Consequences of Loss of Vph1 Protein-containing Vacuolar ATPases (V-ATPases) for Overall Cellular pH Homeostasis*

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In yeast cells, subunit a of the vacuolar proton pump (V-ATPase) is encoded by two organelle-specific isoforms, VPH1 and STV1. V-ATPases containing Vph1 and Stv1 localize predominately to the vacuole and the Golgi apparatus/endosomes, respectively. Ratiometric measurements of vacuolar pH confirm that loss of STV1 has little effect on vacuolar pH. Loss of VPH1 results in vacuolar alkalinization that is even more rapid and pronounced than in vma mutants, which lack all V-ATPase activity. Cytosolic pH responses to glucose addition in the vph1Δ mutant are similar to those in vma mutants. The extended cytosolic acidification in these mutants arises from multiple mechanisms for limiting Pma1 activity when organelle acidification is compromised. pH measurements in early pre-vacuolar compartments via a pHluorin fusion to the Golgi protein Gef1 demonstrate that pH responses of these compartments parallel cytosolic pH changes. Surprisingly, these compartments remain acidic even in the absence of V-ATPase function, possibly as a result of cytosolic acidification. These results emphasize that loss of a single subunit isoform may have effects far beyond the organelle where it resides.

Vacuolar proton-translocating ATPases (V-ATPases) acidify multiple organelles, including mammalian lysosomes, plant and fungal vacuoles, the Golgi apparatus, endosomes, and regulated secretory granules. Through their effects on organelle acidification, V-ATPases impact numerous cellular processes including protein sorting, macromolecular degradation, cytosolic pH and ion homeostasis, and nutrient storage and mobilization (1,2). Consistent with these diverse roles, complete loss of V-ATPase function is lethal in most organisms. Fungi, however, can tolerate a complete loss of V-ATPase function when organelle acidification is compromised. pH measurements in early pre-vacuolar compartments via a pHluorin fusion to the Golgi protein Gef1 demonstrate that pH responses of these compartments parallel cytosolic pH changes. Surprisingly, these compartments remain acidic even in the absence of V-ATPase function, possibly as a result of cytosolic acidification. These results emphasize that loss of a single subunit isoform may have effects far beyond the organelle where it resides.

V-ATPases are highly conserved both at the level of individual subunit sequences and at an overall structural level. A complex of peripheral membrane subunits containing the sites of ATP hydrolysis, V1, is attached to an integral membrane complex, V0, containing the proton pore. In higher eukaryotes, many of the subunits are present as multiple isoforms, encoded as multiple genes and/or splice variants (5). These subunit isoforms exhibit tissue-specific expression and/or organelle-specific localization, and in some cases, impart different biochemical characteristics on V-ATPases, possibly tuning their activity to the requirements of different locales (2). Subunit a of the V0 sector is present as multiple isoforms in many organisms. Humans have four different subunit a genes (designated ATP6V0a1–a4) (2). In S. cerevisiae, subunit a is the only subunit encoded by more than one gene. VPH1 encodes the most highly expressed subunit a isoform, which is localized primarily to the vacuole (6), and STV1 encodes a second isoform that is believed to cycle between endosomes and the Golgi apparatus (7,8). Deletion of VPH1 and STV1 genes individually causes a partial Vma− phenotype, and deletion of both genes mimics the effects of deletion of any of the single isoform V-ATPase subunits (7).

The absence of Stv1-containing V-ATPases in the vacuole creates an opportunity to distinguish the contributions of V-ATPases localized to the vacuole. vph1Δ mutants appear to lose all (6) or most (9) V-ATPase activity in isolated vacuoles. The vph1Δ mutants show strong sensitivity to heavy metals such as Zn2+, reflecting the critical role of the vacuole in metal ion detoxification, but are less sensitive to elevated extracellular pH and Ca2+ than other vma mutants (6). Overexpression of STV1 can partially compensate for loss of VPH1 and reconstitute functional V-ATPases at the vacuolar membrane (7), but these Stv1-containing V-ATPase complexes have significant biochemical differences from Vph1-containing complexes, suggesting that the subunit isoforms are not completely functionally interchangeable (10). In contrast, the stv1Δ mutant has a minimal effect on cell growth, but this has been attributed to VPH1 partially compensating for loss of STV1 as Vph1-containing V-ATPases naturally transit through the Golgi apparatus and endosomes en route to the vacuole (11).

