FEBS Letters 579 (2005) 512–516 FEBS 29169

A role for the non-phosphorylated form of yeast Snf1: tolerance to toxic cations and activation of potassium transport

Francisco Portillo[a,*], José M. Mulet[b], Ramón Serrano[b]

[a] Instituto de Investigaciones Biomédicas, Universidad Autónoma de Madrid-C.S.I.C., Arturo Duperier 4, 28029 Madrid, Spain
[b] Instituto de Biología Molecular y celular de Plantas, Universidad Politécnica de Valencia-C.S.I.C., 46022 Valencia, Spain

Received 22 October 2004; revised 25 October 2004; accepted 9 December 2004

Available online 21 December 2004

Edited by Horst Feldmann

Abstract **The Snf1/AMP-activated protein kinases play a key role in stress responses of eukaryotic cells. In the yeast *Saccharomyces cerevisiae* Snf1 is regulated by glucose depletion, which triggers its phosphorylation at Thr210 and concomitant increase in activity. Activated yeast Snf1 is required for the metabolic changes allowing starvation tolerance and utilization of alternative carbon sources. We now report a function for the non-activated form of Snf1: the regulation of the Trk high-affinity potassium transporter, encoded by the *TRK1* and *TRK2* genes. A *snf1*Δ strain is hypersensitive in high-glucose medium to different toxic cations, suggesting a hyperpolarization of the plasma membrane driving increased cation uptake. This phenotype is suppressed by the *TRK1* and *HAL5* genes in high-copy number consistent with a defect in K^+ uptake mediated by the Trk system. Accordingly, Rb^+ uptake and intracellular K^+ measurements indicate that *snf1*Δ is unable to fully activate K^+ import. Genetic analysis suggests that the weak kinase activity of the non-phosphorylated form of Snf1 activates Trk in glucose-metabolizing yeast cells. The effect of Snf1 on Trk is probably indirect and could be mediated by the Sip4 transcriptional activator.**

Keywords: Budding yeast; Protein kinase Snf1; Plasma membrane potential; Potassium transport

1. Introduction

The plasma membrane electrical potential plays an essential role in all cells, modulating the transport of charged molecules through pumps, channels, uniports and cotransport systems [1]. In *Saccharomyces cerevisiae*, the plasma membrane potential is primarily determined by the two major electrogenic transporters: the H^+-ATPase encoded by the essential *PMA1* gene [2] and the Trk high affinity potassium uptake system encoded by the *TRK1* and *TRK2* genes [3–5]. The proton-pumping activity of Pma1 generates an electrochemical proton gradient and the Trk system is a major consumer of the membrane potential because of the high rates of K^+ uptake. Alterations in the expression and/or activities of Pma1 or Trk1–Trk2 would affect the membrane potential and, consequently, the uptake of nutrients and toxic cations [6,7]. Therefore, the regulation of these two important electrogenic systems is crucial for cell growth and for tolerance to toxic cations.

The yeast Pma1 ATPase is mainly activated at the post-transcriptional level by glucose metabolism [8]. This activation is mediated by downregulation of the inhibitory casein kinase I (encoded by *YCK1* and *YCK2* genes) [9] and upregulation of the activating protein kinase Ptk2 [10]. Trk is also regulated at the post-transcriptional level, being activated by protein kinases Hal4–Hal5 in response to K^+ starvation [11], and inhibited by protein kinase Sky1 [12,13]. Trk is also activated by the calcium-dependent protein phosphatase calcineurin [14] and inhibited by the protein phosphatase Ppz encoded by *PPZ1* and *PPZ2* genes [15]. In addition, the K^+-uptake system is activated by glucose metabolism, but nothing is known about the mechanism [16].

In the present report, we describe a novel modulator of the Trk potassium transporter, the protein kinase Snf1. This kinase belongs to the AMP-activated protein kinase family, which plays a key role in stress responses of eukaryotic cells [17–19]. The yeast Snf1 kinase forms different heterotrimeric complexes comprising the catalytic α subunit (Snf1) [20], the regulatory γ subunit (Snf4) [21] and one of the alternative β subunits (Sip1, Sip2 or Gal83) required for the specific subcellular location of the complex [22] and for interactions with Snf1 targets [23,24]. Extensive work has established that the yeast Snf1 kinase is activated by glucose depletion, being phosphorylated at a conserved threonine (Thr210) by protein kinases Elm1, Tos3 and Pak1 [25,26]. In the presence of glucose, it is inactivated and dephosphorylated by the PP1 phosphatase Glc7 [27]. The phosphorylated form of Snf1 exerts its function by modification of transcriptional regulators of genes involved in a wide spectrum of cell functions, such as adaptation to glucose limitation [28–30], response to different stresses [31,32], meiosis and sporulation [33], invasive growth [34] and life span [35].

