Yeast V-ATPase Complexes Containing Different Isoforms of the 100-kDa a-subunit Differ in Coupling Efficiency and in Vivo Dissociation*

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The 100 kDa a-subunit of the yeast vacuolar (H\(^{+}\))-ATPase (V-ATPase) is encoded by two genes, VPH1 and STV1. These genes encode unique isoforms of the a-subunit that have previously been shown to reside in different intracellular compartments in yeast. Vph1p localizes to the central vacuole, whereas Stv1p is present in some other compartment, possibly the Golgi or endosomes. To compare the properties of V-ATPases containing Vph1p or Stv1p, Stv1p was expressed at higher than normal levels in a strain disrupted in both genes, under which conditions V-ATPase complexes containing Stv1p appear in the vacuole. Complexes containing Stv1p showed lower assembly with the peripheral V\(_1\) domain than did complexes containing Vph1p. When corrected for this lower degree of assembly, however, V-ATPase complexes containing Vph1p and Stv1p had similar kinetic properties. Both exhibited a \(K_m\) for ATP of about 250 \(\mu M\), and both showed resistance to sodium azide and vanadate and sensitivity to nanomolar concentrations of concanamycin A. Stv1p-containing complexes, however, showed a 4–5-fold lower ratio of proton transport to ATP hydrolysis than Vph1p-containing complexes. We also compared the ability of V-ATPase complexes containing Vph1p or Stv1p to undergo in vivo dissociation in response to glucose depletion. Vph1p-containing complexes present in the vacuole showed dissociation in response to glucose depletion, whereas Stv1p-containing complexes present in their normal intracellular location (Golgi/endosomes) did not. Upon overexpression of Stv1p, Stv1p-containing complexes present in the vacuole showed glucose-dependent dissociation. Blocking delivery of Vph1p-containing complexes to the vacuole in \(vps21\Delta\) and \(vps27\Delta\) strains caused partial inhibition of glucose-dependent dissociation. These results suggest that dissociation of the V-ATPase complex in vivo is controlled both by the cellular environment and by the 100-kDa a-subunit isoform present in the complex.

The vacuolar (H\(^{+}\))-ATPases (or V-ATPases) are a family of ATP-dependent proton pumps found in a variety of intracellular compartments that function in both endocytic and secretory pathways (1–8). Acidification of these compartments is essential for many cellular processes, including receptor-mediated endocytosis, intracellular targeting, protein processing and degradation, and coupled transport. V-ATPases are also present in the plasma membrane of certain specialized cells, including osteoclasts (9), renal intercalated cells (10), and neutrophils (11), where they function in such processes as bone resorption, renal acidification, and pH homeostasis, respectively.

The V-ATPases from fungi, plants, and animals are structurally very similar and are composed of two domains (1–8). The V\(_1\) domain is a peripheral complex of molecular mass 570 kDa composed of eight different subunits of molecular mass 70–14 kDa (subunits A-H) that is responsible for ATP hydrolysis. The V\(_0\) domain is a 260-kDa integral complex composed of five subunits of molecular mass 100–17 kDa (subunits a, d, c, c’ and c”’) that is responsible for proton translocation. These structures are similar to that of the ATP synthases (or F-ATPases) that function in ATP synthesis in mitochondria, chloroplasts, and bacteria (12–17). Sequence homology between these classes of ATPase has been identified for both the nucleotide binding subunits (18, 19) and the proteolipid subunits (subunits c, c’, and c’’’) (20, 21). Subunit G has also been shown to have some homology to subunit b of the F-ATPases (22).