In this work, we probe the contributions of V-ATPases at the vacuole to overall pH homeostasis. Using ratiometric fluorescent probes for vacuolar and cytosolic pH, we previously observed striking differences in pH homeostasis between wild-type cells and vma mutants (12). As expected, the mutants had elevated vacuolar pH, but they also exhibited lower cytosolic...
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pH than wild-type cells, particularly upon resumption of glucose metabolism. This may arise in part from internalization of the plasma membrane proton pump, Pma1, in the mutants (12–14). However, perturbation of organelle and cytosolic pH is observed without mislocalization of Pma1 when cells are treated with the V-ATPase inhibitor concanamycin A (12), indicating an unexpected level of coordination between plasma membrane and organelar proton pumps. These results raised the question of how and why Pma1 is mislocalized in the vma mutants and where the signal for mislocalization initiates. Here we show that loss of vacuolar acidification in a vph1Δ mutant is not sufficient to trigger mislocalization of Pma1 but that loss of Vph1 renders the vacuole even more vulnerable to alkalinization than loss of all V-ATPase activity in a vma mutant. Reduced Pma1 activity at the surface may also help to lower the pH of prevacuolar compartments, even in the absence of V-ATPase activity.

EXPERIMENTAL PROCEDURES

Materials—2′,7′-bis-(2-Carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxyethyl ester (BCECF-AM) and Alexa Fluor 488 goat anti-mouse antibody used for immunofluorescence were purchased from Invitrogen. Zymolase-100T was purchased from United States Biological. MES was obtained from GenScript. Pepptone, yeast extract, and glucose were purchased from Fisher, and yeast nitrogen base was from Difco. Concacnamycin A, Ficoll PM400, and other chemicals were from Sigma.

Strains and Media—The wild-type yeast strain used in these experiments was BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0). The mutants were congenic with this strain and included the vph1Δ (same genotype as BY4741 except vph1Δ0::kanMX), stv1Δ (BY4741 genotype except stv1Δ0::kanMX), and vma3Δ (BY4741 genotype except vma3Δ0::kanMX) strains. All of these strains were purchased from Open Biosystems. The vph1Δstv1Δ double mutant (MM112, genotype: MATa his3-200 leu2 lys2 ura3-52 stv1Δ::LYS2 vph1Δ::LEU2) was constructed previously (7). For pH measurement in prevacuolar compartments, wild-type strain SF838-5Aa and congenic vmaΔ and vph1Δ mutants were used because the met15 mutation in the By4741 background precludes use of the MET-inducible promoter. Yeast extract/peptone/dextrose (YPD) medium and synthetic complete (SC) medium were prepared as described (15).

Measurement of Vacuolar, Cytosolic, and Golgi/Endosome pH—Vacuolar pH was measured using the ratiometric fluorescent dye BCECF-AM as described (12, 16). Briefly, wild-type and mutant cells were grown to early to mid-log phase in YEPD buffered to pH 5 and then loaded with BCECF-AM in the same medium. After washing to remove dye, cells were resuspended in YEPD buffered to pH 5 and deprived of glucose on ice for 30 min. For fluorescence measurement, the cell suspension was diluted into 1 mM MES pH 5.0 buffer, and fluorescence intensity at emission wavelength 535 nm and at excitation wavelengths 450 and 495 nm was monitored continuously in a SPEX Fluorolog 3–21 fluorometer. The fluorescence ratio for each sample was calibrated for each strain in every experiment by clamping the pH to a range of values from 5.0 to 7.5 as described (12), and the resulting calibration curve was used to convert the experimental fluorescence ratios to vacuolar pH.

