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Vacuolar H^+-ATPase, but not mitochondrial F_1F_0-ATPase, is required for NaCl tolerance in *Saccharomyces cerevisiae*

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Abstract

Salt tolerance in *Saccharomyces cerevisiae* is a complex trait, involving regulation of membrane polarization, Na^+ efflux and sequestration of Na^+ in the vacuole. Since transmembrane transport energized by H^+-adenosine triphosphatases (ATPases) is common to all of these tolerance mechanisms, the objective of this study was to characterize the responses of the plasma membrane H^+-ATPase, vacuolar H^+-ATPase and mitochondrial F_1F_0-ATPase to NaCl stress. We hypothesized that since the vacuolar ATPase is responsible for generating the proton motive force required for import of cations (such as Na^+) into the vacuole, strains lacking this activity should be hypersensitive to NaCl. We found that strains lacking vacuolar ATPase activity were in fact hypersensitive to NaCl, while strains lacking ATP synthase were not. This effect was specific to the ionic component of NaCl stress, since the mutant strains were indistinguishable from wild-type and complemented strains in the presence of sorbitol.

Keywords: Salt stress; Vacuolar H^+-ATPase; Mitochondrial F_1F_0-ATPase; Salt tolerance; *Vma1,2* mutation; *Atp1,2* mutation

1. Introduction

Salt tolerance is a complex trait involving responses to both osmotic and ionic stresses. The mechanisms by which organisms are able to tolerate high concentrations of salt in their environment have been extensively studied by mutational analysis of the budding yeast *Saccharomyces cerevisiae*. Data obtained thus far indicate that an array of signaling pathways activate salt-responsive genes involved in modulating plasma-membrane polarization, Na^+ influx, Na^+ efflux and sequestration of Na^+ in the vacuole.

One effect of salt stress is a hyperpolarization of the plasma membrane. A low-affinity cation uptake current activated by membrane hyperpolarization (NSC1) has been observed in patch-clamp studies [1] and may contribute to subsequent depolarization of the membrane. Also contributing to depolarization of the plasma membrane are protein kinases HAL4 and HAL5, the induction of which increases the selectivity of the high-affinity TRK1/2 K^+ channel [2]. Accumulation of K^+ and production of glycerol protect cells from the ionic and osmotic components of salt stress, respectively. Influx of K^+ and H^+ (and perhaps Na^+) through the TRK1/2 channel dissipates the driving force for uptake of Na^+ across the plasma membrane. The Ser–Thr protein phosphatase, calcineurin, is required for increased selectivity of TRK1/2 [3]. Calcineurin is a heterotrimer composed of a catalytic subunit (encoded by either *CNA1*or *CNA2*), a Ca^{2+}-binding regulatory subunit (encoded by *CNB1*) and Ca^{2+}/calmodulin [4]. Binding of Ca^{2+}/calmodulin to the catalytic subunit prevents its autoinhibitory activity [5] so that calcineurin is free to interact with a variety of substrates within the cell, regulating the salt response.

Calcineurin is a central regulator of Na^+ influx and efflux. Sodium ions may enter the cell through the TRK1/2 channel or the NSC1 current. Under alkaline or neutral conditions, Na^+ is exported mainly through the action of PMR2/ENA1, a Na^+-adenosine triphosphatase (ATPase) localized to the plasma membrane [6]. Expression and activity of this enzyme is controlled by many factors, highlighting its importance to salt tolerance. Activity of ENA1 is stimulated by calcineurin [7], HAL1,8,9 [8–10], SNF1 [9], and in *Schizosaccharomyces pombe*, AFT1, a transcription factor activated by the *STY1*/*WIS1* MAPK cascade [11]. It is also indirectly activated

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by HAL3, a negative regulatory subunit of the PPZ1/2 protein phosphatases, which normally inhibits ENA1 activity [12]. Carbon source also effects ENA1 activity. The presence of glucose inhibits SNF1 (an activator of ENA1) and activates protein kinase A, an inhibitor of ENA1 [9]. Under acidic conditions such as those used in this study, efflux of Na^+ is mediated mainly by the Na^+/H^+ antiporter, NHA1 [6].