The 100-kDa a-subunit of the V-ATPase is an integral membrane protein possessing an amino-terminal hydrophilic domain and a carboxyl-terminal hydrophobic domain containing multiple putative membrane-spanning segments (23–25). In yeast, the 100-kDa subunit is encoded by two genes, VPH1 and STV1. Vph1p and Stv1p are homologous proteins displaying 54% identity and 71% similarity (23, 24). These proteins show distinct intracellular localization, with Vph1p localized to the vacuole and Stv1p normally localized to some other intracellular compartment, possibly Golgi or endosomes (24). These results suggest that the 100-kDa a-subunit contains information necessary to target the V-ATPase to the appropriate intracellular site. To compare the properties of V-ATPase complexes containing Vph1p and Stv1p, we have taken advantage of the observation that overexpression of Stv1p in a yeast strain deleted in both VPH1 and STV1 results in mislocalization of Stv1p to the vacuole (24). This allowed us to compare the properties of V-ATPase complexes present in the same intracellular compartment (the vacuole) that differed only in the a-subunit isoform present.

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1 The abbreviations used are: V-ATPase, vacuolar proton-translocating adenosine triphosphatase; ACMA, 9-amino-6-chloro-2-methoxyacridine; HA, influenza hemagglutinin; MES, 2-(N-morpholino)ethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; SD medium, synthetic dropout medium;YPD medium, yeast extract-peptone-dextrose medium; YEP medium, yeast extract-peptone medium; HRP, horseradish peroxidase, C\(_{12}\)E\(_9\), polyoxyethylene-9-lauryl ether.
**EXPERIMENTAL PROCEDURES**

**Materials and Strains—Zymolyase 100T** was obtained from Seikagaku America, Inc. *Concanamycin A* was purchased from Fluka Chemical Corp. Protease inhibitors were from Roche Molecular Biochemicals. The monoclonal antibody 3F10 (directed against the HA antigen), which is conjugated with horseradish peroxidase, was also from Roche Molecular Biochemicals. The monoclonal antibody STV1-F3 against the yeast V-ATPase subunit 28, and the monoclonal antibody 10D7 against the 100-kDa a-subunit (27) were from Molecular Probes. *Escherichia coli* and yeast culture media were purchased from Difco Laboratories. Restriction endonucleases, T4 DNA ligase, and other molecular biology reagents were from Life Technologies, Inc., Promega, and New England Biolabs. ATP, phenylmethylsulfonyl fluoride, and most other chemicals were purchased from Sigma.

*Yeast V-ATPase complexes were isolated using a modification of the protocol described by Uchida et al. (31). Yeast were grown overnight at 30 °C to 1 × 10⁷ cells/ml in YEP (2% dextrose, 2% yeast extract, 1% peptone) to allow replication of the cytoplasmic membranes. Cells were pelleted and lysed in phosphate-buffered saline containing 1% C₁₂E₉, protease inhibitors, and 1 mM dithiobis(succinimidyl propionate). An aliquot (corresponding to ~6 × 10⁶ cells) was removed to allow analysis of proteins present in the whole cell lysate. The V-ATPase complexes were immunoprecipitated from the same amount of cell lysate using monoclonal antibodies 3F10 against HA or 10D7-A7 against the yeast V-ATPase A-subunit (26) and the monoclonal antibody 8B1-F3 directed against subunit A followed by a horseradish peroxidase-conjugated monoclonal antibody (B-RIP)-conjugated monoclonal antibodies 3F10 against HA or 10D7-A7 against Vph1p to detect the V₀ domain or antibody 8B1-F3 against the A-subunit and protein G-agarose followed by separation on 8% acrylamide gels. The expression of Vph1p to detect the V₀ domain or antibody 8B1-F3 against the A-subunit and protein G-agarose followed by separation on 8% acrylamide gels.**