Cytosolic pH was monitored using a pH-sensitive GFP, yeast pHluorin (17), expressed from a plasmid under control of the yeast phosphoglycerate kinase promoter. (This plasmid was a generous gift from Dr. Rajini Rao, Department of Physiology, Johns Hopkins University.) Yeast cells were transformed with this plasmid, and transformants were selected on SC medium lacking uracil (SC-uracil). Measurement of cytosolic pH responses was carried out as described (12, 16, 17). Cells were grown to mid-log phase in SC-uracil buffered to pH 5 with 50 mM MES, washed, and deprived of glucose for ~30 min. Fluorescence intensities at emission wavelength 508 nm and at excitation wavelengths 405 nm and 485 nm were measured continuously and calibrated to pH as described (12, 16, 17).

Measurement of pH in prevacuolar compartments was achieved with a pHluorin-halorhodopsin-Gef1 (pHluorin-HR-Gef1) construct. This construct was recently described by Braun et al. (18) and was a generous gift from Blanche Schwappach, University of Goettingen. The reporter is expressed from a URA3-marked plasmid, under control of the inducible MET25 promoter. Wild-type and vph1Δ mutant cells transformed with the plasmid were grown overnight in SC medium buffered to pH 5 with 50 mM MES that lacked both uracil and methionine; the vmaΔ mutant was grown for 2 days under these induction conditions to achieve comparable levels of expression. Induction of probe expression had no effect on growth rate of wild-type or mutant cells used for our experiments. To ensure that cells were in log phase at the time of measurement, they were diluted ~1:2 with fresh medium and allowed to double immediately before the assay. As shown in Fig. 5 and described by Braun et al. (18), the pHluorin-HR-Gef1 is localized to numerous dots in the cytosol, clearly distinct from vacuolar staining in the BCECF-labeled cells and the cytosolic staining of pHluorin-containing cells (17, 19). Fluorescence intensities at excitation wavelengths 400 and 481 nm and at emission wavelength 508 nm were measured in a cell suspension and calibrated to pH as described above for cytosolic pHluorin, except that a fluorescence-matched suspension of untransformed cells was measured in parallel and subtracted to correct for background. (This step was necessary because of lower signal from the pHluorin-HR-Gef1 construct; background signals are ~50% of the total signal for all strains.) A pH calibration curve for the pHluorin-HR-Gef1 construct expressed in wild-type cells is shown in Fig. 5A.

Biochemical Methods—Vacuolar vesicles were isolated and assayed for ATP hydrolysis and proton pumping as described (20, 21). Activity sensitive to 100 nM concanamycin A was taken as V-ATPase activity. For assessment of Pma1 activity, partially purified plasma membrane fractions were isolated and assayed for ATP hydrolysis as described (12); activity in the presence and absence of 100 μM sodium vanadate was compared to obtain Pma1 activity. Specific activity is obtained by expressing vanadate-sensitive activity divided by the total mass of membrane protein in the assay. For immunoblots of Pma1, equal amounts of plasma membrane protein from wild-type and mutant cells were solubilized in cracking buffer (50 mM Tris-HCl, pH 6.8, 8 M urea, 5% SDS, 5% β-mercaptoethanol) at 65°C.
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for 15 min, separated by SDS-PAGE, and transferred to nitrocellulose. Pma1 was detected on immunoblots with rabbit polyclonal antibodies raised against Pma1 that were a generous gift of Amy Chang, University of Michigan, followed by alkaline phosphatase-conjugated goat anti-rabbit antibody.

For measurement of proton export, cells were grown to log phase in YEPD buffered to pH 5. The cells were pelleted, washed, and resuspended at high density in YEP at pH 5 before adding 50 A600 units of cells into 3.6 ml of 1 mM MES, pH 5 buffer (12). In Fig. 3A, glucose was added to 50 mM final concentration at t = 0 min, and extracellular pH was monitored continuously with a pH meter.

Fluorescence Microscopy—For visualization of Pma1, cells were fixed and permeabilized for immunofluorescence microscopy as described (12), 20). Fixed cells were incubated with mouse monoclonal antibody 40B7 (Abcam) against Pma1 followed by Alexa Fluor 488-conjugated goat anti-mouse antibodies and then visualized under fluorescein optics on a Zeiss Imager. Z1 fluorescence microscope. All fluorescence images shown were taken under identical exposure. The pHluorin-HR-Gef1 probe was visualized in live cells using Nomarski optics or a GFP filter set.

