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Increased Expression of Vacuolar Aquaporin and H^+-ATPase Related to Motor Cell Function in *Mimosa pudica* L.[1]

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Mature motor cells of *Mimosa pudica* that exhibit large and rapid turgor variations in response to external stimuli are characterized by two distinct types of vacuoles, one containing large amounts of tannins (tannin vacuole) and one without tannins (colloidal or aqueous vacuole). In these highly specialized cells we measured the abundance of two tonoplast proteins, a putative water-channel protein (aquaporin belonging to the γ-TIPs [tonoplast intrinsic proteins]) and the catalytic A-subunit of H^+-ATPase, using either high-pressure freezing or chemical fixation and immunolocalization. γ-TIP aquaporin was detected almost exclusively in the tonoplast of the colloidal vacuole, and the H^+-ATPase was also mainly localized in the membrane of the same vacuole. Cortex cells of young pulvini cannot change shape rapidly. Development of the pulvinus into a motor organ was accompanied by a more than 3-fold increase per length unit of membrane in the abundance of both aquaporin and H^+-ATPase cross-reacting protein. These results indicate that facilitated water fluxes across the vacuolar membrane and energization of the vacuole play a central role in these motor cells.

One of the most striking features of mature plant cells is the presence of a large central vacuole that can occupy more than 80% of the total cell volume. The constituents of the vacuole are mainly inorganic salts and water (Martinoia et al., 1981; Boller and Wiemken, 1986; Martinoia, 1992). The vacuole, therefore, enables the plant to attain a large size and surface area by accumulating salts from the environment that osmotically drive further water uptake, resulting in minimal energy expenditure for metabolite synthesis (Matile, 1987). Furthermore, the vacuole is also a temporary store for metabolites and nutrients and it plays an important role in cytosolic homeostasis (Matile, 1978, 1987; Boller and Wiemken, 1986; Martinoia, 1992). In contrast to primary metabolites that are stored only temporarily in the vacuole, many compounds of secondary metabolism (Matile, 1987) or modified, potentially toxic xenobiotics (Martinoia et al., 1993) are presumed to be stored indefinitely within the vacuole.

Because of its large volume, the vacuole is the plant cell water reservoir. Changes in the water status of a cell and concomitant water fluxes across the plasma membrane will therefore induce fluxes across the vacuolar membrane. Generally, such water movements are slow and the water permeability of the lipid bilayer is more than sufficient for required fluxes. However, some specialized plant cells, such as stomata cells, the motor cells of the pulvini of *Mimosa pudica*, or of the stamens of *Mahonia* spp. change their volume very rapidly using water exchange with surrounding cells.

During the last few years it has become clear that water flux across biological membranes occurs not only through the lipid bilayer but also across specific proteins, the aquaporins, present in both animals and plants (Chrispeels and Agre, 1994). All known aquaporins belong to the family of the membrane intrinsic proteins. These proteins are very hydrophobic, have a molecular mass of 22 to 27 kD, and usually represent the most abundant membrane protein. The first functional evidence for a plant aquaporin was reported for γ-TIP, the major vacuolar membrane protein in vegetative tissues (Maurel et al., 1993; Chrispeels and Maurel, 1994). This protein is mainly expressed in young, elongating plant parts (Höfte et al., 1992). Recently, α-TIP, the major intrinsic protein of the vacuole-like protein bodies, was identified as a water channel (Maurel et al., 1995). In this case water conductance was enhanced 2-fold after phosphorylation of α-TIP, indicating that some water channels may be regulated in response to the metabolic state of the cell.

