen-activated protein kinase (MAPK) signaling pathway that controls the cell wall integrity. Deletion of the *MPK1/SLT2* gene disturbs the glucose-triggered K_m decrease in ATPase. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: H^+-ATPase; Plasma membrane; Glucose activation; Protein kinase; (*Saccharomyces cerevisiae*)

1. Introduction

The plasma membrane H^+-ATPase of yeast is a proton pump that plays an essential role in the physiology of this organism [1–4]. The activity of the yeast ATPase is regulated at both transcriptional and post-translational levels by a large number of environmental factors [5]. Glucose is the most extensively investigated external signal. The activity of the ATPase is controlled in at least in two ways by the presence of glucose in the medium. First, glucose metabolism increases PMA1 mRNA synthesis [6,7] and second, glucose induces ATPase activation [8]. This activation results from a combined effect on the kinetic parameters of the enzyme, including K_m decrease, V_{max} increase and optimum pH shift to neutral value [8]. These alterations in the kinetic parameters are probably the consequence of more efficient enzyme coupling [9].

Although the molecular mechanism of the glucose-induced activation appears to be based on elimination of an inhibitory interaction of the C-terminus with the enzyme active site [10–12], the signal that triggers this mechanism is unknown. This activation is probably mediated by Ser/Thr phosphorylation [13] by an unknown protein kinase. Two regulatory sites, located at the C-terminus and defined by the phosphorylatable amino acids Ser-899 and Thr-912, are essential for the glucose-induced K_m and V_{max}

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PII: S0005-2736(00)00293-5

changes, respectively [12]. The glucose activation of the enzyme seems to be prevented by a glucose starvation-dependent, YCK-mediated phosphorylation of Pma1 [14].

In an earlier study, we isolated mutants in the Rsp5 ubiquitin–protein ligase that affected the glucose-triggered K_m decrease of the ATPase [15]. In a search for multicopy suppressors of the *rsp5* mutation, we found that overexpression of *WSC2* rescues the *rsp5* mutant ATPase activation defects. *WSC2* has been proposed in the Pkc1-Bck1-Mkk1/2-Mpk1 mitogen-activated protein kinase (MAPK) cascade [16,17]. The Pkc1–Mpk1 cascade is involved in cell wall remodeling [18]. We show that two other components of the pathway, *BCK1* and *MPK1*, are also required for the glucose-triggered K_m decrease of the ATPase.

2. Materials and methods

2.1. Strains and growth conditions

Saccharomyces cerevisiae strains BWG1–7A (*MAT***a** *ade1–100 his4–519 leu2–3,112 ura3–52*) [19], H19 (BWG1–7A *rsp5*) [15], and its derivatives were grown in medium with 2% glucose, 0.7% yeast nitrogen base without amino acids (US Biologicals, Swampscott, MA) and the appropriate requirements [20]. When indicated, medium was buffered with 50 mM Mes adjusted to pH 6.0 with Tris (SD6.0), or with 50 mM succinic acid adjusted to pH 3.0 with Tris (SD3.0).

2.2. Genetic screen

Yeast strain H19 carrying the *rsp5* mutation was transformed [21] with 50 μg of DNA from a YEp24-based library [22]. Approximately 15, 00 transformants were selected in SD6.0 medium. Transformed cells were pooled and plated in SD3.0 medium. After four days at 30°C, five colonies grew on acidic medium. Biochemical analysis of these strains showed that all exhibited wild-type ATPase levels. Plasmids were rescued from yeast [23] and amplified in *Escherichia coli*. Restriction analysis and partial sequencing of these clones revealed that all plasmids contained the same insert (Fig. 1).

Deletion of the WSC2 was performed using the materials and procedure of the EUROFAN (European Functional Analysis Network) [24]. The replacement reporter cassette encoding the Kan^r gene was prepared using specific primers in PCR and used to transform an autodiploid constructed by transformation of strain BWG1–7A with the *HO* gene [25]. Integration of the *wsc2*Δ::KanMX4 construct at the *WSC2* locus was confirmed by PCR. The heterozygous diploid *wsc2*Δ::KanMX4/*WSC2* was sporulated, and tetrads were dissected.