Another mechanism of salt tolerance in yeast is sequestration in the vacuole. Transport of Na^+ into the vacuole is mediated by NHX1, a Na^+/H^+ antiporter localized to a prevacuolar compartment and activated by cytoplasmic acidosis [13,14]. Other proteins required for *NHX1*-mediated salt tolerance are the chloride channel (GEF1) and the vacuolar ATPase [15]. Since transmembrane transport of Na^+ is a key component of salt tolerance, the objective of this study was to characterize the responses of cellular ATPases (P-ATPase, F_1F_0-ATPase and V-ATPase) to salt. The contributions of the V-ATPase and F_1F_0-ATPase were of particular interest because the V-ATPase (with ATP provided by F_1F_0-ATPase) creates the proton motive force (PMF) required for import of cations into the vacuole. Changes in ATPase transcript levels, protein and activity in response to salt were measured, and the phenotypes of V-ATPase and F_1F_0-ATPase mutant and complemented strains were compared.

2. Materials and methods

2.1. Media and culture conditions

Yeast strains (Table 1) were maintained on standard media as described by Sherman [16].

2.2. ATPase activity measurements

Cultures of wild-type yeast were incubated for 24 h at 30°C. Cells were then harvested by centrifugation, washed three times and resuspended in 1 ml of sterile water. This cell suspension was used to inoculate triplicate 3-ml cultures of SC medium containing 0–1000 mM NaCl, which were incubated for 24 h at 30°C (final OD_{600} approximately 0.9). Cultures were harvested by centrifugation and extracts were prepared as described by Hamilton et al. [17]. Quantitation of protein in cell extracts was performed using the Bradford assay [18]. ATP activity was then assayed as described by Briskin et al. [19].

2.3. Complementation of ATPase mutations

Yeast strains carrying *LEU2* insertion mutations in *vma1* and *vma2*, as well as plasmids carrying the complementing genes were provided by T. Stevens (University of Oregon). Strains carrying null deletion mutations of *atp1* and *atp2* and plasmids carrying the complementing genes were provided by D. Mueller (Chicago Medical School). Complemented strains were prepared as described by Hamilton et al. [17].

2.4. NaCl dose–response experiments

Inoculum for each culture was prepared as described above and used to inoculate triplicate 3-ml cultures of SC medium containing 0–1000 mM NaCl. Cultures were incubated for 24 h at 30°C and the final OD_{600} of each culture was measured in a 96-well plate (Costar) using a Spectromax Plus plate reader and Softmax Pro version 1.1 software (Molecular Devices). For comparison, the OD_{600} values for each strain were expressed as a percentage of the control value for that strain. For halotolerance tests, 5-μl aliquots of serial 10^{-1} dilutions of overnight cultures were spotted onto YPD medium containing 1 M NaCl, 1.5 M sorbitol, 100 mM LiCl or 50 μg ml^{-1} hygromycin B and incubated at 30°C for 2–4 days.

2.5. Immunoblotting

Total yeast proteins were separated by SDS–PAGE and electroblotted onto nitrocellulose (0.45 μm, Bio-Rad Laboratories) membranes using the Mini Trans-Blot Cell (Bio-Rad Laboratories) according to the manufacturer's instructions. Membranes were blocked and probed as directed in the Lumi-glo Chemiluminescent Detection System manual (Kirkegaard and Perry Laboratories). A. Lewin, University of Florida, provided polyclonal anti-

