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Chemiosmotic Coupling of Ion Transport in the Yeast Vacuole: Its Role in Acidification Inside Organelles

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Acidification inside the vacuo-lysosome systems is ubiquitous in eukaryotic organisms and essential for organelle functions. The acidification of these organelles is accomplished by proton-translocating ATPase belonging to the V-type H^+-ATPase superfamily. However, in terms of chemiosmotic energy transduction, electrogenic proton pumping alone is not sufficient to establish and maintain those compartments inside acidic. Current studies have shown that the *in situ* acidification depends upon the activity of V-ATPase and vacuolar anion conductance; the latter is required for shunting a membrane potential (interior positive) generated by the positively charged proton translocation. Yeast vacuoles possess two distinct Cl^- transport systems both participating in the acidification inside the vacuole, a large acidic compartment with digestive and storage functions. These two transport systems have distinct characteristics for their kinetics of Cl^- uptake or sensitivity to a stilbene derivative. One shows linear dependence on a Cl^- concentration and is inhibited by 4,4′-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS). The other shows saturable kinetics with an apparent K_m for Cl^- of approximately 20 mM. Molecular mechanisms of the chemiosmotic coupling in the vacuolar ion transport and acidification inside are discussed in detail.

KEY WORDS: Vacuolar acidification; anion transport; chloride transport; protonmotive force; V-ATPase; membrane potential; ΔpH.

INTRODUCTION

Biochemical and cell biological research in the last decade has greatly increased our appreciation of the mechanisms of lumenal acidification of various endomembrane organelles including vacuoles, lysosomes, endosomes, coated vesicles, and the Golgi apparatus. Acidification inside the vacuo-lysosomal systems has been proposed to have crucial roles for numbers of cellular activities such as receptor-mediated internalization, protein sorting, and targeting both in the endocytic and exocytic pathways (Mellman *et al.*, 1986). In the endosomes dissociation of a ligand–receptor complex internalized by endocytosis depends on *milieu acidique* (Mellman *et al.*, 1986; Al-Awqati, 1986). Weak amines or ammonium ions, which dissipate the ΔpH across the membranes of vacuo-lysosomal systems, are known to interfere with the destined localization and posttranslational modifications of many secretory amd lysosomal proteins. The mannose-6-phosphate (M-6-P) receptor that recognizes M-6-P residues on lysosomal glycoproteins binds M-6-P in a pH-sensitive manner (von Figura *et al.*, 1986). It is known that a KDEL-receptor in the ER-Golgi compartment binds at low pH the retention signal KDEL tetrapeptide sequence at the carboxyl termini of the ER residents; thus, the receptor–signal complex fails to dissociate in the acidic lumen of the Golgi apparatus and facilitates retrieval of the ER residents efficiently back to ER (Wilson *et al.*, 1993).

In chemiosmotic aspects, the pH difference

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across the membranes directly provides an electrochemical protonmotive force ($\Delta\tilde{\mu}_{H^+}/F$) with a number of H^+-symport and H^+-antiport systems (Anraku, 1987; Anraku *et al.*, 1989, 1992a, 1992b). For example, accumulation of neurotransmitters into the presynaptic vesicles is catalyzed by $\Delta\tilde{\mu}_{H^+}/F$ as a driving energy (Moriyama and Futai, 1990a,b; Moriyama *et al.*, 1992). There are various organic and inorganic solute transport systems in fungal vacuoles (Ohsumi and Anraku 1981, 1983; Sato *et al.*, 1984a,b; Wada *et al.*, 1987; Kakinuma *et al.*, 1992; Wada *et al.*, 1992a) and in plant vacuoles (Blumward and Poole, 1985; Briskin *et al.*, 1985b; Boller and Wiemken, 1986; Schumaker and Sze, 1986), which all are driven and regulated by the $\Delta\tilde{\mu}_{H^+}/F$ conferred to the membranes via the function of vacuolar H^+-ATPases.

The acidification inside these organelles is accomplished by electrogenic pumping of the cytosolic protons into the lumen of organelles mediated by a vacuolar H^+-translocating ATPase (Anraku *et al.*, 1989, 1992a,b). The members of the V-ATPase family in the vacuo-lysosome systems are highly conserved structurally and functionally in their subunit composition, substrate specificities, and sensitivities for various inhibitors (Al-Awqati, 1986; Schneider 1987; Anraku *et al.*, 1992a; Sze *et al.*, 1992). The V-ATPases in animal, plant, and fungal cells are a multimeric complex comprising at least 9 subunits (Bowman *et al.*, 1985; Mandara and Taiz, 1985; Manolson *et al.*, 1985; Bowman *et al.*, 1986; Randall and Sze, 1987; Rea *et al.*, 1987a; Kaestner *et al.*, 1988; Lai *et al.*, 1988; Kane *et al.*, 1989; Moriyama and Futai 1990a; Matsuura-Endo *et al.*, 1990). Each of the subunits from different sources shows high identity (50–80%) in the primary amino acid sequences (Anraku *et al.*, 1992a,b; Stevens, 1992). Due to these similarities in structure and function, they are classified as the V-type H^+-ATPase, which is distinct from the other classes of H^+-ATPases like F_1F_0-type ATPases in mitochondria, bacteria, and chloroplasts or P-type ATPases in the plasma membrane and ER. Inorganic pyrophosphatase, another proton-pumping hydrolase, also participates in the acidification inside the vacuoles in higher plant cells (Chanson *et al.*, 1985; Rea and Poole, 1985; Maeshima and Yoshida, 1989; Takeshige *et al.*, 1988).