In the case of motor cells, water fluxes are linked to ion fluxes. The most thoroughly investigated motor cells are guard cells of the stomata, where potassium fluxes across inward or outward rectifying channels of the plasma membrane (Schroeder et al., 1987; Ward et al., 1995), along with chloride fluxes and malate synthesis/degradation, are re-

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Abbreviations: HPF, high-pressure freezing; TIP, tonoplast intrinsic protein.

sponsible for rapid changes in the cellular osmolarity (Outlaw, 1983). Hence, water fluxes and volume changes are mainly due to the changes in the vacuolar constituents and volume. Potassium channels have been identified in the vacuolar membrane; and in motor cells of pulvini, potassium channels, similar to those described in the plasma membrane of guard cells, have been demonstrated (Moran, 1990) and the transduction pathway has been studied (Satter and Galston, 1981; Coté, 1995). In both cases, Ca^{2+} is involved in the regulation of the channels; however, at least for stomata, a Ca^{2+}-independent transduction pathway is also being discussed (Ward et al., 1995).

Ion fluxes in plants are secondary energized processes and they depend on the electrochemical gradient generated by the proton pumps of the plasma membrane or vacuolar membrane (Rea and Sanders, 1987; Hedrich and Schroeder, 1989; Serrano, 1989; Martinoia, 1992). Tissues transporting sugars at high rates, such as the phloem, have recently been shown to contain higher amounts of the plasma membrane H^+-ATPase (Bouché-Pillon et al., 1994; Fleurat-Lessard et al., 1995). It is therefore tempting to speculate that motor cells exhibiting rapid ion and water fluxes would also have higher proton pump activities.

Our knowledge of the molecular properties of the aquaporins, channels, and proton pumps has increased considerably lately; however, many physiological questions remain unanswered. To determine whether motor cell movement is linked to an increased expression of vacuolar aquaporins and H^+-ATPase, we compared the density of these proteins between cells types, using immunochemical techniques in *M. pudica* pulvini submitted to either HPF or chemical fixation. Since only mature pulvini move in response to mechanical stimulus, whereas young pulvini are still unable to respond to this stimulus, a direct comparison of identically positioned cortical cells in different functional states was possible. Classically called motor cells in mature pulvini, these cells are described as nonreactive cells in parts unable to move (young pulvini and petiole). Furthermore, they contain two different types of vacuoles (Fleurat-Lessard, 1988), and it was unknown whether only one or both of these vacuoles had developed the property to mediate rapid water flow and ion exchange (Fig. 1, a and b).

MATERIALS AND METHODS

Mimosa pudica L. plants were grown under conditions previously described (Roblin and Fleurat-Lessard, 1983).

HPF Procedures

Young pulvini and mature pulvini were sectioned and put in a device containing hexadecene (Fluka, Buchs, Switzerland) to penetrate the samples and eliminate intercellular air pockets. Then the device was closed and placed in the Balzer HPM 010 (Bal-Tec Products, Selles sur Cher, France) apparatus where the application of pressure (2100 bars) was associated with rapid freezing in liquid nitrogen (−170°C) (Craig et al., 1987; Moor, 1987; Studer et al., 1989). The samples were stored in liquid nitrogen and subsequently cryosubstituted during several days in methanol or acetone at increasing temperatures (−90, −30, and −4°C). London white resin (TAAB Laboratories, J Delville St. Germain en Laye, France) embedding was done at 55°C for 24 h (Fleurat-Lessard et al., 1995).

Chemical Fixation

Young pulvini, mature pulvini, and petioles were cut into pieces 1 or 2 mm thick and fixed for 15 to 30 min in a