Table 1
Yeast strains used in this study

Strain	Genotype	Source
SF838-1D	*MATα, ade6, his4-519, leu2-3,112, ura3-52, pep4-3*	[22]
vma1	Isogenic to SF838-1D except *vma1Δ::LEU2*	[23]
vma2	Isogenic to SF838-1D except *vma2D:LEU2*	[24]
vma1/VMA1	Isogenic to *vma1* except carrying wild-type *VMA1* on plasmid pRS316 (*URA3*)	[17]
vma2/VMA2	Isogenic to *vma2* except carrying wild-type *VMA2* on plasmid pRS316 (*URA3*)	[17]
W303-1A	*MATα, ade2-1, his3-1,15, leu2-3,112, trp1-1, ura3-1*	[25]
W303-Δα	Isogenic to W303-1A except null deletion mutation in *ATP1*	[25]
W303-Δβ	Isogenic to W303-1A except null deletion mutation in *ATP2*	[25]
W303-Δα/*ATP1*	Isogenic to W303-Δα except carrying wild-type *ATP1*on plasmid pRS314 (*TRP1*)	[17]
W303-Δβ/*ATP2*	Isogenic to W303-Δβ except carrying wild-type *ATP2*on plasmid pRS316 (*URA3*)	[17]

bodies raised against the α and β subunits of the yeast F_1-ATPase. M. Maeshima, Nagoya University, provided antibodies raised against the B subunit of the *Vigna radiata* V-ATPase.

2.6. RNA isolation, Northern hybridization and analysis

RNA was isolated from yeast cultures (approximately 5×10^7 cells) using the RNeasy kit (Qiagen) according to the manufacturer's directions. Northern transfers were carried out according to GeneScreen Plus (Amersham Pharmacia) directions, followed by exposure to storage phosphor screens (Molecular Dynamics). Phosphor screens were developed using a Phosphorimager 445SI and the resulting images analyzed using ImagequaNT version 4.2 software. For figure preparation, representative lanes of triplicate samples were selected from the same blot and compiled using Adobe Photoshop® version 5.5.

3. Results and discussion

3.1. Response of yeast ATPases to NaCl

The responses of cellular ATPases (F_1F_0-ATPase, P-ATPase and V-ATPase) to NaCl in wild-type yeast were characterized by measuring ATPase activities, protein and transcript levels. ATP activities were measured after growth in SC medium containing 0–1000 mM NaCl (Fig. 1). F_1F_0-ATPase activity gradually increased from 0.078 nmol Pi mg^{-1} min^{-1} to 0.154 nmol Pi mg^{-1} min^{-1} (197% of control), reaching its peak activity at 600 mM NaCl. P-ATPase activity remained essentially constant from 0 to 800 mM NaCl, but declined to 0.056 nmol Pi mg^{-1} min^{-1} (57% of control) by 1000 mM. At first sight this is surprising, since the main determinant of NaCl tolerance in yeast is ordinarily the ENA1 Na^+ efflux pump (a P-type ATPase). However, under the acidic conditions used here (pH 4.5), Na^+/H^+ antiport mediated by NHA1 would predominate [6]. The presence of glucose in the growth medium would also inhibit the activity of ENA1 by repression SNF1, an activator of ENA1, and by activation of protein kinase A, an inhibitor of ENA1 [9].

In contrast, vacuolar ATPase activity increased sharply from 0.103 nmol Pi mg^{-1} min^{-1} to 0.303 nmol Pi mg^{-1} min^{-1} (296% of control) from 0 to 200 mM NaCl, and decreased slightly at higher salt concentrations. However, even at 1000 mM NaCl, V-ATPase activity was still comparatively high, at 0.194 nmol Pi mg^{-1} min^{-1} (189% of control). Interestingly, these results contrast observations by Persov et al. [20], who examined vacuoles isolated from yeast cells grown in YPD medium containing 800 mM NaCl and found that proton uptake activity was decreased in comparison to activity in vacuoles isolated from cells grown in YPD. However, the differences in medium composition (YPD vs. SC) and pH (neutral vs. acidic) make direct comparison difficult, and highlight the importance of environment in determining which salt tolerance mechanisms are active.