Proton translocation by a protonmotive osmoenzyme is electrogenic, as shown directly using the patch-clamp technique (Hedrich *et al.*, 1989). The yeast V-type ATPase forms the protonmotive force of 180 mV with a contribution of 1.7 pH units interior acidic and 75 mV inside positive (Kakinuma *et al.*, 1981). The H^+-ATPase activity increases under uncoupled conditions, i.e., in the presence of protonophores, and does not require the presence of specific ions (Kakinuma *et al.*, 1981; Uchida *et al.*, 1985), indicating that the enzyme is unlikely to translocate other ionic molecules coupled directly with the protons. This electrogenic nature of V-type ATPase raises the question of how the proton transport establishes the acidification inside each compartment, especially under physiological conditions. In this article, we attempt to focus on molecular physiology on the acidification inside the yeast vacuole as an example for discussing the chemiosmotic energy transduction in the endomembrane systems.

FUNCTIONS OF YEAST ACIDIC COMPARTMENT, VACUOLE

The yeast vacuole is an acidic compartment, which is vitally stained with fluorescent weak amines chloroquine (Kitamoto *et al.*, 1988b) and quinacrine (Umemoto *et al.*, 1990). The vacuole constitutes a large compartment occupying about a quarter of the cell volume, and plays indispensable roles in metabolic storage, in cytosolic ion and pH homeostasis (Kitamoto *et al.*, 1988a,b; Anraku *et al.*, 1989a, 1992a, b; Ohya *et al.*, 1991; Wada *et al.*, 1990; Wada *et al.*, 1992b), and in degradation of macromolecules (Klionsky *et al.*, 1990; Chiang and Schekman 1991; Takeshige *et al.*, 1992; Baba *et al.*, 1994). It contains a number of digestive enzymes (Wiemken *et al.*, 1979; Klionsky *et al.*, 1990) including proteinase A, proteinase B, carboxypeptidase Y (Jones, 1984; Achstetter and Wolf, 1985), dipeptidyl aminopeptidase B (Bordallo *et al.*, 1984), α-mannosidase (Yoshihisa *et al.*, 1988, 1989), and alkaline phosphatase (Kaneko *et al.*, 1982), and serves as a digestive compartment analogous to the lysosome in mammalian cells. Yeast vacuole is also a final destination of the endocytic pathway where degradation of internalized mating factor occurs (Riezman, 1985; Jenness *et al.*, 1986).

The vacuole functions as a storage compartment as well. It provides different metabolically active pools for basic amino acids and Ca^{2+} (Kitamoto *et al.*, 1988a,b; Eilam *et al.*, 1985; Iida *et al.*, 1990). Yeast vacuoles possess several active transport systems for these ionic molecules, which take up amino acids, calcium ions, and polyamines in the cytosol by a