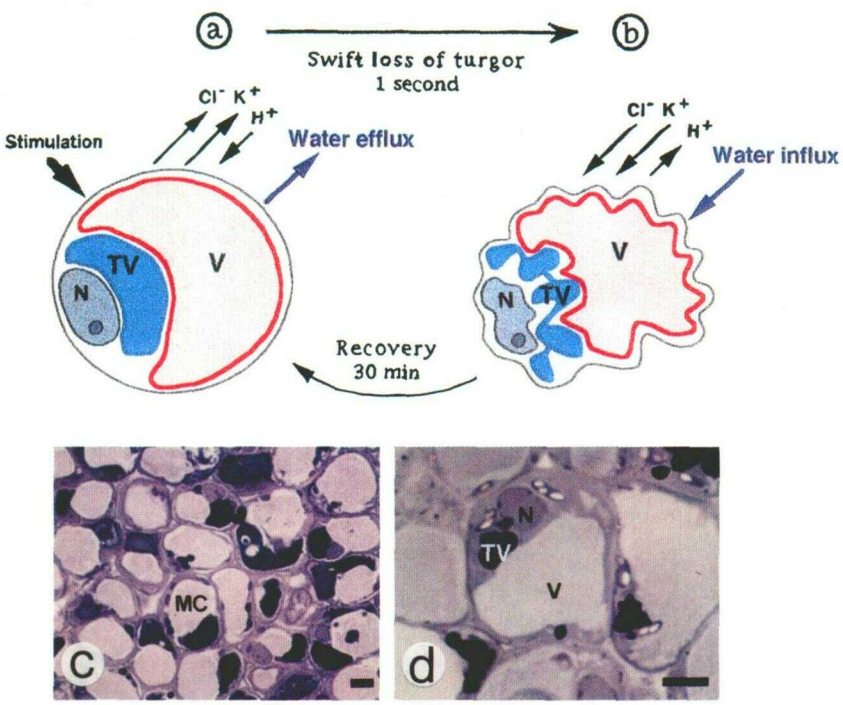

Figure 1. Schematic view of the variation in motor cell shape. The cell remains swollen if pulvini movement is prevented by diethylether (a), whereas it becomes shrunken after pulvini response (b). Motor cells contain two vacuole types, one tannin-rich (TV) located near the nucleus (N), the other aqueous and central (V), corresponding to the vacuole in most mesophyll cells. During shrinkage both vacuoles change their shape: the tannin vacuole forms many tubules that are connected, the aqueous vacuole develops invaginations in its external region. Motor cells (MC) are well-preserved in HPF-treated pulvini (c), in particular their large vacuoles and their dense but narrow cytoplasm (d). Bars, 10 μm.

mixture of 1.5% (w/v) paraformaldehyde, 0.5% glutaraldehyde in 0.05 mM phosphate buffer, pH 7.2. Abundant washing (at least six baths during 2 h) in the same buffer was followed by a postfixation of 4 min in 1% (v/v) OsO_4, rapid dehydration in an ethanol series, and overnight embedding in London white resin. Polymerization was allowed to occur in gelatin capsules at 60°C for 24 h. Semithin sections were used to select cortical cells. Thin sections, carefully spread with toluene vapor, were collected on parlodion-coated gold grids.

Antibodies

Polyclonal antibodies against purified VM23 aquaporin, a γ-TIP of radish vacuolar membrane (Maeshima, 1992), and the α-subunit of the vacuolar H^+-ATPase of *Mesembryanthemum crystallinum* (Ratajczak et al., 1994) were raised in rabbits.

Membrane Isolation and Gel Electrophoresis

Membrane isolation, SDS-PAGE, and western-blot analysis were performed as described (Gallet et al., 1992).

Immunogold Staining

The immunogold reaction was performed at 20°C on thin sections. Solutions were filtered (Millipore MFVCWP, 0.1-mm pores) or centrifuged (500*g*). The sections were hydrated in deionized water and the next steps were performed in the dark. Sections were etched by 0.56 M $NaIO_4$ and 0.1 N HCl, and then were placed for 15 min on PBS, 0.1% (v/v) Triton X-100, 0.2% (v/v) Gly, at pH 7.2. After washing in the PBS-Triton medium, nonspecific sites were saturated for 45 min by goat serum in PBS, 0.2% (v/v) Triton X-100, 0.2% (v/v) Tween, 0.1% (w/v) BSA, and incubated overnight with the antibodies against the water channel protein VM23 and the vacuolar H^+-ATPase in 1/50 dilution. After washing in PBS the sections were placed for 40 min on TBS, pH 8.2, 0.2% (v/v) Tween, 0.2% (v/v) Triton X-100, 1% (w/v) BSA, and goat serum. The secondary antibody (goat anti-rabbit IgG, Biocell TEBU, Le Perray en Yvelines, France), 1/40 diluted and labeled with 15-nm gold particles, was applied for 2 h. The samples were then washed in TBS and deionized water and were subsequently treated for contrast in uranyl acetate at saturation in water (8 min) and in lead citrate (3 min).