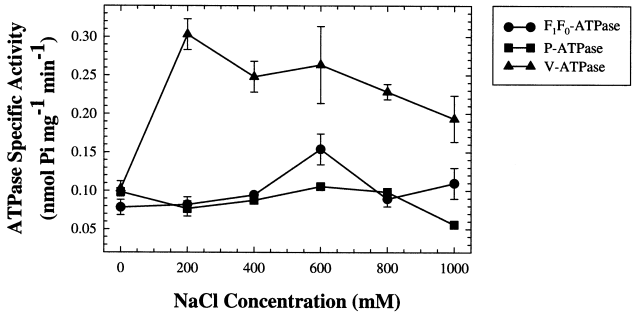

Fig. 1. The effect of NaCl on cellular ATPase activities (F_1F_0-ATPase, P-ATPase, V-ATPase) in wild-type yeast. Cultures were grown 16–24 h in the presence of 0–1000 mM NaCl in SC medium. Values shown are means ± S.E.M. ($n=3$). Results shown are representative of three independent trials.

To determine the mechanism by which ATPase activities change during NaCl stress, expression of V-ATPase and F_1F_0-ATPase subunits was examined by Northern and Western analyses (Fig. 2). Levels of the transcript encoding the F_1F_0-ATPase α subunit increased to 325% of the control level by 800 mM NaCl, followed by a decline to 257% of control by 1000 mM (Fig. 2A,B). This pattern is similar to that observed for increased F_1F_0-ATPase activity (Fig. 1), with the exception that enzyme activity peaks at 600 mM while the transcript level peaks at 800 mM. Protein levels of the F_1F_0-ATPase α and β subunits also increased with increasing NaCl concentration, as determined by Western analysis (Fig. 2C). Interestingly, levels of these subunits continue to increase past 600–800 mM NaCl, the peak concentrations for F_1F_0-ATPase activity and transcript levels.

Levels of the transcript encoding the V-ATPase B subunit also increased gradually over the range of NaCl concentrations tested (Fig. 2A,B), reaching a peak (248% of control) at 600 mM NaCl. The amount of protein also increased in response to NaCl stress. Like the α and β subunits of the F_1F_0-ATPase, protein levels of the V-ATPase B subunit continued to increase from 0 to 800 mM NaCl (Fig. 2C), in contrast to the peak levels of enzyme activity and transcript abundance (200 and 600 mM NaCl, respectively).

3.2. V-ATPase mutants, but not F_1F_0-ATPase mutants, are hypersensitive to NaCl

To test the hypothesis that V-ATPase and F_1F_0-ATPase activities are involved in salt tolerance, yeast mutants lacking subunits of these enzymes were tested to determine whether or not they are hypersensitive to NaCl. The relative sensitivities of V-ATPase mutants, wild-type parent and complemented strains to NaCl were determined by