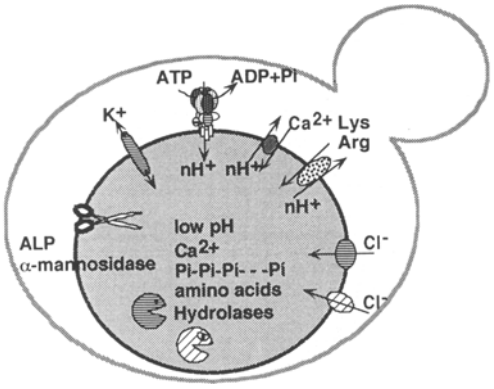

Fig. 1. Function of Yeast Vacuolar Compartment. The vacuole plays an essential role in cellular activities as storage and digestive compartment. The V-type H^+-ATPase generates the protonmotive force ($\Delta\tilde{\mu}_{H^+}/F$) across the vacuolar membrane by transporting protons coupled with hydrolysis of ATP to ADP and inorganic phosphate. Several secondary transport systems driven by the protonmotive force participate in accumulation of basic amino acids (arginine or lysine) or Ca^{2+} by a mechanism of nH^+/substrate antiport. The membrane potential- and Ca_2^+-gated cation channel provides conductivities for monovalent cations including K^+ and Na^+. Two distinct Cl^- transport systems form a large chemical potential difference for protons (ΔpH; interior acidic) by dissipating the membrane potential (ΔΨ; interior positive). An array of hydrolases for macromolecules also plays an essential part in the expression of vacuolar function. Alkaline phosphatase (ALP) and α-mannosidase are membrane proteins. There are other hydrolases including proteinase A (PrA), proteinase B (PrB), and so on in the vacuole present as soluble enzymes. These chemiosmotic and metabolic enzymes together with accumulated molecules in the vacuole are essential for cross-talk and regulation of the cytosolic homeostasis.

mechanisms of nH^+/substrate antiport using the $\Delta\tilde{\mu}_{H^+}/F$ generated by the H^+-ATPase (Ohsumi and Anraku, 1981, 1983; Sato *et al.*, 1984a,b; Kakinuma *et al.*, 1992). In addition, there is a cation-selective, voltage-dependent channel on the vacuolar membrane that participates in the regulation of chemiosmotic balances between the vacuolar and cytosolic compartments (Wada *et al.*, 1987; Tanifuji *et al.*, 1988; Bertl and Slayman, 1990). This diverse array of transport systems coordinately confers the yeast vacuoles the regulatory center of metabolic ion homeostasis of the cells (Fig. 1).

The yeast vacuole accumulates various solutes including basic amino acids and phosphate (Sato *et al.*, 1984a,b; Kitamoto *et al.*, 1988a, b). The inorganic phosphate, in most part, exist as a polymerized from polyphosphate (Urech *et al.*, 1978; Greenfield *et al.*, 1987), presumably in order to decrease osmolarity inside the vacuole. These metabolites compartmentalized in the vacuoles are thought to be mobilized into the cytosol in response to changes of physiological states, such as nutrient starvation, and are utilized for synthesis of cellular macromolecules for adaptation (Kitamoto *et al.*, 1988a; Takeshige *et al.*, 1992).

We should note that these metabolites behave as buffers for protons; thus, a large part of the translocated protons are buffered with these solutes and less would remain as active free protons in the lumen of vacuoles. While the electric charge of protons is translocated into the vacuolar lumen, a ΔΨ is generated. This electro-osmotic event, in principle, must result in the formation of a large ΔΨ with only a small ΔpH in the vacuolar membrane.

Then, how do the organelles convert the $\Delta\tilde{\mu}_{H^+}/F$ to the ΔpH rather than the ΔΨ? We analyzed the effects of various salts on the energization state across the vacuolar membrane and found that transport of anions across the membrane is important virtually for substantial acidification inside the organelles (Wada *et al.*, 1992a). Figure 2 illustrates the generation of ΔpH and ΔΨ upon the addition of ATP to the highly purified vacuolar membrane vesicles with right-side-out orientation. Monitoring of the profiles was made with the fluorescent probes quinacrine, sensitive to ΔpH, and oxonol-V, sensitive to ΔΨ across the membrane. We observed that Cl^- stimulates the initial rate of proton uptake and the extent of acidification, whereas the extent of ΔΨ formation across the vacuolar membrane decreases in the presence of Cl^- salts. These electrochemical events together indicate that Cl^- transport across the energized vacuolar membrane occurs during the acidification inside the vesicles.

The stimulatory effect of Cl^- on the acidification is not due to a direct stimulation of the H^+-ATPase. ATP hydrolysis by the H^+-ATPase is stimulated very little and is essentially unchanged at widely different concentrations of Cl^-; thus, the stimulation of ATPase activity is not sufficient for accounting for the efficiency of the acidification. Consequently, total $\Delta\tilde{\mu}_{H^+}/F$ generated by the H^+-ATPase remains largely unchanged at different concentrations of Cl^-.

This observation turned out to suggest a contribution of vacuolar Cl^- transport to the lumenal acidification. We assessed this more directly by measuring transport of radioactive $^{36}Cl^-$ across the vacuolar membrane to show that $^{36}Cl^-$ is actively taken up by the vacuolar membrane vesicles. This Cl^- uptake is ATP-dependent and protonophore-sensitive, thus the protonmotive force drives this transport.