2.3. Biochemical methods

Yeast plasma membrane was purified from glucose-starved and glucose-fermenting cells by differential and sucrose gradient centrifugation [8]. ATPase activity was assayed at pH 6.5 with ATP concentrations from 0.8 to 6 mM and the concentra-

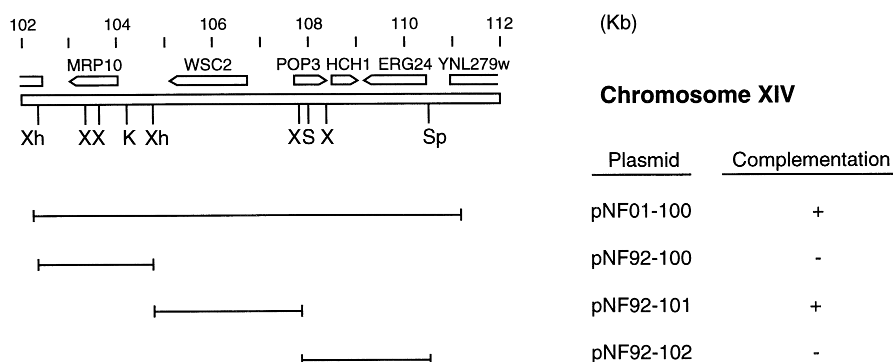

Fig. 1. Chromosomal location and subcloning of the *WSC2* gene. Restriction map of a region of chromosome XIV [16]. Arrows indicate positions of the ORFs. Thin horizontal lines represent DNA subclones used to test the ability to complement the rsp5 mutation. Restriction enzyme sites: K, *Kpn*I; S, *Sal*I; Sp, *Sph*I X, *Xba*I; Xh, *Xho*I.

Table 1
Kinetic parameters of ATPase from glucose-starved and glucose-fermenting wild-type and *rsp5* mutant cells overexpressing *WSC2*

Strain	Relevant genotype	Gene overexpressed	GS		GF	
			K_m (mM)	V_{max} (μmol min^{-1} (mg $protein)^{-1}$)	K_m (mM)	V_{max} (μmol min^{-1} (mg $protein)^{-1}$)
BWG1-7A	Wild type	None	4.0	0.3	0.9	1.2
H19	*rsp5*	None	4.5	0.5	5.0	1.1
BWG/W	Wild type	*WSC2*	4.0	0.3	1.0	1.4
H19/W	*rsp5*	*WSC2*	5.0	0.4	1.5	1.2

GS, glucose-starved cells; GF, glucose-fermenting cells. Cells were grown, collected and treated with glucose as described [10].

tion of free Mg^{2+} was 1 mM [26]. The apparent K_m and V_{max} were extrapolated from double-reciprocal plots fitted using a standard least-squares method. Similar values (within 10%) were obtained with two different plasma membrane preparations isolated independently. Protein concentration was determined by the Bradford method [27] using the Bio-Rad protein assay reagent and bovine IgG as standard.

3. Results and discussion

3.1. Isolation of WSC2

We previously described a *rsp5* mutant defective in the glucose activation-triggered K_m decrease of the ATPase [15]. This strain exhibits slow growth on SD3.0 medium as a consequence of the diminished plasma membrane ATPase activity. To gain insight into the mechanism of ATPase regulation by the Rsp5 ubiquitin–protein ligase enzyme, we screened for genes that confer on the rsp5 mutant the ability to grow on SD3.0 when present in a episomal plasmid. After transformation of the recipient strain with a YEp24-based genomic library, we recovered one plasmid (pNF01–100, Fig. 1). This plasmid complemented the slow growth phenotype of the mutant and restored the wild-type ATPase activity level. Partial sequence analysis showed that the complementing insert corresponds to a region of chromosome XIV (Fig. 1). Subcloning experiments indicated that only the region containing *WSC2* was required for complementation. We next analyzed the effect of *WSC2* carried in a multicopy plasmid on the kinetic parameters of ATPase from glucose-starved and glucose-fermenting wild-type and rsp5 mutant cells. The results showed that WSC2 overexpression in a *rsp5* mutant strain restored the glucose-triggered K_m decrease of the ATPase (Table 1).