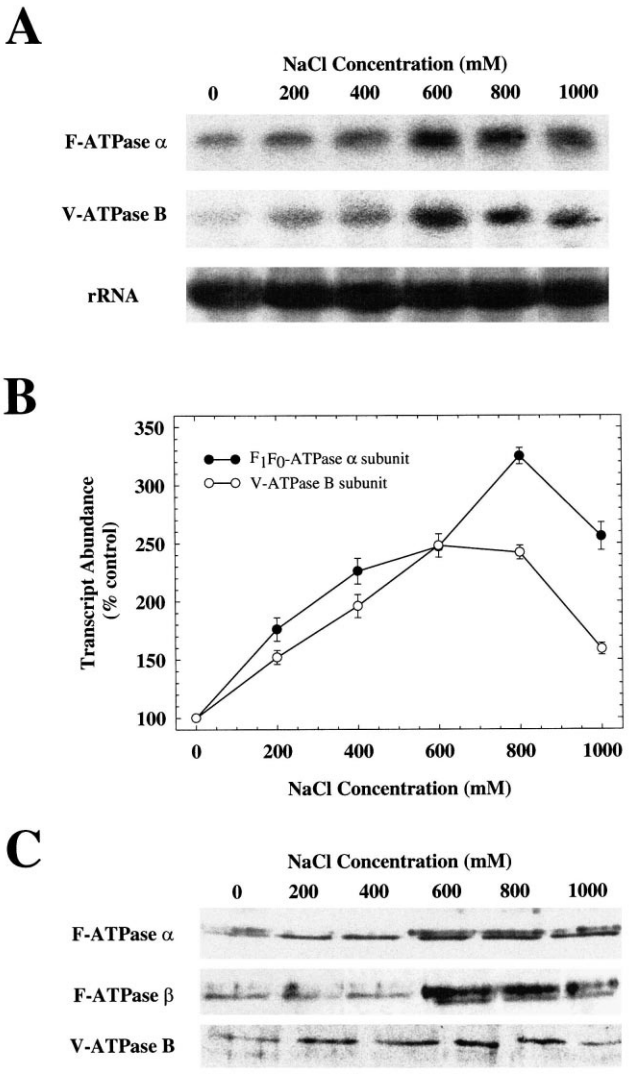

Fig. 2. The effect of NaCl on expression of F_1F_0-ATPase and V-ATPase. A: Levels of the transcripts encoding the F_1F_0-ATPase α subunit and V-ATPase B subunit were measured by Northern analysis. B: Relative abundance of transcripts as measured by densitometry. The ratio of mRNA/rRNA measured at each concentration of NaCl was compared to the control value for each transcript. Values shown are means ± S.E.M. ($n = 3$). C: Determination of protein levels of V-ATPase (B subunit) and F_1F_0-ATPase (α and β subunits) by Western analysis. Cross-reacting bands were visualized using a chemiluminescent detection system. Samples shown are representative lanes selected from triplicates and compiled using Adobe Photoshop® version 5.5. For all panels, data shown are representative of two independent trials.

observing growth in liquid culture. Final OD_{600} values at each NaCl concentration were related to the control values for each strain (Fig. 3A). For each V-ATPase mutant strain (*vma1*, *vma2*), there was a 40–50% greater decrease in growth compared to wild-type at 600–1000 mM NaCl (from control OD_{600} values of 0.49 and 0.48 to OD_{600} values of 0.08 and 0.05, for *vma1* and *vma2* respectively at 800 mM NaCl). In contrast, the complemented strains grew as well as wild-type at these concentrations (control OD_{600} values of 0.82, 0.73 and 0.76 and OD_{600} values of 0.55, 0.46 and 0.46 at 800 mM NaCl for SF838, *vat1*/*VAT1* and *vat2*/*VAT2* respectively).

The salt-sensitive phenotype observed for the V-ATPase mutant strain, *vma1*, was specific to NaCl and LiCl (Fig. 4). This mutant grew as well as the wild-type parent and complemented strains on YPD medium containing other osmotic (sorbitol) and ionic (hygromycin B) stress-inducing agents. In contrast, hypersensitivity of V-ATPase mutants was observed on YPD medium containing 1 M NaCl or 100 mM LiCl (Fig. 4).

Our observation that V-ATPase mutants were inhibited 40–50% more than the wild-type parent and complemented strains at 600–1000 mM NaCl complement those obtained by Gaxiola et al. [8], who found that overexpression of an *Arabidopsis* vacuolar H^+-pyrophosphatase (AVP1) in yeast, which would mimic increased V-ATPase activity, led to increased NaCl tolerance. Haro et al. [21] examined the NaCl tolerance of yeast strains lacking the