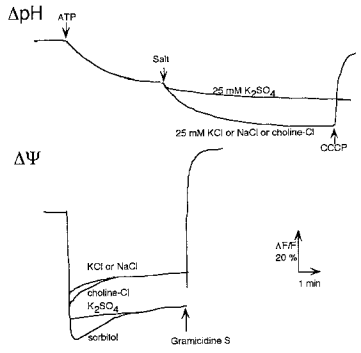

Fig. 2. Formation of ΔpH and ΔΨ across the vacuolar membrane. The formation of ΔpH was measured by quenching of quinacrine fluorescence. Vacuolar membrane vesicles were incubated in a solution containing 5 mM MES-Tris, pH 7.2, 5 mM $MgSO_4$, and 60 mM sorbitol. ATP (final concentration, 0.5 mM) and various salts (25 mM) were added as indicated. The formation of ΔΨ was monitored by quenching of oxonol-V fluorescence. The vacuolar membrane vesicles were incubated with ATP (0.5 mM) in the presence of various salts (60 mM sorbitol, 25 mM K_2SO_4, 50 mM KCl or NaCl or choline-Cl).

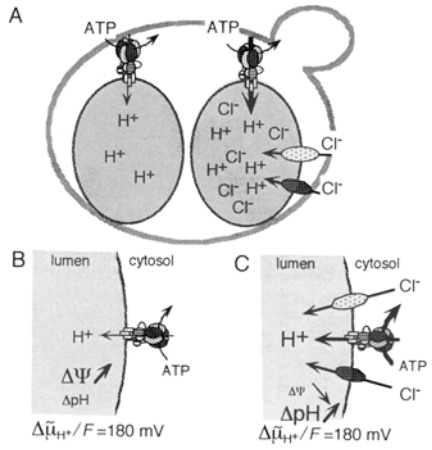

Fig. 3. Conversion of ΔΨ to ΔpH by the Cl^- transport. The chemiosmotic coupling of H^+ and Cl^- transports is shown (Panel A). Intact vacuoles of yeast possess a substantial buffering capacity for protons as they contain polyphosphates and basic amino acids at high concentrations. Thus, only few protons pumped into the lumen are considered to remain as free protons but most would bind to these buffering molecules. The generation of a ΔΨ, inside positive, does inhibit consecutive influx of protons through the V-ATPase complex along with the formation of $\Delta\tilde{\mu}_{H^+}/F$ across the membrane (Panel B). When Cl^- transport occurs in this course, the ΔΨ interior positive is dissipated by net uptake of negative charges, and more protons can be transported into the lumen under the given $\Delta\tilde{\mu}_{H^+}/F$ of 180 mV. This chemiosmotic coupling results in net accumulation of protons in the vacuolar lumen with a small ΔΨ (Panel C).

Anions including Cl^-, NO_3^-, and SCN^- have shown similar effects on the chemiosmotic coupling in vacuolar acidification *in vitro* but SO_4^{2-} or gluconate$^-$ have essentially no effects on either ΔΨ formation or acidification (Wada *et al.*, 1992a). Thiocyanate is a membrane-permeable anion; thus, it translocates electrophoretically across the membrane and does not require any specific physiological transport machinery.

However, the Cl^- transport depends on the specific transporters on the vacuolar membrane. Kinetic analyses on the stimulation of vacuolar acidification reveal that there are two distinct transport systems on the membrane. One shows a saturable kinetics, suggesting the presence of a carrier-type transport on the vacuolar membrane. This saturable component shows an apparent K_m for Cl^- of approximately 20 mM. Another component shows a linear concentration dependence on Cl^-. The possibility that this linear component may reflect a nonspecific permeation of Cl^- across the lipid bilayer is unlikely because we observed that the activity is inhibited after treatment of the vacuolar membrane vesicles with 4,4′-diisothiocyano-2,2′-stilbene disulfonic acid (DIDS), which modifies ε-amino groups of proteins (Wada *et al.*, 1992a). The stilbene derivatives are known to be potent inhibitors for anion transport systems (Bennett and Spanswick, 1983; Bae and Verkman, 1990). The DIDS sensitivity of Cl^- stimulation suggests that the linear component reflects a facilitator for Cl^- diffusion responding to a wide range of [Cl^-] in the cytosol.

We should note here that these transport systems for protons and Cl^- are not directly coupled, since the proton transport occurs in the absence of Cl^- (Kakinuma *et al.*, 1981; Wada *et al.*, 1992a). These two independent transport systems, however, cooperate together for acidifying inside the vacuoles. This chemiosmotic coupling of two independent transport systems results in a net sequestration of HCl from the cytosol into vacuoles (Fig. 3).