**Characterization of Yeast a-subunit Isoforms**

We wished to compare the properties of V-ATPases containing Vph1p and Stv1p, the two isofoms of the yeast 100-kDa a-subunit. Because these isofoms normally reside in different intracellular compartments, it was necessary to devise a strategy that would allow complexes containing these two isofoms to be targeted to the same intracellular membrane. It has previously been shown that overexpression of Stv1p in a strain disrupted in both VPH1 and STV1 causes a significant amount of Stv1p to appear in a V₀ V₁ compartment (24). Because Vph1p has been localized to the vacuole, the question then becomes whether it is possible to directly compare the properties of V-ATPase complexes present in the same intracellular compartment that differed only in the a-subunit isoform present. To detect the expression level of each a-subunit isoform, tandem HA epitope tags were inserted in the amino-terminal region of both Vph1p and Stv1p. Expression of the HA-tagged form of either Vph1p or Stv1p using the
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Figure 1. Western blot analysis of expression levels of HA-tagged 100-kDa a-subunits expressed using low and high copy plasmids. Whole cell lysates prepared from the vph1Δstv1Δ strain MM112 transformed with the single copy plasmid pRS316 alone, HA-tagged Vph1p in pRS316, HA-tagged Stv1p in pRS316, or HA-tagged Stv1p in the high copy plasmid YEp352 were subjected to SDS-PAGE on an 8% acrylamide gel followed by transfer to nitrocellulose and Western blot analysis using the HRP-conjugated monoclonal antibody 3F10 against the HA epitope or the monoclonal antibody 8B1-F3 against the 69-kDa A-subunit followed by an HRP-conjugated secondary antibody. Also shown are the growth phenotypes of cells transformed with each of the indicated plasmids on YPD plates buffered to pH 7.5.

Figure 2. Western blot analysis of levels of HA-tagged Vph1p and Stv1p and the 69-kDa A-subunit on purified vacuolar membranes. Vacuolar membranes were isolated from the vph1Δstv1Δ strain MM112 transformed with pRS316 alone, HA-tagged Vph1p in pRS316, HA-tagged Stv1p in pRS316, or HA-tagged Stv1p in YEp352, an aliquot (0.5 μg protein) was subjected to SDS-PAGE on an 8% acrylamide gel, and the levels of the HA-tagged Vph1p, HA-tagged Stv1p, and the 69 kDa A-subunit were determined by Western blot analysis as described in the legend to Fig. 1. The amount of A-subunit present on the vacuolar membrane is one measure of assembly of the V-ATPase complex.

low copy plasmid pRS316 in the vph1Δstv1Δ double-deletion strain MM112 led to a wild-type growth phenotype. That is, cells showed normal growth at both pH 7.5 and 5.5. This was also true using expression of Stv1p from the high copy plasmid YEp352. Western blotting of whole cell lysates (Fig. 1) indicated that Stv1p is expressed at a lower level from the single copy plasmid pRS316 than is Vph1p. Expression of Stv1p using the high copy plasmid YEp352 led to a significant increase in expression levels of Stv1p.

To confirm previous reports that overexpression of Stv1p led to its presence in the vacuolar membrane, vacuoles were isolated from each of the strains shown in Fig. 1, separated by SDS-PAGE, and analyzed by Western blotting using anti-HA antibodies. As shown in Fig. 2, although Vph1p was detectable in the vacuolar membrane using the single copy plasmid, Stv1p was not. By contrast, when Stv1p was expressed at higher levels using the multicopy plasmid, it was detectable in the vacuolar membrane at levels comparable with Vph1p. Overexpression of the gel shown in Fig. 2 revealed very low levels of Stv1p in the vacuolar membrane when expressed using pRS316. Quantitation using densitometric analysis indicated that although the ratio of Vph1p to Stv1p using pRS316 in whole cell lysates was 3:1 (Fig. 1, second and third lanes) from the left), the ratio in the vacuolar membrane was 30:1 (Fig. 2, longer exposure, not shown). These results confirmed that although Stv1p is normally not targeted to the vacuole, overexpression results in the appearance of a significant amount of Stv1p in the vacuole.

