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# THE PLANT VACUOLE

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## Summary

Plant cells are unique in containing large acidic vacuoles which occupy most of the cell volume. The vacuolar $H^+$-ATPase (V-ATPase) is the enzyme responsible for acidifying the central vacuole, although it is also present on Golgi and coated vesicles. Many secondary transport processes are driven by the proton-motive force generated by the V-ATPase, including reactions required for osmoregulation, homeostasis, storage, plant defense and many other functions. However, a second proton pump, the V-PPase, serves as a potential back-up system and may, in addition, pump potassium. The plant V-ATPase is structurally similar to other eukaryotic V-ATPases and its subunits appear to be encoded by small multigene families. These multigene families may play important roles in the regulation of gene expression and in the sorting of V-ATPase isoforms to different organelles.

## Origin and functions of plant vacuoles

When first formed by cell division in the shoot or root apical meristem, plant cells contain hundreds of small provacuoles, which arise by the budding and fusion of vesicles from the trans Golgi network (Marty, 1978). At this point in development, plant vacuoles resemble animal lysosomes, both in size and in quantity. As plant cell expansion progresses, however, the tiny provacuoles gradually fuse to form the central vacuole, an enormous acidic compartment which can occupy up to 90% of the cell volume. The nucleus and all the other cytoplasmic organelles are displaced into a narrow, rapidly streaming layer between the tonoplast (vacuolar membrane) and the plasma membrane.

What is the function of the central vacuole? Why have plant cells evolved such a dominant structure, which contains little more than a dilute, acidic solution of salts, metabolites and sometimes pigments? Although the vacuolar sap is very dilute, it contains the bulk of the cell's complement of $K^+$, $Ca^{2+}$, sugar, organic acids and other solutes, many of which must be actively transported against their electrochemical gradients. Hence, a considerable amount of energy must be expended to maintain the solute concentration inside the central vacuole. The two enzymes which directly transduce the energy for tonoplast transport are the vacuolar $H^+$-ATPase (V-ATPase) and the vacuolar $H^+$-pyrophosphatase ($H^+$-PPase).

Probably the most important role of the central vacuole is to increase cell size. As solar collectors, plants grow towards sunlight and spread out the surfaces of their photosynthesizing organs (leaves) thereby maximizing light absorption. It is cheaper

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energetically to increase cell size by water uptake than by protein synthesis, the predominant means of cell growth in animals. Plant cells typically undergo a 10- to 20-fold increase in volume during cell expansion, the majority of which consists of water uptake. If the equivalent amount of water were accumulated in the absence of a vacuole, the cytosol would be diluted to toxic levels. For example, in sieve tube members, the cells in the phloem that translocate sugar over long distances throughout the plant, the tonoplast breaks down as a normal part of development. The cytoplasm then forms a watery matrix, called mictoplasm, and most of the other organelles, including the nucleus and mitochondria, degenerate. Mature sieve tube members are kept alive by cytoplasmic connections to the companion cells, which apparently provide the energy and proteins for the maintenance of the integrity of the outer membrane. Although terminally differentiated, sieve tube members are perfectly adapted for their function as conduits for sugar translocation.

Vacuoles play important metabolic roles in addition to growth (Boller and Wiemken, 1986). These roles include the following.

(1) *Storage.* Vacuoles can serve as storage organelles for sugars (Rausch, 1991), polysaccharides (Wagner *et al.* 1983), organic acids (Ting, 1985) and proteins (Chrispeels, 1991). Most of the flavors of fruits and vegetables are due to the compounds stored in the vacuole. When needed, these primary metabolites can be retrieved from the vacuole and utilized in metabolic pathways.

(2) *Toxic avoidance.* Being immobile, plants cannot escape exposure to toxic elements in the environment by moving to another location. Nor do plants have an excretory system for the elimination of such substances. By accumulating heavy metals, such as cadmium (Vogeli-Lange and Wagner, 1990) and sodium (Blumwald and Poole, 1985), the vacuole can be viewed as a micro-kidney inside each plant cell, filtering and sequestering potentially toxic ions from the cytosol.