ROLES OF ANION TRANSPORT IN EUKARYOTIC ENDOMEMBRANE SYSTEMS

The anion-dependent acidification inside the endomembrane systems is common in eukaryotic organisms. Not only in the vacuoles in plant and fungal cells (Bennett and Spanswick, 1983; Martinoia *et al.*, 1986; Kaestner and Sze, 1987; Pope and Leigh, 1987; Pope *et al.*, 1990; Wada *et al.*, 1992a) but also in the Golgi apparatus (Glickman *et al.*, 1983),

lysosomes (Barash *et al.*, 1991), coated vesicles (Arai *et al.*, 1989), endosomes (Bae and Verkman, 1990), and synaptic vesicles (Tabb *et al.*, 1992), does substantial acidification inside the organelles require the presence of millimolar concentrations of Cl^-. In each case studied so far, it has also been shown that the vacuolar H^+-ATPase itself does not require specific anions for its activity of ATP hydrolysis, although there is considerable stimulation on the ATPase activity by the presence of Cl^-, especially in the case of plant vacuolar ATPpases (Bennett and Spanswick, 1983; Churchill and Sze, 1984; Randall and Sze, 1987). The acidification, i.e., a gross accumulation of free protons in the compartments, is highly dependent upon the presence of specific anions such as Cl^- or Br^-, though Br^- is not a physiological halide ion and unlikely to participate *in vivo*.

Plant vacuoles are equipped with H^+-translocating inorganic pyrophosphatase (H^+-PPiase) in addition to the H^+-ATPase, and both H^+-pumps provide the $\Delta\tilde{\mu}_{H^+}/F$ across the vacuolar membrane (Britten *et al.*, 1989; Maeshima and Yoshida, 1989; Sarafian and Poole, 1989; Maeshima, 1991; Sato *et al.*, 1991, 1994). The vacuolar acidification in plants requires Cl^- for ATP- or pyrophosphate-dependent acidification (Bennett and Spanswick 1983; Briskin *et al.*, 1985a; Kaestner and Sze 1987; Pope and Leigh 1987; Schumaker and Sze, 1987), indicating that a similar chemiosmotic coupling of H^+ and Cl^- transport is involved as in yeast and animal vacuolar systems.

In the plant vacuoles, NO_3^- transport also participates in the vacuolar acidification (Kaestner and Sze 1987; Pope and Leigh 1987). Nitrate is known to be a potent inhibitor of the V-type ATPase: at low concentrations of 1–10 mM level, its inhibition is reversible, while higher concentrations of NO_3^- result in dissociation of enzyme complex (Rea *et al.*, 1987b). The H^+-PPiase is insensitive to NO_3^- (Kaestner and Sze, 1987; Maeshima and Yoshida, 1989; Sato *et al.*, 1991). Nitrate is an essential nutrient for plant cells as a nitrogen source for their growth; thus, plant cells may develop those vacuolar NO_3^- transport systems both for the acidification and accumulation of this important anion.

REGULATION OF ANION TRANSPORT

Molecular biological studies on vacuolar H^+-ATPases have shown that the structure and function of the catalytic subunit are highly conserved, implying that the same gene is likely to provide the catalytic subunits for the V-ATPases on the different organelles such as the Golgi apparatus, lysosomes, and endosomes. The extents of acidification in these organelles are, however, shown to be different. Then, how do those organelles properly maintain the ΔpH across their membranes?

Our current understanding on the mechanisms of how each organelle regulates its lumenal pH is restricted; however, a few lines of evidence have suggested that the regulation of anion conductance may provide the differences of ΔpH in different compartments. There are interesting observations that a protein kinase A regulates the Cl^- conductance of endocytic vesicular membranes (Bae and Verkman 1990, Mulberg *et al.*, 1991; Reenstra *et al.*, 1992). Treatment of the vesicles with a purified catalytic subunit of protein kinase A in the presence of ATP enhanced Cl^- transport into the vesicles during the ATP-dependent acidification. In contrast, the vesicles incubated with alkaline phosphatase showed diminished abilities for both acidification and accumulation of Cl^-. These results suggest that phosphorylation/dephosphorylation of the vesicle protein(s) is involved in the regulation of Cl^- conductance in the endomembrane systems.

Other regulatory mechanisms for the acidification are related to assembly and disassembly of the vacuolar H^+-ATPase itself. Ohya *et al.* (1991) have indicated that the *vma* mutations (for *v*acuolar *m*embrane *A*TPase) causes the defects not only in synthesis of the V-ATPase subunits but also in processes for the enzyme assembly and the expression of enzyme activity. The *VMA12* gene encodes a 25-kDa vacuolar membrane protein that is not a subunit of the enzyme complex (Hirata *et al.*, 1993). In the null *vma12* cells, all the peripheral and integral subunits of the ATPase are synthesized but cannot assemble onto the vacuolar membrane, showing that this component is specifically required for the assembly and/or targeting of the enzyme complex. On the other hand, the *VMA13* gene product (Vma13p) is a 54-kDa regulatory subunit of the vacuolar H^+-ATPase complex. A null *vma13* mutant contains a defective H^+-ATPase complex on the vacuolar membrane, which tends to disassemble from the membrane more easily than the wild-type ATPase (Ho *et al.*, 1993). These gene products are considered to serve a regulatory role for *in situ* acidification of different organelles due to

their unique functions for regulating the H^+-ATPase activity.