We report here that Snf1 protein kinase also regulates the Trk1–Trk2 transport system and discuss possible mechanisms for this physiological function.

2. Materials and methods

2.1. Yeast strains, plasmids and growth conditions

The yeast strains used in this study are listed in Table 1. Yeast strains were grown in synthetic media with 2% dextrose and the appropriate requirements [36]. Frozen competent yeast cells were prepared as described [37]. To test the tolerance to different cations, yeasts were suspended in water at a cell density of 2×10^7 cell/ml, serially diluted (10^{-1}, 10^{-2} and 10^{-3}) and about 3 μl was dropped on rich medium

*Corresponding author. Fax: +34 91 5854400.
E-mail address: fportillo@iib.uam.es (F. Portillo).

0014-5793/$22.00
doi:10.1016/j.febslet.2004.12.019

Table 1
Strains used in this study

strain	Genotype	Reference
W303-1A	*Mata ade2-1 can1-100 his3−Δ1 leu2-3, 112 trp1-289 ura3-52*	Wallis et al. [56]
trk1 trk2	W303-1B *trk1::LEU2 trk2::HIS3*	Madrid et al. [6]
mig1	W303-1B *mig1::LEU2*	Nehlin and Rohne [55]
hal4 hal5	W303-1B *hal4::LEU2 hal5::HIS3*	Mulet et al. [11]
snf1	W303-1B *snf1::HIS3*	Östling et al. [51]
cat8	W303-1B *cat8::LEU2*	Hedges et al. [53]
sip4	W303-1B *sip4::TRP1*	Lesage et al. [54]
BY4741	*Mata his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Brachmann et al. [52]
snf1	BY4741 *snf1::KanMX4*	EUROSCARF

(YPD) supplemented with different toxic cations. To test the growth of yeast cells in K^+-limited medium, arginine phosphate plates (AP) [38] were supplemented with 50 μM KCl. Plasmids used in this study were pWS-Snf1, pWS-Snf1-T210A, pWS-Snf1-K84R [44], YEp13-Trk1 [5] and YEp24-Hal5 [11].

2.2. Biochemical methods

Plasma membranes were purified from glucose-metabolizing cells by differential and sucrose gradient centrifugation and ATPase activity was assayed at pH 6.5 with 2 mM ATP [39]. Protein concentration was determined by the Bradford method [40] with the Bio-Rad Protein Assay reagent (Bio-Rad Laboratories, Hercules, CA) and bovine IgG as standard. Proton efflux from yeast cells was determined after starvation at 4 °C and glucose addition at 30 °C [41]. Simultaneous determination of potassium and rubidium content was performed as described [42]. In brief, cells were inoculated in AP medium containing 10 mM KCl and grown until absorbance at OD_{660} nm = 0.4. Then, the cells were washed twice with 20 mM $MgCl_2$ and suspended in AP medium without potassium. 10 ml aliquots were taken, washed with 20 mM $MgCl_2$ and suspended in 0.2 ml buffer containing 2% glucose, 50 mM succinic acid and 20 mM $MgCl_2$. After 5 min, 5 mM RbCl was added and aliquots were taken, washed twice with 20 mM $MgCl_2$, suspended in 0.5 ml MilliQ water, and boiled to extract the intracellular ions. After removal of cell debris by centrifugation, 1/50 dilutions of the supernatant were used for HPLC analysis in Waters equipment with a IC-PAK CM/D column and a Waters 432 conductivity detector. Elution was made in an isocratic flux, using as a mobile phase 0.1 mM EDTA and 3 mM HNO_3. Sample analysis and preparation of rubidium and potassium standards was performed as described by the manufacturer.