(3) *pH and ionic homeostasis.* Reactions in the cytosol are exquisitely sensitive to changes in pH and ionic strength. The concentrations of certain ions, e.g. calcium, are kept extremely low, enabling them to stimulate key regulatory enzymes, such as protein kinases. The pH of the vacuole of higher plants is typically 5.0–5.5, but can reach as low as 2.5 in lemon fruits. However, the record for the most acidic vacuole belongs to the brown alga *Desmerestia*, with a lumenal pH of 0.6 (McClintock *et al.* 1982)! The extremely low pH is due to the accumulation of $H_2SO_4$. In principle, the two proton pumps on the tonoplast can regulate cytosolic pH by pumping massive amounts of protons out of the cytosol into the lumen of the vacuole, although this has been difficult to demonstrate directly (Moriyasu *et al.* 1984).

The vacuolar calcium concentration is typically $1 \times 10^{-3}$ mol $l^{-1}$, whereas the cytosolic calcium concentration is approximately $1 \times 10^{-7}$ mol $l^{-1}$. The steep gradient in calcium is maintained by a proton–calcium exchange mechanism (Schumaker and Sze, 1990). Recent evidence suggests that calcium in the vacuole can be released into the cytosol *via* an inositol 1,4,5-trisphosphate ($InsP_3$)-dependent channel (Schumaker and Sze, 1987; Alexandre *et al.* 1990). Thus, the vacuole may play a key role in regulating intracellular calcium in response to the signal transduction pathway (Fig. 1).

Another inorganic ion whose cytoplasmic concentration is strongly influenced by the

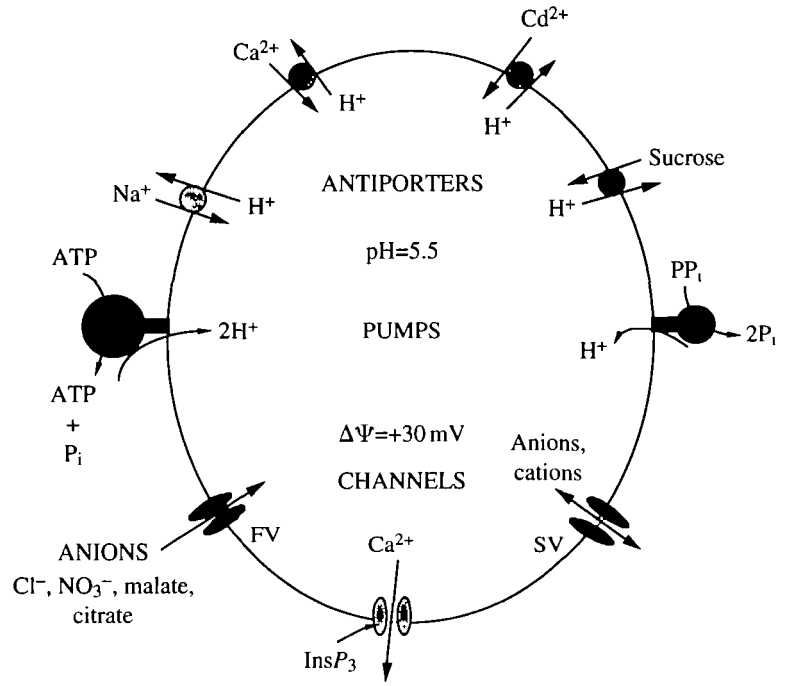


Fig. 1. Transport systems on the tonoplast (vacuolar membrane) of plant cells. Two electrogenic proton pumps driven by ATP and pyrophosphate ($PP_i$) generate the $\Delta$pH and $\Delta\Psi$ for the secondary transport of other solutes into the vacuole. SV, slow vacuolar channel; FV, fast vacuolar channel. See text for discussion. Ins$P_3$, inositol trisphosphate; $P_i$, inorganic phosphate.

vacuole is phosphate. $^{31}P$ nuclear magnetic resonance studies have shown that when plant cells are exposed to varying phosphate levels, cytoplasmic phosphate levels remain constant while vacuolar phosphate concentrations fluctuate widely (Rebeille *et al.* 1985).