3.2. WSC2 gene deletion affects glucose-triggered plasma membrane ATPase activation

WSC2 belongs to a family of genes (*WSC1–*

Table 2
Kinetic parameters of ATPase from glucose-starved and glucose-fermenting wild-type and *wsc2Δ* mutant cells

Relevant genotype	Gene overexpressed	GS		GF	
		K_m (mM)	V_{max} (μmol min^{-1} (mg $protein)^{-1}$)	K_m (mM)	V_{max} (μmol min^{-1} (mg $protein)^{-1}$)
Wild type	None	4.0	0.2	1.1	1.1
wsc2Δ	None	4.0	0.4	4.0	1.0
wsc2Δ	WSC1	4.0	0.3	2.0	1.3
wsc2Δ	WSC2	4.5	0.4	1.0	1.9
wsc2Δ	WSC3	4.0	0.4	1.5	1.2
wsc2Δ	RSP5	5.0	0.3	1.5	1.0

GS, glucose-starved cells; GF, glucose-fermenting cells. Cells were grown, collected and treated with glucose as described [10].

WSC4) that encode predicted integral membrane proteins with a conserved cysteine motif [16]. Genetic analysis suggested that the WSC gene family might mediate intracellular responses to environmental stress through different signaling pathway [16]. This prompted us to further characterize the effect of *WSC2* on activation of the ATPase. To this end, we generated a mutant strain lacking *WSC2*, and analyzed the kinetic properties of the ATPase in purified plasma membrane of glucose-starved and glucose-fermenting wild-type and mutant strain (Table 2). In the *wsc2Δ* mutant, the characteristics glucose-induced K_m decrease was not observed, suggesting that WSC2 is required for ATPase activation. In addition, the fact that WSC1 or WSC3 expressed in multicopy plasmid partially suppressed the phenotype of the *wsc2Δ* mutant (Table 2) also suggests that WSC genes may have overlapping functions.

To further characterize the genetic interaction between *WSC2* and *RSP5*, we tested the phenotype of strains with a *WSC2* gene deletion in combination with *RSP5* expressed in a multicopy vector. Overexpression of *RSP5* suppressed the phenotype of the *wsc2Δ* mutant (Table 2). This epistasis analysis and the fact that *WSC2* suppresses the *rsp5* mutation suggest that *WSC2* and *RSP5* define two independent pathways that may have overlapping functions in the regulation of ATPase activation.

3.3. *MPK1/SLT2 requirement for ATPase activation*

Genetic data suggested that *WSC* genes interact with both the Ras–cAMP and the PKC1–MPK1 signaling pathways to mediate the stress response [16]. Since glucose-induced activation of ATPase is not mediated by the cAMP-dependent protein kinase A [28,29], we studied whether the PKC1–MPK1 kinase cascade is involved in the activation of ATPase. We, first tested the phenotype of strains with a *WSC2* gene deletion that overexpressed protein kinases acting in different signaling pathways, such as Mpk1 and Bck1 (cell wall remodeling), Fus3 (mating), Kss1 (filamentation), and Hog1 (osmolyte synthesis). The results showed that only overexpression of *MPK1* and *BCK1* suppressed the phenotype of the *wsc2Δ* mutant (Table 3). We next studied the phenotype of strains with a deletion of the *MPK1*, *BCK1*, *FUS3*, *KSS1* or *HOG1* gene, and found that *mpk1Δ* and *bck1Δ* mutations abolished the glucose activation-triggered ATPase K_m decrease (Table 3).

3.4. *Phenotype of the wsc2 and mpk1 mutants*

To study the consequences of the *WSC2* and *MPK1* gene deletions on the in vivo ATPase activity, we examined the proton pumping activity and the tolerance to hygromycin B of the *wsc2Δ* and

Table 3
Effect of MAPK cascades genes on kinetic parameters of ATPase from glucose-starved and glucose-fermenting cells