RESULTS

Vph1-containing V-ATPase Complexes Are Essential for Vacuolar Acidification—Although vacuolar vesicles isolated from a vph1Δ strain were initially reported to be completely devoid of bafilomycin-sensitive ATPase activity unless STV1 was overexpressed from a multicopy plasmid (7), a later study suggested that compensatory overproduction of Stv1 protein from its genomic locus provided some V-ATPase to the vacuole in that compensatory overproduction of Stv1 protein from its genomic locus provided some V-ATPase to the vacuole in vacuolar vesicles from one of the wild-type strains used most commonly in the original studies from Manolson et al. (6, 7), we found that the level of concanamycin A-sensitive ATPase activity in vacuolar vesicles isolated from the vph1Δ mutant was very low (0.12 ± 0.08 μmol/min/mg (n = 4) for vph1Δ vacuoles versus 3.5 ± 0.46 μmol/min/mg (n = 3) for the wild-type vacuoles). This is comparable with the level of activity observed in vma mutants lacking single isoform V-ATPase subunits, indicating that there is little or no compensatory activity of Stv1-containing V-ATPase complexes at the vacuole. In contrast, both V-ATPase activity (3.0 ± 0.40 μmol/min/mg (n = 3)) and proton-pumping activities in vacuolar vesicles isolated from the stv1Δ mutant were comparable with those from wild-type cells.

We next tested the effects of the vph1Δ and stv1Δ mutations on vacuolar acidification in vivo. Vacuolar pH can be quantitated using the ratiometric fluorescent indicator BCECF-AM, which localizes to the vacuole in yeast and exhibits a pH-dependent excitation spectrum (12, 19). Wild-type cells were grown to log phase in YEPD buffered to pH 5 with MES and loaded with BCECF-AM and then subjected to an ~30-min glucose deprivation before fluorescence measurement. For measurement of vacuolar pH response kinetics, fluorescence intensities were monitored continuously, and glucose was added to a final concentration of 50 mM after 60 s. As shown in Fig. 1A and reported previously, we saw a drop in pH after glucose addition in wild-type cells after a delay of ~60 s. This drop is completely dependent on V-ATPase activity, and in wild-type cells, is likely to reflect, at least in part, glucose-induced reassembly of the enzyme (12). Consistent with this interpretation, a vma3Δ mutant shows a rise in vacuolar pH after glucose readdition (Fig. 1A), and a mutant lacking both VP1 and STV1 behaved similarly (data not shown). In contrast, the vph1Δ mutant also shows a rise in vacuolar pH after glucose deprivation, but this rise was consistently faster than in the vma3Δ mutant, and the final vacuolar pH was higher (Fig. 1A). Vacuolar pH responses in the stv1Δ mutant were very similar to that of wild-type cells, consistent with the near wild-type V-ATPase activity in isolated vacuoles. Fig. 1A shows representative fluorescence traces for these experiments. In Fig. 1B, the vacuolar pH values at 50 s (just before glucose addition), at 165 s after glucose addition (corresponding to t = 225 s in Fig. 1A), and at 5 min after glucose addition (t = 360 s) are compared across several experiments. From this comparison, it is clear that not only are the vacuoles of the vph1Δ mutant not acidified upon glucose addition, they actually become significantly more alkaline than the vacuoles of the vma3Δ mutant, which has no cellular V-ATPase activity.

Loss of Vph1-containing V-ATPase Complexes Results in Poor Control of Cytosolic pH—Cytosolic pH responses can also be measured in live yeast cells using the expressed ratiometric fluorescence indicator yeast pHluorin (12, 17, 22). For these
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FIGURE 2. Kinetics of cytosolic pH responses in wild-type and mutant cells. Cytosolic pH was monitored continuously in suspensions of wild-type (wt), vma3\(\Delta\), and vph1\(\Delta\) cells transformed with a cytosolic pH-sensitive GFP (pHluorin), as described under “Experimental Procedures.” A, kinetics of cytosolic pH responses for a single representative experiment in each mutant are compared. Cells had been glucose-deprived for \(~30\) min at the beginning of the experiment, and glucose was added to a final concentration of 50 mM at \(t = 60\) s. B, comparison of cytosolic pH measurements at defined times from several independent experiments as those shown in A. The mean cytosolic pH at the indicated time points of 3–6 experiments for each strain is shown. Error bars correspond to S.E. over 3 experiments.