(4) *Defense against microbial pathogens and herbivores.* Plant cells frequently accumulate large quantities of bitter-tasting phenolic compounds, cyanogenic glycosides or alkaloids in their vacuoles which function in discouraging insect herbivores (Taiz and Zeiger, 1991). Chitinase, an enzyme that breaks down fungal cell walls, is specifically synthesized in response to wounding and accumulates in the vacuoles of bean plants (Boller and Vogeli, 1984).

(5) *Pigmentation.* Many plant cells accumulate water-soluble flavonoid pigments called anthocyanins, which range in color from orange-red to purple (Taiz and Zeiger, 1991). In leaf tissue, such pigments are concentrated in the vacuoles of epidermal cells, where they probably function to prevent photooxidation of the photosynthetic apparatus by lowering the light intensity and by screening out ultraviolet irradiation.

In *Petunia hybrida*, flower petal vacuoles contain the pH-sensitive anthocyanin petunidin, which can exist in either a red or blue form, depending on whether the pH is acidic or alkaline. It has been shown that the vacuolar pH is 5.5 for red flowers and 6.0–6.2 for purple flowers. Four complementary genes have been identified which have a

blueing effect on flower color when they are homozygous recessive (De Vlamming *et al.* 1983). It would be interesting to know whether these genes encode subunits of the vacuolar ATPase or PPase and whether they are specifically expressed in flowers.

(6) *Lysosomes.* Like the lysosomes of animal cells, plant vacuoles contain a variety of acid hydrolases, such as proteases, ribonucleases and glycosidases (Boller and Wiemken, 1986). However, there is as yet no convincing evidence that plant vacuoles participate in the normal turnover of macromolecules in the cell. Instead, their main function appears to be to break down and recycle cellular components during senescence, as in the case of leaves or flowers, or during programmed cell death, as in the case of xylem vessel elements.

## Types and distribution of vacuolar transport systems

### *Two electrogenic tonoplast proton pumps*

It is now well-established that the uptake of a wide variety of solutes into vacuoles is driven by the $\Delta\mu H^+$ across the tonoplast (Fig. 1). This $\Delta\mu H^+$ is apparently generated by two separate electrogenic proton pumps: the vacuolar $H^+$-ATPase (V-ATPase) and the vacuolar $H^+$-pyrophosphatase (V-PPase) (Rea and Sanders, 1987; Maeshima and Yoshida, 1989). The exact role of the V-PPase is still somewhat controversial. Plant cells appear to contain substrate levels of pyrophosphate ($PP_i$) in the cytosol (0.1–0.25 mmol $l^{-1}$) sufficient to drive the PPase at half its maximum rate (Chanson *et al.* 1985). In corn root tips, its activity often exceeds that of the V-ATPase (J. Fichmann and L. Taiz, unpublished data). Thus, it seems likely that the V-PPase is an alternative proton pump on the tonoplast, but its precise role during development has not been ascertained. The cDNA for the plant V-PPase has now been sequenced (Sarafian *et al.* 1992). It encodes a polypeptide of about $81\times10^3 M_r$, and is unrelated to any known ATPase. However, it is immunologically cross-reactive with the coupling factor pyrophosphatase of *Rhodospirillum rubrum* (Nore *et al.* 1991), which suggests that it is evolutionarily derived from a eubacterial ancestral gene. Although no V-PPase activity has been reported in animal cells, yeast vacuoles have been reported to contain an active vacuolar $H^+$-PPase (Lichko and Okorokov, 1991). A more complete survey of $H^+$-PPases in other organisms would provide valuable information on the evolutionary history of this enzyme.

