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Phenotypes of Sphingolipid-Dependent Strains of *Saccharomyces cerevisiae*

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To study sphingolipid function(s) in *Saccharomyces cerevisiae*, we have investigated the effects of environmental stress on mutant (*SLC*) strains (R. C. Dickson, G. B. Wells, A. Schmidt, and R. L. Lester, Mol. Cell. Biol. 10:2176–2181, 1990) that either contain or lack sphingolipids, depending on whether they are cultured with a sphingolipid long-chain base. Strains lacking sphingolipid were unable to grow at low pH, at 37°C, or with high salt concentrations in the medium; these environmental stresses are known to inhibit the growth of some *S. cerevisiae* strains with a defective plasma membrane H^+-ATPase. We found that sphingolipids were essential for proton extrusion at low pH and furthermore found that cells lacking sphingolipid no longer exhibited net proton extrusion at normal pH after a 1-min exposure to pH 3. Cells lacking sphingolipid appeared to rapidly become almost completely permeable to protons at low pH. The deleterious effects of low pH could be partially prevented by 1 M sorbitol in the suspension of cells lacking sphingolipid. Proton extrusion at normal pH (pH 6) was significantly inhibited at 39°C only in cells lacking sphingolipid. Thus, the product of an *SLC* suppressor gene permits life without sphingolipids only in a limited range of environments. Outside this range, sphingolipids appear to be essential for maintaining proton permeability barriers and/or for proton extrusion.

We are studying the function(s) of sphingolipids in *Saccharomyces cerevisiae* because this organism is well suited to molecular genetic analysis and because it has only a few sphingolipids, with phosphoinositol as a distinguishing feature of the polar head groups (17, 18). This hydrophobic portion is composed of the long-chain base phytosphingosine, amide linked to an α-OH-C_{26} fatty acid (17, 19).

One strategy that we have adopted for studying sphingolipid function(s) is to compare the phenotype of a strain containing sphingolipids with the phenotype of the same strain that has been manipulated so as to lack sphingolipids. To implement this strategy, we isolated strains that are able to grow without making sphingolipids (5). Such strains have two essential mutations. First, the *LCB1* gene is deleted so that no serine palmitoyltransferase is produced (2, 11). Since this enzyme catalyzes the first step in sphingolipid long-chain base synthesis, the strain cannot make sphingolipids unless supplied with a long-chain base such as phytosphingosine (10, 20). Second, the strains carry a semidominant mutation, termed *SLC* (for sphingolipid compensation), that enables the cell to suppress or bypass the *lcb1* defect and to grow in the absence of exogenous phytosphingosine. Thus, *SLC* strains should be of value in unraveling sphingolipid function(s), since when cultured without phytosphingosine, they grow but make no detectable sphingolipid, whereas when grown with phytosphingosine, they make a normal complement of sphingolipids (5). We demonstrate here that in certain restrictive environments, *SLC* strains exhibit a sphingolipid requirement for the maintenance of a proton permeability barrier and/or for glucose-primed net proton extrusion.

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MATERIALS AND METHODS

Yeast strains and culture conditions. Strains 1Δ4 and 1Δ7, carrying the *lcb1*::*URA3* deletion allele (5) and thus auxotrophic for a sphingolipid long-chain base, were used to select spontaneously arising *lcb1* suppressor strains no longer dependent on a long-chain base for growth. These *SLC* strains were designated 7R4, 7R6 (5), and 4R3. Strains 7R6 and 4R3 have been shown to contain a mutation in the same gene which we have termed *SLC1* (7a).

Liquid medium consisted of 1% yeast extract (Difco Laboratories), 1% Bacto Peptone (Difco), 0.05% KH_2PO_4, 0.005% myoinositol, 50 mM sodium succinate (pH 6.0), 4% glucose, 0.05% tergitol, and where indicated, 25 μM phytosphingosine (prepared as previously described [10]). Where indicated, this medium was supplemented with 0.5 or 1 M sorbitol and the succinate buffer was replaced with 50 mM glycylglycine. All strains were grown aerobically at 30°C with shaking. Turbidity was monitored by measurement of A_{650}, and cells were harvested in the logarithmic phase, at absorbances of 3 to 8.