3. Results and discussion

In a systematic analysis of mutant phenotypes of yeast protein kinases, we found that a null mutation of the *SNF1* gene conferred, in glucose media, a pleiotropic phenotype of sensitivities to toxic cations such as Hygromycin B, Na^+, Li^+ and tetramethylammonium (Fig. 1). This phenotype was also exhibited by mutants in the Snf1-regulatory subunit Snf4 (data not shown). The sensitivity to different toxic cations is suggestive of a hyperpolarized membrane potential, which results in increased cation uptake. An increased electrical potential could be caused by either mutations that upregulate Pma1 [10] or downregulate the Trk K^+ transport system [11]. As indicated in Fig. 1, the phenotype of the *snf1* mutant was similar, but less intense, to that of the *trk1 trk2* mutant (devoid of the Trk system) and of the *hal4 hal5* mutant (devoid of two redundant protein kinases which activate Trk) [11].

Therefore, we next analyzed the activities of H^+-ATPase and K^+ transport in *SNF1* and *snf1*Δ cells growing in glucose media. Under this condition, the activity of H^+-ATPase measured as H^+-pumping in vivo and as ATP hydrolysis in purified plasma membrane in vitro was similar in both strains (Table 2). By contrast, the *snf1*Δ mutation decreased the activity of the Trk system as measured by Rb^+ uptake and by the level of internal K^+ (Table 2). The Rb^+ uptake and intracellular K^+ values in *snf1*Δ mutant cells are similar to those previously reported for mutants affecting Trk regulation [11,13,42,43]. Mutants affected in K^+ uptake such as *trk1*Δ*trk1*Δ and *hal*4Δ*hal*5Δ grow slowly in K^+-limited medium [5] and, accordingly, a null mutation of the *SNF1* gene diminished the ability of yeast to grow in low-K^+ medium (Fig. 1). Moreover, as shown in Fig. 2 the hyperpolarized phenotype of the *snf1*Δ mutant is suppressed by the *TRK1* [3] and *HAL5* [11] genes in multicopy plasmids. Overexpression of *SNF1*, on the other hand, cannot suppress the phenotypes of *trk1 trk2* and *hal4 hal5* mutants (Fig. 2). All the above results strongly suggest that the non-phosphorylated form of the Snf1 protein kinase, predominant in glucose medium, acts upstream of Hal4–Hal5 and Trk to modulate the K^+ uptake system.

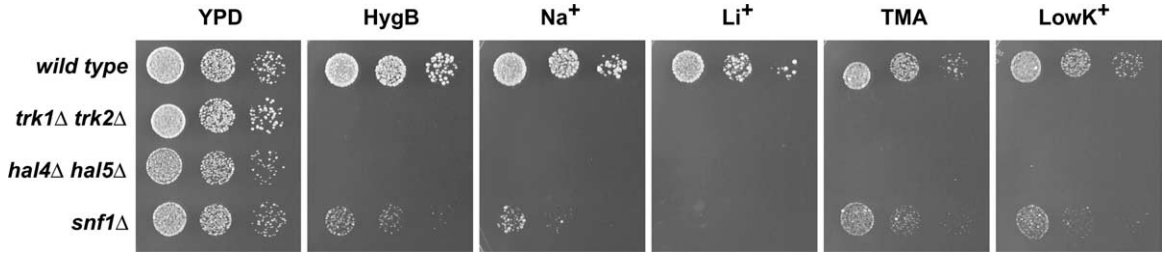

Fig. 1. *snf1*Δ mutation, like *trk1 trk2* and *hal4 hal5* mutations, confers hypersensitivity to different toxic cations and low K^+. Wild-type strain (W303-1B) and derivatives with *hal4 hal5*, *trk1 trk2* and *snf1* mutations were grown in liquid YPD and serial dilutions were dropped on YPD plates with either Hygromycin B (HygB, 50 μg/ml), NaCl (Na^+, 0.8 M), LiCl (Li^+, 0.3 M) or tetramethylammonium chloride (TMA, 0.2 M) or on AP plates with 50 μM KCl ($LowK^+$) as indicated. Growth was recorded after 2 days (YPD, $LowK^+$) and after 3 days (HygB, Li^+, Na^+, TMA). Similar results were observed in two separate experiments. The phenotype of the *snf1* mutant was also observed in a different genetic background (BY4741).