Pma1p is the major exporter of cytosolic protons produced by cellular metabolism. In whole cells, Pma1 is activated by the presence of glucose (24, 25), so acidification of the extracellular medium in response to glucose addition provides an initial measure of Pma1p activity in vivo. As shown in Fig. 3A, wild-type yeast cells show a rapid decline in extracellular pH upon glucose addition and an additional decline upon KCl addition. Entry of K\(^+\) ions depolarizes the plasma membrane and thus stimulates Pma1 activity (26). The vph1\(\Delta\) and vma3\(\Delta\) mutants had a longer delay after glucose addition before external pH began to decline, consistent with the prolonged cytosolic acidification observed (Fig. 2). However, the addition of KCl accelerated export in the vph1\(\Delta\) mutant so that it reached a final pH of 5.2 within another 2 min (data not shown). The vma3\(\Delta\) mutant, which has no cellular V-ATPase activity, continued to export protons very slowly after KCl addition.

FIGURE 3. vph1\(\Delta\) mutants show reduced glucose-activated proton export. A, wild-type (○), vph1\(\Delta\) (●), and vma3\(\Delta\) (▲) cells were grown to log phase, washed, and suspended in a weakly buffered solution lacking glucose. At time 0, glucose was added to a 2% final concentration, and changes in extracellular pH were monitored. KCl (final concentration 50 mM) was added 3 min after the measurements began, and the pH measurement was continued. Results of a single measurement for each strain, representative of three more independent experiments, are shown. B, reductions in Pma1 protein levels in isolated plasma membranes do not fully account for reduced ATPase activity of vph1\(\Delta\). An immunoblot of plasma membrane fractions from wild type, vph1\(\Delta\), and vma3\(\Delta\) mutants probed with anti-Pma1 antibody is shown. Equal amounts of total plasma membrane protein are loaded in each lane. The intensity of Pma1 protein bands was quantitated using the National Institutes of Health ImageJ software, and the intensities of all three samples were normalized to wild type. Relative activities for this plasma membrane preparation are shown for comparison; in this representative experiment, the ATPase activity in the wild-type sample was 1.7 \(\mu\)mol/min/mg of total protein.
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in wild-type cells and shows a similar localization in the vph1Δ or stv1Δ single mutant strains. In contrast, a vph1Δstv1Δ double mutant shows a substantial loss of Pma1p immunofluorescence at the plasma membrane and the appearance of fluorescence in the vacuole and other intracellular compartments (Fig. 4). This pattern of localization is similar to that in a vma2Δ or vma3Δ mutant strain (12). This indicates that the loss of vacuolar acidification observed in a vph1Δ mutant does not result in Pma1 mislocalization. Furthermore, cytosolic acidification in the vph1Δ mutant may arise in part from reduced levels of Pma1p activity at the plasma membrane, but there appears to be little depletion of Pma1 protein from the membrane.