Relevant genotype	Gene overexpressed	GS		GF	
		K_m (mM)	V_{max} (μmol min^{-1} (mg protein)$^{-1}$)	K_m (mM)	V_{max} (μmol min^{-1} (mg protein)$^{-1}$)
Wild type	None	4.0	0.2	1.1	1.1
wsc2Δ	None	4.0	0.4	4.0	1.2
wsc2Δ	*WSC2*	4.0	0.3	1.5	1.3
wsc2Δ	*MPK1*	4.5	0.4	1.0	1.6
wsc2Δ	*BCK1*	4.0	0.4	1.5	1.2
wsc2Δ	*FUS3*	5.0	0.3	4.0	1.0
wsc2Δ	*KSS1*	5.0	0.3	4.5	1.1
wsc2Δ	*HOG1*	4.0	0.4	4.0	1.2
mpk1Δ	None	5.0	0.3	4.5	1.3
bck1Δ	None	5.0	0.3	4.0	1.0
fus3Δ	None	5.0	0.2	1.5	1.4
kss1Δ	None	4.5	0.3	1.0	1.0
hog1Δ	None	5.0	0.4	1.5	1.1
bck1Δ	*MPK1*	5.0	0.3	1.0	1.4

GS, glucose-starved cells; GF, glucose-fermenting cells. Cells were grown, collected and treated with glucose as described [10].

Table 4
Proton pumping activity and tolerance to hygromycin B of the *wsc2*Δand *mpk1*Δ mutants

Relevant genotype	H^+-pump (nmol H^+ min^{-1} (mg fresh weight cells)$^{-1}$	Hygromycin B (MIC) (μg ml^{-1}[a]
Wild type	11	50
*wsc2*Δ	5	80
*mpk1*Δ	7	65

[a]Minimal inhibitory concentration was determined as in [34]. Values are the average of two independent experiments.

*mpk1*Δ mutant cells. Yeast cells actively extrude protons into the medium, an activity caused by plasma membrane H^+-ATPase, which reflects the in vivo activity of the enzyme [30]. On the other hand, mutants with ATPase decreased activity are resistant to the aminoglycoside antibiotic hygromycin B. The antibiotic tolerance results from a decreased uptake of the toxic cation due to depolarization of cellular membrane [31–33]. Table 4 shows that *wsc2*Δand *mpk1*Δ mutant cells exhibited both a lower H^+-pumping activity and an increased resistance to hygromycin B relative to the wild-type strain. These results suggest that the deletion of the *WSC2* and *MPK1* genes cause a decrease of the in vivo ATPase activity.

Overall, the present results suggest that the Wsc2-Pkc1-Bck1-Mkk1/2-Mpk1 MAPK cascade mediates the activation of the ATPase by glucose.

In the fission yeast *Schizosaccharomyces pombe*, the Spm1/Pmk1 is the putative homologue of the budding yeast Mpk1/Slt2 MAP kinase [35]. *SPM1* deletion has no noticeable effect on ATPase activation, but when it is combined with a deletion of the non-essential Pzh1 protein phosphatase gene, a severe decrease in the ATPase activity is observed [36]. Although a kinetic analysis of the ATPase from the double mutant is not available, these data could suggest that Spm1 has a function in controlling ATPase activity in *S. pombe*, resembling that of its counterpart in the budding yeast.

Whether Mpk1 regulates the ATPase directly or indirectly remains to be analyzed. As mentioned above, it has been proposed that the glucose-induced activation of the ATPase to be mediated by Ser/Thr phosphorylation [13]. Ser 899, located in the regulatory domain of the ATPase, is a phosphorylatable site involved in the glucose-induced K_m decrease [12]. One obvious possibility is thus that Mpk1 phosphorylates Ser-899 to induce the K_m decrease; an additional model is that Mpk1 function controls the expression and/or activity of protein(s) involved in ATPase activation. At present, we cannot discern between these two alternatives.

Acknowledgements

We are indebted to Roberto Gaxiola and Maria Molina for their generous gift of plasmids and strains, and Pilar Eraso and María J. Mazón for critical reading of the manuscript. N.d.l.F. is the recipient of a fellowship from the Ministerio de Educación y Ciencia. Spanish Grant DGICYT-PB97-0054 supported this study.