Table 2
Effect of *snf1* null mutation on ATPase activity, intracellular content of K^+ and uptake of Rb^+

Yeast strain	H^+-pump activity (nmol/min mg wet weight)	ATPase activity (μmol/min mg protein)	Rate of Rb^+ uptake (nmol/min mg wet weight)	Internal K^+ (mM)
SNF1	11 ± 1.0	0.95 ± 0.05	2.1 ± 0.1	240 ± 7
*snf1*Δ	10 ± 0.5	1.05 ± 0.10	1.5 ± 0.1	187 ± 2

The values are the average of three independent experiments (±S.D.).

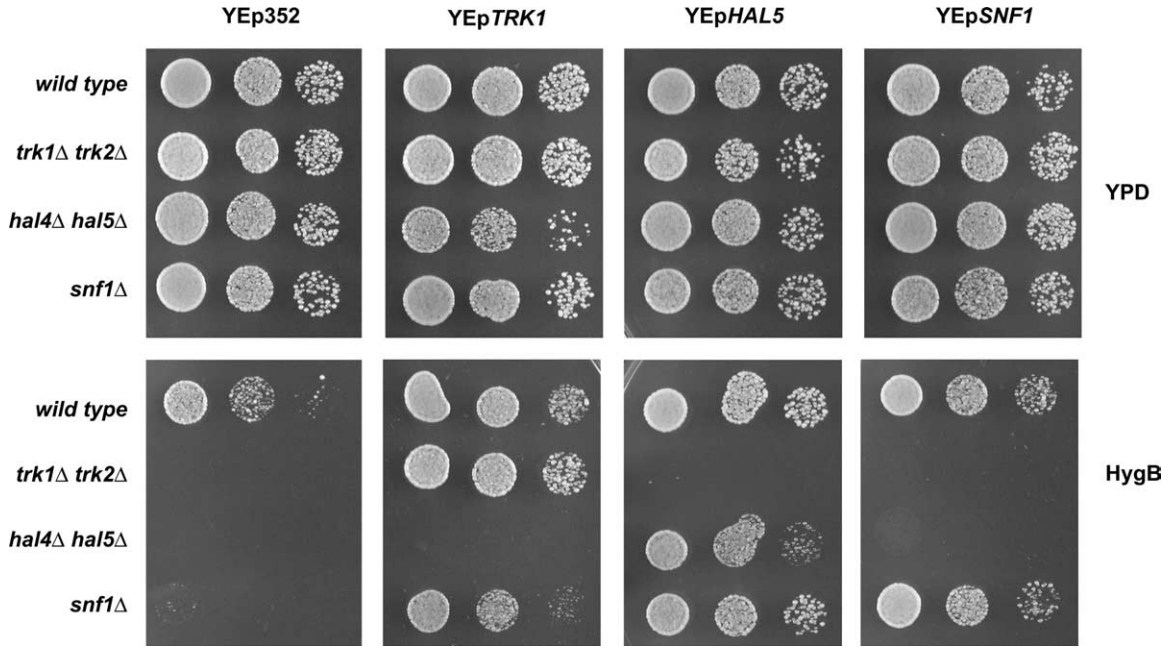

Fig. 2. Effect of gain of function of *TRK1*, *HAL5* and *SNF1* on tolerance of yeast cells to Hygromycin B. Wild-type yeast strain (W303-1B) and derivatives carrying null alleles of either *trk1 trk2*, *hal4 hal5* or *snf1* were transformed with either empty plasmid (YEp352) or multicopy plasmids with *TRK1*, *HAL5* and *SNF1* as indicated. The strains were grown in selective medium and after serial dilutions dropped onto YPD plates with Hygromycin B (HygB, 50 μg/ml). Images were taken after 2 days of incubation at 30 °C. Identical results were obtained with three different colonies.