Although the role of the V-ATPase in driving vacuolar transport has been widely accepted, direct proof has been difficult to obtain. In yeast, it is possible to generate V-ATPase null mutants by gene disruption. Such mutants have been shown to be conditional lethals: i.e. they are strongly inhibited by high external calcium concentration and neutral pH, but grow well at pH 5.5 and low external calcium concentration (Nelson and Nelson, 1990; Ohya *et al.* 1991). Their vacuoles are no longer acidic. Unfortunately, it is not possible to carry out parallel experiments in plants since techniques for specific gene replacement are not yet available. However, as will be discussed in more detail elsewhere in this volume (Gogarten *et al.* 1992*b*), the synthesis of the tonoplast $H^+$-ATPase can be specifically inhibited with antisense DNA to the catalytic subunit (Gogarten *et al.* 1992*a*). The leaves of carrot plants transformed with antisense DNA are

smaller, and the tap root grows at a slower rate. Light microscopy indicated that cell expansion is inhibited in the antisense mutants. These results demonstrate that the V-ATPase does play an important role in facilitating cell expansion. The failure to obtain more drastic inhibition with antisense DNA may be due either to residual V-ATPase activity or to the activity of the V-PPase. In the future, it will be important to construct double antisense mutants, deficient in both enzymes, and determine the effects on growth.

### *Secondary transport systems on the tonoplast*

#### *Antiporters*

The proton-motive force generated by the V-ATPase consists of a $\Delta$pH of about 1.5–2.0 and a $\Delta\Psi$ of about +30 mV relative to the cytosol (Sze, 1985). Several antiporters have been demonstrated which utilize the pH gradient to drive the uptake of $Ca^{2+}$, $Na^+$ and sugars in exchange for protons. As noted earlier, the tonoplast $Ca^{2+}/H^+$ antiporter is very active in most plant cells. Recent evidence indicates that the exchange is electroneutral, i.e. $2H^+$ per $Ca^{2+}$, allowing calcium to accumulate against a positive membrane potential (Blackford *et al.* 1990).

The tonoplast $Na^+/H^+$ antiporter is abundant in halophytic and salt-tolerant plants, such as beets (Blumwald and Poole, 1985) and barley (Garborino and DuPont, 1989), but is also present in glycophytes, such as corn (J. Chiorini and L. Taiz, unpublished data). Accumulation of sodium in the vacuole is one of the principal ways in which salt-tolerant plants avoid the negative effects of salinity, the other being secondary active transport of sodium out of the cell by means of a $Na^+/H^+$ antiporter on the plasma membrane. Exposure of barley roots to salinity results in the rapid activation of the vacuolar $Na^+/H^+$ antiporter, rather than increased synthesis, suggesting that some type of regulation by protein modification is involved (Garborino and DuPont, 1989). The $Na^+/H^+$ antiporter of sugar beet is sensitive to the diuretic agent amiloride, and has recently been purified (Blumwald *et al.* 1987; E. Blumwald, personal communication).

Sugar accumulation in many plant cells is brought about by the combined action of plasma membrane $H^+$/hexose symporters and tonoplast sucrose or hexose/$H^+$ antiporters (Rausch, 1991). However, in sugar-storing cells such as sugar beet, the tonoplast sucrose/$H^+$ antiporter may be the primary driving force for sugar accumulation, since uptake across the plasma membrane appears to be largely passive (Briskin *et al.* 1985).

No $K^+/H^+$ antiporter, similar to the $Na^+/H^+$ antiporter, has yet been identified on plant tonoplasts, despite the fact that certain vacuoles, such as those of guard cells and pulvinar cells, appear to accumulate large amounts of potassium (up to 500 mmol $l^{-1}$) during turgor regulation. Since, in these cases, $K^+$ appears to accumulate against its electrochemical gradient, some type of active mechanism is indicated. Recently it has been suggested that the V-PPase may pump $K^+$ together with $H^+$ into the vacuole (Davies *et al.* 1991). If so, it might explain why the plant vacuolar membrane has two ion pumps.