Solid media used in the phenotyping experiments contained 1% yeast extract, 2% Bacto Peptone, 2% glucose, and 2% agar (except as noted). When indicated, additional ingredients were 25 μM phytosphingosine, 0.75 M KCl, 0.75 M NaCl, and 0.2 M sodium acetate. The pH of the media was adjusted prior to the addition of glucose, phytosphingosine (added as a 4,000× stock solution in ethanol), and agar. In the case of the low-pH plates (3% agar), the agar was dissolved separately and its pH was adjusted to ~4 before mixing of the agar with the other components of the medium. Strains SJ21R (5), 7R4, 7R6, and 4R3 were cultured in liquid medium without phytosphingosine, and strain 1Δ4 was cultured in liquid medium with phytosphingosine. For testing phenotypes, cells were sedimented and resuspended in 1 M sorbitol. Approximately 25,000 cells of each strain were spotted on plates in a volume of 5 μl. The plates were incubated at 30 or 37°C for 4 to 5 days.

Measurement of extracellular pH. Our protocols for studying proton extrusion were based on those of Serrano (13). Cells were harvested, washed twice with 1 M sorbitol, and suspended in 1 M sorbitol to an absorbance of 300 to 400. The cell suspensions (0.5 ml) were added to 9.5 ml of one of the following buffers, some containing 1 M sorbitol: 5 mM glycylglycine (pH 3.0 or 4.0)–100 mM KCl (pH 3 or 4 buffer) or 5 mM sodium succinate (pH 6)–100 mM KCl (pH 6 buffer). The measurements were performed in a 30-ml glass vial at 30°C; pure compressed air was continuously bubbled through the suspension to both stir the sample and remove any CO_2 produced. The pH of the medium was monitored with a Radiometer PHM82 pH meter; readings were taken every 30 s to 1 min. After approximately 2 min, glucose (final concentration, 25 mM) was added to the suspension. Readings of pH were converted to nanomoles of H^+ on the basis of data from a control titration with HCl of wild-type cells suspended in each buffer and monitoring of the subsequent change in pH over the appropriate span. The presence of cells at optical densities of 15 to 20 made little or no difference to the buffering capacity of the suspension.

The experiment involving 2,4-dinitrophenol was performed as follows. A 0.5-ml sample of cell suspension was added to reaction vials containing 9.5 ml of pH 4 buffer, 250 μmol of glucose, and either 200 μl of 20 mM 2,4-dinitrophenol in ethanol (final concentration, 400 mM) or 200 μl of ethanol. The pH of the medium was measured as described above.

Titration of cells with acid. Cells were harvested, washed twice with 1 M sorbitol, and suspended in 1 M sorbitol to an optical density of ~700. Cells (1 ml) were added to 9 ml of either H_2O or 1 M sorbitol. In addition, 1-ml portions of cells were centrifuged, and the pellets were resuspended in 10 ml of 500 mM NaCl–9% *n*-butanol and incubated for 5 min at 40°C in order to permeabilize the cells (12). The titration was carried out at 30°C with compressed air continuously bubbling through the suspension. The cells were allowed to equilibrate for 5 min, at which time the pH was recorded. Known aliquots of HCl were then added to the suspension approximately every 30 s, and pH readings were taken 30 s after each addition. The pH versus added acid profiles were determined for cell suspensions under various conditions and also for the suspending fluids alone (H_2O, 1 M sorbitol, 500 mM NaCl–9% *n*-butanol).