To assess the degree of assembly of V-ATPase complexes containing Vph1p and Stv1p, vacuoles isolated from each strain were also probed using an antibody directed against the 70-kDa A-subunit. It has previously been shown that the A-subunit (and other V1 subunits) only associates with the vacuolar membrane if assembly of the V-ATPase complex is normal (27, 32). As shown in Fig. 2, although the level of Stv1p in the vacuolar membrane using the high copy plasmid is comparable with that of Vph1p using pRS316, the level of A-subunit associated with Stv1p is much lower than that associated with Vph1p. Quantitation by densitometry indicates an 8-fold reduction in the level of A-subunit associated with vacuoles containing Stv1p relative to vacuoles containing Vph1p. This result indicates that, at least when present in the environment of the central vacuole, V-ATPase complexes containing Stv1p are less assembled than V-ATPase complexes containing Vph1p.

To determine whether introduction of the HA epitope tags into Vph1p and Stv1p altered assembly of the V-ATPase complex, vacuolar membranes from strains expressing either tagged or untagged versions of Vph1p and Stv1p were probed by Western blot using antibodies against HA, Vph1p, subunit A, and subunit d (a V0 subunit). No antibodies specific for the untagged version of Stv1p are currently available. As can be seen from Fig. 3, the presence of the HA tag had virtually no effect on the amount of Vph1p, subunit A, or subunit d present in the vacuolar membrane, indicating that introduction of the tag did not disrupt assembly of complexes containing Vph1p. Similarly, for Stv1p-containing complexes, the amounts of sub-
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**TABLE I**

| 100-kDa α-subunit isoform | ATPase activity* | Initial rate of fluorescent quenching‡ | Coupling ratio (fluorescent quenching/ATPase activity)§ |
|---------------------------|------------------|----------------------------------------|------------------------------------------------------|
|                           | $K_m$ (μM) | $V_{max}$ (μmol ATP/min/mg) | ΔF (F/s/mg) | 46 ± 12 |
| Vph1                      | 17944          | 250                                    | 0.11                          | 11 ± 1 |

* ATPase activities were measured on isolated vacuolar membranes containing Vph1p (3 μg of membrane protein) or Stv1p (10 μg of membrane protein) (both untagged) over a range of ATP concentrations from 100 μM to 2 mM ATP, whereas MgSO₄ was maintained at 4 mM as described under “Experimental Procedures.” Activities were measured in the absence or presence of 1 μM concanamycin A, and the results shown represent the concanamycin-sensitive portion of the activity. $K_m$ for ATP and $V_{max}$ were calculated from Lineweaver-Burk plots of ATP concentration versus ATPase activity expressed in μmol/min/mg of protein.

‡ The coupling ratio was calculated from the initial rate of fluorescent quenching (see footnote b), and the ATPase activity was measured at the same ATP concentration (0.5 mM).

**TABLE II**

**Effects of inhibitors on ATPase activity of isolated vacuoles containing Vph1p and Stv1p**

ATPase assays were carried out on isolated vacuolar membranes at 0.5 mM ATP in the presence or absence of the indicated inhibitors as described under “Experimental Procedures.” Activities are expressed relative to the control sample, which received 0.1% Me₂SO. Both Vph1p and Stv1p were untagged.

| Vph1                      | ATPase activity | Stv1p                      | ATPase activity |
|---------------------------|-----------------|----------------------------|-----------------|
|                           | μmol ATP/min/mg |                           | μmol ATP/min/mg |   |
| Control                   | 0.673           |                           | 0.104           |   |
| Concanamycin A            | 1 μM            | 0.069                      | 0.058           | 100 |
| Sodium azide              | 0.5 mM          | 0.864                      | 0.098           | 56  |
| Sodium vanadate           | 0.1 mM          | 0.619                      | 0.078           | 75  |

unit A and subunit d present in the vacuoles was unchanged by the introduction of the epitope tag. Thus, the presence of the HA tag does not alter the assembly of V-ATPase complexes. Interestingly, the ratio of subunit A to subunit d staining in the vacuoles is almost the same for Vph1p and Stv1p (Fig. 3) despite the much lower ratio of subunit A to 100-kDa subunit for Stv1p than for Vph1p (Fig. 2). This result suggests that the lower assembly of V₁ and V₆ observed for Stv1p-containing complexes may be the result of reduced association of Stv1p with subunit d, at least in the environment of the vacuolar membrane.