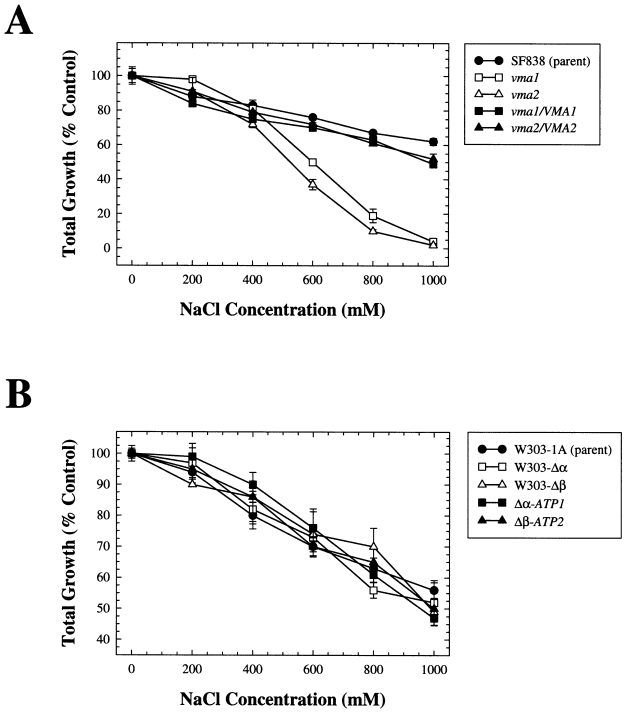

Fig. 3. The effect of ATPase mutations on NaCl tolerance in yeast. A: Yeast strains carrying LEU2 insertion mutations in the *VMA1* and *VMA2* genes (*vma1*, *vma2*), their isogenic parent strain (SF838), and strains carrying wild-type copies of the *VMA1* and *VMA2* genes on low-copy plasmids (*vma1/VMA1*, *vma2/VMA2*) were grown for 16–24 h in SC minimal medium (pH 4.5) containing 0–1000 mM NaCl. B: Yeast strains with null deletion mutations in the *atp1* and *atp2* genes (W303-Δα, W303-Δβ), their isogenic wild-type parent strain (W303-1A) and strains carrying wild-type copies of the *atp1* and *atp2* genes on low-copy plasmids (Δα-*ATP1*, Δβ-*ATP2*) were grown for 16–24 h in SC minimal medium (pH 4.5) containing 0–1000 mM NaCl. Total growth (OD_{600}) of each culture was measured and compared to the control (0 NaCl) values for each strain. Values shown are means ± S.E.M. ($n = 3$). Results shown are representative of three independent trials.

C subunit of the V-ATPase. Although our observation that V-ATPase mutants are hypersensitive to NaCl is in agreement with their results, they also reported that these mutants tolerate only 15 mM LiCl, contrary to our observations. We observed hypersensitivity at 100 mM LiCl but not at 50 mM LiCl (data not shown). It is possible that these differences arose due to the different media used (YPD vs. minimal medium arginine phosphate) or because we performed growth tests on solid medium while Haro et al. used liquid medium [21]. In our experience, V-ATPase mutant strains grow poorly in liquid culture because the cells aggregate and have a slow growth rate. These confounding effects, caused by the pleiotropic nature of V-ATPase mutations, are not apparent when these strains are grown on solid medium.

The relative sensitivities of an F_1F_0-ATPase mutant strain, its parent and complemented strain (W303-Δβ, W303-1A and Δβ-*ATP2*) to NaCl were also measured (Fig. 3B). In contrast to the V-ATPase mutant, the F_1F_0-ATPase mutant was not hypersensitive to NaCl from 0 to 1000 mM (control OD_{600} values of 0.87, 0.79, 0.73, 0.83, and 0.87 for W303-1A, W303-Δα, W303-Δβ, Δα-*ATP1* and Δβ-*ATP2*, respectively). Growth of this mutant was indistinguishable from its parent and complemented strains in the presence of hygromycin B, LiCl, sorbitol and NaCl on YPD solid medium (Fig. 4).

This study highlights the importance of the vacuolar ATPase in yeast NaCl tolerance. In wild-type yeast, NaCl stress causes increased activity of the V-ATPase, with ATP presumably provided by increased activity of the F_1F_0-ATPase. The V-ATPase is thought to contribute to NaCl tolerance by creating the PMF required for sequestration of Na^+ in the vacuole by the Na^+/H^+ antiporter, NHX1 [13]. The PMF is also involved in maintaining homeostasis of other cations, such as K^+ and Ca^{2+}, the disruption of which could contribute to the NaCl-sensitive phenotype of V-ATPase mutants. The electrical potential created by the V-ATPase creates a driving force for uptake of Cl^- through the chloride channel (GEF1).