Three types of controls were done: (a) omission of the first antibody (VM23 or H^+-ATPase), (b) treatment with the preimmune serum (VM23 or H^+-ATPase), and (c) saturation of the antibody with the purified protein (VM23).

Observation and Measures

Observations were made under 80 kV with a 100C microscope (Jeol, Tokyo, Japan). Samples for quantification of gold particles included tissues from three independent chemical fixations or two HPF treatments. For each procedure at least four immunoreactions were performed. The mean number of gold particles was given per 10 μm of vacuolar membrane, with the total measured length obtained from six pulvini for each treatment. Gold particles can easily be detected on tannin vacuoles by their higher electron opacity.

RESULTS

Cross-Reactivity of Antibodies

Immunoblot analysis of microsomes from *M. pudica* pulvini showed that polypeptides in the microsomes specifically cross-reacted with antibodies directed against VM23, a γ-TIP of the radish tonoplast, and with antibodies directed against the catalytic α-subunit of the H^+-ATPase of *M. crystallinum* (Fig. 2). We therefore proceeded to locate and quantify these proteins at the cellular level using protocols that preserve the ultrastructure of the pulvinus as well as the immunoreactivity of the proteins.

HPF Treatment Compared with Chemical Fixation

A conventional chemical fixation method that enables immunolocalization of plasma membrane H^+-ATPase in motor cells has already been developed in our laboratory (Fleurat-Lessard et al., 1995). However, to prevent the possible alteration of membranes by chemical fixatives, we also used the technique of ultrarapid fixation by HPF followed by freeze substitution (Staehelin and Chapman, 1987; Meyer et al., 1988). We established a protocol by infiltrating the aerenchyma with hexadecene, which renders the tissue resistant against the applied pressure (Studer et al., 1989). Using this technique, we succeeded in

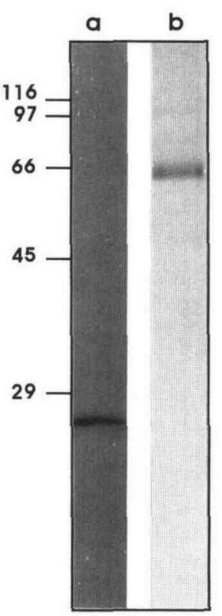

Figure 2. Microsomal fractions isolated from *M. pudica* pulvini were processed for immunoblotting with 1/750 diluted anti-radish VM23 and 1/1000 diluted anti-*M. crystallinum* vacuolar H^+-ATPase antisera as described in "Materials and Methods." A band with an apparent molecular mass of 23 kD corresponds to the aquaporin (a) and the band with an apparent molecular mass of 66 kD corresponds to the vacuolar H^+-ATPase (b). VM23 and H^+-ATPase were detected in 40-μg total proteins of microsomal fractions.