To compare the kinetic properties of V-ATPase complexes containing Vph1p and Stv1p, vacuoles were isolated from the vph1Δ/stv1Δ strain expressing either Vph1p using pRS316 or Stv1p using the high copy plasmid. In this case, the untagged versions of Vph1p and Stv1p were expressed. As indicated in Table I, the $K_m$ for ATP was ~250 μM for complexes containing both Vph1p and Stv1p. Although the $V_{max}$ for ATP hydrolysis for Stv1p-containing complexes was lower than for Vph1p-containing complexes, the 8.6-fold reduction in activity could be entirely accounted for by the lower degree of assembly of V-ATPases containing Stv1p (Fig. 2). Measurement of proton transport using ACMA fluorescence quenching (Table I) indicated an even lower level of proton transport for Stv1p-containing vacuoles relative to Vph1p-containing vacuoles (the ratio of Vph1p:Stv1p was 55:1). This result indicates that the ratio of proton transport to ATP hydrolysis is ~4–5-fold lower for Stv1p-containing complexes compared with Vph1p-containing complexes.

The inhibitor sensitivity of V-ATPase complexes containing Vph1p and Stv1p was also compared. As shown in Table II, ATPase activity for Vph1p-containing vacuoles was 90% inhibited by 1 μM concanamycin A but was only 8% inhibited by 0.1 mM vanadate and was not affected by 0.5 mM azide. By contrast, ATPase activity in Stv1p-containing vacuoles was only 44% inhibited by concanamycin A and 25% inhibited by vanadate. This apparent discrepancy between Vph1p and Stv1p may be partially accounted for by the presence of one or more concanamycin-resistant ATPase activities in the vacuolar membrane, one of which is vanadate-sensitive. Under normal conditions (i.e., with vacuoles containing Vph1p), these other activities are quite minor since the bulk of ATPase activity in the vacuolar membrane corresponds to the V-ATPase. By contrast, because of the much lower levels of V-ATPase activity observed with Stv1p (Table I), the percentage of the total ATPase activity in the vacuolar membrane that corresponds to these "other" ATPases is significantly larger.

To compare the affinity of V-ATPase complexes containing Vph1p and Stv1p for concanamycin A, ATP-dependent proton pumping was measured in vacuoles using ACMA fluorescence quenching. As shown in Fig. 4, the concentration of concanamycin A required for inhibition of 50% of proton transport for both Vph1p and Stv1p-containing complexes was ~0.1 mM. A small residual concanamycin-resistant component of ATP-dependent fluorescence quenching in Stv1p-containing vacuoles was again observed. Nevertheless, the results suggest that Vph1p- and Stv1p-containing complexes have nearly the same affinity for concanamycin A.