Measurement of protein leakage. Cells were grown in liquid media as described above with the further addition of [1-^{14}C]leucine (0.08 μCi/ml). After harvesting, the cells were washed three times with 1 M sorbitol to remove all extracellular [^{14}C]leucine and were suspended to an absorbance of ~400 in 1 M sorbitol. The cell suspension (100 μl) was added to 900 μl of either pH 3 or pH 6 buffer (as defined above) and allowed to incubate at 30°C for 10 min. One milliliter of 0.25 M sodium succinate (pH 6) was added to the pH 3 sample to raise the pH, and the tubes were allowed to incubate for another 20 min at 30°C with shaking. The pH was raised to preclude the possibility that some proteins would not be soluble at pH 3 and hence could not leak from the cell or dissolve even if there were extensive membrane damage. Aliquots (1 ml) of each sample were removed and centrifuged in a microcentrifuge for 5 min. The supernatant fluid was removed, and the cell pellet was suspended in 200 μl of H_2O. Radioactivity in both the pellet (100 μl) and the supernatant (200 μl) was measured by liquid scintillation counting.

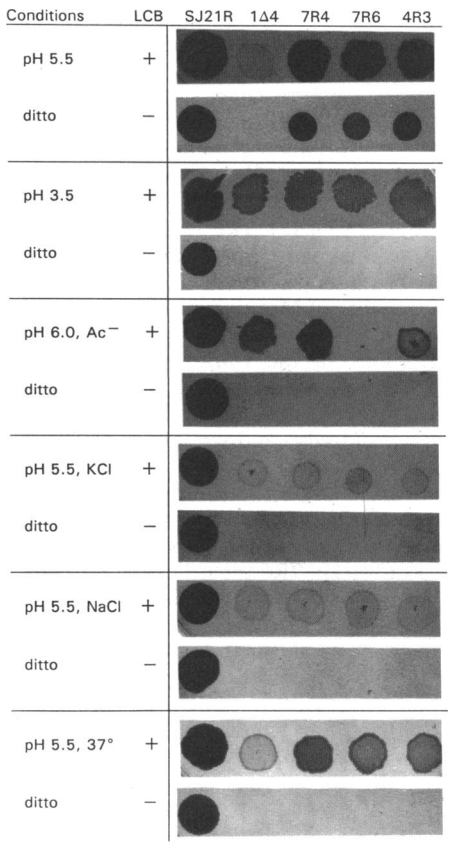

FIG. 1. Evidence that growth of *SLC* strains depends on exogenous phytosphingosine in environmental extremes. Growth on solid media was measured as described in Materials and Methods, using 125,000 cells as an inoculum. LCB, phytosphingosine; Ac^-, sodium acetate. The strains are SJ21R (wild type), 1Δ4 (*lcb1* deleted), and 7R4, 7R6, and 4R3 (*SLC* strains).

RESULTS

Growth of *SLC* strains lacking sphingolipid is restricted by environmental extremes. To begin to identify the physiological function(s) of sphingolipids in *S. cerevisiae*, we searched for culture conditions that allowed growth of *SLC* strains containing sphingolipids (phytosphingosine present in the culture medium) but inhibited growth of strains lacking sphingolipids (phytosphingosine omitted from the culture medium). We were particularly interested in culture conditions that might depend on a function present in the plasma membrane, since this is where the majority of yeast sphingolipids are found (8). The strains examined were the wild type (SJ21R), a derivative (1Δ4) with the *LCB1* gene deleted, and three *SLC* strains (7R4, 7R6, and 4R3) that arose spontaneously from the isogenic strain 1Δ4 or 1Δ7 (5). The data presented in Fig. 1 demonstrate that *SLC* strains did not require phytosphingosine for growth in standard medium but did require phytosphingosine for growth in medium buffered at pH 3.5, on plates incubated at 37°C, with medium containing 0.75 M NaCl or 0.75 M KCl, and in medium containing sodium acetate buffered at pH 6. As expected, under all conditions tested, the wild-type strain grew in the absence of phytosphingosine, whereas strain 1Δ4 required phytosphingosine. The same pattern of results with all of the strains was obtained with liquid media, with strain 1Δ4 exhibiting growth