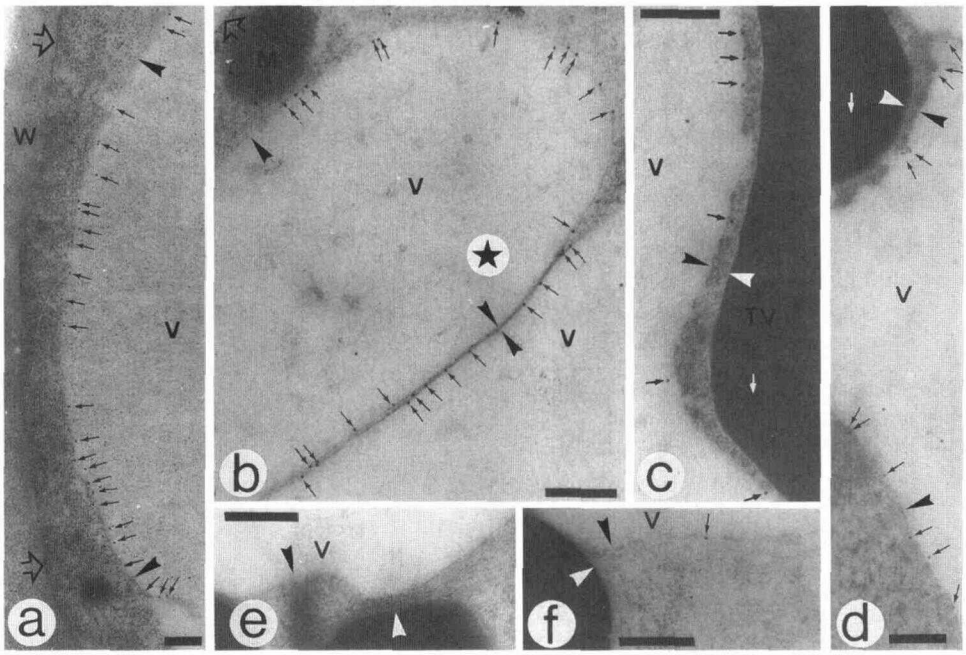

Figure 3. Distribution of vacuolar aquaporin and H^+-ATPase in HPF-treated mature pulvini. Sections were incubated with 1:50 diluted antibodies followed by goat anti-rabbit IgG-15-nm gold (see "Materials and Methods"). a, The immunolabeling (black arrows) for aquaporin is high in the membrane (black arrowheads) of the aqueous vacuole (V), namely in areas enclosing small profiles (asterisk in b). This labeling is denser than in the membrane (white arrowheads) of the tannin vacuole (TV) for both aquaporin (c) and H^+-ATPase (d). Nonspecific labeling using the antibody saturated by the purified VM23 protein (e) or a preimmune serum to vacuolar H^+-ATPase (f). Note the absence of recognition on the plasma membrane (open arrows) and fibrillar material in the aqueous vacuole (a). M, Mitochondria; W, wall. Bars, 0.5 μm.

rapidly freezing the pulvini (after closure) without disintegration of the tissues. As can be seen in Figure 1, c and d, vitrification as well as conservation of the motor cells' ultrastructure were satisfactory. However, under these conditions the petiole did not remain intact, and more work is required to optimize the freezing method for this organ.

Distribution of Aquaporins and H^+-ATPase in the Two Vacuole Types of *M. pudica*

Immunogold labeling of pulvini subjected to HPF revealed that in motor cells, antibodies directed against the VM23 protein (Fig. 3, a–c), as well as those recognizing the A-subunit of the vacuolar H^+-ATPase (Fig. 3d), were observed preferentially in the tonoplast of the aqueous vacuole. In cortical cells of young pulvini, γ-TIP labeling was also detected in the tonoplast of this vacuole, and a pronounced difference in tannin vacuole labeling was observed both for aquaporin and H^+-ATPase (Table I).

This difference of labeling density in the two vacuole types was also observed in organs fixed using conventional chemical methods: mature pulvini (Fig. 4a), young pulvini (Fig. 4c), and petiole (Fig. 4d).

Treatment of the ultrathin sections with VM23 antibodies previously incubated in the presence of a 10-fold excess of

Table I. *Distribution of gold particles in the membrane of aqueous (V) and tannin (TV) vacuoles in Mimosa pudica cortex using γ-TIP and V-H^+-ATPase antibodies*

Counts were made as described in "Materials and Methods" in HPF/freeze-substituted pulvini. Sixty aqueous or tannin vacuoles were analyzed and the total membrane length was at least 400 μm in each vacuole and cell type. Mean is given for 10 μm of membrane ± SE.