Reversible dissociation of the V-ATPase complex has been shown to occur in response to glucose depletion in yeast and has been proposed to represent an important mechanism of regulating V-ATPase activity in vivo (35). To compare glucose-dependent dissociation of V-ATPase complexes containing Vph1p and Stv1p, spheroplasts were prepared from the Vph1p- and Stv1p-expressing strains, incubated in the presence or absence glucose, and solubilized with C₁₂E₉0, and immunoprecipitation was carried out using the antibody SB1-F3 directed against the A-subunit of the V₁ domain. After SDS-PAGE, Western blotting was performed using either SB1-F3 to detect the A-subunit or the anti-HA antibody to detect Vph1p or Stv1p in the Vₒ domain. Dissociation of the V-ATPase is reflected as a decrease in the amount of Vph1p or Stv1p immunoprecipitated using the antibody against subunit A. As shown in Fig. 5a, glucose depletion led to a decrease in the amount of Vph1p immunoprecipitated using the anti-A-subunit antibody (first and second lanes), indicating glucose-dependent dissociation of Vph1p-containing complexes. Because of the lower level of Stv1p expressed using the pRS316 vector, assembled V-ATPase
complexes could not be detected at this exposure (Fig. 5, panel a, third and fourth lanes). A longer exposure, however, revealed that Stv1p-containing complexes do not show dissociation on removal of glucose but instead show some increase in assembly (Fig. 5, panel b, third and fourth lanes). By contrast, when Stv1p was expressed at higher levels (under which conditions a significant amount of Stv1p appears in the vacuole), V-ATPase complexes containing Stv1p showed glucose-dependent dissociation (Fig. 5, panel a, fifth and sixth lanes). By comparing the total expression levels of Vph1p and Stv1p in whole cell lysates (Fig. 5, panel c) with the amounts of Vph1p and Stv1p immunoprecipitated with the anti-A-subunit antibody (Fig. 5, panel b), it is clear that a smaller fraction of Stv1p is assembled with V₁, even when expressed at low levels and in the presence of glucose than for Vph1p. This result is consistent with the data shown in Fig. 2. Quantitation of the results from five independent experiments by densitometry (Fig. 5d) indicates that glucose removal leads to 65% dissociation of complexes containing Stv1p expressed at high levels as compared with 45% dissociation of complexes containing Vph1p. By contrast, V-ATPase complexes formed from Stv1p expressed at low levels show no significant dissociation upon removal of glucose. These results suggest that the membrane environment plays a crucial role in determining whether the V-ATPase undergoes dissociation in response to glucose depletion, with V-ATPase present in the central vacuole undergoing dissociation and V-ATPase present in the Golgi/endosomes remaining assembled.

Dissociation of Vph1p-containing complexes in response to glucose depletion has previously been shown to be reversible upon the readdition of glucose to the media (35). To test whether dissociation of Stv1p-containing complexes that have been targeted to the vacuole is also reversible, spheroplasts were prepared from cells expressing Stv1p from the high copy plasmid, incubated in the presence or absence of glucose for 20 min, or incubated in the absence of glucose for 20 min followed by the addition of glucose and incubation for an additional 20 min. After these incubations, spheroplasts were solubilized with C₁₂E₉, and immunoprecipitation was carried out using the anti-A-subunit antibody followed by SDS-PAGE and Western blotting using antibodies against both HA and subunit A, as described above. As can be seen from the data in Fig. 6, dissociation of Stv1p-containing complexes targeted to the vacuole in response to glucose depletion is also reversible upon the readdition of glucose to the media.

To further test the importance of the membrane environment in affecting in vivo dissociation of the V-ATPase complex, glucose-dependent dissociation was examined in two yeast strains that are disrupted in different steps of the membrane traffic pathway from the Golgi to the vacuole. In *vps21Δ* mutants, Vph1p is found in Golgi-derived vesicles that accumulate as a result of a block in fusion with the prevacuolar compartment, whereas in the *vps27Δ* mutants, Vph1p appears in an exaggerated form of the prevacuolar compartment (36). Glucose-dependent dissociation of Vph1p-containing complexes was examined in these strains relative to the parent strain using an antibody specific for Vph1p. As shown in Fig. 7, glucose depletion appeared to lead to more complete dissociation of Vph1p-containing complexes in the wild-type strain (87%) than was observed for Vph1p-containing complexes in the *vph1Δstv1pΔ* strain described in Fig. 5. When the experiment in Fig. 5 was repeated using the antibody against Vph1p, approximately the same level of dissociation was observed upon glucose depletion (90%) as was seen in Fig. 7. This indicates that the apparent difference in dissociation observed between Fig. 5 and Fig. 7 is due to differences in the ability of the anti-HA and anti-Vph1p antibodies to detect low levels of the 100-kDa proteins. However, it was necessary to use the anti-HA antibody for the experiments described in Fig. 5 because of the absence of antibodies specific for Stv1p.