comparable to that of the other strains at pH 5.5 (data not shown). The solid media were prepared to match those described by McCusker et al. (7), who studied the phenotypes of hygromycin B-resistant strains bearing mutations in the *PMA1* gene coding for the plasma membrane H^+-ATPase. The environmental conditions (except for 37°C) that restricted growth of *SLC* strains lacking sphingolipids paralleled the conditions that restricted growth of some *pma1*-defective strains (7). The *SLC* strains cultured with or without phytosphingosine were sensitive to hygromycin B (300 μg/ml) (data not shown). None of the hygromycin B-resistant mutants of McCusker et al. (7) were sensitive to 37°C; however, Cid and Serrano (4) have produced and located mutations in the *PMA1* gene that result in the inability of *S. cerevisiae* to grow at 37°C. These phenotypic similarities suggested that sphingolipids might be necessary for H^+-ATPase activity, and we therefore examined proton pumping in the *SLC* cells.

Proton extrusion and uptake in *SLC* strains. When proton flux measurements were started at pH 6, little difference was observed in the net proton extrusion rates of wild-type strain SJ21R and *SLC* strain 7R6 regardless of whether strain 7R6 contained or lacked sphingolipids (Fig. 2A). When the measurements were started at pH 4, however, a large alkalinization occurred in the sphingolipid-deficient 7R6 cells, and the subsequent glucose-primed net proton extrusion was almost negligible (Fig. 2B). The initial alkalinization was greater than that which occurred with sphingolipid-sufficient cells or with wild-type cells and was comparable in magnitude to that achieved by adding the protonophore 2,4-dinitrophenol to wild-type cells (Fig. 2C). Identical results were obtained with strain 4R3, and it was further found that inclusion of 1 M sorbitol in the assay medium largely prevented the alkalinization and the loss in proton extrusion ability observed in sphingolipid-deficient cells at the low pH (data not shown). Sorbitol (0.5 or 1.0 M) did not, however, relieve the low-pH growth inhibition (Fig. 1) in sphingolipid-deficient strain 4R3 (data not shown).

Acid titration of sphingolipid-deficient cells indicates increased proton permeability. The rapid alkalinization of the medium by sphingolipid-deficient cells exposed to low pH (Fig. 2) suggests that the cells may have become permeable to protons, causing the external pH to reflect the more neutral cell interior. To examine this point directly, wild-type cells and *SLC* strain 4R3 cultured with and without phytosphingosine were titrated with a strong acid, and the results were compared with those for cells completely permeabilized by treatment with NaCl-butanol (12). The sphingolipid-deficient 4R3 cells required significantly more acid to lower their pH to 3 than did their sphingolipid-sufficient counterpart or the wild-type cells (Fig. 3A), which supports the idea that these cells were more permeable to protons. All cells permeabilized by the treatment with NaCl-butanol gave a similar titration profile (Fig. 3B), which in turn looked similar to the profile of the sphingolipid-deficient 4R3 cells. The suspending fluids without cells were titrated (Fig. 3C) as a control to illustrate their relatively minor buffering capacity.

Protein leakage from sphingolipid-deficient cells at low pH. Because sphingolipid-deficient *SLC* cells exhibited increased permeability to protons at low pH, we wanted to determine whether plasma membrane integrity was compromised enough at low pH to allow macromolecules such as proteins to leak from the cell. To measure protein leakage, wild-type cells and strain 4R3 were cultured with and without phytosphingosine in the presence of [1-^{14}C]leucine. Under all conditions examined, less than 5% of the cellular protein