#### *Channels*

The inside-positive membrane potential across the tonoplast can drive the uptake of various anions *via* channels. Cations can also enter vacuoles through channels down their concentration gradients. The advent of patch-clamp technology has allowed the

characterization of numerous channels in plant membranes (Hedrich and Schroeder, 1989). Two main types of voltage-regulated channels have been identified on tonoplasts: the SV type (slow vacuolar type) and the FV type (fast vacuolar type). SV channels are activated at negative voltages and by relatively high cytoplasmic $Ca^{2+}$ concentrations (>0.3 $\mu$mol $l^{-1}$). FV channels are activated by positive voltages and by low cytoplasmic $Ca^{2+}$ concentrations (<0.3 $\mu$mol $l^{-1}$). Accordingly, FV channels function during active proton pumping by the V-ATPase and V-PPase and probably constitute the principal port of entry for anions such as $Cl^-$, $NO_3^-$, malate and citrate. SV channels would operate under conditions in which the tonoplast was depolarized. Both channels are relatively nonspecific, allowing both cations and anions to pass through, although the permeability to cations is 2–10 times greater than that to anions (Hedrich and Schroeder, 1989). A third type of channel, regulated by Ins$P_3$, has already been mentioned in relation to the release of calcium from the vacuole.

*Other plant endomembrane proton pumps*

V-ATPases have been shown to be present on at least two other organelles of the endomembrane system of plant cells: the Golgi apparatus (Chanson and Taiz, 1985) and coated vesicles (Fichmann *et al.* 1989). However, the functions of the plant Golgi and coated-vesicle ATPases has not yet been determined. Treatment of plant cells with the $Na^+/H^+$ ionophore monensin causes Golgi swelling (Boss *et al.* 1984) and interferes with secretion (Jones and Robinson, 1989). The Golgi apparatus also appears to have $H^+$-PPase activity (Chanson and Taiz, 1985).

## Structure and evolution of V-ATPases

There is now general agreement on the overall structure and subunit composition of V-ATPases, which appear to be similar in all eukaryotes. The enzyme consists of a water-soluble catalytic complex, $V_1$, made up of five subunits, *A–E*, and a hydrophobic integral membrane protein complex, $V_o$, consisting of three to four subunits, one of which is a DCCD-binding $16\times10^3$ $M_r$ proteolipid, the *c* subunit. (The subunit composition of plant V-ATPases is discussed in detail by Sze *et al.* 1992.) In the electron microscope, the plant V-ATPase has the classical ball-and-stalk appearance of the $F_1F_o$-ATPases after negative staining (Taiz and Taiz, 1991; Klink and Lüttge, 1991). However, the $V_1$ catalytic complex is slightly larger than the $F_1$ complex and has a characteristic cleft in the middle which is absent from $F_1$-ATPases, similar to the $V_1$ of *Neurospora* (Bowman *et al.* 1989; Taiz and Taiz, 1991). Plant $V_1$ complexes, like those of other eukaryotes, can be stripped from the membrane by treatment with chaotropic anions, such as nitrate or iodide (Rea and Sanders, 1987; Lai *et al.* 1988), or by low temperatures in the presence of MgATP (Moriyama and Nelson, 1989; Parry *et al.* 1989). Unlike $F_1$ complexes, $V_1$ complexes are inactive when removed from the membrane, and the remaining $V_o$ complex is not leaky to protons (Nelson, 1991; Ward *et al.* 1992).

The physical resemblance of V-ATPases to F-ATPases is consistent with the similarity in enzymatic properties. All eukaryotic V-ATPases, including those of plants, are relatively insensitive to vanadate and hydrolyse MgATP without forming a

phosphorylated intermediate. This property sets them apart from the P-type ATPases of the plasma membrane and sarcoplasmic reticulum. The $H^+$/ATP stoichiometry has been determined to be 2 (Bennett and Spanswick, 1984), a value which favors proton pumping or ATP synthesis under physiological conditions. It has been demonstrated using the patch-clamp technique that the V-ATPase can pump protons against a $10^4$-fold gradient in $H^+$: from pH 7.5 on the cytoplasmic face to pH 3.5 inside the vacuole, more than enough to account for the pH of most vacuoles (Hedrich *et al.* 1989). However, the participation of the V-PPase may be necessary to acidify below this value, as in the case of lemon fruits and *Desmerestia*.