In both the *vps21Δ* and *vps27Δ* strains, dissociation of the V-ATPase in response to glucose depletion was observed but was less complete than for the wild-type strain. Quantitation of three independent experiments (Fig. 7c) revealed dissociation levels of 63 and 76% in the *vps21Δ* and *vps27Δ* strains, respectively, as compared with the 87% dissociation observed in the parental wild-type strain. Western blot analysis of whole cell lysates indicated a slight reduction in the level of Vph1p in the *vps21Δ* strain (Fig. 7b). Glucose-dependent dissociation of the V-ATPase was shown to be reversible upon the readdition of glucose to the media for the wild-type, *vps21Δ*, and *vps27Δ* strains (Fig. 8). Because Vph1p-containing complexes are prevented from reaching the vacuole in the *vps21Δ* and *vps27Δ* strains, the results indicate that complexes containing Vph1p that are present in other intracellular compartments are still able to undergo glucose-dependent dissociation, although to different degrees. It should be noted that because the compartments that accumulate in the *vps21Δ* and *vps27Δ* strains are aberrant (36), it is possible that the reduction in dissociation observed in these strains may be due to some altered property of these compartments. For example, the multimolecular structure of the prevacuolar compartment that accumulates in the *vps27Δ* strain may prevent some essential cytoplasmic signal from reaching a significant fraction of the V-ATPase present in this compartment. Nevertheless, together with the experiments described above, these results suggest that in vivo dissociation of the V-ATPase complex is affected by both the a-subunit isoform present and the intracellular environment in which the complex resides.
Characterization of Yeast α-subunit Isoforms

**DISCUSSION**

V-ATPases have been shown to reside in a large number of different intracellular compartments in eukaryotic cells (1–8). These include clathrin-coated vesicles, endosomes, lysosomes, Golgi-derived vesicles and secretory vesicles such as chromaffin granules and synaptic vesicles. V-ATPases have also been identified in the plasma membrane of numerous animal cell types, including osteoclasts, renal intercalated cells, macrophages, neutrophils, insect goblet cells, and certain tumor cells (9–11, 37, 38). An important question is how V-ATPases are targeted to their correct cellular destination. Recent results suggest that the 100-kDa α-subunit may play a crucial role in this targeting process. Thus, three unique isoforms of the α-subunit have been identified in mouse (α1, α2, and α3) (39, 40). In osteoclasts, the α1 isoform has been shown to localize to intracellular compartments, whereas the α3 isoform localizes to the plasma membrane (40). Consistent with this observation, it has been shown that disruption of the α3 gene in mouse leads to defective bone resorption (41). In yeast, the α-subunit is also encoded by multiple genes whose protein products show distinct cellular localization. Vph1p is localized to the central vacuole, whereas Stv1p is localized to some other intracellular compartment, possibly Golgi or endosomes (24). An important but unanswered question is how the properties of V-ATPases containing unique isoforms of the 100-kDa α-subunit compare.

To address this question, we have overexpressed Stv1p in a strain disrupted in both VPH1 and STV1, under which circumstances a significant amount of Stv1p becomes localized to the vacuole (24). This allowed a direct comparison of the properties of V-ATPase complexes present in the vacuolar membrane that differ only in the α-subunit isoform present. The results suggest that many of the kinetic properties of V-ATPases containing Vph1p or Stv1p are very similar. Thus, these enzymes exhibit the same affinity for ATP and the same (or very similar) inhibitor sensitivity. By contrast, V-ATPases containing the two α-subunit isoforms differ markedly in three properties. First, they show a significant (4–5-fold) difference in the ratio of proton transport to ATP hydrolysis, with Stv1p-containing complexes exhibiting a much lower efficiency of coupling. Changes in coupling efficiency have been proposed to play a role in controlling V-ATPase activity in vivo (42, 43), but this is the first comparison of coupling for two V-ATPases expressed in the same cell. This result also suggests that the V1 and V0 domains may be more loosely connected for Stv1p-containing complexes than for those containing Vph1p.