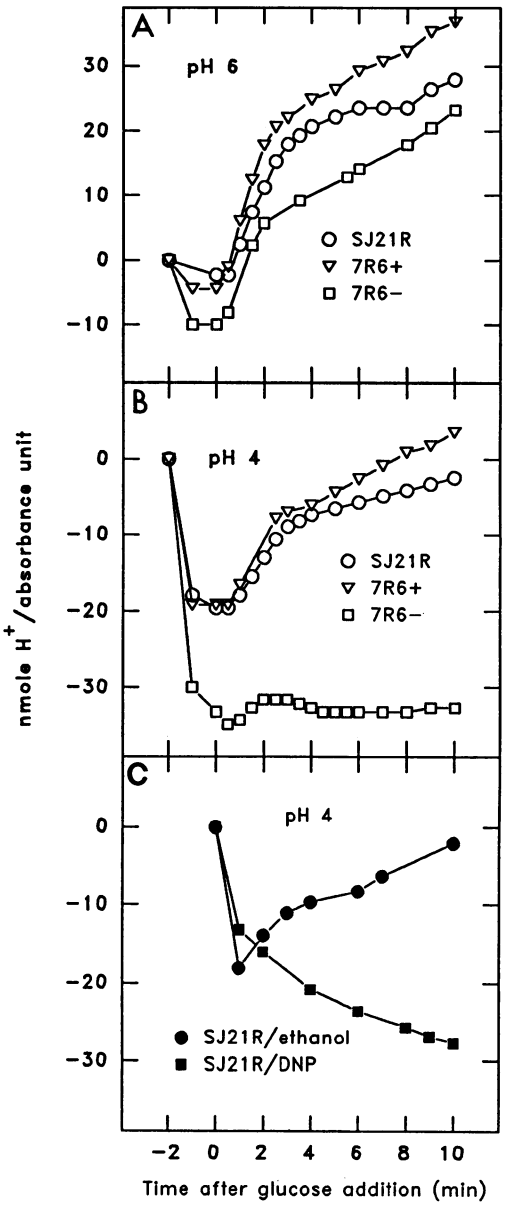

FIG. 2. Drastic reduction in proton extrusion in *SLC* strain 7R6 cultured without phytosphingosine at low pH. The extracellular pH of wild-type strain SJ21R and strain 7R6 cells suspended in weak buffers at pH 6 (A), at pH 4 (B), and at pH 4 in the presence of 2,4-dinitrophenol (DNP) (C) was monitored and converted to net proton flux as described in Materials and Methods. Glucose was added at time zero. Positive numbers indicate proton extrusion (acidification of the medium). 7R6+ and 7R6− refer to strain 7R6 grown in the presence and absence of phytosphingosine, respectively. SJ21R was cultured without a long-chain base.

became soluble (Table 1). These results indicate that the huge increase in proton permeability in sphingolipid-deficient *SLC* suppressor cells at low pH does not extend to macromolecules such as proteins. Hence, the change in proton permeability in sphingolipid-deficient cells was not the result of a massive rupture of the membrane.

Proton pumping is irreversibly damaged by low-pH treatment of sphingolipid-deficient cells. Wild-type cells and strain