Cell Type	Motor Cell (Mature Pulvini)		Nonreactive Cell (Young Pulvini)	
	V	TV	V	TV
Gold particle number (γ-TIP antibody)	9.98 ± 1.02	0.85 ± 0.16	2.90 ± 0.46	0.81 ± 0.56
Control (saturation of the γ-TIP antibody by the purified protein)	0.60 ± 0.18	0.70 ± 0.31	0.19 ± 0.15	0.85 ± 0.42
Gold particle number (V-H^+-ATPase antibody)	5.50 ± 0.88	0.51 ± 0.17	1.53 ± 0.22	0.68 ± 0.21
Control (preimmune serum)	0.40 ± 0.22	0.10 ± 0.05	0.08 ± 0.05	0.14 ± 0.14

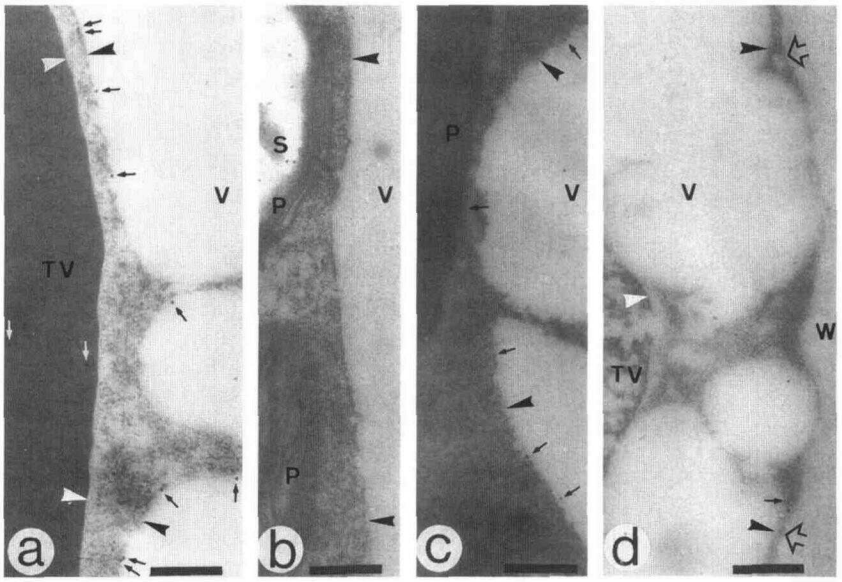

Figure 4. Distribution of aquaporin in the vacuolar membranes of chemically fixed organs. The labeling (black arrows) is denser in the aqueous (V) than in the tannin vacuole (TV) in mature pulvini (a). There is no specific labeling when the antibody is saturated by the purified VM23 protein (b). The number of gold particles is low in young pulvini (c) and very low in petiole (d). White arrows, Nonspecific labeling in the tannin vacuole; P, plastid; S, starch; other legends as in Figure 3. Bars, 0.5 μm.

purified protein results in a very low nonspecific labeling (Figs. 3e and 4b), indicating that the reaction observed in the presence of VM 23 antibody alone can be attributed to a specific interaction of VM 23 antibodies with the corresponding protein in the vacuolar membrane. Furthermore, only very low nonspecific labeling was observed using preimmune serum. The same occurred for H^+-ATPase (Fig. 3f), and no label was observed when the primary antibody was omitted (not shown).

A further control consisted in the determination of gold particles per unit length (10 μm) of plasma membrane in the corresponding cells. The values (0.8 ± 0.2) were similar to that of the other controls (Tables I and II).

Quantification of Aquaporins and H^+-ATPase in Motor and in Nonreactive Organs

In organs prepared by the HPF method, quantification revealed that there was a pronounced difference between the number per length unit of membrane of gold particles in aqueous vacuoles of mature and young pulvini. For the aquaporin (VM 23), in motor cells about 10 gold particles per 10 μm were associated with the tonoplast of the aqueous vacuole, but only 0.8 particle per 10 μm with the tonoplast of the tannin vacuole (Table I). In young nonreactive cells, 3 gold particles per 10 μm were associated with the tonoplast of the aqueous vacuole as compared with 0.8 for the tannin vacuole. The latter was similar to control values.