Consistent with this idea is the second major difference between the two isoforms, namely the degree of assembly of V1 and V0. Thus, V0 complexes containing Stv1p showed a lower degree of assembly with V1 than did V0 complexes containing Vph1p. This may be related to the observation that Stv1p present in the vacuole also shows a lower association with subunit d, which is normally an essential component of the V0 domain (44). Thus, the ratio of subunit d to subunit A in vacuoles derived from the strain overexpressing Stv1p is the same as that observed for vacuoles derived from the strain expressing Vph1p (Fig. 3). The absence of subunit d has been shown to lead to the loss of assembly of V1 subunits with the vacuolar membrane (44), and this may account for the reduced assembly of Stv1p-containing complexes. Nevertheless, it should be noted that Stv1p expressed at high levels appears to
be stable in the vacuolar membrane (Figs. 2 and 3), suggesting that assembly of the entire Vₐ domain is not defective.

It is possible that these differences between Vph1p and Stv1p may in part be due to the presence of Stv1p in an intracellular compartment in which it does not normally reside. However, it is also possible that V-ATPase complexes in the Golgi (which normally contain Stv1p) may not need to be as active or as tightly coupled as V-ATPases in the central vacuole (which normally contains Vph1p). In support of this idea is the observation that in animal cells the pH of the Golgi is generally maintained in the range of 6.0–6.5 (45, 46), whereas the pH of lysosomes (the compartment analogous to the vacuole in higher eukaryotes) is typically 4.0–5.0 (47). Moreover, the lower degree of assembly of Vₐ and Vₐₐ domains was also observed for Stv1p present in its normal cellular location (Fig. 5).

The third property in which V-ATPase complexes containing Vph1p and Stv1p differ is their degree of dissociation in response to glucose deprivation. The Kane laboratory (35) demonstrates that the V-ATPase in yeast undergoes rapid and reversible dissociation in response to removal of glucose from the medium and has proposed that this process represents an important mechanism of regulating V-ATPase activity in vivo. They have shown that many of the signal transduction pathways activated by glucose starvation, including the Ras-cyclic AMP and protein kinase C-dependent pathways, are not involved in this response (48). Dissociation of the Vₐ and Vₐₐ domains has also been shown to occur in insects during molting, under which conditions the need for an active V-ATPase at the apical membrane of goblet cells lining the midgut may be dramatically reduced (49). Pools of free Vₐ and Vₐₐ domains have also been identified in Madin-Darby bovine kidney cells (50), but rapid and reversible dissociation of the V-ATPase in mammalian cells has not yet been reported.

In the present report it is shown that dissociation of V-ATPase complexes in response to glucose depletion appears to be controlled by both the α-subunit isoform present and the intracellular membrane in which the complex resides. Thus, Stv1p-containing complexes show no glucose-dependent dissociation when localized to their normal intracellular site (Golgi/endosomes) but do dissociate when targeted to the vacuole. By contrast, Vph1p-containing complexes appear to undergo dissociation both in their normal cellular environment (the vacuole) and when retained in Golgi or prevacuolar compartments. Nevertheless, dissociation of Vph1p-containing complexes is at least partly affected by the cellular environment in which these complexes reside, although altered properties of the compartments that accumulate in the vps mutants may be partly responsible for the reduced glucose-dependent dissociation. These results suggest that there are signals present both in the sequence of the α-subunit and in distinct intracellular sites that control dissociation of the V-ATPase complex in vivo. One possible reason that V-ATPase complexes present in the vacuole show more complete dissociation in response to glucose depletion than complexes present in other intracellular compartments (such as the Golgi) is that there may be a larger reserve of active V-ATPase in the central vacuole that the cell is able to survive without. Thus, the activity remaining after dissociation may be sufficient to keep the central vacuole relatively acidic while conserving significant amounts of cellular ATP. By contrast, V-ATPases present in the Golgi (or other prevacuolar compartments) appear to be less abundant but may therefore be less dispensable for cell viability. It should be
noted, however, that because Stv1p-containing complexes show a lower degree of assembly with V₁ than Vph1p-containing complexes, even in the presence of glucose, glucose withdrawal may not be able to affect any greater degree of dissociation of these complexes.

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