To test this hypothesis, we next examined the ability of the *SNF1* non-phosphorylatable allele *snf1*-T210A and the poorly active one *snf1*-K84R to suppress the phenotype of the *snf1*Δ mutation. K84R mutates an essential lysine in the ATP binding domain [44] and T210A replaces a threonine in the Snf1 activation loop, which is phosphorylated by the upstream activating protein kinases in response to glucose limitation [45–47]. As shown in Fig. 3, the *snf1*-T210A is clearly able to suppress the hyperpolarized phenotype of the *snf1*Δ mutant cells and, as expected, is also unable to complement the sucrose non-fermentation phenotype of the *snf1*Δ mutant. The results also suggest that the suppression ability required an active protein kinase because the poorly active *snf1*-K84R allele is unable to confer a Hygromycin B resistant phenotype to the *snf1*Δ strain. Therefore, the results obtained so far allow us to propose the non-phosphorylated form of Snf1 as a new component of the mechanisms controlling the Trk1–Trk2 system. To our knowledge, this is the first report suggesting that Snf1 non-phosphorylated at Thr-210 could have a physiological role, nevertheless we cannot discard the possibility that

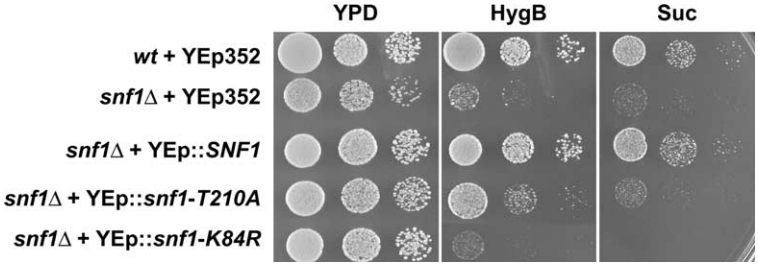

Fig. 3. The *snf1*-T210A allele partially rescues the hyperpolarized-dependent phenotype of *snf1*Δ mutant strain. The *snf1* null mutant was transformed with either empty plasmid or episomal plasmid with *SNF1*, *snf1*-T210A and *snf1*-K84R as indicated. The strains were grown in selective medium and after serial dilutions dropped onto YPD plates with Hygromycin B (HygB, 50 μg/ml) and medium containing sucrose (Suc) as carbon source. Wild-type strain (W303-1B, wt) transformed with empty plasmid served as growth control.

other phosphorylation sites contributed to regulate Snf1 activity. A recent report [48] described that Snf1 is also required for tolerance to hydroxyurea in glucose medium, although in this case both the T210A and the K84R mutants were effective. Apparently, different levels of basal activity of Snf1 are required for these novel functions, while the fully active phosphorylated form of Snf1 is required for sucrose fermentation and expression of genes under stress conditions. Accordingly, our results suggest that the activity of the T210A mutant is higher than that of the K84R mutant.

The Snf1 kinase exerts its function in cell metabolism through the covalent modification of glucose-responsible transcriptional factors. This fact is well documented in the cases of the transcriptional repressor Mig1 and transcriptional activators Cat8 and Sip4 [30]. It was of interest to ascertain whether some of the well known effectors of Snf1 were also implicated in controlling K^+ uptake. To this end, we tested the Hygromycin B resistance phenotype of *mig*1Δ, *cat*8Δ and *sip*4Δ mutant strains. Fig. 4 shows that *sip*4Δ mutant exhibited in glucose medium the same phenotype as that of the *snf1*Δ strain. This result suggests that the non-phosphorylated form of Snf1, predominant in glucose medium, acts through Sip4 to activate the transcription of a protein required for an efficient K^+ transport. It should be noted that Cat8 and Sip4 have been proposed to bind to the same panoply of promoters although with different affinities, being Sip4 less strong activator than Cat8 [49]. Our finding could suggest that at least in glucose medium Sip4 has a separate function from that of Cat8. Two obvious candidates to be regulated by Sip4 are the high affinity Trk1 potassium transporter and the Hal5 activating protein kinase. To determine whether *TRK1* and/or *HAL5* gene expression was modulated by Sip4, we analyzed the expression of *TRK1-LacZ* and *HAL5-LacZ* fusion genes in wild type, *snf1*Δ and *sip*4 Δ strains growing in glucose media but no significant differences were found among them (data not shown). This is in agreement with recent results based on DNA microarrays analysis, in which no alteration of the *TRK1* and *HAL5* gene expression was found in *snf1*Δ mutant strain [50].

One hypothetical model to explain all the above mentioned results would be to consider that the Snf1 protein kinase complex has a dual role in yeast cells depending on glucose availability and, therefore, of the phosphorylation status of Thr-210. In glucose starved cells, the phosphorylation of Thr-210 would lead the fully active kinase complex to act on a set of transcriptional factors devoted to promote the utilization of alternative carbon sources as have been described so far. In glucose fermenting cells, the non-phosphorylated, low activity form of the Snf1 complex could activate a different set of transcriptional factors which in turn would activate the expression of genes implicated in functions that, like K^+ transport, should help to adapt the cell machinery to the high growth rate of sugar-metabolizing yeast.