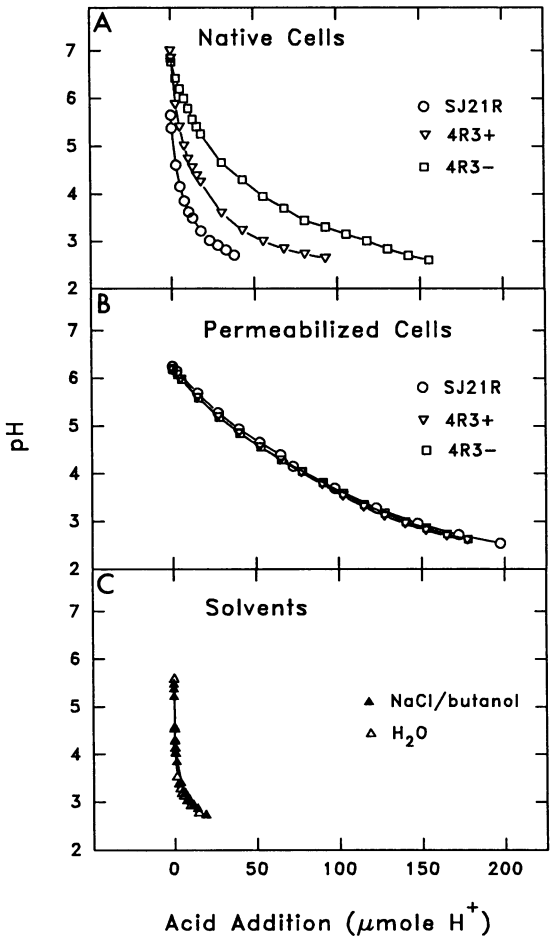

FIG. 3. Acid titration of wild-type and *SLC* cells. Native cells suspended in H_2O (A), cells permeabilized with 0.5 M NaCl–9% butanol (B), and the suspending fluids without cells (C) were titrated as described in Materials and Methods. Symbols: ○, SJ21R cultured without phytosphingosine; ▽, strain 4R3 cultured with phytosphingosine; □, strain 4R3 cultured without phytosphingosine.

4R3 cultured with or without phytosphingosine were exposed to pH 3 for 1 min, and then glucose-primed proton flux at pH 6 was measured. Only the sphingolipid-deficient 4R3 cells completely lost the ability to pump protons, with partial protection afforded by 1 M sorbitol during the pH 3 treatment (data not shown).

Proton efflux in sphingolipid-deficient cells is temperature-sensitive. The proton extrusion activities of wild-type cells and strain 4R3 cultured with and without phytosphingosine were monitored at 30 and 39°C in suspensions buffered at pH 6. The higher temperature had little effect on wild-type cells and sphingolipid-sufficient 4R3 cells, whereas there was an immediate 50 to 100% inhibition of net proton flux in sphingolipid-deficient 4R3 cells (data not shown). Treatment of sphingolipid-deficient 4R3 cells for 5 min at 39°C did not affect proton efflux when immediately measured at 30°C (data not shown). Although both high temperature and low pH inhibit proton extrusion in sphingolipid-deficient cells, the modes of action of these environmental stresses must differ in important aspects.

DISCUSSION

The striking parallel between the conditions that restrict growth of sphingolipid-deficient *SLC* cells (Fig. 1) and the conditions that restrict growth of some strains defective in *pma1*, the gene encoding the plasma membrane H^+-ATPase (7), suggested that the plasma membrane H^+-ATPase could be the target of environmental extremes in cells lacking sphingolipids. Therefore, we measured the ability of sphingolipid-deficient cells to carry out glucose-primed net proton extrusion at a low pH (Fig. 2) or at 39°C. Net proton extrusion was greatly impaired, and in addition, a rapid alkalinization occurred at low pH in sphingolipid-deficient cells (Fig. 2) of a magnitude consistent with almost complete permeability of the cells to protons (Fig. 3). These results indicate that the plasma membrane H^+-ATPase, which is an essential component of glucose-primed proton efflux (for reviews, see references 14 and 16), could be the direct target for the environmental stress-induced inhibition of proton efflux observed in sphingolipid-deficient cells. The simplest mechanism to explain our results is that the *SLC* mutation is in *PMA1* and that the variant H^+-ATPase produced no longer requires sphingolipid for function except at environmental extremes. We have ruled out this possibility because the *SLC1* gene, cloned and sequenced from strains 4R3 and 7R6, is not the *PMA1* gene (7a).