Another possibility is that a peptide-mediated permeation of protons out of the vacuolar lumen might, in theory, participate in the regulation. Interestingly, several homologous *VMA* genes for the major 17-kDa proteolipid, the product of the *VMA3* gene that constitutes a proton channel portion of the V-ATPases, have been reported (Umemoto *et al.*, 1991; Hasebe *et al.*, 1992). If these gene products are destined to localize in specific cellular organelles, then they properly adjust the extents of ΔpH formation among endomembrane systems.

We found also a membrane potential-gated cation channel of yeast vacuole (Wada *et al.*, 1987; Tanifuji *et al.*, 1988). This channel has a large single-channel conductance of 435 pS for K^+, and it exhibits a conductance for Na^+ also. Interestingly, this monovalent cation channel is regulated by the $\Delta\Psi$ across the vacuolar membrane and cytosolic Ca^{2+} (Wada *et al.*, 1987; Tanifuji *et al.*, 1988; Bertl and Slayman, 1990). It may serve the regulatory role for vacuolar acidification, though this possibility should be examined further.

CONCLUSION AND PERSPECTIVES

We have summarized the current knowledge and literature on the mechanisms of the lumenal acidification inside the endomembranous organelles in terms of chemiosmotic energy transduction of the protonmotive force ($\Delta\tilde{\mu}_{H^+}/F$). Studies in the past decade have demonstrated that the lumenal acidification is achieved by the function of both the H^+-translocating pump and anion transport system: the generator of $\Delta\tilde{\mu}_{H^+}/F$ and the energy transducer for converting the $\Delta\Psi$ into ΔpH. Thus, further studies on the molecular mechanics of the chemiosmotic coupling process and their regulation in the vacuolysosomal systems are anticipated to provide a deeper understanding of the physiological significance of *milieu acidique* for dynamic organelle functions.

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REFERENCES

Achstetter, T., and Wolf, D. H. (1985). *Yeast* **1,** 139–157.
Al-Awqati, Q. (1986). *Annu. Rev. Cell Biol.* **2,** 179–199.
Anraku, Y. (1987). In *Bioenergetics: Structure and Function of Energy-Transducing Systems* (Ozawa, T., and Papa, S., eds.), Japan Scientific Societies Press, Tokyo, pp. 249–262.
Anraku, Y., Umemoto, N., Hirata, R., and Wada, Y. (1989). *J. Bioenerg. Biomembr.* **21,** 589–603.
Anraku, Y., Umemoto, N., Hirata, R., and Ohya, Y. (1992a). *J. Bioenerg. Biomembr.* **24,** 395–405.
Anraku, Y., Hirata, R., Wada, Y., and Ohya, Y. (1992b). *J. Exp. Biol.* **172,** 67–81.
Arai, H., Pink, S., and Forgac, M. (1989). *Biochemistry* **28,** 3075–3082.
Baba, M., Takeshige, K., Baba, N., and Ohsumi, Y. (1994). *J. Cell Biol.*, **124,** 903–913.
Bae, H-R., and Verkman, A. S. (1990). *Nature (London)* **348,** 637–639.
Barash, J., Kiss, B., Prince, A., Saiman, L., Gruenert, D., and Al-Awqati, Q. (1991). *Nature (London)* **352,** 70–73.
Bennett, A. B., and Spanswick, R. M. (1983). *J. Membr. Biol.* **71,** 95–107.
Bertl, A., and Slayman, C. L. (1990). *Proc. Natl. Acad. Sci. USA* **87,** 7824–7828.
Blumward, E., and Poole, R. J. (1985). *Plant Physiol.* **78,** 163–167.
Boller, T., and Wiemken, A. (1986). *Annu. Rev. Plant Physiol.* **37,** 137–164.
Bordallo, C., Schwencke, J., and Suarez Rendueles, M. (1984). *FEBS Lett.* **173,** 199–203.
Bowman, B. J., Berensky, C. J., and Jung, C. Y. (1985). *J. Biol. Chem.* **260,** 8726–8730.
Bowman, E. J., Mandara, S., Taiz, L., and Bowman, B. J. (1986). *Proc. Natl. Acad. Sci. USA* **83,** 48–52.
Briskin, D. P., Thornley, R. W., and Wyse, R. E. (1985a). *Plant Physiol.* **78,** 865–870.
Briskin, D. P., Thornley, R. W., and Wyse, R. E. (1985b). *Plant Physiol.* **78,** 871–875.
Britten, C. J., Turner, J. C., and Rea, P. A. (1989). *FEBS Lett.* **256,** 200–206.
Chanson, A., Fichmann, J., Spear, D., and Taiz, L. (1985). *Plant Physiol.* **79,** 159–164.
Chiang, H.-L., and Schekman, R. (1991). *Nature (London)* **350,** 313–318.
Churchill, K. A., and Sze, H. (1984). *Plant Physiol.* **76,** 490–497.
Eilam, Y., Lavi, H., and Grossowicz, N. (1985). *J. Gen. Microbiol.* **131,** 623–629.
Glickman, J., Croen, K., Kelly, S., and Al-Awqati, Q. (1983). *J. Cell Biol.* **97,** 1303–1308.
Greenfield, N. J., Hussain, M., and Lenard, J. (1987). *Biochim. Biophys. Acta* **926,** 205–214.
Hasebe, M., Hanada, H., Moriyama, Y., Maeda, M., and Futai, M. (1992). *Biochem. Biophys. Res. Commun.* **183,** 856–63.
Hedrich, R., Kurkdjian, A., and Flugge, A. I. (1989). *EMBO J.* **8,** 2835–2841.
Hirata, R., Umemoto, N., Ho, M. N., Ohya, Y., Stevens, T. H., and Anraku, Y. (1993). *J. Biol. Chem.* **268,** 961–967.
Ho, M. N., Hirata, R., Umemoto, N., Ohya, Y., Takatsuki, A., Stevens, T. H., and Anraku, Y. (1993). *J. Biol. Chem.* **268,** 18286–18292.