Prevacuolar Compartments Are Mildly Acidic in the Absence of V-ATPase Activity—Other prevacuolar acidic compartments such as the Golgi apparatus may also be affected directly or indirectly by loss of V-ATPase activity but have been inaccessible to direct pH measurement in yeast. We used a new pHluorin-based reporter (pHluorin-HR-Gef1) reported by Braun et al. (18) to assess pH responses in a subset of prevacuolar compartments. This reporter is targeted by fusion to Gef1, which is localized to the Golgi apparatus and other prevacuolar compartments (27, 28). The pHluorin fusion is under control of the inducible MET25 promoter and complemented the growth defects of a gef1Δ strain (18), suggesting that it was functional and that induced overexpression did not dramatically alter cellular pH homeostasis. This is consistent with previous functional studies with overexpressed wild-type GEF1 (28). As shown in Fig. 5, the localization of pHluorin-HR-Gef1 is clearly distinct from the cytosol and the vacuole. Steady state pH measurements with this probe indicated that the compartments occupied maintained a slightly more acidic pH than the cytosol (18). We asked how the pH of these compartments responded to a brief glucose deprivation and readdition. As shown in Fig. 5, B and C, the pH response of the pHluorin-HR-Gef1 probe in wild-type cells resembled the cytosolic pH response, with a transient acidification immediately after glucose addition followed by alkalinization to a level ~0.2 pH units less than the cytosol. We repeated these experiments with a vma mutant (vma2Δ), which lacks all V-ATPase activity (Fig. 5, B and C). Surprisingly, we consistently measured a significantly more acidic pH for the pHluorin-HR-Gef1 in vma2Δ mutants than in wild-type cells. In the vph1Δ mutant, where Golgi-localized Stv1-containing complexes should be active, the pH was intermediate between the vma2Δ and wild-type cells. These results have several implications. First, the pHluorin-HR-Gef1-containing compartment is only slightly acidic in wild-type cells, in agreement with previous measurements (18). Second, in contrast to the vacuole, this compartment becomes more alkaline following glucose readdition to glucose-deprived cells. Third, V-ATPases are not absolutely required for acidification of these compartments because complete loss of V-ATPase activity does not abolish organelle acidification. In fact, these compartments may be even more acidic in vma mutants than in wild-type cells.

DISCUSSION

Consistent with previous work indicating that Vph1-containing V-ATPases are the major determinants of vacuolar pH
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(7, 10, 11), we see no evidence of any vacuolar acidification in the vph1Δ mutant. We were therefore able to examine the consequences of loss of vacuolar acidification on cellular pH homeostasis under conditions where activity of Stv1p-containing V-ATPases should be maintained. We previously showed that the addition of glucose to glucose-deprived cells results in a V-ATPase-dependent drop in vacuolar pH that is replaced by a pH rise in the vma mutants (12). An initial surprise from this work was that the kinetics of vacuolar pH responses, which are very similar among the vma mutants, differ significantly in the vph1Δ mutant. As in the vma mutants, the addition of glucose increases vacuolar pH in the vph1Δ mutant. However, this rise is consistently faster in the vph1Δ mutant than in the vma3Δ mutant. This suggests that factors other than the loss of V-ATPase activity at the vacuole are contributing to the alkalinization.

The increase in vacuolar pH upon glucose addition in both the vph1Δ and the vma mutants likely reflects both the activity of the many transporters that utilize vacuolar pH gradient to drive transport (1) and the buffering capacity of the vacuole. There is no question that the vma3Δ deletion should have a more profound impact on overall organelle pH homeostasis than the vph1Δ mutation because all cellular V-ATPase activity is lost. However, it is possible that the severity of the defect in the vma3Δ mutant induces compensatory effects that are not triggered in the vph1Δ mutant, including loss of Pma1p from the plasma membrane. Combined with this pressure toward proton loss from the vacuole, activation of Pma1p at the plasma membrane in response to glucose could further deplete protons transported from the vacuole into the cytosol. Thus in the vma3Δ mutant, although there is no V-ATPase activity to oppose proton export from the vacuole, proton export from the cell is also slowed when Pma1 is down-regulated. Interestingly, Bowman et al. (30) previously isolated suppressors of concanamycin A sensitivity in Neurospora crassa and found that the suppressor mutations mapped to Pma1 and reduced its activity. Although vacuolar pH was not measured directly, the authors documented little or no restoration of vacuolar acidification in the concanamycin A-resistant N. crassa mutant. This suggests that factors other than the loss of V-ATPase activity at the vacuole. Instead, down-regulation of Pma1 activity in N. crassa by mutation may accomplish the same thing as mislocalization of Pma1 in yeast: reducing proton export to partially normalize overall cellular pH homeostasis. The persistence of Pma1 at the plasma membrane in the vph1Δ cells may help account for the more rapid rise in vacuolar pH in vph1Δ cells. However, the rapid alkalinization of the vacuole in the vph1Δ mutant begins while the cytosol is still as acidic, as in vma mutants. An additional factor may be the profound loss of polyphosphate, the major vacuolar buffering system, in the vph1Δ mutant (31). Although all vma mutants also have low polyphosphate levels (31), vph1Δ has even lower levels than the vma mutants, which may contribute to the rapid initial pH increase.