Other explanations that account for the observed behavior of *SLC* cells lacking sphingolipids can be categorized as direct effects on the H^+-ATPase molecule or effects elsewhere on the plasma membrane that indirectly affect the proton pumping system. Sphingolipids comprise almost a third of the plasma membrane phospholipids of *S. cerevisiae* (8), and some but not all of the unique *S. cerevisiae* sphingolipids (9) can effectively satisfy the well-known (6, 15) phospholipid requirement of the plasma membrane H^+-ATPase. Thus, when the H^+-ATPase is not directly complexed with sphingolipids, its function may be more sensitive to environmental extremes. Alternatively, the ATPase could become a proton channel when complexed with the wrong lipids, converting the membrane to an uncoupled state showing increased proton permeability under environmental extremes. In this regard, the purified H^+-ATPase has been shown recently to undergo a conformational change at low pH (1). Finally, it should be noted that the activity of the plasma membrane H^+-ATPase is strongly regulated in response to nutritional signals, probably via phosphorylation/dephosphorylation (3). It is possible to visualize sphin-

TABLE 1. Minor leakage of protein by sphingolipid-deficient *SLC* cells exposed to pH 3[a]

Strain	pH	% counts in supernatant[b]
SJ21R	3	3.4
	6	1.5
4R3+	3	3.1
	6	0.8
4R3−	3	2.0
	6	2.8

[a] Protein leakage was estimated from L-[^{14}C]leucine-labeled cells as described in Materials and Methods. SJ21R and 4R3− cells were grown in the absence of a long-chain base; 4R3+ cells were grown in the presence of phytosphingosine.

[b] The percentage of total cellular counts found in the supernatant after sedimentation of the cells.

golipid-dependent steps in this process that would be disrupted by environmental stresses in the absence of sphingolipids.

The simplest indirect mechanism to account for reduced proton pumping is one in which low pH triggers a change in the permeability to protons of the sphingolipid-deficient plasma membrane. Thus, no net proton efflux is observed because cells are permeable to protons. As for the mechanism of low-pH alkalinization, one could imagine creation of a proton channel in the sphingolipid-deficient plasma membrane as a result of pH-induced conformational changes in polypeptides ordinarily stabilized by sphingolipid. Another indirect explanation is that alkalinization arises by the action of a sphingolipid-deficient cation/H^+ antiporter, e.g., K^+/H^+ exchange (16), with the protons entering the cell down their concentration gradient. Finally, the loss of net proton extrusion activity in cells lacking sphingolipids could be due to indirect effects that block glucose transport or glycolysis, thereby preventing ATP formation which is necessary for proton extrusion.

In framing hypotheses to account for the low-pH phenomena in sphingolipid-deficient cells, it is important to note that there is no global alteration of membrane permeability, since little or no protein leaked from cells (Table 1). Furthermore, the low-pH-induced inhibition of net proton extrusion in sphingolipid-deficient cells is quite rapid and irreversible, whereas the temperature inhibition of net proton efflux appears to be rapid but reversible, suggesting different underlying mechanisms. Continuing biochemical and genetic studies of *SLC* cells lacking sphingolipids should identify the molecular linkage between sphingolipids and proton permeability and proton efflux in *S. cerevisiae*.

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REFERENCES

1. **Blanpain, J., M. Ronjat, P. Supply, J. Dufour, A. Goffeau, and Y. Dupont.** 1992. The yeast plasma membrane H^+-ATPase. An essential change of conformation triggered by H^+. J. Biol. Chem. **267**:3735–3740.
2. **Buede, R., C. Rinker-Schaffer, W. J. Pinto, R. L. Lester, and R. C. Dickson.** 1991. Cloning and characterization of *LCB1*, a *Saccharomyces* gene required for biosynthesis of the long-chain base component of sphingolipids. J. Bacteriol. **173**:4325–4332.
3. **Chang, A., and C. W. Slayman.** 1991. Maturation of the yeast plasma membrane [H^+]ATPase involves phosphorylation during intracellular transport. J. Cell. Biol. **115**:289–295.
4. **Cid, A., and R. Serrano.** 1988. Mutations of the yeast plasma membrane H^+-ATPase which cause thermosensitivity and altered regulation of the enzyme. J. Biol. Chem. **263**:14134–14139.
5. **Dickson, R. C., G. B. Wells, A. Schmidt, and R. L. Lester.** 1990. Isolation of mutant *Saccharomyces cerevisiae* strains that survive without sphingolipid. Mol. Cell. Biol. **10**:2176–2181.
6. **Malpartida, F., and R. Serrano.** 1980. Purification of the yeast plasma membrane ATPase solubilized with a novel zwitterionic detergent. FEBS Lett. **111**:69–72.
7. **McCusker, J. H., D. S. Perlin, and J. E. Haber.** 1987. Pleiotropic plasma membrane ATPase mutations of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:4082–4088.