Iida, H., Sakaguchi, S., Yagawa, Y., and Anraku, Y. (1990). *J. Biol. Chem.* **265,** 21216–21222.
Jenness, D. D., and Spatrich, P. (1986). *Cell* **46,** 345–353.
Jones, E. W. (1984). *Annu. Rev. Genet.* **18,** 233–270.
Kaestner, K. H., and Sze, H. (1987). *Plant Physiol.* **83,** 483–489.
Kaestner, K. H., Randall, S. K., and Sze, H. (1988). *J. Biol. Chem.* **263,** 1282–1287.
Kakinuma, Y., Ohsumi, Y., and Anraku, Y. (1981). *J. Biol. Chem.* **256,** 10859–10863.
Kakinuma, Y., Masuda, N., and Igarashi, K. (1992). *Biochim. Biophys. Acta* **1107,** 126–130.
Kane, P. M., Yamashiro, C. T., and Stevens, T. H. (1989). *J. Biol. Chem.* **264,** 19236–19244.
Kaneko, Y., Toh-e, A., and Oshima, Y. (1982). *Mol. Cell. Biol.* **2,** 127–137.
Kitamoto, K., Yoshizawa, K., Ohsumi, Y., and Anraku, Y. (1988a). *J. Bacteriol.* **170,** 2683–2686.
Kitamoto, K., Yoshizawa, K., Ohsumi, Y., and Anraku, Y. (1988b). *J. Bacteriol.* **170,** 2687–2691.
Klionsky, D. J., Herman, P. K., and Emr, S. D. (1990). *Microbiol. Rev.* **54,** 266–292.
Lai, S., Randall, S. K., and Sze, H. (1988). *J. Biol. Chem.* **263,** 16731–16737.
Maeshima, M. (1991). *Eur. J. Biochem.* **196,** 11–17.
Maeshima, M., and Yoshida, S. (1989). *J. Biol. Chem.* **264,** 20068–20073.
Mandara, S., and Taiz, L. (1985). *Plant Physiol.* **78,** 327–333.
Manolson, M. F., Rea, P. A., and Poole, R. J. (1985). *J. Biol. Chem.* **260,** 12273–12279.
Martinoia, E., Schramm, M. J., Kaiser, G., Kaiser, W. M., and Heber, U. (1986). *Plant Physiol.* **80,** 895–901.
Matsuura-Endo, C., Maeshima, M., and Yoshida, S. (1990). *Eur. J. Biochem.* **187,** 745–751.
Mellman, I., Fuchs, R., and Helenius, A. (1986). *Annu. Rev. Biochem.* **55,** 663–700.
Moriyama, Y., and Futai, M. (1990a). *Biochem. Biophys. Res. Commun.* **173,** 443–448.
Moriyama, Y., and Futai, M. (1990b). *J. Biol. Chem.* **265,** 9165–9169.
Moriyama, Y., Maeda, M., and Futai, M. (1992). *J. Exp. Biol.* **172,** 171–178.
Mulberg, A. E., Tulk, B. M., and Forgac, M. (1991). *J. Biol. Chem.* **266,** 20590–20593.
Ohsumi, Y., and Anraku, Y. (1981). *J. Biol. Chem.* **256,** 2079–2082.
Ohsumi, Y., and Anraku, Y. (1983). *J. Biol. Chem.* **258,** 5614–5617.
Ohya, Y., Umemoto, N., Tanida, I., Ohta, A., Iida, H., and Anraku, Y. (1991). *J. Biol. Chem.* **266,** 13971–13977.
Pope, A. J., and Leigh, R. A. (1987). *Planta* **172,** 91–100.
Pope, A. J., Jennings, I. R., Sanders, D., and Leigh, R. A. (1990). *J. Membr. Biol.* **116,** 129–137.
Randall, S. K., and Sze, H. (1987). *J. Biol. Chem.* **262,** 7135–7141.
Rea, P. A., and Poole, R. J. (1985). *Plant Physiol.* **77,** 46–52.
Rea, P. A., Griffith, C. J., and Sanders, D. (1987a). *J. Biol. Chem.* **262,** 14745–14752.