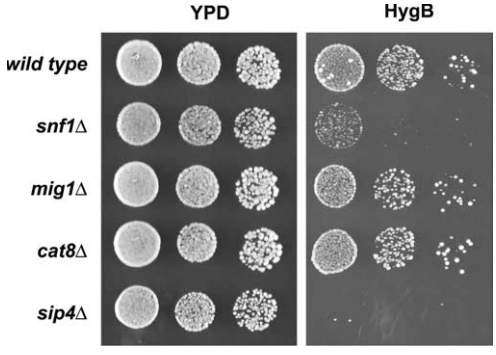

Fig. 4. Effect of Snf1 targets deletions on tolerance to Hygromycin B. The tolerance to the antibiotic (HygB, 50 μg/ml) of wild-type (W303-1B) *snf1*Δ, *mig*1Δ, *cat*8Δ and *sip*4Δ isogenic strains was tested by drop assay as described in the legend to Fig. 1.

Acknowledgments: We are indebted to Marian Carlson, Juana María Gancedo and Pascual Sanz for their generous gift of plasmids and strains, and Pilar Eraso, María J. Mazón and Juana Maria Gancedo for the critical reading of the manuscript. This work was supported by Spanish Grants DGICYT-BMC2001-1517 and BMC2002-04011-C05-02.

References

[1] Stein, W.D. (1990) Channels, Carrier and Pumps. An Introduction to Membrane Transport, Academic Press, New York.
[2] Serrano, R. (1991) Transport across yeast vacuolar and plasma membrane (Broach, J.R., Jones, E.W. and Pringle, J.R., Eds.), The Molecular and Cellular Biology of the Yeast *Saccharomyces*: Genome, Dynamics, Protein Synthesis and Energetics, pp. 523–585, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[3] Gaber, R.F., Styles, C.A. and Fink, G.R. (1988) TRK1 encodes a plasma membrane protein required for high-affinity potassium transport in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 8, 2848–2859.
[4] Ko, C.H. and Gaber, R.F. (1991) TRK1 and TRK2 encode structurally related K^+ transporters in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 11, 4266–4273.
[5] Ramos, J., Alijo, R., Haro, R. and Rodriguez-Navarro, A. (1994) TRK2 is not a low-affinity potassium transporter in *Saccharomyces cerevisiae*. J. Bacteriol. 176, 249–252.
[6] Madrid, R., Gomez, M.J., Ramos, J. and Rodriguez-Navarro, A. (1998) Ectopic potassium uptake in trk1 trk2 mutants of *Saccharomyces cerevisiae* correlates with a highly hyperpolarized membrane potential. J. Biol. Chem. 273, 14838–14844.
[7] Vallejo, C.G. and Serrano, R. (1989) Physiology of mutants with reduced expression of plasma membrane H^+-ATPase. Yeast 5, 307–319.
[8] Portillo, F. (2000) Regulation of plasma membrane H^+-ATPase in fungi and plants. Biochim. Biophys. Acta 1469, 31–42.
[9] Estrada, E., Agostinis, P., Vandenheede, J.R., Goris, J., Merlevede, W., Francois, J., Goffeau, A. and Ghislain, M. (1996) Phosphorylation of yeast plasma membrane H^+-ATPase by casein kinase I. J. Biol. Chem. 271, 32064–32072.
[10] Goossens, A., de La Fuente, N., Forment, J., Serrano, R. and Portillo, F. (2000) Regulation of yeast H^+-ATPase by protein kinases belonging to a family dedicated to activation of plasma membrane transporters. Mol. Cell. Biol. 20, 7654–7661.
[11] Mulet, J.M., Leube, M.P., Kron, S.J., Rios, G., Fink, G.R. and Serrano, R. (1999) A novel mechanism of ion homeostasis and salt tolerance in yeast: the Hal4 and Hal5 protein kinases modulate the Trk1–Trk2 potassium transporter. Mol. Cell. Biol. 19, 3328–3337.
[12] Erez, O. and Kahana, C. (2002) Deletions of SKY1 or PTK2 in the *Saccharomyces cerevisiae* trk1Δ trk2Δ mutant cells exert dual effect on ion homeostasis. Biochem. Biophys. Res. Commun. 295, 1142–1149.
[13] Forment, J., Mulet, J.M., Vicente, O. and Serrano, R. (2002) The yeast SR protein kinase Sky1p modulates salt tolerance, membrane potential and the Trk1,2 potassium transporter. Biochim. Biophys. Acta 1565, 36–40.
[14] Mendoza, I., Rubio, F., Rodriguez-Navarro, A. and Pardo, J.M. (1994) The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. J. Biol. Chem. 269, 8792–8796.