Sequencing of the *A* and *B* subunits of carrot (Zimniac *et al.* 1988), *Neurospora* (Bowman *et al.* 1988) and *Arabidopsis* (Manolson *et al.* 1988) provided the first direct evidence for the evolutionary relatedness of the eukaryotic V-ATPases and the eubacterial-type F-ATPases. Although the overall identity between the two catalytic subunits, *A* and $\beta$, was found to be relatively low (25 %), a number of highly conserved motifs previously implicated in catalysis, including the glycine-rich nucelotide-binding domain, were identified (Zimniac *et al.* 1988). Hence, the eukaryotic and eubacterial enzymes appeared to be distantly related. Surprisingly, a much closer relationship was found between the eukaryotic V-ATPase and the archaebacterial A-ATPases (Gogarten *et al.* 1989). The *A* and *B* subunits of the eukaryotic V-ATPases are 50 % identical to their archaebacterial counterparts. In addition, the catalytic subunits of both types contain a stretch of about 90 amino acids termed the nonhomologous region which is entirely missing from the $\beta$ subunit. The quantitative analysis of this relationship and the profound implications for the evolution of eukaryotes will be discussed elsewhere in this volume.

## Regulation of gene expression

Although V-ATPases are present in nearly all plant cells, regulation of gene expression occurs throughout development and is subject to environmental stress. For example, in corn roots, most of the V-ATPase $H^+$-pumping activity is concentrated in the stelar parenchyma, with only very low activity in the cortex (Walker and Taiz, 1988). Whether this tissue-specific expression is regulated at the transcriptional or post-transcriptional level has not yet been determined. Growth of the roots in 100 mmol $l^{-1}$ NaCl brought about a marked increase in the V-ATPase activity of the cortex, with only a small effect on the stele (Walker and Taiz, 1988), suggesting that expression in the cortex is under environmental control. When the succulent plant *Mesembryanthemum crystallinum* is exposed to salinity, there is a fourfold stimulation of V-ATPase activity which accompanies the shift to Crassulacean acid metabolism. An examination of the subunit compositions at the induced and uninduced stages revealed minor differences, suggesting the possibility of different isoforms (Bremberger *et al.* 1988). Evidence that salinity may increase V-ATPase activity both at the post-translational level (Reuveni *et al.* 1990) and at the transcriptional level (Narasimham *et al.* 1991) was obtained for salt-adapted tobacco cell lines.

The promoter region of a gene for the catalytic subunit of carrot has been sequenced

and shown to be active in enhancing gene expression using the reporter gene β-glucuronidase (GUS) (Struve *et al.* 1990). Interestingly, an ABA box previously identified as sufficient to confer responsiveness to the plant hormone abscisic acid (ABA) is present. This finding is significant since it has been shown that ABA increases V-ATPase gene expression in tobacco cells (Narasimham *et al.* 1991).

## Multigene families

Plant genomes are characterized by large multigene families. Hence it is no surprise that the genes for V-ATPase subunits occur as small gene families encoding isoforms. As described by Forgac (1992), the situation is similar in animals, although fungal V-ATPase subunits are encoded by single copy genes. Thus far, gene families in plants have been demonstrated for the *A* (Narasimham *et al.* 1991; Gogarten *et al.* 1992*a*) and *c* (Lai *et al.* 1991) subunits.

The significance of multiple copies of the V-ATPase genes remains to be conclusively determined. As noted above, tissue-specific and salinity-specific regulation of gene expression could involve separate isoforms. Another possibility is organelle-specific isoforms. As will be discussed elsewhere in this volume, there is evidence in carrot that the catalytic subunits of the Golgi apparatus and the tonoplast V-ATPases are encoded by different genes (Gogarten *et al.* 1992*a*,*b*). This raises important questions about the mechanism of targeting of specific V-ATPases to different organelles.

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