Similar results were obtained with tissues fixed by conventional chemical methods (Table II). For the motor cells of functional pulvini, 9.4 gold particles for the aqueous vacuole and 1.6 for the tannin vacuole, and 3.5 and 0.9 in the case of the young pulvinus, for the tonoplast of the aqueous and the tannin vacuole, respectively. Furthermore, we quantified the labeling in the vacuolar membranes of the petiole and observed an even lower labeling than in cortical cells of young pulvini (2.3 and 1.4 gold particles per 10 μm for the tonoplast of the aqueous and the tannin vacuole, respectively) (Table II).

Observations similar to those for the aquaporin were made for the vacuolar H^+-ATPase. Movement of the pulvinus is accompanied by a 3.5-fold elevation in the amount of vacuolar proton pump protein. As for VM 23, this increase was observed only in the aqueous vacuole but not in the tannin vacuole (Table I). Chemically fixed material showed that the petiole vacuole contains only 60% H^+-ATPase compared with young pulvini.

DISCUSSION

Structural Preservation Using HPF

Fast turgor changes in plant cells are accompanied by structural changes in cellular membrane network. In such a

Table II. *Distribution of gold particles in the membrane of aqueous (V) and tannin (VT) vacuoles of M. pudica chemically fixed cortical cells*

Legend is as in Table I.

Cell Type	Motor Cell (Mature Pulvini)		Nonreactive Cell (Young Pulvini)		Nonreactive Cell (Petiole)	
	V	TV	V	TV	V	TV
Gold particle number (γ-TIP antibody)	9.42 ± 0.43	1.66 ± 0.17	3.56 ± 0.18	0.90 ± 0.13	2.27 ± 0.18	1.39 ± 0.32
Control (saturation of the γ-TIP antibody by the purified protein)	1.03 ± 0.24	0.23 ± 0.16	1.06 ± 0.24	0.34 ± 0.28	0.75 ± 0.21	0.25 ± 0.21

system, conventional chemical fixation procedures may produce artifacts, since penetration and reaction of the fixative is often slower than the structural changes. Rapid freezing techniques preserve biological structures in a form that closely resembles the living state. Furthermore, because of the subsequent freeze substitution step, proteins may remain more intact and therefore show a higher reactivity to antibodies. Because of large intercellular spaces that cannot resist the application of the pressure, this technique may lead to tissue disintegration and has, therefore, not yet been applied routinely to plant tissues. However, in some cases it has been shown that HPF results in excellent structural preservation (Staehelin et al., 1990; Studer et al., 1992; Kaneko and Walther, 1995). Using a modification of the method published by Studer et al. (1989), we were able to freeze *M. pudica* pulvini while preserving good cellular structure (Fig. 1, c and d).

Distinct Role for the Two Types of Vacuoles in *M. pudica* Motor Cells

Cells of *M. pudica* contain two different types of vacuoles. One vacuole, which develops earlier during cell differentiation, contains large amounts of tannins. This vacuole has been postulated to function as a Ca^{2+} store (Toriyama and Jaffé, 1972; Moyen et al., 1995), which, after mobilization, may be the secondary signal for ion fluxes, and subsequently water fluxes, from the vacuole and cytosol to the apoplast. The second aqueous vacuole develops later; it does not contain tannins and, in adult cells, is much larger than the tannin vacuole.

We suggest that water fluxes during leaf movement of *M. pudica* occur mainly across the large, aqueous vacuole, since about 10 times more aquaporins per length unit of vacuolar membrane could be detected as compared with the tannin vacuole. However, the change in cell volume is associated with a change in shape of both vacuole types (Fig. 1, a and b). While the aqueous vacuole shrinks, the tannin vacuole reorganizes into a branched network (Campbell and Garber, 1980). The rapidity of this latter process, which considerably increases the surface of the tannin vacuole, may be associated with the presence of contractile proteins in the cytosol (Fleurat-Lessard et al., 1993).

Furthermore, relative vacuolar volumes should be taken into account. The aqueous vacuole has a diameter at least 3 times that of the tannin vacuole and therefore a volume 27 times larger. Most of the water and solutes are therefore localized in the aqueous vacuole, and it is turgor changes that result in larger volume variations.