[15] Yenush, L., Mulet, J.M., Arino, J. and Serrano, R. (2002) The Ppz protein phosphatases are key regulators of K^+ and pH homeostasis: implications for salt tolerance, cell wall integrity and cell cycle progression. EMBO J. 21, 920–929.
[16] Ramos, J., Haro, R., Alijo, R. and Rodriguez-Navarro, A. (1992) Activation of the potassium uptake system during fermentation in *Saccharomyces cerevisiae*. J. Bacteriol. 174, 2025–2027.
[17] Hardie, D.G., Carling, D. and Carlson, M. (1998) The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell?. Annu. Rev. Biochem. 67, 821–855.
[18] Hardie, D.G. and Hawley, S.A. (2001) AMP-activated protein kinase: the energy charge hypothesis revisited. Bioessays 23, 1112–1119.
[19] Kemp, B.E., et al. (2003) AMP-activated protein kinase, super metabolic regulator. Biochem. Soc. Trans. 31, 162–168.
[20] Celenza, J.L. and Carlson, M. (1986) A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science 233, 1175–1180.
[21] Celenza, J.L., Eng, F.J. and Carlson, M. (1989) Molecular analysis of the SNF4 gene of *Saccharomyces cerevisiae*: evidence for physical association of the SNF4 protein with the SNF1 protein kinase. Mol. Cell. Biol. 9, 5045–5054.
[22] Vincent, O., Townley, R., Kuchin, S. and Carlson, M. (2001) Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism. Genes Dev. 15, 1104–1114.
[23] Schmidt, M.C. and McCartney, R.R. (2000) β-Subunits of Snf1 kinase are required for kinase function and substrate definition. EMBO J. 19, 4936–4943.
[24] Vincent, O. and Carlson, M. (1999) Gal83 mediates the interaction of the Snf1 kinase complex with the transcription activator Sip4. EMBO J. 18, 6672–6681.
[25] Hong, S.P., Leiper, F.C., Woods, A., Carling, D. and Carlson, M. (2003) Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. Proc. Natl. Acad. Sci. USA 100, 8839–8843.
[26] Sutherland, C.M., Hawley, S.A., McCartney, R.R., Leech, A., Stark, M.J., Schmidt, M.C. and Hardie, D.G. (2003) Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex. Curr. Biol. 13, 1299–1305.
[27] Sanz, P., Alms, G.R., Haystead, T.A. and Carlson, M. (2000) Regulatory interactions between the Reg1-Glc7 protein phosphatase and the Snf1 protein kinase. Mol. Cell. Biol. 20, 1321–1328.
[28] Carlson, M. (1999) Glucose repression in yeast. Curr. Opin. Microbiol. 2, 202–207.
[29] Gancedo, J.M. (1998) Yeast carbon catabolite repression. Microbiol. Mol. Biol. Rev. 62, 334–361.
[30] Schuller, H.J. (2003) Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*. Curr. Genet. 43, 139–160.
[31] Alepuz, P.M., Cunningham, K.W. and Estruch, F. (1997) Glucose repression affects ion homeostasis in yeast through the regulation of the stress-activated ENA1 gene. Mol. Microbiol. 26, 91–98.
[32] Thompson-Jaeger, S., Francois, J., Gaughran, J.P. and Tatchell, K. (1991) Deletion of SNF1 affects the nutrient response of yeast and resembles mutations which activate the adenylate cyclase pathway. Genetics 129, 697–706.
[33] Honigberg, S.M. and Lee, R.H. (1998) Snf1 kinase connects nutritional pathways controlling meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18, 4548–4555.
[34] Cullen, P.J. and Sprague Jr., G.F. (2000) Glucose depletion causes haploid invasive growth in yeast. Proc. Natl. Acad. Sci. USA 97, 13619–13624.
[35] Ashrafi, K., Lin, S.S., Manchester, J.K. and Gordon, J.I. (2000) Sip2p and its partner snf1p kinase affect aging in *S. cerevisiae*. Genes Dev. 14, 1872–1885.