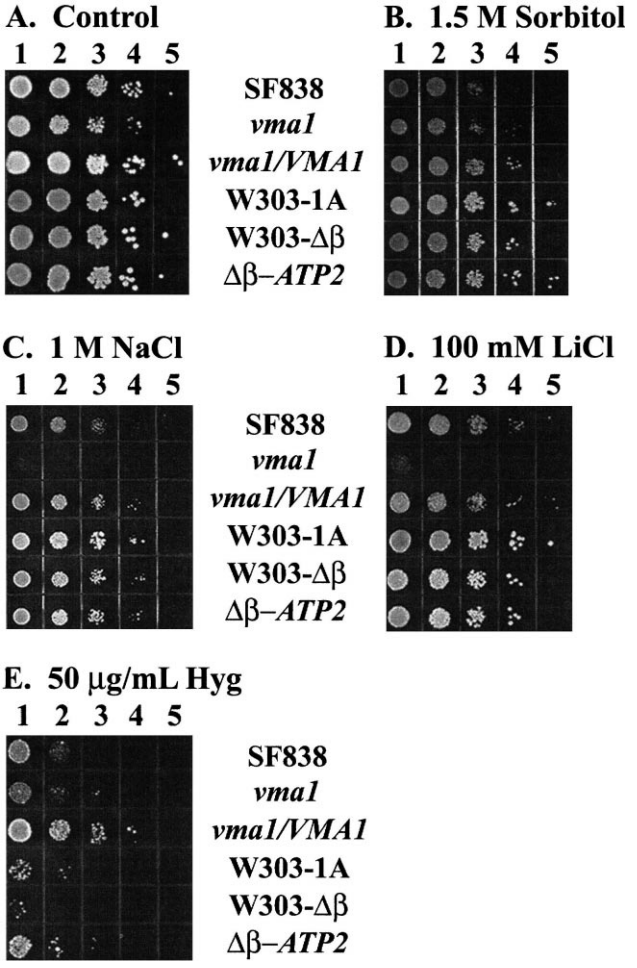

Fig. 4. Halotolerance of V-ATPase and F_1F_0-ATPase mutant strains. Saturated cultures of wild-type parent (SF838, W303-1A), mutant (*vma1*, W303-Δβ) and complemented (*vma1/VMA1*, Δβ-*ATP2*) strains were serially diluted and 5-μl aliquots spotted onto YPD plates containing (A) no added compounds, (B) 1.5 M sorbitol, (C) 1 M NaCl, (D) 100 mM LiCl or (E) 50 μg ml^{-1} hygromycin B. Results shown are representative of two independent trials.

Therefore, it is possible that V-ATPase mutant strains are hypersensitive to NaCl because they are unable to effectively sequester Na^+ and Cl^- in the vacuole, allowing Na^+ to interact with sensitive cytoplasmic targets.