The observation that the aqueous vacuole contains not only a higher density of aquaporin labeling but also about 10 times as much H^+-ATPase as the tannin vacuole is a further hint as to the importance of this vacuole in turgor regulation. ATPase presence indicates the capacity of generating steep electrochemical gradients that can induce rapid fluxes across the vacuolar membrane by secondary energized transport processes. An increase in vacuolar ATPase activity and protein can be observed in response to salt treatment during the transition of *M. crystallinum* from the C3 state to CAM metabolism (Haschke et al., 1989; Ratajczak et al., 1994, 1995). However, the changes were not as dramatic as observed here for *M. pudica* motor cells.

Vacuolar Membrane Aquaporins and H^+-ATPase Related to Pulvinus Movement

It has become increasingly clear that water flux across a biological membrane not only reflects a passive diffusion across the lipid bilayer but is also facilitated by special proteins, the aquaporins, which may play a pivotal role in osmoregulation. In most cases, the diffusional water permeability (permeability coefficient < 0.01 cm s^{-1}) may be sufficient for water transport from one compartment to another. Thus, in Arabidopsis expression of γ-TIP was shown to occur mainly in elongating cells (Ludevid et al., 1992).

The motor cell shrinkage in *M. pudica* requires a fast K^+ efflux through outward rectifying channels, followed by water fluxes from the symplast to the apoplast. Potassium concentrations reported so far for Leguminosae pulvini are in the range of 200 to 500 mM (Satter and Galston, 1981). In our culture conditions, data indicate an overall K^+ concentation of 220 mM for *M. pudica* (Roblin and Fleurat-Lessard, 1983). It has been reported that dark-induced shrinkage of Leguminosae motor cells induces a loss of 1/4 of the K^+ content into the apoplast (Satter and Galston, 1981; Moran, 1990). In *M. pudica* an increase from 27 to 200 mM of K^+ was noted in the apoplast during the bending of pulvini (Kumon and Tsurumi, 1984), and the variation of the osmotic pressure in motor cells may attain 1 MPa (Fleurat-Lessard, 1988). Additionally, observations in the extensor (P. Fleurat-Lessard and N. Franqne, unpublished data), where the motor cell volume decreases by up to 25% during leaflet closure, indicate a high water permeability of both vacuolar and plasma membranes. The membrane in the aqueous vacuole of adult pulvini, able to exhibit fast movements, contains about three times more immunolabeled aquaporins than the tonoplast of the young pulvini and even four times more than the petiole (Tables I and II). This is a hint that fast turgor changes are dependent on the presence of proteins that facilitate water exchanges between different compartments.

Accumulation of ions within the vacuole that lead to motor cell turgor require a large amount of energy. The normal equipment of the vacuole membrane appears to be insufficient to energize these ionic fluxes. Therefore, the concomitant rise of H^+-ATPase to energize ion fluxes, and vacuolar aquaporins, which are responsive for the water fluxes, seems to be a prerequisite to pulvinus differentiation and movement.

In conclusion, our data suggest that the differentiation of motor cells and the motile function of pulvini are accompanied by an increased expression of both vacuolar aquaporin and H^+-ATPase. It appears that the aqueous vacuole type is preferentially responsible for ionic and water fluxes. In our opinion, however, the most intriguing question is how two vacuoles develop differently within the same cell. Therefore, it would be interesting to investigate the mech-

anisms involved in the correct targeting of membrane proteins to the different vacuole types. An analogous case has recently been described where it was shown that in barley two different functionally vacuolar types store lectin and aleurine (Paris et al., 1996). The vacuolar type containing lectin is surrounded by a membrane containing α-TIP, whereas that storing aleurine contained TIP-Ma27, a member of the γ-TIP family. As cells develop large vacuoles, the two compartments merge and the new vacuolar membrane contains only TIP-Ma27. It will be interesting to investigate whether the tannin vacuole contains α-TIPs and therefore belongs to a plant cell, where the two different vacuolar compartments do not fuse during cell development.

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