7a. **Nagiec, M., et al.** Unpublished data.

8. **Patton, J. L., and R. L. Lester.** 1991. The phosphoinositol sphingolipids of *Saccharomyces cerevisiae* are highly localized in the plasma membrane. J. Bacteriol. **173**:3101–3108.
9. **Patton, J. L., and R. L. Lester.** 1992. Phosphatidylinositol phosphate, phosphatidylinositolbisphosphate, and the phosphoinositol sphingolipids are found in the plasma membrane and stimulate the plasma membrane H^+-ATPase of *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. **292**:70–76.
10. **Pinto, W. J., B. Srinivasan, S. Shepherd, A. Schmidt, R. C. Dickson, and R. L. Lester.** 1992. Sphingolipid long-chain base auxotrophs of *Saccharomyces cerevisiae*: genetics, physiology, and a method for their selection. J. Bacteriol. **174**:2565–2574.
11. **Pinto, W. J., G. B. Wells, and R. L. Lester.** 1992. Characterization of enzymatic synthesis of sphingolipid long-chain bases in *Saccharomyces cerevisiae*: mutant strains exhibiting long-chain base auxotrophy are deficient in serine palmitoyltransferase activity. J. Bacteriol. **174**:2575–2581.
12. **Rodriguez-Navarro, A., and E. D. Sancho.** 1979. Cation exchanges of yeast in the absence of magnesium. Biochim. Biophys. Acta **552**:322–330.
13. **Serrano, R.** 1980. Effect of ATPase inhibitors on the proton pump of respiratory-deficient yeast. Eur. J. Biochem. **105**:419–424.
14. **Serrano, R., A. Cid, J. M. Pardo, F. Portillo, and C. J. Vallejo.** 1989. Molecular biology of plasma membrane H^+-ATPase, p. 439–448. *In* J. Dainty, M. I. De Michelis, E. Marre, and F. Rasi-Caldogno (ed.), Plant membrane transport: the current position. Elsevier, Amsterdam.
15. **Serrano, R., C. Montesions, and J. Sanchez.** 1988. Lipid requirements of the plasma membrane ATPase from oat roots and yeast. Plant Sci. **56**:117–122.
16. **Sigler, K., and M. Hoefer.** 1991. Mechanisms of acid extrusion in yeast. Biochim. Biophys. Acta **1071**:375–391.
17. **Smith, S. W., and R. L. Lester.** 1974. Inositolphosphorylceramide, a novel substance and the chief member of a major group of yeast sphingolipids containing a single inositolphosphate. J. Biol. Chem. **249**:3395–3405.
18. **Steiner, S., and R. L. Lester.** 1972. Studies on the diversity of inositol-containing yeast phospholipids: incorporation of 2-deoxyglucose into lipid. J. Bacteriol. **109**:81–88.
19. **Steiner, S., S. Smith, C. J. Waechter, and R. L. Lester.** 1969. Isolation and partial characterization of a major inositol-containing lipid in baker's yeast: mannosyl-diinositol, diphosphoryl-ceramide. Proc. Natl. Acad. Sci. USA **64**:1042–1048.
20. **Wells, G. B., and R. L. Lester.** 1983. The isolation and characterization of a mutant strain of *Saccharomyces cerevisiae* that requires a long chain base for growth and for synthesis of phosphosphingolipids. J. Biol. Chem. **258**:10200–10203.