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References

[1] Bihler, H., Slayman, C. and Bertl, A. (1998) NSC1: a novel high current for cations in the plasma membrane of *Saccharomyces cerevisiae*. FEBS Lett. 432, 59–64.
[2] 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.
[3] Mendoza, I., Rubio, F., Rodriguez-Navarro, A. and Pardo, J. (1994) The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. J. Biol. Chem. 269, 8792–8796.
[4] Stemmer, P.M. and Klee, C.B. (1994) Dual calcium regulation of calcineurin by calmodulin and calcineurin B. Biochemistry 33, 6859–6866.
[5] Cohen, P. (1989) The structure and function of protein phosphatases. Annu. Rev. Biochem. 58, 453–508.
[6] Prior, C., Potier, S., Souciet, J.-L. and Sychrova, H. (1996) Characterization of the *NHA1* gene encoding a Na^+/H^+ antiporter of the yeast *Saccharomyces cerevisiae*. FEBS Lett. 387, 89–93.
[7] Cunningham, K.W. and Fink, G.R. (1996) Calcineurin inhibits *VCX1*-dependent H^+–Ca^{2+} exchange and induces Ca^{2+}-ATPases in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 16, 2226–2237.
[8] Gaxiola, R., de Larrinoa, I.F., Villalba, J.M. and Serrano, R. (1992) A novel and conserved salt-induced protein is an important determinant of salt tolerance in yeast. EMBO J. 11, 3157–3164.
[9] Rios, G., Ferrando, A. and Serrano, R. (1997) Mechanisms of salt tolerance conferred by overexpression of the *HAL1* gene in *Saccharomyces cerevisiae*. Yeast 13, 515–528.
[10] Mendizabal, I., Rios, G., Mulet, J.M., Serrano, R. and Larrinoa, I.F. (1998) Yeast putative transcription factors involved in salt tolerance. FEBS Lett. 425, 323–328.
[11] Nishikawa, T., Aiba, H. and Mizuno, Y. (1999) The cta3+ gene that encodes a cation-transporting P-type ATPase is induced by salt stress under control of the Wis1–Sty1 MAPKK–MAPK cascade in fission yeast. FEBS Lett. 455, 183–187.
[12] Posas, F., Camps, M. and Arino, J. (1995) The PPZ protein phosphatases are important determinants of salt tolerance in yeast cells. J. Biol. Chem. 270, 13036–13041.
[13] Nass, R., Cunningham, K.W. and Rao, R. (1997) Intracellular sequestration of sodium by a novel Na^+/H^+ exchanger in yeast is enhanced by mutations in the plasma membrane H^+-ATPase. J. Biol. Chem. 272, 26145–26152.
[14] Nass, R. and Rao, R. (1998) Novel localization of a Na^+/H^+ exchanger in a late endosomal compartment of yeast. J. Biol. Chem. 273, 21054–21060.
[15] Gaxiola, R.A., Rao, R., Sherman, A., Grisafi, P., Alper, S.L. and Fink, G. (1999) The *Arabidopsis thaliana* proton transporters, AtNhx1 and Avp1, can function in cation detoxification in yeast. Proc. Natl. Acad. Sci. USA 96, 1480–1485.
[16] Sherman, F. (1991) Getting started with yeast. Methods Enzymol. 194, 3–21.
[17] Hamilton, C.A., Good, A.G. and Taylor, G.J. (2001) Vacuolar H^+-ATPase, but not mitochondrial F_1F_0-ATPase, is required for aluminum tolerance in *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. (in press).
[18] Bradford, M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[19] Briskin, D.P., Leonard, R.T., Hodges, T.K. (1987) Isolation of the plasma membrane: membrane markers and general principals. In: Methods in Enzymology (Packer, L., Douce, R., Eds.), Vol. 148, pp. 542–558. Academic Press, New York.
[20] Haro, R., Banuelos, M.A., Quintero, F.J., Rubio, F. and Rodriguez-Navarro, A. (1993) Genetic basis of sodium exclusion and sodium tolerance in yeast. A model for plants. Physiol. Plant 89, 868–874.
[21] Perzov, N., Padler-Karavani, V., Nelson, H. and Nelson, N. (2001) Features of V-ATPases that distinguish them from F-ATPases. FEBS Lett. 504, 223–228.
[22] Rothman, J.H. and Stevens, T.H. (1986) Protein sorting in yeast: the localization determinant of yeast vacuolar carboxypeptidase Y resides in the propeptide. Cell 48, 887–897.
[23] Yamashiro, C.T., Kane, P.M., Wolczyk, D.F., Preston, R.A. and Stevens, T.H. (1990) Role of vacuolar acidification in protein sorting and zymogen activation: a genetic analysis of the yeast vacuolar proton-translocating ATPase. Mol. Cell. Biol. 10, 3737–3749.
[24] Kane, P.M., Yamashiro, C.T., Wolczyk, D.F., Neff, N., Goebl, M. and Stevens, T.H. (1990) Protein splicing converts the yeast TFP1 gene product to the 69-kDa subunit of the vacuolar H^+-adenosine triphosphatase. Science 250, 651–657.
[25] Lai-Zhang, J., Xiao, Y. and Mueller, D.M. (1999) Epistatic interactions of deletion mutants in the genes encoding the F_1-ATPase in yeast *Saccharomyces cerevisiae*. EMBO J. 18, 58–64.