Rea, P. A., Griffith, C. J., Manolson, M. F., and Sanders, D. (1987b). *Biochim. Biophys. Acta* **904,** 1–12.
Reenstra, W. W., Sabolic, I., Bae, H.-R., and Verkman, A. S. (1992). *Biochemistry* **31,** 175–181.
Riezman, H. (1985). *Cell* **40,** 1001–1009.
Sarafian, V., and Poole, R. J. (1989). *Plant Physiol.* **91,** 34–38.
Sato, M., Maeshima, M., Ohsumi, Y., and Yoshida, M. (1991). *FEBS Lett.* **290,** 177–180.
Sato, M., Kasahara, M., Ishii, N., Homareda, H., Matsui, H., and Yoshida, M. (1994). *J. Biol. Chem.* **269,** 6725–6728.
Sato, T., Ohsumi, Y., and Anraku, Y. (1984a). *J. Biol. Chem.* **259,** 11505–11508.
Sato, T., Ohsumi, Y., and Anraku, Y. (1984b). *J. Biol. Chem.* **259,** 11509–11511.
Schneider, D. L. (1987). *Biochim. Biophys. Acta* **895,** 1–10.
Schumaker, K., and Sze, H. (1986). *J. Biol. Chem.* **261,** 12172–12178.
Schumaker, K., and Sze, H. (1987). *Plant Physiol.* **83,** 490–496.
Stevens, T. H. (1992). *J. Exp. Biol.* **172,** 47–55.
Sze, H., Ward, J. M., and Lai, S. (1992). *J. Biomembr. Bioenerg.* **24,** 371–381.
Tabb, J. S., Kish, P. E., Van Dyke, R., and Ueda, T. (1992). *J. Biol. Chem.* **267,** 15412–15418.
Takeshige, K., Tazawa, M., and Hager, A. (1988). *Plant Physiol.* **87,** 211–216.
Takeshige, K., Baba, M., Tsuboi, S., Noda, T., and Ohsumi, Y. (1992). *J. Cell Biol.* **119,** 301–311.
Tanifuji, M., Sato, M., Wada, Y., Anraku, Y., and Kasai, M. (1988). *J. Membr. Biol.* **106,** 47–55.
Uchida, E., Ohsumi, Y., and Anraku, Y. (1985). *J. Biol. Chem.* **260,** 1090–1095.
Umemoto, N., Yoshihisa, T., Hirata, R., and Anraku, Y. (1990). *J. Biol. Chem.* **265,** 18447–18453.
Umemoto, N., Ohya, Y., and Anraku, Y. (1991). *J. Biol. Chem.* **266,** 24526–24532.
Urech, K., Dürr, K., Boller, T., Wiemken, A., and Schwencke, J. (1978). *Arch. Microbiol.* **116,** 275–278.
von Figura, K., and Hasilik, A. (1986). *Annu. Rev. Biochem.* **55,** 167–193.
Wada, Y., Ohsumi, Y., Tanifuji, M., Kasai, M., and Anraku, Y. (1987). *J. Biol. Chem.* **262,** 17260–17263.
Wada, Y., Kitamoto, K., Kanbe, T., Tanaka, K., and Anraku, Y. (1990). *Mol. Cell Biol.* **10,** 2214–2223.
Wada, Y., Ohsumi, Y., and Anraku, Y. (1992a). *Biochim. Biophys. Acta* **1101,** 296–302.
Wada, Y., Ohsumi, Y., and Anraku, Y. (1992b). *J. Biol. Chem.* **267,** 18665–18670.
Wilson, D. W., Lewis, M. J., and Pelham, H. R. B. (1993). *J. Biol. Chem.* **268,** 7465–7468.
Wiemken, A., Schellenberg, M., and Urech, K. (1979). *Arch. Microbiol.* **123,** 23–35.
Yoshihisa, T., and Anraku, Y. (1989). *Biochem. Biophys. Res. Commun.* **163,** 908–915.
Yoshihisa, Y., Ohsmui, Y., and Anraku, Y. (1988). *J. Biol. Chem.* **263,** 5158–5163.