One consequence of Pma1 down-regulation is slow cytosolic pH response to glucose, resulting in prolonged cytosolic acidification. The data presented here with a Gef1-targeted pHluorin raise the possibility that cytosolic acidification could also help to maintain acidification of prevacuolar compartments. The observation that the pHluorin-HR-Gef1-containing compartments are actually more acidic in a vma mutant than in wild-type cells is very surprising. Stv1 has been localized to the Golgi, and Stv1-containing V-ATPases are believed to be responsible for Golgi acidification (7, 8). Stv1-containing V-ATPases do not increase assembly or activity in response to glucose addition as much as Vph1-containing V-ATPases (10), so we would not necessarily expect acidification in response to glucose addition. However, we see little evidence of V-ATPase-dependent acidification of the pHluorin-HR-Gef1-containing compartment.
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Notably, the presence of either Vph1-containing or Stv1-containing V-ATPase complexes is sufficient to prevent the Pma1 mistargeting seen in vma2Δ and vph1Δ double mutants (Fig. 4). Despite elevated vacuolar pH in the vph1Δ mutant, Pma1 remains at the plasma membrane, indicating that loss of vacuolar acidification cannot be the trigger for Pma1 mislocalization. Cytosolic pH is slower to recover after glucose deprivation in both vma2Δ and vph1Δ mutants, arguing that cytosolic acidification is unlikely to be a trigger. Taken together, these data suggest that perturbed pH control in another organelle, possibly a prevacuolar compartment, may signal Pma1 mislocalization. Interestingly, it has been proposed previously that the pH gradient across the multivesicular body might be sensed as part of the ambient pH-sensing pathway (34, 35). We do not believe that the pHluorin-HR-Gef1 construct reaches the multivesicular body because we do not see an accumulation of probe in the elaborated class E compartment (36) adjacent to the vacuole in the vps27Δ mutant (data not shown), so we have not yet measured the pH of this compartment. Both Stv1-containing and Vph1-containing V-ATPases have been detected in the multivesicular body and class E compartment (8, 37). It is intriguing to speculate that loss of the pH gradient across the membrane of the multivesicular body or other endosomal compartments might signal both an intracellular failure of organelle acidification and an extracellular alkalinization. Further experiments will be necessary to test this hypothesis and identify the signaling pathway.

Interestingly, even without mislocalization, activation of Pma1 upon glucose addition appears to be compromised in the vph1Δ mutant (Fig. 3A) and Pma1 activity in isolated plasma membrane fractions is reduced, indicating that activity of these pumps may be coordinated at multiple levels. Proton export from the vph1Δ cells was restored by the addition of extracellular K⁺ ions. Hyperpolarization of the plasma membrane in the vph1Δ mutant could inhibit proton export through Pma1 and be relieved by K⁺ import (38). However, hyperpolarization is unlikely to account for the significantly reduced activity of Pma1 in isolated plasma membranes from the vph1Δ mutant. Instead, it may be that the vph1Δ mutation also compromises glucose signaling to Pma1. Significantly, recent work has suggested that the V-ATPase may play a role in mediating certain glucose signals (29); it is possible that the pathways that activate Pma1 in response to glucose are affected by the vph1Δ mutation. Cytosolic acidification without Pma1 mislocalization was also observed after a brief treatment with the V-ATPase inhibitor concanamycin A (12), also suggesting a second mechanism for down-regulation of Pma1 activity.

There is considerable interest in understanding the contributions of organelle-specific subunit isoforms to V-ATPase function, particularly in higher organisms where isoform composition is more complex. This work indicates that experiments seeking to understand isoform function through deletion or knockdown of individual isoforms must be interpreted with care because loss of pH control in one part of the cell can clearly impact overall cellular pH homeostasis.

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