References

[1] C.W. Slayman, A. Goffeau, Biochim. Biophys. Acta 639 (1981) 197–223.
[2] R. Serrano, in: J.R. Broach, J.R. Pringle, E.W. Jones (Eds.), The Molecular and Cellular Biology of Yeast *Saccharomyces*. Genome Dynamics, Protein Synthesis and Energetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1991, pp. 523–585.
[3] R. Rao, C.W. Slayman, in: R. Brambl, G.A. Marzluf (Eds.), The Mycota, vol. III, Springer, New York, 1996, pp. 29–56.
[4] R. Rao, R.K. Nakamoto, S. Verjovski-Almeida, C.W. Slayman, Ann. N. Y. Acad. Sci. 671 (1993) 195–203.
[5] F. Portillo, Biochim. Biophys. Acta 1469 (2000) 31–42.
[6] R. Rao, D. Drummond-Barbosa, C.W. Slayman, Yeast 9 (1993) 1075–1084.
[7] M. García-Arranz, A.M. Maldonado, M.J. Mazón, F. Portillo, J. Biol. Chem. 269 (1994) 18076–18082.
[8] R. Serrano, FEBS Lett. 156 (1983) 11–14.
[9] K. Venema, M. Palmgren, J. Biol. Chem. 270 (1995) 19659–19667.
[10] F. Portillo, I.F. de Larrinoa, R. Serrano, FEBS Lett. 247 (1989) 381–385.
[11] F. Portillo, P. Eraso, R. Serrano, FEBS Lett. 287 (1991) 71–74.
[12] P. Eraso, F. Portillo, J. Biol. Chem. 269 (1994) 10393–10399.
[13] A. Chang, C.W. Slayman, J. Cell Biol. 115 (1991) 289–295.

[14] E. Estrada, P. Agostini, J.R. Vandenheede, J. Goris, W. Merlevede, J. François, A. Goffeau, M. Ghislain, J. Biol. Chem. 271 (1997) 32064–32072.
[15] N. de la Fuente, A.M. Maldonado, F. Portillo, FEBS Lett. 411 (1997) 308–312.
[16] J. Verna, A. Lodder, K. Lee, A. Vagts, R. Ballester, Proc. Natl. Acad. Sci. USA 94 (1997) 13804–13809.
[17] H.J. Schaeffer, M. Weber, Mol. Cell. Biol. 19 (1999) 2435–2444.
[18] K. Irie, M. Takase, K.S. Lee, D.E. Levin, H. Araki, K. Matsumoto, Y. Oshima, Mol. Cell. Biol. 13 (1993) 3076–3083.
[19] L. Guarente, R.R. Yocum, P. Gifford, Proc. Natl. Acad. Sci. USA 79 (1982) 7410–7414.
[20] M.D. Rose, F. Winston, P. Hieter (Eds.), Methods in Yeast Genetics. A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1990.
[21] H. Ito, Y. Fukuda, K. Murata, A. Kimura, J. Bacteriol. 153 (1983) 163–168.
[22] M. Carlson, D. Botstein, Cell 28 (1982) 145–154.
[23] A.C. Ward, Nucleic Acids Res. 18 (1990) 5319.
[24] A. Wash, A. Brachat, R. Pöhlmann, P. Philippsen, Yeast 10 (1994) 1793–1808.
[25] I. Herskowitz, R.E. Jensen, Methods Enzymol. 194 (1991) 132–146.
[26] R. Serrano, Methods Enzymol. 157 (1988) 533–534.
[27] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[28] M.J. Mazón, M. Behrens, F. Portillo, R. Piñón, J. Gen. Microbiol. 135 (1989) 1453–1460.
[29] J. Becher dos Pasos, M. Vanhalewyn, R.L. Brandao, I.M. Castro, J.R. Nicoli, J.M. Thevelein, Biochim. Biophys. Acta 1136 (1992) 57–67.
[30] R. Serrano, Eur. J. Biochem. 105 (1980) 419–424.
[31] J.H. McCusker, D.S. Perlin, J.E. Haber, Mol. Cell. Biol. 7 (1987) 4082–4088.
[32] D.S. Perlin, C.L. Brown, J.E. Haber, J. Biol. Chem. 263 (1988) 18118–18122.
[33] C.G. Vallejo, R. Serrano, Yeast 5 (1989) 307–319.
[34] D. Sanglard, K. Kuchler, F. Ischer, J.-L. Pagani, M. Monod, J. Bille, Antimicrob. Agents Chemother. 39 (1995) 2378–2386.
[35] T. Zaitsevskaya-Carter, J.A. Cooper, EMBO J. 16 (1997) 1318–1331.
[36] L. Balcells, R. Martin, M.C. Ruiz, N. Gómez, J. Ramos, J. Ariño, FEBS Lett. 435 (1998) 241–244.