[36] Kaiser, C., Michaelis, S. and Mitchell, A. (1994) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, New York.
[37] Dohmen, R.J., Strasser, A.W., Honer, C.B. and Hollenberg, C.P. (1991) An efficient transformation procedure enabling long-term storage of competent cells of various yeast genera. Yeast 7, 691–692.
[38] Ramos, J., Contreras, P. and Rodriguez-Navarro, A. (1985) A potassium transport mutant of *Saccharomyces cerevisiae*. Arch. Microbiol. 143, 88–93.
[39] Serrano, R. (1988) H^+-ATPase from plasma membranes of *Saccharomyces cerevisiae* and Avena sativa roots: purification and reconstitution. Methods Enzymol. 157, 533–544.
[40] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[41] Serrano, R. (1980) Effect of ATPase inhibitors on the proton pump of respiratory-deficient yeast. Eur. J. Biochem. 105, 419–424.
[42] Mulet, J.M., Alejandro, S., Romero, C. and Serrano, R. (2004) The trehalose pathway and intracellular glucose phosphates as modulators of potassium transport and general cation homeostasis in yeast. Yeast 21, 569–582.
[43] Munson, A.M., Haydon, D.H., Love, S.L., Fell, G.L., Palanivel, V.R. and Rosenwald, A.G. (2004) Yeast ARL1 encodes a regulator of K^+ influx. J. Cell Sci. 117, 2309–2320.
[44] Treitel, M.A., Kuchin, S. and Carlson, M. (1998) Snf1 protein kinase regulates phosphorylation of the Mig1 repressor in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18, 6273–6280.
[45] Estruch, F., Treitel, M.A., Yang, X. and Carlson, M. (1992) N-terminal mutations modulate yeast SNF1 protein kinase function. Genetics 132, 639–650.
[46] McCartney, R.R. and Schmidt, M.C. (2001) Regulation of Snf1 kinase. Activation requires phosphorylation of threonine 210 by an upstream kinase as well as a distinct step mediated by the Snf4 subunit. J. Biol. Chem. 276, 36460–36466.
[47] Wilson, W.A., Hawley, S.A. and Hardie, D.G. (1996) Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio. Curr. Biol. 6, 1426–1434.
[48] Dubacq, C., Chevalier, A. and Mann, C. (2004) The protein kinase Snf1 is required for tolerance to the ribonucleotide reductase inhibitor hydroxyurea. Mol. Cell. Biol. 24, 2560–2572.
[49] Hiesinger, M., Roth, S., Meissner, E. and Schuller, H.J. (2001) Contribution of Cat8 and Sip4 to the transcriptional activation of yeast gluconeogenic genes by carbon source-responsive elements. Curr. Genet. 39, 68–76.
[50] Young, E.T., Dombek, K.M., Tachibana, C. and Ideker, T. (2003) J. Biol. Chem. 278, 26146–26158.
[51] Östling, J., Carlberg, M. and Ronne, H. (1996) Multiple pathways are co-regulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8. Functional domains in the Mig1 repressor. Mol. Cell. Biol. 16, 753–761.
[52] Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.
[53] Hedges, D., Proft, M. and Entian, K.D. (1995) CAT8, a new zinc cluster-encoding gene necessary for derepression of gluconeogenic enzymes in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15, 1915–1922.
[54] Lesage, P., Yang, X. and Carlson, M. (1996) Yeast SNF1 protien kinase interacts with SIP4, a C6 zinc cluster transcriptional activator: a new role for SNF1 in the glucose response. Mol. Cell. Biol. 16, 1921–1928.
[55] Nehlin, J.O. and Ronne, H. (1990) Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumor finger proteins. EMBO J. 9, 2891–2898.
[56] Wallis, J.W., Chrebet, G., Brodsky, G., Rolfe, M. and Rothstein, R. (1989) A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase. Cell 58, 409–419.